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The *Current Horticulture* Released

The ***Current Horticulture*** (a journal dedicated for the advancement of Horticultural Science) was released formally on the occasion of the International Conference on '**Recent Trends in Interdisciplinary Sciences: Opportunities and Challenges**', held at MM (Post-graduate) College, Modinagar, Ghaziabad, from 28 February to 1 March 2014. The *Current Horticulture* is being edited by Dr Amar Singh Kashyap, Assistant Professor, Botany Department, MM (Post-graduate) College, Modinagar. At the occasion of its release (from right to left) Dr R C Lal, Principal, MM (Post-graduate) College, Modinagar; Dr G C Sharma, formarly VC, Agra University; Dr H K Malik, IIT, New Delhi; Dr Arun Bhartiya, Vice- Principal and Head, Botany Department, MM (Post-graduate) College, Modinagar; Dr N K Gaur, Convenor of the Conference, along with Dr Amar Singh Kashyap were present on the dias. The issue was appreciated a lot by all the dignitaries on dias along with the audience consisting of eminent scientists, academicians, scholars, teachers etc.

Inaugurated by Mr V C Goyal (IPS), Vice-Chancelor, CCS University, Meerut, the conference was attended by nearly 400 participants. Among the participants, Prof. R C Gupta, University of Louisville, USA; Dr Sujeet Kumar Sharma, Sultan Qaboos University, Oman; Dr Moun Kaushik, Soffia University, Bulgaria; Dr S K Tyagi, CPCB, New Delhi; Dr Rajeev Kr Sharma, Director, PLIM, Ghaziabad; Dr Kavita Tyagi, NMPB, New Delhi; Dr Poonam Mohindra, Delhi University; Dr T Nag, AIIMS, New Delhi; Dr O P Malik from IIT, New Delhi and Dr P Kumar, IUAC, New Delhi, were the prominent participants.

Dr Amar Singh Kashyap

Research Opinion

Current Horticulture **2**(1): 03–04, January–June 2014

Global citrus nutrition research: an incisive analysis

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ABSTRACT

Citrus fruits are produced in many countries around the world, although production shows geographical concentration in certain areas, but still citrus fruits rank first in international fruit trade in terms of value, evolving from a producer- driven to a more consumer-oriented market. In the backdrop of demography-driven diminishing per availability of arable land, plant nutrition has gained phenomenal significance in meeting the challenge of sustaining productivity over changing resource outputs. Indeed, from soil and plant diagnosis to suggestions for appropriate fertilizer applications, current levels of citrus production would never have been possible without the knowledge of plant nutrition. A definite credit in this context could be accredited to developments in analytical techniques in both leaf and juice analysis. Of late, trunk nutrition gained some momentum where conventional methods of nutrient supply have not been able to put forth the desired results spaced over time. In addition, proximal sensing of nutrient stress and spectrum of soil enzymes as dictum of soil fertility changes have further provided some authoritative progress towards precise diagnosis of nutrient stresses. Such breakthroughs will go a long way in developing early warning system in the years to come to enable the redressal to genesis of any nutritional disorder within current growth cycle of crop.

Key Words: Citrus nutrition, Leaf, Juice, Trunk nutrition, Nutrient stress, Nutritional disorder

A guesstimate proclaims over 900 million people in the world are undernourished, and malnutrition alone is responsible for 3.5 million deaths annually. Plant nutrition is a complex process that has developed over the course of plant evolution with the discovery of fundamental importance of plant nutrition, only second to the discovery of photosynthesis as an effective via-medium to bolden plant defence mechanism (Wu *et al*. 2013). The accumulated biochemical and molecular evidences have incredibly confirmed that the nutrient stress is invariably associated with changes in antioxidant system. Under such nutrient-induced stresses, phytophenolic nutrients are first to be affected. Later, with the universal acceptability to the concept of essentiality of nutrients by Aron and Stout, the investigations on the anatomical, histological, and biochemical nutritional disorders became distinctly understandable through a variety of diagnostics (Srivastava and Singh 2001; 2002; 2005; 2008b). For such regulatory systems to function, nutrient conditions need to be sensed, signals need to be transduced, gene expression need to be transcriptionally and post-transcriptionally regulated, transporters be properly trafficked through endomembrane system, and cell cycles need to be coordinated. Such a wide range of responses may be a reflection of very sophisticated systems that have evolved in plants over the time (Tan *et al*. 2005).

Occurrence of nutrient constraints is as old as history of citrus cultivation. Any nutrient constraint at various crop phenophases on nutrient deprived soils has always baffled citrus nutritionists that could well jeopardize the incentives accruing through otherwise balanced fertilization in highly diversified nutrient demanding citrus cultivars (Srivastava and Singh 2008b; 2009). The current state of knowledge on the subject is very fragmentary. The subject becomes still very complex in the absence of knowledge on kinetics and co-kinetics of different nutrients being partitioned across different growth stages so that growth stage-wise nutrient demand is precisely modulated (Srivastava and Singh 2008a). Accordingly, type and source of nutrients are fed synchronising with physiological nutrient demand (Srivastava, 2012). Two major processes of nutrient cycling, viz. mineralization and immobilization of nutrients via litter fall offers a lion's share in meeting

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out the crop nutrient demand in perennial canopy framework of citrus (Srivastava *et al*. 2014).

Development of microbial consortium (microbial reactor) exploiting the native and natural microbial synergisms (with twin role as growth promoter and antagonistic against soil-borne pathogens) is one of the popular methods of providing the desired dynamism to nutrient dynamics within the rhizosphere. Such rhizosphere-specific consortia could further engineer rhizosphere's nutrient demand and supply through loading with organic manures in much value-added form, e.g. biodynamic soil fertility management. The efforts such as these, could only meet their objectivity unless duly supported by methods leading to improved nutrient-use efficiency including the intervention of genomics with metaloenzymes and variable rate fertilization (Wibawa *et al*. 1993; Zaman and Schumann 2006; Zhang *et al*. 2010).

Development of nutrient norms using crop-specific plant parts in citrus cultivars, needs a thorough revisit and to be field validated in order to provide their wider application down to orchard level. However, major point of discontent still remains to be warded off with respect to whether nor not different nutrient norms are required as per cultivar within the same variety. The biggest constraint on the other hand in making soil test ratings more purposeful is the non-redressal of spatial variation in soil fertility. Conjoint use of geoinformatics (Geographical Information System, Global Positioning System and Remote Sensing) with nutriomics, site-specific nutrient management strategy, fertigation comparatively new concept of open field hydroponics, and exploiting nutrient-harmone synergy have collectively yielded definite edge over conventional methods of nutrient management. Ironically, one of the most profoundly researched nutritional disorders, popularly known as lime -induced iron deficiency still needs multi-pronged strategy with regard to management of citrus on calcareous soils. Well-known mycorryizal dependency of citrus still remains an unexploited issue (Wu *et al*. 2013).

The concepts such as organic soil fertility management and integrated nutrient management utilising collective efficacy of organic manures, inorganic fertilizers, and microbial diversity have taken this important issue a step forward towards sustainable nutrient management. Such approaches have given birth to a concept like best management practices , duly validated through economic analysis. The entire gamut of citrus nutrition remains an unfinished exercise unless dealt with the issues like assessing soil salinity and aluminium toxicity (Srivastava 2009) on soluble salt rich (high pH) and divalent bases deprived (low pH) soils, respectively, considering the extreme sensitivity of citrus under both the soil conditions. Despite all these concerns, application of sensor-based technology has further added a new dimension in estimating the fruit yield in an authentic manner so that sustainable productivity vis-à-vis nutrient management strategies go hand-in-hand in offering an alternative source of nutritional security in an era of soils sick of multiple nutrient deficiencies taking their severe toll on human nutrition.

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Research Review Article

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Food anti-microbials: challenges and prospects

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ABSTRACT

Though a number of food preservation strategies are available for the controlling microbial spoilage, most of these are not perfect in terms of quality and safety. Although synthetic antimicrobial and antioxidant agents are approved and being used in a number of countries, the use of natural, eco-friendly, safe and effective preservatives is the latest choice of consumers. Since time immemorial, medicinal plants have proved effective in treating health disorders and cure diseases. The bioactive molecules extracted from medicinal plants have future potential as antimicrobial and antioxidant additives in the food industry. For food packaging materials bio-coat technology integrates antimicrobial protection into any surface or product, reducing microbes by up to 99.99%, making them more hygienic and defending the surface against degradation, odours and staining. A complete range of biocides exist, both organic and non-organic (primarily silver-based) with end use having certain antibiotic properties in diet support immune system and help to defend from certain infectious bacteria. Probiotic vegetables such as Sauerkraut, raw pickles, cultured vegetables, and kim chi etc. rich in lactic acid microorganisms are far superior than synthetic drugs. Use of probiotic in conjunction with antibiotic treatment replaces the health friendly bacteria in the digestive system. Bacteriocins produced by lactic acid bacteria are of keen interest to the food industry for their bio-preservative potential and antimicrobial properties. The increasing demand for high quality 'safe' foods which are not extensively processed has created a niche for natural food preservatives. Future research on anti microbial should focus on health concerned food safety issues that exist or may arise in the time to come.

Key Words: Food, Anti-microbials, Preservatives, Anti-oxidants, Bioactive, Medicinal plants, Probiotic vegetables

Since prehistoric time preservation has become an important part of food processing. Drying, cooling, fermenting, and heating have always been used as the methods of food preservation including use of high concentration of salt and sugar. Microbial spoilage causes losses of up to 40% of all foods grown for human consumption worldwide. Today, consumers expect foods to be readily available and free from food-borne pathogens. Improvements have been made to extend the shelf-life of foods using packaging and processing systems but antimicrobials play a significant role in protecting the food supply (Davidson *et al.* 2005). Antimicrobial food additives are naturally occurring or synthetically produced substances that meet the food additive definition and are used to control microorganisms such as bacteria, viruses, fungi, protozoa, or other microorganisms in or on food or food contact articles.

Food antimicrobials are classified as 'preservatives'. These are of two types, natural and chemical. Natural preservatives include common salt, sugars, vinegars, spices, or plant-based oils. Chemical preservatives are defined by the US Food and Drug Administration (FDA;21CFR 101.22(a)(5), as "any chemical that, when added to food, tends to prevent or retard deterioration thereof, but does not include natural preservatives, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their insecticidal or herbicidal properties". Therefore, preservatives are used to prevent or retard chemical and biological deterioration of foods. The preservatives used to prevent chemical deterioration include antioxidants, to prevent autooxidation of pigments, flavours, lipids, and vitamins; antibrowning compounds, to prevent enzymatic and non-enzymatic browning; and antistaling compounds, to prevent texture changes. Those additives which are used to prevent biological deterioration are termed 'antimicrobials'. According FDA (21CFR 170.3 (0) (2), antimicrobial agents are

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Table 1. Traditional and naturally-occurring food preservatives approved by the Food and Drug Administration

Compound(s)	Microbial target	Primary food applications
Acetic acid, acetates, diacetates, dehydroacetic acid	Yeasts, bacteria	Baked goods, condiments, confections, dairy products, fats/oils, meats, sauces.
Benzoic acid, benzoates	Yeasts, moulds	Beverages, fruit products, margarine
Dimethyl bicarbonate	Yeasts	Beverages
Lactic acid, lactates	Bacteria	Meats, fermented foods
Lactoferrin	Bacteria	Meats
Lysozyme	*Clostridium botulinum* and other bacteria	Cheese, frankfurters, cooked meat and poultry products
Natamycin	Moulds	Cheese
Nisin	*Clostridium botulinum* and other bacteria	Cheese, other products
Nitrite, nitrate	*Clostridium botulinum*	Cured meats
Parabens (alkyl esters propyl,l, methyl, heptyl) of *p*-hydroxybenzoic acid)	Yeasts, moulds, bacteria (gram-positive)	Beverages, baked foods, syrups
Propionic acid, propionates	Moulds	Bakery products, dairy products
Sorbic acid, sorbates	Yeasts , moulds, bacteria	Most foods, beverages, wines
Sulphites	Yeasts, moulds	Fruits, fruit products, potato products, wines

'substances used to preserve food by preventing growth of microorganisms and subsequent spoilage, including fungistats, mold and rope inhibitors'.

Food antimicrobials can be classified as traditional or naturally occurring. Preservatives approved by the US Food and Drug Administration are listed in Table 1. Traditional antimicrobials which are approved by international agencies for regular use includes salts of benzoates, sulfites, sorbates, benzoic acid etc. Naturally occurring are the compounds which are naturally produced and isolated from various sources, including plants, animals and microorganisms, in which they constitute part of host defense systems. (Juneja *et al*. 2012).

Traditionally food preservatives are applied to foods as direct additives during processing, or allowed to develop during processes such as fermentation. Certain preservatives have been used either accidentally or intentionally for centuries, and they include sodium chloride (common salt), sugar, acids, alcohols and components of smoke.

Recently, however, antimicrobials have been used increasingly as primary interventions to inactivate or inhibit the outgrowth of pathogenic microorganisms in foods. Although food antimicrobials have been used for many years, a few of these substances are used exclusively to control the growth of specific food-borne pathogens. Examples of those used exclusively to control specific pathogens are nitrite to inhibit the growth of *Clostridium botulinum* in cured meats, selected organic acid sprays to reduce pathogens on beef carcass surfaces, nisin and lysozyme to inhibit growth of *C. botulinum* in pasteurized process cheese, and lactate and diacetate to inactivate *Listeria monocytogenes* in processed meats. Apart from preventing the growth of microbes, retention of sensory and nutritional quality of food is also important. To maintain the food quality, an application of combined preservative factors (hurdles) is followed. The most important hurdles used on food preservation are temperature, water activity, acidity, redox potential, competitive microorganisms along with preservatives (Leistner 2000). Acceptable daily intake of commonly used chemical preservatives are listed in Table 2.

Table 2. Chemical food preservatives

Agent	Acceptable daily intake (mg/kg body weight)	Commonly used levels (%)
Lactic acid	No limit	No limit
Citric acid	No limit	No limit
Acetic acid	No limit	No limit
Sodium diacetate	15	0.3-0.5
Sodium benzoate	5	0.03-0.2
Sodium propionate	10	0.1-0.3
Potassium sorbate	25	0.05-0.2
Methyl paraben	10	0.05-0.1
Sodium nitrite	0.2	0.01-0.02
Sulphur dioxide	0.7	0.005-0.2

FACTORS INVOLVED IN SELECTION OF ANTI-MICROBIALS

Anti-microbial agents are specific against particular organism growing in the food and accordingly anti-microbial agent is to be selected. There are some factors influencing the selection of anti-microbial agent.

Chemical and Physical Properties

Chemical and physical properties of both food and anti-microbial agents should be known to decide in which form the anti-microbial agent and food to be used and in what concentration to be used because the dissociation property of anti-microbial agents are dependent on pH of the food. In solution, weak acids like acetic, lactic, benzoic and sorbic acid exist in pH dependent equilibrium between dissociated and undissociated state. Preservatives have optimal inhibitory activity at low pH because this favours the uncharged, undissociated state of molecule which is freely permeable across the plasma membrane and is thus able to enter the cell of an organism. Thus the preservative molecule diffuses into the cell until the equilibrium is reached in accordance with the pH gradient across the membrane, resulting in accumulation of anions and protons inside the cell eventually leading to cellular energy depletion (Brul and Coote 1999).

The maximum activity of organic acids used as preservative are in high acid foods and there are very few compounds that are effective at lower concentration in foods with a pH of 5.5 or more. Although anion contributes slightly to anti-microbial activity, the undissociated form of a weak acid has highest anti-microbial activity. A significant decrease in microbial cell load was observed when a mixture of organic acids (sodium lactate 90% and sodium acetate 10%) at different concentration (0-20 g/kg) was added to Marguez sausages of lamb and beef during storage at 8 °C (Ayachi *et al*. 2007).

Solubility

All additives are mostly completely soluble. If anti-microbial agent is fat-soluble, then it should be dissolved in fat and oil to get maximum dispersability and better activity. Nunheimer and Fabian (1940) demonstrated that acetic acid was more effective inhibitor of *Staphylococcus aureus* than lactic acid because of their solubility and it was in the order acetic > lactic > citric > malic >tartaric > HCl. The solubility of nisin A is highest at low pH values and gradually decreases by almost 2 orders of magnitude when the pH of solution exceeds a value of 7. At low pH, nisin Z exhibits a decreased solubility relative to that of nisin A; at neutral and higher pH values, the solubilities of both variants are comparable (Rollema *et al*. 1995).

Storage Condition of Product

Some of the anti-microbial agents may be effective for a short period, while others may be active for a longer period. If the food is to be stored for a longer period then use of antioxidant microbial agent is necessary which is stable for a longer period. Andres *et al*. (2001) has reported that combined effect of chemical preservative and low gas permeability film extended the storage life of unpasteurized Valencia orange juice during storage at 10 °C. A combination of oregano essential oil at 0.9%, nisin at 500 or 1000 IU/g showed a high bactericidal effect against the pathogen (*Salmonella enteritidis*) in minced sheep meat than the oregano essential oil alone. The inhibition percentage against *S. enteritidis* was higher at 10 °C than at 4 °C during storage for 12 days (Govarisa *et al*. 2010). Microorganisms respond differently to a_w depending on a number of factors. Microbial growth, and in some cases production of microbial metabolites, may be particularly sensitive to alterations in a_w. Micro-organisms generally have optimum and minimum levels of a_w for growth depending on other growth factors in their environments. For example, gram (-) bacteria are generally more sensitive to low a_w than gram (+) bacteria (www.fda.gov).

Processing Treatment

Processing treatment may affect the activity of anti-microbials as some may be heat sensitive. So it should be used at the end of the processing. But to get the maximum effectiveness of anti-microbials it should be added at very early stage. It is necessary to use the anti-microbial agents which are very stable during processing to avoid the microbial growth (Murdock and Brokaw 1958). Nazera *et al*. (2005) found that use of more than one combination of either acidic or aromatic compounds can efficiently prevent the growth of *Salmonella sv. typhimurium* ATCC 13311.

MICROBES INVOLVED IN FOOD

Selection of anti-microbial agent depends on the species of microorganism involved in the processed product. Sulphur dioxide and its derivatives can be considered as an "universal" preservative as they have an antiseptic action on bacteria as well as on yeasts and moulds. Benzoic acid and its derivatives have a preservative action which is stronger against bacteria than on yeasts and moulds. Sorbic acid acts on moulds and certain yeast species; in higher dosage levels it acts also on bacteria, except lactic and acetic ones. Formic acid is more active against yeasts and moulds and less on bacteria.

Use of preservatives to extend the shelf-life of food has been common for many years. Preservatives retard

the growth of most spoilage organisms. But preservatives are more effective when added to products that initially have low microbial population and are of little benefits when added to poor quality products with high microbial load. Addition of preservatives to apple juice may also reduce heat resistance of yeast (Beuchat 1981). The *Alicyclobacillus acidoterrestris*, which has been isolated from apple juice, grows at the temperature ranges of 25-60 °C and its spores are able to germinate and grow at pH less than 4 and have D values of 16-23 min at 90 °C which means that they might survive the usual pasteurization treatments used in juice processing and only small numbers are required to contaminate large volumes of juice.

In apple juice at 30 °C 0.1 mg/ml sodium benzoate or potassium sorbate inhibit growth of 10^1 cells/ml *A. acidoterrestris* while 0.5 mg/ml inhibits growth of 10^4 cells/ml. Nisin at 5-10 IU/ml alone, and in combination with either sodium benzoate or potassium sorbate, is also effective in inhibiting multiplication of *A. acidoterrestris*. Another organism *Propionibacterium cyclohexanicum* which was found in orange juice grows at temperatures of 20-40 °C with a reported optimum temperature of 35 °C and a pH range of pH 3.2-7.5. Sodium benzoate (0.5 and 1.0 mg/ml) and potassium sorbate (1.0 mg/ml), both alone and in combination with 2.5, 5 or 10 IU/ml nisin, inhibit growth of *P. cyclohexanicum* in orange juice at 30 °C with no viable cells detected at 29 days, although nisin alone at concentrations up to 1000 IU/ml were not effective in inhibiting multiplication of *P. cyclohexanicum*, suggesting that this organism may be resistant to nisin (Walker and Phillips 2008).

Preservation by Sodium Chloride

The method of food preservation mainly for vegetables and meat has been developed in order to fulfill the requirement of civilians. Preservation of foods by using common salt has led the attention of food technologists as the method of food preservation. Adding salt to foods causes microbial cells to undergo osmotic shock, resulting in the loss of water from the cell and thereby leading to cell death or retarded growth (Davidson 2001). For some microorganisms, salt may limit oxygen solubility, interfere with cellular enzymes, or force cells to expend energy to exclude sodium ions from the cell, all of which can reduce the rate of growth (Shelef and Seiter 2005). In general, there are two methods of preservation of vegetables using salt. In one method, dry salt is added to the food directly whereby water is withdrawn from the tissue and dissolves salt which forms brine. In other method, food is covered by brine and salt is added additionally to maintain the initial concentration of brine. The salt exerts a selective action on the naturally occurring organisms to promote desirable fermentation. These microorganisms produce various compounds mainly lactic acid, and acetic acid, alcohols and also gas (Etchells and Jones 1943).

Reduction of sodium content (by removing both salt and sodium nitrite) in cured meats could allow for rapid growth of lactic acid bacteria and action by proteolytic microorganisms, resulting in a product that spoils more rapidly. Salt not only influences pathogen growth but can also affect survival during heat treatment. Non-proteolytic *C. botulinum* is less able to recover from heat treatment in media containing high salt concentration than media with low concentration (Roberts and McClure 1990; Stringer and Pin 2005).

Sugars

Sugars (sucrose, glucose, fructose and syrups) are used as a preservative in certain food preparations such as jellies, preserves, syrups, juice concentrates etc. The effect of these sugars on microorganisms is related to water activity of the product which is affected by these solutes. Tokuoka and Ishitani (1991) determined minimum water activities for the growth of 35 yeast strains in defined media using fructose or glucose as a_w controlling solute, incubating at 25 °C for up to 120 days. When practically all yeasts were examined, the minimum a_w for growth was somewhat higher in fructose than in glucose which means that fructose was inhibitorier. Chitosan and chitosan-sugar complex on *Staphylococcus aureus* showed that chitosan-lactose complex and chitosan-arabinose complex was the most effective at all concentrations. However, chitosan-galactose complex was the best antimicrobial agent after 24 hours of incubation at all concentrations (Mahae *et al*. 2011).

