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Aims and Scope

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Contents

Nutritional Analysis of Indigenous Vegetable Grown in Marathwada Region of Maharashtra, India

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A B S T R A C T

The total ash and iron and calcium content of common vegetable grown seasonally in marathwada were determined by potassium dichromate titrimetric method. For this study those vegetables are selected which are commonly grown and easily available in market and used frequently in diet. The result of analysis of vegetable shows different variation in ash, calcium and iron content. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. From the above data analysis it concludes that which vegetables are having more amounts of Fe and with ash. From the results it concludes that fenugreek and amaranth contain high amount of Fe and than other vegetables. From these results in future we can design the balanced diet for better health.

Keywords: Minerals, Composition, Vegetable, Analysis of nutrient, Fe, Ca content.

INTRODUCTION

Vegetables are the edible parts of plant that are consumed wholly or in parts, raw or cooked as part of main dish or salad. A vegetable includes leaves, stems, roots, flowers, seed, fruits, bulbs, tubers and fungi (Barrett, et al., 2011). Vegetables are good sources of oil, carbohydrates, minerals and vitamins depending on the vegetable consumed Ihekoronye & Ngoddy, 1985, reported that vegetable fats and oil lower blood lipids thereby reducing the occurrence of disease associated with damage of coronary artery.

Normally, all plants are vegetables. The term vegetable applies to edible part of the plant that stores food in roots, stems, or leaves. Vegetables are green and leafy- like in appearance bearing edible stems or leaves and roots of plants (Sharma, 2004) the food value of vegetable is low owing to the large amount of water present (79–96%). The nutritive value of vegetable is increased greatly because of the presence of mineral salts and vitamins; they also serve as roughages that help in digestion (Sharma, 2004). Vegetables constitute essential diet components by contributing proteins, vitamins, iron, calcium, and other nutrients that are in short supply. Vegetables also contain both essential and toxic elements over a wide range of concentrations. Metals in vegetables may pose a direct threat to human health, plants or vegetables take up elements by absorbing them from contaminated soils and waste water used for

irrigating them as well as from deposits on different parts of the vegetables exposed to the air from polluted environment (Funtua et al, 2008).

It is important that iron is an essential mineral and a vital component of proteins involved in oxygen transport and metabolism. Iron is also an essential cofactor in the synthesis of neurotransmitters such as dopamine, or epinephrine, and serotonin. About 15% of the body's iron is stored for future needs and mobilized when dietary intake is inadequate. (Aberoumand and Deokule, 2008). Epidemiological studies suggest that regular or increased consumption of fruits and vegetables may reduce the risk of chronic diseases and these health benefits are thought to be mainly attributable to their natural antioxidant and dietary fibers content. Calcium, needed for strong bones, is found in dark green leafy vegetables, tofu made with calcium sulfate, calcium-fortified soy milk and orange juice, and many other foods commonly eaten by humans. Although lower animal protein intake may reduce calcium losses, there is currently not enough evidence to suggest that humans have lower calcium needs. Humans should eat foods that are high in calcium and/or use a calcium supplement (Tang, et al., 2007).

The work aimed at determining few nutritionally important minerals like calcium, iron vegetables widely consumed. The objective of the present work was to examine the variability in the mineral content. Plant foods have almost all of the mineral and organic nutrients established as essential for human nutrition. Traditional vegetables contain a number of organic photochemical that have been linked to the promotion of good health. Vegetables hold an important position in well-balanced diets. Green leafy vegetables are believed to occupy a modest place as a source of trace elements due to their high water content (Gibson 1994). Nutritional information is used increasingly by public agencies and agricultural industries to promote fresh product. People are looking for variety in their diets and are aware of the health benefits of fresh fruits and vegetables. They have special interest in food sources rich in antioxidant vitamins (vitamins C, A, and E), calcium (Ca), magnesium (Mg), and potassium (K) and fiber. Most of these nutrient requirements can be solved by increasing the consumption of fresh vegetables. In addition to meeting nutrient intake levels, better consumption of fruits and vegetables is associated with reduced risk of cardiovascular disease and stroke (Gillman 1995).

This study was carried out to evaluate the chemical constituents of vegetables. Proximate analysis was carried out to determine the nutrient content of these vegetables under investigation.

MATERIAL AND METHODS:

For the study the vegetable samples were collected from domestic market, and the study is carried out as follow. The vegetables selected for study are Spinach, Carrot, Garlic, Ginger, Tomato, Amaranths and Fenugreek.

A) Chemical Analysis

The major elements, comprising calcium, phosphorus, potassium and magnesium, and trace elements like iron were determined by different methods. The ash content was determined by combusting the plant materials (AOAC 1990). The ash, thus prepared, was used for estimation of Ca and K by flame photometry method (Baruah and Borah 1998).

a) Determination of Ash content:

90 g of Daucus Corota L (corrot) and 120 g of Lycopersicon Esculentum (Tomato) weighed accurately in a clean sillica dish were first heated over a low Bunsen burner flame to volatilize larger part of organic matter and them transferred to a temperature controlled muffle furnace maintained at 3000 C. The dry ashing is continued till carbon in it has ceased to glow and then the temperature was raised 4500 C and the ignition was continued for 5 to 7 hours. The ash residue so obtained was then cooled in desiccators and weighted. From the weight of ash residue the percentage of ash was calculated by the formula given below:

$$\text{Percentage of Ash vegetable sample (\%)} = \frac{\text{Weight of Dry ash residue (g)}}{\text{Weight of fruit Sample (g)}} \times 100$$

b) Determination of Iron Content:

100 g of each vegetables were accurately weighted in a titrate dish and after dry ashing 0.5 to 1 g of ash residues were transferred with watch glass the ash residues were moistened 40 to 50 ml of dilute Hydrochloric acid (dil HCl) was added. The contents to heat over hot water bath for 30 min subsequently removing the cover and contents to dehydrate titrate (sio2) followed by addition of another 10 ml dil HCl and distilled water to dissolve soluble salts. The undissolved titrate (sio2) transferred to the 100ml standard flask. The residual titrate particles adhering to breaker were transferred completely to filter funnel. The titrate residue in the filter funnel was washed for two to three times with hot distilled water and all the wash used filtrate collected in the standard flask were diluted up to mark of 100ml using distilled water.

A 25ml of aliquot of above ash solution in the standard flask was titrating out into a clean dry conical flask. The solution was then treated with 1-2 ml of concentrated nitric acid oxidiz Fe+1 to Fe +3 and made 5 to 6 l with respect to hydrochloric acid by adding 10 to 50 ml concentrated hydrochloric acid. The content were heated strongly to 70 to 90 c i.e. nearly to boiling and to the hot solution the concentrated tin (II) chloride solution was added drop by drop constant stirring titrate yellow colour of the solution just disappeared ascertaining complete reduction of Fe+3 to Fe+2. Then 1 to 2 drop of tin (II) chloride was added in excess. The content was cooled rapidly under tap water to ^{0}20 C. and excess of tin (II) chloride added was destroyed mercuric chloride solution immediately with vigorous mixing.

Then 200 ml of 2.5 % sulphuric acid followed by 5 ml of 85 % phosphoric acid and few drop of 0.2% aqueous solution of sodium Diphenyl amine sulphonate indicator were added. The Fe+2 solutions were then titrated titrate 0.01 N potassium dichromate solution till color assumes bluish green indicating the nearness of the end point. The titration was continued further by adding drop by drop of titrate maintain an interval of few seconds between each titration until addition of one drop caused the formation of intense purple coloration which permanent indicating the end point of titration. Absorbance readings were measured for each including the standard solutions at 579 nm using UV- Visible spectrophotometer, (Vogel 1983).

c) Determination of calcium content:

100 g of each vegetable were accurately weighed in a silica dish and after dry ashing were 0.5 to 1gm of ash residue transferred to a clean beaker. After covering beaker with watch glass the ash residue moistened with little distilled water. The beaker was covered with watch glass and added 40 to 50 ml of dil HCl with the help of pipette and content were further heated for 30 minutes at low flame after adding 10ml of hydrochloric acid to dehydrate silica and then little water was added to dissolve soluble salts filtered in hot condition through whatmann filter paper No. 41 and then washing were collecting along with filtrate into 100ml standard flask. The silica residue was rejected and filtrate along with washing collected in the 100 ml standard flask was then diluted up to mark of 100ml using distilled water and solution made homogeneous.

A 25ml aliquot of above solution of each fruit was transferred to a small beaker heated to boil and 5 to 1 gm solid Ammonium chloride was added and heating few minutes. Then to hot solution 1:1 ammonia was added slowly with constant stirring so as to precipitate Fe+3 and Al+3 ions as hydroxide. The hydroxide was digested 5 to 10 minute over a low flame and filtered the calcium oxalate monohydrate filtered through a Whatmann filter paper 41 precipitate was then washed with cold distilled water for 3 to 4 times cold distilled water till free chloride ions.

The precipitate along with the filter paper was transferred to beaker and then calcium oxalate mono hydrate precipitate (CaC2 O4, H2O) was dissolved in minimum volume of dil H2 SO4 and transferred to clean dry conical flask. The solution was neutralized by adding 8 M Solution of potassium hydroxide (Tested by PH paper) Around 25 ml of distilled water 4ml of 8 M potassium hydroxide solution were added and allowed to stand for 3 to 5 minutes with occasional stirring them 30mg of hydroxyl ammonium chloride added and 50 mg of slide Patton and readers indicator was then and calcium ion in the solution was titrated against 0.01N EDTA till the color changes from red to blue. The volume of EDTA consumed was recorded. Both the standard solutions and samples solutions were aspirated into a flame photometer one after theother and absorbance readings were obtained and the concentrations of calcium in the samples were obtained by extrapolation from standard calibration plot, (Vogel, 1983).

RESULTS AND DISCUSSION

The results of the percentage ash content and mineral content of the various samples analyzed are presented in Table 1. The results of ash, Ca2+ and Fe2+ concentrations in the samples analyzed is presented in Fig 1, found that fenugreek is the highest source of ca followed by amaranths carrot, spinach, ginger etc. Amaranths is having highest percentage iron followed by fenugreek, spinach, carrot, ginger, garlic and tomato. Calcium is not known to be toxic because it is an essential element for bone development and maintenance and also for reduction of cholesterol level in humans (Ibrahim, 2008). The variation in the nutritional content in this may be due to a number of factors that influence the concentration of mineral elements on and within plants, these factors include climate , atmospheric deposition , nature of soil on which the plant is grown , irrigation with waste water, these observations were made by Anyawu et al,(2004) and Khairah et al, (2004).

Table 1. Nutritional content of selected vegetables.

