APPENDICES

List of Papers Presented in Seminar

1. **Effect of Different Vitamins on Four Plant Pathogenic Fungi**- 99th Session of the Indian Science Congress, Bhubaneshwar, January 3-7, 2012.

2. **Role of microorganisms on seedling growth and survival of *Terminalia bellerica* (Gaertn.) Roxb-** National Conference in Biodiversity Conservation: Challenges Ahead, organized by Botany dept., MSU – 18 – 23rd December, 2010.

3. **Diversity of Arbuscular Mycorrhizal Fungal Spores present in the Rhizospheric soil of four different Grasses and strategies to promote Plantation in degraded Forest area in Gujarat, India**- 1st International Congress: "Mycorrhizal Symbiosis: Ecosystems & Environment on Mediterranean area (MYCOMED)" Marrakesh, Morocco 11 to 13th October 2010.

4. **Ecofriendly Management of Two Fungal Pathogens**- National Seminar on "Air Pollution Management" by Botany Dept., M.S.U. Baroda, 7th Feb, 2010.

5. **Amla Cultivars**- Seminar on Amla : An Indigenous Tree with Enormous Potentialities, organized by Botany dept., MSU and co-ordinated by State Medicinal Plant Board, Gandhinagar- 4th August 2009.

6. **Antifungal property of certain leaf extracts against two phytopathogens -** National Seminar on Emerging areas in Plant Sciences organized by Botany Dept. The M.S.University of Baroda -22nd Feb'09.

Paper accepted for the publication

1. "Diversity of Arbuscular Mycorrhizal Fungal Spores present in the Rhizospheric soil of four different Grasses and strategies to promote Plantation in degraded Forest area in Gujarat, India" in **Science Journal of Agricultural Research.**

2. Two new Leaf spot disease of *Bambusa arundinacea* (Retz.) Roxb. from Gujarat, India in **Journal of Mycology and Plant Pathology.**

3. Phylloplane mycoflora of *Madhuca indica* L. and *Diopsyros melanoxylon* Roxb. In **Internatioal Research Journal of Plant Science.**

4. Antifungal potential of certain leaf extracts against three phytopathogens. In **African Journal of Pathology.**

P – 17: Ecofriendly Management of Two Fungal Pathogens

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Plants suffer with number of fungal of pathogens. The increase in concentration of air pollutants makes the plants more vulnerable to fungal attack. A large number of fungi have been reported causing leaf spot disease in leaves of *Termenalia arjuna*. Isolation from the infected leaves resulted into two culture of fungal pathogens. The pathogenecity of these pathogesn was conformed. The fungal pathogens *Phoma multirostrata* and *Myrothecium roridum* belongs to Fungi imperfecti (Mitosporic) group. An effort has been made to control these two fungi in vitro by seed extract of 5 different plants. The results indicate that out of the 5 plants tested maximum control of P.multirostrata was obtained with seeds of Strychnos nux – vomica followed by *Polyalthia longifolia* and *Annona squomosa*. In case of Myrothecium better control was obtained in case of P. lonmgifolia followed by *Strychnos* and *Syzigium cumini*.

Amla cultivars

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Amla or Indian Gooseberry is indigenous to tropical Southern East Asia, particularly in the central and southern India. Seedling plants are commonly found in Vindhyan hills , North –Eastern hill region and also in North-Western Himalayan region up to an elevation of 1,350m above mean sea level. Amla can be successfully grown in marginal soil, salt-affected soils, ravines, dry and semi-dry regions.

A member of family Euphorbiaceae, amla tree is much branched and grow 9-12m high. Seedling trees may grow beyond 20m. Of the related species, *Phyllanthus acidus* Skeel., syn. *P. distichus* Muella, popularly known as otaheite gooseberry, star gooseberry or country gooseberry. It is mostly grown for ornamental purposes, whereas, wild species *Emblica fischerry* Gamble., syn.*P. fischerry* found in the forest of south India ,bears fruits suitable for pickle making.

In early years there was no improved cultivar of amla. Some popular types were named after the locality of their occurrence, plant type and fruit colour, Banarasi, Chakaiya, Hathi Jhool, Green Tinged, Red Tinged, White streaked and Bansi Red being grown among them. Due to predominance of seedling propagation, India holds rich genetic diversity in cultivated and their wild relatives of amla. Genetic erosion status of these resource are constantly high due to severe deforestation. Natural calamities and adoption of a few popular cultivars in the selected pockets. However enormous variability in amla still remains unexpoites and awaits proper attention on exploration, collection, evaluation and maintaince to conserve genetic diversity available in the nature.

Germplasm survey, collection and evaluation work on amla was initiated at the Narendra Dava University of Agriculture and Technology, Kumarganj, Faizabad, during 1982-83. Ninteen germplasm including 10 in fruiting stage are being maintained at this

University. Considerable variability in fruit shape (round, flattend-round, oval-round and triangular) ; length(3.91-2.90cm);width(4.50-3.50cm);fruit weight (50.55-26.91g); number of segments(6); fruit colour (whitish-green to apricot yellow, yellowish green, light green with pink tinge, pink.) ; seed size small to large ;flesh colour whitish green to pink; fibre more or less ; TSS(8.0-14%) ; acidity (1.9-2.5%) and ascorbic acid (422-600mg/100g pulp have been observed with available genotypes. Diversity in vegetative growth (upright, spreading, dropping) ; flowering (February end to first week of April); sex ratio (1:28-1:355) ; fruit maturity and productivity have also been observed with these genotypes.

Selection of promising types suitable for processing industry, high yield potential, tolerance to abiotic and biotic stresses as well as selection for better quality fruits would be of immense value for commercialization of this valuable fruit.

Star gooseberry (*Phyllanthus acidus* Skeel, syn *P. distichus* Muell.-Arg.) Family; Euphorbiaceae : Known as paramlakhi, the star gooseberry grows in the backyard gardens throughout Assam. It prefers subtropical climate and grows well in highlands. The tree is deciduous in nature, 4.56.0m tall with erect branches at the top. The leaves are obliquely ovate with a rounded base, acute tip, light green in colour. Flowers are very small, pink in colour, borne on branches and on the main stem. Inflorescence is a raceme. The fruits are round, deeply ribbed and creamy yellow in colour when ripe .They are harvested during May-June. Fruits are generally used for making pickles and other preservatives .The sun dried slices are fed in dysentery.

VARIETIES

There are 3 main varieties of amla – Banarasi, Francis (Hathijhool) and Chakaiya. These varieties have theirown merits and demerits. Banarasi, an early – maturing amla, is a shy-bearing, prone to heavy dropping of fruits with poor shelf-life. Francis suffers from severe incidence of fruit necrosis. Chakaiya fruits are fibrous, smaller in size and also have a tendency to bear heavy crop in alternate years. Other varieties identified and released for commercial cultivation are :

Kanchan (NA 4)

A seedling selection from Chakaiya, it is heavy and regular bearer (7.7 female flowers / brachlet), with medium- sized fruits, having higher fibre content. It is preferred by industries for pulp extraction and manufacturing of various products. This has been adopted very well in the semi-arid regions of Gujarat and Maharashtra.

NA 6

A seedling selection from Chakaiya, it is prolific and heavy-beared (10.8 female flowers/branchlet). It is deal for preserve and candy, owing to low fibre content.

NA 7

A seedling selection of Francis, it is precocious, prolific and regular-bearer (9.7 female flowers/branchlet). This is an ideal variety for preparation of products and has a great promise.

Besides, Anand 1, Anand 2 and Anand 3 have been selected as promising strains in Gujarat.

Diversity of Arbuscular Mycorrhizal Fungal Spores present in the Rhizospheric soil of four different Grasses and strategies to promote Plantation in degraded Forest areas of Baria division in Gujarat, India

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Abstract

Arbuscular Mycorrhizal Fungi (AMF) are most important members of soil microbial community in natural ecosystem. These useful organisms form an indispensable component of any fertile soil. It is said that a good quality of soil is almost equal to 1 kg of gold. Symbiotic relationships of AMF provide an alternative for survival of plants in highly stressed areas. This association helps in uptake of P as well as other minerals, maintain better water balance, increase plant biomass and produce growth promoting substances. Use of AMF is found to be helpful in avoiding the build up of toxic concentration in sensitive sites within the cells and thus prevent any damaging effect. By application of such organisms use of synthetic fertilizers can be reduced. Strategies to promote plantation in poor / degraded soil includes introduction of native AM Fungi in the soil as well as certain plant growth promoting bacteria like *Pseudomonas* fluorescent *and Rhizobium etc.* Pelleting of seeds will be tried with small root pieces to incorporate AM fungi and bacterial broth medium to incorporate suitable bacteria. The use of such dual culture technique is expected to yield desired results.