MAJOR CHEMICAL ANTI-MICROBIALS

Sulphur Dioxide and Sulphites

Sulphur dioxide (SO_2) has been used for many centuries as a fumigant. It is a colourless, suffocating, pungent-smelling and non-flammable gas. Sulphur dioxide and its various sulphites dissolve in water (85 g in 100 ml at 25 °C), and at low pH levels yield sulphurous acid, bisulphite and sulphite ions. Various sulphite salts contain 50-68% active sulphur dioxide. At pH values less than 4.0 the antimicrobial activity reaches its maximum. Activity of sulphur dioxide also depends on whether it is in bound form or free form. In gaseous form, it is very effective but there may be some cases in which sulphurous acid (H_2SO_3) is more active as compared to sulphur dioxide. It is bactericidal at high concentration and bacteriostatic at lower concentration.

Sulphur dioxide is used as a gas or in the form of its sulphite, bisulphite and metabisulphite salts which are powders. The gaseous form is produced either by burning sulphur or by its release from the compressed liquefied form. Metabisulphite is more stable to oxidation than bisulphites. The antimicrobial action of sulphur dioxide against yeasts, moulds and bacteria is selective, in case of wine, sulphur dioxide does not affect desired type of microorganism but retard the activity of undesirable microorganisms, eg. wild yeast, which is involved in contamination of wine. In addition to its antimicrobial effects, sulphur dioxide is added to foods for its antioxidant and reducing properties, and to prevent enzymatic and non-enzymatic browning reactions.

Sulphur dioxide and its salts potassium sulfite and sodium sulfites are used in the preservation of a variety of food products. In addition to wines these include dehydrated/dried fruits and vegetables, fruit juices, acid pickles, syrups, semi-processed fruit products, pastries, margarine, cheese fishes, sweets, ground beef etc.

Fruit juices are preserved by adding potassium metabisulphite. Dehydrated/dried fruits and vegetables, acid pickles, syrups, semi-processed fruit products, etc. are also preserved by using potassium metabisulphite. It can be used in liquid form also by exposing fruits and vegetables to the vapors of liquid SO_2.

Since antimicrobial preservatives are used rarely alone, their use is combined with other processing treatments like pasteurization, partial dehydration, refrigeration and packaging. Sulphur dioxide reacts with vitamin B and makes it unavailable. Therefore, its use should be avoided in foods which serve as a major source of this vitamin in the diet. Also, sulphur dioxide decolorizes foods. Beech (1958) has reported that addition of sulphur dioxide to pure cultures of yeasts inactivated the fermenting yeast *(C. Pulcherrima)* but the fermenter *(Saccharomyces uranum)* was unaffected. Reed and Peppler (1973) have reported that wine yeast may be acclimatized to ferment in the presence of 50-100 mg of SO_2/ ℓ for commercial and home juice. Warth (1985) has reported that sulphur dioxide extends lag time of yeast growth rather than slowing its growth rate.

Veiga and Madeira-Lopes (2000) studied the effect of weak acid preservatives on the growth and thermal death of the yeast *(Pichia membranifaciens)* in a commercial apple juice. *Pichia membranifaciens* exhibited a dissociative temperature profile (the temperature range of thermal death was distinct from the temperature range of growth) when incubation took place either in a commercial apple juice (AJ) or in a synthetic mineral medium with glucose and vitamins (MGV). In AJ, the maximum temperature for growth (T max) was 38.6 °C, which decreased to 36 °C in the presence of either 1 mM sorbic or 1 mM benzoic acid. The minimum temperatures of thermal death (T min) were, respectively, 40 and 38 °C with either of the acids. The yeast could grow with up to 2 mM sorbic or 3 mM benzoic acid, at 25 °C, which is close to the optimum temperature for growth (Top). At temperatures slightly above T min, sorbic acid was an actual enhancer of death rather than benzoic, the latter conferring some protection. However, these effects were reversed at higher temperatures (above 43 °C), at which benzoic acid was the most operative, in contrast to sorbic which was highly protective of the yeast against thermal death. The addition of acetaldehyde to sulphur-dioxide-containing juice reduced the lag phase and increased the overall specific growth rates.

Duo Toit and Pretorius (2000) reviewed the multiple stages at which microbial spoilage can occur in the process of wine-making, altering the quality and hygienic status of the wine and rendering it unacceptable. During alcoholic fermentation stage the addition of sulphur dioxide to the juice exerts selective pressure on the development of yeasts and bacteria. During post-fermentation stage, spoilage may occur in the bottle or during storage in oak barrels. At this usage of correct dosage of antimicrobial agents is very critical to ensure a stable product that will withstand attack from spoilage yeasts and bacteria. Some wine spoilage yeasts like Zygosaccharomyces are highly resistant to preservatives (SO_2, sorbic and benzoic acid) used in grape juice and wine.

Oppermann *et al.* (1999) has conducted that the occurrence of *Botrytis cineril* and SO_2 bleaching on table grapes are the main causes of deterioration in fruit quality during post-harvest storage.

Benzoic Acid

In most countries, benzoic acid and its sodium and potassium salts are permitted for food preservation. The maximum permissible limit varies from 0.15 to 0.25%. In US A, benzoic acid and sodium benzoate are considered GRAS (Generally Recognised As Safe) up to a maximum concentration of 0.1%. In UK, benzoic acid and sodium benzoate are permitted on a wide scale. In India, benzoic acid and its salts are permitted up to a maximum level of 0.075% in selected food products. Brachfeld (1969) has reported that benzoates are effective inhibitors of yeasts and moulds occurring in acidic foods below 4.5 pH. Use of benzoic acid is mainly directed against control of yeast and moulds. Benzoic acid has been found to act synergistically with both sorbic acid and sulphur dioxide, and combination of benzoic acid and sorbic acid has been reported to inhibit many bacterial strains better than either benzoic acid or sorbic acid alone.

Benzoic acid exist in granular form, has a sweet astringent taste and its solubility is very low. It is mainly used for preservation of processed fruit products and pickled vegetables, e.g. jam, jellies, sauces, ketchup, syrup, and sprays of benzoic acid are used for fruit storage. It is used in these products on a wider scale than sorbic acid because of its lower price. Fruit pulp can be preserved by the addition of 0.1-0.13% sodium benzoate. It is used widely to preserve fruit juices and is used alone or in combination with sorbate in the preservation of margarine, peanut butter, mayonnaise and fish marinades.

Benzoic acid and sodium benzoate were among the first additives used to preserve apple products. Important considerations for successful use of benzoates for apple cider preservation were reviewed by Fellers (1924) which still remains applicable. Fellers emphasized that sodium benzoate should be used only with acid foods and is effective only with products with low microbial loads.

Casareigo *et al.* (2000) have reported incapable growth of *Zygosaccharomyces bailli* in banana pulp after storage for 7 days at ambient temperature that treated with benzoate concentration of 800-1000 ppm.

Perez-Diaz *et al*. (2008) found that supplementation of sweet potato puree with 0.06% (wt/vol) sorbic acid or benzoic acid plus mild acidification of sweet potato puree with citric acid to pH 4.2 prevented growth of *L. monocytogenes* during storage at 4 °C.

Vijayanand *et al*. (2001) studied the preservation of papaya fruit chunks preserved by hurdle technology using the hurdles pH, mild heat treatment preservatives and packaging. Papaya chunks treated with increases in levels of preservatives to 680 mg potassium metabisulphite/kg and 826 mg sodium benzoate/kg exhibited good storage stability up to 90 days at 2 °C and ambient temperature. Canned tomato soup with added 750 ppm sodium benzoate retained high ascorbic acid, β-carotene and lycopene content than soup without added preservative (Vashista *et al.* 2003). Priya *et al*. (2013) reported that high quality paste of ginger-garlic could be prepared by with added sodium chloride, xanthan gum and sodium benzoate (0.2 g/ℓ).

The combination of antioxidant stabilizer and preservative are very important for the preparation of high quality ginger-garlic paste. The storage life of grape pomace treated with 0.1% sodium benzoate and an exposure to 2.0 kGy dose of γ-irradiation could be extended up to 16 days as compared to 8 days of control grape pomace. And the microbial study indicated that 0.1% sodium benzoate + 2.0 kGy treatment reduced the total aerobic bacterial count, yeast and mould count during 16 days of storage period compared to the control (Augustine *et al.* 2013).

Sorbic Acid

Sorbic acid is mainly used against yeast and moulds including aflatoxin-producing fungi. Bacteria are only partially inhibited; catalase positive are more sensitive than catalase negative. Microbial inhibitory action of sorbic acid is pH dependent and decreases with rise in pH. However, even at higher pH, sorbic acid is more effective than propionates and benzoates and therefore it is widely used for bakery products like cakes, filling for chocolates and various types of cheese and cheese spreads. Sorbic acid has been successfully employed in the development of shelf stable ready to eat chapaties in our country.

In cheese industry, sorbic acid is applied in many ways. It may be directly added to fresh or processed cheese as sorbic acid or potassium sorbate. Fungistatic wrappers containing calcium sorbate are also very effective for the prevention of fungal growth of cheese and bread. Sorbic acid is used in some countries in sweetened wines to inhibit re-fermentation by *S. cerevisiae* but it is not an effective inhibitor of LAB, AAB or yeasts such as *Brettanomyces, Saccharomycodes* and *Zygosaccharomyces*. The effectiveness of sorbic acid is directly related to the wine pH, alcohol levels, SO_2 concentrations and numbers of spoilage yeasts (Duo Toit and Pretorius 2000).

Sorbates have been found useful for prevention of surface mould growth on hard sausages. Recently, the use of potassium sorbate has been recommended along with small quantity of nitrite for the preparation of cured ham and bacon. Inclusion of potassium sorbate in the curing mixture prevents the growth of *Clostridium* spp. and the formation of botulinum toxin.

Sorbates are used in the preservation of fermented vegetable products and vegetables pickled in vinegar. Presence of sorbate inhibits lactic acid fermentation slightly but suppresses the growth of film forming yeast and moulds. Interest in the use of sorbic acid, sodium and potassium sorbate for the preservation of apple juice began in the 1950s. Ferguson and Powrie (1957) reported that addition of ascorbic acid to apple juice improved the inhibitory properties of sorbic acid.

Ough and Ingraham (1960) have reported that inhibitory effect of sorbic acid is increased when it is used in combination with sulphur dioxide and ethanol. Thus, effective levels of sorbic acid in wine are generally lower than those required for sweet cider.

Castro *et al*. (2002) studied the effect of several additives (EDTA, ascorbic and acetic acids) frequently present in the formulation of salad dressings on sorbate stability concerning *Z. bailii* growth in aqueous model systems of pH 3.5. The addition of EDTA, ascorbic acid and use of acetic acid protected sorbic acid from destruction. In particular, the presence of 0.075 g/kg of

EDTA was essential for minimizing sorbates destruction and keeping, after 90 days of storage at 33°C, its residual concentration above 0.400 g/kg. This value is the minimal inhibitory concentration (MIC) for inhibiting the growth of *Z. bailii* when systems were packed in PET flasks and citric, and acetic acids were used as acidulants. Packaging material, acidifying agent and EDTA level showed a strong influence on the effect of EDTA on sorbates stability. As an example, in the case of systems acidified only with citric acid and containing 0.075 g/kg or 0.500 g/kg of EDTA, it was necessary the use of glass flasks and the lowest concentration of EDTA to get a residual preservative concentration above the MIC after 90 days of storage at 33°C.

Rajshekhara *et al*. (2000) has reported that acidulants and other chemical preservatives (sodium benzoate, potassium sorbate) can be used to reduce the thermal processing times of fruit juices, thereby maintaining product quality .

In a hurdle technology approach in which factors pH and water activity other than preservatives (potassium sorbate, calcium propionate, and sodium benzoate) are evaluated to prevent spoilage by *Aspergillus niger*, *A. flavus* and *Penicillium corylophilum* in analogs of a bakery product. Potassium sorbate has been found to be the most effective in preventing fungal spoilage at the maximum concentration tested (0.3%). Suboptimal doses (0.03%) of all preservatives tested led to an enhancement of growth of *Aspergillus* and *Penicillium isolates* (Marin *et al.* 2002)

Marin *et al*. (2003) tested the effect of sorbic acid and potassium sorbate on growth of different *Eurotium* isolates in a bakery product when added in concentrations ranging from 0.025 to 0.2%. It was observed that 0.025 and 0.05% concentrations always enhanced the isolates growth, while 0.1% had little preservative effect. Finally, even the highest concentration (0.2%) was not suitable as it only controlled fungal growth under certain water activity and temperature levels. Hence, these weak-acid preservatives are not useful when added to bakery products with near to neutral pH.

A mathematical model was developed by Battey *et al.* (2002) to predict the probability of yeast spoilage of cold-filled ready-to-drink beverages considering five variables such as pH, titratable acidity, sugar content, sodium benzoate and potassium sorbate at different concentrations. The samples inoculated with *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Candida lipolytica* (~5.0 × 104 CFU/ml each) were plated on malt extract agar after 0, 1, 2, 4, 6 and 8 weeks. Logistic regression was used to create the predictive models. The pH and sodium benzoate and potassium sorbate concentrations were found to be significant factors controlling the probability of yeast growth. Interaction terms for pH and each preservative were also significant in the predictive model. Neither the titratable acidity nor the sugar content of the model beverages was a significant predictor of yeast growth in the ranges tested.

Asehruou *et al*. (2002) studied on control of 'bloater' spoilage of fermented green olives of the cv. Picholine which were brined in 5% NaCl solution adjusted to pH 4 or 5 with lactic acid and with or without 0.05% potassium sorbate, and with or without inoculation with *Lactobacillus plantarum* strain 1159. All samples were inoculated with *P. anomala* strain. Results showed that bloater spoilage of the olives was considerably reduced by adjustment of the brine to pH 4, addition of potassium sorbate and inoculation with *L. plantarum*. These treatments did not impair sensory quality of the olives.

The chemical preservatives, potassium sorbate and calcium propionate, are found effective in the control of poultry chicken spoilage causing bacteria like *Shigella sonnei*, *Vibrio parahaemolyticus*, *Staphylococcus aureus* and *Salmonella typhimurium* at 1400 μg/mℓ and the higher mean inhibition zone was recorded on potassium sorbate than calcium propionate (Jageethadevi *et al*. 2012).

Acetic Acid

Acetic acid is a general preservative inhibiting many species of bacteria, yeasts and to a lesser extent moulds. It is also a product of the lactic-acid fermentation, and its preservative action even at identical pH levels is greater than that of lactic acid. The main applications of vinegar (acetic acid) include products such as pickles, sauces and ketchup.

Use of vinegar containing 0.1% acetic acid inhibited the growth of *E. coli* O157:H7 and other food-borne pathogens and that the bactericidal effect of acetic acid was synergistically enhanced by sodium chloride but attenuated by glucose (Entani *et al*. 1998).

Statistical analysis on the death and survival of food-borne pathogens in commercial mayonnaise, dressing, and sauces was reported by Smittle (2000) shows that the most important and significant factor in destroying pathogenic bacteria is pH as adjusted with acetic acid, followed by the concentration of acetic acid in the water phase. The reported highest manufacturing target pH for dressings and sauces is 4.4, which is below the 4.75 pK_a of acetic acid and below the reported inhibitory pH of 4.5 for food-borne pathogens in the presence of acetic acid.

Fleming *et al*. (1995) has reported that replacement of potassium sorbate in air purged fermenting cucumbers with acetic acid on commercial level or

laboratory scales. Use of acetic acid may be preferred to sorbate when chemical preservatives are undesirable or cost is an issue.

Ponce de Leon *et al.* (1993) investigated the use of acetic and citric acids for control of fish spoilage bacteria *Pseudomonas* sp. and *Moraxella* sp. in culture media. A concentration of 0.05% acetic acid in nutrient broth containing 4% NaCl clearly inhibited *Pseudomonas* sp. and to some degree *Moraxella* sp. The effect of salt was much greater in the presence of acids than when it works alone. VB-N production of both microorganisms was inhibited by 0.05% of acetic acid, *Moraxella* sp. was found also to be sensitive in the presence of 0.05% of citric acid. The degree of inhibition decreased as the acid concentration decreased. The extent of anti-microbial activity of these acids coincided with their degree of undissociation.

Yong Su and Ick Jong (1994) studied the effect of dipping treatment in acetic acid, lactic acid and potassium sorbate on vacuum packed pork loin cut during refrigeration. Results indicated that initial spoilage occurred in the control after 16 days of storage, whereas in potassium sorbate treatments spoilage initiated after 23 days of storage.

Citric Acid

Citric acid is the main acid found naturally in citrus fruits; citric acid has been used as flavoring and preservative agents in traditional foods since antiquity. It is widely used in carbonated beverages and as an acidifying agent of foods because of its unique flavour. It has an unlimited acceptable daily intake and is highly soluble in water. It is a less effective anti-microbial agent than other acids. Treatment of sliced fruits such as apples with organic acids (e.g. dipping in a 3.4% solution of ascorbic acid) enhanced destruction of *E. coli* O157:H7 during domestic drying (Burnham *et al.* 2001). Citric acid at 0.5-1% applied on the surface of peeled oranges (pH ≥ 6.0) extended product shelf-life at 4-21°C (Pao and Petracek 1997). The application of citric acid on growth and metabolism of anaerobic *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* cultures showed increased growth inhibition of both yeast species with increasing pH values. In *S. cerevisiae*, citric acid shifted the primary energy metabolism resulting in lower ATP production. These metabolic changes in *S. cerevisiae* were pH-dependent; i.e. the higher the pH, the lower the ATP production, and thus the growth of *S. cerevisiae* is more inhibited by citric acid at higher pH values. In *Z. bailii*, citric acid caused virtually no changes ATP production (Neilsen and Arneborg 2007).

EFFECT OF CHEMICAL PRESERVATIVES ON HUMAN HEALTH

Sulphites or sulphate agents (in the forms of sodium sulphite, sodium bisulphite, sodium metabisulphite, potassium bisulphite, and potassium metabisulphite) are widely used as preservatives in a variety of food and beverages. Sulphites have been used for centuries, mainly as food additives, but can also be present naturally in foods such as fermented beverages and wines. Symptoms of sulphite sensitivity include asthma, urticaria, angio-oedema, abdominal pain, nausea, diarrhoea, seizures, and anaphylactic shock resulting in death. Sulphites cause few to no problems in most people without allergies and asthma, even when large amounts are consumed. There is no clear understanding of the mechanism by which inhaled sulphites trigger bronchospasm. It may be due to the formation of sulphur dioxide (SO_2) within the airways that affects the airway mucosa and to some extent this activates both the IgE mechanism and the cholinergic reflex resulting in bronchoconstriction. Two studies were undertaken to assess sulfite reactivity in wine sensitive asthmatics. Only a small number of wine sensitive asthmatic patients responded to a single dose challenge with sulfited wine under laboratory conditions. This may suggest that the role of sulfites and/or wine in triggering asthmatic responses has been overestimated. Alternatively, cofactors or other components in wine may play an important role in wine induced asthma (Vally and Thompson 2001)

But there are reports that sodium sulfite, sorbic acid and sorbates have a very low level of mammalian toxicity. Even in long-term exposure studies of up to 10% of the diet showed no carcinogenic activity. Both compounds have been subjected to extensive tests, including acute, short-term and chronic toxicity/ carcinogenicity tests, two-generation reproduction and teratogenicity studies, studies showing that they are non-mutagenic and non-clastogenic *in vitro* and *in vivo*. Walker (1990) observed no toxicity in the experimental setting. *In vivo*, the low toxicity of sorbic acid was explained by the fact that it is metabolized rapidly by pathways similar to other fatty acids. However, sulfite oxidase which exists in mammalian tissues and cells is species specific. These differences in sulfite oxidase activities have an impact on the sensitivity to sulfite (Beck-Speier *et al*. 1985). Especially macrophages and granulocytes show very low activities of sulfite oxidase and are therefore susceptible to sulfite in energy metabolism, production of lipid mediators and reactive oxygen species (Beck-Speier *et al*. 1993; Beck-Speier *et al*. 1998; Beck-Speier *et al*. 2003).

Sulfites that enter mammals via ingestion, inhalation, or injection are metabolized by sulfite oxidase to sulfate. Sorbic acid, an unsaturated fatty acid, is metabolized in a manner similar to that of other fatty acids and thus can serve as an energy source. In the

presence of adequate metabolizable carbohydrate, the major end products are CO_2 and H_2O. This rapid catabolism is also the reason for its low toxicity (Walker 1990). There are some evidences that food additive intolerance resulted in some cases of chronic generalized pruritus. Studies found that sodium nitrate was the most frequently involved substance (Asero 1999, 2000, 2005), and one case of sodium metabisulphite-intolerance was also reported by Asero (2005). A case of sodium benzoate-induced chronic pruritus was observed in a 75-year-old woman. About 24 h after taking 100 mg sodium benzoate, the patient experienced a relapse of diffuse pruritus that lasted for 48 h. This finding was confirmed by a second series of two double blind, placebo-controlled challenges including sodium benzoate and placebo. Again, the additive induced pruritus that appeared about 24 h after the administration and that this time lasted for 5 days and further confirms that food additives intolerance should be always considered in the differential diagnosis of patients with chronic pruritus (Asero, 2006).

Benzoates (E210-E219) used mainly in marinated fish, fruit- based fillings, jam, salad cream, soft drinks and beer, have been found to provoke urticaria, angioedema and asthma. Furthermore, they have also been directly linked with childhood hyperactivity. Sulphites (E220-E227), used mainly in dried fruits, fruit juices and syrups, fruit-based dairy deserts, biscuit doughs, cider, beer and wine, have been linked with pruritus, urticaria, angioedema and asthma. When fed to animals, sulphites have also been found to have a mutagenic action (Tuormaa 1994).

NOVEL NATURAL ANTI-MICROBIALS

There is an increasing awareness regarding the ill effects of using synthetic antimicrobials and chemical preservatives in food items and the preference for natural preservatives has been growing in the food market. Natural anti-microbial agents of plant origin such as essential oils and spices (eg. basil, thyme, clove, cinnamon, mustard, garlic, ginger and mint), enzymes obtained from animal sources (e.g. lysozyme, lactoferrin), bacteriocins from microbial sources (nisin, netamycin) and naturally occurring polymer (chitosan) have been used to prevent growth of spoilage and pathogenic microorganisms in foods. The antimicrobial activity of plant essential oils is due to their chemical structure, the compounds with phenolic groups as oils of clove, oregano, rosemary, thyme, sage and vanillin are found to be most effective against gram-positive than gram-negative bacteria (Lucera *et al*. 2012).

