RESEARCH ARTICLE

Skin Disease in Companion Animals: A Nutritional Impact

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ABSTRACT

The skin can provide cues and clues to underlying systemic health. The appearance of skin and coat is influenced by nutrients such as proteins, fats, minerals and vitamins. Any imbalance in these can disrupt the barrier function and immune protection provided by the skin. Polyunsaturated fatty acids such as omega 6 and omega 3 are found to be an important dietary essential. Omega 3 fatty acids had more effect on pruritic skin disorders compared to omega 6 due to its more anti-inflammatory effect. For improvement of both quality and quantity of fur, supplementation of lysozyme, Zn along with linoleic acid can be done. When combinations of nutrients such as B complex vitamins along with amino acids are given can improve skin barrier properties and reduce TEWL in dogs. Topical preparation of PUFA and essential oil was found to be safe treatment compared to oral supplementation due to its less adverse effects to correct cutaneous changes. Through chemical analysis if the diet is not adequate or nutritionally balanced various supplements can be included if owner wishes a healthy coat for their pets. Healthy pets being fed high quality commercial foods may not benefit from additional supplementation however additional research is needed to determine optimum nutrition for dogs and cats with various types of diseases.

Keywords: Omega fatty acids, Vitamin, Protein, Healthy skin

INTRODUCTION

The skin is the largest organ of the body and depending on species and age, representing 12-24% of an animal's body weight. The skin has many functions, including serving as an enclosing barrier and providing environmental protection, regulating temperature, producing pigments and Vitamin D and sensory perception. The skin and the coat accordingly are the mirror of animal health and nutrition provided.The health of a pet's skin and hair coat can be affected by a variety nutrient most importantly protein, vitamin A, vitamin E, the essential fatty acids (EFAs) and zinc. Dogs and cats that are consuming high quality, complete, balanced pet foods are unlikely to suffer from a serious deficiency or excess of any of these nutrients. However, feeding a poorly formulated or stored commercial food, or preparing a homemade diet that is not correctly balanced, can contribute to skin disorders. In addition, any metabolic or functional disease that affects a pet's ability to digest, absorb, or use nutrients can cause secondary nutrient imbalances that may manifest as dermatoses. Failure in the skin's immunity can lead to a variety of problems, ranging from a low-grade skin infection or infestation to severe microbial disease and life-threatening neoplasia. The maintenance of a healthy skin and coat is therefore a primary objective in the maintenance of bodily health and warrants special attention both from owners and veterinary surgeons.

Anatomy of Skin

Adult skin is composed of three layers:
- The epidermis
- The dermis
- The hypodermis or sub cutis

Epidermis

The epidermis is the outermost layer of the skin. All epidermal cells are derived from the basalmembrane *(stratum basale)*, which is composed primarily of keratinocytes although other cells,including melanocytes, are also present. Keratinocytes produced by the basal membranedifferentiate sequentially to form:
- The prickly cell layer *(stratum spinosum)*
- The granular layer *(stratum granulosum)*
- The clear layer *(stratum lucidum)* only in the footpads and, to a lesser extent, the nose
- The outermost horny layer *(stratum corneum)*, which consists of non-nucleated, fully keratinized cells

Keratinocytes have many functions, including:
- Production of keratin, a fibrous, sulphur-containing protein
- Production of a lipid secretion which has an integral role in the regulation of the *stratumcorneum*barrier function and desquamation.

Skin Barrier Complex

The epidermis of skin barrier especially stratum corneum is composed of two main parts i.e., the cells or corneocytes and the intercellular lamellar lipids. The epidermal lamellar lipids comprise mainly of ceramides, sterols and fatty acids. Nutrition helps to improve ceramide production and therefore strengthen the skin barrier function. This lipid and cellular barrier not only serves to maintain hydration but also help to keep chemicals, allergens, microbes out. Transepidermal water loss (TEWL) describes the total amount of water lost through the epidermis by passive diffusion. It is a normal process but when it rises too high it can lead to skin dehydration and cause further infection

Dermis

The dermis supports the epidermis and consists of a matrix of collagen and reticular and elasticfibers in a ground substance of chondroitin sulfate and hyaluronic acid. Cells present in this layerare fibroblasts, mast cells, and histiocytes, although other cell types may be present in certaindisease conditions. The tensile strength and elasticity of the skin is largely attributable to the dermis, which is also responsible for the maintenance and repair of the skin and modifies the structure and function of the epidermis.

Hypodermis

The underlying hypodermis is made up of loose connective tissue, elastic fibers, and variableamounts of fat. This layer acts as energy reserve, as an insulator, and as protective padding andmaintains the body contours. (Fig. 1)The hair is housed in an epithelial pit called a hair follicle and is attached, via the hair bulb, to thedermal papilla in the base of the follicle. It is here that mitotic activity occurs, which leads to theproduction of the hair matrix.

Parameters Used To Assess Skin And Coat Condition In Dogs And Cats

There is a strong perception that skin and coat condition is an indicator of an animal's general well-being and the nutritional adequacy or superiority of its diet.There have been many reports in the literature of long termnutritional deficiencies causing a myriad of problems that include excess scale, crusting, erythema, pruritus, greasy skin, and alopecia(Marsh, 1999) (Table 1) One of the most commonly used methods of assessment of healthy skin and coat is sensory evaluation panel. The sensory panel selected for the evaluation of animal coat condition is known as the Quantitative Descriptive Analysis (QDA). To facilitate accurate and unbiased assessments of a group of test animals, a number of considerations regarding thepresentation of animals to the panelist exist. These include:

- Prior to evaluation, the coat should be combed evenly all over in a standardized manner in order to eliminate the influence of the way in which the coat is lying. A clean comb should be used for each animal, which should then be degreased in alcohol or methylated spirits.
- Each animal should be assessed under identical conditions, preferably indoors, to provide an evenly and consistently lit area.
- Between each assessment the assessorsshould wipe their hands with an alcohol tissue or wash them with soap and water.
- Each animal should be examined by the assessors as a group but, to avoid any effect of changes in coat texture during handling, the assessors should touch each animal in the same sequence.
- Each animal should be identified only by an unmemorable number, not by name. This prevents any subconscious favouritism and prevents recall of previous score.
  Table 2 describes the influence of various nutrients on skin health.

Essential Fatty Acids

Polyunsaturated fatty acids(PUFA) are needed to maintain normal structure and function of the skin. As components of cell membrane phospholipids and precursor for variety of regulatory compounds, the EFAs maintain the health and integrity of epithelial tissue in the body (Bauer *et al.*, 2008). The omega 6 fatty acids that are considered to be essential nutrients include linoleic acid in dogs and linoleic acid and arachidonic acid in cats. In addition, cats exhibit low D-6 desaturase activity and cannot meet their physiologic requirement for arachidonic acid through biotransformation from linoleic acid (Rivers *et al.*, 1975). Consequently, both linoleic acid and arachidonic acid are considered essential nutrients for cats. LA is specifically responsible for maintaining the cutaneous water permeability barrier, while AA, as a prostaglandin precursor, is needed for normal epidermal proliferation (Lenox and Bauer,2013).The long chain omega 3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), support normal cell membrane fluidity and also have anti-inflammatory and immunostimulatory property. Rees *et*

al., 2001 conducted a study which involved supplementing 18 normal dogs with flax seed (FLX) and sunflower seed (SUN) and evaluating their effects on skin and hair coat condition scores and serum polyunsaturated fatty acids (PUFA) concentrations. Skin and hair coat were evaluated in a double-blinded fashion using a numeric scoring system and serum PUFA concentrations were determined. The purpose of this study was to determine whether FLX vs. SUN supplementation under controlled conditions of total dietary fat would improve skin and hair coat condition scores and dogs were randomly allotted to either the sunflower or flax seed group (nine dogs each) and food was withheld overnight prior to any blood sample collections. The dogs were fed a calculated amount of the diets based on their beginning body weight. All dogs were fed the basal diet for a two week acclimation period. Immediately after this time(day 0), the dogs were fed using the same staggered feeding schedule with the basal diet now supplemented with its respective oilseed for 84 additional days. All dogs were weighed regularly during the feeding period and finally it was concluded that a 1- month supplementation with either flax seed or sunflower seed in dogs provides temporary improvement in skin and hair coat. These changes appeared to be associated with increased serum 18 carbon PUFA.

Protein

Hair is composed of 95% protein, which is rich in the sulphur-containing amino acids, methionine and cysteine. Normal growth of hair and keratinization of the skin thus create a high demand for protein and may account for between 25 and 30% of the animal's daily protein requirement (Scott *et al.*, 2001). Failure to meet this demand results in the cutaneous manifestations of protein malnutrition including brittle, depigmented hair, which is easily shed and slow to regrow, excessive scaling and thin, inelastic and hyperpigmented skin. Aromatic amino acids like tyrosine and phenylalanine are essential for synthesis of melanin responsible for hair pigmentation- pheomelanin (red, brown) and eumelanin (black). An insufficient intake of tyrosine results in change of colour. A diet that does not contain enough tyrosine and/or phenylalanine to ensure optimum melanin synthesis causes reddening of the hair in black cats (Yu *et al.*, 2001).

Zinc

Zinc is an important mineral for many critical biological functions, such as cellular metabolism, which is crucial for the maintenance of a healthy coat and skin (Patrick, 2010). Zinc is particularly important in the rapidly dividing cells of the body, such as the epidermis. It is essential for the biosynthesis of fatty acids, has a role to play in the body's inflammatory and immune systems, and is also involved in the metabolism of vitamin A. Julia *et al.*, 2009 through their studies hypothesised that Zn act as a cofactor for enzyme required for fatty acid metabolism.Significantly increasing the concentrations of particular nutrients above the minimal requirements will have on the skin and hair coat quality of dogs receiving a complete balanced diet. Based on these Marsh *et al.*, 2000 conducted a study at Waltham Centre for Pet Nutrition using 32 adult dogs ranging from 1 to 10.5 years of age. The study consisted of 9 week prefeed phase followed by 9 week test feed phase. During prefeed phase all dogs were fed standard diet. During the test phase all the dogs were divided into four panels based on coat condition.
Panel 1: Standard diet alone
Panel 2: Standard diet plus additional linoleic acid (total dietary linoleic acid=3.6 g/MJ)
Panel 3: Standard diet plus additional zinc (total dietary zinc=23.9 mg/MJ)
Panel 4: Standard diet plus additional linoleic acid (3.6 g/MJ) plus zinc (23.9mg/MJ)
After test phase they were assessed for skin and coat condition.Finally concluded that supplementation of diet with zinc or LA alone does not show much significant in coat gloss and coat scale when compared to control diet while combination of zinc and LA through diet shows significant difference. Zinc and LA combination able to change either the rate of production of sebum or physical properties or chemical composition of substance leading to an increase in perceived gloss.

Vitamins

Vitamin A

Vitamin A (retinol and its derivatives) as reported by David *et al.*, 1999 has many physiological functions that are involved in normal epithelial cell differentiation and particularly important for keratinisation process. Cats require a dietary source of preformed retinol because unlike dogs, they are unable to utilize the retinol precursor beta carotene. Green *et al.*, 2012 through their study reported that cats are capable of converting beta carotene to active vitamin A by administering them oral dose of 5mg/kg BW or 10mg/kg BW beta carotene. The appearance of retinol in plasma indicates that cats are capable of converting beta carotene to active vitamin A.

Vitamin E

Vitamin E is a natural antioxidant and together with Se is important for maintaining stability of cell membrane. As a free radical scavenger, it protects cells from potentially damaging effect of toxic oxygen radicals whose major source is lipid metabolism. Vitamin E is a generic term covering two classes of fat soluble vitamins: tocopherol and tocotrienols. Alpha- tocopherol is the most widespread form of vitamin E in animal foods and organism. It is the form with greatest biological antioxidant activity in cell membrane.

Vitamin B Complex

Involved as a cofactor of enzymes required for metabolic functions like carbohydrate, proteins, and fats. Brewer's yeast constitutes a natural source of B complex vitamins. All B group vitamins help at different level to enhance the lustre of hair coat. Biotin is essential for skin integrity. Insufficient intake of folic acid leads to hair loss. Reduced activity of biotin dependent propionyl CoA carboxylase enzyme indicates biotin deficiency that substantiate biotin requirement by the cats (Carrey and Morris, 1977). Biotin deficiency produced mainly by feeding a diet containing dried egg white. After receiving it shows characteristic dermal lesions. Frank *et al.*, 2014 shown that certain nutritional components when fed in combinations can influence skin barrier properties as well as lipid synthesis. These components stimulate ceramide synthesis and reduce TEWL. It was said that B complex vitamins along with amino acids reduce TEWL in dogs. According to Watson *et al.*, 2006 combination of nutrients could be used to improve skin barrier function i.e., diet supplemented with pantothenate, choline, nicotinamide, histidine, inositol significantly decreases TEWL in dogs which is measured by Evaporimeter EP-2.

Nutritional Deficiencies

Fatty Acid Deficiency

Dietary deficiencies of essential fatty acids are uncommon but may occasionally occur in dogs and cats that are fed poor quality, low fat dry foods or inappropriately formulated home-prepared diets. Levels of PUFA may also be depleted in food after oxidative damage resulting from prolonged storage or in cases in which antioxidants such as vitamin E are included in inadequate amounts. Cutaneous signs may be apparent within 2–3 months when a deficient diet is fed. Initially, surface lipid production is decreased to produce a dull, dry coat with accompanying fine scale. Prolonged deficiency results in alopecia, a greasy skin particularly on the ears and between the toes, and secondary pyoderma (Watson, 1998). Correction of the deficiency may be achieved by changing to a higher fat, premium quality diet, by the addition of food oils to the diet or by the administration of proprietary fatty acid supplements. One teaspoon (5 mL) of a mixture of vegetable oil and animal fat or fish oil per can or cup (225 g) of food is an effective supplement (Scott *et al.*, 1995). However, increasing the dietary PUFA content simultaneously increases the requirement for vitamin E and may also increase the requirement for other vitamins and minerals involved in fatty acid utilization. According to Muller and Kirk, 2012 when dietary fat supplementation is impossible,

topical application of EFAs may be of some benefit. Studies show that topically applied fat can correct the cutaneous changes of fatty acid deficiency. Spot-on formulation containing polyunsaturated fatty acids and essential oils are effective for treating skin conditions like atopic dermatitis, feline miliarydermatitis

Vitamin A Deficiency

Both deficiency and excess of vitamin A can give rise to cutaneous lesions of hyperkeratinisation and scaling, alopecia, poor hair coat and increased susceptibility to microbial infections. Hyperkeratinization of the sebaceous glands can result in occlusion of their ducts and the formation of firm, popular eruptions(Watson, 1998). Vitamin A-responsive dermatosis in dogs is a rare condition characterized by an abnormality of cornification that occurs in adult dogs, predominantly Cocker Spaniels, but also in Labrador Retrievers and miniature Schnauzers. The skin lesions typically presentas multifocal areas of alopecia, erythematous plaques,scaling, crusting, follicular plugging with frond-likekeratinous debris mainly localized on the ventral andlateral chest. The histopathologic hallmark of vitamin A-responsive dermatosis is orthokeratotic epidermal and predominantly follicular hyperkeratosis. Therapy consists of oral supplementation of 10,000 IU of vitamin A (retinol), once daily with food. A response and clinical resolution to therapy should be seen within 3 to 8 weeks(Patrick, 2010).

Vitamin E Deficiency

Vitamin E is a natural antioxidant and, together withselenium, is important for maintaining stability of cell membranes.As a free radical scavenger, it protects cells from the potentially damaging effects of toxic oxygen radicals, whosemajor source is lipid metabolism. The dietary requirement ofvitamin E, therefore, is linked to the dietary intake of PUFA,and high fat diets can induce a relative deficiency of vitaminE. Similarly, levels of vitamin E may be depleted after theoxidation of fat during processing or prolonged storage of food. Pancreatitis or steatitis is associated with a deficiency of vitamin E incats that are habitually or exclusively fed high fat diets, particularlycanned red tuna or other oily fish(Watson,1998). To prevent vitamin E deficiency in cats fed commercial sold diets, the Association of American Feed Control Officials (AAFCO) recommends that fish oil containing diets for cats should be supplemented with 10 IU of vitamin E for every gram of fish oil per kg diet (Hendriks*et al.*, 2002).

Vitamin B Complex

Biotin Deficiency

Biotin deficiency can result in facial and periocular alopecia in dogs that can progress to more generalized crusting lesions. This condition mayoccur due to feeding large amounts of raw egg whites which contain avidin, a protein that bindsbiotin and prevents its gastrointestinal absorption.

Riboflavin Deficiency

Produces cheilosis in addition to seborrhea but willnot occur if the diet contains meat or dairy products (Scott *et al.*, 2001).

Niacin Deficiency

Niacin issynthesized from tryptophan by all animals except cats, and a deficiency is possible only when the diet islow in animal protein and high in corn or other cereals thatare a poor source of tryptophan. A deficiency results in pellagra(humans) or "black tongue" (dogs), with ulceration of mucousmembranes, pruritic dermatitis of the hind legs and ventral abdomen.Table 3 describes the minimum requirement of nutrients to maintain good health & coat condition

CONCLUSION

One of the most powerful methods of establishing andmaintaining good skin and coat condition is to ensure that the doghas a well-structured diet. Most commercially available preparedpetfoods provide a complete and balanced diet with respect to thenutrients required to maintain the health of the animal. Nutritional imbalances can occur in animals fed high-qualitycomplete diets when the diet is stored under inappropriateconditions, when it is supplemented with individual nutrients, or inanimals fed home-prepared diets. Problems with the skin and coat condition of companion animals are among the most frequent reasons for veterinary consultation. On presentation of signs indicative of nutritionalimbalance such as scaling or a dull brittle coat, it is important toestablish what the animal is routinely fed. The ownersshould understand the implications of supplementing their pet's dietin any way and the possible impact that this may have on skin andcoat condition. Most of the owners commonly give treat and nutritional supplements to their dogs and cats. Many supplements include a variety of vitamins, minerals, fatty acids and others contain probiotics, herbs, and antioxidants. Healthy pets being fed high quality commercial foods may not benefit from additional supplementation however additional research is needed to determine optimum nutrition for dogs and cats with various types of diseases. A balanced diet with adequate amino acids, vitamins, minerals and fatty acids is essential for maintenance of healthy skin and is important for complete cure during the clinical management of most of the dermatological disease conditions in dogs and cats.

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Table 1: **Parameters used to assess skin and coat condition**

QDA Parameter	Parameter Definition	Method of Evaluation
Gloss	Reflection of light from the coat	Visual assessment performed before touching the animal, as texture can influence the assessor
Softness	Flexibility and compliance of the hair	Run fingers through the full thickness of the coat
Optimum Coat Feel	The absence of a greasy or a dry feel of the coat	Run fingers through the full thickness of the coat
Scale	Visible dandruff on the skin or hair	Lift hair in the opposite direction of growth and examine skin and hair for the appearance of white flakes

(Marsh, 1999)

Table 2: Nutrients that can influence skin barrier function

Polyunsaturated fatty acids (PUFA)	Included in sebaceous gland lipids produced by glands that forms the surface film hydrolipidic
Proteins	Sufficient intake of all indispensable amino acids required for maturation of keratinocytes.
Vitamin A	Essential for the differentiation of keratinocytes and corneal layer formation.
Biotin	Metabolism essential for PUFA.
Vitamin C	Also play a key role in lipid corneal layer film
Zinc	A zinc supplement reduces fluid loss and zinc deficiency causes abnormal formation of the cornea.
Nicotinamide	Increase the concentration of ceramide and free fatty acids in the corneal layer.
Water soluble Vitamins	Participate in the metabolism of polyunsaturated fatty acids.
Vitamin E	Secreted by the sebaceous glands, allows limiting fatty acid oxidation

(Patrick, 2010)

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Table 3: Requirement of Nutrients to Maintain Good Health & Coat Condition

Nutrients	Minimum Requirement (Per MJ)
Proteins	9.6g
Lipid	3.3g
Linoleic acid	0.66g
Zinc	3mg
Copper	0.3mg
Vitamin E	1.8 IU
Vitamin A	245.5 IU
Niacin	0.72mg
Riboflavin	0.15mg

(Source: WCPN nutritional guidelines for dogs)
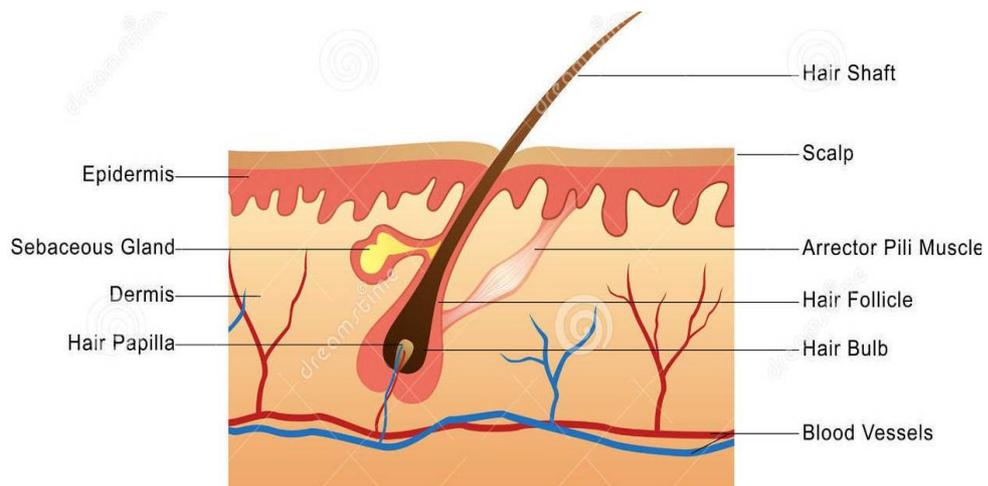
Figure 1: Anatomy of skin
(Stanley, H.D and Stanlet. D. 1996)

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RESEARCH ARTICLE

Nutritional Composition of the Egg Fruit (*Pouteria campechiana* (Kunth) Baehni): A Potential Alternative Source of Nutraceuticals

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ABSTRACT

Characterization of the nutrient composition and nutraceutical potential of the egg fruits, *Pouteria campechiana* was analyzed. Fruits were collected from the Palode hills of Thiruvananthapuram district, Kerala and analyzed for carbohydrates, proteins, lipids, essential micronutrients, vitamins and antinutrient factors. Proximal analysis revealed substantial amount of reducing sugar (1.38 mg/g) and total carbohydrate   (3.73 mg/g). Total proteins (2.01 mg/g), lipids (0.99mg/g) and essential vitamins (ascorbic acid, β-carotene and tocopherols) were found in optimal quantity. Further, fruits of *P. campechiana* appeared to be potential sources for selected micronutrients such as copper, iron, manganese, zinc, potassium, calcium, magnesium and also essential amino acids. Specifically, compared to common fruits consumed in many parts of India, *P. campechiana* appears to be potential source of iron (0 .126mg/g), magnesium (1.3 mg/g) and copper (0.004 mg/g) and would contribute towards meeting the recommended daily requirement of these against malnutrition. Zinc content (0.005 mg/g) was marginally lower. The proven antioxidant such as flavonoid (3.37 mg/g), phenols (2.26 mg/g) and antinutrients like phytic acid (1.5μg/g) and tannic acid (0.69 μg/g) at marginal levels suggest the nutraceutical potentials of the fruit. Significant level of nutrients, micronutrients and antioxidants suggest that egg fruits may potentially be used as food source or fortifying ingredient for locally processed foods to reduce deficiencies in selected, commonly deficient nutrients. Thus, *P. campechiana* is a promising species for cultivation as backyard planting especially farming systems suffering from crop loss, food shortage and chronic malnutrition.
Key words: Antioxidants; mineral; protein; phenol; egg fruit

INTRODUCTION

Consumption of traditional types of fruit provides ideal health benefits because of their potential source of phytochemicals, nutrients and prevents many diseases. Many epidemiological studies have proved that fruit and leafy vegetable consumption was associated with health benefits through reducing the risk of cardiovascular disease, Parkinson's disease and cancer. Kerala is known for its diverse tropical and sub-tropical agro-climatic conditions, which are conducive to grow different types of fruits and vegetables. India stands second (27.8 million metric tons) in fruit cultivation and production after China. Knowledge available on the edible as well as therapeutic characters associated with wild fruits were isolated and complete data on their nutritional composition were negligible [1]. Since the food and phyto-resources were diminishing globally with the population explosion, it is the need of the hour to find novel alternatives for enriching the resource base of natural indigenous food basket. It is in this aspect the present study focuses attention. Out of 118 wild edible fruits reported from the forests of Kerala, 32 fruits were popular based on their individual merit and desirability due to their nutritional and nutraceutical values. Current research has shown that a wide range of indigenous fruit trees have the potential to provide rural households with a means to meet nutritional and medicinal needs [2]. Previously several reports have been published on the nutritional properties of wild fruits and vegetables growing in various parts of the country and also their role on combating malnutrition and poverty in the continent [3]. For example the star fruit was a good source of reducing sugars, ascorbic acid, minerals (K, Ca, Mg and P) and amino acids (serine, glutamic acid and alanine). Canistel (*Pouteria campechiana*) is an evergreen native tree of Southern Mexico and Central America belongs to Sapotaceae.It is cultivated in other countries, such as Brazil, Taiwan, Vietnam and the Philippines for its fruit. In India, egg fruit grows in Maharashtra throughout the Western Ghats, Kerala, some parts of Tamil Nadu, and are reported as hobby fruit in a few gardens of Auroville. The fruit does not have any serious farming issues, and it generally sulks in the shadow of its immensely popular the sapota. The present study, aims to analyze the nutrient and nutraceutical potential of the egg fruits.

MATERIALS AND METHODS

Many leafy vegetables and fruits that are widely consumed in rural areas in Kerala especially along the forest fringe villages. To estimate the nutritive values, healthy and disinfected ripe fruits of *Pouteria campechiana* were collected from Palode areas of Thiruvananthapuram district, Kerala.

Analysis of samples

All samples were washed thoroughly to remove any attached soil, other impurities and were blotted dry. Various standard methods as mentioned below were employed for analyzing the nutrient parameters.

Moisture

The moisture content of the samples was determined by using moisture balance.

Total carbohydrate

Fresh fruit extract was prepared by hydrolyzing the test sample in 2.5 N HCl for 3 h in boiling water bath, followed by neutralizing it with sodium carbonate. It was then centrifuged and the supernatant was collected for analysis. The analysis was carried out using standard protocol [4].

Total sugar (TS)

The TS was estimated using anthrone reagent [5]. 1 ml of methanolic extract was taken in a test tube and chilled. After a while 4 ml of anthron reagent was carefully added along the walls of the test tube. The test tubes were

thereafter immersed in ice water. The tubes were brought to ambient temperature and boiled in water bath for 10 min. After proper cooling, the absorbance was measured at 625 nm.

Reducing Sugar (RS)

RS was estimated using Dinitrosalicylic acid (DNS) reagent [6]. 3 ml of DNS reagent was added to 3 ml of sample in a lightly capped test tube. The mixture was heated at 90° C for 5-15 mins to attain a reddish brown colour. Then, 1 ml of Rochelle salt solution was added to stabilize the colour. After cooling to room temperature in cold water bath, absorbance was recorded at 575 nm.

Non Reducing Sugar (NRS)

NRS was calculated by subtracting the amount of reducing sugar from that of total sugars.

Protein

Protein was estimated using Bradford method [7].

Amino acid

Proximal composition of amino acid was calculated as per the protocol of Moore and Stein [8].

Phenol

Total Phenol was estimated using standard protocol of Mayer *et al* [9].

Acidity

The acidity of fruit pulp was determined by titration of a known weight of sample with NaOH using Phenophthalein indicator. The value was calculated with reference to percent tartaric acid[10].

Ascorbic acid

Ascorbic acid was estimated following titration method [11].

Carotenoids

Carotenoid was evaluated following standard method of Arnon [12].

Mineral elements

0.5 g of fine powdered fruit sample of the fruit was digested following wet digestion procedures using conc. HNO_3 and 30% H_2O_2. The digested samples were used for elemental analysis. Iron (Fe), Copper (Cu),Magnesium (Mg), Manganese (Mn) and Zinc (Zn) was determined using Atomic Absorption Spectrophotometer. Potassium (K) and Calcium (Ca) using Flame photometer.

Estimation of crude fibre

1 gram of dried fruit was boiled with 200 ml of 0.255N sulphuric acid for 30 min and then washed twice and thrice with distilled water. It was then boiled with 200ml Sodium hydroxide fo 30 min.Content was dried filtered using Whatman No 1 filter paper and weighed.residue was again dried and incinerated. The difference between the weight of dried material and that of ash obtained was taken and from that percentage of fibre content was calculated.

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Estimation of Ash content

1g dried fruit was taken in a dried silica crucible and incinerated in a electric Bunsen burner and heated until feed from carbon. It was then cooled and weighed and percentage of ash was calculated. Acid soluble ash was also determined using 25N Hydchloric acid.

Lipid content

Lipid extraction was carried out with 2 g of homogenized fruit flesh with Soxhlet extractor with 250 ml of petroleum ether and then the solvent was removed by evaporation. Result was expressed as the percentage of lipids in the dry matter of fruit powder.

Anti-nutritional factors

The flavanoid was estimated following standard method [13]. Tannins level in the methanolic extracts was assessed using the vanillin-HCl method [14]. Similarly, phytic acid was also determined by the protocol of Vaintraub and Lepteva [15].

RESULTS AND DISCUSSION

The average proximate composition of *P. campechiana* pulp was summarized in Table 1. Carbohydrate was the predominant component with value of 3.73 mg/g followed by moisture, along with protein, and fat. Moisture content was 33.33%.

Carbohydrate analysis

Sugar was the most important constituents of *P. campechiana*, making them a rich source of energy for the human diet. The amount of reducing sugar and non-reducing sugar in *P. campechiana* were 1.38 mg/g and 2.35 mg/g tissue respectively. The amount of carbohydrate obtained was high and this was useful for getting the energy for metabolic processes. High carbohydrate contents in *Rubus* berries which give sweet taste of these fruits and consumption of fruits rich in carbohydrates should be promoted for the specific purpose of satisfying starvation and hunger [16].

Moisture content

The high moisture content facilitates spoilage of fruits and therefore not acceptable to consumers. In this study, the moisture content of egg fruit was 33.33%. This value was slightly different from the observation of moisture content in other fruits at different stages of development and was about 50-60%. Usually, decline in water content persisted throughout the ripening process in some fruit, whereas in others it reversed during the progression of the ripening process. Total acid content was recorded as 0.24%. Acidity in the fruit facilitate digestion and absorption processes in the small intestine[17,18].

Lipid content

The low level of lipids content (0.99) mg/g with high contents of sugars means that *P. campechiana* is safe for the heart and high blood pressure patients because it contains a low level of fatty acid and glycerol.

Protein content analysis

P. campechiana was also considered as good source of protein .i.e., 2.01 mg/g. Eleven Tunisian cultivars of date palm were estimated for protein and found the highest protein content of 2.85 mg/100 g dry matter [19]. This shows that egg fruit is rich in protein than those found in dates.Similar values of proximate composition have also been reported along many tropical plants of Nigeria [20] while other reports reveal values more or less at par with the conventional food and vegetables of different plant species [21, 22]. Crude protein content in common fresh leafy vegetables range between 2.2 to 7.3% was also reported, similar to the present results [23].

Amino acid profile

Naturally occurring food products have amino acids having potential antioxidant, antimicrobial and anti-inflammatory properties. It can also stimulate the immune system [24, 25]. The aminoacid pofile of several fruits have been investigated. The quantitative evaluation of amino acid pulp of *B. aegyptiaca* [26] showed a low dietary intake of 4.1 g/ 100 g, against 36 g/100 g for FAO reference [27]. Cysteine to be the dominant amino acid detected in unripe kundang fruit (13.59 g/100 g) with methionine being the lowest (1.26 g/100 g). But in ripe fruit, alanine was found to be dominant (7.52 g/100 g) whereas tyrosine was detected to be the lowest (1.37 g/100 g) [28]. Overall, the amino acids contents in unripe fruit were higher than the ripe fruit. This can be correlated to the higher protein content in unripe fruit compared to ripe one. In *P. campechiana* the concentration of aspartic acid was found to be higher (624.89 µg/g), serine being represented by comparatively lower value (36.73 µg/g). Essential amino acids like phenylalanine and tyrosine were also present in significant levels (Table 2). The fruit pulp also shows high proline content. The dominant levels of proline in fruit pulps is often assigned to its role in plant stress tolerance [29].

Mineral element analysis

The mineral composition of traditional fruits have been analysed by several researchers [30, 31]. The relative amounts of most of the elements (Table 3) and especially that of iron is found to be higher in *Pouteria*. The value is much higher than the well known dietary supplement of iron i.e., dates [32]. Anaemia was a serious public health problem in India affecting all segments of the population affecting infants, young children, adolescent boys, girls and pregnant women [33, 34]. In this scenario, *Pouteria* fruits can be effectively exploited as a natural dietary supplement of iron. Calcium concentration was the highest (11.5 mg/gm), followed in descending order by potassium (8.6 mg/gm), magnesium (1.3 mg/gm), iron (0.126 mg/gm), zinc (0.005 mg/gm) manganese (0.004 mg/gm) and copper (0.004 mg/gm). The mineral composition suggests that the *P. campechiana* fruits are also rich source of calcium and potassium. The relatively high calcium content was essential for healthy bone development and energy metabolism. When compared to the mineral nutrients constitution of fruits like apple, banana, grapes and guava, the egg fruit showed high concentration of the minerals zinc and copper. Copper and zinc minerals are essential to the proper functioning of organs and metabolic processes. Its high concentration in egg fruit when compared to common edible fruits shows its importance in human diet.Ash content was an index to the nutritive value of foods. The ash value was generally index of the purity as well as identity of the drug. The ash content obtained in the fruit was 1.4 % which is nearer to the ash range mean value of legume which were 2.4 -5.0% [35]. Contamination with sand and dirt may be determined by acid insoluble ash. Acid insoluble ash was 0.3018% and Water insoluble ash was 0.800%. In some wild fruits fund in Nigeria, shows high ash values that reflects the high mineral values specifically the macro minerals in the plant [36]. Similar trend in the medicinal species of genus *Sesbania* was reported [37].

Egg fruit can be considered as a good source of dietary fibre such as cellulose, hemicellulose, lignin and pectin. The crude fibre obtained was 13.86% .Dietary fibre was known to influence digestion and absorption processes in the small intestine [38, 39]. Since the crude fibre value of *P.campechiana* fruit is moderately high, if consumed it would aid digestion and absorption. Like the carbohydrate concentration in egg fruit the fibre concentration is also depend on ripening stage. The total dietary fibre contents of 13 date varieties from various countries found that the percentage

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of total dietary fibre was in the range of 6.4-11.5, depending on variety and degree of ripeness [40].Egg fruits may be considered as an almost ideal food providing a wide range of essential nutrients and potential health benefits. Vitamins were essential micro-nutrients for organisms that were used for multiple biochemical reactions. The results showed that the fruit was rich in β-carotene, vitamin C, tocopherol followed by riboflavin and thiamine (Table 4). Further, the fruit under investigation are good sources of ascorbic acid or vitamin C and are comparable to contemporary cultivars such as papaya and strawberry.These results are at par with those reported by many studies [41, 42, 43, 44, 45, 46, 47]. Anti-nutritional factors were widely distributed in wild plants used as food and medicine which were responsible for several health complications [48]. Several anti-nutritional factors including oxalate, tannins, phytic acid, lignins, saponins, alkaloids, cyanogens and enzyme inhibitors whose presence greatly impair the digestion of various nutrients [49,2,50], therefore, reducing the nutritional value of such plants and limiting their utilization as food [51,52]. Results presented in Table 5 revealed that the investigated fruit had lower level of phytic acid whereas moderate level of tannins. Total phenols and flavanoids were found to be high, 2.26 mg/g and 3.37 mg/g respectively. Phytic acid was marginal (i.e., 1.55 µg/g) and tannins level is 0.694 µg/g. Similar pattern of anti-nutritional contents of some of the unconventional food plants were previously reported [53]. The high levels of polyphenols suggest its role as antioxidants.

The consumption of the leaves of *Fagus sylvatica*, the seeds of *Pinus radiata*, and the shoots of *Pteridium aquilinum* were reported [54]. The eating of the fruits of *Quercus robur*, and *Q.ilex* was common until some decades ago and was still remembered by the informants. However, the consumption of those fruits has now a social stigma. Ethnobotanical survey and reports from 17 plants belonging to 14 families were separately used as food and medicine by the indigenous population [55]. Significant level of proximate composition, mineral profile, vitamin C, β-carotene with minimal anti-nutritional factors revealed the importance of the fruit as nutraceutical. The fruits of *Berberis asiatica* were evaluated as good potential food supplement or cheaper alternative against commercial fruits across the hilly regions of Uttarakhand [56]. The egg fruit (*P.campechiana*) are easy to cultivate and are remarkable in nutrition. Considering sugar, fibre and protein contents of fruit flesh, the food scientist could be encouraged to develop new source of food supplements. Also, the mineral contents in the fruit sample have the potential to provide a good source of zinc, potassium, calcium and sodium in the diet. The findings suggest further investigations into nutritional profiles and processing methods of the species reported and study of the pharmacological properties for the nutraceutical components, since they are also used for medicinal applications.

ACKNOWLEDGMENTS

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Table 1. Analysis of various nutritional parameters in P. *campechiana*

Nutrient factors	Values (mg/g)
Total carbohydrate	3.73
Reducing sugar	1.38
Non reducing sugar	2.35
Fat	0.99
Total protein	2.01
Moisture content	33.33%
Acidity	0.24%
Total ash	1.40%
Acid insoluble ash	0.3018%
Water insoluble ash	0.800%
Crude fibre	13.86%

Table 2. Aminoacid profile in the fruit

Amino acids	Values(µg/g)
Tyrosine	49.65
Phenylalanine	264.01
Serine	36.73
Glycine	276.01
Aspartic acid	624.89
Proline	360.51
Cysteine	48.62
Isoleucine	319.01

Table 3. Mineral composition in *P. campechiana*

Minerals	Values (mg/g)
Iron	0.126
Copper	0.004
Manganese	0.004
Zinc	0.005
Magnesium	1.3
Calcium	11.5
Potassium	8.6

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Table 4.Analysis of vitamins in *P. campechiana*

Vitamins	Values (mg/g)
ß-Carotene	1.58
Thiamine	0.81
Riboflavin	3.7
Ascorbic acid	0.086
αTocopherol	0.22

Table 5. Estimation of anti nutritional factors

Antinutritive factors	Values (mg/g)
Total phenol	2.26
Flavonoids	3.37
Tannic acid	0.0006
Phytic acid	0.0015

RESEARCH ARTICLE

Variations in the Fibre Morphology of Plantation Grown Non-Infected Agarwood (*Aquilaria malaccensis* Lamk.).

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ABSTRACT

The variations in fibre characteristics namely fibre length, fibre diameter, fibre wall thickness and fibre lumen width of *Aquilaria malaccensis* non-infected wood age 3, 5, 7 and 9 year old respectively were investigated. Six trees from each even aged stand and similar class diameter selected from Assam Forestry plantations were used for the study. Wood samples were systematically collected from the periphery, middle and pith wood sections of the radial and longitudinal positions. The mean middle values of fibre length were 943.50 (µm), 1121.25 (µm), 1324.28 (µm) and 1565.55 (µm); fibre diameter were 38.28 (µm), 43.11 (µm), 54.14 (µm) and 68.27 (µm); fibre wall thickness were 4.49 (µm), 5.27 (µm), 7.34 (µm) and 9.52 (µm); while the mean values for fibre lumen width were 23.07 (µm), 27.18 (µm), 41.03 (µm) and 47.84 (µm) for 3, 5, 7 and 9 year old non-infected *Aquilaria malaccensis* wood respectively. Fibre length, fibre diameter and cell wall thickness increased with increase in age while fibre lumen width decreased with increase in age. However all the fibre characteristics examined in this study is increased from innerwood to outerwood. The effects of age and radial positions contributed significantly to variations in fibre characteristics of non-infected *Aquilaria malaccensis* wood. Analyses of variance used for mean separation were significant (p <0.05).

Key words: *Aquilaria malaccensis*, Agarwood,Anatomical properties, Fibre Length, Fibre Diameter, Fibre Wall Thickness, Fibre Lumen Thickness and Radial position.

INTRODUCTION

Aquilaria malaccensis is one of 15 tree species in the Indo-Malaysian genus (Oldfield *et al.*, 1998). It is also known as Agallochum belongs to the Thymelaeaceae family. It is a large evergreen tree, growing over 15-40 m tall and 0.6-2.5 m in diameter and has white flowers (Chakrabarty*et al.,* 1994). It grows best in undulating terrain from 200 – 700 meters, with an annual rainfall of 1500 – 6500mm, a mean annual maximum temperature of 22-28ºC and a mean annual minimum temperature of 14 – 21ºC. it is propagated through seeds (Mabberley, D J, 1997).Agarwood formation is a pathological process taking place in the stem or main stemwhere an injury has occurred. It forms an association with endotrophicmycorrhiza fungi. In natural forests, only 7-10% of the trees are infected by the fungus (Ng. *et al*, 1997). The fragrance produced by the burning of infected wood. However the Agarwood oil is an essential oil obtained by water and steam distillation. It is used in luxury perfume, insect repellent and for medicines (Chakrabarty*et al.,* 1994).

The timber of undiseased Agarwood trees is known as 'Karas', is very light and is used for making indoor construction, jewellery box andveneer (Angela Barden *et al.*, 2000). Apart from the infected wood, the non-infected woods are used for interior wood work. For that purpose the mechanical properties is must to know clearly, which can directly studied from the anatomical properties of the wood.Considering the anatomical properties, it varies from pith to bark, from tree base to the top, from stem to branchesanddepending upon the age (Rupert *et al.,* 2002).The variations in wood properties are attributable to the different distribution patterns of its micro structures, its arrangement, size anddimension of components cells. In hard wood, the cells that make up the anatomical organization are the vessels, fibres, parenchyma cells and the wood rays. Fibres are the principal element that is responsible for the strength of the wood (Panshin*et al.,*1980;Desch and Dinwoodie, 1983).The fibre properties have a positive correlation on the strength characteristics of wood(Ocloo and Ling, 2003). Onilude, 2001 discovered a positive correlations between fibre length and mechanical properties from the pith to the bark which increases respectively for plantation grown *Terminaliasuperb.*The present investigation was, therefore undertaken to study the fibre morphology of the non-infected Agarwood. The main objective of the study was to examine the variation in fibre morphology of different aged Agarwood (*Aquilaria malaccensis* Lamk.) of plantation (Figure 1.) is need in an hour.

MATERIALS AND METHODS

*Aquilaria malaccensis*Lamk.plantation at different age gradation *viz.,* three, five, seven and nine year old located at Hojai district, Assam were chosen for studying the fibre morphology of wood samples at different age gradations. From each age gradations six trees were randomly selected and wood samples were obtained from the trees by destruction for the study purpose.Maceration of the wood samples was done using Jeffrey's method (Sass, 1971). For maceration, Jeffrey's solution was used and it is prepared by mixing equal volumes of 10 per cent potassium dichromate and 10 per cent nitric acid. Radial chips of wood shavings were taken from the 1 cm^3 wood blocks separately from the three radial positions viz., pith, middle and periphery. These chips were boiled in the maceration fluid for 15-20 minutes so that the individual fibres were separated. Then these test tubes were kept for 5-10 minutes so that the fibres settled at the bottom. The solution was discarded and the resultant material was thoroughly washed in distilled water until traces of acid were removed. The samples were stained using saffranin solution and mounted on temporary slides using glycerin as the mountant. Temporary slides were made by staining these sections with saffranin stain and subjected to measurements and photography using Image analysis system (Motic) under 10x, 20x and 40x lens.The observations were recorded on minimum three fields on each section of different aged wood samples. The observations are fibre length (µm), fibre diameter (µm), fibre wall thickness (µm) and fibre lumen width (µm) were measured from macerated wood samples at cross section (µm: Micrometer).

RESULTS AND DISCUSSION

Fibre length

The variation in the fibre length was found to be statistically significant at 5 per cent among the different age gradations of wood sample. The mean values of fibre length obtained in the radial position (Pith, Middle and Periphery) was lowest in third year [pith (921.25 µm), middle (943.50 µm), and periphery (963.25 µm)] and highest in ninth year [pith (1525.10 µm), middle (1565.55 µm), and periphery (1590.25 µm)] of wood sample (Table 1).The maximum mean fibre length was observed in the nine year old *A.malaccensis*wood sample (Figure 2). This result establishes that wood fibre length increases with increase in age. The same line of findings had been reported by Jorge *et al.*, (2000) who found that with increase in age there was an increase in fibre length from innerwood to outerwood. This is an indication that age, radial position from where the wood samples were collected contributed to the variation in fibre length. Therefore wood fibre length increased with increasing in age. Generally, there was decrease in fibre length from the base to the top and an increase from inner wood to outer wood (Izekorand Fuwape 2011).

Fibre diameter

Fibre diameter of ninth year shows significant different from the seventh, fifth and third year sample, In the radial positions (Pith, Middle and Periphery), the mean values of fibre diameter obtained was minimum in third year [pith (36.24 µm), middle (38.28.75 µm), and periphery (32.15 µm)] and maximum in ninthyear [pith (63.14 µm), middle (68.27 µm), and periphery (70.25 µm)] wood sample (Table 1). Fibre diameter increased with age, from third to ninth year old wood samples.The observed increase in fibre diameter associated with the increasing age of the tree may be due to many molecular and physiological changes that occur in the vascular cambium as well as the increase in the wood cell wall thickness during the tree aging process (Plomion*et al.*, 2001 and Roger *et al.*, 2007).

Fibre wall thickness

The fibre wall thickness increases with age of the tree. The mean values of fibre wall thickness recorded in the radial position (Pith, Middle and Periphery) was lowest in third year [pith (3.15 µm), middle (4.49 µm), and periphery (5.05 µm)] wood sample and highest in ninth year [pith (9.12 µm), middle (9.52 µm), and periphery (9.74 µm)] wood sample (Table 2). Analysis of variance at 5 per cent probability showed that, the effects of the different age classes and radial positions from where the samples were collected contributed significantly to variations in cell wall thickness. Current findings are in concurrence with studies on *T. grandis* by Izekor and Fuwape (2011). Akachuku (1980) also attributed the increase in cellwall thickness if *Gmelinaarborea* to changes in cell size that are associated with annual and periodical growth cycles and the increase in the age.

Fibre lumen width

Fibre lumen width values for pith, middle and periphery was highest in ninth year wood [pith (46.28 µm), middle (47.84 µm), and periphery (48.50 µm)] and lowest in third year wood sample [pith (21.25 µm), middle (23.07 µm), and periphery (24.52 µm)] from the radial position (Table 1). The ninth year value of fibre lumen width is significantly different from the lumen width of other year wood samples.The fibre lumen width ranged from23.07 (third year) to 47.84 µm (ninth year) and also lumen width increases with increases in age gradation at middle portion of the wood (Table 2.).This showed that fibre lumen width increases with age, which may be attributed to the increase in the length of fibre initial associated with increasing age of the cambium (Jorge et al., 2000). The observed differences in lumen width with increasing age of the tree may also be due to increase in cell size and physiological development of the wood as the tree grows in girth. Frimpong-Mensah, F. (1992) reported positive relationship between variations in lumen width and age of the cambium.The differences in anatomical characteristics, fibre length, fibre diameter, fibre

wall thickness and fibre lumen width of 3, 5, 7 and 9 yearold *Aquilaria malaccensis* wood were discovered significant ($p < 0.05$). There were also significant differences within and between trees of the same and different age classes. Fibre characteristics such as fibre length, fibre diameter, fibre wall thickness and fibre lumen width increases with increases in age. Generally anatomical characteristics increased from innerwood to outerwood from where the wood samples were collected. The effects of age, and radial positions contributed significantly to variations in fibre characteristics of non-infected *Aquilaria malaccensis* wood. This helps in understanding the mechanical strength of the non-infected *Aquilaria malaccensis* wood used for house interior construction and decorative purpose.

ACKNOWLEDGEMENTS

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Table 1. Mean fibre morphology of pith, middle and periphery of *Aquilaria malaccensis* at different ages

Anatomical properties		Fibre length (µm)			Fibre diameter (µm)		
Radial position		Pith	Middle	Periphery	Pith	Middle	Periphery
Age in years	3	921.25	943.50	963.25	36.24	38.28	32.15
	5	1089.25	1121.25	1145.50	40.52	43.11	48.47
	7	1302.45	1324.28	1355.25	51.52	54.14	57.85
	9	1525.10	1565.55	1590.25	63.14	68.27	70.25
Mean		1209.51	1238.66	1263.56	47.86	50.95	52.18
SEd		30.61	28.96	41.49	0.21	0.15	0.18
CD (P = 0.05)		68.21	64.54	92.45	0.47	0.34	0.40

Table 2. Mean fibre morphology of pith, middle and periphery of *Aquilaria malaccensis* at different ages

Anatomical properties		Fibre wall thickness (µm)			Fibre lumen width (µm)		
Radial position		Pith	Middle	Periphery	Pith	Middle	Periphery
Age in years	3	3.15	4.49	5.05	21.25	23.07	24.52
	5	4.98	5.27	5.78	26.95	27.18	28.95
	7	6.95	7.34	7.58	40.47	41.03	41.57
	9	9.12	9.52	9.74	46.28	47.84	48.50
Mean		6.05	6.66	7.04	33.74	34.78	35.89
SEd		0.25	0.22	0.20	0.32	0.76	1.64
CD (P = 0.05)		0.55	0.50	0.44	0.70	0.70	3.66

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Figure 1. Different aged plantation of *Aquilaria malaccensis*

3 years old plantation

5 years old plantation

7 years old plantation

9 years old plantation

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Figure 2. Fibre morphology of third and fifth year old *Aquilaria malaccensis*
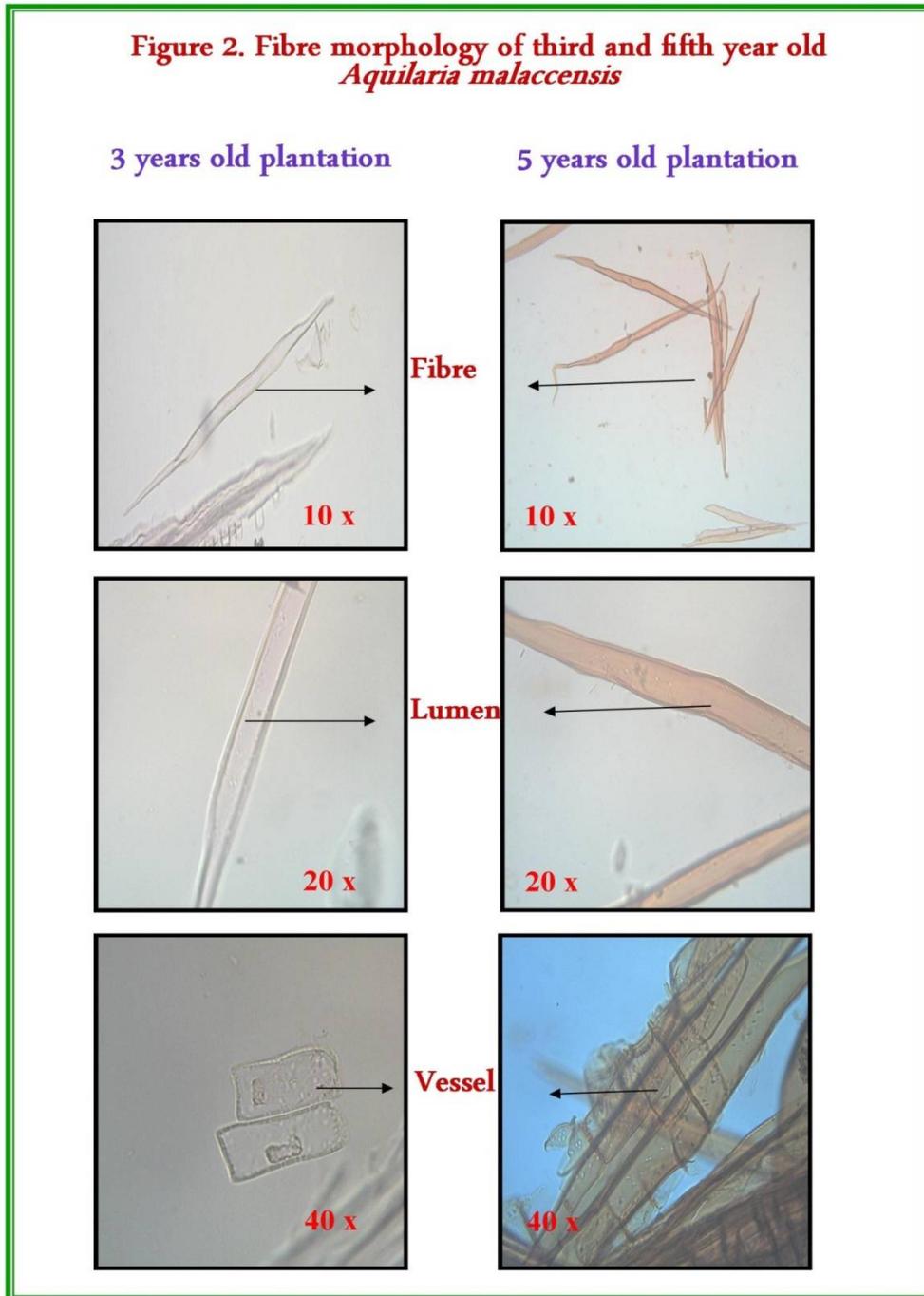
Figure 3. Fibre morphology of seventh and ninth year old *Aquilaria malaccensis*
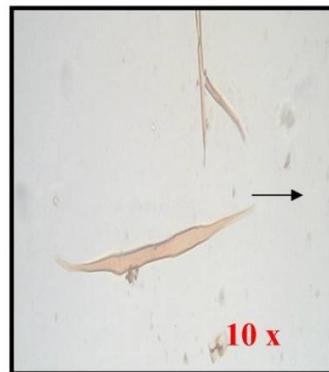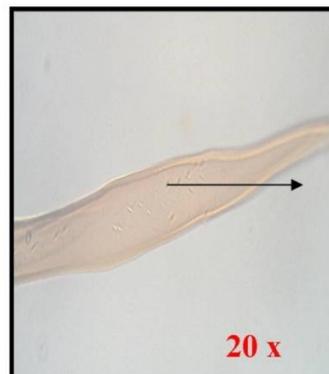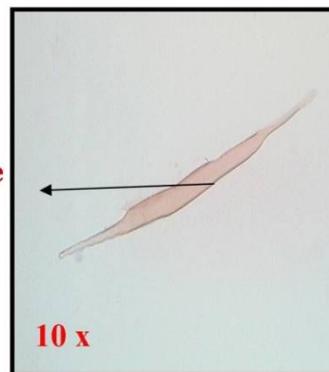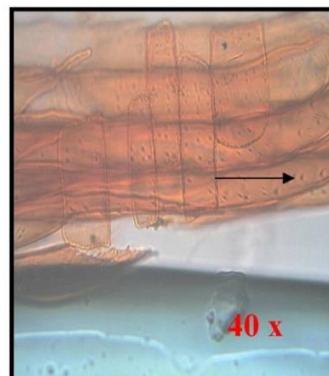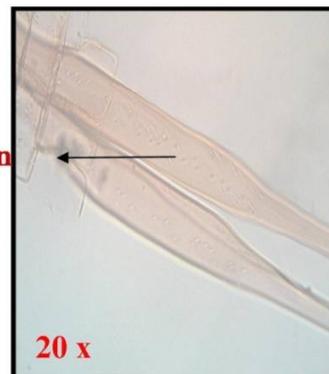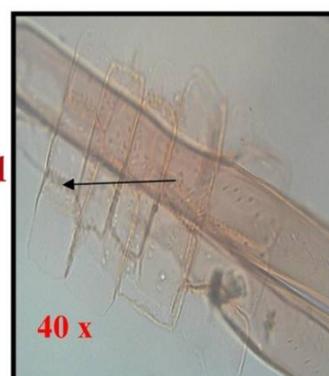
RESEARCH ARTICLE

Seasonal Abundance of Lady Bird Beetle, *Coccinella septempunctat* (Linn.) in Mustard Crop

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ABSTRACT

A field experiment was conducted at C. P. College of Agriculture, SDAU 2012 revealed that the average population of *Cocconella septempunctata* (Linn.) range 0.00 to 7.2/sq. m. area with an average of 2.03/sq. m. area when an average minimum and maximum temperature was 8.4°C to 27.3°C, respectively. The mean relative humidity was 74 per cent in morning and 25 per cent in evening. The predator appeared to build up from second week of January. Its population increased with increase of the population of mustard aphid and peak (7.2/sq. m. area) in second week of February. Correlation coefficient between *C. septempunctata* and weather parameters revealed positive association between *C. septempunctata* and morning (r = 0.483) and evening humidity (r = 0.379), maximum temperature (r = 0.372) and sunshine hours (r = 0.178), whereas minimum temperature showed negative correlation (r = - 0.141). The highly significant positive correlation (r = 0.914) was recorded between *C. septempunctata* and aphid population.

Key Words: Aphid, *Coccinella septempunctata*, mustard

INTRODUCTION

Brassica (rapeseed- mustard) is the second most important edible oilseed crop grown in India after groundnut and accounts for nearly 30 per cent of the total oilseeds produced in the country. The aphid, *Lipaphis erysimi* is an important pest of mustard and has become a serious pest; the crop is severely attacked by aphid at the flowering stage. Different types of predators were found among that the *Coccinella septempunctata* are considered to be efficient predators on mustard aphid and keep the aphid infestation naturally checked. The *Coccinella septempunctata* are

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popularly called as the lady bird beetle. They are of great economic importance because a large majority of them are predaceous both in their grub as well as adult stages on the various small bodied insects including aphid (Rawat and Modi, 1969).

MATERIALS AND METHODS

Field survey was carried out to know the seasonal abundance of *Coccinella septempunctata* predators on mustard grown in 20 m x 20 m^2 plots at the college farm and kept unsprayed in winter season during 2011-2012. In order to determine the seasonal abundance of coccinellid beetle (*C. septempunctata*), the populations of *C. septempunctata* and its host, *L. erysimi* were observed on 10 plants which were randomly selected from a plot of the mustard field. Five spots (1 m x 1 m) were observed throughout the crop season at weekly interval. The population of coccinellids both larvae and adults were recorded on the whole plant carefully. Correlation study was carried out with weather parameters.

The aphid index was recorded as under:
0 -Plant free from aphid.
1- Aphid present but colonies not built up. No injury due to pest appearance on plant.
2 -Small colonies of aphids present on leaves of plant. Such leaves exhibit slight curling due to aphid feeding.
3 -Large colonies of aphids present on leaves and other parts, damage symptoms visible due to aphid feeding.
4 -Most of the leaves covered with aphid colonies. Counts are not possible and the plant shows more damage symptoms due to aphid feeding.
5- The plant completely covered with aphid colonies, plant growth hindered due to pest feeding.

The average aphid index was worked out by using following formula:

$$\text{Av. aphid index} = \frac{0N + 1N + 2N + 3N + 4N + 5N}{\text{Total no. of plants observed}}$$

Where,
0, 1, 2, 3, 4, 5 are the aphid index, and N= Number of plants showing respective aphid index.

RESULTS AND DISCUSSION

The result presented in Table 1 showed that the aphid incidence continued throughout the crop season except fourth week of November and first week of December. The infestation of aphid commenced from second week (5th week after sowing) of December with initial population of 0.42 aphid index/plant. Thereafter, it increased at steady rate. The peak population of 3.80 aphid index/plant was recorded in first week (13 week after sowing) of February. After that the aphid population gradually declined and remained up to first week of March (17 week after sowing). Data also indicated (Table 1) that the population of predator (*C. septempunctata*) ranged from 0.00 to 7.20 per sq. m. area with an average of 2.03/sq. m. area during study period. The population of *C. septempunctata* commenced from the second week of January (10 week after sowing) with 0.60 Coccinellids per sq. m. The Coccinellid population gradually increased and reached to a peak level of 7.20 Coccinellids per sq. m. during second week of February (14 week after sowing) and population gradually decrease with aphid population decreased. Thus, the result clearly indicated that the *C. septempunctata* were active during the aphid population present in crop. Looking to the fluctuated population of aphid and predators as well as weather parameters, it was apparently happened that the population of *C. septempunctata* was increased with the increase of aphid population. It meant that the activity of the predator was positively related with its host density. The present findings are in agreement with those of Kulkarni and Patel (2001) and Bilashini and Singh (2010).The data presented in Table 2 showed that the population of *C. septempunctata* had highly significant positive correlation (r = 0.914) with aphid population. The aphid population

was increased the *C. septempunctata* population also increased. The correlation between aphid and weather parameters (Table 2) revealed that the relative humidity morning (r = 0.670*) and evening (r = 0.577*) had significant positive correlation, which indicated that with increase in the relative humidity, the aphid population was also increased. Further, it can be seen from the data that the aphid population had positive correlation (r = 0.052) with minimum temperature and negative correlation (r = - 0.436) with maximum temperature, while the sunshine hours showed negative effect on the population of aphid. Thus, the results showed that when relative humidity (Mor. and Eve.) was increased, the aphid population also increased. Similarly, the correlation study of *C. septempunctata* revealed that (Table 2) the relative humidity morning (r = 0.483) and evening (r = 0.379), maximum temperature (r = 0.372) and sunshine hours (r = 0.178) had positive correlation, while minimum temperature had negative correlation (r = -0.141) with *C. septempunctata*. Similar results have been reported by Bhangare *et al.* (2010).

CONCLUSION

From the ongoing discussion, it can be seen that the population of *C. septempunctata* was increased with the increase of aphid population. It means the activity of the predator was positively related with its host density. It was also noted that with increase of relative humidity (Mor. and Eve.), the aphid population also increased.

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Table 1: Seasonal abundance of mustard aphid, *L. erysimi* and *C. septempunctata* and weather parameters

Date	Weeks after sowing	Aphid index/plant	*C. septempunctata* /sq. m.	Average temperature		RH. (%)		Sunshine hrs./day
				Max.	Min.	Mor.	Eve.	
26-11-11	3	0.00	0.00	32.8	12.0	82	29	9.2
03-12-11	4	0.00	0.00	31.1	13.2	85	26	7.1
10-12-11	5	0.42	0.00	28.4	10.8	64	19	9.3
17-12-11	6	0.64	0.00	26.0	4.9	68	22	8.4
24-12-11	7	0.70	0.00	28.7	7.0	66	21	7.9
31-12-11	8	0.90	0.00	26.8	6.4	68	22	7.5
07-01-12	9	1.12	0.00	24.9	6.6	70	28	8.2
14-01-12	10	1.54	0.60	24.5	4.6	76	21	9.0
21-01-12	11	1.80	1.80	23.1	5.2	82	28	7.9
28-01-12	12	2.18	3.60	25.6	5.3	84	31	7.5

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04-02-12	13	3.80	5.40	24.6	6.4	84	35	7.4
11-02-12	14	3.50	7.20	23.8	9.4	76	24	8.9
18-02-12	15	2.92	5.60	27.6	10.9	72	21	9.6
25-02-12	16	1.90	3.80	30.4	11.6	65	24	9.8
03-03-12	17	0.80	2.40	30.7	11.7	70	22	10.2
Average		1.48	2.03	27.27	8.4	74.13	24.87	8.53

Table 2: Correlation coefficient between population of mustard aphid (*L. erysimi*), *C. septempunctata* and weather parameters

Particular	*C. septempunctata*	Weather parameters				
		Temperature (°C)		Relative Humidity (%)		Sunshine hrs.
		Min.	Max.	Mor.	Eve.	
Aphid	0.914**	0.052	-0.436	0.670*	0.577*	-0.117
C. septempunctata	-	-0.141	0.372	0.483	0.379	0.178

*, ** Significant at 5 per cent and 1 per cent levels of significance, respectively.

RESEARCH ARTICLE

Investigation of Secondary Metabolites from Annatto Seed Extract (TNBi – 13) through GC-MS

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ABSTRACT

Today 80% of the world population depends on natural products for their well being. Annatto is one such wonder plant which is native to Central and South America whose economic parts are its bright red seeds. The term "annatto" in industrialized countries is commonly referred to *Bixaorellana* seed extract containing carotenoid-type pigments, widely used to dye an assortment of foods, textiles and body care products. GC-MS analysis of dye extracts obtained from the seeds of Bixa genotype (TN Bi - 13) using hexane solvent led to the identification of various compounds. The GC-MS method confirmed the presence of 25 compounds among which trans-Geranylgeraniol, Spathulenol, Methylhydrogen-(9'Z)-6,6',-dioate, Germacrene-d, á-Cubebene, Himachalol and á-cadinene. The compounds like trans-Geranylgeraniol (60.33%) and Spathulenol (11.97%) showed the highest percentage composition which have been reported for anticancer and antispasmodic activity.

Keywords: Annatto, Dye, Soxhlet Apparatus, GC-MS, Bixin, Carotenoids, Sequiterpenes, Anticancer activity

INTRODUCTION

Plants are man's friend in survival, giving him food, fuel and medicine from the days beyond dawn of civilization [1]. During the twentieth century, when exploring the natural environment, man has made great discoveries that have enabled him to use a considerable number of natural resources [2]. According to World Health Organization,

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about 80% of the world population depends on the natural product for their health due to minimal side effects and cost effectiveness [3]. In recent period the gas chromatography and mass spectroscopy studies have been increasingly applied for the analysis of most of the medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids and lipids [4].

*Bixaorellana*L. (Bixaceae), a shrub native to Central and South America, also known as annatto, uruc`u, or achiote,is a symbol for the Amazonian tribes who traditionally use its seeds as coloured ink to paint their bodies for religious ceremonies [5]. The term "annatto" in industrialized countries is commonly referred to *B. orellana*seed extract containing carotenoid-type pigments, widely used to dye an assortment of foods, textiles, and body care products [6]. Recently, *B. orellana*extracts have also been shown to be a viable option for commercial exploitation as a replacement for synthetic dyes and pigments in dyeing and finishing of leather [7]. Two carotenoids give annatto its unique red colour: bixin and norbixin. Bixin (methyl (9-cis)-hydrogen-6,6-diapo- W,W-carotenedioate) is the main pigment responsible for the orange red colour of the seeds and extracts of annatto represent approximately 80% of its total carotenoids [6, 8]. Therefore, *B. orellana*is a well-known commercial crop. *B.orellana* L., is an important natural food dye yielding plant which yields non toxic food dye called Bixin and   Nor - Bixin also called as "Annatto". Annatto is one of the 13 basic food pigments approved by US FDA and ranks 2nd in the world. Annatto is used in dairy industry for colouring butter, cheese, ice-creams and cosmetics for colouring hair oils, lipsticks and in textile industry for dyeing cotton, silk clothes [9].

MATERIALS AND METHODS

Collection of seed

The mature fruits of Bixagenotype (TN Bi– 13) were collected from the Bixa gene park established at Horticulture College and Research Institute, Periyakulam, Tamil Nadu. The collected material was shade dried and seeds were separated from the dry pods (Figure 1).

Preparation of sample extract

A sample of 5g seed of Bixagenotype (TN Bi – 13) was extracted in 25 mL of Hexane (HPLC grade) in Soxhlet Apparatus (SOXTEC 2043 FOSS). It was kept overnight to remove the traces of hexane and the obtained extract was kept in airtight vials at 4^{0}C for further use.

GC - MS analysis

The chemical composition of the seed extract was analysed using Thermo GC - Trace Ultra Ver: 5.0 and Thermo MS DSQ II fitted with a DB 35 - MS capillary standard non - polar column (30 m, ID: 0.25 mm and film thickness of 0.25 µm). 0.5 µl of methanol extract was injected for analysis and Helium was used as a carrier gas at 1 mL/ min. The instrument was set as follows, injector port temperature set to 250^{0}C, source kept at 220^{0}C. The oven temperature was programmed from 70^{0}C to 260^{0}C at the 6^{0}C/ min rate. The MS was set to scan from 50 - 650 Da. The MS also had inbuilt pre - filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum, NIST4 and WILEY9 containing more than five million references.

Identification of compounds

Interpretation of mass spectrum of GC - MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9 [10]. The spectrum of the unknown component was compared with the spectrum of the known components stored in the inbuilt library.

RESULTS AND DISCUSSION

GC-MS analysis of dye extracts obtained from the seeds of Bixa genotype (TN Bi - 13) using hexane solvent led to the identification of various compounds. The chromatograph of hexane extract is shown in the figure 2. The various (major and minor) compounds detected by GC-MS in the extracts are shown along with their respective retention time and area percentage in table 1 and 2. The presence of various components with different retention (RT) times was confirmed by GC-MS spectra. The mass spectroscopy analyzed the components eluted at different times to identify the structure and the nature of the compounds. The fragmentation of large compound into small ones gives rise to appearance of peaks at different m/z ratios. These mass spectra act as a fingerprint of the very compound that can be identified from the library. The results of the GC-MS analysis showed the presence of variouscaretenoids, free fatty acids, alcohols, aldehydes, ketones, furans, esters, hydrocarbon, mono- di- terpenes, sequiterpenesand can be further analyzed for more specifications.The GC-MS method confirms the presence of 25 compounds among which trans-Geranylgeraniol(60.33%), Spathulenol(11.97%), Methylhydrogen-(9'Z)-6,6',-dioate (2.51%), Germacrene-d (1.93%), á-Cubebene (1.93%),Himachalol (1.01%)are having a area %above 1% whereas á-cadinene (0.33), farnesol (0.35), Campherenone (0.69) etc are the compounds having a area % below 1%. The data are presented in Table 1. and Table2.

The main compounds found in *Bixaorellana* plant are carotenoids and apocarotenoids. Most of the carotenoids have been isolated from seed and seed coats. Bixin [methylhydrogen-(90 Z)-6,60 -diapocarotene-6,60 -dioate] is the major carotenoid compound present in *B. orellana* seed coat and accounts for 80% in addition to the presence of other carotenoids in trace amounts [11,12]. Terpenoids mainly C_{20}-terpene alcohol (all-geranylgeraniol) as a major chemical component in *Bixaorellena* were isolated by Jondiko and Pattenden [13].trans-Geranylgeraniol have been reported for anticancer activity on B16F-10 melanoma cell line[14].Sesquiterpenes are also a major group of volatile compounds found in annatto extracts [15]. The majorsequiterpenecompounds characterized from bixaseed oil reported (Z, E)-farnesyl acetate (11.6%), occidentalol acetate (9.7%), spathulenol (9.6%) and ishwarane (9.1%) [16]. Thespathulenolis a colorless, viscous oil coupled with earthy and aromatic odour which has immunosuppressive effect[17].Another sesquiterpenehimachalol (1.01%) which is present mainly in *Cedrus deodar* is an excellent insect repellant.Several othersesquiterpenes found usually as water-soluble and in oil-soluble extracts include δ-copaene[18].Minor sesquiterpenes found in *B. orellana*extracts that have distinctive aromas include cubebene, β-, and δ-cadinene. Cubebene has been described as having a fruity, sweet, citrus-like smell. β- and δ-cadinene are compounds occasionally used as fixatives in candy flavours and have a dry-woody, slightly medicinal-tarry odour with some similarities to spices in the cumin-thyme family [19].Some of the monoterpenes and sesquiterpenes found in the extracts have been previously described as having antimicrobial properties or as being associated with pharmacological properties [20]. D- germacrene,mainly found in dried seeds (1.93%), is known for its antimicrobial activity [21].The results of the present study indicates Bixa as a source of caretenoids, alcohols, aldehydes, ketones, furans, esters, hydrocarbon, sequiterpenes which posses natural properties as antioxidants, antimicrobial, anti – inflammatory, anti carcinogenic, analgesic, antispasmodic and as insect repellants too.Despite being widely used and studied, little is known about the volatile organic compounds' (VOCs) composition of *B. orellana*, especially regarding the chemical compound profile from each individual genotype. These results suggest that there are chances of variation among the secondary metabolites not only at a quantitative level but also at a qualitative level. Hence profiling of each and every screened genotype is required in order to attain the best selection. Further, critical study is needed to establish the relationship between geographical localities with the dye content.

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Table1.Major chemical compounds identified through GC - MS in seed extract of Bixagenotype (TN Bi – 13)

S.No.	Retention Time	Compound Name	Molecular Formula	Molecular weight	Area %
1.	6.78	Methylhydrogen-(9'Z)-6,6',-dioate	$C_{25}H_{30}O_4$	394	2.51
2.	9.53	à-Terpinolene	$C_{10}H_{16}$	136	1.38
3.	12.60	à-Guaiene	$C_{15}H_{24}$	204	2.54
4.	12.60	Aromadendrene	$C_{15}H_{24}$	204	2.54
5.	12.91	GERMACRENE-D	$C_{15}H_{24}$	204	1.93
6.	12.91	á-Cubebene	$C_{15}H_{24}$	204	1.93
7.	12.91	à-Copaene	$C_{15}H_{24}$	204	1.93
8.	15.96	Ledene oxide-(II)	$C_{15}H_{24}O$	220	2.79
9.	17.12	Spathulenol	$C_{15}H_{24}O$	220	11.97
10.	18.52	Himachalol	$C_{15}H_{26}O$	222	1.01
11.	18.52	Globulol	$C_{15}H_{26}O$	222	1.01
12.	18.52	Veridiflorol	$C_{15}H_{26}O$	222	1.01
13.	26.49	Geranyl-à-terpinene	$C_{20}H_{32}$	272	1.11
14.	27.80	Trans-Geranylgeraniol	$C_{20}H_{34}O$	290	60.33

Table 2. Minor chemical compounds identified through GC - MS in seed extract of Bixageno type (TNBi – 13)

| S.No.	Retention Time	Compound Name	Molecular Formula	Molecular weight	Area %
1.	6.78	Methyl 4-oxodecanoate	$C_{11}H_{20}O_3$	200	0.79
2.	9.09	3-Methyl-2,3-dihydro-benzofuran	$C_9H_{10}O$	134	0.88
3.	13.70	á-Cadinene	$C_{15}H_{24}$	204	0.33
4.	14.92	1,6- imethylthieno [2',3':3,4] bicycle [3.3.0] oct-6(7)- en-2-one	$C_{12}H_{12}OS$	204	0.42
5.	14.92	(Z)-3-(5-Trimethylsilyl-2-penten-4 ynyl) furan	$C_{12}H_{16}OSi$	204	0.42
6.	19.06	Longiborneol	$C_{15}H_{26}O$	222	0.61
7.	21.71	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.55
8.	28.54	Farnesol	$C_{15}H_{26}O$	222	0.35
9.	30.03	Campherenone	$C_{15}H_{24}O$	220	0.69
10.	35.71	6-Bromo-4-[2-[(trifluoroacetyl) amino] phenyl]-5,8-d imethoxyquinoline	$C_{19}H_{14}Br F_3N_2O_3$	454	0.39
11.	35.71	2,6,9-Tribromo-4-methoxy-furo[3,2-g] coumarin	$C_{12}H_5Br_3O_4$	450	0.3

Figure 1. Bixa Genotype TN Bi - 13

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Figure 2. GC-MS chromatograph of seed extract of Bixa genotype (TN Bi – 13)

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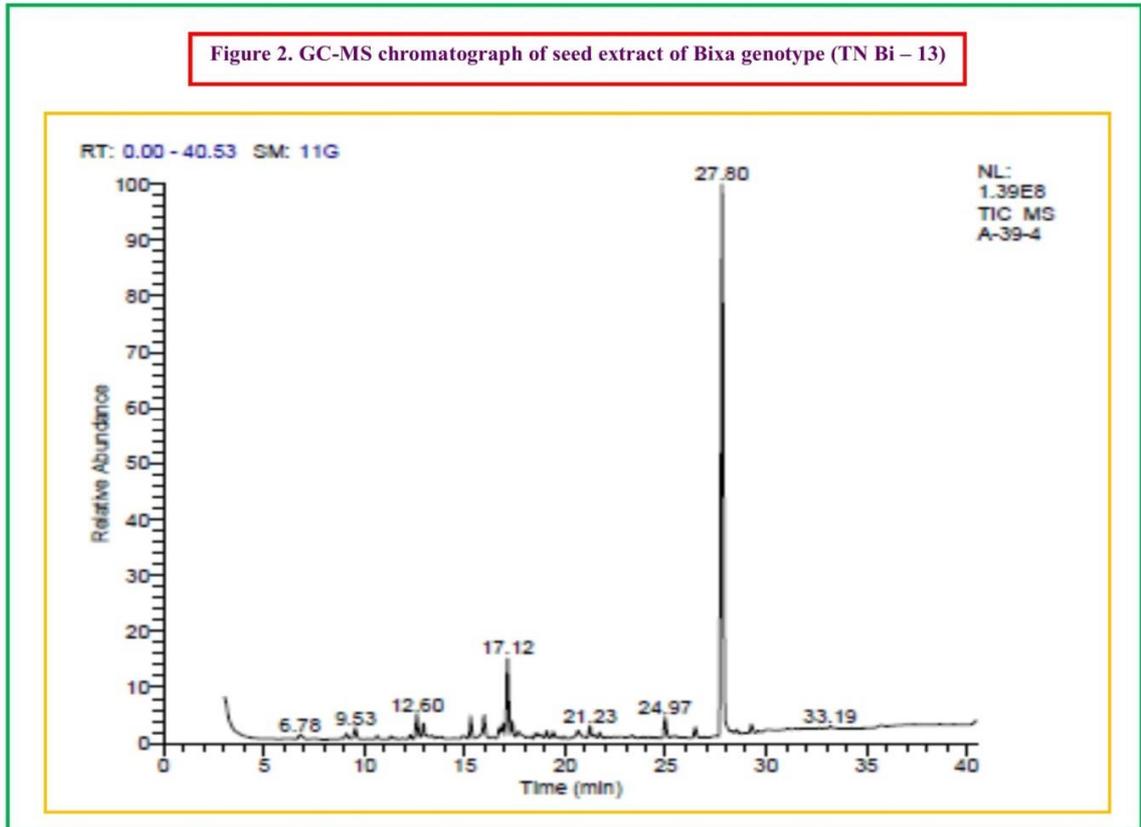
GC - MS Analysis of Withered *Tectona grandis* Leaves

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ABSTRACT

Tectona grandis Linn. f. belongs to the lamiaceae family and is cultivated around the world for its valuable timber but is predominantly found in tropical regions like India and other South-East Asian countries. It is now one of the most important species of tropical plantation forestry. The whole plant is also medicinally important as it contains enormous number of phytoconstituents which helps in curing the ailments. The results of GC - MS analysis for yellow withered leaves of *Tectona grandis* showed a total of 31 compounds, of which phthalic acid butyl 4-octyl ester, squalene, 2-methoxy-6-(3′, 5′-dimethoxyphenyl) methyl benzoic acid, pyrido [2,3-b] indole, geranyl-p-cymene, (-)-caryophyllene oxide and m-nitrobenzaldehyde acetylhydrazone showed the greatest contribution to the percentage of the total area.

Key words: *Tectona grandis*, withered leaf, methanol, GC-MS, metabolites, squalene.

INTRODUCTION

Natural plant based phytochemicals are known to contain active principle that can be used for therapeutic purposes. Use of plants as a source of medicine has been inherited and is an important component of health care system [1]. In plants, phytochemicals act as a natural defence system for host plants and provide colour, aroma and flavour to them. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is significant in the development of new drugs [2]. Phytochemical analysis of plants which were used in folklore has yielded a number of compounds with various pharmacological activities, therefore standardisation of the plant material is the need of the

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day [3]. Hence, modern methods describing the identification of active components in the plant material may be useful for newer drug formulations.*Tectona grandis* Linn. f. also known as "the King of Timber" belongs to the lamiaceae family. It is a large, deciduous tree growing 30 - 35 meter in height with light brown bark, leaves simple, opposite, broadly elliptical or acute or acuminate with minute glandular dots, flowers are white in colour, small and have a pleasant smell [4]. It is cultivated around the world for its valuable timber, but predominantly found in tropical regions like India and other South-East Asian countries. The tree is found adapted to a variety of habitats and climatic conditions from arid areas with only 500 mm of rain per year to moist forests with upto 5,000 mm. It is now one of the most important species of tropical plantation forestry [5]. The whole plant is also medicinally important as it contains enormous number of phytoconstituents which helps in curing the ailments [6, 7, 8, and 9]. It is known that the mature leaves of teak contains numerous secondary metabolites such as acetovanillone, E-isofuraldehyde, evofolin, syringaresinol, medioresinol, balaphonin, lariciresinol, zheberesinol, 1-hydroxypinoresinol together with two new compounds tectonoelin A and tectonoelin B [10] whereas no literatures are available regarding the chemical constituents of withered leaves of *Tectona grandis* and hence the present investigation is undertaken. The main objective of the present study is to identify the chemical constituents present in the withered leaves of *Tectona grandis.*

MATERIALS AND METHODS

Plant collection

The fresh yellow withered leaves of *Tectona grandis* were collected from the litter fall in the plantations across the western zone of Tamil Nadu and shade dried at room temperature with constant turning to inhibit fungal growth. The dried leaves were later crushed to obtain a coarse powder to ease extraction using soxhlet apparatus.

Preparation of methanol leaf extract

Exactly 5.0 grams of the crushed yellow leaves of *Tectona grandis* were extracted with 25 mL of methanol in an automated soxhlet apparatus (SOXTEC 2043 FOSS). The extraction was performed at 60^0 C for 2 hours and 30 minutes completing three cycles. All the phytoconstituents were extracted from the leaves at the end of the third cycle. The extract was then dried at room temperature and the dried extracts were stored at 4^0C in air tight sterile vials in refrigerator.

GC - MS analysis

The chemical composition of the leaf extract was analysed using Thermo GC - Trace Ultra Ver: 5.0 and Thermo MS DSQ II fitted with a DB 35 - MS capillary standard non - polar column (30 m, ID: 0.25 mm and film thickness of 0.25 µm). 0.5 µl of methanol extract was injected for analysis and Helium was used as a carrier gas at 1 mL/ min. The instrument was set as follows, Injector port temperature set to 250^0 C, source kept at 220^0 C. The oven temperature was programmed from 70^0C to 260^0C at the 6^0C/ min rate. The MS was set to scan from 50 - 650 Da. The MS also had inbuilt pre - filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum, NIST4 and WILEY9 containing more than five million references.

Identification of compounds

Interpretation of mass spectrum of GC - MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9 [11]. The spectrum of the unknown component was compared with the spectrum of the known components stored in the inbuilt library.

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RESULTS AND DISCUSSION

The GC - MS analysis results of yellow withered leaves of *Tectona grandis* are summarised in Table 1, of which phthalic acid butyl 4-octyl ester, squalene, 2-methoxy-6-(3′, 5′- dimethoxyphenyl) methyl benzoic acid, pyrido [2,3-b] indole, geranyl-p-cymene, (-)-caryophyllene oxide and m-nitrobenzaldehyde acetylhydrazone showed the greatest contribution to the percentage of the total area. The chromatograph of methanolic extract from *Tectona grandis* leaves by GC - MS is given in Figure 1. Additionally, some 3 compounds (area <1%) were identified as can be seen in Table 2. Among the pythochemicals identified, squalene chemically an isoprenoid, is a powerful antioxidant. This component is of major importance concerning the protective action against cancer [12]. The compound geranyl-p-cymene is a monopene which finds its place in the traditional medicines. The compound m-nitrobenzaldehyde has been found to be the parent compound for nitrendipine which is an effective antihypertensive agent [13]. Also, caryophyllene oxide, an oxygenated terpenoid is a well-known preservative in food, drugs and cosmetics [14].*Tectona grandis* apart from possessing a valuable heartwood, is also the unique source of secondary metabolites with many pharmacological utilities. The present result also justifies the use of leaves for various purposes. Therefore, the leaves considered as a waste in the teak plantation can also be utilised for extracting the metabolites. However, the uses of the other compounds have to be studied for its usage in different fields in the future.

ACKNOWLEDGEMENTS

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Table 1: Major compounds identified in *Tectona grandis* leaf extract

S.No	RT (min)	Compound name	Molecular formula	Molecular weight	Area %
1	6.74	Dodecane	$C_{12}H_{26}$	170	2.83
2	9.69	5- Tetradecene	$C_{14}H_{28}$	196	1.66
3	9.69	5- Eicosene	$C_{20}H_{40}$	280	1.66
4	**11.78**	**m-Nitrobenzaldehyde acetylhydrazone**	**$C_9H_9N_3O_3$**	**207**	**3.07**
5	13.42	1- Octadecene	$C_{18}H_{36}$	252	1.46
6	**16.09**	**(-)- Caryophyllene oxide**	**$C_{15}H_{24}O$**	**220**	**3.43**
7	16.82	2- (1-phenanthryl) benzaldehyde	$C_{21}H_{14}O$	282	1.29
8	17.66	1- Hexadecanol	$C_{16}H_{34}O$	242	2.27
9	19.27	4,4,5,8- Tetramethylchroman-2-ol	$C_{13}H_{18}O_2$	206	1.33
10	19.90	2- Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	1.03
11	20.39	Epiglobulol	$C_{15}H_{26}O$	222	1.08
12	21.71	Hexadecanoic acid	$C_{17}H_{34}O_2$	270	2.36
13	22.17	2,4- Di-p-chloroanilino cyclopent-2-enone	$C_{17}H_{12}D_2Cl_2N_2O$	332	2.35
14	**22.94**	**Geranyl-p-cymene**	**$C_{20}H_{30}$**	**270**	**3.96**
15	23.99	1-Aza-4,5-(2,3-furyl) bicyclononan-9-one	$C_{10}H_{11}NO_2$	177	1.94
16	**24.97**	**Phthalic acid butyl 4-octyl ester**	**$C_{20}H_{30}O_4$**	**334**	**17.78**
17	25.51	Heptadecanoic acid 16-methyl- methyl ester	$C_{19}H_{38}O_2$	298	1.43
18	27.55	Geranyl linalool isomer	$C_{20}H_{34}O$	290	2.15
19	29.70	1-(2-Methylsulfanylbenzoyl) pentylidene phosphorane	$C_{31}H_{31}OPS$	482	1.63
20	30.20	Hexanoic acid 5-tridecyl ester	$C_{19}H_{38}O_2$	298	1.22
21	**31.39**	**2- Methoxy-6-(3′, 5′- dimethoxy phenyl) methyl benzoic acid**	**$C_{17}H_{18}O_5$**	**302**	**9.76**
22	31.81	2,4-Octadienoic acid, (acetyloxy)- decahydro-dihydroxy-3-tetramethyl - 5 - oxo - 1H - cyclopropabenz azulen - 9 - yl ester	$C_{30}H_{40}O_8$	528	2.29
23	**32.22**	**Pyrido[2,3-b]indole**	**$C_{19}H_{14}N_2O_2$**	**302**	**6.68**
24	33.21	Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	1.53
25	34.66	Dioncopeltin a	$C_{23}H_{25}NO_4$	379	1.65
26	34.98	4-Ethyl-5-hydroxybenzofuran-2-yl acetic acetic Acid	$C_{12}H_{12}O_4$	220	2.77
27	**36.66**	**Squalene**	**$C_{30}H_{50}$**	**410**	**16.24**
28	38.67	Deoxyartonin R	$C_{31}H_{30}O_9$	546	2.17

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Table 2: Minor compounds identified in *Tectona grandis* leaf extract

S. No	RT (min)	Compound name	Molecular formula	Molecular weight	Area %
1	3.07	N-m- Tolyl- succinamic acid	$C_{11}H_{13}NO_3$	207	0.92
2	28.13	Effusanin B	$C_{22}H_{30}O_6$	390	0.94
3	35.63	Halcion (triazolam)	$C_{17}H_{12}Cl_2N_4$	342	0.81

Figure 1. The chromatograph of methanolic extract of *Tectona grandis* leaves by GCMS

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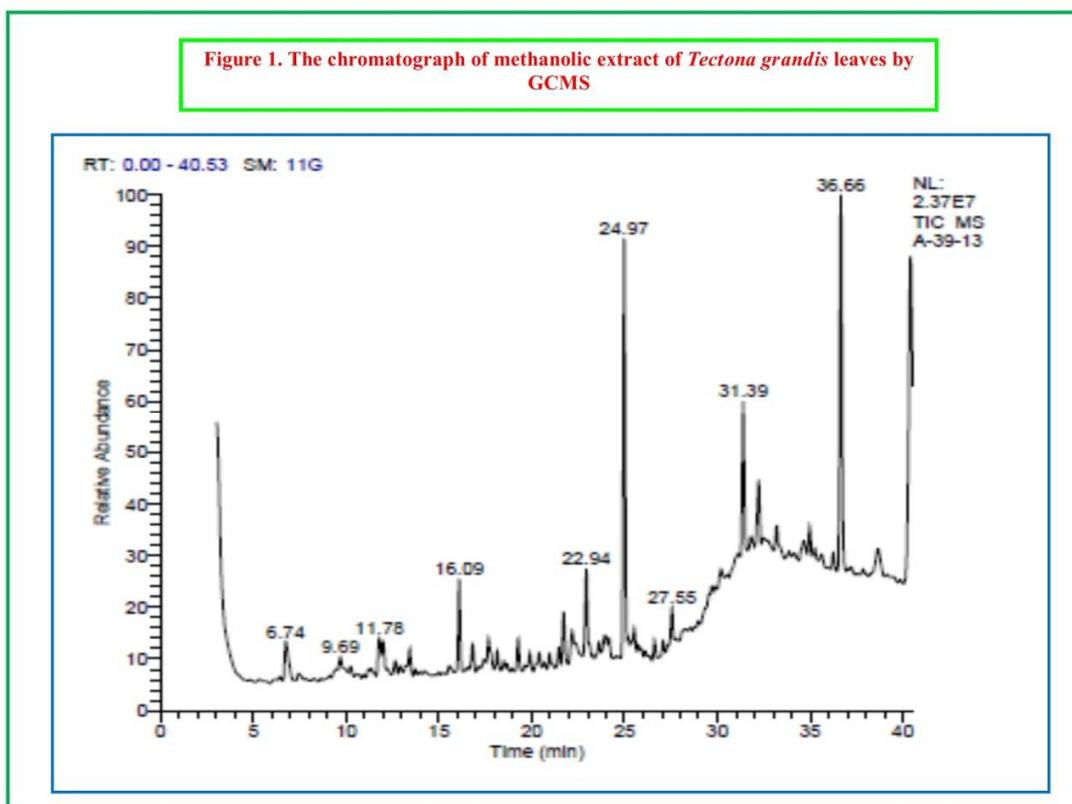
Association between Lung Function and Basal Metabolic Rate in Young Adult

Noorzaid Muhamad*, Than Htun, Resni Mona Abdullah, Nazatul Afifah Kamaruzaman,Nur Afiqah Hasnul Hadi , Nurul Syuhada Abu Bakar, Khairunnisa Mohd Aman and S. Jannathul Firdous

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ABSTRACT

Basal Metabolic Rate (BMR)is the rate of energy utilization in the body during absolute rest when the patient is awake. We aimed to study the association of lung function and basal metabolic rate-BMR among male and female medical students of 19-26 years of age in UniversitiKualalumpur Royal College of Medicine Perak (UniKL RCMP). On average, 100 (54 male, 46 female) medical students took part in this study and their lung function was measured using a Spirometry with single use sterile antibacterial-viral filters with integrated mouthpiece "TRANSLAB Electronic Spirometer Model: Chestgraph (HI-101)" whereas students BMR were measured using Karada machine "OMRON Body Composition Monitor with scale (HBF-362)". There was no significant correlation between lung function and gender but a significant correlation exist between BMR and gender as ventilatory function and its related anthropometric parameters differs by gender.However, the studyshowed nosignificant relation between lung function and BMR among young adult. To explain further, studies on serum hormone levels with larger sample size are to be undertaken to explain the relationship between BMR and lung function.

Keywords: Basal metabolic rate, forced vital capacity, forced expiratory volume, lung function and spirometry.

INTRODUCTION

Spirometry is the most frequently used measure of lung function and is a measure of volume against time. It is a simple and quick procedure to perform: patients are asked to take a maximal inspiration and then to forcefully expel air for as long and as quickly as possible[1]. Measurements that are made include Forced Expiratory Volume in one second (FEV1) where the amount of air breathed out as forcefully as possible in 1 second. The FEV1 value can help to estimate the severity of COPD. With normal lungs and airways, we can normally blow out most of the air from our lungs within one second. Measurements also include Forced Vital Capacity (FVC) wherethe amount of air that can be forcibly breathed out after taking a deep breath and the ratio of the two volumes whereFEV1 divided by FVC (FEV1/FVC). Spirometry and the calculation of FEV1/FVC allow the identification of obstructive or restrictive ventilatory defects. A FEV1/FVC < 70 % where FEV1 is reduced more than FVC signifies an obstructive defect. An FEV1/FVC > 70% where FVC is reduced more so than FEV1 is seen in restrictive defects such as interstitial lung diseases and chest wall deformities[1]. BMR generally decreases with age and with the decrease in lean body mass (as may happen with aging). Increasing muscle mass increases BMR, although the effect is not significant enough. Illness, previously consumed food and beverages, environmental temperature, and stress levels can affect one's overall energy expenditure as well as one's BMR. BMR can be measuredby using Karada Scan. It is a full body sensing technology to measure one's weight, to know your body composition, basal metabolic rate, body fat percentage, body age, body mass index, visceral fat level, subcutaneous fat and skeletal muscle percentage in whole body, trunks, legs and arms.Tissues containing much water such as muscles, blood vessels, and bones are highly conductive with electricity, but fat tissues are not. Therefore, by using bioelectrical impedance principle, it is possible to determine the ratio of fat tissue compared to other tissues in the body by measuring the electric resistance of the body tissues, using extremely weak electric current applications to the body.