During present study a survey was undertaken in degraded forest areas with certain grasses in Bandheli (Godhra division) and Kaltalai and Rampura (in Baria div.) about 115 km from Vadodara city (Gujarat) in India. Rhizospheric soil samples of 25 different places were collected. Soil samples of *Heteropogon contortus* showed more percentage (220) as compared to *Themada triandra* (165). In Kalitalai *Chloris cruciata* showed more number of spores (150) while it was 110 only in soil sample analyzed from Rampura. The percentage occurrence of AM spores was more in Bandheli. The amount of fine sand particles was more in this sample. Analysis of AM spores resulted into identification of different species belonging to 4 genera *i.e. Glomus, Gigaspora, Sclerocystis* and Acaulospora. Since Glomus was present predominantly it was selected for further studies. Seed pelleting and other studies like change in percentage germination and change in plant biomass are in progress

The sharp fall in the fungal population in the late phases is understandable because of rapid rate of decomposition and obvious decrease in nutrients availability.

Effect of AM Fungi and Flyash on Gladiolus

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Keywords: *Gladiolus, AM fungi, Fly ash*

Gladiolus occupies prime position in the floriculture industry and becoming popular day by day for cut flower trade. However, many of the imported varieties require a heavy input of cultivation reduce the margin of profit. Development of any technology which may curtail the cost of cultivation of such varieties may go a long way to help the farmers. In the present investigation efforts were made to study the synergistic effects of AM fungi and different concentrations of fly ash was used on Gladiolus cultivar Tambree. 45% fly ash and AM fungi treated gladiolus showed the best result.

Effect of Different Vitamins on Four Plant Pathogenic Fungi

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Keywords: *Vitamins, Pestalotiopsis disseminata, Phomopsis tectonae, Pestlotiopsis maculans and Lasiodiplodia theobromae.*

Vitamins are organic molecules required in small amount and not used as a source of either energy or structure materials of protoplasm. Fungi like other organisms require minute amounts of these specific organic compounds for their normal development. It is believed that they are needed for metabolic reactions and functions as coenzymes or constituent parts of coenzymes. Our current knowledge of vitamin requirements of fungi indicates that they generally need only water soluble vitamins .

In the present study an attempt has been made to study the effect of 10 different vitamins on the growth and sporulation of Phomopsis *tectonae* and *Lasiodiplodia theobromae* from the leaves of *Tectona grandis, Pestalotiopsis disseminata* from *Terminalia arjuna, P. maculans* from the leaves of *Bambusa arundinacae.* The growth of these fungi was recoded after 15 days of incubation. It was observed that all the four organisms showed lesser growth as compared to control sets. In *P. disseminata* the growth decreased with increase in concentration of thiamine. At 200µm the growth of *P. disseminata* was lowest. In *P. maculans* and *L. theobromae* the growth remained almost constant even with varied concentration of thiamine. In *P. tectonae* highest growth was observed at 50 µm. Growth of *P. maculans* was highest among the four organisms, in pyredoxine, however, the growth of this organism was less at 200 µm compared to mycelia weight obtained at 50 µm. The growth of *L. theobromae* remained same at all concentrations. In case of riboflavin *L. theobromae* exhibited highest growth at 100µm followed by *P. disseminata.* In case of nicotinic acid it was observed that this vitamin accelerated the growth of *P. disseminata, P. maculans* and *L. theobromae,* contrary to this the increasing concentrations from 25µm to 100µm reduced the growth of *P. tectonae.* Ascorbic acid inhibited the growth of all the four organisms with increase in concentration.

Two new Leaf spot disease of *Bambusa arundinacea* (Retz.) Roxb. from Gujarat, India

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Keywords :*Bambusa arundinacea, leaf spot, Pestalotiopsis maculans, Drechslera rostrata.*

Abstract

Survey was conducted in Pavagadh forest region of Panchmahal District of Gujarat. An area with dense Bamboo cover was located at a distance of 50 km from Vadodara and in the Arboretum of M.S. University campus during August – September 2009. Leaves with spots at the tip and leaves with lesions in the centre of lamina of *Bambusa arundinacea* were collected. After isolation on PDA medium, two new fungal pathogens *Pestalotiopsis maculans* (Corda) Nagraj and *Drechslera rostrata* (Drech) Rich & Fras were recorded. These are new host record for the country.

Introduction

Pavagadh is a Hill Station, situated about 46 km away from Vadodara in the municipality of Panchmahal district in Gujarat state in western India. It is known for a famous Mahakali temple which draws thousands of pilgrims everyday. It is situated at an elevation of 823 m above sea level at 22 ° 28'00 N 73 ° 30'02''E. Area of this locality Chapaner – Pavagadh Archaelogical Park has been in scripted by UNESCO as World Heritage site in 2004.

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Besides *Tectona grandis*, bamboos are planted by forest department in Pavagadh forest area. Bamboos are tall perennial, arborescent grasses belonging to Bambucaceae, a tribe under Poaceae comprising of four sub – tribes: Arundinaceae, Eubambuseae, Dendrocalamceae and Melocannceae, generally inhabiting humid tropical, sub tropical regions of the world. They form an important constituent of many deciduous and evergreen forests. Areas under *Bambusa arundinacea* with 75 clumps/ha are classified as dense forest. (Varma and Bahadur, 1980).

In India one third of the entire population of bamboos is being utilized for constructional purposes. Single mat and veneer boards have been made from the green culms of *B. arundinacea*. It is well suited for making bamboo - ply. It is used for supporting crops such as sugarcane, betel vines and other climbing crops. Since the culms and rhizomes contain nutritive elements N,P,K, and Ca, they are suitable for preparing compost or manure. Trays made from this species are used for rearing silkworms in sericulture industry. Charcoal from bamboo is reported to have more calorific value than wood – charcoal, it is used by goldsmiths. *B. arundinacea* yield a wax with low melting point which is used as a base for shoe polishes in place. Leaves of *B. arundinacea* used as are much valued as fodder, particularly during scarcity. In India seeds of *B. arundinacea* are extensively eaten by the poor during famines. Seeds resemble paddy but are bigger in size. Seeds are pickled, used for making beer (Anonymous, 1965).

Materials and Methods

Diseases leaves were collected from Pavagadh forest area, an World Heritage site located near Vadodara and from Arboretum, M.S. University of Baroda campus. Infected leaves were washed in tap water then surface sterilized with freshly prepared 0.1 % $HgCl_2$ solution for 1 min.

Both fungal pathogens *D. rostrata and P. maculanas* were isolated and maintained on Potato Dextrose Medium.

Result and Discussion

Hino (1938) first use the term "*Fungorum bambusicolorum*" (bambusicolous fungi), but did not give any fungi growing on any bamboo substrates, which include leaves, culms, branches, rhizomes and roots. The genera of bamboo with the highest numbers of fungi recorded globally are *Arundinacea, Bambusa, Phyllostachys* and *Sasa*. Species of *Bambusa* in particular have been found to support a high fungal diversity. This is probably due to a larger number of collections, as it is one of the most widespread genera in tropical and subtropical Asia (Dransfield and Widija, 1995).

Disease Symptoms: Leaves with yellowish brown tips appeared in case of infection with *Drechslera rostrata* (Drech) Rich & Fras. and in case of infection caused by *Pestalotiopsis maculans* (Corda) Nagraj symptoms appeared as brown oval lesions in the mid rib region of the older leaves.

Identification of both pathogens is done based on morphological characters (Fig.a, b). Cultures were identified and confirmed from Agharkar Institute, Pune and IARI respectively. The fungi were inoculated on healthy leaves and Koch's postulates were confirmed. Since the two pathogens are not cited in literature (Jamalludin *et al.*,1989, Bilgrami *et al.*, 1981). Hence the two pathogens constitute new host record on the leaves of bamboo.

Fig. Showing conidia of

Pestalotiopsis maculans (Corda) Nagraj

Drechslera rostrata (Drech) Rich & Fras

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Phylloplane mycoflora of *Madhuca indica* L. and *Diopsyros melanoxylon* Roxb.