Cinnamon, cloves, and cumin showed the strongest antimicrobial effects against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Mycobacterium smegmatis*, *Micrococcus luteus* and *Candida albicans* as test strains, with inhibition zones between <10 and >30 mm by the disc-diffusion method (Agaoglu *et al*. 2007).*Thymus eigii* essential oil was particularly found to possess stronger antimicrobial activity when compared with vancomycin (30 mcg) and erytromycin (15 mcg) standards and in combination with other essential oils (Toroglu 2007). The essential oils of eighteen plants, namely, *Artemisia judaica*, *A. monosperma*, *Callistemon viminals*, *Citrus aurantifolia*, *C. lemon*, *C. paradisi*, *C. sinensis*, *Cupressus macrocarpa*, *C. sempervirens*, *Myrtus communis*, *Origanum vulgare*, *Pelargonium graveolens*, *Rosmarinus officinalis*, *Syzygium cumini*, *Schinusmolle*, *S. terebinthifolius*, *Thuja occidentalis* and *Vitex agnus-castus*, were isolated and the major constituents of the isolated oils were limonene, alpha -pinene, 1,8-cineole β-thujone, sabinene, alpha-phellandrene, 4-terpeneol, trans-caryophyllene and β-citronellol.

The isolated oils were more effective against *E. carotovora* var. carotovora than *A. tumefaciens*. The oil of *T. occidentalis* revealed the highest antibacterial activity among the tested oils showing the lowest MIC values of 400 and 350 mg/ℓ, on *A. tumefaciens* and *E. carotovora* var. *carotovora*, respectively. In mycelial growth inhibition assay, most of the essential oils showed pronounced effect and the oil of *A. monosperma* was the most potent inhibitor with EC50= 54, 111, 106 and 148 mg/ℓ against *A. alternata*, *B. cinerea*, *F. oxysporum* and *F. solani*, respectively. On the other hand, the oils caused strong reduction in spore germination of fungi compared with the control. The oils of *A. judaica* and *A. monosperma* caused the highest spore germination inhibition of *F. oxysporum* and their EC50 values were 69 and 62 mg/ℓ, respectively. Among the tested fungi, *F. oxysporum* was the most susceptible fungus to all of the tested oil except the oil of *S. molle* (Badawy and Abdelgaleil 2014).

The *in vitro* antibacterial activity of ethanolic extracts of cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and cumin (*Cuminum cyminum*, CMN) against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) showed bactericidal effect after 6 h of incubation by cinnamon and clove, while cumin showed bactericidal activity after 24 h (Mandal *et al*. 2011). Antimicrobial enzymes from natural sources are being effectively used for active antimicrobial packaging as an emerging alternative solution to prevent spoilage and pathogenic microorganisms in fresh foods. Tests on thin meat slices laid on paper sheets containing antimicrobial proteins either lysozyme or lactoferrin or both indicated that lysozyme was most effective in preventing growth of *Listeria innocua* microbiota (Barbiroli *et al*. 2012). Zein films incorporated with partially purified lysozyme showed antimicrobial

Table 3. Application of bacteriocins in food biopreservation

Bacteriocin	Culture producer	Target microorganism	Food	Reduction (log CFU/g)	References
Nisin	*Lactococcus lactis*	*Brochothrix thermophacta*	Pork	3.5	Nattress, Yost and Baker 2001
Nisin	*L. lactis*	*Listeria monocytogenes*	Fermented milk	6.0	Benekerroum *et al*. (2002)
AcH Pediocin	*Lactobacillus plantarum*	*L. monocytogenes*	Cheese	1.0-2.0	Loessner 2003
Enterocin	*Enterococcus faecalis*	*L. monocytogenes*	Milk	2.0	Elotmani *et al*. 2002
Enterocin	*Enterococcus faecalis*	*Staphylococcus aureus*	sausage	5.3	Ananou *et al.* 2005
Nisin Z	*Lactococcus lactis*	*S. aureus*	Afuega;l pitu cheese	2.0	Rilla *et al*. 2004

effect on *Bacillus subtilis* and *Lactobacillus plantarum* (Mecitoflu *et al*. 2006).

Another form of natural anti-microbials used for food preservation is from microorganisms. Biopreservation refers to the extension of shelf, life and improvement of the safety of foods using microorganisms and or their metabolites (Ross *et al*. 2002). Bacteriocins are ribosomally synthesized, extracellularly released low-molecular-mass peptides or proteins which have a bactericidal or bacteriostatic effect on other bacteria. Bacteriocin production has been found in numerous species of bacteria, among which, due to their "generally recognised as safe" (GRAS) status, LAB have attracted great interest as natural antimicrobial preservative. So far, bacteriocins nisin and pediocin PA-1 are licensed as food preservatives (Settanni and Corsetti 2008; Balciunas *et al*. 2013).

The synergistic antimicrobial effect of nisin and allyl isothiocyanate (AITC) against *Listeria monocytogenes, Staphylococcus aureus, Salmonella Typhimurium* and *Shigella boydii* was observed showing the fractional inhibitory concentrations <1. Nisin and AITC as synergistic inhibitors could be an effective approach to achieve satisfactory antimicrobial activity against a wide range of food borne pathogens (Zou *et al*. 2013). Another naturally occurring antimicrobial compound is chitosan, a heteropolysaccharide obtained commercially by deacetylation of chitin, which is an abundant constituent of crustacean shells and fungi (Rhoades and Roller 2000; Sebti *et al*. 2005). Since last decade, chitosan has received increasing attention for its applications in food industries. It is considered as GRAS (Generally Recognized as Safe) by Food and Drug Administration (FDA) (Luo and Wang 2013).

Sangsuwan *et al*. (2008) reported that populations of *E. coli* inoculated on fresh-cut cantaloupe was reduced by more than 5 log CFU/piece in 8 days at 10 °C packed in a chitosan (1.5% w/v)/methyl cellulose (0.5% w/v) film. Populations of *S. cerevisiae* on fresh-cut cantaloupe melon and pineapple coated with film were reduced about 3 log CFU/piece in 4 d of storage at 10 °C. Coating of chitosan on jujube fruit to control post-harvest disease inhibited the spore germination, germ tube length and mycelial growth of *P. expansum* significantly in a concentration-dependent mode. Further, observation by electron microscopy revealed that plasma membrane of *P. expansum* was gradually disrupted after chitosan application (Wang *et al*. 2014).

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Geospatial assessment and mapping of soil properties of horticultural farm

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ABSTRACT

This study was carried out to evaluate the heterogeneity of selected soil quality properties in surface and subsurface soil layers in an agricultural farm under tuber crops cultivation and to determine the correlation between these soil properties. A total of 260 soil samples were collected in March 2011 from agricultural farm of Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala, India. The samples were collected from 0-15 and 15-30 cm soil depths and each sampling locations was noted using a global positioning system. The collected samples were analyzed for soil moisture per cent, soil colour, turbidity ratio, soil pH, organic matter and labile carbon. After data normalization, descriptive statistical analysis of the data was carried out. Correlation between different parameters was analyzed. The spatial analysis was done using geostatistical analyst extension of ArcGIS 10.0 software. Spatial distributions of soil physico-chemical properties in the farm were estimated using kriging interpolation. The results showed that soils were generally acidic in pH. About 55% samples were yellowish red in colour. Among soil properties studied, very high variability was noticed in case of lability of carbon, followed by turbidity ratio and the lowest variation was observed for soil pH and labile carbon. Gaussian and exponential models fitted well with the experimental semivariograms of most of the soil properties. The nugget to sill ratios, which gives the degree of spatial dependence, was observed to be weak to strong for the soil properties. The kriged interpolation maps of the soil properties can be used as very good tools for farm planning at regional scale.

Key Words: Spatial variability, Global positioning system (GPS), Geographical information system (GIS), Geostatistics, Kriging

Soil management systems play an important role in sustainable horticultural development. The soil management systems such as soil tillage, fertilizers and extreme irrigation often create unsuitable changes in soil quality. The most important effect of soil tillage is the decrease in soil organic matter and an increase in soil pH (Paz-Gonzalez *et al*. 2000). Soil quality cannot be measured directly, but soil properties that are sensitive to changes in management can be used as indicators. Soil organic matter (SOM) and related soil properties are probably the most widely acknowledged indicators of soil quality. Labile organic substances regulate the productivity of ecosystems in short time intervals and they are influenced by the way of farming (Jandl and Sollins 1997). The amount of labile carbon influences the activity and mass of microorganisms in the soil. The capacity of microorganisms to release plant-available nitrogen is influenced by the quality of organic matter inputs. Understanding the spatial variability and mapping of soil quality properties are very important and it makes a useful tool for comprehensive soil management and environmental assessment.

Geostatistics has been widely used in soil science and the aim of which is to use point information to estimate spatial variability and it uses sampled point information to interpolate the non-sampled areas. Geostatistics provides a set of statistical tools for incorporating the spatial coordinates of soil observations in data processing, allowing for the description and modeling of spatial patterns, predictions at unsampled locations with certain and exact errors and assessment of the uncertainty attached to these predictions (Goovaerts 1998). This useful tool generates interpolation maps with varying levels of precision (Burgess and Webster 1980). Kriging is a geostatistical method for spatial interpolation, which uses the semivariance to measure the spatially correlated component, a component

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that is also called spatial dependence or spatial autocorrelation (Chang 2012).

Therefore, study was carried out to determine the spatial variability of some important soil quality properties for two soil depths using geostatistics from irregularly sampled soil properties of horticultural farm and to prepare spatial maps for both soil depths using ordinary kriging.

MATERIALS AND METHODS

The study was carried out in the experimental farm of CTCRI, Thiruvananthapuram, Kerala, India (Latitude: 80 321 N; Longitude: 760551 E; Altitude: 50 m above msl). The experimental farm was under the cultivation of different tuber crops, viz. cassava, sweet potato, yams, Amorphophallus, taro, tannia, Chinese potato and arrowroot. The soil type was laterite soil which comes under the soil order Ultisols and the soil series was Trivandrum series (Soil Survey Organization, 2007). The soils of the study site were low in available nitrogen, high in available phosphorus and potassium contents (Soman and Byju 2013).

Soil Sample Collection and Laboratory Analysis

Georeferenced soil samples were collected from 130 locations of CTCRI farm covering all the five blocks using a global positioning system (GPS) receiver (Garmin GPS 12). The Fig. 1 shows the locations of different sampling points of CTCRI farm. The sample locations were about 50-60 m apart in an irregular grid and soil samples were collected from two different depths, 0-15 and 15-30 cm. The samples were air dried and ground to pass through a 2-mm sieve and analyzed for the physico-chemical properties, viz. soil colour, oven dry soil moisture per cent, turbidity ratio, soil pH, organic matter and labile carbon. The basic colour of the soil was determined according to standardized charts (Munsell Soil Colour Charts 1975).

The soil moisture per cent was determined by gravimetric method (Byju 2001), turbidity ratio of the sample was estimated by using turbidimetric method proposed by Williams *et al*. (1966). Soil pH was determined in 1:2.5 soil water suspension, using pH meter (Page *et al*. 1982). The organic matter content was determined by Walkley and Black titration method (Walkley and Black, 1934). Labile carbon content was determined by using permanganate method given by Weil *et al*. (2003). The non-labile carbon and the lability of carbon were estimated using the following equations given by Blair *et al*. (1995).

$$\text{Non-labile carbon} = \frac{\text{Total carbon}}{\text{Labile carbon}}$$

$$\text{Lability of carbon} = \frac{\text{Labile carbon}}{\text{Non-labile carbon}}$$

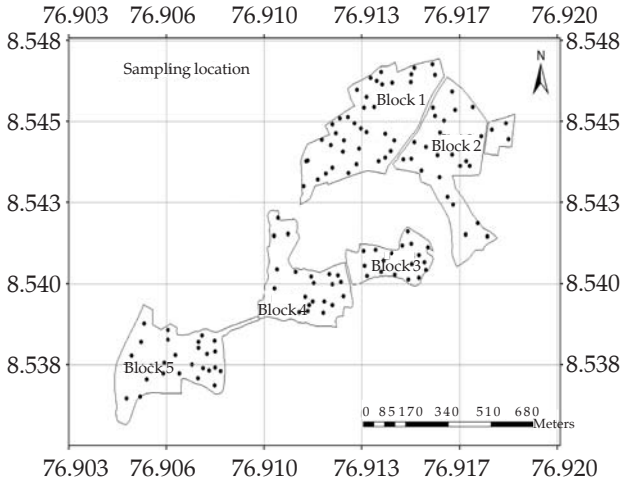


Fig. 1. Locations of sampling points in experimental farm of CTCRI, Thiruvananthapuram, India

0-15 cm	15-30 cm

Descriptive Statistical Analysis

Before descriptive statistical analysis, the data were tested for their normality using Kolmogorov-Smirnov (K-S) test as well as normal quantile-quantile (Q-Q) test. Since all data sets were found to be normally distributed, no transformation was done. The data sets were analyzed for their descriptive statistical parameters such as mean, minimum, maximum, median, coefficient of variation (CV), skewness and kurtosis. Of these different parameters, the CV is the most discriminating factor; when CV is < 10.00, the property shows low variability, and if CV is > 90.00, the property shows great variability (Xing-Yi *et al*. 2007). The data with a range of -1 to +1 skewness were considered as normally distributed (Virgilio *et al*. 2007). If kurtosis of the data is < 3, the distribution is more peaked than the Gaussian distribution, if kurtosis is equal to 3 it is as peaked as the Gaussian and if it is > 3, it is less peaked than Gaussian. The descriptive statistical analysis was performed using Excel 2007.

Correlation Analysis

Relationships between soil physico-chemical properties were established by using correlation analysis. The correlation coefficients between the different soil variables in surface and subsurface soil layers significant at 1 and 5% probability levels were calculated using Excel 2007.

Geostatistical Analysis

Geostatistical analysis was carried out using the Geostatistical Analyst extension of ArcGIS 10.0. Geostatistical analysis was done for different soil properties like soil pH, organic matter, labile carbon, oven dry soil moisture and turbidity ratio to produce

semivariograms with a best-fitted model that would quantify the spatial structures and derive the input parameters for spatial interpolation using kriging (Krige 1951). Spatial variability is expressed by a semi-variogram Y (*h*), which measures the average dissimilarity between data separated by a vector h (Goovaerts 1998). It was computed as half the average squared difference between the components of data pairs.

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[z(x_i) - z(x_i + h) \right]^2$$

where, $N(h)$ is the number of data pairs within a given class of distance and direction, $z(x_i)$ is the value of the variable at the location x_i and $z(x_i+h)$ is the value of the variable at a lag of h from the location x_i.

Experimental semivariogram value for each soil property was computed using ArcGIS 10.0 and plotted with a lag distance *h*. The computed semivariogram values ($\gamma(h)$) for corresponding lag (h) were fitted with available theoretical semivariogram models. Best-fit model with lowest value of residual sum of squares was selected for each soil property and each soil depth. Four commonly used semivariogram models were fitted for each soil property. These are the spherical, circular, Gaussian and exponential models. These models provide information about spatial structure and spatial attributes such as nugget (C_0), partial sill (C), sill ($C+C_0$) and range (a).

Expressions for different semivariogram models used in this study are given below:

Spherical model

$$\gamma(h) = C_0 + C \left[1.5\frac{h}{a} - 0.5\left(\frac{h}{a}\right)^3 \right], \; if \; 0 \le h \le a$$

Circular model

$$\gamma(h) = C_0 + C \left(1 - \frac{2}{\pi} \cos^{-1}\left(\frac{h}{a}\right) + \sqrt{1 - \frac{h^2}{a^2}} \right), \; 0 < h \le a$$

Gaussian model

$$\gamma(h) = C_0 + C \left[1 - \exp\left(\frac{-h^2}{a^2}\right) \right] for \; h \ge 0$$

Exponential model

$$\gamma(h) = C_0 + C_1 \left[1 - \exp\left(\frac{-h}{a}\right) \right] for \; h \ge 0$$

Ordinary Kriging

Surface maps of soil properties were prepared using semivariogram parameters through ordinary kriging. Ordinary kriging estimates the value of soil attributes at unsampled locations, $z(u)$ using weighted linear combinations of known soil attributes $z(u_a)$ located within a neighbourhood $W(u)$ centered around u.

$$z^*(u) = \sum_{\alpha=1}^{n(u)} \lambda_\alpha \; z(u_\alpha),$$

where, λ_α is the weight assigned to datum $z(u_\alpha)$ located within a given neighbourhood $W(u)$ centered on u. Kriged map for each soil property was prepared using Geostatistical Analyst tool of ArcGIS 10.0.

Interpolation Criteria

The kriged values were evaluated using cross-validation statistics and four parameters viz. mean error, root mean square error (RMSE), average standard error and RMSE standardized were calculated using the following formula:

$$\text{Mean error (ME)} = \frac{1}{n} \sum_{i=1}^{n} \left| Z*(x_i) - Z(x_i) \right|$$

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{n=1}^{n} \left\{ Z(x_i) - Z^*(x_i) \right\}^2}$$

$$\text{Average standard error} = \sqrt{\frac{1}{n} \sum_{n=1}^{n} \sigma^2(x_i)}$$

$$\text{RMSE standardized} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left(\frac{ME}{\sigma^2(x_i)} \right)^2}$$

The smaller the values and the closer to zero, the higher the precision of interpolations with any technique will be.

RESULTS AND DISCUSSION

Descriptive Statistical Analysis of Soil Properties

According to the classification by Landon (1984), the soils of the study area were generally acidic with pH ranged from 4.0-5.60 with a mean value of 4.49 in the surface soil layer and from 3.99 to 5.40 with a mean value of 4.39 in the subsurface soil layer (Table 1). The organic matter content was higher in the surface soil layer, which ranged from 0.43 to 3.31% with a mean value of 1.63%, whereas in subsurface soil layer, it ranged from 0.28 to 2.76% with a mean value of 1.19%. The labile carbon content was also higher in surface layer (mean = 0.1431) than the subsurface layer (mean = 0.1429). Soil labile organic carbon is the microbial degradable carbon associated with microbial growth. Labile carbon in soil is used as a metric of soil quality (Mirsky *et al.* 2008), and is directly linked to plant available nutrient turnover and is a sensitive indicator of management impacts on soil carbon sequestration (Cole *et al*. 1997). Among the two carbon fractions estimated data showed that labile carbon declines faster than non-labile carbon.

Table 1. Descriptive statistics of soil properties (n = 130)

Soil property	Unit	Depth (cm)	Mean	Min.	Max.	Median	CV*	Skewness	Kurtosis
pH		0-15	4.49	4.00	5.60	4.46	6.65	0.91	1.31
		15-30	4.39	3.99	5.40	4.38	5.11	0.90	2.18
Organic matter	%	0-15	1.63	0.43	3.31	1.60	31.16	0.27	0.42
		15-30	1.19	0.28	2.76	1.20	33.35	0.34	1.34
Labile carbon (LC)	%	0-15	0.1431	0.1427	0.1436	0.1431	0.10	-0.01	0.64
		15-30	0.1429	0.1426	0.1433	0.1429	0.08	-0.02	0.52
Non-labile carbon	%	0-15	0.80	0.11	1.78	0.79	36.69	0.27	0.43
		15-30	0.55	0.02	1.46	0.55	42.01	0.34	1.34
Lability of carbon		0-15	0.21	0.08	0.81	0.18	51.70	2.56	8.97
		15-30	0.34	0.10	1.85	0.26	72.66	3.17	13.13
Oven dry moisture	%	0-15	1.38	0.35	2.33	1.38	28.46	-0.09	0.13
		15-30	1.38	0.30	2.33	1.35	27.38	-0.04	0.18
Turbidity ratio		0-15	0.34	0.12	0.99	0.31	51.34	2.04	4.30
		15-30	0.41	0.12	0.96	0.33	55.64	1.19	0.31

CV, Coefficient of variation

Similar result was reported by Blair *et al*. (1995) in three different soils. The oven dried soil moisture ranged from 0.35 to 2.33% in 0-15 cm layer with a mean value of 1.38%. In 15-30 cm layer, oven dried soil moisture ranged from 0.30 to 2.33% with a mean value of 1.38%. The turbidity ratio ranged from 0.12 to 0.99, the average being 0.34 in surface layer and from 0.12 to 0.96 with a mean value of 0.41 in subsurface layer. The turbidity ratio is used as a measure of aggregate stability and is an important index of soil quality which influenced the retention and mobility of water and nutrients (Swift *et al*. 2004). Islam and Weil (2000) reported a small aggregate stability value in cultivated soils than natural forest land.

Among soil properties studied, very high variability was noticed in the case of lability of carbon as indicated by very high coefficient of variation (51.70% in surface soil layer and 72.66 in the subsurface soil layer), followed by turbidity ratio. According to the classification by Hillel (1980), soil pH and labile carbon values showed a 'low' variation (CV < 10%). A 'low' variation of soil pH was reported by many authors (Afshar *et al*. 2009; Yuqi Li *et al*. 2012).

Thus, all the variables studied except oven dry soil moisture and labile carbon presented positive skewness values. Among soil properties, the kurtosis values of turbidity ratio (0-15 cm) and lability of carbon were greater than 3.0 and all other variables showed a kurtosis less than 3.0 (Table 1).

Correlation Matrix of Soil Properties

There was correlation matrix of soil properties in the surface (0-15 cm) soil layer (Table 2). Soil pH in surface soil layer was significantly and positively correlated with soil moisture content (r = 0.34). Organic matter showed significant positive correlation with labile carbon (r = 0.66) and moisture content (r = 0.31) and showed significant negative correlation with lability of carbon (r = -0.83) and turbidity ratio (r = -0.35). Labile carbon showed significant positive correlation with non-labile carbon and moisture content and showed negative correlation with lability of carbon and turbidity ratio (P<0.01). Non-labile carbon showed positive correlation with moisture (r = 0.31) and negative correlation with lability of carbon and turbidity ratio (P<0.01). Lability of carbon was positively correlated with turbidity ratio and negatively correlated with soil moisture.

