1. Introduction

The northern part of West Bengal commonly called as North Bengal is endowed with diverse natural resources. It comprises old alluvial, terai and hill zones distributed in six districts of West Bengal viz. Coochbihar, Jalpaiguri, Darjeeling, Malda, Dakshin Dinajpur and Uttar Dinajpur. Location of North Bengal is between $24^0$74' N to $27^0$25' N latitudes and $83^0$07'E to $84^0$40'E longitudes (Plate-1). The most important economic plantation crop of the region is tea. Other than tea the region is also famous for cultivation of solanaceous plants like brinjal and tomato. All the three plants mentioned above are affected by several fungi. The production of the crops depends largely upon improved technologies befitting to the agro-climatic and socio-economic characteristics of this region.

In order to control the diseases, huge amount of chemical fungicides are used indiscriminately every year in the fields of North Bengal. These fungicides often persist on leaves and soil and polluting the adjacent water bodies. The residual effect of the synthetic fungicides on edible parts like leaves and fruits may be harmful for human consumption. Thus the social and environmental costs of synthetic fungicides are high. At present there is an all-round compulsion among the multinational companies and other agencies to go in for bio-rational alternative arsenal, which can be eco-friendly and benign to environment.

Botanical fungicides broadly comprise of secondary metabolites from microbes and higher plants. Based on their mode of action secondary metabolites of higher plants are classified as phytochemicals with antifungal action, phytoalexins and systemic acquired resistance (SAR) inducers. There are evidences of applying phytochemicals in various plants to check different fungal diseases (Rana *et al.*, 1999a,1999b; Khan and Kutiyar, 2000; Natarajan *et al.*, 2001; Hu *et al.*, 1999). The present work aims at exploiting the potential phytochemicals with antifungal action from various sources in checking the damage caused by various fungal diseases in leaves. This will help to broaden the scientific base on which total control of the

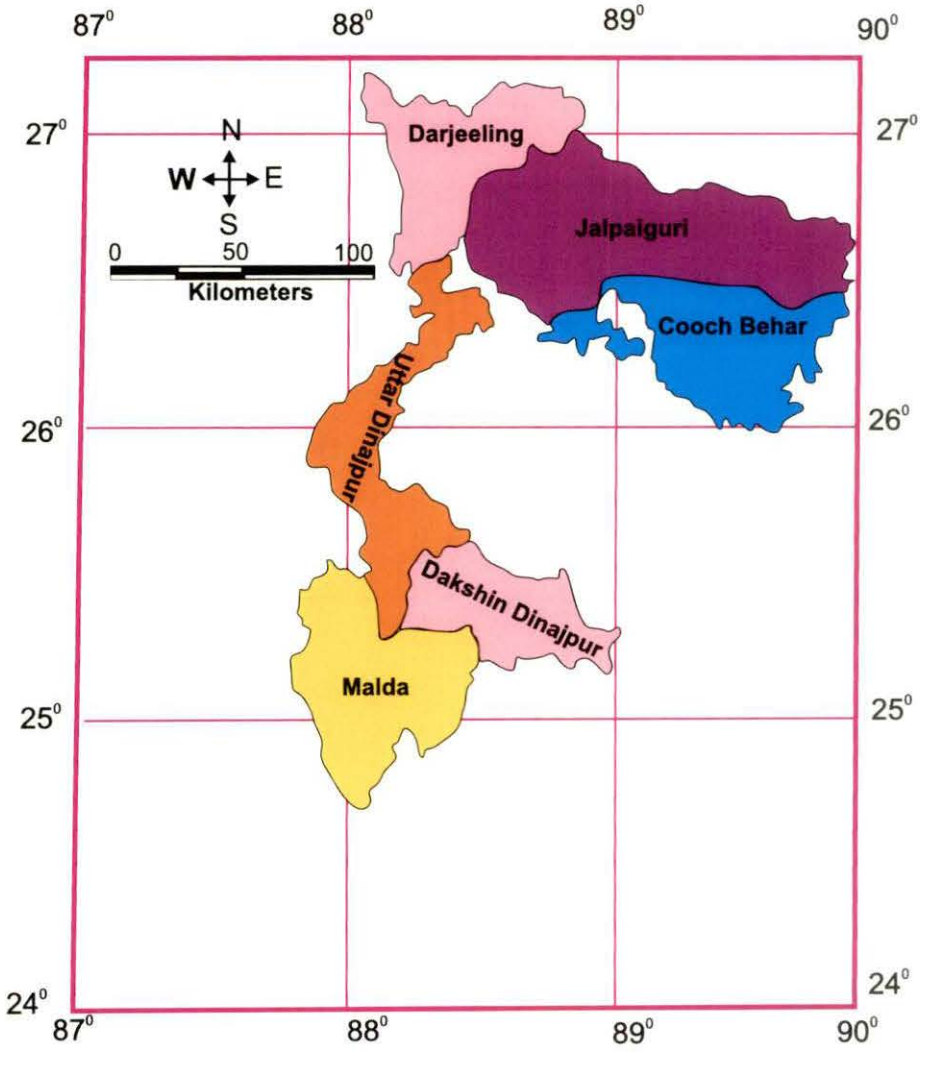

Plate 1: Map of North Bengal showing six Districts (Present study area)

predominant fungal diseases without causing any environmental hazard may be established.

Certain parts of various plant species harbours secondary metabolites, which represent a variety of chemical structures (Bell and Charlwood, 1980). Their roles are mostly unknown though many of them have been found to posses anti-fungal properties. Various types of preformed anti-fungal chemicals such as saponins, unsaturated lactones, cyanogenic glycosides, oils and phenolic compounds are found to be present in relatively large quantities in the tissues of some plant species. Their occurrence, distribution and possible functions have been reviewed by Schlosser (1980, 1988).

Antifungal activities of essential oils from cultivars of *Brassica juncea* have been reported by Shin and Kang (2001). Antifungal agent from the roots of *Cudrania cochinchinensis* against *Candida* and *Aspergillus* sp has been reported by Fukai *et al.* (2003). The plant extracts of *Glycosmis calciocola* and *G. rupestris* was reported as antifungal by Rahamani *et al.* (2004). AL-Howiriny *et al.* (2005) reported three new diterpenes and biological activity of different extracts of *Jasonia monata.* Chemical composition and *in vitro* anti fungal properties of essentil oils from leaves and flower of *Erigeron floribundus* has been studied by Kuiate *et al.* (2005). Antifungal activity of selected species of *Terminalia* and *Curcuma* has been studied by Fyhrquist *et al.* (2004) and Gritsanapm *et al.* (2000). Saxena *et al.* (2003) and Flach *et al.* (2002) showed anti-fungal activity of some plants and also analyzed the antifungal properties chemically. Anti-fungal action of essential oil and other components have been reported by several scientists (Mann and Markhan, 1998; Deena and Thopil, 2000; Demirci *et al.*, 2000; Mathpal *et al.*, 2002 and Simic *et al.*, 2004). Anti-fungal activity of *Tagetes patula* extracts have been shown by Mares *et al.* (2002). Anti-fungal sesquiterpenes from stem bark of *Guarea macrophylla* has also been demonstrated by Logo *et al.* (2002)

Pant *et al.* (1997) found *Oxalis*, *Melia* and *Ageratum* as effective as Dithane M-45 against hill bunt disease but *Tagetes* was not. Aqueous and methanolic extracts of different plants are reported as anti-fungal by Watanabe *et al.* (2001), Chouskey and Srivastava (2001), Digrak *et al.* (1999), Ali *et al.* (2000), Shukla *et al.* (2000),

Khan *et al.* (1999), Bhandari *et al.* (2000), Jetty and Lyengar (2000), Ali *et al.* (1999), Cavin *et al.* (1999), Mouhajir *et al.* (1999), Digrak *et al.* (2001) and Adedeyo *et al.* (2001). Garlic extract has been shown to be a potent anti-microbial substance (Yoshida *et al.* 1999a, 199b). Several authors have reported anti-fungal activity in different crops (Mittal *et al.* 2002, Yasmin and Saxena, 1990, Sharma *et al.* 2002, Yoshida *et al.* 1987, Singh *et al.* 1990, Reimers *et al.* 1993, Singh *et al.* 1995, Parimelazhagan and francis,1999)

Solanum melongena is an economically important and beneficial crop and is grown widely in tropical Asia and Mediterranean countries (Sunseri *et al.* 2003). The plant was cultivated in the Middle Ages, mainly for medical reasons, in the area of South and East Asia. It is a very well appreciated vegetable all over India as well as in the state of West Bengal. It is believed that it has been domesticated in north-east India (Kluza *et al.,* 2000). It grows in almost all the districts of northern part of West Bengal, the present study area. Several brinjal varieties are cultivated throughout the year. With the increase of population, market demand of brinjal is increasing rapidly leading to an increase in the cultivation of the crop. As the cultivation of brinjal is increasing, disease problems associated with the crop are also increasing. A main factor limiting the yield and commercial value of horticultural crops is their susceptibility to diseases. Climatic conditions (high rainfall and very high humidity) are optimally favourable for the pathogens to infect host plant in the present study area. Like many other vegetables brinjal is also subject to attack by many fungi, bacteria, viruses and nematodes. At the onset of the study, several fungi were isolated from infected parts of brinjal plants from fields situated at Phansidewa near Siliguri. Among the isolated fungi two fungi were found to be associated consistently with the brinjal plants grown in the farmer's fields in the present study area. These are *Colletotrichum gloeosporioides* and *Fusarium equiseti.* Although much work has been done in *Alternaria solani* and *Fusarium solani* found elsewhere in the study area but very little studies were done with *Colletotrichum gloeosporioides* and *Fusarium equiseti.*

Tea (*Camellia sinensis*(L.) O. Kuntze) plants are cultivated in three distinct zones in India viz. 'North-East India', 'South India' and 'Himachal Pradesh-Uttaranchal'. Tea plants are exposed to a number of pathogens, mostly fungi that

cause diseases in tea. Among the fungal diseases, the important diseases are blister blight, gray blight, brown blight, black rot and diplodia disease caused by *Exobasidium vexans, Pestalotiopsis theae, Colletotrichum camelliae, Corticium invisum and Lasiodiplodia theobromae* (Pat.) Griffon & Mauble (=*Botryodiplodia theobromae* Pat.) respectively (Sarmah 1960). Among the diseases mentioned grey blight caused by *Pestalotiopsis theae* is most common and reduce production by damaging large number of maintenance leaves. In severe condition the production leaves are also attacked. In the present study measures have been taken to control *Pestalotiopsis theae* by use of botanicals.

The tomato is native to South America and probably originated in the highlands of the west coast of South America (Smith, 1994). In 1753 the tomato was placed in the genus *Solanum* by Linnaeus as *Solanum lycopersicum* L. (derivation, 'lyco', wolf, plus 'persicum' peach, i.e. "wolf-peach"). However, in 1768 Philip Miller placed it in own genus, and he named it *Lycopersicon esculentum.* In 2005, about 125 million tons of tomatoes were produced in the world. China, the largest producer, accounted for about one quarter of the global output. The tomato is now grown worldwide for its edible fruits (Anonymous, 2005).

Large quantity of tomato is produced in north Bengal. Tomatoes are warm season plant growing well with 7 hours of sunlight a day. The consumption of tomato is believed to benefit the heart. They contain lycopene, one of the most natural antioxidants, to help prevent prostate cancer and protect against harmful UV rays. Tomato plants are exposed to a number of pathogens, mostly fungi that cause variety of diseases in tomato. *Alternaria* leaf spot was found consistently in the tomato fields of north Bengal along with other fungal diseases. In the present study, attempts have been taken to control *Alternaria alternata* caused leaf spot disease of tomato.

The application of broad-spectrum chemical fungicides is the common practice in most of the horticultural crops for controlling fungal diseases. The fungicides are extremely hazardous to our health and environment. Therefore, it is essential to adopt eco-friendly methods to control fungal diseases of our vegetable crops. With these observations the present work have been undertaken and it is likely that the results will broaden the scientific base upon which total control of the fungal

diseases of brinjal, tea and tomato may be established through ecofriendly disease management programmes.

On the basis of the above study the present work has been planned with the following objectives.

1. To identify potential plants for the presence of different antifungal compounds, effective against major phyto-pathogens.
2. Screening of plants for potential antifungal properties.
3. Solvent Extraction of plant parts for partial isolation of the active principles.
4. Isolation and identification of major pathogen(s).
5. Bio-assay of the plant extracts.
6. Comparison of different extraction processes for obtaining the antifungal compounds from their sources.
7. Application of selected bio-products in the plants as anti-fungal agents to control the pathogen(s) and assessment of disease reduction.

2. Literature Review

Plants are exposed to a variety of pathogens but they protect themselves from invasion by pathogens using various defense mechanisms. The defense mechanisms include defense barriers (such as cuticle, cell wall, deposition of lignin, callose, phenolic compounds), formation of papillae, synthesis of pathogenesis related proteins (Aist, 1976; Benhamou *et al.*, 1990). Plant responses to fungal attack lead to resistance or susceptibility following interaction between plant and the pathogen. Pathogenesis and disease resistance are closely related to each other. Pathogenesis is related to compatible interactions while resistance is related to incompatible ones. Presently, biological control and botanicals play important role in case of plant disease management as they are environment-friendly. Plants have tremendous ability to synthesize aromatic substances. Most of which are phenols or their oxygen substituted derivatives (Geissman, 1963). Most of them are secondary metabolites and out of which at least 12,000 have been isolated. The isolated number is estimated to be less than 10% of the total aromatic substances (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Some of the substances provide odour or flavor (such as terpenoids) and pigments (such as quinines and tannins). Many other compounds are responsible either for food preservation or used for their medicinal properties.

At the onset of the present study it was considered worthwhile to review the reports presented by the earlier workers. However, the present review will be focused towards use of botanicals in the economically important crops. The observations of the previous workers in concord with the present line of investigation have been presented in the following paragraphs. The review has been done in a selective manner rather than comprehensive one. For convenience, the observations have been grouped into some aspects. The different aspects of this review are:

- Diseases of tomato
- Diseases of tea
- Diseases of brinjal
- Disease control by botanicals.
- Disease control by selected natural chemicals.

Diseases of tomato: It is important to detect pathogens that cause diseases in tomato plants. A number of pathogens cause disease in plants. Among the fungal diseases, the important diseases are early blight disease of leaf, *Septoria* leaf spot, Anthracnose rot, stem canker, Black mold rot, *Cercospora* leaf mold, stem rot, late blight, leaf mold, and wilt diseases caused by *Alternaria solani*, *Septoria lycopersici*, *Colletotrichum gloeosporioides*, *Alternaria alternata* f.sp. *lycopersici*, *Stemphylium botryosum*, *Pseudo cercospora* leaf mold, *Didymella lycopersici*, *Phytopthora infestans*, *Cladosporium fulvum*, and *Fusarium oxysporum*. Leaf blight disease caused by *Alternaria alternata* is one of the common diseases of tomato in North Bengal.

Mercure of University of cunnecticut showed that tomato was attacked by several pathogens like *Oidiopsis taurica*, *Phytophthora infestans*, *Cladosporium fulverum*, and *Botrytis cinerea* (Mercure, 2009). Mercure (2009) reported that gray mold and ghost spot diseases of tomato were caused by the fungus *Botrytis cinerea*. They also reported that older plants were more susceptible. Plants with dense or succulent foliage were most heavily damaged. The disease caused a loss of leaf area and fruit quality (Jones and Jones 1991). They also reported appearance of light tan or gray spots on leaves due to fungal growth. The fungal growth was dense and resembled felt. Elliptical spots formed on the stem where infected leaves met the stem. The cankers were tan with concentric rings.

Jones and Jones (1991) also reported that dying flower petals were very susceptible. The fungus could either kill the flower or, if fruit was set, might grow into the developing fruit and caused a soft rot. The spots were one inch in diameter and were usually greyish or yellowish green with lighter edges. The skin of the fruit usually broke over the decayed areas but remained intact over the rest of the fruit. A dark gray growth of fungus appeared over the spot.

Although, leaf mold disease of tomato occur all over the world, but it has been reported as a greenhouse disease in Connecticut (Mercure 2009; Jones, and Jones 1991). It caused tomato leaves to fall off, which resulted in lower yield. High humidity was required for the fungus to grow successfully. They also described that leaves were the only part of the plant that was affected. Lower leaves were affected first, then younger leaves. Pale-green or yellowish areas appeared on the upper leaf surface which later became distinctly yellow. The mold was more deeply coloured in the centre of the areas. The leaf spots turned yellowish brown with maturity.

Mercure (2009) and Sherf and MacNab (1986) reported a powdery mildew disease caused by the fungus *Leveillula taurica.* The fungus affected tomato, potato, eggplant and cotton. The disease affected outdoor tomatoes, especially those grown in shady areas with poor air circulation, but the disease was more common in the green houses. The shrivelling of the leaves caused a loss of yield.

Late blight is a very devastating disease of tomato, potato and eggplant. It was caused by the fungus *Phytopthora infestans.* In favourable weather the disease progressed very quickly destroying plants and fruits in 2 weeks (Mercure, 2009; Hausbeck, 1997).

Several authors have isolated *Alternaria alternata* consistently from tomato or other solanaceous plants. They identified the fungal pathogen on the basis of morphological characters (Ellis, 1971, Shakir *et al.*1997, Sultana, 1981).

Although *Alternaria alternata* was previously reported in Pakistan as a saprophytic pathogen of tomato causing post harvest losses in high frequency but in 2004 it was reported as one distinct pathotype, producing leaf blight symptoms of tomato (Akhtar *et al.* 2004). They reported that leaf blight disease symptom of tomato (*Lycopersicon esculentum*) on affected plants started with yellowing and browning of the lower leaves. Symptoms often developed from the leaf tips and along the margins of the leaf petiole. Under severe infection, lesions enlarged and coalesced. Concentric circles with dark layers of spores were reported under moist conditions on blighted leaves. Infection under favorable condition was reported to cause severe defoliation with considerable yield losses.

Disease of tea: The parasitic microorganisms cause disease on tea. In 1964, Agnihothrudu reported 385 species of fungal pathogens on tea plants. In 1989, Chen and Chen revised the situation and listed 507 fungi as fungal pathogens. In the same year (1989) Barua reported 190 fungal pathogens from north-east India. However, in the following paragraphs the works on some of the major diseases of tea are being presented.

Wang *et al.* (1990) studied diseases of tea in 6 zones of Zheijiang province of China from 1985 to 1988. Symptoms and biology of pathogens of 20 diseases were described. Among them, 5 of the 20 diseases caused by *Colletotrichum camelliae, Monochaetia camelliae, Pestolotia guepini, Phyllosticta theicola* and *Fusarium ventricosum* were distributed more widely and caused more severe damages.

Barthakur (1994) reported that root diseases caused by *Ustulina zonata, Fomes lamaoensis, Rosellina arcnata* and *Armillaria mellea* and stem diseases caused by *Tunstallia aculeate* and *Poria hypobrunnea* were also common in Darjeeling tea-gardens but they were difficult to control. He also reported that among the common diseases of tea in the hills of Darjeeling, blister blight caused by *Exobasidium vexans* was the most serious.

Park *et al.* (1996) reported the occurrence of gray blight of tea in several tea plantations in Boseung, Chonnam Province, Korea Republic, during 1992-94 after harvesting and pruning of the second crop. Circular to irregularly shaped dark brown spots developed in concentric rings on leaves and black, dot-like acervuli formed randomly on them. The causal fungus of gray blight was identified as *Pestalotiopsis longiseta.* Typical symptoms by *P. longiseta* appeared 11 days after inoculation.

During 1991-1993, Khodaparast and Hedjaroude (1996) surveyed several tea plantations in the north of Iran in order to determine the main fungal diseases of tea. The result of the survey and pathogenicity tests showed that *Botrytis* sp., *Glomerella cingulata, Fusarium solani, Botryodiplodia theobromae, Pestalotiopsis longiseta, P. nattrassii, P. theae, Phyllosticta theacearum* and *Corticium rolfsii* were pathogens of tea.

Among the 12 tea growing districts of Kenya, *Armillaria* root rot disease was most severe in the district, east of rift valley. Investigations showed that infection of tea bushes were primarily by the mycelial growth from residual tree roots and from infected tea roots rather than from rhizomorphs. Inoculum from residual tree in debris in the soil was the most important source of infection in plantations of seed origin (Onsando *et al.*, 1997).

In 1999, Chandramouli gave a brief account on abiotic and biotic problems in the nurseries in India, and suggested several remedial measures against the diseases like stalk rot caused by *Pestalotia theae,* brown blight by *Colletotrichum camelliae*, root rot caused by *Pythium* sp./*Cylindrocladium* sp. or *Fusarium* sp., blister blight caused by *Exobasidium vexans* and leaf spot caused by *Cercosporella theae.* Hu-shuXia (1996) found two highly resistant cultivars to *Pestalotiopsis theae* among the 18 cultivars tested in Anhui Province of China. Based on productivity, disease and pest resistance, the superiority of Fushan variety of green tea over Yabukita (green tea) was reported by Yamaguchi *et al.* (1992).

Diseases of Brinjal: Some common fungal diseases of brinjal are damping-off (caused by *Pythium* sp/ *Fusarium* sp. or *Rhizoctonia solani*), *Phomopsis* blight (caused by *Phomopsis vexans*), anthracnose (caused by *Colletotrichum gloeosporioides*), fruit rot (caused by *Pythium* sp.), southern blight (caused by *Sclerotium rolfsii*), early blight (caused by *Alternaria solani*) and wilt of brinjal (caused by *Fusarium solani*). Fungal diseases are considered as a major factor that adversely affects successful cultivation of the crop.

Anthracnose disease caused by *Colletotrichum gloeosporioides* (penzig) Sacchardo is one of the most common fungal diseases of brinjal. The pathogen attacks leaves and fruits and produces typical anthracnose lesion. Characteristic symptoms are sunken black lesion and blackening of the inner tissues of the affected areas. Fernandes *et al.* (2002) reported 34 isolates obtained from naturally infected garden egg, sweet peeper and eggplant-fruits. Wijesekara *et al.* (2005) isolated twenty *Colletotrichum* isolates from different crops comprising of the species *C. capsici, C. dematium, C. falcatum,* and *C. lindemuthianum* from different geographical location of India. They isolated *C. gloeosporioides* from brinjal plant of Solan region.

Bletos *et al.* (1997) reported that wilt caused by *Verticillium dahliae Kleb.* caused an estimated yield reduction of up to 50%. According to him, it was one of the most destructive diseases of eggplant. Symptoms of the disease were reported to be yellow bronze wilted areas and vescular discolouration. Bueno *et al.* (2000) also conducted studies on wilt caused by *Verticillium dahliae* on eggplant. Kennet et al. (1970) and Kishi (1974) reported that *Fusarium oxysporum f. sp. melongenae* induces vascular wilt disease in egg-plant that led to major yield losses in Asian countries. The symptoms are often confused with those of *Verticillium* wilt (Stravato *et al.*, 1993).

Fusarium equisiti has been isolated by Baird and Carling (1998) from intact senescent cotton primary roots from Georgia.

Gilbert *et al.* (2003) isolated *Fusarium equisiti* from F.H.B.(*Fusarium* head blight) affected wheat plants. Around 8.1% of leaf sections showed presence of *Fusarium equisiti* during 1998-2000.

Disease control by botanicals

Plants naturally synthesize several carbon compounds, basically for their physiological functions or for use as chemical weapons against pathogens, insects and predators (Fatope, 1995). It has been estimated that 70-80% of total world population

largely depend on traditional herbal medicine to meet their primary health care need (Hamayun *et al.*, 2006). Plants have been proved as useful source of several antifungal molecules that are harmless and benign to the environment. There are certain advantages in the deployment of botanical pesticides. These are biodegradable, safe to non-target organisms, renewable and suit to sustainability of local ecology and environment. Moreover, repeated application of fungicides to attain desirable level of disease control has been discouraged by some of the farmers (Singh and Singh, 2005).