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Abstract

Indian region is one of the most diverse biogeographic regions of the world, embracing a wide range of topography from perpetually snow covered high Himalayas to plains at sea level. A great variety of climatic and altitidinal variations coupled with varied ecological habitats has contributed immensely to the rich diversity. National Forest Policy 1988 advocates for increasing forest productivity and meet the demands of timber, fuel, fiber, paper etc.

Phylloplane fungi have been poorly studied as compared to endophytes, saprobes and pathogenic fungi. Nutrients stimulatory for microorganism on phyllosphere and phylloplane regions consist primarily of plant materials *(e.g.* pollen grains, old petals, etc.) or insect excreta. Stimulation of necrotrophic (destructive) fungi by pollen grains (Fokkema, 1981) and aphids honey dews (Fokkema *et al,* 1983) is well documented. Fungi may be blown from near or for off places and settle on leaf lamina. These fungi may grow when conditions are favorable and can cause diseases, the fungi may be necrotrophic and obligate parasites. Such a study is required to find out ecological niches in order to control them. A study was under taken on leaves of *Madhuca* and *Diospyros* to find out phylloplane fungi. Results indicated that more number of viable fungi were recorded by serial dilution technique.

Introduction

Numerous investigations have been carried out on the fungal flora of leaf surfaces of several plants growing or cultivated in many parts of the world by several researchers (Abdel-Fattah *et al.*, 1977; Abdel-Hafez, 1981, 1984, 1985; Abdel-Hafez *et al.*, 1995; Eicker, 1976; Khallil and Abdel-Sater, 1993; Mazen *et al.*, 1985; Nagaraja, 1991; Perez and Mauri, 1989; Sharma, 1974).

Tropical forests in Jambughoda have large number of *Madhuca* and *Diospyros* trees. *M. indica* suffers from, *Cylindroncladium scorpariu, Pestalotia dichaeta, Pestalotia* sp., *Phyllachora madhuca, Polystictus steinbelianus, Scopela echinulata, Sarcinella* sp. *Sphaceloma madhucae* ***Diospyros melanoxylon*** Roxb. is attacked by *Aecidium rhytismodieum, A. miliar, Cercospora kaki, Pseudocercospora kelleri, Sarcinella gorakhpurensis.* It is essential that diseases must not occur in *D. melanoxylon* tree as its leaves are used for making local cigarette (Bidi) and Madhuca flowers and oil obtained from seeds is used for different purposes.

Phyllosphere is the immediate vicinity of leaf surface, where microbial communities are constantly in dynamic state due to exo-and endogenous sources of nutrients. It is a complex terrestrial habitat that is characterized by presence of a variety of microorganisms including bacteria, filamentous fungi and yeasts. Phylloplane fungi are the mycota growing or the surface of leaves. There are two groups of phylloplane fungi: residents and casuals. Residents can multiply on the surface of healthy leaves without noticeably affecting the host. Whereas, casuals land on the leaf surface but cannot grow.

Most of the methods used for the study of phylloplane fungi have advantages and disadvantages. Direct observation for example, enables one to determine the distribution of the phylloplane population and provides useful information such as the growth forms and spatial distributions of a variety of microorganisms. Direct microscopy gives a more precise estimate of population size than plate count methods (Baker, 1981). Direct count methods give higher densities than impressions because some organisms may remain on the plant when the adhesive tape is removed. It is not however, easy to determine the viability and identity of propagules unless the groups are separated by their morphological differences using microscopy (Last and Warren, 1972; Baker 1981; Parbey *et al.,* 1981). The irregular nature of the plant surface, moreover, makes the examination difficult at high magnification (Paton, 1982).

Leaf washing facilitates the 'identification of colonizers' from chance initiators (Last and Warren, 1972). Culture condition may also affect the subsequent growth of the propagules (Parberry *et al.,* 1981) and some organisms may not grow on the medium provided (Baker, 1981).

In the leaf impression method, fungal spores on the leaf surface stick onto the agar. This may give rise to very dense fungal and bacterial growth on the agar plate (Lee *et al.*, 2002). When the spore technique is used only fungal colonies with mature fruiting bodies will be isolated and immature fungal colonies, or species that need water for spore release, will be neglected.

Rai and Singh (1981) have investigated the antagonistic activities of some phylloplane fungi (of mustard and barley) against *Alternaria brassicae and Drechslera graminea.* The antagonists are *Aureobasidium pullulans, Epicoccum purpurascens, Cladosporium cladosporioides* and *Alternaria alternata.* The most significant effects were observed when the spores of leaf surface fungi or their metabolites were sprayed on leaves prior to inoculation of the pathogens.

Materials and Methods

There are several methods for investigating phylloplane fungi such as tape impressions followed by culturing (Langvad, 1980) and spore – fall techniques (Lamb and Brown 1970, Dickinson 1973, Langvad 1980, Vardavakis 1988). With tape impressions a thin agar film is pressed on to the leaf surface that can be incubated in Petri dishes containing filter paper moistened with glycerol. The spore fall technique involves attaching the leaf onto the inside of the Petri dish lid above agar, using the premise that fungal spores can fall or are short onto the agar surface.

Three methods: direct observations, leaf washing method, cellotape method were followed to study phylloplane fungi on *Diospyros melanoxylon, Madhuca indica* leaves.

The above two plants were chosen to study because they come in abundant source, easily available *D. melanoxylon* is one of the Minor Forest Produce and has high economic value.

Leaves of *D. melanoxylon* and *M. indica* were sampled and brought to laboratory in polythene bags. The leaves were washed in sterile distilled water and collected in conical flask. 1 ml of water after washing the leaves was placed on PDA containing plates and were incubated at 25^0C and examined until fungal colonies appeared. Sterile mycelia were subcultured to PDA medium.

The fungi were identified on basis of taxonomic keys when fruiting occurred.

Results and Discussion

The fungi present in infection court may be influnced by other microbes and environmental conditions. A survey conducted on phylloplane microflora by cellotape and serial dilution plate method revealed presence of 13 different fungi in the *D. melanoxylon* and eight in case of *M. indica* it is intersting to note that leaves when infected by these fungi may produce toxic compounds. The fungi like *Aspergillus, Candidus, Alternaria alternata, Nigrospora sphaeraca* and *Phyllactinia* sp. Were recovered by dilution plate technique only. *A niger* and *Alternaria alternata* are most common airbone fungi these are common aero allergens.

Table 1: List of fungi isolated from the phylloplane of *Diospyros melanoxylon* and *Madhuca indica*

Sr. No.	Fungi	*Diospyros melanoxylon*	*Madhuca indica*
1.	*Alternaria* sp.	√	√
2.	*Aspergillus awamori*	√	√
3.	*Aspergillus fumigatus*	√	√
4.	*Aspergillus niger*	√	√
5.	*Cladosporium cladosporoides*	√	-
6.	*Colletotrichum capsici*	√	√
7.	*Curvularia lunata*	√	-
8.	*Fusarium oxysporum*	√	-
9.	*Penicillium citrinum*	-	√
10.	*Pestalotiopsis* sp.	√	-
11.	*Pestalotiopsis versicolor*	√	-
12.	*Phyllactinia* sp.	√	-
13.	*Rhizopus stolonifer*	√	√
14.	*Trichoderma* sp.	√	√

Note - Presence of fungi based on 5 inoculms placed on PDA plates.

Table 2: List of fungi recorded and their percentage frequency of occurence by two different methods.

Sr. No.	Species	*Madhuca indica*		*Diospyros melanoylon*	
		Cellotape method	Dilution	Cellotape	Dilution
	Zygomycetes				
1.	*Mucor heimalis*	2	5	2	2
2.	*Rhizopus stolonifer*	2	5	5	5
	Ascomycetes				
3.	*Chaetomium globosum*	1	5	2	5
4.	*Phyllactinia*	-	-	-	2
	Anamorphic fungi				
	Hyphomycetes				
5.	*Aspergillus candidus*	-	2	-	3
6.	*A. niger*	2	10	2	10
7.	*A. awamori*	-	-	2	2
8.	*Alternaria alternata*	-	5	-	10
9.	*Cladosporium cladosporiodes*	1	2	-	2
10.	*Curvularia lunata*	2	8	4	10
11.	*Fusarium oxysporum*	-	5	2	6
12.	*Nigrospora sphaerica*	-	2	-	2
13.	*Penicillium citinum*	2	10	2	4
14.	*Trichoderma viride*	4	10	2	10
	Coelomycetes				
15.	*Pestalotiopsis* sp.	2	2	2	6
16.	*Colletotrichum capsici*	1	2	-	2

NOTE: Presence (-) absence of colonies based on average of 3 replicates. Numericals show percentage frequency.