There was correlation matrix of soil properties in subsurface soil layer (Table 3). Soil pH and moisture content at subsurface soil layer was negatively correlated (r = -0.36). Organic matter showed significant positive correlation with labile carbon (r = 0.62) and moisture (r = 0.21) and significant negative correlation with lability of carbon (r = -0.78) and turbidity ratio (r = -0.48). Labile carbon was positively correlated with non-labile carbon and moisture content and negatively correlated with lability of carbon and turbidity ratio. Non-labile carbon showed positive correlation with moisture (r = 0.21) and negative correlation with lability of carbon (r = -0.78) and turbidity ratio (r = -0.48). In subsurface soil layer, positive correlation was observed between lability of carbon and turbidity ratio (r = 0.53) and moisture content and turbidity ratio (r = 0.21) (Tables 2 and 3).

Geostatistical Analysis of Soil Properties

The semivariogram parameters (nugget, partial sill, sill and range) for soil quality properties of CTCRI farm with the best-fitted model are presented in Table 4. The experimental semivariograms of soil properties are shown in Fig. 2. The optimal theoretical model for

Table 2. Correlation matrix of soil properties in surface soil layer (n = 130)

Soil property	pH	Organic matter	Labile carbon (LC)	Non LC	Lability of carbon	Moisture	Turbidity ratio
pH	1						
Organic matter	0.10	1					
Labile carbon (LC)	0.10	0.66**	1				
Non LC	0.10	1.00	0.66**	1			
Lability of carbon	0.10	-0.83**	-0.61**	-0.83**	1		
Moisture	-0.34**	0.31**	0.25*	0.31**	-0.36**	1	
Turbidity ratio	-0.10	-0.35**	-0.40**	-0.35**	0.36**	-0.05	1

* and ** correlations significant at 0.05 and 0.01 probability levels

Table 3. Correlation matrix of soil properties in the subsurface soil layer (n = 130)

Soil property	pH	Organic matter	Labile carbon (LC)	Non LC	Lability of carbon	Moisture	Turbidity ratio
pH	1						
Organic matter	-0.03	1					
Labile carbon (LC)	-0.15	0.62**	1				
Non LC	-0.03	1.00	0.62**	1			
Lability of carbon	-0.04	-0.78**	-0.38**	-0.78**	1		
Moisture	-0.36**	0.21*	0.23*	0.21*	-0.04	1	
Turbidity ratio	-0.17	-0.48**	-0.26**	-0.48**	0.53**	0.21*	1

* and ** Correlations significant at 0.05 and 0.01 probability levels

organic matter (0-15 cm), oven dried soil moisture (in two soil depths) and turbidity ratio (in two soil depths) was exponential, whereas soil pH and labile carbon were best fitted to Gaussian model. The organic matter in 15-30 cm soil layer was best fitted to circular model.

The level of spatial dependence varied from weak to strong for different soil properties. All the parameters showed a very low nugget value. Labile carbon in 0-15 cm showed high nugget value (4.533) compared to other soil properties studied. The range of all soil physical properties varied from 29.53 to 4080.94 m. Organic matter showed high range values than other properties studied. The maximum variation between any two neighboring samples, the sill variance, was lowest for all the properties except for labile carbon.

Variography analysis indicated different spatial dependence level for measured soil physical properties. Measurement error, which can be calculated using semivariograms, is an important tool that can be used to describe the properties of the spatial structure, nugget to sill ratio, showing strong, moderate and weak (< 0.25, 0.25 - 0.75 and > 0.75) spatial dependence or autocorrelation (Cambardella *et al*. 1994). The nugget value represents the random variation which was derived from the inaccuracy of measurements or variations of the properties that cannot be detected in the sample range (Trangmar *et al*. 1985). The values of nugget effect for most of the properties studied were small which suggest that the random variance of variables is low in the study area. A small nugget effect, and close to zero indicates a spatial continuity between the neighboring points. A small range value implies a distribution pattern composed of small patches (Al-Omran *et al*. 2013). High range values of the properties was due to some impact of intrinsic processes on these soil properties. Kavianpoor *et al*. (2012) reported a high range value for organic matter (Table 4 and Fig. 2).

Interpolation Criteria

The cross - validation results of selected models for mapping soil properties are presented in Table 5. Among four different models tested, the Gaussian and exponential models were found as the best fits in case of most of the soil properties. The organic matter in 15-30 cm depth was best described by the circular model (Table 5).

Kriging Interpolation of Soil Properties

The spatial interpolation maps of soil chemical properties are shown in Fig. 3. Spatial interpolation maps of soil pH in the surface and subsurface soil layer showed that the entire farm had acidic pH ranging from 4 to 5.60 in surface and from 3.99 to 5.10 in the subsurface soil layers. In the surface soil layer, high organic matter content (> 2.25) was observed only in the eastern part of block 3. For majority of the farm area, the organic matter content was in the range of 1.26 to

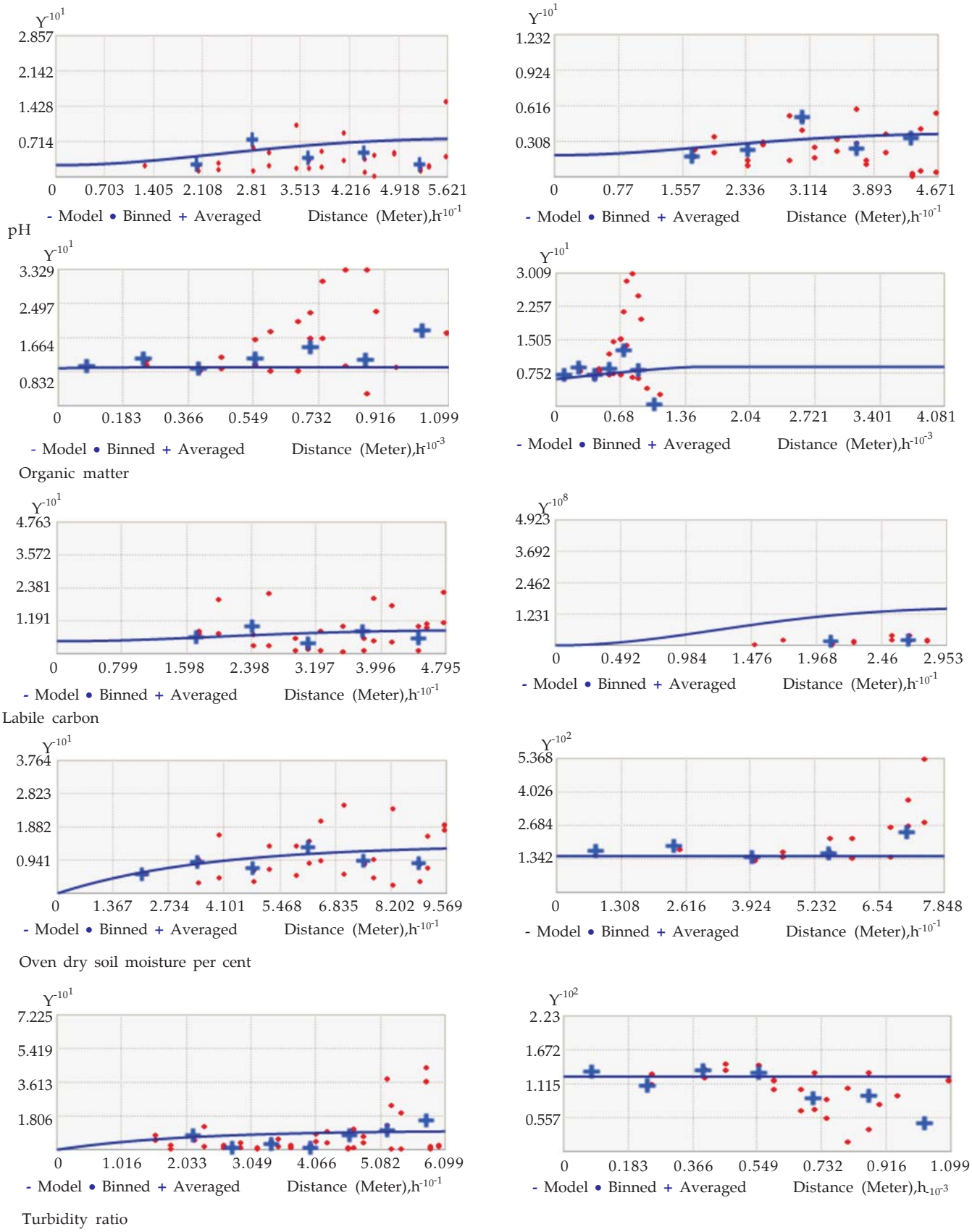


Fig. 2. Experimental and model semivariograms of soil properties

Table 4. Semivariogram parameters of soil properties of CTCRI farm

Soil property	Unit	Depth (cm)	Model	Nugget C_0	Partial sill, C	Sill C_0 +C	Range (m)	$\frac{C_0}{C_0+C}$	Spatial dependency
pH		0-15	Gaussian	0.012	0.069	0.081	57.088	0.15	Strong
		15-30	Gaussian	0.017	0.0197	0.037	46.431	0.46	Moderate
Organic matter	%	0-15	Exponential	0.088	0.0071	0.095	1569.59	0.93	Weak
	%	15-30	Circular	0.062	0.028	0.09	4080.94	0.69	Moderate
Labile carbon	%	0-15	Gaussian	4.533	4.15	8.683	47.95	0.52	Moderate
	%	15-30	Gaussian	1.524	1.524	3.048	29.53	0.5	Moderate
Oven dry moisture	%	0-15	Exponential	0	0.137	0.137	109.36	0	Strong
	%	15-30	Exponential	0.015	0	0.015	784.795	1	Weak
Turbidity ratio		0-15	Exponential	0	0.01	0.01	60.989	0	Strong
		15-30	Exponential	0.0123	0	0.0123	1098.71	1	Weak

Table 5. Cross validation statistics of kriged values for soil properties

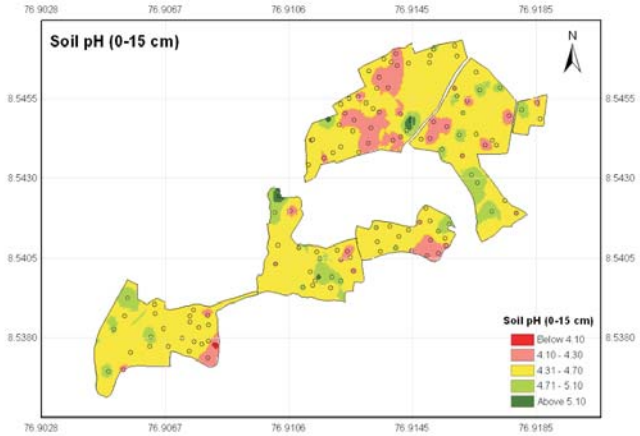
Soil property	Unit	Depth (cm)	Model	Mean error	RMSE*	Average standard error	RMSE standardized
pH		0-15	Gaussian	-0.0033	0.333	0.324	1.010
		15-30	Gaussian	-0.0014	0.233	0.204	1.140
Organic matter	%	0-15	Exponential	0.007	0.491	0.308	1.590
	%	15-30	Circular	0.009	0.378	0.259	1.459
Labile carbon	%	0-15	Gaussian	-7.886	0.0001	9.755	1.369
	%	15-30	Gaussian	-1.281	0.0001	0.0001	0.879
Oven dry moisture	%	0-15	Exponential	0.006	0.315	0.338	0.948
	%	15-30	Exponential	0.004	0.309	0.124	2.490
Turbidity ratio		0-15	Exponential	-2.321	0.136	0.104	1.303
		15-30	Exponential	-0.002	0.182	0.114	1.598

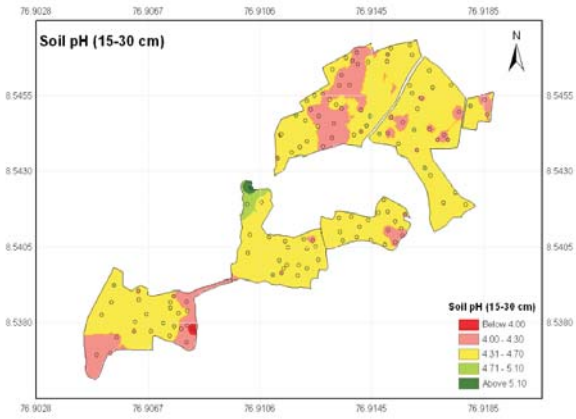
RMSE*, Root mean square error

2.25. In the subsurface soil layer, the organic matter was in the range of 0.55 to 1.50 for most of the farm area. Labile carbon content of surface soil layer was highest in the eastern part of block 3 and lowest in the northern part of block 4 and eastern part of block 5. In the subsurface soil layer, the labile carbon content was in the range of 0.1429 to 0.1431 for most of the farm area. In the surface and subsurface soil layers, high moisture content (> 1.75) was observed in the north eastern part of block 2 and south eastern part of block 3. In the study area, high values of turbidity ratio were observed in some parts of block 3 and block 5 (Fig. 3).

CONCLUSION

The soils of the study area were generally acidic in pH. Highest variability was noticed in case of lability of carbon followed by turbidity ratio. The lowest





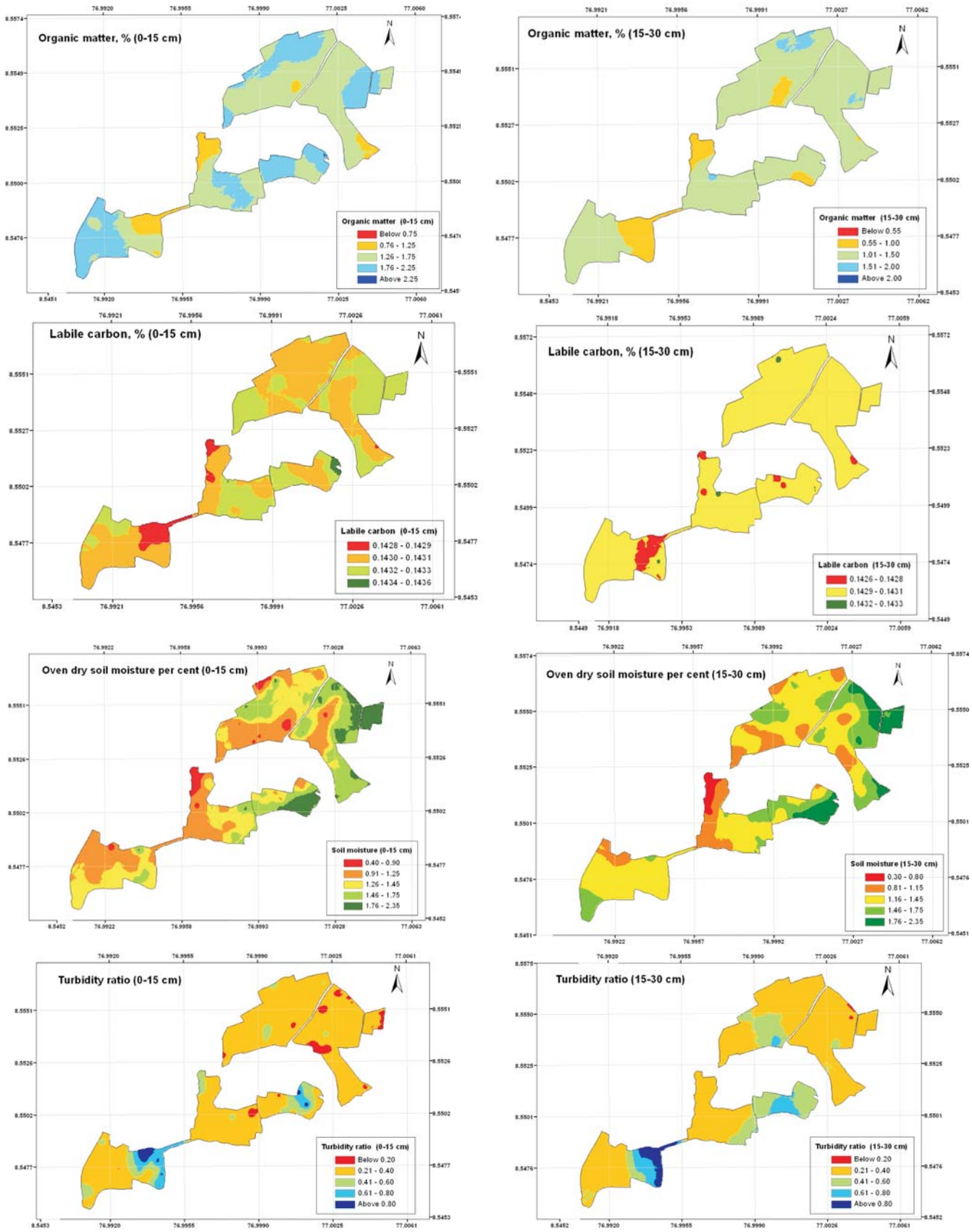


Fig. 3. Kriged maps of soil chemical properties

variation was observed for soil pH and labile carbon. The organic matter and labile carbon contents was higher in surface layer than the subsurface layer. The turbidity ratio (aggregate stability) was low in most of the farm area which may be due to different soil management systems such as soil tillage, fertilizers and irrigation The labile carbon content in soil was in strong positive correlation with organic matter content in soil ($P<0.01$). Of the four different semivariogram models used, Gaussian and exponential models fitted well with the experimental semivariograms of most of the soil properties. Soil properties showed a weak to strong spatial dependence. The semivariogram parameters were used for kriging that produced interpolation maps of soil properties which can be used as very good tools for farm planning at regional scale. The kriged maps of different soil properties helps to understand the distribution of these properties in the farm area and it have implications for crop selection and site specific land management.

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Morphological characterization in interspecies of *Bauhinia* species

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ABSTRACT

An experiment was conducted to characterize the variability in morphology as well as foliar epidermal micromorphology of five species of *Bauhinia,* belonging to family Fabaceae to determine the suitability for interspecies taxonomic delimitation and identification. Generally, these species are perennial shrub and trees with apparent differences in diagnostic features and have recorded differences in their growth habit, branching pattern, leaf shape, size, petiole size and veination pattern. The tree habit was observed from drooping small tree to erect bushy tree, whereas veination pattern was actinodramous in *B. racemosa, B. tomentosa* and B. *malabarica,* and it was campylodromous in *B. blakeana* and *B. purpurea*. Mostly, epidermal cells in all species were polygonal with straight walls excluding *B. tomentosa* L. which had an undulating outline. The stomata types were mainly anisocytic, anomocytic and paracytic. Among five species, *B. blakeana* L. was hypostomatic with anisocytic and paracytic stomata. Among different species, frequency of stomata differed markedly, were noticed with highest frequency in *B. malabarica* L. (30/mm^2), followed by *B. blakeana* (24/mm^2) and it was lowest in *B. tomentosa* L. (13/mm^2), followed by *B. racemosa* (14/mm^2). All the species had trichomes which were either unicellular or multicellular or both the types, but *B. malabarica* had notable unicellular hooked trichome. The highest trichome index (20%) was observed in *B. tomentosa,* followed by *B. malabarica.* However, it was recorded lowest in *B. purpurea* (0.51%), followed by *B. racemosa*.

Key Words: Bauhinia, Veination, Epidermal micromorphology, Stomata, Trichomes

The *Bauhinia* L. is an extremely variable genus of shrubs and medium-sized or large trees of more than 200 species of subfamily Caesalpinioideae. It belongs to large flowering family Fabaceae, with a pantropical distribution. The genus, named after the twin *Bauhin* brothers, is characterized by bilobed leaves with a cleft at the apex that forms 2 rounded lobes. From the base, veins spread out fan-wise, and the leaf is more or less folded along the centre rib. Bauhinia is also known as mountain ebony or simply orchid tree and *kachnar* in India and Pakistan. Its trees reach up to a height of 6-12 m, and their branches spread 3-6 m outwards. The lobed leaves are usually 10-15 cm across. Generally, flowers have five petals in shades of red, pink, purple, orange or yellow with diameter of 7.5-12.5 cm and are often fragrant. The tree begins flowering in late winter and often continues to flower up to early-summer (Cooke 1903). Some of the *Bauhinia* species have a long history of traditional and medicinal applications. The entire *B. purpurea* L. plant has been used in cases of dropsy, rheumatism, convulsions, delirium and septicaemia (Asolker *et al*. 2000). The *B. purpurea* L. possesses potential antiproliferative and antioxidant activities (Zakaria *et al*. 2011).

Various extracts of leaves of *B. racemosa* L. have been studied to develop a new pharmaceutical drug for the prevention of enteric infections (Dahikar *et al*. 2011). The stem bark of *B. racemosa* L. is astringent and used in the treatment of headache, fever, skin diseases, tumor, blood diseases, dysentery, and diarrhoea (Prakash and Khosa 1976). Looking into the utilization of different *Bauhinia* species for specific purpose, its distinctness and specificity of characters are required to differentiate various species in terms of morphological characters. Kotresha and Seetharam (1995) performed epidermal studies in some species of *Bauhinia* L. Lusa and Bona (2009) conducted comparative morphological and anatomical analyses of *B. forficata* L. and *B. variegata* L. The foliar epidermis is one of the most noteworthy

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taxonomic characters from a biosystematic point of view, taxonomic studies of a number of families have been conducted on the basis of leaf epidermis (Bhatia 1984). However, not much work has been done on the micromorphology of *Bauhinia*. Hence, an attempt was made to examine the phytomorphology as well as micromorphological characters of 5 species of *Bauhinia* L., so that the quantifiable characters of trichomes and stomata could be combined with other morphological characters to produce a valid set of characters for recognizing *Bauhinia* species.

MATERIALS AND METHODS

The leaves of different species of *Bauhinia* were collected during March and April in 2010 and 2011 from Junagadh Agricultural University, Junagadh, and Vadodara (Gujarat, India). The *B. racemosa* (Lal Dhori hill Junagadh), *B. blakeana* (Campus of Junagadh Agricultural University), *B. malabarica* (Motibaug, Junagadh Agricultural University), *B. tomentosa* (opposite Hansa Mehta Library garden, MSU Baroda) and *B. purpurea* (Science Faculty Garden, MSU Baroda) were collected. Photographs of relevant and accessible morphological characters were taken on living plants in the field during sampling. All species were identified with the help of Gujarat Flora (Shah 1978) and The Flora of the Presidency of Bombay (Cooke 1903) except *B. blakeana*, which is a hybrid between *B. variegata* and *B. purpurea*, grown in Junagadh.