Terras *et al.* (1993) noticed synergistic enhancement of antifungal activity of wheat thionins by 2 to 72-folds when combined with albumins of radish or rape. They found antifungal activity against filamentous fungi and some gram-positive bacteria. Permeabilization of the hyphal plasmalemma of thionins was shown to be the mode of action. Soil amendments with crop residues led to build up of allelochemicals and plant nutrients. In a comparative study, it was shown that incorporation of straw was found more effective than burning of straw in containing the symptoms of eye spot disease (*Pseudocercosporella herpotrichiodes*) and sharp eye spot disease (*Rhizoctonia cerealis*) of wheat (Prew *et al.*, 1995).

Kirkegaard *et al.* (1996) while evaluating rape and Indian mustard as companion crop showed that the latter was more effective in minimizing the incidence not only of take-all disease of wheat but also *Rhizoctonia solani, Pythium* and *Cochliobolus sorokiniana.* The tissue extract of Indian mustard was equally effective and hence the role of volatile isothiocyanates was implied. Certain phytochemicals like gallic acid and absicic acid have been shown to be antifungal. Absicic acid was shown to inhibit mycelial growth and sporidial formation and also germination of teliopores (Singh *et al.*, 1997)

Bianchi *et al.* (1997) tested *Fusarium solani, Colletotrichum lindemuthianum, Pythium ultimum* and *Rhizoctonia solani* and found that garlic extracts inhibited mycelial development *in vitro.* They also used aqueous extract of powdered oven-dried (35 °C) garlic bulbs incorporated into the growth medium and reported that the hyphae of *R. solani* and *C. lindemuthianum* showed collapse and for *F. solani* hyphae appeared thinner than in controls.

Ali *et al.* (1999) screened hexane and methanol extracts of sixteen plants of the family Caesalpiniaceae, of Pakistan and tested their antibacterial and antimicrobial activity. As compared to hexane extracts, the methanol extracts of all the examined

plants showed stronger growth inhibition against bacteria and fungi. *Cassia* species was reported to be more active among the plants tested. Ethanol extract of *Melia azadirachta* fruit showed fungistatic (MIC 50-300 mg/ml) and fungicidal (MFC60-500 mg/ml) activity against *Aspergillus flavus, Fusarium moniliforme, Microsporum canis* and *Candida albicans* (Carpinella *et al.*, 1999).

Digrak *et al.* (1999) studied the antimicrobial activities of *Valex* (the extract of Valonia), the extracts of *Mimosa* bark, gullnut powders, *Salvia ancheri* Var. *ancheri* and *Phlomis bourgeie*. The results of the study indicated that *Mimosa* bark extracts had the maximum antibacterial activity, followed by the *Valex,* gullnut powders, *Salvia ancheri* var. *ancheri* and *Phlomis bourgeie* extracts. Furthermore, it was found that gullnut powders and the extracts of *Mimosa* bark contained high amounts of tannins and showed antifungal activity.

Ke *et al.* (1999) collected two hundred and four species of traditional Chinese herbal medicines belonging to 80 families from Yunnan Province of China and tested for antifungal activities using a *Pyricularia oryzae* as a test organism. Twenty-six herbal medicines of 23 families were active against *P. oryzae* and the ethanol extract of *Dioscorea camposita* (dioscoreaceae) exhibited best bioactivity among the samples tested.

Baka (2010) used six medicinal plant extracts (*Amaranthus spinosus, Barbeya oleoides, Clutia lanceolata, Lavendula pubescens, Maerua oblongifolia* and *Withania somnifera*) of Saudi Arabia to control diseases of vegetable crops caused by fungi viz. *Alternaria brassicae, Alternaria solani, Botrytis fabae, Fusarium solani* and *Phytophthora infestans*. Among the extracts *L. Pubescens* was reported to be best in controlling all the phytopathogenic fungi tested. Similarly, Mdee *et al.* (2009) made acetone extracts of seven common plant species of South Africa and tested against ten different phytopathogens. They also determined minimum inhibitory concentrations of the extracts and suggested use of the extracts in the crops subject to verification of nondeleterious effects of the compounds.

Yoshida *et al.* (1999a) isolated three thiosulfinates with antimicrobial activity from oil-macerated garlic extract and their structures were identified by them as 2-propene-1-sulfinothioic acid S-(Z,E)-1-propenyl ester [AIIS(O)SPn-(Z,E)], 2-propene sulfinothioic acid S-methyl ester [AIIS (O)SMe] and methane sulfinothioic acid S-(Z,E)-1-propenyl ester [MeS(O)SPn-(Z,E)]. Antimicrobial activities of AIIS (O) SPn-

(Z, E) and AIIS (O) SMe against gram positive and gram negative bacteria and yeasts were compared with 2-propene-1-sulfinothioicacids-2-propenylester [AIIS(O)SAII, allicin]. Antimicrobial activity of AIIS (O) S Me and All S(O)S Pn-(Z,E) were comparable and inferior to that of allicin, respectively. In another study, Yoshida *et al.* (1999b) isolated and identified an organosulfur compound from oil-macerated garlic extract by silica gel column chromatography and preparative TLC. The antimicrobial activity of isoE-10-DA was inferior to those of similar oil-macerated garlic extract compounds such as E-ajoene, Z-ajoene and Z-10-DA.

Demirci *et al.* (2000) collected the leaves of five *Betula* species, *B. pendula, B. browicziana, B. medwediewii, B. litwinowii* and *B. recurvata* from different parts of Turkey. The leaves were hydro distilled to yield the consequent essential oils. The essential oils showed antifungal activity against various phytopathogenic fungi like *Cephalosporium aphidicola, Drechslera sorokiniana, Fusarium solani* and *Rhizoctonia cereals.*

Limonene is the major constituent of essential oil of exocarpic part of *Citrus sinensis* which possessed strong and broad-spectrum antifungal activity against important fungal pathogens of sugarcane (Rao *et al.*, 2000). Limonene could completely inhibit the mycelial growth of *Ceratocystis paradoxa* at 2000 ppm and that of *Fusarium moniliforme* and *Curvularia lunata* at 3000 ppm concentration. Rao *et al.*, 2000 also reported that the minimum concentrations which could inhibit the growth of the fungi was non-phytotoxic to sugarcane germination and growth.

Ogunwande *et al.* (2001) analysed methanol extracts from leaves, stem bark, root bark, fruits and seed kernels of *Butyrospomum pradoxum* (*Vitellaria paradoxa*) and reported the presence of alkaloids (in leaves and stem barks), flavones (in stem and root bark), saponins (in root bark), steroids (in stem bark, fruits and seed kernels) and tannins (in leaves and root bark) which have antimicrobial activity against bacteria (*Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Ralstonia solanacearum* and *Bacillus cereus*) and fungi (*Fusarium oxysporum* and *Candida albicans*).

Jaspers *et al.* (2002) tried to control *Botrytis cinerea* leaf colonization and bunch rot of grapes with essential oils in laboratory and field. In detached lateral experiments, the essential oils from thyme (*Thymus vulgaris* L.) and clove (*Syzygium aromaticum* L.), as well as massoialactone (derived from the bark of the tree

Cryptocarya massoia R.Br.) were not phytotoxic on leaves at concentrations of 0.33% or less. *B. cinerea* sporulation on artificially induced necrotic leaf lesions was significantly reduced by thyme (Thyme R) and masoialactone oils at 0.33%. A single application of either compound at concentrations of 0.33% controlled bunch rot and necrotic leaf lesion colonization by *B. cinerea.* Spray applications of Thyme R oil (0.33%) at 8-10 day intervals from flowering to harvest controlled *B. cinerea* bunch rot but also made senesce to floral tissues.

Bautista-Banos *et al.* (2003) evaluated the *in vitro* fungicidal effect of chitosan and aqueous extracts of custard apple leaves, papaya leaves, papaya seeds, and the combination of chitosan and plant extracts on the development of *Colletotrichum gloeosporioides,* causative agent of anthracnose on papaya. They found that chitosan had a fungicidal effect on *C. gloeosporioides.* Extracts alone did not show any fungicidal effect while the combination of 2.5% chitosan with all the tested extracts had a fungistatic rather than fungicidal effect. Changes in the conidial morphology of *C. gloeosporioides* were observed with 1.5% chitosan concentration after 7 h incubation. For *in situ* studies, control of anthracnose disease was obtained with 1.5% chitosan applied before *C. gloeosporioides* inoculation.

Almada-Ruiz *et al.* (2003) evaluated antifungal activities of four polymethoxylated flavons, isolated from cold-pressed orange oil against *Colletotrichum gloeosporioides,* a major plant pathogen of fruits that causes significant damage to crops in tropical, sub-tropical and temperate regions. They noticed that methoxylated flavones were effective in inhibiting mycelial growth of the fungus. Complete inhibition of the growth of the pathogenic fungus *C. gloeosporioides* was observed at a concentration of 100 $\mu g\ ml^{-1}$

Curtis *et al.* (2004) reported that garlic extract showed activity against the plant pathogenic bacteria *Agrobacterium tumefaciens, Erwinia carotovora, Pseudomonas syringae* pv. *maculicola, P.s.* pv. *phaseolicola, P.s.* pv. tomato, *Xanthomonas campestris* pv. *campestris,* the fungi *Alternaria brassisicola, Botrytis cinerea, Plectosphaerella cucumerina, Magnaporthe grisea,* and the oomycete *Phytophthora infestans.*

Peraza-Sánchez *et al.* (2005) screened seven Yucatecan plant extracts to look for fungicidal activity for the control of *C. gloeosporioides.* Bioassay-directed purification of the root extract of one of the most active plants, *Acacia pennatula,*

resulted in the isolation of the new compound 15,16-dihydroxypimar-8(14)-en-3-one (1). The isolated compound showed inhibitory activity on growth, sporulation, and germination of the fungus in "agar dilution" bioassay *in vitro*.

Deepak *et al.* (2005) used methanolic extracts of forty plant species commonly growing across India and screened for antisporulant activity against *Sclerospora graminicola*, the causative organism of pearl millet downy mildew. The methanolic extracts of nine species did not show any effect, whereas the activity of the extracts of *Clematis gouriana, Evolvulus alsinoides, Mimusops elengi, Allium sativum* and *Piper nigrum* were commensurable to that of the marketed botanical fungicides. The extracts of 11 species (*Agave americana, Artemisia pallens, Citrus sinensis, Dalbergia latifolia, Helianthus annus, Murraya koenigii, Ocimum basilicum, Parthenium hysterophorus, Tagetes erecta, Thuja occidentalis* and *Zingiber officianale*) exhibited remarkable antisporulant effect even after 10-fold dilution of the crude extracts. But in the case of remaining 15 plants the crude extracts lost activity after 10-fold dilution. The antisporulant activity of commercialised *Azadirachta* preparation (Nutri-Neem) was more pronounced than that of Reynutria based on (Milsana) and Sabadilla (veratrin).

Guleria and Kumar (2006) searched for bioactive compounds from lipophilic leaf extracts of medicinal plants used by Himalayan people. They screened antifungal properties by direct bioautography. *Alternaria alternata* and *Curvularia lunata* were used as test organism in bioautography. The results were evaluated by the diameter of the fungal growth. They showed five effective plant species with antifungal activity among the 12 investigated. They used $CHCl_3$: CH_3OH (1:9, v/v) as a solvent to develop silica gel TLC plates. Clear inhibition zones were observed for lipophilic extracts of *Vitex negundo* (R_f 0.85), *Zantoxylum alatum* (R_f 0.86), *Ipomea carnea* (R_f 0.86), *Thuja orientalis* (R_f 0.80) and *Cinnamomum camphora* (R_f 0.89). The best antifungal activity was shown by lipophilic leaf extract of *T. orientalis*.

Kiran *et al.* (2006) screened thirty plant extracts (aquous extract) against the pathogen *Sclerotium rolfsii in vitro* to examin the inhibitory effect on mycelial growth and sclerotial production. Maximum inhibition (74%) of mycelial growth was recorded at 10% concentration of plant extract (*Prosopis juliflora)*. Other two antifungal plant extracts were from *Agave americana* (showed 68% overall inhibition) and *Nerium indicum* (showed 54% overall inhibition). The inhibition (94%) of sclerotial production was exhibited by *Agave americana* and almost similar inhibition was shown by

Clerodendrum inerme leaf extracts. Leaf extract of *Riccinus communis* and fruit extract of *Riccinus communis* also gave 72% inhibition.

Reddy *et al.* (2007) reported the antifungal component of cloves. They isolated, characterized and tested the efficacy of cloves against *Aspergillus* spp. The major component, eugenol was identified on TLC plate as dark coloured spot with R_f 0.5 along with standard. In TLC plate bioautography test, TLC plates were spray inoculated with four species of *Aspergillus* (*A. flavus*, *A. paraciticus*, *A.niger*, *A. ochraceus*) and eugenol on TLC plates inhibited mycelia growth of all four species of *Aspergillus*.

Meena *et al.* (2007) evaluated partly purified seven plant extracts viz. mahua flowers, Satyanashi leaves, bitter temru fruits, neem gloy stems, amaltas leaves, tichoma pods and crusted apple seeds and four antimicrobial chamicals were tested against bacterial plant pathogens namely *Pseudomonas solanacearum*, *Xanthomonas campestris* pv. *Campestris*, *Xaxonopodis* and *Xanthomonas* pv. *Citri* by disc diffusion technique. Product componantes from mahua flowers and Satyanashi leaves were found effective against *Pseudomonas solanacearum* at 1000 ppm.

Mewari *et al.* (2007) screened two mosses viz. *Entodon plicatus* C. Muell and *Rhynchostegium vagans* jaeg for their antimicrobial activity against *Bipolaris sorokiniana* (Sacc. and Sorok), *Fusarium solani* (Mart.) Sacc.(fungi) and *Pseudomonas sclanacearum*, *Xanthomonas oryzae* pv.*oryzae* (bacteria). Aquous extracts of the two mosses were found to be ineffective. Ethanolic extracts of *E. plicatus* showed maximum inhibition (42%) and petroleum ether extract of *R.vagans* exhibited maximum inhibition (45%) of *B. Sporokiniana.* Extracts of *R. vagans* were found to be more effective inhibitors of *F. solani* than those of *E. plicatus*. Ethanolic extract of *R.vagans* showed maximum inhibition (44%) of *F. solani* whereas alcoholic extracts of both the mosses showed more effective antimicrobial activity.

Raghavendra *et al.* (2009) reported that Phyton-T, an extract of seaweed (*Sargassum wightii*) reduced late blight disease incidence and induced defense enzymes against *Phytophthora infestans* (late blight pathogen of potato) and enhanced quality of potato. Tuber soaking and foliar spray in combination with Phyton-T (0.4%) and mancozeb (0.3%) for thrice at 15 days interval reduced the disease incidence up to 80%.

Phytochemical compound and antimicrobial properties of methanolic extracts of *Aspilia mossambicensis* (Compositae) were evaluated by Musyimi *et al.* (2008) against clinical strain of *Streptococcus pyogenes* (gram positive) and *Salmonella typhi* (gram negative) bacteria and one strain of fungi *Aspergillus niger*. Methanolic plant extract of leaves was found to be more active against the three microorganisms than the root extract.

Ravindra and Kumar (2007) evaluated the antifungal activities of acetone, hexane, dichloromethen and methanol extracts of leaves of four plant species (*Acacia pennata*, *Anaphylis wightiana*, *Capparis pepiaria* and *Catunaregum spinosea*) against pathogen viz. *Candida albicans*, *Kluyeromyces polysporus*, *Aspergillus niger*, *Aspergillus fumigantus*. High antifungal activity was observed with methanolic extract of *Anaphylis wightiana* against all the test pathogens with the MIC values ranging from 0.02 to 0.06. Methanolic extract of *Acacia pennata*, *Anaphylis wightiana*, *Capparis pepiaria* have very stroung antifungal activity against tested pathogens particularly *C. albicans* and *K. polysporus*.

Essential oils of peppermint *Mentha piperita* L. (Lamiaceae), which were used in flavours, fragrances, and pharmaceuticals, were investigated by Iscan *et al.*, (2002) for their antimicrobial properties against 21 human and plant pathogenic microorganisms. The bioactivity of the oils (menthol and menthone) was compared using the combination of *in vitro* techniques such as micro dilution, agar diffusion, and bio-autography. It was shown that all of the peppermint oils screened strongly inhibited plant pathogenic microorganisms, whereas human pathogens were only moderately inhibited. Chemical compositions of the oils were analyzed by GC and GC/MS. Using the bio-autography assay, menthol was found to be responsible for the antimicrobial activity.

Vukovic *et al.* (2007) examined the chemical composition of essential oil and the *in vitro* antimicrobial activities of essential oil and methanol extract of *Teucrium montanum*. The inhibitory effects of essential oil and methanol extracts of *T. montanum* were tested against 13 bacterial and three fungal spcies by using disc-diffusion method. GC/MS analyses revealed that essential oil contains mainly delta-cadinene (17.19%), β-selinene (8.16%), α-calacorene (4.97%), 1,6-dimethyl-4-(1-methylethyl)-naphthalene (4.91%), caryophyllene (4.35%), copaene (4.23%), torreyol (3.91%), 4-terpineol (3.90%), cadina-1, 4-diene (3.39%), β-sesquiphellandrene (3.34%), τ-cadinol (3.12%)

and γ-curcumene 93.18%). The essential oil has antibacterial as well as antifungal effect.

Gupta *et al.* (2008) assessed the antimicrobial activities of cinnamon (*Cinnamomum zeylanicum*) extract (50% ethanol) and its oil, and compared their effectiveness against ten bacteria (seven Gram-positive and three Gram-negative) and seven fungi by agar well diffusion assays. Cinnamon oil exhibited a broad spectrum of antagonistic activity against bacteria and fungi, as compared to its extract. The oil was found to be very effective with a lowest minimum inhibitory concentration (MIC) of 1.25% (v/v) against *Bacillus* sp., *Listeria monocytogenes*, *E. coli* and *Klebsiella* sp. Amongst the fungi, *Rhizomucor* sp. was found to be highly sensitive to the oil. They showed that cinnamon oil was a more potent antimicrobial agent than cinnamon extract and they suggested it to be a potential bio preservative.

Wang *et al.* (2003) isolated from the bulbs of the onion *Allium cepa*, a novel antifungal peptide distinct from the antimicrobial peptide previously reported from onion seeds. The antifungal peptide, designated as allicepin, was purified with a procedure that involved aqueous extraction, ion exchange chromatography on DEAE-cellulose, affinity chromatography on Affi-gel blue gel and FPLC-gel filtration on Superdex 75. Allicepin was unadsorbed on DEAE-cellulose and adsorbed on Affi-gel blue gel. The molecular weight of allicepin was estimated to be 10K by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and gel filtration of Superdex 75. Allicepin exerted an inhibitory activity on mycelial growth in several fungal species including *Botrytis cinerea, Fusarium oxysporum, Mycosphaerella arachidicola* and *Physalospora piricola.*

Zhang *et al.* (2009) identified essential oils of the aerial parts of *Ocimum basilicum,* an endemic medicinal plant growing in China. They obtained the oil by hydrodistillation and then analyzed by GC-MS. Fifteen compounds were found representing 74.19% of the total oil. The main components were as follows: linalool (29.68%), Z-cinnamic acid methyl ester (21.49%), cyclohexene (4.41%), alfa-cadinol (3.99%), 2,4-diisopropenyl-I-methyl-I-vinylcyclohexane (2.27%), 3,5-pyridine-dicarboxylic acid, 2,6-dimethyl-diethyl ester (2.01%),bita-cubebene (1.97%), guaia-1(10), 11-diene (1.58%), cadinene (1.41%) (E) cinnamic acid methyl ester (1.36%) and beta-guaiene (1.30%). The essential oils showed significant antifungal activity against some plant pathogenic fungi.

Bajwa *et al.* (2007) determined antifungal activities of shoot and root extracts of two Asteraceous plant species viz, *Parthenium hysterophorus* L. and *Ageratum conyzoides* against *Macrophomina phaseolina* (Tassi) Goid., the pathogen of charcoal rot disease of sunflower (*Helianthus annus* L.). A measured reduction in *M. phaseolina* biomass was observed due to aqueous extracts of different concentrations. Lowest concentration of 2% of both root and shoot extract of *P. hysterophorus* markedly suppressed the biomass. But 4% root and shoot extract of *A. conyzoides* was most effective.

Ahmed and Beg (2001) Studied ethanolic extracts of 45 Indian medicinal plants traditionally used in medicine for their antimicrobial activity against certain drug-resistant bacteria and a yeast *Candida albicans* of clinical origin. Of these, 40 plant extracts showed varied levels of antimicrobial activity against one or more test bacteria. Anticandidal activity was detected in 24 plant extracts. Overall, broad-spectrum antimicrobial activity was observed in 12 plants (*L. inermis*, *Eucalyptus sp.*, *H. antidysentrica, H. indicus, C. equistifolia, T. belerica, T. chebula, E. officinalis, C sinesis, S. aromaticum* and *P. granatum*). No correlation was observed between susceptibility of test strains with plant extracts and antibiotic resistance behaviour of the microbial strains (*Staphylococcus aureus, Salmonella paratyphi, Shigella dysenteriae, Escherichia coli, Bacillus subtilis, Candida albicans*). Qualitative phytochemical tests, thin layer chromatography and TLC-bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents.

Shafique and Shafique (2008) investigated antimycotic potential of *Datura metel* (syn. *Datura alba* Nees.) *in vitro* against *Ascochyta rabiei*, the cause of chickpea blight disease. The pathogen was exposed to four different concentrations (1%,2%,3% and 4% w/v) of shoot and root extracts (made in n-hexane) of *D. metel* in an experiment, performed following poison food technique. All the employed concentrations of both root and shoot extracts significantly suppressed the growth of the target fungal pathogen. There was 28-34% and 23-41% reduction in colony diameter of *A. rabiei* due to different concentrations of n-hexane shoot and root extracts of *D. metel*.

Nagaraja *et al.*, (2008) assessed the antimycotic activity of *Acacia catechu* Willd. extracts using three different solvents (ethanol, acetone and hexane).

Antimycotic activity was screened against chosen fungi like *Asp;ergillus niger, Fusarium oxysporum, Alternaria alternata, Rhizopus stolonifer* and *Macrophoma phaseolina* following agar well diffusion technique. The maximum inhibition was recorded in ethanol, acetone and hexane roots extracts. Growth of *A. niger* was controlled by acetone extract of bark, where as *F. oxysporum, A. Alternata, R. stolonifer* and *M. phaseolina* by acetone extracts of *A. catechu* extract. They suggested that the extracts could be utilized for the management of the fungi mentioned. They recommended isolating, identifying and integrating the bioactive principle in the management programme of the pathogens mentioned.

Irobi and Daramola (1993) investigated antifungal activities of *Mitracarpus villosus* leaves and inflorescence by agar-diffusion and tube-dilution techniques. Ethanolic extracts produced definite antifungal activities against *Trichophyton rubrum, Microsporum gypseum, Candida albicans, Aspergillus niger* and *Fusarium solani.* The zones of inhibition produced by the ethanol extracts ranged from 10 to 20.5mm while ketoconazole control ranged from 9 to 19 mm. The minimum inhibitory concentration of the extracts ranged from 0.50 to 4.0 mg/ml while their minimum fungicidal concentration values ranged from 1 to 8 mg/ml. Their results indicated that the extracts were fungistatic at lower concentrations and fungicidal at higher concentrations.