Concerning about BMI and lung function, obesity is known to contribute to other respiratory illnesses including asthma, sleep apnea, pulmonary embolism and hypoventilation syndrome making it logical to investigate obesity as a risk factor for loss of lung function. In 650,000 subjects evaluated as part of the Canadian National Health Survey, the prevalence of obesity was significantly higher in COPD subjects when compared to those without COPD (24.6% and 17.1%, at *P*<0.001)[2]. Total energy expenditure is the one that influence body weight and body composition. It consists of basal metabolic rate, thermal effect of food and energy expenditure of physical activity. BMR shows the rate of energy expenditure of the body to sustain basic life process such as respiration and blood circulation. It is basically the result of energy exchange in all cell of the body. BMR is different on each individual making it hard to use as a tool of measurement. Those with higher BMI normally have higher BMR. Therefore, the aim of this study is to assess the association between lung function and BMR using the spirometry technique and Karada scan.

MATERIALS AND METHODS

The study was conducted among 100 medical students in which there are 54 male students and 46 female students. A cross-sectional study involving the medical students of Royal College of Medicine Perak (RCMP) regarding the relation between lung function and basal metabolic rate.

Methods of measurement

For spirometry test, the patient inhaled maximally and then exhales into the spirometer with maximum expiratory effort as rapidly and completely as possible [3]. The patient were asked to closed their nose using their hand and the mouthpiece were ensured to be fit into the patient mouth to prevent any breathing from nose and the mouth that may cause error in the reading. In addition patient was relaxed and wore loose fitting clothing to prevent any difficulty in breathing. Heavy exercise and smoking were avoided in the test. For BMR measurement, the students

were asked to wear light clothes, and step on the CA Radar machine bare footed or wearing thin socks. The students were asked to stand straight with their hand extended at 90°. All measurements were recorded.

Data collection and processing

Written consents were obtained including questionnaire about lifestyle, physical activity, family history, stress and disease experienced. All the information about the test was explained beforehand. The printed test of lung function was obtained with the result show the FEV /FVC percentage. The ranges in determining the lung function were normal (80% - 100 %), mild (60%-80%), moderate impairment (40%-60%) and severe (<40%). Consent letters need to be signed by each participant involved in this study to prove their agreement in participating the research. Participant's responses and information were kept strictly confidential. Ethical approval was obtained from the Ethics Committee of UniKLRCMP.

RESULTS

Association of lung function and basal metabolic rate (BMR)

Out of 100 respondents, 87 have normal lung function which involved 50 male and 37 female. While for mild impairment lung function test, 9 samples were affected. This involved 3 male students and 6 female students. Table 1 was shown with moderate impairment found in 4 students, which involved 1 male student (1.0%) and 3 female students (3.0%). The results were insignificant ($t = 3.324$, $df = 2$, $p > 0.05$). Out of 100 respondents, 41 samples have BMR >1500, giving a prevalence of 41.0%, and involved 36 male students and 5 female students. While for BMR <1500, 59 samples giving a prevalence 59.0% which involved 18 male students and 41 female students. This result was significant ($t = 31.970$, $df = 1$, $p < 0.01$).Out of the 41 samples with BMR>1500, 37 of them (90.24%) showed normal lung function test, 2 samples (4.88%) showed mild impairment and another 2 samples (4.88%) were having moderate impairment. Out of 59 samples with BMR<1500, 50 of them (84.75%) showed normal lung function test, 7 samples (11.86%) had mild impairment and another 2 samples (3.39%) had moderate impairment. This result was insignificant ($t = 4.215$, $df = 2$, $p > 0.05$). There was no significant relationship between LFT and BMR as shown in Table 1.

Correlation between other variables and LFT/BMR

The lung function test with BMR was correlated with other variables such as medical history, playing, sleeping duration, eating fast food and visceral fat composition as shown in Table 2.

DISCUSSION

Association of lung function and basal metabolic rate (BMR)

Our study found no correlation between lung function and basal metabolic rate. This is in contrast to other study that shows that there is a significant correlation between lung function and BMR. However, studies were conducted among elderly patients and postmenopausal woman, andsubjects with increased pulmonary function were more frequently observed in the group of elderly men with elevated BMR than in the group of elderly men with lower BMR[4]. Our studies were conducted among young adults.Our study also found no correlation between gender and lung function. Women generally have a higher percentage of body fat than men[5].However, earlier study has shown that body fat distribution has independent effects on lung function that are more prominent in men than women[6] and shows that lung function does not have any correlation with any of the body composition parameter[7].

Association of lung function with BMR and other variables

Although there is a number of factors that does effect lung function and BMR individually and long term exposure to this entire factor can affect both BMR and lung function. These factors were asked in the questionnaire provided, to know the lifestyle, habits and genetic disease and family history of the subject.There was no significant relationship between LFT and gender since p > 0.05. Beyond gender, height, and age, there are a lot of other factors significantly influencing lung function such as asthma, genetic factor, smoking and others.The complexity of factors influencing lung function uncovers difficulties in establishing the proper, reliable predicted values for FEV1 and FVC [8]. However, there was a significant and direct relationship between BMR and gender (p < 0.01). The males (54.0%) had a significantly higher prevalence of BMR compared to the females (46.0%). One of the factors that increase BMR is stress or in this case easily distracted condition. Subjects were asked in the questionnaire if they are easily stressed and the type of stress they have. Most of them stated that they were emotionally stressed as a student. Stress release hormone causes rapid increase in BMR, so the body is able to breakdown energy stores to supply the body need during stress.In basal metabolic rate, result is significant between gender and BMR as female has lower BMR perhaps due to the fact that women is smaller in size and has more body fat than male. This also can be shown in our result as the number of male of BMR higher than 1500 is more, compared to the number of female student.

BMR also shows significant value with body fat distribution, muscle and visceral fat. Body fat will cause decrease in BMR while skeletal muscle will increase the BMR. This is because muscle tissue is active when resting while fat is inactive, thus, a person with high muscle will have high BMR while a person with high fat will have a low BMR[6]. Asthma, heart attack, tuberculosis, and sinusitis can give rise to constrictive lung disease. However, allergic rhinitis and sinusitis are associated with more severe asthmatic symptoms and, in patients with poorly controlled asthma, more exacerbations but are not associated with low lung function[9]. Our result proves that asthma has effect on lung function as it shows that there is a significant relation between asthma and lung function. In case of asthma and basal metabolic rate, patients with COPD and bronchial asthma have increased metabolic rates. These increased rates were suggested to be directly correlated to the severity of the diseases and the impairment in the respiratory function[10]. However, our study found no significant relation between asthma and BMR. Previous study show that the athletes were shown to have a significantly higher BMR[11] and our result support this by showing a positive relation between playing sports/physical fitness with BMR. Physical fitness is associates with high skeletal muscle percentage and the significant result between BMR and skeletal muscle also proves that playing sports does have a significant relation with BMR.Most of the male subjects play sports such as rugby, football and basketball. And most of them spend 1-2 hour playing it for a couple times in a week, which is enough for an active lifestyle. Half of the female students play sports actively, while the rest of them of them are living an active healthy lifestyle, walking as a mode of transportation to the college. Hence, most of the subject has a healthy range of BMR and high lung function. However, while previous suggest that there is a significant dose/response relationship between lung capacity (FVC and FEV1) and level of physical exercise[12]. Our result showed a negative correlation between lung function and playing sports and alsothere was an association between BMR and fast food consumption but not lung function. Majority of the subjects spend more than 4 hours on their gadgets or television. The students nowadays have their own smartphones, tablets and laptops that they used and spend time on most of the time. Our study showed that as there is no significant association between gadgets usage and BMR or lung function.

Our results showed a negative correlation between sleep deprivations and BMR. However, we only have one female subject with obesity, and though shehas decrease in lung function compared to the other subject that has normal BMI,it is not enough for comparison. Sleep deprivation is uncommon among the student as most of the subject had a good night sleep of 5-7 hours.In conclusion, Basal metabolic rate (BMR) has a significant association with gender, body fat distribution, skeletal muscle, visceral fat, physical fitness, and fast food consumption. Also, while asthma has a significant effect on lung function, the other variables mentions above do not. Further studies need to be done with bigger sample sizes.

ACKNOWLEDGEMENTS

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Table 1: Correlation between Lft and Bmr

BMR	Percentage rate	LFT normal	LFT mild impairment	LFT moderate impairment	Total
BMR <1500	Count	50	7	2	59
	% within BMR	84.7%	11.9%	3.4%	100.0%
	% within LFT	57.5%	77.8%	50.0%	59.0%
	% of Total	50.0%	7.0%	2.0%	59.0%
BMR >1500	Count	37	2	2	41
	% within BMR	90.2%	4.9%	4.9%	100.0%
	% within LFT	42.5%	22.2%	50.0%	41.0%
	% of Total	37.0%	2.0%	2.0%	41.0%
Total	Count	87	9	4	100
	% within BMR	87.0%	9.0%	4.0%	100.0%
	% within LFT	100.0%	100.0%	100.0%	100.0%
	% of Total	87.0%	9.0%	4.0%	100.0%

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Table 2: Correlation between Other Variables and Lft/Bmr

Other variables		LFT Normal	LFT Mild impairment	LFT Moderate impairment	BMR <1500	BMR >1500
Medical history	normal	66%	8%	-	44%	29%
	asthma	15%	1%	4%	10%	20%
	sinusitis	6%	-	-	5%	6%
Gadget time	<1 hour	14%	1%	-	8%	7%
	1-2 hours	18%	1%	-	13%	6%
	3-4 hours	30%	5%	2%	24%	13%
	>4 hours	25%	2%	2%	14%	15%
Playsport	Yes	61%	8%	1%	36%	35%
	No	25%	1%	3%	24%	5%
Sleep duration	4-5 hours	47%	8%	3%	32%	14%
	5-7 hours	36%	3%	1%	26%	14%
	>7 hours	4%	-	-	1%	3%
Fastfood	Yes	73%	8%	3%	54%	30%
	No	14%	1%	1%	5%	21%
Easily distracted	Yes	36%	5%	3%	29%	14%
	No	51%	2%	4%	30%	27%
Transportation	Vehicle	77%	8%	3%	52%	36%
	Walking	10%	1%	1%	7%	5%
Genetic disease	Yes	20%	1%	-	13%	8%
	No	67%	8%	4%	46%	33%
Visceral fat	Low	77%	9%	3%	59%	30%
	High	10%	-	1%	-	11%

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RESEARCH ARTICLE

Study of Some Mechanical Properties for Epoxy Resin Reinforced With Copper Powder

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ABSTRACT

A hand lay- up method was used to prepare Epoxy / Cu composite by adding different weight percentages (2, 4, 6 and 8 wt%) from copper particle of particle size (30μm) .The experimental results showed that , In natural condition (N.C) , the hardness value and the compression value increase with increasing weight percentages of copper particles in the composite for all samples .After immersion the samples in chemical solutions , the value of hardness decreases with increasing the immersion time and weight percentage of (Cu) particle in composites , but the value of compression decreases at comparison with (N.C) , and increases with increasing the weightpersonages of (Cu) particles in composites .

Key words: Epoxy resin , Copper powder , Hardness , Compression .

INTRODUCTION

Epoxy resins are basically thermosetting resins , which can reacted with curing agent to form a cross – linked polymeric structures [1,2].Their most outstanding property is their excellent adhesion to both metallic and non – metallic surface . Cured epoxy resin are characterized by their good mechanical properties , thermal and electrical insulation properties [3] . Many particle types of fillers are used to improve the other properties of matrix materials such as mechanical , thermal and electrical conductivity [3].Low cost particulate fillers are added to epoxy in commercial production primarily for reasons of economy and improvement in molding characteristics [4-6] . Metal particle such as (Cu) particle is added to epoxy resins lead to composite with higher density , improved electrical , thermal conductivity and mechanical properties and are therefore of particle interest for specific applications [7].

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Theoretical part

Hardness

Hardness is one of the important mechanical properties . It is defined as the resistance of material surface to penetration or deformation [8].The material hardness depends on the type of coupling forces between atoms and molecules , type of surface and the temperature .The hardness was measured by shore D method [9].

Compressibility

Compressibility is defined as the maximum stress on the solid material surface uncle Normal load [10].The compressibility or compression strength (C.S) is given by the relation [11].

$$C.S = F/A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where:

F: Applied force (N).

A: Cross – section are of sample (mm^2).

Experimental part

Preparation Epoxy /Cu composites

The materials used to prepare the test samples were epoxy resin (Ep105 Quickmast) of density $(1.2)g/cm^3$ supplied by Jordan company with hardener aliphatic amine (HY 956) as a matrix .The hardener was added to epoxy resin in ration (1:3) to transform it to solid state . Copper particle of density 8.9 g/cm^3 , average particle size (15.0 µm) and purity 99.5 % was added to epoxy in weight percentages of (2,4,6 and 8 wt %) and mixed carefully in a circular dies to make the composite samples .

Hardness test

Shore D hardness product of Time Group Inc. Company was used to measure the Hardness by using dibbing tool , the pointed dipping tool penetrates in to the sample surface by the pressure applied on the instrument .

Compressibility test

Hydrolic compressor was used to measure compressibility by applying relation (1).

RESULTS AND DISCUSSION

Hardness results

Fig (1,2,3) represent the shore D hardness values as a function of copper particles concentration in composite samples before and after immersion in (HCl) and (NaOH) solutions for (15) days .From fig (1) can be seen that the shore (D) hardness values increased with increasing of (Cu) concentration at (N.C) .This result may be related to the copper particle will be entire the cavities between epoxy resin particle , and then reduce these cavities and this behavior leads to increase the resistance samples to indentation .After immersion all samples in (HCl) or (NaOH) solution (0.2N) for (15) days (Fig. 1,2) . The values of shore (D) hardness decreases for all samples because of the (HCl) or (NaOH) solution will be entire in to composite samples through crack capillary tubes .close to copper particle zone and then separate the interface between (Cu) particles and epoxy resin . From fig .3 can be seen that the values of

hardness for samples immersed in (HCl) solution is less than that for samples immersed in (NaOH) solution at the same normality (0.2N) and the same immersion time (15) days . This belong to ability of (HCl) solution to damage the bonds and penetrate in to the interface and causing swelling.

The results of hardness tests after immersion different periods (0,1,3,5,10,15) days in (HCl) and (NaOH) solution (0.2N) are shown in fig.(4,5) . From these figures noticed that the values of hardness decrease with increasing the immersion time , this belong to the shrinkage and unsaturation between epoxy and (Cu) powder which happen during the manufacturing process for composite samples . So this process perhaps produces cracks and capillary tubes help the solution to diffuse through them inside the samples and became nearly elastic.

Compressibility results

Fig .6. Shows that the values of compressibility increases with increasing the (Cu) particle (wt%) at (N.C) . The reason is: at low Cu – particle concentration in composite, the inter distance between (Cu) particle will be big and causes in creament in mean free path, So the compressibility of composite material will be small. But at increasing (Cu) concentration, the inter distance between (Cu) particle decreases which cause increasing in compressibility [12]After immersion the samples (10) days in (HCl) or (NaOH) solution (0.2N) , show from Fig.(6,7) that the compressibility decreases with increasing Cu – particle wt% . This belongs to the penetration of the solution inside the composites and decreasing the compressibility. From Fig.8. shown that the compressibility of samples immersed in (HCl) solution is less than that for samples immersed in (NaOH) solution . Because of the ability (HCl) solution to damage the bonds and penetrates inside the sample more than that for (NaOH) solution of (0.2N) which is the same for (HCl) solution.

CONCLUSION

1. At natural condition (N.C) . the hardness values and the compressibility values increase with increasing Cu-particle wt% in composites for all samples .
2. After immersion in (HCl) or (NaOH) solution (0.2N) for different periods (0,1,3,5,10,15) day , we conclude :
A. The hardness values and compressibility value decrease with increasing the immersion time in (HCl) or (NaOH) solutions .
B. The values of hardness and compressibility at (N.C) is more than that at immersion in (HCl) or (NaOH) solutions for all samples .
C. The values of hardness and compressibility for samples immersed in (HCl) solution (0.2N) are less than that for samples immersed in (NaOH) solution of the same normality (0.2N) and the same immersion time interval .

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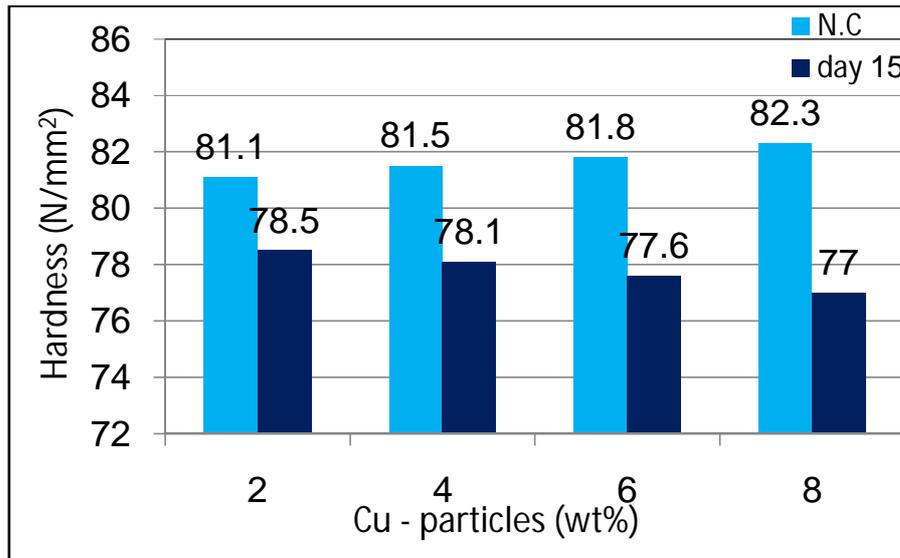
Fig.1. Shows the hardness ageist Cu – particle (wt%) for sample at (N.C) and immersion (15) day in HCl solution
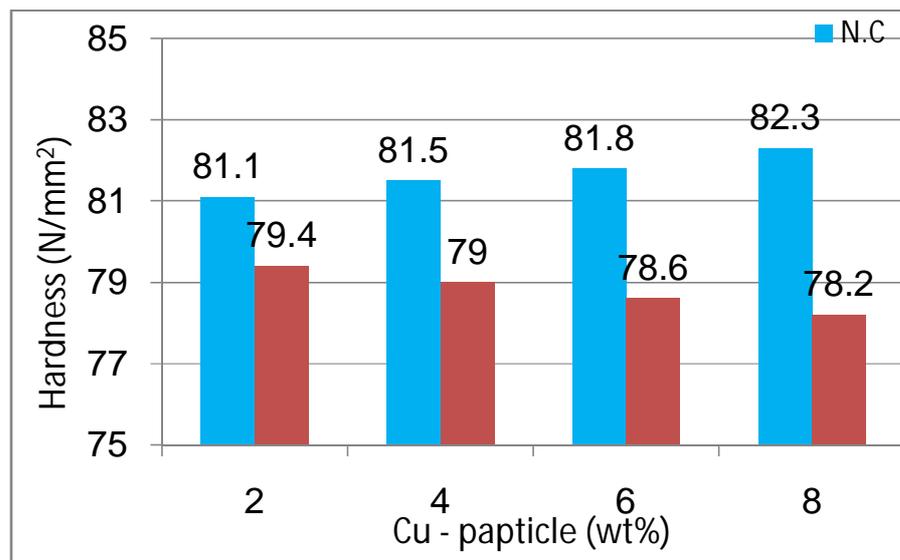
Fig.2.Shows hardness values agaist cu – particles (wt%) for samples at (N.C) and immersion (15)day in NaOH solution
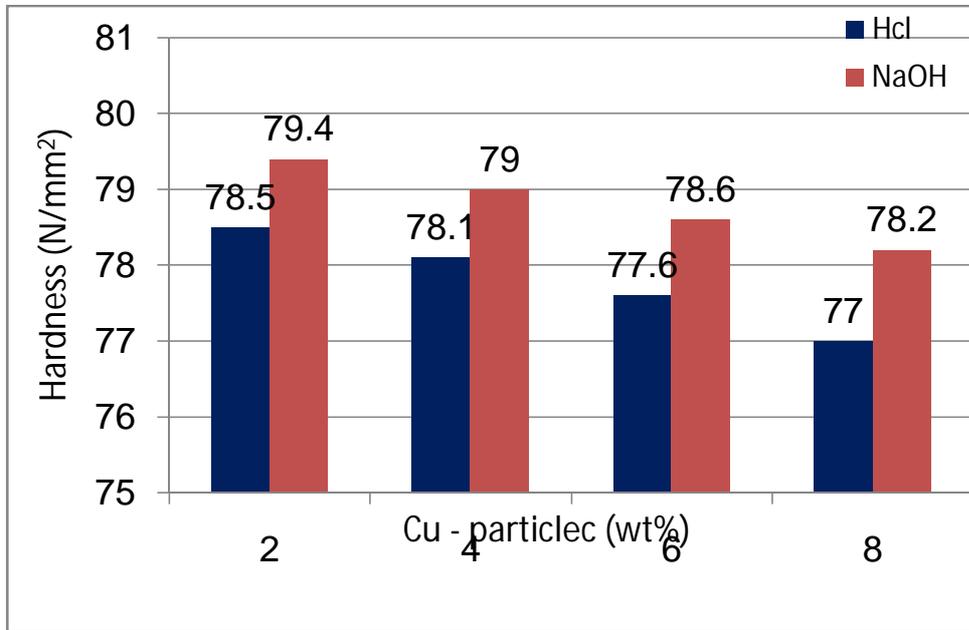
Fig.3. Hardness values comparison between (HCl) and (NaOH) solutions with Normality (0.2)
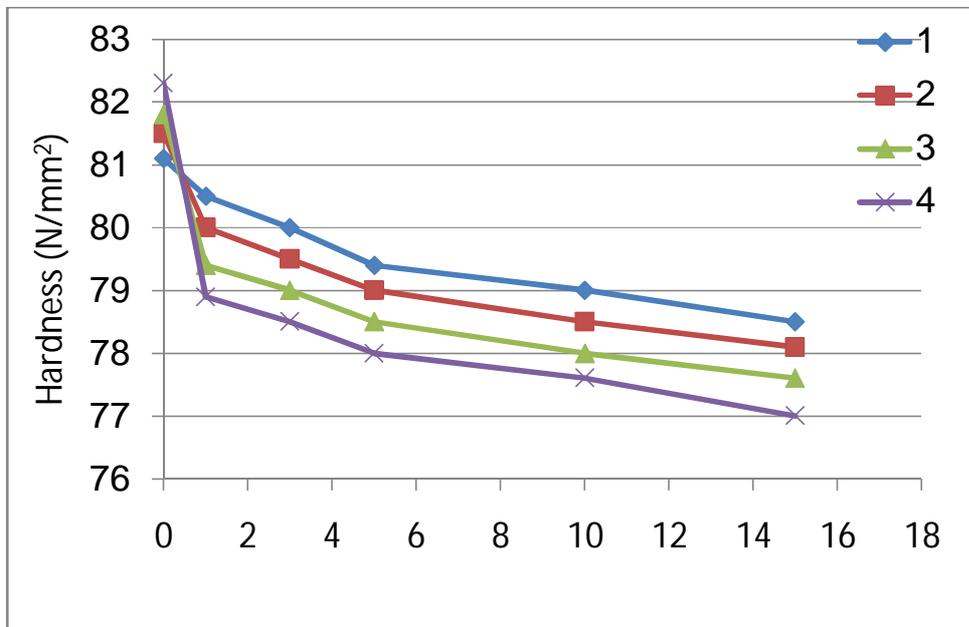
Fig .4. Hardness values as afunction of immersion time (day) in (HCl) solution (0.2N)

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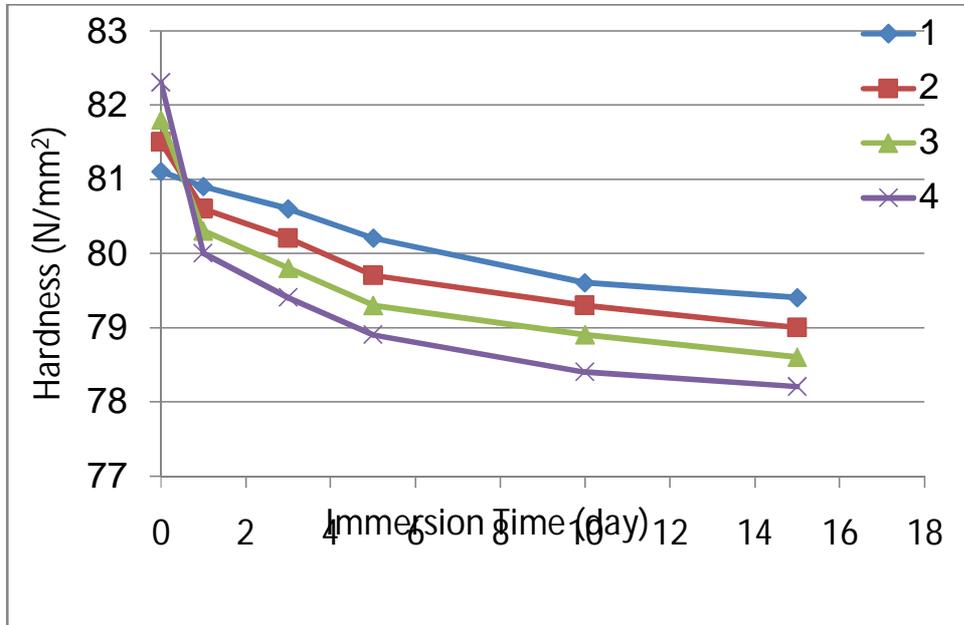
Fig .5.Hardness values of sample as a function of immersion time (day) in (NaOH) solution (0.2N)
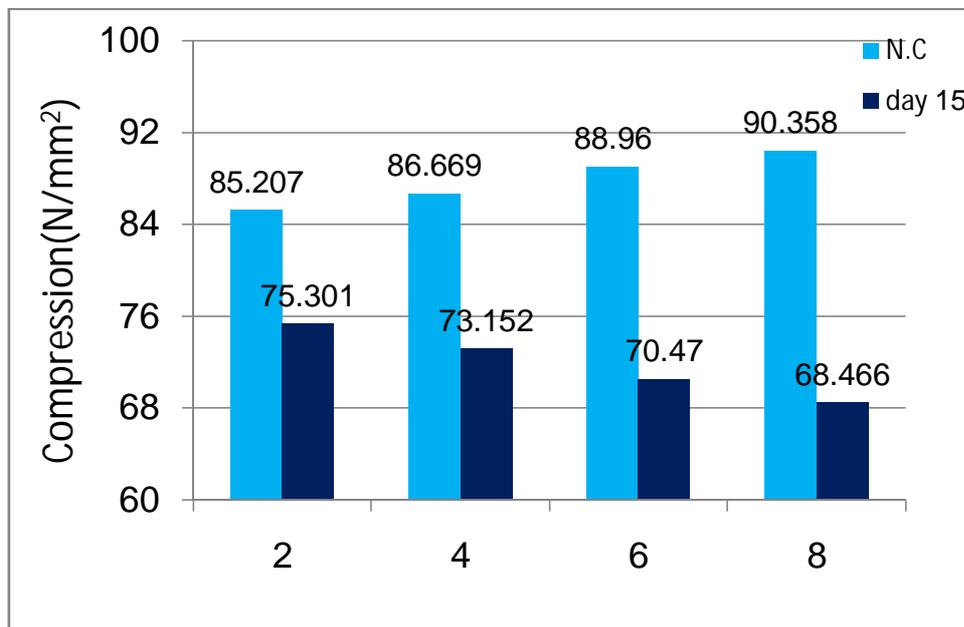
Fig . 6. Compressibility values as a function of Cu – particle (wt%) at (N.C) and immersion (10) days in (HCl) solution (0.2N)

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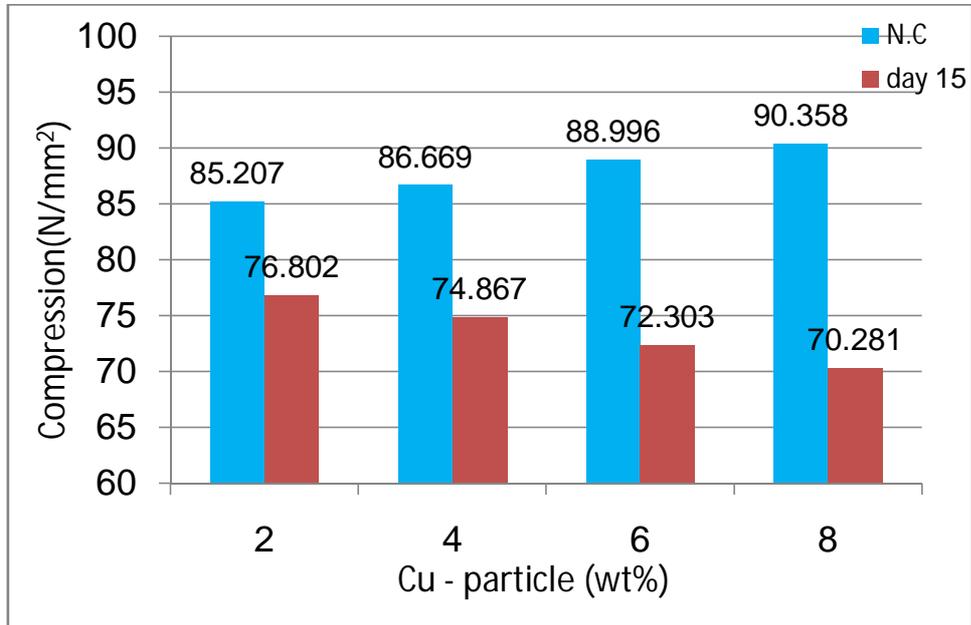
Fig .7. Compressibility values as a function of Cu – particle (wt%) at (N.C) and immersion (10) days in (NaOH) solution (0.2N)
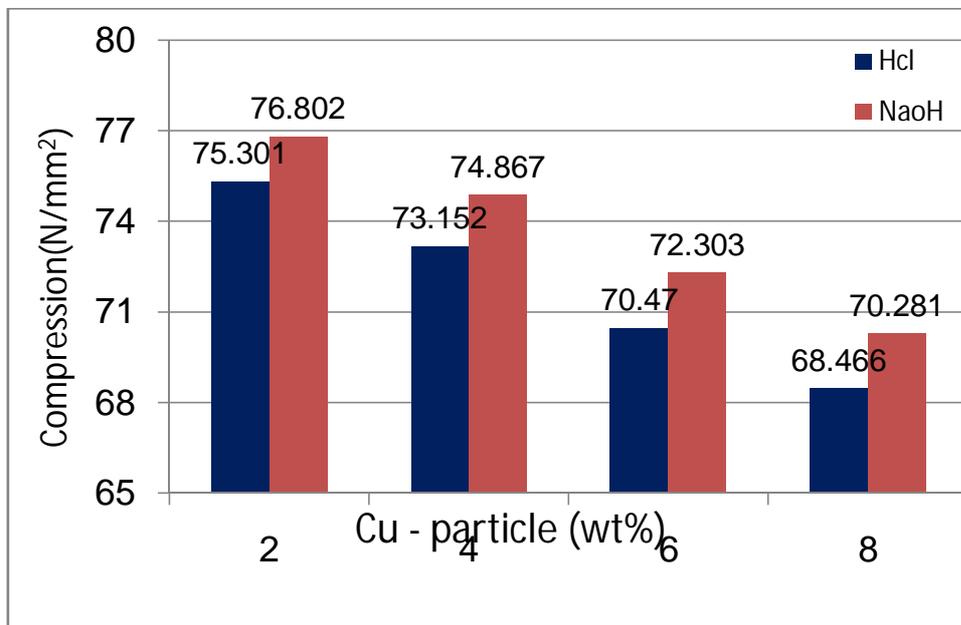
Fig .8. Compressibility values as a function of Cu – particle (wt%) after immersion (10) days in (HCl) and (NaOH) solution

RESEARCH ARTICLE

Green Roofing System - Mitigation of Urban Heat Island Effect in Coimbatore

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ABSTRACT

Green roofs provide environmental benefits by protecting buildings against solar radiation and temperature fluctuations and by reducing building's energy consumption by direct shading. Green roofs are used as aesthetic elements as well as their ecological benefits for the city and urban environment. Comparative measurements were performed through field study in Tamil Nadu Agricultural University (TNAU) to produce quantitative data on this subject and investigate the thermal properties of a typical extensive green roof. This paper analyses the thermal properties of a typical extensive green roof in comparison with a concrete roof (reference roof). During measurement period, it has been confirmed that a typical extensive green roof with 0.15 m thick growing media provided a thermal protection to the building envelope against extreme temperature effects. The maximum temperature and minimum temperature of green roof was recorded in the study period from 20.4ºC in the month of February, 2015 and 16.2ºC in the month of November respectively, whereas the maximum and minimum temperature of reference roof was obtained from 34.2ºC in the month of February, 2015 and 20.2ºC in the month of November respectively. The temperature differences between green roof and reference roof slabs had reached to 14.2ºC for maximum temperature and 16ºC for minimum temperature. In this study, the rate of decrease in temperature of green roof for maximum and minimum temperature was found from 0.4ºC and 0.2ºC. Results obtained from the field measurements show that green roofs are a sustainable choice in Coimbatore climate conditions.
Keyword: Urban Heat island Effect, Green Roof, Temperature fluctuations

INTRODUCTION

A green roof is a vegetative layer grown on a rooftop. As with trees and vegetation elsewhere, vegetation on a green roof shades surfaces and removes heat from the air through evapotranspiration. These two mechanisms reduce temperatures of the roof surface and the surrounding air. The surface of a vegetated rooftop can be cooler than the ambient air, whereas conventional rooftop surfaces can exceed ambient air temperatures by up to 90°F (50°C) (Liu K.and Bakaran B., 2003). There are several different types of green roof: intensive, and extensive. The main difference between each of these types of green roof is the depth of the substrate and the vegetation used (with a thicker layer of substrate allowing for a greater variety of plants) (Urbis limited. 2007). Assessment of temperature distributions in roof layers and defining the role of extensive green roofs in terms of sustainability were investigated within the scope of this research.

MATERIALS AND METHODS

The green-roof experiment was conducted at Coimbatore, which is located in the tropical climate zone at latitude 11º1.1' North and longitude76º 58.5' East in the state of Tamilnadu, The average annual rainfall of the region is 612 mm received in 45 rainy days. About 55 per cent of annual rainfall is received during North East Monsoon season and 30 percent during south west monsoon. The south-west monsoon contributes rain in the months from June to August. A humid September is followed by an October-November rain from the retreating North east monsoon. The green roof system and allocation of instrumentation was shown in Fig. 1. From bottom to top, the installed green roof consists of: (1) Concrete based roof deck, (2) a water proofing membrane, (3) a Root barrier , (4) a drainage layer, (5) a filter membrane , (6) a growing medium The properties of growing medium and (7) Marigold (scientific name: *Calendula officinalis*) as a vegetation layer.

Temperature profile

The temperature data analysis of different layers (Vegetation, Growing medium and onside roof slab) of an extensive green roof was investigated to provide a synoptic summary of temperature variations by experimental green roof and Reference roof.

RESULT AND DISCUSSION

Temperature fluctuations in roof layers

In the study period, temperature measurements were made in the bottom layers of roof systems to analyze the temperature change between the surface and roof slabs. Green roof protected the roof slab and waterproofing membrane from extreme temperature effects that occurred on the surface of the roof. Extreme temperature fluctuations on the roof surface have caused rapid temperature changes on the roof slab of the reference roof. The fluctuations of temperature (Fig 2) for roof slab was obtained very low compared to vegetation and growing media. In the study period, temperature measurements were made in the bottom layers of roof systems to analyze the temperature change between the surface and roof slabs. Roof slab temperature values were measured to determine the heat attenuation ability of roof systems. Green roof protected the roof slab and waterproofing membrane from extreme temperature effects that occurred on the surface of the roof. Extreme temperature fluctuations on the roof surface have caused rapid temperature changes on the roof slab of the reference roof. The fluctuations of temperature for roof slab was obtained very low compared to vegetation and growing media. The temperature fluctuations for the study period, the maximum temperature and minimum temperature were obtained by 28.1ºC at 14 hr and 10.8ºC at 1 hr respectively.

Temperature profile for Green roof and Reference roof

In the study period, the temperature fluctuations in both green roof and reference roof were same but the temperature of green roof was minimum compared to reference roof. In this study, the rate of decrease in temperature of green roof for maximum and minimum temperature was found from 0.4ºC/hr and 0.2ºC/hr. This illustrates the ability of green roof growing medium to decrease surface temperatures due to the evaporation of moisture from the soil and also the vegetation can also reduces the surface temperature by evapotransipiration. In measurement period (Sep 2014 – Feb 2015), the temperature fluctuations in the vegetation layer were regularly monitored. The average temperature variations of vegetation layer for the study period, starting from midnight, begins to drop slowly, the reaching of daily minimum of 13ºC at 1 hour. Thereafter, it rises rather quickly to reach the maximum of 30.7º C at 14 hr. It then drops quickly to 22ºC at 18 hr, and cools at a slow and rather constant rate of about0.15ºC/h throughout the night. The temperature fluctuations for the study period, the maximum temperature and minimum temperature were obtained by 33.5ºC in the month of September, 2014 at 14 hr and 10.8ºC in the month of November, 2014 at 1 hr respectively.

Temperature values occurring on the surfaces of the roofs affect the roof layers beneath. Presence of plant canopy shades the roof and decreases temperature values occurring on roof surface. While bringing an ecological solution to the structural surfaces with green roof systems, it is also aimed to mitigate urban heat island effects in urban scale and reduce extreme temperature effects in building level. In entire measurement period the temperature of growing medium (substrate) was investigated. The temperature variations of growing medium was monitored, at midnight the average temperature begins to drop slowly, thereafter it was attained maximum of 29.5ºC at 14 hr. It then delineate quickly to 21.1ºC at 18hr and cools at a slow and steady rate. In the month of September, 2014 and October, 2014 evaporation rate in the growing media was remarkable due to moderate precipitation and moisture content. In a study of Jim and He, 2010, it was highlighted that the substrate moisture is effective in regulating substrate thermal behavior. As a similar result, inadequate soil moisture and shallow substrate has caused overheating of the growing media and the surface of the green roof in this study. In addition, the result was agreed well with some research studies as well as Jim and Peng,2012 which shows the shallow substrates allows solar energy to heat up the entire layer and increases ET due to moisture content. In the month of December, 2014 to February, 2015 severe temperature fluctuations were observed on the growing medium layer and reached up to 30.3°C. When increasing evapotranspiration rate, it attributed to reduce heat intensity over the growing medium. In the study period, temperature measurements were made in the bottom layers of roof systems to analyze the temperature change between the surface and roof slabs. Roof slab temperature values were measured to determine the heat attenuation ability of roof systems. Green roof protected the roof slab and waterproofing membrane from extreme temperature effects that occurred on the surface of the roof. Extreme temperature fluctuations on the roof surface have caused rapid temperature changes on the roof slab of the reference roof. The fluctuations of temperature for roof slab was obtained very low compared to vegetation and growing media. The temperature fluctuations for the study period, the maximum temperature and minimum temperature were obtained by 28.1ºC at 14 hr and 10.8ºC at 1 hr respectively.

CONCLUSION

Thermal impacts of a typical green roof in climate conditions of Coimbatore have been analyzed in terms of their expected benefits for the building and the surrounding urban environment in a case study (TNAU). Findings obtained from this study confirm that a typical extensive green roof contributes to reduce the extreme thermal effects in urban environment. Also, it can be said that a typical green roof is a sustainable choice for Coimbatore climate conditions.

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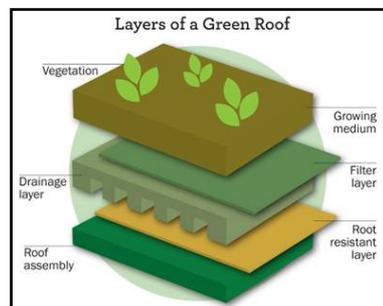
Fig.1. Layers of a Green Roof
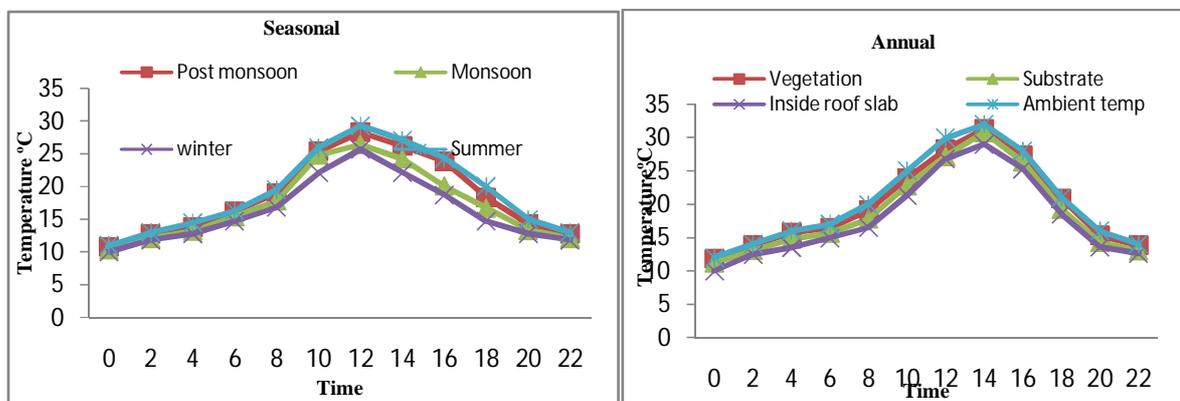
Fig 2. Fluctuations of temperature for roof slab

RESEARCH ARTICLE

Effect of Withania Somnifera against Cadmium Induced Testicular Toxicity in Rats

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ABSTRACT

The present study was carried out to assess the protective effect of ethanolic extract of Withania somnifera (Ashwagandha) whole plant powder on cadmium induced testicular toxicity (Micrometry changes) in reproductive organs of male Wistar rats. Cadmium in the form of cadmium chloride was administered to Wistar albino rats for 45 days (n=48). Upon this cadmium treated rats ethanolic extract of Withania somnifera was administered orally for 30 days to observe its ameliorative effect on male reproductive system. The results revealed that, there was a significant (P<0.01) improvement in micrometry changes in testis and accessory sex organs, thereby reducing the severity of Cdcl$_2$ toxicity in male rats.

Keywords: Withania somnifera, Micrometry, testis, accessory sex organs, Cdcl$_2$ toxicity.

INTRODUCTION

Cadmium (CAS registry number 7440-43-9) is a naturally occurring element of relatively less abundance in the earth's crust (0.1-0.5 ppm), which occurs in air, water, soil, tissues of plants and animals. Cadmium is present primarily in ores of zinc, copper or lead, the extraction and processing of which releases large quantities into the atmosphere, hydrosphere and soil. It is toxic, nonessential and classified as a human carcinogen by the North Carolina National Toxicology Program (NTP, 2000). Cadmium is used to produce colorants, stabilizers of plastics and electroplating protective coatings, solders, alloys and cadmium rods. It is also used for the production of alkaline nickel-cadmium batteries, fireworks and fluorescent paints (Martelli, 2006). Cadmium impairs reproductive capacity by causing

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severe testicular degeneration, seminiferous tubular damage and necrosis in rats (Xu et al., 2005) also enhances intracellular reactive oxygen species (ROS) production and lipid peroxidation, which may lead to tissue damage (Gupta et al., 2004 and Kara et al., 2005). Withania somnifera (Solanaceae), commonly known as Ashwagandha, Indian ginseng and Winter cherry (Andallu and Radhika, 2000), has been an important herb in the Ayurvedic and Indigenous medical systems for over 3000 years. Many studies indicate that Ashwagandha possesses Antioxidant (Dhuley, 1998), Antitumor (Jayaprakasam et al, 2003), Anti-inflammatory (Begum and Sadique, 2004), Immunomodulatory (Rasool and Varalakshmi, 2006), Antistress (Dadkar et al, 1987), Adaptogenic (Bhattacharya and Muruganandam, 2003), Antiulcer (Bhatnagar et al, 2005) and Rejuvenating properties (Patil et al, 2012). W. somnifera used for its aphrodisiac, liver tonic, astringent, emaciation, insomnia, senile dementia (Pattipati et al, 2003), analgesic effect (Kulkarni and Ninan, 1997), memory-improving effects (Schliebs et al, 1997), exhibit antibacterial and anti-fungal (Devi et al., 1993). Hence the present study was carried out to evaluate protective effect of ethanolic extract of Withania somnifera against cadmium induced testicular toxicity.

MATERIALS AND METHODS

Collection and identification of plant

Fresh plants of Withania somnifera were collected from district of Parbhani during the months of May – June. From the collected plant, herbarium was prepared for identification and authentication from the Department of Botany, Marathwada Agricultural University and voucher specimen was deposited in the department.

Preparation of *Withania somnifera* ethanolic extract

The whole plant of *Withania somnifera* was shade dried and grinded to powder. The powder was extracted with 70% ethanol using soxhlet apparatus for 48 hrs and the residue was concentrated and dried at 37°C using Rota vacuum evaporator and the extract was stored under refrigeration until further use.

Animals:

Wistar albino rats (48 male animals) weighing 160g to 180g of 8 weeks old, were obtained from animal house facility of Raj Biotech Wing, Satara, Maharashtra. The research work was approved by the IAEC (Institutional Animal Ethics Committee) with IAEC No. 04/PHARMACOLOGY/ 2007/ MAFSU- COVAS/PBN/PG. Food and water to rats were provided ad libitum (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages. The rats were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22±20C with 12 h light/dark cycle.

Chemical

Cadmium chloride (CdCl2) purchased from Qualigens, Mumbai, India. All other chemicals used were of analytical grade.

Qualitative phytochemical screening

Ethanolic extract of Withania somnifera whole plant powder were analyzed qualitatively for various phytochemical constituents as per standard procedures (Harborne, 2005).

Evaluation of acute oral toxicity

Acute oral toxicity testing of ethanolic extract of whole plant was carried out according to the Organization for Economic Co-operation Development guidelines (OECD, TG-420, 2001).

Experimental design

The animals were grouped into 4 groups of 12 rats each. Group 1 was Control group (n=12) to which no treatment was given and was designated as healthy control. Group 2 animals were administered with Cadmium chloride at the dose of 200 ppm in drinking water for 45 days. Upon these cadmium chloride pre-treated groups, Withania somnifera ethanolic whole plant extract was administered at the dose of 250 and 500 ppm to the Group 3 and Group 4 orally daily for 30 days. At the end of the treatment, animals were anaesthetized and animal dissected, organs were collected and fascia is removed for further examination. The histological sections of testis and accessory sex organs were subjected to micrometry to study the size of important structures by means of an ocular micrometry. A stage micrometer was mounted on microscope slide that does have units (millimeters (mm) or micrometers (µm)). When calibrating, line up the stage micrometer with the ocular micrometer and count the number of divisions on the ocular micrometer per millimeter or micrometer on the staged micrometer. The micrometry included the following aspects viz. thickness of the capsules of testis, diameter of seminiferous tubules, thickness of interstitial space, height of sertoli cells, diameter of various stages of germ cells, diameter of lumen of seminal vesicles, height of secretary epithelial cells of seminal vesicles and diameter of lumen of prostate gland. The micrometry was carried out by examining the maximum possible histostructures in many slides of each tissue. The data was analyzed by using Equal Completely Randomized Block Design and all the measurements were expressed as Mean ± Standard Error (Mean±SE).

RESULTS AND DISCUSSION

The highly sensitive cellular composition of the spermatogenic epithelium and the high rate of mitotic activity make the test is more vulnerable to environmental and occupational hazards than other tissues (Queiroz and Waissmann, 2006). In recent years, heavy metal pollution, particularly cadmium, lead, arsenic and chromium (Daud et al., 2013) has threatened living beings, causing adverse effects on human health such as renal and testicular dysfunction and pulmonary problems (Rukhsanda et al., 2014). In the present study, testicular toxicity was induced by administering cadmium chloride and protective effect of Withania somnifera was evaluated with reference to the micrometry of reproductive organs in wistar albino rats. The phytochemical screening revealed the presence of major phytochemicals like Alkaloids, Flavonoids, Glycosides, Tannins and Phenolic compounds. Similar observations were found by Asha rani et al, (2012). Ethanolic extract of W. somnifera plant were tested for acute oral toxicity in rats, the extract possessed no toxicity up to 2000 mg/kg respectively. The animals were healthy, feed and water intake were normal. There was no untoward behavioural change during entire period of study.

The micrometry of testis is depicted in table 03, there was no significant difference in thickness of capsule of testis between the control and all treated groups. The diameter of seminiferous tubules and height of sertoli cells were significantly ($P<0.01$) reduced and thickness of interstitial space was significantly ($P<0.01$) increased in cadmium treated groups. A significant ($P<0.01$) improvement in the above mentioned parameters were noticed in the rats treated with ethanolic extract of W. somnifera @ 500 ppm. Cadmium exposure has been reported to be a risk factor for infertility. Studies have shown that exposure to cadmium causes lipid peroxidation, which is associated with cadmium toxicity in testis (Shuenn et al., 2004). Cadmium can directly damage the testis. Its effects on the testis appear to be manifested mainly in the sertoli cells, which present more morphological changes under scanning electron microscopy; It also causes derangement in spermatogenesis and spermiogenesis (Boscolo et al, 1985). Withania somnifera is traditionally used to increase vital fluids, much fat, blood, lymph and cell production. It helps

to counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, rejuvenating the reproductive organs (Vaidyaratnam, 1994).

The diameter of various stages of germ cells were depicted in table 04, the mean diameter of different stages of germ cells were significantly reduced (P<0.01) among the rats treated with cadmium chloride compared to control group. However, the diameter in the plant treated @ 500 ppm was at par with control except primary spermatocyte. There was no significant difference in diameter of secondary spermatocyte in all the four groups. Cadmium induces profound and irreversible injury to the mammalian testis by disrupting endothelial cells of microvessels, oedema and haemorrhage by morphological analysis (Mason et al., 1964). Withania somnifera causes marked restoration in seminiferous tubule, primary and secondary spermatocytes and spermatogonia cells denotes normal functioning of the spermatogenesis against endosulfan exposed mice (Ranjit Kumar et al., 2012). Micrometric measurement of seminal vesicles and prostate gland were shown in table 05, there was an significant (P<0.01) decrease in the lumen of seminal vesicles and prostate gland among the rats intoxicated with cadmium chloride as compared to control group of rats. However, cadmium chloride with ethanolic extract of W. somnifera treated rats (500 ppm) showed significant (P<0.01) increased diameter as compared to cadmium treated group. The lumen of the seminal vesicles also showed a significant (P<0.01) improvement in the rats treated with plant extract as compared to cadmium chloride alone. The amelioration of male reproductive function by W.somnifera is mainly due to its antioxidant activity, with its active constituent's sitoindosides VII-X and withaferin present in the secondary metabolites may attributed to the underlying pharmacological activities (Ahmad et al., 2010, Bhatnagar et al., 2005, Gupta et al., 2003, Bhattacharya et al., 2001, Bhattacharya et al., 1997).

CONCLUSION

Thus it can be concluded from the study that, *Withania somnifera* causes restoration of testicular parameters and can be used as rejuvenative tonic and as male reproductive booster evidenced by the huge reversal of cadmium toxicity in testicular, seminal vesicle and prostate glands functions thus these plant not only possess antioxidant and rejuvenating property but also maintains the cellular integrity of testicular cells leading to the normal functioning.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

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Table.1. Phytochemical screening of Ethanolic extract of *W. Somnifera*:

SI No.	Phytoconstituents		Crude formulation
1	Alkaloids	Mayer's test	P
		Wagner's test	P
		Hager's test	P
		Dragendroff's test	P
		Tannic acid test	P
2	Flavonoids	Ferric chloride test	P
		Lead acetate test	P
3	Glycosides	Sodium hydroxide reagent test	P
		Benedict's test	P
4	Steroids	Salkowski test	P
		Leiberman Burchardt test	P
5	Tannins	Ferric chloride test	P
		Gelatin test	P
6	Phenolic compounds		P
7	Diterpenes	Salkowski test	A
		Leiberman Burchardt test	A
8	Saponins		P
9	Gums and mucilages		A
10	Carbohydrates – Molish's test		A

Table.2. Acute oral toxicity of Ethanolic extract of *W.somnifera*:

SI No.	Group	No. of animals	Dose (mg/kg)	Results
1	G-I	3	Control	No death
2	G-II	3	5	No death
3	G-III	3	50	No death
4	G-IV	3	300	No death
5	G-V	3	2000	No death

Table.3. Effect of cadmium chloride and Ethanolic extract of *W.somnifera* whole plant powder on micrometry of testis of rats:

Group	Treatment	Thickness of capsule (µ) (Mean±SE)	Diameter of seminiferous tubules (µ)(Mean±SE)	Thickness of interstitial space (µ)(Mean±SE)	Height of Sertoli cells (µ)(Mean±SE)
I	Control	59.64±5.39	388.30±2.50 [a]	44.03±1.37 [a]	61.60±2.03 [a]
II	Cdcl₂ (200ppm)	51.58±2.14	255.50±2.28 [b]	51.58±4.01 [b]	33.95±1.81 [b]
III	Cdcl₂+ *W.somnifera* @250 ppm	54±2.09	289±2.64 [c]	47±1.08 [a]	39±1.90 [a]
IV	Cdcl₂+ *W.somnifera* @ 500 ppm	53.20±3.43	324.53±3.45 [c]	36.97±0.96 [a]	43.05±2.09 [c]

*Each group contained twelve rats, Mean values in columns with different superscript are significantly variable (P<0.01).Mean values in columns with similar superscript are not significantly variable (P<0.01).

Table.04. Effect of cadmium chloride and Ethanolic extract of *W.somnifera* whole plant powder on micrometry of germ cells of rats.

Group	Treatment	Diameter (µ) (Mean±SE)			
		Spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids
I	Control (Normal feed)	4.28±0.05 [a]	12.25±0.58 [a]	5.23±0.64	5.17±0.23 [a]
II	Cdcl₂ (200ppm)	3.96±0.05 [b]	8.40±0.57 [b]	3.61±0.08	3.34±0.11 [b]
III	Cdcl₂+ *W. somnifera* @ 250 ppm	4.07±0.04	9.56±0.32	4.12±0.09	4.08±0.13
IV	Cdcl₂+ *W.somnifera* @ 500 ppm	4.19±0.03 [a]	10.02±0.15 [c]	4.98±0.20	4.69±0.09 [a]

*Each group contained twelve rats, Mean values in columns with different superscript are significantly variable (P<0.01). Mean values in columns with similar superscript are not significantly variable (P<0.01).

Table.5. Effect of cadmium chloride alone and *W.somnifera* extract on Micrometry of seminal vesicle and prostate gland of rats.

Sl No	Treatment	Diameter of lumen of Seminal Vesicles (µ)	Height of secretory epithelial cells of Seminal Vesicles (µ)	Diameter of lumen of Prostate gland (µ)
1	Control	319.50±3.94 [a]	16.10±0.57 [a]	285.32±5.76 [a]
2	Cdcl₂ (200ppm)	175.71±2.25 [b]	6.30±0.87 [b]	117.68±2.36 [b]
3	Cdcl₂ + W.somnifera(250ppm)	247.41±2.98 [c]	9.87±0.62 [c]	156.37±2.76 [c]
4	Cdcl2 +W.somnifera(500ppm)	282.10±3.67 [d]	11.90±0.5 [d]	198.11±3.11 [d]

*Each group contained twelve rats, Mean values in columns with different superscript are significantly variable (P<0.01).Mean values in columns with similar superscript are not significantly variable (P<0.01).

RESEARCH ARTICLE

In vivo Evaluation of Wound Healing Activity of *Tamarindus indica* Seed Coat Crude and Aqueous Extract in Rats.

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ABSTRACT

Wound healing activity of Tamarindus indica seed coat crude and aqueous extract was assessed in albino Wistar rats using excision wound model. Rats were anaesthetized under diethyl ether and skin excision of approximately 200 mm was made aseptically on dorsum of the body. Ointment containing 10% extract along with positive and negative control was applied topically for 14 days and readings were noted. The results revealed a significant wound healing activity of the test substance compared with standard 10% boric acid ointment.

Key words: Excision method, crude, Acqueous extract, diethyl ether, 10% boric acid.

INTRODUCTION

According to the Wound Healing Society, wounds are 'physical injuries that result in an opening or break of the skin causing disturbance in the normal skin anatomy and function (Strodtbeck, 2001). It involves structural and functional restoration of an injured tissue (Berlanga-Acosta et al., 2010). Although the healing process is continuous, it can be arbitrarily divided into four main phases: hemostasis, inflammation, proliferation, and remodeling (Mendonc et al., 2009; Velnar et al., 2009). These phases rely on complex biological and molecular events involving cell migration and proliferation, extracellular matrix deposition and angiogenesis and are regulated by growth factors (Faler et al., 2006). Tissue damage also triggers a robust influx of inflammatory leukocytes to the wound site that play key roles in clearing the wound of invading microbes but also release signals that may be detrimental to repair leading to fibrosis. Inflammatory stimuli upregulate the expression of some endothelial adhesion molecules such as E-selectin or ICAM-1 (McLaughlin et al. 1998). TNF-α (Tumor Necrosis Factor-Alpha), a well-known pro-inflammatory pleiotropic

cytokine, elicits numerous responses including proliferation, apoptosis, inflammation, and reactive oxygen species (ROS) generation (Woo et al., 2000, Li et al., 2002). In endothelial cells, TNF-α leads to an enhanced expression of vascular adhesion molecule-1 (VCAM-1) and ICAM-1(Osborn et al., 1989), thereby is enabling leukocytes to enter the inflammatory sites. Moreover, recent studies suggest that ICAM-1 induction by TNF-α is mediated via Rac1 in endothelial cells (Wung et al., 2005). Interleukin-10 (IL-10) has been proposed to have an inhibitory effect on the production of several inflammatory cytokines including TNF-α. Acute wound healing effects were indicated by inhibition of pro-inflammatory cytokines such as ICAM-1 and TNF-α and up-regulation of anti-inflammatory cytokine IL-10 (Sharma et al., 2013). Medical treatment of wound includes administration of drug either locally (topical) or systematically (oral or parental) in an attempt to aid wound repair. Herbal medicines have been used extensively to treat a wide range of medical conditions. The widespread availability and use of herbal medicines in today's world indicates an increased need to evaluate objectively their effectiveness for specific conditions (Jung, 2007) and their use is widespread in both developing and developed countries. The demand for natural remedies is growing in developing countries (Abdel-Azim et al., 2011) as such natural substances may be safer and cheaper (Narayan et al., 2011) and useful in both human (Sharp, 2009) and veterinary practices (Jaiswal et al., 2004). Hence the present study was conducted to evaluate the wound healing activity of Tamarindus indica seed coat crude and aqueous extract using excision wound method in adult albino Wistar rats.

MATERIALS AND METHODS

Test materials

The seeds of *T. indica* were collected from Vythri taluk of Wayanad district, Kerala and identified by a botanist, MSSRF, Kalpetta. The crude seed coat was separated and powdered whereas, aqueous seed coat extract was done with boiling water and extracted according to standard protocol. Before dermal application the extract was mixed with 10% white soft paraffin (medical grade).

Phytochemical analysis

The crude seed coat and aqueous extracts were tested qualitatively for different phyto-constituents like steroids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes, saponins and alkaloids using standard methods described by Harborne, 2005.

Experimental design

The animal study was carried out with the Institutional Animal Ethical Committee Clearance (IAEC). Nulliparous and non-pregnant female Wistar albino rats between 8 and 12 weeks old and weighing around 150-180g ± 20 % of the average body weight were used for the study. The temperature of the experimental animal room was maintained at 22±3ºC and the relative humidity of 60-70%. The sequence of 12 hours light and 12 hours dark cycle was maintained through artificial lighting. For feeding, conventional laboratory animal feed was used with *ad-libitum* drinking water. Animals were randomly selected for use in the study and marked to provide individual identification. Approximately 24 hours before the study, hairs on the dorsal surface of the body was shaved and skin was removed by shaving, with care to avoid abrasion. Approximately 10% of the body surface area was cleared for the application of the test substance. The seed extracts were made into ointment using 10% white soft paraffin as vehicle.

Acute oral toxicity

Acute toxicity study was carried out according to the Organization of Economic Corporation Development Guidelines [OECD, TG-420 (2001)] guidelines along with the principles & criteria summarized in the Humane

Endpoints Guidance Document [OECD, TG-423 (2001)]. The extract dissolved in distilled water was administered orally in doses of 5, 50, 300 and 2000 mg/kg to the group of three each rats and the animals were observed for 24 hours for morbidity and mortality. During the first 1 h after the drug administration, the rats were observed for any gross behavioral change and the parameters observed were hyperactivity, grooming, convulsions, sedation and loss of righting reflex, respiration, salivation, urination and defecation (Vogel, 2002).

Wound healing activity

Excisional wounds are created according to the method described by Kokane *et al.,* (2009) with slight modifications. The Wistar rats were anesthetized with diethyl ether intranasal using anesthetic chamber. The dorsal neck of the animals was shaved and an excision wound of 200 mm was made on the shaved surface. The wounding day was considered as day 0. A 10% of extract was applied on the wound till the wound was healed. The negative control animals were treated with white soft paraffin (vehicle) and 10% boric acid was used in positive control animals. The wounds were monitored and the area of wound was measured on 14 post-wounding days and the mean % wound closure was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound. Wound healing rate was calculated (Muthusamy *et al.,* 2008)

$$\text{Percentage of wound closure} = \frac{(\text{wound area on day 0} - \text{wound area on day n}) \times 100}{\text{Wound area on day 0}}$$

Where, *n* =number of days (4th, 8th, 12th, and 16th day).

RESULTS

Tamarindus indica seeds were identified and authenticated by MS Swaminathan Research Foundation, Wayanad, Kerala and specimen voucher has been deposited in the foundation. The results of qualitative phytochemical analysis were shown in table 01. A preliminary phytochemical screening on the active extracts showed the presence of alkaloids, tannins, phenolic compounds in both the extracts, saponins present in aqueous seed extract and diterpenes in seed coat crude
.
Acute toxicity study

The acute toxicity test after oral administration of 2000 mg/kg of extracts revealed no toxicity. There was no significant alteration in water or food consumption or body weight during the experiment. The LD_{50} of both the extracts were found to be >2000 mg/kg body wt.

Wound healing activity

Wound healing activity presented in image 01 can be attributed for the presence of secondary plant metabolites and pharmacological activity of the test substance was comparable to standard 10 % boric acid.Secondary metabolites like phenolic compounds and flavonoids as biologically active ingredients in the treatment of wound healing particularly due to their antioxidant, antibacterial and anti-inflammatory properties (Leo *et al.,* 2010). Alkaloids attributes to their inhibition of a number of inflammatory molecules including lipooxygenase, cyclooxygenase, leukotrienes and prostaglandins (Omprakash *et al.,* 2007). Tannins are reported to possess anti-inflammatory property evident by the inhibition of prostaglandin synthesis (Timothy *et al.,* 2011). Extracts rich in phenolic compounds can provide additional benefits over the individual components in inhibiting oxidative stress, (Manca *et al.,* 2013).

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DISCUSSION

Wound healing involves structural and functional restoration of an injured tissue (Berlanga-Acosta *et al.*, 2010). It occurs as complex, inherent cellular responses to injury involving harmonized interactions between various immunological and biological systems (Tarameshloo *et al.*, 2012). The diverse processes of healing may be integrated into a succession of dependent phases like hemostasis, inflammation, proliferation, and remodeling. The time course of acute wound healing usually ranges from one to four weeks (Velnar *et al.*, 2009).

The topical application of both crude and aqueous extracts, as well as the reference drug, increased the percentage of wound contraction, inhibited infections, and accelerated re-epithelialization. The results also showed the presence of well-structured epidermal layer, which was close to normal skin. This indicated that the re-epithelialization process in the wounds treated with the extract preparation was in a more advanced stage, which was further confirmed by the good reorganization of the derma layers. Infact, the wound healing properties of plants has generally been attributed to their antioxidant, anti-inflammatory activities (Suntar *et al.*, 2012). Antioxidants have been shown to reduce oxidative stress due to their ability to scavenge free radicals, reduce lipid peroxidation and inflammatory damages, promote the proliferation of fibroblast adhesion during wound healing, and stimulate the re-epithelialization, neovascularization and maturation of extracellular matrices (Weeks *et al.*, 2007).Although the exact mechanisms and modes of action of the *T. indica* seed coat crude and aqueous extracts described in this work have not yet been fully determined, their wound healing effects could presumably be attributed to their bioactive molecules and their associated anti-inflammatory, antimicrobial and antioxidant activities. Further detailed study is required to elucidate the exact mechanism. Hence from the present study it can be concluded that *T. indicus* seed coat crude and aqueous extract possess wound healing activity without any significant toxic effects.

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Conflict of Interest

None declared.

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Positive control	210	200.	196.	176.	157.	137.	121.	107.	93.5	54.2	33.5	21.2	10.5	4.25
Negative control	189	171.	162.	150.	138	131.	121	113.	105.	102.	98.2	91.6	89.1	77.3
T. seed crude	181.	167.	154.	136.	147.	138.	130.	116.	90.5	56.5	44.7	34	22.5	15.2
T.seed aqueous	187.	173	160.	147.	131	117.	90.7	56.2	40.7	35.8	29	23.7	13	9.5
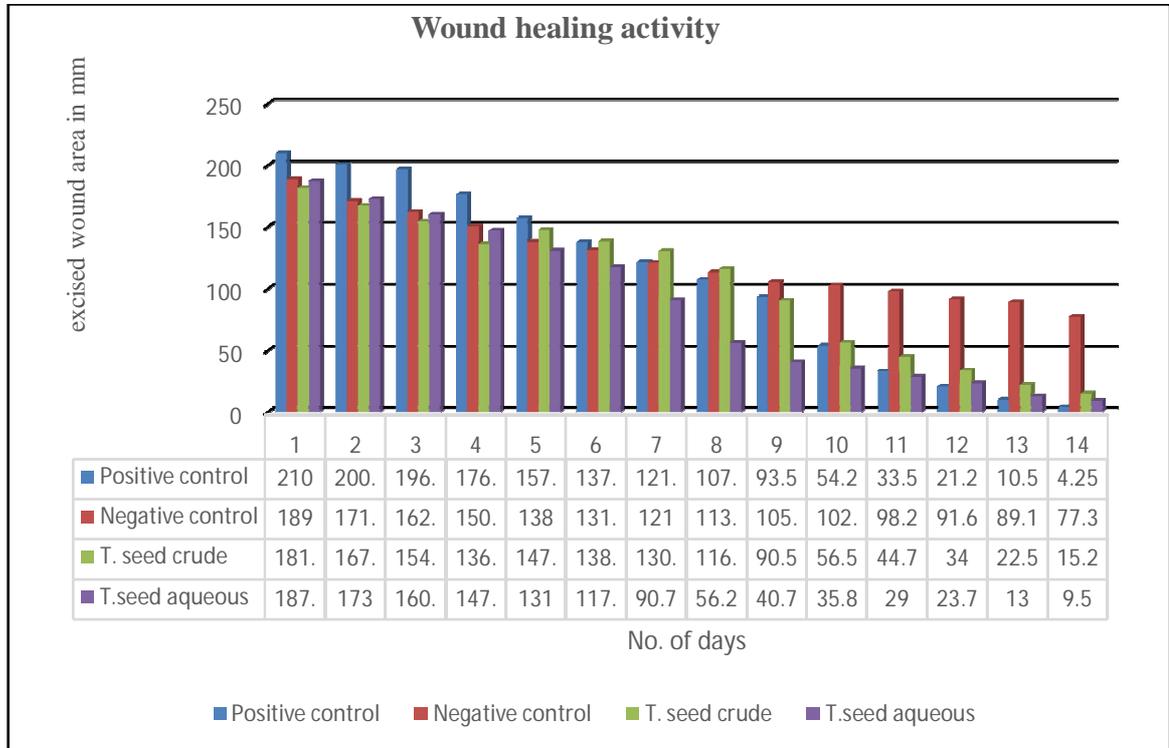
Fig. 1. Wound healing activity of T. indica seed coat crude and aqueous extracts for 14 days – excision wound model in rats.

RESEARCH ARTICLE

Evaluation of Antagonist Potential of *Bacillus spp.* against Plant Pathogenic Fungus

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ABSTRACT

Wheat (*Triticum aestivum)* belonging to family *Poaceae* is theleading essential food crop around the world. Several biotic and abiotic factors cause significant loss of production of wheat. Among biotic agents, one of the major plant pathogen *Tilletia indica* (smut fungus) causing Karnal Bunt, attributes more than 40% yield losses and it is spread by soil borne spores i.e. Teliospores. Considering the harmful effects of chemical fungicides on man and the environment, many countries in the world are developing biological control as the better alternative to chemical control. Biological control may be safe, effective and ecofriendly method for plant disease management.Present study aims to determine *in vitro* antagonistic efficiency of different gram positive and gram negative bacteria against wheat pathogenic fungus *T. indica*. Measurement of mycelial growth of *T. indica* was done on every 7th day interval up to 60th day by dual culture technique.Among various strains, *Bacillus subtilis*was found to cause morphological changes in the pathogenic fungus under *in vitro* culture condition.In dual culture assay and assay for volatile compounds *Bacillus subtilis* revealed significantly higher inhibition on tested pathogen. Protease activity was further checked to confirm the antagonistic effect of *Bacillus subtilis*. Antifungal compounds (secondary metabolites) were extracted from bacteria to determine the inhibitory activity against pathogenic fungus using poison food technique.These experimental results showed the fungicidal effects of various bacterial species against pathogenic fungi and future prospects of *B. subtilis* as a biocontrol agent.

Keywords - *Tilletia indica*, Karnal Bunt, *Bacillus spp.,* biocontrol

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INTRODUCTION

Karnal bunt of wheat (*Triticumaestivum*) is caused by the smut fungus *Tilletiaindica*. It was first discovered in 1930 at the Botanical research station, Karnal, Haryana, in Northwest region of India [1]. It has been reported in all major wheat growing states of India, Pakistan, Iraq, Mexico, and Afghanistan (1). *T.indica* is a basidiomycetous pathogen which belongs to the order Ustilaginales. The prime effect of KB is to reduce yield and confer bad taste to wheat flour, ultimately reducing the quality of the flour [2]. Low temperature and high humidity significantly favors this disease. *T.indica* spores distribute through seed, soil and wind [1]. Germination of *T.indica* teliospores begins after 5-6 days of incubation under optimal conditions. The KB pathogen infection occurs on the floral parts,entering into the glumes, rachis, ovary and then entire kernel [3]. Teliospores of *T.indica* are very tough to eradicate as they are resistant to extremes of heat, cold and harsh chemical treatment. Chemical control of the disease is very challenging and is not cost effective as teliospores of *T. indica*have staggered germination and can stay viable for 5–6 years and maximum numbers of the fungicides tested against teliospores are fungistatic and not fungicidal [4]. It has been suggested that the use of different strains of genus *Bacillus* or use of their metabolites could be alternative method to chemical method of plant [5]. *Bacillus* spp. are of great choice as they are antibiotics and endospore producers and extremely tolerant to heat and desiccation, they also have ability to produce variety of antibacterial and antifungal antibiotics such as zwittermicin-A [6], kanosamine [7]. In the present work an attempt has been made to focus on interaction of *T.indica* with different gram positive and gram negative bacteria to evaluate their antifungal activity. Some conventional approaches have been used for controlling KB which includescultural practices such as rotation of crop, sowing of disease-free seeds, and adjustment of the time of irrigation to minimize the effect of disease infection [8,9].

MATERIALS AND METHODS

Microorganism and culture condition

Culture of *B. pumilus*(MTCC 8743*)*and*B.subtilis*(MTCC 8601)was purchased from IMTECH, Chandigarh whereas culture of *P. aeruginosa,*was obtained from Centre of Biotechnology,University of Allahabad, and all the culturesweremaintained on nutrient agar at 37° in Centre of Biotechnology, University of Allahabad. Pathogenic fungi *T.indica* was obtained from G.B Pant University of Agriculture and Technology, Pantnagar and maintained on potato dextrose agar (PDA) at 21° in Centre for Biotechnology, University of Allahabad, Allahabad.

Antagonistic activity test

The dual culture technique was conducted to test the antagonistic effect of different bacteria against *T.indica.* Pairings were made between *P. aeruginosa, B. pumilus, B. subtilis*and *T.indica*using pour plate method [10]. 5 ml (10^8 cfu) of culture from *P. aeruginosa*, *B. subtilis, B.pumilus* were properly mixed in PDA for pour plate method and agar disc of size 4 mm of fungi was cut from an actively growing culture plate with the help of sterile cork borer and placed in the centre of PDA plate [11].The mycelia disc on PDA without bacteria served as control .The experiment was done in triplicates. Inhibition of fungal growth was calculated by using formula (12)   % of inhibition = C-T/C ×100
Where, C= Diameter of fungal disc in control (cm), T = diameter of fungal disc in treated (inoculated with antagonist).

Microscopic Observation

Scanning electron microscopy

The mycelia from the control plate and the treated plate with the bacteria were used for the microscopic study after incubation of 21 days. The specimens were fixed with 2% glutaraldehyde and alcoholic dehydration treatment [13].

Further coating with gold particles was done on the specimens to observe the structural changes in fungal mycelia (control and treated).

Microscopy

After 14th day of incubation, fungal mycelia were taken from control, and treated plates and their morphological changes were observed by staining with lactophenol cotton blue [14]. Structural changes were observed by inverted microscope (Leica).

Protease Activity

Proteolytic activity of all three bacteria were tested on plates containing .5% skimmed milk, 2%agar and 50mM Tris HCL (pH 8) [15]. 4mm diameter wells were made in the medium and 50 μl of enzyme source (supernatant obtained after centrifugation at 12000rpm, 4º) was dispensed for 48 hours at 37º. A Clear zone around the well due to degradation of the substrate confirms the positive reaction [16].

Test for Volatilemetabolites

Sealed plate method

The antagonism due to antifungal volatile organic compounds (VOCs) was tested, where the bacterial lawn was prepared by spreading 100μl of a suspension of *B.subtilis* cells on the nutrient agar plate. After incubation at 37º for 24 hours, the upper lid of the petri dish was replaced by a plate having 4mm fungal disc of tested pathogen on PDA in inverted position [17]. The two plates were sealed together in order to maintain the similar environment. The plate having no antagonist was considered as control.

Aerated plate method

5mm of wide strip was removed near the edge in sterilized condition in order to provide physical barrier between the two half of the petri plate. 50μl of bacterial culture was spread on first half and the fungal disc was placed on the other half of the plate. Plates were incubated at 37º [17].

Secondry Metabolite Extraction

All the bacterial culture was maintained in nutrient broth. The culture was centrifuged at 10,000 rpm for 15 min to obtain the cell free filtrate. The metabolites extracted from *B.subtilis* culture filtrate was mixed with different organic solvents (ethyl acetate, hexane, methanol) in the ratio of 1:1:1 and studied their bio-efficacy against wheat pathogenic fungi [18]. Antifungal compounds were extracted by mixing equal volume of solvents with cell free supernatant, and extract was separated from the aqueous fraction with the help of separating funnel and then evaporated in rotary evaporator at 65º C at 120rpm. The extracted metabolites were tested for their antifungal activity against pathogen with the help of Poison food technique [19].

RESULTS AND DISCUSSION

It is well reported that some species of *Bacillus* produce many antibiotics which show antifungal activity [20]. The prime achievement of this work was screening of *B.subtilis* as a biocontrol agent against *T.indica*which could be an efficient approach in inhibiting Karnal Bunt and improvement of the quality of the soil [14]. The inhibition in the growth of fungal pathogen by *B. subtilis*confirms that this bacteria has potential to produce some secondary metabolites.*In vitro* evaluation of inhibitory effect of three bacteria against *T.indica* was studied on 14thand 21st day. In

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the case of*B.pumilus* and *P.aeruginosa,* there was growth and proliferation of fungal mycelia like the control. On the other hand, growth of *T.indica*treated with *B.subtilis*was greatly reduced as shown in Fig 1. At 21st day of incubation *B.pumilus* and *P.aeruginosa*showed 4.25% and 0% inhibition respectively whereas *B.subtilis*showed 89.36% of inhibition as reported in Table(1.).Structural changes like swollen orbicular structurecalled theca was observed on the fungal mycelial head with thickening of the hypha on interaction with *B.subtilis* and *T.indica*while, the interactions of *T. indica* with *P. aeruginosa* and *B. pumilus*showed insignificant changes in the morphology as compared to the control fungus as shown in fig.2(a) (14).These observations were further confirmed by using scanning electron microscopy as shown in fig 2(b). These effects on the hyphal structure could be an indication of the amassing of various toxinsin that area [21].Among the three *B. subtilis* strain showed strong protease activity as shown in fig (3.).Proteases plays major role in cell destruction process. They exposed the protein structure, and uncover innermost glucan layers and chitin microfibrils in the result of the binding to the outer mannoprotein layer of the cell wall[22].Production of volatile compound (antifungal) by *B.subtilis* here suggests that there is more than one mode of action of antifungal activity by this bacterium against fungal pathogen [17].There was greater reduction in growth of *T. indica* in sealed plate method in comparison to aerated plate as shown in fig 4(a,b) and 4(c) asit would provide less dilution of the internal atmosphere inside the plates and less gaseous escape to the external environment. The aerated method of volatile bioassay removed possible fungal-bacterial interactions in anaerobic condition that may have ensued in the sealed plate bioassay [17]. The production of inhibitory volatiles may have the potential to increase the survival rate of bacteria in soil by eliminating possible competitors for nutrients [23].

We have demonstrated that the crude extracts from culture filtrate of *B. subtilis* showed strong inhibition. *In vitro* estimation of antifungal activity of hexane extract of secondary metabolites of *Bacillus subtilis*showed probably complete inhibition of mycelial growth of test pathogen in comparison to methanol and ethyl acetate extracts as shown in figure(5).It has been proven that numbers of Bacillus strains are safe due to their nonpathogenicity and they have been consumed in ton quantities in various commercial food arrangements also[24].*Bacillus* strains have various benefits over other bacteria (biocontrol) in several manners such as they are mostly soil inhabitants, have capability for sporulation, easy to cultivate, has long shelf life, and also some of them are able to produce growth hormones[25].

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Table 1: Calculation of % inhibition of growth of *T.indica* at 21st day of incubation with bacterial strains (*Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas aeruginosa*)

Species	Radial growth (cm)			%Inhibition (21st day)	%Inhibition (VOCs)
	7th day	14th day	21st day		
T. indica	3.7	4.7	4.7	0.0	0.0
T.indica+ B.subtilis	0	0.5	0.5	89.36	53.01
T.indica+ B. pumilus	3.0	4.0	4.5	4.25	0.0
T.indica+ P.aeruginosa	3.2	4.7	4.7	0.0	0.0

Figure 1.Antagonistic effect of the three bacteria against *T. indica* using pour plate method (a) *T. indica*(control) (b) *T. indica + B. subtilis* (c) *T. indica+B. pumilus* (d) *T.indica+P. aeruginosa*

Figure 2(a). Morphological observation of the three bacteria against *T. indica* on 21st day of incubation (mycelia stained with lactophenolcotton blue) (a) *T. indica*(control) (b) *T. indica + B. subtilis* (c) *T. indica+B. pumilus* (d) *T. indica+P. aeruginosa*

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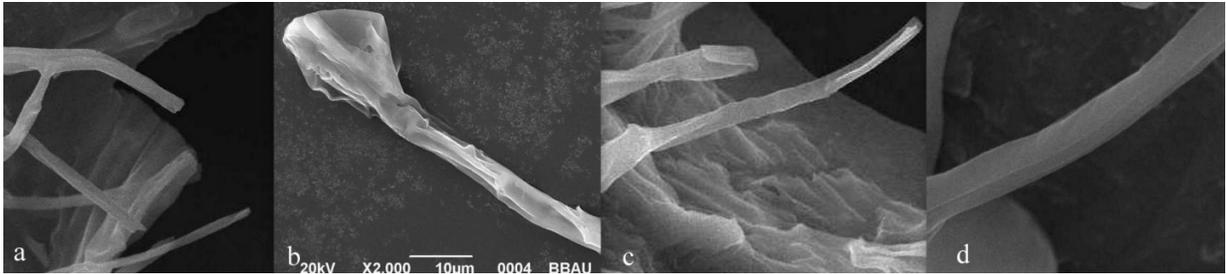
Figure 2(b). Scanning electron micrographs showing changes in fungal hyphae (a) Mycelia of *T. indica*
without any treatment (b) *T. indica + B.subtilis* (c) *T. indica+B. pumilus* (d) *T. indica+P. aeruginosa*

Figure 3. Presence of clear zone shows the proteolytic activity of *B. subtilis*
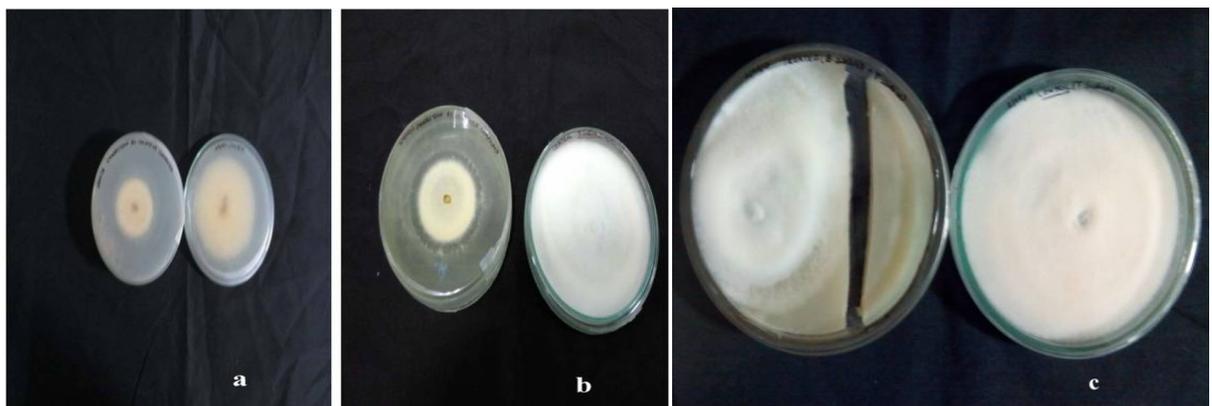
**Figure 4(a, b). Production of volatile compounds by sealed plate method at 14th and 21st day, (c) aerated
plate method at 21st day of incubation**.

Figure 5. Poison food technique showing the antifungal activity of hexane extract of *Bacillussubtilis*

RESEARCH ARTICLE

Prevalence and Diversity of Different Fly Species in Poultry Farms of Bangalore Districts

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ABSTRACT

A study was conducted to observe the prevalence of flies in eleven different poultry farms of Bangalore district in three seasons during one year period under different systems of rearing. A total of six different flies were found to occur viz. *Musca domestica, Chrysomya megacephala, Hydrotaea aenesence, Hydrotaea capensis, Hermetia illucens* and *Sarcophaga ruficornis.*In the rainy season all six variety of flies were prevalent, where as in winter and summer only *M. domestica, C. megacephala* and *Hermetia illucens* were prevalent. In deep litter system of rearing only *M. domestica* was found to be prevalent where as the in case of cage system of rearing all six different flies species were found to be prevalent viz, *M. domestica, C. megacephala, H. aenesence, H. capensis, Hermetia illucens* and *Sarcophaga ruficornis. M. domestica* was found to be predominant species present in all poultry farms. This constituted the first systematic and extensive study in Bangalore district and no reports were available from other parts of Karnataka state.

Keywords: Seasonal prevalence, Systems of rearing, Bangalore

INTRODUCTION

India's poultry industry represents a major success story. It is one of the fastest growing segments of the agricultural sector today in India with annual growth rates of 5.57 percent and 11.44 percent in egg and broiler production respectively. While agricultural production has been rising at the rate of 1.5- 2 percent per annum over the past two to three decades, poultry production has been rising at the rate of around 8-10 percent per annum, with an annual

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turnover of US$ 7500 million (Rajesh Mehta and Nambiar). It accounts for about one percent of India's GDP and 11.70 percent of the GDP from the livestock sector. Livestock population of India is among the highest in the world, it contributes approximately 4 % to GDP and 27 % to agricultural GDP (Ali, 2015). South India accounts for majority of total poultry production and consumption in the country (Abroader Consultancy India Pvt Ltd, 2015). The high growth has placed India at 3rd position after China and USA with a production of 59.8 billion eggs and 5th after USA, China, Brazil, and Mexico (Central Avian Research Institute 2011). Fly menace in poultry farms is one of the significant problems. Several species of flies are found associated with poultry production facilities such as houseflies and their relatives; flesh flies, blow flies, bottle flies, filter flies, soldier flies and vinegar or fruit flies. These flies cause annoyance, discomfort and are also harmful to human and animal health.These flies are usually scavengers in nature and are capable of transmitting many diseases to human and animals. In view of the severity of the problem, these flies need to be studied and effective control measures have to be implemented. There is very scanty information on the prevalence of different flies in poultry farms in most parts of India including Karnataka. A detailed study is essential to know the different fly species. In view of this scenario and lack of seasonal prevalence study in Karnataka, the present study was undertaken in Bangalore district to observe the prevalence of flies in poultry farms under different managemental conditions.

MATERIALS AND METHODS

Eleven different poultry farms in Bangalore were selected and flies were collected by using sweep net, different flies were preliminarily identified based on wing pattern, color of flies, head and thorax pattern and separated. Some adult flies were dry preserved to preserve their natural colour and bristles, and the rest were preserved in 70% ethanoland larvae were collected by handpicking from poultry manure of the different larvae. Larvae of different flies were separated and stored in 70% ethanol.The adult flies and larvae were confirmed subsequently by mounting several specimens on glass slides after clearing in liquefied phenol solution for 24 hr (Wirth and Martson 1967).

Morphological identification

Morphological identification of adult flies was done by using keys of White *et al.*, 1940; Sabrosky, 1949; James, 1960; Van Emden, 1965;Zumpt, 1965; Fonseca 1968; Walker, 1994; Triplehorn and Johnson, 2005; Carvalho and Mello-Patiu, 2008; Whitworth 2010; Lin *et al.*, 2010; Szpila, 2010; Meiklejohn, 2012; Ramaraj, *et al.* 2014; Irish *et al.* 2014; Akbarzadeh*et al.*, 2015; Oliveira *et al.* 2015. The characters for morphological identification of fly included length of fly, eyes, antennae, wing pattern, and colour of flies, thorax and abdominal pattern. Morphological identification of larvae were done by using keys of Zumpt, 1965; Holloway 1991; Wells*et al.*, 1999; Sukontason*et al.* 2003; Sukontason*et al.*, 2004; Brink 2009; Thyssen, 2010; Szpila 2010 .,Velasquez *et al.* 2010. Basic morphological features of larva used for identification purpose included length of larvae, integument, spines, cephalopharyngeal skeleton, respiratory structures such as anterior and posterior spiracles.

RESULTS

A total of six different flies were found to prevalent viz. *Musca domestica, Chrysomya megacephala, Hydrotaea aenesence, Hydrotaea capensis, Hermetia illucens* and *Sarcophaga ruficornis.* In the rainy season all six variety of flies were prevalent, where as in winter and summer only *M. domestica, C. megacephala* and *Hermetia illucens* were prevalent. In deep litter system of rearing only *M. domestica* was found to be prevalent where as the in case of cage system of rearing all six different flies species were found to be prevalent viz, *M. domestica, C. megacephala, H. aenesence, H. capensis, Hermetia illucens* and *Sarcophaga ruficornis.*

DISCUSSION

In the present study total of six different flies were identified and found to be prevalent in various poultry farms viz *Musca domestica, Chrysomya megacephala, Hydrotaea aenesence, Hydrotaea capensis, Hermetia illucens* and *Sarcophaga ruficornis.*In different parts of world many authors reported the prevalence of various flies in poultry farms viz. house fly *Musca domestica*, little house fly *Fannia canicularis*, blackgarbage *fly Ophyra leucostoma*, black blow fly *Phormia regina*, stable fly *Stomoxys calcitrans* and green blow flies *Phaenicia spp.* was reported by Axtell (1970) in United States. *Musca domestica, Stomoxys calcitrans, Ophyra chalcogaster, Fannia pusio,Phaenicia cuprina, Chrysomya megacephala, Chrysomya rufifaces, Parasarcophaga ruficomis, Parasarcophaga argyrostoma, Seniorwhitea orientaloides, Volucella obese, Eristalis arvorum, Hermetia illucens* were reported by Toyama and Ikeda (1976) in Leeward and Central Oahu. *Simoides pachymera, Sepsis lateralis, Coproica, Fannia leucosticte, Fannia canicularis, F. femoralis, Musca domestica, Chrysomya putoria, Muscina stabulance* were reported by Hulley (1986) in Grahamstown. House fly (*Musca domestica*), little house fly (*Fannia annicularis*), dumb flies (*Hydrotaea aenescense, H. capensis, H. ignava)*, black soldier fly, *Hermetia illucens, Drosophila replete*, blow flies such as *Lucilia, Phaenicia, Phormia, and Calliphora. L. cuprina and L. sericata* is also sometimes associated with poultry farms. Biting flies such as stable fly, *Stomoxys calcitrans, Culex quinquefasciatus* and *C. pipiens* are also known to occur. Moth flies such as the meal moth, *Pyralis farinalis*, mediterranean flour moth, *Anagasta kuehniella*, and the Indian meal moth *Plodia interpunctella*, cockroaches such as German, *Blattella germanica*, American, *Periplaneta americanum*, and Oriental, *Blatta orientalis* was reported by Axtell (1999) in USA. *Musca domestica, Fannia canicularis, Hydrotaea aenescens*, and small dung flies of the family Sphaeroceridae were reported by Rutz (2000) in United States. House fly – *Musca domestica*, little house fly – *Fannia sp.* black garbage fly – *Hydrotea* (Ophyra) *aenescens*, numerous species of blow flies (green or blue bottle flies), small dung fly – Sphaeroceridae have been reported by Williams (2010) from US. House flies *Musca domestica*, little house flies *Fannia cannicularis*, blow flies of Calliphoridae, flesh flies Sarcophagidae, dung flies Sphaeroceridae, fruit flies Drosophilidae were reported by Saif et al. (2011). *Musca domestica, Stomoxys calcitrans, Muscina stabulans, Fannia spp, Chrysomya putoria* and *Hermetia illucens* were reported by Tucci (2011) from Brasil. *Muscina stabulans, Fannia canicularis, Musca domestica*, soldier fly, *Hermetia illuscens, Stomoxys calcitrans, Alphitobius diaperinus*, also known as lesser mealworm were recorded by Levot (2013) from State of New South Wales. Little housefly, (*Fannia cannicularis*) the common housefly (*Musca domestica*) and the false stable fly (*Muscina stabulans*) wasreported by James (1960) in Australia.

In India, the prevalence of flies in poultry farms was reported by various authors viz. house fly (*Musca domestica*), little house fly (*Fannia annicularis*) coastal fly (*F. femoralis*), latrine fly *(F. scalaris)*, false stable fly (*Muscina stabulans*), blow flies, flesh flies, and filth flies reported by Muniyellappa (2010). House fly *Musca domestica* as a major pest species, little house fly, *Fannia canicularis* were reported by Harikrishnan (2009) in Namakkal, Tamil Nadu. *Musca domestica*, little house fly *Fannia canicularis*, black garbage fly *Hydrotaea aenescens* was again reported by Harikrishnan (2011) in Namakkal, Tamil Nadu. House flies as a major pest, also other flies with less importance such as Chrysomya and Fannia were reported by Ponnudurai (2013) in Namakkal, Tamil Nadu. Among the six species of flies which were recorded in the present study, they have been reported by previous workers wherein *Musca domestica* was reported in poultry farms in all the reports in India and also in other countries representing it as one of the major fly species. *Chrysomya megacephala* was reported by Toyama and Ikeda (1976), Ponnudurai (2013). *Hydrotaea aenesence* was reported by Axtell (1999), Rutz (2000), Williams (2010), Harikrishnan (2011). *Hydrotaea capensis* reported by Axtell (1999). Hermetia illuscence reported by Toyama and Ikeda (1976), Axtell (1999), Tucci (2011), Levot (2013) and *Sarcophaga ruficornis* was reported by Toyama and Ikeda (1976). General prevalence of flies in the present study was reported by various authors in India viz, *Musca domestica* by Nandi and Sinha (2004) from Sundarbans biosphere, Bharti (2008) from Punjab, Bharti (2009) from Punjab, Muniyellappa (2010) from Bangalore, Harikrishnan (2009 and 2011) in Namakkal, Tamil Nadu, Roy et al (2012) from West Bengal, Ponnudurai (2013) in Namakkal, Tamil Nadu, Subaharan and Verghese (2014) from Bangalore. *Chrysomya megacephala* was reported by Nandi (2000) from Sikkim, Singh and Bharti (2000) from Punjab, Bharti (2009), Nandi (2004) in Kolkata, Bharti (2011) from Punjab, Ponnudurai (2013) in Namakkal, Tamil Nadu, Subaharan and Verghese (2014) from Bangalore, Ramaraj et al. (2014) from Tamil Nadu, Divya and Sathe (2015) from Western Ghats, Maharastra, India.