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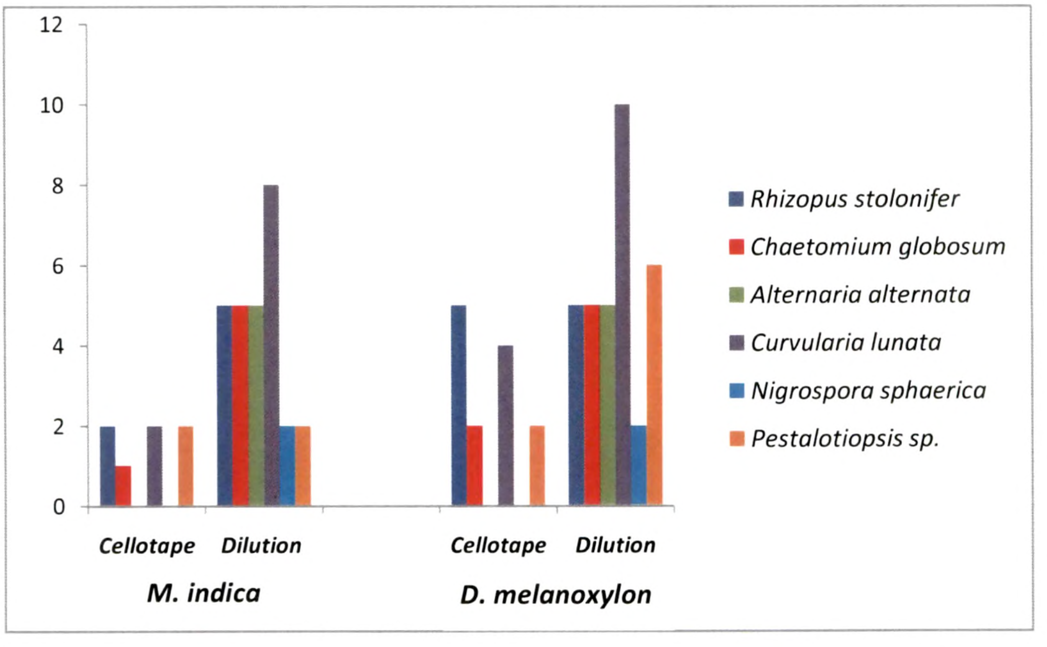
Fig: Graph showing occurence of phylloplane fungi by Cellotape and dilution plate methods



Antifungal potential of certain leaf extracts against three phytopathogens

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Plants diseases play a major role in the destruction of natural resources in agriculture and forestry. Among the Foliicolous fungi members of fungi imperfecti appear to be most aggressive. Usually chemical compounds are used to control leaf spot diseases, but they in fact help pathogens in developing resistance against fungicides. Unfortunately more the specific effect of a chemical on an organism, greater the probability of decreasing the effect through genetic shifts in population, whereas broad spectrum fungicides produce undesirable consequences on non – target organisms (Tjamos *et al.,* 1992) to reduce the synthetic fungicides, a few botanicals *viz.* neem oil, need seed extract, mahua and pomgam oil have been tested and found t o possess fungicidal properties against certain pathogens (Rajappan *et al.,* 1997) Arya (1988) found aqueous shoot extract of *Ephedra foliata* and leaf extract of *Eucalyptus occidentalis* effective against *Phomopsis viticola* at 25%. At higher concentration (75%) leaf extract of neem was most effective causing 82.3 % spore inhibition. Tripathi (2005) tested 24 angiospermic plants. Leaf extract in ethyl acetate) of *Citrus aurantifolia, Murraya koenigii, Nerium indicum, Prunus persica, Ocimum gratissimum* and (ethyl alcohol) extract of *Acacia nilotica* showed good activity. Benzene extracted leaf extract of *Ipomoea fistulosa* – a profusely growing weed was found inhibitory to *Aspergillus niger, Colletotrichum gloeosporiodes* and two species of *Fusarium* (Mittal *et al.*, 2004)

Table no:10 Percentage inhibition of *Fusarium pallidoroseum* at different concentration of leaf extracts

Sr. no.	Plant Selected	Methanolic extract			Aqueous extract		
		1 ml	5 ml	10 ml	1 ml	5 ml	10 ml
1.	*Alangium salviifolium*	16	34	66	36	25	30
2.	*Grevillea robusta*	23	29	53	17	15	18
3.	*Heterophragma adenophyllum*	19	34	39	11	24	25
4.	*Polyalthia longifolia*	27	37	51	9	16	20
5.	*Terminalia arjuna*	12	28	39	50	29	39
6.	*Dalbergia sisso*	-	12	22	-	-3	12
7.	*Eclipta alba*	-	15	24	-	3	17

Table no: 11 Percentage inhibition of *Thelevia subthermophila* at different concentration of leaf extracts

Sr. no	Plant Selected	Methanolic extract			Aqueous extract		
		1 ml	5 ml	10 ml	1 ml	5 ml	10 ml
1.	*Alangium salviifolium*	16	34	66	23	25	36
2.	*Grevillea robusta*	31	53	83	18	20	25
3.	*H. adenophyllum*	15	37	53	0	9	20
4.	*Polyalthia longifolia*	13	52	65	10	15	25
5.	*Terminalia arjuna*	28	56	48	20	17	50
6.	*Calotropis procera*	60	75	80	50	53	51
7.	*Datura metel*	12	48	51	73	33	29
8.	*Cynadon dactylon*	65	12	71	57	63	20
9.	*Withania somnifera*	13	36	56	18	35	31

Table no: 12 Percentage inhibition of *Drechslera rostrata* at different concentration of leaf extracts

Sr. no.	Plant Selected	Methanolic extract (Conc)			Aqueous extract (Conc)		
		1 ml	5 ml	10 ml	1 ml	5 ml	10 ml
1.	*Calotropis procera*	52	31	42	48	37	21
2.	*Datura metel*	10	68	62	59	38	64
3.	*Polyalthia longofolia*	13	31	52	13	20	25
4.	*Withania somnifera*	28	62	72	13	43	31

Zafar *et al.*, (2002) reported that chloroform extracts of leaves of *M. azedarach* was active against *Fusarium chlamdosporum* while hexane, ethanol and water extracts were not. In a similar kind of work Bajwa et al., (2006) reported the antimycotic activity of aqueous and dichloromethane fractions of *Cicer arietinum* against *Drechslera tetramera* and *D. hawaiiensis.*

Leaf decoction of *Calotropis procera, Datura stramonium* were found to be effective in suppressing uredospore germination on detached leaves of wheat. (Bhatti, 1988). Hasa *et al.,* 91992) that leaf extracts of *Datura stramonium* reduced the development of rust pustules on the leaves of wheat. Mughal *et al.,* (1996) observed that aqueous extracts of *Datura alba* and *Withania somnifera* inhibited the growth of *Alternaria alternata, A. brassicola* and *Myrothecium roridum.*

According to Karim *et al.,* (2008) *Calotropis procera* leaf extract showed maximum fungal growth inhibition of *Candida albicans and Microsporum boulardi.* The bacteriocidal activity of *Calotropis procera* could be due to presence of calactin, mudarin and protein called calotropin which are active constituents of *C. Procera* (Parotta, 2001).

Fig. 1 Percentage inhibition of *F. pallidoroseum* in metahnolic and aqueous extracts of certain plants.