Adiguzel *et al.* (2007) reported the *in vitro* antimicrobial activities of the essential oil and methanol extract from *Satureja hortensis* as well as the content of its essential oil. The chemical composition of hydrodistilled oil of *Satureja hortensis* was analysed by means of GC-MS. Thirty constituents was identified. The main constituents of the oil were thymol (40.54%), gama-terpinene (18.56%), carvacrol (13.98%) and p-cemene (8.97). The essential oil of *Satureja hortensis* exhibited the activity against 25 bacteria, 8 fungi, and one yeast, *C. albicans*; exerting the MIC ranging from 15.62 to 250 µl/ml. Similarly, methanol extract of the plant also showed antimicrobial activity.

Dongmo *et al.* (2008) studied that growth inhibitory effect of *Eucalyptus saligna* and *Eucalyptus camaldulensis* essential oils on *Phaeoramularia angolensis* on agar medium. The mycelium growth was completely inhibited at 6000 ppm and 6500 ppm for *E. saligna* and *E. camaldulensis* respectively. The fractionation of the two oils by column chromatography allowed the abstention of fractions with very few components which were also tested on the mycelium growth. The chemical

composition carried out by GC/MS revealed 1,8-cineole as the major component of these two oils. For *E. saligna*, fraction 5 rich in 1,8-cineole as the major component of these two oils. For *E. saligna*, fraction 5 rich in 1,8-cineole (92.05%) and fraction 10 rich in terpinen-4 ol (75.5%) showed total inhibition at 6000 ppm and 1500 ppm respectively. The fraction 4 and 5 of *E. camaldulensis*, relatively poor in 1,8-cineole (11.20 and 19.20 respectively) with a total absence of others oxygenated monoterpens did not show total antifungal potential of *Eucalyptus Saligna* and *Eucalyptus Camaldulensis* essential oils from Cameroon against *Phaeoramularia angolensis*.

Iqbal *et al.*, (2001) assayed antifungal activity from water extracts of some common weeds. The water extracts from the weed species (*Ageratum conyzoides, Oxalis corniculata, Phyllanthus debilis, Vernonia cinerea* and *Desmodium trifolium.* The extract from *Ageratum conyzoides* inhibited the mycelial growth of *Rhizoctonia solani, Aspergillus niger* and *Phomopsis theae*. The extract from *Oxalis corniculata* was active against *A. niger* while, *Phyllanthus debilis* suppressed the growth of *P. theae*. The activity generally declined after three days of incubation, while *A. conyzoides* remained active up to nine days after incubation.

Wanchaitanawong *et al.*, (2005) investigated antifungal activity of Thai herb and spice extracts against food spoilage fungi *Aspergillus niger, A. oryzae* and *Pencillium* sp. by using agar well diffusion method. Thirteen plant extracts were tested. Crude ethanol extracts of three plants (*Piper betel, Boesenbergia pandurata, Andrographis paniculata*) exhibited antifungal activity against the test microorganisms. *Penicillium* sp. was more resistant to the extracts than *A. niger* and *A. oryzae*. The antifungal index of the selected plant extracts with various concentrations against test fungi was measured by agar dilution assay. With the increase of the concentration, the antifungal activity also increased. A complete fungal inhibition was observed when Piper betel extract concentration 1.5% (v/v) was used. The antifungal index from the other plants ranged between 30 to 60%.

Dabur *et al.* (2005) isolated a novel compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2yl)-1- methylethyl pentanoate from the plant *Datura metel* L. The *in vitro* activity of this dihydrophrrole derivative against *Aspergillus* and *Candida* species was evaluated by using standard methods approved by the National Committee for Clinical Laboratory Standards. The compound was found to be active against all the species tested, namely *Candida albicans, Candida tropicalis, Aspergillus fumigatus,*

Aspergillus flavus and *Aspergillus niger*. The MIC at which more than 90% of growth was inhibited by the compound ranged from 21.87 to 43.75 μg ml^{-1} against various fungal species by micro broth dilution assay.

Dabur *et al.* (2007) screened antimicrobial activity of some Indian medicinal plants potential of seventy-seven extracts from twenty-four plants against eight bacteria and four pathogenic fungi, using micro broth dilution assay. Water extracts of *Accacia nilotica, Justicia zelanica, Lantana camara* and *Saraca asoca* exhibited good activity against all the bacteria tested and the MIC was recorded in range of 9.375-37.5 g/ml and 75.0-300.0 g/ml against the bacterial and fungal pathogen, respectively. The other extracts of *Phyllanthus urinaria, Thevetia nerifolia, Jatrpha gossypifolia, Saraca asoca, Tamarindus indica, Aegle marmelos, Acacia nilotica, Chlorophytum borivilianum, Mangifera indica, Woodfordia fruticosa* and *Phyllanthus embelica* showed antimicrobial activity in a range of 75-1200 μg/ml.

Prusky *et al.* (1982) isolated from peels of unripe avocado fruits and identified as 1-accetoxy-2-hydroxy-4-oxo-heneicosa-12, 15-diene. The compound inhibited *in vitro* vegetative growth of *Colletotrichum gloeosporioides* and totally inhibited spore germination at 790 μgml^{-1}. Concentration of the diene in peels of unripe fruits were as high as 1,200 $\mu g.g^{-1}$ fresh weight of peel (~1,600 $\mu g.ml^{-1}$), but these decreased during ripening to about 120 $\mu g.g^{-1}$ fresh weight of peel (~160 $\mu g.ml^{-1}$). Concentrations of the compound decreased differentially during the ripening of two cultivars of avocado fruits that differed in rates of development of symptoms caused by *C. gloeosporioides*. The evidence supports the hypothesis that the antifungal compound is the basis for latent infection of *C. gloeosporioides* in unripe avocado peel and that subsequent active infections result from its metabolism during ripening.

Hassanein *et al.*, (2008) studied of leaf extracts of neem (*Azadiracta indica*) and chinaberry (*Melia sp.*) against two tomato pathogenic fungi *Alternaria solani* and *Fusarium oxysporum*, the causal agents of early blight and wilt diseases of tomato plant respectively. Leaf extracts of different concentrations of aqueous, ethanol and ethyl acetate of neem and chinaberry were done. In case of neem inhibition percentages were 17.88%, 23.66, 52.77% and 70.55% for *A. solani* in the four concentrations used, while those for *F. oxysporum* were 14.77%, 23.88%, 31.22% and 100%, respectively. The corresponding values with chinaberry leave extracts were 3.11%, 5.22%, 5.33% and 5.77%, recorded for *A. solani* and 5.44%, 6.11%, 6.35 and 6.55% and 6.55% for *F.*

oxysporum. Both ethanol and ethyl acetate extracts of neem leaves assayed at a concentration of 20%, completely suppressed the growth of *F. oxysporum* and inhibited *A. solani* at 52.44% and 62.77% concentrations respectively. The same extracts from chinaberry (20%) slightly inhibited the growth of both pathogenic fungi and values of inhibition not exceeded the 7%. All used concentrations of neem extract effectively suppressed the mycelial growth of both pathogenic fungi and this effect gradually increased with increasing concentration. Tomato plants Sprayed with 20% aqueous neem leaf extracts lowered the disease incidence to 42.54% in pathogenicity (*in vivo*) test of *A. solani*. Highest percentage (100%) of seed germination was recorded in neem extract supplemented experimental set in presence of *F. oxysporum* in comparison to nonsupplemented experimental set where germination was observed as 70%.

Prakash (2006) tested five plant extracts for the presence of microbicidial activity by Kirby-Bour method in agar gel plates using poultry pathogenic *E. coli* as microbes. Among the five herbs used two herbs (*Piper betel* and *Cassia auriculata*) found to have microbicidal activity. The potency of microbicidal activity of that two herbs were tested quantitatively by using different dilutions and in different solvents, clinical experiments conducted in birds with infection and found that the plant *Cassia auriculata* have had more potent microbicidal activity when compared to *Piper betel*.

Pelegrini *et al.* (2006) studied an antifungal peptide from passion fruit (*Passiflora edulis*) seeds with similarities to 2s albumin proteins. Authors purified and characterized a novel plant peptide of 5.0 kDa, pe-AFP1, from the seed of passion fruit (*Passiflora edulis*). Purification was achieved using a Red-Sepharose Cl-6B affinity column followed by reversed-phase chromatography on Vydac C18-TP column. *In vitro* assays indicated that Pe-AFP1 was able of inhibiting the development of the filamentous fungi *Trichoderma harzianum, Fusarium oxysporum,* and *Aspergillus fumigatus* with IC_{50} values of 32, 34, and 40 $\mu g\ ml^{-1}$, respectively, but not of *Rhizoctonia solani, Paracoccidioides brasiliensis* and *Candida albicans*. The protein was also subjected to automated N-terminal amino acid sequence, showering high degree of similarities to storage 2S albumins, adding a new member to this protein-defence family.

Park *et al.* (2008) performed *in vivo* fungicidal activity of some medicinal plant extracts (at concentration of 0.5, 1 and 2 mg/ml) against six phytopathogenic fungi. Their efficacy varied with plant pathogen, tissue sampled and plant species. Very

strong fungicidal activity was produced by extracts fo *Boswellia carterii, Saussurea lappa, Glycyrrhiza uralensis, Piper nigrum, Rheum coreanum, Lysimachia foenum-graecum, Evodia officinalis, Santalum album* and *Curcuma longa* at 2 mg/ml. At 1 mg/ml, *S. album, P. nigrum* and *L. foenum-graecum* showed potent fungicidal activity against *Blumeria graminis* f.sp. *hordei, Puccinia recondita* and *Magnaporthe grisea,* respectively. *Lysimachia foenum-graecum* exhibited strong fungicidal activity against *M. grisea* at 0.5 mg/ml.

Arwa *et al.* (2008) studied phytochemical compounds and antimicrobial properties of the methanol extracts of *Rhoicissus revoilli* (Vitaceae) between January and December 2007 at Maseno University, Kenya. The bacterial use for the antimicrobial analysis consisted of clinical strains of *Streptococcus pryogenes* (gram positive) and *Salmonella typhi* (gram negative) bacteria and one strain of fungi (*Aspergillus niger*). The methanol extract was active against the 3 microorganisms. Root extracts showed greater microbial growth inhibition in comparison to leaf extracts. They reported the presence of active compounds of flavonoids, alkaloids, saponin, steroids and antharaquinones in the plant extracts.

Tomczykowa *et al.,* (2008) tested the antibacterial and antifungal properties of the extracts, and essential oils of *Bidens teripartita* flowers and herbs. Twelve extracts and two essential oils were investigated for activity against different bacteria (*Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Escherichia coli,Klebsiella pneumoniae, Pseudomonas aeruginosa*) and fungi (*Candida albicans, C. parasilosis, Aspergillus fumigates, A. terreus*) using a broth micro dilution and disc diffusion methods. The results indicated antimicrobial activity of the tested extracts (except butanolic extracts), which however did not inhibit the growth of fungi used in this study. Bacteriostatic effect of both essential oils was insignificant but they had strong antifungal activity.

Parekh and Chanda (2007) tested *in vitro* antimicrobial activity of *Trapa natans* L. fruit. They extracted antifungal properties in different solvents with increasing polarity such as 1,4-dioxan, chloroform, acetone, dimethylformamide, ethanol and water. The extractive yield ranged from 0.62-12.62%. The antibacterial activity of all the extracts was determined by agar disc diffusion method. Maximum antibacterial activity was observed against Gram negative bacteria. The resistant Gram negative strains were *C. fruendii, E. aerogenes, E. coil, P. vulgaris, P. aeruginosa* and *S.*

typhimurium. Amongst Gram positive bacteria, *M. flavus* was the most susceptible bacteria and *B. subtilis* was most resistant fungal strain. The best antimicrobial activity was observed with 1,4-dioxan extract and the least activity was observed with petroleum ether extract. The inhibitory effects of the extracts were comparable with the standard antimicrobials used.

Kim *et al.*, (2002) studied crude extracts of *Xanthium strumarium* inhibited mycelial growth and zoospore germination of *Phytophthora drechsleri*, the causal agent of Atractylis rot, *in vitro*. Fresh sap from *X. strumarium* at 50-fold dilution was highly effective in controlling the disease incidence in pot and field trials. Purified extracts from cocklebur inhibited mycelial growth and zoospore germination *in vitro* at a concentration of 12.5µg/ml and 15.6µg/ml respectively. Hyphal tips affected by the compound showed malformation. The antifungal compound purified from *X. strumarium* was identified as 4-oxo-1 (5), 2,11, (13)-xanthatriene-12, 8-olide, known as 'deacetyl xanthumin'.

Abere *et al.*, (2007) assessed antimicrobial activity of the extract of *Mitracarpus scaber* leaves using agar plates and concentrations of the extract varying between 25mg/ml and 300mg/ml to determine the minimum inhibitory concentration at 37^0C against bacterial and fungal organisms viz. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Sarcina lutea, Candida albicans* and *Klebsiella pneumoniae.*

Rispail *et al.*, (2005) Worked on secondary metabolites including a very wide variety of compounds with different structures and chemical properties. In order to obtain an overview of the secondary metabolite content of a plant species such as *Lotus japonicus*, a profiling technique coupling sequential extraction with different chromatographic methods (GS-MS, HPLA-DAD/MS) was established. The method allowed the qualitative analysis of ionic (charged) compounds such as alkaloids and non-ionic (neutral) compounds such as terpenoids and phenolic compounds.

Bansod and Rai (2008) screened medicinal plants for their antifungal activity due to essential oils against *A. funigatus* and *A. niger*. Minimum inhibitory concentrations (MICs) of oils (%v/v) against *Aspergillus fumigatus* and *Aspergillus niger* done by agar dilution method and minimum inhibitory concentration (MIC) and minimum cidal concentration (MCCs) data (%v/v) obtained by the broth micro dilution method. The result showed that the maximum antimycotic activity was demonstrated by oils of *Cymbopogon martini, Eucalyptus globulus* and *Cinnamomum jeylenicum* as

compared to control, followed by *Cymbopogon citratus* which showed activity similar to control by Miconazole nitrate. The oils of *Mentha spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera* and *Zingiber officinale* exhibited moderate activity. The oils *Cuminum cyminum, Allium sativum, Ocimum sanctum, Trachyspermum copticum, Foeniculum vulgare* and *Elettaria cardamomum* showed comparatively low activity against *A. niger* and *A. fumigatus* as compared to control. Mixed oils showed maximum activity as compared to standard.

Salar and Suchitra (2009) evaluated antimicrobial activity of the aqueous and organic solvent extracts of different parts (roots, stems, leaves and fruits) of *Solanum xanthocarpum* against bacteria and fungus. Plant extracts of *S. xanthocarpum* were prepared in distilled water and in organic solvents viz. ethanol, benzene, acetone and methanol. Agar well diffusion technique was used to assess the antimicrobial activity of various extracts against Gram-positive (*Staphylococcus aureus, S. epidermidis*), Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria and the fungus *Aspergillus niger*. The diameter of zone of inhibition was taken as an indicator of antimicrobial effect. Except aqueous extracts of different parts of *S. xanthocarpum*, organic solvent extracts showed antimicrobial activity against the test organisms. A strong inhibition of *P. aeruginosa* was caused by the ethanolic and methanolic extracts of *S. xanthocarpum.*

Malabadi and Kumar (2007) reported antifungal activities of acetone, hexane, dichloromethane and methanol leaf extract of four plant species (*Acacia pennata, Anaphalis wightiana, Capparis pepiaria* and *Catunaregum spinosea*) of Belgaum (situated in Karnataka of India). They determined antifungal activities against pathogens like *Candida albicans, Kluyeromyces polysporous, Aspergillus niger* and *Aspergillus fumigates.* Highest antifungal activity was observed with methanolic extracts of *Anaphalis wightiana* against all the tested pathogens with MIC value ranged from 0.02 to 0.06. Methanolic extracts of *Acacia pennata, Anaphaklis wightiana, Capparis* pepiaria have very strong antifungal activity against tested pathogens particularly *C. albicans* and *K. polysporus.*

Phongpaichit *et al.* (2004) investigated antifungal activities from leaf extracts of *Cassia alata L. Cassia fistula L.* and *Cassia tora* L. on three pathogenic fungi (*Microsporum gypseum, Trichophyton rubrum* and *penicillium marneffei*). Among 3 species, *C. alata* was the most effective leaf extract against *T. rubrum* and *M. gypseum*

with the 50% inhibition concentration of hyphal growth at 0.5 and 0.8 mg/ml, respectively, whereas the extract of *C. fistula* was the most potent inhibitor of *P. marneffei* with the IC_{50} of 0.9 mg/ml. In addition, they found that all three *Cassia* leaf extracts affected *M. gypseum* conidial germination. On the basis of microscopic observation they reported that the treated hyphae and macroconidia with leaf extracts were shrunken and collapsed, which was correlated with cell fluid leakage.

Carrot seed oil is the source of the carotene sesquiterpenes carotol, daucol and caryophyllene. Jasicka-Misiak *et al.* (2004) evaluated antifungal activity of carrot seed oil and its major sesquiterpene compounds. The strongest antifungal activity was observed for the main constituent of carrot seed oil, carotol, which inhibited the radial growth of the test fungi up to 65%.

Teak (*Tectona grandis* L.f., Verbenaceae) sawdust extract inhibited the growth of *Aspergillus niger*. Sumthong *et al.*, (2006) isolated the active compounds quinines from teak (*Tectona grandis*) effective on fungal cell wall stress. By (1)H-NMR the active compounds were identified as deoxylapachol and tectoquinone. Two *A. niger* transgenic strains which showed induction of 1,3-alpha-D-glucan syntheses were used as a cell wall damage model. The result showed that deoxylapachol from *T. grandis* extract induced fungal cell wall stress.

Thomson, 1978 reported that the roots of ginseng plants contained the active saponins and essential oils. He also mentioned that leaves of *Eucalyptus* contain essential oils and tannins.

Mathur and Gurjar (2002) reported that leaf of *Calotropis procera*, *Eucalyptus* sp. *Catharanthus roseus*, *Trogonella foenumgraecum*, bulbs of *Allium cepa*, bulblet of *Allium sativum*, powder of *Carcuma longa*, seeds of *Plantago ovata* and fruits of *Momordica charantia* significantly inhibited the growth of *Rhizoctonia solani.* They also exhibited that aqueous extracts of oil cakes of *Azadirachata indica*, *Cucumis melo*, *Gossypium* sp. *Sesamum indicum* and *Brasica juncea* completely inhibited the sclerotial production of *R. Solani in vitro.*

Mares *et al.* (2002) tested the methanol extracts of 10 cultivars of *Tagetes patula* on two phytopathogenic fungi, *Botrytis cinerea* and *Fusarium moniliforme. B. cinerea* showed a high dose dependent inhibition, with a marked difference between light and dark treatment. *F. moniliforme* was more resistant and not affected by the different treatment conditions (light–dark) even at higher dose.

Bandyopadhayay *et al.* (2002) reported that aqueous alcoholic extract of *Bauhinia scandens* had strong antifungal activity against some pathogens namely *Curvularia* sp, *Rhizoctonia oryzae*, *Candida albicans* and *Alternaria* sp. Their experiment established a new dimension of replacement of chemical drugs and manipulation of fungal growth by herbal products.

Abou-Jowdah *et al.* (2002) showed that petroleum extracts gave better result than that of methanolic extracts against some fungal pathogen. Wild marjoram (*Origanum syriacum*) petrolium ether extract showed complete inhibition of mycelial growth of *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp, *Cladosporium* sp., *Fusarium oxysporum* f. sp. *Melonis*, *Verticillium dahliae*, *Inula viscosa* and *Mentha longifolia.* Extracts of *Centaurea pallescens*, *Cichorium intybus*, *Eryngium creticum*, *Salvia fruticosa* and *Melia azedarach* showed more than 95% inhibition of spore germination of the fungal species tested.

Mahasneh (2002) exhibited that aqueous, ethanol and butanol crude extract of aerial part of *Avicennia marina* which showed a moderate antifungal activity. Chouksey and Srivastava (2001) reported that root extract of *Terminalia arjuna* has antifungal activity.

Ramejani *et al.* (2002) reported that volatile oil extracted from the leaves of *Eucalyptus citriodora* had a wide spectrum antifungal activity against *Aspergillus fumigatus* and *Candida albicans*. At 400 mg/ml concentration growth of both fungi was inhibited and considerable reduction in size of cells and hyphae were reported. Sporulation was also decreased.

Doohan *et al.* (2000) investigated antifungal activity in soluble extracts from seeds of a range of wheat cultivars named WEK0609, CM 820036 and Arina. Antifungal activity was assessed in terms of B-D-glucoronidase (GUS) activity of a *Fusarium culmorum* GUS transformant using a sensitive laboratory assay.

Rana *et al.* (1999a) tested the antifungal activity of an aqueous extract of the leaves of garlic creeper (*Adenocalyxmna alliaecum* Miers) against *Alternaria brassicae* (Berk) Succ. The extract could strongly inhibit the spore germination.

Carpinella *et al.* (1999) showed that the ethanol extract of *Melia azedarach* ripe fruit had fungistatic and fungicidal activity against *Aspergillus flavus*, *Fusarium moniliformae*, *Microsporum canis* and *Candida albicans.*

Irobi and Adedayo (1999) tested aqueous extract of the dormant fruits derived from *Hyphaene thebaica* for *in vitro* antifungal activity using agar dilution method. They found that the extract showed significant antifungal activity against a wide range of fungal isolates including *Candida albicans, Microsporum gypseum, Trichophyton rubrum, Mucor sp., Fusarum solani* and *Aspergillus niger.*

Xu *et al.* (1998) reported a plant protein present in the orchid, *Gastrodia elata* BL. f. *flavida* S. Chow, and also reported the activity of the protein against the fungi *Valsa ambiens, Rhizoctonia solanii, Gibberella zeae, Ganoderma lucidum* and *Botrytis cinerea.*

Merali *et al.* (2003) reported that extract of *Echinacea* sp. had activity against the fungi *Trichophyton tonsur*ans, *T. Mentagrophytes, Microsporum gypseum* and *Pseudallescheria boydii.*

Fukai *et al.* (2003) isolated four antifungal agents from the roots of *Cudrania cochinchinenisis* using bioassay guided fractionation method. These compounds showed antifungal activity against *Cryptococceous neoformans, Aspergillus fumigatus* and *A. nidulans.*

Passos *et al.* (2003) analysed the antifungal activity of the seed and leaf essential oils of *Caryocar brasiliensis* by GC/MS. The most significant result of bioassay was obtained against *Cryptococcus neoformans* and *Paracoccidioides brasiliensis.*

Joshi *et al.* (2003) partitioned the methanol extract of *Ailanthus excelasa* with chloroform. The chloroform extract showed fungistatic and fungicidal activity against *Aspergillus fumigatus, Penicillium frequentence, Penicillium notatum* and *Botrytis cinerea.*

Nino *et al.* (2003) reported that methanol extract of *Solanum* spp., *Tibouchina grossa, Hyeronima macrocarpa, Miconia lehmannii* and *Sapium stylare* inhibit fungal growth of *Aspergillus fumigatus, Candida albicans* and *Fusarium solani.*

Rocha *et al.* (2004) reported that ethanol extract of *Clytostoma ramentaccum* and *Mansoa hirsute* inhibit the growth of *Aspergillus niger* and *Fusarium oxysporum.*

Rahmani *et al.* (2004) evaluated the antifungal activity of *Glycosmis calcicola* and *G. rupestris* extracts using poison food and spore germination technique. Their experiment showed that the chloroform extract of *G. calcicola* was most effective in inhibiting mycelial growth, sporulation and spore germination.

Logo *et al.* (2004) showed that *Piper crassinerving, P. aduncum* contained new benzoic acid derivative which has antifungal activity against *Cladosporium* species.

Jain *et al.*, (2007) showed curcumin of *Curcumin longa* possessed anti-microbial activity.