To obtain epidermal surfaces, portions of trimmed leaf samples were soaked in Jeffrey's fluid for 24 h at 58 °C in an oven. Upper and lower epidermises were separately stripped off gently with the help of needles and forceps. The epidermal peels were washed thoroughly with water 2-3 times, stained in 0.05% aqueous toluidine blue in 1% borax, mounted in 50% glycerine, and observed under a light microscope. Microphotographs were taken using a digital camera fitted onto a Leica DME microscope. Qualitative and quantitative features of epidermal cells, stomata and trichomes from 10 different peels were assessed under uniform magnification (Salisbury 1927).

RESULTS AND DISCUSSION

The growth habit of different plant species was significantly different in all the evaluated species (Table 1). The *B. racemosa* was small drooping branched tree, *B. blakeana* and *B. purpurea* were erect branched and *B. tomentosa* was erect slender shrub, whereas *B. malabarica* was erect andbushy tree. Mostly the shape of leaves of different species was similar in shape, i.e. bilobed, more or less halfly cleaved, but size of leaves and petiole length were observed considerably different. The maximum leaf size (11.00 cm × 14.78 cm) was recorded

Table 1. Vegetative morphology of *Bauhinia* spp.

Species	Growth habit	Bark colour	Leaf shape	Leaf colour		Leaf size (cm)	Leaf petiole length (cm)	Leaf veination
				Dorsal side	Ventral side			
B. racemosa	Small, drooping tree	Creamish white	Small, bilobed rounded slightly cleved	Green	Grayish	3.7×5.5	1.58	Actinodramous
B. tomentosa	Erect, slender shrub	Blackish brown	Medium, bilobed rounded slightly cleved	Green	Green	4.8×5.2	2.00	Actinodramous
B. blakeana	Erect, branched	Creamish white	Small,bilobed rounded halfly cleved	Pale greenish yelleow	Pale greenish yelleow	10.0×13.0	4.00	Campylodromous
B. malabarica	Erect, bushy tree	Brown	Small,bilobed rounded slightly cleved	Pale green	grayish	3.5×4.5	2.60	Actinodramous
B. purpurea	Erect, branched	Creamish white	Big, bilobed rounded, halfly cleved	Pale yellowish dark green	Yellowish light green	11.00×14.78	4.38	Campylodromous

in *B. purpurea*, followed by *B. blakeana* (10 cm × 13 cm), whereas minimum (3.5 cm × 4.5 cm) leaf size was measured in *B. malabarica*. The petiole length ranged between (1.58-4.38cm) among all the different species. The maximum petiole length (4.38 cm) was observed in *B. purpurea,* followed by *B. blakeana* and it was found to be minimum in *B. racemosa* (1.58 cm) followed by *B. tomentosa*. The bark showed wide variation in their colour, creamy white in *B. racemosa*, *B. blakeana* and *B. purpurea*, blackish brown in *B. tomentosa* and brown in *B. malabarica*. The veination pattern was observed campylodromous in *B. blakeana* and *B. purpurea* and actinodramous in *B. racemosa*, *B. tomentosa* and *B. malabarica*.

Internal Micromorphology of Leaves

The foliar micromorphology is based on the epidermal characters of five species of *Bauhinia.* The qualitative and quantitative micromorphological features of *Bauhinia* species are presented in Tables 2, 3 and 4. All epidermal cells are polygonal with straight anticlinal wall patterns; however, anticlinal walls are irregular and wavy with an undulating outline in *B. tomentosa*. In *B. racemosa*, epidermal cells have anticlinal walls and anisocytic and anomocytic stomata (Figs. A. 9-11); *B. blakeana* has anticlinal walls and anisocytic and paracytic stomata (Figs A. 3-4); *B. malabarica* has an anticlinal wall pattern with anisocytic stomata (Fig A. 5-7); in *B. tomentosa*, irregular, undulating epidermal walls are found with paracytic stomata (Figs B. 5-8); and *B. purpurea* has anticlinal walls and paracytic and anisocytic stomata (Figs B. 1 and 2). The cell size also varied among species.

The maximum size was recorded in *B. tomentosa* (60.56 µm × 66.00 µm) and minimum size was observed in *B. malabarica* (24.50 µm × 30.32 µm). *B. tomentosa* differs from other species by its irregularly shaped epidermal cells with undulating walls. All the other species had polygonal epidermal cells with straight or beaded walls, with the exception of the lower epidermis of *B. racemosa* which had rectangular epidermal cells. All 5 species are hypostomatic except *B. blakeana*, which is amphistomatic. All species had paracytic and anisocytic stomata except *B. racemosa* where the paracytic type of stoma was absent and only anomocytic and anisocytic stomata were present (Figs A. 9 and 11). *B. blakeana* had an abnormal type of stoma with only a single subsidiary cell or two adjacent stomata with common subsidiary cells (Fig A. 4). In *B. malabarica* and *B. tomentosa*, subsidiary cells were normal (Figs. A. 7 and B. 8). Distribution of stomata was restricted and typically crowded near the leaf margin and midvein in *B. purpurea* (Figs. B 1 and 3). There was great variation in stomatal index (Table 3), which was the highest in *B. malabarica* (24.65%) and the lowest in *B. tomentosa* (8%).

Table 2. Micromorphological characters of epidermal cells

Species	Epidermal cell shape and nature of cell wall		Epidermal cell frequency (mm^2)		Epidermal cell size (l×b) (µm)	
	Dorsal surface	Ventral surface	Dorsal surface	Ventral surface	Dorsal surface	Ventral surface
B. tomentosa	Irregular , undulated	Irregular ,undulated	517	539	60.56 × 66.00	59 × 69.00
B. blakeana	Polygonal, straight	Polygonal ,straight some beaded	430	400	33.40 × 66.00	42.84 × 69.00
B. malabarica	Polygonal, straight beaded,	Polygonal,straight	78	76	24.50 × 30.32	23.96 × 29.70
B. purpurea	Polygonal ,beaded	Polygonal, straight	432	496	26.48 × 27.58	34.80 × 28.00

Table 3. Micromorphological charactors of stomata and their parameters

Species	Stomata type		Stomal frequency (mm^2)		Guard cell size (l×b) (µm)		Subsidiary cell size		Stomatal index	
	Dorsal surface	Ventral surface	Dorsal surface	Ventral surface	Dorsal surface	Ventral surface	Dorsal surface	Ventral surface	Dorsal Surface	Ventral surface
B.racemosa	Absent	Anisocytic, Anomocytic	_	14	_	14.54 × 3.80	_	21.76 × 17.48	_	16
B.tomentosa	Absent	Paracytic	_	13	_	12.20 × 3.00	_	54.00 × 60.00	_	8
B.blakeana	Anisocytic& Paracytic	Anisocytic , Paracytic	8	24	10.3 × 3.00	12.77 × 3.32	20.98 x11	25.43 × 12.82	11	23
B.malabarica	Absent	Anisocytic	_	30	_	13.26 × 2.80	_	13.22 × 24.00	_	24
B.purpurea	Absent	Paracytic, Anisocytic	_	18	_	12.00 × 5.10	_	26.50 × 17.50	_	19

Table 4. Micromorphological charactors of trichomes and their parameters

Species	Trichome type		Trichome length and breadth (mm^2)		Trichome frequency (mm^2)		Tricohome index (%)	
	Upper surface	Lower surface	Upper surface	Lower surface	Upper surface	Lower surface	Upper surface	Lower surface
B.racemosa	Absent	Unicellular, covering	_	56.00 × 14.35	_	3	_	1.53
B.tomentosa	Unicellular, covering	Unicellular, covering	90.00 × 10.90	96.00 × 10.90	11	15	18	20
B.blakeana	Absent	Multicellular, uniseriate, unicaellular, covering	_	89 ×13.7	_	11	_	7.6
B.malabarica	Multicellular,uniseriate, unicaellular, hooked	Multicellular, uniseriate, unicaellular, covering	89.00 × 13.70	100 × 11.30	2	9	1.89	11
B.purpurea	Absent	Multicellular, uniseriate,covering	_	87.00 × 13.10	_	1	_	0.51

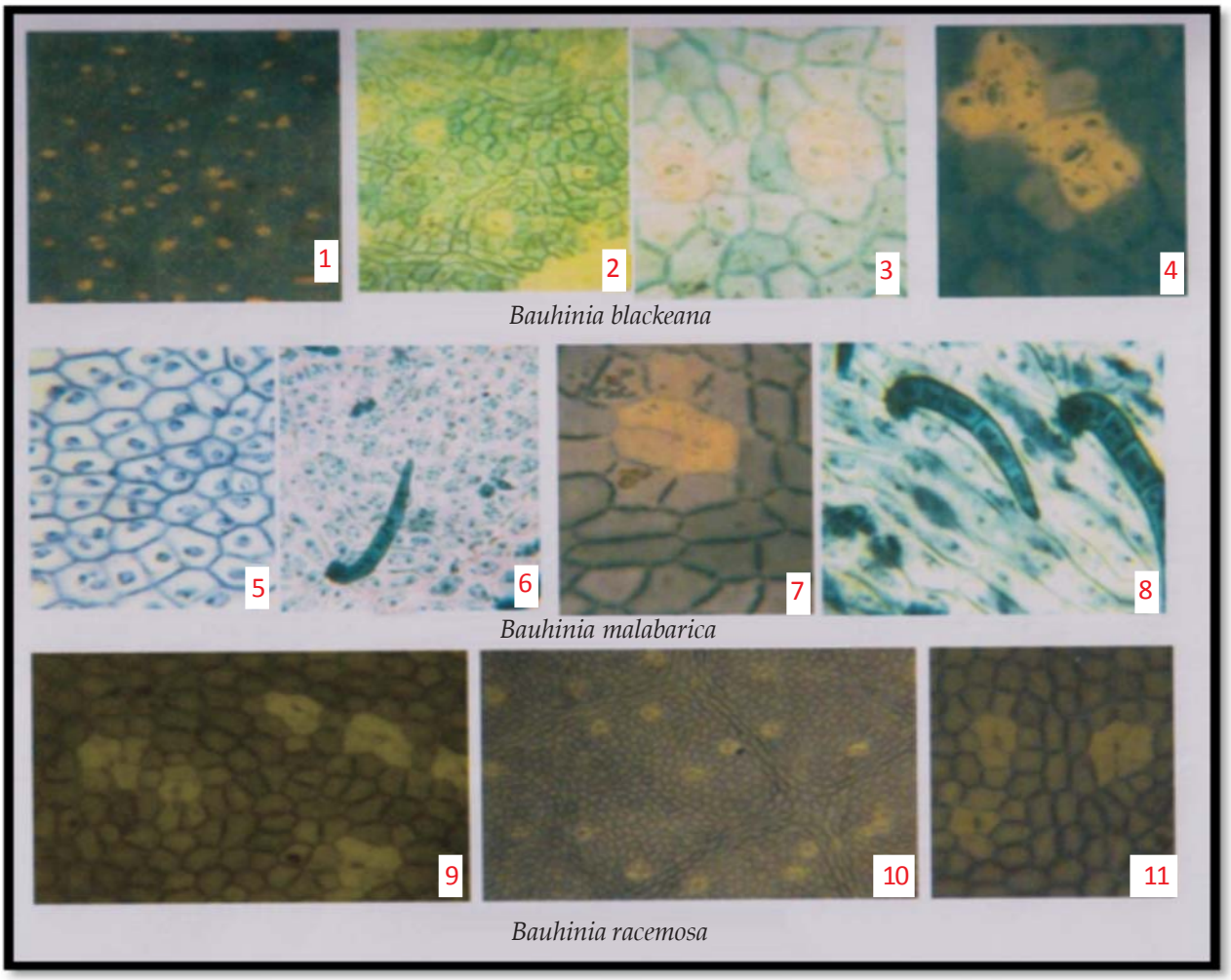

Fig. A. Micro-morphological features of *Bauhinia* spp.

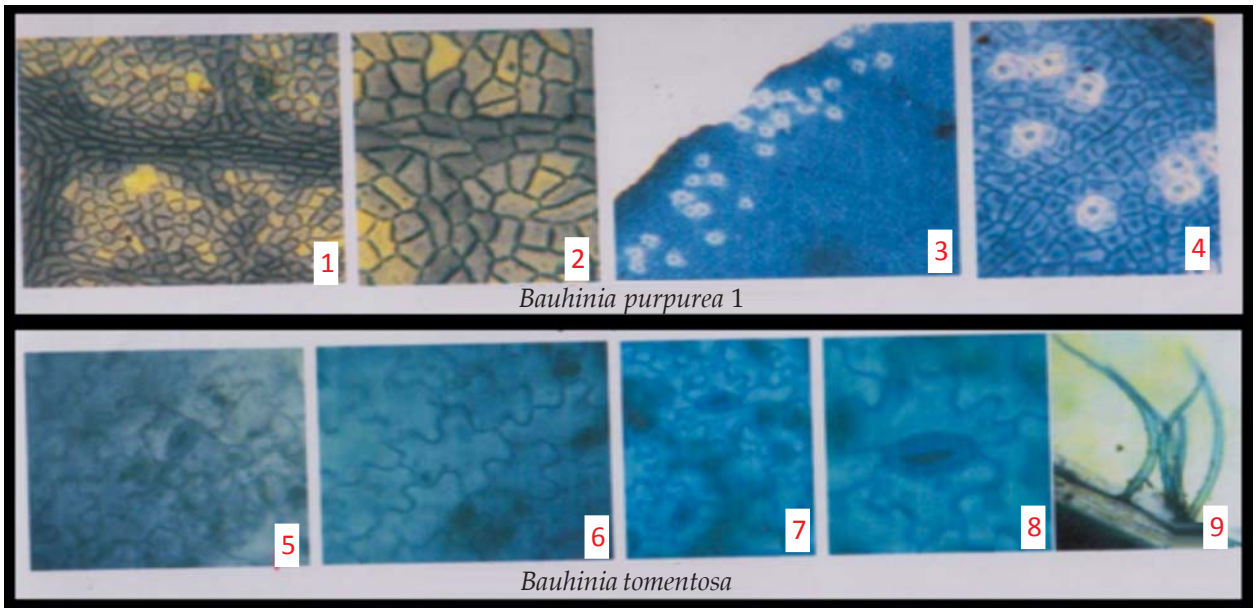

Fig. B. Micro-morphological features of different *Bauhinia* spp.

The highest subsidiary cell size was observed in *B. tomentosa* (54.00 µm × 60.00 µm), while the lowest was found in *B. racemosa* (21.76 µm × 17.48 µm). The length of guard cell was observed maximum in *B. racemosa* (14.54 µm) and the same was minimum in *B. blakeana* (10.30 µm). Guard cell breadth was recorded the highest in *B. purpurea* (5.10 µm) and it was the lowest in *B. malabarica* (2.80 µm) (Table 3). Foliar trichomes were non-glandular and covering, two types of trichomes could be distinguished, i.e. covered, unicellular, uniseriate, and covered, multicellular, uniseriate. Trichomes were absent on the dorsal surfaces of leaves in *B. racemosa* and *B. blakeana*. There was a covered multicellular trichome observed in *B. racemosa* and *B blakeana*; *B. malabarica* had hooked and multicellular trichome, *B. tomentosa* had covered unicellular trichome (Fig. B.9) and *B. purpurea* had covered multicellular trichome.

Multicellular trichomes are 2-4 celled and thick walled. In *B. malabarica*, multicellular and unicellular trichomes (Fig. A.8) were present on both surfaces. In *B. blakeana* and *B. purpurea*, multicellular trichomes were seen only in the ventral surfaces. Trichome frequency varied in all the species. The highest frequency (15/ mm^2) was found in *B. tomentosa* and the same was lowest (1/ mm^2) in *B. purpurea* (Table 4). The length of trichome varied in different species. It could be categorized into long trichomes (95 µm and above) and short trichomes (below 95 µm). The longest trichomes (100 µm) were observed in *B. malabarica* and the shortest in *B. racemosa* (56.00 µm). The breadth was observed highest (14.35 µm) in *B. racemosa* and it was lowest in *B. malabarica* (10.80 µm). The trichome index was calculated for all species which showed variation among species. It was recorded the highest (18 and 20%) in both dorsal and ventral surface in *B. tomentosa* and the lowest in *B. purpurea* (0.51%).

CONCLUSION

The observed species showed difference in tree habit, leaf size, petiole size, epidermal cells, stomata and trichomes. The differences in cell walls were confirmed, which were of only 3 types, i.e. straight, wavy, and sinuate, whereas epidermal cell walls in all *Bauhinia* species were polygonal and straight, except in *B. tomentosa*, where it was wavy and undulating. Major variations in stomatal frequencies of *B. malabarica* and *B. tomentosa* were also notable; the distribution of stomata was also very specific in *B. blakeana,* which had amphistomatic (other species were hypostomatic). The *B. purpurea* also differs due to the presence of crowded stomata near the leaf margins and veins. The ratio of average length and breadth of guard cells and subsidiary cells showed some dissimilarity; thus, the species can be differentiated on the basis of all of the characters studied. Thus, it may be inferred that *Bauhinia* species have both long and short hairs, but the size and the morphology of hairs differ. In *B. racemosa,* hairs are unicellular, long and taper to a pointed tip; in *B. malabarica* hairs were multicellular and hooked. Therefore, different species of the same genus may also be identified by their distinct stomatal and trichome characters.

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Effect of drip irrigation and fertilizer application on yield of baby corn (*Zea mays*)

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ABSTRACT

The field experiments were conducted during consecutive seasons of 2010- 2011 at the Research Farm of Water Technology Centre, Indian Agricultural Research Institute, New Delhi, India, to study the effect of drip fertigation and fertilizer application in furrow irrigation on yield of baby corn hybrid, HM-4. The recommended dose of fertilizers was applied in all the treatments. In drip irrigation, fertilizer solutions were applied through venture injector. However, broadcasting method was used for fertilizer application under furrow irrigation. Different treatments includes three fertigation frequencies (twice weekly, weekly and fortnightly), three dripper discharges at different systems operating pressures were the main treatments under drip fertigation and fertilizer application under furrow irrigation. Yield attributes of baby corn were significantly ($P<0.05$) affected by fertigation frequencies. Fertigation frequency schedule as twice weekly recorded significantly highest yields of baby corn (22.5 q/ha) and fodder (633.3 q/ha) with dripper discharge at system operating pressure of 1.0 kg/cm during second season. However, lowest yields of baby corn (10.7 q/ha) and fodder (413.0 q/ha) were recorded under fertilizer application in furrow irrigation during third season.

Key Words: Drip irrigation, Fertilizer, Yield, Baby corn, Fertigation, Green fodder, Cob

Baby corn (*Zea mays* L.) is dehusked maize ear harvested within 2-3 days of silk emergence, but prior to fertigation. It has prime place as a safe and high nutritive quality vegetable and has huge potential for commercial production. The tender cobs are consumed raw as a natural food. It is very tasty sweet and easy to consume because of its tenderness and sweetness with nutritive value. There is a potential demand for high quality baby corn in national and international markets (Thavaprakaash *et al*. 2005). Cultivation of baby corn generates employment for the rural poor since 3-4 crops can be raised in a year, giving good profit per unit area per unit time, besides its fodder yield is also high. Baby corn production is a profitable cultivation as it provides good quality green fodder, avenues for crop diversification, value-addition and enhanced revenue generation (Pandey *et al*. 2002).

Water is basic need for every crop to enhance the production. In India, agriculturel sector alone consumes almost 85% of available fresh water resource and its share is expected to reduce to 69% by 2025 (Sivannapan, 2009) due to every increasing demand of different sectors. Therefore, it is paramount important that irrigation methods that save water should be followed in order to maximize crop yield. Surface irrigation methods result in poor application efficiency (30-35%). However, highest water application efficiency (90% and above) can be achieved through microirrigation (drip or sprinkler irrigation). Drip irrigation is one of the advanced and innovative irrigation methods that can save 30-40% water as compared to surface method of irrigation. In drip irrigation, 20-60% higher yield is obtained with drip irrigation in some studies, while in other studies slightly lower or equal to that of conventional irrigation along with reduction in irrigation requirement of 30-60%. Phene *et al*. (1986) reported that significant yield increased in tomatoes with the use of high frequency SDI and precise fertility management.

Water-use efficiency has also been significantly improved through the use of SDI (Phene *et al*. 1986).

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Drip irrigation not only saves water but also increases yields of vegetable crops as water and nutrients are directly applied to the plants (Tiwari *et al*. 1998; Tiwari *et al*. 2003). Phene *et al*. (1986) conducted a study on root distribution of sweet corn under drip irrigation and fertilization treatment and reported differences between surface and subsurface drip irrigation on sweet corn rooting system in the top 0.45 m. High root length density was observed below 0.30 m in the subsurface drip irrigation than in the surface drip (Al-Omranand *et al*. 2004). Paul *et al*. (2011) reported that highest yield of tomato (397.4 q/ha) was recorded under 100% net irrigated volume with drip irrigation and lowest yield (308.4 q/ha) was recorded under surface irrigation. Sivanappan *et al*. 2009 reported that fertigation enhanced the yield potential by 3 times from the same quantity of water, i.e. by saving about 45-50% of irrigation water and increasing the productivity by about 40%. The information on baby corn cultivation with drip irrigation would be very useful to the Indian farmers for better understanding of water application. Thus, an experiment was conducted to compare the yield of baby corn under drip fertigation and fertilizer application in furrow irrigation.

MATERIALS AND METHODS

The experiment was conducted at the Research Farm of Water Technology Centre, Indian Agricultural Research Institute, New Delhi, India (Latitude $28^0$37'30"-$28^0$30'0" N, Longitude $77^0$88'45"-$77^0$81'24" E and AMSL 228.61 m) during October 2010-July 2011 for 2 seasons (October 2010-January 2011 and April- July 2011). Soil samples from 0 to 60 cm at an interval of 15 cm from the soil depth were collected to determine major nutrients and physical and chemical properties of soils (Table 1). The sampling was done using a core sampler for the determination of the bulk density and hydraulic conductivity. Hydrometer method was followed to determine the sand, silt and clay percentages of soil. The soil of the experimental area was deep loam soil comprising 37.57% sand, 40.67% silt and 21.59% clay. The bulk density of soil was 1.47 g /cm^{-3},field capacity 0.20 and saturated hydraulic conductivity 1.19 cm/h, respectively.