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Hydrotaea aenesence was reported by Harikrishnan (2011) from Namakkal, Tamil Nadu. *Hydrotaea capensis* was reported by Bharti et al (2001) from Uttar Pradesh and Bharti (2008) from Punjab. *Hermetia illucens* was reported by Roy et al (2012) from West Bengal, Gayatri and Madhuri (2013) from Maharashtra. Sarcophaga ruficornis was reported by Roy and Dasgupta (1977) from West Bengal, Nandi (1992) from Gujarat, Nandi (1993) from Maharashtra, Patil (2013) from Ahmednagar, and Subaharan and Verghese (2014) from Bangalore. Among different flies only the larvae of *Musca domestica, Chrysomya megacephala* and *Hermetia illucens* were found to be present in poultry manure whereas larvae of other flies such as *Hydrotaea aenesence, Hydrotaea capensis* and *Sarcophaga ruficornis* were not found in the poultry manure. *Sarcophaga ruficornis* flies usually breed in dead carcass and were hence not found in manure. In case of cage rearing system all flies were found to be prevalent whereas in deep litter system of rearing only *Musca domestica* was as the heaping of poultry droppings under cages and accumulation of manure with moisture was favourable for breeding of different kinds of flies where as in deep litter system of rearing the dry condition of manure and constant disturbance of the deep litter was not favourable for fly breeding. This constituted the first systematic and extensive study in Bangalore district and no reports were available from other parts of Karnataka state and could not be compared.

ACKNOWLEDGEMENTS

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RESEARCH ARTICLE

A Study on the Time Dependence of the Matter Content of theExpanding Universe in the Framework of Brans-Dicke Theory

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ABSTRACT

In the framework of Brans-Dicke theory, a cosmological model regarding the expanding universe has been formulated by considering an inter-conversion of matter and dark energy. A function of time has been incorporated into the expression of the density of matter to account for the non-conservation of the matter content of the universe. This function is proportional to the matter content of the universe. Its functional form is determined by using empirical expressions of the scale factor and the scalar field in field equations. This scale factor has been chosen to generate a signature flip of the deceleration parameter with time. The matter content is found to decrease with time monotonically, indicating a conversion of matter into dark energy. This study leads us to the expressions of the proportions of matter and dark energy of the universe. Dependence of various cosmological parameters upon the matter content has been explored.

Key Words: Matter to dark energy conversion, Brans-Dicke theory, Time variation of matter and dark energy, Gravitational constant, Density of matter, Cosmology.

INTRODUCTION

It has been established beyond doubt, by a number of recent astrophysical observations, that the universe is expanding with acceleration (Bennet *et al.*, 2003; Riess *et al.*, 1998; Perlmutter *et al.*, 1999). It has been shown by several studies on cosmology that nearly seventy percent of all constituents of the universe have a large negative pressure and is referred to as dark energy (DE), which is found to have a major role in driving the expansion process with acceleration. It has not yet been possible to determine its true nature. The cosmological constant (λ) is a widely known parameter of the general theory of relativity, which is regarded as one of the most suitable candidates acting

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as the source for this repulsive gravitational effect and it conforms to observational data reasonably well, despite its own limitations (Sahni and Starobinsky, 2000). So far the researchers have proposed a large number of models regarding dark energy and their characteristics have been studied extensively (Padmanabhan, 2003; Sahni and Starobinsky, 2000). It is important to note that this accelerated expansion is a very recent phenomenon and it follows a phase of expansion of universe with deceleration. For the successful nucleosynthesis and also for the structure formation of the universe, this is important. On the basis of observational findings, beyond a certain value of the redshift (z) (i.e. z > 1.5), the universe certainly had a decelerated phase of expansion (Riess *et al.*, 2001). Therefore, the evolution of the dark energy component has been such that its effect on the dynamics of the universe is appreciable only during the later stages of the matter dominated era. On the basis of an analysis of supernova data, a recent study conducted by Padmanabhan and Roy Choudhury (2003), has shown that there has certainly been a change of sign of the deceleration parameter (q) of the universe, from positive to negative, implying that a decelerated expansion preceded the present state of accelerated expansion.

Apart from the models regarding the accelerated expansion, involving the cosmological constant (λ), many other models of dark energy have been proposed and applied for the sake of a proper explanation of findings (Copeland *et al.*, 2006; Martin, 2008). A negative value of the deceleration parameter, implying accelerated expansion, has been successfully generated by all these models. One of the most significant of these models is a scalar field with a positive potential which produces an effective negative pressure if the kinetic term is dominated by the potential term. One refers to this scalar field as the *quintessence scalar field*. In scientific literature there are a large number of models regarding quintessence potentials and they have been extensively used. One may go through a study by V Sahni (2004) on this field, to have detailed information in this regard. A proper physical explanation or background is lacking about the origins of models of most of the quintessence potentials. In order to ascertain the behaviors and roles played by these entities, one has to take into account the possibility of an interaction between the different components of the constituents of universe, such as the matter and dark energy, which is expected to give rise to a transfer of energy from one field to another. Researchers have proposed many models where a transfer of energy takes place from the component of dark matter to the component of dark energy (Zimdahl, 2012; Reddy and Kumar, 2013), in such a manner that during the later period of evolution, the dark energy predominates over matter and an accelerated expansion of universe is caused. But the interactions between two components, upon which the models were constructed, were chosen to be arbitrary in most cases, without a strong logical foundation of a physical theory. For a proper interpretation of the role of dark energy in causing accelerated expansion, a prolonged search has been going on for the theory of an interaction between matter and scalar field, on the basis of which a cosmologically viable model can be formulated.

In order to avoid the difficulties due to the arbitrariness of these models in the formulation of a particular Q-field, non-minimally coupled scalar field theories have been used to formulate models which can very effectively account for the transition from the decelerated phase to the accelerated phase of cosmic expansion. This has been made possible by the presence of the scalar field in the framework of the theory and it does not have to be incorporated separately. The most natural choice in this context is the Brans-Dicke (BD) theory which is regarded as the scalar-tensor generalization of general relativity (GR), because of its simplicity and a possible reduction to GR in some limit. Thus the Brans–Dicke theory or its modified versions have been shown to account for the present acceleration of universe (Banerjee and Pavon, 2001a; Brunier *et al.*, 2005). An observation regarding the BD theory is that it can potentially generate sufficient acceleration in the matter dominated era even without taking into consideration an exotic Q - field (Banerjee and Pavon, 2001b). However, the researchers have been looking for a theory which can explain the change of cosmic expansion state from deceleration to acceleration. In most of the models the dark energy and dark matter components are considered to be non-interacting and are allowed to evolve independently. Due to the unknown nature of these two components, one is expected to get a relatively generalized framework for study by assuming an interaction between them. It has been shown by Zimdahl and Pavon (2004) that the interaction between dark energy and dark matter can be used effectively to solve the coincidence problem. This idea may lead to the formulation of an interaction or inter-conversion of energy between the dark matter and the Brans - Dicke scalar field

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which is a geometrical field. Amendola (1999) had made a prediction earlier that there is a possibility of an inter-conversion of energy between the matter content of the universe and the non-minimally coupled scalar field.It has been found from several applications of the Brans-Dicke theory that, in most of the models, the Brans-Dicke dimensionless parameter ω is required to have a very low value, typically of the order of unity, for an accelerated expansion of the universe (Das *et al.*, 2014). It was once demonstrated in one of these studies that, considering the Brans-Dicke scalar field to be interacting with the dark matter, a generalized form of the Brans-Dicke theory can lead to an accelerated expansion even with a high value of ω (Banerjee and Das, 2006). For these studies, either one makes a modification of the Brans-Dicke theory to account for the findings properly or a quintessence scalar field is chosen to cause the required acceleration. Clifton and Barrow (2006) have shown in a recent study, which has also been shown by another group (Banerjee and Das, 2006), that no additional potential is necessary to generate the signature flip of the deceleration parameter from positive to negative. To explain the observational findings in this regard, they considered an interaction between the Brans-Dicke scalar field and the dark matter.

In the present model, a generalized form of Brans-Dicke theory has been used. In this form, the dimensionless parameter ω of Brans-Dicke theory is no longer treated as a constant. It is regarded as a function of the scalar field φ which is a time dependent quantity. This form of Brans-Dicke theory was first proposed by Bergman (1968), and Nordtvedt (1970) expressed it in a more useful form. The present study is not based upon any particular theoretical formulation regarding the mechanism of interaction between matter and the scalar field, causing an inter-conversion between matter and dark energy. A simple model has been proposed here by only taking into consideration a fact that the matter content of the universe is not conserved. This model has inherently kept open the possibilities of an inter-conversion between matter and some other form of energy, which might be regarded as dark energy, which is held responsible for generating the accelerated expansion of the universe, following a phase of deceleration. To express the density of matter (ρ) as a function of time, a function, denoted by $f(t)$, has been incorporated in its expression in a manner such that it accounts for the non-conservation of matter content of the universe. The modified expression of the density of matter (ρ) shows that if one chooses $f(t) = 1$ at all values of t, it would be conservation of the matter content of the universe represented by the expression of ρ. Empirical expressions of the scale factor (a) and the scalar field parameter (φ) have been used in Brans-Dicke field equations, in order to determine the functional form of $f(t)$. According to its definition, $f(t)$ is equal to the ratio of the matter content of the universe at any time (t) to the content of matter at the present epoch. This function $f(t)$ has been used here to formulate relevant expressions to determine the time variations of the proportions of matter and dark energy of the universe, assuming the mater content to be the only source of dark energy. The present study also explores the time dependence of the density of matter (ρ) and the gravitational constant (G). The purpose of formulating this model is to find out the effect of the change of matter content on the characteristics of the cosmic expansion. The effect of the dark energy content of the universe at any instant of time and the rate of its increase, upon the observational findings like gravitational constant, Hubble parameter and the deceleration parameter has been studied here. One finds that, the faster the generation of dark energy, more rapid would be the changes in these parameters.

Theoretical Model

For a spatially flat Robertson-Walker space-time, the following differential equations are the field equations of the generalized Brans-Dicke theory (Banerjee and Ganguly, 2009).

$$3\left(\frac{\dot{a}}{a}\right)^2 = \frac{\rho}{\varphi} + \frac{\omega(\varphi)}{2}\left(\frac{\dot{\varphi}}{\varphi}\right)^2 - 3\frac{\dot{a}}{a}\frac{\dot{\varphi}}{\varphi}, \tag{1}$$

$$2\frac{\ddot{a}}{a} + \left(\frac{\dot{a}}{a}\right)^2 = -\frac{\omega(\varphi)}{2}\left(\frac{\dot{\varphi}}{\varphi}\right)^2 - 2\frac{\dot{a}}{a}\frac{\dot{\varphi}}{\varphi} - \frac{\ddot{\varphi}}{\varphi}. \tag{2}$$

Combining (1) and (2) one gets,

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$$2\frac{\ddot{a}}{a} + 4\left(\frac{\dot{a}}{a}\right)^2 = \frac{\rho}{\varphi} - 5\frac{\dot{a}}{a}\frac{\dot{\varphi}}{\varphi} - \frac{\ddot{\varphi}}{\varphi}. \tag{3}$$

From equation (1), the expression of $\omega(\varphi)$ is obtained as,

$$\omega(\varphi) = 2\left[3\left(\frac{\dot{a}}{a}\right)^2 - \frac{\rho}{\varphi} + 3\frac{\dot{a}}{a}\frac{\dot{\varphi}}{\varphi}\right]\left(\frac{\dot{\varphi}}{\varphi}\right)^{-2} \tag{4}$$

In many theoretical models the content of matter (dark + baryonic) of the universe has been assumed to remain conserved (Banerjee and Ganguly, 2009). Following equation expresses the conservation of matter content of the universe.

$$\rho a^3 = \rho_0 a_0^3 = \rho_0 \quad \text{(taking } a_0 = 1) \tag{5}$$

There are some studies on Brans-Dicke theory of cosmology where one takes into account an interaction between matter and the scalar field. A possibility of an inter-conversion between dark energy and matter (both dark and baryonic matter) is taken into consideration in these studies. Keeping in mind this possibility, we propose the following relation for the density of matter (ρ).

$$\rho a^3 = f(t)\,\rho_0 a_0^3 = f(t)\,\rho_0 \quad \text{(taking } a_0 = 1) \tag{6}$$

In the present study we have not considered any theoretical model to explain or analyze the mechanism of interaction between matter and the scalar field. We have only considered a simple fact that the right hand side of equation (5) cannot be independent of time when one takes into account non-conservation of matter due to its generation from dark energy or its transformation into dark energy. We propose to introduce a function of time $f(t)$ in equation (5) to get a new relation represented by equation (6). This function $f(t) = \frac{\rho a^3}{\rho_0 a_0^3}$, at any instant of time t is the ratio of matter content of the universe at the time t to the matter content at the present instant ($t = t_0$). Thus $f(t)$ can be regarded as proportional to the total content of matter (dark+baryonic) $M(t)$ of the universe at the instant of time t. We have denoted this ratio by R_1 where $R_1 \equiv f(t) = M(t)/M(t_0)$. We have defined a second ratio $R_2 = \frac{1}{f}\frac{df}{dt} = \frac{1}{M}\frac{dM}{dt}$ which represents fractional change of matter per unit time. If, at any instant, R_2 is negative, it indicates a loss of matter or a change of matter into some other form due to its interaction with the scalar field. We have also defined a third ratio $R_3 = f - 1 = \frac{M(t) - M(t_0)}{M(t_0)}$ indicating a fractional change of matter content from its value at the present time.

One may assume that the process of conversion of matter into dark energy started in the past at the time of $t = \gamma t_0$ where $\gamma < 1$. Hence, $M(\gamma t_0) = M(t_0) R_1(\gamma t_0)$ is the total matter content of the universe, at $t = \gamma t_0$, when no dark energy existed. Thus $M(\gamma t_0)$ is the total content of matter and dark energy at all time. Assuming matter to be the only source of dark energy, the proportion of dark energy in the universe at any time t is given by the following ratio

$$(\backslash R_4 = \frac{M(\gamma t_0) - M(t)}{M(\gamma t_0)} = \frac{f(\gamma t_0) - f(t)}{f(\gamma t_0)} \quad \gamma < 1 \tag{7}$$

Thus $(R_4 \times 100)$ is the percentage of dark energy present in the universe. Nearly 70% of the total matter-energy of the universe is dark energy at the present time (Das and Mamon, 2014). For a proper choice of γ and k (to be defined later), we must have $R_4(t_0) \times 100 = 70$ approximately.

The proportion of matter (dark + baryonic) in the universe is therefore given by,

$$R_5 = 1 - R_4 = 1 - \frac{M(\gamma t_0) - M(t)}{M(\gamma t_0)} = \frac{M(t)}{M(\gamma t_0)} = \frac{f(t)}{f(\gamma t_0)} \tag{8}$$

Thus $(R_5 \times 100)$ is the percentage of matter (dark + baryonic) present in the universe.
The purpose of the present study is to determine a functional form of $f(t)$ to explore the time dependence of the ratios R_1, R_2, R_3, R_4 and R_5.

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Using these parameters, the density of dark energy can be expressed as,

$$\rho_d = \frac{R_4}{R_5}\rho = \frac{f(\gamma t_0) - f(t)}{f(t)}\rho \tag{9A}$$

Thus, the density of total matter and energy is, $\rho_t = \rho_d + \rho = \frac{\rho}{R_5} = \frac{f(\gamma t_0)}{f(t)}\rho \tag{9B}$

To formulate the expression of $f(t)$ we have used the following relation which is based on equation (6).

$$f(t) = a^3 \frac{\rho}{\rho_0} \tag{10}$$

Here, the density of matter (ρ) can be obtained from equation (3). For this purpose one needs to choose some suitable functional form of the Brans-Dicke scalar field φ. In the present study we have chosen an empirical forms of φ, following some recent studies in this regard (Banerjee and Ganguly, 2009; Roy *et al.*, 2013). The proposed ansatz for φ is expressed as,

$$\varphi = \varphi_0 \left(\frac{a}{a_0}\right)^k = \varphi_0 a^k \tag{11}$$

Here k is a constant which determines the rapidity with which the parameter $\varphi\left(\equiv \frac{1}{G}\right)$ changes with time.

Combining equation (11) with equation (3), one gets the following expression of density of matter of the universe (ρ).

$$\rho = \varphi H^2 [k^2 + (4 - q)k + (4 - 2q)] \tag{12}$$

Using equation (12), ρ_0 can be written as,

$$\rho_0 = \varphi_0 H_0^2 [k^2 + (4 - q_0)k + (4 - 2q_0)] \tag{13}$$

Substituting from equations (12) and (13) into equation (10) we get,

$$f(t) = a^3 \frac{\varphi H^2 [k^2 + (4-q)k + (4-2q)]}{\varphi_0 H_0^2 [k^2 + (4-q_0)k + (4-2q_0)]} \tag{14}$$

In our derivation of the equations (12) we have used the standard expressions of Hubble parameter (H) and deceleration parameter (q), which are, $H = \dot{a}/a$ and $q = -\ddot{a}a/\dot{a}^2$ respectively. In the expression of $f(t)$, in the equation (14), the parameters φ, H and q are all functions of time . Their time evolution depends upon the time variation of the scale factor from which they are calculated. To calculate $f(t)$ using equation (14), we have used an empirical scale factor. This scale factor has been chosen in order to satisfy a recent observation regarding the deceleration parameter $q(\equiv -\ddot{a}a/\dot{a}^2)$. According to this observation the universe had a state of decelerated expansion before the present phase of acceleration began (Banerjee and Ganguly, 2009; Banerjee and Das, 2006; Das and Mamon, 2014). Thus, the deceleration parameter had a positive value before reaching the present stage of negative values. The functional form of our chosen scale factor is such that the deceleration parameter, calculated from it, shows a change of sign as a function of time. This scale factor, also used by Roy *et* al. (2013) and Pradhan *et al.* (2015), is expressed as,

$$a = a_0 \ (t/t_0)^\alpha \ Exp[\beta(t - t_0)] \tag{15}$$

Here, the constants $\alpha, \beta > 0$ to ensure an increase of scale factor with time. The scalar field parameter (φ), Hubble parameter (H) and deceleration parameter (q), based on this scale factor are given by,

$$\varphi = \varphi_0 \left(\frac{a}{a_0}\right)^k = \varphi_0 \ (t/t_0)^{k\alpha} \ Exp[\beta k(t - t_0)] \tag{16}$$

$$H = \dot{a}/a = \beta + \frac{\alpha}{t} \tag{17}$$

$$q = -\ddot{a}a/\dot{a}^2 = -1 + \frac{\alpha}{(\alpha + \beta t)^2} \tag{18}$$

Here, for $0 < \alpha < 1$, we get $q > 0$ at $t = 0$ and, for $t \to \infty$, we have $q \to -1$.

It clearly means that the chosen scale factor generates an expression of deceleration parameters which changes sign from positive to negative as time goes on. The values of constant parameters (α, β) have been determined from the following conditions.

Condition 1: $H = H_0$ at $t = t_0$ (19 A)
Condition 2: $q = q_0$ at $t = t_0$ (19 B)

Combining the equations (19A) and (19B) with the equations (17) and (18) respectively, one obtains,

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$$\alpha = (1 + q_0)(H_0 t_0)^2 = 4.76 \times 10^{-01} \tag{20A}$$

$$\beta = \frac{H_0 t_0 - \alpha}{t_0} = \frac{H_0 t_0 - (1 + q_0)(H_0 t_0)^2}{t_0} = 1.25 \times 10^{-18} \tag{20B}$$

The values of different cosmological parameters used in the present study are,

$$H_0 = 72 \left(\frac{Km}{Sec}\right) per \ Mega \ Parsec \ = 2.33 \times 10^{-18} sec^{-1}$$

$t_0 = 14 \ billion \ years = 4.415 \times 10^{17} sec$

$\varphi_0 = \dfrac{1}{G_0} = 1.498 \times 10^{10} m^{-3} Kgs^2$

$\rho_0 = 2.831 \times 10^{-27} Kg/m^3$ [present density of matter (dark+baryonic)]

$q_0 = -0.55$

To determine the value of $f(t)$ from equation (14), one must apply the expressions of (16), (17), (18), (20A, B) and use the above mentioned values of cosmological parameters.

The function $f(t)$ is defined by the relation $\rho a^3 = f(t) \rho_0 a_0^3$. According to this relation, the value of $f(t)$ is always positive and, $f(t) = 1$ at $t = t_0$ (taking $a_0 = 1$). The functional form $f(t)$ in equation (14) ensures that $f(t) = 1$ at $t = t_0$. The values of k for which $f(t)$ is positive over the entire range of study (say, from $t = 0.5 t_0$ to $t = 1.5 t_0$) is given below.

$k < (k_-)_{min}$ or $k > (k_+)_{max}$ over the entire range of study. Here,

$$(k_-)_{min} = (q - 2)_{min} \quad \text{over the range from } t = 0.5 t_0 \text{ to } t = 1.5 t_0 \tag{21}$$

$$(k_+)_{max} = (q - 2)_{max} \quad \text{over the range from } t = 0.5 t_0 \text{ to } t = 1.5 t_0 \tag{22}$$

For our range of study, i.e. from $t = 0.5 t_0$ to $t = 1.5 t_0$, we find,

$(k_-)_{min} = -2.64$ and $(k_+)_{max} = -2.33$

Thus we have a lower and an upper range of permissible values for k which are $k < (k_-)_{min}$ or $k > (k_+)_{max}$ respectively. The upper range, $k > (k_+)_{max}$, includes both positive and negative values of k and the lower range, $k < (k_-)_{min}$, has only negative values. According to equation (11), the parameter φ is a decreasing function of time for negative values of k, causing the gravitational constant $(G = \frac{1}{\varphi})$ to increase with time. Therefore we find that the upper range of k allows G to be both increasing and decreasing function of time, although the lower range of k causes G to be an increasing function of time. To choose between these two ranges of k, we have to determine the values of ω_0 at different values of k and compare them with those obtained from other studies. Using equation (4) we can write the following expression (eqn. 23) regarding ω for this model.

$$\omega = \frac{2}{k^2} \left[3(1 + k) - \frac{\rho}{\phi H^2} \right] \tag{23}$$

Using equations (23) we get the following expression of ω_0 (the value of ω at the present epoch).

$$\omega_0 = \frac{2}{k^2} \left[3(1 + k) - \frac{\rho_0}{\phi_0 H_0^2} \right] \tag{24}$$

According to several studies on Brans-Dicke theory ω_0 has a negative value close to (-1) (Das and Mamon, 2014).

To have $\omega_0 < 0$, the condition to be satisfied by k is given by,

$$k < \frac{\rho_0}{3 \ \phi_0 H_0^2} - 1 \quad \text{or,} \quad k < -0.9884 \tag{25}$$

For the entire lower range of k values and for a part of its upper range, the above condition is satisfied.

The gravitational constant (G), which is reciprocal of the Brans-Dicke scalar field parameter (φ) is given by,

$$G = \frac{1}{\varphi} = \frac{(a/a_0)^{-k}}{\varphi_0} = \frac{1}{\varphi_0} Exp \left[k\alpha t_0^\beta \right] Exp[-k\alpha t^\beta]$$

Thus, $\dfrac{G}{G_0} = Exp \left[k\alpha t_0^\beta \right] Exp[-k\alpha t^\beta] \tag{26}$

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An experimentally measurable parameter $\frac{\dot{G}}{G}$ is given by,

$$\frac{\dot{G}}{G} = \frac{1}{G}\frac{dG}{dt} = -k\frac{\dot{a}}{a} = -kH \qquad (27)$$

Using equation (27) we get,

$$\left(\frac{\dot{G}}{G}\right)_{t=t_0} = -kH_0 \qquad (28)$$

The value of k should be so chosen that $\left(\frac{\dot{G}}{G}\right)_{t=t_0} < 4 \times 10^{-10}\ Yr^{-1}$ (Weinberg, 1972).

Graphical Analysis of Theoretical Findings

We have plotted ω_0 as a function of k in Figure 1. For the lower range of k values, the values of ω_0 are negative and also close to the values obtained from other studies (Das and Mamon, 2014). For the upper range of k, we have both positive and negative values of ω_0, the positive values being totally contrary to the findings of other studies in this regard (Das and Mamon, 2014). According to equation (28), the gravitational constant increases with time for negative values of k. Thus, for the entire lower range of k values, G increases with time. Only for the negative values of the upper range, which constitute a small part of this range, G increases with time. There are experimental observations and theoretical models where G has been shown to be increasing with time (Pradhan *et al.*, 2015). In Figure 2 we have plotted the Brans-Dicke parameter ω as a function of time for a value of k in the lower range and for a negative value in its upper range. This is found to be an increasing function of time, with all negative values, for the lower range values of k and this behavior is quite consistent with other studies (Das and Mamon, 2014). But its behavior is different for the upper range of k values. For the positive values of the upper range of k, the values of ω are all positive, as obtained from equation (23), which is inconsistent with the findings of other studies On the basis of observations in the last three paragraphs, we have found it logical to choose values from the lower range of k to determine the time dependence of $f(t)$ and other relevant parameters connected to it in the present study.

Figure 3 shows the variation of $R_1(\equiv f)$ as a function of time for three different values of k in its lower range. It shows that the matter content of the universe $[M(t) = f(t)M_0]$ decreases with time and the rate of its change becomes faster for more negative values of k. The variation of R_2 and R_3 as functions of $R_1(\equiv f)$ has been shown in Figure 4. From Figure 3 we know that R_1 decreases with time monotonically. By definition, R_2 is simply proportional to the rate of change of R_1. As R_1 approaches its present value of unity, R_2 becomes less and less negative, implying slower rate of production of dark energy from matter. R_3 is found to be positive in the past ($R_1 > 1$) and negative in future ($R_1 < 1$), as expected from its definition and the behavior of R_1. Plots of R_4 and R_5 as functions of time are shown in Figure 5. The proportion of dark energy (R_4) in the universe is found to increase with time. Since it is assumed to be generated from matter (dark + baryonic), the proportion of matter in the universe (R_5) decreases with time. Thus, the sum of R_4 and R_5 is unity since the beginning of the conversion process at $t = \gamma t_0$. With $k = -4$ and $\gamma = 0.6$ these curves give nearly correct values at the present time $t = t_0$.

Figure 6 shows the plots of density of matter (ρ_m), dark energy (ρ_d) and total density (ρ_t) as functions of time. Equations (6), (9A, B) have been used for these plots. With $k = -4$ and $\gamma = 0.6$, one obtains approximately correct values from these curves for $t = t_0$.

Figure 7 shows plots of H and q as functions of R_1. As R_1 approaches its present value of unity, the dark energy content of universe becomes larger although its rate of generation from matter becomes slower. It can be inferred from these curves that larger proportion of dark energy causes faster changes in Hubble parameter and deceleration constant.

Figure 8 shows the variations of G/G_0 and $\dot{G}/G$ functions of R_1. As R_1 approaches its present value of unity, the dark energy content of universe becomes larger although its rate of generation from matter becomes slower. It appears from these curves that, as dark energy increases, the gravitational constant increases. One may also infer that, as $|R_2| \equiv |\dot{f}/f|$ becomes smaller with time, the values of $\dot{G}/G$ decreases. As the parameter k is made more and more negative, the fractional rate of depletion of matter content ($|R_2|$) increases and the rate of change of the gravitational constant, Hubble parameter and deceleration parameter increases. Therefore the dark energy content and its rate of production is found to have a role in governing the behavior of various cosmological parameters connected to the expansion of universe.

CONCLUSION

The purpose of the present study is to find out the time evolution of the matter content of the universe by using standard empirical expressions of scale factor and the Brans-Dicke scalar field parameter. The density of matter has been assumed to depend upon a function of time $f(t)$, which is proportional to the matter content of the universe. No specific form of this function has been assumed in the present model. Its form has been determined from the field equations. One may choose a particular form of this function $f(t)$ to get a better model. This function decreases with time, indicating a conversion of matter into dark energy. It is found through graphical analysis that, as the proportion of dark energy content of the universe increases with time, the deceleration parameter becomes more and more negative and the gravitational constant is found to increase with time. The implications of all these observations are that there is a possibility of a dependence of the gravitational constant and the deceleration parameter on the content of dark energy and the rate of its generation. It has been found in the present study that if the matter of the universe is regarded as the only source of dark energy, the present proportions of both of them must depend on how long this process of conversion has been continuing. The experimentally determined values of the present proportions of matter and dark energy can be obtained from this model by a proper tuning of parameters. As a future plan to continue this study, one may improve this model by changing the functional form of the empirically chosen Brans-Dicke scalar field parameter (φ) and also by choosing a specific form of $f(t)$ in terms of φ and the scale factor. The choice of the functional form of $f(t)$, which accounts for the non-conservation of matter content of the universe, should be such that its value must be unity at the present time, as per the requirement of its defining relation expressed by equation (10). From different functional forms of $f(t)$, one may determine the nature of dependence of the cosmological parameters like gravitational constant, Hubble parameter, deceleration parameter etc. upon the dark energy content of the universe and also the rate of conversion of matter into dark energy.

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Figure 1: Plot of the Brans-Dicke dimensionless parameter ω **at the present epoch** $\left(t = t_0 \right)$ **as a function of** k.

Figure 2: Plot of ω **as a function of time, for a value of** k **in the upper range (*dotted*) and one in the lower range (*solid*), shown along the right and left vertical axes respectively.**

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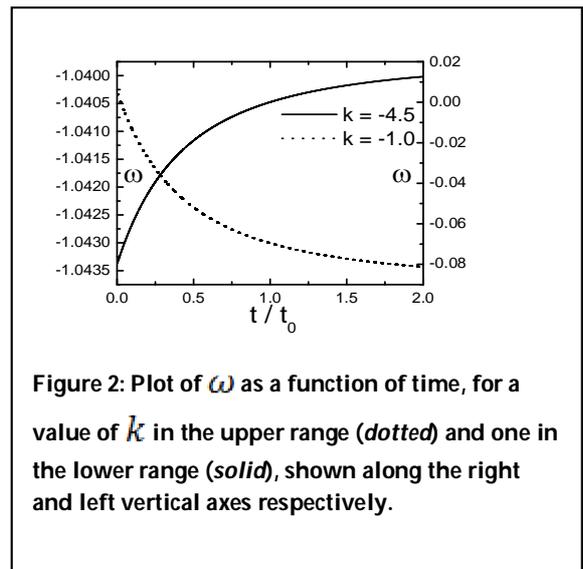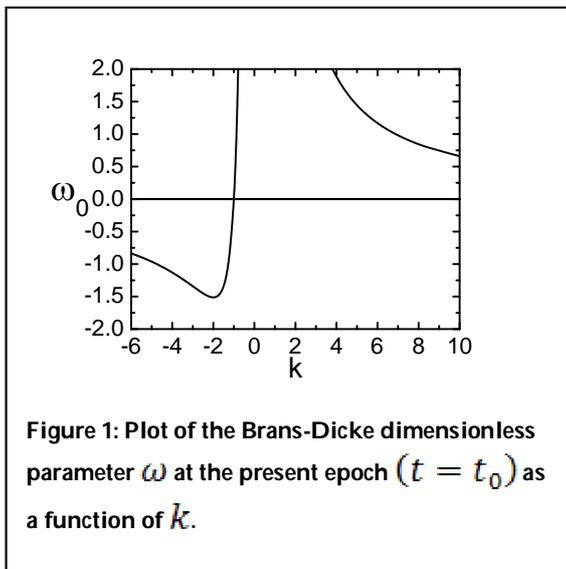
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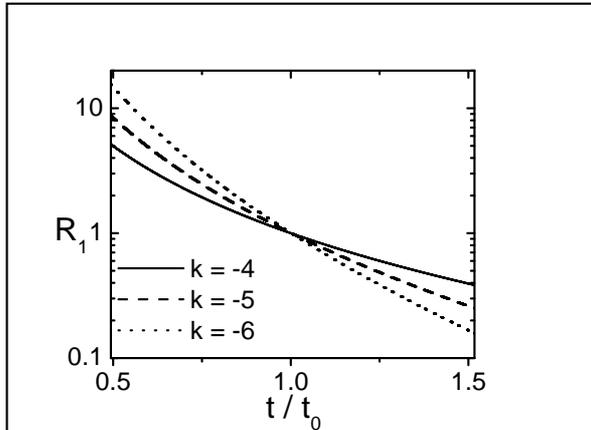
Figure 3: Plot of $R_1\left[\equiv f(t)\right]$ as a function of time for three different values of k in its lower range.
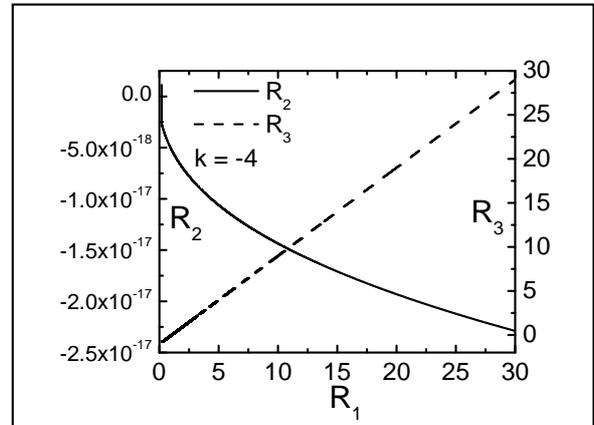
Figure 4: Plot of R_2 and R_3 as a function of R_1 time for three different values of k in its lower range.
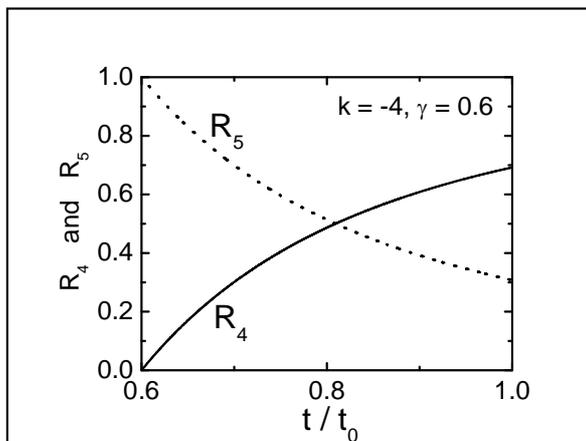
Figure 5: Plot of R_4 (*solid*) and R_5 (*dotted*), the proportions of dark energy and matter respectively, as functions of time. Matter to energy conversion began at $t = \gamma t_0$.
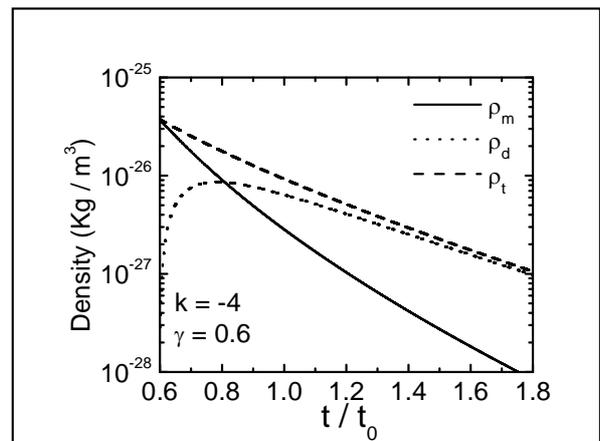
Figure 6: Plot of density of matter ($\rho_m \equiv \rho$), dark energy (ρ_d) and total density (ρ_t) as functions of time.

Figure 7: Plot of H and q as functions of R_1 along the left and right vertical axes respectively.

Figure 8: Plot of G/G_0 and $\dot{G}/G$ as functions of R_1 along the left and right vertical axes respectively.
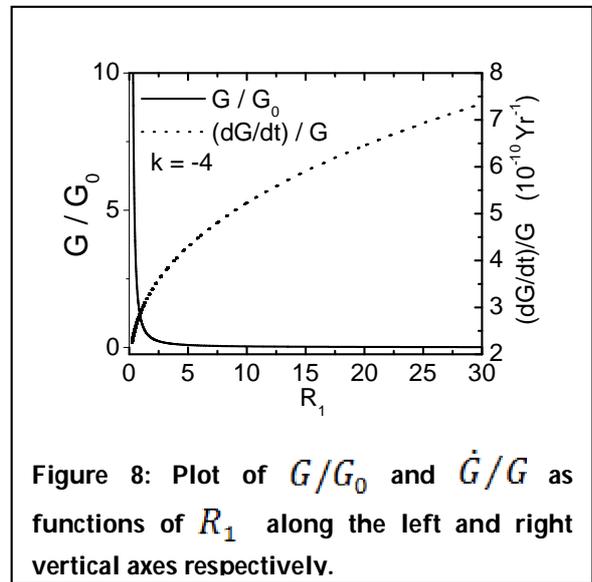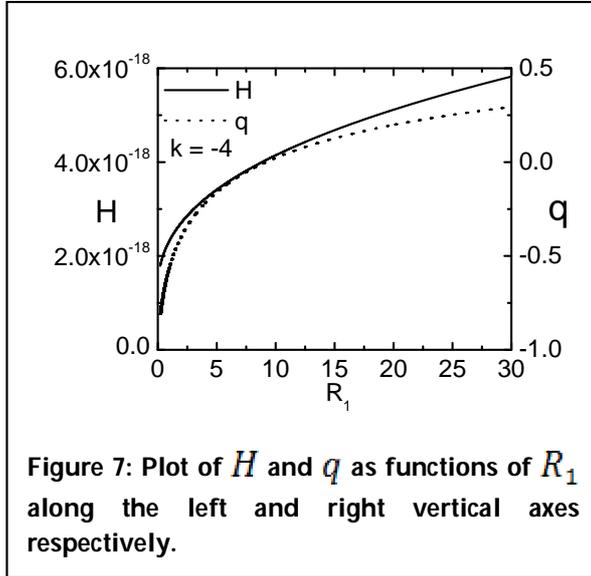
RESEARCH ARTICLE

Primary Production in Tropical Wetlands of Aligarh Region, Northern India: A Limnological Study

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ABSTRACT

Primary production has been used as a potential index of productivity of a given aquatic ecosystem. Rich wealth of primary producers constitutes a significant position in trophic levels of wetlands. Primary production studies in these wetlands showed fluctuations in net and gross primary production along with community respiration and chlorophyll content. These parameters showed bimodal fluctuations in primary productivity showing peaks of higher rates of net primary productivity during summer and post monsoon seasons except at one wetland during investigations where it shows peaks during summer, monsoon and post-monsoon periods. Seasonal fluctuations in the rate of primary production do not remain same throughout the year.

Key words: - Primary production, wetlands, trophic levels and seasonal fluctuations.

INTRODUCTION

The inland freshwater ecosystem of India harbor a rich wealth of primary producers both microphytes and macrophytes which constitute a significant position in the trophic levels of aquatic ecosystem. Biological productivity of any wetland depends largely on its ability to support the growth of photoautotrophic organisms consisting mainly of higher plants and algae (Kumari and Kumar,2002). An accurate knowledge of primary production in natural water bodies is central concern of limnology (Schwoerbel, 1987). Primary production studies are of paramount important in understanding eutrophic nature, nutrient status, and standing crop of any wetland ecosystem. Solar energy is trapped by chlorophyll bearing plants, which transform it into chemical energy, which is stored by green plants in the form of plant tissues and is called as primary productivity. Thus, the primary productivity is the basis of whole

metabolic cycle in the wetlands or any natural ecosystem. Based on production potentialities, wetlands can be categorized as *oligotrophic*, *mesotrophic*, *eutrophic* and *dystrophic* (Goldman and Horne, 1983; Untoo *et.al.*,2002). Many workers have correlated nutrients availability in determining the trophic status of wetlands. It is well known that these nutrients help in synthesis of chlorophyll and act as carriers of essential substances. It becomes necessary in limnological studies to estimate the amount of major nutrients and their role in determining the aquatic primary productivity (Paul and Verma, 1999). A deficiency or excess of these nutrients leads to destruction of healthy status of wetlands. A considerable work has been done in this regard so far within and outside India. Ferris and Tyler (1985) documented relationship between chlorophyll and phosphorus. Comita (1985) reported seasonal cycles of primary production. Khan *et al.*, (1988, 2002) and Untoo *et.al.*, (2002) also reported relationship between nutrients and primary productivity. High rate of production both in natural and man-made ecosystems occur when physico-chemical factors are favorable. Biological production is the key to the extent to which natural wetland resources may be utilized for whatever purpose (Bohra and Kumar, 2002). Due to galloping increase in population of India, fish production in wetlands and other water bodies is gaining importance to combat and fulfill animal protein needs. This demand can only be fulfilled by increasing the fish production in several utilized wetlands and other water habitats with diverse geological and climatic features of India. This can be achieved only by increasing the primary production in these wetlands as it forms the basis of increasing production at the next trophic level. The present study gives an account of primary production in three tropical wetlands of Aligarh region of northern India. The study of primary production includes gross primary productivity (*G.P.P*), the rate of transformation of radiant energy to chemical energy and is the total production i.e production as well as respiration, net primary productivity (*N.P.P*), the net production left after expenditure in respiration and community respiration (*C.R*), the rate of loss of fixed energy in respiration. Organic matter is accumulated when *G.P.P* exceeds *C.R* and therefore *N.P.P* can also be used as an index of secondary productivity (Brylinsly, 1980).

MATERIALS AND METHODS

Studies were made at monthly basis on three wetlands of Aligarh i.e. Chaarat Pond-1 (CP-1), Chaarat Pond-2 (CP-2) and Medical Pond (MP). Primary production was estimated by measuring the changes in the dissolved oxygen (D.O.) concentration in light and dark bottles after following methodology of Gaarder and Gran (1927) and Vollenweider (1969). D.O. was determined on the site in the field itself using Wrinkler's modified technique as described in APHA (1992). Chlorophyll was estimated after following methodology given by Trivedy and Goel (1984).

RESULTS

Wide ranges of fluctuations were noted in the net and gross primary production along with community respiration and chlorophyll, 'a' pigment content. The values of the results are shown in (Table 1).At CP-1, the values of net primary production, NPP were found to vary between 0.6870gC/m³/hr as recorded in January, 2014 and 1.7320gC/m³/hr recorded in November, 2014.At CP-2, the values of net primary production, NPP were found to vary between 0.5640gC/m³/hr as recorded in January 2014 and 1.6660gC/m³/hr recorded in the month of August, 2014.Further, at MP ranges of net primary production, NPP results varied between 0.5640gC/m³/hr as recorded in February, 2014 and 1.8360gC/m³/hr recorded in July, 2014.

The monthly observations of gross primary productivity, GPP showed temporal fluctuations.
At CP-1, the values of gross primary productivity, GPP were found to vary between 0.8230gC/m³/hr as recorded in January, 2014 and 2.1300gC/m³/hr recorded in June, 2014.
At CP-2, the values of gross primary productivity, GPP were found to vary between 0.7260gC/m³/hr as recorded in January, 2014 and 1.9530gC/m³/hr recorded in November, 2014.
Whereas in case of MP, gross primary productivity, GPP were found to vary between 0.6750gC/m³/hr as recorded in February, 2014 and 2.2400gC/m³/hr recorded in July, 2014.

Similarly community respiration (CR) rates were also found to fluctuate in all the three wetlands of Aligarh region in different months of investigations (Table 1).

At CP-1, community respiration (CR) rates were found to vary between 0.0470gC/m³/hr as recorded in March, 2014 and 0.4050gC/m³/hr recorded in June, 2014.

At CP-2, community respiration (CR) rates were found to vary between 0.0081gC/m³/hr as recorded in December, 2013 and 0.9260gC/m³/hr recorded in June, 2014.

In the case of MP, community respiration (CR) rates were found to vary between 0.0110gC/m³/hr as recorded in November, 2014 and 0.6781gC/m³/hr recorded in August, 2013.

Variations in chlorophyll, 'a' pigment content have been noted from all the three studied wetlands of Aligarh region under present study showing variations are shown in (Table 18).

At CP-1, chlorophyll, 'a' pigment content shows variation from 0.562mg/L in February, 2014 and 3.262 mg/L in October, 2013.

At CP-2, chlorophyll, 'a' pigment content varies from 0.7220mg/L in January, 2014 and 3.2410 mg/L in August, 2013.

In the case of MP, chlorophyll, 'a' pigment content shows variation from 0.7860mg/L in February, 2014 and 3.7951 mg/L in October, 2014.

DISCUSSION

The primary production involves chaemo-autotrophic processes, forming the base of energy flow in the ecosystem. In the present study of primary production in these wetlands of Aligarh region of Northern India, *Gross Primary Productivity* (GPP), *Net Primary Productivity* (NPP) and *Community Respiration* (CR) are more or less similar at CP-1, CP-2 and at MP. Seasonal fluctuations in the rate of primary production were recorded, which appears that the production rate does not remain same throughout the course of study similar finding was reported by other workers too in tropical waters (Hulbert, *et al.*, 1960; Menzel and Ryther, 1961; Prasad and Nair, 1963 and Ali and Khan, 1979). As it is clear from the (Table 1), the values recorded of gross production were always found higher than the values of net primary production. It was due to the fact that phytoplankton cells lose an appreciable amount of assimilated amount of carbon during different metabolic activities particularly through respiration and excretion (Fogg *et al.*, 1973 and Haque, 1991). A large population under unfavorable conditions may have a low rate of production, whereas a small population under favorable conditions may have high rate of production (Bohra and Kumar, 2002).

In the present investigations bimodal fluctuations were recorded in the primary productivity showing peaks of higher rates of NPP during summer, and post monsoon seasons at all the three wetlands except at MP wetlands where it shows peaks during summer, monsoon and post monsoon periods. The variation in the rates of production as noted might be due to favorable and unfavorable physico chemical conditions during different months of investigations in these wetlands of Aligarh region. A higher rate of production indicates that these wetlands are primarily rich in nutrients with enough lighted zone and energy content. The maximum rate of NPP, during summer periods of investigation was probably due to high temperature and appreciable phytoplankton density. Prasad and Nair (1963), Khan and Siddique (1971) and Gaur (1998) have reported high value during summer and at the time of good plankton production. Higher values of primary production during some months of post monsoon and monsoon, in these wetlands were found to be due to increased concentration of nutrients added along with the sewage and surface run-off. Sreenivasan (1964) and Ayyappan and Gupta (1985) have also reported higher rates of primary production during summer and monsoon months. Low values of primary production in these three wetlands of Aligarh region of northern India may be due to low temperature, less photoperiod and low intensity of light due to dense fog cover and less sunshine or visibility.

The photosynthetic rate of phytoplankton and other green algae has been noticed to be in higher value at some intensity between extremes of mid-day irradiance at the surface (Lewis Jr.,1974). When statistically analyzed, GPP and NPP values were found to be significant positive with chlorophyll 'a' (Fig.1) and with water temperature (Table

2) at all the three wetlands under present study but with phytoplankton, the values showed a non-significant positive correlation at CP-1 and negative correlation at CP-2 and MP wetlands of Aligarh region of northern India (Table 2). Community Respiration (CR), the rate of plankton respiration was also estimated in terms of $gC/m^3/hr$. The values of C.R. were found to vary from season to season and from one wetland to another wetland of Aligarh region of northern India during the course of this study. It may be because of high rate of decomposition of organic matter in these wetlands and some turbid conditions during different months of investigations.

Further, all the three wetlands showed wide fluctuations in the study of Chlorophyll 'a' content (Table 1). Looking at the variations in Chlorophyll 'a' readings which is a measure of standing crop of phytoplankton, Welch (1952). The spatial variations in the values of Chlorophyll 'a' showed almost the same trend as exhibited by NPP. Hutchinson (1975c) considered transparency as an index of productivity, according to Clarke (1941) the availability, extent and intensity of light are the most important factors governing the photosynthetic activity of chlorophyll bearing organisms in aquatic ecosystems. Higher values of Chlorophyll 'a' occurred when transparency was low and vice-versa. The high values of GPP and NPP in these wetlands of Aligarh region of northern India were obtained at the time of high concentration of Chlorophyll 'a' and vice-versa. A correlation analysis also showed significant direct relationship between Chlorophyll 'a' and GPP and NPP in these wetlands of Aligarh region of northern India (Table 2).

CONCLUSION

These wetlands were found to be highly productive showing peaks during summer and post monsoon seasons. Higher rates in productivity as compared to reported ones, indicates that these wetlands are primarily rich in nutrients with enough lighted zones and energy content. Being productive in nature and free from pollution load, except sewage input. These wetlands of Aligarh region of northern India can very well be used intensively for pisciculture or even for integrated fish farming after following the modern technology used and recommended by CIFA, Kaushalyaganga, Bhubneshwar, Odissa for their proper management.

ACKNOWLEDGMENTS

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Table 1 Statically Briefs of Various Parameters of GPP, NPP and Chlorophyll 'a' in CP-1, CP-2 and MP, Wetlands of Aligarh, northern India.

Parameters	Parameters	Wetlands/Sites	Coefficient of Correlation (r Value)	Sinificant at (p<0.05)
Gross Primary Productivity (GPP)	Chlorophyll 'a'	CP-1	0.887	√
		CP-2	0.704	√
		MP	0.536	√
	Phytoplankton	CP-1	0.005	-
		CP-2	0.570	√
		MP	0.138	-
	Water Temperature	CP-1	0.663	√
		CP-2	0.693	√
		MP	0.703	√

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Net Primary Productivity (NPP)	Phytoplankton	CP-1	0.044	-
		CP-2	0.445	-
		MP	0.162	-
	Chlorophyll 'a'	CP-1	0.865	√
		CP-2	0.742	√
		MP	0.497	√
	Water Temperature	CP-1	0.611	√
		CP-2	0.633	√
		MP	0.526	√
Chlorophyll 'a'	Phytoplankton	CP-1	0.133	-
		CP-2	0.333	-
		MP	0.031	-

Chaarat Pond-1 (CP-1), Chaarat Pond-2(CP-2) and Medical Pond (MP)

Table 2: Monthly Variations in Primary Productivity, Community Respiration and Chlorophyll a in CP-1, CP-2 and MP Wetlands of Aligarh region of northern India.

Months↓ Wetlands→	**Net Primary Productivity** NPP (g C/m³/hr)			**Gross Primary Productivity** GPP(g C/m³/hr)			**Community Respiration** CR(g C/m³/hr)			**Chlorophyll 'a'** (mg/L)		
	CP-1	**CP-2**	**MP**	**CP-1**	**CP-2**	**MP**	**CP-1**	**CP-2**	**MP**	**CP-1**	**CP-2**	**MP**
August,2013	1.628	1.567	1.437	1.717	1.806	2.115	0.089	0.239	0.678	2.452	3.241	3.317
September,2013	1.329	1.367	1.322	1.492	1.511	1.473	0.163	0.149	0.151	2.708	2.591	2.683
October,2013	1.482	1.482	1.472	1.693	1.514	1.561	0.211	0.032	0.089	3.262	2.816	3.685
November,2013	1.632	1.371	1.622	1.952	1.731	1.734	0.320	0.360	0.112	3.252	2.146	2.945
December,2013	1.121	1.453	1.351	1.242	1.416	1.442	0.121	0.008	0.091	1.348	2.322	2.545
January,2014	0.687	0.564	0.892	0.823	0.726	1.064	0.136	0.162	0.172	0.831	0.722	2.075
February,2014	0.923	0.675	0.564	1.023	0.783	0.675	0.100	0.108	0.111	0.562	0.892	0.786
March,2014	1.372	1.264	1.320	1.419	1.375	1.421	0.047	0.111	0.101	2.610	2.590	2.832
April, 2014	1.572	1.464	1.532	1.713	1.642	1.604	0.141	0.178	0.072	2.920	2.846	2.715
May,2014	1.638	1.575	1.746	1.823	1.713	1.834	0.186	0.138	0.088	3.130	2.824	3.146
June,2014	1.725	1.214	1.260	2.130	2.140	1.673	0.405	0.926	0.413	3.210	3.360	3.340
July,2014	1.523	1.428	1.836	1.813	1.924	2.240	0.290	0.496	0.404	3.070	3.035	2.315
August,2014	1.617	1.666	1.448	1.816	1.917	2.106	0.199	0.251	0.658	2.561	3.139	3.328
September,2014	1.219	1.288	1.331	1.501	1.602	1.462	0.282	0.314	0.131	2.819	2.703	2.784
October,2014	1.391	1.372	1.482	1.581	1.703	1.572	0.190	0.331	0.090	3.151	0.915	3.795
November,2014	1.732	1.280	1.732	1.831	1.953	1.842	0.099	0.673	0.011	3.261	2.064	1.735
December,2014	1.142	1.343	1.242	1.231	1.354	1.286	0.089	0.011	0.044	1.437	2.465	2.473

RESEARCH ARTICLE

Arsenic Induced Alteration in Macromolecule Concentration and Antioxidant System in Two Improved Rice Varieties

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ABSTRACT

Arsenic contamination of rice has been highlighted as major issue throughout the world as it is a staple food for millions. The aim of study was to analyze the effect of different concentration of arsenic on the germination, physiology, macromolecules concentration and antioxidant enzymes in improved varieties of rice. *In vitro* study indicates that the lower concentration of arsenate had a stimulating effect on germination, chlorophyll content as well as respiratory content while an inhibitory effect at higher concentration. Roots were more affected than shoots. Further, the content of macromolecules (carbohydrate and protein) was elevated while the activities of their hydrolyzing enzymes (α, β amylase and protease) were declined on arsenic stress. The significant elevation in the activity of superoxide dismutase and peroxidase enzymes also proved the generation of reactive oxygen species due to the arsenic toxicity
.

Keywords: Arsenic; α-amylase; β-amylase; peroxidase; rice; superoxide dismutase

INTRODUCTION

Arsenic (As) is one of the most toxic metalloid naturally present in the environment (Fitz and Wenzel, 2002) with an average concentration of about 3 mg/kg in earth crust and about 1-8 µg/L in sea water (Smedley and Kinniburgh, 2002). Arsenic contamination received higher attention worldwide because it causes considerable harmful effects to plants and humans, including inhibition of growth in plants (Stoeva et al., 2003), physiological disorders in humans (Stoeva et al., 2005). Arsenic contamination of groundwater has been reported throughout the world but the situation is severe in Asian countries like Bangladesh, India and China (Chowdhury et al., 2000; Williams et al., 2006). In these

regions, arsenic in drinking water exceeds the WHO recommended value of 10µg/L (WHO 2001) and even 50µg/L specified in India and Bangladesh (Ahamed et al., 2006; Ahmed et al., 2004). This potentially toxic element exits in both inorganic and organic form in the soil where, inorganic form is more toxic than organic form (Smith et al., 1998). The rice crop is one of the major staple food throughout the world and has been reported as hyper accumulator of arsenic (Meharg et al., 2009). The rice crop is more prone to arsenic accumulation than other cereals as it is generally grown under flooded condition where arsenic mobility is high, thus posing a potential health risk to humans and animals (Xu et al., 2008). When rice plants are grown in arsenic treated soil, decrease in yield and fruit production, as well as in fresh and dry biomass of roots and shoots and morphological changes have been observed (Mokgalaka-Matlala 2008; Shaibur et al., 2008; Srivastava et al., 2009). Previous experiments carried out and the plethora of information obtained reveals that metabolism of plants is adversely affected under arsenic stress, which results in more accumulation of soluble sugars and starch in many plants species (Dubey and Singh 1999; Dubey 1997) Further, it was observed that when plants were exposed to inorganic arsenic, there was generation of reactive oxygen species as a result of oxidative stress (Flora 1999). A plant gets protection against the oxidative damage by antioxidant enzymes including ascorbate peroxidase, superoxide dismutase and glutathione reductase (Hartley-Whitaker et al., 2002).

In the present study, two improved rice varieties PR122 and PUSA1121 have been used to investigate the potential risks of arsenic. These two varieties are cultivated in Malwa region of Punjab, India which has been reported to have high concentration of As in irrigation water and soil ranging from 11.4- 688 ppb (Jain and Kumar, 2007). The selected rice varieties PR122 and PUSA1121 has not been studied previously for the effect of As. So, the main objective of this study was to investigate the alteration in growth parameters, effect on chlorophyll, effect on macromolecules and their hydrolyzing enzymes and effect on antioxidant enzymes under arsenic toxicity in both the varieties PR122 and PUSA1121.

MATERIALS AND METHODS

The experiment was performed at the Central University of Punjab, Bathinda (India). The seeds of rice were procured from Punjab Agricultural University; Ludhiana. Two varieties of rice PR122 and PUSA1121 were used in the study. Seeds were surface sterilized with 5% sodium hypochlorite and subsequently rinsed with distilled water. The arsenic solution was prepared by dissolving sodium arsenate (Loba chem Pvt Ltd) in distilled water. Four different concentrations of sodium arsenate i.e. 50µM, 100µM, 250µM, 500µM which is equivalent to 12µM, 24µM, 60µM and 120µM of arsenic, were used for experiment. Control was maintained by moistening the filter paper lined in the tray.

Germination studies

Forty seeds of each variety were soaked in their respective concentrations of arsenic i.e. 12µM, 24µM, 60µM and 120µM for 18 hours. After 18 hours, trays were lined with thin layer of sterilized cotton and one filter paper and moistened with 20mL of different concentration of arsenic and incubated at 35^0 temperatures under dry and hygienic conditions. On 7th day, the numbers of germinated seeds were counted and the results were expressed as percentage over control.

$$\text{Germination} = \frac{\text{Total no. of germinated}}{\text{Total no. seeds taken for}} \times 10$$

The seedlings were harvested on 7th day and the shoot and root length was measured using metric scale in centimeter. The roots and shoots were oven dried at 60^0 for 48 hours for dry weight biomass estimation.

Chlorophyll content

Total chlorophyll content was measured by spectrophotometric method (Hiscox and Israelstam 1979) and calculations were done by (Arnon, 1949) and modified by (Rani, 1991). For this 100mg of test plant material was taken in test tube and 4mL of DMSO reagent was added to it and kept in water bath for 1 hour. After cooling, absorbance was taken at wavelength of 645nm and 663nm using DMSO as blank.For estimation of percent cellular respiratory ability (Steponkus and Lanphear, 1967), 100mg of tissue from fully expanded leaves of test plant material was dipped in 1.5mL of freshly prepared TTC solution. The samples were incubated in the dark at room temperature for 18 hours during which the colour of the plant tissue in tubes turned red. After 18 hours, TTC solution was drained off from test tubes then rinsed twice with distilled water. After washing, samples were extracted with 5mL of absolute alcohol and kept in water bath for 20 minutes. After cooling, absorbance was recorded at 530nm and expressed in terms of dry weight equivalent. The cellular respiratory ability was expressed as a percent with respect to control.

Preparation of extracts

100mg of plant material (root and shoot) was homogenized in pestle mortar with 5mL of 0.1M of phosphate buffer (pH 7). The homogenates were centrifuged at 15,000 rpm for 15 minutes. The supernatant was used for determining the activities of total protein, carbohydrates, protease, α amylase, β amylase, superoxide dismutase and peroxidase.

Estimation of carbohydrate and protein content

For carbohydrate estimation (Loewus, 1952), 200µL freshly prepared plant extract was taken in test tube and made to a final volume of 1mL by adding 800µL distilled water. Added 4mL anthrone reagent in each test tube, simultaneously blank was prepared by adding distilled water and heated for eight minutes in boiling water bath. Cooled and when green to dark green colour appeared, the reading was taken on UV-Visible spectrophotometer at 630nm. For protein content (Lowery, 1951), 2.5mL of reagent C (50mL 2% sodium carbonate in 0.1N sodium hydroxide and 1mL of 0.5% $CuSO_4.5H_2O$ in 1% sodium hydroxide) was taken in test tube and then 0.5mL of plant enzyme extract was added to it; simultaneously blank was prepared by adding distilled water. The samples were mixed thoroughly and then the reaction mixture was allowed to stand for 10 minutes at room temperature. After 10 minutes, 0.25mL of reagent D (Folin Ciocalteau's reagent) was added in the test tube and mixed properly. After 30 minutes, the reaction mixture in the test tube turned blue and the reading was taken on UV-Visible spectrophotometer at 660nm.

Assays for sugar metabolizing enzymes

α amylase activity was estimated (Muentz, 1977) (EC 3.2.1.1) by using starch as a substrate . 0.5mL of plant extract was taken in test tube and then 1mL of substrate solution was added to it, simultaneously blank was prepared by adding distilled water. The reaction mixture was incubated for half an hour and then 0.1mL of EDTA was added. 0.2mL reaction mixture was taken in another test tube and 3mL of iodine solution was added, absorbance was recorded on UV-Visible spectrophotometer at 630nm using starch (50µg/mL) as standard.β amylase activity was estimated by (Bernfeld, 1951) (EC 3.2.1.2) using maltose as a substrate. 0.5mL of plant extract was taken in a test tube followed by the addition of 0.7mL of substrate and 0.1mL of 0.1M EDTA, simultaneously blank was prepared by adding distilled water. The reaction mixture was incubated for half an hour at 30^0 followed by the addition of 1mL DNSA. Boiled the reaction mixture in water bath for 20 minutes and then 3mL of distilled water was added. The absorbance was recorded on UV-Visible spectrophotometer at 560nm using starch (50µg/mL) as standard.

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Protease activity was estimated by (Basha and Beevers, 1975) (EC 3.4) using casein as a substrate. 0.5mL of enzyme extract was taken in test tube and 0.5mL of casein solution was added to it. The reaction mixture was incubated for 1 hour at 37^0 to which 1mL of TCA solution was added. It resulted in precipitation of the proteins. The reaction mixture was centrifuged at 10,000 rpm for 5 minutes. After centrifugation, supernatant was collected and Lowery method was performed for the estimation of enzyme activity. Specific activity was calculated against 50µg/mL tyrosine as standard and expressed as g/hour/mg protein.

Assays for antioxidant enzymes

For superoxide dismutase assay (SOD) (Marklund and Marklund, 1974) (EC 1.15.1.1), the reaction mixture was prepared by adding 1.5mL of Tris HCL buffer (pH 8.2) and 0.5mL of EDTA.1mL of pyrogallol solution and 0.1mL of enzyme extract was added in a cuvette immediately and the absorbance was recorded spectrophotometrically at 420nm after every 30 seconds up to 3 minutes. A blank was prepared without pyrogallol solution. A unit of enzyme has been defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank. For peroxidase assay (POD) (EC 1.11.1.7) (Shannon et al., 1966), 3mL of guaiacol was taken in test tube. 0.1mL of H_2O_2 and 0.1mL of enzyme extract was added in a cuvette immediately. The reaction was initiated by H_2O_2 addition and the absorbance was recorded spectrophotometrically at 470nm after every 30 seconds up to 3 minutes. A blank was prepared without H_2O_2 solution. Peroxidase activity has been expressed as change in absorbance/minute/g of tissue.

Statistical analysis

All the experiments were performed in a completely randomized block design and performed twice. Three replicates were maintained for each treatment. The data collect from dose response study were subjected to one way ANOVA with Tukey's test.

RESULTS

Effect of arsenic on germination, root and shoot length and biomass dry weight

Arsenic toxicity was evaluated by carrying out germination studies in seeds of rice varieties and the interpretation of results showed significant decrease in germination with the increase in arsenic concentration (Table 1 and 2). Plant growth in terms of root and shoot length was significantly inhibited at 12-120µM arsenic treatment. In addition, biomass (DW) significantly declined in the range of 22%- 69% for PR122 and 23%-63% for PUSA1121 at 12-120µM arsenic treatment.

Effect of arsenic on total chlorophyll content

Presence of arsenic had an inhibitory effect on growth and photosynthetic pigments in rice seedlings. Chlorophyll content of PR122 declined by 19%, 21%, 34% and 70% in response to 12, 24, 60 and 120µM arsenic treatments, respectively, over the control (Fig. 1). Initially, PUSA1121 showed an elevation of 10% in chlorophyll content at 12µM arsenic cocentration, that further declined by 4%, 6% and 25% at 24-120 µM arsenate treatment, as compared to control (Fig. 1). Chlorophyll content in PR122 was more affected as compared to PUSA1121.

Effect of arsenic on percent cellular respiration

The percent cellular respiration content decreased by 11% to 54% with the increase in arsenic concentration from 12-120µM, while PUSA1121 initially showed an increase of 12% at 12µM arsenic treatment and then, decreased to 21%, 46% and 60% for 24, 60, 120µM arsenic treatments (Fig. 2). These results led to the conclusion that lower

concentration of arsenic has stimulating effect on cellular respiration content while higher concentration has inhibitory effect on cellular respiration content.

Effect of arsenic on carbohydrate content and the activity of their hydrolyzing enzymes - α and β amylase in roots and shoots

Carbohydrate content in PR122 roots increased from 18%-39% in response to 12-60µM arsenic treatment while PUSA1121 showed an increase of 55%, 31% and 45% over the control, at 12, 24 and 60µM arsenic treatments (Fig. 3a). Similarly, shoots of PR122 showed an elevation in carbohydrate content by 18%-91% while PUSA1121 showed an increase of 110%, 80%, 94% and 104% over the control, with the increasing arsenic concentration (12-120µM) (Fig. 3b). In contrast, α-amylase activity showed a decline in roots as well as shoots of PR122. The decline was reported to be 25-68% in roots (12-60µM) (Fig. 3a) and 24-79% in shoots in response to (12-120µM) arsenic treatments of PR122 (Fig. 3b). Similarly, β-amylase activity declined by 27%-77% in roots and 35%-88% in shoots in response to (12-60µM) arsenic treatment (Fig. 3a, b). Roots and shoots of PUSA1121 showed 20%-74% and 14%-71% decline in α-amylase activity over the control, for µM and 12-120µM arsenic concentration, respectively. Similar trend was observed for PR122 where the decline in β-amylase activity of roots (12-60µM) and shoots (12-120µM) was reported to be 27%-77% and 35%-88% respectively, over the control.

Effect of arsenic on protein content and the activity of hydrolyzing enzyme - protease in roots and shoots

Protein content increased in roots and shoots of both the rice varieties. Protein content in roots of PR122 and PUSA1121 varieties increased by 6%-29% and 19%-22%, respectively, over the control, for 12-60µM arsenic treatments (Fig. 4a). Increasing trend in protein content was also reported in shoots of PR122 and PUSA1121, with an increase of 14%-62% and 17%%-31% over the control, for 12-120µM arsenic concentrations, respectively (Fig. 4b). Roots of both the varieties i.e., PR122 and PUSA1121 showed decline in protease activity by 45%-85% and 53%-89% over the control, at 12-60µM arsenic treatment (Fig. 4a). Likewise, protease activity in shoots was reported to decline by 40%-90% and 44%-91% over the control for PR122 and PUSA1121, at 12-120µM arsenic treatments, respectively (Fig. 4b).

Effect of arsenic on SOD (Superoxide dismutase) activity in roots and shoots

Excess of arsenic altered the activity of antioxidant enzymes. The increase was significant at all treatments as compared to the control. SOD activity increased in roots of both the varieties i.e. PR122 and PUSA1121 by 47%- 67% and 80%- 133%, in response to 12, 24 and 60µM arsenic treatments, respectively, over the control (Fig. 5a). Shoots of both the varieties revealed an increasing trend in SOD activity, whereby the increase for PR122 and PUSA1121 was about 110%-212% and 86-154% respectively, as compared to control, at 12-120µM arsenic treatments (Fig. 5b).

Effect of arsenic on POD (Peroxidase) activity in roots and shoots

Significant increase was observed in the activity of peroxidase (POD) in roots and shoots of both the rice varieties. Percent enhancement in POD activity in roots of PR122 and PUSA1121 was observed to be 5%-48% and 28%-38% over the control, respectively, at 12-60µM arsenic treatment (Fig. 6a). Similarly, at 12-120µM arsenic concentration, percent elevation in POD activity in shoots of both the varieties i.e., PR122 and PUSA1121 was observed to be 36%-99% and 3%-23%, respectively, over the control (Fig. 6b).

DISCUSSION

Arsenic exposure significantly affects the growth parameters and physiology of rice seedlings. In the present study, different concentrations of arsenic were studied on two rice varieties i.e. PR122 and PUSA1121. It was observed that

both the varieties were radically affected in response to the increased concentration of arsenic. Germination studies revealed that low concentration of arsenic stimulates the growth of rice seedlings, whereas higher concentration inhibits the seed germination. Moreover, similar results were also reported by (Hoffmann and Schenk 2011; Shri et al., 2009) in rice varieties. The inhibitory effect was also observed in root and shoot length as compared to control in both the rice varieties. The effect was more prominent on root length than shoot height since roots are the first site of contact with the heavy metals. Earlier, (Choudhury et al., 2011; Das et al, 2004; Abedin et al., 2002a; Abedin et al., 2002b) reported the pronounced inhibition in roots than shoots. It was also observed that as the plant height decreases, it automatically affects biomass of plant, and in turn the dry biomass of plant decreased with increased concentration of arsenic. (Panaullah et al., 2009; Pigna et al., 2009; Srivastava et al., 2009) also reported reduction in dry biomass at higher concentration of arsenic. It was observed that arsenic interferes with plant metabolic processes such as phosphorylation and ATP synthesis and thus no ATP are formed which inhibits the plant growth and ultimately plant death (Garg and Singla 2011). The effect of arsenic was studied on photosynthetic pigments of the test plants PR122 and PUSA1121 and the results showed that reduction in chlorophyll content was more significant at higher concentration of arsenic than at lower level of arsenic. Similar results were also reported by various researchers (Azad et al., 2013; Hoffmann and Schenk 2011; Rahman et al., 2007; Shaibur et al., 2006). The reason is attributed to the fact that As binds with –SH base of protein after it enters and gets accumulated in the cell which ultimately leads to disruption of chloroplast, due to which chlorophyll content is reduced. Cellular respiratory ability declined in both the rice varieties i.e. PR122 and PUSA1121. Similar results were reported by Shao et al., 2011; Singh et al., 2007. Excess of heavy metals disturbed the physiological and biochemical processes of plant hence disturbing the cellular respiratory ability of the plant. According to (Shao et al., 2011), each plant has its own protective mechanism towards stress so they can adjust according to the environmental changes. Carbohydrate content increased with the increase in the concentration of arsenic in both the varieties i.e. PR122 and PUSA1121. The results obtained are in accordance with previous studies of Choudhury et al., 2010; Dubey and Singh, 1999; Dubey 1997. A study led by Dubey and Singh 1999 states the fact that plant needs more energy to combat metallic stress which, in turn is provided by the production of more soluble sugars. Thus, to overcome cellular dehydration, the cells produce and accumulate more soluble sugars under As stress, thus resulting in increased carbohydrate content. However, carbohydrate content was more in roots than shoots thus affecting the roots more than shoots. High levels of arsenic in plants also affect the hydrolyzing enzymes amylases and proteases. Starch is the main reserve food material of plants and mobilization of starch reserves depends on α and β amylase and their hydrolyzed products are absorbed by scutellum which is used by growing embryo for the growth of seedlings. Presence of any environmental stress may disturb the mobilization of reserves which could significantly affect germination (Kaneko et al., 2002). The activities of both α and β amylase declined in both the varieties i.e. PR122 and PUSA1121 with increased concentration of arsenic. Same trend of observation was reported by (Jha and Dubey, 2005; Jha and Dubey, 2004). High content of arsenic affects the protein levels of the plant. Proteins of cells will not be used up due to reduced plant growth but will accumulate which results in increase in protein content of seedling (Yu et al., 1995). Another reason for increase in protein content was decrease in activity of protease enzyme. Protease activity decreased with increased concentration of sodium arsenate in both varieties PR122 and PUSA1121. The decreased activity of protease indicates that the plant is inefficient to hydrolyze proteins under arsenic stress (Bhattacharya et al., 2013; Mishra and Dubey, 2006). Significant increase was observed in superoxide dismutase (SOD) and peroxidase (POD) activity with increased arsenic. Similar observations were reported by (Bhoomika et al., 2013; Dave et al., 2013; Geng et al., 2006). Plant gets protection against the ROS by releasing scavenging enzymes superoxide dismutase (SOD) and peroxidase (POD).

CONCLUSION

From the present study, it can be concluded that different concentration of arsenic affects growth and physiology of PR122 and PUSA1121 rice varieties. Arsenic in lower concentrations may have growth promoter effects. Roots accumulate more arsenic than shoots. Lower concentration of arsenic had a stimulating effect on chlorophyll content as well as respiratory content while an inhibitory effect at higher concentration. Carbohydrate and protein content

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was increased with increase concentration of arsenic while the activity of their hydrolyzing enzymes α, β amylase and protease was decreased with increased concentration of arsenic. Significant increased was observed in the activity of SOD and POD at higher concentration of arsenic.

ACKNOWLEDGEMENTS

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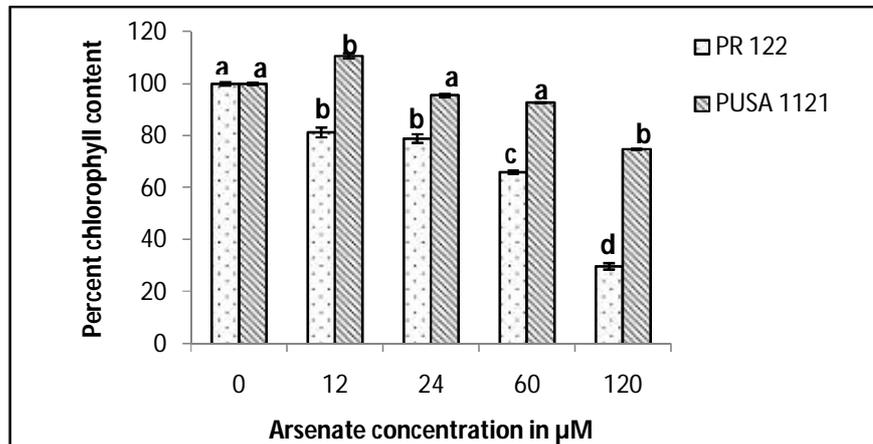
Fig. 1 Percent chlorophyll content of rice *cvs*.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E represents standard error.
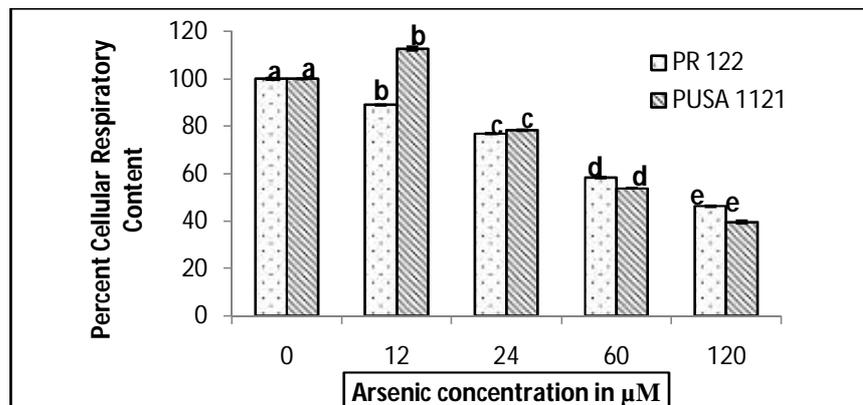

Fig. 2 Percent cellular respiratory content of rice *cvs*.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E represents standard error.

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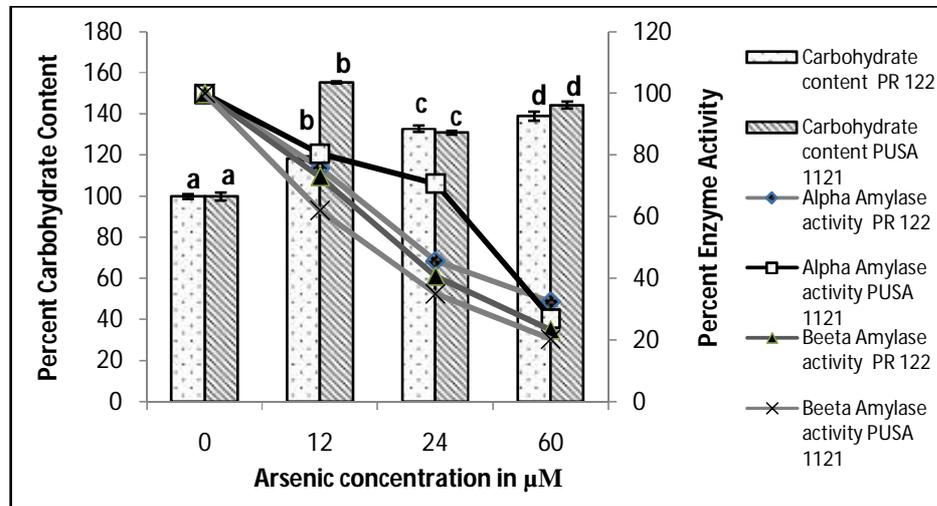
Fig. 3a Carbohydrate content and the activity of their hydrolyzing enzymes - α **and** β ***amylase*** **in roots of rice** *cvs.***PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.**
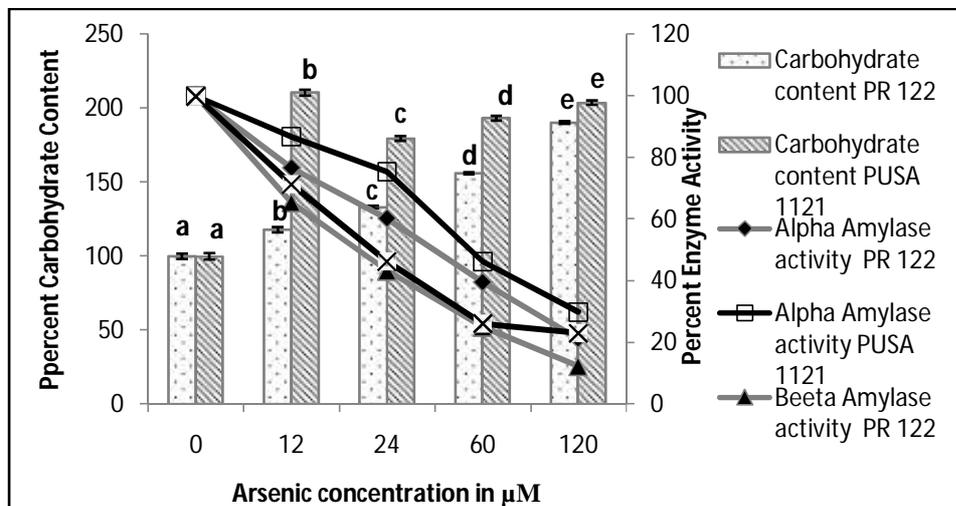
Fig. 3b Carbohydrate content and the activity of their hydrolyzing enzymes - α **and** β **amylase in shoots of rice** *cvs.***PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.**

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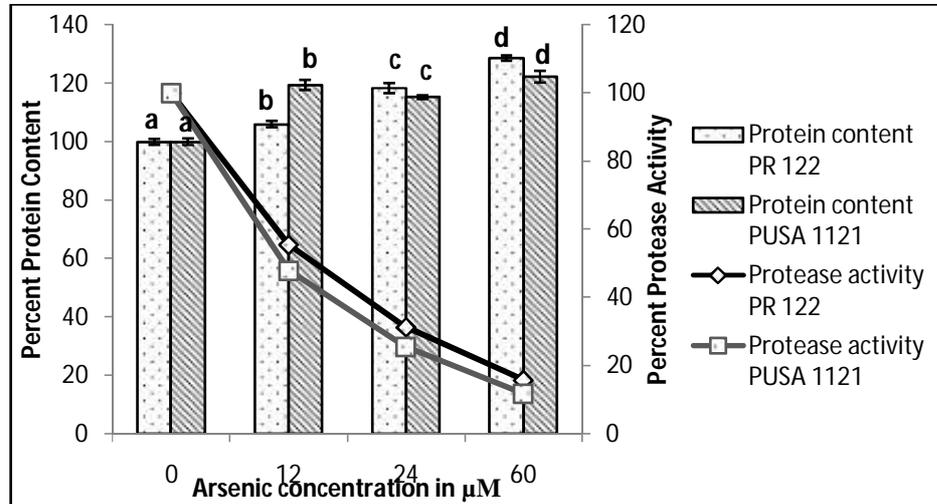
Fig. 4a Protein content and the activity of their hydrolyzing enzymes-protease in roots of rice *cvs.*PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.
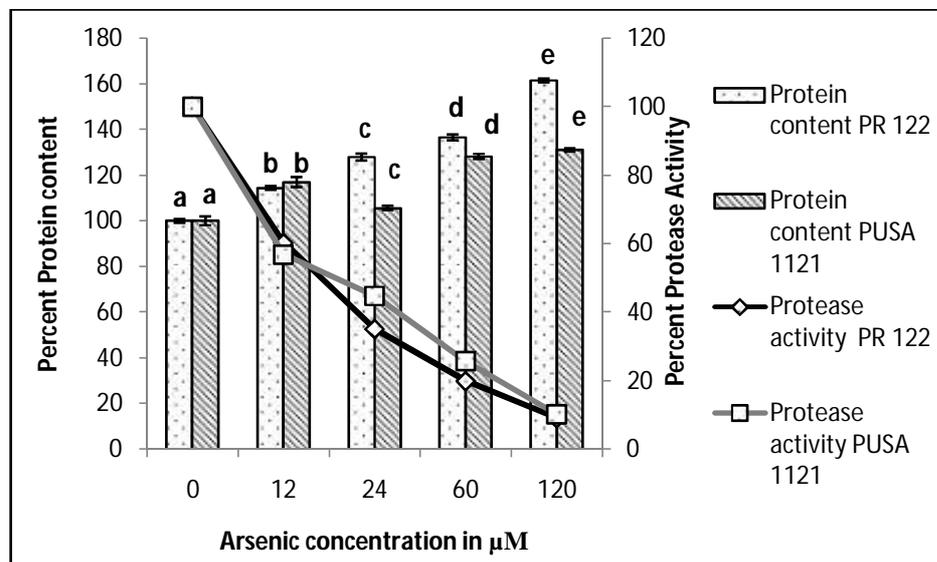
Fig. 4b Protein content and the activity of their hydrolyzing enzymes-protease in shoots of rice *cvs.*PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.

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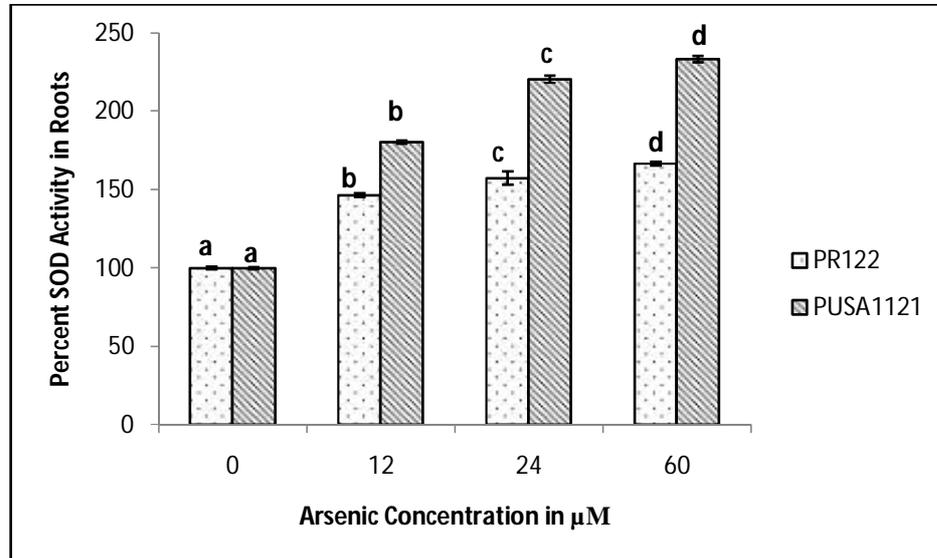
Fig. 5a Percent SOD activity in roots of rice *cvs.***PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Me ans followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.**

Fig. 5b Percent SOD activity in shoots of rice *cvs.***PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Mens followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.**

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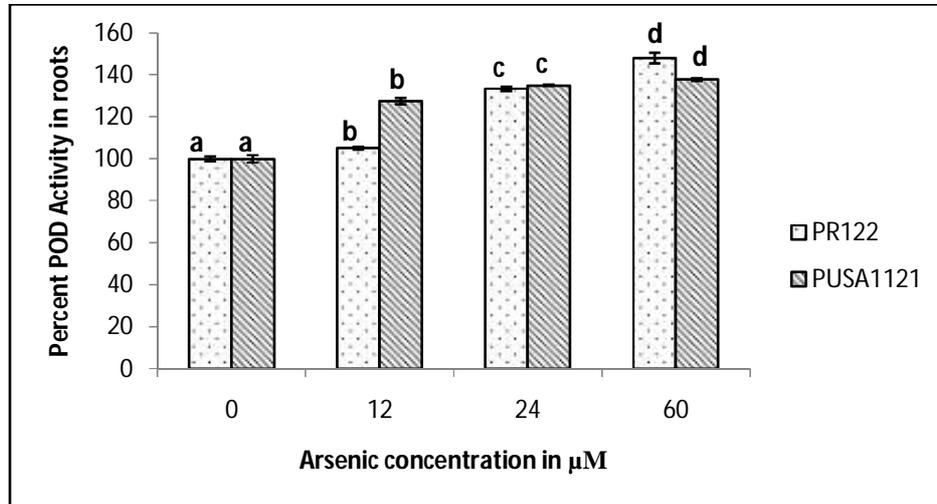
Fig. 6a Percent POD activity in roots of rice *cvs*.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Me ans followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.
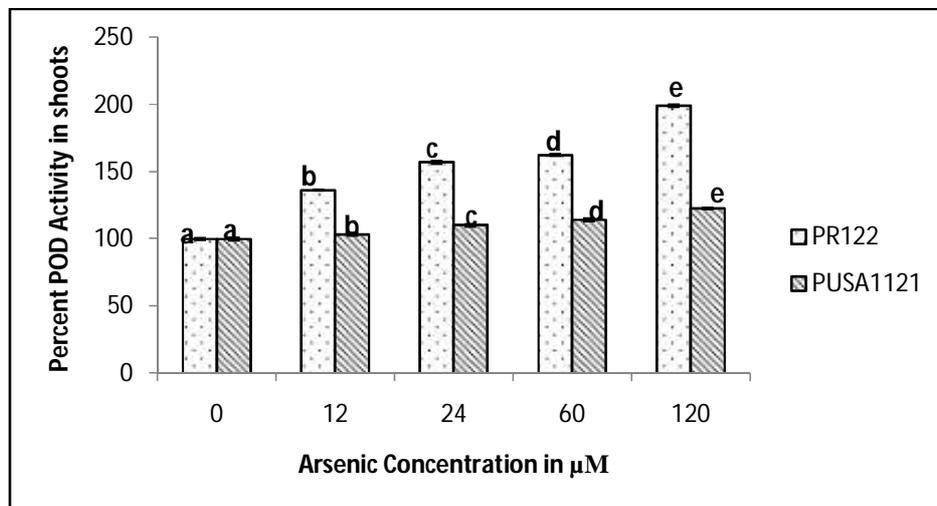
Fig. 6b Percent POD activity in shoots of rice*cvs*.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.

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Table 1 Effect on germination; shoot length, root length and dry weight in PR122 and PUSA1121 with increasing the concentration of arsenic

Conc. (µM)	PR122				PUSA1121			
	Germination	Shoot length (cm)	Root length (cm)	Dry weight (g)	Germination	Shoot length (cm)	Root length (cm)	Dry weight (g)
00	40.00[a] ±0.00	4.84[a] ±0.09	6.26[a] ±0.19	0.036[a] ±0.0007	40.00[a] ±0.00	7.41[a] ±0.16	7.94[a] ±0.36	0.043[a] ±0.0001
12	39.00[a] ±0.33	4.53[a] ±0.10	3.05[b] ±0.18	0.028[b] ±0.0008	40.00[a] ±0.00	6.41[b] ±0.18	5.15[b] ±0.25	0.033[b] ±0.0008
24	38.33[ab]±0.33	3.91[b] ±0.15	2.32[c] ±0.03	0.025[b] ±0.0007	38.66[a] ±0.33	5.47[c] ±0.22	2.68[c] ±0.14	0.028[c] ±0.0008
60	36.33[b] ±0.88	3.67[c] ±0.06	0.62[d] ±0.20	0.016[c] ±0.0009	36.00[b] ±0.57	4.49[d] ±0.07	1.35[d] ±0.16	0.020[d] ±0.0004
120	30.66[c] ±0.88	2.70[d] ±0.12	0.21[e] ±0.02	0.011[d] ±0.0011	30.66[c] ±0.88	3.36[e] ±0.12	0.21[e] ±0.02	0.016[e] ±0.0008

Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificantly at the 0.05% level and S.E represents standard error.

Table 2 Percentage germination, shoot length, root length and dry weight in PR122 and PUSA1121 with increasing the concentration of arsenic

Conc. (µM)	PR 122				PUSA 1121			
	Germination	Shoot length	Root length	Dry weight	Germination	Shoot length	Root length	Dry weight
0	100	100	100	100	100	100	100	100
12	97.5	93.59	48.72	77.77	100	86.5	64.86	76.74
24	95.82	80.78	37.06	69.44	96.65	73.81	33.75	65.11
60	90.82	75.82	9.9	44.44	90	60.59	17	46.51
120	76.65	55.78	3.35	30.55	76.65	45.34	2.64	37.2

RESEARCH ARTICLE

Roosting Ecology of the Indian Flying Fox *Pteropus giganteus* in Southern Tamil Nadu, India

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ABSTRACT

The characteristic of the roost environment is closely related to reproductive success and survival. Disturbance and destruction of day roost sites is a major threat to bat population. Roosting activity and breeding is the first step for studying bat conservation. We studied a total of 47 day roost trees belonging to 10 species and seven families were spread over seven places was identified. Our study showed that the colony size ranged from 60 to 420 in tree (*Pongamia glabra*) more than 400 individuals were roosting in a single roost tree (*Ficus religiosa*). Large trees like *Ficus religiosa, Terminalia arjuna, Pithecellobium dulce*, have more individuals ranged from 120 to 420 in a single roost tree. We also found, 60% of day roosting sites were closer to water bodies like rivers, pond and water channel. A maximum of 18 roosting trees with more than 500 individuals were located in Vaigai dam and Allinagaram temple along with water bodies and rivers. Nearly 50% of *P. giganteus* roosting sites are closely with association to human beings. It is clear that individuals of *P. giganteus* preferred open area and majority of the roosts are located nearer to human habitations.

Keywords bats, roosts, climatic factors, diversity, human habitations

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INTRODUCTION

Selection of specific roost can result in fitness benefits for animals by providing shelter from the adverse weather, places to rear young, protection from predators and opportunities for mating (Alcock 2001). Bats show diverse roosting behaviours and are accustomed to use variety of roosts according to the difference in their roosting requirements during different seasons of the year (Kunz 1982; Kurta 1986). Based on utilization bat roosts are categorized in to three types: day roosts, night roosts and hibernation sites. Day roosts are diurnal shelter and night roosts are feeding & resting place during foraging. Hibernation roosts are occupied during longer periods of cold environment (Fenton 1983). Bats exhibit a great diversity in their habitat selection by making use of a wide variety of naturals structures like rocks, bamboo culm and tree cavities, underneath exfoliating bark or foliage, as well as many synanthropic roosts such as deserted buildings and tunnels (Cryan 2003; Kunz and Lumsden 2003). The roost sites are varies from dense foliage (Ayensu 1974), to open conspicuous areas of trees (Bradbury 1977; Kunz 1982). Roost sites are essential for bats to exhibit variety of social activities like copulation, hibernation, maternal care, escape from adverse weather and predators (Altringham 1996; Kunz and Lumsden 2003). Diurnal roosts are in fact important component of bat ecology as they are reported to spend more than half of their lives there (Kunz 1982; Barclay and Kurta 2007). Roost sites are playing an important in the life of history of bats. Knowledge about their roosts, factors influencing roost selection and ecological significance of these roost sites is important to understand sowal life, physiology and behaviour of bats. Roost selection is influenced by microclimatic condition, predator risk, parasitic infection and distance to foraging areas. These factors strongly influence the bat's survival and fitness (Vonhof & Barclay 1996). Selection of roost may vary between sexes and reproductive stages of animals especially females (Mahson et al., 1996 & Anderson 2000). *Pteropus giganteus* is one of the largest fruit bats and has a widespread distribution in the south-east Asia. It is a colonial species, living in groups. They usually prefer large trees as a roosting place. They feed on variety of fruits and leaves. Foraging range of this bat is about 50km. They roost on the branches of large trees and directed exposed to sunlight & rain (Neuweiler 1969). The roosting nature of this bat, make them more vulnerable to predation, hunting and environmental function. Bats spend about 15 hours per day in their day roost & involved in many activities like mating nursing youngones. (Barclay and Kurta 2007). When bats are excluded from their preferred roosts, they are forced to select alternative roosts and in such situation reproductive success will decline (Brigham and Fenton 1986).

The specific roost sites selected by various bat species may be determined in part by the factors such as morphology, flight, echolocation capabilities, proximity to other resources (e.g. food, water and hibernation sites) and climatic factors. Due to urbanization and development of economic zones, trees in the forest area and sub-urban are steadily declined, which ultimately affect many living things especially bats & birds. Decline in the bat population might have great impact on environmental stability. In the recent past, bat populations in the south Tamil Nadu have been disappeared significant. Only limited numbers of studies have been carried regarding the roosting behaviour of P. giganteus in Tamil Nadu. In this study we made an attempt to address the roost preference, diversity, population dynamics and threads to their roosts of *P. giganteus* in the southern Tamil Nadu, India.

MATERIALS AND METHODS

The present study was conducted at seven locations in and around Madurai (Figure 1), namely (i) Alagarkovil, (78º12'45.59"E, 10º04'31.22"N) 30 km northeast of Madurai Kamaraj University (MKU) campus, (ii) Sourashtra school, Madurai (78º08'07.81"E, 9º 55'00.32"N) 16 km east of MKU campus, (iii) VC's residence inside the MKU campus (78º01'01.21"E, 9º56'24.11"N), (iv) Vidyalaya school, Virudhunagar (77º57'45.85"E, 9º 35'14.48"N) 40 km south of MKU campus, (v) Allinagaram temple, Theni (77º29'23.14"E, 10º00'24.40"N) 70 km west of MKU campus, (vi) Vaigai dam, Andipatti (77º35'22.08"E, 10º3'5.64"N) 40 km west of MKU campus, (vii) Nallachchampatti, Usilampatti (77º48'59.83"E, 10º3'10.79"N) 32 km west of MKU campus. This study was carried out between October 2008 and September 2009. A field survey was made to locate the trees with P. giganteus in the above mentioned area. Once the

trees with bats were identified the following parameters were measured and noted; species of trees, height of tree, number of bats per tree and maximum and minimum height where bats hanging in each tree species. Physical parameters Temperature & humidity was also recorded. In order to understand their diurnal roost behaviours visual observations were made with support of binoculars twice in a month (Balileo 20 × 50 Gross feld) from 16:30 h (day 1) to 14:30 h (day 2). Hence we obtained a total of 528 hours of observation sessions from seven the locations. Potential disturbances and predation pressures at the day roosts were also noted. Taxonomical identification of the tree species were made with help of floral parts such as leaves, fruits, seeds and flowers from the Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Trivandrum, Kerala. The measurement of tree heights was made by visual comparison method (Muñoz-Romo *et al.* 2008). In each roost, we attempted to measure the maximum and minimum height of the branches at which bats roost in each tree. Population size of the colony was counted by performing direct bat count during daytime. To minimize the level of disturbance, bats were not captured during this survey that precluded the determination of sex, age, reproductive condition and body mass.