Anbhule *et al.* (2005) reported that *Adhatoda vasica* has antifungal activities against *Aspergillus niger, Trichoderma spp.*and *Rhizoctonia solani.*

Disease control by selected natural chemicals

Simple phelnols and phenolic acids: Phelolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as antimicrobial. Eugenol is considered both as antifungal (Soatthiamroong *et al.* 2003) and antibacterial (Thomson, 1978). Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and Caffeic acid are common representatives of a wide group of phenyl propane derived compounds which are in the highest oxidation state. Catechol and pyrogallol both are hydroxylated phenols, show toxicity to micro-organisms. Catechol has two–OH groups, and pyrogollol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Geissman, 1963). In addition, some authors have found that more highly oxidized phenols are more inhibitory (Urs and Dunleavy, 1975). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987).

Quinones: Quinones are aromatic ring with two ketone substitutions. They are ubiquitous in nature and are characterized as highly reactive. These compounds, being coloured, are responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin (Schmidt, 1988). Their presence in henna gives that material its dyeing properties (Fessenden and Fessenden,1982). Quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern *et al.* 1996), often leaking to inactivation of the protein and loss of function. For that reason, the potential range of quinine group has great antimicrobial effects. Kazmi *et al.* (1994) described an anthraquinone from *Casia italica*, a Pakistani tree. Anthraquinone was bacteriostatic for *Bacillus anthracis,*

Corynebacterium pseudodiphthericum and *Pseudomaonas aeruginosa* and bacteriocidal for *Pseudomonas pseudomalliae.*

Catechins: Catechins, the most reduced form of the C_3 unit in flavonoid compounds deserve special mention. Toda *et al.* (1989) noticed that tea exerted antimicrobial activity due to presence of catechins.

Flavones: Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinines). The addition of a 3-hydroxyl group yields flavonol (Fessenden and Fessenden, 1982). Flavonoids are also hydroxylated phenolic substances but occur as a C_6-C_3 unit linked to an aromatic ring. Dixon *et al.* (1983) reported that flavonoids were known to be synthesized in plants in response to microbial infection. They reported *in vitro* antimicrobial activity of flavonoids against a wide range of microorganisms. Tsuchiya *et al.* (1996) reported that lipophilic flavonoids may disrupt microbial membranes. Aerial part of *Centaurea raphanina*, belongs to Asteraceae, contain an anticicrobial lactone (Panagouleas *et al.* 2003).

Tannins: Scalbert (1991) reviewed the antimicrobial properties of tannins. He listed 33 studies which had documented the inhibitory activities to filamentous fungi, yeast and bacteria causing different plant diseases. 'Tannins' is general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Their molecular weights range from 500 to 3,000 and they were divided into two groups, hydrolysable and condensed tannins (Haslam, 1996). Hydrolyzable tannins were based on gallic acid, usually as multiple esters with D-glucose; while the more numerous condensed tannins were derived from flavonoid monomer. This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannins-containing beverages, especially green teas and red wines, can cure or prevent a variety of ills (Serafini *et al.,* 1994).

Coumarins: O'Kennedy and Thornes (1997) reported that coumarins are phenolic substances made of fused benzene and α-pyrole rings. Several coumarins have antimicrobial properties. Coumarin was found *in vitro* to inhibit *Candida albicans.* Hydroxy cinnamic acid, related to coumarins seem to be inhibitory to gram positive bacteria (Fernandez *et al.*1996). Phytoalexins, which were hydroxylated derivatives of coumarins were produced in carrot in response to fungal infection and possessed antifungal activity (Hoult and Paya, 1996)

Terpenoid and Essential oils: The terpenes or terpenoids are active against bacteria (Barre *et al.*,1997), fungi (Shafi *et al.*, 2004) and viruses (Xu *et al.* 1996). The essential oil of *Juglans regia* var *Kumaonica* has antifungal activity (Chowdhury *et al.* 2003). The essential oil of *Centella asiatica* show antifungal and antibacterial activity (Minija and Tropill, 2003). The general chemical structure of tannin is $C_{10}H_{16}$ and they occur as diterpenes, triterpenes and triterpenes and teraterpenes (C_{20}, C_{30} and C_{40}) as well as hemiterpenes (C_5) and sesquiterpens (C_{15}). When the compounds contain additional elements, usually oxygen, they are termed as terpenoids. Terpenoids are synthesized from acetate units and as such they share their origin with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Examples of common terpenoids are methanol, camphor and artimism.

Alkaloids: Heterocylic nitrogen compounds are called alkaloids. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum* (Fessenden and Fessenden,1982). Alkaloid Barberine has strong antimicrobial activity (Bhandari *et al.* 2002). Alkaloid Daturin present in *Datura metal* has antifungal activities against *Rhizoctonia solani* and antifungal activity against *Xanthomonas oryzae* pv *oryzae* (Kagale *et al.* 2004).

Polypeptides: Peptides are inhibitory to microorganisms were first reported in 1942 (Cichewicz and Thorpe, 1996).

Chu and Ng, (2003) reported that thaumatin like protein of *Castanea mollissima* can inhibit the mycelial growth of *Fusarium oxyporum, Mycospharella arachidicola* and *Physalopora piricola.*

An alkaloid, carpaine, is also antimicrobial (Burdick, 1971). Terpenoids present in the sap also contribute to its antimicrobial properties (Thomson, 1978). Osato *et al.*(1993) reported the bacteriostatic property of the latex.

Several plant extracts have antimicrobial properties even to the human pathogenic microbes. Some of them have also been included here.

In growth chamber experiments, Mahboubi *et al.* (2008) reported that plant-derived antimycotics were important to the mycologists due to increased resistance of fungi to azoles. They studied anticandidal activity of *Zataria multiflora* (thyme), *Pelargonium graveolens* (geranium), *Artemisia sieberi baser* (Artemisia), *Rosmarinus officinalis* (rosemary) and *Lavandula stoechas* (lavender) oils against some clinical isolates of *C. albicans.* Disk diffusion method and macro broth dilution assay were

employed to evaluate the antifungal activity of these oils. Essential oils were analyzed by GC which led to identification of these main components. Carvacrol (39.8%), Citronellol (45.2%, alfa-pinene (23.7%), 1,8-cineol (30.2%) and alfa-athujone (38.8%) are the main components of thyme, Geranium, Rosemary, Lavender and Artemisia oils respectively. Thyme oils showed strong antifungal activity (34-40mm, MIC≤62.5 µg/ml) but lavender, rosemary and Artemisia oils showed only a moderate effect (zone inhibition <12mm). The inhibition Zone of thyme oil is larger than Amphotricin B.

Verstegui *et al.* (1996) investigated activity of several widely distributed plants from the vegetation of Mexico and the southern USA. The plants were evaluated on the growth of yeast and moulds; *Candida albicans, Candida krusei, Candida rugosa, Cryptococcus neoformans, Cryptococcus laurentis, Cryptococcus albidus, Microsporum canis, Microsporum gyspeum , Trichophyton tonsurans, Epidermophyton flocosum* and *Sporotrix schenckii.* The extracts analyzed showed good antifungal activity against more than one organism.

Tadeg *et al.* (2005) investigated the antifungal activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders, Hydro - alcohol extracts of *Acokanthera schimperi* (d.c). Benth et Hook (Apocynaceae), *Calpurna aurea* L.(Leguminoseae), *Kalanchoe petitiana* (Engl.) Cufod. (Crassulaceae), *Lippia adonensis* Hochst. (Verbenaceae), *Malva parviflora* L.(Malvaceae), *Olinia rochetiana* L. (Oliniaceae), *Phytolacca dodecandra* L'Herit (Phytolaccaceae), *Verbascum sinaiticum* Bentham (Scrophulariaceae) were screened for antifungal activity against different strains of fungi which were known to cause different types of skin infections. Of all the plants tested, *L. adoensis* and *O. rochetiana* were most active species against fungal strains.

Zaidi and Crow (2005) reported the antifungal activity of four important medicinal plants from Balochistan, Pakistan. The plants were *Grewia erythraea* Schwien f. (Tiliacee), *Hymenocrater sessilifolius* Fisch. (Lamiaceae), *Vubcetixicum stocksii* Ali & Khatoon (Asclepidaceae) and *Zygophyllum fabago* L.(Zygophyllaceae). The extracts of *Z. fabago* and *V. stocksii* showed good activity against *Candida albicans.*

De Leo *et al.* (2004) reported that leaves of *Baseonema aeuminatum* had antifungal activity against *Candida albicans.*

Loizzo *et al.* (2004) investigated the antifungal activity of methanol, ethyl acetate, dichloromethane, n-hexane , n-butanol and choloroform extract of *S. vulgaris* showed significant activity against *Trichophyton tonosurans* (IC50 of 0.031 mg/ml.)

DeCampose *et al.* (2005) investigated the crude methanol extract and some fractions (hexane, dichloromethane and ethyl acetate) from *Piper solmsianum* var, *solmsianum* (Piperaceae) for possible antifungal activity against twelve pathogenic fungi. The experiments showed that the crude extract exhibited antifungal action against all the tested dermatophytes, with IC_{50} values between 20-60 μgml^{-1}. Similar activity was also reported for the hexane, dichloromethane and ethyl acetate fractions.

Angioni *et al.* (2006) invested the chemical composition and antifungal activity of the essential oil from the stems/leaves and flowers of *Lavandula* species growing wild in southern Sardinia, Italy. The essential oils tested were effective on the inactivation of *Rhizoctonia solani* and *Fusarium oxysporum*, and less effective on the inactivation of *Aspergillus flavus*. Among the components tested, fenchone, limonene and myrtenol reported to be the most effective on the growth inhibition of *Rhizoctonia solani.* The chemical composition of the essential oil of the Sardinian *R. officinalis* was obtained by hydro-distillation. The major compounds in the essential oil were α-pinene, borneol, camphene, camphor, verbenone and bornyl acetate.

Singh *et al.* (2004) investigated the chemical constituents and antifungal effects of essential oil. The oil exhibited a broad spectrum of fungitoxic behaviour against all tested fungi like *Aspergillus niger, Fusarium moniliforme* and *Curvularia lunata.* They obtained absolute inhibition zone of mycelial growth at 6µl dose of the oil. Analysis of ajwain essential oil showed the presence of twenty six identified components, which account for 96.3% of the total. Thymol was found to be a major component along with p- cymene, γ-terpinene,β-pinene and terpinen-4-ol. The essential oils from different tissues of Japanese cedar, *Cryptomeria japonica* D.Don (Taxodiaceae) were active against four wood decay fungi and six tree pathogenic fungi, while essential oil isolated by hydro-distillation from the aerial parts of *Chenopodium botrys* L. (Chenopodiaceae) showed significant fungicidal Activity.

Ali *et al.* (2008) screened and evaluated antimicrobial activities of *Mimusops elengi.* Different solvent extracts of bark, fruits and leaves of *Mimusops elengi* were tested for their antibacterial and antifungal activities. The activities of extracts were not significantly enough against most of the tested organisms. Fruit extracts were less

potent against most of the tested organisms compared to those obtained from bark and leaves and were inactive against the fungus *Trichoderma viride*. Leaves extracts displayed good activity against *Bacillus subtilis* and *Trichoderma viride* and were inactive against *Helminthosporium sativum.*

Abdulrahman and Aba Alkahil (2005) carried out the antifungal activity of some extracts against some plant pathogenic fungi to determine the antifungal activity of five plant extracts viz, *Allium stivum, Cymogopogon proxims, Carum carvi, Azadirachta indica* and *Eugenia carryophyllus* extracted with either cold distilled water (CDW), boiling distilled water (BDW) or cold ethanol (CET) as well two culture filtrates of *Trichoderma* sp. antagonistic against *Fusarium oxysporum* f. sp. *Lycopersici, Botritis cinerea* and *Rhizoctonia solani.* Plant extracts specially those extracted with CDW had strong antifungal activity with significant inhibition on the growth of the three tested fungi. They also reported that the inhibitory magnitude of the tested plant extracts to the tested fungal pathogens were proportional to the applied concentration. The most effective plant extracts were *Allium sativum, Carum carvi* and *Eugenia carryophyllus.*

Muthuvelan and Raja (2008) assessed for a best solvent for extracting the active constituents from 10 plant extracts. Thin layer chromatography (TLC) was used to separate and establish the active constituents present in each of the medicinal plants. Active constituents from each plant were extracted by using three different solvent systems namely diethyl ether, chloroform and hexane and were tested against three species of gram negative and three species of gram positive bacteria (*Escherichia coli, Pseudomonas aureginosa, Streptococcus peneumoniae, Aeromonas hydrophila Staphylococcus aureus, Bacillus cereus*) by means of agar well diffusion assay. Studies on the antioxidant activity were also carried out for these plant extracts by using Diphenylpicryl-hydrazyl (DPPH) method. For the antimicrobial activity, the study revealed that among the selected plants, *Azadirachta indica, Pongamia pinnata* and *Aloe barbadensis* had the maximum antibacterial activity. Among the extraction procedures diethyl ether was found to be the best solvent.

Shalini and Srivastava (2009) investigated antifungal activity screening and HPLC analysis of crude extracts from *Tectona grandis*, Shilajit, *Valeriana wallachi* against *Alternaria cajani, Curvularia lunata, Fusarium* sp., *Bipolaris* sp. *and Helminthosporium* sp. at different concentrations (1000,2000,3000,4000 and 5000

µg/ml). Better antifungal activity was observed with the extracts of *Valeriana wallachi*, that showed excellent inhibitory activity against *Helminthosporium* sp. (96.15%) followed by Shilajit against *Alternaria cajani* (95.12%) and *Helminthosporium* sp. (95.00%) at concentration of 5000 µg/ml. Among different fungi tested *Bipolaris* sp. and *Fusarium* sp. were found to be more sensitive to crude extract when compared to other. The increase in the production of phenolics in the extract can be correlated with the induction of resistance in tested plants against phytogenic fungi. HPLC analysis of the crude extract of the plants showed four different phenolic acids.

Mahindra and Mohan (2006) screened antibacterial activity against methanol extracts of 22 plants of India. Out of the plants tested *Blumea lacera* (Burm f.) DC (Asteraceae), *Canscora diffusa.* (vahl) R. Br. (Gentianaceae), *Cassia alata.* L., *C. biflora* L., *C. fistula* L., (Ceasalpiniaceae), and *Putranjiva roxburghii* wall. (euphorbiaceae) extracts were found to be more effective against both gram positive and gram-negative bacteria. *Staphylococcus aureus* was found to be susceptible to 68% of the tested extracts, whereas *Pseudomonas aeruginosa* showed resistance to most of the plant extracts.

Att-ur-Rahman and Choudhary (1997) worked on antifungal phytochemicals of medicinal plants. They isolated a number of interesting compounds. The agar diffusion method was used to determine the antifungal activity of the extracts against the pure fungal isolates. They determined the structure of the antifungal principles by using modern spectroscopic and/or X-ray diffraction technique.

Vaughn *et al.* (1984) identified antifungal activity of natural compounds against thiabendazole-resistant *Fusarium sambucinum* strains. Several natural compounds (which were effective potato sprout inhibitors) were examined for antifungal activity against three thiabendazole(TBZ)–resistant strains and a TBZ-sensitive strain of *Fusarium sambucinum*. Salicylaldehyde was the most inhibitory compound and completely inhibited fungal growth. Cinnmaldehyde, salicylaldehyde, and thymol prevented all growth when incorporated in media at 0.1% (v/v) levels. TBZ-resistant strains were only partially inhibited by the wild-type strain. Treatment of tubers with compounds after wounding and inoculation with fungal spores was generally ineffective in suppressing dry rot, possibly due to lack of direct contact between the fungi and the compounds.

Bhakshu *et al.* (2008) worked with the antimicrobial and phytochemical studies of *Crotalaria madurensis* Wt. var. *kurnoolica* Ellis et Swaminathan (Fabaceae), an endemic medicinal plant found in the forest of Nallamallias of Eastern-ghat of India. The ether and ethyl acetate extracts of the plant material exhibited a broad spectrum of antimicrobial activity on human pathogenic microorganisms of six bacteria and two fungal strains.

Kotkar *et al.* (2002) screened foliar extract of *Annona squamos*a (Family Annonaceae) for antimicrobial and insecticidal activity. Flavonoids isolated from aqueous extracts of *A. squamosa* showed antimicrobial activity against all the common microbial contaminants of pulse and 80% insecticidal activity against *Callosobrunchus chinensis* at a concentration of 0.07mg/ml. Various physico-chemical tests, chromatographic and spectroscopic studies with partially purified aqueous extract indicated the presence of flavonol type flavonoids.

Kordali *et al.* (2005) investigated the chemical composition and antifungal activity of the essential oils from three Turkish *Artemisia* species (Asteraceae). The results showed that all the three oils had potent inhibitory effects over several fungi tested tested by them. Pure camphor and 1,8-cineole were the major components of the oils and the oils were tested for antifungal activity against some of the fungal species.

3. Materials and Methods

3.1: Plant materials

3.1.1: Collection of diseased samples of some important fungal pathogens of economically important crops of north Bengal.

North Bengal is endowed with diverse natural resources. It is surrounded by Nepal and Bihar in the west, Assam and Bangladesh in the east, Sikkim and Bhutan in the north and Bangladesh in the south. Five districts of north Bengal are in plains and one district is in the hills. The sub-Himalayan region of north Bengal is enriched with cultivation of tea plants. The region is also renowned for cultivation of tomato and brinjal (Plate 2 & 3). These three plants were selected for the present study, although several other plants are also growing in the region. Diseased samples of the three plants were collected from several places of north Bengal and has been mentioned in the following table 3.1

Table 3.1: Isolated fungal cultures of tea, tomato and brinjal

Host Plants with Number of fungal organisms isolated	Fungal organism(s) isolated	Collection places of the diseased samples*
Tea 1	(*Pestalotiopsis theae*)	Garubathan (Jalpaiguri), Chalsa (Jalpaiguri), Bagdogra (Darjeeling), Naxalbari (Darjeeling)
Tomato 1	(*Alternaria alternata*)	Haldibari (Cooch Behar), Raiganj (Uttar Dinajpur), Balurghat (Dakshin Dinajpur), Falakata (Jalpaiguri)
Brinjal 2	(*Colletotrichum gloeosporioides*)	Khoribari(Darjeeling), Phansidewa (Darjeeling) Rajganj(Jalpaiguri), Belakoba (Jalpaiguri), Itahar (Malda)
	Fusarium equiseti	Panjipara (Uttar Dinajpur), Dinhata(Cooch Behar), Barobisha (Jalpaiguri),Gangarampur (Dakshin Dinajpur)

*Name of the places with district in parentheses.

3.1.2. Collection of plants for experiments:

Plate 2: fig.a. Field showing cultivated variety of brinjal. **fig. b:**Showing brinjal production in farmer's field.

Plate 3: Healthy tomato fields in North Bengal.
fig.a. Field showing cultivation of tomato.
fig.b. Showing tomato production in farmers field.

Tomato plants: Seeds of six different tomato varieties (Karan, Romeo, Pasuja, Trishul, US1080, Mahyco, Avinash 2) were collected from Leuchipukri Krishi Bhander, District Darjeeling.

Brinjal plants: Six different brinjal varieties (Green round, Supriya Variety, Pant Rituraj, Kanha Hybrid, Pant Samrat, Muktakeshi local) were selected for present study. Selection was done on the basis of the seed germination and growing suitability in the environmental and field conditions of north Bengal. Selected seeds of different brinjal varieties were collected from 'New Basundhara Seed Shop', Barobisha, District Jalpaiguri.

Tea plants: Nursery tea plants of six selected varieties (TV-9, TV-18, TV-22, TV-25, TV-26 and TV-30) were procured from tea nurseries of Kharibari, Siliguri, West Bengal and were planted in the experimental garden as well as in pots.

3.1.3. Maintenance of the plants for experiments

Tomato: Tomato is a warm season crop, it requires warm and cool climate. It thrives well in temperature 10^0C to 30^0C. The optimum range of temperature is 21-24^0C. A pH range of 5.5-6.0 was maintained in the field for growth of tomato plants. The soils with proper water holding capacity, aeration, free from salts were selected for cultivation.

As the fruit production and quality depends upon nutrient availability, balanced fertilizers were applied. Mono ammonium phosphate (MAP) was used as a starter fertilizer to supply adequate phosphorous during germination and seedling stages.

The seedlings were sprayed with starter solution of micronutrient. Before planting farm yard manure 50 ton per hectares was applied. Normally tomato crop requires 120 kg N_2, 50 kg phosphorus (P_2O_5), and 50 kg potash (K_2O). Nitrogen need to be given in split doses. Half nitrogen and full P_2O_5 was given at the time of transplanting and remaining nitrogen was given after 30 days and 60 days of transplanting. General culturing operations like hand hoeing, weeding, mulching, staking etc. was followed during maintenance of the tomato plants. Weeds were controlled by mulching and also by application of herbicide (oxyflorophen) at the rate of 12 kg/hectare.

Brinjal: Brinjal seeds were sown 4-6 weeks before the plants were set in the garden. Seeds were sown 1.25 cm deep in the seed-bed (on pot or tray or directly on soil) and medium moist condition was maintained. Temperature was 25^0-30^0C and pH was maintained at the range of 5.5 to 6.8. Soil was kept moist during the period of

germination. After completion of germination seed-bed was watered regularly. Excess watering was avoided. Plants were replanted in desired places (either in pots or in soil) for experimental purposes. A basal dressing of 60 kg of P_2O_5 and K_3O were applied per hectare. Application of 100 kg of nitrogen/ha were done in three splits.

Tea: Several tea varieties (viz. TV-9, TV-18, TV-22, TV-25, TV-26, and TV-30) approved by Tocklai Experimental Station, Jorhat, Assam were used during the present study. All the varieties mentioned above were selected for plantation in the experimental garden, Department of Botany, University of North Bengal, based on their growing suitability as observed under field conditions and environmental aspects, over the years at Tocklai Experimental Station, Jorhat, Assam, India and was recommended by Bezbaruah and Singh, 1988. All the seed varieties were raised in the nursery of the experimental garden. Several pits of 40 cm^3 were dug at approximately 60 cm and 90 cm intervals between plant to plant and row to row respectively. Planting mixtures per pit were formulated in the following ratios: Well rotten dry cattle manure: superphosphate: rock phosphate: thimate::4000:25:25:2.5. Procedures for mixing are as follows. Rock phosphate was placed at the bottom of the pit following which half portion was covered with cattle manures-soil mixture. Thimate was mixed with a portion excavated soil and was applied with upper part of the pit. Superphosphate was placed approximately 5 cm below the ground level. Finally, plantation was done with a total of 20 plants of all the six varieties. Some of the plants were also grown in earthen pots (one plant per pot, 25 cm diameter) each containing 4 kg of soil: planting mixture (1:1).

3.1.4. Collection of plants for extraction of botanicals

On the basis of easy availability in the growing areas of sub-Himalayan north Bengal eighty plant materials were collected from forests and adjoining areas of the region. Different parts of these plants (leaf, bark, root, rhizome as applicable) were extracted and screened for their fungi toxic properties against four fungal pathogens (*Alternaria alternata, Fusarium equiseti, Colletotrichum gleosporioides* and *Pestalotiopsis theae*). Collected Plants were identified and voucher specimens were deposited in the laboratory herbarium, Department of Botany, University of North Bengal. Name of the plants along with their traditional uses have been presented in the table 3.2 and photograph of some of the potential plants/plant parts have been shown in the plates 4, 5, 6 & 7. Traditional uses reported in the table 3.2 have been collected from Rastogi and Mehrotra (1995), Chopra *et al.* (1996) and Chatterjee and Pakrashi (1997)

Table 3.2: List of plants used for extraction of botanicals with their traditional use.