Sr no.	Name of common vegetables (Local)	Botanical name	Actual weight of sample taken for Analysis (g)	Weight of ash obtained (g)	Ash content (mg/100mg)	Iron content (mg/100mg)	Calcium content (mg/100mg)
1	Spinach	*Spinacia olerecea*	100	1.1	1.1	7.884	45.76
2	Carrot	*Daucus carota*	90	0.925	1.027	1.841	78.93
3	Fenugreek	*Trigonella foenum*	80	0.925	1.156	14.504	384.8
4	Tomato	*Lycopersicon esculentum*	120	0.61	0.508	0.455	40.34
5	Amaranth	*Amaranthus tricolourl.*	75	1.81	2.41	25.407	377.32
6	Ginger	*Zingiber officinales*	100	1.21	1.21	2.439	19.36
7	Garlic	*Allium sativum*	120	1.125	0.937	1.26	27
8	Cucumber	*Cucumis sativus*	150	0.51	0.34	2.37	16.02

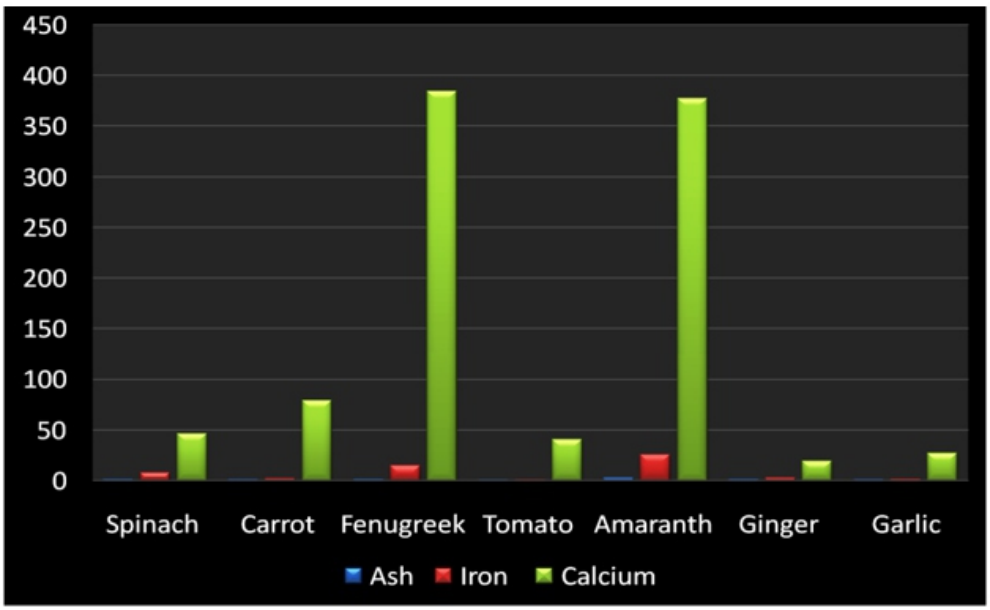

Fig.1 Comparison between nutritional content of mentioned vegetables

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Effect of Cyanobacterial Biofertilizer on Soil Nutrients and Mulberry Leaf Quality and Its Impact on Silkworm Crops

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ABSTRACT

: The present field experiment was conducted to study the effect of cyanobacterial biofertilizer (CBB) along with and without chemical (NPK) fertilizer on soil nutrients and leaf quality traits of mulberry. Eleven CBB treated mulberry plots (Tl to Tl2) and one control plot (T12) were maintained and data on soil pH, soil nutrients and leaf quality traits were recorded in each of these experimental plots for every crop. The soil nutrients were increased whereas; the soil pH was decreased in different CBB treated plots as compared to control plot. Among different CBB treatments, T9 (Av + Nm + Sm (120kg/ha/crop)+ 50% NPK) exhibited superior performance with regard to leaf quality traits and was found on par with control 772 (100% NPK). Bioassay study also revealed no significant difference in silkworm growth and cocoon characters between treatments T9 and TI2. Overall results showed that T9 was very effective in terms of improvement in soil nutrients and leaf quality traits in mulberry. Further, it is also clear from the study that 50% reduction in the dose of chemical fertilizer can be compensated by the addition of higher dose of CBB. Therefore, CBB can be recommended to the sericulture farmers with an intension to improve the soil fertility condition and leaf quality traits besides saving 50% cost of chemical fertilizers.

Keywords: Cyanobacteria, mulberry leaf quality, leaf yield, soil nutrients and cocoon characters

INTRODUCTION

Cyanobacteria are a diverse group of prokaryotes, widely distributed in fresh water, marine and terrestrial environments.They are free living photosynthetic bacteria which exist singly or in colonies and some of them are in filament forms (Curtis, 1992; Jacobson et al., 1993). The most common filament forms of Cyanobacteria are Anabaena and Nostoc species as these Cyanobacteria possess normal vegetative cells and specialized heterocyst cells which are involved in photosynthesis and nitrogen fixation respectively (MeckerraseJal., 1990; Jacobson etal., 1993; Ehiraetal., 2003; Huang et al., 2005). Cyanobacteria are capable of forming symbiotic association with many plants and fungi (Perter, 1990; Bergman et al., 1992; Meeks and Elahi, 2002). In association with plants, Cyanobacteria fix atmospheric nitrogen and release organic matter, which is absorbed by plants (Gantar et al., 1995; Rai et al., 2000). Cyanobacteria were also used as biofertilizers in several crop plants (Venkataraman, 1986; Subba Rao, 1988; Gangawar and Thangavelu, 1992; Bose and Majumdar, 1999; Dasappa, 2000).

For the last two decades, biofertilizers like Azotobacter, Azospirillum, Vesicular arbuscular mycorrhiza (VAM) and Cyanobacteria etc., were used extensively to minimize the frequent use of chemical fertilizers, to improve the soil status and plant growth (Venkataraman, 1986; Subba Rao, 1988; Gangawar and Thangavelu, 1992; Das et al., 1995; Bose and Majumdar, 1999). These biofertilizers were reported to be ecofriendly, economical andbeneTicial in mulberry (Das et al., 1995; Reddy et al., 2000 and Dasappa, 2000). However, very few reports are available exclusively on the use of cyanobacteria as biofertilizers for mulberry (Bose and Majumdar, 1999 and Dasappa 2000). Also the earlier scientists have not emphasized much on the influence of CBB on soil nutrients and leaf quality traits of mulberry. Therefore, the present investigation was carried out to assess the efficiency of cyanobacterial biofertilizers along with or without chemical fertilizer on soil status, leaf yield and leaf quality traits of mulberry and their influence on silkworm rearing parameters under tropical conditions of Mysore.

MATERIALS AND METHODS

Field experiment was conducted in the farmer's field of Budiguppe village, Kanakapura Tq Ramanagara district, Karnataka, India, to analyze the effect of cyanobacterial biofertilizer (CBB) along with and without chemical fertilizer (NPK) on leaf yield and leaf quality traits ofmulberry. Initially, the soil nutrient status viz., soil pH, organic carbon (OC), total nitrogen (N), available phosphorus (P), available potassium (K) and cyanobacteria spp. were recorded before conducting the experiment. For the study, three different'doses of CBB (i) 90 kg/ha/crop, (ii) 105 kg/ha/crop and (iii) 120 kg/ha/crop were selected based on the earlier experimental works (Bose and Majumdar, 1999; Dasappa, 2000). Likewise three different doses of chemical fertilizers i.e., (i) 25% NPK: 15:6:6 kg/ha/crop, (ii) 50% NPK: 30:12:12 kg/ha/crop (iii) 100% NPK: 60:24:24 kg/ha/crop (recommended dose of CSR&TI, Mysore) were used. Three species of cyanobacteria viz.,Anabaena variabilis (Av), Nostoc muscorum (Nm) and Scytonema millei (Sm) were used as CBB in the study. The pure cultures of these cyanobacteria in slants were obtained from Indian Agricultural Research Institute (IARI), New Delhi; they were sub-cultured and multiplied in the laboratory of the Department of Botany, University of Mysore, India. The large volume of multiplied cyanobacteria were lyophilized and made into powder to avoid secondary contamination. These lyophilized cyanobacteria were mixed individually with farmyard manure (FYM) and made as cyanobacterial biofertilizer (CBB). The field was kept wet for few days immediately after the application of CBB. Further, irrigation schedule was properly maintained in all the experimental plots. The application of CBB to the experimental plots was done by following the standard procedure (Venkataraman, 1972; Anonymous, 1978).

For the study, single and combination treatments of CBB and chemical fertilizers were computed such as:

Tl: Av (90kg/ha/crop) + 50% NPK;
T2: Nm (90kg/ha/crop) + 50% NPK;
T3: Sm (90kg/ha/crop) + 50% NPK;
T4: Av + Nm (90kg/ha/crop) + 50% NPK;
T5: Av + Sm (90kg/ha/crop) + 50% NPK;
T6: Nm + Sm (90kg/ha/crop) + 50% NPK;
T7: Av + Nm + Sm (90kg/ha/crop) + 50% NPK;
T8: Av + Nm + Sm (105kg/ha/crop) + 50% NPK;
T9: Av + Nm + Sm(120kg/ha/crop + 50%NPK);
T10: Av + Nm + Sm (120kg/ha/crop + 25%NPK);
T11: Av + Nm + Sm (120kg/ha/crop) without NPK and
T12: 100% NPK (Control).

Two years old (0.20 ha.) M5 mulberry garden was selected and from this garden a total of 2400 plants grown with a spacing of 60cm x 60cm were taken for the study. For each treatment, 4 replications with 50 plants /replication were maintained in randomized block design (RED). Initially the plants were pruned 30 cm above the ground level and CBB was applied 15 days after pruning. Cultural operation and application of FYM was done as per the norms of CSR&TI, Mysore (Krishnaswami, 1978). After 70 days of pruning, 20 plants were randomly selected replication- wise, from each of twelve treatments and leaf yield data were recorded for ten crops. The average of ten crops data on leaf yield was calculated treatment wise and converted to ton/ha/year.

From different CBB treated and control plots mature leaves (12th - 14th leaf from top) were collected replication / treatment wise and different leaf quality traits were estimated. Leaf Moisture Content (LMC%)and Moisture Retention Capacity (MRC%) after 6 hrs from harvest were estimated by following the method of Vijayan el al. (1996). Total chlorophyll content (mg/g of fresh weight) was estimated by adopting the procedure of Hiscox and Israelstam (1979). Nitrate reductase (NR) activity was also estimated in mature leaves (Scot and Neyra, 1979). Leaf samples collected were dried and used in triplicate for analysis of total protein content following Lowry et al. (1951), total amino acid content estimated by adopting ninhidrin method using leucine as standard (Spies, 1955). Nitrogen content was estimated by micro-kjeldahl method (Jackson, 1973). Sugar and starch contents (soluble carbohydrate) were estimated by the method of Dubios et al. (1956) and Me. Cready et al. (1960) respectively. Further, to confirm the efficiency of CBB on leaf quality traits, a bioassay study was conducted with silkworm race PM * NB4D2 for three different seasons. Leaves from CBB treated (T9) and control (T12) plots were utilized for feeding the silkworm until spinning. At three different seasons, rearing data viz., larval

duration, weight of 10 mature larvae (g), Effective Rate of Rearing (ERR) by number and by weight, Single cocoon weight, Single shell weight and Shell ratio (%) were recorded separately, later average of three seasons data was calculated and analyzed statistically (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The influence of different treatments of CBB with and without chemical fertilizers on soil nutrient parameters and on leaf quality traits was assessed and summarized in Table I a; i II. The soil pH in control (T12) plot was 8.2, but it was decreased to 7.3 to 8.0 in different CBB (T1 - T11) treated plots. It was reported that soil pH decreased in the cyanobacteria treated plot (Dasappa 2000). Cyanobacteria can change the soil pH towards neutrality (Hashem, 2001). Likewise, in control plot, the soil nutrients i.e., Organic carbon (OC), Nitrogen (N), Phosphorus (P) and Potassium (K) were 0.36%, 0.033%, 7.35kg/ha and 174.20kg/ha respectively. Whereas, in different CBB treated plots, the nutrients were found increased and the range of increase in the nutrients are, OC: 0.38 - 0.52%; N: 0.034 - 0.054%; P: 7.46- 11.46kg/ha and K: 151.83 - 194.37kg/ha. The increase in soil nutrients was certainly due to the influence of CBB. Cyanobacteria produce extra cellular polymers of diverse chemical composition, especially exopolysaccharides that enhance microbial growth and as consequence, improve soil structure and exoenzyme activity (Caire et al., 1997). In the present study, soil nutrients were high in T9; this was mainly due to the higher dose of CBB with 50% NPK fertilizers. In contrast, all the nutrients were low in Tl 1 plot, probably due to total avoidance of chemical fertilizer in the treatment.lt was observed that application of higher dose of CBB alone (Tl 1) proved less effective in improving the soil nutrients. Cyanobacteria not only maintain the soil pH but also help to enhance soil nutrients, retain soil moisture and liberate different hormones that stimulate plant growth (Hashem, 2001; Meeks and Elahi, 2002).