RESULTS

We have located a total of 47 day roosting trees of *P. giganteus* were identified. They were distributed at seven places in and around Madurai (Fig. 2). Roosts were found in well-established large trees than that of the medium sized trees. Ten species of roost trees belonging to 7 families were identified (Table 1). Colonies with large number of *P. giganteus* were observed in   Moraceae family members such as *Ficus religiosa* (n = 238),*Ficus benghalensis* (n = 214). Other potential roosting trees preferred by bats are belonging to   families of Annonaceae (*Polyalthia longifolia*, n = 422), Melliaceae (*Azadirachta indica*, n = 322), Fabaceae (*Tamarindus indica*, n = 397), Combretaceae (*Terminalia arjuna*, n = 240), Fabaceae (*Pithecellobium dulce*, n = 270), Anacardiaceae (*Mangifera indica* n = 320), Myrtaceae (*Eucalyptus* sp. n = 340) and Fabaceae (*Pongamia glabra* n = 60) (Table 2). The study covered the three districts of southern Tamil Nadu. Among them more number of roost trees observed in Theni district (n = 8), followed by Madurai district (n = 5) and Virudhunagar district (n = 3). Among the 47 day roost tree species, *Ficus religiosa* was observed as a day roost in 2 districts (n = 3). Eight tree species such as *Ficus religiosa, Tamarindus indica, Pongamia glabra, Pithecellobium dulce, Polyalthia longifolia* and *Azadirachta indica* were observed only in the Theni district. The colony size of *P. giganteus* ranged between 60 and 420 individuals. The minimum number of individuals (n = 60) was observed in *Pongamia glabra* (Fabaceae) at (Vaigai dam) Theni district and maximum number of individuals (n = 420) was observed in *Ficus religiosa* (Moraceae) at Nallachchampatti, Madurai district (Table 2; Fig. 1 & 2). The height of the tree ranged between 17 and 22 m, and the highest range was observed 22 m in *Ficus religiosa* (Moraceae) consisting of 140 individuals located in the Sourashtra school, Madurai (Table 3; Fig. 1, 2 & 3). The lowest range was observed 17 m in *Pongamia glabra* (Fabaceae) consisting of 60 individuals at Vaigai dam. The shortest height at which bats were ranged between 10 m and 17 m (Table 3). The minimum roosting height of bat was observed in *Azadirachta indica* (Melliaceae) at 10 m range in the Vidaylaya, the maximum roosting height was observed in *Terminalia arjuna* (Combretaceae) at 17 m range in Allinagaram temple, Theni. The disturbances in the roost of *P. giganteus* colonies were same in all roosts at daytime (Table 4). Birds were the major disturbance factor in Alagarkovil and Allinagaram temple. However anthropogenic activity were the major disturbance in the colonies at VC' residence, Kshathriya Vidyalaya, Sourashtra school, Vaigai dam, and Nallachchampatti. Vehicular movement was observed to be disturbance in the Kshathriya Vidyalaya and Sourashtra school. . In southern regions of Tamil Nadu, the Indian flying fox, *P. giganteus* populations face potential threats mainly because of hunting pressure and felling of roost trees.   In addition, in many rural areas and in the remote villages people engaged in hunting *P. giganteus*. Human are the potential thread among risk factors in Alagarkovil, Sourashtra school, Vidyalaya, Vaigai dam and Nallachchampatti (Table 5). Other potential predators include mongoose in the Allinagaram temple and owl in the VC's residence, MKU, Madurai.

DISCUSSION

A total of 47 day roost trees belonging to 10 species and seven families was spread over seven places were identified. It is clear that *P. giganteus* preferred open area and majority of the roosts are located nearer to human habitations. In previous studies it was shown *P. giganteus* as "large, noisy, squabbling colonies on trees" (Prater 1971) and "huge noisy colonies of many hundreds" (Banks and Banks 1995). Most flying foxes (genus *Pteropus*) that have been studied are moderately or strongly colonial (Rainey and Pierson 1992). A few *Pteropus* colonies consist of a few thousands to millions of individuals (Nowak 1999, (Mickleburgh *et al.* 1992; Goveas *et al.* 2006; Maruthupandian and Marimuthu 2013), to 800-1000 (Neuweiler 1969). Bats living in groups provide a number of fitness benefits (Kunz 1982). It may reduce the costs of rearing young via co-operative breeding (Kerth *et al.* 2001) and may reduce individual predation risk via clustering during emergence from roosts (Speakman *et al.* 1999). Information transfer at roost sites may reduce costs associated with finding foraging areas (e.g. *Myotis bechsteini*-Kerth *et al.* 2001). Khan (1985) reported that the largest colony in Bangladesh contained 2500 individuals. In Sri Lanka, the largest known aggregation of the Indian flying fox in Peradeniya Gardens (Krystufek 2009). The Madagascar flying fox, *Pteropus rufus* was found in dense with colonies of up to 5000 individuals (Mackinnon *et al.* 2003). Our study showed that the colony size ranged from 60 to 420 in tree (*Pongamia glabra*) to more than 400 individuals were presented in a single roost tree (*Ficus religiosa*). Large trees like *Ficus religiosa, Terminalia arjuna* and *Pithecellobium dulce*, have more inidividuals ranged from 120 to 420 in a single roost tree. These three trees are relatively having more number of bats, where water source near proximity. . Most *Pteropus* species roost in the emergent trees often in areas with topographic features that offer protection from strong wind, assist in thermoregulation and provide access to updrafs for easier flight (Pierson and Rainey 1992; Richmond *et al.* 1998). *P. giganteus* are usually located in well-established trees (Neuweiler 1969). In India, most of the earlier reports indicate that *P. giganteus* roosts in *Ficus* spp. (*F. benghalensis*- Koilraj *et al.* 2001; Srinivasulu and Srinivasulu 2004; Goveas *et al.* 2006; Chakravarthy *et al.* 2009; *F. religiosa*- Maruthupandian and Marimuthu 2013). Our results showed that *P. giganteus* uses a total of different plant species (including *Ficus* spp.) belonging to seven family trees as day roost. The roost preference may be mainly due to the height of the tree (Brooke *et al.* 2000) and trees with large circumference (Barclay *et al.* 1988). Such preference to roosting trees was observed in *P. mariannus* on *Artocarpus* species (Wiles *et al.* 1991) and *P. giganteus* on *Eucalyptus* species in North India (Goyal *et al.* 1993). In the present study, *Ficus religiosa, Terminalia arjuna, Pithecellobium dulce* are comparatively larger than other trees (tree height, diameter) which may facilitate bats to gain protection, enable them to become airborne and land more easily and also to evade predators rapidly. Similar observations were made on *P. mariannus* in Pacific Islands (Wiles *et al.* 1997) and *P. livingstonii* on the trees of *Tambourissa, Ficus, Anthoclesita* (Granek 2002). Thus, large size of trees is also one of the factors for colony size, larger size of trees having larger colony size of bats. Brooke *et al.* (2000) reported that Samoan flying fox, *Pteropus samoensis* selects its roosting trees that were forming part of the bats diet and trees with leaves and branches that camouflage the bats and protect from enemies. However, in *P. giganteus,* leaves of *F. benghalensis, F. religiosa, M. indica* and *B. latifolia* were consumed. *T. indica* and *A. lebbeck* were not part of the diet (Sudhakaran and Doss 2012). Nelson (1965) reported that colonies of *P. scapulatus* and *P. gouldii* are found along the river banks or water holes and occasionally in open forests in Australia. *P. poliocephalus* colony sites were located near lakes, river or along the coast and in the range of habitat types such as riparian forest and mangroves as well as urban and sub-urban areas (Nelson 1965; Hall 2002). *Pteropus livingstonii* prefers to live closer to valleys containing water, which are therefore more humid and may indeed be a further indication of their temperature sensivity (Granek 2002). Similar observations have also been noted for *P. alecto* (Palmer and Woinarski 1999). In Sri Lanka, it is particularly abundant in the wet area of the South western corner of the Island (Krystufek 2005). In addition, riparian forests had larger trees and exposed root systems on riverbanks that provided key roosting sites for bats (Bernad and Fenton 2003). 60% of day roosting sites were closer to water bodies like rivers, pond and water channel. A maximum of 18 roosting trees were located in Vaigai dam and Allinagaram temple along with water bodies and rivers & these roosting trees containing more than 500 individuals. Vidyalaya school containing more than 600 individuals along with pond, Nallachchampatti containing more than 400 individuals along with pond and agricultural lands. Temperature and humidity play a role in determining camp (roost) selection in *P. poliocephalus*

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(Stephanie Snoyman and Culum Brown 2010). Frugivorous bats are often exposed to high ambient temperature while roosting in trees during day time (Nelson 1965; Jones 1972). For heterothermic bats that live in cold climates, roosts with a relatively warm microclimate are particularly important (Bell *et al.* 1986). , Roost temperature is important during the period of both gestation and lactation for pre and post natal growth and development of young bats (Racey 1973; Tuttle 1976; Racey and Swift 1981; Hoying and Kunz 1998).

During summer months, most of the individuals roost in core area of the canopy to keep away themselves from scorching sunlight, Individuals of *P. giganteus* roost in large trees and directly exposed to day light and are able to control and maintain their body temperature by exhibiting wing fanning and salivating their bodies. Based on this we also found some relationship between roosting behaviour of *P. giganteus* with temperature and humidity on roosting trees. During the study between October and September 2009 the average temperature in each roosting trees was at a maximum of 29°C and humidity at a maximum of 69%. It clearly shows that environmental factors like temperature and humidity are important factors to determine roosting behaviour in *P. giganteus.* Lower temperature and higher humidity limit the energy expenditure of bats especially during gestation and lactation females. In India, *P. giganteus* colonies are usually located in close association with man and tend to be found in well-established trees in cities and villages (Bates *et al.* 1994; Bates and Harrison 1997). Nearly 50% of *P. giganteus* roosting sites are closer to association with human settlements . Out of seven field sites these five sites (Alagarkovil, Allinagaram temple, Sourashtra school, Vaigai dam, Vidyalaya school) were frequently disturbed by human beings and remaining two sites (Nallachchampatti and VC' residence, MKU) were relatively not much disturbed by human beings. This result clearly shows that *P. giganteus* always prefer to be in close association with man habitats.

In most of the rural areas, *P. giganteus* individuals were hunted for medicinal purpose and as a source of protein. People believe that the meat of *P. giganteus* is good for curing 'asthma'. Our observation clearly indicates that five out of seven field sites, each roosting trees of *P. giganteus* was hunted by human being except VC's residence, MKU and Allinagaram temple, Theni. So humans are the major predator for *P. giganteus.* Fruit bats are excellent seed dispersers, pollinators and indicators of habitat diversity (Agoramoorthy 2002; Agoramoorthy and Hsu 2002, 2004). At least 300 plant species of nearly 200 genera mainly rely on large populations of Old World fruit bats for their propagation (Marshall 1983, 1985; Fujita and Tuttle 1991). These plants produce approximately 500 economically valuable products including fruits, dyes, tannins, timber, medicines and fibres (Fujita and Tuttle 1991). The Indian flying fox (*P. giganteus*) is listed as 'Least Concern' species by the IUCN Red data book and mentioned their population trend as 'decreasing'. If fruit bats of India are not protected, their populations will be drastically reduced in view of their low reproductive rate. Such a situation may have a cascading effect on ecosystems with potentially serious ecological sequences and economic disadvantages (Fujita and Tuttle 1991; Elmqvist *et al.* 1992).

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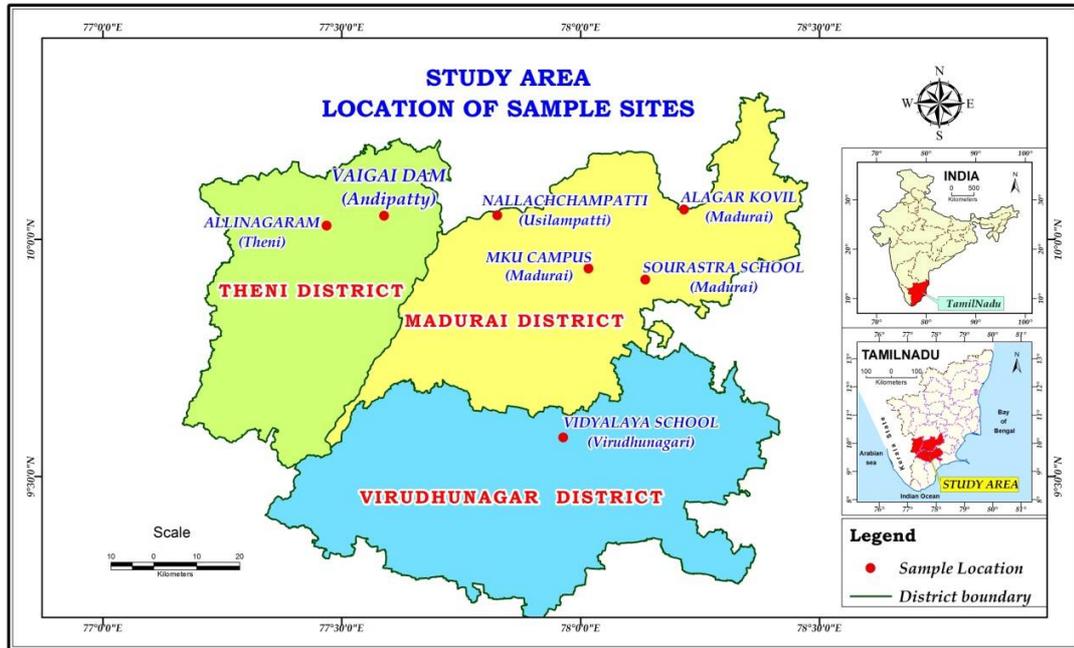
Figure 1. Day roost sites of *P. giganteus* located at various regions of southern Tamil Nadu

Figure 2. *P. giganteus* occupied *Ficus religiosa* as a day roost tree

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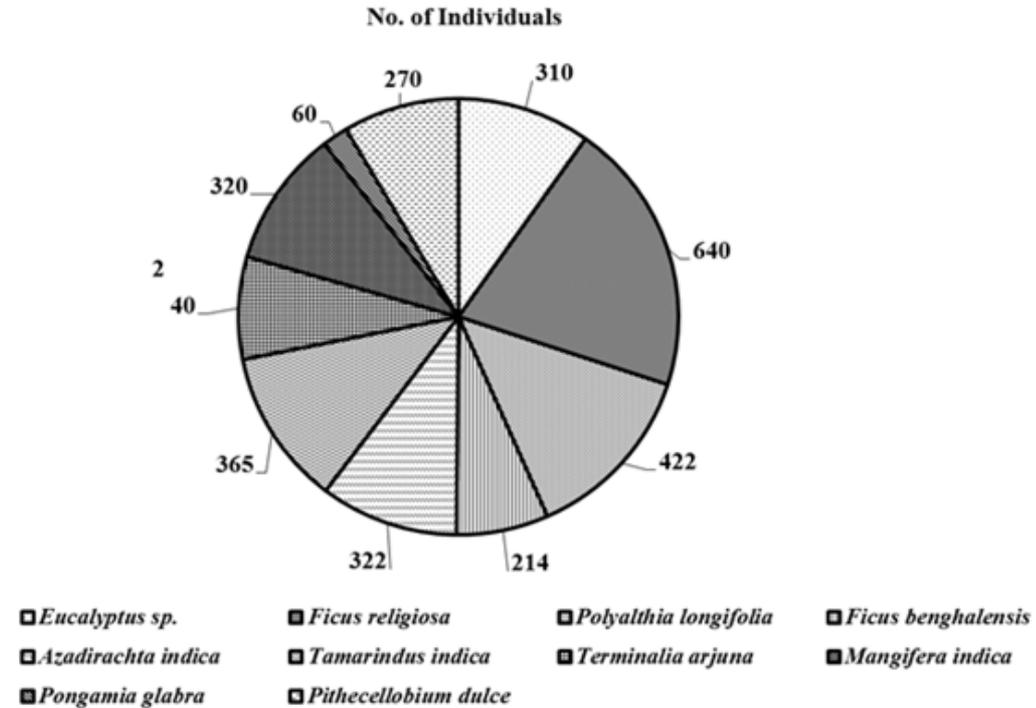
Fig 3. Tree preferences by *P. giganteus* as a day roost trees and their heights
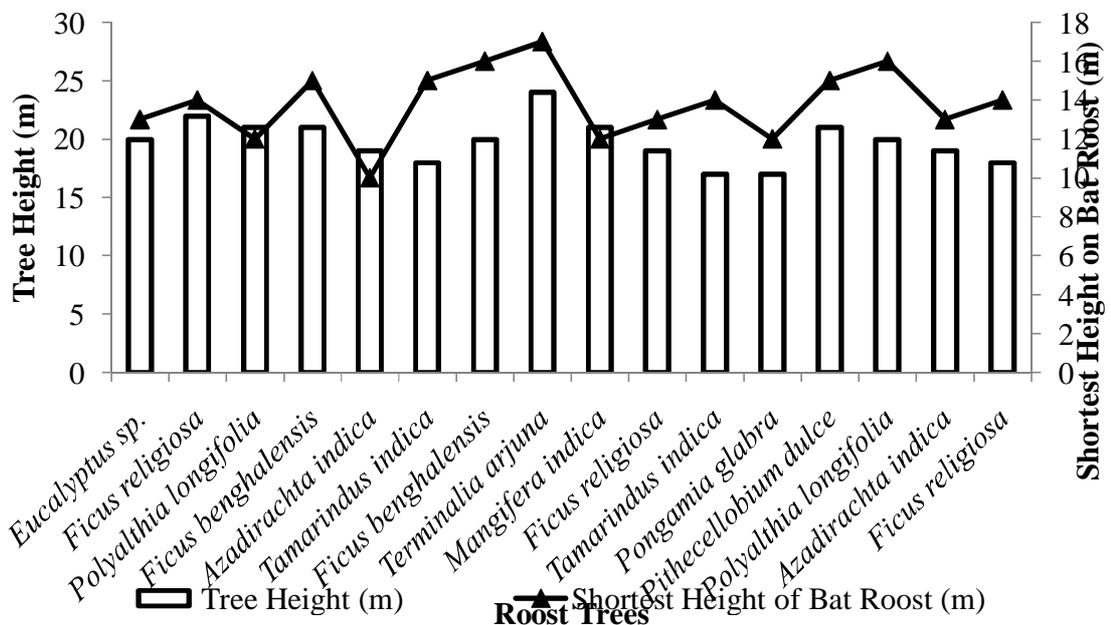

Fig. 4 Day roost trees of *P. giganteus* their heights and shortest height on bat roost

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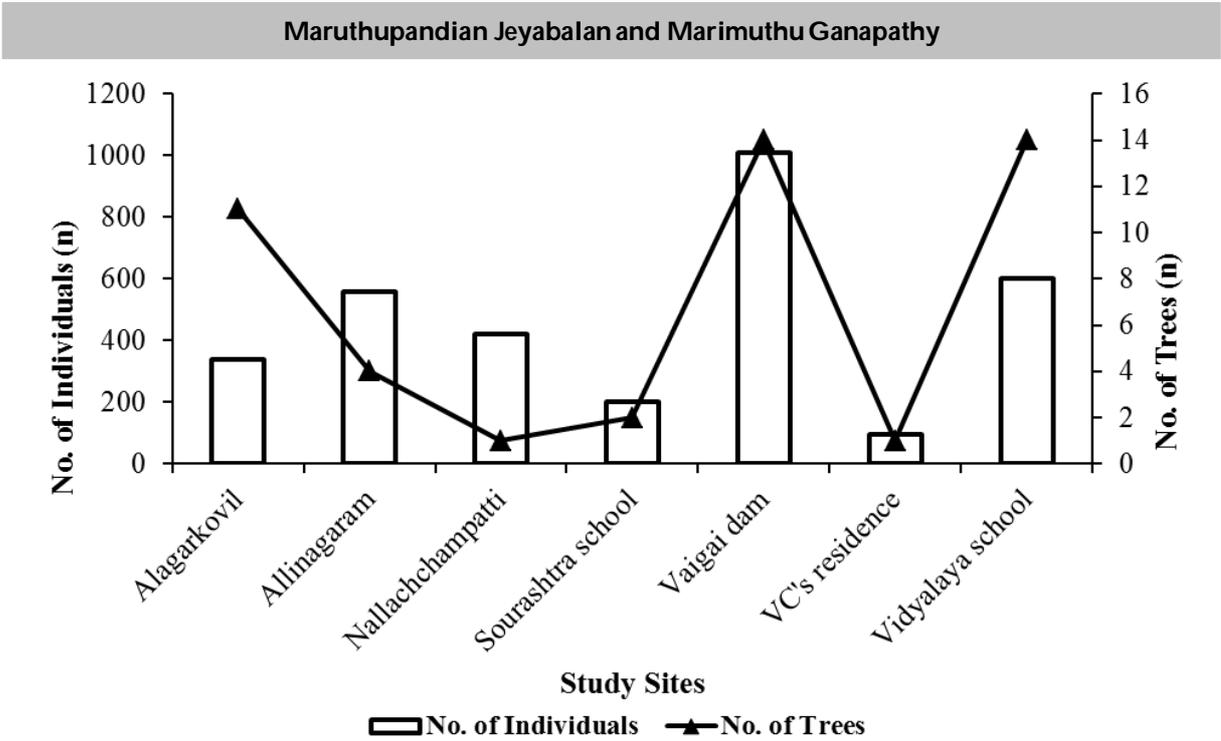
Fig 5. Study site preferences by *P. giganteus* as a day roost trees

Table 1. Number of day roost trees at species and family levels

No.	Day roost trees			
	Species name	No.	Family name	No.
1	*Ficus religiosa*	3	Moraceae	5
2	*Ficus benghalensis*	2	Fabaceae	4
3	*Azadirachta indica*	4	Melliaceae	2
4	*Polyalthia longifolia*	7	Annonaceae	2
5	*Tamarindus indica*	12	Myrtaceae	1
6	*Eucalyptus* sp.	11	Combretaceae	1
7	*Terminalia arjuna*	2	Anacardiaceae	1
8	*Pongamia glabra*	1	--	--
9	*Pithecellobium dulce*	3	--	--
10	*Mangifera indica*	2	--	--

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Table 2. Day roost trees of *P. giganteus* their family names, heights, location, etc

Name	Family	Height of the tree (m)	No. of trees	No. of bats	Sites and distance, direction from MKU campus
Eucalyptus sp.	Myrtaceae	20	11	340	Alagarkovil, Madurai, ~ 30 km, northeast
Ficus religiosa	Moraceae	22	1	140	Sourashtra school, Madurai, ~16 km, east
Polyalthia longifolia	Annonaceae	21	1	62	
Ficus benghalensis	Moraceae	21	1	94	VC's residence, MKU campus
Azadirachta indica	Melliaceae	19	2	176	Vidyalaya School, Virudhunagar, ~40 km, south
Tamarindus indica	Fabaceae	18	11	305	
Ficus benghalensis	Moraceae	20	1	120	
Terminalia arjuna	Combretaceae	24	2	240	Allinagaram temple,Theni, ~ 70 km, west
Mangifera indica	Anacardiaceae	21	2	320	
Ficus religiosa	Moraceae	19	1	80	Vaigai dam, Andipatti ~40 km, west
Tamarindus indica	Fabaceae	18	1	92	
Pongamia glabra	Fabaceae	17	1	60	
Pithecellobium dulce	Fabaceae	21	3	270	
Polyalthia longifolia	Annonaceae	20	6	360	
Azadirachta indica	Melliaceae	19	2	146	
Ficus religiosa	Moraceae	18	1	420	NallachchampattiUsilampatti, ~34 km, west

Table 3. Day roost trees of *P. giganteus* their heights and shortest height on bat roost

Name	Family	Height of the tree (m)	Shortest height on bat roost (m)
Eucalyptus sp.	Myrtaceae	20	13
Ficus religiosa	Moraceae	22	14
Polyalthia longifolia	Annonaceae	21	12
Ficus benghalensis	Moraceae	21	15
Azadirachta indica	Melliaceae	19	10
Tamarindus indica	Fabaceae	18	15
Ficus benghalensis	Moraceae	20	16
Terminalia arjuna	Combretaceae	24	17
Mangifera indica	Anacardiaceae	21	12
Ficus religiosa	Moraceae	19	13
Tamarindus indica	Fabaceae	18	14
Pongamia glabra	Fabaceae	17	12
Pithecellobium dulce	Fabaceae	21	15
Polyalthia longifolia	Annonaceae	20	16
Azadirachta indica	Melliaceae	19	13
Ficus religiosa	Moraceae	18	14

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Table 4. Day time disturbances in the study sites

No.	Study sites	Disturbances
1	Alagarkovil (Madurai)	Birds, Anthropogenic activities
2	Allinagaram temple (Theni)	Birds
3	Nallachchampatti (Usilampatti)	Anthropogenic activities
4	Sourashtra school (Madurai)	Anthropogenic activities
5	Vaigai dam (Andipatti)	Anthropogenic activities , Birds
6	VC's residence (Madurai Kamaraj University, Madurai)	Anthropogenic activities , Birds
7	Vidyalaya school (Virudhunagar)	Anthropogenic activities , Birds

Table 5. Predation risk in day roost of *P. giganteus*

No.	Study sites	Predation risk
1	Alagarkovil (Madurai)	Humans hunting
2	Allinagaram temple (Theni)	Mangoose, Humans hunting
3	Nallachchampatti (Usilampatti)	Humans hunting
4	Sourashtra school (Madurai)	Humans hunting
5	Vaigai dam (Aundipatti)	Humans hunting
6	VC's residence (Madurai Kamaraj University, Madurai)	Owl
7	Vidyalaya school (Virudhunagar)	Humans hunting

RESEARCH ARTICLE

Developing a Software Safety Analysis Model for Nuclear Imaging Device Based on Markov Chain

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ABSTRACT

Using software in instrumentsandequipment in the worldis growingrapidly and it can be said of the new generation of software development, safety and reliability applications, such as other engineering products is an important issue in many countries. Softwarefor use in a device must be safe and reliableand able to prevent unsafe conditions and high maintenance costs and even life events. For example, if you can imagine that a software error leading to an imaging device, that can lead to excessive radiation exposure to the patient and cause irreversible damage.According to the importance of the topic and also the few studies conducted in this area,in thisarticle,a software safety analysis model based onMarkov chainis developed. Then the model for nuclear imaging device in a state medical imaging center implemented andrisk rates, probability, reliability, mean time to failure and the severity-repeated index have been calculated on the software immune system.

Key words: Softwaresafety, reliability, severity-repeated index, nuclear imaging device.

INTRODUCTION

In many systems and equipment, including medical equipment, correct function of the software is important becausesimple incorrect function may be because a large percentage of losses such as death, injury or environmental

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damage.For example, heart patients due to improper software programs available in the pacemaker, have lost their lives. So we have to increase the reliability of the software. However, it should be noted that all software errors do not lead to safety problems and all software functions are not safe of what they are defined, but the goal of design, construction and implementation of the software safety program is eliminating hazards or reduce the risk to an acceptable level, and the safety engineering systems reducerisks by identifying, monitoring and evaluation,and reach the potential effects of adverse incidents to a minimum. Accidents that are associated with medical devices are classified into seven distinct groups as shown in Figure 1.

Safe area, include necessities such as:
1. Reliability
2. Warn or prevent highrisk output
3. Accuracy of measurement

This research aims to discuss software safety in nuclear medical devices.Nuclear imaging is one of the imaging methods in medicine that ispowerful and non-invasive tool that can give information to doctors for physician's diagnosis through metabolic and functional changes in defect organ.Imaging or nuclear scan is a technique that viasafe and a very small amount of radiation provides detailed images of the body. It should be noted that the amount of radiation that patients receive through imaging is equal with common CT-scan and amount of ray that ordinarily receive through 2 or 3 years. Small amounts of radioactive material inter into the bloodstream are called radionuclides, radionuclides attached to red blood cells and with them circulate in the heart. A special gamma camera that is sensitive to the return ray of radionuclides, receives gamma rays. (The gamma camera detects and counts photons originating from the target organ and takes an imagefrom singleScintillation created). Nuclear imaging device can be divided into different types, that in thisstudy, Spect CT and PTC modelsare briefly mentioned.SpectCT is a technology in nuclear medicine and radiology and thefusion of tomography and Spect.SpectCT systems take simultaneously functional and anatomical images from the patient.Spect CTacquires functional information fromSpect and anatomical information from CT.Spect CT is composed of CT scanner, separate gamma camera and common board.The PTC is a device that enables to provide simultaneous imaging of anatomical lesions (such as location, size and shape) and the metabolic changes that occur mainly in cells.There is a number of software in the nuclear imaging device that control with main software.This study presents a mathematical model based on Markov chain to evaluate and improve the level of safety in the above software. Numerous studies have been conducted in the field of security software that some of them are mentioned below.

InBorcsok and Schaefer (2007)research has mentioned that reliability analysis of the software systems safety often needs to add expert information; because little information is valuable basis. In this study, information expert finds ananalysis process byusing data. This extra information isable to calculate reliability characters with the most accurate count. [1] Adler and Kemmann(2009) explained that the software safety guarantee hope to approach the development of safety software. The software evaluation by the ability of its independence is a challenge. They could design Argument software through combine general software with an engineering model. [2]Paul et al (1999) in their paper discussed the analysis of the danger that includes safety aspects of the software, the reliability of the programs and data safety necessity and finally designeda model and test confidence coefficient(tolerance)of the system.[3]Valentiniet al (2008) conducted a study to examine the safety testin Petri Nets to access the system LHC.[4]Sun et al (2009) a method for enhancing the reliability of software on molding SRGM for safety critical softwarepresented whichhelp how to use the software reliability growth model that becomes NPP PSA. [5] Park et al (2009) believe that each section of the four additional networks that built base on DRPS have four main processors such as:BP processor, CP processor, ATIP processor and the COM^3 processor. Each operation is done by BP and CP and other processors have an indirect relationship with the operator. So any adverse action in BP (or CP) software, can change the result DRPS. They believe that decreasing error in the software development is so important because software error is due to design failure. [6]Menon et al (2009) in their study evaluate the safety standards and the results are discussed in the data obtained basis of providing evidence.[7]Habliand Kelly (2010) in their paper

suggested that warranty process can range from an operator by a clear definitionof the software between perfect and without default software. This operator confirms the status change ofperfect software until itswarranty.[8] Yongchaoet al (2010) in their paper on the safety of the existing software reliability test had gained by technology (which is aeronautical) were examined.[9]

Medikonda et al (2010) in their study analyzed integrated safety, which critical system control software (which is safe FTA test) for determining the input data was checked.[10]PanesarWalawegeet al (2010) were able to obtain the chain descriptions of existingevidence for safety software.[11]Ploeschet al (2010) were successful to provide a way to continue a code quality manage by using safety analysis software.[12]Smith and Simpson (2011) were successful to provide a simple guide for safety software that emphasizes on standard automatic measurement tool to integrateexamination for safety in the development of a software project.[13]Mayr (2011) in his paper safety standard operating software for the operating system is presented.[14]Denney and Pai (2011) described evaluation of the softwaresafety because of having trustable Argument. [15] Knight et al (2011) in their study tried to invent a new way to improve the safety[16].Jang and Park (2012) presenteda method for estimating the reliability in nuclear software safety. This method is based on reliability software resulted of SRGM model. They investigated the capability of the following:

1) Mold design based on a special method to ensure highly accurate estimates
2) The ability to predict software errors [17].
Park et al (2013) evaluated probability failure of the Bayesian software.The purpose of this method isinsuring the reliability of the software[18].

Hawkins et al (2013) demonstrated that in the software safety, there is an assurance to each of the rules proportional to system risks distribution in software, which is dependent onpreparing trustableArgument.[19]Hawkins et al (2013) developed a prototype of a software safety Argument for the aircraft wheel brake system.[20]Habliet al (2013) studied on the principles of the software safety assurance.[21]Thus, according to the above mentioned,need for software safety assessment in nuclear imaging device to protect the life and health of the patient, the safety of personnel and property of the medical center seems necessary.

Analysis of Safety Software

Software safety analysis is divided into three levels: system level, model level, functional level.

Analysis of the system level

"Risk" is a safety feature. The combination of the risk and the probability of its occurrence are index value of it.The system level usually includes fault tree analysis, failure mode, effects and analysis of the crisis and event tree analysis based on risk.

Analysis of the model level

In the process of analyzing the model level, "risk" is the important safety feature. Acceptable level of risk is usually used to measure the system safety.

$$THR = \frac{10^{-8}}{hour \times system\ basic\ function}$$

Analysis of thefunctional level

In this level "safe fail" is base of safety features. Using similar technology that is used to estimate the safety is the features of reliability. Most of the time assumes any risk of failure, is only two possible failure modes with different possibility:

Safe fail and unsafe fail.Dangerous failure rate(FRDS = λ_d) and relative risk($\delta = \frac{\lambda_d}{\lambda} = \frac{\lambda_d}{\lambda_{S+\lambda_d}}$)andprobability of safe keeping ($P_{N,S}$)and mean time to dangerous sidefailure(MTTF$_{DS} = \int_0^\infty P_{N,S}(t)dt$) arefour quantitative indexes for measuring the system safety. Analysis of the functional levelneeds a clear definition of possible consequences of two different results of failure.Until the safe and unsafe boundaries are clearly defined, we can construct the confidence hypothesis by using probability theory. But risk is only arelative concept that this method can prevent analyzingof layer unit, thus we need to consider two factors, severity and frequency of our risk. As a result, there are advantages and disadvantages of these three levels of analysis. So over system safety analysis, system futures must be considered. Using this method, continuously with system analysis, wecan select one of the three-levelsor different combination of them.

Survey method (Development of safety analysis model)

In this study a safety analysis model base on Markov chains (transition from one stage to another)has been developed. Markov chain is a series of random variables that all random variables havethe same sample space, but their probability distributionscan be different.Each random variable in a Markov chain is only dependent on justpriorvariable. The sequence of random variables represents as follows:

$$X^{(0)}, X^{(1)}, X^{(2)}, \dots$$

The sample space of random variables of Markov chain can be continuous or discrete, limited or unlimited.Due to the nature of the problem under consideration, we will assume discrete and finite sample space. But the subject is also extended to the case of continuous and unlimited.Assuming discrete finite sample space, we can show any random variable with a probability distribution. We present this distribution with a vector (P)that includes the probability values of each sample space. The other display of the Markov chain is:$P_0, P_1, P_2, \dots$

According to the definition of the Markov chain, knowing the first component of the chain and the relationship that makes$i$componentfrom$i-1$ component is enough.$P_i = \left[p(X^i = x_1), \dots, p(X^i = x_n) \right]$

This relation callsconversion function (T) andthis is how to obtain the probability vector components by the following function:

$$p(X^{i+1} = x) = \sum_i p(X^i = \tilde{x})T_i(\tilde{x}, x)$$

Graph of different software systems

For an applicationunder study considered three different modes that were shown in the figure below. This graph also mentions the use of Markov chain in safety analysis and software reliability.

Model assumptions

For the graph above, the following assumptions are considered:
• Imaging machine is composed of a software system and an immune system
• System(machine)fails when the software system fails to work.
• All fails are independent constantly.
• All failure rates are constant.
In the following table indexes and variables used in this model are presented.

Reliability

The reliability of a system is the possibility of safe and perfect operation for specified time according to the existing and predetermined conditions.

$$R(t) = 1 - \sum_0^t f(t)$$

$f(t)$ is equal to the probability density function that obtains froma Poisson distribution.

$f(t) = e^{-\alpha}\frac{\alpha^t}{t!}$ α= the number of failures occurred during the time t

Failure rate

To obtain a failure rate in relatively high equipment fromduring the performance, until the disability or failure to act of the machines a survey has done and equipment failure rate at time t is the probability density of failure in the next time interval, provided it is perfect at the beginning of the period. $\lambda(t) = \frac{F(t)}{R(t)}$

Repair rate

The mode of failure of the device at any given time determines the principles and methods.$\mu(t) = \int_0^t \frac{1}{\theta} e^{\frac{t}{\theta}} dt$ In formula θ is equal to the average length of service.

The mean time to failure of the system software

This is a good measure forestimating the average time that a software system working before the error in each of the states is defined in the graph above.

Safety analysis model equations

According to the different transitions of the Markov chain and a software system and also taking into consideration the variables and parameters are defined, the following calculation is performed using the technique of differential equations:

$$\frac{dP_0(t)}{dt} + (\lambda_u + \lambda_s)P_0(t) = 0$$

$$\frac{dP_1(t)}{dt} - \lambda_s P_0(t) = 0$$
$$\frac{dP_2(t)}{dt} - \lambda_u P_0(t) = 0$$

$$t = 0, P_0(0) = 1, P_1(0) = P_2(0) = 0$$

By solving the equations we get:

$P_0(t) = e^{-(\lambda_u + \lambda_s)t}$

$P_1(t) = \frac{\lambda_s}{\lambda_u + \lambda_s}(1 - e^{-(\lambda_u + \lambda_s)t})$

$P_2(t) = \frac{\lambda_u}{\lambda_u + \lambda_s}(1 - e^{-(\lambda_u + \lambda_s)t})$

Similarly, the reliability of the software system is:

$R(t) = e^{-(\lambda_u + \lambda_s)t}$

R (t) is the reliability of the software system at time t.

The mean time to failure of a software system is:

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$$MTTF_{xm} = \int_0^\infty R(t)\, dt = \frac{1}{\lambda_u + \lambda_s}$$

$MTTF_{xm}$is the mean time to failure of the system software.The table below shows the types of failures, time and duration of the service providedBased on the above table and equationsobtained, we can present the calculationsin the table below.The results suggest the probability that a software system works normally is equal to 0.924, the probability that a software system fails safely is equal to 0.063 and also the possibility that software system failsunsafelyis equal to 0.0129.

Calculation of FSI index for modes 1 and 2

In relation to the failures above mentioned,indexes AFR, ASR and FSI in modes 1 and 2 calculates as below (over 3 years):In the calculation of the FSI index for the failures mentioned in modes 1 and 2 of the diagram of the transmission system of software system that is the appropriate index in comparative studies, we can say that the failures in which the software immune systemdoes not work can play important role in estimating safety related to thesoftware. Althoughthenumber of failures that the immune system does not work is less thanthat immune system work and fewer lost hours are allocated to them, buttheir FSI index almost half.Based on Gerami and Rocky (2010), if the FSI index in a year is less than 0.1 indicates the safety level is relatively high. [22]Consequently, according to calculations have been done for 3 years, it can be said imaging device under consideration is not desirable for safety level.

CONCLUSION

Nuclear imaging devices because of the wide workplace, unpredictable movements and the nature of the control computer program of them have certain characteristics. Thus, the existing software on nuclear imaging devices causes unique challenges in terms of safety. In this study baseon Markov chain a software safety analysis model forthe nuclear imaging device was developed. This model can be used in conjunction with other safety analysis techniques, to enhance the security of the software. Also fordeveloped model, based on data sampled at a government medical imaging center, hazard rates, possibilities, confidence capabilities, mean time to failure, and also intensity-repeated indices related to software immune system were calculated and results presented in tables 3 and 4.Based on these results, strongly recommend that imaging center managers immediately take action to develop effective programs to enhance the software safety level.For future research suggests,the effectiveness of the modelin comparison with other safety analysis techniques can be calculated and this model can be usedfor other devices and sensitive equipment such as control softwareof fighter aircraft or surgical robot.

ACKNOWLEDGMENTS

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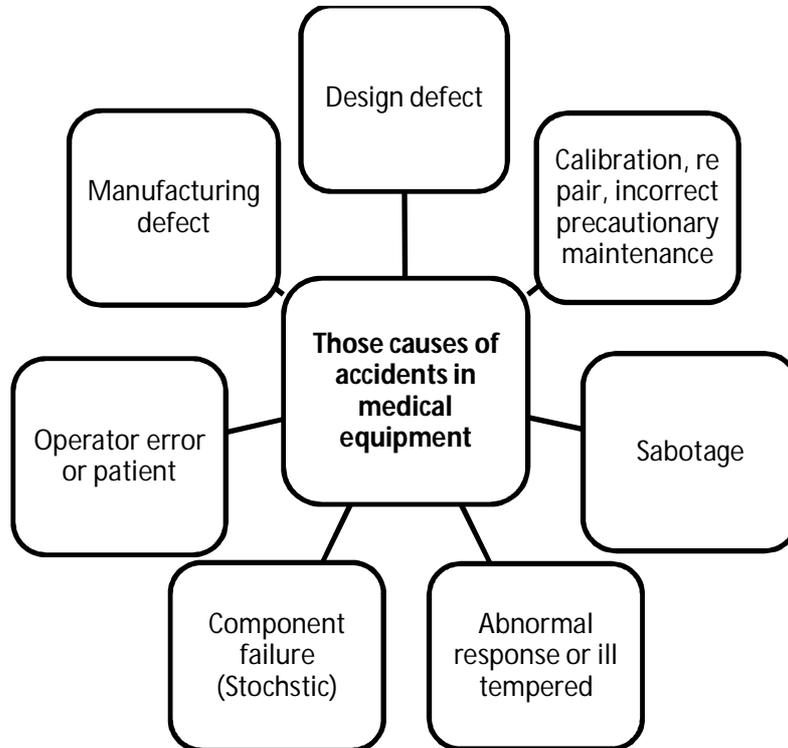
Figure 1- Causes of accidents in medical equipment
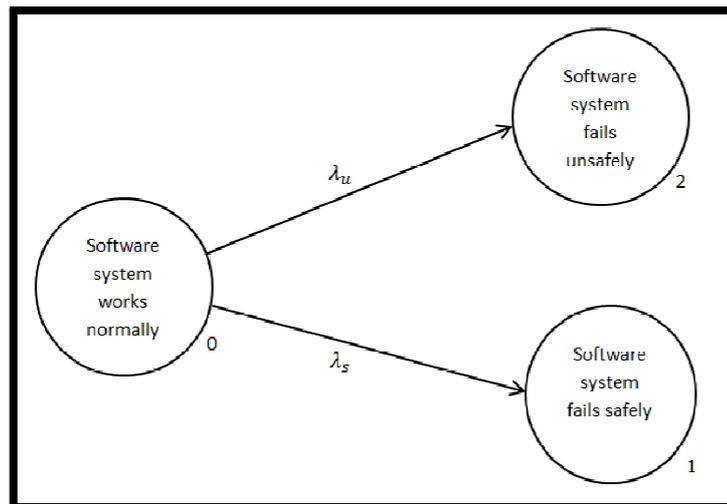
**Figure 2- software system transition from one stage to the next**

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Table1- indexes and variables

Description	Variable
i is the i thstate of the system i =0: Softwaresystemworks normally i =1: Software system has failed safely i =2: Software system has failed unsafely	i
$P_i(t)$: Thepossibility thatRabat system isinstate i and time t	$P_i(t)$
λ_i: The i -th constantfailurerate $i = s$: The transition fromstate0to1 $i = u$: The transition fromstate0to2	λ_i

Table 2 - Types of failures, time and duration of the service

Row	Fail / Potential risk	Occurrence time	Length of service
1	The existence of magnetic waves	Every four months	30 minutes
2	Login incorrect data	Every six months	1 day
3	System board failure	Every four months	8 days
4	Operator error	Rarely Once a year	1 day
5	Data redundancy	Rarely Once a year	2 days

Table3. Calculated values

Definition	The amount	Record
Danger rate from 0 to 1	0.52	λ_s
Danger rate from 0 to 2	0.107	λ_u
System reliability	0.925	$R(t)$
The mean of fail time	1/594	$MTTF_{xm}$
Possibility to work normally	0.924	P_0
Possibility of safe failure	0.063	P_1
Possibility of unsafe failure	0.0129	P_2

Table4. Calculation ofintensity-repeat index for modes 1 and 2 (FSI)

State of	Number of events	Lost work days	Lost work time	Useful work time	AFR	ASR	FSI
1	13	93	12875	116700	111.396	48.50	2.324
2	8	55	7140	112500	71.11	23.75	0.884

RESEARCH ARTICLE

A Case Report on Acquired Idiopathic Megaesophagus in a Labrador Retriever and its Management

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ABSTRACT

A three year old maleLabrador Retriever was presented with complaint of "vomiting" averaging two months and was under treatment with antacids and antiemetics, with no improvement.The dog was lethargic, slightly dehydrated with poor body condition. Detailed enquiry revealed that the dog had been regurgitating following feeding. Plain radiographs of the neck and thorax revealed dilated air-filled oesophagus. "Tracheal stripe" sign was evident in plain radiographs of thorax. Esophagography with barium swallow confirmed megaesophagus. Since the dog exhibited the condition at their adulthood and there was no historycausing trauma of the oesophagus, the condition was diagnosed as idiopathic megaesophagus. Medical and dietary management, and change in feedingpattern were resorted to. Elevated feeding of small, frequent mealswas adopted and Neostigmine @ 0.05 mg/kg orally for seven days was started initially. Later the dose was reduced and adjusted according to response, and continued for two to three weeks. Dramatic improvement was noticed in the dog over a period. Later, Neostigmine was discontinued while elevated feeding was continued. Telephonic enquiry and follow up presentation revealed that medical and dietary management were found very much effective in the management of the condition.

Keywords: Megaesophagus, Neostigmine, Labrador

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INTRODUCTION

Megaesophagus is a common cause of regurgitation in dogs, characterised by esophageal hypomotility, dilatation and progressive loss of body condition (Washabau, 2003).The disease can either be congenital or acquired. The congenital cases have been described in a number of breeds, including Great Dane, GSD, Irish setter, and Siamese cats. Where, acquired cases can either be idiopathic (unknown origin), or can arise secondary to an underlying disorder like myasthenia gravis, polymyositis, polymyopathies, dermatomyositis,polyneuropathies, dysautonomia, botulism, distemper, neoplasia, brain stem disease, lead and thallium toxicity, Addison's disease, hypothyroidism, pituitary dwarfism, and thymoma(Gaynor*et al.,* 1997).Congenital and idiopathic acquired megaesophagus disorders are suspected due to a combination of neurologic dysfunction within the afferent arm of swallowing reflex, altered esophageal viscoelastic properties, and poor vagal responsiveness to intraluminal esophageal distention. Secondary acquired esophagus can be caused by any disease that inhibits esophageal peristalsis by disrupting central, efferent, or afferent nerve pathways or by any disease of the esophageal musculature, including immune mediated infections, and preneoplastic etiologies(Hopper*et al.,* 2001). Esophageal dysmotility is the term used to describe defective esophageal motility without overt dilation of the esophagus. The same diseases that cause megaesophagus are also responsible for esophageal dysmotility. The main primary clinical sign of megaesophagus is regurgitation (without pain), whilst secondary signs (pyrexia, coughing, dyspnoea, weight loss) may also be present and are usually due to nasal reflux, inhalation pneumonia, and malnutrition. There is no curative medical or surgical therapy for idiopathic megaesophagus and all methods are supportive (German, 2005).

MATERIALS AND METHODS

A three year old maleLabrador retriever was presented to Veterinary Dispensary, Kembalu, with acomplaint of chronic "vomiting" for 2 months, gradual weight loss, weakness and deterioration in body condition.Past history revealed proper scheduled vaccination and present history revealed that the dog had signs of regurgitation of food material immediately after feeding and signs of pneumonia including coughing and nasal discharge and was treated with antacids and antiemeticswithout any significant clinical improvement. On general clinical examination, the animal was weak, emaciated and lethargic (Fig. 1). All the physiological parameters were under the normal limit. Cervical and thoracic plain radiography revealed entrapment of air indifferent segments of esophagus and tracheal stripe signs (Fig. 2). Contrast radiography with barium swallow confirmed dilatation of esophagus (Fig. 3). Since the dog exhibited the symptoms in adulthood with an unknown etiology, the condition was diagnosed as idiopathic megaesophagus.The dog wastreated with neostigmine at the rate of 0.05mg/kg orally for 7 days and dose was reduced to 0.025 mg/kg body weightorally and continued for three weeks. Also, advised semi-solid diet in small quantity at frequent intervals from an elevated/upright position (Fig. 4) and maintaining the animal in elevated posture immediately after feeding for few minutes.

RESULTS AND DISCUSSION

The dog responded to the medical, dietary and elevated feeding pattern therapy and almost regained its body condition with in a period of three months.The management of megaesophagus includes, treatment of underlying disease if possible and is otherwise performed according to the general therapeutic guide lines of esophageal diseases. Broad spectrum antibiotics are indicated when aspiration pneumonia occurs (Spillmann, 2007).Medical managementof generalized megaesophagus involves modification of feeding practices. Dogs with megaesophagus generally tolerate a liquid or semi-liquid gruel better than solid food. Feeding from an elevated position allows gravity to help move the liquid into the stomach. If possible the animal should be held in a vertical position for 5 - 10 minutes after eating. Multiple feedings rather than one large single meal may also help minimize food accumulation in the esophagus. Low-profile-gastrostomy tubes can be fixed for feeding in dogs with idiopathic megaesophagus in an effort to minimize aspiration pneumonia. The silicon tubes used are extremely durable and are usually replaced

on a yearly basis. The frequency of aspiration pneumonia has been markedly reduced in comparison to oral feeding and this therapeutic modality should be considered when a client is willing to dedicate the time to tube maintenance and feeding. The prognosis for dogs with megaesophagus is very variable depending upon the underlying etiology, the degree of dysfunction and the systemic status of the dog. The long-term prognosisis poor in most cases, although some cases can be managed successfully for years. The prognosis is improved if treatment of an underlying disease is possible (Marks, 2013).

CONCLUSION

Adult dogs presented with history of "vomiting" and unresponsive to routine therapy should be suspected for idiopathic megaesophagus, which could be confirmed by radiography and effectively managed by oral neostigmine for few weeks initially, along with elevated feeding pattern. Follow up radiographs also shall be taken for confirming persistence or deterioration of the condition.

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Fig.1: Animal presented with history of regurgitation, lethargy and weakness

Fig. 2: Entrapment of air in esophagus (Tracheal stripe sign)

Fig.3: Contrast radiography – showing dilatation of oesophagus

Fig.3: Elevated/upright feeding pattern

RESEARCH ARTICLE

Cervical Esophageal Perforation Due to Fish-Bone in a Cat and its Surgical Management

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ABSTRACT

Oesophageal foreign bodies were common in cats and mainly caused by bones, needles or fish hooks. A case of oesophageal perforation by a fish-bone in a cat and its surgical management was presented. A one-year-old tom cat was presented with the history of an open wound on left lateral aspect of neck, through which food materials were found leaking. Examination revealed a bone piece projecting through the wound. Under general anaesthesia, the bone (fish bone) was removed and the oesophageal wall was apposed, after scarification of the edges. The skin wound was debrided and closed in routine manner. Advised alternate day dressing and semisolid diet for a week. Routine postoperative antibiotic and analgesic therapy were provided for five days. After 10 days, the sutures were removed and animal had an uneventful recovery.

Keywords: Cat, Esophageal Perforation, Fish Bone

INTRODUCTION

Esophageal perforation is a serious disorder that is difficult to diagnose and associated with significant morbidity and mortality. Early diagnosis and treatment are essential and reduce the mortality rate. Esophageal foreign bodies

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are a common problem in cats and dogs. The most common foreign bodies are bones, fish hooks, needles, string foreign bodies and others include balls, wooden sticks, toys, and trichobezors (Michels et al., 1995).All esophageal foreign bodies are an emergency requiring immediate removal.Delay of even a few hours can greatly increase the chance of esophageal stricture following removal. Foreign material present for an extended time in the esophagus, foreign bodies that have sharp points, and foreign bodies that expand resulting in pressure necrosis, are all at increased risk of esophageal perforation (Michels et al., 1995).Esophageal perforation is a rare, difficult and challenging clinical event and there are many causes such as endoscopic examinations, surgical procedures, placement of tubes and intubation and others include penetrating wounds, trauma, swallowing foreign bodies or acid or caustic substances, and spontaneous rupture due to infections (*Silviuset al.,* 1976). Mostly,esophageal perforation in cats are secondary to esophageal foreign bodies(Beitzel and Brinker, 1956). Perforations can occur in the cervical, thoracic, or abdominal esophagus. Abdominal perforations should be surgically repaired, while cervical and thoracic perforations can be managed either by repair or conservative treatment. Cervical perforations are usually more nonthreatening, but intrathoracic wounds present higher morbidity and mortality, especially when the diagnosis is made late (**Hasimoto et al., 2013).**

MATERIALS AND METHODS

A one year old tom cat weighing two kg was presented to Veterinary Dispensary, Kembalu with the history of swelling at cervical region since one month, which ruptured three days back and had an open wound on left lateral aspect of neck (Fig. 1), through which food materials were found leaking. Physical examination revealed a bone piece projecting through the wound. Surgical correction was resorted to.The animal was anaesthetized using a combination of ketamine hydrochloride at the rate of 10 mg/kg body weight and xylazine at the rate of 1 mg/kg body weight intramuscularly. Meloxicamwas administered at the rate of 0.3 mg/kg intramuscularly. The area around the site of perforation was shaved and prepared aseptically. The fish bone was removed using artery forcep (Fig. 2). The area was flushed with saline solution and scarified the wound edges. The oesophageal wall was apposed in a simple interrupted pattern using polyglactin 910 absorbable suture material in single layer (Fig. 3). The skin edges were sutured in a horizontal mattress pattern using non absorbable suture material nylon (Fig. 4). Routine post operative care was provided for five days with cephalexin at the rate of 20 mg/kg body weight orally and meloxicam at a rate of 0.2 mg/kg body weight orally.

RESULTS AND DISCUSSION

The sutures were removed on 10th post-operative day and animal had an uneventful recovery. Oesophageal perforation was seen occasionally in cats, but it was always an emergency condition.Usually it happened, when cats feed on sharp foreign bodies like fish or chicken bones.The persistence of a foreign body in the esophagus stimulates peristaltic activity. If the foreign body puts excessive pressure on the esophagus or if it remains at one location for several days, pressure necrosis may occur and can cause perforation. The major complications of perforation of cervical esophagus include esophagitis, local abscessation, and subcutaneous emphysema formation and thoracic perforation includes spontaneous pneumothorax, acute myocardial infarction, perforated peptic ulcer, acute pancreatitis, dissecting aortic aneurysm, and pneumonia *(Nesbitt and Sawyers, 1987).*

The literature suggested many procedure for treating perforation but that there is no single surgical procedure considered to be gold standard for treating perforations. Usually line of treatment include surgical approach with primary repair or even esophagectomy with an immediate or delayed reconstruction of the removed segment and extensive mediastinal drainage *(Zumbroet al.,* 2002), and clinical treatment with thoracic drainage, broad-spectrum antibiotics, and total parenteral nutrition.

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CONCLUSION

It was concluded that any draining wound on the cervical region in cat should be suspected for oesophageal perforation and immediate surgical intervention was necessary.

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Fig. 1: Esophageal perforation

Fig. 2: Recovered fish bones

Fig. 3: Esophageal wall suturing

Fig. 4: Skin suturing

RESEARCH ARTICLE

Medical Management of Transmissible Venereal Tumour in a Labrador

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ABSTRACT

A three year old male Labrador weighing 41 kg was presented toVeterinary Dispensary, Kembalu, with a complaint of occasional haemorrhagic discharge from the prepuce since one month. The animal was found to be alert and active. Retraction of the prepuce revealed presence of pedunculated multi lobular hyperaemic masses around the base of the penis. Physiological parameters were within normal range.Haemogram revealed mild leucocytosis with neutrophilia. Giemsa stained impression smear revealed the presencenumerous discrete round tumour cells with uniform chromatin and pale blue cytoplasm containing multiple clear vacuoles. Histopathology revealed presence of large polyhedral cells with round nucleus with prominent nucleoli, basophilic cytoplasm with numerous vacuoles suggestive of transmissible venereal tumour. Animal was treated with vincristine sulphate at the rate of 0.025mg/kg intravenously diluted in normal saline once in week for 4weeks. Animal had an uneventful recovery.

Key words: Labrador, cytoplasm, haemorrhagic,

INTRODUCTION

Canine Transmissible venereal tumour or venereal granuloma is a reticulo-endothelial tumour which occurs as single or multi-nodular cauliflower shaped pendular, nodular, papilar, or multilobular, poorly circumscribed, invasive tumour generally attached to prepuce and base of the penis in males and vagina or labia in females (Das *et al.,* 2000).

The haemorrhagic discharge producedin transmissible venereal tumour accompanies with offensive odour (Tella *et al.,*2004). TVT is a contagious tumour and the transmission occurs by inoculation of the neoplastic cells through damaged mucosa or skin commonly during coitus and also occur by licking, biting or by scratching tumour affected area.Metastasis of the TVT have been countered in eyes, skin, brain, subcutaneous tissue, lung, lymph nodes, tonsils,liver, spleen, oral mucosa, hypophysis, peritoneum, and bone marrow (Prasad *et al.,*2007; Santos *et al.,*2008).

MATERIALS AND METHODS

A three year old male Labrador weighing about 41 kg was presented to Veterinary Dispensary, Kembalu, with a complaint of pain during passage of urine and voiding blood tinged urine. Present history revealed the animal was treated with antibiotics, styptics and other supportive therapy with unsuccessful result.On clinical examination the dog was active with normal mentation. All the visible mucous membranes were blanched. Temperature (102.2ºF), respiration (32/min), pulse (98/min), heart rate (103/min) were found to be in the normal range.No abnormalities were detected on physical examination of abdomen. Haemogram revealedRBC 6.2 x 10^6 cells/mm^3, haemoglobin 10 g%, PCV 40%, MCV 66.4 fl, MCHC 33.3 g/dl, MCH 21pg, platelet count 4.57 lakhs/L, TLC 16.1 x 103cells/mm^3 and neutrophils 65%. Serum biochemical parameter creatinine 0.56mg/dl. Urine sample was collected by catheterization for urinalysis. On gross examination the urine colour was normal, which on urinalysis in COBAS strip revealed a pH of 6, RBC +, WBC +, protein ++ and constituents like glucose, ketones and bilirubin were absent. Direct microscopic examination showed epithelial sloughing and presence of polyhedral cells. Animal was subjected to abdominal radiograph, where no abnormalities were detected.Palpation of the urinary and genital tract upon retraction of prepuce revealed presence of predunculated multi-lobular hyperaemic mass around the bulbus glandis of penis.Multiple impression smears stained with Giemsa revealed presence of large polyhedral cells with round nucleus and prominent nucleoli, basophilic cytoplasm with numerous vacuoles.The case was diagnosed as transmissible venereal tumour from the above findings.

Treatment and results

The animal was treated with vincristineat the rate of 0.025mg/kg body weight at an interval of two weeks for four successful treatments. The animal had an uneventful recovery after fourth successive treatment.

DISCUSSION

Thisstudyshowsregimenofintravenousadministrationofvincristinesulphateat0.025mg/kgbodyweightintravenously alonefor 4 subsequent weeks was very effective in the completeremission of TV Tindogs.Canine transmissible venereal tumour (CTVT) or Sticker's sarcoma is a contagious naturallyoccurring benign reticulo-endothelialtumour of dogs that can be transmitted between dogs via inoculation of the live tumor cell during mating,without prevalence breed or sex(Park*et al.*, 2006). Transmissible venereal tumor (TVT) is the only tumor in dogs that can be transmitted by mating. The mutated tumour cell acts as an etiology for the development and progression of TVT.Impression smearof TVT mass consists of round, ovoid or polyhedral cells with vacuoles. Histopathology of the TVT cells consists of large, round cells with hyper chromatic nuclei and centrally situated unique nucleoli with a moderately abundant eosinophilic cytoplasm (Igor *et al.,*1992). A number of mitotic features can be observed in parenchymal cells that make up these tumours. The tumor cells are rich in stromal which contains the blood vessels which are relatively narrow and can be observed as thin lines within the clusters of tumor cells. The management of transmissible venereal tumouris not veryeasy in dogs, especially in developing countries like India, as most owners cannot afford thecost of surgical intervention and/or radiotherapy. This has made the control of this tumour almost impossible in this region. The use of anti-tumour drugs have been described as a more effective method of treatmentin TVT (Brown *et al.*, 1980; Idowu *et al.*, 1984; Oni, 1994).

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Fig.1. TVT at the root of the penis

Fig. 2. Impression smear for microscopic examination
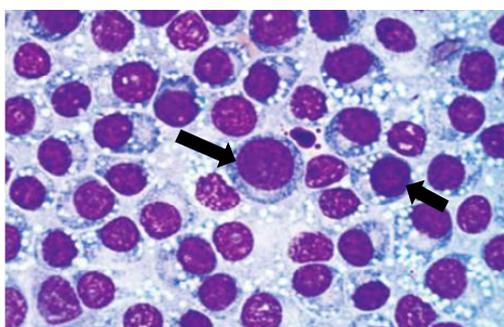
Fig.3. TVT cells in Giemsa stain
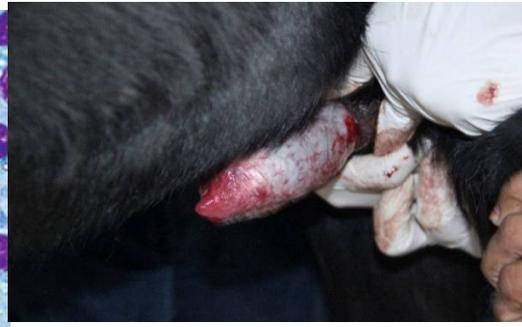
Fig. 4. Regressed TVT after chemotherapy

RESEARCH ARTICLE

Impacts of Ultraviolet-B and Photo synthetically Active Radiation on *Anabaena cylindrica* and *Synechocystis* PCC 6803: a Comparative Study

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ABSTRACT

The effects of ultraviolet-B (UV-B) and photosynthetically active radiation (PAR) on photosynthetic efficiency and photoprotective role of MAAs have been studied in cyanobacteria such as *Anabaena cylindrica* and *Synechocystis* PCC 6803. UV-B radiation was found to have an adverse effect on photosynthesis performance on both cyanobacteria. Growth and photosynthetic pigment were continuously declined in UV-B radiation as compared to PAR and PAR+UV-B exposure after 48 h of exposure. There was mark reduction of chlorophyll fluorescence recorded under UV-B radiation while negligible changes observed in PAR. Interestingly, it was found that an accumulation of mycosporine-like amino acids (MAAs) when organism exposed under UV-B and PAR+UV-B irradiation. HPLC analysis was revealed that two peaks at retention time 2.7 and 2.8 min with absorption maxima at 334 nm in *Anabaena cylindrica* and *Synechocystis* PCC 6803 respectively. This result shows that UV radiation induces the accumulation of MAAs in cyanobacteria and overcome the deleterious effects on photosynthetic pigments.

Key words: Cyanobacteria, Mycosporine-like amino acid (MAAs), Photoprotection, Pulse Amplitude Modulation (PAM), Ultraviolet-B (UV-B)

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INTRODUCTION

Cyanobacteria are largest group of Gram-negative photosynthetic organisms, having cosmopolitan distribution in nature. They are primary producer in both terrestrial and aquatic ecosystems. The continued release of atmospheric pollutants such as chloroflurocarbons (CFCs), chlorocarbons (CCs), organobromides (OBs) and reactive nitrogen species (RNS) induces rapid depletion of stratospheric ozone layer which is responsible for the increase in solar ultraviolet-B radiation (UV-B: 280-315 nm) on the earth surface [1- 3]. UV-B radiation is highly energetic and tendency to damage cellular proteins, nucleic acids and other biologically active compounds in organisms. Cyanobacteria are simultaneously exposed by solar radiation mainly UV-A and UV-B in their natural climate [4]. The continuous exposure of solar radiation induces the production of highly toxic reactive oxygen species which are counter inhibited by the accumulation of carotenoids and photo protective pigments [5-7]. Cyanobacteria have been developed several defense strategies such as avoidance, DNA repair mechanism, scavenging of free radicals, photorepair and programmed cell death to mitigate the damaging effects of ultraviolet and photosynthetically active radiation [8]. Ultraviolet absorbing/screening compounds such as mycosporine-like amino acids (MAAs) and scytonemin have been subject of much attention during recent year for possible role in photoprotection [9]. Mycosporine-like amino acids (MAAs) are small (<400 Da) colorless water-soluble compounds characterized by cyclohexenone or cyclohexinamine chromophores conjugated with the nitrogen substituents of an amino acid or its amino alcohol, which have absorption ranging between 310 nm to 360 nm [9]. MAAs arrests three out of ten photons from being absorbed by genetic material (DNA) or protein [10]. The MAAs concentration inside a cyanobacterial cell is capable to absorb 10-26 % photon energy of UV-B radiation, which provides more resistance to UV-B radiation against damage [4]. Due to their UV-absorption capacity, they act as strong photoprotective agent for cyanobacteria from harmful ultraviolet radiation, thus they may use as possible candidature for natural sunscreen [11].The aim of this study was to investigate the ability of the cyanobacteria *Anabaena cylindrica* and *Synechocystis* PCC 6803 to produce UV protecting / absorbing compounds that act as photoprotective agent to protect the photosynthetic system by dissipating harmful doses of UV radiation. This study may provide counter mechanism of photoprotection of photosynthetic pigments in morphologically dissimilar cyanobacteria.

MATERIALS AND METHODS

Experimental organisms and culture conditions

The experimental organism *Anabaena cylindrica* and *Synechocystis* PCC 6803, were grown axenically in the presence of nitrogen and nitrogen free BG-11 medium respectively at $27 \pm 2°C$ [12] under white fluorescent light (72 μmol m^{-2} s^{-1}) with a photoperiod of 14:10 (light/dark).

PAR and UV radiation

The culture of *Anabaena cylindrica* and *Synechocystis* PCC 6803 was kept in open glass Petri dishes (75 mm in diameter), were continuously exposed for 0, 12, 24 and 48 h of time duration under artificial radiation of ultraviolet-B (UV-B; 280-315 nm), and white fluorescent light (PAR; 400-700 nm). The desired wavelength of radiation was restricted by using 295 nm and 395 nm cut-off filter foils (Ultraphan, UV Opak Digefra, Munich, Germany) for UV-B radiation and PAR respectively [13].

Determination of growth and photosynthetic efficiency

Equal volumes of test samples (control) as well as irradiated samples under PAR, PAR+UV-B and UV-B radiations were taken at fixed time intervals (0, 12, 24 and 48 h). The growth and chlorophyll a (Chl *a*) content were measured as describe earlier [14].

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To determine the photosynthetic efficiency of *Anabaena cylindrica* and *Synechocystis* PCC 6803 have been adapted in dark for 20 min after exposure of PAR, UV-B and PAR+UV-B irradiation. The modulated chlorophyll fluorescence kinetics was obtained by a flash of actinic light with an intensity of 100 µmol m^{-2} s^{-1} and measured with a Pulse Amplitude Modulation Fluorometer (Super Head Fluorometer FL, 3500/F, Photon System Instruments, Czech Republic). The initial fluorescence (F_0) was measured by applying an analytic modulated flash of light (all PS II reaction centers are open). A saturating flash light was applied to measure the maximum fluorescence F_m, where all reaction centers are closed. The ratio of variable to maximum fluorescence (*Fv/Fm*) of photosystem II (PS II) was used as maximum quantum yield of the photosynthetic apparatus.

MAAs extraction and partial purification

The irradiated and non-irradiated cyanobacterial cells were harvested by centrifugation and pellets were dissolved in 5 ml of 100 % methanol (HPLC grade Spectrochem, Mumbai) and MAAs was extracted by overnight incubation in dark at 4°C. Thereafter, aliquots were centrifuged and supernatants were evaporated at 40 °C in a vacuum evaporator up to dryness. Dry sample was redissolved in 2 ml of Mili Q distilled water and filtered through 0.22 µM pore sized filters (Axiva Biotech, New Delhi). The purification of MAAs was done by HPLC method (Water 2998, 515 PUMP, Milford, USA) equipped with ODS-2 column and Empower-2 software. A 50 µl sample volume was used for injection in the presence of 0.02 % acetic acid (v/v) as mobile phase in double-distilled water, at a flow rate of 1.0 ml min^{-1}. The MAAs was detected at wavelength of 330 nm by PDA detector (Photodiode Array) equipped with HPLC. The identification and quantification of MAAs retention times were performed as described [15, 16].

Absorption spectroscopy

Absorption spectra of all samples were recorded in a single beam spectrophotometer (Beckman DU 70, Instruments, Palo Alto, CA, USA). The absorption spectra were analyzed using the software provided by Manufacturer.

Statistical analysis

All the experiments were carried out in three replicates. The data were analyzed by ANOVA and multivariate analysis in SPSS 16.0 for windows.

RESULTS AND DISCUSSION

Growth and photosynthetic performance

Growth response of cyanobacteria *Anabaena cylindrica* and *Synechocystis* PCC 6803 was measured after 48 h exposure of PAR, UV-B and PAR+UV-B irradiation and shown in Fig. 1 (A, B). Both *Anabaena cylindrica* and *Synechocystis* PCC 6803 were showed maximum growth rate up to 47 and 29 % respectively in PAR exposure. Growth pattern of *Anabaena cylindrica* and *Synechocystis* PCC 6803 declined tremendously by 42 and 44 % respectively upon 48 h exposure of UV-B irradiation as compared to PAR. The decreasing trend in growth of cyanobacteria after UV-B irradiation was due to the different degree of cellular damage either directly or indirectly on various structural as well as various components of genetic material [17-19]. Fig. 2 shows the changes in photosynthetic pigment Chl *a* content after exposure of PAR, PAR+UV-B and UV-B. There was an increasing trend in Chl *a* after PAR exposure in both tested cyanobacteria *Anabaena cylindrica* and *Synechocystis* PCC 6803. However, a gradual decline in Chl *a* content was found in above cyanobacteria after exposure under PAR+UV-B radiation as compared to PAR. Furthermore, a tremendous decline in Chl *a* content was shown after 48 h exposure of UV-B irradiation in both cyanobacteria *Anabaena cylindrica* and *Synechocystis* PCC 6803. In addition, Chl *a* content was more affected in *Anabaena cylindrica* as compared to *Synechocystis* PCC 6803. This result concluded that photosynthetic pigment is greatly affected under UV-B radiation in comparison to PAR and PAR+UV-B. It has been reported that the damaging

effect of UV-B radiation on photosynthetic pigment was due to photobleaching effects or ROS mediated peroxidation [20, 21].

The photosynthetic efficiency of above mentioned cyanobacteria was measured by photosynthetic quantum yield of PS II (*Fv/Fm*) after 12, 24 and 48 h exposure under different radiation condition PAR, PAR+UV-B and UV-B (Fig. 3 A, B). In comparison to PAR, optimum quantum yield of PS II was declined under PAR+UV-B; while more decrease in quantum yield of PS II (*Fv/Fm*) was observed in the sample exposed with UV-B radiation. It was a recorded decrease of 64 and 36 % of quantum yield of PSII after 48 h of UV-B exposure in *Anabaena cylindrica* (Fig. 3 A) and *Synechocystis* PCC 6803 (Fig. 3 B) respectively in comparison to PAR and PAR+UV-B radiation. The damage of PSII proteins such as D1 may be a possible explanation for the damage by UV-exposure [22-24] in test organism *Anabaena* sp. BI42. The decrease in optimum photosynthetic yields (*Fv/Fm*), growth and photosynthetic pigments under UV radiation have also been reported in several cyanobacterial species [19, 25].This result proves that UV-B radiation affects the electron transport system *via* the Quenching analysis of both tested cyanobacteria. The photosynthetic efficiency (*Fv/Fm*) ratio decreases, relative to dark-adapted cyanobacteria when cyanobacteria are exposed with UV-B and PAR+UV-B irradiation.

Analysis of UV-absorbing compounds

From previous studies, it is confirmed that a number of MAAs have been identified from different groups of micro/macro algal species [8, 26-27] and play a potential role in photoprotection. In this present study, occurrence of UV protecting / absorbing compounds was analyzed in two cyanobacteria *Anabaena cylindrica* and *Synechocystis* PCC 6803 after PAR, UV-B and PAR+UV-B irradiation (Fig. 4 A, B). We have recorded UV-absorption maximum (λ_{max}) at 334±2 nm and 310±2 in *Anabaena cylindrica* and *Synechocystis* PCC 6803 respectively after UV-B and PAR+UV-B irradiated samples. The synthesis of MAA is stimulated by continuous irradiation of UV-B and PAR+UV-B. The present study shows that the two tested cyanobacteria *Anabaena cylindrica* and *Synechocystis* PCC 6803 are able to synthesize MAAs in response to UV-B radiation. HPLC chromatogram obtained from purified aqueous solution from showed peaks at retention time 2.8 min and 2.7 min isolated from *Anabaena cylindrica* and *Synechocystis* PCC 6803 respectively (Fig. 5 a, b and Fig.6 a, b) with absorption maxima at 334 nm. However, HPLC chromatogram was devoid of any peaks at the absorbance of 310 nm, which may be result of degradation of MAAs. Biosynthesis of P-334 is also reported in certain unicellular and filamentous cyanobacteria [15, 28-29]. This result obtained from the HPLC analysis revealed that an increase in biosynthesis of MAAs content in the cyanobacteria irradiated by PAR+UV-B and UV-B. There was negligible induction of MAAs recorded in sample irradiated with PAR. The present study shows that *Anabaena cylindrica* and *Synechocystis* PCC 6803 was able to induce the production of MAAs in response to UV-B radiation. A significant increase in MAAs was recorded at 48 h of UV-B radiation. The cells which contain high concentration of MAAs are 25 % resistant to UV-B radiation in comparison to those which contains less amount of MAAs [10].

CONCLUSION

In this present investigation, along with conventional parameters such as growth and photosynthetic pigment measured by spectrophotometer, we have also studied the photosynthetic efficiency and synthesis/occurrence of MAAs in two cyanobacteria such as *Anabaena cylindrica* and *Synechocystis* PCC 6803 after 48 h exposure of PAR, UV-B and PAR+UV-B radiation. UV-B absorbing compounds have been playing a significant role in photoprotection by reducing the deleterious effects of UV-B radiation. In this study, we have shown the comparative analysis of photosynthetic performance in morphologically dissimilar cyanobacteria under various light regimes to elucidate the role of UV-absorbing pigments in photoprotection. The tested organism could be a model organism for synthesis of MAAs and understanding the relative fluorescence stability in chlorophyll which may be applicable in the field of pharmaceutical and cosmetic industries.

ACKNOWLEDGEMENTS

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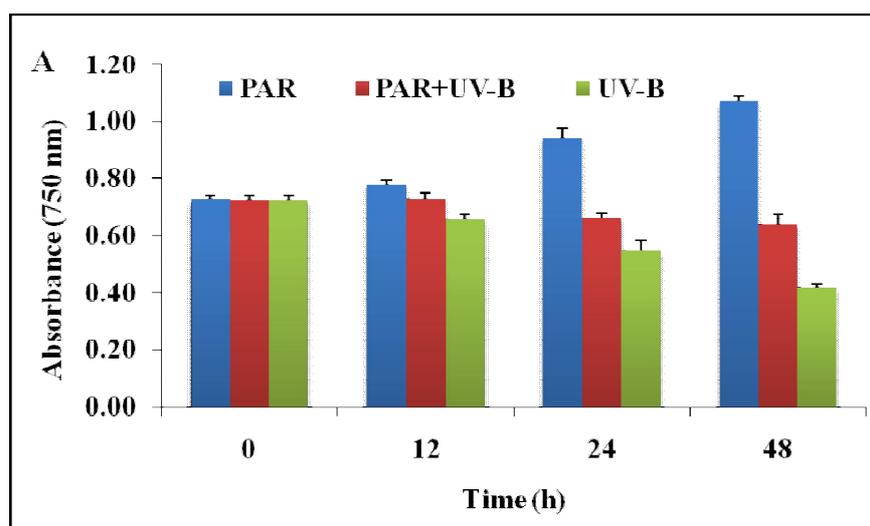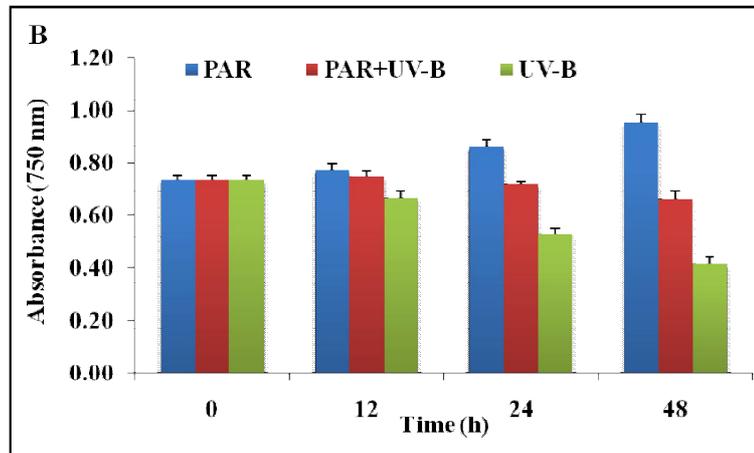
Fig. 1 Effect of PAR, PAR+UV-B and UV-B radiation on growth content of *Anabaena cylindrica* (A) and *Synechocystis* PCC 6803 (B).
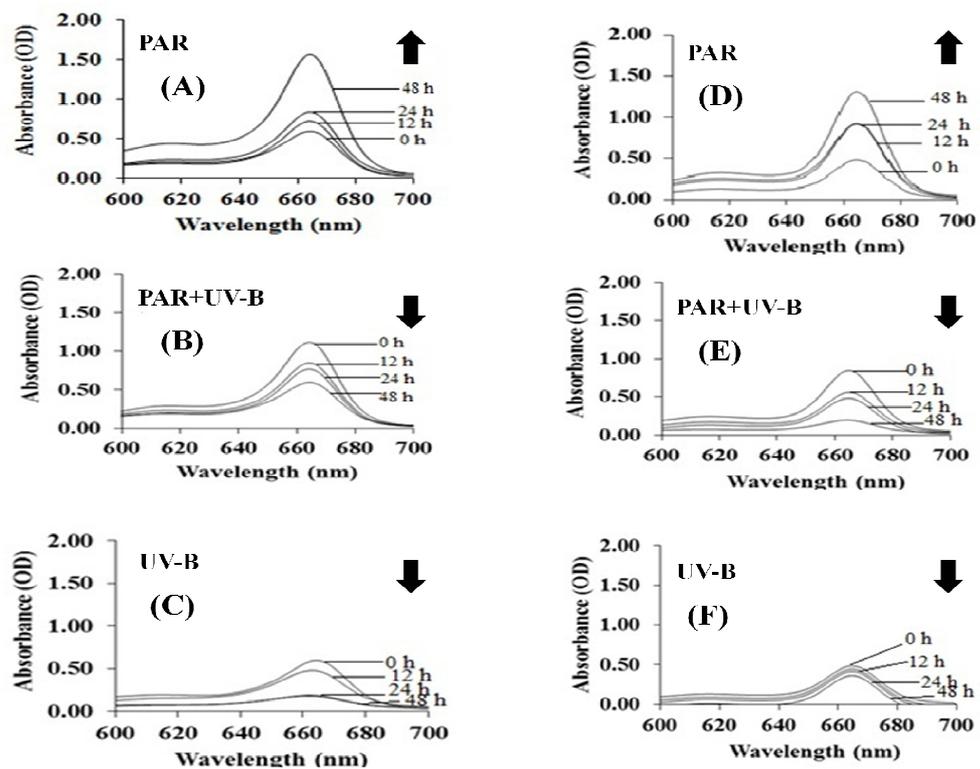

Fig. 2 Effect of PAR, PAR+UV-B and UV-B radiation on photosynthetic pigment content of *Anabaena cylindrica* (A, B, and C) and *Synechocystis* PCC 6803 (D, E, and F). The up arrow denotes the increase in absorption of pigment content and down arrow denotes the decrease in absorption of pigment content respectively.
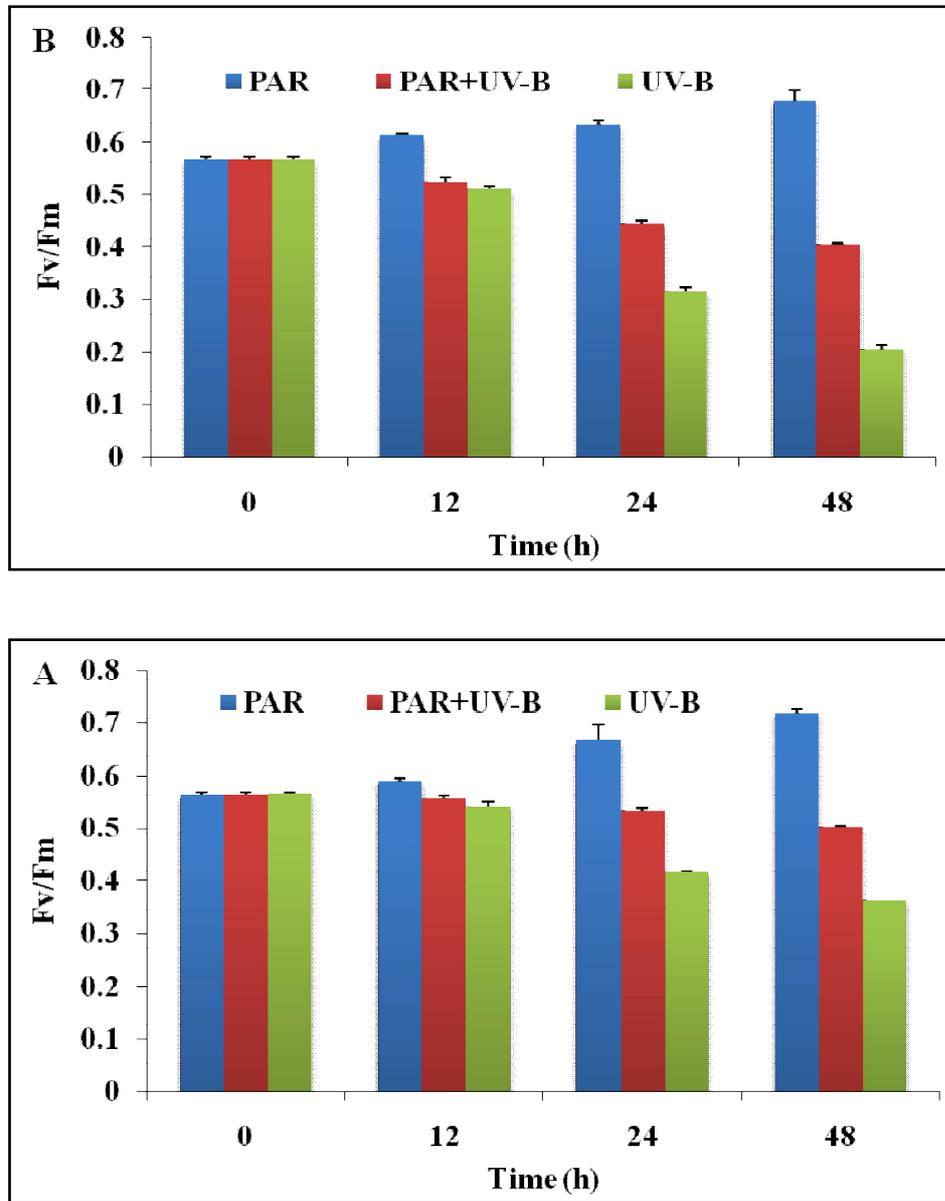
Fig. 3 Measurement of photosynthetic efficiency of PS II reaction centers measured as the ratio *Fv/Fm* in *Anabaena cylindrica* (A) and *Synechocystis* PCC 6803 (B).

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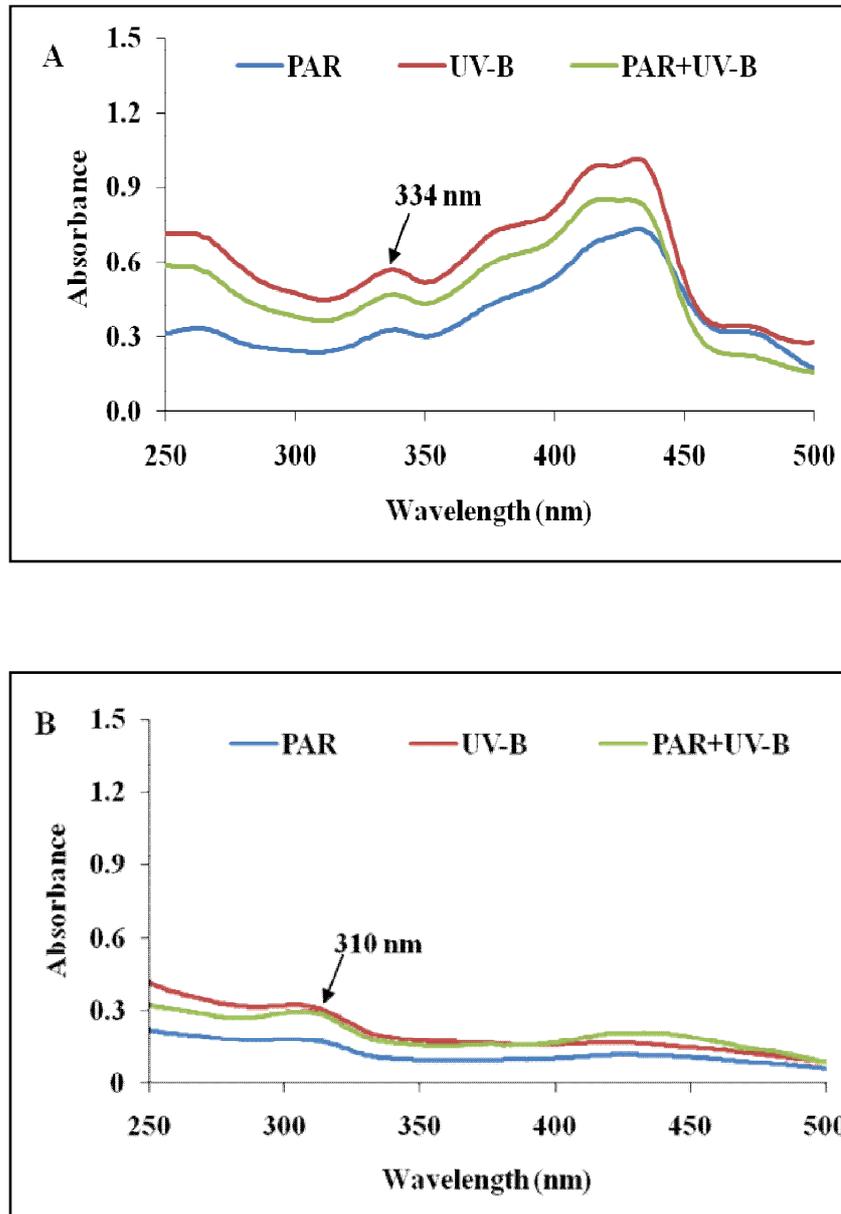
Fig. 4 UV-Vis absorption spectra of methanolic extract of *Anabaena cylindrica* **(A) and** *Synechocystis* **PCC 6803 (B).**

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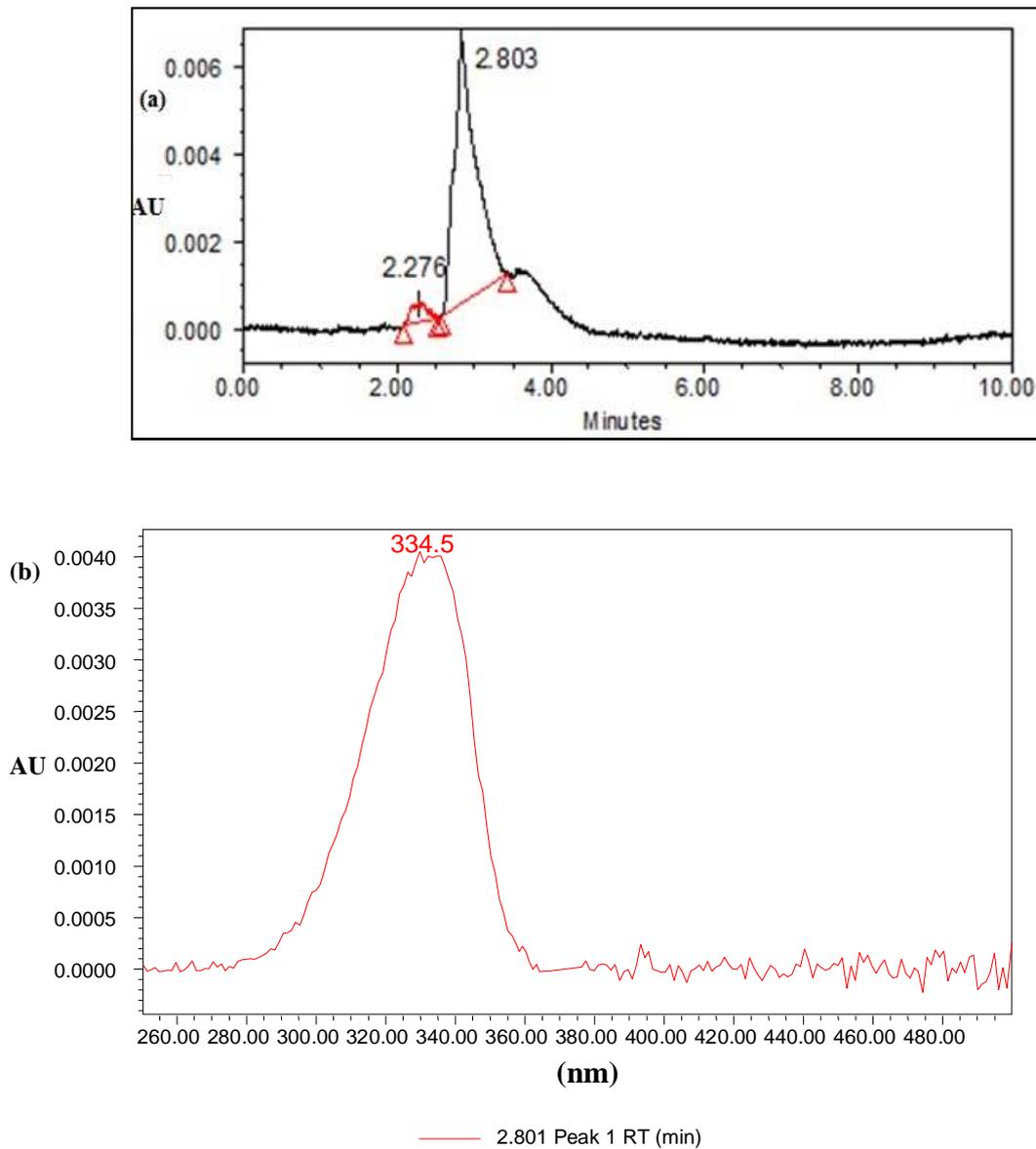
Fig. 5 HPLC chromatogram (a) and absorption spectra (b) of partially purified MAAs from *Anabaena cylindrica* showing peak   at 2.8 min with absorption spectrum at 334 nm, identified as P-334.