To meet the crop nutritional requirement, 8 t/ha of farmyard manure, 150 kg/ha of N, 60 kg/ha of P_2O_5 and 60 kg/ha of K_2O ware applied (Dass *et al*. 2009). Nitrogen, potassium and phosphorus were applied in the form of urea, murate of potash and phosphoric acid respectively. Fertigation was started 15 day after sowing and was stopped 15 day prior to the end of the crop period. During the fertigation, the solutions of different fertilizers were applied separately and were not mixed together to avoid precipitation due to non-compatibility of two chemicals.

A hybrid maize crop (baby corn- HM4) was sown at a spacing of 60 cm × 20 cm during October 2010 and April 2011 and August 2011. The treatments comprised two methods of irrigation, viz. furrow irrigation and drip irrigation with three levels of fertigation nitrogen (weekly twice, weekly and fortnightly). Each treatment was replicated thrice (R_1, R_2 and R_3). The details of different treatments are given below:

T_1 = Fertigation biweekly and dripper discharge 0.94 ℓ/h at 0.5 kg/cm^2 system operating pressure
T_2 = Fertigation weekly and dripper discharge 0.94 ℓ/h at 0.5 kg/cm^2 system operating pressure
T_3 = Fertigation fortnightly and dripper discharge 0.94 ℓ/h at 0.5 kg/cm^2 system operating pressure
T_4 = Fertigation biweekly and dripper discharge 1.41 ℓ/h at 1.0 kg.cm^2 system operating pressure
T_5 = Fertigation weekly and dripper discharge 1.41 ℓ/h at 1.0 kg/cm^2 system operating pressure
T_6 = Fertigation fortnightly and dripper discharge 1.41 ℓ/h at 1.0 kg/cm^2 system operating pressure
T_7 = Fertigation biweekly and dripper discharge 1.71 ℓ/h at 1.5 kg/cm^2 system operating pressure
T_8 = Fertigation weekly and dripper discharge 1.71 ℓ/h at 1.5 kg/cm^2 system operating pressure
T_9 = Fertigation fortnightly and dripper discharge 1.71 ℓ/h at 1.5 kg/cm^2 system operating pressure
C= Control (fertilizer application with furrow irrigation)

The operating pressures of drip irrigation system was maintained at 1.0 kg/cm^2. Each treatment has three replications. The fresh yields were taken as fresh cob weight (husked baby corn) and fresh baby corn (unhusked baby corn) at harvesting and fresh weight of the fodder. Statistical analysis was done by using

Table 1. Soil characteristics of the experimental field

Soil depth (cm)	Soil texture			Soil			BD			
	Sand (%)	Sit (%)	Clay (%)	Texture (%)	FC (%)	PWP (%)	g/c m^3	Ks, (cm/h)	pH	EC, (dS/m)
0-15	38.72	40	21.28	Loam	19.84	6.78	1.57	1.65	7.98	0.904
15-30	36.72	42	21.28	Loam	20.37	7.53	1.65	1.74	8.05	0.475
30-45	34.72	42	23.28	Loam	22.56	7.60	1.63	1.58	8.26	0.394
45-60	37.44	40	22.56	Loam	22.84	8.10	1.58	1.74	8.37	0.398

standard analysis of variance (ANOVA). Two probability levels, 0.01 and 0.05 were considered for determination of significance. Analysis of variance was conducted for cob, baby corn and fodder yield.

The amount of irrigation water needed was calculated on daily basis according to the crop coefficient (Kc) and daily reference evapotranspiration (ET_o) by using Penman-Monteith's semi-empirical formula (Allen *et al*. 1998). Baby corn is about 114 and 103 days duration crop when sown in October and April respectively. This may be divided into four stages, namely initial, developmental, middle and maturity. The actual evapotranspiration (ET_c) was estimated by multiplying ET_o with crop coefficient for different months based on crop growth stages. The crop coefficient during the crop season was adopted as 0.15, 1.15 and 1.00 as initial, middle and maturity stages respectively (Allen *et al*. 1998). The crop water requirement was estimated as follows:

$$I = ET_c + DR + RO - P$$

where I, water requirement of crop, mm/d; ET_c, actual crop evapotranspiration ($ET_c = ET_o \times K_c$), mm/d ; ET_o = potential evapotranspiration, mm/d; K_c= crop coefficient; DR = deep percolation, mm/day (assumed zero); RO run-off, mm/d (assumed zero) and P effective rainfall, mm/d.

Irrigation efficiency of drip irrigation system was assumed as 95% (Rajput and Patel 2006) and 60% in furrow irrigation method. The water requirement of baby corn ranged from 0.1 to 3.5, 1.2 to 8.7 and 0.6 to 5.8 mm/d from early stage to peak demand period during October 2010-February 2011, April 2011-July 2011 and August 2011-November 2011, respectively. Total rainfall during the crop seasons of October 2010- February 2011, April 2011-July 2011 and August 2011-November 2011 were 33.3, 138.4 and 163.6 mm, respectively. The required amount of water was applied through drip irrigation with alternative day irrigation and system was operated based on dripper discharge at system operating pressure of 1 kg/cm^2. In furrow irrigation treatment, required amount of water was applied on weekly basis.

RESULTS AND DISCUSSION

Baby corn and fodder yields were recorded during all three consecutive seasons (October 2010-February 2011, April 2011- July 2011 and August - November 2011). Highest yields were recorded during second season (April 2011- July 2011) in all the nine treatments compared to other seasons (Figs 1 and 2). The lowest yields of baby corn and fodder were recorded during third season. Statistical analysis revealed that baby corn and fodder yields were significantly (P<0.01) affected with fertigation frequency (Table 1). The yield of baby corn was significantly different during different seasons. The variation in yields during different seasons could be attributed to the variation in weather parameters. The results showed that weather conditions were best suited during second season for baby corn cultivation.

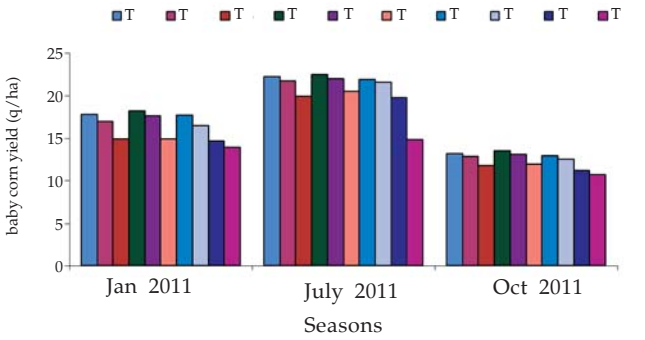

Fig. 1. Cob yield under different treatments

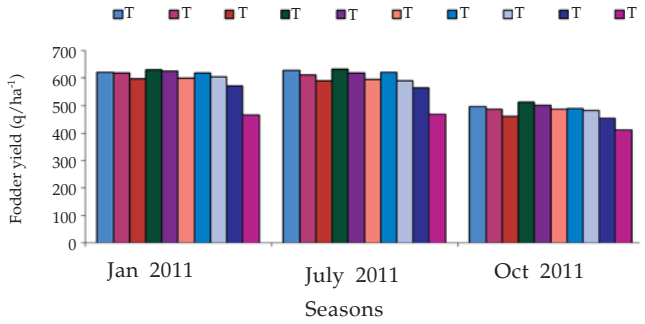

Fig. 2. Fodder yield under different treatments

Fertigation at different system operating pressures did not show any significant effect on yield characters. Yield of baby corn under biweekly and weekly fertigation schedules were at par. However, it was significantly different statistically at 5% and 1% level with fortnightly fertigation frequency schedule (Table 1). Yields of baby corn with interaction of season and fertigation frequency and fertigation at different system operating pressures were statistically significant.

A trend of lower yields of baby corn attributes was observed in all the treatments during third season as

Table 2. CD values at 5% and 1% significant level for different yield attributes of baby corn

Yield	Season 1		Season 2		Season 3	
attributes	(P<0.01)	(P<0.05)	(P<0.01)	(P<0.05)	(P<0.01)	(P<0.05)
Baby corn yield	12.69	5.14	9.33	3.77	7.68	3.11
Fodder yield	11.68	4.85	6.70	2.71	6.91	2.79

compared to first and second seasons. During second season (April 2011- July 2011), 39.9% higher baby corn yields were recorded compared to third season in treatment T_4, i.e. biweekly fertigation schedule with 1.41 ℓ/h dripper discharge at system operating pressure of 1.0 kg/cm^2. The average highest yield of baby corn and fodder were recorded in treatment T_4, i.e. biweekly fertigation schedule with 1.41 ℓ/h dripper discharge at system operating pressure of 1.0 kg/cm^2 (22.5, 633.3 q/ha) during second season while average lowest yield of baby corn and fodder was recorded in the control treatment, i.e. fertilizer application in furrow irrigation during third season (10.7 and 413.0 q/ha). Higher yields were observed at higher fertigation frequencies. It might be due to sufficient amount of nutrients available as per plants requirement during peak uptake time result in no nutrients stress in plants.

The fertigation frequency schedules had statistical significant effect on yield attributes of baby corn. Fertilizers applied through fertigation in biweekly and weekly fertigation schedule resulted in higher yield compared to fortnightly fertigation schedules with the same quantity of fertilizers applied. Higher baby corn yields, i.e. 31.17, 52.22 and 26.39% were recorded under treatment T_4 (biweekly fertigation schedule with 1.41 ℓ/h dripper discharge at system operating pressure of 1.0 kg/cm^2) compared to the control treatment (fertilizer application in furrow irrigation) during season 1, 2 and 3 respectively. Higher fodder corn yields, i.e. 35.75%, 35.17% and 23.97% were recorded under treatment T_4 (biweekly fertigation schedule with 1.41 ℓ/h dripper discharge at system operating pressure of 1.0 kg/cm^2) as compared to the control treatment (fertilizer application in furrow irrigation) during first, second and third season, respectively. The lower yield in fortnightly fertigation schedule may be attributed to non-availability of nutrients to plants at the time of need, leading to nutrient stress resulting in subsequent poor plant growth and lower crop yield. In frequent nutrient application, plants were not subjected to nutrient stress and hence higher crop yields were realized.

Similar results were reported by Binder *et al*. (2000), Rajput and Patel (2006), Kumar (2012), Kumar *et al*. (2012), Das *et al*. (2009) and Sampathkumar and Pandian (2010). Rajput and Patel (2006) conducted a study on onion with different fertigation frequencies and suggested yield of onion was not affected significantly in daily, alternative day and weekly fertigation frequency. However, there was a decrease with monthly fertigation. Sampathkumar and Pandian (2010) investigated the effect of fertigation frequencies and levels on growth and yield of maize and reported that fertigation frequency schedule once in 6 days registered higher grain yield and it was on a par with other fertigation frequencies.

CONCLUSION

Thus, it is clear that method of fertilizer application and fertigation frequency had significant effect on yield of baby corn irrespective to quantity of fertilizer used. The higher yield of baby corn can be achieved with drip irrigation and with more frequent application of fertilizers through fertigation. The crop seasons also have significant effect on baby corn yields.

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Growth and flowering behaviour of gladiolus (*Gladiolus hybrida*) hybrid under different plant spacings and corm sizes in midhill areas of Himachal Pradesh

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ABSTRACT

An experiment was conducted to find out the effect of corm size and plant spacings on growth, flowering and corm production in a newly-developed hybrid of gladiolus (*Gladiolus hybrida* L.) (Hb 1-9). Three corm sizes (3.0-3.5 cm, 3.6-4.0 cm and 4.1-4.5 cm diameter) at three spacings (30 cm × 6, 30 cm × 10 and 30 cm × 14 cm) were tested in a randomized block design replicated thrice. The gladiolus hybrid, Hb1-9, with S_3 and P_3 showed better performance, number of days taken for sprouting (15.27 days), duration of flowering (12.86 days), weight of spike (53.49 g), plant height (87.75 cm), number of days taken for bud break (100.58 days), number of days taken for first floret opening (104.09 days), size of florets (9.52 cm), spike length (72.96 cm), size of corm/plant (4.73 cm), number of corms/ plant (1.49), number of cormels (30.16) and days taken to harvesting stage (102.05).

Key Words: Growth, Flowering behaviour, Spacings, Corm size, Bud break, Cormels, Floret

Gladiolus (*Gladiolus hybrida* L.) belonging to family Iridaceae, is very important flower that stands for its beauty of flowers and long vase-life. The highly decorative and attractive spikes are predominantly used for cut flowers and the plant is also excellent for beds, pots and herbaceous borders. The availability of different species and varieties enabled the use of gladiolus for different purposes. The *Gladiolus primulinus*, being very attractive, is most suitable for mixed borders, whereas *Gladiolus grandiflorus* is quite ideal for exhibition purposes. The *pixiola* type like nanus, colvillei, byzantinus etc. are accredited to be very useful for forcing under glass houses besides for pot culture (Misra *et al*. 2006). As a landscape plant, it improves the aesthetic look of the garden. The spikes of gladiolus are very popular in various flower arrangements and are also used for preparing high class bouquets (Mukhopadhyay, 1995)

Gladiolus has gained prominence both in domestic and international floriculture trade. It ranks next to tulip in bulbous cut flowers in the flower markets of Holland (Misra *et al*. 2006). Presently, as a cut flower crop, gladiolus is being cultivated in US (Florida and California), Holland, Australia, Japan, Italy, France, Poland, Iran, Brazil, China, Malaysia, Singapore, Indonesia, Sri Lanka and Thailand besides in Israel. In India, it is grown commercially in over 6,000 ha (Nair and Singh 2004), and has been registered as an important cut flower in the domestic markets of Bangalore, Delhi, Hyderabad, Kolkata and Mumbai. The quality as well as quantity of flower spikes and daughter corms depend on several factors such as growing environmental conditions, size of corm and cormel, depth of planting, time of planting and fertilizer management, including other cultural operations (Arora and Khanna 1990). Gladiolus is mainly propagated by corms and size of mother corms and planting density play very important role in production of quality spikes and propagation index. The size of corm influences the growth, development, yield and quality of flowers and propagules. Similarly, spacing affects the photosynthetic activities as well as availability of nutrients. Therefore, present study was undertaken to find out the optimum spacing between plants and effect of different corm sizes on growth, flowering and multiplication.

MATERIALS AND METHODS

The experiment was conducted at the experimental farm of Department of Floriculture and Landscaping,

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College of Horticulture, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, during the cropping season (March 2009 to October 2009). The experimental farm is located at 1276 m above mean sea-level at a latitude of 30°52'N and longitude of 77°11'33"E. The climate is typically semi-temperate. Maximum temperature during the investigation ranged from 24.9°C to 33.3 C and minimum temperature ranged from 8.47° C to 19.5°C. Maximum and minimum relative humidity were 85 and 18%, respectively. Maximum rainfall was measured 408 mm during experimental period. The newly-developed hybrid of gladiolus, Hb1-9, is hybrid progeny of Interpid × White Dream.

The field was ploughed to a depth of 30-40 cm to get a fine tilth. The clods were broken and weeds as well as stones were removed. A basal dose of fertilizers and manure comprising 5 kg FYM, 30 g N, 20 g P_2O_5 and 20 g K_2O/m^2 were incorporated in soil. The nitrogen was supplied in the form of calcium ammonium nitrate, P_2O_5 in the form of single super-phosphate and K_2O as muriate of potash at the time of field preparation, respectively. The nitrogen was applied in two split doses. Second dose of nitrogen was applied at the time of six-leaf stage. The hybrid with corms of varied sizes, i.e. 3.0-3.5 cm, 3.6-4.0 cm, 4.1-4.5 cm were treated with Dithane M-45(0.2%) and Bavistin (0.1%) before planting. Corms were planted at a spacing of 30 cm × 6 cm, 30 cm × cm and 30 cm × 14 cm, there by adjusting 16, 10 and 7 corms/m^2, respectively.The depth of planting was twice the size of the corms. The experiment was laid out in open field on 26 March 2009 in a Randomized Block Design (Factorial) using 9 treatment combinations, and replicating them thrice. There were seven plants per replication. After 40 days of planting, earthing-up was carried out and half dose of nitrogen was given in the form of CAN. After planting pre-emergence spray of Paraquat (6 ml/ℓ) was applied to control the weeds.

The weeds, viz. *Cyprus rotundus* and *Oxalis latifolia* were most prominent. Hoeing was done manually. This helped in controlling the weed population as well as for better aeration and moisture conservation. Throughout the experimentation from planting till the termination of the experiment, field was irrigated depending upon requirement of plants. Generally, plants were watered daily during the first week of transplanting and later on alternate days. Crop was sprayed with Rogor (1.5 mg/ℓ) to control the crop against thrips. Drench application of Dithane M-45 (0.2%) and Bavistin (0.1%) was carried out at regular interval of 15-20 days to check fungal diseases. The observations were recorded on days taken to sprouting (days), plant height (cm), days taken to bud break (days), days taken to first floret opening (days), duration of flowering (days), spike length, total number of florets/spike, weight of spike (g), days taken for harvesting stage (days), size of florets (cm), number and size of corms/plant, number and weight of cormels/plant. Disease and insect pest incidence was checked at regular intervals. The statistical analysis of data was carried out as per the method described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The interaction effect of corm size (S) and plant spacing (P) was significant on most of the parameters (Table 1). Earlier sprouting (15.27 days) was observed with S_3P_3 which was found to be statistically at par with S_3P_2 and S_3P_1. However, maximum number of days for sprouting was observed with S_1P_3 which took 21.35 days. This might be due to that with wider spacing, large-sized corms received more space and nutrients at the initial stage and resulted in early sprouting. Shalini *et al*. (2004) also reported similar results with gladiolus cv. 'Debonair'.

The interaction effect of corm size and spacing (S × P) revealed significant differences in plant height. Maximum plant height (87.75 cm) was recorded in S_3P_3 interaction. Smallest plants were recorded (61.88 cm) for S_1P_1. This might be due to more food reserves in large corms and more photosynthetic activities when raised at wider spacing. The beneficial effect of large corms and greater planting distance confirmed earlier findings of Mukhopadhyay and Yadav (1984) and El-Gamassy and El-Gendy (1962).

There were significant differences in interactions between corm size and plant spacing (S × P) with respect to as early bud break in S_3P_3 which took minimum number of days (100.58), whereas S_1P_1 showed delayed bud break taking (115.23 days) (Table 1). This might be due to more food reserves in large corms and more photosynthetic activities when raised at wider spacing.

The data elucidated that S_3P_2 took lesser days for commencement of flowering which occurred in 104.08 days (Table 1). However, S_3P_2 interaction was found to be statistically similar to S_3P_3 interaction which took 104.09 days for flowering. In contrast, S_1P_3 showed delayed flowering taking 119.02 days.

It is evident that S_3P_3 interaction resulted in maximum duration of flowering, bearing flowers for 13.46 days, whereas S_1P_1 showed minimum flowering duration of 6.81 days (Table 2). The results further support the findings of Singh (2000) who also reported increase in duration of flowering with increase in corm size. Beneficial effects of larger corm size and wider plant spacing on duration of flowering have been reported by Kumar and Yadav (2006), Sharma and Gupta (2003).

The data on corm size and plant spacing (S × P)

Table 1. Effect of spacing and corm size on vegetative characters of gladiolus

Treatment	Days taken for sprouting			Plant height (cm)			Days taken to bud break			Days taken for first floret opening		
	P_1 (30cm ×6 cm)	P_2 (30cm ×10 cm)	P_3 (30cm ×14 cm)	P_1 (30cm ×6 cm)	P_2 (30cm ×10 cm)	P_3 (30cm ×14 cm)	P_1 (30cm ×6 cm)	P_2 (30cm ×10)	P_3 (30cm ×14 cm)	P_1 (30cm ×6 cm)	P_2 (30cm ×10 cm)	P_3 (30cm ×14 cm)
S_1(3.0-3.5 cm)	20.74	17.45	21.35	61.88	62.78	65.01	115.23	114.24	112.87	117.45	118.11	119.02
S_2 (3.6-4.0 cm)	19.08	18.83	17.87	70.92	73.07	74.04	105.96	108.74	107.47	113.11	111.26	110.22
S_3 (4.1-4.5 cm)	15.52	15.53	15.27	80.22	81.99	87.75	105.96	101.86	100.58	105.41	104.08	104.09
CD (5%)	Hybrid × corm size ×plant spacing (H × S × P) = 0.87			Hybrid × corm size × plant spacing (H × S × P) = 0.58			Hybrid × corm size × plant spacing (H × S × P) = 0.87			Hybrid × corm size X plant spacing (H × S × P) = 1.19		

Table 2. Effect of spacing and corm size on various characters of gladiolus

Treatment	Duration of flowering			Spike length			Weight of spike			Days taken for harvesting stage		
	P_1 (30 cm ×6 cm)	P_2 (30 cm ×10 cm)	P_3 (30 cm ×14 cm)	P_1 (30 cm ×6 cm)	P_2 (30 cm ×10 cm)	P_3 (30 cm ×14 cm)	P_1 (30 cm ×6 cm)	P_2 (30 cm ×10 cm)	P_3 (30 cm ×14 cm)	P_1 (30 cm ×6 cm)	P_2 (30 cm ×10 cm)	P_3 (30 cm ×14 cm)
S_1 (3.0-3.5 cm)	6.94	7.36	7.66	47.09	47.99	50.22	23.66	33.53	35.49	116.15	115.40	117.73
S_2 (3.6-4.0 cm)	8.21	8.94	9.69	56.13	58.28	59.25	38.96	40.48	42.13	110.98	108.90	108.05
S_3 (4.1-4.5 cm)	10.53	11.72	12.86	65.43	67.20	72.96	47.13	51.21	53.49	103.81	102.32	102.05
CD (5%)	Hybrid × corm size × plant spacing (H × S × P) = 0.35			Hybrid × corm size × plant spacing (H × S × P) = 0.58			Hybrid × corm size × plant spacing (H × S × P) = 1.44			Hybrid × corm size × plant spacing (H × S × P) = 1.61		

revealed that maximum spike length was recorded in S_3P_3, producing 72.96 cm spike length (Table 2). In contrast S_1P_1 produced smaller spikes of 47.09 cm length. Wider spacing might have influenced spike length as plants may have received sufficient sunlight for photosynthesis. Beneficial effect of larger corm size and wider spacing have been documented by Shiraz and Maurya (2005), Shalini *et al*. (2004), Sharma and Gupta (2003), Patil *et al*. (1995), Mukhopadhyay and Yadav (1984).