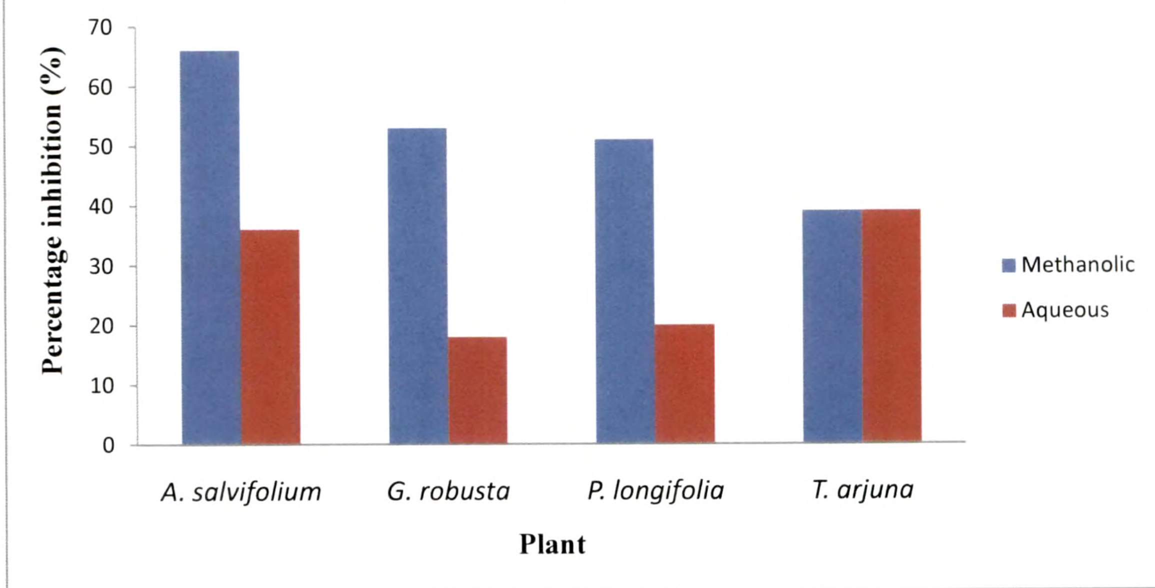


Fig. 2 Percentage inhibition of *T. subthermophila* in methanolic and aqueous extracts of certain plants

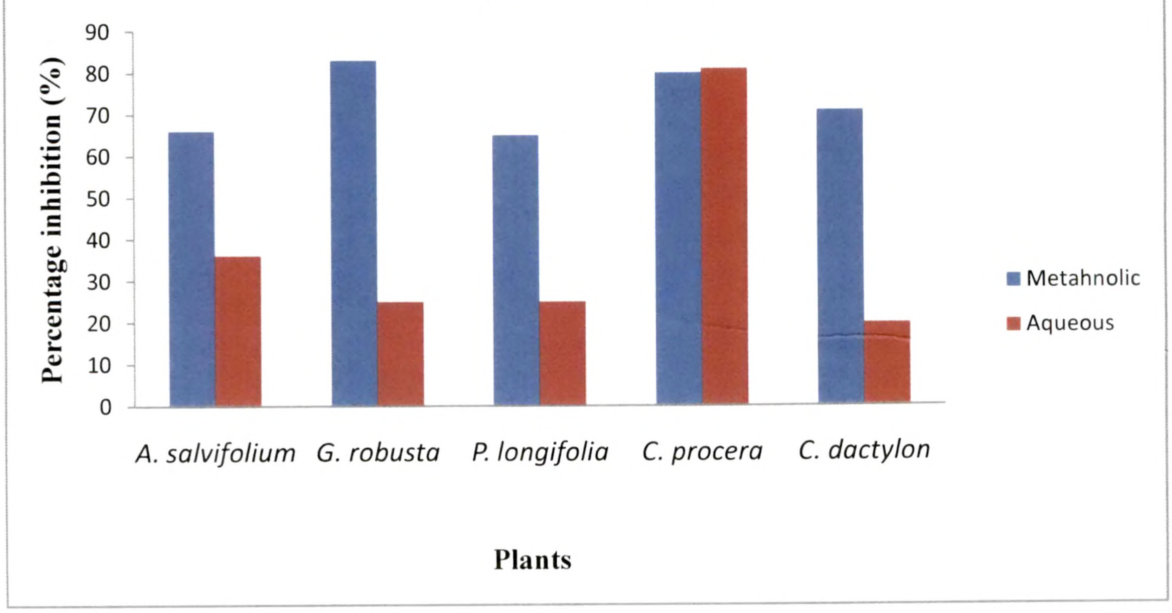
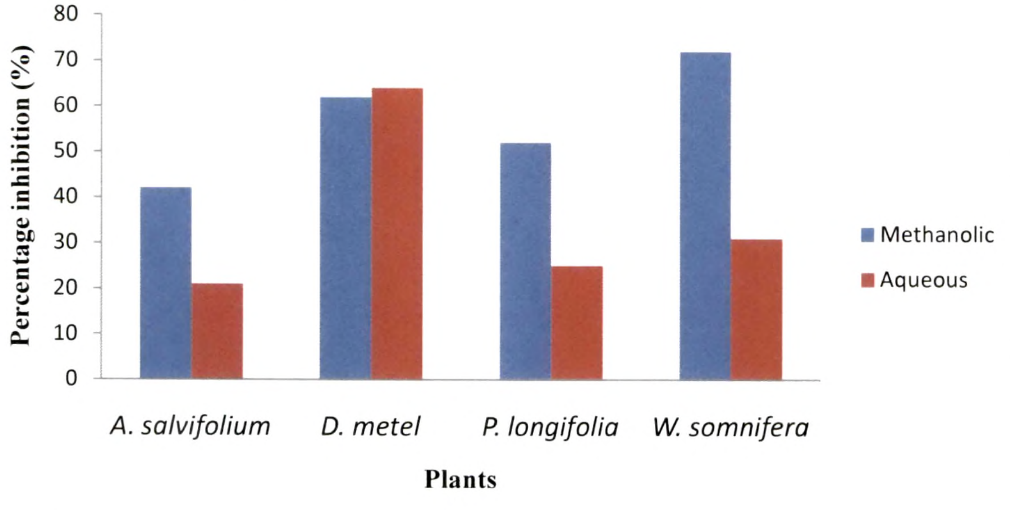
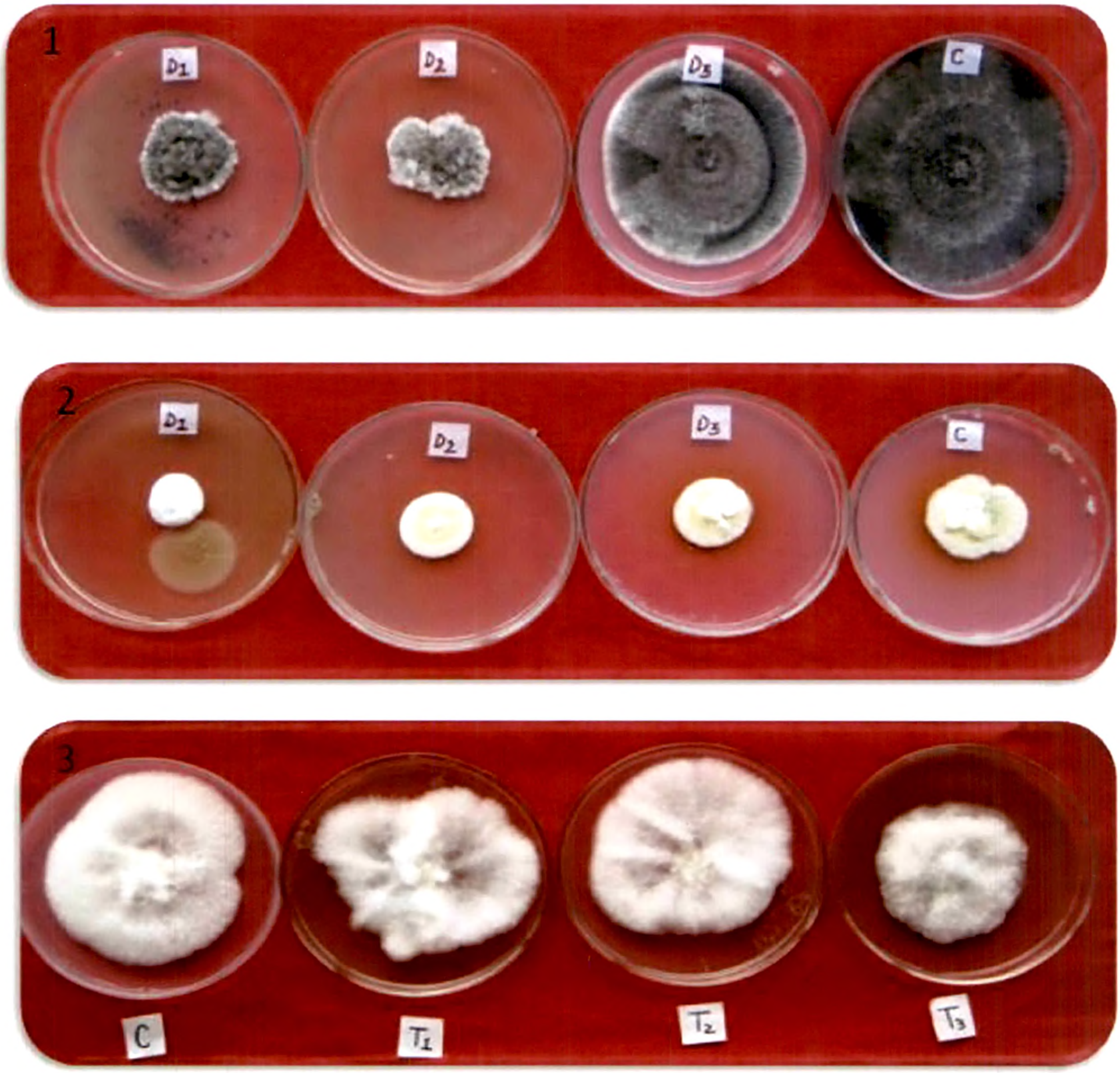