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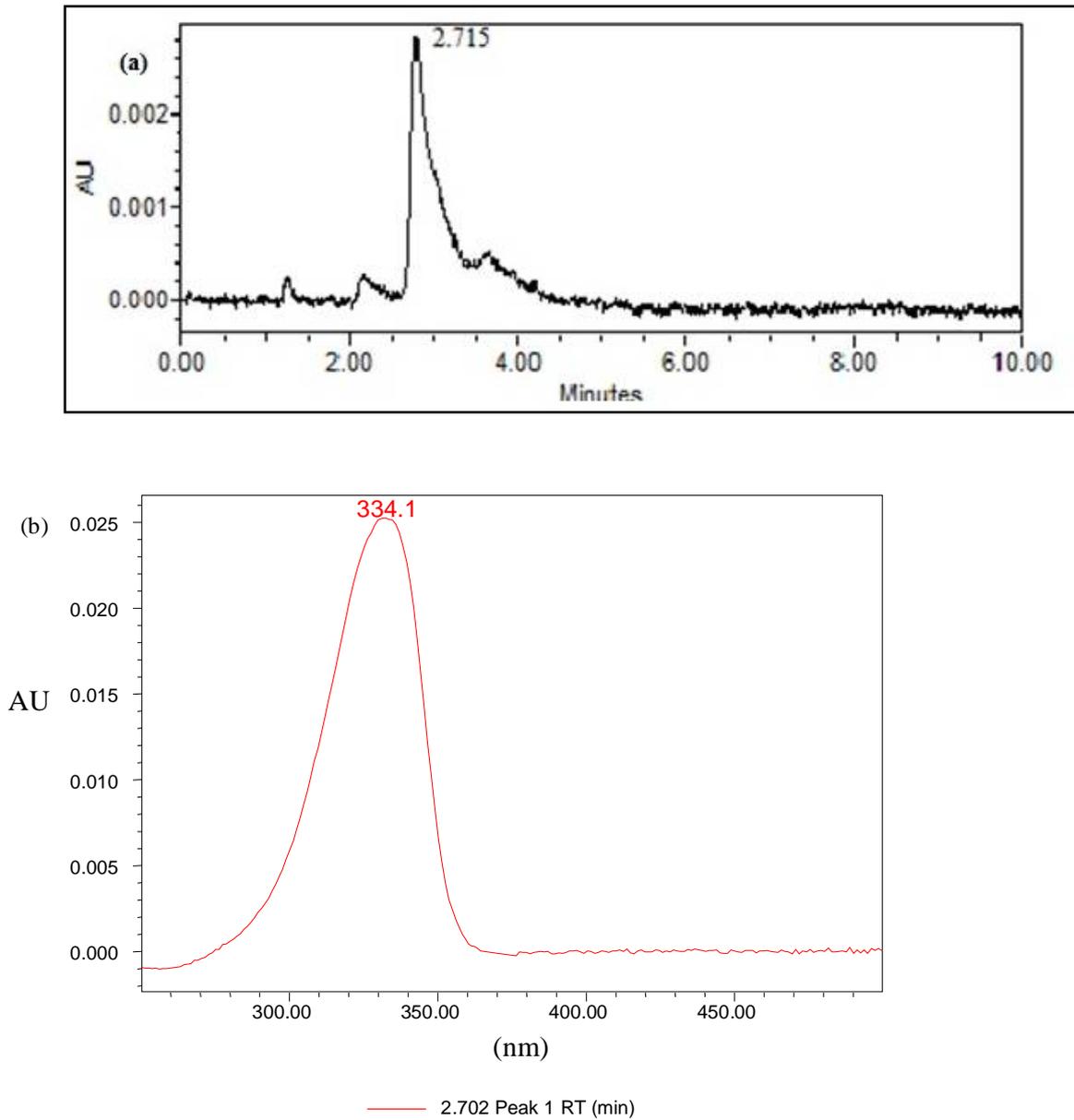
Fig. 6 HPLC chromatogram (a) and absorption spectra (b) of partially purified MAAs from *Synechocystis* **PCC 6803 showing peak   at 2.7 min with absorption spectrum at 334 nm, identified as P-334**

RESEARCH ARTICLE

Evaluation of Starch and Sugar Content of Different Rice Samples and Study their Physical Properties

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ABSTRACT

Rice is one of the main dietary foods widely consumed by Kurdish people in the Kurdistan Region. In this research, five different types of rice samples have been studied. The starch content of rice samples was found to be in the range of 81.23-92.73%. The high starch content of 92.73% was obtained by sample-5 and the low and standard of starch content of 81.23% was obtained by sample-1. The total sugar content of rice samples was determined by using a phenol-sulfuric acid method at different hydrolysis time under constant acid concentration (2% H_2SO_4) and temperature (25°C). The physical properties including length, width, thickness, equivalent diameter, surface area, sphericity, aspect ratio, volume, bulk density, true density, porosity, and thousand kernel weight was studied for rice samples. The physical properties are necessary for designing appropriate equipment for process operations such as handling, transporting, sorting, and designing storage structures in food processing industry based on their properties.

Keywords: Rice, Starch, Polarimetric method, Reduced Sugar, Phenol-Sulphuric method, Physical Properties.

INTRODUCTION

Rice is the major food to the most people around the world. It is providing 35-60% of the caloric intake of three billion people in Asian countries (Guyer, et al., 1998) including Kurdistan people. In the Kurdistan region, there are several types of rice available, but Kurdi rice is an only rice cultivated in the Kurdistan region and each of them have their

own physical properties which are useful in sizing grain hoppers and storage facilities. Rice consists of different components but starch is the main component of rice and it contains 80-90% of the total constituents. Starch is a polymer of glucose (Li and Yeh, 2001; Singh, et al., 2003) linked together by α-D-(1-4) or α -D-(1-6) glycosidic bonds. The starch granule mass comprises 70% amorphous regions, which consists of amylose and branching points of amylopectin molecules, and 30% crystalline which is mainly composed of the outer chains of amylopectin (Kodandaram and Bhotmange, 2014). Starch is the major dietary source of carbohydrates and it is the most abundant storage polysaccharide in plants. Therefore, rice becomes one of many potential sources of starch. The rice starch is a unique starch among available commercial starches due to its small granule size and its hypoallergenic residual protein (Schoch, 1967). The shape and size of starch granules depend on the source and the ambient condition of the growing area. Starch shapes have the forms of globular, ellipse, oval, lenticular and amorph. The sizes of starch granules are about 3 to 30μm for cereals about 10 to 100μm for tubers (Muhammad, et al., 2014). In this research, the polarimetric (or Ewers') method used to determine the amount of starch content and Phenol-Sulphuric method for total reduced sugar content. This method used due to its advantages such as low cost reagents, readily available and most importantly, can be used to quantify monosaccharides, oligosaccharides and polysaccharides. Therefore, it has been used to quantify the total sugar content of rice samples by constructing the calibration curve from glucose. At the same time, their physical properties have been studied.

Experiment

MATERIALS AND METHODS

All the chemicals that used in this research were provided by Koya University in Kurdistan Region-Iraq. Hydrochloric acid (HCl), Carrez I solution (30% ZnSO4), Carrez II solution (15% K4[Fe(CN)6]), Phenol, (96% H_2SO_4). All solutions were prepared from distilled water.

Determination of starch content

Five rice samples were available in the Kurdistan Region market were selected in this research, namely: sample-1; Kurdi, sample-2 Royal; sample-3 Knooz; sample-4 Mahmood and sample-5 Zer. A portion of 5 g of a homogenized sample is weighed in a 100 ml Kohlrausch volumetric flask and its content is mixed with 25 ml of 1.124% HCl solution. After addition of another 25 ml of 1.124% HCl solution, the suspension is heated on a boiling water bath for 15 min (after 3 min the content of a volumetric flask is mixed to avoid coagulation). Once the hydrolysis is finished, 20 ml of 1.124% HCl solution is added. After fast cooling (using a stream of flowing water), clarification using 5 ml of Carrez I and Carrez II solutions. Finally, a volumetric flask is filled up with detailed water, its content is properly mixed, and filtrated using a filtration funnel. The obtained filtrate is then transferred to a polarisation tube (2 dm) and starch content measured by using a Polax-2L. The obtained value is firstly corrected for a temperature (20°C) by using equation 1

$$\alpha_{\text{corrected}} = \alpha_{\text{meaured}} - 0.0144 \, (t - 20) \qquad (1)$$

And the amount of starch (*X*) in the rice samples was calculated by using equation 2

$$X = \frac{10^4 \, \alpha_{\text{corrected}}}{[\alpha]_\lambda^t \, \ell \, m} \qquad (2)$$

Where α calculated value of optical rotation is, $[\alpha]_\lambda^t$ is the optical activity (specific rotation) (+ 185.9° rice starch) depending on the discharge lamp and wavelength of light used and variety of starch, ℓ is the path length (2 dm), and *m* is the sample weight (5 g). For a mercury discharge lamp and a wavelength (λ) of 546.1 nm.

Standard and stock solution of glucose

Stock glucose solution was made by dissolving 5 g of glucose in 100 ml of distilled water. Various dilutions of the stock glucose solutions were made separately by pipetting a known volume of the stock solution (1, 2, 3, 4, 5, 6 and 7 ml) into a 100 ml volumetric flask and filling the volume with distilled water up to the mark. The concentrations made for this study were: 0.05, 0.15, 0.2, 0.25, 0.3 and 0.35 g/ml. To determine the calibration curve for standard glucose, 2 ml of each of the standard solutions was pipetted out and taken into a separate test tube. Then 0.4 ml of a 5 % aqueous solution of phenol reagent and 2 ml of 96% sulfuric acid was added. The mixture was kept for 10 min at room temperature, and placed in a water bath at 25°C for 20 min. Then the absorbance was read at 540 nm using a UV-visible spectrophotometer. Blank solutions were prepared in the same way as above, except that the 2 ml of the standard solution was replaced by distilled water. Then, the amount of total reduced sugar content present in the sample solution was calculated using the standard graph and expressed as gram glucose equivalents (GE) per 10 g of sample (Albalasmesh, et al., 2013; Miliauskas, et al., 2004).

Determination of total sugar content

10 g of flour of each rice samples were hydrolyzed under constant concentration of sulfuric acid (2) % at temperatures (25°C) and the different hydrolysis time (20, 40, 60, 80, and 100) minutes for optimal. The mixture was added in glass bottles and sealed to prevent contamination and vaporization of acid due to heat. After hydrolysis, the liquid fraction of the hydrolyzate samples was filtered and collected. A 2 ml aliquot of a sample solution was mixed with 0.4 ml of a 5 % aqueous solution of phenol in a test tube. Subsequently, 2 ml of concentrated sulfuric acid was added rapidly to the mixture. The test tubes were allowed to keep for 10 min at room temperature, and placed in a water bath temperature (25°C) for 20 min for color development. The total sugar concentration was determined by using UV-visible spectrophotometer at 540 nm wavelength of glucose absorbance and the quantification was made from a calibration curve using glucose as standard and calculation were performed by an equation of the linear regression obtained from the calibration curve.

Physical prosperities

The grain moisture content of each of the samples was determined by selecting 100 grains at random from the chamber, dried down to the desired moisture content. The rice grains were randomly selected from each sample for measuring their dimensions length (L), width (W) and thickness (T) a vernier calliper reading to 0.01mm was used.

Equivalent diameter (De)

The equivalent diameter (De) in mm considering a prolate spheroid shape for each rice samples was determined by equation3 (Mohsenin, 1986; Jain and Bal, 1997).

$$D_e = \left(\frac{L(W \times T)^2}{4}\right)^{1/3} \qquad (3)$$

Sphericity (Ø)

The sphericity (Ø) is a ratio of the surface area of the sphere having the same volume as that of grain to the surface area of the grain was determined by equation 4 (Mohsenin, 1986).

$$\emptyset = \frac{(L\text{WT})^{\frac{1}{3}}}{L} \qquad (4)$$

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Grain volume (V) and surface area (S)

Grain volume (V) and surface area (S) of each sample rice were calculated by using equation 5 and 6 (Jain and Bal, 1997).

$$V = 0.25[\frac{\pi}{6} L(W + T)^2] \qquad (5)$$

$$S = \frac{\pi B L^2}{(2L - B)} \qquad (6)$$

Where, $B = \sqrt{WT}$

Aspect ratio

The aspect ratio (Ra) was determined by using equation 7 (Varnamkhasti, et al., 2008).

$$R_a = \frac{W}{L} \qquad (7)$$

Bulk density (ϱb) and true density (ϱt)

The bulk density (ϱb) was determined by dividing the mass per unit volume (Fraser, et al., 1978) and the true density (ϱt) was determined by the water displacement method (Mohsenin, 1986).

The thousand kernel weight

The thousand kernel weight was determined by randomly selecting one thousand grains from each rice samples and weighed (Varnamkhasti et al, 2008).

Porosity

Porosity (ϵ) is a ratio of intergranular void space volume and the volume of the bulk grain and calculated by equation 8 (Jain and Bal, 1997; Thompson and Isaacs, 1967; Mohsenin, 1970).

$$\epsilon = \left(\frac{Pt - Pb}{Pt}\right) \times 100 \qquad (8)$$

RESULTS AND DISCUSSION

The starch content of the five different rice samples was available in the Kurdistan Region measured by polarimetric method. The amount of starch content (%) was 81.23 in sample-1; 86.26 in sample-2; 88.96 in sample-3; 91.43 in sample-4 and 92.73 in sample-5, and minimum and maximum amount of starch content can be observed for sample-1 and sample-5 as shown in fig. 1.The total reduced sugar content of different rice samples was determined through the hydrolysis process at a different hydrolysis time under constant acid concentration (2% H_2SO_4) and temperature (25°C). The amount of sugar produced was determined by using phenol-sulfuric acid. The glucose equivalent (GE) was calculated from the calibration curve of glucose standards. The concentrations of unknown sugar samples were determined from a standard curve of glucose (fig. 2), (Y=7.5864285714286x-0.45328571428571; R^2= 0.9876).At 20 and 100 min hydrolysis time reduced sugar content achieved 12.15 and 12.22%, and sugar content decreased to 10.04% at 80 min, 9.83% at 60 min and 9.53% at 40 min, probably due to degradation of forming monosaccharides in the presence of hot concentrate acid, and the maximum sugar for sample-1 was 12.22% observed at 100 min as shown in fig. 3. The sample-2 shows almost the same amount of sugar content at 60 and 80 min hydrolysis time, which was

18.26 and 18.25%, and because of degradation which form inhibitors, the sugar content observed in lower percentage at and 13.90% at 100 min, 14.34% at 40 min, 16.27% at 20 min as shown in fig. 4.

For sample-3, the maximum sugar content obtained 19.72% at 20 min of hydrolysis time. It seems 20 min are a sufficient hydrolysis time to cleaved most of the glycosidic linkages compare to hydrolysis times, which was obtained sugar content 10.24% at 80 min, 11.30% at 40 min, 12.49% at 60 min and 17.52% at 100 min, probably due to degradations as shown in fig. 5. A 20 min of hydrolysis time obtained 7.93% for sample-4. It was not sufficient for cleaved of the glycosidic linkagestherefore, lower sugar content achieved, due to degradations of monosaccharides which can be observed at 60 and 100 min sugar content was 13.83 and 12.4%. But at 40 and 80 min hydrolysis time sugar content was 22.84 and 23.34% due to sufficient time to hydrolyze all linkages with lower degradation as shown in fig. 6. The sample-5 at 20 to 100 min hydrolysis times obtained sugar content from 12.86, 12.26, 15.38, 13.90 and 13.98%. The maximum sugar content was 15.38% observed at 60 min from hydrolysis time as shown in fig. 7.

The summary of the physical properties of different type rice samples is represented in Table I. The moisture content of rice samples was varied from 4.24% (sample-3) to 12.78% (sample-4) on a wet basis. The moisture content provides valuable information *to suggest the stability in storage of rice samples.*The equivalent diameter and sphericity for rice samples varied from 2.51 (sample-4) to 3.16 (sample-1), however, the lowest sphericity value were observed 34.89% (sample-4) and the highest 55.64% (sample-1). This might be due to the typical shape of the sample-1 which has pointed tips along the width axis, thereby increasing the characteristic width compared to other samples. The aspect ratio distribution is important to classify the grains and determine the extent of off-size in market grade (Varnamkhasti, et al., 2008). The aspect ratio was found to be lowest in sample-1,2,3,and 4 (0.23) and highest in sample-5 (0.24).

The lowest porocity value was observed in 33.34% (sample-1), which might be due to high thickness value and the highest porocity value was 40% (sample-4), due to low thickness value.The kernel volume and surface area of samples were observed, the mean kernel volume values were ranged from 11.80 (sample-5) to 14.33 mm3 (sample-1), while the surface area was observed from 23.17 (sample-4) to 26.82 mm2 (sample-2). The surface area effects on drying rates of samples which can be characterized by using the surface to volume ratio and the ratio of surface area to volume affects drying time and energy requirements. The bulk densities of rice samples were ranged from 814.64(sample-4) to 884.52 kg/m3 (sample-2). The values for the true densities were varied from 1357.79 (sample-4) to 1419.29 kg/m3 (sample-5). The bulk density provides useful information for the design of silos and hoppers for grain handling and storage (Nalladulai, et al., 2002).The thousand kernel weight was observed in the range from 18.74 (sample-5) to 19.91 (sample-2) for rice samples. The knowledge of thousand kernel weight is a useful index to milling outturn.

CONCLUSION

The polarimetric method used for determination of starch in different types of rice samples. The standard amount of starch content observed in sample-1, which is only cultivating rice available in the Kurdistan region, and compared to other samples which were imported from India and Thailand. The total sugar content determined at different hydrolysis times under the constant acid concentration and temperature, the maximum sugar achieved for sample-3 was 19.72% at 20 min, sample-5 and sample-2 were 15.38%, and 18.26% at 60 min, sample-4 was 23.34% at 80 min and sample-1 was 12.22% at 100 min due to sufficient time to hydrolyze and cleave all glycosidic linkage in the rice samples with occurring minimum degradations and the minimum total reduced sugar content of rice samples observed were Sample-4 was 7.93% at 20 min, sample-1 and sample-5 were 9.56% and 12.28% at 40 min, sample-3 was 10.27% at 80 min and sample-2 was 13.90% at 100 min, probably due to insufficient of hydrolysis time or degradations of forming monosaccharides in the presence of hot concentrated acid. The physical properties of rice samples were revealed the variation of physical dimensions of rice sample grains varying from short to long varieties

and this information is useful for designing storage and optimizing milling processes to prevent the post-harvest and milling losses.

ACKNOWLEDGMENTS

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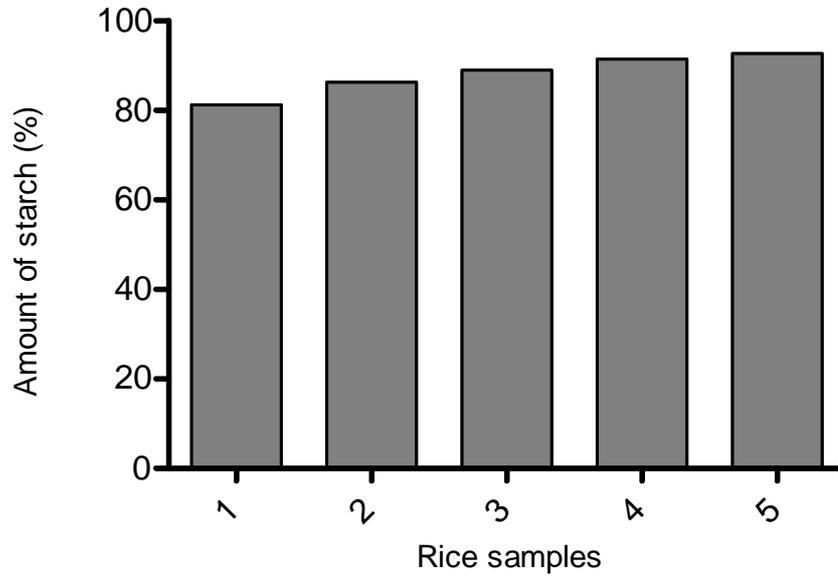
**Fig. 1: Amount of starch content (%) in flour of different rice samples**
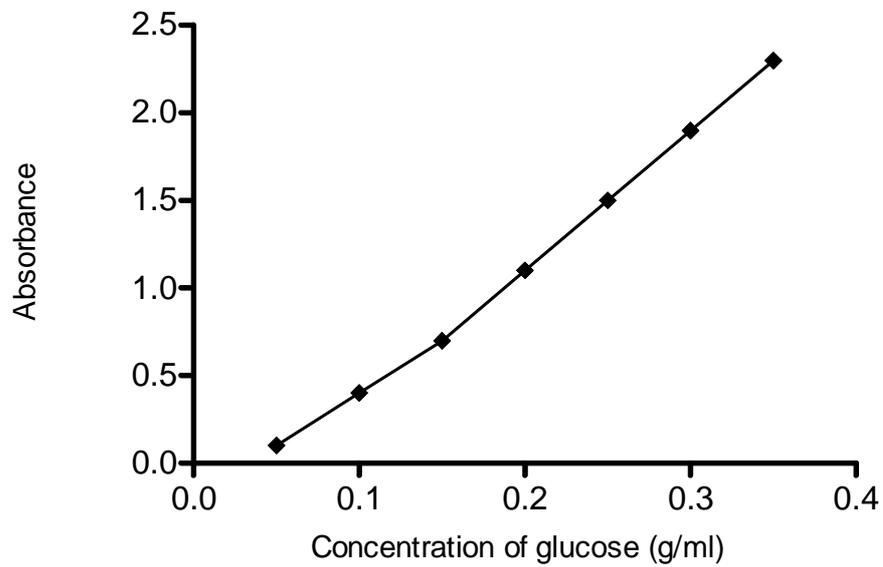
Fig. 2: Calibration curve of standard glucose for determination of total sugar content.

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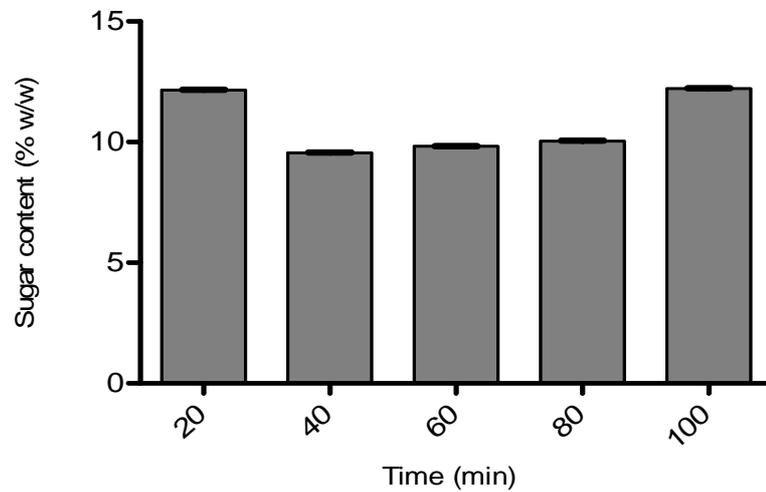
Fig. 3: Amount of sugar content (% w/w) of the rice sample-1 at different hydrolysis time of 10% biomass under constant concentration of (2% H_2SO_4) and temperature (25°C).
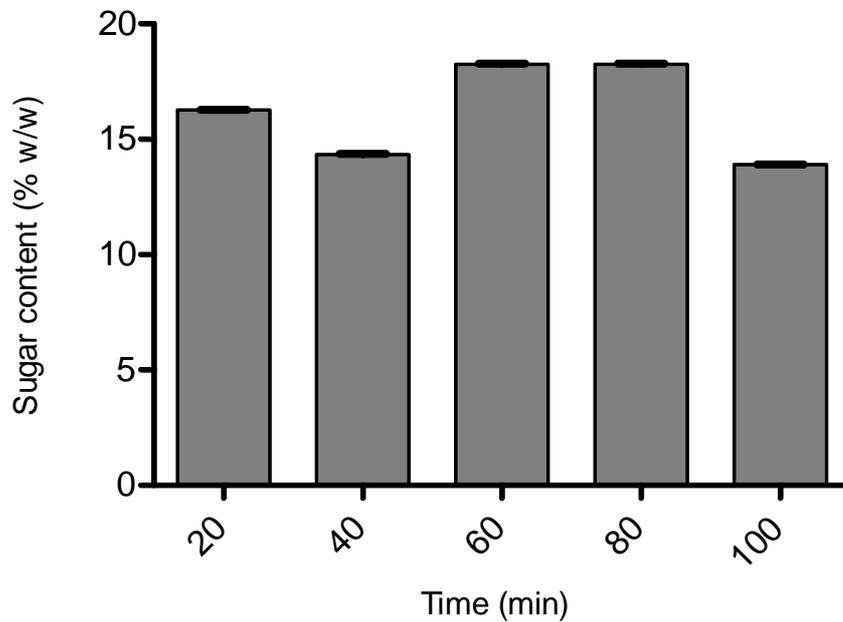

Fig.4: Amount of sugar content (% w/w) of the rice sample-2 at different hydrolysis time of 10% biomass under constant concentration of (2% H_2SO_4) and temperature (25°C).

Fig. 5: Amount of sugar content (% w/w) of the rice sample-3 at different hydrolysis time of 10% biomass under constant concentration of (2% H_2SO_4) and temperature (25°C).
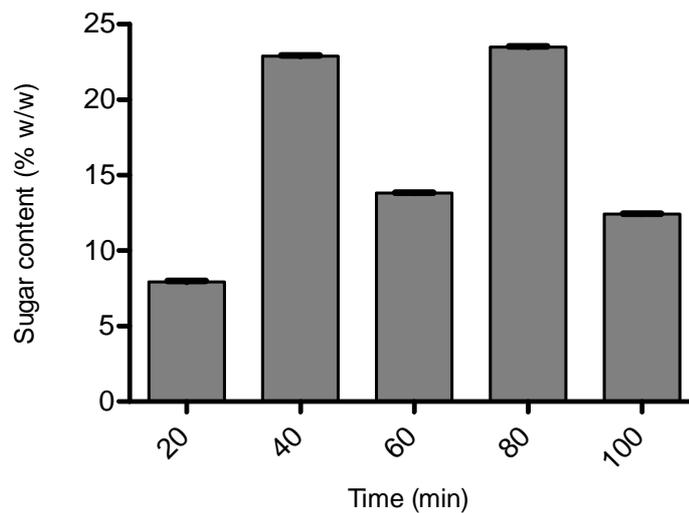
Fig. 6: Amount of sugar content (% w/w) of the rice sample-4 at different hydrolysis time of 10% biomass under constant concentration of (2% H_2SO_4) and temperature (25°C).

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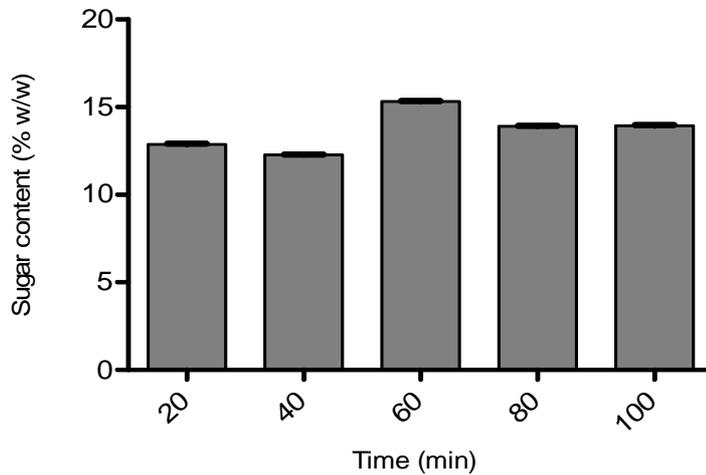
Fig. 7: Amount of sugar content (% w/w) of the rice sample-5 at different hydrolysis time of 10% biomass under constant concentration of (2% H_2SO_4) and temperature (25°C).

Table I physical property of different type rice samples.

Property	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5
Length (mm)	5.34±0.25	8.34±0.35	8.55±0.39	8.41±0.49	7.80±0.54
Width (mm)	2.75±0.16	1.95±0.07	1.94±0.07	1.94±0.05	1.88±0.17
Thickness (mm)	1.77±0.25	1.70±0.17	1.52±0.04	1.41±0.14	1.51±0.06
Moisture (wet basis)	12.78±1.42	7.47±0.66	4.24±0.48	8.03±0.97	7.07±0.63
Equivalent diameter (mm)	3.16±0.36	2.85±0.24	2.66±0.12	2.51±0.14	2.51±0.20
Sphericity (%)	55.64±3.38	36.00±0.71	34.38±1.16	33.89±1.73	36.14±2.45
Aspect ratio	0.23±0.02	0.23±0.01	0.23±0.02	0.23±0.02	0.24±0.03
Volume (mm3)	14.33±2.26	14.15±1.93	13.50±1.14	12.41±1.08	11.80±1.43
Surface area (mm2)	23.27±2.70	26.82±2.22	25.75±1.51	24.26±1.63	23.17±1.78
Bulk density (kg/m3)	881.00±3.37	884.52±9.17	856.39±21.03	814.64±12.64	875.23±21.70
True density (kg/m3)	1362.46±14.96	1386.21±41.52	1404.00±24.93	1357.79±13.51	1419.29±10.76
Porosity (%)	35.33±0.57	36.17±1.25	39.00±1.32	40.00±1.00	38.33±2.44
Thousand kernel weight	19.72±0.02	19.91±0.01	19.53±0.01	19.83±0.02	18.74±0.03

RESEARCH ARTICLE

Standardization of Scale to Measure Attitude of Extension Workers towards Programme and Activities of ATMA

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ABSTRACT

An attempt was made to develop and standardize the measuring the attitude of EWs towards programmes and activities of ATMA based on Likert's (1932) methods of summated ratings.In all, sixty two statements related to attitude towards programme and activities of ATMA were initially framed. These 62 statements included 55 positive and seven negative statements. These statements were mailed to a panel of 100 Judges and appropriateness (relevancy) of the statements for inclusion in the scale to measure attitude of extension workers towards programme and activities of ATMA. The responses of judges were secured on a three point continuum were assigned to the responses for positive statements. The scoring was reversed for negative statements. Applying the criteria of more than 75 relevancy percentage, 28 statements were selected and others were rejected. A list of 28 statements identified on the basis of relevancy percentage, was administration to 25 extension workers from non sample area. The responses from them were elicited on five point continuum for positive statements. The procedure was reversed for negative statements. The scores for each individual on the scale were computed by summing up the score of the individual item responses. Two criterion groups were formed by taking 25 per cent of the respondents having highest scores and 25 per cent of the respondents having lowest scores were selected. The value of critical ratio (t) for all the 28 statements were computed and arranged in descending orders. The statements having greater than 2.14't' value were then selected for inclusion in the final format of the scale. The test-retest and split-half techniques for testing reliability were used. The criteria of content and construct validity were applied for testing validity of attitude of extension workers

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towards programmes and activities of ATMA scale. The final format of attitude scale with 28 positive statements can be administered to the EWs with a five point response continuum .The attitude score for each respondent was calculated by adding up the scores on all statements in the scale.

Key words: Attitude, Extension Workers ,ATMA, Programme,

INTRODUCTION

The government of Maharashtra has introduced the innovation in technology transfer the agricultural technology Management Agency (ATMA) on experimental basis in four districts since 1998-99.The farm information and advisory center (FIAC) at the block is under control the ATMA Organisation .It works through Block Technology Team (BTT) consisting of extension workers of development departments and the farm advisory committee (FAC) having farmers representatives on it. It is responsible for programme planning and implementation. The FIAC play vital role in effective transfer of technology to the farming community. It also co-ordinates the work of supply and services agencies for effective implementation of farm production plans. The EWs motivate, educate & guide farmers to adopt new ideas and practices. The block level extension workers are middle level functionaries and form the strategies linkage between governing board & farmers interest groups. The efficiency effectiveness of the ATMA programme mainly depends upon the favourable attitude of the block technology team members in the programme planning and implementation. No comprehensive scale was available to measure the attitude of extension workers towards programme & activities of ATMA. In order to arrangement this attempt was made for measuring the attitude of extension workers towards programme & activities of ATMA. Thurstone and Chave (1946) referred to attitude as the degree of positive or negative affect associated with some psychological object. In the present study, was operationalized as the degree of positive or negative reaction of an individual extension worker towards the programme and activities of ATMA.

Procedure for Standardization of attitude of Extension Workers

In order to develop and standardize the measuring instrument the methods of summated ratings suggested by Likert (1932) was followed for construction of attitude scale. The detail procedure adopted for this purpose is described here under

Framing and preparation of item pool

The purpose of framing and preparation of item pool was to develop a set of statements which reveal the agreement or disagreement with each statement indicating different degree of favourable or unfavourable attitude of respondents towards programmes and activities of ATMA. Statements were collected by referring books, journals, theses and other relevant literature on the topic. The experts engaged in extension work in the state Department of Agriculture and Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola were also consulted. In all, sixty two statements related to attitude towards programme and activities of ATMA were initially framed. These statements were examined in the light of the criteria suggested by Edwards (1957) for screening the statements. All these 62 statements were fulfilling those criteria and hence were retained for further analysis. These 62 statements included 55 positive and seven negative statements.

Determining item relevancy

It is quite possible that 62 statements retained initially may not be relevant equally in measuring attitude of extension workers towards programmes and activities of Agricultural Technology Management Agency. Hence, these 62

statements were subjected to scrutiny by an expert panel of judges to determine the relevancy and their subsequent screening. Lists of these 62 statements were mailed to a panel of 100 Judges in the field to extension education and field extension service. These judges were requested to indicate appropriateness (relevancy) of the statements for inclusion in the scale to measure attitude of extension workers towards programme and activities of ATMA. The responses of judges were secured on a three point continuum namely, most relevant, relevant and not relevant and scored as 3, 2, and 1 respectively were assigned to the responses for positive statements. The scoring was reversed for negative statements. In 62 judges could respond in stipulated time. These judgments were used for working out the relevancy percentages of the each statements by using the procedure given by Patil *et al.* (1996).Applying the criteria of more than 75 relevancy percentage, 28 statements were selected and others were rejected.

Item analysis

Further, it was considered essential to delineate the item that discriminates between persons holding different attitudes. A list of 28 statements identified on the basis of relevancy percentage, was administration to 25 extension workers from non sample area. The response from them were elicited on five point continuum namely, strongly agree, agree, undecided, disagree and strongly disagree and were recorded as 5,4,3,2, and 1, respectively for positive statements. The procedure was reversed for negative statements. The scores for each individual on the scale were computed by summing up the score of the individual item responses. The respondents were arranged in the descending order according to their total score. Two criterion groups were formed by taking 25 per cent of the respondents having highest scores and 25 per cent of the respondents having lowest scores were selected. These two groups provided the criterion groups as 'high' and 'low' groups to evaluate the individual items. The critical ratio (+_) for each item was worked out by the formula given by Edwards (1957) The't' value is a measure of the extent to which a given items differentiates between the high group from the low group.

Item selection

The value of critical ratio (t) for all the 28 statements were computed and arranged in descending orders. The value of critical ratio 2.14 was observed to be significantly differentiating between high and low group. The statements having greater than 2.14't' value were then selected for inclusion in the final format of the scale. By this procedure 28 statements were retained and included in final format of attitude scale (Table .1).All of these statements were positive

Testing reliability of the scale

The test-retest and split-half techniques for testing reliability were used

a) Test-retest method

The format of scale having 28 items was administered twice to 25 EWs with an interval of fifteen days. The responses were obtained on five point continuum as strongly agree, agree, undecided, disagree and strongly disagree with a score of 5, 4, 3, 2 and 1, respectively. The total scores of all 25 EWs for each item were calculated separately. The scores were then correlated. The value of 'r' was 0.8453 and found significant at 0.01 level of probability. It has indicated that the scale was stable and measured the attitude of extensions workers over time.

b) Split-half method

The format of scale containing 28 items was administered twice to 25 extension workers from non-sampled area. The responses were obtained on five point continuum as strongly agree, agree, undecided, disagree and strongly disagree with a score of 5, 4, 3, 2, and 1, respectively for working out split half reliability the scores earned by all the EWs on odd and even items were added together separately and were correlated. The reliability coefficient calculated for the

attitude scale by following this procedure was 0.8262 and found significant at 0.01 level of probability. It was indicative of high reliability of the constructed attitude scale.

Testing Validity of the scale

The criteria of content and construct validity were applied for testing validity of attitude of extension workers towards programmes and activities of ATMA scale.

a) Content validity

The contents of the scale were derived from the list of statements developed in consultation with literature, experts in the field and judges opinion on appropriateness of items included in the scale. It was assumed that the scores obtained by administering the scale measured the attitude of extension workers towards programme and activities of ATMA and nothing else.

b) Construct validity

The construct validity was tested by using't' test. The items were grouped into two groups high and low group and difference in means was tested by't' test. The items with significant't' values among high and low groups were than selected in inclusion of scale. Thus the construction validity of attitude scale developed for measurement of extension workers' attitude towards programme and activities of ATMA.

Norms for use of scale

The final format of attitude scale with 28 positive statements can be administered to the EWs with a five point response continuum namely, strongly agree, agree, undecided, disagree and strongly disagree with the scores of 5, 4, 3, 2 and 1, respectively. The attitude score for each respondent was calculated by adding up the scores on all statements in the scale. The attitude score range from a minimum of 28 to a maximum of 140.The categorization of the respondents was done on the basis of four quartile considering the obtainable index range was made as Highly unfavourable (up to 25 index), Unfavaourable (26 to 50 index),Favourable(51 to75 index) and Highly favourable(Above 75 index).

CONCLUSION

The scale was found to be reliable and valid .Therefore; it can correctly measure the attitude of extension workers towards programme and activities of Agricultural Technology Management Agency to maximum precision possible and can yield consistent results when used on different occasions involving the same/different respondents. This scale could also be used to measure attitude of EWs in the other organization even beyond study area with necessary modification in the wordings of the scale items.

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Table No.1. Final format of Attitude scale Constructed and Standardization for Measuring the Attitude of EWs towards Programme and activities of ATMA. Response continuum: Strongly agree (5) Agree (4) Undecided (3) Disagree (2) and Strongly disagree (1)

Sr. No.	Statements
1.	ATMA is a programme of people, for the people and by the people
2.	The training to extension functionaries in innovative areas is well taken care of by SAMETI (State level Agricultural Management and Extension Training Institutes) in ATMA
3.	ATMA organizes various programmes for the development of women
4.	New research information becomes quickly available to the farmers due to ATMA
5.	ATMA organization at the block level has increased accountability of key stakeholders
6.	ATMA organization is the real decentralization of day-to-day management of Agricultural Technology system
7.	The farmer's participation is highly ensured in identification of problems in ATMA
8.	Effective co-ordination between the research, extension and supply and service agencies is ensured only in ATMA organization
9.	An excellent work is always recognized in ATMA organization
10.	Farmer's Advisory Committees (FACs) comprising of key stakeholders and farmers representatives exert considerable influence in the preparation and scrutiny of action plan in ATMA
11.	Farmers need based research work is strengthened in the ATMA organization
12.	ATMA is a programme that takes every care for allround development of rural areas
13.	NGOs and voluntary organizations are involved in identification and solving the farmers problems
14.	It is due to ATMA the advice through various NGOs is made available to farmers
15.	ATMA provides check at various levels while planning and implementation of developmental activities for rural people
16.	The Block Technology Team plays an active role in preparation of block action plan
17.	The autonomy of ATMA provides much needed flexibility to quickly responding to demands from the field
18.	The promising and proven technology is transferred to farmers in ATMA
19.	The Farmers Advisory Committee at block is a means to provide regular feedback about the programmes implemented through ATMA
20.	ATMA organization emphasizes more on bottom-up planning procedures for setting the research-extension agenda
21.	Increase in agricultural production in the area is because of advice provided to the farmers through ATMA
22.	Problems of farming are jointly tackled by different departments only in ATMA
23.	ATMA is a helping new institutional arrangement for technology dissemination at the district level
24.	Programmes prepared in ATMA are based invariably on farmers needs and problems
25.	The extension workers have certainly a scope to improve their professional competencies in

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	ATMA
26.	It is because of ATMA organization the gap between the research, extension and the supply and service agencies has been narrowed
27.	Extension activities are planned systematically and supervised adequately at all levels in the ATMA organization
28.	ATMA is a blessing to the farmers

RESEARCH ARTICLE

Impact of Low to High Intensity of Resistance Training Program in Enhancing Leg Strength among Males

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ABSTRACT

Leg strength is very essential for sports persons, and especially for athletes. Leg strength is the capacity of the lower limbs to exert muscular force (Baumgartner and Jackson, 1991), (3). A Study pertaining to two days of training per week had shows improvement in the strength (Bell, 1990, Faigenbaum et al., 2002), (2,7). The purpose of this study was to investigate the impact of low to high intensity of resistance training program in enhancing leg strength among untrained males. A group of (N=30) untrained subjects were selected randomly for this study from the various classes of physical education college course, age of the subjects between 18-22 years. The training program was employed for 12 weeks, five resistance training exercises considered for the legs, 25 minutes of training per session, two days of training per week. The selected leg strength test considered for this study was sitting calf raises, standing leg curls, adductors, abductors, and leg extensions. The scores were recorded in kilograms. To find out the mean differences from pre to post test, mean, S.D and t-tests were computed by means of Statistica Software.The analyzing of data reveals that the mean and standard deviation with regard to sitting calf raises performance among training group from pre to post test were (25.70, 12.70) and (64.83, 14.59) increased by 39%. Standing leg raises with mean and S.D were (23.12, 7.86) and (45.87, 14.92) increased by 50%. Abductor exercise with mean and S.D were (59.50, 13.86) and (109.83, 23.14) increased by 54.17%. Adductors with mean and standard deviation were (61.40, 25.27) and (113.10, 26.08) increased by 54.29%. Leg extension exercise with mean and S.D were (29.17, 15.43) and (49.80, 16.37) increased by 58.57%. Twelve weeks of low to high resistance training program have a significant effect in enhancing leg strength among males. Similar results were obtained Hawkins and et.al, (2009) indicate that the high velocity and high force training programs on untrained college males, consisting of weight lifting, plyometric, improved the lower body performance, especially in the area of jump height and power (5).

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In the present study the selected college males were untrained and their scores were very low in the pre test pertaining to all the selected resistance exercises for the lower body. In the post test the participants had shows an improved performance in enhancing leg strength in all the selected resistance exercises.It was concluded that the impact of low to high resistance training program in enhancing leg strength among the males had shows greater performance from pre to post test in all the selected exercises, which is very encouraging and significant.

Key words: Resistance, strength, Intensity, enhances.

INTRODUCTION

Resistance training is a form of strength training in which each effort is performed against a specific opposing force generated by resistance i.e. pushing or pulling or stretched or bent. Exercises are isometric when a body part is holding still against the force. Isotonic exercises are shortening and lengthen of the muscle simultaneously. Resistance exercise is very useful in improving strength and increasing hypertrophy of the skeletal muscles. A benefit of the resistance training includes increased muscle, tendon and ligament strength, bone density, flexibility, tone, metabolic rate and postural support.   Lot of research had been done earlier on the leg strength enhancement of the athletes. Still lots of areas are not addressed. The training which develops strength in shortest possible time in preparing the athletes for the matches will be the first preference for the coaches and athletes to include in their schedule. An athlete wants to improve their performance without any side effects or injuries during the training and during the matches. Always sensible approach is very important in any training program for enhancing strength performance. Starting the resistance training program with low intensity is a levelheaded approach for untrained participants to avoid injuries and for the adaptation.

Resistance training is defined as works to increase the muscle strength and endurance by performing repetitive exercises with weights, weight machines, or resistance bands, (Scott, 2008). Strength is defined as a force in which a muscle or a group of muscles will be exerting against resistance in ones maximum effort. It is the ability to overcome resistance or to act against resistance (Singh, 1991).Leg strength is very important for sports persons, especially athletes. The strength of the muscles is related to its cross sectional area of girth. Strength training increases the contractile protein that gives the muscle pulling power. Leg strength is the capacity of the lower limbs to exert muscular force (Baumgartner and Jackson, 1991). To develop strength, one should apply the basic principles of progressive resistance training, specifity with the right intensity with proper mode (Faigenbaum et al., 2002; Fleck & Kreamer, 1997; MacArdle et al., 1996; National strength & conditioning Association, 1985). According to the American Sports Medicine Institute (ASMI), the goal of resistance training is to gradually and progressively overload the musculoskeletal system to get stronger. In this study two days of training per week had shows improvement in the strength (Bell, 1990, Faigenbaum et al., 2002; Flanagan et al., 2002). Untrained participants (less than 1 year of consistent training) experience maximal strength gains with an average training intensity of 60% of their 1 RM or approximately a 12 RM, training each muscle group 3 days per week. Novice's weight training 2 times per week may make approximately 80% of the strength gains as compared to training 3 times per week. Rhea et al (2003) suggested caution when prescribing multiple-set programs to those who have not been training consistently for at least 1 year. Adequate time is required to become accustomed to the stress of resistance exercise and avoid over-stress injuries in the early phases of training. Novice trainees may also lack the desire to commit to a training program requiring the additional time needed to perform multiple sets and thus reduce adherence to the exercise regimen. The purpose of this study was to investigate the impact of low to high intensity of resistance training program in enhancing leg strength among untrained males.

MATERIALS AND METHODS

Selection of subjects: A group of 30 subjects were selected for this study from the various sections of college undergoing physical education classes at King Fahd University of Petroleum & Minerals, Saudi Arabia during the year 2012-13. The age of the subjects was between 18-22 years. The purpose of this study was explained and doubts were addressed to the participants.

Experimental Design: The subjects (N=30) were selected for this study randomly. The resistance training program was employed for 12 weeks, 25 minutes of training per session, two days of training program in a week. The resistance training exercises which was employed on the participants was, (A1: sitting calf raises, A2: Standing leg raises, A3; Adductors, A4: abductors and A5: leg extensions). The low to high intensity exercise program which was executed on the participants is presented in the below table-1.

Procedure of testing: The selected leg strength test considered for this study was sitting calf raises, standing leg curls, abductor, adductor, and leg extension for 10 reps x lifting max weight, (kgs). A Pre and post test was conducted before and after the 12 weeks training program. The scores were recorded in kilograms. The training was given at the Gymnasium at stadium, King Fahd University of Petroleum & Minerals, Saudi Arabia. All the scores for pre and post test were recorded in kilo grams for analyzing the data.

Statistical Analysis

To compare the mean differences between pre to post test, mean, standard deviation and t-tests were computed by means of Statistica Software. A significance level at 0.05 level was adjusted.

RESULTS

The analyzing of data for selected variables i.e. (sitting calf raises, standing leg curls, abductor, adductor, sitting leg extension), performance from pre to post test among participants is presented in the table -3 by the help of statistical tools i.e. mean, standard deviation and t test.The analyzing of data reveals that the mean and standard deviation with regard to sitting calf raises performance among training group from pre to post test were (25.70, 12.70) and (64.83, 14.59) respectively. Standing leg raises performance shows by the training group from pre to post test with mean and standard deviation were (23.12, 7.86) and (45.87, 14.92) respectively. The resistance training group had showed performance with regard to abductor exercise from pre to post test were the mean and standard deviation were (59.50, 13.86) and (109.83, 23.14) respectively. The participants of training group had showed performance with regard to adductors with mean and standard deviation were (61.40, 25.27) and (113.10, 26.08) from pre to post test respectively. The mean, standard deviation with regard to leg extension exercise performed by the training group from pre to post test were (29.17, 15.43) and (49.80, 16.37) respectively. From pre to post test pertaining to sitting calf raises improved by 39%, standing leg raises performance was improved by 50%, regard to abductor exercise improved by 54.17%, adductor performance was improved by 54.29%, and leg extension exercise had improved with 58.57% strength respectively.

DISCUSSION

The results of this study suggested that twelve weeks of low to high resistance training program have a significant effect in enhancing leg strength among males. This is evident from the earlier studies that the resistance training increases the strength and also it depends on the intensity of the training schedule. This study is agreement with the findings of this study. It was investigated to determine EUR was sensitive to different types of resistance training in

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untrained college males. Results indicate that the high velocity and high force training programs consisting of weight lifting, plyometric, improved the lower body performance, especially in the area of jump height and power (Hawkins and et.al, 2009). In one of the study it was studied on acute and long term effects of resistance training regimens with varied combinations of low and high intensity Exercises, found that the maximal isokenetic strength and muscular endurance of leg extension showed significant result (p>0.05) greater increase in the HC group than the HS group (Nagasawa, et al, 2004). Strength development was the result of right resistance or intensity to the targeted muscle group with an emphasis on other training principles such as frequency, duration, and repetition (Fleck & Kreamer, 1997). In the present study the selected college males were untrained and their scores were very low in the pre test pertaining to all the selected resistance exercises for the lower body. In the post test the participants had shows an improved performance in enhancing leg strength in all the selected resistance exercises. This is evident that the low to high intensity of training program, two days of training per week, is also useful in enhancing leg strength in untrained participants.

CONCLUSION

It is concluded that the impact of low to high resistance training program in enhancing leg strength among the untrained participants had shows greater performance from pre to post test in all the selected exercises, which is very encouraging and significant.

ACKNOWLEDGEMENTS

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Table.1: Low to high intensity exercise program

	Pre- test (Test Items : A1, A2, A3, A4, A5)			
Week : 1	Intensity:20%, of body weight	Sets 2	Reps 25	Rest between the sets : 1 min
Week : 2,3	Intensity : 20% and 40%	Sets 2	Reps 20	Rest :1 min
Week : 4,5,6	Intensity : 30% and 60%	Sets 2	Reps 15	Rest :2 min
Week : 7,8,9	Intensity (20 %, 40%, 80%)	Sets 3	Reps 15,12,10	Rest :3 min
Wk,10.11,12	Intensity (20%, 60%, 100%)	Sets 3	Reps15,12,6	Rest 3 min
	Post Test : (A1, A2, A3, A4, A5)			

Table.2: Table showing the details of the selected variables for the pre and post test

Sl.no	Variables	Test
1	Sitting calf raises	To find out the strength of the calf
2	Standing leg curls	To find out the strength of Hamstrings
3	Abductors	To find out the strength of hip abductor group
4	Adductors	To find out the strength of hip adductor group
5	Leg extension	To find out the strength of quadriceps

Table-3 showing mean, standard deviation and t-value of the selected variables (sitting calf raises, standing leg curls, adductor, abductor, sitting leg extension), performance from pre to post test among participants

Variables	Test	Group (N=30)		
		Mean	S.D	P-value
Sitting calf raises	Pre	25.70	12.70	0.000
	Post	64.83	14.59	
Standing leg curls	Pre	23.12	7.86	0.000
	Post	45.87	14.92	
Abductors	Pre	59.50	13.86	0.000
	Post	109.83	23.14	
Adductors	Pre	61.40	25.27	0.000
	Post	113.10	26.08	
Leg extensions	Pre	29.17	15.43	0.000
	Post	49.80	16.37	

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Table: 4: Table showing the improved performances from pre to post in means of percentages

Variables	A1	A2	A3	A4	A5
Improved performance from pre to post test in percentages	39%	50%	54.17%	54.29%	58.57%

RESEARCH ARTICLE

Management of *Alternaria alternata* of Blond Psyllium (*Plantago ovata* L.) through Plant Extract

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ABSTRACT

The experiments were conducted at Department of Plant Pathology, S.K.N. College of Agriculture, Jobner (Rajasthan). *Alternaria alternata* was isolated from leaves of isabgol and observed to be pathogenic under artificial conditions. An attempt was more find out the efficacy of different plant extracts against *Alternaria alternata* incited by leaf blight of isabgol (*Plantago ovata* L.) extracts of different plants; garlic (*Allium sativum* L.), neem (*Azadirachta indica*, lantana (*Lantana camara*), turmeric (*Curcuma longa*), ashwaganda (*Withania somnifera*) in different concentration were studied by poisoned food technique.Among the five plant extracts maximum inhibition of mycelial growth was observed with *Allium satvium* (83.69) followed by *Azadirachta indica* (81.11), *Curcuma longa* (70.74) and *Withania somnifera* (63.70). Higher concentration of plant extracts were more effective in reducing mycelial growth as compared to lower concentrations plant extracts.

Keywords: Alternaria alternata, plant extract and plantago ovate

INTRODUCTION

Blond psyllium (*Plantago ovata* Forsk.) commonly known as *isabgol*, is an annual herb with narrow linear rosette like leaves belonging to the family Plantaginaceae. Isabgol belongs to a large genus of Herbs or sub shrubs distributed mostly in temperate regions and a few in the tropics. It comparises about 200 species of which 10-14 are native to India Rahn ((1996). *Isabgol* is an important cash crop cultivated for its export and being of important medicinal value

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is reported to have larger demands and is traded in major medicinal drug markets of the world. *Isabgol* has pharmaceutical importance to treat dysentery, chronic constipation and chronic diarrhoea and as laxative demulcents, emollients and diuretics. India commands nearly monopoly in the production and export of the seed and husk to the world market. India is earning about Rs. 1600 million as foreign exchange from the export of blond psyllium products to countries like USA, Germany, France, England, Spain and Belgium (Maiti, 2000).

In India, the *isabgol* crop is mainly grown as commercial crop in Gujarat, Rajasthan and Madhya Pradesh. However, the crop is spreading to other non-traditional parts of the country such as Haryana, Uttar Pradesh and Karnataka. In Rajasthan, it is being cultivated in 190081 hectare area with a total production of 99950 tonnes of seeds with an average productivity of 525 kg/ha (Anonymous, 2012-13). In Rajasthan, Isabgol mainly cultivated in Barmer, Jalore, Nagaur, Jodhpur and Jaisalmer districts. Presently, Rajasthan is on the top in productivity in India. Fungus produces muriform conidia and usually formed in chains. The conidia are broadest near the base and toper gradually to an elongate beak (Dube 2002).Mandal (2010) reported a number of pathogens viz., Fusarium wilt (*Fusarium oxysporum),* damping off (Pythium ultimum trow), leaf blight (Alternaria alterata (Fr.) Keissler), downy mildews (Peronospora plantaginis) and powdery mildew (Erysiphe cichoracearum D.C.) affecting this crop. Alternaria blight has become a serious problem in recent years. It has been found that downy mildew affected crop is more prone to be attacked by A. alternata. It causes considerable damage every year and sometimes become very severe which results in total loss of yield (Patel et al., 1982). Hence, present investigations were carried out to test efficacy of plant extracts against leaf blight of isabgol pathogen in vitro conditions.

MATERIALS AND METHODS

Following five different plant extracts were tested *in vitro* against *Alternaria alternata* by Poisoned Food Technique.

Efficacy of plant extracts on mycelial growth of *Alternaria alternata* (*in vitro*)

The effect of each plant extract viz., garlic (*Allium sativum* L.), neem (*Azadirachta indica,* lantana (*Lantana camara*), turmeric (*Curcuma longa*), ashwaganda (*Withania somnifera*) were tested by PFT at three different concentrations i.e. 5, 10 and 15 per cent. To get these, the required plant parts were thoroughly washed with sterilized water and grinded separately in electric grinder using equal amount of sterilized distilled water. The mixture was squeezed with double layered sterilized cheese cloth. The extracts thus obtained were considered as of 100% concentration. Required amount of stock solution was added to PDA to get desired concentration.The effect of plant extracts against mycelial growth of *Alternaria alternata* were tested by PFT. Required quantity of each plant extracts were mixed thoroughly in melted PDA, to get desired concentration, just before pouring in sterilized Petriplates and was allowed to solidify for 12 hours. Each plate was inoculated with 5 mm disc of 7 days old culture of *Alternaria alternata* with the help of sterilized cork borer. The inoculated Petriplates were incubated at 25 ± 1^0C for 10 days. A control was also maintained where medium was not supplemented with any plant extracts. The experiment was conducted in completely randomized design with four replications. Colony diameter (two diagonals) was measured after 10 days of incubation. The per cent growth inhibition was calculated by Vincent's (1947) formula.

$$\text{Per cent growth inhibition} = \frac{C-T}{C} \times 100$$

Where,

C	=	Diameter of colony in check (Average of both diagonals)
T	=	Diameter of colony in treatment (Average of both diagonals)

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RESULTS AND DISCUSSION

Efficacy of plant extracts on mycelial growth of *Alternaria alternata* (*in vitro*)

Five plant extracts i.e. garlic cloves (*Allium sativum*), neem leaves (*Azadirachta indica*), turmeric rhizomes (*Curcuma longa*), lantana leaves (*Lantana camara*) and ashwagandha leaves (*Withania somnifera*) were evaluated at three concentrations (5, 10 and 15%) against *Alternaria alternata* on PDA by poisoned food technique. Data presented as per cent inhibition of radial mycelial growth are presented in Table 1. The data clearly shows that increase in concentrations of the plant extracts caused a decrease in mycelial growth of the fungus thereby, resulting in increased inhibition. Among these plant extracts, garlic was found most effective showing 78.88, 82.22 and 89.99 per cent inhibition of mycelial growth of *Alternaria alternata* at 5, 10 and 15 per cent concentrations, respectively followed by neem which caused 72.22, 80.00 and 91.11 per cent inhibition of linear growth of *Alternaria alternata,* respectively.

Leaf extract of *Lantana* resulted in 33.33, 38.88 and 42.22 per cent inhibition at 5, 10 and 15 per cent concentrations respectively and it was found to be least effective against *Alternaria alternata.* In general, higher concentrations of plant extract were more effective in reducing the mycelial growth as compared to lower concentrations. Plant extract (P) x concentration (C) interaction was also found significant. This suggests that plant extracts offer a better alternative to fungicides as they are safer and effective too. Similar results were obtained by Bochalya (2012) and Meena et al. (2013), Meena et al. (2014).

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Table: 1 *In vitro* fungitoxicity of plant extracts against *Alternaria alternata* by poisoned food technique after 7 days of incubation at 25 + 1 °C

Plant extract	Part used	Per cent growth inhibition at different concentration (%)			Mean
		5	10	15	
Garlic	Clove	78.88 (62.64)	82.22 (65.06)	89.99 (71.56)	83.69 (66.42)
Neem	Leaves	72.22	80.00	91.11	81.11

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		(58.19)	(63.43)	(72.65)	(64.75)
Turmeric	Rhizome	60.00	77.77	74.44	70.74
		(50.77)	(61.87)	(59.63)	(57.42)
Ashwagandha	Leaves	42.22	70.00	78.88	63.70
		(40.52)	(56.79)	(62.64)	(53.30)
Lantana	Leaves	33.33	38.88	42.22	38.14
		(35.26)	(38.57)	(40.52)	(38.11)
Check	-	00.00	00.00	00.00	-

		SEm+	CD (p=0.05)
	F	0.59	1.67
	Con.	0.48	1.36
	F x Con.	1.17	3.34

*   Average of four replications

Figures given parenthesis are angular transformed values

Plate: Fungitoxicity of different plant extracts against *Alternaria alternata* **(***in vitro***)**

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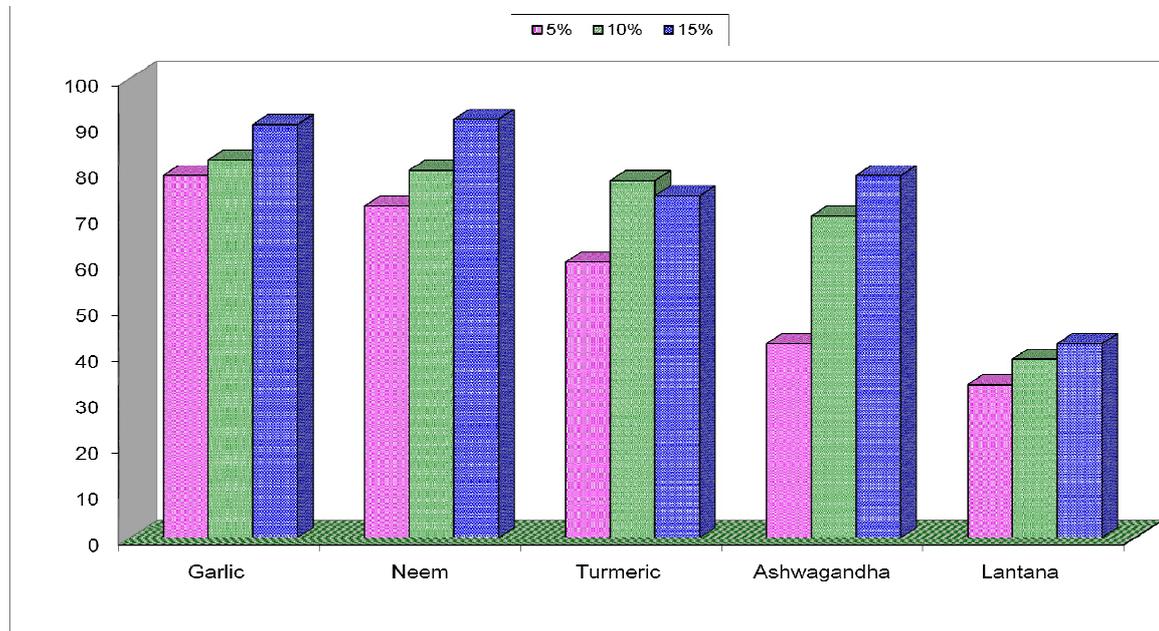
Fig. 1 *In vitro* **fungitoxicity of plant extracts against** *Alternaria alternata* **after 7 days of incubation at 25 ± 1 °C**

RESEARCH ARTICLE

Antibacterial Activity of Zn/Nylon Nanocomposite against *Escherichia Coli* and *Staphylococcus aureus* Bacteria

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ABSTRACT

Objectives: Synthesis of the Zinc/Nylon nanocomposite and testing their activity against the positive and negative bacteria. Methods/Statistical Analysis: Nylon 6,10 prepared by the condensation polymerization method between 1,6 hexamethylenediamine and sebacoyl chloride. Nylon 6, 10 was coated with different concentration of Zinc nitrate to form zinc/Nylon nanocomposite and using sodium borohydride as reducing agent. The polymer and nanocomposite were an analysis by FT-IR and XRF. The antibacterial activity was carried out against *Escherichia coli* *(ATCC 25922)* and *Staphylococcus aureus(ATCC 25923)* bacteria (Gram-positive and negative bacteria). Findings: The result shows that the zinc/Nylon nanocomposite was sensitive for both types of bacteria and more sensitive for gram positive and indicated that the Zn/nylon 6,10 antibacterial activity, it's very useful in food systems as a preservative agent after further required investigations and risk assessments. Application/Improvements: The application of Zn/ Nylon could be recommended as a preservative agent against foodborne pathogens, in food production and processing, in medical plastic materials, Nylon bags for keeping foods in order to avoid contamination with bacteria pathological.

Keywords: Nylon, Zn/Nylon nancomposite, *Escherichia coli* and *Staphylococcus aureus* bacteria

INTRODUCTION

Recently, in preparation polymers with the structurally controlled was interested in particular using. Our group attached to the polymers was interesting for investigations of polymer properties [1]. Polymer nanocomposite is synthesis by adding inorganic fillers to the polymer matrix, the products having at least one dimension in the nanometer range [2,3]. Application of polymer nanocomposite is found in wide fields such as mechanics, optics and flame retardants [4,5]. However nanofibers have been used in different areas [6] such as sensors [7] catalysis energy storage and biomedical materials [8]. Nanofillers are a common method to improve properties of polymeric materials like giving antimicrobial properties using in medical applications [9]. In the last few years have been numerous research composite materials such as nano-silver was abilities to immune fungal and bacterial infections [10]. Polyamide synthesis and properties have attracted wide attention [11]. Nylon is polyamides, constitutes a family of compounds which has a potential mechanical property, is widely used in the industry [12-14]. Nylon is polymers consist of polyethylene $(CH_2)n$ segments and separated by peptide (NH-CO) units, These peptides unite in nylon giving some of its unique properties [15,16]. Nylon is a polar synthetic and electronic rich polymer (polyamide), one of the important kinds nylon is nylon 6, 6 usually prepare from hexamethylene diamine and adipoyl chloride [17]. Silver nanoparticle was used as antibacterial activity against at low concentration [18]. Nylon 6, 6 was loaded with silver nanoparticles to produce Ag/ nylon nanocomposite it has exhibit antibacterial activity against E. coli. The antibacterial efficiency of Ag/ nylon nanocomposite was investigated by introducing the particles of nanocomposite into the media contain E.coli. After 24 hours the result shows that they exhibited antibacterial effect [19] which is indicating that the synthesized metallic ZnO has antibacterial activity [20, 21].

The development and improvement of accurate and efficient methods of rapid antibiotic susceptibility testing are important for public health. Antimicrobial susceptibility information about pathogens may significantly reduce morbidity and mortality, the cost of treatment, and duration of hospitalization if this information can be provided to clinicians in a rapid and timely fashion [22]. The risk of infection from pathogenic microorganisms on environmental surfaces derives not only from their presence but also from their ability to survive on many surfaces. The persistence of pathogenic microorganisms has been established in studies of their survival on surfaces in institutional, commercial, and domestic settings. Bacteria are a major cause of disease and even human death [23-25]. Many metallic elements have an ability to inhibit the growth of bacteria and to inactivate enzymes. This antimicrobial effect is shown by metals such as mercury, silver, copper, lead, zinc, gold, aluminum and other metals, and the concentration of the metal needed for this antimicrobial effect is extremely low [26]. Thus, zinc oxide (ZnO) and copper oxide nanomaterials are incorporated into a variety of medical and skin coatings because of their antimicrobial and/or antifungal properties. The ZnO nanoparticles (NPs) have been added as antimicrobials to wallpaper for use in hospitals [27] some data from hospitalized patients demonstrates that Staphylococcus aureus, Enterococcus sp, coagulase negative *Escherichia coli* and Pseudomonas aeruginosin are the most important pathogen involved in the skin and soft tissue infections [28].

The aim of the present study is synthesis nylon 6, 10 from 1,6 hex methylene diamine and sebacoyl chloride and loaded with zinc nitrate at different concentration to form Zn/nylon 6, 10 nanocomposites by condensation polymerization method and study their antibacterial activities against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) by Kirby-Bauer method.

MATERIALS AND METHODS

Materials

Sebacoyl chloride, 1,6 hexamethylene diamine, Zinc nitrate $Zn(NO_3)_2$ and sodium borohydride were purchased from Sigma-Aldrich. All chemicals were used without further purification. The distilled water was used for all preparation

and measurements. An FT-IR spectrum was used for the analysis, polymer to a sample of the scanning range of 450 to 4000 cm-1. The sample was prepared for FT-IR as a plate. The Zn/nylon 6,10 nanocomposite was analyzed by Rigaku NEX CG X-ray fluorescence (XRF) spectrometer. The XRF is an effective method for measured zinc metals in a sample. The polymer nanocomposite samples were placed in the chamber and measured by 20 mm diaphragm in a vacuum. X-ray spectra were obtained using RX9, Cu, Mo and Al conditions. In these analyses, the X-ray tube current was set to approximately 1 mA for the RX9 target and into 0.5 mA for other targets. The X-ray tube voltage has been set to 25 kV only for the RX9 and 50 kV for Cu, Mo, and Al targets. The X-ray measuring time was only 200s for the Al target and 100s for other targets. The zinc metals were higher energy (8.5 Kcv) and appear in Mo targets.

Synthesis of Nylon 6, 10 Rebaz A. Omar

0.5gm of sebacoyl chloride and 0.5gm of 1,6-hexanediamine prepared in 100 ml of distilled water. The first solution added to the second solution to form a mixture and both of them are acid chloride ends of the sebacoyl chloride react with both amine ends of the 1,6-diaminohexane to form new amide linkages. The polymer was collected from the interface of the two phases, it which forms are made up of alternating 1,6-diaminohexane and sebacoyl groups which known as an alternating copolymer. This particular nylon is made of units of 6 carbons between two nitrogen atoms, an amide linkage and another unit of 10 carbons which are repeatable until it forms nylon-6,10.

Synthesis of Zinc/Nylon 6, 10 nanocomposite

For synthesis Zn/nylon 6,10 nanocomposite a piece dry per weight nylon 6,10 was used and it put in the distilled water for (24 h) to remove all solvent and interference. The nylon was swollen and inset in the different concentration of zinc nitrate (0.1, 0.2 and 0.3) mM for (48h). To produce Zn/nylon 6,10 nanocomposite 0.2mM sodium borohydride used as a solution reducing agent at room temperature for 48h. The final products were illustrated zinc was loaded on the nylon and sample is dried in an oven for 12h at 40 ºC. Finally, the sample was ready to antibacterial test.

Antibacterial performances

Staphylococcus aureus (ATCC 25923) and *Escherichia coli (ATCC 25922),* microorganisms isolated from clinical materials from Azadi hospital in Erbil city. Cultures of bacteria were grown overnight in blood and MacConkey agar at 37°C. The primary identification of bacterial isolates was made based on colonial appearance, pigmentation, Gram reaction, motility test and standard biochemical tests. These cultures are transferred to Tryptic Soya broth medium overnight at 24hrs. The overnight broth culture of the organism was diluted in nutrient broth to an inoculum load of approximately 1.0x106 cfu/ml. It was standardized according to National Committee for Clinical Laboratory Standards [29] by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0×106 cfu/ml.

The antibacterial performance carried out using Kirby-Bauer method as described by Lalitha [30] and by Kim [31]. Fresh plates of individual isolate were prepared and incubated at 350C for 24hours. A discrete colony of each isolate was transferred into a test tube of 5ml of TSB broth and incubated overnight at 37C° and turbidity of prepared inoculums were adjusted equal to that 1.0×106 CFU/ml (standardized by 0.5 McFarland standard) and inoculum were spread on Muller-Hinton agar medium by using a sterile glass spreader, this procedure was repeated by streaking two more times, rotating the plate approximately 60o each time to ensure an even distribution of the inoculums. Sterile plastic agents (Sterilized by UV light) were placed on the culture medium. For control negative used plastic with standard values and also standard antibiotic discs (ciprofloxacin 10μg/disc, Ampicillin & Cloxacillin 10μg/disc, Tobramycin 10μg/disc, Vancomycin 10μg/disc) for control positive. The plates were incubated at 37C° for 18 to 24 hours and were observed for growth inhibition. After 16 to 18 hours of incubation, each plate was examined and circular zones of inhibition observed. The diameters of the zones of inhibition (as judged by the unaided eye) were measured. The zones were measured from the upper surface of the culture medium illuminated

with reflected light, with the cover removed. The zone margin was taken as the area showing no obvious, visible growth that can be detected with the unaided eye.

RESULTS AND DISCUSSION

Characterization of Zn/Nylon nanocomposite

FTIR spectroscopy studies of the Zn/Nylon6, 10 nanocomposite shows spectra of prepared sample (Fig. 1a), exhibit a wide absorption band at 3415.79 cm^{-1} which are assigned to N-H stretching vibrations. The bands at 2934.73 and 2851 cm^{-1} correspond to symmetric CH$_2$ stretching vibrations. The located bands at 1698.29, 1539.29, 1254.89 and 1465.20 cm^{-1} attributed to Amid I, II, III, and N-H deformation, and the absorption bands at 931.88, 678.58 and 551.23 assigned to C-C stretching, C-C bending and C-C deformation. The absorption band observed at 668 and 472 cm^{-1} are formed by the stretching vibration modes of Zn-OH, Zn bond as shown in fig. 1b, 1c, 1d. The peaks of pure nylon 6,10 shifted to lower wavenumber and base on the results of IR, hybridization between Zn and nylon molecules is expected, which causes in an intense interaction. The chemical-adsorbed monolayer of nylon structure caused an interface hybrid effect between Zn and nylon.

Fig. 2 shows that the Zn element was present in the different samples with high intensity. The X-ray fluorescence was working on the principle of absorbing fluorescence by detectors, its proportion conductance was a change in the energy of the fluorescence which is processed by the electronic. The signal of fluorescent was measured in kilo-electron volts on the horizontal axis, the vertical axis was an intensity occurrence per second. The energy of the fluorescent determined the elements while the intensity of the fluorescence was identified the concentration of the elements in a sample.

The nylon sample without coatings exhibits no peak for the zinc element as shown in fig. 2a. At the same time the coated nylon by different concentration of zinc. When the nylon coated with (0.3mM) of zinc the higher intensity observed, which was above 500.000cps as shown in fig. 2b. However, the coated sample at (0.2, 0.1 mM) showed almost lower intensity for zinc element, which is indicates the intensity of zinc decreases to 200.000, 50.000 cps respectively, with decreasing concentration of zinc element in the sample as shown in fig. 2c and 2d.

Bactericidal activity

The appearances of zones of inhibition on the microbial growths were indications of the efficacy of the test agents on the inhibition of bacterial growth [32]. It was noted that the nylon saturated material zinc has appeared effect as inhibition and influential on both bacteria tested *Staphylococcus aureus* and *Escherichia coli* in standard conditions and sterile, which was appeared as zone around the material selected and its measuring the diameter ranging approximately from 8 to 12 mm to *Staphylococcus aureus* while it was little effect on *Escherichia coli* about approximately from 6 to 9 mm and the increased was associated with increasing of concentration of the substance and respectively, show fig. 3 and 4. The current studies are consistent with a previous study [32] which was the use of silver with nylon material against bacteria *E.coli* and had an effect on bacteria growth. Zn/nylon 6,10 completely inhibited the growth of all the tested pathogens and most of them were inhibited at a concentration of 0.1Mm to 0.3Mm of Zn. It has concluded [33] that Zinc has an excellent antibacterial activity against enteric bacterial pathogens common in our setup which may provide a basis for treatment of diarrhea. The antibacterial activity of the agent tested was much stronger than that of Zn powder. This could be simply explained as smaller particles normally have a larger surface to volume ratio, which provides a more efficient mean for antibacterial activity [34]. Also In a study conducted by Ahmed [35] that the Zn has been affected by bacterial growth, indicated that Gram-positive bacteria were more sensitive than Gram-negative bacteria to Zn nanocomposite with nylon and this is consistent to a large extent to our study with the difference in the concentration of zinc and material additives show fig. 3 and 4. There are many explanations about the reasons for the difference in the effect of the material in Gram-positive bacteria more

than Gram-negative of the most important the outer thick peptidoglycan layer and its amino acid constituent, surface proteins (e.g., adhesions) and teichoic acids plus lipoids (forming lipoteichoic acids) which act as chelating agents and also execute certain types of adherence. Another hypothesis is some components found in Gram-negative bacteria, and not in Gram positives, which can oppose Zn attachment onto cell walls; the possible nominees include the extra outer membranes and the pathogen-associated molecular patterns which include lipopolysaccharide (consisting of lipid A, core polysaccharide, and O antigen), porins and particular fragments of peptidoglycan. The third supposition is that the Gram-negative cell wall, according to its structure and thickness, may prevent Zn from penetrating into the cells and interacting with their internal components. Also, it was demonstrated [36] that Zn was more effective for killing Gram-positive than Gram-negative bacteria because they have simpler cell membrane structure. Another proposed way in which the membrane can be compromised is the alteration of membrane lipid components [37].

It was proposed that nanomaterials that can physically attach to a cell can be bactericidal if they come into contact with this cell. Liu et al [38] indicated that Zn nanocomposite may distort and damage bacterial cell membrane, resulting in a leakage of intracellular contents and eventually the death of bacterial cells; the inhibitory effects against E. coli O157:H7 increases as the concentration of Zn increased and this is consistent to a large extent to our study, which used three different concentrations as shown in fig. 4.

CONCLUSION

The Zn/nylon 6,10 nanocomposite has been prepared by the condensation polymerization method and zinc ion was reduced by sodium borohydride. The concentration of nanocomposite was controlled and characterized by FT-IR and XRF. The FT-IR spectra showed the characterization peak for different concentration of Zn bonded. Also, XRF analysis showed that the peak was represented for zinc (8.5 Kcv) and the intensity of the peaks. The Zn/nylon 6,10 nanocomposite used against both Gram-positive and negative bacteria. It showed an effective and powerful antibacterial. The treatment with Zn/ Nylon resulted in bacterial cell lysis, and its antibacterial action was more against Gram-positive bacteria in comparing to Gram-positive due to its resistance to the nanocomposite sample.

The application of Zn/ Nylon could be recommended as a preservative agent against foodborne pathogens, in food production and processing, in medical plastic materials, Nylon bags for keeping foods in order to avoid contamination with bacteria pathological and others. We are recommending studying the impact of these materials on other pathogenic bacteria. Make sure health and safety standards with the use of these materials in various industries and its suitability for humans.

ACKNOWLEGEMENT

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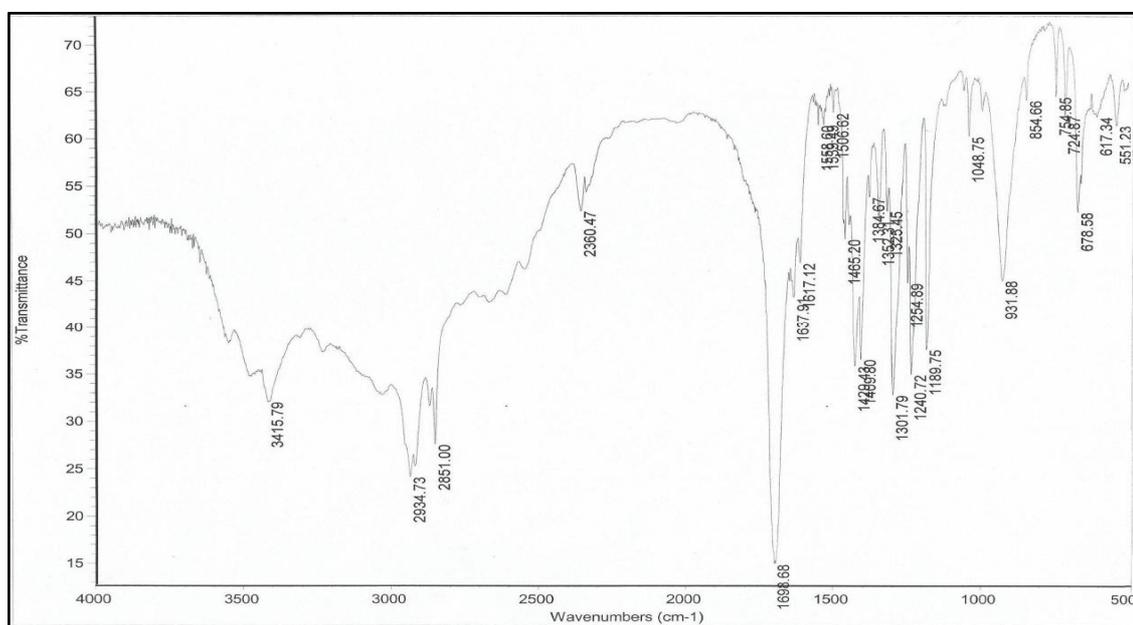
Fig. 1a: FTIR spectra of a Nylon

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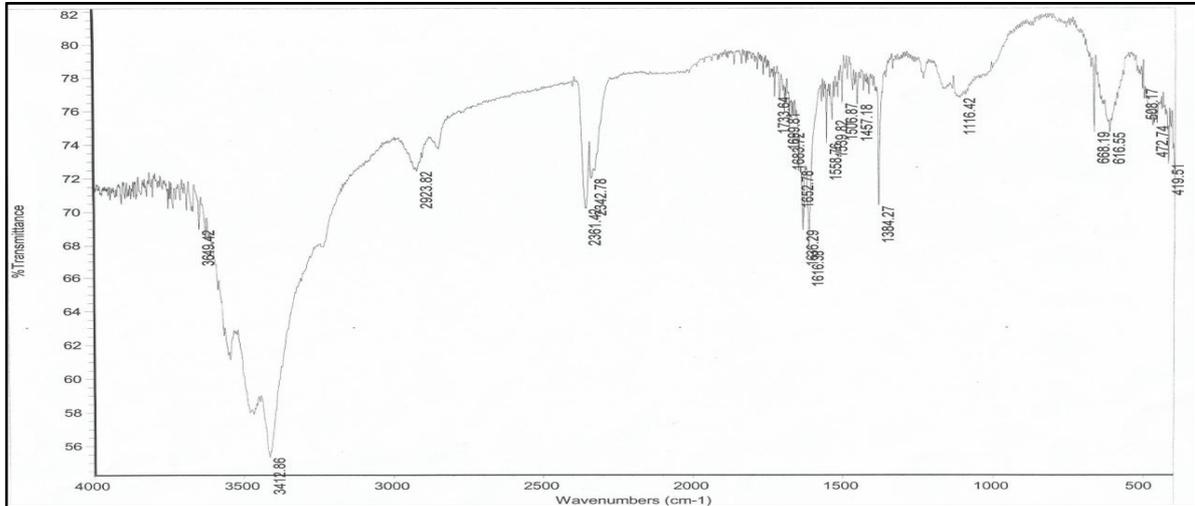
Fig. 1b: FTIR spectra of a 0.1M ZnO/ Nylon 6, 10 nanocomposites
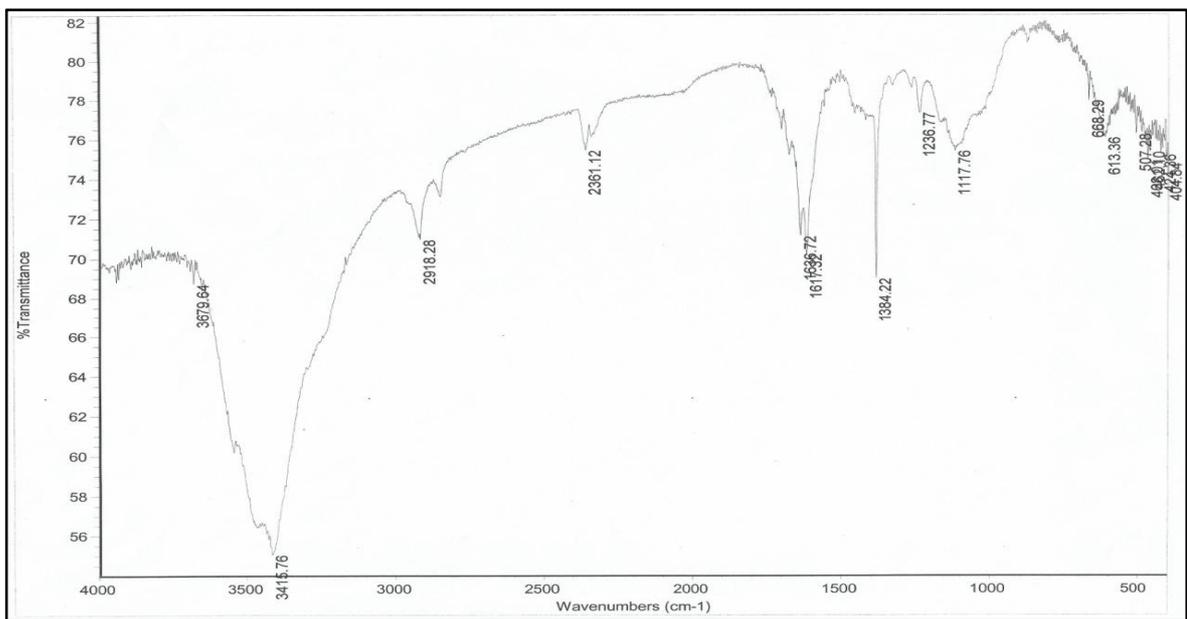
Fig. 1c: FTIR spectra of a 0.2M ZnO/ Nylon 6, 10 nanocomposites

Fig. 1d. FTIR spectra of a 0.3M ZnO/ Nylon 6, 10 nanocomposites
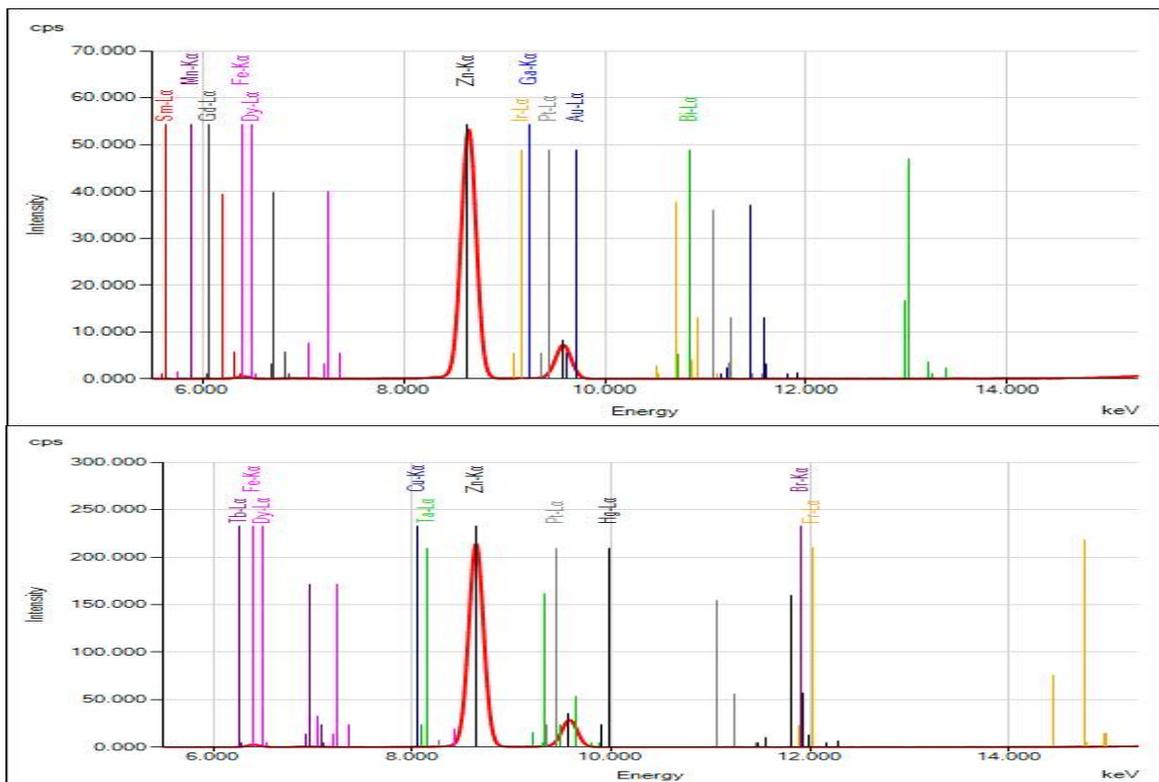
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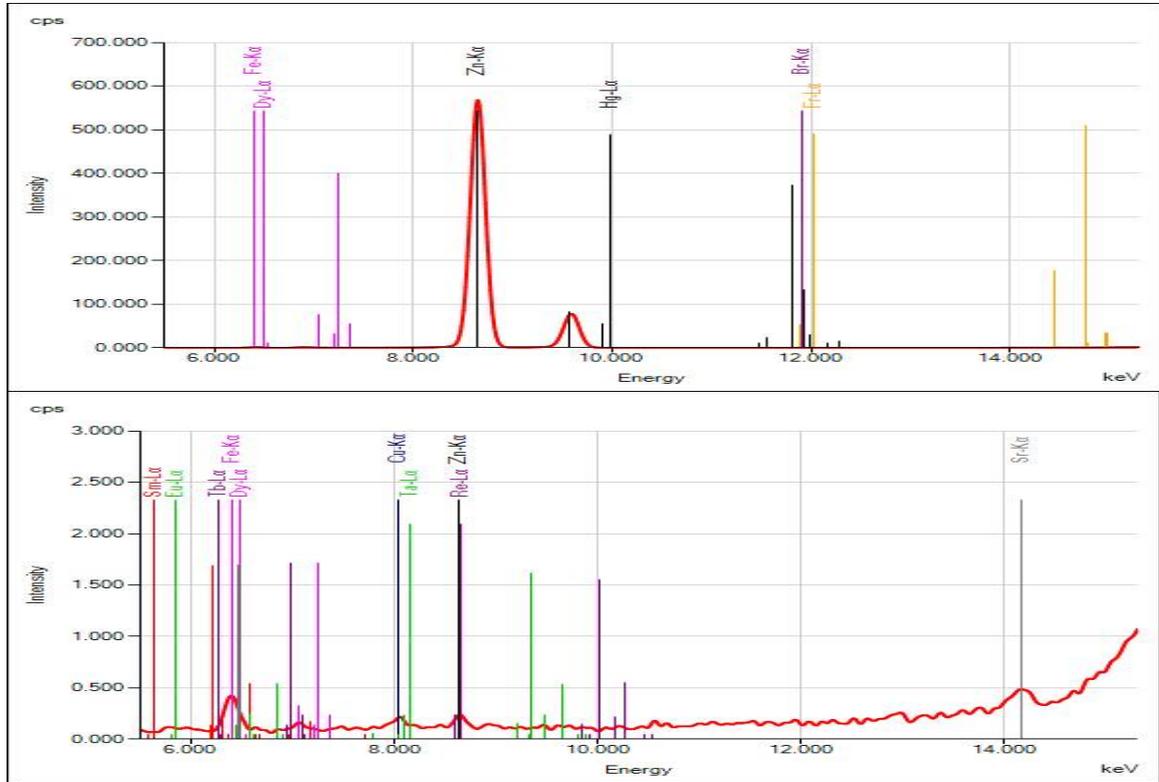
Fig. 2: X-ray Spectra for Nylon a) Nylon without Zn b) Nylon with 0.3mM AgNO3 c) Nylon with 0.2mM AgNO3 d) Nylon with 0.1 mM AgNO3

**Fig. 3: Inhibition effect to Zn/nylon 6,10 on *Escherichia coli* bacteria**

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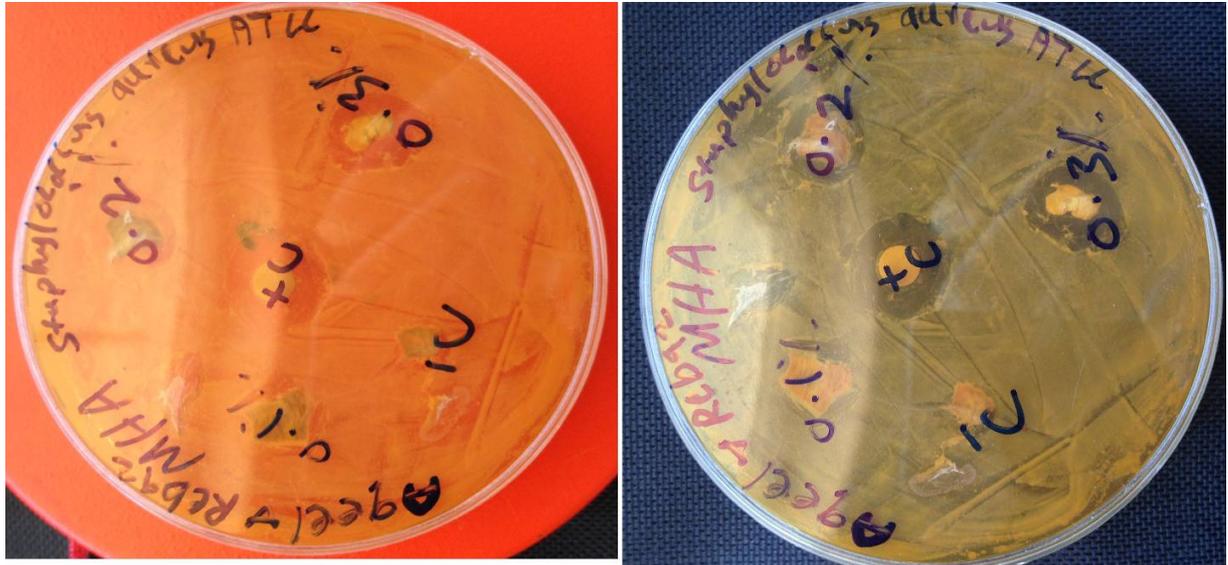
Fig. 4: Inhibition effect to Zn/nylon 6,10 on *Staphylococcus aureus bacteria*

RESEARCH ARTICLE

Cognitive Appraisal of Stress, Its Etiopathological Implication and Management

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ABSTRACT

Cognitive impairment is the major health problem in normal as well as in diseased individual. Stress is the major culprit involved directly or indirectly in the cognitive dysfunction. Cognition is the physiological process of knowing, including awareness, perception, reasoning, and judgment. Cognitive functions are mainly categorized into memory, attention, creativity and intelligence. Cognition dysfunction is a result of complex interplay between stress, immune system and cognitive function.Ayurveda is one of the oldest systems of traditional medicine which offers holistic approach for promotion and preservation of health by addressing the physical, mental and spiritual aspects in unison. Ayurveda described several strategies including pharmacological (medicinal dietary supplementation and dietary modification) and non-pharmacological (lifestyle modification in the form of daily and seasonal regimen) to impede stress and to enhance cognitive function.The present review article has discussed about the stress, cognitive function and impact of stress on cognitive health.

Keywords: Cognition, Stress, Rasayana, Cognitive Function, Prakriti.

INTRODUCTION

Cognition can be defined as the processes; an organism uses to organize information. This includes acquiring information i.e. perception, attention, understanding and retaining i.e. memory and using it to guide behavior (reasoning and co-ordination of motor outputs). Interventions to improve cognitive function may be directed at any one of these core faculties [1] Stress is a descriptive term used in the context of both the behavioral and biological sciences to cover conditions which are physical, biological, or psychological in nature that typically cannot be controlled by organisms [3]. Stress is mainly defined as time pressure. We feel stressed when we do not have the time to perform the tasks we want to perform within a given period of time. This time pressure usually triggers a set of physiological reactions that give us the indication that we are stressed. Stress results from an actual or threatened loss of resources.A resource represents physical, psychological, social or organizational aspects of an individual that serve multiple purposes.Selye defined stress as "the non-specific response of the body to any demand made on it′ and viewed stress as the common denominator of all adaptive reactions in the body and complete freedom from stress as death.Later, he used the term stressor to designate the stimulus that provoked the stress response. To derive a conceptualization of stress, Selye chose to delineate what it (stress) was not and wrote that stress is not [2]

1. Simply nervous tension; it can occur in organisms without nervous systems or in anesthetized or unconscious patients.
2. An emergency discharge of hormones from the adrenal medulla.Although catecholamines are a part of the stress reaction, they are not the only hormones activated, and they play no role in generalized inflammatory diseases or local stress reactions.
3. Everything that causes a secretion from the adrenal cortex (i.e. secretion of corticoids).Adreno-corticotropichormone (ACTH) can stimulate the release of corticoids without producing a stress response.
4. Always the nonspecific result of damage.Normal activities, such as tennis or a passionate kiss, can produce a stress response without conspicuous damage.
5. The same as a deviation from homeostasis, the body's steady state. Reactions to loud noises, blinking of the eye, or contracting a muscle may cause deviations from the resting state without evidence of a generalized stress reaction.
6. Anything that causes an alarm reaction. It is the stressor that is the stimulus and not the stress itself.
7. Identical with the alarm reaction: These reactions are characterized by certain end-organ changes caused by stress and, hence, cannot be stress.
8. A non specific reaction: The pattern of the stress response is specific, although its cause and effects may vary.
9. Necessarily bad: The stress of success,challenge, and creativity is positive, whereas that of failure, anxiety, and infection can be negative.
10. To be avoided: Stress cannot be avoided. It is ubiquitous; it is an essential ingredient of life.

IMPACT OF STRESS ON COGNITIVE FUNCTION AND HEALTH

Stress related phenomena are often classified as physical, emotional and cognitive with respect to their effect on human performance. There are numerous examples of the effects of stressors in all three categories affecting human performance. Physical stress is particularly interesting in the light of the contemporary concern with the quality of the natural environment. Urban areas represent several stressors in the form of noise and air pollution and disturbance of daily and seasonal regimen. Worry and emotions are personality traits and are negatively correlated with proficiency in a simple motor work. Cognitive stress, resulting from the need for coordination of multitasking in nearly all daily activity, is perhaps the most common stress condition. Heightened mental load resulting from multitasking typically slows responding [4]. Stressors are environmental, biological, and/or cognitive events that, among other things, challenge or threaten the well-being of an organism, increase its arousal or activation level, and deplete its resources.Stress also affects the human immune system. Although chronic stress typically produces suppression of a wide range of immune system parameters, acute stress has been found to stimulate certain aspects

of immune functioning [5]. Emotion and stress share many characteristics. A stressful experience will often cause a particular emotion (e.g. surprise, fear, joy, etc.), and particular emotions can create stressful situations (e.g., blushing due to extreme timidity can cause a stressful situation for an individual).Because of these similarities between emotion and stress, most of the literature on emotion,stress and memory intermixes the effects of emotion and those of stress upon memory function. However, emotion and stress are two different entities. Although a stressful experience will almost always trigger a specific emotion but in contrast a particular emotion does not always elicit a stress reaction[6].