Name of the plant	Family	Parts used	Traditional uses
Acacia catechu (L.f)Wild	Mimosaceae	Leaf	Astringent and useful in passive diarrhoea.
Acalypha indica L.	Euphorbiaceae	Leaf	Laxative, beneficial of scabies, maggot infested wounds and other skin diseases.
Adhatoda vasika Nees.	Acanthaceae	Leaf	Antihelmintic,antiseptic, antispasmodic, expectorant and sedative
Aegle marmelos (L.) Corr	Rutaceae	Leaf	Leaves are used in diarrhoea and dysentery.
Ageratum conyzoides L.	Asteraceae	Leaf	Cuts and sore,for fomentation in leprosy and other skin diseases.
Allium sativum L.	Liliaceae	Bulb	Juice used as rubefacient in skin disease, atonic dyspepsia, flatulence, and colic
Alstonia scholaris (L.) R.Br.	Apocynaceae	Leaf	Used in beriberi, in congetion of liver, dropsy,and applied to ulcers.
Amaranthus spinosus L.	Amaranthaceae	Leaf	Medicinal effect like in lukorrhoea, menorrhagia,and idigetion.
Anisomeles indica (L.) O.Kuntze.	Lamiaceae	Leaf	Astringent,Carminative,tonic used in gastritis and fever.
Annona squamosa L.	Annonaceae	Leaf, Bark	Hair tonic antidiabetic diarrhea, sedative expectorant and stimulant.
Argemone mexicana L.	Papavaraceae	Leaf	Acrid, diuretic,ophthalmic and stomachic.
Artocarpus heterophylus Lam.	Moraceae	Leaf	Used in skin disease and antidote to snake bite.
Asparagus racemosus Wild	*Aasparagaceae*	Leaf	Antibacterial, antiprotozoal antiulcer, andimmunomodulatory activity.
Azadirachta indica L.	Mmelicaceae	Leaf	Useful as stomachic febrifuge, antihelmintic, in dysentery and dyspepsia
Borreria alata (Aublet) De Candolle.	Rubiaceae	Leaf	Diarrhoea, dysentery and kill teeth worm.
Caesalpinia pulcherrima (L.) *swartz.*	Caesalpiniaceae	Leaf	Abortifacient antiperodic, purgative,ulcers, asthma and malaria.
Calotropis gigantean (L.) R.Br.Aiton	Apocynanceae	Leaf	Leaves applied to painful joints or swelling; eczema, skin erutions, ulcers and wound.
Cannabis sativa L.	Cannabaceae	Leaf	Stimulant, decreased blood pressureand antiulcers activity Mimosaceae

Contd.....

Table 3.2 (contd.): List of plants used for extraction of botanicals with their traditional use.

Name of the plant	Family	Parts used	Traditional uses
Cassia tora L.	Caesalpiniaceae	Leaf	Cure ring worm, itches and other skin diseases; laxative.
Centella asiatica L.Urban.	Umbelliferae (Apiaceae)	Leaf	Antidysentric, diuretic and tonic, treatment of leprosy and syphilitic ulcers.
Catharanthus roseus (L.) G. Don	Apocynaceae	Leaf	Diabetis, Hemostatics, Treatment for leukaemia.
Citrus limon (L.) Burm.	Rutaceae	Leaf	Juice refrigent and antidiarrhoeal; antidysentric and gout.
Clausena excavata Burm.f.	Rutaceae	Leaf	Anticeptive activity, fragrance
Clerodendrum viscosum Vent.	Verbanaceae	Leaf	Applied externally for tomours and skin disease.
Clitoria ternatea L.	Fabaceae	Seed	Powerful purgative.
Crotalaria mucronata Desv.	Fabaceae	Leaf	In scabies, impetigo and diminishes salivation.
Datura stramonium L.	Solanaceae	Leaf	Antiseptic, narcotic.
Datura metel L.	Solanaceae	Leaf	Narcotic, Hypnotic, Halucinogenic
Datura innoxia Mill.	Solanaceae	Leaf	Narcotic, Hypnotic, Halucinogenic
Elephantopus scaber L.	Solanaceae	Leaf	Astringent, cardiotonic and febrifuge.
Phyllanthus emblica L.	Euphorbiaceae	Leaf	Juice applied externally to ulcers, infusion useful in chronic dysentery.
Eucalyptus globosus Labill.	Myrtaceae		Antimicrobial essential oil
Euphorbia hirta L.	Euphorbiaceae	Leaf	Juice is beneficial in colic dysentery, cough and worm infestation.
Heliotropium indicum L.	Boraginaceae	Leaf	Antitumor, Wound healing.
Hibiscus rosa-sinensis L.	Malvaceae	Leaf	Emollient and aperients,juice beneficial in gonorrhoea.,and used for blackening hair.
Holarrhena antidysentrica Wall.	Apocynaceae	Leaf	Piles, dropsy, astringent,diarrohea,fever,carminative,bronchitisandskin disease.

Contd...

Table 3.2(contd.): List of plants used for extraction of botanicals with their traditional use.

Name of the plant	Family	Parts used	Traditional uses
Hyptis suaveolens (L.) Poit.	Lamiaceae	Leaf	Carminative, stimulant uterine disease, infusion anticephalagic, epistaxis.
Lantana camara L.	Verbenaceae	Leaf	Leaf juice is used as antimicrobial in skin disease.
Lagerstroemia speciosa L. Pers.	Lythraceae	Leaf	Diabetes, ailments, purgative and diuretic
Mangifera indica L	Anacardiaceae	Leaf	Anthelmentic,asthma, laxative,inflammation, constipation, andrheumatismand dysentery.
Melastoma malabathricum L.	Melastomaceae	Leaf	Used in diarrhoea and dysentery.
Melia dubia Cav.	Meliaceae		Antilithic, diuretic, emmen. Applied as poultice, to relieve nervous headaces.
Mimosa pudica L.	Mimosaceae	Leaf	Juice used in sinus, sores, piles, and fistula., paste applied to glandular swelling and hydrocele.
Mitracarpus verticillatus (Schumach&Thonn) Vatke	Rubiaceae	Leaf	Agrihorticulture &Medicine
Moringa oleifera Lamk.	Moringaceae	Leaf	Used as purgative and for cough.
Murraya koenigi (L.) Spreng	Rutaceae	Leaf	Bruised and applied locally to eruptions and poisonous bite.
Nyctanthes arbor-tristis L.	Nytanthaceae	Leaf	Fever, rheumatism, and obstrinate sciata.
Ocimum gratissimum L.	Lamiaceae	Leaf	Gonorrhoea, headache and abdominal colic in children.
Ocimum sanctum L.	Lamiaceae	Leaf	Fresh leaves used for curing ring worm and other skin diseases
Oldenlandia corymbosa L.	Rubiaceae	Leaf	Artharitis, Rheumatism, Stomach troubles

Contd...

Table 3.2 (contd.): List of plants used for extraction of botanicals with their traditional use.

Name of the plant	Family	Parts used	Traditional uses
Oxalis corniculata L.	Oxalidaceae	Leaf	Antidiarrhoeal, antidyspeptic, astringent, antipyretic and appetizing.
Phyllanthus fraternus Webster	Euphorbiaceae	Leaf	Diuretic, swelling, ulcers in the form of poultice, oil used in opthalmia.
Piper betle L.	Piperaceae		Treatment of wound, eczema and chest cure cough.
Plumeria rubra L.	Apocynanceae	Leaf	Febrifuge and paste applied as a poultice.
Polyalthia longifolia Sonnerat	Annonaceae	Leaf	Antibacterial, antifungal activity, and antitumour activity
Pouzolzia indica L.	Utricaceae	Leaf	Antimicrobial activity and diarrhoea.
Psidium guajava L.	Myrtaceae	Leaf	Used in swollen gum and ulcers.
Raphanus sativus L.	Brassicaceae	Root	Urinary complaints, piles and pains.
Rauvolfia tetraphylla L.	Apocynanceae	Leaf	Sedative ,toxic and skin diseases.
Ricinus communis L.	Euphorbiaceae	Leaf	Applied externally to boils and sores.
Saraca asoca (Roxb.) De Wilde	Caesalpiniaceae	Bark	Bark is used to treat menorrhoea,weakness hemorrhage, dropsy and uterine sedative.
Scoporia dulcis L.	Scrophulariaceae	Leaf	Antidiabetic., used in bronchitis, cough, fever, headache, gout and eye etc.
Sida acuta L.	Malvaceae	Leaf	Febrifuge.
Smilax zeylanica L.	Smilaceae	Leaf	Used in rheumatism and pain, used in bloodless dysentery
Solanum khassianum Clark	Solanaceae	Leaf	Steroid producing, skin diseases and leprosy.
Solanum nigrum L.	Solanaceae	Leaf	Applied painful and swollen testicles, rheumatic joints and skin diseases.
Solanum torvum Sw.	Solanaceae	Leaf	Treatment of hyperactivity, skin diseases and leprosy.
Solanum xanthocarpum Schrad & Wendl.	Solanaceae		Steroid producing, skin diseases and leprosy.
Tridax procumbens L.	Asteraceae	Leaf	Antidiarrhoeal; antidysentric and controls bleeding wounds.

Contd...

Table 3.2(contd.): List of plants used for extraction of botanicals with their traditional use.

Name of the plant	Family	Parts used	Traditional uses
Syzygium cumini (L.) Skeels	Myrtaceae	Bark	Astringent,used for sore throat, and diarrhea.
Tectona grandis L.f.	Verbenaceae	Leaf	Astrigent, diuretic, hepatic and stimulant.
Terminalia arjuna (Roxb). W&A	Combretaceae	Leaf	Juice used earache externally used as a cover on sores and ulcers,
Vitex negundo L.	Verbenaceae	Leaf	Antipyretic,astringent,digestive,expectorant, febrifuge, cholera, cough and stomachic
Xanthium strumarium L.	Asteraceae	Leaf	Antisyphilitic, astringent and diuretic.
Zingiber officinale Roscoe	Zingiberaceae	Rhizo -me	Used in dyspepsia and flatulent colic.

3.2. Fungal cultures used

Various fungal pathogens were isolated from the infected tea, tomato and brinjal plants of North Bengal. Although several fungal pathogens were isolated and identified it was considered to select at least one pathogen of the three hosts for the present study.

Thus, four fungal cultures originally isolated from the naturally infected leaves/stems of the three plants (tea, brinjal and tomato) were grown in potato dextrose agar medium and maintained in the laboratory. The pathogens were subjected to Koch's postulates and have been listed in table 3.3.

3.3. Verification of the disease through Koch's postulates

For verification of a particular pathogen Koch's postulates were done. Young plants were collected from experimental garden and leaves were inoculated with the conidial suspension of the pathogen prepared from 7 to 10 days old culture. Spores of *Fusarium equiseti* and *Alternaria alternata* were grown in PDA, spores of *Colletotrichum gleosporioides* were grown in 'Oat meal agar' medium and spores of *Pestalotiopsis theae* were grown in Richard's solution. Inoculation was done following the inoculation technique of Dhingra and Sinclair (1995). After 72 to 96 hours of inoculation, when disease symptoms appeared on the leaves, the respective pathogens were re-isolated from infected host leaves. Healthy plants were maintained separately for comparison. For re-

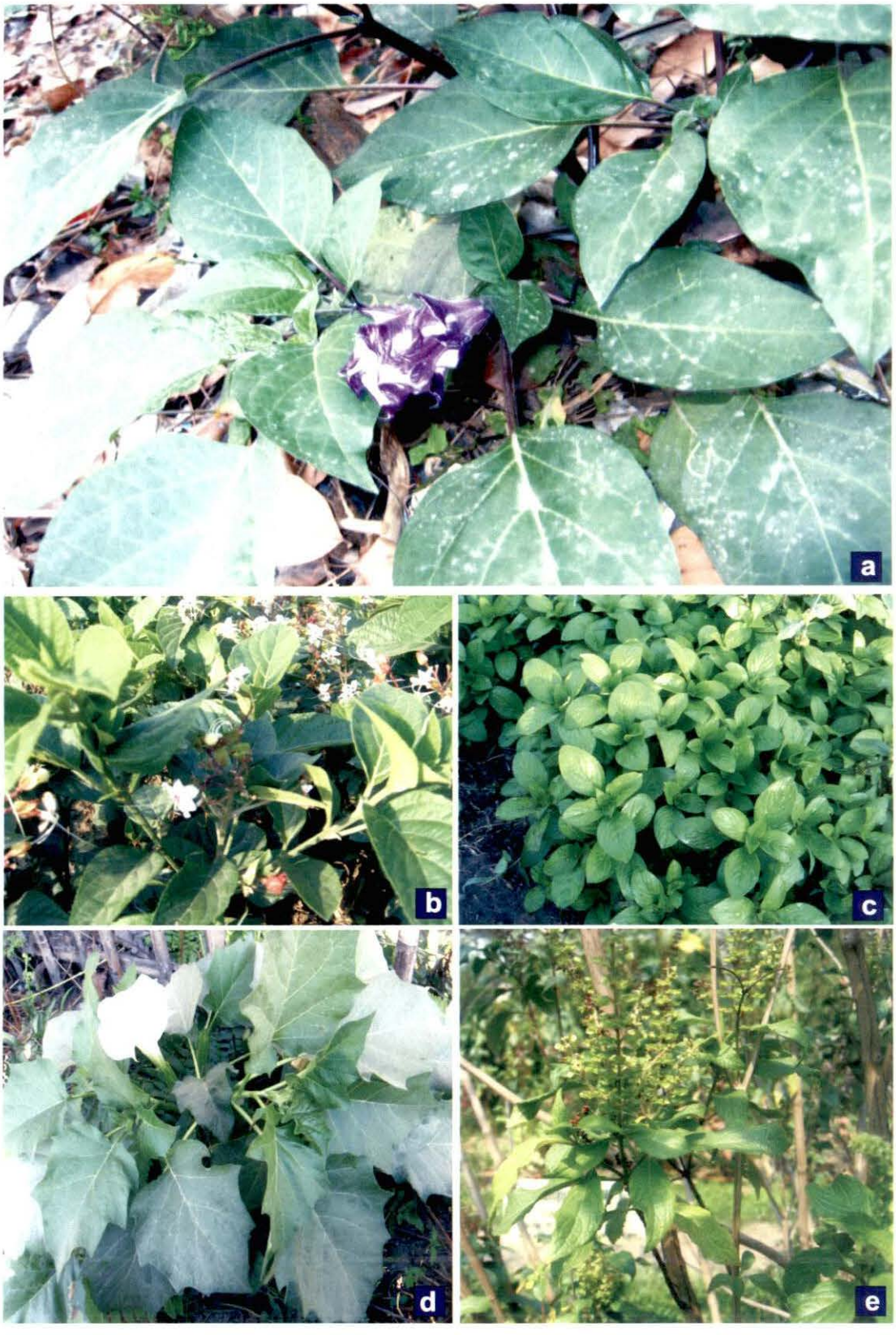

Plate 4 : Some potential plants with antifungal properties against *Alternaria alternata*. **fig.a.** *Datura innoxia*; **fig.b.** *Clerodendrum viscosum;* **fig.c,** *Borreria alata*; **fig.d.** *Datura stramonium;* **fig.e.** *Ocimum gratissimum.*

Plate 5: Some potential plants/plant parts with antifungal properties against *Pestalotiopsis theae*. **fig.a.** *Ginger officiniale*; **fig.b.** *Allium sativum*; **fig.c.** *Xanthium* strumarium; **fig.d.** *Polyalthia longifolia;* **fig.e.** *Dryopteris filixmas.* **fig.f.** *Datura metel.*

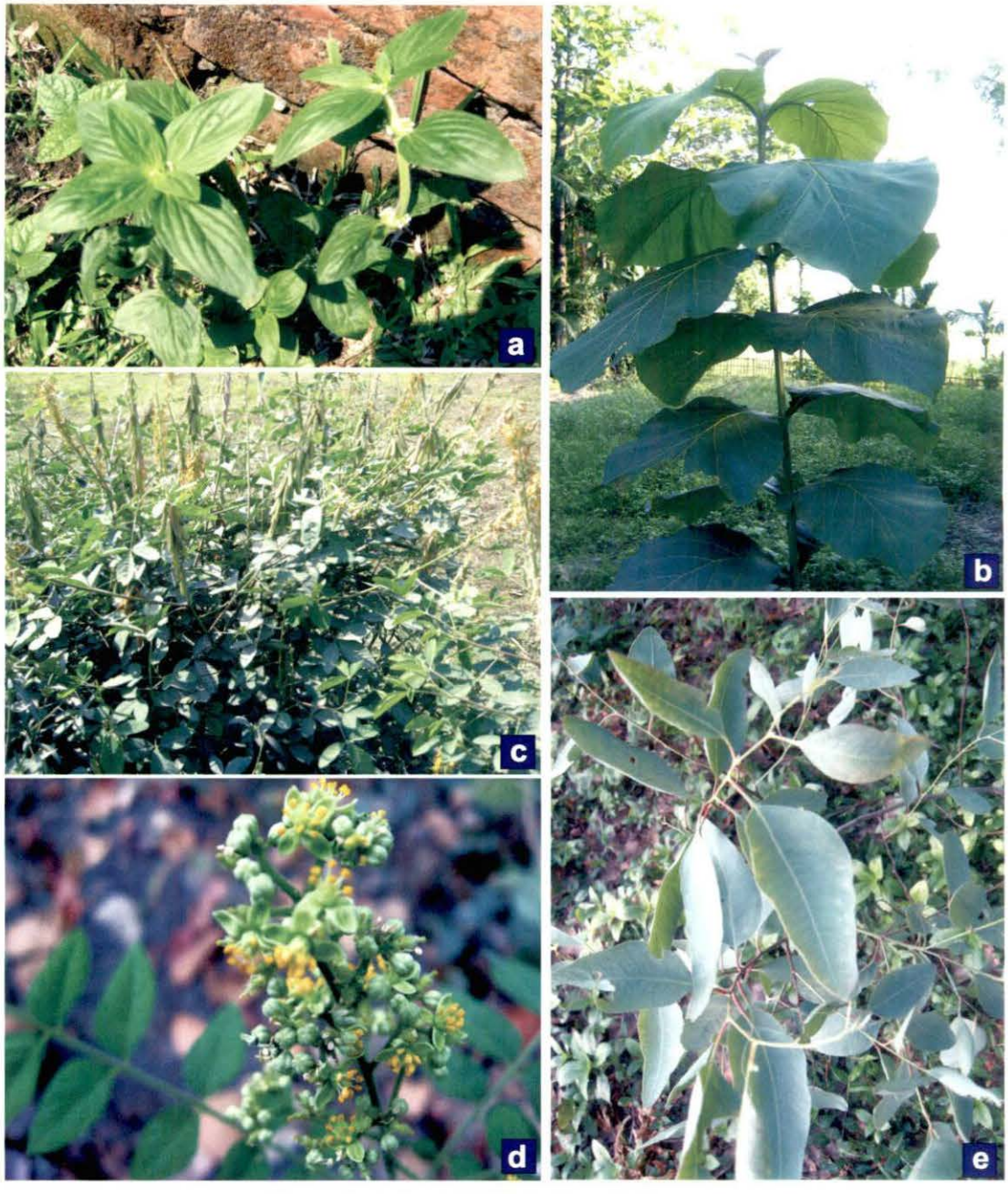

Plate 6: Some potential plants with antifungal properties against *Fusarium equiseti*. **fig.a.***Mitracarpus verticillatus*; **fig.b.***Tectona grandis*; **fig. c.** *Crotalaria mucronata;* **fig. d.** *Clausena excavata*; **fig. e.** *Eucalyptus globosus.*

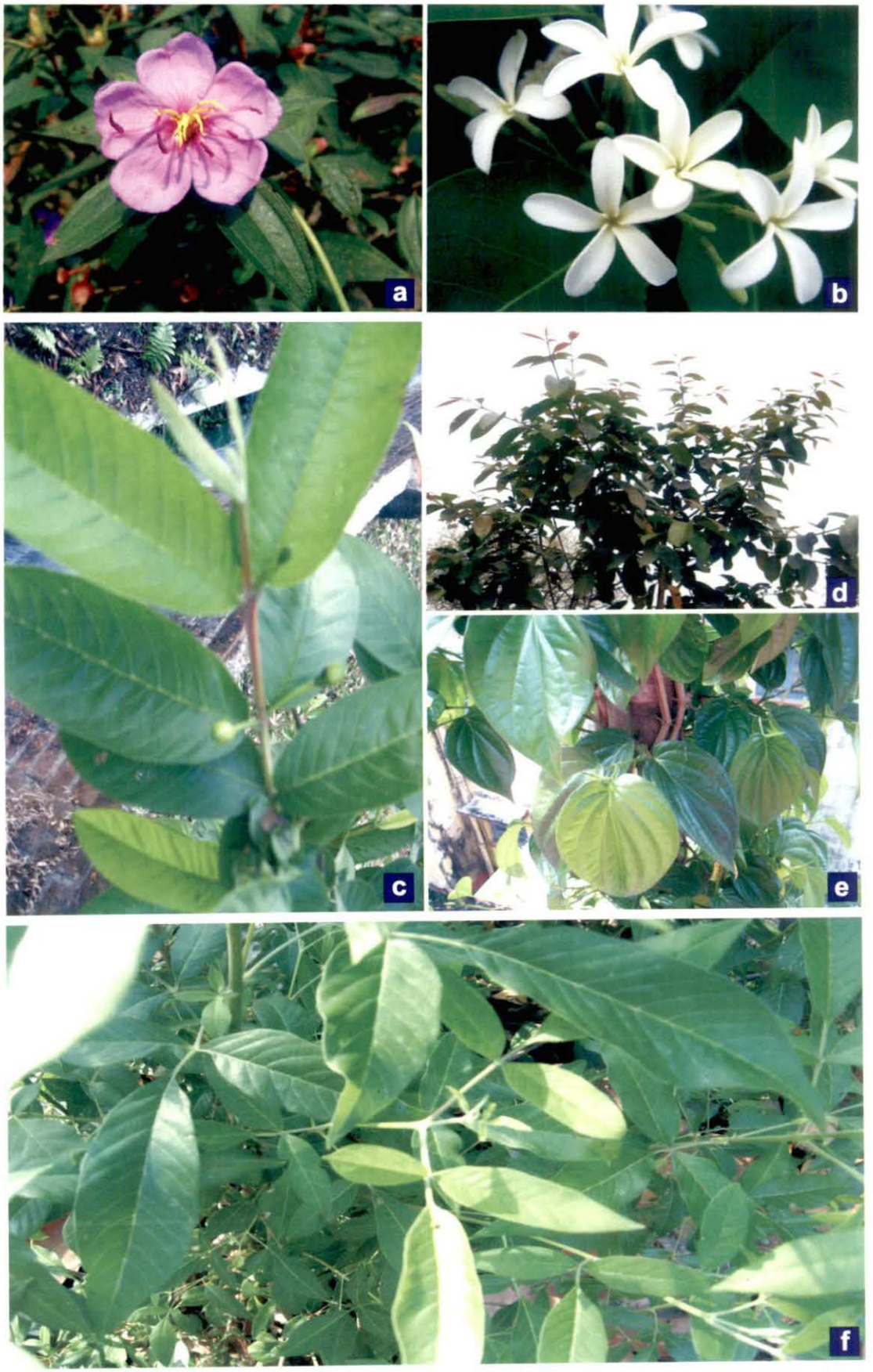

Plate 7: Some potential plants with antifungal properties against *Colletotrichum gloeosporioides*. **fig.a.** *Melastoma malabathricum*; **fig.b.***Holarrhena antidysentrica*; **fig. c.** *Psidium guajava*; **fig.d.** *Syzygium cumini*; **fig.e.** *Piper betel*; **fig.f.** *Vitex negundo*.

isolation, infected portions of the leaves were cut into small pieces (1-2cm), washed thoroughly with the sterile distilled water, surface sterilized with 0.1% Mercuric Chloride ($HgCl_2$) for 1-2 minutes, washed thrice with sterile distilled water and finally transferred aseptically into sterile slants of PDA medium. The inoculated slants were incubated at 28°C and were observed till sporulation. Sporulated cultures were used for microscopic studies. The organisms were confirmed after comparing them with the respective stock cultures. If an organism was consistently re-isolated then it was treated as a pathogen and was identified in the laboratory or elsewhere as mentioned in table 3.3.