LEAF QUALITY TRAITS:

The growth of silkworm (Bombyx mori L.) and production of quality cocoons depends on timely feeding of good quality mulberry leaves to silkworm. Therefore, it is most imperative to analyze different leaf quality traits such as leaf moisture content (LMC), moisture retention capacity (MRC), total chlorophyll, nitrogen, protein, amino acids and carbohydrate contents. The leaf quality traits estimated in different CBB treated (Tl -Til) and control (T12) plots were represented in Table II. LMC and MRC are two important factors in maintaining the nutrition level in mulberry leaves, which in turn improve its palatability for silkworm. These two traits are influenced by genetic and environmental factors (Vijayan et al., 1997) and are also positively related with an increased growth of silkworm larvae (Paul etal., 1992). In the present study, LMC and MRC were high in T9 (73.5% and 75.0% respectively) and were on par with control T12 (73.6^% and 75.2%).

Table I. Effect of different CBB Treatments on soil nutrients

Treatment	Soil Status (pH)	Organic Carbon (%)	Total Nitrogen (%)	Available Phosphorus (Kg/ha)	Available Potassium(Kg/ha)
T1 : Av (90kg/ha/crop) + 50% NPK	7.6	0.42	0.042	8.43	178.41
T2: Nm (90kg/ha/crop) + 50% NPK	7.9	0.44	0.044	8.44	180.56
T3 : Sm (90kg/ha/crop) + 50% NPK	7.8	0.43	0.042	8.32	176.77
T4 : AV + Nm (90kg/ha/crop) + 50% NPK	7.5	0.44	0.044	8.22	175.53
T5 : AV + Sm (90kg/ha/crop) + 50% NPK	8.1	0.43	0.043	8.66	176.33
T6 : Nm + Sm (90kg/ha/crop) + 50% NPK	7.4	0.45	0.056	8.72	177.22
T7 : Av + Nm + Sm (90kg/ha/crop) + 50% NPK	7.3	0.56	0.047	8.6	176.55
T8 : Av + Nm + Sm (105kg/ha/crop) + 50% NPK	7.6	0.66	0.055	9.24	189.66
T9 : Av + Nm + Sm (120kg/ha/crop) + 50% NPK	7.5	0.54	0.045	11.22	195.32
T10 : AV + Nm + Sm (120kg/ha/crop) + 50% NPK	7.8	0.66	0.044	9.45	178.56
T11 : Av + Nm + Sm (120kg/ha/crop)	7.4	0.48	0.043	7.66	152.56
T12 : 100% NPK (Control / Temoin)	8.1	0.35	0.056	7.45	173.23
CD at 5%	**NS**	**0.08**	0.047	**2.05**	**9.12**

Table II. Effect of different CBB Treatments on leaf quality traits of mulberry

Treatment	LMC (%)	MRC (%) (after 6 hrs of harvest)	Total Chlorophyll (mg/g.f.wt0	Protein (%)	Nitrogen (%)	Sugar (%)	Starch (%)	Total amino acid (mg/g/dry wt.)	NR activity (µg NO hr/g)	Leaf yield (ton/ha/yr)
T1 : Av (90kg/ha/crop) + 50% NPK	70.4	72.66	2.43	18.56	3.12	9.74	10.66	202.5	7.6	28.67
T2: Nm (90kg/ha/crop) + 50% NPK	71.4	72.66	2.43	17.56	3.12	8.74	10.66	202.5	7.6	30.67
T3 : Sm (90kg/ha/crop) + 50% NPK	72.4	71.66	2.43	19.56	3.12	10.74	10.66	205.5	7.6	30.67
T4 : AV + Nm (90kg/ha/crop) + 50% NPK	70.4	72.66	2.43	18.56	3.12	10.74	10.66	202.5	7.6	28.67
T5 : AV + Sm (90kg/ha/crop) + 50% NPK	72.4	72.66	2.43	17.56	3.12	8.74	10.66	202.5	7.6	30.67
T6 : Nm + Sm (90kg/ha/crop) + 50% NPK	72.4	71.66	2.43	19.56	3.12	10.74	10.66	205.5	7.6	31.67
T7 : Av + Nm + Sm (90kg/ha/crop) + 50% NPK	72.4	72.66	2.43	17.56	3.12	8.74	10.66	202.5	7.6	30.67
T8 : Av + Nm + Sm (105kg/ha/crop) + 50% NPK	72.4	71.66	2.43	19.56	3.12	10.74	10.66	205.5	7.6	31.67
T9 : Av + Nm + Sm (120kg/ha/crop) + 50% NPK	73.5	75.2	2.87	20.55	4.12	12.66	11.22	234.6	9.5	35.6
T10 : AV + Nm + Sm(120kg/ha/crop) + 50% NPK	71.4	72.66	2.43	17.56	3.12	8.74	10.66	202.5	7.6	30.67
T11 : Av + Nm + Sm(120kg/ha/crop)	72.4	71.66	2.43	19.56	3.12	10.74	10.66	205.5	7.6	30.67
T12 : 100% NPK (Control / Temoin)	70.4	72.66	2.43	18.56	3.12	10.74	10.66	202.5	7.6	28.67
CD at 5%	NS	0.31	0.12	0.06	0.07	0.05	0.2	2.3	1.21	0.6

Table III. Rearing performance and Cocoon characters of silkworm in T9 and T12.

Treatment	Larval duration (days)	Wt. of 10 mature larvae (g)	ERR		Single Cocoon Wt.	Single Shell Wt.	Shell Ratio (%)
			By No.	By Wt.			
T9 : Av + Nm + Sm (120 kg/ha/crop) + 50% NPK	28.07 ± 0.98	40.97 ± 0.62	8197 ± 32.6	13.95 ± 1.96	1.687 ± 0.09	0.298 ± 0.09	17.66 ± 1.36
T12 : 100% NPK (control / temoin)	28.12 ± 1.16	40.55 ± 1.54	8015 ± 54.6	13.32 ± 2.32	1.625 ± 0.16	0.284 ± 0.08	17.48 ± 2.56
t value /	NS	NS	2.14*	2.47	NS	NS	NS

Av : Anabaena variabiliso; Nm : Nostoc muscorumo, Sm : Scytonema milleio; NPK : Chemical fertilizers / Engrais chimiquet, Kg / ha / crop / kg / ha /, ERR : Effective rate of rearing.

The leaf quality is usually determined based on higher LMC and MRC (Bongale and Chaluvachari, 1995; Sujathamma and Dandin, 2000). Further in T9, the reduced dose (50%) of chemical fertilizer did not affect the LMC and MRC. This may be due to the influence of CBB, which had mediated the moisture availability in the soil rhizosphere, thereby maintaining normal growth, water uptake and other metabolism in plants.

Chlorophyll content is an important quality trait, which determines the photosynthetic efficiency of a variety. Total chlorophyll content was high in T9 (2.87mg/gf.wt) and found on par with TI2 (2.87mg/gf.wt.), while it was low in Tl 1 (2.23mg/gf.wt.). The increased chlorophyll content in T9 was mainly due to higher dose of CBB along with 50% NPK fertilizers. It was reported that higher chlorophyll content in leaves indicates the photosynthetic efficiency in mulberry (Sujathamma and Dandin, 2000). Low chlorophyll content in Tl 1 clearly indicates that higher dose of CBB alone may not be able to supply the required nutrients for the normal growth and physiological activities of plants. Next to leaf moisture content, total nitrogen and amino acid contents are the two important traits, which determine the leaf quality in mulberry (Machii, 1989). These two traits were high in T9 (4.12% and 234.6mg/g.dry wt.) and T12 (2.43% and 202.5mg/g.dry wt.), while these traits were low in Tl 1 (2.84% and 176.9mg/g dry wt.). It was reported that varieties possessing higher nitrogen and amino acid contents in leaves are nutritively superior and positively related to growth and development of silkworm (Machii and Katagiri, 1991; Suryanarayana and Shivashankar Murthy, 2002). In case of T9 though the recommended dose of NPK fertilizers was reduced to half, the total nitrogen and amino acids contents were not affected, this may be due to attributed influence of CBB on the availability of mineral elements in the soil and their translocation in to the plant system, which later in turn enhance the synthesis of amino acids and other hormones etc., in the treatments.

Among the eleven CBB treatments, total protein content was found comparatively high in T9 (20.55%) and was on par with control Tl2 (20.56%). According to Horie (1980), dietary protein level should be

20-25%, which is required for optimal growth of silkworm larvae. The lower or reduced use of chemical (NPK) fertilizer in T9 did not affect the available quantity of protein content in the leaves; this may be due to influence of CBB which had compensated the reduced dosage of chemical fertilizers in the treatments. In mulberry leaves, carbohydrates are available in plenty and it was reported to be the main source of energy for silkworm (Hiratsuka, 1917 and Horie, 1978). The quantity of carbohydrate is determined based on the quantity of total sugar and starch available in leaves (Bose and Bindroo, 2001).Sugar and starch content were high in treatment T9 (12.66% and 11.22%) and was almost equal to that of control (12.44% and 11.48%). The results revealed that CBB had pronounced influence on biosynthesis of carbohydrates in the leaves. The low dose of NPK fertilizers in T9 was compensated efficiently by the addition of higher dose of CBB in the treatment.

Nitrate reductase (NR) activity is one of the most important regulatory enzymes in nitrogen metabolism, which catalyses the reduction of nitrate to nitrite, a rate limiting step in the utilization of nitrates in plants (Deckard et al, 1973). NR activity was the highest in T9 (9.5ugNo2/hr/gf.wt.) and lowest in Tl (7.6 ugNo2/hr/gf.wt). NR activity in T9 was almost equal to that of control T12 (10.4ugNo2/hr/gf.wt). In the present study, 50% curtailment in the recommended dose of chemical fertilizer did not affect the NR activity in T9 (Table I). This may be probably due to higher dose of CBB in T9, which had compensated the lower (50%) use of chemical fertilizer. Similarly, among the CBB treatments, leaf yield was found high in T9 (35.6 ton/ha/yr), while it was low in Tl 1 (28.67 tons/ha/yr). In the present study, T9 besides showing high NR activity also showed high leaf protein, nitrogen and amino acids. It was reported that NR activity is significantly correlated with leaf protein, leaf yield and other yield attributing characters, thus it can be used as an additional parameter for identifying the superior mulberry genotypes (Rao et al., 2000).