Cognitive activation theory of stress (CATS)

Cognitive activation theory of stress include mainly four aspects which are as follows [7]
- Stress stimulus i.e. stressor or load.
- Stress experience
- Stress response
- Feedback from stress response

There are several types of physical, physiological, psychological and emotional loads or demands felt by the individual that are reported as stress to the extent that they are deemed a loss or a threat.It is argued that it is not the physical characteristics of a stimulus that elicits the stress response but is a person's appraisal based on previous experiences and future expectations that translates a situation into a stressful experience.A stressor triggers an alarm reaction which occurs prior to adaptation and is termed as stress response. In this phase the individual has an increase in arousal, and there is a specific response to handle the cause of the alarm. Individual and situational differences play a role in the alarm reaction. After responding to a stressor, the individual receives feedback regarding the results of his or her response. The feedback can influence the feeling of being stressed and the individual can alter the perception of the stressor and or the outcome[8].i.e. the stress response always depends upon the Prakriti (psychosomatic constitution of the body).

Here, in this context Ayurveda propounds the concept of TrividhaSatva (i.e. Pravara, Madhyam and Heen). The individual having the Pravara Satva (good mental stamina/strength) does not fear from a stressful event and handles the situation very well, the individual with Madhyam Satva (medium type of mental stamina/strength) fears a little but able to handle the stressful event up to some extent and the individual with Heen Satva (lowest/minimum mental stamina/ strength) fears from a stressful event and is not able to handle the situation [9].A variety of stressful conditions impede a variety of memory measures. A study showed a significant impact of stressful noise on immediate verbal memory [10]. Another study reported asimilar effect of hypoxia on the executive function of working memory [11]. Mandler was one of the first cognitive psychologists to speculate theoretically about the effects of stress on memory [12].

Information overload threatens our limited cognitive capacity and can quickly degrade performance in many daily tasks. In most experiments that have examined time pressure, the decline in performance is gradual rather than catastrophic.Burrows has examined how workload variables such as amount of information, speed of information presentation and secondary task requirements, interact to create a condition of overload and how they influence recognition memory [13]. In general, theories of stress account for its effects on cognition and on human performance in terms of multiple psychological and biological processes. These processes include, (but are not limited to) arousal or activation (stress intensity is directly and linearly related to arousal level), attention allocation(stress controls directly or indirectly the distribution of attention across points of environmental and internal input and can overload attentional capacity), and plans or strategies for the deployment of attention and other resources. The theories may differ in their assumptions about these processes.

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Objective measurement of stress

The psychological effects of stress have been measured in various ways which include psychosomatic changes in the individual experiencing stress, self-report by individuals and performance or behavioral changes. Several physiological responses are reliably correlated with the experience of stress and with the occurrence of stressful physical stimuli. One response arises in the autonomic sympathetic nervous system, which controls both neural and hormonal processes. Second principal stress-response system is the hypothalamic-pituitary-adrenal axis, which regulates the release of glucocorticoids from adrenal cortex. Two of the most salient hormonal responses to stress are increase in the serum levels of nor-epinephrine and cortisol.Third stress response system is immune system. Although chronic stress typically produces suppression of a wide range of immune system parameters, acute stress has been found to stimulate certain aspects of immune system function. Interleukin-6 (Il-6) level is elevated in response to a variety of stressors, which is an important marker of stress measurable in saliva. Modified from [14].

Key features of model of stress and coping

Tache&Seyle defined the coping as adapting to stress situations and summarized the essential points of Selye's model of stress which are as follows [15]

- All life events cause some stress.
- Stress is not bad per se, but excessive or unnecessary stress should be avoided whenever possible,
- The stressor is the stimulus eliciting a need for adaptation and stress is the response.
- The nonspecific aspects of the body's reaction to an agent may not be as obvious as the specific effects. Sometimes, only disease or dysfunction will make an individual realize that he or she is under stress.
- Stress should be monitored through a battery of parameters.
- Stress should not be equated with only ACTH, corticoid, or catecholamine secretions. These seem to manifest the main pathways of nonspecific adaptation.
- Removal of the stressor eliminates stress.

The stress coping can be categorizes into two groups, self-generated or active which include confrontation, fight, and escape, which are evoked when stressors are controllable or escapable and passive which include quiescence, immobility and freezing, which are elicited when the stressor is inescapable. Active and passive coping styles are related to the way in which a performer interprets the task at hand and appraises the stress that it entails.Coping style mediates not only conscious verbal appraisal of a stressful situation, but also concomitant physiological activity.

Strategies to enhance cognitive function and stress management

Different types of strategies including pharmacological as well as non-pharmacological are proposed to enhance cognition. Most interventions target either disease pathologies or the processes underlying normal cognition. The strategies are as follows

- Natural medicines
- Lifestyle modification including environmental enrichment and exercise
- Dietary supplementation
- Pharmaceutical drugs
- Advanced techniques
- Rasayana (medicinal dietary supplementation).

Ayurveda propounds the concept of medicinal dietary supplementation in the form of Rasayana which rejuvenate both body and psyche by its nutraceutical action and thus improve the cognitive function [16].

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Education and training, as well as the use of external information processing devices may be designate as conventional means of enhancing cognition. They are often well established and culturally accepted. The spectrum of cognitive enhancements includes not only medical interventions, but also psychological interventions (such as learned ''tricks'' or mental strategies), as well as improvements of external technological and institutional structures that support cognition. A distinguishing feature of cognitive enhancements, however, is that they improve core cognitive capacities rather than merely particular narrowly defined skills or domain specific knowledge.

CONCLUSION

Stress is a major etio-pathological factor involved in the cognitive dysfunction, a major health problem of 21st century and is one of the most functionally debilitating aspects of many neuropsychiatric disorders and neurodegenerative disorders.In general, cognitive activation theory of stress account for stress effects on cognition and on human performance in terms of multiples of processes.Different stressors have different effects and there are significant variations in the effects of stress on different individuals depending upon their Prakriti.Despite of several years of scientific efforts, still there is no satisfactory therapeutic strategy to cure cognitive impairment in modern medical system. Ayurveda is a science of healing which have holistic approach towards the promotion and preservation of cognitive functions. Ayurveda described several measures like lifestyle management by using principles of daily & seasonal regimen, Sadvratta (ideal routins), dietary modification and Rasayana in the form of medicinal dietary supplements, etc. for enhancing cognitive functions. In this way, Ayurveda offers a holistic approach for the management of stress and cognitive dysfunction.

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RESEARCH ARTICLE

Antibacterial Activities of Ethanol and Aqueous extracts of some Perennial Plants against three Gram Negative Pathogenic Bacteria from Koya city – Kurdistan Region - Northern Iraq

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ABSTRACT

Aim of this investigation was to determine whether both ethanol and aqueous extracts of different parts of three Perennial plants (*Dodonaea viscosa*,*Eucalyptus* sp. and *Pinus* sp.) Could provide the biological activity to inhibit the growth of three gram negative pathogenic bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi).*20 grams of the plant parts powder were extracted with 200 ml of ethanol and 40 grams were extracted with 160 ml of distilled water. MIC was estimated for ethanol and aqueous .Antibacterialactivity assay of the ethanol and aqueous extracts was carried out disk diffusion method. Pure colonies of test bacteria were transferred to nutrient broth and incubated overnight at $37C°$ and turbidity of prepared inoculums were adjusted equal to that 10^6 CFU/ml (standardized by 0.5 McFarland standards) and 100µl of inoculum was spread on Muller-Hinton agar medium by using a sterile glass spreader. For control, discs were impregnated with sterile water or absolute alcohol (control negative) and also standard antibiotic discs (ciprofloxacin 10µg/disc, Ampicillin &cloxacillin10µg/disc, Tobramycin 10µg/disc) as a control positive.

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Interestingly in our study we have observed promising antimicrobial activity against studied bacteria. It is worth mentioning that most of the results of aqueous and ethanolic extracts were distinct because they are, even if it was cannot appearing in some large inhibition zone, but it was interesting because it was close to the positive control , where inhibition percentage arrived to more than 90%.Suggest both extracts of *D.viscosa* shoots against *P.aeruginosa*, *Eucalyptus* sp.leavesagainst *E.coli* and *S. typhi* , ethanolic extract of pine cone against *P.aeruginosa* and and aqueous extract of pine leaves against *E.coli* ,as a good antibacterial .

Keywords: Antibacterial, gram negative bacteria,*Dodonea viscosa*,*Eucalyptus* sp.,*Pinus* sp.

INTRODUCTION

Plants have attracted researchers all over the world as a source of medicinal treatment because of the active compounds that present in their parts. Recently these plants of medicinally importance are increasingly being investigated by researchers because of their antimicrobial activity. The antimicrobial activities attributed to compounds synthesized by plants which are known by their active ingredients for instance the phenolic compounds which are part of the essential oils [1]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [2]. In addition 'ethno-directed sampling' of species used in traditional medicine has proven far more fruitful in the identification of new drugs compared to random screening [3].

Several studies have been conducted on antimicrobial activity of plants in different parts of the world in an effort to discover new antimicrobial compounds from various plants and their species. These novel compounds may represent an alternative to synthetic chemicals such as drugs and antibiotics, which may exhibit side effects. The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. The widespread and indiscriminate prescription of antibiotics has resulted in the emergence of a number of drug-resistant bacteria [4]. *Escherichia coli*strainsare examples of multi resistant bacteria that are becoming an alarming problem within the healthcare system [5 and 6]. There is a strong necessity for the development of new drugs for the cure of infections provoked by these resistant and multi-resistant bacteria species [7].These bacteria are associated with a number of infections including, but not limited to UTIs, lower and upper respiratory tract infections (*E. coli*, *K. pneumonia* and*P.mirabilis*) and typhoid fever (*S. typhi*). Moreover, these pathogenic bacteria are capable of elaborating several virulent factors including the formation of biofilms on colonized surfaces [8 and 9].The aim of this investigation was to determine whether both ethanol and aqueous extracts of different parts of three Perennial plants (*Dodonaeaviscosa* , *Eucalyptus* sp. and *Pinus* sp.) could provide the biological activity to inhibit the growth of three gram negative pathogenic bacteria (*Escherichia coli* , *Pseudomonas aeruginosa*and *Salmonella typhi*) .

MATERIALS AND METHODS

1- Plant materials.

Scientific Classification of Selected plants.

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***Eucalyptus* sp.(10)**	***Dodonaea viscose (11)***	***Pinus* sp.(12)**
Kingdom: Plantae Order: Myrtales Family: Myrtaceae Genus: *Eucalyptus* Species :sp. *Binomial Name: Eucalyptus* sp.	Kingdom: Plantae Order: Sapindales Family: Sapindaceae Genus: *Dodonaea* Species: *viscosa* *Binomial Name: D.viscosa*	Kingdom: Plantae Order: Pinales Family: Pinaceae Genus: *Pinus* Species: sp. Binomial Name: *Pinus* sp.

Preparation of plant material

The fresh leaves, inflorescence and shoots of (Dodonaeaviscosa and Eucalyptus sp.) , the fresh leaves, cones and shoots of (Pinus sp.) were harvested , rinsed with tap water and air dried under shade and reduced to coarse powder and then micronized to fine powder using the electric blender .The powder was stored in an airtight paper bag until required .

Preparation of the ethanolic extracts

The preparation of the different parts extracts were performed following the methods described by (13). 20 grams of the powder were extracted with 200 ml of solvent (ethanol) contained in a 500 ml sterile conical flask and covered with cotton wool plug and wrapped with aluminum foil. Extraction was allowed to proceed for 24 h in cooler. The extract was filtered using a clean muslin cloth and then Whatman No. 1 filter paper. The filtrate was then evaporated to dryness using a rotary evaporation attached to a vacuum pump.

Preparation of the aqueous extracts

The preparation of the different parts extracts were performed following the methods described by (14). 40 grams of the powder were extracted with 160 ml of solvent (distilled water) contained in a 500 ml sterile conical flask and covered with cotton wool plug and wrapped with aluminum foil. Extraction was allowed to proceed for 24 h in cooler. The extract was filtered using a clean muslin cloth and then Whatman No. 1 filter paper.

Pathogenic Bacteria

Pathogenic bacteria used.
1. Escherichia coli(ATCC: 25218)2. Salmonella typhi(ATCC: 14028)
3. Pseudomonas aeruginosa (ATCC: 27853).
   Those isolated bacteria obtained from laboratory of general microbiology and
a laboratory of medical bacteriology / department of Medical Microbiology / Faculty of Science and Health / Koya university .

Determination of MIC value of ethanol and aqueous extract

Minimum Inhibitory Concentration (MIC) of the ethanol and aqueous extract against the tested bacteria was determined using serial twofold dilutions of ethanol plats extract with 100 μl. of fresh cultures ($1.5 * 10^5$ CFU/ml standardized by 0.5 McFarland standard) in each well from Micro titer plate (BRAND plates®, Germany) . The concentration of the ethanol extracts were ranged from 1000 mg/ml to 62.5 mg/ml, but the concentration of the

aqueous extracts were ranged from 1250 mg/ml to 78.13 mg/ml. Each assay was run in triplicates. The inoculated plates were incubated for 37 °C for 24 hours. After incubation period, the MIC values were determined by observed the turbidity of the wells in the micro titer plate. Well of the micro titer plate that showed no turbidity was interpreted as no growth of the tested bacteria. The MIC was defined as the lowest concentration of plantextracts that can inhibit the growth of the tested bacterial.

Antibacterial Assay

Antibacterialactivity assay of the ethanol and aqueousextracts was carried out disk diffusion method against test bacteria according to (15). Pure colonies of test bacteria were transferred to nutrient broth and incubated overnight at 37C° and turbidity of prepared inoculums were adjusted equal to that 10^6 CFU/ml (standardized by 0.5 McFarland standard) and 100µl of inoculum was spread on Muller-Hinton agar medium by using a sterile glass spreader. Sterile filters paper (Watchman No. 1, diameter 5 mm) was impregnated in 40 µlfrom both extracts and placed on the culture medium (MHA). For control, discs were impregnated with sterile water or absolute alcohol (control negative) and also standard antibiotic discs (ciprofloxacin 10µg/disc, Ampicillin &cloxacillin10µg/disc, Tobramycin 10µg/disc) .The prepared disks were placed on lawn cultures of the bacteria. The plates were left at room temperature for one hour to allow the diffusion of extract into the medium, and then were incubated at 37°C for (24-36) hours to allow maximum growth of the microorganisms. The inhibition zone diameter around each disk was measured (mm). The assay was repeated twice and mean of the experiments was recorded.

Statistical analysis

All determinations were carried out in twice replicate and the values are mean ± standard error (S.E).

RESULTS AND DISCUSSION

Getieet al., 2003 (16) reported the absence of D.viscosa activity against gram negative organisms, but interestingly in our study (Table 1) we have observed promising antimicrobial activity against studied bacteria .Antibacterial activity of ethanolic and aqueous extracts of different parts of D.viscosa are presented in table 1 which showed that there some differences efficiency among different data of ethanolic extract were the height data showed with ethanolic extract of shoots against P.aeruginosa which arrived to 14.5 mm , were there are some observations with aqueous extractshowed attractive results when gives inhibition zone arrived to 13 mm compared with positive control 11 mm by shoots extract against P.aeruginosa. So (17) reported the promising activity against gram negative bacteria.The crude ethanolic extract and aqueous fractions of D.viscosa were analyzed for antibacterial potential against three gram negative bacteria: E.coli, S.typhi, and P.aeruginosa. Preliminary screening showed inhibition against E.coli and P.aeruginosa(18 and 19).

Table2 showed the biological activity of Eucalyptus sp., there are more attractive results showed, where the observation data appeared height inhibition of ethanolic and aqueous extract ofleaves against E.coli which arrived to 17.5 and 16 mm respectively compared with standard antibiotic which arrived to 17 and 10 mm respectively. The ability of the crude extracts to inhibit the growth of recalcitrant bacteria as those used in this study is in agreement with previous reports of the antibacterial activities of other Eucalyptus species (9 and20). Natural products, such as a plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for control of microbial growth owing to their chemical diversity. Besides antimicrobial, several plants are being used in different areas of human health such as traditional medicine, functional foods, dietary supplements and recombinant protein manufacturing. Phytochemicals, especially flavonoids, polyphenols, anthocyanin and carotenoids, share the major market (21). Antibacterial activity of ethanolic and aqueous extracts of different parts of Pinus sp. are presented in Table 3. Highly significant antibacterial activity was observed in ethanolic extract of cones against P.aeruginosa compared with other results, but the result which observed in aqueous extract of leaves showed highest inhibition

zone against E.coli compared with positive control and others (22). These results show that the aqueous extract of pine leaves are the best among the other extracts and the aqueous extract better than ethanolic and this is the most security when compared the water with ethyl alcohol.

In order to more discuss the findings and to clarify the percentage of inhibition, the charts 1-3 shows that there are clear differences between the alcoholic and aqueous extract against studied bacteria. The maximum zone of inhibition against S.typhiwas recorded by leaves and inflorescence extract of Eucalyptus sp.(chart1)compared with other extracts and positive control which arrived to (78.95 & 73.68) % for ethanolic extract respectively and (73.68 & 71.00) % for aqueous extract respectively , at the same time there are other activity of ethanolic extract of Eucalyptus shoots and Dodonaea leaves (73.68 & 71.00) % respectively (23).The ethanolic extract of D. viscosaleaf has anti-bacterial effect against gram negative bacteria (24) , therefore to follow the inhibition percentage of biological effectiveness of the studied plants against E. coli the chart2 shows that the highest percentages of inhibition against it was at ethanolic extract of Eucalyptus leaves (92.11 %) , but there are attractive results compared with positive control , where the ethanolic extract of inflorescence and shoots of same plant appeared good inhibition percentage (86.84 & 84.21) % respectively , so the highest inhibition percentage of aqueous extract arrived to (84.21&76.32) % respectively for leaves and inflorescence extract of Eucalyptus , the aboveresults is considered as attractive results compared with positive control which arrived to 71.05% at its best inhibitory cases. According to (16and 25)the crude extract of Dodoneaviscosahas no activity against E. coli. Studedplants were appeared different activity against P.aeruginosa as chart3showed, where the highest zone of inhibition showed by leaves aqueous extract and inflorescenceethanolic extract of Eucalyptus (81.58 %) compared with other data included positive control. It is worth mentioning that most of the results of aqueous and ethanolic extracts were distinct because they are, even if it was cannot appearing in some large inhibition zone, but it was interesting because it was close to the positive control. May be attributed the observed antimicrobial activities to the presence of some bioactive compounds like alkaloids tannins, saponins, terpenes, essential oils and amongst others, several authors have linked the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (9 , 26 , 27, 28and29). Effectiveness of plants as antimicrobial agents is hinged on their mode of action in the body , generally, plant products have been demonstrated to have tropism for specific organs or systems in the body with resultant multiple effects on the body (9 and 30).

CONCLUSION

Suggest both extracts of D.viscosa shoots against P.aeruginosa, Eucalyptus sp. leaves against E.coli and S. typhi , ethanolic extract of pine cone against P.aeruginosa and aqueous extract of pine leaves against E.coli , as a good antibacterial , and the fact that the studied plants possesses many medicinal factor makes it a very useful plants, and the extracts could be useful in therapeutic treatment, but this has to be substantiated by in vivo experiment.

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Table 1. Biological activity of plant parts extract of Dodonaeaviscosa against pathogenic bacteria.

Plant Parts	Pathogenic Bacteria	Ethanolic Extract	Control type		Aqueous Extract	Control type	
			Positive	Negative		Positive	Negative
Leaves	*S. typhi*	13.5 ± 0.51	16	7	9 ± 1	10	N.I.
	E.coli	11 ± 1	17	6	11.5 ± 1.5	9	N.I.
	P.aeruginosa	14.5 ± 0.51	19	6	12± 2	11	N.I.
inflorescence	*S. typhi*	12 ± 0	16	7	0 ± 0	10	N.I.
	E.coli	11 ± 1	17	6	7 ± 1	9	N.I.
	P.aeruginosa	10 ± 0	19	6	12 ± 0	11	N.I.
Shoots	*S. typhi*	13 ± 1	16	7	7.5 ± 0.51	10	N.I.
	E.coli	10 ± 0	17	6	8.5± 1	9	N.I.
	P.aeruginosa	8 ± 0	19	6	13 ± 1	11	N.I.

Data given are mean of two replicates ± S.E., N.I= No Inhibition.

Table 2. Biological activity of plant parts extract of *Eucalyptus* sp. against pathogenic bacteria.

Plant Parts	Pathogenic Bacteria	Ethanolic Extract	Control type		Aqueous Extract	Control type	
			Positive	Negative		Positive	Negative
Leaves	*S. typhi*	15 ± 3	14	16	14 ± 1	12	N.I.
	E.coli	17.5 ± 0.51	17	6	16 ± 1	10	N.I.
	P.aeruginosa	12 ± 2	18	7	15.5 ± 0.51	10	N.I.
inflorescence	*S. typhi*	14 ± 1	14	16	13.5 ± 0.51	12	N.I.
	E.coli	16.5 ± 1.5	17	6	14.5 ± 0.51	10	N.I.
	P.aeruginosa	15.5 ± 0.51	18	7	13 ± 1	10	N.I.
Shoots	*S. typhi*	14 ± 2	14	16	11 ± 1	12	N.I.
	E.coli	16 ± 2	17	6	11.5 ± 0.51	10	N.I.
	P.aeruginosa	12 ± 0	18	7	12 ± 1	10	N.I.

Data given are mean of two replicates ± S.E., N.I= No Inhibition.

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Table 3. Biological activity of plant parts extract of *Pinus* sp. against pathogenic bacteria.

Plant Parts	Pathogenic Bacteria	Ethanolic Extract	Control type		Aqueous Extract	Control type	
			Positive	Negative		Positive	Negative
Leaves	*S. typhi*	N.I.	15	7	11 ± 1	11	N.I.
	E.coli	10 ± 1	18	6	11 ± 1	9	N.I.
	P.aeruginosa	11 ± 1	17	6	9.5 ± 1	12	N.I.
Cones	*S. typhi*	N.I.	15	7	9 ± 1	11	N.I.
	E.coli	12 ± 2	18	6	9 ± 1	9	N.I.
	P.aeruginosa	13 ± 1	17	6	10 ± 0	12	N.I.
Shoots	*S. typhi*	N.I.	15	7	10 ± 1	11	N.I.
	E.coli	10 ± 1	18	6	9.5 ± 1.5	9	N.I.
	P.aeruginosa	10 ± 0	17	6	10.5 ± 0.51	12	N.I.

Data given are mean of two replicates ± S.E., N.I= No Inhibition.
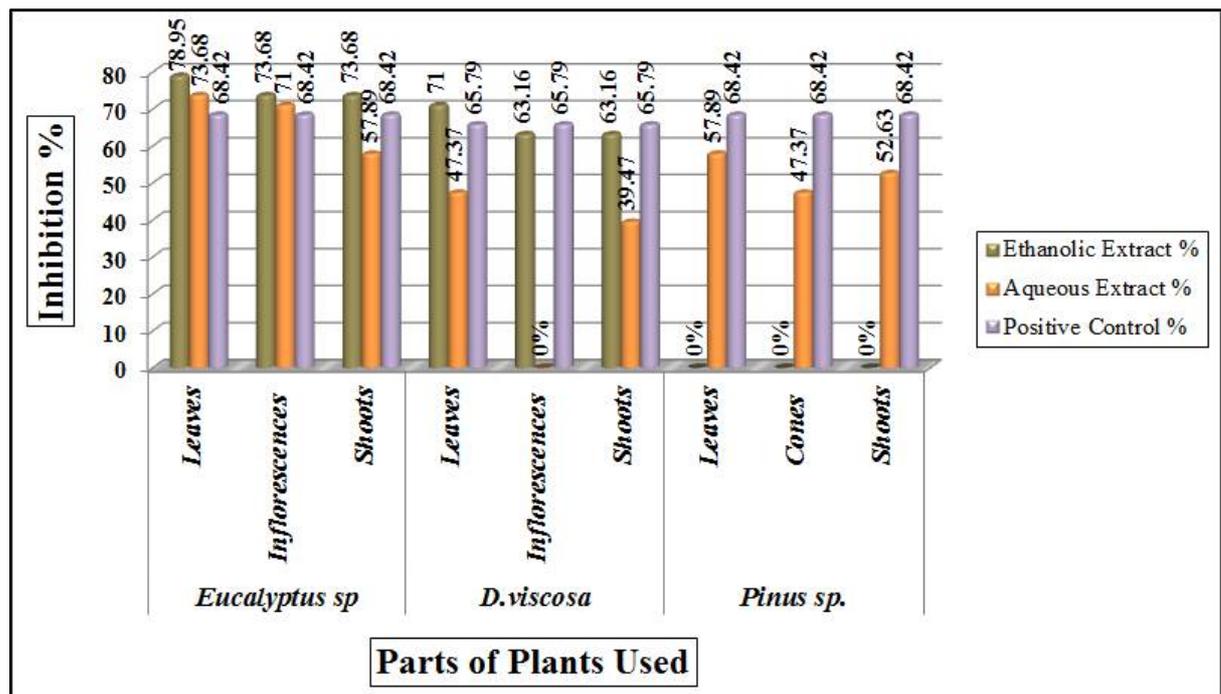
Fig.1. Inhibition percentage of three different partsextractfrom plant used against *Salmonella typhi*.

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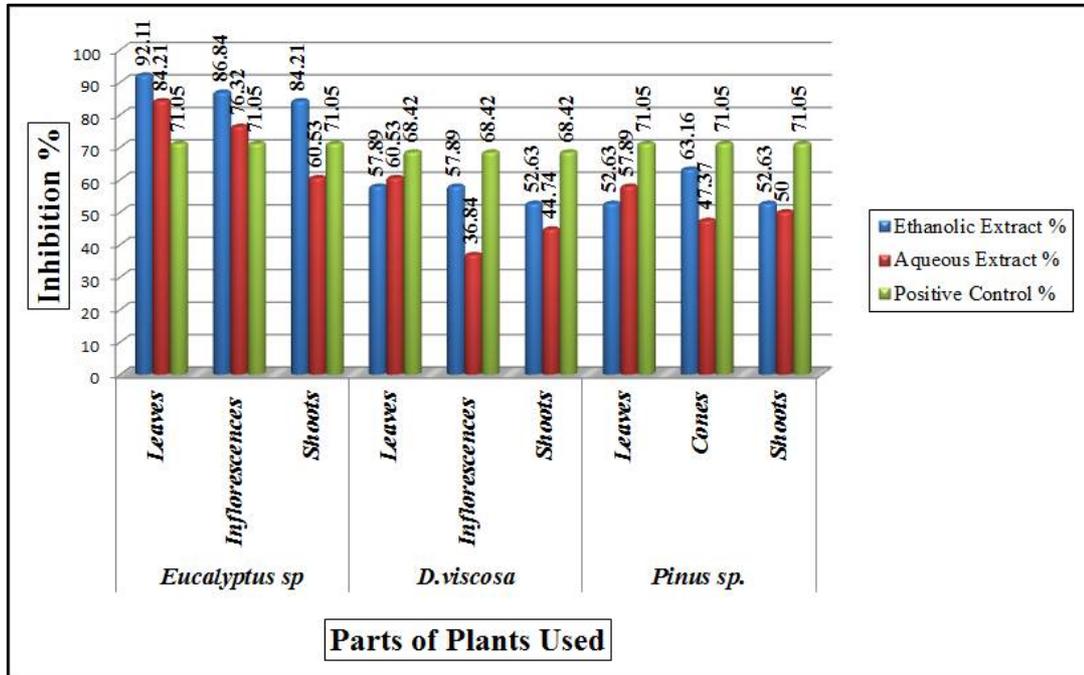
Fig.2. Inhibition percentage of three different partsextractfrom plant used against *Escherichia coli.*
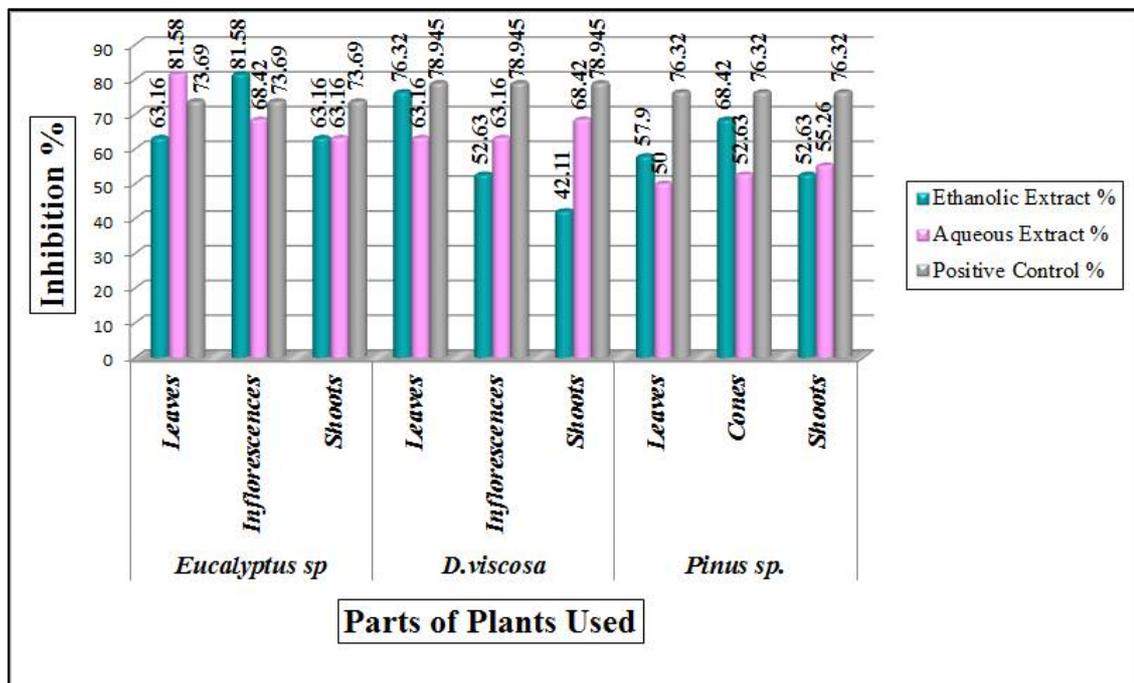
Fig.3. Inhibition percentage of three different partsextractfrom plant used against *Pseudomonas aeruginosa.*

RESEARCH ARTICLE

Variation of Chlorophyll, Carotenoid Content and Chlorophyll Fluorescence in Banana Cultivars as a Function of their Efficiency

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ABSTRACT

Banana are the principal basic fruit for 400 million people in tropical regions and represent the fourth important global food in terms of production value and worth after rice, wheat, and milk. The total production of crop exceeds 106 million ton/year. They are cultivated in more than 120 countries. The present study aims to analyse the variation of pigment composition and FTIR finger printing of four cultivars of banana. The investigation was carried out in the cultivars obtained from the natural habitats. The content of chlorophyll *a*, chlorophyll *b,* and total chlorophyll (Chl *a+b)* was measured and carotenoid content was determined on each cultivar. During the whole period of investigation the range of average content of chlorophyll in fresh weight was constant among the cultivars. The levels of total chlorophyll and carotenoids were determined in banana *(Musa sapientum* L.) leaves of four commercial cultivars, such as ethan, kappa, kadali and padathi i.e. 0.43-3.0 and 0.25-0.41 mg/g. In all the cultivars chlorophyll content was significantly higher. Similarly, the level of carotenoids among the different cultivars varied significantly and maximum of 0.41 mg/g as observed in padatti and minimum of 0.25 mg/g in kadali. The ratio chlorophyll to carotenoid pigments was also different among the cultivars. Fluorescence variables (Fo, Fm, and Fv) were greater in the cultivar padatti and least in kadali.

Key words: chlorophyll, carotenoids, cultivars, banana, fluroscence.

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Banana represents the largest herb, cultivated in most of the tropical countries. Generally, bananas are known as plantains and are mostly evolved from the edible varieties of two species *Musa acuminata* and *Musa balbisiana* [1]. Based on the nutritional values, bananas represent the world's promised agricultural crop. Bananas are rich in nutrients, starch, sugar and provitamin A and C, minerals such as potassium, calcium, sodium and magnesium. Further, bananas are nutritionally with low protein but relatively rich in carbohydrates, vitamins and minerals [2]

The plant pigmentation is one of the core research areas of botanists. The diverse pigment groups are located at different regions in plant parts. Flavonoids dominated almost most of the tissues; carotenoids are present in different parts of the plants such as leaves, roots, seeds, fruits and flowers. Anthocyanins or chlorophylls have specific cellular or subcellular location. Anthocyanins are usually found in epidermal cells of flower petals, whereas chlorophylls and carotenoids are in plastids in subepidermal photosynthetic cells of leaves. Similarly, betalains are water-soluble and appear in vacuoles [3]. Photosynthetic pigments in plants comprise chlorophylls a and b and these pigments forms the light harvesting complex of photosystem II, with consequent electron transport system. Pigments such as carotenoids are also found in plants, and are considered as additional components in the photosynthetic complex by providing photoprotection and stability of proteins present in the photosystem [4][5][6]. Plants experience different types of stresses viz; photo-oxidaive stress, deficiencies of minerals and drought during their yearly growth periods. Light stress is frequent under tropical conditions, and chlorophyll and carotenoid level are indicators of plant response to light intensity. Chlorophyll tends to be photooxidized at high irradiance and carotenoids can inhibit chlorophyll photooxidation, the relationship between chlorophyll and carotenoids may be used as a potential indicator of photooxidative damages caused by high irradiation [7].

Fluorescence variables used for analyzing the functioning of PS II include: Fo (initial fluorescence), Fm (maximum fluorescence) and Fv (variable fluorescence) and Fv/Fm ratio [8]. Fv represents the difference between Fm and Fo (Fv = Fm-Fo). The Fo parameter is the minimal fluorescence yield when all reaction centers are in the oxidized or open state. When leaves are briefly exposed to a saturating light level, all PS II centers are closed i.e., quinone A is reduced and a maximum yield of fluorescence (Fm) is observed. The Fv/Fm ratio is a fluorescence variable directly correlated with the physiological efficiency of the photosynthetic machinery. This ratio is been considered to be proportional to the quantum efficiency of PS II [9].

As photosynthesis is the basic process during which light energy is absorbed and converted into organic matter, the importance of the plant pigment chlorophyll (a and b forms) as an intermediary in transformation of the absorbed solar energy and its activity in the process of photosynthesis and synthesis of organic substances in plants are crucial. Therefore, this paper provides an overview of methods for monitoring the optical activity of chlorophyll molecules and methods (non-destructive and destructive) for quantification of chlorophyll in plants. These methods are used to estimate the effects of different stress factors (abiotic, biotic and xenobiotic) on the efficiency of photosynthesis and bioproductivity, aiming to assess the impact that these limiting factors have on the yield of various cultivars. Also, those methods for analysis of chlorophyll optical activity and/or content are appropriate for assessing the reaction of weed species to different agricultural practices (mineral nutrition, treatment by herbicides, etc.) and studies of different aspects of weed ecophysiology and their influence on crop harvest. The present study focused on the following objectives such as diversity of photosynthetic pigment concentrations and chlorophyll fluorescence in leaves of the four cultivars of banana, such as ethan, kappa, kadali and padatti.

MATERIALS AND METHODS

Plant materials

All the 4 banana cultivars were collected from different natural sites of Thiruvananthapuram district, Kerala.

Quantification of pigments

Chlorophylls

1 g of leaf sample was ground in a mortar and pestle with 20 ml of 80% acetone. The supernatant was collected. The pellet was re-extracted with 5 ml of 80% acetone each time, until it became colourless. All the supernatants were pooled and utilized for chlorophyll determination. The chlorophyll content in the 80% acetone extract was determined [10] . Absorbance was read at 645 nm and 663 nm in a Spectrophotometer [11].

Chlorophyll a (mg/g) = 12.7 A663-2.69 A645
Chlorophyll b (mg/g) = 22.9 A645-4.68 A663
Total Chlorophyll (mg/g) = 20.2 A645+8.02 A663

Total carotenoid content (TCC)

TCC was determined by visible absorption spectrophotometry at an absorbance of 450 nm using the absorption coefficient of β-carotene.

Fluorescence analysis

Fluorescence emission was assessed in fully expanded, *in situ* leaves between 9:00 and 11:00 am with a plant efficiency analyzer (PEA, MK2 - 9600, UK). After 15 min dark adaptation, each leaf disc was exposed to a saturation pulse of high light intensity (2250 mmol m^{-2} s^{-1}) for five seconds and fluorescence variables (Fo, Fm, Fv, Fv/Fm) were determined.

Statistical analysis

Data were analyzed by using the analysis of variance and the means compared by Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

Plant pigments are the key regulators of photosynthetic mechanism [12].However; these pigments were liable to degradation in plants when exposed to biotic or abiotic stress [13]. Chlorophyll content showed significant variation among the cultivars ($P < 0.05$). During the investigation, the mean content of chlorophyll in fresh matter of banana varied from 0.4316 in kadali to 3.01 in padatti cultivar (Table 1). Lefsrud et al reported that chlorophyll pigment in kale leaf tissue increased and then decreased in response to leaf age among the variants [14].

The recorded quantities of carotenoids level was 0.246 to 0.405/g compared with cabbages Brussels sprouts, green lettuce or spinach carotenoid showed an increased level[15] but was at par with to that of parsley[16] and in dill [17] . Similarly, in cabbage the content of carotenoids is found in highest level in the oldest leaf buds [18] .However, decrease in β- carotene with the course of maturation among lettuce was noted [19]. In the present analysis, it was observed that the carotenoid contents were highest in padatti. The level of carotenoid variation was significant among the cultivars ($P < 0.05$) [20].Variation in chlorophyll and carotenoids among cultivars were reported in response to harvesting dates in Lactuca [21]. Similar trend of increase in carotenoids with harvesting dates of Kale is also reported by Korus and Kmiecik[22].The ratio of chlorophyll and carotenoid pigments varies from 3.93 mg/g in the case of ethan cultivar to 11.06 for the cultivar padatti. It is possible, that the usual role of protective screen of the carotenoid pigments may be taken over by other pigments present in large quantities in the leaves as well as in the shoots.

The lower content of chlorophyll b compared to chlorophyll a in padatti is against the concepts of the classic physiology, something particular of the sun plants i.e., the banana was planted mostly in intense sunlight. It is possible that the cultivated banana need a much consistent protection screen since they bloomed better there than in the shade as noticed in papaya [23].Physiologically, the chlorophyll and carotenoid pigments selectively absorb the radiations of light. Chlorophyll a presents a maximum absorption of the radiations with a wavelength of 700 and 435 nm respectively whereas; chlorophyll b has a maximum absorption of the radiations with the wavelength of 644 and 453 nm respectively. Meanwhile, the carotenoid pigments have a maximum absorption of the radiations within the wavelengths 400-480 nm. With this reality as a starting point, the current banana cultivars have more intense absorption of blue radiation [24] [25].

The content of chlorophyll and levels of other leaf biochemical constituents can be used as indicators of crop physiology under conditions of deficiency of nutrition [26]. Such deficiencies leading to chlorosis can be alleviated through application of nutrients, thereby improving crop yields and the quality [27]. Application of minerals also promotes better utilization of assimilates in metabolic and growth processes.Chlorophyll fluorescence is an ideal technique to evaluate the functioning of photosystem II [28]. Likewise, in the present study evaluated the photosynthetic efficiency among selected banana cultivars.Chlorophyll fluorescence analysis such as Fo and Fm were significantly higher in padatti when compared with other cultivars (Table 2). Further, the values of Fv/Fm, Y, Qp and QN were significantly higher in padatti when compared to others. The cultivar kappa showed an optimal value. Meanwhile, kappa the ratio of Fv/Fm was low i.e., 0.645. The values were statistically significant at 1% level. Morphological and physiological characteristics of *in vitro* or *ex vitro* strawberry plants were analyzed [29] and reported similar Fv/Fm values. In the present analysis, the Fv/Fm ratios were between 0.697 and 0.819. Generally, chlorophyll a fluorescence technique is considered as a physiological marker of plant response to abiotic (environmental) or pathogenic stress. The photosynthetic potentiality of two strawberry cultivars was evaluated in terms of the chlorophyll fluorescence for accessing the drought stress in plants [30] [31]. Desiccation and rehydration stress on chlorophyll fluorescence characteristics in strawberry cultivars were correlated and screened for heat tolerant Fragaria accessions [32,33]. Fv and Fv/Fm parameters are the proven markers of thermotolerance of photosynthetic pathway in *Fragaria* species exposed to three temperature conditions were also reported and the decreasing level of Fm, Fv/Fm with intensity of light has noticed [34, 35].The stomatal conductance with photosynthetic efficacy of six walnut cultivars from the national assortment were related and the decrease in Fo suggests the loss of energy transfer from antenna complex to reaction centers of photosystem [36, 37]. Qp indicates the proportion of inactivated photosystem II reaction centers [38] [39]. Similarly, the salt-induced decrease in this attribute may be due to the separation of light harvesting complex II from the PSII reaction center [40]. Further, the reduction in Y implies impairment of the ability of plants to repair the damage if any to photosystem II [41]. Fv/Fm value corresponds to the decrease in maximum fluorescence (Fm) displaying the disruption of antenna complex of PSII, enhancement in dissipation of energy and destruction of photosystem II reaction center [42].

In addition, the decrease in Fv/Fm indicates that regaining activity of RUBP could have been disrupted [43]. So, it tempts to say that increase in photorespiration in C_3 plants is the main reason for the enhancement in the rates of electron transport [44]. Increase in NPQ exhibits an adaptive energy dissipation process protecting the photosynthetic apparatus against photo-damage [45]. Kitao et al [46] examined the effects of intense light in combination with high temperatures on the photosynthetic system in four dipterocarp species and also observed marginal difference in Fv/Fm ratio values among species. The increased reversible photoinhibition observed in sun exposed leaves probably reveals a dynamic regulatory process protecting the photosynthetic apparatus from severe damage by high light [47]. Fv/Fm ratio 0.83 has been reported for unstressed plants [48]. In the banana cultivars, the values were almost near 0.8 revealing the unstressed nature of the plants.

CONCLUSION

The banana cultivars show an overall similarity in terms of photosynthetic pigment and their efficiency. Chlorophyll a is predominant, the chlorophyll a and b ratio varying between 1.1 to 2.05 similar to the normal photophysiology as being specific to plants. Chlorophyll and carotenoid pigments selectively absorb the light radiations for carrying out photosynthesis. The carotenoid pigments content is low for kadali cultivar (0.246 mg/g fresh material), while for the others it ranges within normal limits, with the values of 0.31 to 0.4 mg/g per fresh material. Most probably, together with the carotenoid pigments acting as protectors of the chlorophylls, there are other pigments such as anthocyanin present in large quantities in plants use different strategies with respect to the mechanism of accumulation and uses of photosynthetic pigments. Fv/Fm values were also range between 0.645 to 0.81. Further studies are warranted to analyze the photosynthetic efficiency with different environmental parameters among the banana cultivars.

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Table 1. Pigment analysis of banana cultivars in terms of chlorophyll and carotenoids

	Ethan	Kappa	Kadali	Padatti
Chlorophyll a (mg/g)	0.85	0.93	1.43	3.01
Chlorophyll b (mg/g)	0.68	0.84	1.01	1.47
Total chlorophyll (mg/g)	1.53	1.77	2.44	4.48
Chlorophyll a/b	1.25	1.11	1.42	2.05
Carotenoids (mg/g)	0.389	0.31	0.246	0.405
Chlorophyll/Carotenoids	3.93	5.70	9.92	11.06

Table 2. Chlorophyll fluorescence parameters in banana cultivars Ethan, Kappa, Kadali and Padatti

Characters	Ethan	Kappa	Kadali	Padatti
Minimal fluorescence of light adapted leaf [Ft]	289	355	301	377
Minimal fluorescence of dark adapted leaf [Fo]	178	217	185	120
Non-photochemical fluorescence [Qn]	0.201	0.248	0.244	0.289
Maximum quantum yield of primary	0.697	0.645	0.71	0.819

photochemical reaction (Fv/Fm)				
Maximum fluorescence at steady state [Fms]	488	502	542	589
Photochemical fluorescence quenching [Qp]	0.7	0.734	0.8	0.81
Maximum fluorescence [Fm]	589	612	638	665
Fluorescence at steady state of light adapted leaf [Fs]	234	266	274	297
Quantum yield of electron transport [Y]	0.459	0.5	0.543	0.588
Electron transport rate [ETR]	11	20	23	27
Non-photochemical chlorophyll fluorescence quenching [NPQ]	0.31	0.27	0.26	0.24

RESEARCH ARTICLE

Spatial and Temporal Analysis of Rainfall Variability in Amaravathi Basin of TamilNadu

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ABSTRACT

Water is a key anxiety for life, any development and planning activities. As India is a monsoon reliant country for its major portion of rainfall, it is necessary to analyze the occurrence and distribution of rainfall. In this regard, a detailed study of monthly, seasonal and spatial variation of rainfall for the study area has been carried out. Therefore, the present study deals the rainfall characteristics of the Amaravathi basin of Tamil Nadu State, which includes the spatial distribution and variability through different seasons, precipitation ratio and frequency occurrences. The study is based on 43 years of the monthly rainfall data for 33 rain gauge stations. While analyzing the long term average of monthly and annual rainfall, the annual rainfall of the basin is 978.82 mm, of which the winter, summer, southwest and northeast monsoon record 18.27, 148.42, 411.46 and 388.94 mm respectively. The station Anaimalai and Valparai receives the highest rainfall of around 3500 mm whereas Sulur records the lowest of 403 mm. The annual variability ranges from 19.67 percent to 44.50 percent. The distribution of rainfall revealed that the basin is more influenced by North east monsoon than south west monsoon.

Keywords: Annual and seasonal rainfall, rainfall variability, precipitation ratio and frequency.

INTRODUCTION

Rain is a vital natural phenomenon which can persuade the human life. In fact, the rain that falls into certain area can viewed as a result of many factors, which can divided into three segments: space, time and other factor. Jagannadhasarma (2005) has analysed the rainfall pattern of the coastal zone of Krishana Godavary River Basin Andhra Pradesh, India. He has made analysed the annual, monsoon and non monsoon rainfall and spatial and frequency distribution of rainfall intensity and G.Vennila (2007) has analysed rainfall variation analysis of Vattamalaikarai subbasin, Tamil Nadu, India. She has interpreted monthly, seasonal variation, intensity and frequency of rainfall.Although the annual average rainfall of Amaravathi basin exceeds 980 mm, there is a wide gap among rainfalls, which are not evenly distributed spatially and temporally. Temporally, most of rainfalls are distributed between June to November, about 54% of the annual rainfall, and the rainfall of flood season is significantly different from that of dry season. Spatially, the mountainous area has more rainfalls than plain area, and the slopping land would cause the water conservation ability of watershed to drop sharply.

To prepare a proper crop and water management plan and to design irrigation drainage, erosion control and flood control structure, the knowledge of rainfall pattern, total rainfall, its distribution and daily/monthly or annual maximum and minimum rainfall are essential. The present study made an attempt to gain knowledge about the spatial and temporal distribution of rainfall and its trend in Amaravathi basin. This includes the spatial variation, variability through different seasons, precipitation ratio and frequency.

Study area description

The River Amaravathi is one among the main tributaries of the river Cauvery in its mid reach. It raises from Naimakad at an elevation of 2300 m above MSL in the Western Ghats (Anaimalai) in Idukki district of Kerala state. It flows towards north east till the confluence with the river Cauvery on its right bank. Throughout its course of 256 km, the Amaravathi receives a number of small streams. The Amaravathi basin lies between the latitudes $10^{\circ} 06' 51''$ N and $11^{\circ} 02' 10''$ N and longitudes $77^{\circ} 03' 24''$ E and $78^{\circ} 13' 06''$ E. It has a catchment area of 8280 km² constituting four districts *viz.,* Coimbatore, Dindigul, Karur and Tiruppur in Tamil Nadu. The basin is covered by survey of India top sheets 58F 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 58J 1, 2, 11, 13 and is bounded by the Vaigai basin on the south, Noyyal basin on the north, Parambikulam and Aliyar basin on the west and Ayyalur basin and Kadavur hills on the east. Thirty six rain gauge stations were lying in the study area (Table 1 and Figure 1). The study area falls in the sub-basin of Cauvery River basin.

Climate

The basin has four distinct seasons, south west monsoon from June to September, north east monsoon from October to December, the winter season from January to February and summer from March to May. The highest monthly mean of daily maximum temperature is around 40.6ºC in April and the lowest monthly mean of daily minimum temperature is 22.4ºC during January at Coimbatore (Peelamedu). The maximum average wind speed at Coimbatore is 32.6 kmph in the month of June. The minimum average wind speed is 9.5 kmph in November at Coimbatore (Peelamedu). The mean relative humidity is low in dry weather and high in the monsoon season. The sky is very cloudy during the monsoon season and is lightly clouded during non-monsoon season. The monthly potential evapotranspiration value varies from as low as 66 mm in November to as high as 130.9 mm in May.

MATERIALS AND METHODS

The daily rainfall data for the period of 1971-2014 have been collected from Public works Department, Chennai and tabulated as to calculate the monthly and seasonal rainfall for the respective rain gauge stations. The average annual

rainfall of the study area is calculated for the period of forty four years (1971-2014) from the records of the thirty six rain gauge stations. To achieve the framed objectives, the collected rainfall data are categorized into four major seasons viz., Winter, summer, South west, North east. Finally, the data are interpreted by preparing various charts and diagrams using geographical information system.

Trend Analysis

The magnitude of the trend in the seasonal and annual series was determined using the Sen's estimator (Sen, 1986) and statistical significance of the trend in the time series was analysed using Modified Mann-Kendall (MMK) test (Gajbhiye, 2015). The Z values for seasonal and annual rainfall clearly exhibit the trends in spatial dimension over the study area (Sushant *et al.*,2015). Sen's estimator (Partal, 2006) widely used for determining the magnitude of trend in hydro-meteorological time series (Kumar, 2010). In this method, the slopes (T_i) of all data pairs are first calculated by

$$T_i = \frac{(X_j - X_K)}{(j - k)} \quad \text{For } i = 1, 2, 3\ldots\ldots$$

where X_j and X_k are data values at time j and k (j>k) respectively. The median of these N values of T_i is Sen's estimator of slope which is calculated as

$$b = \begin{cases} T_{\frac{N+1}{2}} & \text{N is odd} \\ \frac{1}{2}\left(T_{\frac{N}{2}} + T_{\frac{N+1}{2}}\right) & \text{N is Even} \end{cases}$$

A positive value of b indicates an upward (increasing) trend and a negative value indicates a downward (decreasing) trend in the time series. To ascertain the presence of statistically significant trend in hydrologic climatic variables such as temperature, precipitation and stream flow with reference to climate change, nonparametric Mann-Kendall (MK) test had been employed by a number of researchers (Singh ,2008). The MK method searches for a trend in a time series without specifying whether the trend is linear or non-linear.
The statistics (S) is defined as

$$S = \sum_{i=1}^{N-1} \sum_{J=1+1}^{N} sgn (X_j - X_k)$$

Where, N is number of data points. Assuming $(X_j - X_k) = \theta$, the value of *sgn(θ)* is computed as follows:

$$Sgn (\theta) = \begin{pmatrix} 1 & f & q > 0 \\ 0 & f & q = 0 \\ -1 & f & q < 0 \end{pmatrix}$$

This statistics represents the number of positive differences minus the number of negative differences for all the differences considered. For large samples (N>10), the test is conducted using a normal distribution (Helsel, 1992) with the mean and the variance as follows:

$$E(S) = 0$$

$$Var(S) = \frac{N(N-1)(2N+5) - \sum_{k=1}^{n} t_k(t_k - 1)(2t_k + 5)}{18}$$

Where, n is the number of tied (zero difference between compared values) groups, and t*k* is the number of data points in the *k*th tied group. The standard normal deviate (Z-statistics) (Hirsch, 1998) is then computed as

$$Z = \begin{cases} \dfrac{S-1}{\sqrt{Var(S)}} & f & S > 0 \\ 0 & f & S = 0 \\ \dfrac{S+1}{\sqrt{Var(S)}} & f & S < 0 \end{cases}$$

If the computed value of %Z %> $z_{\alpha/2}$, the null hypothesis (Ho) is rejected at a level of significance in a two-sided test. In this analysis, the null hypothesis was tested at 99 % and 95% of confidence level.

Statistical procedure for rainfall variability Analysis

Variability of rainfall can be expressed by statistical parameters of coefficient of variability (CV). Co efficient of variation (CV) is a statistical measure of how the individual data points vary about the mean value. A greater value of CV is the indicator of larger spatial variability, and vice versa. According to Hare (1983), *CV* is used to classify the degree of variability of rainfall events as less, moderate and high. When *CV* < 20% it is less variable, *CV* from 20% to 30% is moderately variable, and *CV* > 30% is highly variable. Areas with *CV* > 30% are said to be vulnerable to drought. Australian Bureau of Meteorology, (2010) used rainfall index for analysis of rainfall variability in Australia given as (P90 - P10)/P50 where, Pn is nth percentile of the data. The threshold value is given in the Table 2.In this study, annual rainfall variability has been analyzed for 36 stations of the Amaravathi river basin using co efficient of variation. The monthly rainfall charts and annual variability charts are also prepared.

Spatial analysis

The spatial distribution of rainfall was analysed using the Inverse Distance Weighted (IDW) interpolation method. Pingale *et al.,* (2014) mentioned that IDW interpolation technique is an effective method and having the assumption that the variables at a point to be predicted are similar to the values in the nearby observation stations. The results of the studies showed that IDW method is a good tool for displaying the spatial distribution of rainfall**.**

RESULTS AND DISCUSSION

Rainfall Analysis

The average annual rainfall of Amaravathi basin is 978.82 mm. The fig 3 shows the distribution of annual rainfall over the basin. Some parts of Coimbatore, Tiruppur and Karur districts received less rainfall i.e. 600 mm while comparing with other part of the basin. Anamalai block and Kodaikanal receives the highest rainfall i.e. greater than 2000 mm. less amount of rainfall received by sulur block i.e. 404 mm. So, there is a wide gap among rainfalls, which are not evenly distributed spatially and temporally. Anaimalai rain gauge station shows the maximum average annual rainfall of 3500 mm followed by Valparai (2500 mm).

Seasonal distribution of rainfall

In seasonal distribution of rainfall south west monsoon contribute high rainfall i.e., 28% of total rainfall followed by north east rainfall (26%). Most of the rainfall is distributed between June to November, about 54% of the annual rainfall (Fig 4).The north east monsoon is very high in southwest side of Amaravathi basin i.e. Kodaikanal and Anaimalai having NE rainfall of 761and 520 mm respectively. The lowest NE rainfall observed in Northwest and north east side of the basin. The blocks Sultanpet, Sulur, Dharapuram, K.Paramathy and Karur blocks are having very low rainfall(less than 250 mm) during North east monsoon (Fig 5). The South-west monsoon is very high in Anaimalai block having 2600mm followed by Kodaikanal (465 mm) and Natham (420 mm) respectively. The lowest south-west rainfall observed blocks of Pongalur, Kundadam, Sultanpet, Sulur, Dharapura (Less than 200 mm) (Fig 6).

Temporal spread of rainfall in Amaravathi basin

The average annual rainfall of Amaravathi basin is 978.82 mm. The highest amount of rainfall received during 1977 (1419 mm) followed by 2005(1273 mm) (Fig 7). The lowest amount of rainfall received during 2012 (583 mm) followed

by 1982 (641 mm). From rainfall variability analysis (IMD method), 8 excess years, 29 Normal years, 7 Deficit years were observed.

Trend of annual and seasonal mean rainfall

The trend analysis was done using the Mann-Kendall test. Mann-Kendall test is a non-parametric test which is commonly used for hydrological data analysis. The results of the Mann-Kendall analysis to deduct the trend in annual and seasonal rainfall for the study area are given in Table3. And their analyzed graphical representations are shown in Fig.8 to 12 with their Zc statistics value below The increasing trend of rainfall was observed in North East, Winter and summer seasons with significant increase during winter (α = 0.05). The decreasing trend was observed in south west monsoon with 0.1 % of level of significance. Overall Annual rainfall showed increasing trend.

Rainfall Variability Analysis

Australian Bureau of Meteorology, 2010 stated that rainfall index lies below 0.5 then it shows less variability of rainfall. From the analysis the rainfall index was worked out as 0.41. Hence the annual variability of Rainfall is very less in Amaravathi basin.In Indian condition; CV is the best indicator to explain the variability of rainfall. The percentage of CV varies from 19.67 to 44.49 %. High value of CV indicates the greater the variability in rainfall. The average value of CV in Amaravathi basin is 32.13 %. According to Hare (1983), if the CV> 30%, then the rainfall is highly variable.

CONCLUSION

The average annual rainfall of Amaravathi basin is 978.82.34 mm. From the distribution of annual rainfall analysis over the basin it was observed that, some parts of Coimbatore, Tiruppur and Karur districts received less rainfall i.e. 600 mm while comparing with other part of the basin. Anamalai block and Kodaikanal receives the highest rainfall i.e. greater than 2000 mm. less amount of rainfall received by sulur block i.e. 404 mm. So, there is a wide gap among rainfalls, which are not evenly distributed spatially and temporally. Anaimalai rain gauge station shows the maximum average annual rainfall of 3500 mm followed by Valparai (2500 mm).The north east monsoon is very high in southwest side of Amaravathi basin i.e. Kodaikanal and Anaimalai having NE rainfall of 761and 520 mm respectively. The lowest NE rainfall observed in Northwest and north east side of the basin. The blocks Sultanpet, Sulur, Dharapuram, K.Paramathy and Karur blocks are having very low rainfall(less than 250 mm) during North east monsoon. The South-west monsoon is very high in Anaimalai block having 2600mm followed by Kodaikanal (465 mm) and Natham (420 mm) respectively. The lowest south-west rainfall observed blocks of Pongalur, Kundadam, Sultanpet, Sulur, Dharapuram (Less than 200 mm). The increasing trend of rainfall was observed in North East, winter and summer seasons with significant increase during winter (α = 0.05). The decreasing trend was observed in south west monsoon with 0.1 % of level of significance. Overall Annual rainfall showed increasing trend. The percentage of CV varies from 19.67 to 44.49 %. High value of CV indicates the greater the variability in rainfall. The average value of CV in Amaravathi basin is 32.13 %. According to Hare (1983), if the CV> 30%, then the rainfall is highly variable.

ACKNOWLEDGEMENT

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Table 1. Location of Rain Gauge stations in Amaravathi Basin

Station name	Latitude	Longitude	Station name	Latitude	Longitude
Peranai	10.08889	77.85833	Palani	10.4533	77.5144
Vedasandur	10.53889	77.96806	Anaipalayam	10.8756	77.9597
Dindugal	10.37444	77.99361	Virupatchi	10.4664	77.6986
Kuthiraiyar dam	10.38694	77.36667	Marudhanadhi dam	10.25	77.6833
Karur	10.95833	78.07833	kodangnar dam	10.5944	77.9731
Kankeyam	11.00111	77.56222	Kudadam	10.8461	77.4353
Palladam	10.99472	77.28222	Pongalur	10.9728	77.3814
Aliyar nagar	10.48639	76.96722	Negamam	10.7444	77.1031
Sultanpet	10.87528	77.19639	Pollachi	10.6572	77.0108
Udumulpet	10.57361	77.24389	Chatrapatti	10.3583	77.975
Thirupur	11.10472	77.34389	Kamachipuram	10.5125	77.8619
Komaralingam	10.48806	77.35	Nilakottai	10.1625	77.8542
Kodaikanal	10.22861	77.4625	Anaiamali	10.3019	76.9464
Amaravathi Nagar	10.42528	77.26056	Sarkarpathi	10.48	77.8706
K paramathy	10.9575	77.91083	Valparai	10.3344	76.9625
Aravankurichi	10.77333	77.91	Vellakovil	10.9472	77.7108

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Mulanur	10.79306	77.70667	Dharapuram	10.7322	77.5292
Natham	10.11111	78.12056	Palaviduthi	10.6469	78.2383
Nilakottai	10.16639	77.8525	Kodumudi	11.0814	77.8858

Table 2. Rainfall variability index and Coefficient of variation classes

Australian Bureau of Meteorology, (2010)		Hare (1983)	
Class	Index	CV (%)	Class
Very high	1.50-2.00	>30	Highly variable
High	1.25-1.50	20-30	Moderate
Moderate to high	1.00-1.25	<20	Low
Moderate	0.75-1.00		
Low to moderate	0.50-0.75		
Low	<0.50		

Table 3. Mann-Kendall's test result

Time series	Test Z	Significance	Q
SW	-1.87	+	-3.045
NE	0.84		1.346
Winter	2.06	*	0.186
Summer	1.41		0.759
Annual Rainfall	-0.84		-2.490
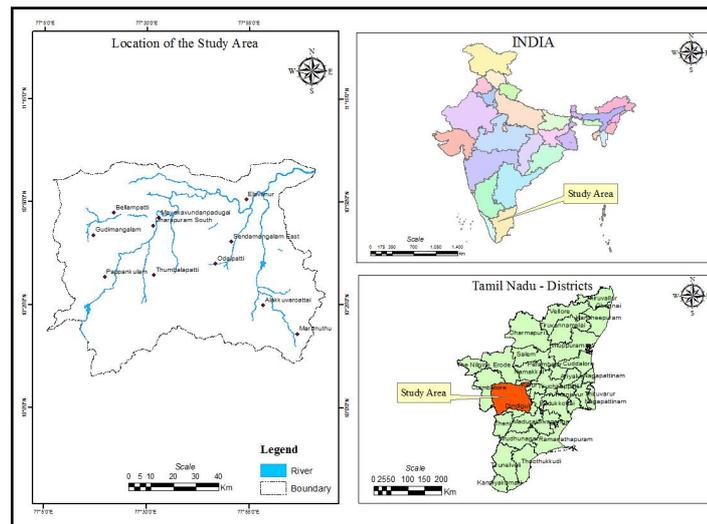
Fig.1. Location of the study Area

11151
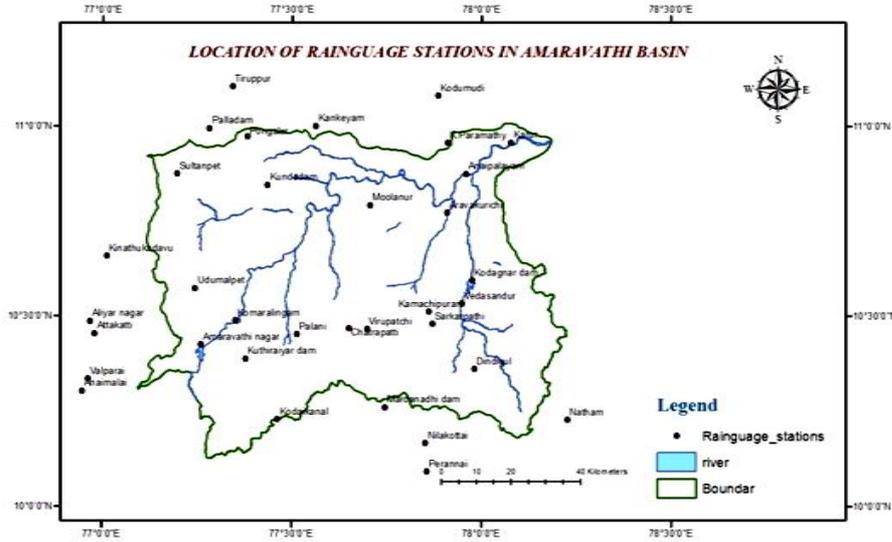
Fig 2. Location map of rain gauge stations in the study area
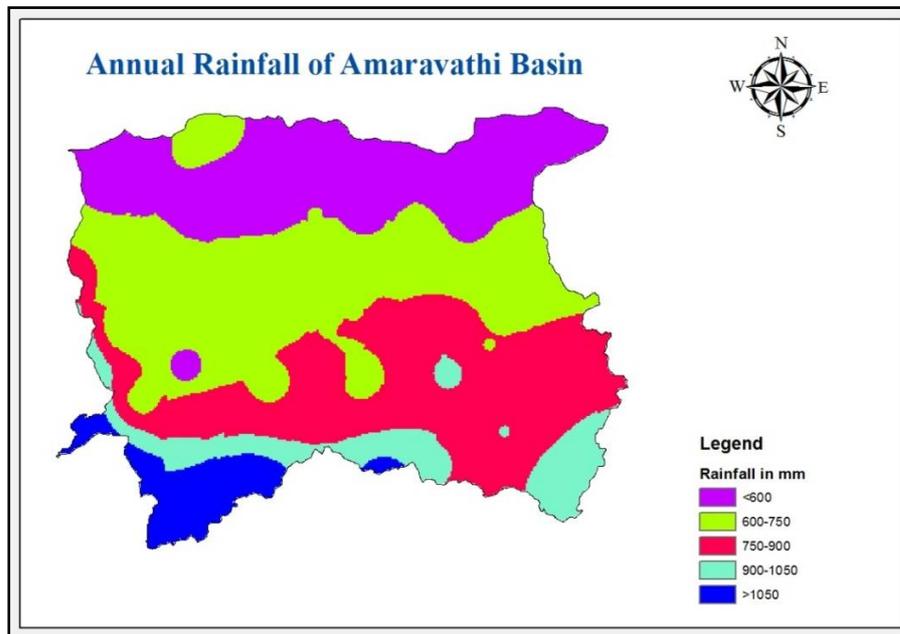
Fig.3. Annual Rainfall of Amaravathi basin

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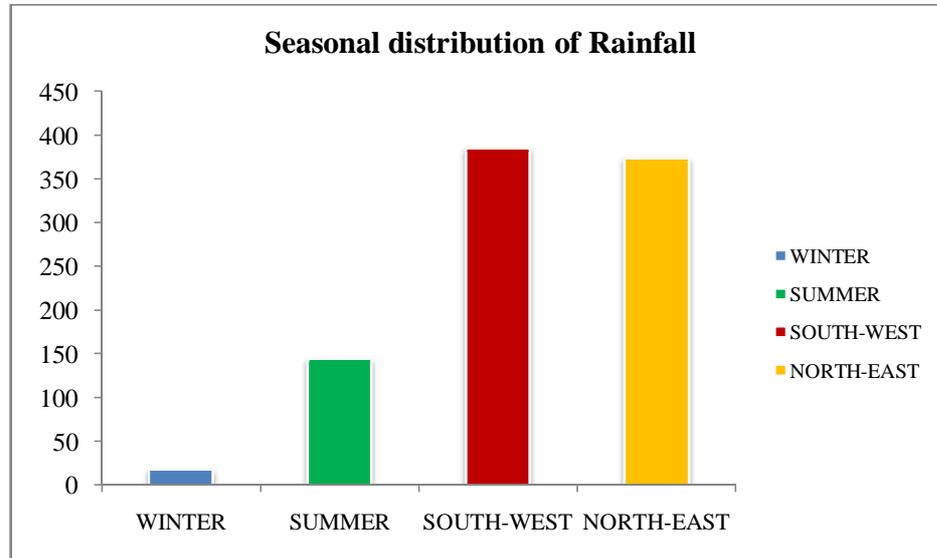
Fig 4. Seasonal distribution of rainfall
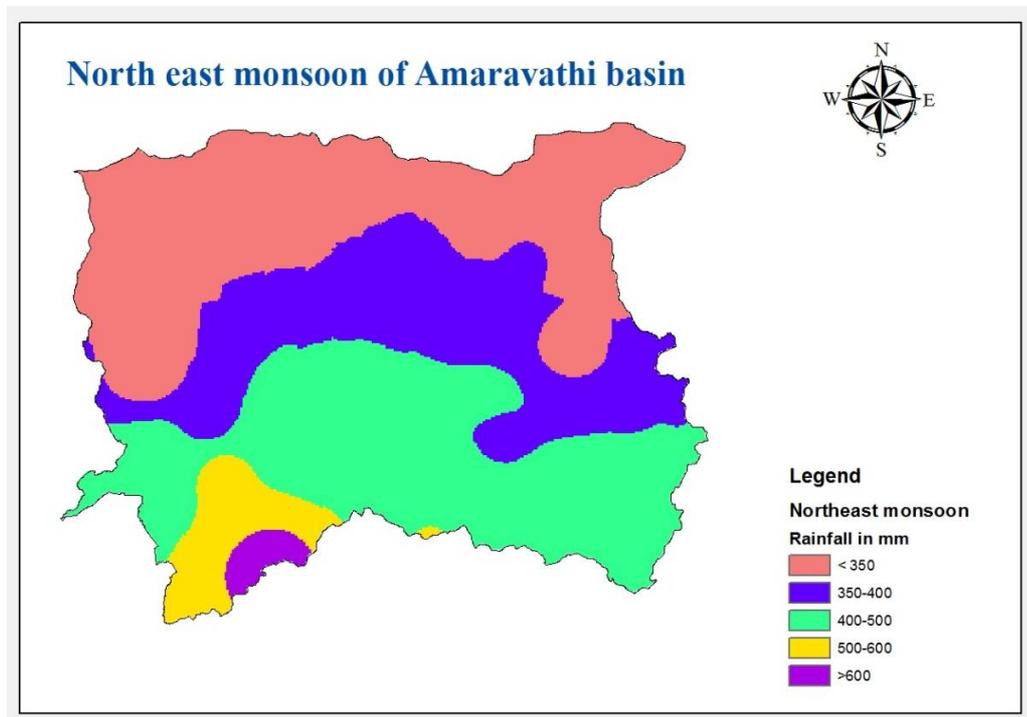
Fig.5. North East Monsoon of Amaravathi basin

Thangamani and Raviraj
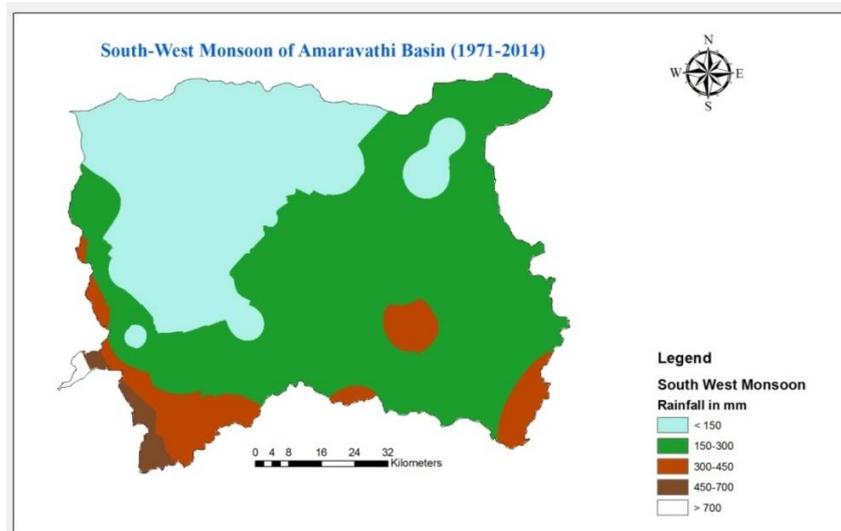
Fig.6. South-west Monsoon of Amaravathi basin
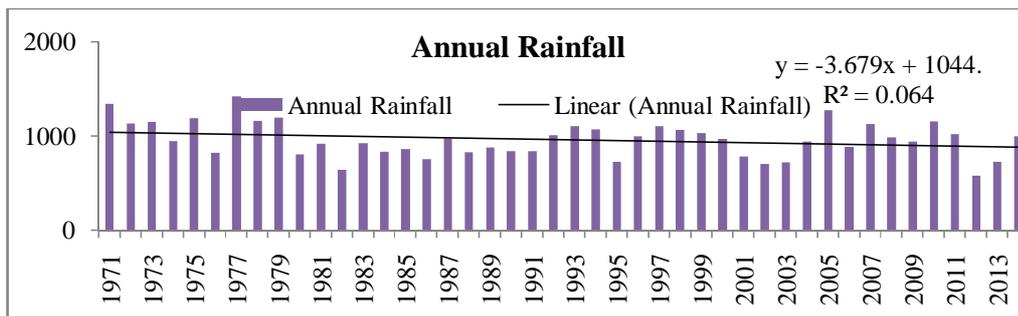
Fig 7 Trend of season wise Rainfall
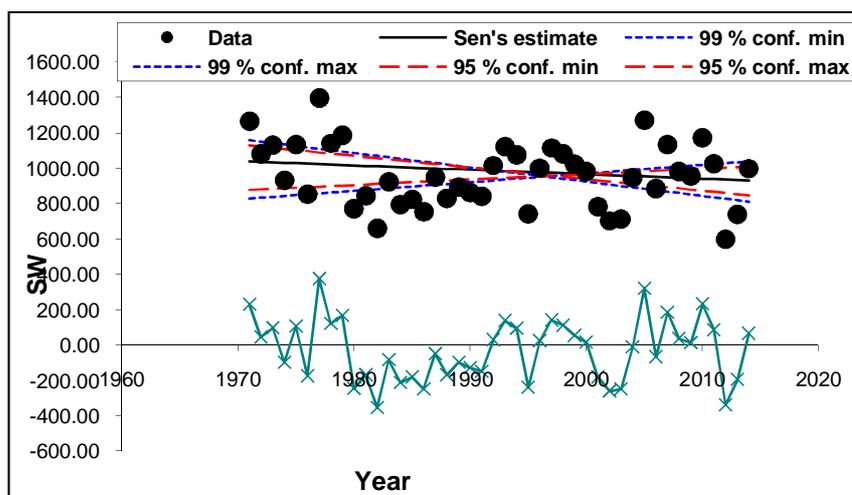
Fig 8.Trend of South West monsoon
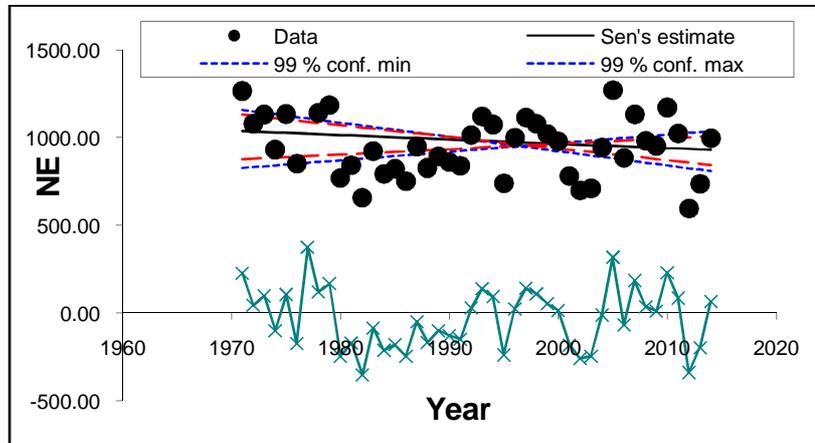
Fig 9.Trend of North East Monsoon
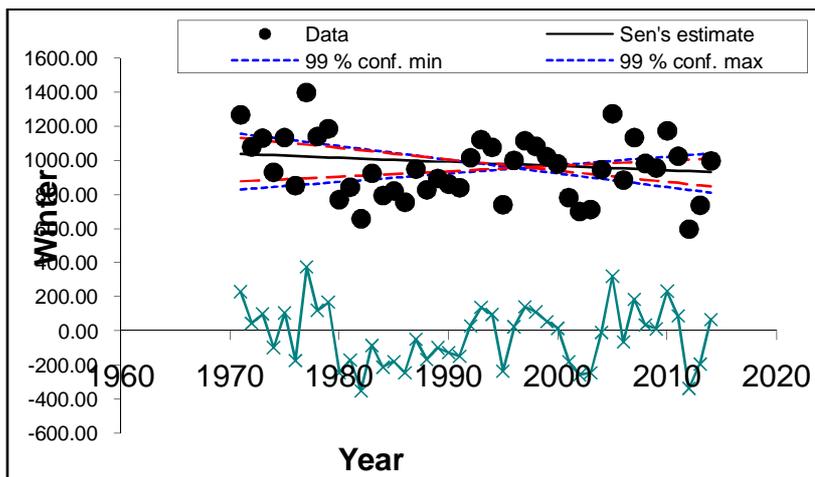
Fig 10.Trend of Winter
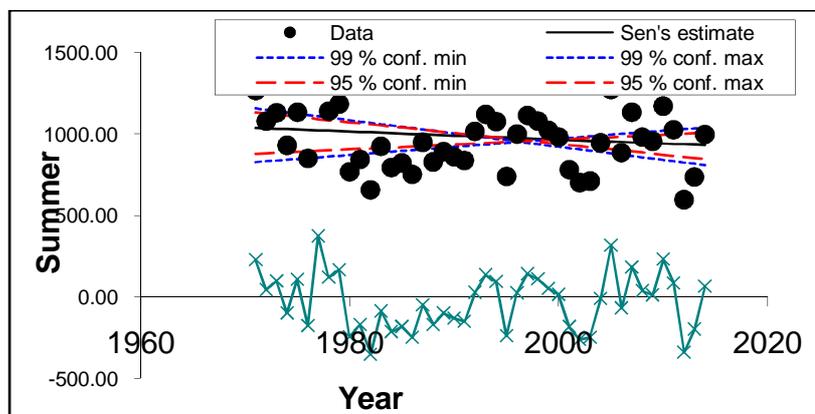
Fig 11.Trend of summer
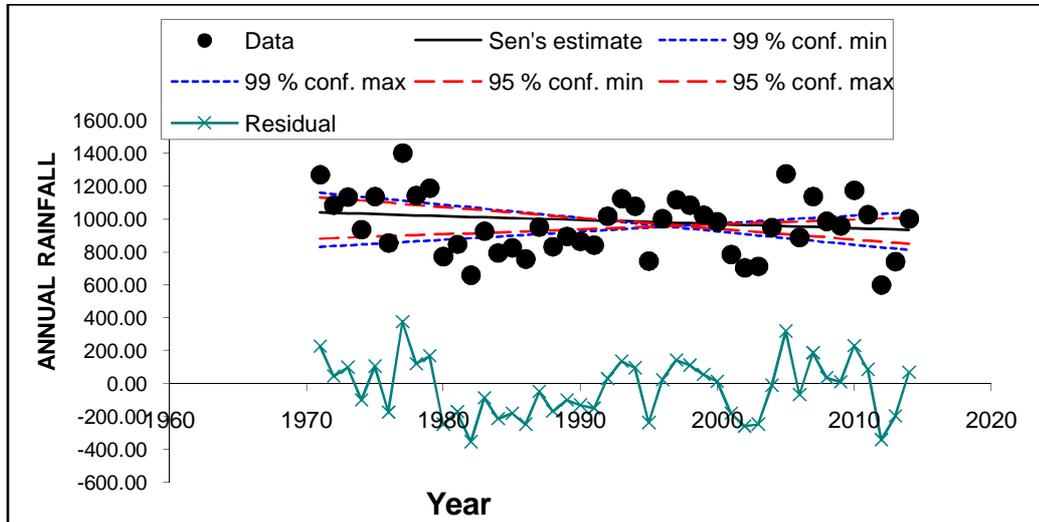
Fig.12 Trend of Annual Rainfall
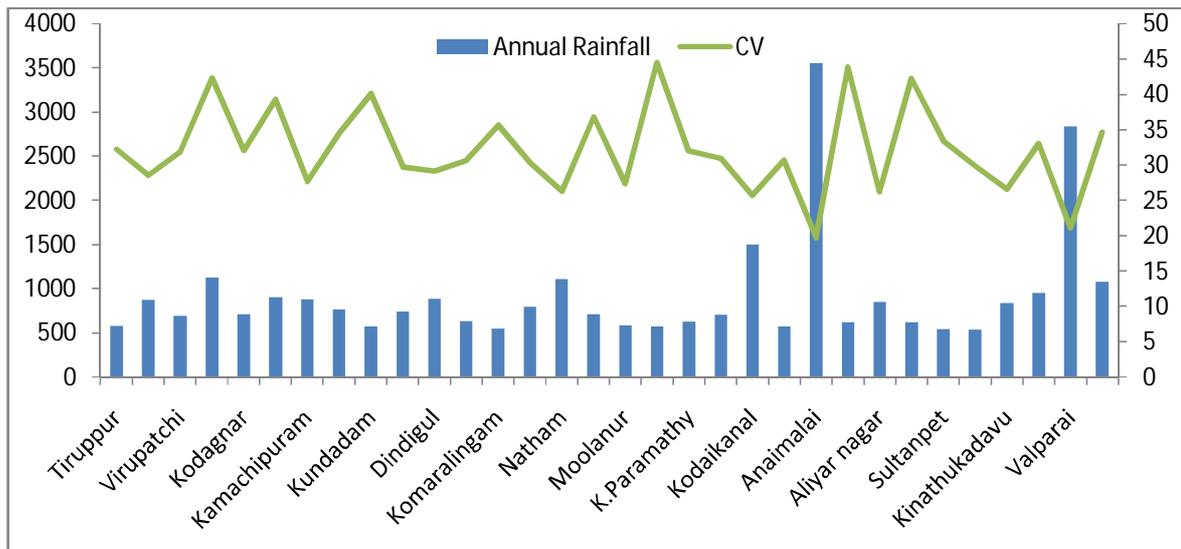
Fig 13 Station wise coefficient of variation in Amaravathi basin

RESEARCH ARTICLE

A Novel Phage based Formulation for the Biocontrol of the Pathogen *Pseudomonas aeruginosa* Causing Soft Rot in Onion (*Allium cepa* L.)

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ABSTRACT

Pseudomonas aeruginosa is a Gram-negative soil organism and the causative agent of bacterial soft rot in higher plants like Arabidopsis thaliana and Lactuca sativa. Bacterial rot of onion in the field and storage has been a major problem in onion growing areas all over the world. The rise of multidrug resistant strains has necessitated renewed interest in lytic bacteriophages. The impracticality of phage as biocontrol agents because of the phage-resistant bacteria that develop at high phage:bacterium ratios, the instability and reduced activity of the phage under *in vivo* conditions led to combination therapies involving other antimicrobial agent or phage cocktail instead of single phage. In the present study, a virulent soft rot pathogen *Pseudomonas aeruginosa* OB-6 isolated from the infected field in Cuddalore District was made to interact with OP-3 Lytic bacteriophage isolated from the rhizosphere soil of the infected field of Coimbatore. Based on the broad host range, optimum latent period and higher burst size the OP-3 Lytic bacteriophage was selected for the study among the other phage isolates. The antimicrobial properties of fatty acids prompted us to study on the combination of Linoleic acid with Bacteriophage. *In silico* analysis and *in vitro* experiments revealed that Linoleic acid can interrupt the AHL signal molecule synthesis and control the expression of the virulent genes regulated by Quorum sensing. To ensure activity and survival of the phage under *in vivo* conditions, protective formulation is inevitable. An attempt was made to synthesize Linoleic acid enriched Liposome vesicle for delivery of Bacteriophage. The results revealed that the antibacterial activity of liposomal bacteriophage was more compared to free phage.
Keywords: *Pseudomonas aeruginosa*, lytic bacteriophages, Linoleic acid, liposomes, soft rot, onion

INTRODUCTION

Onion (*Allium cepa* L.) is an important vegetable crop for internal consumption and also a highest foreign exchange earner among the other fruits and vegetables in India. Onion extract has been reported to be effective in cardiovascular disease and also possess antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory, and prebiotic activities [1]. In terms of area and production of Onion, India ranks second after China. Productivity in India is however very low at around 17.01 metric tonnes per hectare and holds only fifteenth position among the eighteen major onion producing countries in the world [2]. Thus there is a wide gap between the yields obtained in India and other developing countries reflecting the huge scopes to increase the yield in India. One of the major reasons for lower productivity of onion in India could be attributed to the susceptibility of onion to pests and diseases. Annual production and storage losses in garlic and onions as result of diseases can range from a trace to 50% or more depending upon the location, environment, and the causal agent involved [3]. Most onion diseases begin on plants growing in the field and continue to develop on the bulbs during storage and transit, when symptoms become evident. Soft rot is one of the significant spoilage diseases of vegetables caused by both pectinolytic bacteria and fungi that break down the pectic substances (pectin) of the middle lamella. Abd-Alla, M.H. *et al.* (2011) first reported on the soft rot disease caused by *Pseudomonas aeruginosa* in storage Onions. The effectiveness of this organism in causing infection is multifactorial, involving both secreted and cell-associated bacterial products, such as proteases, lipase, polygalacturonase, and pyocyanin. Expression of these virulence factors appears to be controlled by signal molecule dependent cell-cell communication system known as quorum-sensing. No efficient antibiotics or chemicals or bio control methods are available to manage losses to bacterial soft rot. Hence there is a strong need for effective control measures.

There has been renewed interest in bacteriophage as Bio control agent with increasing reports of antibiotic-resistant bacteria [4]. Since single phage usage has lot of demerits, the development of new techniques of combining bacteriophage with suitable antimicrobial agent has been attempted. The potentially high beneficial effect of combined bacteriophage-Essential oil (trans-cinnamaldehyde) therapy was reported by treating enterohemorrhagic *E. coli0157:H7* with BEC8 (an Enterohaemorrhagic *E. coli0157:H7* specific phage preparation) bacteriophage cocktail [5]. Likewise, the increased efficacy of phage therapy in combination with antibiotics [6] and with metallic nano particles [7] has been reported.Free fatty acids affect the expression of bacterial virulence factors, which are important or essential for the establishment of an infection, probably by disrupting cell-to-cell signalling. Linoleic acid is a naturally occurring omega-6 essential long chain unsaturated fatty acid that has been reported to inhibit the growth of *Staphylococcus aureus* [8] and also effective as a biofilm inhibitor of *Klebsiella pneumoniae* at 0.0312 mg/ml, which is 32-fold lower than its MIC [9]. Liposomes are vesicles composed of concentric phospholipid bilayer molecules and used as a vehicle for administration of drugs, bioactive agents and pesticides to the plant. They directly fuse with bacterial membranes, releasing their contents either within the bacterial cell membranes or into its interior. Recently, the use of liposome encapsulated bacteriophage oral therapy against *Salmonella spp.* was reported [10]. Bacteriophages are extremely large and require giant liposomes for its encapsulation. In another study, liposome mediated intracellular bacteriophage therapy was reported for the first time against multi-drug-resistant intracellular pathogens like *Mycobacterium tuberculosis* [11]. In this study Soy lecithin that has higher percentage of linoleic acid (64%) was used as the Phospholipid for use as a carrier for Bacteriophage delivery.

MATERIALS AND METHODS

Inoculants and chemicals used

Based on the virulent potential, the bacterial isolate *Pseudomonas aeruginsa* OB-6 isolated from soft rot infected onion bulb in the field (Plate.1 and 2.) was selected for the study. Bacteria were grown to mid-log phase in Luria–Bertani (LB) broth (15.0 g tryptone, 0.5% yeast extract, 0.5% NaCl) at 37°C and were washed once in phosphate-buffered

saline (PBS). Cells were re-suspended In PBS to a final concentration of 1×10^8 cfu ml^{-1} and were diluted accordingly in PBS to The required inoculums size for each experiment. Inoculation of the mid log phase cultures (10^8 cells ml^{-1}) of the selected isolates was done at the rate of 50 ml broth pot^{-1} by soaking the wounded bulb in the bacterial suspension before planting. Based on the broad host range one phage isolate of *Pseudomonas viz.,* OP-3 obtained from rhizosphere soil of infected onion plant was used for the study. Soya lecithin was obtained from Sigma, Aldrich, Mumbai and Alpha-tocopherol was purchased from Sigma, Aldrich, Mumbai. Cholesterol was obtained from SD Fine Chemicals and all other chemicals and reagents were of analytical grade or superior.

Pot culture study and seed material

Pots (30 x 28 cm size) were filled with 10 kg soil. Onion seeds of Co-4 variety obtained from the Horticultural College and Research Institute, Tamil Nadu Agricultural University were used in the experiment. The treatments were replicated thrice in a completely randomized design (CRD).

Treatment schedule

T1	-	Antibiotic @100µg ml^{-1}
T2	-	Bacteriophage @10^9 PFU ml^{-1}
T3	-	Linoleic acid @300µM ml^{-1}
T4	-	Liposome encapsulated bacteriophage @10^5 PFU ml^{-1}
T5	-	T1 + T2
T6	-	Healthy control (Sterile water alone)
T7	-	Inoculated control @ 50ml broth pot^{-1}(10^8 cells ml^{-1})

Isolation and purification of bacteria

The rotten bulbs of the onion seedlings collected from farmer's field and the rotten bulbs collected from the market were used for isolation of Bacteria following standard procedures of serial dilution agar plate method [12]. Rotten onion bulbs were washed by tap water and disinfected by soaking in bleach at 1% active sodium hypochlorite for 3 min, followed by rinsing in sterile distilled water. The diseased fleshly scale tissues were cut into small pieces by using sterile surgical blade. These pieces of onion scale were ground in 1 ml of sterilized saline phosphate buffer (pH 7) using mortar and pestle. A 10-fold-dilution series was prepared from suspensions of each sample extract and 100 µl of each dilution and the undiluted extract were spread on Nutrient agar medium and kept for 24 hours incubation at 28°C.

Bacterial growth pattern

Growth curve of the selected virulent isolate was plotted in order to determine the generation time of bacteria in Nutrient Agar broth. The 24 h grown bacterial isolate was used as inoculum for conducting the growth experiments. The mother culture was prepared by inoculating a loopful of culture in 10ml of nutrient broth and incubated. One ml of the 1.0 OD culture (the optical density absorbance of the isolate was adjusted to 1.0 at 600 nm) was inoculated into 100 ml nutrient broth and incubated over rotary shaker at 30±2°C. The growth was determined by measuring the absorbance at 610 nm at every 12 h up to 144 h and blank was also maintained. The growth curve was drawn using OD value against time intervals.

Isolation of bacteriophages from rhizosphere soil of soft rot disease infected onion field Bacteriophage enrichment from rhizosphere soil

Enrichment was done to increase the number of phage virions in rhizosphere soil of soft rot infected onion seedling. Bacteriophages were isolated by modified method of Smith and Huggins (1982) [13]. For the isolation of phages, 150 ml of phage broth was mixed with 50 ml of LB broth (supplemented with 0.2 % maltose and 10Mm $MgSO_4$) and 50 ml of Tryptone soy broth (supplemented with 4mM $CaCl_2$) and incubated at 37ºC for 1 hr. About 5 ml of 24 hours grown culture was added to the sample mixture. After incubation at 37ºC for overnight, the culture was centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered through 0.45µm membrane syringe filter to remove bacterial cells resulting in bacteria free phage filtrate.

Confirmation of bacteriophages by plaque formation

To confirm the phages present in the crude phage lysates, the double layer technique [14] was used. Dilutions of crude phage lysates were prepared with Sterile SM buffer (0.05 M Tris-HCl (pH 7.5), 0.1 M NaCl, 10 mM $MgSO_4$ and 1% (w/V) gelatin). About 100µl of 24hrs grown bacterial culture was mixed with 100µl of each dilution of phage lysates. This was then mixed for 30 minutes to facilitate the absorption of phages onto the bacterial cells. The mixed culture was inoculated into 5ml of soft agar which was maintained at 45ºC in water bath. The inoculated soft agar was poured onto the tryptone hard agar plates. The plates were rotated to spread the soft agar on hard agar. Plates were incubated at 37ºC overnight for plaque formation.

Purification and storage

Phages were purified by three subsequent single plaque isolations. Single plaque isolations were carried out by transferring phages from isolated plaques to a fresh lawn of the host bacterium using sterile toothpicks and then quadrant streaking them with sterile plastic transfer loops. Following purification the phages were propagated by mass streaking on fresh lawns of the host. After 24-h incubation at 37ºC, the phages were eluted by pouring 5 mL sterilized tap water into the 100 mm×15 mm Petri dishes and gently shaking the plates (~20 rpm) for 30 min. The elute was centrifuged (10,000 g for 10 min), treated with chloroform and filter-sterilized and then quantified as described below, and stored in 2-ml plastic vials at 4°C in complete darkness. The concentrations of these suspensions were approximately 10^9 plaque forming units (PFU) per ml.

Preparation of Giant Unilamellar liposome

Liposomes were prepared using a modified method described by Alexander Moscho *et al.,* 1996 [15]. Accordingly about 40 mM of Soy lecithin, 10 or 20 mM of cholesterol (Chol) and 0.04 mM of α-tocopherol (Vitamin E acetate) were dissolved in 980 µl of chloroform and 100-200 µl of methanol in a round bottom glass tube. Chloroform was removed under a Nitrogen stream and the rotary movement of the glass tube promoted the formation of a thin lipid film on the glass wall. The lipid film was then hydrated with 7ml of bacteriophage solution (approximately 10^5 PFU/ml in Saline Magnesium buffer) by adding them carefully along the glass tube prior to evaporation of the organic solvent. After evaporation for 2 min, an opalescent fluid was obtained with a volume of approximately 6.5ml (Plate 6.).

Liposome encapsulation –a better choice for delivering viable organisms in protected microenvironments

A formulation, for treatment of a bacterial infection, comprises liposome encapsulated bacteriophage, water and adhesive to adhere the bacteriophage to a surface. One such formulation comprising water: 85% - 99.98% by weight; bacteriophage: 0.01 % - 5% by weight; and adhesive: 0.01 % - 10% by weight. In the present work, the bacteriophage is encapsulated in liposomes and the final product is a complex that combines liposomes and bacteriophage. The complex is dispersed in water and starch that is used as adhesive. When the liposomal bacteriophage formulation is

applied to the root, the starch helps the bacteriophage to adhere to the root where it is applied and when it dries the adhesive holds the particles in place. Thereby enhanced fixing of the encapsulated bacteriophage is achieved, giving resistance to loss of the bacteriophage and improved anti-bacterial activity *in situ.* Delivery of the liposomal bacteriophage is achieved through root application by spraying or watering plant roots with Liposomal bacteriophage formulation.