An inquisition of Table 2 revealed that maximum weight of spike (53.49 g) was recorded in S_3P_3 interaction. However, minimum spike weight (23.66 g) was recorded in S_1P_1 interaction. This might be due to availability of more food reserves in larger corms which help plants to put better growth and flowering and more photosynthetic activities in wider spaced plants as they receive more sunlight.

The minimum number of days for harvesting stage (102.05) was recorded in S_3P_3 interaction; however, S_3P_3 was found statistically at par with S_3P_2 (102.32) interaction (Table 2). Maximum days were registered by S_1P_3 (117.73) which was at par S_1P_1 (116.15). Larger-sized corms may have provided sufficient food material to the plants due to which they might have reached harvesting stage earlier than plants raised from smaller corms. Wider spacing provides sufficient space to plants which helps the plants to utilize more water, nutrition, air and light to put better growth.

The maximum floret size (9.52 cm) was noticed in S_3P_2 interaction (Table 3). However, this was statistically at par with S_3P_3 (9.30) interaction. Minimum floret size was noticed in S_1P_1 (6.13 cm) interaction. Beneficial effects of larger corm size and wider plant spacing has been documented by Shiraz and Maurya (2005), Shalini *et al*. (2004), Patil *et al*. (1995), Mukhopadhyay and Yadav (1984).

The S × P interaction was found to be significant as S_3P_3 produced maximum corms/plant, i.e. 1.49, whereas minimum number of corms/plant was recorded in S_1P_2 (1.01) interaction (Table 3). Sharma and Gupta (2003) observed maximum number of corms/plant (1.51) with wider plant spacing (40 cm × 40 cm), whereas minimum number of corms/plant (1.00) were produced when plants were raised under closer spacing (10 cm × 40 cm). The results are in the accordance to the findings of Kumar and Yadav (2006), Shiraz and Maurya (2005), Nair and Singh (2004), Singh and Singh (2000), Singh and Bijimol, (1999), Mukhopadhyay and Yadav (1984).

Spacing and size (S × P) interaction revealed that significantly bigger size of corms was noticed with S_3P_3 (4.73 cm), whereas, smallest corm size was noticed in S_1P_1 (3.09 cm) interaction (Table 3). Beneficial effect of large corm size and wider plant spacing have been

Table 3. Effect of spacing and corm size on various characters of gladiolus

Treatment	Size of florets (cm)			Number of corms/plant			Size of corms/plant			Number of cormels/plant		
	P_1 (30 cm ×6 cm)	P_2 (30 cm ×10 cm)	P_3 (30 cm ×14 cm)	P_1 (30 cm ×6 cm)	P_2 (30 cm ×10 cm)	P_3 (30cm ×14)	P_1 (30 cm ×6 cm)	P_2 (30 cm ×10 cm)	P_3 (30 cm ×14 cm)	P_1 (30 cm ×6 cm)	P_2 (30 cm ×10 cm)	P_3 (30 cm ×14 cm)
S_1 (3.0-3.5 cm)	6.13	6.95	7.30	1.02	1.01	1.14	3.09	3.26	3.43	3.39	5.04	8.23
S_2 (3.6-4.0 cm)	7.67	7.75	7.89	1.19	1.26	1.30	3.65	3.72	3.85	10.36	12.92	14.46
S_3 (4.1-4.5 cm)	8.17	9.52	9.30	1.34	1.37	1.49	4.03	4.24	4.73	18.95	22.99	30.16
CD (5%)	Hybrid × corm size × plant spacing (H × S × P) = 0.38			Hybrid × corm size × plant spacing (H × S × P) = 0.05			Hybrid × corm size × plant spacing (H × S × P) = 0.16			Hybrid × corm size × plant spacing (H × S × P) = 2.90		

reported by Kumar and Yadav (2006), Shiraz and Maurya (2005), Dilta *et al*. (2004), Nair and Singh (2004), Sharma and Gupta (2003), Sharma and Talukdar (2003), Singh and Singh (2000).

It is evident that S_3P_3 resulted in maximum number of cormels/plant (30.16), whereas S_1P_1 produced minimum number of cormels/plant, i.e. 3.39 (Table 3). Wider plant spacing reduced competition for nutrients among plants and allowed them to utilize more water, aeration and sunlight for photosynthetic activities which may have contributed in cormel production. The results are in accordance with the findings of Dalvi *et al*. (2008). They observed maximum number of cormels /plant (61.40) from wider spacing (30 cm × 30 cm), whereas minimum number of cormels/plant (52.38) was recorded in closer plant spacing (30 cm × 10 cm). These results further can be explained in the light of findings by Kumar and Yadav (2006), Nair and Singh (2004), Shalini *et al*. (2004), Sharma and Gupta (2003), Sharma and Talukdar (2003), Singh and Singh (2000), Mukhopadhyay and Yadav (1984) who also observed increase in cormel production with increase in size of mother corm.

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Estimation of leaf area model in hooker chives (*Allium hookeri*) and chollang (*Allium chinense*) using non-destructive method

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ABSTRACT

An experiment was conducted during 2011-12 on hooker chives (*Allium hookeri*) and chollang (*Allium chinense*), to find out the best method of estimation of leaf, at Horticultural Research Farm, Andro, Central Agricultural University, Manipur. In this study, a leaf area estimation model was developed using linear measurement such as laminar length and breadth individually and together with the product of length and breadth by step-wise regression analysis. *Allium* species are commercially used by the people of Manipur as spice crops. However, their cultivation has not been commercialized so far. *In-situ* leaf area estimation of these crops is important for studying the relationship between leaf area development and plant growth. The proposed leaf area (LA) estimation model of regression equation based on leaf length, $Y=6.426 + 2.051\times_1$ having correlation of co-efficient of determination ($r^2=0.91$) were suited for the estimation of leaf area of hooker chives, while for chollang the proposed leaf area (LA) estimation model of regression equation based on dry weight of leaf, $Y=3.636+4.605X_3$ having the co-efficient of determination ($r^2 = 0.94$) were most suited for the estimation of leaf area. However, dry weight of leaf method being destructive, the non-destructive method of regression equation in chollang based on leaf breadth, $Y= 0.214 + 3.772X_2$ having the co-efficient of determination ($r^2=0.93$) will be better suited for the estimation of leaf area estimation in chollang.

Key Words: Hooker chives, Chollang, Leaf area, Estimation, Linear measurement, Regression analysis

Hooker chives (*Allium hookeri*) and chollang (*Allium chinense*) are traditional herbal medicine with thick and fleshy leaves belonging to the Alliaceae family. These grassy, perennial, bulbous plants are popularly grown in Manipur as spice crops and are available through the year. The leaves of Hooker chives are linear to broadly with 0.5-1.0 cm wide and midvein distinct and blossoms during July-October, whereas chollang leaves are fistular, 2-4 in number and flower in June-July. People depend more and more on both bulbous herbs for cooking as spices by using their leaves and bulbs. Although no specific mention of medicinal uses has been seen for these species, members of this genus are in general very healthy additions to the diet and also use as medicinal herbs in Manipur. Besides, they contain sulphur compounds which help in reducing blood cholesterol levels and act as a tonic to the digestive system and also notify the circulatory system. John (2010) also reported medicinal properties of chollang for preventing cardiac problems. Moreover, among *Allium* species, particularly hooker chives is resistant to powdery mildew, which can be used in the crop improvement programme (Brezhnev and Korovina, 1981). Besides, Yumnam and Tripathi (2012) also reported that hooker chives is used as a medicine for aphrodisiac purpose. However, these perennial bulbous herbs are still being neglected in research. Meitei and Devi (2005b) also reported about the importance of leaf area estimation for economically important crops in Manipur.

Measurement of leaf area is often necessary for horticultural, agronomic and physiological studies. Non-destructive methods of estimation of leaf area are useful in studying the relationship between leaf area development and plant growth. These methods provide repeated sampling of the same plants over times, thus facilitating the study of leaf dynamic not possible with destructive sampling procedure. Measurement of leaf area by Digital Planimeter is very costly and availability of the equipment is limited due to financial constraints. But various workers reported about the accurate non-

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destructive methods of estimation of leaf area by these device on various horticultural crops like onion (Glenn 1971), pungent chilli (Meitei *et al*. 2005a), Chinese chives (Meitei *et al*. 2005b), faba bean (Peksen 2007) and clary sage (Kumar and Sharma 2010). There is not much systematic research work done on estimation of leaf area related to hooker chives and chollang in India or elsewhere. So, in order to overcome these problems studies were carried out to test whether a leaf area estimation model can be derived for hooker chives and chollang from the linear measurement of leaf length and breadth alone or from the product of length and breadth.

MATERIALS AND METHODS

The estimation of leaf area of hooker chives and chollang was done during 2011-12 at Horticulture Research Farm, Andro, Central Agricultural University, Imphal, Manipur. About 40 leaf samples of different sizes were used for the estimation of leaf area. The collected leaf samples from each hooker chives and chollang were then traced on a transparent sheet for the determination of leaf area by Placom Digital Planimeter. Leaf length and width were also determined subsequently for each type. The respective leaves were then dried in an electric oven at 60 ^{0}C for 24 h in order to get constant weight and the individual dry weights were recorded. The regression equations of actual leaf area without petiole were obtained along with their correlation co-efficient (Y). From the above relationship, following type of regression equation $Y= a + bX_1$, $Y= a + bX_2$, $Y= a + bX_3$ and $Y= a + bX_4$ were developed by calculating the regression parameters a and b. Y in the above expression represents leaf area and X_1, X_2 X_3 and X_4 represent the linear parameters like length, breadth, product of length and breadth and dry weight of leaf respectively. The regression models having a co-efficient of determination more close to 1.0 were suitable and good fit for the application in estimating leaf area. The leaf area obtained with these models was compared with actual leaf area and the significance of difference between them was determined by the help of paired t-test.

RESULTS AND DISCUSSION

The relationship obtained between actual leaf area, product of length and width and leaf dry weight of hooker chives and chollang are presented in Tables 1 and 2 along with relationship of graphic form of predicted and observed value of leaf area in Figs 1 and 2 respectively. Correlation co-efficient (γ) and co-efficient of determination (r^2) were calculated for finding out the components of leaf area of each crop. The co-efficient of determination (r^2) varied from 0.10 to 0.91 in chollang and 0.87 to 0.94 in hooker chives respectively. The co-efficient of determination, closer to 1.0 was observed with dry weight of leaf (r^2=0.94) method, followed by leaf breadth (r^2= 0.93), indicating that these equations are good fit for the estimation of leaf area in hooker chives. The r^2 value between the predicted and observed value (Fig 2) is 0.93, with standard of error of 7.33 cm^2. Since the predicted value is less than 1.0, there is statistically significant relationship between the variables 99% confidence level. In chollang, leaf length (r^2= 0.91) followed by the product of leaf length and breadth (r2=0.90), indicted these equations are good fit for the estimation of leaf area in chollang. Further, r^2 value between the predicted and observed value (Fig 2) is 0.91, with standard of error of 1.69 cm^2 having the predicted value of less than 1.0, there is statistically

Table 1. Relationship between actual leaf area and various parameters of hooker chives

Parameter	Regression equation	Correlation co-efficien (γ)	Co-efficient of determination (r^2)	Calculated value of 't'
Leaf length (X_1)	$Y=16.084 + 0577X_1$	0.93	0.87	15.61
Leaf breadth (X_2)	$Y=0.14 + 3.772X_2$	0.96	0.93	23.55
Dry weight of leaf (X_3)	$Y=3.636 + 4.605X_3$	0.97	0.94	24.87
Leaf length × leaf breadth (X_4)	$Y=-1.623+ 1.495X_4$	0.94	0.89	17.80

Table 2. Relationship between actual leaf area and various parameters of chollang

Parameter	Regression equation	Correlation co-efficien (γ)	Co-efficient of determination (r^2)	Calculated value of 't'
Leaf length (X_1)	$Y=6.426 + 2.051X_1$	0.95	0.91	19.92
Leaf breadth (X_2)	$Y=4.666+1.882X_2$	0.88	0.77	11.47
Dry weight of leaf (X_3)	$Y=-1.635 + 9.471X_3$	0.35	0.10	2.30
Leaf length × leaf breadth (X_4)	$Y= -1.187+0.738 X_4$	0.95	0.90	18.81

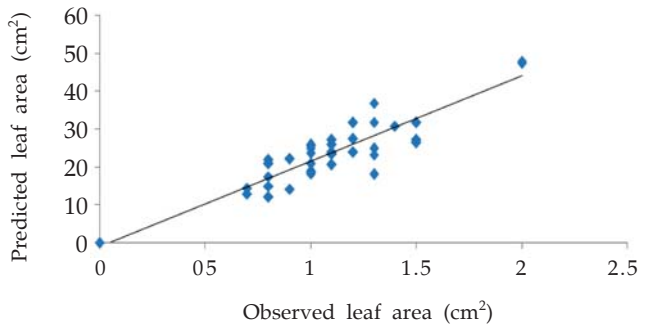

Fig. 1. Relationship between observed area and predicted area of leaf hooker chives

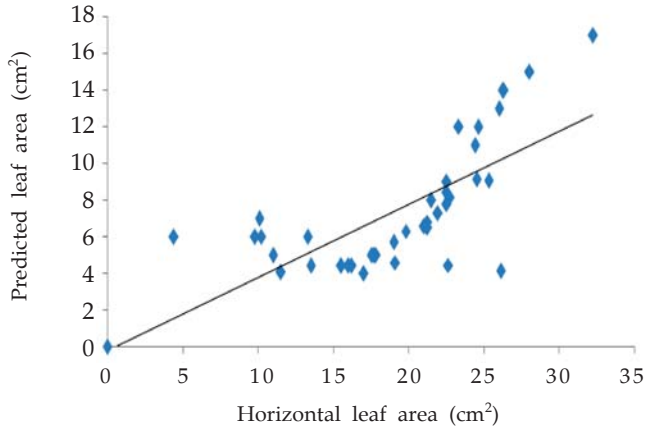

Fig. 2. Relationship between the observed area and predicted area of leaf chollang

significant relationship between variables 99% confidence level.

There was no significant difference between actual leaf area and area estimated using different models based on parameters like plant length, breadth and dry weight. The co-efficient of determination more closer to 1.0 were observed in larger t-values based on dry weight of leaf in hooker chives, indicated good suited for estimating the leaf area but the model based on dry weight of leaf being a destructive method, non-destructive estimation of leaf area based on leaf breadth having the co-efficient of determination (r^2=0.93) will be better suited for the estimation of that area of hooker chives. Similarly, leaf length based on model of non-destructive method having co-efficient of determination (r^2=0.91) are indicated good fit for estimating the leaf area of chollang. However, there was no significance between actual leaf area and area estimated by using the respective models based on the data. These findings are in conformity with the findings of various horticultural crops like onion (Glenn 1971), pungent chilli (Meitei *et al*. 2005a), Chinese chives (Meitei *et al*. 2005b), faba bean (Peksen 2007) and clary sage (Kumar and Sharma 2010) which use the co-efficient of determination more closer to 1.0 as the best suited for estimating the leaf area. Therefore, for most accurate estimation of leaf area, regression equation based on leaf breadth for hooker chives and the product of length and breadth for chollang can be used in the future as non-destructive method of leaf area estimation for studying the relationship between leaf area growth and development.

CONCLUSION

The results indicates that estimation of leaf area in hooker chives and chollang can be performed relatively quickly and with precision under field conditions using a non-destructive methology. To estimate the leaf areas of them precisely, variables that are required are sufficient number of leaves with smallest and largest leaf on each type to predict the leaf area of hooker chives and chollang.

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Current Horticulture **2**(1): 47–49, January–June 2014

Correlation analysis in different varieties of mango (*Mangifera indica*)

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ABSTRACT

The studies were undertaken to correlate various physical properties, chemical qualities and yield characters in mango (*Mangifera indica*). The length and width of fruit were positively correlated with pulp percentage, it means that edible portion of fruits increased along with size of the fruits. Negative association in length and width was observed with specific gravity, peel percentage and stone percentage. The reducing sugar was not associated significantly with other chemical qualities of fruits. Positive correlations were observed between non-reducing sugars, total sugars and T S S. Average number of fruits/tree was found positively correlated with average fruit yield/tree, but it was non-significant and negatively correlated with average weight per fruit.

Key Words: Physial properties, Yield parameters, Mango, Correlation analysis, Stone, Pulp, Chemical qualities

Mango (*Mangifera indica* L.) is the member of family Anacardiaceae. It is most important commercial fruit grown in India. There is huge variation in growth, yield and quality characters in mango cultivars (Rajput and Pandey 1997; Sharma *et al*. 2001; Shrivastava *et al*. 1987). It has great adoptability in a wide range of climatic and soil conditions. It is utilized at all stages of its development both in its immature and mature stages. It can be grown under humid and dry conditions. It requires good railfall for its growth (June-October) and rain-less dry weather from November onwards during flowering and fruit setting. Mango is grown in all the districts of Madhya Pradesh but the maximum acreage is in Rewa, Satna and Jabalpur (Shrivastava *et al*. 1987). The maximum areas under mango production is in Vindhya regions of Madhya Pradesh. A number of cultivars have been developed through seeding selection and superior cultivars have been recommended for various regions but meagre efforts have been made so for to determine the correlation between physical properties, chemical qualities and yield characters. Therefore, a study was undertaken to correlate various physical properties, chemical qualities and yield characters of mango varieties.

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MATERIALS AND METHODS

The study was undertaken at the Fruit Research Station, Kuthulia, Rewa, under Jawaharlal Nehru Krishi Viswa Vidyalaya, Jabalpur, Madhya Pradesh, during 2005. Thirty varieties of mango, viz. Khirama, Kakaria, Karela, Kelam, Sunderja, Sunderja (Kithwaria), Dilsad, Bangalora, Rumani, Krishnabhog, Himsagar, Baneshan, Bombay green, Shukul, Amrapali, Aswaniya, Dashehari, Mankurad, Safeda, Erbin, Langra, Chausa, Mallika, Fazli, Swarnarekha, Kesar, Taimuriya, Kalapahar, Mahmood Bahar and Dashehari (Chhotee) were replicated thrice in a randomized block design having a single tree per treatment per replication. The physical properties were fruit width (cm), average length (cm), specific gravity, fruit peel (%), fruit pulp (%) and fruit stone (%). Fruit size (length and width) recorded by measuring fruits with the help of measuring tape.

The fruits were weighed and volume of fruits was determined by water displacement method. Specific gravity of fruits was calculated by dividing weight of fruits with its volume. Peel, pulp and stone were separated, weighed and calculated on fresh-weight basis. The chemical qualities, reducing sugar (%), non-reducing sugar (%), total inverted sugar (%), acidity (%) and T S S were calculated. The T S S of fruit pulp was determined with the help of zeiss hand refract meter. However, quality parameters such as reducing sugar

Table 1. Correlation between physical properties of mango

Character	Fruit width (cm)	Specific gravity	Fruit peel (%)	Fruit pulp (%)	Fruit stone (%)
Average length (cm)	0.667*	-0.597*	-0.221	0.486*	-0.092*
Fruit width(cm)		-0.546*	-0.296	0.582*	-0.147*
Specific gravity			0.110	-0.705	0.134
Fruit peel (%)				-0.152*	0.364
Fruit pulp (%)					-0.329

* Significant at 5 % level of probability

Table 2. Correlation between chemical qualities of mango

Character	Non reducing sugar (%)	Total (%)	Acidity (%)	TSS (%) (^{0}Brix)
Reducing sugar (%)	-0.0779	0.039	-0.048	0.066
Non-reducing sugar (%)		0.895*	-0.516*	0.739*
Total sugar (%)			-0.668*	0.895*
Acidity (%)				-0.513*

* Significant at 5 % level of probability

(%) and non-reducing sugar (%) and acidity (%) were analyzed by using standard methods (A O A C, 1970). The yield characters, average number of fruits/tree, average yield/tree (kg) and average weight of fruit (g) were studied. The yield was recorded by weighing the fruits at the time of each picking. Fifty uniform mature fruits from each tree were used for recording various observations of fruits. The coefficients of correlation between different characters were calculated as per the method of Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The simple correlation coefficient between physical properties of mango fruits are presented in Table 1. The length and width of fruits was positively correlated with pulp percentage. It means that edible portion of fruits increased along with size of fruits. Negative association of length and width was observed with the specific gravity, peel percentage and stone percentage. It revealed that with increase in fruit size the percentage weight of non-edible portions and specific gravity decreases. Specific gravity of fruits was positively associated with peel and stone percentage and negative associated with pulp percentage. This showed that fruits with higher specific gravity had poor physical properties with higher percentage of stone and lower percentage of pulp. Pulp percentage was negatively associated with peel and stone percentage, perhaps it might be due to completion strees. Similar associations between these traits have been reported by Singh and Bajwa (1985), Prasad (1987), Attri *et al*. (1999), Singh *et al*. (2004), Bhowmick and Banik (2008) and Majumder *et al*. (2012).

Table 3. Correlation between yield parameters of mango

Character	Average number of fruits/tree	Average yield (kg/tree)	Average weigh (g/fruit)
Average yield (kg/tree)	0.998	0.811*	0.0733*
Average number of (fruits/tree)	_	-0.995*	-0.388*
Average weight per fruit (gm)		_	-0.459

* Significant at 5 % level of probability

The simple correlation coefficient studies were made between chemical qualities of fruits (Table 2). It is evident from the data that the reducing sugar were not associated significantly with other chemical qualities of fruits. Positive correlations were observed between the non-reducing sugar, total sugars and T S S. It indicated that if non-reducing sugar content is higher, the total sugar and T S S content of fruits will also be higher. Percentage of acidity was negatively correlated with other chemical qualities. These findings are in agreement with Singh *et al*. (1985) and Prasad (1987), Attri *et al*. (1999), Singh *et al*. (2004), Bhowmick and Banik (2008).

The simple correlation coefficient studies were made between yield of fruits (Table 3). Average number of fruits/tree was found positively correlated with average fruit yield/tree, but it was non-significant and negatively correlated with the average weight/fruit. Average fruit yield/tree was less associated with the average weight /fruit. This might be because of the fact that average weight/fruit is higher. The total number of fruits/tree remained less and vice versa. Similar associations between above traits have been reported by Attri *et al*. (1999), Singh *et al*. (2004), Uddin *et al*. (2007), Bhowmick and Banik (2008) and Majumder *et al*. (2012).