From the study, it is understood that application of reduced (50%) dose of chemical fertilizers can be compensated by applying the required dose of CBB and due to the reason, leaf quality traits in T9 was found on par with T12 (control). In cyanobacteria, nitrogen / carbon fixation mechanism is a unique biochemical process wherein the N fixation is mediated by the enzyme nitrogenase, which is well protected in the heterocyst cell (Hashem, 2001 and Ehira el al., 2003). It is a thick walled, large transparent cell, occurs at regular intervals amidst series of vegetative cells of Cyanobacteria. In symbiosis with plants, heterocyst cell formation in cyanobacteria increases 3 to 10 fold (Meeks and F.lahi, 2002) and this leads to the enhancement of nitrogen fixation process. The fixed nitrogen is first reduced to ammonium in the heterocyst, later it is converted to glutamate and then passes as amino acids to adjacent vegetative cells (Thomase 1977). In return fixed carbon, probably sucrose flows from vegetative cells to heterocyst cells (Wolk, 1968; Schillinig and Ehrensperger, 1985). Cyanobacteria during their intercellular biochemical processes secrete several nutrients, hormones and vitamins etc., which are absorbed by the plants.

SILKWORM REARING PERFORMANCE:

The leaves from CBB treated (T9) and control (T12) plots were collected and used for bioassay study to analyze the influence of CBB on silkworm growth and cocoon characters. The average of three seasons rearing data recorded on silkworm growth and cocoon characters are summarized in Table III. In general, significant differences were not observed in rearing parameters between T9 and T12. However, a marginal improvement on silkworm growth and cocoon characters was recorded in T9 as compared to control (Table III). This clearly indicates the efficiency of CBB, which had compensated the lower use of fertilizers by not only fixing the atmospheric N, but also synthesizing required nutrients, vitamins, amino acids, hormones etc., which helps to improve the growth, metabolism and physiological activity of the host plants, with the result leaf quality might have been improved and thus feeding on such quality leaves maintained the normal growth and cocoon characters of silkworm.

Overall study reveals that mixture of three combination species of CBB treatment was found more effective than combination of two or single species CBB treatment. The highest dose of CBB (120kg/ha/crop) with 50% NPK fertilizers in T9 was found effective and showed superior performance with regard to leaf yield, leaf quality and cocoon parameters. In case of T10, the similar highest dose of CBB (120kg/ha/crop) with 25% NPK fertilizers exhibited moderate performance. Finally, the highest dose of CBB (120kg/ha/crop) without NPK fertilizers in Tl 1 showed the least performance with respect to leaf yield and leaf quality traits probably due to total avoidance of chemical fertilizers in the treatment. In case of T8, CBB dose was slightly reduced (105kg/ha/crop) but chemical fertilizer dose was increased when compared to T10 and due to the reason T8 showed better performance than T10. It is clear from the study that higher dose of CBB along with NPK fertilizers to an extent of 50% is essential for maintaining the normal growth, yield and metabolism in plants. The present study confirms the earlier reports that mixture of three species of CBB combination proved to be the most effective in terms of maintaining the soil pH and improvement in soil environment, leaf yield, leaf quality and also in economizing the chemical fertilizer application by 50 % (Dasappa, 2000). Cyanobacteria cheap source of N do not cause pollution, improve soil fertility condition, water-holding capacity and maintain biodiversity of soil (Hashem, 2001)." Therefore, CBB can be recommended to the sericulture farmers as cost effective and eco-friendly approach to sustain the productivity of both mulberry as well as cocoons. However the major constraint lies in culturing/ production of CBB in large quantities in the laboratory and supply to the farmers.

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Study on Some Medicinal Plants Used By The Tribal and Rural People of Chitrakoot, Satna District, Madhya Pradesh, India

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A B S T R A C T

Chitrakoot is also well known for its beautiful hill ranges, historical caves, perennial streams and varied flora and fauna. In the present study, totally 84 species of plants belonging to 39 used as an ethno medicine on different diseases (pyretics, skin, diabetes, ulcer, gastrointestinal, diarrhoea and dysentery) by the tribal and rural peoples of study area. This paper deals with an information's about such plants with respect to their local name, botanical name, parts used and name of diseases on which they are practiced.

Keywords: Medicinal plants, Chitrakoot, diseases

INTRODUCTION

Medicinal plants play an important role in human life to combat diseases since time immemorial. Plants provide us food, clothes and other necessities and amenities for comfortable and safe living. Herbal drugs are comparatively safer and modern drugs can produce serious side effects. In India, the sacred and Vedas dating back between 3500 B.C. and 800 B.C. give many references of medicinal plants.

The study of ethnobotany showed that plants used by aboriginal people for their different purpose (Harshberger, 1996). Ethnobotany is the interrelationship between the primitive men and plants (Jones, 1941). A good number of plant species are being used by tribal and rural people for the treatment of diarrhoea and dysentery (Sikarwar et al., 2008).

WHO (1985) recommended the search for beneficial use of medicinal plants for the treatment of diabetes mellitus. More than 1200 plants are used around the world in the control of diabetes mellitus. Present time, hundreds of millions of people, in developing countries, derive a major part of their subsistence needs and income from collected medicinal plants and their products (Walter, 2001).

The present study was performed with the aim of producing an inventory of the herbal plants used by tribal and rural peoples of chitrakoot for treating pyretics, skin, ulcer, gastrointestinal, diabetes, diarrhoea and dysentery diseases.

MATERIALS AND METHODS

Chitrakoot is situated in the northern region of satna district of M.P. and surrounded on North, Northwest and Northeast by Karwi (Chitrakoot) district of U.P. and west by Panna district of M.P. It lies between 800 52' to 80 73'N latitude, covering an area of 1,584 sq km. Several tribal communities like Kol, Gond, Mawasi, etc. reside in Chitrakoot forest area of Majhgawan block of Satna District, Madhya Pradesh.

An ethnobotanical survey in different remote areas of chitrakoot District, Satna Madhya Pradesh was made from 2013-2014. Data are based on personal contact and observation and interview with local traditional healers and villagers of different localities of the study area. The plant identified by published literature.

RESULT AND DISCUSSION

Medicinal plant study was carried out in the chitrakoot region (M.P.) with several traditional healers and local tribal people. The different plants species were known to be effectivelyused for treating pyretics, skin , ulcer, gastrointestinal, diabetes, diarrhoea and dysentery diseases by the tribal and rural peoples of chitrakoot.

Use for Antipyretics

The extract prepared from the Azadirachta indica (leaves), Ocimum sanctum (leaves), Emblica officinalis (Fruits), Vitex negundo (roots, flower, fruits and bark) etc. reported to have antipyretic activity. The Table-1 recorded that name of plants used as Antipyretics activity.

Totally 17 species of plants belonging to 14 families were known to reported to have antipyretic activity (fig.1).

Table -1. Medicinal plants used for antipyretics activity.

sr. No.	Common name	Botanical name	Part used	Family	Uses
1	Neem	*Azadirachtaindica*	Leaves	Meliaceae	Antipyretic
2	Bhindi	*Abelmoschus esculentus*	Seed	Malvaceae	Antipyretic
3	Australian fever tree	*Eucalyptus globules*	Dried leaves; Gum; Oil	Myrtaceae	Antipyretic
4	Tulsi	*Ocimum sanctum*	Leaves	Labiatae	Antipyretic; Antitussive
5	Satavari	*Asparagus adscendens*	Tuberous Roots	Liliaceae	Antipyretic; Demulscent; Nutritve Tonic
6	Lahusan	*Allium sativum*	Bulb; oil	Liliaceae	Antipyretic; Antiseptic
7	Brahmi	*Centella asiatica*	Whole Plant	Umbelliferae	Antipyretic; Blood purifier

8	Dhaniya	*Coriandrum sativum*	Leaves;Seeds	Umbelliferae	Antipyretic; Carminative
9	Amla	*Emblica officinalis*	Fruits	Euphorbiaceae	Antipyretic
10	Biiter gourd	*Momordica charantia*	Fruit; Leaves; Seeds	Cucurbitaceae	Antipyretic;
11	Palwal	*Trichosanthes dioica*	Fruits	Cucurbitaceae	Antipyretic;
12	Bahera	*Terminalia belerica*	Fruit	Combretaceae	Antipyretic;
13	Imli	*Tamarindus indica*	Fruits	Caesalpiniaceae	Antipyretic
14	Ganja	*Cannibis sativa*	Leaves; Dried Flourerscence	Cannabaceae	Antipyretic; Analgesic
15	Nirgandi	*Vitex negundo*	Roots; Flower Fruits; Bark	Verbenaceae	Antipyretic; Astringent
16	Wild mint	*Lantana involucrate*	Whole Herb	Verbenaceae	Antipyretic
17	Bambo	*Bambusavulgaris*	Shoot; Seeds; Roots; Leaves	Graminae	Antipytretic; Diuret ic

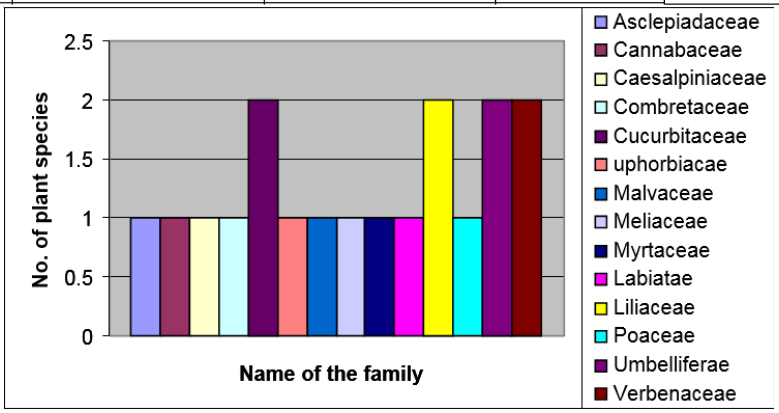

Fig. 1. plants family (species) used as Antipyretics activity.

Use for the treatment of gastrointestinal diseases (anti ulcer activity , diarrhoea and dysentery)

Gastrointestinal disorders include the condition caused by eating indigestible, excessive or irregular foods, imbalanced and spicy diet, adulteration in food and contamination of drinking water, resulting the symptoms like abdominal pain, acidity, constipation, dyspepsia, indigestion, flatulence,etc. Diarrhoea, dysentery, colic and colitis also occur due to digestive complaints. Peptic ulcer dieses encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder. The leaves of Lantana camara used to treat gastrointestinal diseases (Mishra and Singh, 2009: Mishra, 2014). The Parthenium hysterophorus has many ethenomedicinal properties (Mishra and Singh, 2009).

Totally 30 medicinal plants species such as Ficus benghalensis, Abutilon indicum, Syzygium cumin, Acacia leucophloea, Asparagus racemosus etc belonging to 26 families have been a valuable source of therapeutic agents to treat various disorders including Antiulcer diseases etc (Table-2, Fig-2).

Table- 2 Medicinal plants used for the treatment of gastrointestinal diseases.