Fig. 3 Percentage inhibiton of *D. rostrata* in aqueous and methanolic extract of certain plants





1 - *Drechslera rostrata on methanolic leaf extract of Datura stramonium*

2 - *Thelevia subthermophila on D. stramonium*

3 - *Fusarium pallidoroseum on methanolic leaf extract of Terminalia arjuna*

Differences in growth rate were exhibited with respect to the concentration employed. The periodic assays revealed significant reduction in fungal growth rate in all higher concentrations. The comparison between different aqueous and methanolic leaf extract concentrations showed that the percentage colony growth inhibition was significantly greater in methanolic extract in contrast to aqueous extract.

Diversity of Arbuscular Mycorrhizal Fungal Spores present in the Rhizospheric soil of four different Grasses and strategies to promote Plantation in degraded Forest area in Gujarat, India

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SUMMARY

Arbuscular Mycorrhizal fungi (AMF) are the most important members of soil microbial community in ecosystem. These useful organisms form an indespensible component of any fertile soil. It is said that a good quality of soil is almost equal to 1kg of gold. Symbiotic relationships of AMF provide an alternative for survival of plants in highly stressed areas, this association helps in uptake of P as well as other minerals, maintain better water balance, increase plant biomass and produce growth promoting substances. By application of such organisms use of synnthetic fertilizers can be reduced. Stratagies to promote plantation in poor or degraded soil includes introduction of native AMF in soil. Pelleting of different seeds will be tried with small root pieces to incorporate AMF. During present study a survey was undertaken in degraded forest areas with certain grasses in (Godhara and Baria divisions) 115 kms from Vadodara city in India. Rhizospheric soil samples from 25 different places were collected. Soil samples of *Heteropogon contortus* showed more number of AM spores (220) as compard to *Themeda triandra* (165). In Kalitalai *Chloris barbata* showed more number of spores (150), while it was only 110 in soil sample analyzed from Rampara. The percentage occurence of AM spores was more in Bandheli. Analysis of AM spores resulted into identification of different species belonging to 3 genera i.e. *Glomus*, *Gigaspora,* and *Acaulospora*. Since *Glomus* was present predominantly in most of the cases it was selected for further study.

Key words: AM Fungi, *Heteropogon contortus*, *Themada triandra, Chloris barbata, Glomus, Gigaspora*

Introduction:

Mycorrhizas are symbiotic association between plant roots and certain fungi. These associations have evolved with plants since the colonization of dry land by plants began as a survival mechanism for fungi and higher plants. Both endure the existing environments of low soil fertility, periodic drought, diseases, extreme temperature and other natural stresses. AM fungi cannot complete their life cycle without host plants. Benefits derived by plants from this relationship includes increase nutrient uptake, more water absorption, increased nutrient mobilization, production of feeder roots, longevity as well as stress tolerance etc. (Manoharachary *et al.*,2008). AM fungi are naturally occurring fungal component of soil microbiota in most of terrestrial ecosystems as well as in some aquatic plants (Stenland and Charvat 1994, Manoharachary *et. al.,* 2008). However, it is important to distinguish between specificity (the ability to colonize), effectiveness (plant response to colonization), and ineffectiveness (the amount of colonization) because AM are widely different in these abilities depending on the environment. They do have wide host ranges, however and are capable of long term relationship with many different plants. In order for this partnership to work at atleast four elements must be in place: a) Appropriate root morphology, b) Fungal structures able to penetrate the plant cell, c) Extra radical mycelia which are root like vegetative fungal structure growing in the soil and d) Soil condition.

AM fungi are the dominant component of the rhizosphere soil and transfer many assimilates to the roots. This alters the root exudation pattern and hence changes the microbial population dynamics of the rhizosphere and rhizoplane regions (Brundrett 2004). The fungi responsible are classified in the phylum Glomeromycota, of order Glomales. They are assumed to be unculturable and, except for germination, wholly dependent on photosynthetic plants. AM fungi are used to be classified as Vesicular Arbuscular Mycorrhizae (VAM) but research uncovered that a major suborder did not form thin walled, lipid filled vesicles, so they are referred to as AM associations today. There is no evidence for specificity between plants and AM fungi (Smith and Read 1997). Mycorrizal fungi act as providers and protectors for plants. For example N,P,K are deficient in certain soils and can be increased in plant intake by mycorrhizae (Norland, 1993) . Other essential nutrients such as Ca, Mg, S ,Fe, Zn, Al & Na have been shown to increase in plants with AM fungi (Daft & Hacskaylo, 1976) .

Baria taluka of Dahod district in Gujarat has 29353.39 ha. as reserved forest area. Possibility of raising trees and grass is very higher here and in certain other areas of the state. Survey was conducted in different areas of Rampara, Kali Talai areas of Baria division Forest in Central Gujarat region, India.The study area falls between in 22° 41' 60 N latitude and 73° 54' 0 E Longitude.

Materials and Methods

Common Grasses selected for the Study include:

1. *Dicanthium annulatum* (Forsk.) Stapf. (Zinzvo)

Stems of this perennial grass usually woody at the base with strong wiry roots and tufted leaves then geniculately ascending. Nodes may be beared or not. Leaves rigid, glaucous, glabrous or hairy above with tubercle- based hairs, sheath beared at tip, ligule oblong, obtuse. Spiklets variable in size 2.5 to 6.2 cm, pinkish or nearly white.

2. *Heteropogon contortus* L. (Nani-Shukali)

Plants are perennial, stems erect from a decumbent rooting base, 50-70 cm tall, branched, compressed, glabrous, seriate and leafy. Leaves are flat, glabrous, acuminate, sheaths shorter than internodes, ligule short, membranous, ciliate. Racemes 8-15 cm long, few to many, axillary and terminal. Sessile spiklets 0.4 -0.5 cm long, small, naked.

3. *Themeda triandra* Forsk. (Butani, Marar)

It is perennial, densely tufted herb. Stem is stout or slender, erect or geniculate and ascending, subsimple or branched, nodes glabrous. Leaves are linear, flat. Sheaths compressed, keeled, smooth. Panicle narrow, reaciform, branched. Involucral spikelets are whorled, sessile, persistent, lanceolate, acute, oblong, glabrous, male, glumes-3. Pedicellate spiklets are linear-lanceolate.

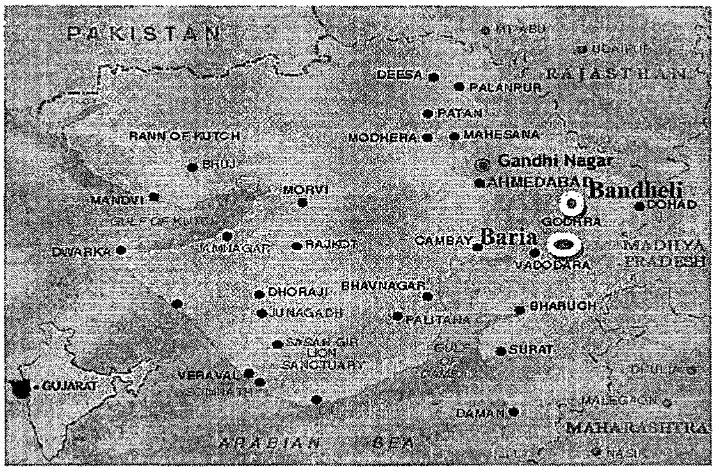
4. *Chloris barbata* L. (Mindadiu, Kaliu)

A member of family Poaceae this grass is perennial. Leaf-sheaths glabrous on surface. Ligule is a ciliolate membrane, leaf-blades involute. Inflorescence is composed of racemes, racemes 2–3; paired or digitate, spreading; unilateral. Spikelets pendulous, solitary, sessile. Spikelets comprising 1 fertile floret; with diminished florets at the apex. Spikelets cuneate, laterally compressed. Glumes persistent; similar; shorter than spikelet; thinner than fertile lemma gaping. Lower glume lanceolate, upper glume has primary vein smooth or scaberulous, in upper glume lateral veins are absent.