Observations

The crop was harvested by observing maturity indices. Plants were uprooted from each pot separately. The loss due to soft rot was assessed from the number of infected plants from each pot. The severity of the infection in all the experiments were graded according to a grade chart with grade specifications based on the area of the scales involved in spoilage. Each grade was assigned a category value as an index of infection [16]. Symptoms of soft rot were graded from 0 to 5 as follows: 0, no rotting of the scales; 1, Infection occurs at the neck of the bulbs small area of outer scale shows the oozing carrying an area of 1-5%; 2, Oozing from the second inner scale of bulbs and 5-10% affected; 3, bulb size reduced to 1/4th of its size and area of infection increased to 10-21%; 4, bulb size reduced to half of its size (21-50% affected); and 5, greater than 50% bulbs affected. The loss in weight due to soft rot was computed and expressed in percentage (w/w) [17]. Soil particles adhering to the bulbs were removed; yield parameters like weight, diameter and circumference of the bulb were measured.

Infection(%) = No. of infected bulbs / Total number of bulbs x 100

Loss of weight % = Initial weight - weight after discarding the rotten tissues/Initial weight x 100.

Percentage of disease reduction (PDR) was calculated [18].

PDR = Ack – Atr / Ack × 100

where Ack and Atr represent the severity of the disease in control and treated specimens, respectively.

Virulence test to determine the efficacy of different control agents *in vivo*

This study was conducted to determine the antibacterial effect of different control agents on the soft rot incidence *in vivo.* Healthy onion bulbs were used for the test. A triangular section of 1 cm on each side of the sterilized onion bulbs was cut to a depth of three layers and inoculated with 100 μl of bacterial suspensions. The bacterial inoculum was prepared from mid log phase cultures on nutrient broth incubated at 30°C. Bacterial cells were collected in sterile demineralised water and adjusted to10^8 cfu / ml. For negative control 100 μl of sterile water was used. Positive control was maintained with bacteria alone. The bulbs were left to rest until the fluid was absorbed into the tissue. For the assay, 100 μl of different control agents were added and allowed to rest for fluid absorption and the cap was secured using a tooth pick. The efficacy of combined effect of antibiotic and free phage was determined by adding 100 μl of LB broth with Streptomycin (0.5 μg/ml) and phage (10^9 pfu/ml). Dissected bulbs were placed in a sterilized plastic container containing sterile moistened filter paper, covered with plastic wrap and incubated for 70 h. The extent of rot in each case was measured (as length) with transparent meter ruler. Also the extent of growth inhibition of the pathogen was measured in relation to the level of rot caused in the control (without control agents). Each experiment consisted of three replicates per treatment and was carried out at least three times. Likewise the amount of rotten tissue produced in each bulb was determined and the percentage of rotten tissue was taken as criterion of treatment effect. Every bulb was weighed before and after removing the rotten portion. Rot severity = (W1-W2)/W1 x 100 where, W1 = Weight of whole tuber and W2 = weight of the tuber after removal of the rotten tissue [19].

Effect of different control agents on the bio control of soft rot disease in onion under pot-culture condition:

A pot culture experiment was conducted to study the efficacy of lytic phage in single and in combination with other biocontrol agents to control soft rot disease in onion (CO-4) caused by *Pseudomonas aeruginosa.* Onion bulbs were sanitized before testing with pathogen by washing them in 0.5% NaOCl household grade, for 10 minutes and subsequent washing with tap water, air dried and stored at 16°C. Pots were filled with 10 kg of field soil from onion

field and the bulbs were planted at the rate of 4 bulbs / pot. For negative control, plants were watered with sterile deionized water with no phage. Positive control was maintained by inoculating with bacteria alone. All the treatments were replicated three times in a completely randomized design. The remaining treatments were inoculated with 24 h old broth cultures of the selected isolate at the rate of 50 ml broth / pot (containing 10^8 cells / ml broth) after wounding the bulb. On the 10th day after the bacterial challenge, youngest leaves appeared bleached and wilted. After the symptoms were noticed, different bio control agents were applied as per the treatment schedule. At an interval of fifteen days the application of control agents were repeated again. Disease incidence and disease severity and yield parameters were assessed two weeks after the application of control agents and the experiment was conducted until the maturity indices were observed and the plants were harvested.

Statistical analysis

Experimental data were subject to a one-way analysis of variance using a computer program (AGRES software). Means were compared to test significance between treatments using the LSD at 5% probability.

RESULTS AND DISCUSSION

Isolation of bacterial isolates

After incubation period, morphologically distinct, single, well-separated 50 number of colonies growing on the plates were serially numbered, purified by streaking on NA medium. About 22 bacterial isolates selected based on the pathogenicity test were further subjected to screening for the selection of the most virulent isolate based on their extracellular enzyme activity (data not shown). The virulent isolate OB-6 was identified as *Pseudomonas aeruginosa* based on biochemical and Molecular identification (Plate 3 and 4).

Growth kinetics of *Pseudomonas aeruginosa* strain OB-6

Growth kinetics of the strain was studied to find the best culture time for an inoculum preparation and to know how long it takes for each bacterium to enter into exponential phase. The strain was monitored for 144 hours by measuring the O.D at 600 nm every hour 37°C. From figure 1, *Pseudomonas aeruginosa* OB-6 reached the mid exponential phase after 10 hours of culture. Therefore, the best culture time chosen for the inoculum preparation of *Pseudomonas aeruginosa* OB-6 is 10 h (Figure 1).

Isolation of Bacteriophage

About six numbers of bacteriophages, namely OP-1, OP-2, OP-3, OP-4, OP-5 and OP-6 that showed different plaque morphology were tested for their host range against the *Pseudomonas aeruginosa* strains isolated from different location. One step growth curve was determined to know the optimum latent period and the burst size of the broad range phages OP-1, OP-3, OP-6. (Plate 5 and Figure 2). Based on the bacteriophage and host interactions, the elite bacteriophage OP-3 with potent antibacterial efficiency was chosen for further experiment (Data not shown).

Determination of optimum dose of the pathogen

In a preliminary experiment, infection conditions for the pathogen, *Pseudomonas aeruginosa* strain OB-6, was determined. A concentration of 10^8 colony forming units (cfu) infiltrated per bulb combined with incubation at 25°C were determined as ideal positive control conditions, since this ensured visible infection of the onion bulbs in more than 90% of the cases.

Preparation of Giant Unilamellar Liposomes

In silico analysis revealed that Linoleic acid are capable of blocking the synthesis of AHL signal molecule thereby repressing the virulent genes that are responsible for causing soft rot. The same was confirmed experimentally. The need for protective formulation for maintaining the stability and activity of the bacteriophage under field conditions necessitated the preparation and application of Linoleic acid enriched liposomes (Plate 6. and 7.). Soy lecithin, a by-product of the soybean oil production has been reported to be comprised of phospholipids such as phosphatidylcholine, Phosphatidylethanolamine and Phosphatidylinositol that are made up of fatty acids Palmitic (14%), stearic (4%), oleic (10%), Linoleic (64%), Linolenic acid at 7% [20]. Hence Soy lecithin, a natural source of Phospholipid that is having high percentage of Linoleic acid was used in the experiment. Moreover unsaturated fatty acids have been reported to cause biofilm dispersal and also act as chemoattractant to *Pseudomonas aeruginosa.*

In vivo **and Greenhouse experiment to determine the efficacy of different control agents**

Virulence test to determine the efficacy of different control agents under *in vivo* **condition using Onion bulbs**

From the *in vivo* experiment, the activity of the different control agents showed that the pathogen *Pseudomonas aeruginosa* strain OB-6 reacted to the control agents to different levels as shown by mean inhibition diameter of rot caused on Onion bulbs and per cent inhibition of the rot severity which are recorded as shown in Table 1. The control agents showed variation in the reduction level of rot caused by the pathogen. With respect to inhibition of rot zone diameter, liposome encapsulated bacteriophage formulation was more effective (84.5%) when compared to control group which was only infected with bacteria and the value differed significantly ($p \leq 0.05$) from other control treatments against soft rot while the use of antibiotic speedomycin was the least effective against the pathogen with an inhibition of 35.08% for *Pseudomonas aeruginosa* strain OB-6. The other control agents exhibited varying level of inhibition between these two levels (35.08% - 71.05%). Likewise with regard to the reduction of percentage of rotten tissue, liposome encapsulated bacteriophage formulation was very effective compared to other treatments.

Effect of different control agents on the control of soft rot in onion bulbs under pot culture condition

On the basis of the results of the confrontation test on Onion bulbs, the potential of different control agents were assessed for their ability to reduce *in vivo* soft rot incidence on onion bulb (CO-4) in greenhouse (Plate 8). According to the results, they showed different spectra of activity. The highest incidences of rots were observed on wounded samples during the first two weeks. After the application of biocontrol agents, a progressive decline was observed in subsequent weeks.

Effect of different control agents on disease incidence

Looking at the number of rotten bulbs for the positive control with inoculated pathogen 9 out of 12 bulbs displayed rot. Significant decreases in the number of decayed bulbs were observed in the treatments with antibiotic Speedomycin that displayed 7 rotten bulbs out of 12 bulbs followed by the combined treatment of antibiotic and bacteriophage displaying 5 rotten bulbs. But in liposomal encapsulated phage treated bulbs, only 2 bulbs displayed rot thereby significantly inhibiting the growth of soft rot bacteria *in vivo.* The results (Table 2) of the pot trial showed that the soft rot disease incidence was effectively controlled by the application of liposome encapsulated phage formulation @ 50ml per pot to the root of the plant. This treatment recorded the least disease incidence accounting for 88.89 % decrease in the incidence of soft rot over control respectively. This was followed by T_3 and T_2 in the decreasing order of merit. The maximum disease incidence was recorded in the inoculated control (T_7) (75.00 %). In the healthy control plants, no symptoms were observed throughout the experiment. The treatment of onion bulbs with Liposomal encapsulated bacteriophage successfully reduced the initial infection and multiplication of soft rot bacteria.

Effect of the different control agents on soft rot severity

Different control agents were tested for their ability to reduce the soft rot disease severity on Onion bulbs in a pot culture experiment. The control agents reduced the severity of *Pseudomonas aeruginosa* strain OB-6 mediated soft rot of the bulb significantly *in vivo* when the pathogen and the control agent were co inoculated although they differed in their extent of reduction. The treatment *in vivo* with the control agents antibiotics, linoleic acid, free phage and liposome encapsulated bacteriophage has shown a protection against the development of *Pseudomonas aeruginosa* strain OB-6. For the liposomal encapsulated bacteriophage, both the number of rotten bulbs and the extent of bulb rotting caused by *Pseudomonas aeruginosa* strain OB-6 was significantly reduced with the protection reaching 85.2%. Whereas for other control agents the protection varied between 37.24% and 72.00% when compared to the positive control (Table 3).

Effect of different control treatments on the yield parameters of onion bulb

The results indicated that there is a difference among the different treatments with respect to yield related parameters. The results revealed that in T_4 treatment minimum disease incidence of 8.33% and increased mean diameter of onion bulbs (21.49mm) and mean Circumference of onion bulbs (67.55mm) were observed when compared to all the other treatments. In next best treatment T_3, disease incidence of 25% along with increase in bulb diameter and circumference of bulb were obtained (Table 4). The mean diameter and circumference of the bulb were observed in decreasing order for T_5, T_2 and T_1 treatments and were (18.25mm, 63.12mm), (17.68mm, 59.21mm) and (16.84mm, 51.54mm) respectively. The results of the activity of the different control agents against *Pseudomonas aeruginosa* strain OB-6 showed that all physiologic parameters had a significant difference; this signification has varied from one agent to another. Furthermore, the analysis of mean comparisons of the evaluated factors (diameter of the bulb and circumference of the bulb) has revealed an effect of the treatment on the biological control experiment. In particular, the treatment by the liposomal encapsulated bacteriophage has demonstrated that this can significantly inhibit the growth of soft rot bacteria *in vivo*. The treatment of the onion bulbs at the initial stage of infection had significantly reduced soft rot disease of onion bulb together with effective control on the multiplication of soft rot bacteria.

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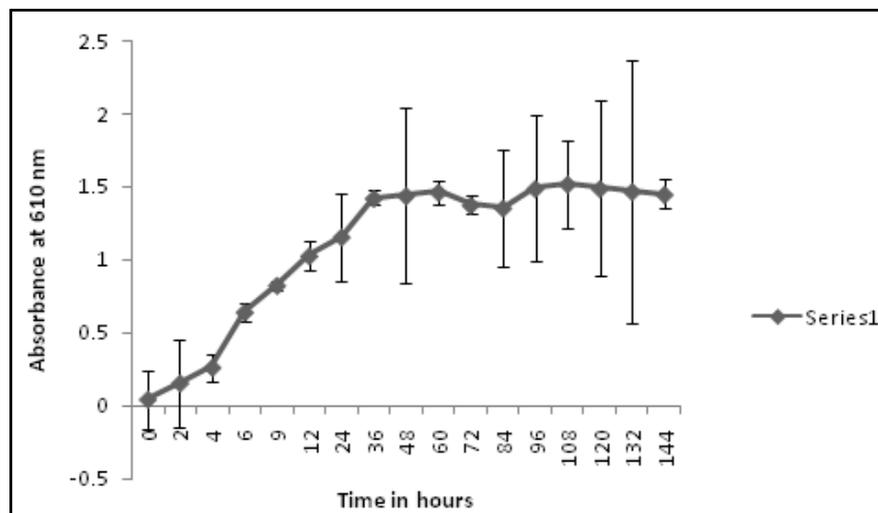
Figure 1. Growth kinetics of *Pseudomonas aeruginosa* strain OB-6 in nutrient broth at 37°C, with shaking at 75 rpm/min
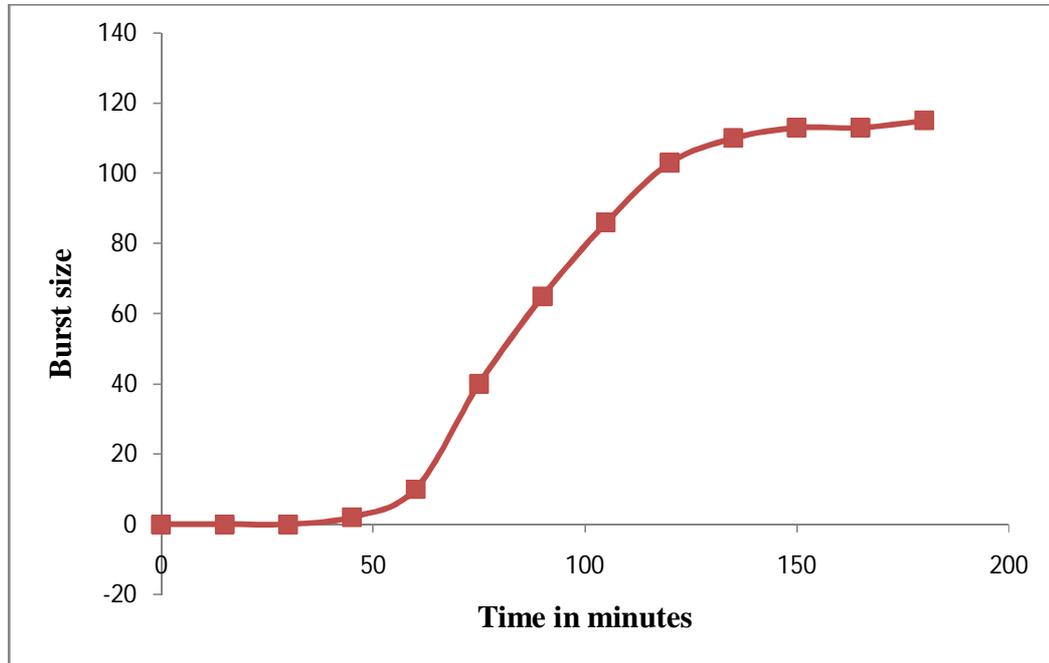
Values are the means of three determinations.
Figure 2. One step growth curve of OP- 3 phage on *Pseudomonas aeruginosa* OB-6 strain.

Plate 1. Soft rot symptom in the Onion plant under field condition

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Plate 2. Soft rot infected onion plants from the field


Plate 3. *Pseudomonas aeruginosa* **OB-6**

**Plate 4.** *Pseudomonas aeruginosa* **colonies under UV illumination**

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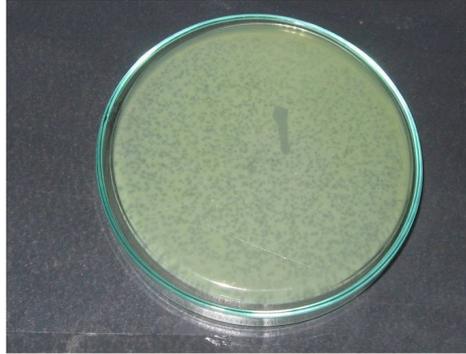
1. OP-1 phage
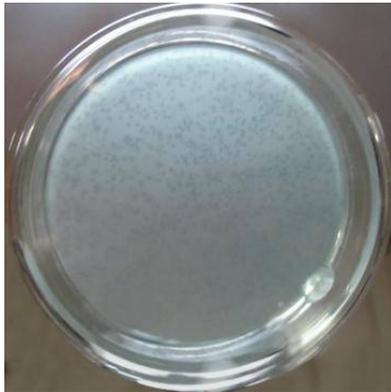
2. OP-2 phage

3. OP-3 phage
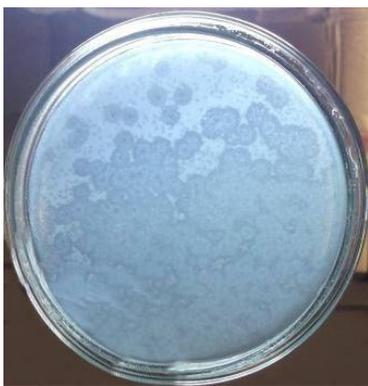
4. OP-4 phage

5. OP – 5 phage

Plate 5. Plaque morphology of different isolated phages

Plate 6 - Thin layer lipid hydration method for liposome synthesis

Plate 7. Pelleted liposomes on centrifugation

Plate 8. Pot culture experiment to determine the efficacy of different control agents

Table 1. Virulence test to determine the efficacy of different control agents under *in vivo* condition by using onion bulbs

Tr. No.	Control agents	Rot zone (cm)	Rot weight (g)	Rot severity (% of rotted tissue)
T1	Antibiotic	2.22 ± 0.088	5.69 ± 0.029	11.00 ± 0.113
T2	Bacteriophage	2.05 ± 0.088	3.26 ± 0.014	5.14 ± 0.180
T3	Linoleic acid	0.99 ± 0.033	0.75 ± 0.031	1.13 ± 0.007
T4	Liposome encapsulated bacteriophage	0.53 ± 0.003	0.39 ± 0.004	0.75 ± 0.004
T5	T1+T2	2.00 ± 0.012	3.00 ± 0.062	3.55 ± 0.070
T6	Healthy control	0.00 ± 0.00	0.00 ± 0.000	0.00 ± 0.000
T7	Inoculated control	3.42 ± 0.055	11.0 ± 0.198	30.20 ± 0.190
Grand mean		1.6014	3.4414	7.3957
SEd CD(0.05)		SEd = 0.0342 CD (0.05) = 0.0733	SEd = 0.1640 CD (0.05) = 0.3519	SEd = 0.1646 CD (0.05) = 0.3530

Table 2. Effect of different control agents on the control of bacterial soft rot disease incidence

Tr. No	Control agents	Percent incidence of soft rot	Percent decrease of incidence over control
T1	Antibiotic	58.33±0.471	22.22 ± 0.210
T2	Bacteriophage	33.33±0.351	55.56 ± 0.676
T3	Linoleic acid	25.00±0.135	66.66 ± 0.126
T4	Liposome encapsulated bacteriophage	8.33±0.165	88.89 ± 0.103
T5	T1 + T2	41.66±0.826	44.45 ± 0.232
T6	Healthy control	0.00±0.00	0.00

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T7	Inoculated control	75.00±0.270	0.00
Grand mean		34.5214	36.5086
SEd CD (0.05)		SEd =1.2269 CD (0.05) =2.6318	SEd = 0.7854 CD (0.05) = 1.6848

Table 3. Effect of different control agents on the severity of bacterial soft rot disease

Tr. No	Control agents	Percent severity of soft rot	Percent decrease of severity over control
T1	Antibiotic	20.15±0.254	37.24 ± 0.338
T2	Bacteriophage	18.55±0.284	42.22 ± 0.195
T3	Linoleic acid	8.99±0.008	72.00 ± 0.875
T4	Liposome encapsulated bacteriophage	4.75±0.158	85.20 ± 0.611
T5	T1+T2	14.00±0.555	43.60 ± 0.669
T6	Healthy control	0.00	0.00
T7	Inoculated control	32.11±0.784	0.00
Grand mean		14.0786	34.7171
SEd CD(0.05)		SEd = 0.3025 CD (0.05) = 0.689	SEd = 0.3518 CD (0.05) = 0.7546

Table 4. Effect of different control agents on the yield parameters of Onion bulbs

Tr. No	Control agents	Mean diameter of the bulb (mm)	Mean circumference of the bulb (mm)
T1	Antibiotic	16.84±0.434	51.54±0.114
T2	Bacteriophage	17.68±0.589	59.21±0.867
T3	Linoleic acid	19.37±0.661	64.68±0.690
T4	Liposome encapsulated bacteriophage	21.49±0.716	67.55±0.923
T5	T1+T2	18.25±0.746	63.12±0.754
T6	Healthy control	21.30±0.316	66.56±0.773
T7	Inoculated control	16.24±0.637	49.13±0.638
Grand mean		18.7386	60.2557
SEd CD(0.05)		SEd = 0.4497 CD (0.05) = 0.9647	SEd = 1.2155 CD (0.05) = 2.6074

RESEARCH ARTICLE

Machine Learning Optimization Model using Green's Theorem

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ABSTRACT

Optimization in the field of machine learning is one of the issues that experts are trying to use different approachesto provide a way to improve the machine performance.However, some mathematical tools mentioned in other sciences are very valuable to current issues in engineering science, who can enter and illuminate the field of engineering.In this paper, after a brief description of the issue raised by Green in microeconomic theory, tries to use from concept of "Separating Hyper plane" and "Supporting Hyper plane" to optimize the Support Vector Machine (SVM).Finally, a mathematical method is presented for use in such cases to maximizing margins in SVMs.

Key words: Machine learning, Support vector machine, Separating Hyper plane, Supporting Hyper plane

INTRODUCTION

Due to the special place of the machine in different industries and its profound impact on the productivity of an enterprise,in recent years theorists have addressed issues related to the field of machine learning.Because of the importance of the issue, part of these researchesis related to optimization. Olkopf and Smola (2001) with linear and nonlinear kernel and Shalev and Srebro (2008) with a reverse dependency approach, optimized SVMs.Also Xu et al. (2001) offered a method for clustering margin maximization. [1], [2], [3]Yang et al. (2016) optimized the SVM by a global stochastic optimization technique, particle swarm optimization (PSO) algorithm, it makes VW-SVM to be an adaptive parameter-free method for automated unmixing of protein subcellular patterns. [4]Aich and Banerjee (2016) inverse solution procedure is elaborated to find the near-optimum setting of process parameters in EDM machine to obtain the specific need based MRR-ASR combination. [5]Linn et al. (2016) proposed approach in the context of group classification using structural MRI data and showed that control-based normalization leads to better

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reproducibility of estimated multivariate disease patterns and improves the classifier performance in many cases. [6]In Ebrahimiand Khamehchi's(2016) research support vector machine (SVM) was used to overcome the problem. The reservoir simulation software was replaced by the trained SVM. [7]In this study, using Green's theorem in the theory of microeconomics, a method have been developed to maximize margins in SVMs.

Green's theorem in the theory of microeconomics

In a matter of maximizing profit (UMP[1]), the profit function (U(x, y))is maximized due to some constraints such as budget $(1 - P_X * X - P_Y * Y = 0)$.Also, in a cost minimization problem (EMP[2]), the cost function ($E(x, y)$) is minimized due to some constraints such as profit, where the total cost is$E = P_X * X + P_Y * Y$.Using Lagrange multipliers, optimal vector in the UMP is synchronized with optimal integration in the EMP. Or in other words UMP and EMP are the dual problem.On the other hand Hessian matrix should be quite negative for a maximization problem and quite positive for a minimization problem. Mass-Colell et al. (1995) presented a different concept of duality. [8]

In the mathematical appendix of this book, Green suggests the following two important cases:

a. Separating Hyper plane Theorem
b. Supporting Hyper plane Theorem

The following are briefly discussed.

Separating Hyper plane Theorem
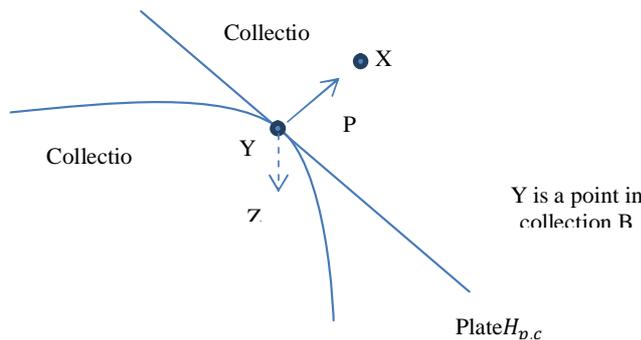
Assume A ,$B ⊂ R^n$ $and A ∩ B = ∅$. Also assume collection B is a convex and closed collection as $x ∈ B$ and $y ∈ B$. There is $∈ R^n$, $p ≠ 0$ per x, y and there is $c ∈ R^n$ as $p.x > c$,$p.y < c$.There is therefore a top plate ($H_{p,c}$) that separates the A and B, that they (A and B) are opposite each other on either side.

Supporting Hyper plane Theorem
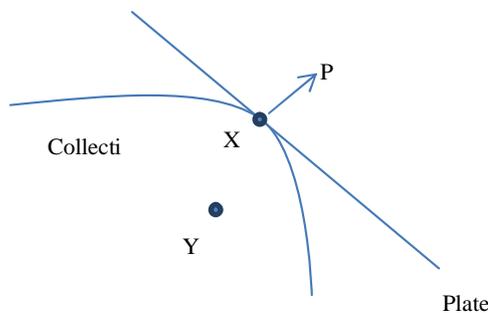
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Assume $B \subset R^n$ is a convex and $x \in B$. There is $\in R^n$, $p \neq 0$ per y as $p.x \geq p.y$. There is therefore a top plate ($H_{p,c}$) that support B.

Optimizationmathematical model in SVMs

According to the two cases and Green's approach in microeconomic,we can suggest a method to optimize the SVMs. The use of this theorem is that, based on a mathematical method, a SVM should be able to choose the best plate.If we have the following definition:

Separator plate: $x.w + b = 0$
Supporting plate: $x.w + b = -1$
Margin: $1/||w||$
Normal vector: w

Then, based on this mathematical method, SVMs select the plate that maximizes the margin of separation between the two classes from all the separatorplates. A SVM classifies the inputs into two classes, using a plate in a multi-dimensional space. A SVM includes vector b and vector w that use them to training data {x, y}. It is described as follows:

$x_i.w + b \geq +1 \, for \, y_i = +1$
and
$x_i.w + b \leq -1 \, for \, y_i = -1$
or
$y_i(x_i.w + b) - 1 \geq 0 \;\; \forall i$

Margin is defined using vector geometry1/ ||w||, where w is the normal vector to a separatorplate which is equidistant from the supporting plate,so that the supporting plates support various classes or series of observations in the training data.

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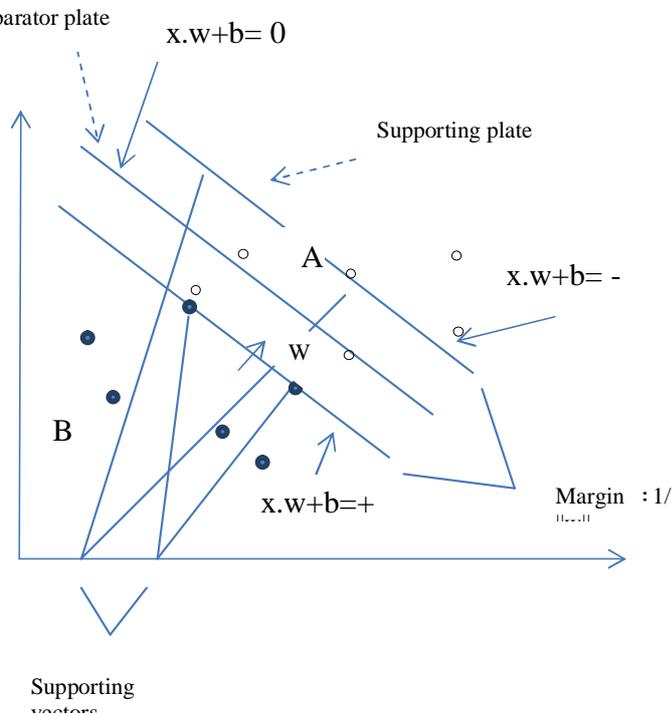
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Maximization of margins is an optimization problem, which involves the use of Lagrange multipliers, which is common in economics. In Lagrange settings, where $H_{ij} = y_i y_j x_i x_j$, the term $\propto^T H \propto$ is formulated. In the study, "Linear Kernel" is intended as a point production, $K(x_i, x_j) = x_i^T x_j$. If the data are not linearly separable, other kernel functions such as polynomial and annular kernel functions is recommended for non-linear separable data.

CONCLUSION

In this study, after two brief description of Green's theorem, was tried to use from mathematical concepts in the field of machine learning. The findings of this study show that Green's theorem in microeconomic theory can be used to maximize margins SVMs. This research could open the window to develop new approach to optimization of SVMs. Based on the results of this study, it is recommended for future research an algorithm be designed and implemented.

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RESEARCH ARTICLE

Study of Port Flow Maldistribution and Heat Transfer in Different Sets of Chevron Type Plate Heat Exchanger

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ABSTRACT

Experiments has been carried out to study the flow maldistribution and heat transfer of single pass U-type chevron plate heat exchanger by using water as working fluid in both the channel. Results are predicted for a high chevron angle, $\beta=60^o$ and fixed port size, d_p =25.4 mm at different set of platesviz., 15, 21 and 27. The present results are verified with the available results of Bossiouny and Martin [3]and Rao and Das [18]. The variation of different parameters like effectiveness, temperature effectiveness, flow maldistribution parameter with NTU and mass flow rate are studied for different set of plates. The static pressure distributions along the port for both isothermal and non-isothermal conditions are presented. A correlation among flow maldistribution, NTU, heat capacity ratio and number of channels has also been developed.

Keywords: Plate heat exchanger, flow maldistribution, chevron, temperature effectiveness, NTU

INTRODUCTION

Plate heat exchangers are widely used for mainly heating, cooling and heat recovery processes in industrial applications. Initially, the gasketed plate heat exchangers were specially designed for hygienic application such as dairy, brewery, pharmaceuticals and food processing industries, it has also found place in the modern power industries due to its favorable characteristics, such as high overall heat transfer coefficients, easy maintenance, compact size, convenient to increase the heat transfer area, compactness, less fouling, smaller hold up volume and hence quicker response to control operations, capability to recover heat with extremely small temperature difference. In plate heat exchangers every plates rotated by 180º in the plane to separate and produce cross corrugated flow

channels in which the fluid flow is in an opposite direction. The cross-corrugated channel generates a highly turbulent flow for increasing the heat transfer even with the low Reynolds number. The pressure drop as well as thermal performance of plate exchanger depends critically on distribution of fluid and geometrical properties of the chevron plates, namely on corrugation angle, area enlargement factor and channel aspect ratio. Mueller and Chiou [1] presented the good review of the work devoted the problem related with maldistribution. Bassiouny and Martin [2, 3] have derived the axial velocity, total pressure drop, pressure distributions in both the inlet and outlet conduits of plate heat exchangers as well as the flow distribution in the channels between the plates. From the analysis a general flow maldistribution characteristic parameter (m^2) has been derived from the mass and momentum formulation for inlet and exit port flows for all the plate heat exchangers. Bajura [4] has presented the analytical investigation of the performance of flow distribution systems for both intake and exhaust manifolds. A mathematical model describing the flow behavior at a discrete branch point was formulated in terms of a momentum balance along the manifold. Division of a fluid stream into parts by means of a manifold is accompanied by fluid pressure changes owing to wall friction and to the changing fluid momentum have been presented by Acrivos et al. [5].Martin [6] considered the combined effects of the longest flow path and the competition between crossing and longitudinal flow to derive a relatively simple but physically reasonable equation for the friction factor as a function of chevron angle and the Reynolds number.

Mulley and Manglik [7] experimentally investigated the turbulent flow heat transfer and pressure drop in a plate heat exchanger for different corrugation angle. Their analyses are based on the assumption of equal flow rate in all the channels. Tereda et al. [8] investigated the port-to-channel flow maldistribution for a fixed number of plates, corrugation angle and varying port diameter. Gulenoglu et al. [9] experimentally studied the thermal and hydraulic performance with three different plate geometries and developed the new correlation for Nusselt number and friction factor. Faizal and Ahmed [10] have studied the pressure drop and heat transfer in PHE with different spacing between the corrugated plates. Han et al. [11] numerically and experimentally investigated the temperature, pressure, and velocity fields in chevron, corrugated-plate heat exchanger and found the highest temperature appears around the upper port, while the lowest temperature appears in the cold fluid inflow around the lower port. In pressure field, the fluid pressure is gradually reduced along the flow direction. Focke et al. [12] experimentally investigated the effect of the corrugation inclination angle on the thermohydraulic performance of plate heat exchanger. They have considered equal flow in each channel, indicating an ideal case of no flow maldistribution. Khan et al. [13] experimentally investigated heat transfer for single phase flow at Reynolds numbers range (500 – 2500), different chevron angle, corrugation depths and configurations.Nilpueng and Wongwises [14] investigated the heat transfer coefficient and pressure drop of water flow inside a plate heat exchanger with a rough surface and compared with that of smooth surface. Rao et al. [15, 16] experimentally investigated the port flow maldistribution in plate heat exchangers for small and large plate package with high and low corrugation angle. They found that flow maldistribution increases with overall pressure drop. Fernandes et al. [17] studied flow characteristic in corrugated type PHE for varying corrugation angle, aspect ratio and high viscosity fluids at low Reynolds number. They have found the friction factor correlations increase with the increase of the aspect ratio and the decrease of the chevron angle.Out of the cited literatures [1-17] on plate heat exchanger, theoretical hydraulic studies has been done by [1-6] while [7-17] focused on the thermal aspects without taking into account the effect of flow maldistribution on heat transfer. Thus the literature absences in the combined experimental study with flow maldistribution and heat transfer which has been conducted in the present study. A correlation for effectiveness has also been developed from the experimental data.

Experimental Setup and Procedure

The layout of the experimental set-up is shown in Fig.1 consisting of temperature transmitters 1,3,5 and 8, pressure transmitters 2,4,6, and 8, digital flow meters 9 and 10, primary PHE 11, secondary PHE 12, hot water tank 13, cold water tank 14, boiler water tank 15, boiler 16, ground water tank 17, pumps 18, 19, and 20, water treatment plant 21 and 22, pressure gauge 23, condenser 24, valves 25,26 and 27, and programmable logic control unit (PLC). A

temperature controller is used to maintain a constant temperature in the insulated hot water storage tank. Variable frequency drive is used to control the pumps, motor RPM (hot and cold side pump).The maximum operating steam pressure and temperature of boiler are 7.5 bars and 160°C. The steam enters in primary PHE at 2 bars and 135°C which heats the water at desired temperature. The desire hot water is used in secondary PHE for heating the water coming from cold water tank. The steam leaves from the primary PHE and condenses in the condenser. In addition to these, external pressure transmitters (range from 0 kPa to 500 kPa) having a copper tube connected with inlet and outlet ports are used to measure the pressure drop along the channels in Fig. 2.The typical connection of this tube acting as pressure tap with the experimental chevron plate is shown in Fig. 3.

Data reduction

Thermal analysis

The e–NTU method,P–NTU method and θ-P method are used to study the thermal performance of any heat exchanger.The methods of analysis are explained as follows

3.2.1 The e-NTU method

The mathematical formulae used in the present work for its various parameter calculations are
Rate of heat transfer between hot and cold fluids

$$Q = m^{.} C_{ph} (T_{hi} - T_{he}) = m^{.} C_{pc} (T_{ce} - T_{ci}) \qquad (1)$$

The maximum heat transfer rate is calculated from

$$Q_{max} = C_{min} (T_{hi} - T_{ci}) \qquad (2)$$

The effectiveness is calculated from

$$\varepsilon = \frac{Q}{Q_{max}} \qquad (3)$$

Number of transfer unit is defined as

$$NTU = \frac{UA}{C_{min}} \qquad (4)$$

Eq. (4) is compared with the developed correlation of the effectiveness by Rao and Das [18] for plate heat exchanger stated as,

$$\varepsilon = 0.7630 \, (NTU)^{(0.4788 - 0.1084 \ln(NTU))} \exp(-0.4961 \, R) \exp(-0.0521 \, m^2) \qquad (5)$$

3.1.2 The p-NTU method

The heat transfer rate from the hot fluid to cold fluid in heat exchanger in p-NTU method, is expressed as

$$q = T_{p1} C_1 \Delta T_{max} = T_{p2} C_2 \Delta T_{max} \qquad (6)$$

Temperature effectiveness of the cold and hot fluid is given by

$$T_{p1} = \frac{T_{co} - T_{ci}}{T_{hi} - T_{ci}}, T_{p2} = \frac{T_{hi} - T_{ho}}{T_{hi} - T_{ci}} \qquad (7)$$

3.1.3 The θ-P method

The heat transfer rate in θ-P method is expressed as

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$$q = UA\theta(T_{hi} - T_{ci}) \qquad (8)$$

The non-dimensional temperature as

$$\theta = \frac{\varepsilon}{NTU} \qquad (9)$$

Hydraulic analysis

The pressure drop due to friction in the corrugated passage is calculated based on the flow rate by using an empirical formula:

$$\Delta P_{ch} = f_{ch} \frac{L_{ch}}{d_h} \rho \frac{v^2_{chm}}{2} \qquad (10)$$

The maldistribution parameter m^2 is obtained from Bossiouny and Martin equation [3] for identical inlet and exit port dimension.

$$m^2 = (\frac{nA_c}{A_p})^2 \frac{1}{\zeta_c} \qquad (11)$$

The non-dimensional channel velocity is obtained by using channel pressure drop and mean channel pressure drop of a plate heat exchanger.

$$u_c = (\frac{\Delta P_{ch}}{\Delta P_{mch}})^{\frac{1}{2-a}} \qquad (12)$$

Eq. (12) is compared with analytical results developed by Bossiouncy and Martin [3] for U-type flow arrangement stated as,

$$u_c = (\frac{A_p}{nA_c})m \frac{\cosh m(1-z)}{\sinh m} \qquad (13)$$

Uncertainty in measurements

An analysis is performed to determine the uncertainty in the measurement data.The uncertainties independent variable of flow rate, pressure measurement and temperature measurement are estimated to ±2%, ±0.25% and ±0.25%. The uncertainty in dependent variable is calculated from Moffat [20] procedure.

If R is a function of the independent variables X_1, X_2, X_3, …..X_n and W_1, W_2, W_3, ……W_n. The uncertainty in the dependent variable σ_R is given by

$$\sigma_R = [(\frac{\partial R}{\partial X_1} W_1)^2 + (\frac{\partial R}{\partial X_2} W_2)^2 + ... + (\frac{\partial R}{\partial X_n} W_n)^2]^{\frac{1}{2}} \qquad (14)$$

The uncertainties in dependent variables viz., channel pressure drop and temperature effectiveness are found as ±5.5% and ±0.011%.

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RESULTS AND DISCUSSION

Validation

Comparison of experimental effectiveness with Rao and Das [18] theoretical model of plate heat exchanger
The comparison of experimental and theoretical results of effectiveness of plate heat exchanger is shown in Fig. 4. Both the experimental and Rao and Das [18] theoretical results are found in good agreement. It is seen from the figure that the effectiveness increases with increases the NTU due to heat capacity ratio decrease at higher flow rate in PHE.Comparison of experimental non-dimensional channel velocity with Bossiouncy and Martin [3] analytical model of three different sets of PHE Comparison between experimental non-dimensional channel velocity with the analytical model developed by Bossinouny and Martin [3] of three different sets of PHE is shown in Fig.5, which shows that experimental results are in good agreement with the theoretical model. The presence of small deviations may be due to sudden change in cross-section of the inlet and outlet ports. It can also be observed that the non-dimensional channel velocity decreases with increase in the number of plates, resulting in non-uniformity of flow in the channel.

Typical experimental results

Variation of temperature effectiveness with mass flow rate of different set of plate
Variation of temperature effectiveness with mass flow rate of different set of plate is shown in Fig.6. This reveals that the temperature effectiveness increases with mass flow rate because of increment in difference of fluid temperature. Also at higher flow rate convective heat transfer is high. At lower flow rate, fluid motion is highly ordered, when flow rate increases motion of fluid becomes highly irregular with the fluid moving to and fro in all directions. This results in higher fluid mixing, which ultimately enhances heat transfer resulting in increment in temperature effectiveness. The temperature effectiveness from 15 sets of plate is higher than that of 27 sets of plates at same mass flow rate due to flow maldistribution is increased of 27 sets of plate.

Variation of non-dimensional temperature with temperature effectiveness of different sets of plate
Fig. 7shows that the variation of non-dimensional temperature with temperature effectiveness of different sets of plate. It is seen that the non-dimensional temperature is decreased with temperature effectiveness at higher flow rate in PHE. At lower flow rate, as non-dimensional temperature goes on remains constant and then starts decreasing to increase the flow rate. At higher flow rate, the residence time for cold fluid was not sufficient enough to approach the hot fluid inlet temperature and hence there is decreasing trend in non-dimensional temperature difference with increasing in temperature effectiveness.

Variation of flow maldistribution parameter with mass flow rate of different sets of plate
Variation of flow maldistribution parameter with mass flow rate of different sets of plate is shown in Fig. 8. It is observed that the flow maldistribution parameter increases with mass flow rate of different sets of plate for fixed port size because the overall friction resistance decreases at higher flow rate. It is also seen that the flow maldistribution parameters, m^2 is higher for higher number of plates because of flow maldistribution parameters, $m^2$is a function of number of channel. Therefore, flow maldistribution parameter is larger for higher number of plates. As number of plates increases, higher number of channels are formed which ultimately leads to higher flow branching. Higher flow branching causes larger momentum changes. Momentum gain due to decrease in flow rate is higher for a large number of plates, which ultimately leads to higher inlet pressure along the port. On the other hand, pressure in the outlet decrease gradually due to higher momentum and friction losses for large number of plates. As a result of this flow maldistribution parameter is higher for a large number of plates.

Variation of flow maldistribution with temperature effectiveness for different sets of plate

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Fig. 9presents the variation of flow maldistribution with temperature effectiveness for different sets of plate. It shows that the flow maldistribution parameter m^2, increases with temperature effectiveness because of change of temperature difference increases at higher flow rate in PHE.At higher flow rate for all cases, temperature effectiveness is increased due to motion of fluid highly irregular with the fluid moving to and fro in all directions. As a result of this, which ultimately enhances heat transfer resulting in increment in temperature effectiveness. It is also observed that the flow maldistribution parameter increases with number of plate as well temperature effectiveness. Since flow maldistribution parameter is a strong function of number of channel.

Variation of temperature effectiveness with NTU of different set of plate heat exchanger
Variation of temperature effectiveness with NTU for different set of plates in a heat exchanger is illustrated in Fig.10.Itshows the effect of the number of plates for a given plate heat exchanger on its thermal performance. In this case, it is observed that the temperature effectiveness increases with NTU of all cases. At a given NTU,the thermal effectiveness increases with decrease in the number of plates due to low value of m2 for less number of plates. On the other hand, an increase in number of plates increases m2, and the rate of decrease of performance due to maldistribution is faster. Ultimately, the net effect is a deterioration in performance of PHE with large set of plates. Variation of static pressure drops along the inlet port at isothermal and non-isothermal condition

The variation of static pressure drop along the inlet port at isothermal and non-isothermal conditions for different set of plate is shown in Fig. 11. The static pressure profile for both conditions shows a similar trend. Static pressure increases with increase in number of plates. It can be observed that static pressure for non-isothermal condition is higher due to rise in the temperature of the working fluid. As the thermal content of water decreases due to heat loss to the surface of plate, depletion in the enthalpy of fluid results in gradual drop in pressure in comparison to isothermal test. The fluid between the plates of the heat exchanger expands with increase in temperature of plates. The inner surface of the plates restricts the expansion of the fluid as a result of which, the pressure between the plates increases enormously with a slight enhancement in temperature. So, higher pressure drop for the hot fluid stream as compared to cold fluid stream at the same flow rate is observed.

Variation of static pressure along the outlet port at isothermal and non-isothermal condition
Fig.12 shows the pressure profile along the outlet port for different set of plates at both isothermal and non-isothermal condition. It is observed that the static pressure increases along the port for all the conditions due to increase in outlet port velocity. The static pressure is higher for small set compared to large set of plates because the port velocity decreases with increase in number of plates, for same mass flow rate. Under non-isothermal condition, the viscosity of fluid decreases, resulting in increase of static pressure.

Correlation

Based on experimental data (185 data points), the experimental correlation for the effectiveness is developed as follows.

$$\varepsilon = \frac{NTU e^{-0.3182\,NTU}}{R^{0.4789}} n^{-0.4367} (m^2)^{-0.01} \qquad (15)$$

The above correlation shows that the effectiveness of PHE is decreased with the increase in heat capacity ratio, number of channels, flow maldistribution parameter and decrease in NTU. Eq. (14) shows that the effectiveness of PHE is a function of NTU, R, n and m2. The goodness of the fit is indicated by value of correlation coefficient of 0.94 as shown in the parity plot (Fig 13).

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CONCLUSION

The flow maldistribution and heat transfer for different sets of plate are experimentally investigated under steady state condition. Effect of mass flow rate and number of plates on flow maldistribution and heat transfer have been studied. The effectiveness increases with mass flow rate, NTU and decreasing number of plates in a PHE. The flow maldistribution enhances with number of plates, temperature effectiveness and mass flow rate in PHE. Temperature effectiveness is found to be increasing with mass flow rate but decreasing with number of plates. Non-dimensional temperature decreases with temperature effectiveness and number of plates. For the same mass flow rate, static pressureincreases along the inlet and outlet ports but channel pressure drop decreases. The pressure drop in the channel decreases with increase in number of plates in PHE.Higher flow maldistribution is observed for non-isothermal working condition. Based on flow maldistribution and temperature effectiveness, the set of 15 plates gives better performance than other set of plates used in PHE. A new correlation in term of effectiveness of PHE has been developed. Also, the present investigation suggests that uniform flow distribution in a set with more number of plates is a challenging task.

Nomenclature

A	heat transfer area, [m^2]
A_p	cross- sectional area of the port, (mm^2)
A_c	cross- sectional area of the channel, (mm^2)
a	exponent of the Reynolds number ($f_{ch} = CRe^{-a}$)
b	plate spacing, b= p+t, (mm)
C_p	fluid specific heat at constant pressure, [J/kg°C]
D_h	hydraulic diameter of the corrugated channel, (mm)
d	diameter of port (25.4 mm)
f_{ch}	channel friction factor
h	convective heat transfer coefficient, [W/m^2°C]
k	fluid thermal conductivity, [W/m°C]
L	vertical distance between the two ports, (650 mm)
m^2	flow maldistribution parameter
n	number of channels per fluid
P_{in}	inlet port pressure, (pa)
P_o	outlet port pressure, (pa)
P_t	total pressure drop in PHE, (pa)
Re	Reynolds number, Re= $(\rho u_{ch} D_h)/\mu$
Q	rate of heat transfer between fluids, [W]
Q_{max}	rate of heat transfer [W]
T	temperature,[°C]
T_p	temperature effectiveness
$T_{m,c}$	coldbulk mean temperature, [°C],
$T_{m,h}$	hotbulk mean temperature, [°C],
ΔT_m	logarithmic mean temperature difference, [°C]
T_m	bulk mean temperature (°C)
t	thickness of plate, (0.5 mm)
u_{ch}	dimensionless channel velocity
V_{chm}	mean channel velocity, m/s
W	width of the plate,(108 mm)
W_{in}	inlet port velocity (m/s), flow rate in port (m^3/s)/ area of port (m^2)

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W_o	outlet port velocity (m/s), flow rate in port (m^3/s)/ area of port (m^2)
Z	axial co-ordinate along the port, (mm)
z	dimensionless co-ordinate along the port (Z/Lp)
NTU	number of transfer unit
PHE	plate heat exchanger

Greek letters

β	corrugation angle of the plate (60°)
ρ	density of the fluid, (kg/m^3)
μ	dynamic viscosity of fluid, [Pa s]
ζ_c	overall friction coefficient (f_{ch}L/d_h +other miner losses, including turning losses)
ε	effectiveness
θ	non-dimensional temperature

Subscripts

c_i	cold fluid inlet
c_o	cold fluid outlet
h_i	hot fluid inlet
h_o	hot fluid outlet

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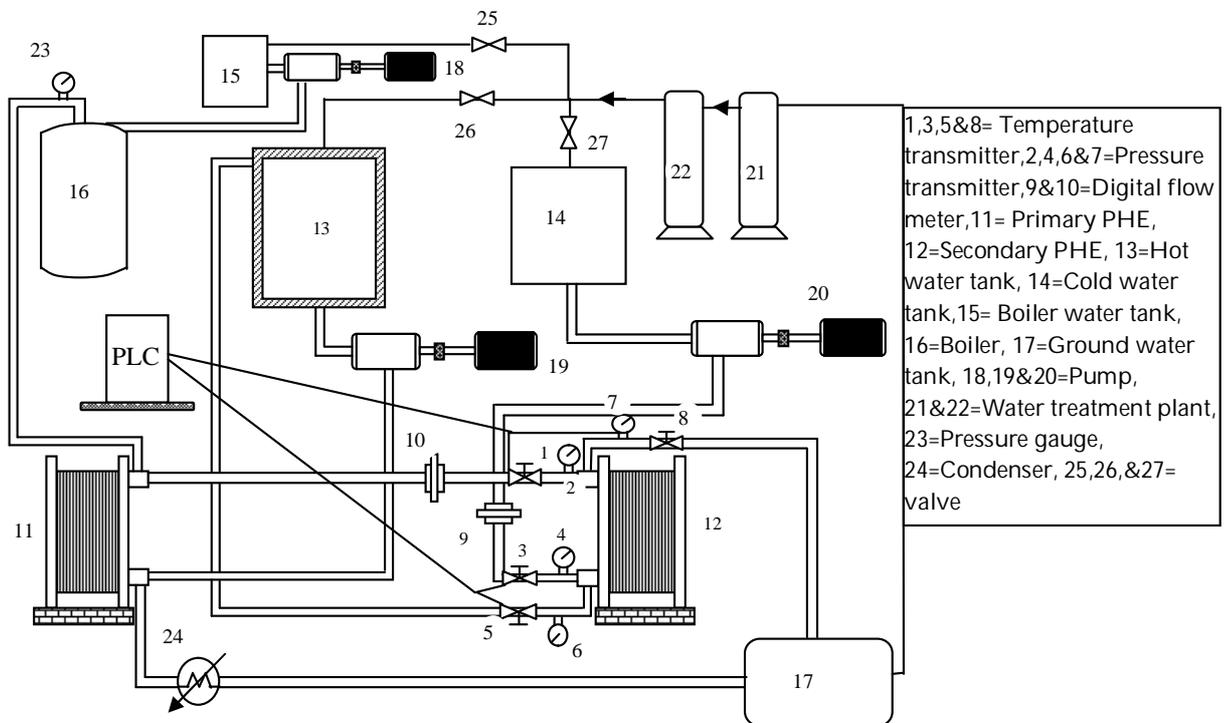
Fig.1. Schematic layout of experimental set-up

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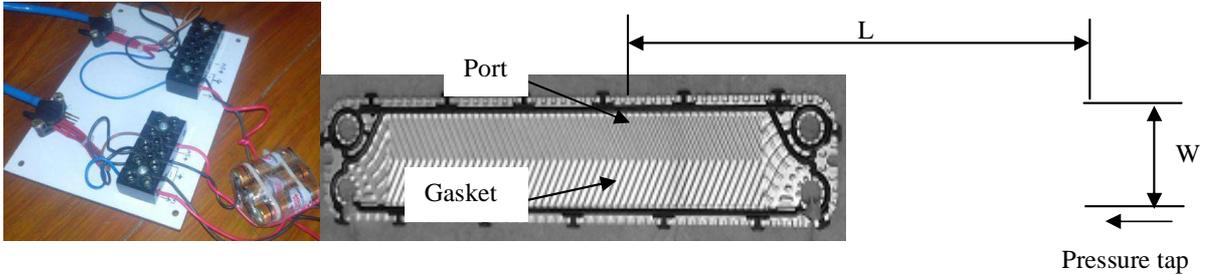
Fig. 2. External pressure transmitter Fig. 3 Pressure tap fixed at top and bottom port of
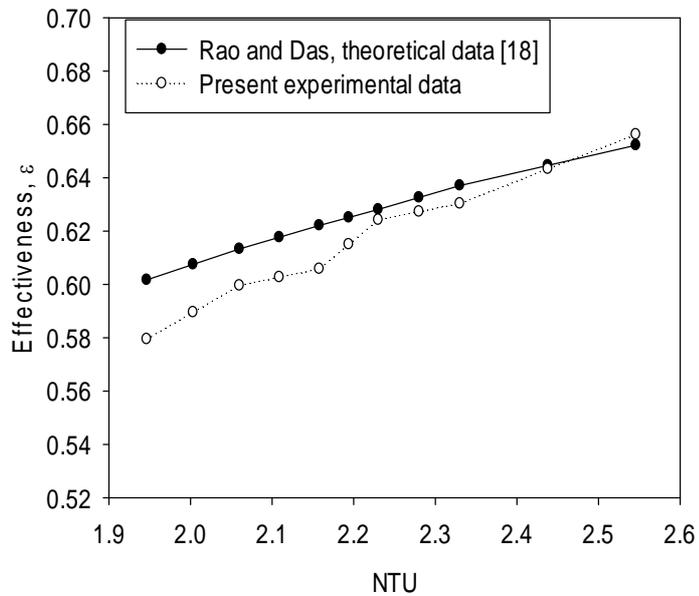
Fig. 4.Comparison of experimental effectiveness with Rao and Das [18] theoretical model of plate heat exchanger

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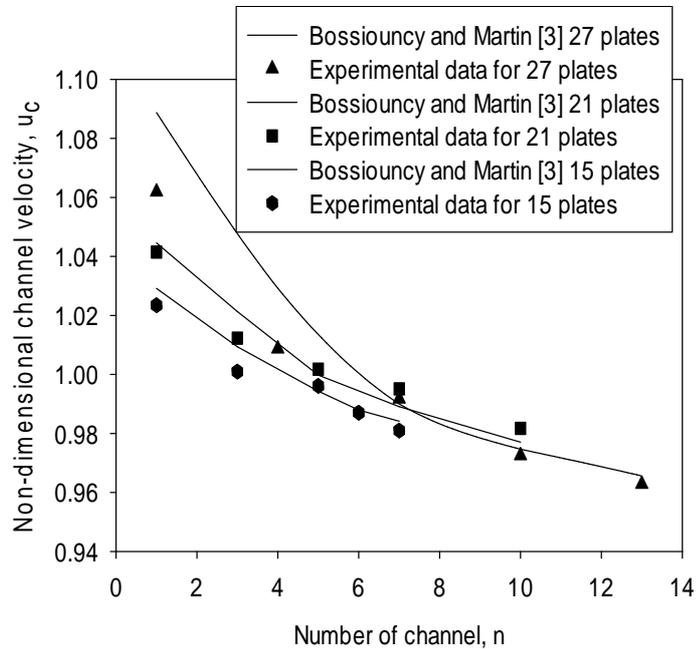
**Fig. 5. Comparison of experimental non-dimensional channel velocity with Bossiouncy and Martin [3] analytical model of three different sets of PHE**
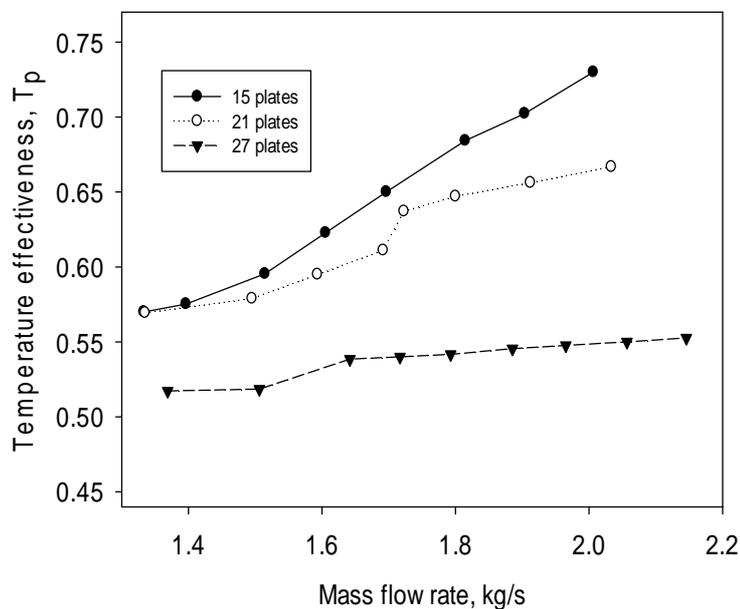

Fig. 6.Variation of temperature effectiveness with mass flow rate of different set of plate

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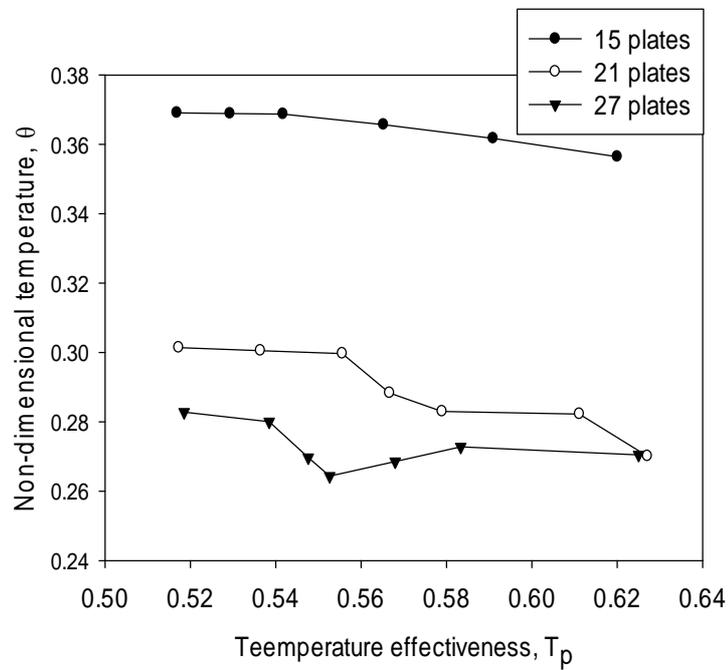
Fig.7.Variation of non-dimensional temperature with temperature effectiveness of different sets of plate
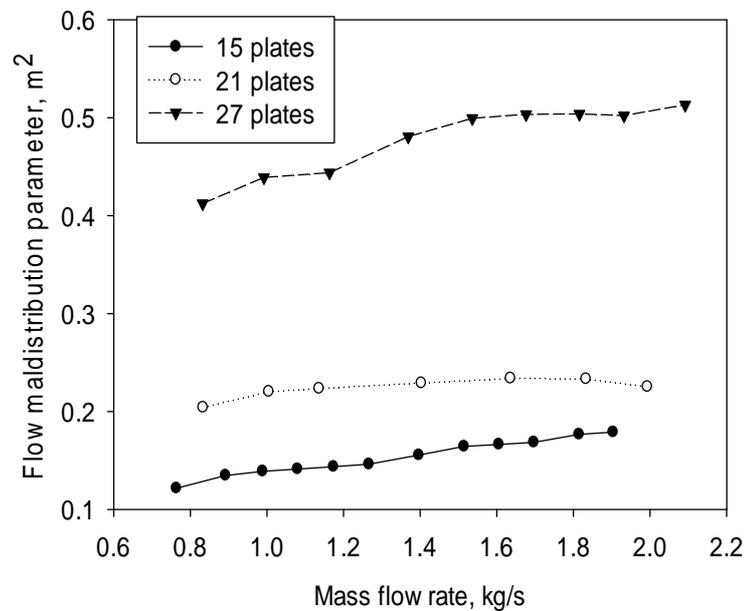
Fig. 8.Variation of flow maldistribution parameter with mass flow rate of different sets of plate
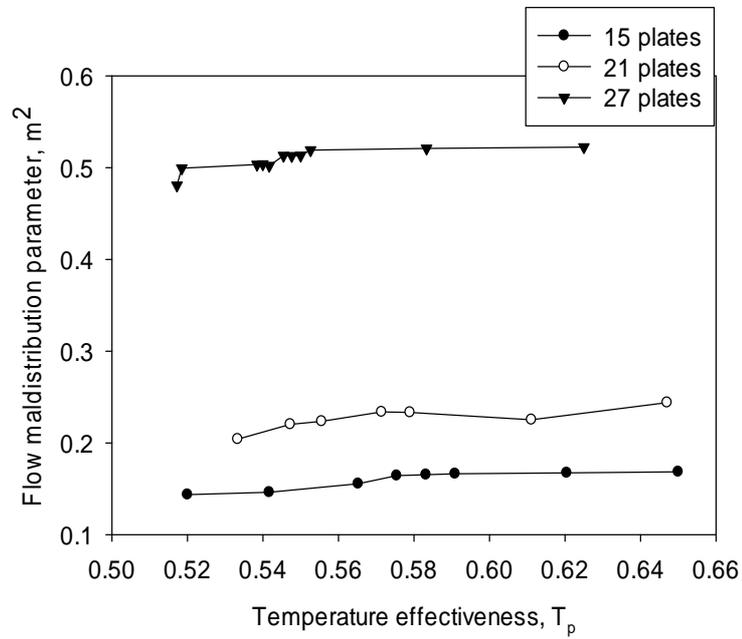
Fig. 9.Variation of flow maldistribution with temperature effectiveness for different sets of plate
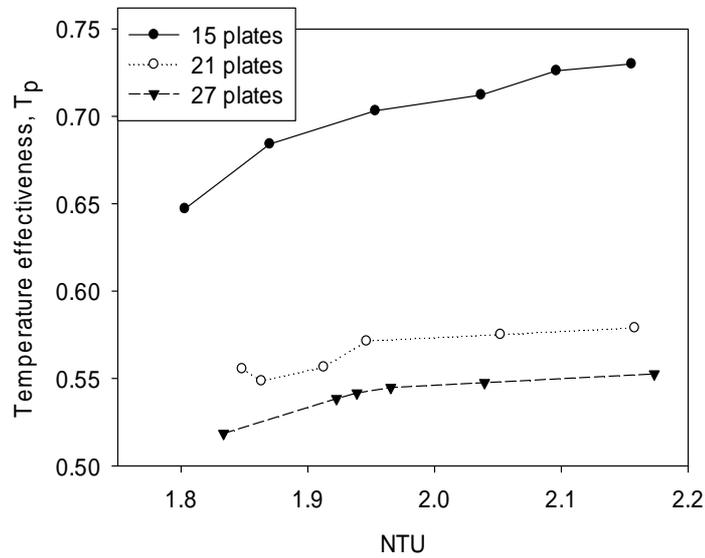
Fig. 10.Variation of temperature effectiveness with NTU of different set of plate heat exchanger

Bhupal Kumar and Singh
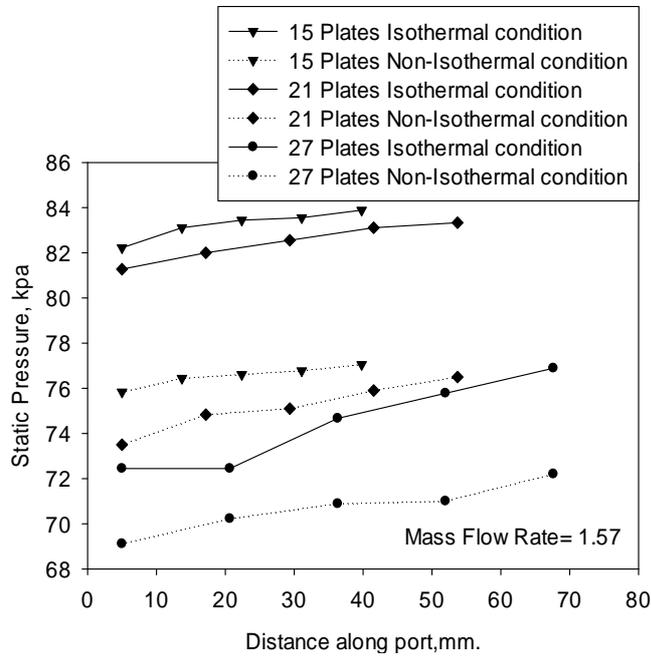
Fig. 11.Variation of static pressure along the inlet port at isothermal and non-isothermal condition
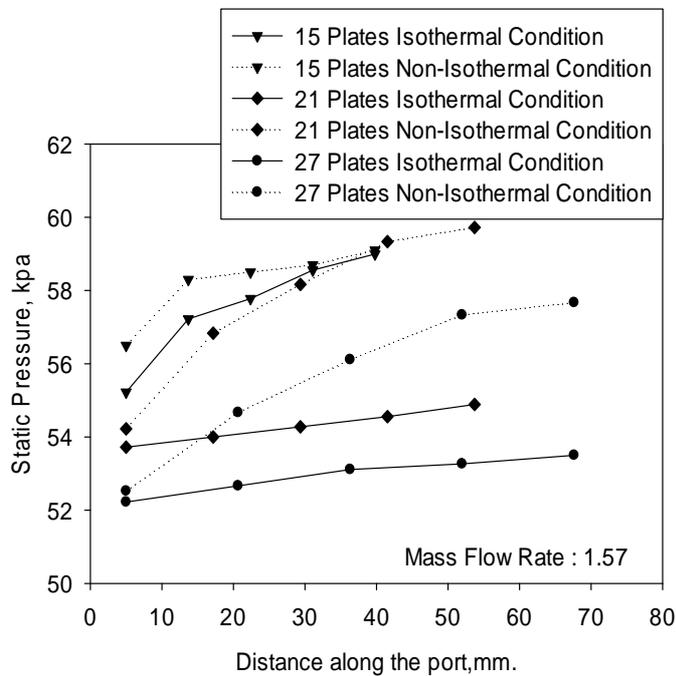
Fig. 12.Variation of static pressure along the outletport at isothermal and non-isothermal condition

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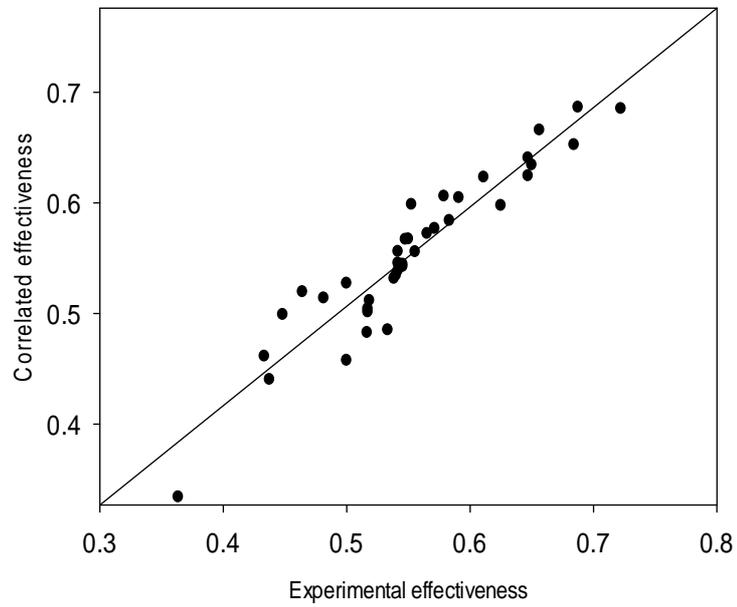
Fig. 13.Parity plot of effectiveness of PHE

RESEARCH ARTICLE

Dermatoglyphics: Analysis of Finger Patterns among Varsity Throwers

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ABSTRACT

Dermatoglyphic is the scientific study of fingerprints. Dermatoglyphics has been a useful tool in understanding basic questions in scientific studies especially in sports and physical education may give answer for it. The present study aims to examine the dermatoglyphic finger patterns among all India varsity Throwers. 32 throwers, who are participated in the 75th All India Inter University Athletic Championship held at Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka during January 2015. The selected subjects who are qualify for finals in the throwing events such as Shot put, Discuss throw, Javelin throw and Hammer throw for this study. The age of the subjects ranged from 18 to 28 years. According to their category, the subjects are classified into four groups such as group-I Shot-put (n=8), group-II Discus throw (n=8), group-III Javelin throw (n=8) and group-IV Hammer throw (n=8). The dermatoglyphic finger patterns like Whorls, Loops and Arches are select as dependent variables. The identifying finger patterns are follows by the standard procedure using Cummins ink method. The collected data analyzed statistically by using analysis of variance (ANOVA) to find out the significance among groups. Further, the Scheffe's post hoc test applied to know the paired mean difference between groups if they obtained 'f' value found significant. The level of confidence fixed at 0.05. The result shows that, there was a similar association of finger pattern among different varsity throwers on whorls and loops. And there was a significant difference found on arches among different varsity throwers.

Key Words: Dermatoglyphics, Finger Pattern, Various Varsity throwers.

INTRODUCTION

The type of fingerprint is unique based on the genetical characteristics of each individual. In the recent decades, a considerable improvement has achieved in the concept of relation between the types of pattern of lines on the fingers and psychiatric disorders. Fingerprint patterns are genotypically de-termined and remain unchanged from birth till death (Vij, 2005). The term dermatoglyphics was coined by Cummins method (Cummins, 1926). Fingerprint has been used as a biometric for the gender and age identification because of its unique nature and do not change throughout the life of an individual (Maltoni *et,al.* 2003). In fingerprint, the primary dermal ridges (ridge counts) are formed during the gestational weeks 12-19 and the resulting fingerprint ridge configuration (fingerprint) is fixed permanently (John,et.al.,1969). Fingerprints are static and its size and shape changes may vary with age but basic pattern of the fingerprint remains unchanged. Also, the variability of epidermal ridge breadth in humans is substantial (Kralik and Novotny, 2003).

Fingerprints are also used for the gender and age identification because of its unique nature and do not change throughout one's life (Pankanti, Prabhakar, and Jain, 2002). The sporting arena has become so much competitive, that the talents in different games and sports need to be identified at a very early age, so as to give them much specialised coaching from a much younger age (Singh & Kumar, 2013). Attempt to find out if the athletes in track and field differed from non-athletes in palmer dermatoglyphics revealed significant difference between athletes and non athletes in almost all the selected dermatoglyphic traits (Sharma and Shukla's ,1981).The Conducted of a comparative study of dermatoglyphics in sportsmen and non-sportsmen. The findings revealed the dominance of loop finger patterns and significantly greater inter tri-radial distances in sportsmen (Verma and Kumar ,1986) The Dermatoglyphic features may be used as a suggestive diagnostic tool to make a provisional diagnosis to identify the persons who are at risk of some ailments and to check the performance among athletes of different sports activities. However, it requires more extensive and studies in a large number of patients as well as athletes (Sharma and Sharma, 2012). Besides the patterns of dermatoglyphics among athletes of different sports activities may help in finding specific variations among different sports groups and so the study of dermatoglyphics may help to prepare a criterion for talent selection (Lahiri et al., 2013). Hence, it is a well-known fact that Dermatoglyphics patterns are reflected does the genetic properties of a person in relation to certain qualities. Keeping all these facts in mind the present study conducted to analyze the study of (dermatoglyphics) pattern among different varsity throwers.

MATERIALS AND METHODS

The subjects of the study were 32 throwers, who are participated in the 75th All India Inter University Athletic Championship held at Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka during January 2015. The selected subjects who are qualify for finals in the throwing events such as Shot put, Discuss throw, Javelin throw and Hammer throw for this study. The age of the subjects ranged from 18 to 28 years. According to their category, the subjects are classified into four groups such as group-I Shot-put (n=8), group-II Discus throw (n=8), group-III Javelin throw (n=8) and group-IV Hammer throw (n=8). The dermatoglyphic finger patterns like Whorls, Loops and Arches are select as dependent variables. The identifying finger patterns are follows by the standard procedure using Cummins ink method. The number of patterns from all 10 fingers counted and added separately. Thus, an individual's total of all patterns will be ten, which may or may not show whorls, loops and arches. The collected data analyzed statistically by using analysis of variance (ANOVA) to find out the significance among groups. Further, the Scheffe's post hoc test applied to know the paired mean difference between groups if they obtained 'f' value found significant. The level of confidence was fixed at 0.05.

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RESULTS

The table I shows that there was no significant difference among varsity throwers between loops and arches of both right and left hand. The result also shows that that there was a significant difference found on left hand arch andright hand arch among varsity throwers. Further, the scheffe's post hoc test was applied for left and right hand arches to find the significance between the groups

The table II shows that there was no significant difference found between SP and DT, SP and JT, DT and JT on both right and left hand arches. The result also shoes that there was a significant difference found between SP and HT, DT and HT, JT and HT on both right and left hand arches.

RESULT AND DISCUSSION

Te result of the study shows that there was no significant difference among varsity throwers on whorls and loops of both right and left hand. The right and left hand arches found significant among varsity throwers. Further, the hammer throwers have arches when compare with other throwers and they did not have single arches in both hand fingers.The various studies found relation with based on the present result were discussed below. However, minimum numbers of study in association with sports. A fingerprint is an individual characteristic, no two yet been found to possess identical ridge characteristics. Fingerprints are a reproduction of friction skin ridges found on the palm of the fingers and thumbs. Dermatoglyphics deals with the study of the epidermal ridges and their configurations on the fingers, palms and soles. The study on Dermatoglyphics patterns of athletes and non-athletes found significant differences among throwers, jumpers, runners and non-athletes in males as well as in females in a number of selected Dermatoglyphics variables (Bharadwaj, 1986). In the other study investigated the factor structure of dermatoglyphic variables on national level men gymnasts (Verma and Sexena, 1988). Here the finger patterns are unchanged among the different versity throwers. The archs are very low in all subjects when compared with loops and whorls.

CONCLUSION AND IMPLICATION

The study concluded that the whorls and loops are similar among all throwers however the shot-put throwers, discuss throwers, Javelin throwers have more whorls and loops when compare with hammer throwers. The arches are present only for hammer throwers when other throwers did not have a single arch in both hand fingers. Based on the result of the study the dermatoglyphic variable may useful for selection of throwers based on finger pattern. .

ACKNOWLEDGEMENTS

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Table.I: ANOVA on Finger Patterns of Among Varsity Throwers

Finger Patterns	Test	Shot Put	Discuss Throw	Javelin Throw	Hammer Throw	SOV	SS	Df	MS	F
Left Hand Whorl	Mean	2.62	2.50	2.50	2.25	B	0.59	3	0.19	0.16
	SD	1.18	1.19	1.30	0.46	W	33.37	28	1.19	
Right Hand Whorl	Mean	3.12	2.87	3.12	2.50	B	2.09	3	0.69	0.59
	SD	1.24	1.12	1.24	0.53	W	32.62	28	1.16	
Left Hand Loop	Mean	2.25	2.75	2.62	1.87	B	3.75	3	1.25	0.92
	SD	1.38	1.16	1.30	0.64	W	37.75	28	1.34	
Right Hand Loop	Mean	2.00	1.19	1.75	1.87	B	0.25	3	0.08	0.07
	SD	1.87	1.12	1.28	0.35	W	31.25	28	1.11	
Left Hand Arch	Mean	0.00	0.00	0.00	0.87	B	4.59	3	1.531	14.91*
	SD	0.00	0.00	0.00	0.64	W	2.87	28	0.103	
Right Hand Arch	Mean	0.00	0.00	0.00	0.62	B	2.34	3	0.781	11.66*
	SD	0.00	0.00	0.00	0.51	W	1.87	28	0.067	

The table value of 3 and 28 is 2.95.

Table.2: Scheffe's Post Hoc Test of Arches among Throwers

Hand	SP vs DT	SP vs JT	SP vs HT	DT vs JT	DT vs HT	JT vs HT	CI
Left Hand Arches	0.00	0.00	0.87*	0.00	0.87*	0.87*	**0.48**
Right Hand Arches	0.00	0.00	0.62*	0.00	0.62*	0.62*	**0.39**

*Significant SP - Shot put DT - Discuss Throw JT - Javelin Throw HT - Hammer Throw
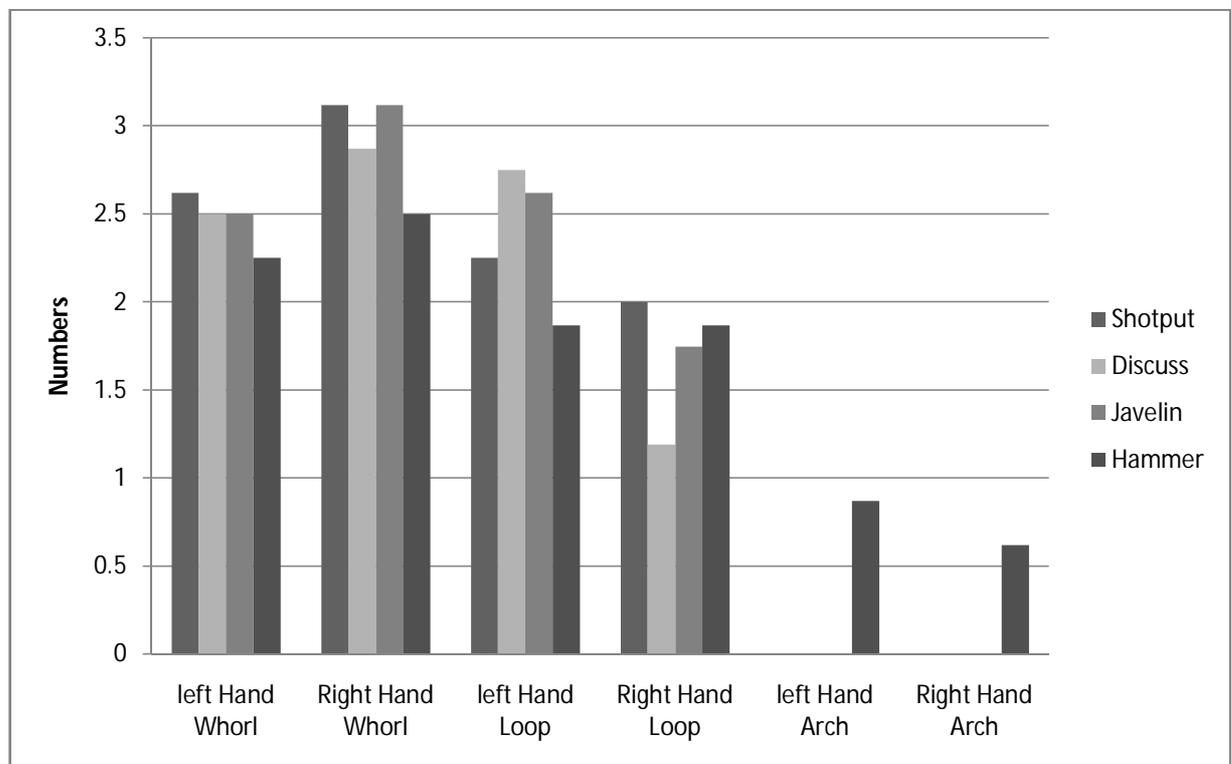
Fig.1. Finger Pattern among Varsity Throwers

RESEARCH ARTICLE

Emission of Volatile Organic Compounds during the Melting Process of Mechanical Recycling of Agricultural Polymers

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ABSTRACT

The scope of adoption of plastics in agriculture is very wide in agricultural system including conserving the natural resources, enhancement of production, productivity and quality of produce. In the developed countries, plastics have become an inevitable part of agricultural production to utilisation system. More than half the plastics are disposed by burning on-farm, with most of the remainder buried or dumped on-farm. Due to inefficiencies of open combustion, emissions from open burning are much greater per mass of material burned than emissions from controlled incineration (*e.g.,* 20 times as much dioxin, 40 times as much particulate matter). These emissions pose risks to human health. The aims of this study are to clarify the species and the amount of compounds emitted in air or N2 atmosphere and at several temperature during the melting process of plastic mechanical recycling and to suggest using an anoxic atmosphere to reduce emission of these compounds. Based on results obtained, lower temperature and lower oxygen level is recommended to reduce hazardous or malodour compounds during the plastic melting process of mechanical recycling.

Keywords: LDPE, Melting Process, Volatile Organic Compounds.

INTRODUCTION

Plastics (LDPE, HDPE, polystyrene resins) have become ubiquitous in agriculture: In dairy farming they are used as silage bags, bunker silo covers, bale wraps and twines; in nurseries and ornamental horticulture they are used as hoop-house covers, trays and containers; in fruit and vegetable production, as row covers and mulch films. Plastic pesticide containers are used in all sectors of agriculture. Increasingly, plastics are substituted for the longer lasting materials previously used in agriculture (*e.g.,* silage bags in place of concrete silos, plastic hoop houses in place of glass green houses) because of production efficiency and economics. More than half the plastics are disposed by burning on-farm, with most of the remainder buried or dumped on-farm. Due to inefficiencies of open combustion, emissions from open burning are much greater per mass of material burned than emissions from controlled incineration (*e.g.,* 20 times as much dioxin, 40 times as much particulate matter). These emissions pose risks to human health. Hence, recycling is required for waste plastics by mechanical process. In this mechanical process, wastes plastics are melted and then recycle products are manufactured. Additionally, the amount of plastics recycled by this process has increased year-by-year, so mechanical recycling will become more and more important in the future. Meantime, hazardous compounds might be emitted during the melting process. To date, many studies were conducted to investigate emissions of volatile compounds from the plastics during the melting process [2-6]. Almost all reports indicated that the compounds from the process are products by polymer degradation. When waste plastics were melted, other compounds originated from various factors such as additives besides the polymer degradation would be emitted. The aims of this study are to clarify the species and the amount of compounds emitted in air or N2 atmosphere and at several temperature during the melting process of plastic mechanical recycling and to suggest using an anoxic atmosphere to reduce emission of these compounds.

MATERIALS AND METHODS

Sample

FT5230 Industrial Grade LDPE material from Borouge Pte Ltd, Singapore was used. The waste plastic pellets obtained from the local recycler were also used.

Experimental apparatus

The experimental apparatus is shown in Figure 1. The sample pellets were placed in a tubular furnace (Ceramic Tubular Furnace ARF Series, Asahi Rika Corporation) with a temperature controller (Digital Temperature Controller AMF-N, Asahi Rika Corporation) and heated at the controlled temperature in air or N2. The gas flow was 300 mL/min. At the downstream of the furnace, the line was branched into 2 directions, and an ATD tube (referred to hereinafter) was connected to one of the following lines. VOCs contained in the out gas were collected via the tube pumped at 100 mL/min (Pocket Pump 210-1002, SKC) for 10 min. prior to sampling; 2-min preheating was conducted.

Adsorbents

ATD tubes (PerkinElmer) filled by 100 mg of Tenax TA (Tenax TA 60/80 mesh, Analytical Columns) and 70 mg of Carboxen 1000 (Carboxen 1000 60/80 mesh, SPELCO) were used for sampling. The tubes were preheated at 320°C for 3 hr in N2 flow.

Experimental condition

In this study, we aimed to qualitatively and quantitatively clarify the volatile compounds from the polymer. To investigate the effect of temperature on emission of volatile compounds, LDPE was heated at the temperature of 150, 200 and 250 °C. This temperature range was set on the assumption of operational conditions. The species of volatile compounds emitted

from LDPE and waste plastics in both air and N_2 atmosphere at the temperature of 200 °C were characterized. 0.3 g of plastic pellets was tested. Triplicate measurements were performed for each experimental condition.

Instrument	Condition
Desorption Instrument	
Primary Desorption	300 Deg Cel, 15 min
Secondary Desorption	5 Deg Cel – 300 Deg Cel, 45 min
GC / MS	
Column	HP-1 Methyl Siloxane Capillary, 60 m x 250 um x 1 um
Carrier Gas	He (1 mL/min)
Column Temperature	40 Deg Cel, 4 min ; 280 Deg Cel, 10 min
Detection Mode	Scan

RESULTS AND DISCUSSION

Effects of temperature

During the melting process of the Low density polymer materials, it clearly indicated that the emission of volatile organic compounds had an increasing trend (Figure.2). Higher temperature leads to higher emission of volatile organic compounds. 2230% of increase was observed between the emission quantities at 150 C and 250 C which accounted for 32 µg/g and 745 µg/g. During the process at 200 C, the emission of VOCs was 131 µg/g. Detailed classification of the emitted VOCs were also obtained and expressed in the Figure 3. Higher amount of oxidative compounds were generated due to rapid degradation of the polymer chain due to the higher processing temperature. VOCs content in the outgas was observed higher as the result of higher amount of volatile compounds gets emitted with higher temperature. At 200 C, amount listed under the category 'others' was found higher than the emission at 150 C and 250 C. Butylated hydroxytoluene forms the major component under the "others" category. It is one of the antioxidants present in the polymer materials. It suggests that BHT will diffuse independently of temperature in this range. Melting point of BHT is 69-71 °C and boiling point is 265 °C, BHT in LDPE polymer would also evaporated without reaction with other compounds. Hence it's clear that the Butylated hydroxytoluene will independently diffuse at its own properties resulting in the lower classification of the component "others". Aliphatic Hydro Carbon is the larger component during the higher temperature processing.

Effects of atmosphere

A large part of these VOCs emitted from these plastics are likely originated from the degradation of the polymers. A clear difference was observed in the results of LDPE and waste plastics between the atmospheres. As shown in Figure 4, the number of the peaks obtained in N2 was considerably smaller than that in air. Compared to the chromatogram obtained in air, the peak heights observed in the early retention time were negligible small in N2. According to the temperature condition of GC, these peaks correspond to the compounds with lower boiling points than 180 °C. Because most of VOCs were produced in the degradation of polymer, it is suggested that N2 inhibits the degradation procedure and therefore prevents production of lower molecular weight compounds. Comparison of the groups of VOCs emitted from LDPE in both the atmospheres is shown in Figure 5. Larger amounts of oxygenated organic compounds such as aldehydes, ketones and carboxylic acids were produced in air than in N2. The ratio of the amounts of these matters produced in N2 to in air was only 2.5 %. In this case, a large part of "Others" was occupied by BHT. As mentioned above, these BHT were considered to be emitted from the polymer by evaporation without reaction with other compounds. It indicates that the diffusion of BHT isn't predominant by atmosphere. The same result was observed in the case of waste plastics. N2 atmosphere prevented the emission of VOCs with the lower

boiling point than 250 °C (Figure 6). The larger amounts of oxygenated compounds were detected in air than in N2 (Figure 7). The amount of oxygenated productions detected in N2 was 38 % of that in air.

CONCLUSION

The result showed the higher temperature caused more TVOC emission. VOCs emitted from virgin plastics were likely to be polymer degradation products as written in literatures2-5. Besides the polymer degradation, additives were emitted from melting LDPE. Furthermore,VOCs which were likely to be derive from food residue attached to waste plastics and un-separated plastics such as PVC were emitted from waste plastics. More TVOC and more oxygenated organic compounds considered to be hazardous to human health and the cause of malodour were emitted in air atmosphere than in N2. Based on these results, lower temperature and lower oxygen level is recommended to reduce hazardous or malodour compounds during the plastic melting process of mechanical recycling.