S.No	Common name	Botanical name	Part used	Family	Uses
1	Neem	*Azadirachta indica*	dried bark extract	Meliaceae	Gastrointestinal dieses, Antiulcer,
2	Bargad	*Ficusbenghalensis*	Bark, buds	Moraceae	Diarrhoea
3	Kanghi	*Abutilon indicum*	leaves	Malvaceae	Diarrhoea
4	Reunjha	*Acacia*	Bark	Mimosaceae	Diarrhoea
5	Jamun	*Syzygium cumin*	Leaves	Myrtaceae	Dysentery, Diarrhoea

6	Tulsi	*Ocimum sanctum*	All parts	Labiatae	Antiulcer, Antibacterial,
7	Satavari	*Asparagus racemosus*	Extract of fresh root	Liliaceae	Anti -diarrhoeal, Antibacterial, Antiulcer
8	Jangali piyaz	*Urginea indica*	Bulb	Liliaceae	Diarrhoea
9	Indian Sarsaparilla	*Hemidesmus indicus*	Extract	Asclepiadaceae	Antidiarrhoeal, mucoprotective, Antiulcer
10	Kutaja	*Holarrhena pubescens*	Leaves	Apocynaceae	Diarrhoea
11	Achar	*Buchanania lanzan*	Gum	Anacardiaceae	Diarrhoea
12	Aamla	*Emblica officinalis*	Fruit Extract	Euphorbiacae	Antiulcer
13	Punarnaba	*Boerhavia diffusa*	root	Nyctaginaceae	Gastrointestinal dieses
14	Marorphalin	*Helicteres isora*	Fruit	Sterculiaceae	Gastrointestinal dieses
15	Brahmi	*Bacopa monniera*	Fresh Juice	Scrophulariaceae	Antiulcer
16	Kamraj	*Selaginella bryopteris*	Whole plant	Selaginellaceae	Dysentery, Diarrhoea
17	Bel	*Aegle marmelos*	Fruit	Rutaceae	Dysentery
18	Ghumaiya	*Argemone maxicana*	Root	Papaveraceae	Dysentery
19	Doob	*Cynodon dactylon*	Whole plant	Poaceae	Dysentery, Diarrhoea
20	Arjun	*Terminalia arjuna*	leaves	Combretaceae	Diarrhoea
21	Dhawa	*Anogeissus latifolia*	Bark	Combretaceae	Diarrhoea
22	Papeeta	*Carica papaya*	Seeds	Caricaceae	Anti-helmintic, antiamebic, Antiulce
23	Kachnar	*Bauhinia variegate*	leaves	Caesalpiniaceae	Diarrhoea
24	Sal	*Shorea robusta*	Stem bark, seed	Dipterocarpaceae	Dysentery, Diarrhoea
25	Bijahra	*Pterocarpus marsupium*	Gum	Fabaceae	Dysentery, Diarrhoea
26	Dhak, Palas	*Butea monosperma*	Stem bark	Fabaceae	Dysentery, Diarrhoea
27	Amaltas	*Cassia fistula*	Fruit pulp	Fabaceae	Gastrointestinal dieses
28	Chilla	*Casearia elliptica*	Root	Flacourtiaceae	Dysentery
29	Carrot grass	*Parthenium hysterophorus*	Root	Composite	Dysentery
30	Gandheriya	*Lantana camara*	Leaves	Verbenace	Gastrointestinal dieses

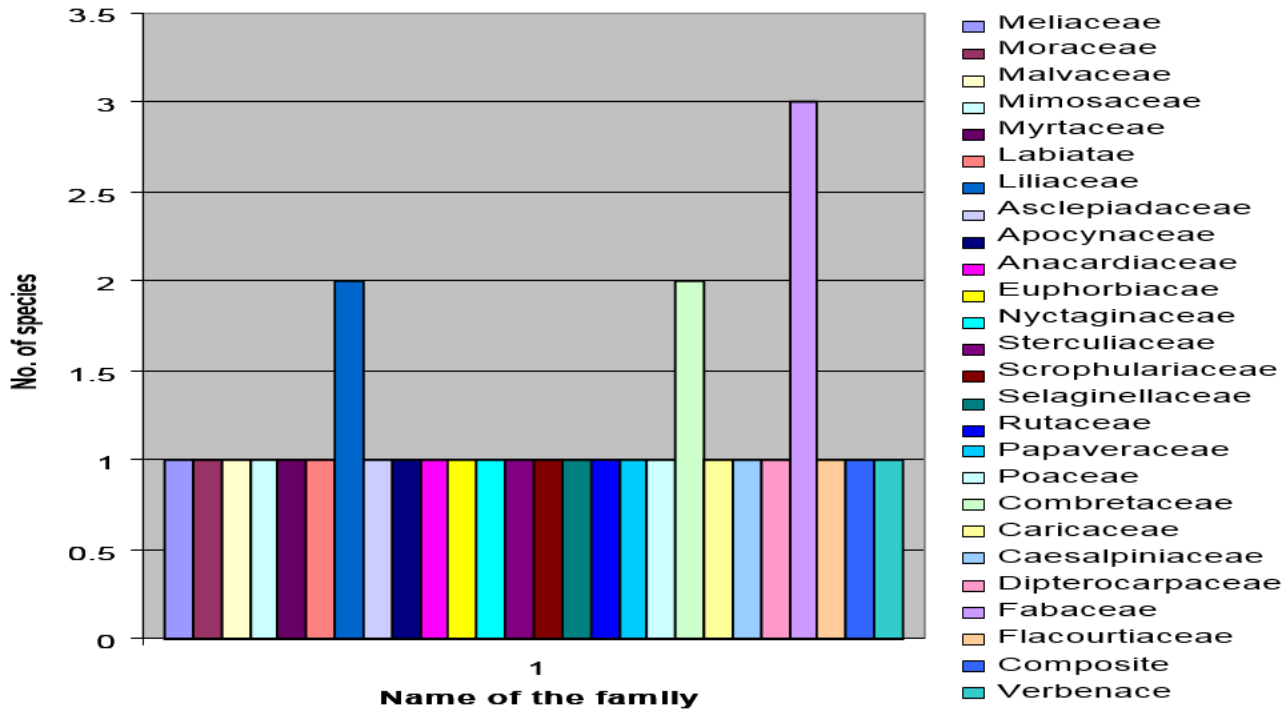

Fig. 2. plants family (species) used as Anti gastrointestinal diseases activity Use for Antidiabetic activity

Diabetes mellitus is the most common disorder in human beings and is caused by inherited or acquired deficiency in production of insulin by the pancreas, which results in an increased concentration of sugar in blood.Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug.

Totally 22 species of plants belonging to 17 families were known to reported to have antipyretic activity (Table- 3, fig.3).

Table-3 Medicinal plants used for antidiabetic activity.

S. No.	Common name	Botanical name	Part used	Family	Uses
1	Holy Basil	*Ocimum sanctum*	leaf extract	Lamiaceae	Antidiabetic
2	Onion	*Allium cepa*	bulb	Liliaceae	Antidiabetic
3	Satavari	*Asparagusracemosus*	Extract of fresh root	Liliaceae	Antidiabetic
4	Rice	*Oriza sativum*	Root	Poaceae	Antidiabetic
5	Ginger	*Zingiber officinale*	rhizome	Zingiberaceae	Antidiabetic
6	Gudmar	*Gymnema sylvestrae*	Leaves	Asclepiadaceae	Antidiabetic
7	Mango	*Mangifera indica*	leaf extract	Anacardiacea	Antidiabetic
8	Aloe	*Aloe vera*	Leaf pulp extract	Aloaceae	Antidiabetic
9	Wrightia tinctoria	*Safed korea*	seed	Apocynaceae	Antidiabetic
10	Garlic	*Allium sativum*	bulb	Alliaceae	Antidiabetic

11	Aamla	*Emblica officinalis*	Fruit Extract	Euphorbiacae	Antidiabetic
12	Binbi	*Coccinia grandis*	Root	Cucurbitaceae	Antidiabetic
13	Karela	*Momordica charantia*	Fruit	Cucurbitaceae	Antidiabetic
14	Guduchi	*Tinospora cordifolia*	Leaf, Stem & Whole plant	Menispermaceae	Antidiabetic
15	Jamun	*Syzygium cumin*	Leaves	Myrtaceae	Antidiabetic
16	Neem	*Azadirachta indica*	plant extract	Meliaceae	Antidiabetic
17	Bargad	*Ficus benghalensis*	Bark, buds	Moraceae	Antidiabetic
18	Dhaniya	*Coriandrum sativum*	Leaves;Seeds	Umbelliferae	Antidiabetic
19	Aparajita	*Clitoria ternatea*	Flower	Fabaceae	Antidiabetic
20	Indian Gum	*Acacia arabica*	seeds	Fabaceae	Antidiabetic
21	Amaltas	*Cassia fistula*	Fruit	Fabaceae	Antidiabetic
22	Mahua	*Madhuca longifolia*	Flower and bark	Sapotaceae	Antidiabetic

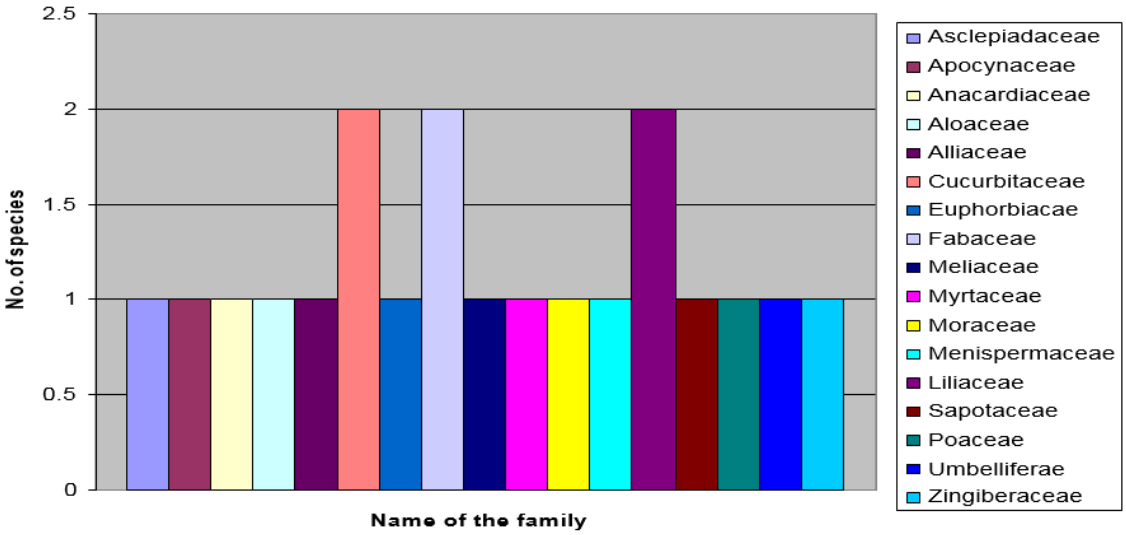

Fig. 3. plants family (species) used as antidiabetic activity

Uses as skin diseases activity

Eczema is mostly formed in children and infants and is seldom seen among adults. Also called dermatitis, eczema is a non contagious disease. Totally 17 species of plants belonging to 13 families were known to reported to have antipyretic activity (Table- 4, fig.4).

Table-4 Medicinal plants used for the treatment of skin diseases.