Table 3.3: List of fungal cultures isolated from infected plant parts of three economically important plants of North Bengal.

Fungal culture*	Source	Identified by	Identification no.
Fusarium equiseti	Naturally infected tender brinjal stem	IARI, New Delhi.	6566.07
Colletotrichum gloeosporioides	Naturally infected brinjal leaf	IARI, New Delhi.	5446.02
Alternaria alternata	Naturally infected leaves of Tomato	IARI, New Delhi.	7065.08
Pestalotiopsis theae	Naturally Infected leaves of tea	Dr. A. Saha, Molecular Plant pathology and Fungal Biotechnology lab., Department of Botany, Univ. North Bengal	

*All the four fungi were used as test pathogens in the present study.

3.4. Maintenance of stock cultures

Freshly prepared sterile PDA slants were used for the maintenance of the fungal cultures. Pathogens grown on sterile PDA media were stored in two different conditions, viz. at low temperature in refrigerator (at 5°C) and at room temperature. At the interval of two weeks subculture was done for preparation of inoculum for different experiments.

3.5: Scanning electron microscopy (SEM)

Preparation of sample for electron microscopy and observation:

Fungal mycelia were fixed with 2.5% glutaraldehyde solution for 1 hour. Glutaraldehyde was removed by slight decanting. Then 50% ethanol was added to the fungal material on the cover glass and allowed for 5 minutes incubation. After 5 minutes 50% ethanol was replaced by 70% ethanol and it was also incubated for 5 minutes. In the similar way 70% ethanol was replaced by 90% ethanol. After 15 minutes again 90% ethanol was added and incubated for another 15 minutes. Finally the cover glass was dipped in absolute alcohol. After the absolute alcohol treatment the cover glass was air dried and coated with gold. Gold coating was performed in IB2-ion coater (Japan). The cover glass was then observed in scanning electron microscope [Model : Hitachi S-530 (Japan) 1986] with appropriate magnification. Necessary, measurements were also done and have been shown on some of the photographs taken from the microscope.

3.6. Pathogenicity test

3.6.1. Pathogenicity following detached leaf inoculation technique

Pathogenicity test was done by artificial inoculation of detached leaf with test pathogen following the detached leaf inoculation technique proposed by Dhingra and Sinclair (1995). To perform the experiment fresh young fully expanded and detached host leaves were placed on plastic trays lined with moist blotting papers the leaves in the trays were inoculated with spore suspensions of the pathogen. Initially 4-6 wounds (light scratch of 2 mm length) were made on the adaxial surface of each leaf with the help of a sterile sharp needle. Spore/ conidial suspension bearing approximately $1x10^6$ conidia / ml of a test pathogen were prepared from 7-10 d old cultures. Twenty micro litre drops (4-12 drops / leaf based on leaf size) of conidial suspension of a pathogen were placed on its host leaf (where wounds were made) with a hypodermic syringe. In control sets, drops of sterile distilled water were placed on the leaves. Each tray was covered with a glass lid and sealed with petroleum jelly in order to maintain the required moistures inside the trays during incubation. For each experiment at least 100 inoculation drops were mounted on the surface of leaves of desired hosts.

3.6.2. Pathogenicity following whole plant inoculation

Following the whole plant inoculation method of Dickens and Cook (1989), plants were treated with the test pathogen. Inoculation was done by spraying spore suspension of the pathogen prepared from 7-10 d old cultures of test pathogen grown on sterile Petri plates containing media. In control sets, plants were sprayed by sterile distilled water. The plants were kept for 48 hours in transparent polythene chamber. The polythene chambers

were previously mist-sprayed with sterile distilled water to maintain high humidity. Ten plants of each variety were inoculated in each treatment.

3.6.3. *In vivo* control of foliar pathogens by application of plant extracts.

In vivo bioassay on whole plants: To estimate the antagonistic activity of different plant materials, fresh aquatic extracts (0.5g/ml distilled water) were prepared and was mixed with 0.05% tween 20 (used as a wetting agent). Selected plants (of brinjal/tomato/tea) in pots of 27 cm diameter were sprayed with the extracts and were kept for 24 hours in a transparent poly house under normal conditions of light and temperature. Ten plants were sprayed with each treatment. Then the plants were inoculated with the test pathogen as mentioned in section 3.6.2. The assessment of disease was performed as described in the following section (Section-3.7.2).

3.7. Disease assessment

3.7.1. Assessment of disease in detached leaf inoculation technique

Symptoms (Grey blight lesions) produced at the inoculation sites were counted. The number of lesions produced out of the total number of spore-suspension drops multiplied by 100 gave the percentage of lesions produced. Percentages of lesion production were calculated after 24, 48 and 72 hours of inoculation.

3.7.2. Assessment of disease in whole plant inoculation

Symptoms (anthracnose lesions/ leaf spot lesions/ Grey blight lesions or leaf blights lesions as applicable) produced were observed. The number of lesions developed on the leaves after 2, 4, 6, 8 and 10 days of inoculation were counted and diameters of each lesion were measured. The results were computed following the method of Sinha and Das (1972). The diameters were categorized into four groups and a value was assigned to each group as follows:

- Very small-restricted lesions of 1-2 mm diameter: 0.1
- Lesions with sharply defined margins of 2-4 mm diameter: 0.25
- Slow spreading lesions of 4-6 mm diameter: 0.5
- Spreading lesions of variable size (beyond 6 mm in diameter) with diffused margin: 1.0

The number of lesions in each group was multiplied by the value assigned to it and the sum total of such values was noted and disease index was computed as the mean of observations on ten plants per treatment or as mentioned in the respective experiments.

3.8. Preparation of plant extracts

3.8.1. Aqueous extract (from fresh plant parts): Following the method of Mahadevan and Sridhar (1982) with some modifications, the extracts of the plant parts were made. Fresh plant materials were collected and washed thoroughly with sterile distilled water and allowed to dry at room temperature. After drying the materials were weighed, ground and extracted separately with sterile distilled water and ethanol (0.5g/ml). The extracts were filtered through double-layered cheese cloth and then centrifuged at 20,000 g for 15 minutes for 2-4 times until clarification as suggested by Kagale *et al.* 2004. The supernatants of the aqueous extracts were sterilized by passing through a Millipore filter (0.2µm). All extracts were stored at 4^0C. The extracts were used for spore germination bioassay and TLC plate bioassay.

3.8.2. Alcohol extract and ethyl acetate extract (from fresh plant parts): Alcohol extract: Fresh plant parts were washed thoroughly with sterile distilled water and surface water was soaked by blotting paper at room temperature. The materials (3 gm) were ground in a 'mortar & pestle' with 6 ml 50% ethanol with autoclaved water to make 0.5g/ml concentration. The extracts were filtered through double-layered muslin cloth and centrifuged at 10,000 g for 30 min. The supernatant of alcohol extracts were collected in plastic vials and all extracts were stored at 4^0C until used for bioassay.
Ethyl acetate extract: Ethyl acetate extract was prepared using ethyl acetate instead of alcohol following the technique as stated above.

3.8.3. Extract from dry leaves/plant parts

Plant parts were dried, ground to a fine texture, and then soaked in desired solvent for extended periods. The slurry was then filtered after which it was dried under reduced pressure (in rotary vacuum evaporator) and re-dissolved in the alcohol or in the desired solvent to a determined concentration. Crude products were then used in spore germination bioassay, disc diffusion bioassay, agar cup bioassay, poisoned food technique and TLC plate bioassay for antifungal properties and also in a variety of experiments to screen antifungal properties of plants.

3.8. 4. Isolation of phytochemicals by column chromatography from selected plants for their antifungal properties.

The crude extract was fractionated by different methods including silica gel column chromatography using various solvent system following standard methods

(Harborne, 1991; Evans, 2002). The fractions were analysed using Thin Layer Chromatography. The exaction procedure of each extract depended on the chemical constituents of that extract and has been mentioned in following section 3.17.

3.8.5. Soxhlet extraction of antifungal compounds

Soxhlet is an equipment which is used to extract of photochemical exhaustively in different solvents. A fine powder of dried leaves (150g of a potential plant) was extracted in a suitable solvent (Benzene/ hexane/ chloroform/ methanol) in soxhlet for 24 hrs. Soxhlet apparatus model HP-6-500 and rotary vacuum evaporator model Eyela-MS 1000 was used for extraction of the plant parts. The extracted fractions were subjected to different bioassays for their antifungal activity.

3.8.6. Extraction by chemical fraction method:

Ten gram fresh leaf was air dried and powdered. Dried powder (10 gm) was mixed thoroughly in 100 ml solvent mixture of methanol: water (4:1) ratio. After 5 min it was filtered by filter paper in a flask. Thus, two fractions, aqueous phase and residue were separated. The aqueous phase was evaporated up to $1/10^{th}$ volume at 40^0C. The evaporated solution was acidified by adding H_2SO_4 [2M] and mixed with three volume of chloroform. The mixture was separated by separating funnel into two flasks, aqueous acid layer and chloroform layer. The chloroform layer contained moderately polar compounds such as terpenoids / phenolic compounds etc. The aqueous acid layer was basified to 10 pH with NH_4OH and then dissolved in chloroform : methanol (3:1) ratio in twice volume and again separated. The aqueous basic may have the alkaloids. The residue formed during first filtration was mixed with ethyl acetate (5 volume) and then filtered. One filtered residue may have polysaccharide and other may have fat wax or neutral compounds. Each chemical fraction was checked for antimicrobial properties by spore germination bioassay. This extraction procedure has been shown schematically in the following table 3.3

3.9.Preparation of spore suspension:

Ten days old sporulated fungal culture was taken and approximately 3-5ml sterile distilled water was poured in the culture tube aseptically. Gentle scrapping was done by an inoculating needle on the agar surface. After the scrapping, the tube was shaken and the resultant mixture was strained through cheesecloth. The filtrate was used as spore suspension. The concentration of the spores in the suspension was adjusted by adding sterile distilled water following Haemocytometer count.

Table 3.3 :Chemical fraction method followed from Harborne, 1973,1991

<table>
<tr><td colspan="4">Dry powdered leaves</td></tr>
<tr><td colspan="4">↓
Homogenised for 5 min. in CH_3OH:H_2O(4:1)
(10 X vol/wt.), filtered</td></tr>
<tr><td colspan="3">↓
Aqueous phase evaporated(40°C) to $^1/_{10}$ th vol. Acidified with H_2SO_4 + 3 vol. chloroform, separated by separating funnel into two flasks</td><td>↓
filtrate was mixed with ethylacetate (5 volume)and then filtered ,</td></tr>
<tr><td colspan="2">↓
aqueous acid layer The aqueous acid was basified to pH-10 with NH_4OH and then dissolved in chloroform: methanol (3:1) ratio in twice volume and again separated</td><td rowspan="2">↓
The chloroform extracted layer has moderately polar extract such as terpenoids/pH enolics compound.</td><td>↓
Filtrated residue may have polysaccharide</td><td>↓
Filtrate evaporated neutral extract may have fat, waxs, neutral compounds.</td></tr>
<tr><td>↓
$CHCl_3$:CH_3OH Basic extract contained most alkaloids</td><td>↓
aqueous basic phase evaporated and extracted with CH_3OH (CH_3OH extract in polar extract contained quartanary alkaloids).</td><td></td><td></td></tr>
</table>

3.10. Spore germination bioassay

Inhibitory effects of botanicals against four fungi were tested. In experimental sets, plant extract/ chemical fraction (30 µl) was placed on the centre of a grease free microscopic slide and allowed to evaporate. For rapid evaporation air was blown by a blow drier. After evaporation of the solvent (ethanol), spore suspension was mounted on the slides in the same place where the extract was applied. In case of control, the spores of the pathogens were allowed to germinate in sterile distilled water drops mounted on sterile grease free slides kept in a humid chamber. In solvent control set, fresh solvent was placed and subsequently evaporated before application of spore suspension. This was done to avoid residual effects of the solvents used for extraction of the antifungal compounds. The slides were then incubated at 28±1°C in a humid chamber. Two small glass rods (60 mm in length) were placed in a 90 mm petridish and a slide was placed on the rods in a uniformly balanced position. Sterile distilled water was carefully poured in the petridish so that the bottom of the slide remained just above the water surface. The petridish was then covered and incubated at 28±1°C. Following 48 h of incubation, the

slides were stained with lacto phenol-cotton blue and observed under the microscope. Approximately, 200 spores were observed in each slide for germination. The entire experiment was repeated thrice.

3.11. Bioassay by poisoned food technique

Two milliliter of plant extract was added to 18 ml of the molten PDA medium, mixed well and poured in a sterile petridish (90 mm diameter) under aseptic condition and was allowed to solidify. In control sets 2 ml of sterile distilled water was added instead of plant extracts. The both experimental and control plates were inoculated with a pathogen and incubated for required period. Radial growth of the pathogen was measured from both plant extract supplemented and non supplemented petridishes.

3.12. Bioassay by disc diffusion method: Filter paper discs (4 mm in diameter) were prepared from whatman no-1 filter paper using ordinary punching machine. Plant extracts of different concentrations were made and the discs were soaked in different concentrations, air dried and was placed aseptically on the surface of spore suspension supplemented PDA medium in a petidish. In control set discs were soaked in pure solvent only. Inocula were supplemented on the surface of the PDA media by using a sterile glass spreader. The diameter of the inhibition zones surrounding the filter paper discs were measured for the antifungal activity of the extracts tested.

3.13. Agar cup bioassay and determination of Minimum inhibitory concentration (MIC)

For screening of inhibitory effect of botanicals against test pathogens, the spore germination bioassay technique and agar cup bioassay technique was followed. Minimum inhibitory concentration (MIC) of all active components was measured following the techniques of Suleman *et al*, 2002.

At first, 2 ml of spore suspension was added in petridish and subsequently 18ml of molten PDA medium was poured in the Petridish. The medium and the spore suspension were mixed by rotating the petridish carefully. After solidification, by means of a cork borer (5 mm in diameter) cups or wells were made on the spore suspension supplemented agar plate. Then various concentrations of the plant extracts were prepared and poured into the cups. Each cup contained 50µl of plant extracts. Finally, the plates were incubated at 28 ± 1^0 C. The plates were observed after 48h.

In another set of experiments different concentration of fungicides were prepared and poured into the agar cups instead of plant extracts. The lowest concentration of a

fungicide which could control the growth of a fungus was considered as minimum inhibitory concentration (MIC) of that fungicide against the test fungus.

3.14. Bioassay of different soxhlet extracts of plants made in different solvents (following agar cup bioassay technique)

Bioassay of different soxhlet extracts of plants (made in different solvents) were done following agar cup bioassay as described by Suleman *et al.* (2002). Details of the technique have been described in section 3.13. The plates were incubated at 28 ± 1^0C. After the required incubation period, the radial growth of the mycelia was measured and percent inhibition over control was calculated.

3.15. Thin layer chromatography (TLC) plate bioassay

TLC originally developed from a need to separate chemical mixture. In TLC, the stationary phase is coated as a thin (0.25-2.0 mm) layer on a glass or metal plate foil plate that is then placed in a reservoir of mobile phase. The various substances move from the spot at different rate and is deposited in the absorbent layer in the different position. The movement of a given analytic was characterized by retardation factor (R_f).

3.15.1. Preparation and activation of TLC plates

The stationary phase (silica gel G as slurry) was applied to glass plates, using a plate spreader (applicator) that made a uniform layer of 1 mm thickness. Glass plates (20cm×10cm) were dipped into chromic acid to remove all dirt and grease from the glass plates. The glass plates were washed in distilled water and thereafter, they were arranged on a platform of 100cm x 20cm length. Seventy gram silica gel G was added in 100ml distilled water, shaken vigorously and then poured in the slot of TLC applicator. A coat of silica gel (1mm thick) was applied on TLC plates using the applicator. Then the plates were dried at room temperature and kept for the further use.

TLC plates were activated at 70^0C for 45 minutes in a hot air oven. Activated plates were allowed to cool down at room temperature. The extracted test samples were applied as spots at one end at 1.5cm from the edge of the plate. The sample loaded plates were kept in a glass chamber containing solvent [either (Hexane:Ethylacetate:Methanol :: 60:40:1) or (Chloroform:Methanol::9:1)] . When solvent moved up to 15cm from the point of sample application, plates were taken out from solvent chamber and air dried until solvent evaporated completely. Spore suspension was prepared as described in section 3.9 using Richard's solution in place of distilled water and poured in an

automizer. The TLC plates were placed in upright position and sprayed with suspended spores in media. The plates were incubated in humid chamber for 48 to 72 hrs. The spores germinated on plates keeping visible inhibition zones on the plates if antifungal properties were present. The diameter and R_f values of inhibition zones were recorded if any.

3.16. Phytochemical analysis

Different extracts of plant samples as mentioned in section 3.8 were spotted (10µl containing 10 µg crude extract) on precoated silica gel 60 F254 aluminum TLC plates (SRL Silica gel 60 F254). TLC plates were subjected to linear ascending of solvent system of hexane: ethyl acetate: methanol in the ratio of 60:40:1(v/v). When solvent moved up to 8 cm from the point of sample application, plates were taken out from solvent chamber and air dried until solvent evaporated completely. The chromatograms were dried by flowing hot air to remove the remaining solvents. The plates were then sprayed with different chromogenic spray reagents. The detection of phytochemical constituents under UV-light and spray reagents were performed. The developed chromatograms were seen under UV254nm and UV365nm wavelength. The spray reagents were prepared in laboratory following techniques of Wagner and Bladt (1996). Suggested colour reactions have also been presented in the following table 3.4.

3.16.1 Preparation of spray reagents

3.16.1.1 Vanillin –sulphuric acid (VS)

1% ethanol vanillin (solution-I)

10% ethanolic suphuric acid (solution-II)

Plates were sprayed with 10 ml of solution I and immediately 10 ml solution II were sprayed. After the spray the plates were heated at 110^0 C for 7 min. Finally, the plates were evaluated visually. Components of essential oils (terpenoids, phenylpropanoids) were detected if any.

3.16.1.2 Anisaldehyde–sulphuric acid (AS)

Anisaldehyde (0.5 ml) was mixed with 10ml glacial acetic acid followed by 85ml methanol and 5ml concentrated sulphuric acid. The TLC plates were sprayed with 10 ml anisaldehyde–sulphuric acid (heated at 100^0 C for 7 min) and then evaluated visually or under UV light (UV-365nm). The reagent was freshly prepared and used. Terpenoids, propylpropanoids, pungent and bitter principles, saponins were detected if any.

3.16.1.3 Iodine reagent: About 10gm solid iodine was sprinkled in a chromatographic glass tank. The developed TLC plates were placed into the tank and exposed to iodine vapour. All compounds containing conjugated double bonds gave yellow–brown visible zones.

3.16.1.4 Potassium hydroxide reagent (KOH)

10% ethanolic potassium hydroxide was sprayed and observed visually (Borntrager reaction).

3.16.1.5 Dragendorff reagent

Solution A =Bismuth nitrate (0.85 g) was dissolved in 10ml glacial acetic acid and 40ml water in hot condition. **Solution B** = Potassium iodide solution (8 gm in 30 ml distilled water). **Stock reagent**: Solution A and Solution B was mixed in equal volume.

Before spraying 1 ml stock solution was mixed with 2 ml of glacial acetic acid and 10 ml of water (**Dragendorff reagent**). After reagent spray, 10% ethanol-H_2SO_4 was sprayed. Finally colour reactions were observed.

Table 3.4: Colour reaction after chromogenic spray and identification of the compounds on TLC plates (following Wagner and Bladt, 1996)

Spray reagents	Phytochemical constituents	Colour appeared
Vanillin-sulphuric acid (VS)	Monoterpene alcohol Bitter principle Saponin	Blue Blue-green Blue
Anisaldehyde-sulphuric acid (AS)	Triterpene Terpenes Essential oil Bitter principle	Red-violet Violet Green Dark green
10% KOH	Anthraquinones Coumarins	Red Light blue
Dragendorff reagent (DRG)	Alkaloids	Brown or orange-brown

3.17 Column separation techniques

3.17.1 Column separation technique for *Datura stramonium* leaf extract.

A clean glass column (60 cm in length and 1.5 cm in diameter) was filled with petroleum ether. The silica powder (60-120 mesh) was dropped slowly from top of the column. Thus 3/4th part of whole column was filled with silica gel in petroleum ether. The remaining 1/3rd part of column was filled with silica powder mixed with plant crude extract. The elution of solvent was performed following the schedule as mentioned in following table. Each fraction collected was of 20 ml. The elution began with a single non polar solvent followed by combination of gradually increasing polar solvent along with no polar solvent commonly known as elution gradient.

Name of the solvent	Volume(ml)	Fraction no.
Petroleum ether	100	1-5
5% ethyl acetate +Hexane	100	6-10
10% ethyl acetate +Hexane	100	11-15
15% ethyl acetate +Hexane	100	16-20
20% ethyl acetate +Hexane	100	21-25
25% ethyl acetate +Hexane	100	26-30
30% ethyl acetate +Hexane	100	31-35
35% ethyl acetate +Hexane	100	36-40

3.17.2 Column separation technique for *Polyalthia longifolia* leaf extract.

The leaves of the plant were collected, washed and dried at room temperature (30-32°c) for 7 days. The material was powdered in a grinder and soaked in 300ml methanol for 72 hrs. The extract was filtered with whatman No. 1 filter paper and the filtrate was concentrated in vacuum in a rotary evaporator. The residue was collected in diethyl ether and resuspended in methanol. The concentrated extract (0.1gm/ml) served as the stock solution.

Two grams of the residue of the methanol extract were subjected to chromatographic separation on a silica gel column (SRL) and eluted with solvents by gradually increasing the polarity in the following way: Hexane, Hexane ethyl

acetate(3:1,1:1,1:3),ethyl acetate, ethyl acetate: methanol(3:1, 1:1, 1:3) and methanol. Twenty fractions of 100ml each were collected and concentrated by evaporation. Each extract was assayed by the spore germination bioassay technique using spores of *P.theae*.

3.17.3 Specific solvent extraction technique for fractionation of *Polyalthia longifolia, A. sativum and D. stramonium* plant part extracts.

This was done to get antifungal properties of the above mentioned plant parts in a concentrated and partially purified form. The fractions thus prepared were tested following different bioassay techniques and have been presented in Section 4.3 (chapter III).

Extraction of *Polyalthia longifolia* : A fine powder of air dried leaves was extracted in methanol for 5days. The extracts were concentrated under reduced pressure to form a dark green residue (fraction PL1). This fraction was extracted with Hexane soluble fraction was evaporated (fractionPL2) while the non soluble residue was again extracted with ethyl acetate in a similar way to give the ethyl acetate soluble fraction which was evaporated (fractionPL3). The final residue was soluble in methanol (fraction PL4). MIC of each fraction was determined and thin layer chromatograms of the fractions were used for bioassay.

Extraction of *A .sativum:* Fresh bulbs of A. sativum were peeled macerated in a grinder with methanol and the extract was squeezed through cheese cloth. The solvent was evaporated to form an off white residue (fractionAS1). The fraction was extracted with hexane (fractionAS2) ethyl acetate (fractionAS3) and finally with methanol (fractionAS4) in similar way. MIC was determined with all the fractions.

Extraction of *D. stramonium*: The leaves were air dried and grinded to a fine powder. This material was extracted in methanol for five days. The extract was concentrated in a rotary evaporator in vaccum and a dark green residue (5g) was obtained (DS1). It was extracted separately with benzene (DS2). Chloroform (DS3) and diethyl ether (DS4).The ether extract was further separated into acidic (DS5), basic (DS6) and neutral fraction (DS7). All the fractions were subjected to bioassay and their MIC values were determined.