Isolation of AM fungi by Wet Sieving and Decanting Method:

Wet Sieving and Decanting method as suggested by Gerdemann and Nicolson (1963) was used to obtain AM spores from rhizospheric soil. The method includes mixing of 100g soil with 1litre of tap water and then decanting it in a stack of sieves. In the present case 5 sieves ranging from 63 to 500 μm were used. The AM spores were separated on different sieves, based on their size. They were then picked up by hypodermic syringe using a stereo microscope. Permanent slides were prepared in polyvinyl alcohol glycerol. Similar types of 5 spores were placed in a vial containing

0.05% streptomycin sulphate for further use. VAM spores were collected, photographed and on the basis of their colour, spore size, identification has been done.



RESULTS:

Table 1: Number of AM spores per 100g isolated from different soil samples of Baria division and economic importance of these grasses.

Sr. No.	Plants	Location	Sample No.	No. of spores/100g soil	Economic Importance
1.	*Heteropogon contortus*	Bandheli, Godhra	A	220	Used as fodder. Roots are diuretic and sometimes used in rheumatism.
		,,	B	137	
		,,	C	205	
		,,	D	150	
		,,	E	110	
		Kalitalai, Baria	F	130	
		,,	G	98	
		Rampara, Baria	H	105	
2.	*Chloris barbata*	Kalitalai, Baria	A	150	Used as fodder in young stage.
		,,	B	97	
		Rampara, Baria	C	85	
		,,	D	110	
3.	*Themada triandra*	Bandheli, Godhra	A	157	Palatable when young and unpalatable when mature. Shows some amount of tolerance to drought.
		,,	B	165	
		Rampara, Baria	C	95	
		,,	D	115	
		Kalitali, Baria	E	97	
		,,	F	120	
4.	*Dicanthium annulatum*	Rampara, Baria	A	85	Highly esteemed fodder grass. Palatable both when young and mature. Controls soil erosion because of its elaborate root system.
		,,	B	125	
		Kalitalai, Baria	C	140	
		,,	D	100	

Table 2: Identification of AM spores based on different morphological characters of 4 different grasses

Sr. No.	Name of AM Fungi	Colour of the Spore	Size (µm)	Wall layers	Thickness of wall (µm)	Thickness of Hyphae (µm)
1	*Glomus glomerulatum* Sieverding	Yellow-Brown	64	2	6.4	---
2	*G. claroides* Schenck& Smith	Yellow-Brown	76.8	2	12.8	---
3	*G. glomerulatum* Sieverding	Yellow-Brown	64	2	6.4	---
4	*G. clarum* Nicolson & Shank.	Yellow-Brown	92.8	2	9.6	---
5	*G. macrocarpum* (Tul.&Tul.) Berch& Fortin	Yellow-Brown	86.4	2	6.4	---
6	”	Yellow-Brown	88.2	2	19.6	---
7	*G. claroides* Schenck& Smith	Yellow-Brown	70.4	2	6.4	---
8	*G. clarum* Nicolson & Shank.	Hyaline	85.7	2	6.4	---
9	*G. etnicatum* Becker & Gerd.	Yellow	83.2	2	6.4	---
10	”	Yellow	73.6	2	6.4	---
11	*G. citricola* Tang. & Zang.	Hyaline-Yellow	57.6	2	6.4	---
12	*G. geosporum* (Nicol. & Gerd.)Walker	Yellow-Brown	294.4	2	9.6	---
13	*G. fasciculatum* (Thaxter) Gerde.& Trappe emend. Walker & Koske	Hyaline-Yellow	124.8	3	9.6	---
14	”	Yellow-Brown	112	2	9.6	---
15	*G. agrregatum* (Schenck Smith) emend. Koske	Yellow-Brown	131.2	2	12.8	---
16	*G. fasciculatum* (Thaxter) Gerde.& Trappe emend. Walker & Koske	Yellow-Brown	92.8	2	9.6	---
17	*Gigaspora albida* Scenck& Smith	Brown	86.4	2	6.4	6.4
18	*Glomus mosseae* Nicol.& Gerd.	Hyaline Yellow	70.4	2	6.4	---
19	*Gigaspora ramisporophora* Spain, Siverding & Schenck	Brown	284.8	2	12.8	12.8

20	*Gigaspora albida* Scenck& Smith	Yellow-Brown	220.8	2	16	12.8
21	*Glomus fuegianum* (Spegazzii)	Yellow-Brown	67.2	2	6.4	---
22	*G. hoi* Berch. & Trappe	Yellow-Brown	73.6	3	9.6	---
23	,,	Yellow-Brown	92.8	3	12.8	---
24	*G. macrocarpum* (Tul. & Tul.) Berch & Fortin	Hyaline-Brown	121.6	2	9.6	---
25	*Gigaspora decipiens* Hall & Abbot.	Brown	259.2	2	9.6	9.6
26	*G. fasciculatum* (Thaxter) Gerde. & Trappe emend. Walker & Koske	Hyaline-Yellow	124.8	3	9.6	---
27	*Gigaspora candida* Bhattacharjee,Mukherji, Tiwari & Skoropad	Brown	204.8	2	16	16
28.	*Acaulospora laevis* Gerde.& Trappe	Yellow-Brown	183.15	3	6.66	6.66

Note: - size of hyphae not recorded
--- = Absent

Table 3: Identification of different spores of *Glomus* based on different morphological characters

Slide No	Name of AM Fungi	Colour of spore	Size (µm)	Wall layers
1	*Glomus fasciculatum* (Thaxter)	Yellow to brown	108.8	3
2	*G. aggregatum* (Shenck. & Smith) emend. Koske	Brown	105.6	2
3	*Glomus etunicatum* Becker & Gerdemann	Yellow to brown	128	2
4	*Glomus monosporum* Gerdemann & Trappe	Yellow to brown	169.7	2
5	*Glomus convolutum* Gerde.& Trappe.	Yellow to brown	83.2	2
6	*G. macrocarpum* (Tul. & Tul) Berch & Fortin	Yellow to brown	108.8	2
7	*G. maculosm* Schenck & Smith	Hyaline to yellow	121.2	2
8	*G .claroides* Schenck & Smith	Yellow to Brown	92.8	2
9	*G. melanosporum* Gerdemann & Trappe	Yellow to brown	157.6	2
10	*G. caledonium* (Nicol. & Gerd) Trappe & Gerdemann	brown	139.3	2
11	*G. intraradices* Schenek & Smith	Yellow to Brown	109.1	2
12	*G. tenerum* (Tandy) Mc Gee	Orange	150.1	2
13	*Glomus geosporum* (Nicol. And Gerd.) Walker	Yellow to Brown	196.9	2
14	*G. hoi* Berch. & Trappe.	Yellow to Brown	112	2
15	*G.mossae* (Nicol & Gerd) Gerdemann & Trappe	Yellow to brown	127.3	2

Conclusions:

It is evident from Tables above that rhizospheric soil of different grasses harbored –

- 15 species of *Glomus*
- 4 species of *Gigaspora*
- Only one species of *Acaulospora*