S.No.	Common name	Botanical name	Part used	Family	Uses
1	Latjira	*Achyranthes aspera*	Plant	Amaranthaceae	Eczema
2	Cholai	*Amaranthus spinosus*	Root, leaves	Amaranthaceae	Eczema
3	Aloe	*Aloe vera*	Leaf pulp extract	Aloaceae	Eczema

4	Anantamul	*Hemidesmus indicus*	root	Asclepiadaceae	Eczema
5	Mandara	*Calotropis gigantea*	Latex	Asclepiadaceae	Eczema
6	Ashok	*Polyalthia longifolia*	Flower	Annonaceae	Eczema
7	Bakus	*Justicia ocumbens*	Leaves	Acanthaceae	Eczema
8	Amaltas	*Cassia fistula*	Bark	Fabaceae	Eczema
9	Dhak, Palas	*Butea monosperma*	Flower	Fabaceae	Eczema, wormring,
10	Khaksi	*Crotalaria medicaginea*	Flower	Fabaceae	Eczema
11	Karanja	*Pongamia pinnata*	Seed	*Fabaceae*	Eczema
12	Tulsi	*Ocimum basilicum*	Leaves	Labiatae	Eczema
13	Neem	*Azadirachta indica*	leaves	Meliaceae	Eczema
14	Guduchi	*Tinospora cordifolia*	Stem	Menispermaceae	Eczema
15	Kaya	*Lxora coccinia*	Flower	Rubiaceae	Eczema
17	Doob	*Cynodon dactylon*	Whole plant	Poaceae	Skin disorder

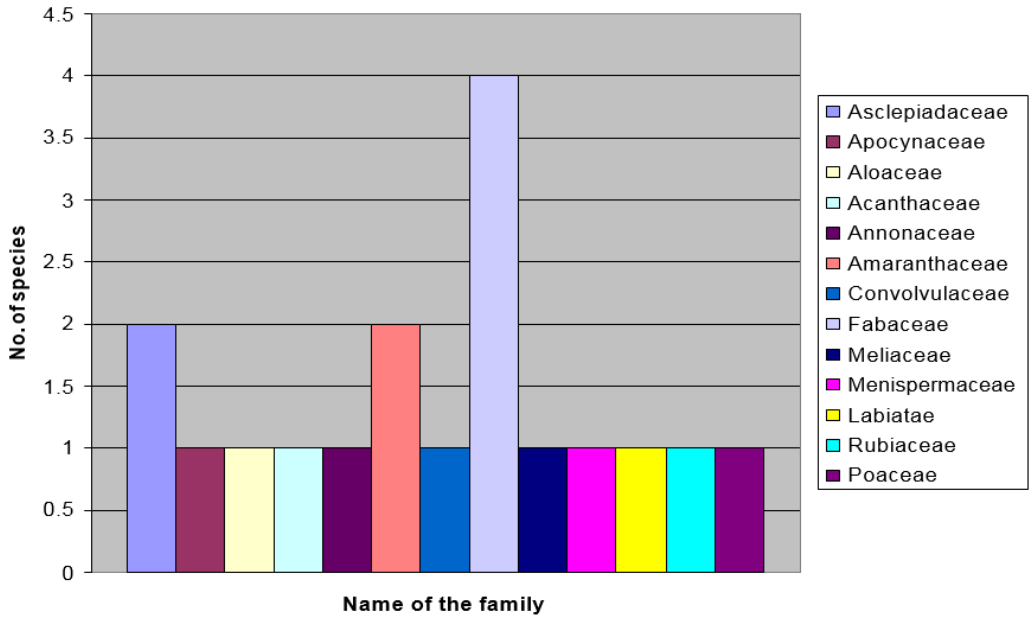

Fig. 4 plants family (species) used as antieczematic activity

CONCLUSION

Medicinal plants contain so many chemical compounds which are the major source of therapeutic agents to cure human diseases . The plants species were known to be effectively used for treating

pyretics (17 species, 14 family), gastrointestinal (30 species, 26 family), skin (17 species, 13 family), diabetes (22 species, 17 family) diseases by the tribal and rural peoples of chitrakoot region(M.P.)The traditional knowledge on the properties of plants and the medicinal plants uses a vital role against various diseases. Various medicinal plants and plants extracts uses to fever, antiulcer, antipyretic, anti diabetic and anti skin activity.

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Effect of Integrated Use of Farm Yard Manure (FYM) and Chemical Fertilizers (NPK) on Productivity of Bread Wheat Under Arid Conditions

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A B S T R A C T

The effect of integrated use of chemical fertilizers (NPK) and farm yard manure (FYM) on grain yield, straw yield, grain protein content and net profit of wheat was carried out in the Experimental Farm of the Faculty of Agriculture, South Valley University at Qena during two seasons on a sandy soil. The recommended NPK and FYM were applied alone and in various combinations among them. A randomized complete block design, with three replications, was used in this study. Treatments significantly affected grain and straw yields, as well as grain protein content. The highest values of previous traits were obtained from treatment T4 (50% chemical NPK + 6 tons FYM per ha). Also, this treatment gave the maximum return and net profit per ha compared with the other treatments. Therefore, integrated plant nutrient supply system could help in meeting the goals of balanced fertilization and reduce environmental pollution.

Keywords: FYM, grain yield, straw yield, grain protein content, profitability.

1. INTRODUCTION

Wheat is one of the most important cereal crops in the world and it has the widest distribution among cereal crops. The crop is primarily grown for its grain, which is consumed as human food. Wheat is also the most important cereal crop of Egypt and accounts for about 40.2 % of the total cereal production with acreage of 1.26 million hectares. Bread wheat (Triticum aestivum, L.) account for about 92.14 % of the wheat cultivated area in Egypt [1]. Increasing grain yield of wheat is an important national goal to face the continuous increasing food needs of Egyptian population.

Sustainable agricultural productivity might be achieved through a wise use of integrated nutrient management. It enhanced plant growth, water, and soil and land management [2]. The use of organic soil amendments has been associated with desirable soil properties including higher plant available water holding capacity and cation exchange capacity and lower bulk density, and can foster beneficial microorganisms [3], [4]. Organic fertilization was found to be favorable for enhancing growth and productivity of wheat [2], [5]-[7]. Application of Farm yard Manure helps to increase the DMP, yield and nutrient uptake by wheat [8]. Also, the application of organic fertilizer increased grain protein

content [9], [10]. The combination of mineral fertilizers, with organic manures, helped in increasing the grain yield of wheat and implied a saving of 50% cost, compared to a system with only mineral fertilization [11]. The present work was carried out to study the effect of integrated use of chemical and organic fertilizer on productivity and profitability of wheat at newly reclaimed sandy soils as well as reduce environmental pollution.

2. MATERIALS AND METHODS

2.1 Experimental site description

The investigation was carried out at the experimental farm of the Faculty of Agriculture, South Valley University at Qena Governorate, Egypt, during two seasons (2010-11 and 2011- 12). The farm is located at an altitude of 79 m above mean sea level and is intersected by 26°10′ N latitude and 32°43′ E longitude. The soil of the experimental site is sandy-loam throughout its profile (74.4 % sand, 15.8 % silt and 9.8 % clay), with a pH value of 7.77, 2.62 EC (dSm-1), 0.42% organic matter content, 0.34% total N, 7.98, 186 ppm available P and K, respectively.

2.2 Experimental treatments and design

The experiment was carried out in a randomized complete block design with three replications. Experimental unit measured 3.0 m in width and 4 m in length. The treatments details are presented in Table 1.

Table 1: Details of various treatments

Treatment	Detail
T1	Recommended NPK (238: 78: 100 kg ha^{-1})
T2	12 tons FYM (Farm yard manure)
T3	25% NPK + 9 tons FYM
T4	50% NPK + 6 tons FYM
T5	75% NPK + 3 tons FYM
T6	Control (without NPK or FYM)

2.3 Cultural practices

Bread wheat (Giza 168 cv.) was sown on the 15th of November in both seasons. Whole of phosphorus and potassium were applied basally before sowing in all treatments. Nitrogen fertilizer was applied in three equal doses; the first, during soil preparation, and the second and third after 21 and 63 days from sowing, respectively. The other cultural practices were carried out as recommended for the crop.

2.4 Measured traits

At harvest time, grain and straw yields were estimated at plot basis. Grain protein content on dry matter basis was determined by the Kjeldahl method according to AOAC [12]. For economic evaluation, a variable and fixed costs (land preparation, irrigation, harvesting, land rent, etc.), as well as total return,

included price of grain and straw yields were estimated from tables of Agricultural Statistics, Economic Affairs Sector (EAS), Ministry of Agriculture and Land Reclamation, Egypt.

2.5 Statistical analysis

The data were analyzed by analysis of variance using MSTAT-C statistical software. Treatment means were compared using Duncan's multiple tests [13]. Probability levels lower than 0.05 or 0.01 were held to be significant. Since data followed the homogeneity test, pooling was done over the seasons and mean data are given.

3. RESULTS AND DISCUSSION

3.1 Grain and straw yields

From the pooled data of grain (4.475 tons ha-1) and straw (7.212 tons ha-1) yields per ha, it is inferred that application of NPK fertilizers in integrated treatment T4 (50% NPK + 6 tons FYM) was found to be statistically and numerically superior to other treatments (Table 2). The minimum and significantly lower grain (2.450 tons ha-1) and straw (4.186 tons ha-1) yields per ha were recorded in control (without NPK or FYM) than other treatments. Grain and straw yields under organic treatment alone (T2) were significantly higher than control (T6) and lower than chemical (T1) and integrated treatments (T3, T4, T5). Such increase in grain and straw yields, due to application of T4, might be due to the role of organic fertilizer in enhancing soil biological activity, which improved nutrient mobilization from organic and chemical sources. These results are in harmony with the findings of Zahoor [2], Regar [5] and Nawab [7]. Also, Kiani [11] found that combination of mineral fertilizers with organic manures helped in increasing the grain yield of wheat and implied a saving of 50% cost, compared to a system with only mineral fertilization.

Table 2: Effect of integrated use of organic and chemical fertilizers on grain yield, straw yield and grain protein of wheat (data over two seasons)

Fertilization treatments	Grain yield (ton ha-1)	Straw yield (ton ha-1)	Grain protein content (%)
T_1- Recommended NPK	4.475 b	7.212 b	11.91 a
T2- 8 tons FYM (Farm yard manure)	3.850 c	6.425 c	11.65 a
T_3- 25% NPK + 6 tons FYM	3.975 c	6.685 c	11.83 a
T_4- 50% NPK + 4 tons FYM	4.750 a	7.510 a	11.98 a
T_5- 75% NPK + 2 tons FYM	4.350 b	7.150 b	11.95 a
T_6- Control (without NPK or FYM)	2.450 e	4.180 e	11.01 b

The same letters within columns means not significant differences at 5% level.

3.2 Grain protein content (%)

The results in Table 2 indicated grain protein content under organic treatment alone (T2), chemical fertilizer (T1), and integrated treatments (T3, T4, T5) were significantly higher than control (T6). The minimum and significantly lower grain protein content (11.01 %) was recorded in control (without NPK or FYM) than other treatments. The results obtained by El- Bagoury [9] and Yakout [10] agreed with these results and they concluded that grain protein contents responded to organic matter application.

3.3 Economic evaluation

The results in Table 3 indicated that maximum return and net profit per ha of 17273 and 8198 L.E., respectively, were obtained from treatment T4 (50% NPK + 6 tons FYM). The minimum return and net profit per ha of 9161 and 2259 L.E., respectively were recorded in control (without NPK or FYM) than other treatments. Also, the highest value of return-cost ratio (1.90) was obtained by the application of T4, while, the lowest (1.33) was obtained from T6 (control). The highest return and net profit values observed in the T4 treatment can be attributed to the increases in grain and straw yields produced per unit area under this treatment (Table 2). These results are in agreement with those reported by Shah and Ahmad [14] who found that integrated use of urea and FYM at 75:25 or 50:50 ratios (N basis) had produced maximum yields and was, then, recommended for profitable wheat grain yield.