3.18 : Major chemicals used

In addition to the common laboratory reagents, following chemicals were used during the work:

Chemicals	Company
Acetic acid glacial	SRL Pvt. Ltd., Mumbai, India
Anisaldehyde	SRL Pvt.Ltd. Mumbai, India
Benzene	SRL Pvt.Ltd. Mumbai, India
Bismuth nitrate	SRL Pvt. Ltd., Mumbai, India
Chloroform	E. Merck (India) Ltd., Mumbai, India
Diethyl ether	SRL Pvt. Ltd., Mumbai, India
Ethyl acetate	SRL Pvt.Ltd. Mumbai, India
Hexane	E. Merck (India) Ltd., Mumbai, India
Iodine	SRL Pvt.Ltd. Mumbai, India
Mercury(II) chloride	E -Merck (India) Ltd., Mumbai, India
Methanol	SRL Pvt. Ltd., Mumbai, India
Nickel chloride	Sigma Chemicals Co., USA
Petrolium ether	SRL Pvt. Ltd., Mumbai, India
Potassium hydroxide	Merck (India) Ltd., Mumbai, India
Potassium nitrate	E. Merck (India) Ltd., Mumbai, Ind
Potassiun dihydrogen phosphate-	Merck (India) Ltd., Mumbai, India
Potassiun Iodide	E. Merck (India) Ltd., Mumbai, India
Silica Gel (60-120 mesh for column)	SRL Pvt. Ltd., Mumbai, India
Silica Gel GF-254	SRL Pvt. Ltd., Mumbai
Sodium Chloride	Merck (India) Ltd., Mumbai
Sodiun nitrate	SRL Pvt.Ltd. Mumbai
Sucrose	Ranbaxy Fine chemicals Ltd Mumbai,
Sulphuric acid	SRL Pvt. Ltd., Mumbai
Vanillin	SRL Pvt. Ltd., Mumbai
Tween 20	SRL Pvt. Ltd., Mumbai

3.19. Chemcial fungicides used

Trade name	Chemical name
Bavistin	Carbendazim (2-methoxycarbamoyl)-benzimidazole)
Nystatin	Mycostatin nickel chloride
Captaf	Captan 50% wettable powder

3.20. Media and solution used

A number of culture media and solutions were used during the present study, the name and compositions of these media and solutions are given below.

Potato Dextrose Broth (PDB)

Peeled Potato	:	40 g
Dextrose	:	2g
Distilled water	:	100ml

Peeled potato in required amount was boiled in distilled water. The potato broth was collected by straining through cheese cloth and then required amount of dextrose was added. Finally the medium was sterilized at 15 lb p.s. i. for 15 minutes.

POTATO DEXTROSE AGAR (PDA)

2% agar powder was added to the final potato dextrose broth solution to prepare potato dextrose agar. The agar was melted by heating the media before sterilization.

OAT MEAL AGAR (OMA)

Oat meal	:	40g
Agar agar	:	15g
Distilled water	:	1000ml

Required amount of powdered oat was boiled in distilled water in a water bath, stirred occasionally and strained through cheese cloth. The agar powdered was added to it and melted by heating before the medium was sterilized at 15 lb p.s. i. for 15 minutes.

LEAF EXTRACT AGAR (LEA)

Brinjal leaf	:	20g
Agar agar	:	2g
Distilled water	:	100ml

Fresh brinjal leaves of 20g were boiled in distilled water. Leaf decoction was collected by staining through cheese cloth. Required amount of agar powder was then added and melted by boiling. Finally the medium was sterilized at 15 lb p.s. i. for 15 minutes.

Czapek Dox Agar (CDA)

$NaNO_3$	:	3 g
K_2HPO_4	:	1 g
KCI	:	0.5 g
$MgSO_4$, $7H_4O$	:	0.5 g
$FeSO_4$	:	0.01 g
Sucrose	:	30 g
Agar agar	:	15 g
Distilled water	:	1000 ml

(Initially, all the ingredients except agar and K_2HPO_4 were dissolved. Next, agar was added and dissolved by steaming. Finally K_2HPO_4 was added to the molten solution, mixed thoroughly and sterilized at 15 Ib p.s.i. for 15 minutes).

Richard's Solution / Medium (RM)

KNO_3	:	10 g
KH_2PO_4	:	5 g
$MgSO_4$, $7H_2O$	:	2.5 g
$FeCI_3$	:	0.02 g
Sucrose	:	50 g
Distilled water	:	1000 ml

(Required amount of all the constituents were taken and mixed with required distilled water. All the constitutes were dissolved by stirring and sterilized at 15 Ib p.s.i. for 15 minutes).

HOAGLAND AND KNOP'S SOLUTION

KNO_3	:	0.61 g
$Ca(NO_3)_2$, $4H_2O$	:	0.95 g
$MgSO_4$, $7H_2O$	:	0.49 g
$NH_4(H_2PO_4)$	:	0.12 g
$MnSO_4$, $4H_2O$	:	3.00 g
$ZnSO_4$, $7H_2O$	:	0.500 mg
H_3PO_3	:	0.5 ml
$CuSO_4$, $5H_2O$	:	0.025 mg
Na_2MoO_4, $2H_2O$	:	0.025 mg
H_2SO_4	:	0.5 µl
$FeC_6O_5H_7$, $5H_2O$	:	0.2 g
Distilled water	:	1000 ml

Required amount of all the constituents were taken and they were mixed thoroughly in distilled water.

4. Results

Northern part of West Bengal separated by river Ganges is commonly called as north Bengal. North Bengal stretches to the east up to the border of Bangladesh & Assam and to the west up to Bihar & Nepal. Its ecological and climatic conditions help in the ample production of tropical and subtropical crops. Some of the principal agriculture proceeds of north Bengal are brinjal, tomato, tea etc. During the past few decades there has been remarkable improvement in cultivation as well as in the production of agriculture products. Although, modern technology and advancements have helped immensely in sowing, seedling development and cultivation of many tropical and subtropical crops but still there is lack of application of environment friendly processes for the diseases of the crops. In the present study, four different pathogens of three different crops were taken in to consideration for the purpose of their control by botanicals. The detailed experimental results and observations have been presented in the following three chapters.

Chapter I :

Pathogens (*Fusarium equiseti* and *Colletotrichum gloeosporioides*) of brinjal and their control.

Chapter II :

Pathogen (*Alternaria alternata)* of tomato and its control.

Chapter III :

Pathogen (*Pestalotiopsis theae*) of tea and its control.

CHAPTER I

4.1 Chapter I : Pathogens (*Fusarium equiseti* and *Colletotrichum gloeosporioides*) of brinjal (*Solanum melongena*) and their control.

At the onset of the present study two pathogens of brinjal were isolated from severely affected brinjal plants from the farmer's field situated at Phansidewa in the district of Darjeeling (Plate 8). The two pathogens were grown in tubes as well as in plates. The morphology of the fungal colonies has been presented photographically in plates- 8, 9 & 10.

Study of *F. equiseti* : On maturity mycelial colony of *Fusarium equiseti* produced reddish brown colour in tubes and plates (Plate 8 & 9). The morphology of the fungus was studied in light microscope. For microscopic observation, mycelia were taken in microscopic slides from pure culture and stained using cotton-blue in lacto phenol. The slides were mounted with cover glass, sealed and observed under microscope. Comma-shaped micro conidia in aerial mycelium were evident. Macro conidia were strongly septate and sickle shaped (plate 9). The basal cell is distinctly foot shaped. Conidiophores were also evident. The length and breadth of the mature macro conidia were 15-27micrometer and 3-5 micrometer respectively.

Study of *C. gloeosporioides*: The morphology of the pathogen C. gloeosporioides was observed in PDA and OMA (slants and plates) and PDB (Erlenmeyer flask). When the fungus was cultured in PDA or PDB, the mycelia was white in colour, which gradually turned pale yellow and further darker to gray (plate 10). In OMA, mycelia were pale yellow coloured but growth was not as profuse as PDA. Huge masses of pinkish acervuli were produced in OMA, which was very less when grown in PDA. Mycelia and conidia of the fungus were light colored. The length and breadth of the mature conidia were 12-15micrometer and 3.5-5.5 micrometer respectively. The mature conidia were light, one-celled and hyphae were septate, the diameter of the mature hyphae was between 3-5 micrometer.The scanning electron microscopic studies were also performed to understand the details of the surface morphology of the fungal pathogen as well as to get accurate measurement of the spore and hyphae. The measurements have been shown directly on the electron microscopic photographs presented in plate 11.

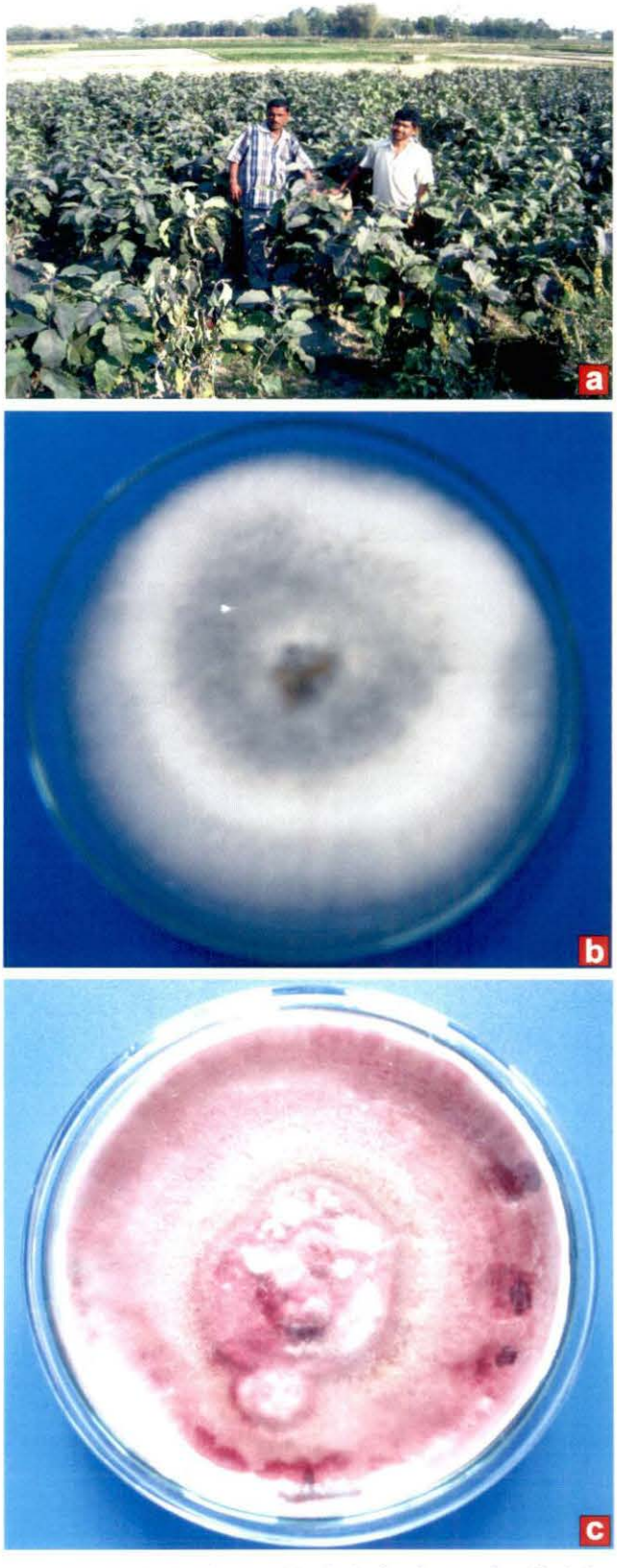

Plate 8: fig. a. Naturally infected brinjal plants in field of Phansidewa near Siliguri, **fig.b.** *Fusarium equiseti* isolated from farmer's field **fig.c.** Sporulated culture of *F. equiseti* in PDA.

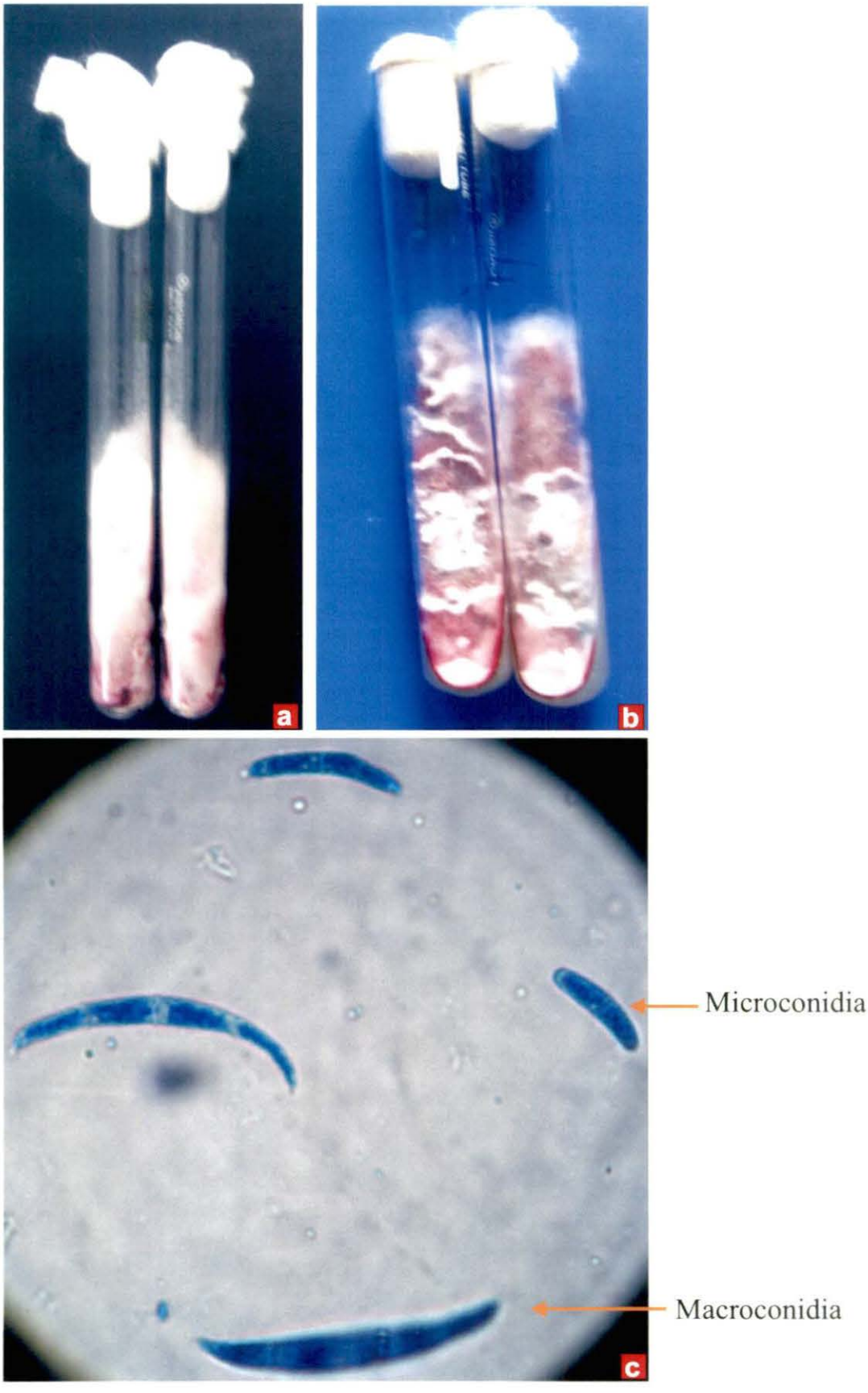

Plate 9: fig. a. Mycelia of *Fusarium equiseti*.
fig.b. Mycelia with conidiospores.
Fig.c. Macroconidia and microconidia.

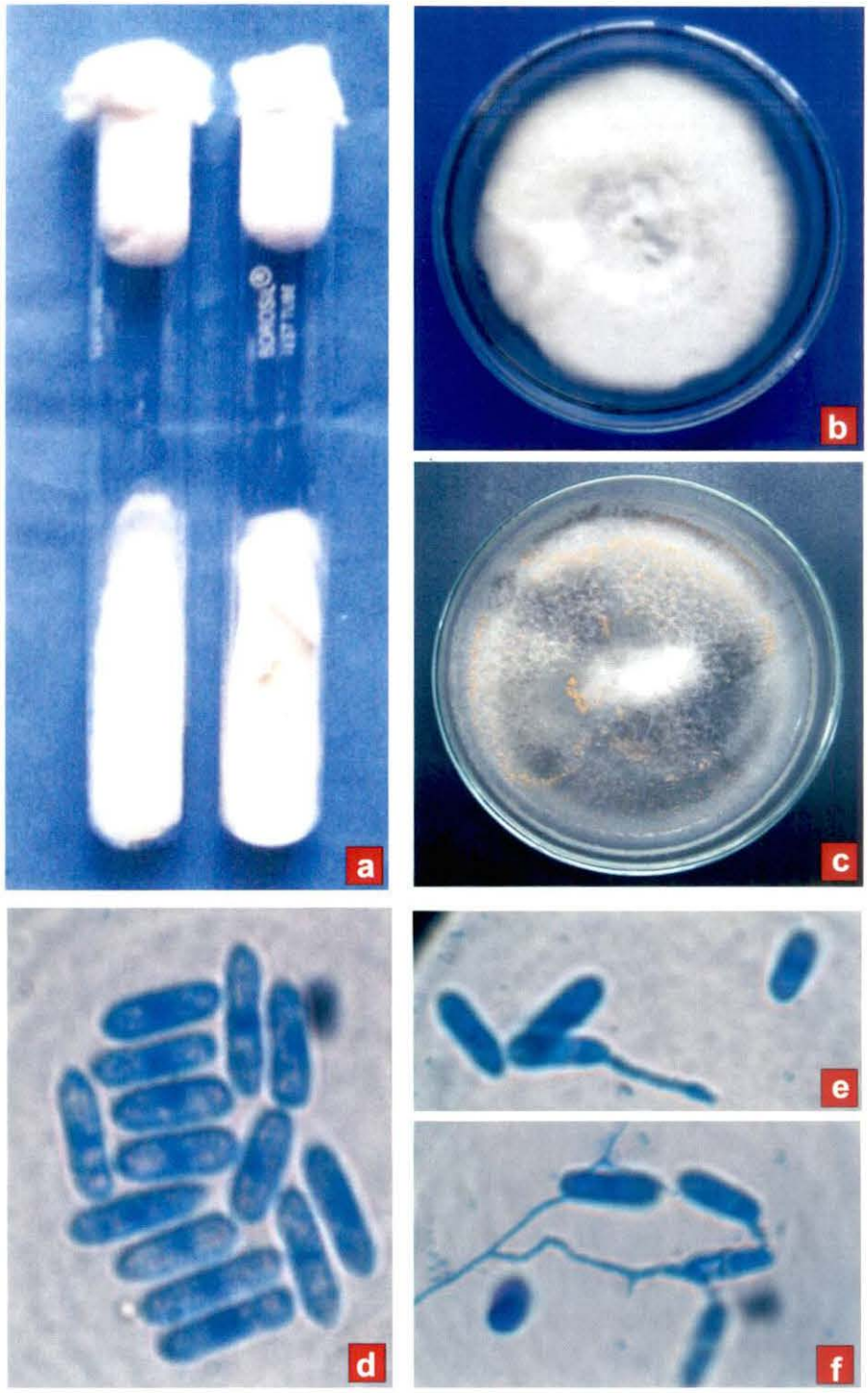

Plate 10 : fig.a. Mycelia of *Colletotrichum gloeosporioides* in PDA slants **fig.b.** Mycelia of *C.gloeosporioides* in PDA plate **fig.c.** Sporulated culture of *C.gloeosporioides* in OMA plate **fig.d.** Spores of *C.gloeosporioides*.**fig.e&f.** Germinating spores of *C.gloeosporioides*.

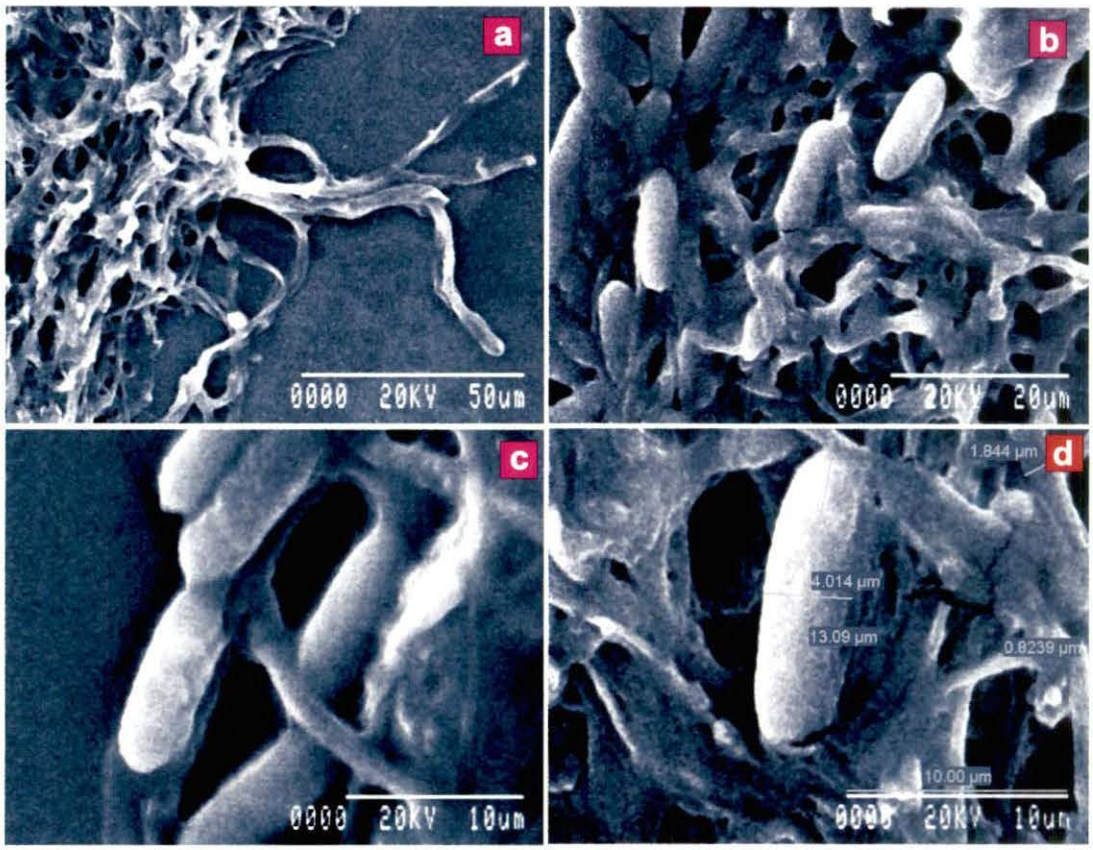

Plate 11 : Scanning electronmicroscopic photographs of Colletotrichum gloeosporioides **fig.a.** Hyphae of the fungus **fig.b.** Spores with mycelia **fig.c.** Spores with measurment in µm.

4.1.1: Pathogenicity test of *Colletotrichum gloeosporioides* and *Fusarium equiseti* in different Brinjal varieties.

Pathogenicity of *Colletotrichum gloeosporioides* and *Fusarium equiseti* was tested following whole plant inoculation technique. Six brinjal varieties (viz. Green round supriya, variety, Pant rituraj Kanha hybrid, Pant samrat and Muktakeshi) were selected for the experiments. The details of the experiment and disease assessment procedures have been mentioned in the materials methods (section 3.7). From the data (represented in the table-4.1.1.) it is quite clear that Green round variety showed maximum disease development (mean disease index /plant was 8.4 after 10 days of inoculation) against *Fusarium equiseti.* Therefore brinjal plants of green round variety were considered as the most susceptible variety towards the pathogen among the varieties tested. Varieties like supriya, Pant rituraj, Kanha hybrid and Pant samrat were also considered as susceptible as their mean disease index values /plant were 5.5, 5.4, 4.8, and 4.8 respectively after 10 days of inoculation. On the other hand Muktakeshi variety was the most resistant as it produced minimum mean disease index value/plant (1.2). Muktakeshi variety was used as resistant plant throughout the present study as and when required.