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Microbial resistance in arsenic rich environment : isolation and characterization of arsenic resistant bacteria from soil

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ABSTRACT

In a experiment, arsenic resistant bacteria were isolated from soil of Varanasi region in India. All the 16 species isolated from the soil showed resistance in Arsenic containing medium. The YEPG medium was used which allowed growth of bacteria. By the serial dilution plate technique, pure isolates were obtained. Then RAPD were used for characterization. Thus, the results showed that microorganisms were capable to resist arsenic rich environment. The isolated microorganism was identified as *Bacillus sonorensis* strain, which showed 99% sequence similarity to *Bacillus sonorensis*.

Key Words: Microbial resistance, Arsenic, Isolation, Bacteria, Soil, Environment

Arsenic is the 20th most abundant element in the earth's crust and is widely distributed throughout nature as a result of weathering, dissolution, fire, volcanic activity and anthropogenic input. The anthropogenic input includes the use of arsenic in pesticides, herbicides, wood preservatives, and dye stuffs as well as production of arsenic-containing wastes during smelting and mining operations. Arsenic mainly occurs in two inorganic forms, arsenate As(V) and arsenite As(III). The arsenite is 100 times more toxic than As(V). Due to the natural abundance of arsenic in the environment, representatives from various bacterial genera have developed different resistance mechanisms for arsenic compounds (Mukhopadhyay *et al*. 2002).

The bacterial resistance with regard to reduction of arsenate or oxidation of arsenite can be divided into two basic categories consisting of either detoxification reactions that confer arsenic resistance, or redox reactions that conserve the energy gained by the reactions for cell growth (Silver and Phung 2005). Several bacteria involved in arsenic transformation processes, featuring reduction, oxidation and methylation mechanisms, have been reported. Developing efficient and environment-friendly technologies to remove arsenic from soil and water systems is of great importance to many countries including India. Bioremediation of heavy or toxic metal contaminated sites have been often shown to be more efficient than chemical and physical methods, especially when stimulating indigenous microbial communities.

MATERIALS AND METHODS

Soil samples were collected from different regions of Varanasi, India. The samples were dried and made serial dilution of up to 10-fold. Arsenic resistant bacteria were isolated from soils by the serial dilution spread plate technique. Dilution were plated on to YEPG media having different concentrations of arsenic (0.25 mM-2 mM) and dilution plates were incubated at 37°C for 2-8 days to allow the bacteria to sporulate and then colonies were picked and streaked onto YEPG plates for purification. Pure colonies were transferred from these plates to slant YEPG incubated at 37°C until sporulated and stored at 4°C until used. Stock cultures were transferred every 3-4 weeks.

Genomic DNAs were isolated from As resistant bacteria and amplified using the random primers. A degree of polymorphism was obtained in each RAPD profile. The universal forward and reverse primer of 16s rRNA were used for PCR amplification. Samples were then sent to M/S GCC Biotech, Kolkata, India, for sequencing. Analysis of DNA sequences and homology searches were completed with a MEGABLAST using the BLAST algorithm for comparison of a nucleotide query sequence against a nucleotide sequence database.

RESULTS AND DISCUSSION

The pH of soils from different regions ranged from

[1, 2, 3, 4 and 5]Assistnt Professor

6.18-7.4. The isolates were identified by 16s rRNA gene sequence (~15 bp) and one was only sequenced in this study. The sequences were compared to sequences available in the GenBank using BLAST application. The DNA sample named 3SSSS had 99% sequence similar to the bacteria (*Bacillus sonorensis*). The sequence of this sample (3SSSS) in NCBI GenBank named as *Bacillus sonorensis* 3SSSS (Accession No. KF574385).

The 16s rRNA sequence has hypervariable regions, where sequence have diverged over evolutionary time. These are often flanked by strongly conserved regions. Primers were designed to bind to conserved regions and amplify variable regions. The degenerate primer contains two or more than two nucleotides used to amplify the DNA sequence of 16s rRNA gene. Our study confirms the presence of As resistant bacteria in As content in soils of Varanasi.

The soil samples from Varanasi were checked for the presence of arsenic resistant bacteria. About 16 different bacteria were found in soil samples. In the RAPD analysis, all the bacteria showed different band patterns. This highlighted the difference in their lineages and therefore the differences in their homology with other bacterial strains. The PCR for 16S rRNA amplified this region in all the samples. One out of sixteen 16S rRNA PCR products were sent for sequencing (outsourced) and the sequences were obtained. The BLAST SEARCH was performed to find the homology (Figs 1 and 2).

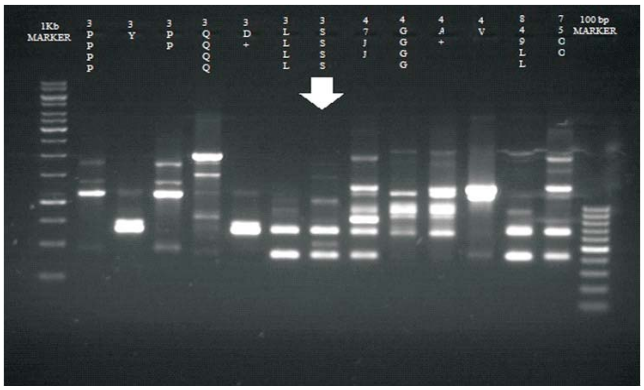

Fig 1. RAPD profile of genomic DNA of arsenic resistant bacteria

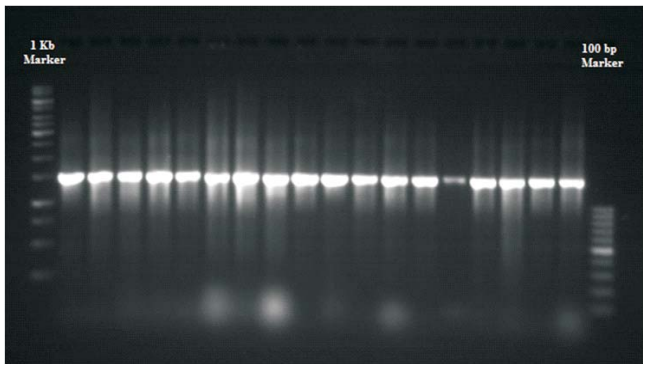

Fig 2. PCR amplification using 16S rRNA, Product size ~1500bp

Our study confirms the presence of arsenic-resistant bacteria in arsenic content in soils of Varanasi. Arsenic compounds have been widespread in environment at near toxic levels since the origin of life. In response to arsenic, microorganisms have developed mechanisms of arsenic resistance and enzymes that oxidize As(III) to As(V) or reduce As(V) to As(III), involving the formation and degradation of organoarsenicals such as methyl arsenic compounds (Mukhopadhyay *et al*. 2002).

Microorganisms have a variety of ways to cope with high levels of arsenic, ranging from reduced uptake, methylation and adsorption to dissimilatory arsenate respiration. Conventional methods for removing metals from Industrial effluents include chemical precipitation, oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery (Ahluwalia and Goyal 2007; Mouedhen *et al*. 2009).

These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1-100 mg/ℓ (Nourbakhsh *et al.* 1994). Therefore, it is important to develop an innovative, low-cost and eco-friendly technique for metal removal from soil which is carried by a large number of microorganisms that have capability to grow in the presence of high concentrations of heavy metal (Nies 1992). Anderson and Cook (2004) have reported strains of *Aeromonas, Exiguobacterium, Acinetobacter, Bacillus* and *Pseudomonas*, that can tolerate high concentrations of arsenic species (up to 100 mM arsenate or up to 20 mM arsenite).

Several bacteria (Cervantes *et al*. 1994) belonging to the genera *Acidithiobacillus, Bacillus*, *Deinococcus, Desulfitobacterium* and *Pseudomonas* (de-Vicente *et al.* 1990; Dopson *et al*. 2001; Niggemyer *et al*. 2001; Suresh *et al*. 2004) have also been reported to be resistant to arsenic. Since heavy metals are ubiquitously present in the environment, microorganisms have developed mechanisms to resist the toxic effects of these metals (White and Gadd, 1986).

The bioremediation of arsenic from contaminated sites involves reduction and oxidation of arsenic with the use of arsenic resistant microorganisms.

The successful exploitation of these bacterial strains with proper biotechnology for bioremediation of arsenic will be beneficial. Therefore, more advanced research is required for a deeper understanding about these bacterial strains to improve arsenic bioremediation process.

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Morphological variations in culture growth rate and pattern of different isolates of *Colletotrichum capsici* causing fruit rot and die back in chilli (*Capsicum annuum*) on different solid media

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ABSTRACT

Five *Colletotrichum capsici* isolates belonging to two groups: (i) four from fruit rot and (ii) one of die back were collected from different conventional chilli-growing areas (blocks) of Ghaziabad adjacent district of NCR Delhi. Their morphological cultural growth were studied on different solid media. The maximum growth of isolate Ccfrs on CMA and OMA and rapid growth on all seven solid media, more virulent strain.Cc_{FRBR} showed the least growth in all tested media .Both two strains Cc_{FRR} and Cc_{FRD} resulted in less difference in growth for all the media and the same in morphologically resemblance in culture plates and natural fruits surface in field condition.

Key Words: Morphological cultural variations, Solid media, Isolates, Fruit rot, Die back

Fruit rot and die-back caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is one of the economically important disease of Capsicum and possess a serious threat to it successful cultivation. This disease is prevalent in almost all major chilli-growing areas in India. The disease is distructive on mature and ripe fruits leading to huge losses in yield and quality of fruits. The disease was first reported by Sydow (1913) from Madras (India). Later on, it was reported by Chowdhury (1957) from Assam, Rai and Chohan (1966) in Punjab, Bansal and Grover (1969), Sujathabai (1922) in Tamil Nadu and Datar (1995) in Maharashtra; Rai and Chohaan (1966); Gupta (1988); Thind and Jhooty (1990); Palarpawar (1987); Thind and Jhooty (1990); Jeyalakshmi and Seetharaman (1999) and Khirbhal *et al*. (2004). The existance of pathogenic and morphological variants of the pathogen *(C. capsici)* for most conventional chilli-growing areas in Uttar Pradesh and morphological pattern for the region of NCR and Delhi has not been so far studied. Therefore, an attempt was made to study the vareability among isolates of *C. capsici* of new suitable media for best growth and search out the physiological races/strains suitable media for growth, samples collected from most conventional chilli-growing areas in northern Uttar Pradesh especially NCR and Delhi (India).

MATERIALS AND METHODS

The plants of chilli having symptoms of fruit rot and die-back caused by *Colletotrichum capsici* were collected from important chilli-growing districts (five blocks) of Ghaziabad, NCR and Delhi (India). The *C. capsici* isolates were isolated from chilli fruit showing typical fruit rot and die-back symptoms. Followed one by one separately, based on type of symptoms on affected chilli fruits, the sample were placed into five broad categories in two groups: (i) four from fruit rot and (ii) one from die-back. In thease categories (i) (S) the affected fruit area under lessions were on whole matured fruits of full red with scattered form of acervuli. In the second category (D), lesions were less growth on fruits with acervuli in densly aggrigated form . In third category (R), lesions were in concentric rings without bleaching and more growth covered fruit area in comparision of dense, in fourth category (BR), the lesion were bleached and acervuli appeared in concentric rings (ii) die-back (S), lesions start with back narrow side fruit infection and narrow tissue become shrinked black dry but resemble scattered acervuli in middle region of fruit and named as the isolates-Cc_{FRS},Cc_{FRD}, Cc_{FRR}, Cc_{FRBR} and Cc_{DBS}.

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All these five categories of isolates of chilli fruits were washed in running tap water and dried with tissue paper. The infected fruit tissue was cut out in a size of approximately 3 mm^2 separately with scalpel. A surface disinfection was made in a series of sodium hypochlorite (1%) (Clorox 10%)for 5 min, then dipped into sterilized water for 2 min and once again dried on sterilized filter paper. The tissues were placed on potato dextrose agar (PDA), 4 pieces per plate, at 25°C and 7.0 pH incubation for 6-10 days, then culture are ready after proved the koch's postulate for cultural growth rate of best solid media.

These isolates were separately on PDA (potato dextrose agar), CA (Czapeck's dox agar), RA (Richard's Agar), OMA (oat meal agar), CMA (corn meal agar), CFA (chilli fruit extract agar) and PMA (pectin mineral agar) of solid media. The pH of each media adjusted at 7.0 pH by using 0.1 N HCl and 0.1 N NaOH by digital pH meter. Laboratory experiment were conducting in 15 cm × 15 cm petri-dishes. The 3 mm disc of each culture of isolates *cuted* by cork borer of 10 day old culture were placed at the centre of the solid media separately isolates on all seven media each isolate has triple replication and incubate (stability chamber) at 28 + 1°C. The linear growth in each case was determined by taking average of colony diameter in two directions after 9 days of incubation.

RESULTS AND DISCUSSION

The results were tabulated for five replications were made of each isolates in each media (Table 1). Among seven solid media separately tested and observed, maximum radial growth of colony of each isolates was in OMA and CMA, followed by PDA and RA. In RA, slightly more than CFA and CA. In case of isolates the Cc_{FRS} recorded highest growth in all solid media followed by Cc_{FRR}. The isolates Cc_{FRD} and Cc_{DBS} resulted very less difference in growth. Lowest growth was resulted in Cc_{FRBR} in all the media. The best growth of all five isolates of *Colletotrichum capsic*i grew on OMA and CMA supported by a number of workers Parbery (1981); Mathur *et al*. (2000); Khirabha *et al*. (2004); Jamil and Nicholson (1989), working with *C. graminicola*. They found oat meal agar gave the best result on corn meal agar was not tested till now by any worker, third medium was tested for best growth and resulted high growth on PDA. Many workers supported the best growth in OMA medium for *C. capsici*.

Jeyalakshmi and Seetharaman (1999) and Prakasam (1983) all the tested media produced conidia singly measured 18.03-22.9µ × 3.70-3.95 µ agreed with the original descriptions given by Sydow (1913) and by Sutton (1980) is one of the main characters of identification key for the species *C. capsici* with sickle shaped in structure.The rapid growth of Cc_{FRS} in all five isolates on all solid media the highly virulent strains support the reports of Koolman (1927) who observed that highly virulent strains were exemplified and high growth development of *in vitro* and *in vivo*. Difference of five isolates, sympatologically cultural morphologically needed further confirmation through molecular based. The strains of *C. capsici* for fruit rot and die back needs more deep study for seperation these two diseases through DNA fingerprinting techniques for further description.

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Table 1. Growth of different isolates of *Colletotrichum capsici* on different solid media

	Radial growth of colony (cm*) *of C. capsici*							
Isolate	PDA	CA	RA	OMA	CMA	CFA	PMA	Mean
C_{CFRS}	8.08	7.80	7.93	9.64	9.20	7.76	6.08	8.07
C_{CFRD}	7.62	7.37	7.45	9.18	8.77	7.27	5.63	7.61
C_{CFRR}	7.85	7.55	7.63	9.39	8.97	7.47	5.69	7.79
C_{CFRBR}	6.91	7.04	6.97	8.78	8.53	6.85	5.09	7.16
C_{CDBS}	7.35	7.25	7.33	9.01	8.67	7.10	4.93	7.37
Mean	7.56	7.40	7.46	9.20	8.82	7.29	5.48	

Cc_{FRS}, *Colletotrichum capsici* fruit rot; S, scattered; D, dense; R, rings; BR, bleach rings

Cc_{DB}, *Colletotrichum capsici* die back; S, scattered

*Each value is average of 3 replicates

CD= (P=0.05) Isolates (A)=0.176 Medium (B)=0.131 A × B =0.360

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Effect of planting time on growth, flowering and corm production in gladiolus (*Gladiolus hybrida*)

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ABSTRACT

An experiment was conducted to find out the effect of different planting dates on vegetating growth, flowering and corm production in gladiolus (*Gladiolus hybrida* L.) cv. Novalux at Department of Horticulture, Allahabad Agricultural Institute, Allahabad. Maximum plant height, number of leaves/plant, length of spike, size of floret and duration of spikes in field were recorded under 30th October planting, whereas minimum days to spike initiation and days to basal floret opening were observed with 14th December planting. There were maximum number of corms/plant and weight of corm under 30th October planting, whereas 15th October planting proved beneficial in corm size and weight of cormels/plant.

Key Words: Corm, Cormels, Gladiolus, Spike, Growth, Flowering, Plantint time

Gladiolus (*Gladiolus hybrida* L.) is the most important ornamental bulbous plant grown for its elegant cut spikes and garden display. Flowering period in gladiolus can be extended by planting its corms at regular intervals of 10-15 days so as to produce an array of blooms for a longer period. By regulating the date of flowering, flower production can be spread more evenly, especially for catching off-season market in plains as well as for export market.

MATERIALS AND METHODS

A field experiment was conducted in a randomized block design with six treatments and four replications during 2003-2004. Treatments were six planting dates, viz. 15 October (T_1), 13 October (T_2), 14 November (T_3), 29 November (T_4), 14 December (T_5) and 29 December (T_6). The cultivar, Novalux, was planted at a spacing of 30 cm × 30 cm. The individual plot size was 1.2 cm × 0.9 m. A uniform dose of FYM@ 4kg/m^2 was applied to all the plots a week before planting. Each plot was surrounded by a bund to prevent the movement of water and nutrients.

One-third of urea and CAN with full doses of single superphosphate and muriate of potash was applied as basal dose before planting of corms. Remaining dose of urea and CAN was applied in two split doses, i.e. half at four-five leaf stage and the remaining at spike emergence stage. Observations on various vegetative, flowering and corm parameters were recorded. The data were analyzed statistically.

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RESULTS AND DISCUSSION

The effect of planting dates on growth, flowering and corm parameters are presented in Table 1. Among the dates of planting, 30 October planting recorded maximum plant height (91.29 cm). Minimum plant height (61.56 cm) was observed under 29 December, number of leaves/plant were highest (10.0) in 30 October planting and lowest (7.51) being in 29 December planting. Among dates of planting, average length of spike was maximum (74.16 cm) in 30 October planting and minimum (58.33 cm) in 29 December planting. Average size of floret was recorded maximum (12.17 cm) in 30 October and minimum (8.62 cm) in 29 December planting. Maximum duration of spike in field (13.2 days) was observed in 30 October planting and minimum (8.88 days) in 29 December. Minimum days of spike initiation (61.67 days) were observed under late planting done on 14 December and maximum (72.33 days) under 30 October planting. Minimum days taken to basal floret opening (73.66) were found in 14 December and maximum (82.83) in 15 October planting.

Table 1. Effect of planting dates on growth, flowering and corm field of gladiolus cv. Novalux

Planting date	Plant height (cm)	No of leaves/ plant	Days to pike initiation	Days to basal floret opening	Length of spike (cm)	Size of floret (cm)	Duration of spike in field	No. of corms / plant	Weight of corm (g)	Corm size (cm)	Weight of cormels (g)
15 October	85.75	8.61	70.33	82.83	70.25	11.33	12.13	2.50	24.49	5.28	23.99
30 October	91.29	10.00	72.33	80.83	74.16	12.17	13.20	2.75	28.28	5.2	15.08
14 November	78.43	8.32	69.00	78.17	68.58	10.66	11.28	2.38	23.15	4.49	11.07
29 November	75.00	7.75	64.83	76.91	66.66	10.41	10.81	1.91	22.28	4.34	8.83
14 December	66.75	7.58	61.67	73.66	60.33	9.62	10.04	1.74	25.63	4.02	6.48
29 December	61.50	7.51	63.50	75.57	58.33	8.62	8.88	1.23	20.56	3.87	5.07
CD (P = 0.05)	3.87	0.66	1.83	1.82	4.49	0.35	1.39	0.30	0.79	0.17	6.47

Maximum number (2.75) of corms/plant was observed in 30 October planting and minimum (1.23) in 29 December. Maximum weight of corm (28.28 g) was observed in 30 October and minimum (20.56 g) in 29 December planting. Corm size was found maximum (5.28 cm) in 15 October planting and minimum (3.87cm) in 29 December. Maximum weight (23.99 g) of cormels/ plant was found in 15 October planting and minimum (5.07 g) in 29 December. The plant height increased successively from first to second planting, i.e. 15 October-30 October and started declining as the planting was delayed beyond 30 October. This may be due to prevailing useful temperature range in days as well as nights (Dhankar *et al.* 1997). The average number of leaves may be due to optimum photoperiods, which in turn influenced the vegetative growth of plant (Dod *et al.* 1989).

The study clearly indicates that planting dates had a significant effect on length of spike. Corms planted on 30 October produced longest spikes, whereas corms planted on 29 December produced shortest spike. Variations due to planting dates has also been reported (Saini *et al.* 1988; Dod *et al.* 1989; Dhankar *et al.* 1997). The size of floret increased with delay in planting. The 30 October planting produced large-sized florets and 29 December planting produced small-sized floret. The results are in close agreement with those of Sharma *et al.* (2002). Duration of spike in field was maximum under 30 October planting, followed by early planting dates, i.e. 15 October, whereas it was minimum under late planting dates, i.e. 29 December. A significant variation in duration of spike in field has been reported by various workers (Leinfelder and Gruber (1985) and Misra and Saini (1988). Minimum days to spike initiations were observed under late planting done on 14 December and maximum days were observed under 30 October planting. Days taken to basal floret opening was found minimum in 14 December and maximum 15 October planting. These results were supported by Sindhu and Verma (2007).

Corm production differed markedly with different planting dates. Maximum number of corms/plant was observed under 30 October and minimum under 29 December planting. The results are in close conformity with the finding of Pant and Lal (1991). All planting dates significantly influenced weight of corm. Maximum weight of corm was observed under 30 October planting and minimum under 29 December. Average size of corm was maximum in 15 October planting and minimum in 29 December planting (Vitra 1981). The maximum weight of cormlets was produced under 15 October planting and minimum under 29 December planting. Similar variations were also observed by Banker and Mukhopadhyay (1980).

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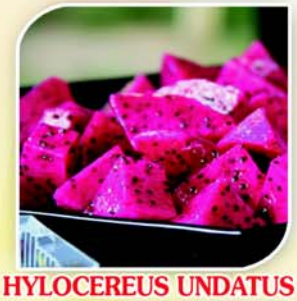

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