Table 3: Some economics of wheat productivity per ha at various treatments (data over two seasons)

Treatments	Total costs (L.E ha^{-1})	Return (L.E* ha-1)			Net profit (L.E ha-1)	Return-cost ratio
		Grain	Straw	Total		
T_1- Recommended NPK	9106	10472	5914	16386	7280	1.8
T_2- 8 tons FYM (Farm yard manure)	9048	9009	5268	14277	5229	1.58
T_3- 25% NPK + 6 tons FYM	9060	9302	5482	14784	5724	1.63
T_4- 50% NPK + 4 tons FYM	9075	11115	6158	17273	8198	1.9
T_5- 75% NPK + 2 tons FYM	9090	10179	5863	16042	6952	1.76
T_6- Control (without NPK or FYM)	6902	5733	3428	9161	2259	1.33

*1 L.E. (One Egyptian pound) = $ 0.143

4. CONCLUSION

Generally, it cane be concluded that application of 50% of chemical NPK + 6 tons FYM per ha on wheat gave the highest values of grain and straw yields as well as grain protein content. Also, this treatment gave the maximum return and net profit per ha. Thus, 50 % of chemical NPK was replaced by FYM.

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An Investigation of Biodiversity of Endophytic Fungi Associated with Some Medicinal Plants

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ABSTRACT

The present investigations of Endophytic fungi residing in medicinal plants have not been systematically characterized. In this study, we isolates from 5 medicinal plant species using traditional morphological methods. The colonization rate, isolation rate, and relative frequency of these endophytes were investigated. The relationship between the composition of endophytic fungi and the chemical constituents of host plants was also explored for the first time. The results showed that endophytic fungi from these medicinal plants exhibited high biodiversity, host-recurrence, tissue-specificity, and spatial heterogeneity. Taxa of Colletotrichum spp, Aspergillus spp, Bispora spp., Geotrichum spp., Trichoderma spp. Cladosporium spp. Aspergillus spp. Gliocladium spp., Trichoderma spp. Fusarium spp. Trichosporoniodes spp Trichoderma spp and mycelia sterilia were the dominant fungal endophytes. Some Aliphatic, Aromatic, Amines, Aldehyde, Ester, Ketone and Phenol compounds were found to more likely coexist with certain endophytic fungi in the same plants. Our systematic investigation reveals that traditional medicinal plants are a rich and reliable source of novel endophytic fungi. This study was the first step towards understanding host-endophyte relationships based on the plant chemistry.

Keywords: Biodiversity, endophytic fungi, host-endophyte relationships, host-preference, medicinal plants, plant chemistry,

INTRODUCTION

Endophytic fungi frequently demonstrate single host-specificity at the plant species level but this specificity could be influenced by environmental conditions (Cohen, 2004). Some researchers use 'partial heterogeneity' or 'geographic variation' to indicate the endophytic fungal segregation impacted by environmental differences (Yahr et al., 2006). Endophytes are also able to colonize multiple host species of the same plant family within the same habitat, and the distribution of some endophytes can be similar in closely related plant species. For example, most endophytic fungi belonging to Balansieae and originally isolated from the grass family Poaceae were also detected in its closely related families Cyperaceae and Jun-caceae (Marks et al., 1991). On the other hand, differences in endophytic fungal assemblages have been found in different tissues of the same plant species, or even in different tissues of a single plant, which is a reflection of tissue specificity (Collado et al., 2001; Frohlich et al., 2001; Ganley and Newcombe, 2006). Endophytic fungi represent an important and quantifiable component of

fungal biodiversity, and are known to affect plant community diversity and structure (Sanders, 2004; Gonthier et al., 2006; Krings et al, 2007). To date, only about 80,000-100,000 fungal species have been described (Hawksworth and Rossman, 1987; Kirk et al,, 2001), out of a conservative estimate of 1.5 million (Hawksworth, 1991). Recent studies of endophytic fungi from tropical and temperate forests support the high estimates of species diversity (e.g., Kumar and Hyde, 2004; Santamaria and Bayman, 2005; Santamaria and Diez, 2005; Sanchez Marquez et al, 2007). These estimates do not include several additional sources of fungal diversity (Ganley and Newcomb , 2006).

Endophytic fungi are a group of fungi that colonize living, internal tissues of plants without causing any immediate, overt negative effects (Hirsch and Braun, 1992). Many recent studies have revealed the ubiquity of these fungi, with an estimate of at least 1 million species of endophytic fungi residing in plants (Dreyfuss and Chapela, 1994) and even lichens (Li et al, 2007). A variety of relationships exist between fungal endophytes and their host plants, ranging from mutualistic or symbiotic to antagonistic or slightly pathogenic (Schulz and Boyle, 2005; Arnold, 2007). Because of what appears to be their contribution to the host plant, the endophytes may produce a plethora of substances of potential use to modern medicine, agriculture, and industry, such as novel antibiotics, antimycotics, immuno-suppressants, and anticancer compounds (Strobel and Daisy, 2003; Mitchell et al., 2008). There is great potential of finding new drugs from endophytes for treating new diseases in humans and animals (Kumar et al., 2005). In addition, the studies of endophytic fungi and their relationships with host plants will shed light on the ecology and evolution of both the endophytes and their hosts: the evolution of endophyte-plant symbioses; the ecological factors that influence the direction and strength of the endophyte-host plant interaction (Saikkonen et al, 1998, 2004).

The relationships of endophytes with single or multiple plant hosts can be described in terms of host-specificity, host-recurrence, host selectivity, or host-preference (Zhou and Hyde, 2001; Cohen, 2006). Host-specificity is the relationship in which a fungus is restricted to a single host or a group of related species, but does not occur in other unrelated plants in the same habitat (Holliday, 1998). The frequent or predominant occurrence of an endophytic fungus on a particular host or a range of plant hosts is often defined as host- recurrence, but the fungus can also occur infrequently on other host plants in the same habitat (Zhou and Hyde, 2001). A single endophytic fungal species may form relationships with two related plant species but demonstrate a preference for one particular host, and this phenomenon is categorized as host-selectivity (Cohen, 2004, 2006). The term 'host-pre- ference', however, is more frequently used by mycologists to indicate a common occurrence or uniqueness of the occurrence of a fungus on a particular host, and the term is also used to indicate the differences in fungal community compositions and isolation frequencies from different host plants (Suryanarayanan and Kumaresan,

2000; Bettucci et al., 2004). The differences in endophyte assemblages from different hosts might be related to the chemical differences of the hosts (Paulus et al., 2006).

Since natural products are likely adapted to a specific function in nature, the search for novel secondary metabolites should concentrate on organisms that inhabit novel biotopes (Schulz et al., 2002)., Schulz et al. (2002) isolated about 6500 endophytic fungi from herbaceous plants and trees over a course of 12 years, screened them for biologically active compounds, and found a correlation between biological activity and biotope, e.g. a higher proportion of the fungal endophytes, in contrast to the soil isolates, inhibited at least one of the test organisms for anti-algal and herbicidal activities. Medicinal plants have been recognized as a repository of fungal endophytes with novel metabolites of pharmaceutical importance (Strobel et al, 2004; Wiyakrutta et al., 2004; Kumar et al., 2005; Tejesvi et al.,2007). The various natural products produced by endophytic fungi possess unique structures and great bioactivities, representing a huge reservoir which offers an enormous potential for exploitation for medicinal, agricultural and industrial uses (Tan and Zou, 2001; Zhang et al., 2006).

The investigation study was to investigate qualitatively and quantitatively the biodiversity of endophytic fungi from 5 medicinal plant species occurring in Dhanvantri Vana, a unit of Government Ayurvedic College, Bangalore. Although there are limitations in classifying endophytes using the traditional morphological methodology, at present there are no workable alternatives (Duong et al., 2006; Hyde and Soytong, 2007) since molecular phylogenetic identification is still not applicable to all fungal taxa on a large scale.

MATERIALS AND METHODS

Plant material

Five medicinal plant species such as Caesalpinia sappan, Alternanthera sessil, Sapindus laurifolius, Basala alba and Acalypha indica were collected The root hairs were collected from the five different plant. The sample was collected from Dhanvantri Vana, a unit of Government Ayurvedic College, Bangalore. The samples were collected from June 2013 to September 2014. The plants with no visible symptoms of disease were carefully selected after physical examination. The samples were kept in sterile containers and processed within few hours after sampling.

Sample collection:

The root hairs were collected from the five different plants. The sample was collected from Dhanvantri Vana, a unit of Government Ayurvedic College, Bangalore. The samples were collected from June 2013 to September 2014. The plants with no visible symptoms of disease were carefully selected after physical examination. The samples were kept in sterile containers and processed within few hours after sampling.

Root hair processing

The root hairs of selected 5 medicinal plants were collected and cut into 1 cm long pieces. It was disinfected with 75% alcohol for 1 minute followed by immersion in 1-5% sodium hypo chloride for 3-10 minutes. They were again immersed in 75% alcohol for 30 seconds. The root bits were then rinsed in sterile distilled water and blot dried on sterile blotting paper.The root bits were placed on Potato Dextrose Agar (PDA) supplemented with Streptomycin. The plates were sealed with parafilm and incubated at 28ºC for 4-6 weeks.

Microscopic features:

The morphology of different endophytic fungi was studied by staining with methylene blue of the isolates from Potato Dextrose Agar (PDA) after 4-6 week incubation at 28ºC.

Organic Analysis of Fungal broth:

The crude extract of broth was studied for the presence of antibacterial compounds like phenols, aldehydes, esters, etc. the tests were performed under laboratory conditions for organic analysis. Organic compounds may be aliphatic of aromatic. They may be saturated or unsaturated. Depending upon the functional group they contain, they show different solubility and give characteristic reactions. Solubility tests were performed with dilute HCl, 1% NaOH and concentrated sulfuric acid.