ACKNOWLEDGEMENTS

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Plate- I

Plate-II

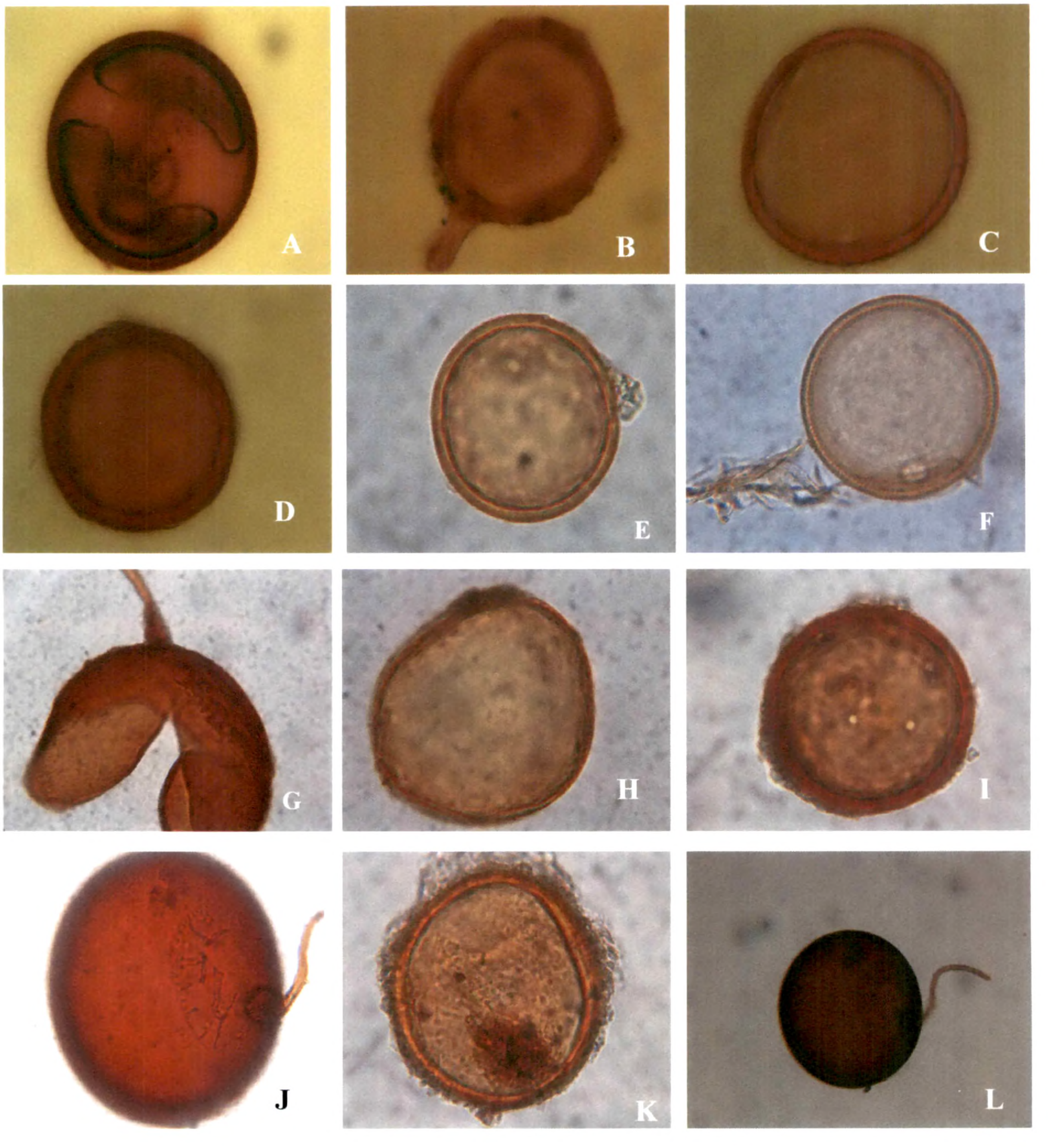

Plate- I

A) & B) - Grasslands of Baria Division
C) *Heteropogon contortus*
D) *Themada triandra*
E) *Dicanthium annulatum*
F) *Chloris barbata*
G) *Glomus aggregatum*
H) *Glomus clarum*
I) *Gigaspora albida,*
J) *Glomus fasciculatum*,
K) *Sclerocystis microcarpa*
L) *Glomus aggregatum*

Plate-II

A) *Glomus melanosporum*
B) *Glomus mosseae*
C) *G.glomerulatum*
D) *G.claroides*
E) *G.citricola*
F) *G. macrocarpum*
G) *Gigaspora candida*
H) *G. etunicatum*
I) *G.geosporum*
J) *Gigaspora ramisporophora*
K) *G.hoi*
L) *Gigaspora decipiens*

P. 14 Role of microorganisms on seedling growth and survival of *Terminalia bellerica* (Gaertn.) Roxb

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Association of Mycorrhizal fungi is known to help the plants in eleviating the stress from deficiency of nutrients, and availability of water. A well known medicinal plant *Terminalia bellerica* locally known as 'Baheda' belongs to the family Combretaceae. The study was conducted over a period of 1 year to explore the effects of different seed treatments on germination and growth of the seedlings. Seeds were subjected to 10 different treatments by using three different gums of *Anogeissus*, *Prosopis* and Gum Arabic. Seed germination was observed after 15 days of sowing. The highest percentage of germination and biomass was observed in the seeds treated with AM fungi and bacteria as compared to the seeds treated with only bacteria or AM fungi. The percentage of increase in biomass was more in microorganism treated seeds.

An effort was made to check the survival rate of *T. bellerica* seedlings by reducing water supply. When the plants were not watered for 10 days, the plants which were treated with AMF survived while all other died.

with TPD also have been studied as the ethephon application is a common practice in rubber plantations to stimulate the latex yield. In healthy trees, stimulation resulted in an increment in the cell divisions and length of fusiform cambial cells. The stimulation on affected bark resulted in triggering of structural aberrations in the cambium. The effect of permanently altered cambial activity on the differentiation of laticifiers with respect to TPD has also been discussed.

TH02-16

Edible Vaccines

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Edible vaccines hold great promise as a cost-effective, easy-to-administer, easy-to-store, fail-safe and socioculturally readily acceptable vaccine delivery system, especially for the poor developing countries. It involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. Introduced as a concept about a decade ago, it has become a reality today. A variety of delivery systems have been developed. Initially thought to be useful only for preventing infectious diseases, it has also found application in prevention of autoimmune diseases, birth control, cancer therapy, etc. Edible vaccines are currently being developed for a number of human and animal diseases. There is growing acceptance of transgenic crops in both industrial and developing countries. Resistance to genetically modified foods may affect the future of edible vaccines. They have passed the major hurdles in the path of an emerging vaccine technology. Various technical obstacles, regulatory and non-scientific challenges, though all seem surmountable, need to be overcome.

TH02-17

Antifungal property of certain plant extracts against two leaf spot pathogens

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Tectona grandis L.f. is economically important forest tree. Teak trees are found prominently in dry decidous forests and produce high quality of wood. Two fungi i.e., *Fusarium*

pallidoroseum (Cooke) Sacc. and *Thielavia subthermophila* Mouch. were isolated from infected leaves of teak. Pathogenecity test for both these fungi resulted in appearance of disease symptoms within 5 – 7days after inoculation. Re – isolation from different inoculated leaves confirmed that both fungi were pathogenic. Considering the non pollutive and biodegradable nature of ecofriendly plant based alternatives, various plant extracts have been tried as botanical pesticides.The inhibitory effects of methanolic and aqueous extracts of plants were assessed against both the pathogens by poisoned food technique. Observations were recorded after 7 days of mycelia growth. Out of 5 plants tested against fungus *T. subthermophila*, *Grevillea robusta* A. Cunn. (83%) recorded highest mean inhibition of mycelial growth followed by *Alangium salviifolium* (L.f.) (66%). Extract of *Polyalthia longifolia* (Sonn.) Thw. (65%) *Heterophragma adenophyllum* Seem. (53%) *Terminalia arjuna* Roxb. (48%) were also effective. For *F. pallidoroseum* maximum inhibition was obtained in leaf extract of *A. salviifolium* (66%) followed by *G. robusta* (53%), *P. longifolia* (51%), *T. arjuna* (39%) *H. adenophyllum* (39%) leaf extracts of *Grevillea* and *Alangium* can be used at commercial scale to control leaf infecting pathogens.

Key words: *Tectona grandis* L., *Fusarium pallidoroseum, Thielavia subthermophila,* leaf extract, *Grevillea robusta*

TH02-18

Comparative study of the effect of bio-fertilizer (AM fungi) on yield of selected crops

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Traditional agriculture relies on the use of chemical fertilizers to provide the soil with the nutrients needed to grow plants. Such use has often proved to be hazardous. Farmers frequently apply more fertilizer nutrients than that are used by the plants, leading to excess nitrogen and phosphate causing various ecological problems. In the contrary, beneficial role of Biofertilizers, (*Rhizobium*, AM fungi) has already been established in nitrogen fixation, mineral cycling, and energy flow enhancing plant growth, plant succession in disturbed and undisturbed ecosystems without causing any harm to the environment. Mycorrhizal association under adverse environmental conditions or in physiologically stressed soil benefits plants by manipulating plants micro-climate. It helps plants by solubilizing and absorbing phosphate, sulphur, potassium, calcium, magnesium, manganese, zinc and copper, essential for plants growth. It increases availability and uptake efficiency of plants for