ACKNOWLEDGEMENTS

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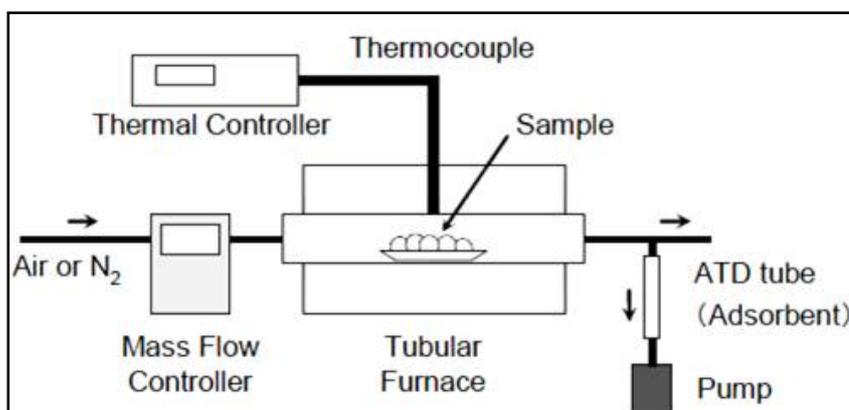
**Figure. 1. Experimental Apparatus**
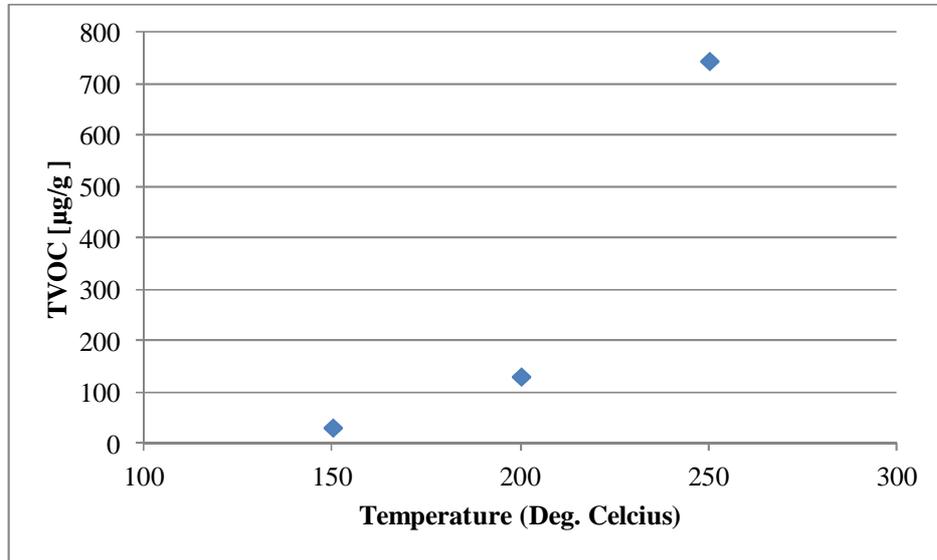
Figure. 2. The amounts of TVOC from melting LDPE in Air at 150, 200 and 250 °C
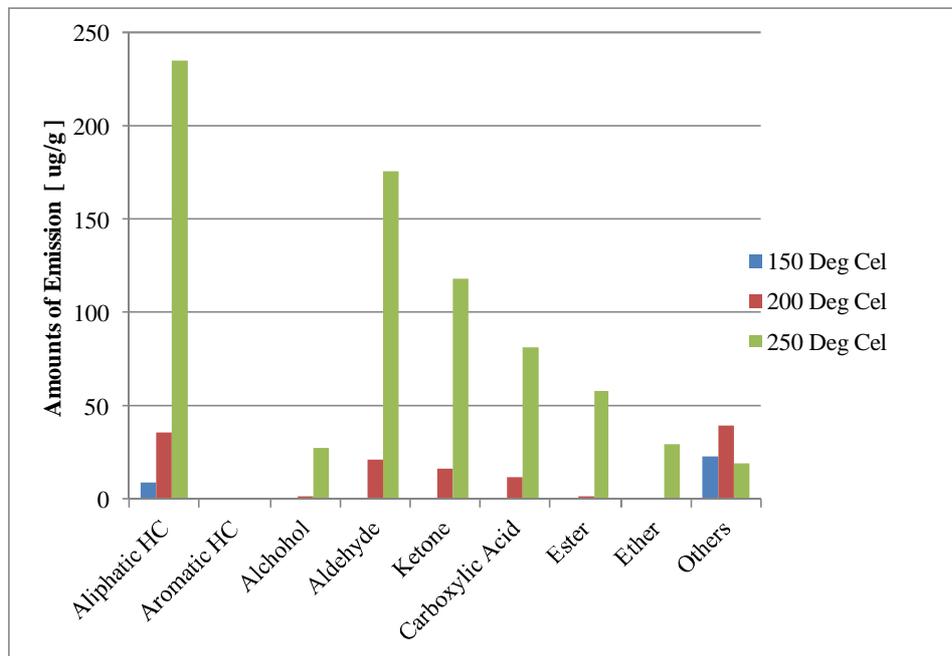
**Figure. 3. Classified compounds from melting LDPE in Air at 150, 200 and 250 °C**
HC = Hydrocarbon
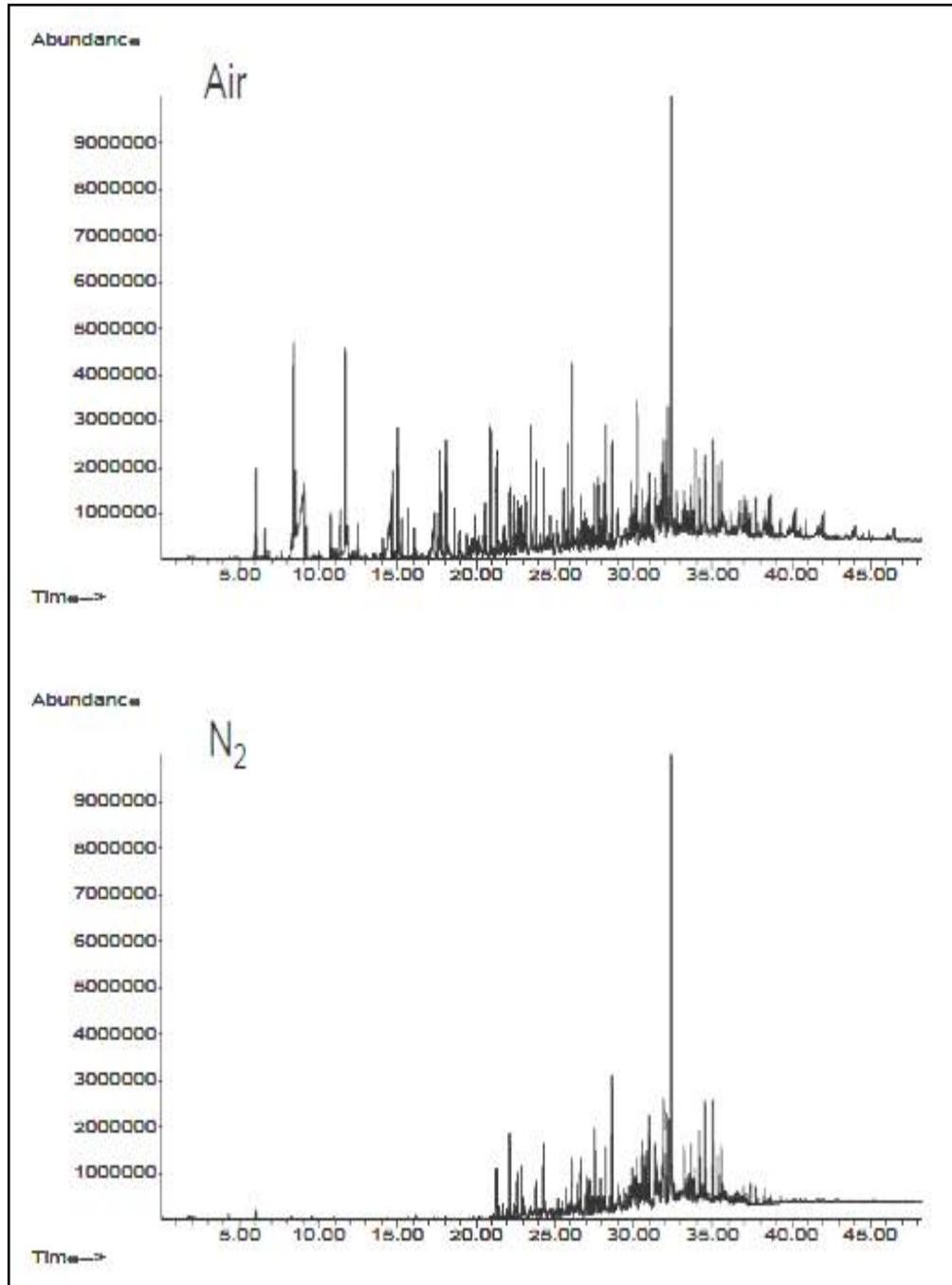
Figure 4. Total ion chromatograms of VOCs from melted LDPE in the Air and N2 atmosphere at 200 °C – Computer Generated Image from Equipment
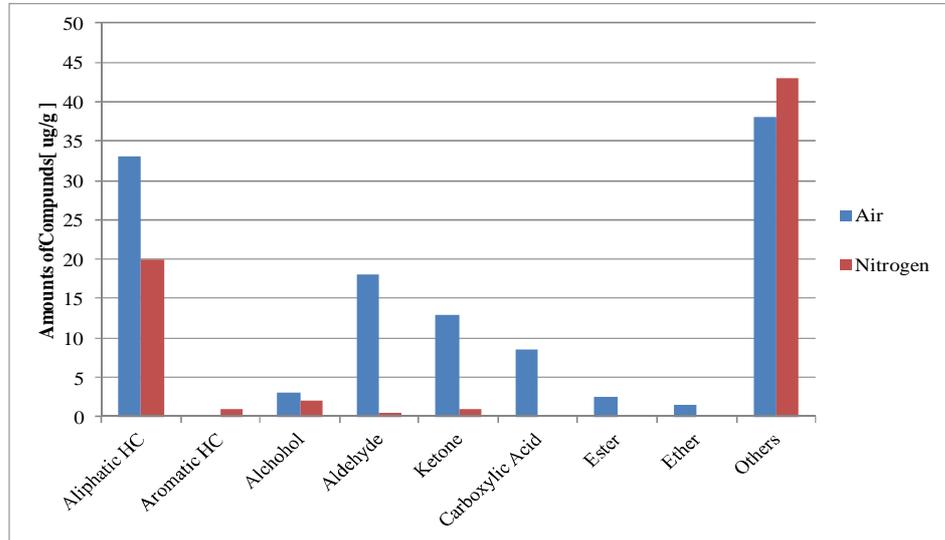
Figure. 5. Classified compounds from melting LDPE in Air and N2 atmosphere at 200 °C
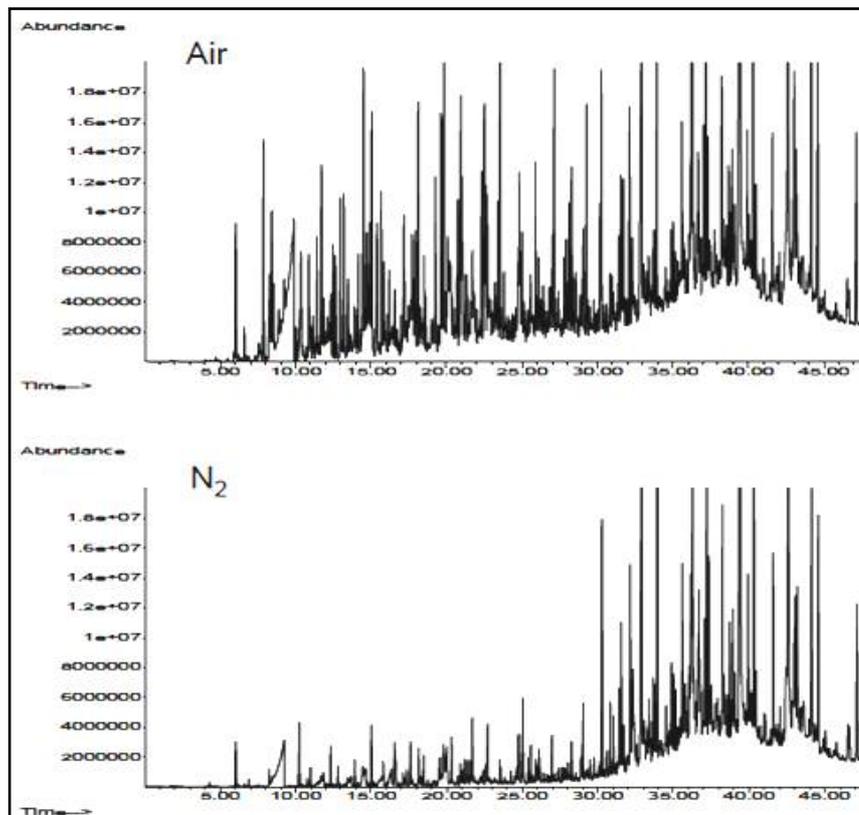
**Figure 6. Total ion chromatograms of VOCs from melted waste plastic in the Air and N2 atmosphere at 200 °C – Computer Generated Image from Equipment**
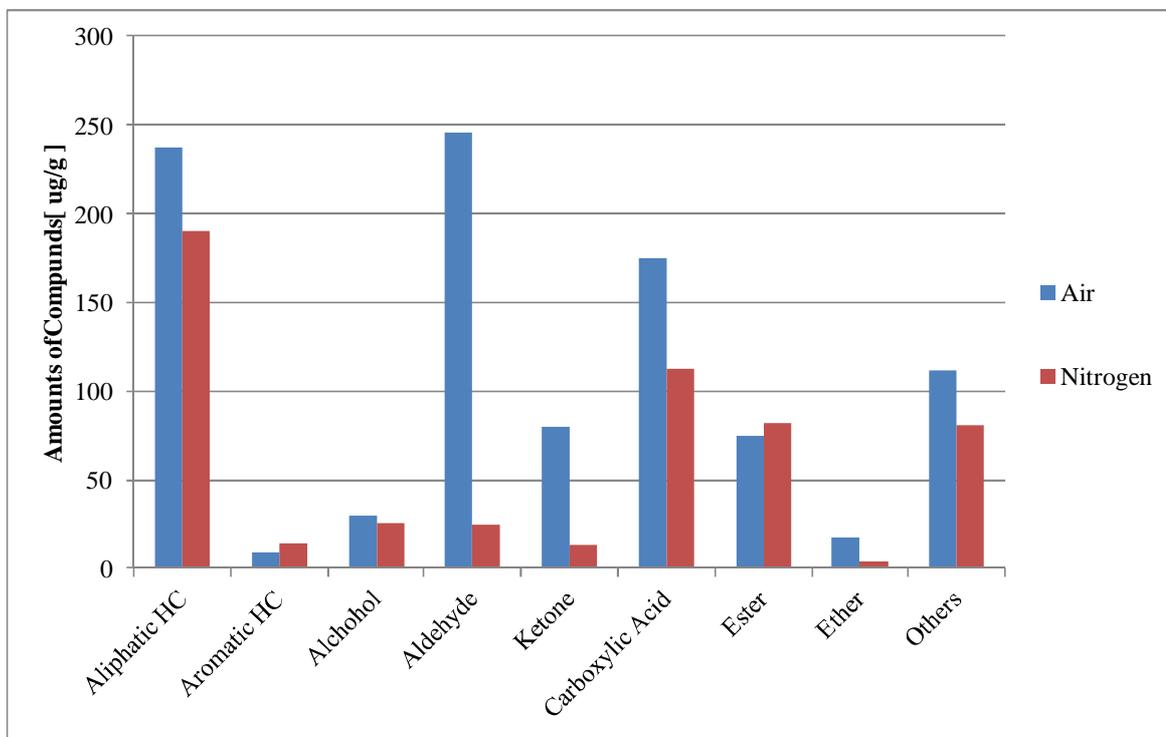
Figure. 7. Classified compounds from melting waste plastic in Air and N2 atmosphere at 200 °C

RESEARCH ARTICLE

Isolation, Evaluation and Characterization of *Pseudomonas* sp. from Rhizospheric Soil of Marigold Plant with Biocontrol Activity against *Rhizoctonia solani* under *In vitro* Conditions

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ABSTRACT

Twenty two isolates of *Pseudomonas* sp. were isolated from rhizospheric soil of marigold and characterized on the basis of morphological and biochemical properties. These isolates were evaluated for their biocontrol property against *Rhizoctonia solani*, various plant growth promoting traits, seed bacterization and plant protection assay of maize. All isolates produced indole acetic acid whereas the production of HCN, siderophore, ammonia and solubilized phosphate was 81.81%, 72.73%, 63.63% and 50% respectively. Twelve isolates inhibited the growth of *R. solani* in dual culture assay. Seeds of Kanchan-25 variety of Maize were used for seed bacterization and plant protection assay. Out of twelve active isolates, six isolates viz BD-7, BD-8, BD-10, BD-12, BG-4 and BG-5 were selected and screened for their potential role as PGPR. Selected isolates enhanced the rate of seed germination and also suppressed the growth of fungal pathogen, *R. solani,* when co-inoculated with fungal pathogen and isolate.

Keywords: *Pseudomonas*, PGPR traits, Anti-rhizoctonial activity, Seed bacterization, Maize

INTRODUCTION

Chemical fertilizers and pesticides are used in agriculture to attain higher yields. This dependency on these organic compounds have raised problems such as environmental pollution, health hazards, interruption of natural ecological nutrient cycling and destruction of biological communities that support crop production. Therefore, bio-resources such as plant growth promoting microorganisms can be used in place of chemical fertilizers and pesticides, which are

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novel and potential tools to provide substantial benefits to agriculture [1].*R. solani* is a ubiquitous soil-borne plant pathogenic fungus that causes significant yield losses in many agriculturally important crops and is responsible for various plant diseases such as collar rot, root rot, damping off and wire stem [2, 3]. In corn (*Zea mays*), reddish-brown lesions on roots, seedlings may be stunted or killed and grown-up plant may lodge symptoms that appear due to the infection of different anastomosis groups of *R. solani,* resulting in crown and brace root rot disease in plant [4, 5, 6].

The rhizosphere is the volume of soil surrounding and under the influence of plant roots [7]. In the rhizosphere, very important and intensive interactions take place between the plant, soil, microorganisms and soil microfauna. In fact, biochemical interactions and exchanges of signal molecules between plants and soil microorganisms have been studied [8, 9, 10]. These interactions can significantly influence plant growth and crop yields. Rhizobacteria are rhizosphere competent bacteria that aggressively colonize plant roots; they are able to multiply and colonize all the ecological niches found on the roots at all stages of plant growth, in the presence of a competing microflora [11]. Microorganisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some being pathogens and some displaying beneficial effects. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed PGPR [12]. So, this study was carried out to determine effect of isolated bacteria on *R. solani* and also the influence of bacteria on germination of maize seeds infected with *R. solani*.

MATERIALS AND METHODS

Sampling, selective isolation and biochemical characterization of isolate

Rhizospheric soil sample of marigold were collected from Dehradun (30° 19' N, 78° 04' E) and Ghurdauri (30°182'N, 78°695'E) regions of Uttarakhand, India. The samples were collected in sterile polybags and stored at 4°C until use. *Pseudomonas* was isolated from the root adhering soil of marigold through serial dilution and selective isolation method in which 9 ml of sterile distilled water was added to 1 g of rhizospheric soil and the mixture was shaken at 120 rpm for 5 min. Serial five-fold dilutions were prepared from the extract and 0.2 ml of each dilution was seeded onto King's B medium. All plates were incubated at 28 ± 2^0C for 2 days. *Pseudomonas* was biochemically characterized as per standard methods of Cappuccino and Sherman (1992) [13] that includes Grams reaction, starch hydrolysis, hydrolysis of gelatin, casein hydrolysis, catalase test, oxidase test, citrate utilization test, Methyl Red and Voges-Proskauer (MR-VP) test and H_2S production test.

Plant growth promoting traits of isolates

Quantitative estimation of Indole Acetic Acid (IAA)

All the isolates were screened for IAA production. A loopful culture was inoculated and incubated into pre-sterilized nutrient broth containing 1% of tryptophan for 5 days at 28 ± 2^0C. After incubation, broth were centrifuged at 3,000 rpm for 30 min and 2 ml of the supernatant was mixed with two drops of ortho-phosphoric acid and 4 ml of Salkowaski reagent (50 ml, 35% perchloric acid; 1 ml 0.5 M $FeCl_3$). Appearance of pink color indicated IAA production and OD was measured at 535 nm [14].

Phosphate Solubilization Activity

All the isolates were screened for inorganic phosphate solubilization. A loop full culture was streaked onto Pikovskaya's agar medium containing inorganic phosphate and plates were incubated at 28 ± 2°C for 3 days. After 3 days, the colonies showing the clear halo zone around them indicated solubilization of mineral phosphate [15]. Phosphate solubilization activities were screened by measuring the clearing zone surrounding the developed bacterial colony via calculation of phosphate solubilization index (PSI):

PSI= A/B × 100
Where A= total diameter (colony+ halo zone)
B= diameter of colony

Siderophore production assay

Isolates were streaked on the center of Chrome Azurol S (CAS) agar media and incubated at 28 ± 2°C for 48h. After incubation, orange halos around the colonies indicate the occurrence of siderophores in blue-colored CAS media which shows the consumption of iron in CAS media [16].

Hydrogen cyanide production

It was detected by spreading 1 ml of 24 h old broth culture of *Pseudomonas* on the Kings B medium and incubating plates with Whatman filter paper no.1 flooded with the solution containing 0.5% picric acid in 2% sodium carbonate located in the upper lid of Petri plate. To avoid the escape of the gas, the plates were sealed with the parafilm. After 24-48 h incubation, yellow to orange change in the color of the filter paper was observed [17].

Ammonia Production

Isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water and incubated for 48-72 h at 25±2°C. Nessler's reagent (0.5, ml) was added in each tube. Development of blue to light yellow colour was a positive test for ammonia production [13].

VI) In vitro antirhizoctonial activity

The isolates were preliminary screened for antifungal activity against *R. solani*. Eight mm agar plug of pure culture of *R. solani* was prepared by using sterile cork borer and plug was placed at the centre of a Petri dish containing PDA (Potato Dextrose Agar). A loopful bacterial isolates were point inoculated on PDA 1.5 cm from the edge of each plate [18]. Plate was incubated for 72h at 28°C followed by calculating percent inhibition of radial growth (PIRG) by the following formula:
PIRG= (R1-R2/ R1) ×100
Where R1 = Radial growth of fungus in control plate
R2 = Radial growth of fungus interacting with antagonistic bacteria

Maize seed bacterization and Plant growth

The experiment was conducted to assess the influence of six selected efficient isolates for the germination efficiency and antagonism against fungal plant pathogen, *R. solani*. Isolates were grown in nutrient broth medium on a shaker (150 rpm) for 2 days and centrifuged at 10,000 rpm for 5 minutes. Kanchan-25 variety of Maize procured from local Krishi Vigyan Kendra was used. The seeds were rinsed with distilled water and then surface sterilized with 5% sodium hypochlorite solution for 10 minutes. Then, seeds were washed with distilled water for two- three times to remove the residual sodium hypochlorite. The seeds were soaked in sterile 1 % carboxy methyl cellulose (CMC) solution for 24 hrs. Eight sterilized seeds were placed in each sterilized Petri plates. The plates were seeded aseptically with the following sets in triplicate. The seeds germination was observed after 7 days of incubation.
Set1- Seed control- seeds were coated with carboxy methyl cellulose (CMC).
Set2- Seed coated with CMC and isolates.
Set3- Seed coated with CMC and *R. solani.*
Set 4 -Seed coated with CMC, isolate and *R. solani.*

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RESULTS AND DISCUSSION

Isolation and biochemical characterization of isolate

The present study was focused on the isolation of *Pseudomonas* from rhizospheric soil of Marigold, anti-rhizoctonial activity and maize seed bacterization and its growth. Twenty two bacterial isolates were effectively isolated from the rhizosphere of Marigold. On the basis of morphological and biochemical characterization, the isolates were found to belong to genus *Pseudomonas*. All isolates were Gram negative in nature and had rod shape. They were catalase and oxidase positive. 19 isolates were utilizing citrate as sole source of carbon and energy. 18 isolates were hydrolyzing casein and gelatin while only four isolates were capable to hydrolyzing starch. Isolates were not capable to produce H_2S gas and acid to become MR-VP negative.

Plant growth promoting activity of isolates

Several researchers are working on isolation of bacteria from various niches, exhibiting plant growth promoting activity and biocontrol activity, to improve and increase the fertility of soil. Twenty two isolates were isolated from rhizosperic soil of marigold and more than 50% of isolates were having different plant growth promoting activity and biocontrol activity. Recently, Przemieniecki *et al.*, 2016 [19], isolated *Pseudomonas* sp. SP0113 from potable water of closed well. The isolate had several plant growth promoting traits such as phosphatase activity, phosphate solubilization activity, capability to produce ammonia and also possess antifungal activity against the *R. solani*, *Fusarium* sp. and *Microdochium nivalis*. Similarly, Elkahoui *et al.*, 2014 [20], isolated endophytic bacteria P2, identified as *Pseudomonas* sp., from leaves of olive tree. The strain P2 had antifungal activity and specifically inhibited the growth of mycelia of *R. solani* upto 86%. Strain P2 also had capability to produce siderophore.

Phosphate solubilization

Eleven isolates (50%) among 22 isolates solubilized phosphate on solid Pikovskyaya's agar and showed their potential to solubilize phosphate by formation of clear halo zone on Pikovskyaya's agar. Maximum zone was observed in isolate BD-9 (21 mm) and phosphate solubilizing activity was ranged between 21-15mm. According to phosphate solubiliztion index, the maximum amount of soluble phosphate from tricalcium phosphate $[Ca_3(PO_4)_2]$ was released by BD-9 (262.20) and the lowest by BG-7 (155.55) (Table-1). Phosphorus is second largest element required by plant after nitrogen. It is present in soil as insoluble form and cannot be utilized by plant. The key role is played by microorganism present in soil to solubilize these insoluble phosphates [21]. The microorganism which are present in rhizosphere (some of them called as PGPR) and free living phosphate solubilizing microorganism makes phosphorus available to plants by releasing phosphate from inorganic and organic phosphate compounds present in soil [16]. These phosphates solubilizing microorganisms (*Pseudomonas*, *Rhizobium*, *Bacillus*) can be used as inoculants and are helpful in increasing crop yield [22].

Quantitative analysis of IAA Production

All the twenty two isolates were producing indole acetic acid by degrading tryptophan. Maximum concentration of IAA was produce by BD-1 (48.6 µg/ml) after 96 h of incubation (Table-1). Production of phytohormones such as indole acetic acid may be contributed generally by plant associated microorganism which resides in rhizosphere of host plant [23]. Their excretion leads to root establishment either by primary root elongation or proliferation of lateral and adventitious roots, which increases their ability to make bonding with soil and to water and nutrient uptake from the soil.

Siderophore Production

All twenty two isolates were screened for siderophore production on solid CAS blue agar and showed a clear zone of decolorization (blue to orange) representing iron chelation and 72.73 % of isolates (16) produced siderophore. The microorganism that produced siderophore was capable of suppressing the growth of some soil borne fungal pathogen. *Bacillus subtilis* BN1 was isolated from rhizospheric soil and inhibited the growth of *Macrophomina phaseolina* up to 60% [24]. This shows that siderophore acts as biocontrol agent.

Ammonia production

Fourteen isolates produced ammonia gas among twenty two isolates after 72 hours of incubation. Ammonia released by diazotrophs is one of the most important traits of PGPR's which benefits the crop. It is supported by ammonia production in 95% of isolates of *Bacillus* followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45%) isolated from rhizospheric soil [25] and twenty six *Pseudomonas* isolates were producing ammonia, which were isolated from the rhizosphere of apple and pear plants [26]. These can be economical and helpful in crop improvement by providing nitrogen to plant.

HCN production

Eighteen isolates were capable to produce HCN after 72 h of incubation. HCN production can play an important role in antagonistic potential of PGPR in controlling fungal diseases in wheat seedlings under in-vitro conditions [27]. Sclerotia germination of *M. phaseolina* was suppressed by HCN producing PGPR [28].

Anti-rhizoctonial activity

All the twenty two isolates were preliminary screened for antifungal activity against phytopathogen R. solani and out of which twelve isolates were able to inhibit the growth of *R. solani* (Fig.1). Maximum inhibition index was observed in isolate BG-4 (55) (Table-1). Similarly, Toppo and Tiwari, 2015 [29], isolated 28 isolates from rhizospheric soil, and identified strains as *Pseudomonas* sp.. Of these isolate four isolates (PKS10- *Pseudomonas syringae*, PKM11- *P. syringae*, PKJ25- *P. alcaligenes* and PKB27- *P. alcaligenes*) displayed anti-rhizoctonial activity. Rhizospheric bacteria were used for production of compounds which inhibited the growth of plant root pathogens including *R. solani* and *Fusarium oxysporum* [30]. Similar work was supported by Ramayasmruthi et al., 2012 [31]. They isolated eighteen isolates from the rhizosphere of *Solanaceae* family. Out of these, six isolates were active against different phytopathogens viz *Collectotrichum gleosporioides*, *Alternaria brassicola*, *Alternaria brassiceae*, *Alternaria alternate*, *Fusarium oxysporum*, *R. solani* and *Phytophthora* sp. Eleven isolates were isolated from rhizospheric soil of rice field of which four isolates having high efficiency inhibited the mycelia of *R. solani* [32].

Seed bacterization and plant protection study

Isolate BD-7, BD-8, BD-10, BD-12, BG-4 and BG-5 were selected on the basis of their antagonistic activity for seed bacterization and plant protection activity. Selected PGPR isolates significantly affected the maize seedling (Kanchan -25). Germination parameters were observed to know the extent of completeness of germination. Recording of germination was carried up to 7 days in plate method (fig. 2) and at the end of 7 days all the seeds that had not germinated were taken out. These germinated seeds were counted to calculate germination percentage (fig. 3). Treatment of maize seeds with different isolates showing variable germination percentage ranges from 43.75 to 75 and four isolates viz BD-7, BD-8, BD-12 and BG-5 have similar or more germination percentage with respect to control. The treatment of seeds with co-inoculation of fungal pathogen (*R. solani*) with different isolates showed growth in seeds as compared to seeds treated with fungal pathogen. The result also revealed that the isolates BD-7, BD-10, BG-4 and BG-5 promoting growth of seeds treated with co-inoculated isolate and pathogen were equally or

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more as compared to seeds inoculated with isolates alone. This is suggested that isolates were capable to promote the growth of maize seeds and also suppressed the growth of fungal pathogen.The isolates significantly affected the length of maize seedlings (figure 4). Results revealed that the shoot length increased in isolate treated plants as compare to uninoculated control except in BD-8 isolate. The highest shoot length was 16.33 ± 2.54 cm (plant^{-1}) was recorded in treatment of BG-10 isolate followed by BG-4 (15.66 ± 1.31 cm, plant^{-1}). BD-12, BG-5 and BD-7 showed significantly higher shoot length over control. The increase in shoot length was observed in the seeds which were co-inoculated with isolates and pathogen over fungal inoculated seeds alone. Root length of maize plant was ranged from 15 to 27.03 cm plant^{-1}. The isolate BD-10 produced the highest root length (27.03 ± 1.40 cm plant^{-1}) in comparison to control and other isolates. Significant increase in root length was observed in seeds co-inoculated with isolate and pathogen over fungal inoculated seeds alone (fig.4). Similar work was done by Ramayasmruthi et al., 2012 [31], observed that the bacteria isolated from the rhizosphere of brinjal, capsicum and chilli can produce various traits of PGPR. Seed bacterization of chilli seeds with isolate R (*P. fluorescens*) and along with pathogen, *Colletotrichum gloeosporoides,* reduced 50% seed mortality. This treatment also showed 100% growth index indicating that isolate R can have the property of both as PGPR and as biocontrol.From the present study, it can be concluded that twenty two bacterial isolates that belong to genera *Pseudomonas* had different PGPR traits. Of these, six isolates were having good antirhizoctonial activity as they enhanced the biomass of maize. Therefore, we suggest that these isolates can be commercialized as biofertilizers as well as biocontrol agents against *R. solani.*

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Table 1: Plant growth potential and biocontrol potential of isolates.

| Sr. No.	Isolates	IAA quantity (µg/ml)	Phosphate solubilization index	Anti-Rhizotonial activity	Inhibition index = (A-B) X 100 /A
1	BD-1	48.6	250	-ve	-
2	BD-2	17.2	-	-ve	-
3	BD-3	21.2	200	-ve	-
4	BD-4	15.8	257.14	+ve	22
5	BD-5	21.6	-	-ve	-
6	BD-6	20.4	-	-ve	-
7	BD-7	22.2	-	+ve	51.11
8	BD-8	28.4	-	+ve	46.67
9	BD-9	45.2	262.2	-ve	-
10	BD-10	17.4	-	+ve	40
11	BD-11	21.8	-	-ve	-
12	BD-12	21.0	-	+ve	46.67
13	BG-1	17.2	181.82	+ve	26.67
14	BG-2	17.2	222.22	+ve	22
15	BG-3	17.2	180	+ve	22
16	BG-4	20.6	175	+ve	55.56
17	BG-5	20.2	-	+ve	44.44
18	BG-6	19.6	200	-ve	-
19	BG-7	20.2	155.55	+ve	28.89
20	BG-8	18.4	177.77	+ve	51.11
21	BG-9	20.2	-	-ve	-
22	BG-10	21.2	-	-ve	-
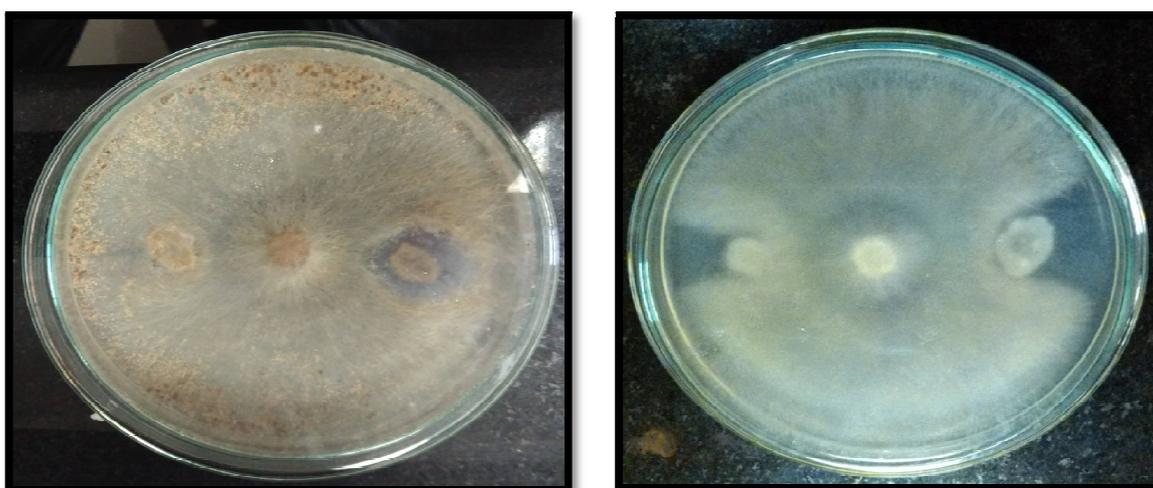
**Fig.1.Screening of isolates for anti-rhizoctonial activity.**
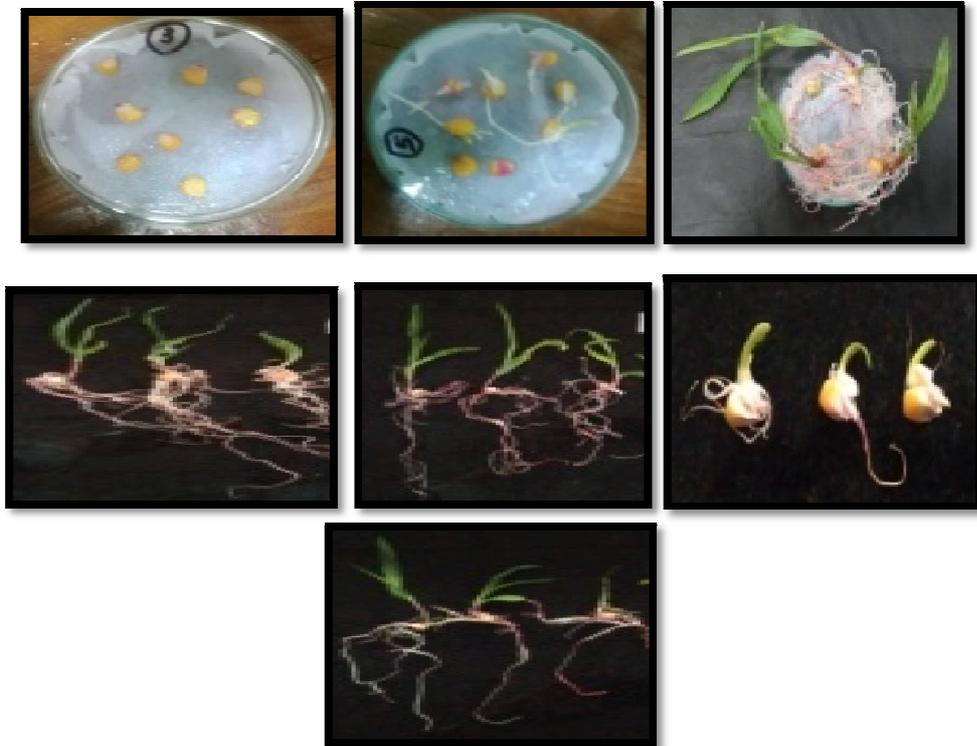
Figure-2: (a, b, c) Showing seed germination on 0, 3 and 7 day. (d-g) Showing root and shoot length of germinated maize seeds i.e. (d.) Control (e.) Seeds inoculated with isolate (BD-7) (f.) Fungal control (g.) Seeds inoculated with isolate along with *R.solani* (BD-10 + Fungus).
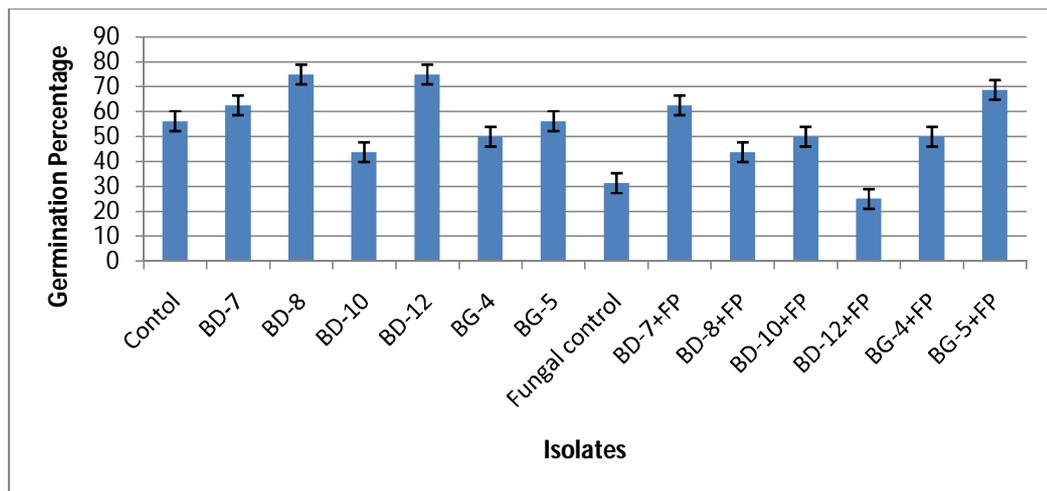

Fig.3. Germination percentage of maize seeds coated with different active isolate and co-inoculated with isolate and fungal pathogen. * FP denotes for fungal pathogen

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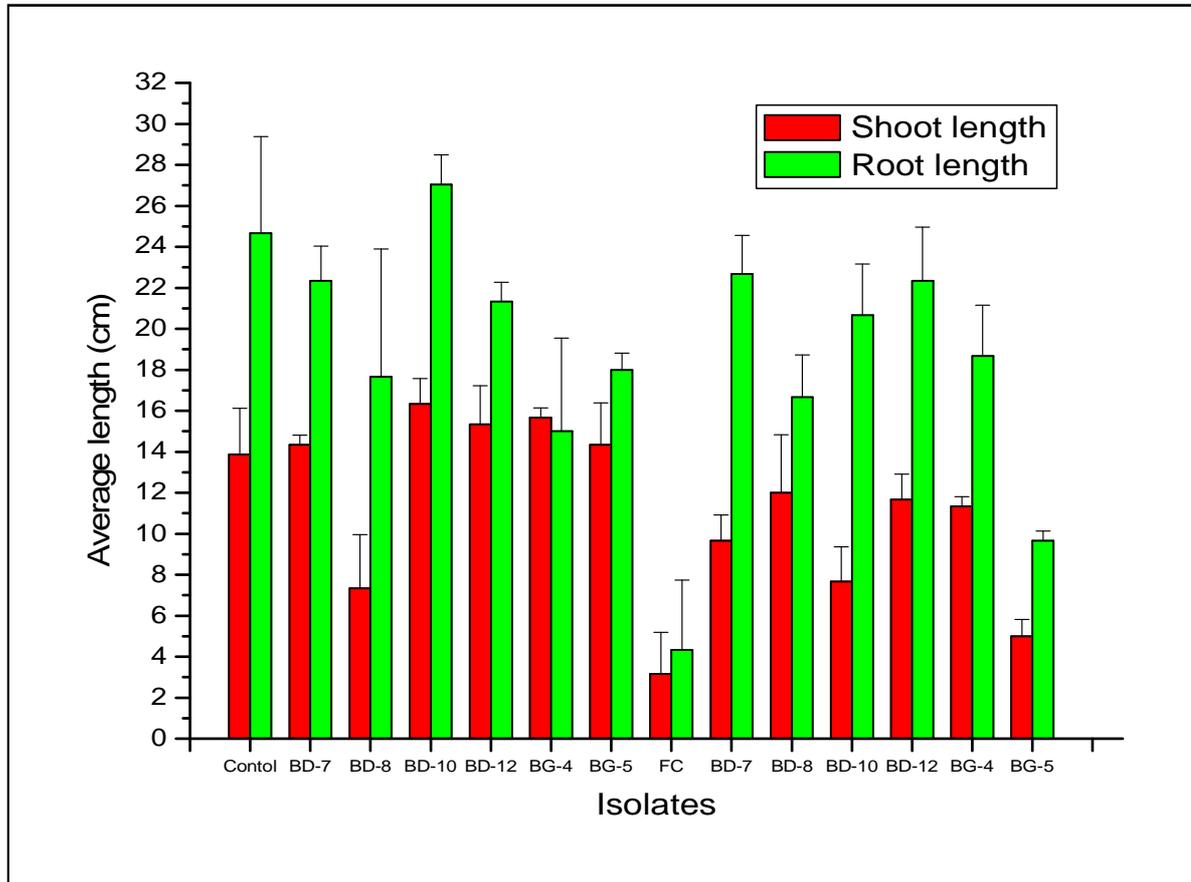
Fig.4. Root and shoot length of maize seeds grown in the presence of active isolates and along with fungal pathogens.

RESEARCH ARTICLE

Kinetic Uptake and Isothermal Dynamics for the Removal of a Malachite Green (Aniline Green) from Simulated Wastewater using Natural Adsorbents

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ABSTRACT

The uptake of malachite green by onion membrane was studied by using a batch technique. The investigations were conducted to indicate the effect of different experimental parameters such as initial concentration of malachite green, adsorbate dose, contact time, pH and temperature. The optimum conditions were determined from these parameters. Study of adsorption isotherms was achieved at the optimum condition which gives us the best fitting adsorption isotherm model. The equilibrium data of adsorption of malachite green onto onion membrane was fitted to Freundlich isotherm and pseudo-second-order kinetic models. The results showed that onion membrane can be used as an alternative adsorbent for decolorizing of wastewater.

Keywords: Adsorption Kinetic, Dye removal, Natural adsorbent, Waste water treatment

INTRODUCTION

The effluents from industries are highly polluted with large amounts of pollutants such as dissolved and suspended solids[1-2]. The release of such effluents has a harmful influence on environment and aqueous systems. Further, the presence of even small amounts of dyes in water is highly visible[3-4]. The removal of toxic chemicals from wastewaters

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before discharging into the environment is of great importance, since many dyes and their degradation products are toxic and carcinogenic, a serious hazard to the environment. The classical methods used to treat colored effluents include photocatalytic degradation, microbiological decomposition, electrochemical oxidation, membrane filtration, and adsorption techniques[1]. Among these, adsorption is the most widely used method because of its efficiency, easy operation, low cost, less energy intensiveness, simple design, and non-toxicity. It has been demonstrated that 14% of the artificial textile dyes utilized consistently are released to water streams. Wastewater treatment plants are the real wellsprings of these to the environment, because of the obstinate and complex nature in structure of dyes, it is extremely hard to decolorize dyes, which make it mandatory to dispose them from waste stream before being tossed out into the main stream. Most industrial systems use activated carbon as an adsorbent for removal of dyes in sewage due to its remarkable adsorption capability, because of the high price of activated carbon; adsorption is favored by utilizing cheaper materials. Previously conducted research shows that many cheap adsorbent can be successfully used to remove dyes from aqueous solutions. This study attempts to check the potential of onion membrane which is accessible in abundance in KOYA city, Kurdistan-region, to treat the industrial effluent containing MG dye.

MATERIALS AND METHODS

The membrane of onions was purchased from local markets in Kurdistan, Koya. Hydrochloric acid 37% (Merck),sodium hydroxide (Aldrich), potassium chloride (Aldrich), potassium hydrogen phthalate (Merck),sodium hydrogen carbonate (Aldrich), and potassium hydrogen phosphate (Merck) were used to prepare the buffer solutions with different pH values. Finally, MG (Aldrich) with a molecular formula of $C_{23}H_{25}ClN_2$ was used without further purification.

General procedure

Preparation of Adsorbent

Onion membrane was obtained approximately 1g/250g onion. The onions were peeled, chopped and then the membranes were removed from the leaves. They were rinsed and washed with distilled water to remove dusts. Then, it was dried at 65°C in an oven over night. After that, the dried adsorbent was kept in the desiccators to avoid moisture adsorption.

Swelling Measurements

The swelling property of the membranes were investigated by immersing 0.06 g of membrane (15 ×15 mm2) in 250 ml of distilled water at room temperature (±2°C) in atmospheric conditions until swelling equilibrium was reached. Following the removal from the water, they were blotted with filter paper and weighed. Then, the swelling capacity was calculated using the following equation[5-6]:

$$\text{Swelling \%} = \frac{W_2 - W_1}{W_1} \times 100 \qquad (1)$$

Where W1 (g) and W2 (g) are the weights of the dried and swollen membranes, respectively.

Dye Adsorption Process

The initial concentration of MG solutions was prepared by dissolving 0.25g of MG in 250 ml of deionized water. Then, the 0.06 g of dried onion membranes were immersed in a prepared MG solution and shaken at 180 rpm for 8 hours at room temperature. During this period, 5 ml of solution was taken for further analysis frequently. Finally, the collected solutions were filtered and the amount of non adsorbed dye ions in the solutions was determined

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spectrophotometrically using a UV-visible spectrophotometer (Agilent Carry-100 UV-Visible Spectrophotometer) at a wavelength of 617nm[7]. The MG adsorption amount, efficiency, and capacity were calculated by the following equations:

Adsorption amount (g.L^{-1}) = $C_i - C_e$ (2)

Adsorption capacity (g:g^{-1}) = qt = $\dfrac{C_i - C_e}{W} \times V$ (3)

Adsorption efficiency (%) = $\dfrac{C_i - C_e}{C_i} \times 100$ (4)

Where W is the mass of adsorbent in g, V is the volume of MG solution in L, and C_i and C_e are the initial and equilibrium concentrations in g.L^{-1}, respectively. Moreover, 0.06 g of onion membrane was immersed in each solution at 20°C and was allowed to equilibrate in an isothermal shaker. After a contact time of 24 hours, the suspensions were filtered through filter paper and the final pH values of supernatant were measured again using a pH meter. Lastly, the final pH values were plotted against the initial pH values. The pH at which the curve crosses the line final pH equal initial pH was taken as the pHzpc of the onion membrane[9].

RESULTS AND DISCUSSION

Malachite Green Stock SolutionVarious concentrations were prepared from stock solution of MG by dissolved 1000μg/ml, Fig.1. Shows calibration plotted of malachite green.

Swelling Properties of Onion Membrane

The functional swelling conducted of the onion membranes over 8 hours of soaked in deionized water is shown in Fig.2. The swelling percentage increased up to 1,106% and then plateaued, with no big differences in water uptake with further increases in time. Initially, the water molecules were in contact with the membrane, then, they attacked and permeate into the onion membrane cells. Clearly, this swelling system cannot continue forever, and by the increasing membrane-water interaction.

Dye Adsorption Properties of Onion Membrane

The adsorption capacity of MG onto onion membranes was studied. Fig.3. Shows the preparation of the membranes for MG adsorption (Figure.3a) and color changes in the membrane and dye solutions before and after adsorption during the first and eight hours of contact time. Thus, as can be seen before and after adsorption MG onto the membrane.

Characterization of FTIR

The membrane of onion contains proteins (with–COOH and –NH2 groups), sugars, carbohydrate, and vitamins C, B6 and A (with–OH groups), minerals, and over 80% water[10-11]. Fig.4. (a) showed that the membrane has several anionic groups such as –OH and–COOH. In addition, MG is a cationic dye consisting of–N (CH3)$_2$ can become charged species and have ionic and dipole–dipole interactions with anionic groups in the surface of the onion membrane[12]. In addition, the = N–and–N (CH3)$_2$ groups in the structure of MG have hydrogen bonds with hydrogen atom of–COOH and–OH groups of the onion membrane. Thus, the adsorbent can uptake MG very fast with high efficiency through the strong electrostatic attraction between the surface groups on the membrane and the cationic MG. Fig.4. (b) indicate the dye loaded successfully onto the membrane as can seen the FTIR spectra the peak at 1161.15 cm^{-1} for the C-N stretching vibrations and peak at 2918.30 cm^{-1} for C-H stretching of asymmetric - CH3 group gives the perception of structure of malachite green. The FTIR spectra of adsorbed dye showed peak at 1240.23 cm^{-1} for -C-N-

stretch with supporting peak at 1012.63 cm^{-1}, the peak at 2848.86 cm^{-1} indicate C-H stretching and peak around 3500 cm^{-1} for N-H stretch represents the formation of primary and secondary amines.

Effect of Contact Time

Achievement of the removal process was evaluated by a batch equilibration technique as a function of time. As shown in Fig.5 the adsorption capacity of MG dye on the membrane increased rapidly during the first hour of contact and then became slower until equilibrium was reached after eight hours. The maximum adsorption was 1.168 g.g^{-1} with 83.60% efficiency after the first hour and 1.198 g.g^{-1} with 96.3% efficiency after eight hours. This behavior can be referring to the larger surface area of the onion membrane at the initial stage of the adsorption process. Thus, as the available sites became saturated, adsorption did not increase significantly with further contact time.

Adsorption Kinetics

For the study of the potential using an adsorbent for a specific separation function and to investigate the adsorption efficiency as well as the adsorption rate, the kinetic model of the adsorption removal was considered. The adsorption kinetics of the malachite green ions with the onion membranes was examined using two kinetic models: pseudo first-order and pseudo-second-order, which are given in the following equations, respectively

$$\log(q_{e-}q_t) = \log q_e - \frac{K_1}{2.303}t \tag{5}$$

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \tag{6}$$

Where t is the time (min) and q_e, q_t, (g.g^{-1}) and q_e^2 is the amounts of MG adsorbed by the onion membrane at equilibrium, at time t, and at maximum adsorption capacity, respectively. K_1(min^{-1}), K_2(g.g^{-1}.min^{-1}) are the adsorption rate constants of the pseudo-first-order and pseudo-second order models, respectively. As shown in Table1, the theoretical equilibrium adsorption capacity (1.2879g.g^{-1}) using the pseudo-second-order model fitted well with the experimental data (1.813g.g^{-1}), with a better R^{2}value. Fig.6 shows the agreement between the experimental adsorption capacities with the calculated values of pseudo-second-order, which were obtained using the data in Table1. Therefore, the agreement between experimental data and pseudo-second-order can prove the physical adsorption of MG on a highly heterogeneous onion membrane.

Effect of Adsorbent Dosage

It is clear that by increasing the adsorbent dose the percentage of dye removal increases, but adsorption density, the amount adsorbed per unit mass, decreases. It is easily understood that the number of available adsorption sites increases by increasing the adsorbent dose and it therefore results in an increase in the percentage of dye adsorbed. Table 2 and Fig.7 showed the adsorption of MG 50 ppm as a function of dosage of membrane.

Effect of pH on the Adsorption Process

The values of pH of the solution have a significant effect in the adsorption process of the MG onto the adsorbent. Fig.8. showed that with an increase in the initial pH of the MG solution, adsorption capacity and removal increases rapidly and then increases slowly with a further increase in the pH. The maximum adsorption capacity was resulted at 1.202 g.g^{-1}, with 94.7% removal at pH 10. This result can be explained by the electrostatic interaction between the cationic MG species and the surface of the adsorbent, which should be a negatively charged species. The lower adsorption at acidic pH levels was probably due to the presence of an excess of H$^+$ ions competing with the dye cations for adsorption sites[13-14-15]. In order to confirm these results, the pHzpc of the onion membrane was

determined. In this study, pHzpc value was 5.9, which at this pH the adsorbent surface has net electrical neutrality. At a pH below the pHzpc, the surface of the adsorbent is positive, and at a pH above the pHzpc, the surface of the adsorbent becomes more negatively charged by losing protons. Thus, the adsorption of the MG reached its maximum value in the higher pH because of strong electrostatic attractions between the negatively charged surface of the onion membrane and the cationic MG.

Isothermal study

The main importance to design adsorption system is study adsorption isotherm. It is used to indicate the adsorption capacity of the adsorbent and also describe the interaction of an adsorbate with the adsorbent. Hence, two isotherm models, Langmuir and Freundlich were studied to find a more suitable model for the design process. The Langmuir model, and it is the most popular based on the assumption that the adsorbate molecules monolayer coverage (Chemisorption) on the surface of the adsorbent within uniform energies which is structurally homogeneous [17-21]. The Freundlich isotherm describes the adsorption process as non-uniform distribution of the adsorbate molecules onto surface of the adsorbent through multilayer adsorption (Physisorption) occurs on heterogeneous system[19].

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m k_L} \qquad (7)$$

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \qquad (8)$$

Where q_e (g.g^{-1}) is the amount of MG adsorbed at equilibrium time, q_m (g.g^{-1}) is the maximum adsorption capacity, and C_e (g.L^{-1}) is the equilibrium MG concentration. k_L and k_F (L.g^{-1}) are the Langmuir and Freundlich adsorption equilibrium constant. 1/n is the empirical Freundlich constant[20]. Fig.9. clearly indicate the calculated values of q_e belong to the Freundlich isotherm is in agreement with the experimental value, which revealed that the Freundlich isotherm is more suitable than the Langmuir isotherm for describing the adsorption. (Table 3) This is confirmed by the correlation coefficient (R^2) of the Freundlich isotherm model (0.9828), which is greater than R^2 (0.9714) of the Langmuir model. The empirical Freundlich constant (1/n) which can be obtained from the linear plot of log qe versus log C_e, is an indicator of the favorability and surface affinity for the solute. When the 1/n values are in the range 0.1-1, the adsorption process is favorable[22]. Furthermore, if the n is below1, then the adsorption is a chemical process; otherwise, the adsorption is a physical process. In this research, the value of 1/n is 0.24, false between 0.1 and 1, elucidate that adsorption of MG ions by an onion membrane is favorable with physisorption.

Effect of Temperature

The effect of temperature on the adsorption of MG with initial concentration of 50mg/ L at temperatures 20, 30, 40, 50 and 60 °C on onion membrane (0.06 g) has been investigated. Fig.10. showed that increasing the temperature leads to a decrease in the dye adsorption capacity of the onion membrane (0.160 g.g^{-1}, with 14. 5% uptake) after eight hours of contact time. This can be attributed to a weakening of the adsorptive forces between the active sites on the adsorbent and the dye molecules due to the degradation of the onion membrane in the high temperature[22-23]. The results propose that a high temperature is not suitable for adsorbing MG when the onion membrane is adsorbent. Thus, it is preferable to let the temperature of industrial wastewater decrease to 20°C to perform maximum adsorption capacity.

CONCLUSION

Removal of MG from industrial wastewater by adsorption with onion membrane has been experimentally determined and the percentage of color removed increase with increasing adsorbent dosage, increase with increasing contact time and varied with dye solution pH. In addition, the removal percentages decrease with increasing temperatures. Optimum temperature was found to be 20 °C. Therefore, adsorption capacity of onion membrane for

the MG decreased with temperature. The results propose that a high temperature is not suitable for adsorbing MG when the onion membrane is adsorbent. Further, it is preferable to let the temperature of industrial wastewater decrease to 20°C to perform maximum adsorption capacity. The maximum removal found to be at pH =10. The adsorption of the positively charged dye group on the adsorbent surface is primarily influenced by the surface charge on the adsorbent which in turn is influenced by the solution pH. Optimum adsorbent dosage for the dye is 0,06g/250ml. It is obvious as with increasing amount the active sites for adsorption of MG dye increase which results in an increase in removal efficiency. Finally, the adsorption of MG by the onion membrane agreed with the second-order kinetic model. Moreover, analysis of the equilibrium isotherms using the Langmuir and Freundlich isotherms showed that the Freundlich model fitted well with the experimental data.

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0.015x + 0.154y = 0.997= ^{2} R
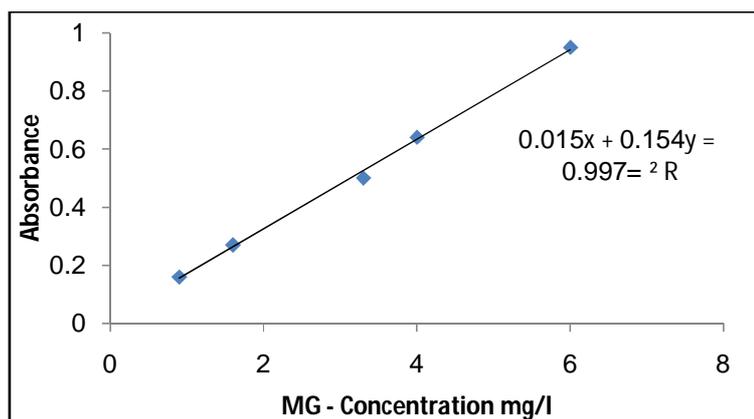
Fig.1. Calibration plot of MG
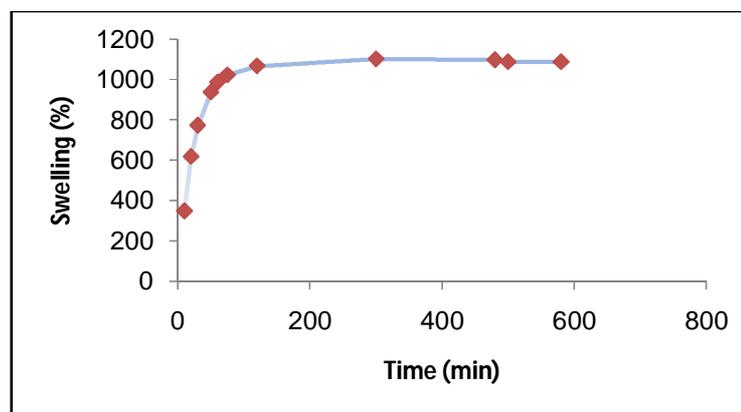

Fig.2. Conducts swelling of the onion membrane was plotted as a function of time

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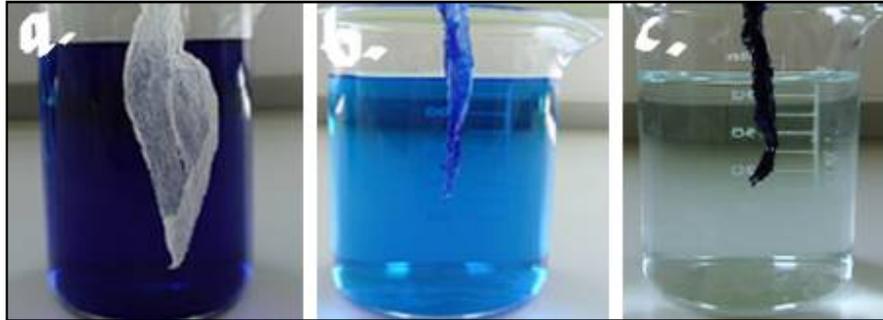
Fig.3. Adsorption process, (a) Onion membrane used as adsorbent, (b) Onion membrane after 2h, (c) Onion membrane after 8 h
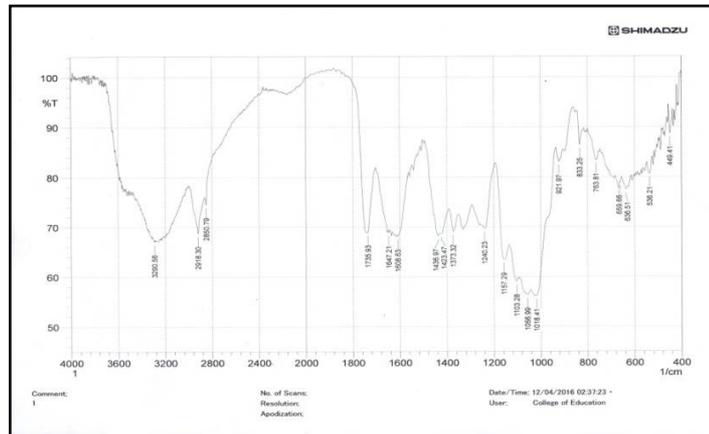
Fig 4.a. FTIR analysis for onion membrane
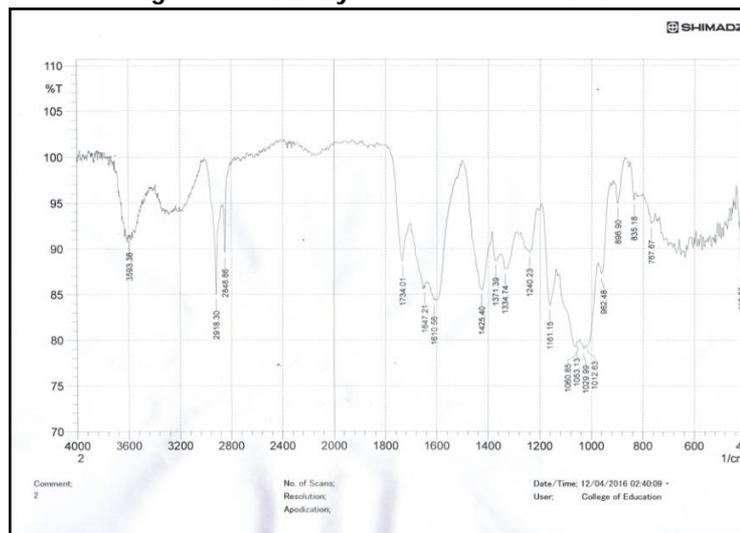
Fig.4.b. FTIR analysis after dye loaded onto onion membrane

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$$q_t = \frac{C_i - C_e}{W} \times V$$
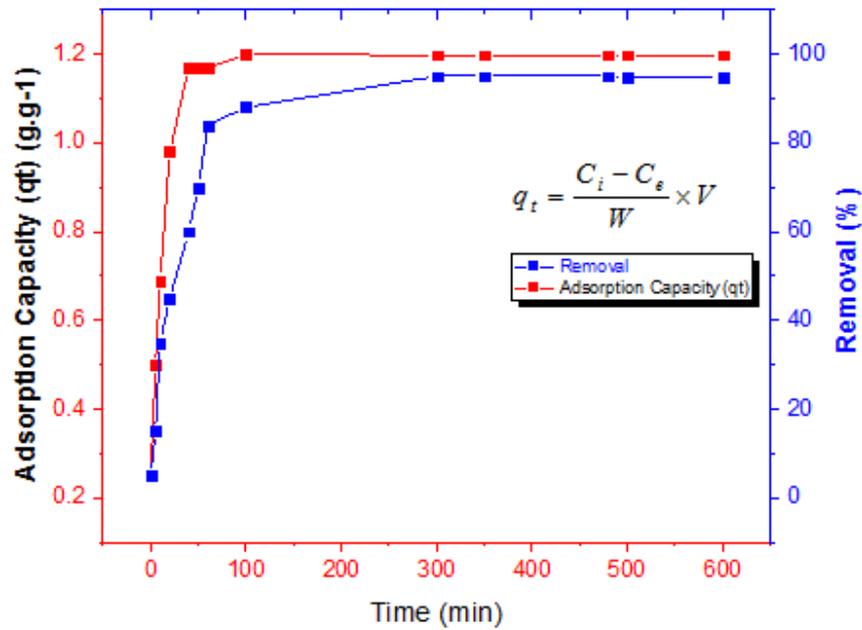
Fig.5. Effect of contact time on MG adsorption capacity (g.g⁻¹) and removal (%)
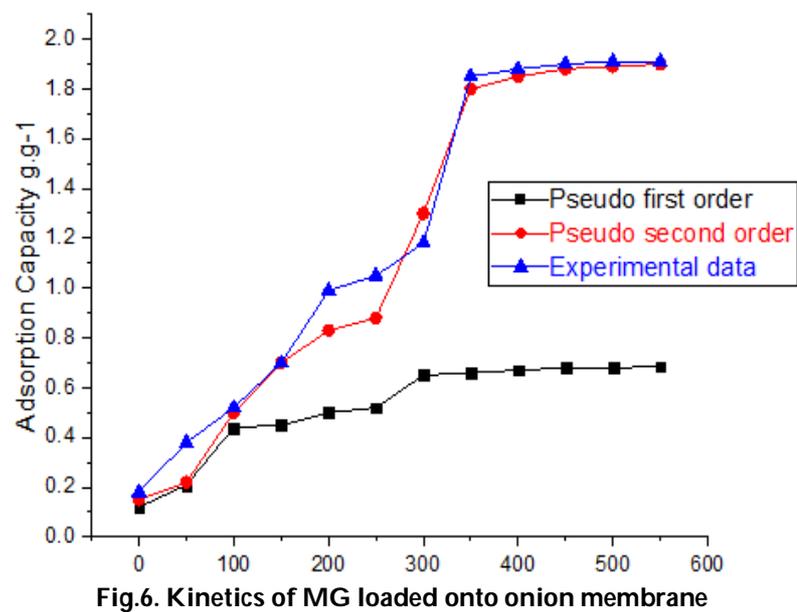
Fig.6. Kinetics of MG loaded onto onion membrane

Table 1: Experimental data of kinetic models for MG loaded onion membrane

| Kinetic models and parameters	Malachite green
q_e exp. (g.g^{-1})	1.813
Pseudo-first order K_1(min^{-1})	0.0198
q_e cal. (g.g^{-1})	0.5929
R^2	0.8360
Pseudo-second order K_2 (g.g^{-1}.min^{-1})	0.0195
q_e cal. (g.g^{-1})	1.2879
R^2	0.9793

Table 2: Experimental comparability of adsorbent dosage, Removal percentage of MG and adsorption capacity

Adsorbent dosage(mg)	Removal %	q_e (mg/g)
50	68.1	2.604
100	90.0	1.882
150	95.2	1.295
200	98.9	0.983
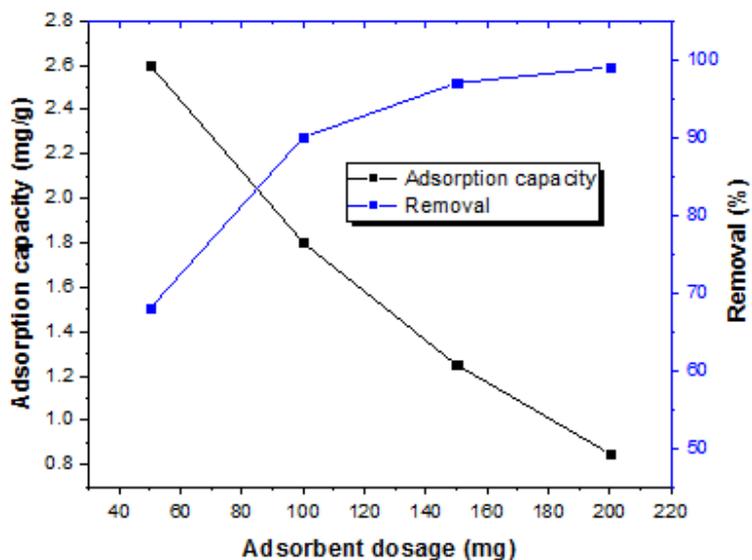
Fig.7. Effect of adsorbent dosage and removal percentage of MG

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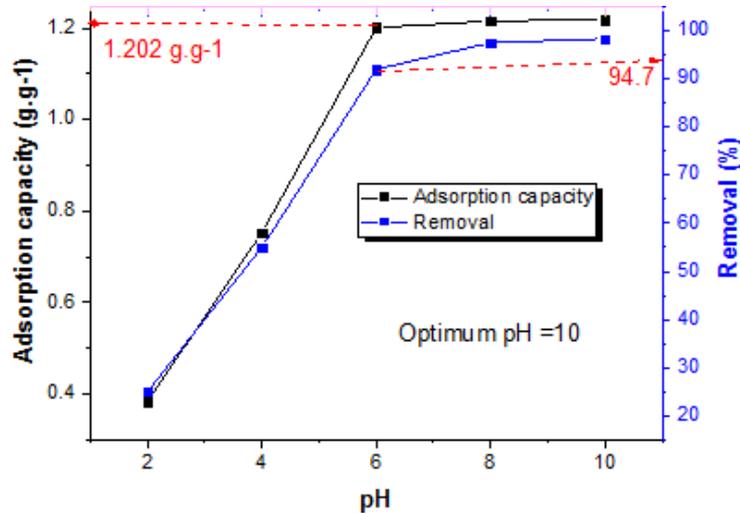
Fig.8. Effect of pH of the removal process
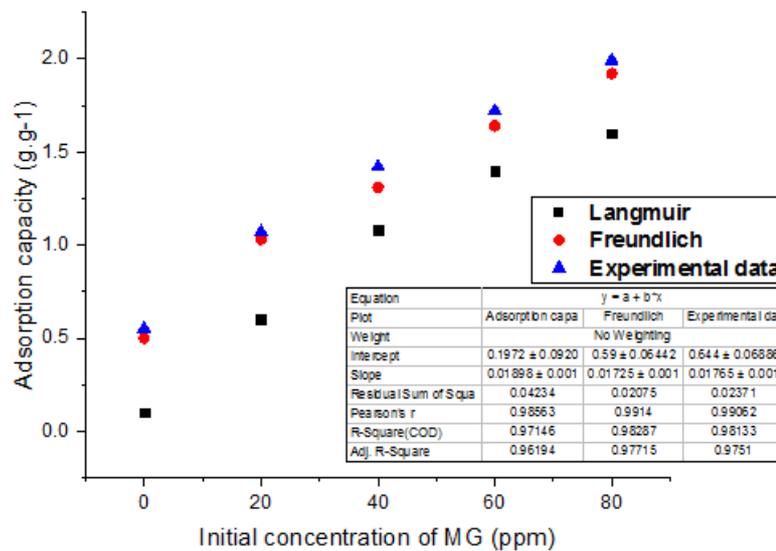
Fig.9. Isothermal analysis of MG onto onion membrane by Langmuir and Freundlich

Table 3: Isothermal analysis for adsorption process of MG

Langmuir		Freundlich	
q_{max} (g.g^{-1})	1.8994	q_e cal. (g.g^{-1})	1.9107
q_e cal. (g.g^{-1})	1.7982	K_F (L.g^{-1})	2.1917
K_L (L.g^{-1})	70.164	n	5.9841
R^2	0.9714	R^2	0.9828

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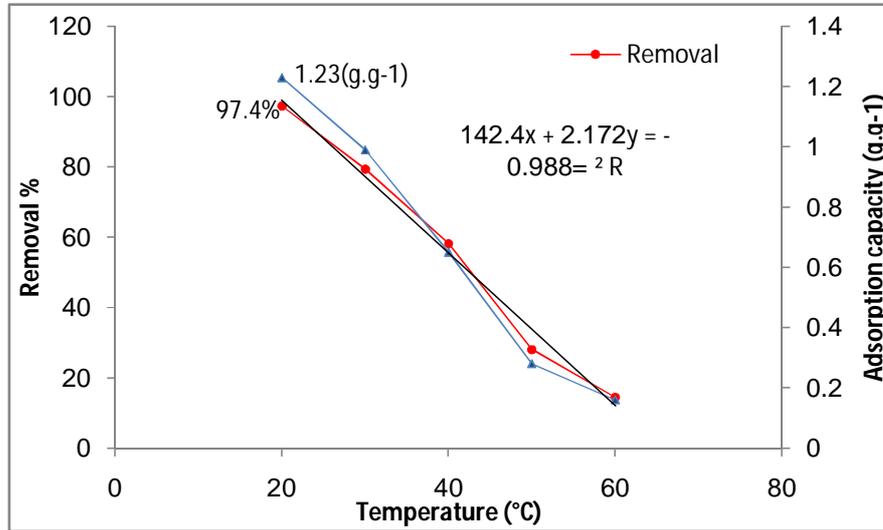
Fig.10: Effect of temperature on the adsorption of MG onto onion membrane

RESEARCH ARTICLE

Influence of HCV on Electrolyte and some Liver Parameters in Chronic Renal Failure Patients on Hemodialysis in Erbil Governorate

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ABSTRACT

The present study aims to measure the electrolyte and some liver parameters in chronic renal failure patients infected with HCV and non-HCV infected. This study comprised 75 patients with chronic renal failure on hemodialysis, (15) of them tested positive for HCV by ELISA test. Patients were divided into two groups (HCV-positive and HCV-negative).The data of the current study was expressed as (Mean ± S.E.M) and the SPSS using samples T-test for comparing between pre and post hemodialysis for both HCV-positive and HCV-negative patients. P value(P<0.05)level was considered to be statistically significant.The results showed a significant increase in serum potassium, ALT and AST and non-significant increase in sodium in HCV-positive patients as compared to HCV-negative patients in pre-hemodialysis and post hemodialysis patients. Furthermore, non-significant decrease in calcium in HCV-positive patients as compared to HCV-negative patients in pre-hemodialysis and post hemodialysis patients. Hepatitis C virus affected on serum transaminase activity (ALT and AST) in chronic renal failure patients on hemodialysis. Chronic renal failure patients infected with HCV on hemodialysis have higher level potassium as compared to non-infected HCV patients.

Keywords: electrolyte, HCV infection and chronic renal failure.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major health problem among hemodialysis (HD) patients in developing countries [1]. Chronic renal disease is a progressive loss in renal function over a period of months or years. Infection with HCV is associated with a poor prognosis for survival among dialysis patients [2]. Chronic renal failure is associated with disorders of the extra and intracellular electrolyte homeostasis. The damage of renal function may be responsible for various electrolyte abnormalities including negative balances of sodium, potassium, calcium, phosphorus, and magnesium [3]. Infections with hepatitis C Virus (HCV) can cause rapidly progressive renal disease and their recognition and management are critical in patients with ESRD [4]. Chronic viral infection is associated with a 57% higher risk of death in patients with CKD undergoing hemodialysis when compared to non-viral infected subjects [5]. Viral infections do not only affect the liver or heart but have been implicated in the pathogenesis of kidney disease.

The kidneys play a critical role in regulating electrolytes, they control the levels of sodium and potassium; therefore, a disturbance in blood levels of these electrolytes may be related to kidneys functions [6]. Electrolytes regulated by changes in urinary excretion for Na^+, K^+, Cl^- and Ca^{2+}. Sodium transport activity is regulated by many factors, including protein kinase-dependent phosphorylation, which can increase both activity and channel numbers. Distal tubular Na^+ and K^+/H^+ transport is regulated by the action of aldosterone, which increases the synthesis of apical Na^+ and K^+ channels, Na^+-K^+ ATPase, along with the activity of Na^+-H^+ exchange and the H^+-ATPase [3].There are several common causes of hyponatremia some of them are: renal loss, or cellular shift. Aldosterone deficiency increases renal loss of sodium and water, with sodium loss in excess of water loss. Hypovolemic hyponatremia accompanied by urinary sodium is caused by extra renal loss of hypotonic fluid as with prolonged vomiting, diarrhea, sweating or trauma [7]. The HCV infected patients on hemodialysis had significantly higher serum ALT levels [8].The frequency of raised serum alanine aminotransferase (ALT) concentrations in patients who are infected with the hepatitis C virus (HCV) and have chronic renal failure (CRF) that requires hemodialysis (HD) therapy has been reported to be between 4 and 67% [9-11]. The present study aims to measure the electrolyte and some liver parameters in chronic renal failure patients infected with HCV and non-HCV infected.

MATERIALS AND METHODS

The present study included 75 patients (39 women and 36 men) in CRF requiring maintenance hemodialysis, who were recruited from Hawler Teaching Hospital, kidney hemodialysis Center from May 2015 to November 2015. 15 patients tested positive for HCV by third generation ELISA test. They have attended to the hospital in Hawler Governorate. The age of the patients was in the range of 20-73 years. A questionnaire form was filled for each subject by direct interview. The data requested included demographic data and causes of disease. The patients received HD thrice a week for 2-3 hours per session at blood flow rates of 250-350 ml/min using polyflux hollow-fiber filter.

The dialyzer consists of bicarbonate (32mmol/L), sodium (140mmol/L), potassium ion (2 mmol/L) and calcium (1.50 mmol/L). Blood samples were collected from CRF patients before and after HD session to measured electrolyte change using (OPTI LION electrolyte analyzer) and the serum calcium, ALT and AST was estimated by using full automated chemical analyzer (Biolabo, KENZA 240TX).

Statistical analysis

The data of the current study was expressed as (Mean ± S.E.M) and the SPSS using samples T-test for comparing between pre and post hemodialysis for both HCV-positive and HCV-negative patients. P value(P<0.05)level was considered to be statistically significant.

RESULTS AND DISCUSSION

Among 75 (39 women and 36 men) chronic renal failure patients on hemodialysis, 39 (52%) were men and 36 (48%) were women who had been followed in our study in Hawler Teaching Hospital, kidney hemodialysis Center units, 60 (80%) of them were HCV-negative and 15 (20%) were positive for HCV by third generation ELISA test. There was no significant difference in duration of hemodialysis between HCV-negative and HCV-positive patients on hemodialysis. The characteristics of the subjects are shown in (Table 1). The mean age in the study population was 44.81±16.63 (range: 23–75) years. The most prevalent causes of kidney disease were unknown aetiology (38.3%) and around (30%) had diabetes and (16.7%) caused by kidney infection and kidney stone (15%).

In this study the levels of sodium, potassium, calcium, ALT and AST were compared in HCV positive patients and HCV negative patients on hemodialysis. The results showed a significant increase in serum potassium, ALT and AST, and non-significant increase in sodium in HCV positive patients as compared to HCV negative patients in pre-hemodialysis and post hemodialysis patients. In addition, non-significant decrease in calcium in HCV positive patients as compared to HCV negative patients in pre-hemodialysis and post hemodialysis patients as shown in (Table 2 &3).

We examined the change of electrolytes levels and compared in HCV-positive and HCV-negative hemodialysis patients. In patients with CRF, serum potassium level revealed highly significant increase in CRF patients infected with HCV, this increase may be due to arise from true excess or imbalance in the distribution between potassium inside and outside of cells.Cotler et al. [12] found that the serum ALT levels were significantly lower in patients with CRF. The exact reason for the lower ALT in patients with CRF needs to be fully elucidated. One possibility is due to liver cells protection by hepatocyte growth factor (HGF), which is higher concentration in patients with CRF for whom HD is recommended [13]. The lower ALT activity in HD patients may also be a consequence of a smaller serum HCV viral load, due to the adsorption of the virus genome in the dialyzer membrane or to the induction of endogenous interferon caused by the HD [14]. In the present study, the ALT levels also increased significantly after the HD session in both patient groups (with and without anti-HCV). This increase may be a consequence of the loss of liquid during the session, thereby correcting the prior hemodilution.

Data found in the presented study showed a significant decrease in serum calcium level, these decrease may be due to decrease renal tubular reabsorption of calcium causing decrease in calcium ion level these results are in agreement with [15] found that hypocalemia and decrease glomerular filtration rate lead to reduction in filtered calcium and increase in proximal tubular reabsorption of sodium and calcium.The results showed a significant increase in serum ALT and AST in HCV infected patients as compared to non-infected HCV patients in pre-hemodialysis and post hemodialysis patients. These increase due to liver disease on long term hemodialysis.

CONCLUSION

Hepatitis C virus affected on serum transaminase activity (ALT and AST) in chronic renal failure patients on hemodialysis. Chronic renal failure patients infected with HCV on hemodialysis have higher level potassium as compared to non-infected HCV patients.

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Table 1: Demographic data and underlying disease of the study patients with and without HCV infection

	HCV-negative patients	HCV- positive patients	P value
Age (years)	**(23-75)**	**(33-74)**	
Gender			
Male	32	7	
Female	28	8	
Duration of dialysis (months)	32.80±4.055	38.80±6.331	**NS**
Causes of disease			
Unknown aetiology	23 (38.3%)		
Diabetes	18 (30%)		
Kidney infection	10 (16.7%)		
Kidney stone	9 (15%)		

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Table 2: level of some serum electrolytes and some liver parameters in renal failure patients and renal failure infected with (HCV) virus in pre hemodialysis.

Parameters	CRF patients		P value
	Pre hemodialysis HCV-negative patients	Pre hemodialysis HCV-positive patients	
Sodium (mg/dl)	136.8±1.222	139.7±1.430	0.163
Potassium (mg/dl)	4.529±0.219	5.671±0.279	0.007
Calcium (mg/dl)	7.425±0.509	7.126±0.525	0.703
ALT (IU/L)	9.720±1.354	15.00±1.652	0.043
AST(IU/L)	17.56±2.313	27.75±4.103	0.048
Results expressed as Mean ±S.E.			

Table3: level of some serum electrolytes and some liver parameters in renal failure patients and renal failure infected with (HCV) virus in post hemodialysis.

Parameters	CRF patients		Statistical evaluation
	Post hemodialysis HCV-negative patients	Post hemodialysis HCV-positive patients	
Sodium (mg/dl)	138.7±1.063	138.9±1.046	0.938
Potassium (mg/dl)	3.483±0.255	4.071±0.108	0.046
Calcium (mg/dl)	9.000±0.577	8.638±0.259	0.521
ALT (IU/L)	11.94±1.463	22.50±5.258	0.048
AST(IU/L)	21.38±2.367	33.60±3.326	0.011
Results expressed as Mean ±S.E.			

RESEARCH ARTICLE

The Trend Analysis of Maximum of Daily Precipitation of Iran Hydrometric Stations using Non-Parametric Methods

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ABSTRACT

Trend analysis is one of the most statistical methods which are widely used to evaluate the potential effects of climate change on hydrological time series as a series of observations rainfall and river flow is used around the world. In this research, the maximum daily rainfall data were identified from 41 synoptic stations for a period of 40 years from 1965 to 2005.Common statistical period was considered for the desired station and then reconstruction of statistical errors for stations that have a defect in some of the data were performed using represent stations. Using three methods of Mann-Kendall nonparametric, Spearman correlation, Autocorrelation was significant study at confidence level of 0.95 and 0.99.Stations that were significant the review process were investigated.The results showed that in all the stations studied rainfall does not follow the trend. Total stations 0.95 and 0.99 were significant at confidence levels in Mann-Kendall test and Maximum of daily precipitation at 19 stations during 1965-2005 have increasing trend in the maximum of daily precipitation. Only 4 stations in Spearman test, Khoy, Isfahan, Bushehr and Dezful at confidence levels of 0.95 were significant which stations of Khoy with decreasing (negative) trend and stations in Isfahan, Bushehr and Dezful have increasing (positive) trend, respectively. After Autocorrelation nonparametric test, the Maximum rainfall selected stations, the results did not indicate any significant change at confidence levels of 0.95 and 0.99 for the entire station, and the outcome of the trend study was not applied.
Keywords: Mann-Kendall Test, Daily rainfall, Trend Analysis, Significance Level, Hydrological Stations

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INTRODUCTION

Precipitation events are expected to become substantially more intense under global warming, but few global comparisons of observations and climate model simulations are available to constrain predictions of future changes in precipitation extremes.Changes in global climate and alteration of Earth's hydrological cycle [1,2,3] have resulted in increased heavy precipitation with consequent increased surface runoff and flooding risk [4],which is likely to continue in the future [5]. Analysis of trend, is the most important statistical methods which widely used to assess the potential effects of climate change, on hydrological time series such series of observations of rainfall and river flows have been used in different parts of the world [6]. There is a trend in the time series of climate and climate change may be the result of gradual changes of natural or caused by human activity.The existence of significant trends in a time series of rainfall alone can not be the proof of climate change in the region, but strengthens its occurrence hypothesis.This is due to several factors control the climate system [7].There are methods that identify the analyze of trend .

Among the methods of studied this research, Man Kendall nonparametric methods, is the most common and most used in the analysis of time series. Generally, climate change will often be slow and may not be perceptible in a short period of several years [8].Studies show that a significant positive trend for global precipitation However, it is somewhat different behaviors in terms of total precipitation amounts have been observed at regional scales.The results of some studies are suggestive of existence of the increasing trend in regional scale. In a study was conducted in Australia during 1951-2003 where rainfall index tends to be wetter conditions than in the twentieth century [9] .According to natural variability of climate and the fact that human activity due to the increasing of greenhouse gases can not be neglected [10]. Anthropogenic climate change is expected to change the distribution, frequency and intensity of precipitation and result in increased intensity and frequency of floods and droughts, with damaging effects on the environment and society [11,12,13,14,15].Changes in the frequency and intensity of extreme rainfall are a serious problem for human lives [16], often resulting in strong winds, lightning and floods [17]Many extreme events will become more numerous in the 21st century [15], influencing the policy determination in many sectors, such as economic development , agriculture management, infrastructure (construction management), and others [18]. As a result of greenhouse gas (GHG) build-up in the atmosphere, global mean near-surface temperature shows an increasing trend since the beginning of the twentieth century [19,20,21]with greater increases in mean minimum temperature than in mean maximum temperature [22].The purpose of this study was to analyze the maximum of daily rainfall in country hydrometric stations using non-parametric methods.

MATERIALS AND METHODS

Study area

Iran, with an area of 1645000 square kilometers between 25 and 40 degrees north latitude and 44 ° 64 'east longitude located. Annual rainfall minimum in the Lut Desert least millimeter or even zero in some years and it's maximum of Bandar Anzali and more than 1500 mm per year. The distribution of the time, the highest rainfall in the cold season in the Alborz and Zagros mountains as snow descends. Mainly rainfall associated with Siberia cold fronts and sides of the monsoon in south. The highest amount of rainfall is North Slope which is located by the front Mediterranean. In central and southern areas, which do not have sufficient altitude and it located in the shelter of the Alborz and Zagros Mountains ranges, low rainfall and arid regions where they occur. The average rainfall is approximately 240 mm per year which is distributed irregularly across the country.Figure 1 shows the geographical location of the study area.

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The maximum of daily rainfall data from 41 synoptic stations for a period of 40 years from 1965 to 2005 was received from the Meteorological Agency and the maximum of daily rainfall data was extracted from the data. After the review period, the station that has the largest number during the forty years of the study, as well as good distribution in all latitudes and high altitudes were selected to obtain acceptable results of the investigation. Common statistical period for desired stations in 1965-2005 were considered.Then reconstruction of the statistical errors for stations that have defects of statistics in some years were performed using represents stations .To assess trends in the data series, usually used Parametric and Nonparametric methods. In this study, we used nonparametric three methods of Mann-Kendall, Spearman correlation and Autocorrelation was significant study at confidence level of 0.95 and 0.99.Stations that were significant, trend study was discussed.

Mann-Kendall test

Statistical calculation of the test is as follows:
Calculating the difference between individual observations another and symbols function actions and parameter extraction S is obtained from the following equation:

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} \text{sgn}(x_f = x_k)$$

Where: n the number of observations and Kx, Xj is given $K\,th$ and $J\,th$ of the series. Symbols Function has following formula:

$$sgn = \begin{cases} 1 & if\ (Xj - Xk) > 0 \\ 0 & if\ (Xj - Xk) = 0 \\ -1 & if\ (Xj - Xk) < 0 \end{cases}$$

The variance calculated is obtained from the following equation:

$$\text{var} = \frac{n(n-1)(2n+5) - \overset{n>10}{\underset{j=1}{\sum^{m}}} t_i(t_i-1)(2t_i-1)(2t_i+5)}{18}$$

$$var = \frac{n(n-1)(2n+5)}{18} \quad if\ \ n < 10$$

n is the number of observed data,
m represents the number of series in which at least one duplicate data exists and is equal to the value of t represents the data.
Finally, the z value is determined by the following equations:

$$Z = \begin{cases} \dfrac{S-1}{\sqrt{var\ S}} & if\ \ S > 0 \\ 0 & if\ \ S = 0 \\ \dfrac{S+1}{\sqrt{var\ S}} & if\ \ S < 0 \end{cases}$$

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S represents Standard deviation
Var ,represents variance.

Spearman test

In statistics, the Spearman's rank correlation coefficient is shown by the Greek letter of ϱ. It is nonparametric statistics to measure the correlation coefficient between two random variables. The value of this coefficient indicates the ability to express a uniform variable as a monotonic function of other variables. The Spearman's rank correlation coefficient is defined as the Pearson correlation coefficient between the ranking data.
Spearman correlation coefficients are calculated as follows:

$$\rho = \frac{\sum_i (x_i - \overline{x})(y_i - \overline{y})}{\sum_i (x_i - \overline{x})^2 (y_i - \overline{y}) \sum_i (y_i - \overline{y})^2}$$

Where *i* = paired score

Autocorrelation

The autocorrelation describes a random process of correlation between process values at different points of time as two strokes or subtracting time.
If X is a repeatable process and i as a point in time after the beginning of the process (*i* may be integer of time or a real number to process with continuous-time).
So Xi is generated value by the implementation process at the time of *i*.
Suppose the process, the mean μi and variance $\sigma^2{}_i$ is defined for all *i* times.
Thus, the correlation between both the *S* and *t* are defined as follows:

$$R(s,t) = \frac{E\left[\left(X_t - \mu_t\right)\left(X_S - \mu_s\right)\right]}{\sigma_t \sigma_s}$$

Where, E is the expected value operator.
This expression for all processes or time series is not well-defined, since the variance may be zero (for a constant process) or infinite.
If the function of R is well defined, its value should be placed in the range of [-1, 1], where 1 indicates perfect correlation and -1 indicating perfect anti-correlation.
If Xt is a constant process of the second order, thus mean μ and variance σ 2, is independent of time and the autocorrelation depends only on the difference between t and s. The correlation depends only on the time interval between two values, but the situation does not depend on time.
This study suggests that this relationship can be expressed as a function of delay time and also has an even function of τ = s-t.

$$R(\tau) = \frac{E\left[\left(X_t - \mu\right)\left(X_{t+\tau} - \mu\right)\right]}{\sigma^2}$$

And according to a pair of this function, we can say:

$$R(\tau) = R(-\tau)$$

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In some other fields of statistics and time series analysis, to normalize by σ 2 and is used the "autocorrelation" which is synonymous with "Auto-covariance". However, normally it is important for two reasons. Due to its interpretation of the relationship as a relationship that no amount of scale " The power of statistical dependence" provides and the normalization is effective on the statistical properties of the estimated autocorrelation.

RESULTS AND DISCUSSION

Table 1 comparing of stations shows in the three methods Mann-Kendall , Spearman and Autocorrelation test that the three stations in Mann-Kndal test Khoy, Isfahan and Bushehr station at significant level of 0.95 have meaningful confidence level and 38 stations were not meaningful confidence level.As well as, all stations were not significant at the significant level of 0.99 and maximum of daily rainfall of two stations as Isfahan and Bushehr had increased during the years 1965-2005 and Khoy station was a decreasing trend.Only 4 stations in Spearman test, Khoy, Isfahan, Bushehr and Dezful at confidence levels of 0.95 were significant and 37 stations were not significant and at confidence levels of 0.99 all stations was significant and Khoy station with decreasing (negative) trend and available stations in Isfahan, Bushehr and Dezful have increasing (positive) trend, respectively. After Autocorrelation nonparametric test, on the Maximum rainfall selected stations, the results did not indicate any significant change at confidence levels of 0.95 and 0.99 for the entire station, and the outcome of the trend study was not conducted.The Mann-Kendall test, maximum value of z is related to Khoy, Tabriz and Bandar Anzali stations and minimum value of z is related to Isfahan, Bushehr and Abadan stations, respectively. In the Spearman test, , maximum value of z is related to stations of Bushehr, and minimum value of z is related to Bandare-Lenge, Arakand and Zanjan respectively.In the Autocorrelation test, the maximum value of z is related to stations of Zahedan, Iranshahr and Tabriz.

CONCLUSION

Generally, after collecting data on the maximum daily precipitation, the study of climate change 41 stations in the country, during the period 1965-2005, several tests were performed to evaluate the data. To investigate the possibility of any sudden changes and significant trends was used Mann-Kendall, Spearman and Autocorrelation test. To detect the presence and quantity of the results of this study showed which all stations were not specific trends in precipitation. Total station at confidence levels of 0.95 and 0.99 were significant in Mann-Kendall test, and maximum of daily precipitation at 19 stations during 1965-2005 have increasing trend the maximum of daily precipitation, 8 stations have decreasing trend and 14 stations were found during the study period, no trend in the maximum of daily rainfall stations in significant levels tested. Only 4 stations in Spearman test, Khoy, Isfahan, Bushehr and Dezful at confidence levels of 0.95 were significant which stations of Khoy with decreasing (negative) trend and stations in Isfahan, Bushehr and Dezful have increasing (positive) trend, respectively. After Autocorrelation nonparametric test, the Maximum rainfall selected stations, the results did not indicate any significant change at confidence levels of 0.95 and 0.99 for the entire station, and the outcome of the trend study was not applied. Since climate change is a very complex phenomenon and the need for comprehensive studies, it is recommended to more accurate planning and decisions of statistical methods and other various models to examine all elements of climate change on the regional scale.

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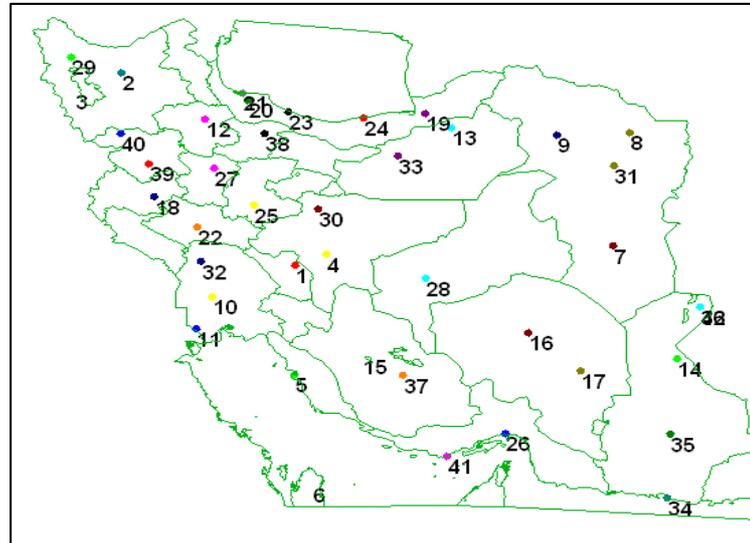
Figure 1: Location map of the synoptic stations

Table 1. Comparison of stations among in three methods of Mann -Kndal, Spearman and Autocorrelatin tests.

Autocorrelatin		Spearman			Mann-Kendall			
Significant level	z	Significant level		z	Significant level		z	Stations
0.95-0.99		0.99	0.95		0.99	0.95		
NS	1.09	NS	NS	0.48	NS	NS	3.63	Shahrkurd
NS	1.21	NS	NS	1.32	NS	NS	5.52	Tabriz
NS	1.6	NS	NS	0.56	NS	NS	4.92	Urumiye
NS	0.35	NS	S	1.88	NS	S	6.15	Khoy
NS	0.89	NS	S	1.77	NS	S	2.24	Isfahan
NS	-0.19	NS	NS	0.01	NS	NS	3.97	Kashan
NS	0.12	NS	S	1.99	NS	S	2.25	Bushehr
NS	-0.5	NS	NS	0.95	NS	NS	3.14	Tehran
NS	-1.49	NS	NS	0.13	NS	NS	4.13	Birjand
NS	-1	NS	NS	0.21	NS	NS	4.36	Mashhad
NS	0.37	NS	NS	0.77	NS	NS	4.81	Sabzvar
NS	0.07	NS	NS	1.03	NS	NS	3.14	Torbate-Heyderiye
NS	1.03	NS	NS	0.25	NS	NS	4.4	Ahvaz
NS	-1.93	NS	NS	1.5	NS	NS	2.69	Abadan
NS	1.49	NS	S	1.75	NS	NS	2.72	Dezful
NS	-0.87	NS	NS	0.095	NS	NS	5.05	Zanjan

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NS	-1.89	NS	NS	0.17	NS	NS	3.95	Semnan
NS	0.24	NS	NS	0.66	NS	NS	4.81	Shahrood
NS	1.51	NS	NS	0.64	NS	NS	4.85	Zahedan
NS	0.02	NS	NS	0.03	NS	NS	4.11	Chabehar
NS	1.24	NS	NS	0.12	NS	NS	4.13	Iranshahr
NS	-1.02	NS	NS	0.77	NS	NS	3.63	Zabul
NS	0.28	NS	NS	1.18	NS	NS	2.89	Shiraz
NS	0.53	NS	NS	1.52	NS	NS	2.78	Fasa
NS	-1.22	NS	NS	0.75	NS	NS	3.39	Qazvin
NS	-1.58	NS	NS	0.53	NS	NS	4.66	Sanandaj
NS	0.96	NS	NS	0.11	NS	NS	3.99	Saqez
NS	-1.05	NS	NS	0.43	NS	NS	3.62	Kerman
NS	0.25	NS	NS	0.13	NS	NS	4.31	Bam
NS	0.36	NS	NS	0.59	NS	NS	3.29	Kermanshah
NS	-0.96	NS	NS	0.14	NS	NS	4.21	Gorgan
NS	-2.04	NS	NS	0.12	NS	NS	4.26	Rasht
NS	-0.08	NS	NS	1.13	NS	NS	5.33	Bandare-Anzali
NS	-0.24	NS	NS	1.19	NS	NS	2.99	Khoramabad
NS	-0.97	NS	NS	0.92	NS	NS	3.24	Ramsar
NS	-0.6	NS	NS	1.07	NS	NS	3.07	Babolsar
NS	-0.69	NS	NS	0.032	NS	NS	4.35	Arak
NS	0.24	NS	NS	0.94	NS	NS	3.32	Bandare-Abbas
NS	-0.89	NS	NS	0.009	NS	NS	4.17	Bandare-Lengeh
NS	-0.46	NS	NS	1.04	NS	NS	3.05	Nozhe
NS	0.69	NS	NS	0.59	NS	NS	4.84	Yazd