Isolation of endophytic fungi

A total of 5 samples of plant roots were cut into small pieces (10 mm in length). Surface sterilization and isolation of endophytic fungi followed a modified procedure as described by Schulz et al. (1993), and the details of the procedure were also given in our previous study (Huang et al., 2007b). Antibiotics penicillin G and streptomycin (Sigma, St. Louis, MO, USA) were added to the cultures to suppress the growth of bacteria. The pure endophytic fungal strains were photographed and preserved in the Biotechnological laboratory Indian Academy, Bangalore

RESULTS AND DISCUSSION

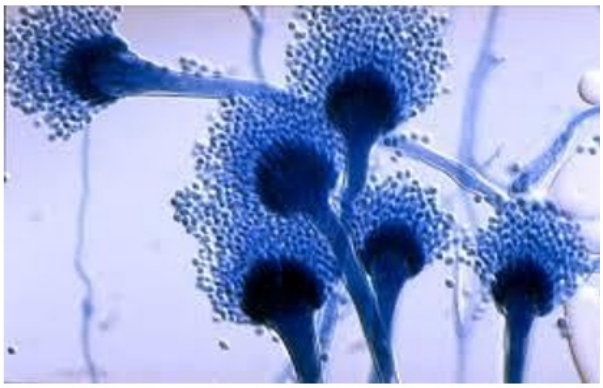
Biodiversity of endophytic fungi

The 12 endophytic fungal strains isolated from the 5 medicinal plants were collected from Dhanvantri Vana, a unit of Government Ayurvedic College, Bangalore. classified into 12 distinct morphospecies (Table 1). Mycelia sterilia consists of various morphological fungal types, but not forming true spores. This group of fungi is considerably prevalent in endophyte studies (Lacap et al., 2003). In the 12 frequently encountered endophytic fungal groups, Branched septet mycelium, conidiophores erect, globosa vesicle with uniseriate sterigmata which produces chain of conidia by basipetal succession.

mycelia sterilia had the highest relative frequency (10.16%) in the 5 medicinal plants. Colletotrichum which are frequently identified as endophytes (Photitia et al., 2005;Devarajan and Suryanarayanan, 2006) was the second most frequent endophytic group, followed by Aspergillus spp, Bispora spp., Geotrichum spp., Trichoderma spp. Cladosporium spp. Aspergillus spp. Gliocladium spp., Trichoderma spp. Fusarium spp. Trichosporoniodes spp Trichoderma spp with relative frequencies (Fig. 1). These were the dominant genera or order of endophytic fungi found in this study, similar to the findings reported previously for many tropical endophytic fungi (Corrado and Rodrigues, 2004; Krohn et al., 2007). The 18 infrequent endophytic fungal species or genera included Aureobasidium pullulans, Botryo-sphaeria, Chaetomella, Chaetomium, Clado-sporium, Coelomycetes, Drechslera-Yike, Ellisembia, Ephelis Flagellospora, Helmin-thosporium, Pestalotiopsis, Physalospora, Pyrenochaeta, Pyriclilariopsis, Rhizosphaera, Spiropes, and Verticillium. Among them, Spiropes, Ellisembia, and Helminthosporium are reported here for the first time as endophytic fungi.

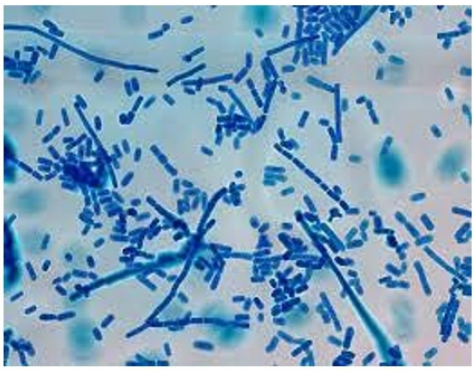
The endophytic fungi are very unique in their properties, which infect only the plant parts like root. They differ from the rhizosphere soil in their qualitative microflora niche. They can be considered obligatory parasites infecting only the plants. Very few can be facultative in existence surviving in the rhizosphere soil and also as an endoparasite in plants. The isolated fungi were maintained as a stock on PDA slants. The fungi isolated in endophytic are as follow:

Sr. no.	Plant name	Medicinal Properties	Endophytic Fungi
1	*Caesalpinia sappan*	Diarrhoea	*Colletotrichum spp. Aspergillus spp.Bispora spp. Geotrichum spp.*
2	*Alternanthera sessilis*	Skin rashes, Liver disorders	*Trichoderma spp. Cladosporium spp. Aspergillus spp.*
3	*Sapindus lourifolius*	Diarrhoea, White patches	*Gliocladium spp.Trichoderma spp.*
4	*Basala alba*	Mouth Ulcer, body energy	*Fusarium spp. Trichosporoniodes spp*
5	*Acalypha indica*	Skin disease , cough	*Trichoderma spp.*



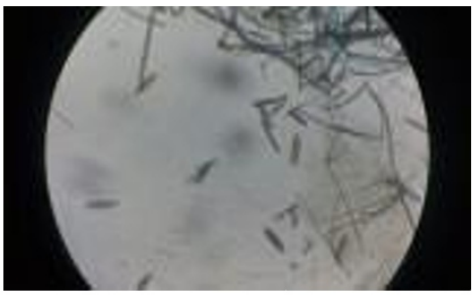
Aspergillus *spp.*

Bispora

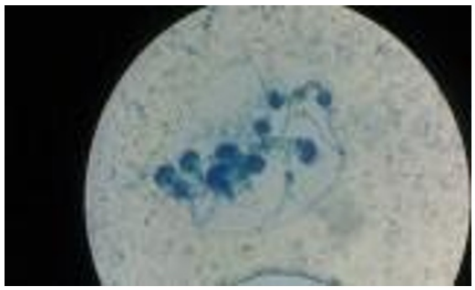


Geotrichum

Fusarium



Helminthosporium



Gliocladium spp

Morphological characteristic:

The morphology of isolated fungi were studied under microscope after staining with methylene blue stain.

The characteristics of fungal isolates are tabulated as below:

Fungal Isolate	Characteristic features
Aspergillus spp.	Branched septet mycelium, conidiophores erect, globosa vesicle with uniseriate sterigmata which produces chain of conidia by basipetal succession.
Bispora spp.	Mycelium dark; conidiophores dark, short simple, sparingly branched; conidia dark, oblong to ellipsoid, 2-celled or less with thick black septa, saprophytic.
Colletotrichum spp.	Acervuli is disc shaped, waxy. Conidiophores are simple, elongated, conidia hyaline, ovoid and parasitic.
Cladosporium spp.	Septate mycelium, conidiophores tall, dark and clustered. Conidia (blastospores) dark, 1-2 celled, ovoid to cylindrical, lemon shaped. Saprophytic or parasitic.
Fusarium spp.	Mycelium extensive with slender conidiophores, whorl of phialids grouped as sporodochium, micro conidia 1-celled ovoid, macor conidia is 2-3 celled, canoe or sickle shaped.
Gliocladium spp.	Conidiophores like penicillate brushes, conidia in mucilaginous droplets. Shows a stage Verticillium. Saprophytic in soil.
Geotrichum spp.	Mycelium white, septate; conidiophores absent; conidia hyaline, single celled, short cylindrical with truncate ends, mostly saprophytic.
Trichoderma spp.	Conidiophores branched, non verticillate, conidia in terminal clusters. Saprophytic in soil and wood.
Trichosporonoides spp.	True and pseudo mycelium, conidiophores simple with swollen globose apex bearing conidia (botryo blastospores) on sterigmata, conidia hyaline single celled yeast like.

Previous studies reported distinct endophyte community compositions in different host plants suggesting host preferences (Cannon and Simmons, 2002; Cohen, 2006). This study also found the significant differences in both presence /absence and relative abundance of fungal endophytes in the medicinal plants occurring in Bangalore. The colonization rate and the isolation rate of endophytic fungi from these plants varied greatly. Some medicinalplants harbored more endophytic fungi than others. The number of fungal taxa colonizing these hosts ranged from three to 15, and many of the fungal taxa had different isolate frequent-cies in different hosts. Some of the common endophytes not only existed in more plant hosts but also had higher relative frequencies within each of the hosts.-In contrast; some other endophytic fungi were detected in only one given plant host. These descriptions of host-preference were consistent with previous reports (Arnold et al., 2001; Bettucci et al., 2004).

The endophytic fungi in these 5 medicinal plants exhibited tissue-specificity. Some fungal endophytes were more likely found in the roots while others in the stems. Some infrequent fungal endophytes were found in only one type of tissue (leaf /stem/root). The difference in endophyte assemblages from various tissues indicated that some fungal endophytes have an affinity for different tissue types and this might be a reflection of their capacity for utilizing or surviving within a specific substrate (different tissue texture and chemistry) (Rodrigues, 1994; Photita et al., 2001). Many previous reports also discovered tissue-specificity in endophytic fungi (e.g., Taylor et al., 2001; Ganley and Newcombe, 2006).

Spatial variability has not been thoroughly explored for endophytic fungi and may be difficult to discern because stratum, substrate, or host preferences confound spatial patterns (Arnold et al, 2000). In this study, among-site differences were found in endophytic composition and abundance, but this spatial variation may contain the component of host- preferences as the collection sites also differ in plant composition. Some sites harbored more fungal endophytes than the others, and some endophytes were found in only one location. Spatial heterogeneity in the distribution of endophytes was also reported in previous studies (Arnold et al., 2001; Gallery et al., 2007). Such spatial heterogeneity may be partly due to differences in environmental conditions, including humidity, temperature, rainfall and potential inoculum sources (Photita et al., 2001; Santamaria and Dayman, 2005). The host plants in our study possessed different phenolic compounds (e.g., Aliphatic compound phenolic acids, Aromatic compound , Acid, Amine, Aldehyde, Ester, Ketone and these different hosts were colonized by various different endophytic fungi. Some correlation apparently exists between the endophytic fungal assemblages and the host chemistry. Unfortunately, it was impossible to identify or quantify neither all the chemical compounds nor all the endophytic fungi present in the plant hosts. In this study, only the total contents of phenolics and 5 hosts were investigated and their major phenolic compounds and fungal endophytes identified. However, the results suggested that the total contents of phenolics and

flavonoids of the host plants influence both the quantity of endophytic fungal taxa and the number of endophytic isolates. Moderate TPC and TFC appear to favor the growth of endophytic fungi. The plants with too low or too high TPC and TFC were colonized with fewer endophytic fungi. Based on the established matrix, some phenolic compounds were found to more likely coexist with some endophytic fungal taxa, such as chlorogenic acid with mycelia sterilia, Colletotrichum, and Phomopsis, rutin with Colletotrichum, and Aspergillus spp, Bispora spp.,Although some endophytic fungi may prefer the hosts with specific compounds, the presence of a given compound could not guarantee the presence of a given endophyte. The quantity of the specific compounds could also play a role in fungal colonization. Furthermore, the colonization of endophytes can induce the plants to produce certain compounds, and some of the special compounds with small quantity might be actually produced by the endophytes within the host plants. There exist other hypotheses that microbial symbionts could affect plant nutrition, defensive chemistry, and biodiversity (Rudgers et al, 2007). The host-endophyte relationship is complex and involves many factors, but studying the presence-absence of a given endophyte in the presence of a given compound of the host plant is an important first step toward our understanding of this intricate relationship. Further studies are needed to reveal the interaction between the host plant and its endophytes.

In conclusion, this study investigated endophytic fungal diversity and host-endophyte relationship based on traditional metholody. The 12 endophytic fungal isolates from 5 medicinal plants were identified and classified. Colletotrichum spp, Aspergillus spp, Bispora spp., Geotrichum spp., Trichoderma spp. Cladosporium spp. Aspergillus spp. Gliocladium spp., Trichoderma spp. Fusarium spp. Trichosporoniodes spp Trichoderma spp were the dominant fungal taxa. The evidence for host-preference, tissue-specificity, and spatial heterogeneity was found in the endophyte distribution based on fungal community compositions and isolation frequencies. Certain correlations exist between the endophytic fungal assemblages and the host plant chemistry.

The Studying. endophytes as we have done here is a method-dependent process (Quo et al.,2001) and thus the fungi isolated are dependent on our methodology. We were also unableto identify the mycelia sterilia. Future investigation should be use molecular sequence data to identify mycelia sterilia (e.g. Wang et al., 2005; Promputtha et al., 2005; Sanchez Marquez et al., 2007). Total fungal communities should be detected by extracting the entire host DNA (for example from roots) with various methods to sequence individual taxa. Potentially

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