Table 4.1.1: Pathogenicity of *Fusarium equiseti* on brinjal varieties following whole plant inoculation technique

Brinjal varieties	Incubation period (Days)				
	2	4	6	8	10
	MDI/ plant*	MDI/ plant	MDI/ plant	MDI/ plant	MDI/ plant
Green round	0	0.8	3.9	5.8	8.4
Supriya Variety	0	0	2.6	4.7	5.5
Pant Rituraj	0	0	2.4	4.6	5.4
Kanha Hybrid	0	0	2.2	3.9	4.8
Pant Samrat	0	0	2.1	3.7	4.8
Muktakeshi local	0	0	0	0.7	1.2
CD at 5%	-	-	0.18	0.33	0.41

MDI= Mean disease index
*Mean of 3 replications.

Similarly, from the data (represented in the table 4.1.2.) it is evident that Green round variety showed maximum (mean disease index /plant = 13.56) disease development after 10 days of inoculation (with *Colletotrichum gloeosporoides*). Varieties like supriya, Pant rituraj, Kanha hybrid and Pant samrat were also considered as susceptible as their mean disease index values/ plant were 7.25 5.49, 4.80, and 3.22 respectively after 10 days of inoculation. Muktakeshi variety showed resistance against *Colletotrichum gloeosporoides* like the *Fusarium equiseti*. The minimum mean disease index/plant against *Colletotrichum gloeosporoides* was 2.42. Therefore, in the following studies Green round variety and Muktakeshi variety were used as susceptible plant and resistant plant respectively for the experiments performed in the present study (wherever applicable).

Table: 4.1.2 Pathogenicity of *Colletotrichum gloeosporoides* on brinjal varieties following whole plant inoculation technique

Brinjal varieties	**Incubation period (Days)**				
	2	**4**	**6**	**8**	**10**
	MDI/ plant*	**MDI/ plant**	**MDI/ plant**	**MDI/ plant**	**MDI/ plant**
Green round	1.5	3.24	8.36	11.56	13.56
Supriya Variety	0.9	1. 65	2.18	5.30	7.25
Pant Rituraj	0.6	1. 46	2.18	4..43	5.49
Kanha Hybrid	0.32	1. 27	2.14	4.38	4.80
Pant Samrat	0.12	0.90	1.76	2.16	3.22
Muktakeshi local	0.0	0.6	0.98	2.10	2.42
CD at 5%	0.24	0.13	0.77	0.16	0.15

MDI= Mean disease index
*Mean of 3 replications.

4.1.2: Screening of extracts of different plants for antifungal activity against *F.equiseti* and *C. gloeosporioides*.

To screen potential antifungal activity eighty (80) plants were collected from different parts of North Bengal. Extracts of the leaves (50% ethanolic) were prepared and were used for their antifungal properties against *F. equiseti* and *C. gloeosporioides*. Spore germination technique described by Suleman *et.al.* (2002) was followed for the screening. A detailed procedure of spore germination bioassay and preparation of extracts have been described in materials and methods (section 3.10). Data presented in the table were calculated on the basis of the percent inhibition of spore germination of the two different fungi in different plant extracts tested. It was evident from the result (Table-4.1.3) that 50% ethanolic extract of *Xanthium strumarium*, *Crotalaria mucronata* and *Datura stramonium* were effective in controlling spore germination (cent percent control) of both the fungal pathogens of brinjal. Among the other 50% ethanol extracts about seventeen plants have shown their antifungal potentiality. The percent inhibition of the plant extracts have been presented in table- 4.1.3. Inhibition percentage of each of the plant extracts have been shown in the following lines against *F. equiseti*. Above 95% inhibition was recorded in two plants such as *Mitracarpus verticillatus* (showed 98% inhibition) and *Scoporia dulcis* (showed 97% inhibition). Two plants extract *Datura metel* and *Polyalthia longifolia* inhibited spore germination within the range of 90 - 95%. Twelve plants extract (*Allium sativum, Annona squamosa, Melia dubia, Piper betle, Plumeria rubra, Clausena excavata, Datura innoxia, Phyllanthus emblica, Eucalyptus globosus, Psidium guajava, Tectona grandis* and *Borreria alata*) inhibited spore germination within the range of 80 - 90%. Similarly, fifteen different ethanolic (50%) plant extracts showed inhibition of spore germination against *C. gloeosporioides*. The differential antifungal activity of the potential plants has been presented in the following lines along with percent inhibition of spore germination. The fifteen potential plants are *Catharanthus roseus* (80%), *Clerodendrum viscosum*(92%), *Holarrhena antidysentrica* (95%), *Allium sativum* (95%), *Aegle mermelos*(88%), *Annona squamosa* (87%), *Piper betle* (83%), *Polyalthia longifolia* (90%) *Clausena excavata* (90%), *Datura innoxia* (87%), *Melastoma malabathricum* (85%), *Psidium guajava* (90%), *Scoporia dulcis* (95%), *Syzygium cumini* (80%) and *Borreria alata* (85%).

Table 4.1.3 Effect of different plant extracts on spore germination of *Fusarium equiseti* and *Colletrotrichum gloeosporioides*

Name of plants	Family	*F. equiseti* (50 % alcoholic extract)		*C. gloeosporioides* (50 % alcoholic extract)	
		% Germination	% Inhibition	% Germination	% Inhibition
Acacia catechu (L.f)Wild	Mimosaceae	80	20	18	78
Acalypha indica L.	Euphorbiaceae	22	78	85	15
Adhatoda vasika Nees.	Acanthaceae	74	26	65	35
Aegle mermelos (L.) Corr	Rutaceae	40	60	12	88
Ageratum conyzoides L.	Asteraceae	22	78	40	60
Allium sativum L.	Liliaceae	15	85	05	95
Alostonia scholaris (L.) *R. Br.*	Apocynanceae	25	75	90	10
Amaranthus spinosus L.	Amaranthaceae	99	01	100	00
Anisomeles indica (L.) kuntze	Laminaceae	25	75	25	75
Annona squamosa L.	Annonaceae	15	85	13	87
Argemone mexicana L.	Papavaraceae	25	75	82	18
Artocarpus heterophylus Lam.	Moraceae	86	12	70	30
Asparagus racemosus Wild	*Asparagaceae*	65	35	65	35
Azadirachta indica L.	Melicaceae	43	57	44	56
Borreria alata (Aublet) De Candolle.	Rubiaceae	20	80	15	85

Contd...

Table 4.1.3. contd. : Effect of different plant extracts on spore germination of *Fusarium equiseti* and *Colletrotrichum gloeosporioides*

Name of plants	Family	*F. equiseti* (50 % alcoholic extract)		*C. gloeosporioides* (50 % alcoholic extract)	
		% Germination	% Inhibition	% Germination	% Inhibition
Caesalpinia pulcherrima (L.) Swartz.	Caesalpiniaceae	60	40	70	30
Cannabis sativa L.	Cannabinaceae	20	80	75	25
Calotropis gigantean (L.) R. Br.ex Aiton	Asclepiadaceae	00	100	75	25
Cassia tora L.	Caesalpiniaceae	95	05	75	25
Catharanthus roseus (L.) Don	Apocynanceae	92	08	20	80
Centella asiatica L.	Apiaceae	40	60	80	20
Citrus limon (L.) *Burm.*	Rutaceae	10	90	100	00
Clerodendrum viscosum Vent.	Verbenaceae	70	30	08	92
Clausena excavata Burm.f.	Rutaceae	12	88	10	90
Clitoria ternatea L.	Fabaceae	100	0	100	00
Crotalaria mucronata Desv.	Fabaceae	17	83	28	72
Datura stramonium L.	Solanaceae	00	100	00	100
Datura metel L.	Solanaceae	10	90	76	24
Datura innoxia Mill.	Solanaceae	12	88	13	87
Elephantopus scaber L.	Asteraceae	100	00	100	00
Phyllanthus emblica officinalis L.	Euphorbiaceae	12	88	86	14
Eucalyptus globosus. Labill.	Myrtaceae	13	87	95	05
Euphorbia hirta L.	Euphorbiaceae	*97*	*3*	*95*	*5*
Heliotropium indicum L.	Boraginaceae	85	15	100	00

Contd...

Table 4.1.3 contd. : Effect of different plant extracts on spore germination of *Fusarium equiseti* and *Colletrotrichum gloeosporioides*

Name of plants	Family	*F. equiseti* (50 % alcoholic extract)		*C. gloeosporioides* (50 % alcoholic extract)	
		% Germination	% Inhibition	% Germination	% Inhibition
Hibiscus rosa-sinensis L.	Malvaceae	66	34	100	00
Holarrhena antidysentrica Wall.	Apocynanceae	32	68	05	95
Hyptis suaveolens (L.) Poit.	Lamiaceae	66	34	37	63
Lantana camara L.	Verbenaceae	78	22	68	32
Lagerstroemia speciosa L. Pers.	Lythraceae	77	23	81	19
Mangifera indica L.	Anacardiaceae	78	22	73	27
Melastoma malabathricum L	Melastomaceae	35	65	15	85
Melia dubia Cav.	Meliaceae	14	86	17	83
Mimosa pudica L.	Mimosaceae	98	02	50	50
Mitracarpus verticillatus (Schumach&Thonn) Vatke	Rubiaceae	02	98	13	87
Moringa oleifera Lamk.	Moringaceae	28	72	55	45
Murraya koenigi (L) Spreng	Rutaceae	88	12	90	10
Nyctanthes arbor-tristis L.	Nytanthaceae	90	10	90	10
Ocimum gratissimum L.	*Lamiaceae*	35	65	47	53
Ocimum sanctum L.	Lamiaceae	84	10	90	10
Oldenlandia corymbosa L.	Rubiaceae	75	25	75	25
Oxalis corniculata L.	Oxalidaceae	85	15	85	15
Phyllanthus fraternus Webster.	Euphorbiaceae	82	18	95	05
Piper betel L.	piperaceae	20	80	17	83
Plumeria rubra L.	Apocynanceae	14	86	28	72
Polyalthia longifolia Sonnerat	Annonaceae	09	91	10	90
Pouzolzia indica L.	Utricaceae	84	16	100	00

Contd...

Table 4.1.3 contd. : Effect of different plant extracts on spore germination of *Fusarium equiseti* and *Colletrotrichum gloeosporioides*

Name of plants	Family	*F. equiseti* (50 % alcoholic extract)		*C. gloeosporioides* (50 % alcoholic extract)	
		% Germination	% Inhibition	% Germination	% Inhibition
Psidium guajava L.	Myrtaceae	12	88	10	90
Raphanus sativus L.	Brassicaceae	60	40	45	55
Rauvolfia tetraphylla L.	Apocynanceae	08	02	25	75
Ricinus communis L.	Euphorbiaceae	99	01	76	24
Saraca asoca (Roxb.) De Wilde	Caesalpiniaceae	80	20	66	32
Scoporia dulcis L.	Scrophulariaceae	3	97	15	85
Sida acuta L.	Malvaceae	100	0	100	0
Smilax zeylanica L.	Similaceae	45	55	45	55
Solanum khassianum Clark.	Solanaceae	10	90	30	70
Solanum nigrum L.	Solanaceae	100	00	90	10
Solanum torvum Sw.	Solanaceae	30	70	70	30
Solanum xanthocarpum Schrad & Wendl.	Solanaceae	35	65	36	64
Tridax procumbens L.	Asteraceae	55	45	100	00
Syzygium cumini (L.) Skeels	Myrtaceae	80	20	20	80
Tectona grandis L.f.	Verbenaceae	15	85	30	70
Terminalia arjuna (Roxb). W&A	Combretaceae	90	10	85	15
Vitex negundo L.	Verbenaceae	34	66	22	78
Xanthium strumarium L.	Asteraceae	00	100	00	100
Zingiber officinale Roscoe	Zingiberaceae	38	61	43	57

Data are average of 300 spores and rounded to nearest whole number

4.1.3: Study of antifungal activity of potential extracts at different concentration by spore germination bioassay.

The dried powdered of plant materials (aerial parts) were extracted with methanol using soxhlet apparatus for 48 hrs. The solvent was distilled off at lower temperature under reduced pressure in rotary flash evaporator and concentrated on water to get the soxhlet extract which was stored in room temperature for future use. Details of extraction procedure have been presented in the section 3.8.5.

Seven different soxhlet extracts which showed preliminary antifungal activity *in vitro* against *Fusarium equiseti* were used to test their antifungal activity in different concentrations. Stock solution (5000 µg /ml) of the soxhlet extract was prepared by dissolving 5 mg of the soxhlet extract in 1 ml of distilled water. Required concentrations (1000, 2000, 3000, 4000 and 5000 µg /ml) were prepared from each stock solution by dilution with distilled water. One drop (40 µl) of extract of each concentration of each plant was placed on a sterile grease-free slide. Spore suspension (10^5 ml^{-1}) from 7-10 days old culture was prepared following the method as mentioned in Section 3.9. The slides were placed in moist chambers in Petri dishes. The slides with spores were then incubated at 28 ± 1^0C for 24 hr. Germination was observed after staining with cotton blue prepared in lactophenol under microscope. Spores mixed in sterile distilled water only served as control. All the experiments were conducted in triplicate.

Five different concentrations of seven different plant extracts (viz. *Datura innoxia, Azadirachta indica, Xanthium strumarium, Tectona grandis, Clausena excavata, Polyalthia longifolia* and *Crotalaria mucronata*) were tested for their efficacy against *Fusarium equisiti*. From the results (table- 4.1.4) it is evident that *Tectona grandis* and *Crotalaria mucronata* could inhibit spore germination completely at a concentration of 5000 µg /ml. The next effective plant extract was *Xanthium strumarium* which showed 99% inhibition of spore germination. The other plant extracts like *Datura innoxia, Azadirachta indica, Clausena excavata* and *Polyalthia longifolia* showed 91%, 68%, 97% and 87% inhibition of spore germination respectively. At concentration of 1000 µg /ml almost all the seven plant extracts could inhibit spore germination of *Fusarium equiseti* at the range of 45 – 55 %.

Table 4.1.4 Effective concentration of leaf extracts for antifungal activity against germination of spores of *Fusarium equiseti.*

Extracts made from plants	Percent inhibition of spore germination				
	Plant extract concentration (µg/ml)				
	1000	2000	3000	4000	5000
Datura innoxia	52	56	65	78	91
Azadirachta indica	53	55	61	65	68
Xanthium strumarium	57	68	81	91	99
Tectona grandis	45	58	63	87	100
Clausena excavata	48	72	84	85	97
Polyalthia longifolia	55	60	69	81	87
Crotalaria mucronata	52	62	74	86	100

Inhibition in control (sterile distilled water) was recorded as zero [0]

4.1.4: Study of antifungal activity of potential extracts at different concentrations by poison food technique.

On the basis of spore germination bioassay five plants (*Xanthium strumarium, piper betle, Crotalaria mucronata, Mitracarpus verticillatus,* and *Datura stramonium*) were selected for further experiments. Antifungal activity of the selected plant extracts (aqueous, ethanolic and ethylacetate extracts) were tested for mycelia-growth inhibition of *Fusarium equiseti* and *Colletotrichum gloeosporioides*. In these experiments plant extracts were mixed with potato dextrose agar media and after solidification mycelia blocks were placed on the plant extract supplemented media. Details of the experiments have been discussed in the section 3.11 of materials and methods. Results of the radial growth of the fungi were noted and have been presented in Table 4.1.5.

From the result's it was evident that leaves extracted in distilled water, ethanol and ethylacetate exhibited significant inhibitory effect against *Fusarium equiseti* and *Colletotrichum gloeosporioides*. Aqueous and ethanolic leaf extracts of *Xanthium strumarium, piper betle, Crotalaria mucronata, Mitracarpus verticillatus,* and *Datura stramanium* showed less activity than the leaf extracts made in ethyl acetate. In antifungal

assay agar *Piper betle* leaf extract (ethyl acetate extract) showed 90% inhibition of radial mycelia growth against *C. gloeosporioides* in comparison to control. Percent growth inhibition of *C. gloeosporioides* were 78.8, 75.0, 86.6 and 83.3 respectively when ethyl acetate extracts of *Xanthium strumarium, Crotalaria mucronata, Mitracarpus verticillatus,* and *Datura stramonium* were tested in poisoned food technique. Ethyl acetate extracts of *Xanthium strumarium, Crotalaria mucronata, Mitracarpus verticillatus* and *Datura stramanium* also showed antifungal growth inhibitory activity of *Fusarium equiseti* (Percent inhibition was 86.6, 73.3, 81.1, 86.6 and 86.6 respectively).

Table 4.1.5: Effect of antifungal activity of selected plant extracts on the growth of *the two pathogens (F. equiseti & C. gloeosporioides)* of brinjal. (following poisoned food technique)

Plants Used	Leaves extracted in the Solvent	*Fusarium equiseti*		Colletotrichum gloeosporioides	
		*Radial growth (mm)	% inhibition	Radial growth* (mm)	% inhibition
Xanthium strumarium	Aqueous	17	81.1	27	70.0
	Ethanol	15	83.3	22	75.5
	Ethyl acetate	12	86.6	19	78.8
Piper betle	Aqueous	45	50.0	17	81.1
	Ethanolic	28	68.8	14	84.4
	Ethyl acetate	24	73.3	09	90.0
Crotalaria mucronata	Aqueous	35	61.1	40	55.5
	Ethanolic	26	71.1	28	68.8
	Ethyl acetate	17	81.1	22	75.5
Mitracarpus verticillatus	Aqueous	39	56.6	27	70.0
	Ethanolic	23	74.4	22	75.5
	Ethyl acetate	12	86.6	12	86.6
Datura stramonium	Aqueous	24	73.3	23	74.4
	Ethanolic	14	84.4	18	80.0
	Ethyl acetate	12	86.6	15	83.3
Control	-	90	-	90	-

Control diameter = 90 mm; Data are mean of three replications; PDA : extract = 9:1; Incase of control sterile distilled water was added instead of extract. *Data were taken after 7 days of incubation. Percent inhibition were calculated in relation to control (where no inhibition of growth was recorded)

4.1.5: Study of antifungal properties in three different solvents and antifungal assay

Soxhlet gives us opportunity to extract substantial amount of active principle directly. Soxhlet extract can be used for preparation of field applicable formulations. Selection of the solvent is one of the important aspects for soxhlet extraction. In the present

section three different solvents were used to know the best solvent to be used in future for extraction of the active antifungal principles. Six plants were selected separately for each of the two pathogens of brinjal and the extracted compounds were subjected to antifungal bioassay at same concentration. On the basis of the results the suitable solvents were selected for future extraction purpose. From the table 4.1.6 it is clear that benzene was the best solvent for extraction of the anti fungal properties from six plants as shown in table 4.1.6. *Crotalaria mucronata* and *Datura stramonium* extracted in chloroform showed similar antifungal activity against *F. equiseti*.

In case of antifungal assay against *C. gloeosporioides* benzene (as a solvent in soxhlet extraction) showed the best result and in some cases chloroform also showed promising results (in case of *Borreria alata* and *Piper betle*) (table-4.1.7).

Table 4.1.6: Antifungal activity of six plant extracts (prepared in soxhlet using three different solvents) against *F. equiseti* (following agar cup method)

Plant Extract*	Different solvent	Diameter of Zone(mm)
Xanthium strumarium	Benzene	12
	Chloroform	11
	Hexane	07
Mitracarpus verticillatus	Benzene	14
	Chloroform	13
	Hexane	11
Crotalaria mucronata	Benzene	10
	Chloroform	10
	Hexane	00
Eucalyptus globosus	Benzene	15
	Chloroform	13
	Hexane	10
Tectona grandis	Benzene	10
	Chloroform	07
	Hexane	00
Datura stramonium	Benzene	12
	Chloroform	12
	Hexane	08
Control	Benzene	0
	Chloroform	0
	Hexane	0
	Distilled water	0

*concentration 100mg/ ml was used in all the cases except control

Table 4.1.7: Antifungal activity of six plant extracts (prepared in soxhlet using three different solvents) against *C. gloeosporioides* (following agar cup method)

Plant Extract*	Different solvent	Diameter of Zone
Xanthium strumarium	Benzene	20
	Chloroform	17
	Hexane	15
Datura stramonium	Benzene	12
	Chloroform	10
	Hexane	08
Melastoma malabathricum	Benzene	08
	Chloroform	09
	Hexane	06
Borreria alata	Benzene	18
	Chloroform	18
	Hexane	10
Vitex negundo	Benzene	08
	Chloroform	07
	Hexane	00
Piper betle	Benzene	14
	Chloroform	14
	Hexane	11
Control	Benzene	00
	Chloroform	00
	Hexane	00
	Water	00

*concentration 100mg/ ml was used all the cases

4.1.6: Determination of minimum concentration of potential plant extracts by agar cup bioassay

Following the preliminary results of spore germination bioassay, some plant extracts were made in soxhlet using benzene as solvent. Extraction procedure in soxhlet has been described in the materials and methods section 3.8.5. These plant leaf extracts were subjected to agar cup bioassay against *Fusarium equiseti* and *Colletotrichum gloeosporioides.* The minimum inhibitory concentration of the extracts was also determined against the two pathogens of brinjal. The details of the procedures of agar cup bioassay have been presented in the materials and methods section section 3.13. Diameter of inhibition zones of different extracts at different concentrations were noted in the table 4.1.8. Different concentrations of the extracts were prepared and poured in agar cups or wells (five mm. in

diameter) and the lowest concentrations which showed inhibition were considered as MIC of respective extract.

Results presented in table-4.1.8 clearly indicated the antifungal activity of *Xanthium strumarium* leaf extract against *Fusarium equiseti* and *Colletotrichum gloeosporioides.* The antifungal activity of *C. gloeosporioides* has been presented in (plate-12). Eight different concentrations (0.1, 0.08, 0.06, 0.04, 0.02, 0.01, 0.006 and 0.004 gm/ml) of the *Xanthium strumarium* leaf extract were prepared by serial dilution of the stock. All the concentrations were tested for their MIC values. The MIC values of *Xanthium strumarium* leaf extract were 0.04gm/ml and 0.006gm/ml respectively against *Fusarium equiseti* and *Colletotrichum gloeosporioides.*

Table 4.1.8: Agar cup bioassay of *Xanthium strumarium* leaf extract (extracted in benzene in soxhlet) against mycelia growth of *Fusarium equiseti and Colletotrichum gloeosporioides.*

Plant extract	*Fusarium equiseti*		*Colletotrichum gloeosporioides*	
	Extract Concentration (g/ml)	Inhibition zone diameter (mm)	Extract Concentration (g /ml)	Inhibition zone diameter (mm)
Benzene soxhlet extract of: *Xanthium strumarium*	0.1	12	0.1	16
	0.08	8	0.08	15
	0.06	6	0.06	13
	0.04	4	0.04	12
	0.02	0	0.01	8
	-	-	0.008	7
	-	-	0.006	7
	-	-	0.004	00
	control	00	control	00

Three different plant extracts showed their antifungal potentiality to inhibit spore germination of *Fusarium equiseti.* Those three plant extracts were extracted in benzene using soxhlet. Finally, their antifungal activity has been tested against mycelia growth of the fungus. The results of the activity of the three plant extracts (*Eucalyptus globosus, Mitracarpus verticillatus* and *Borreria alata*) have been presented in Table-4.1.9, 4.1.10 and 4.1.11 respectively.

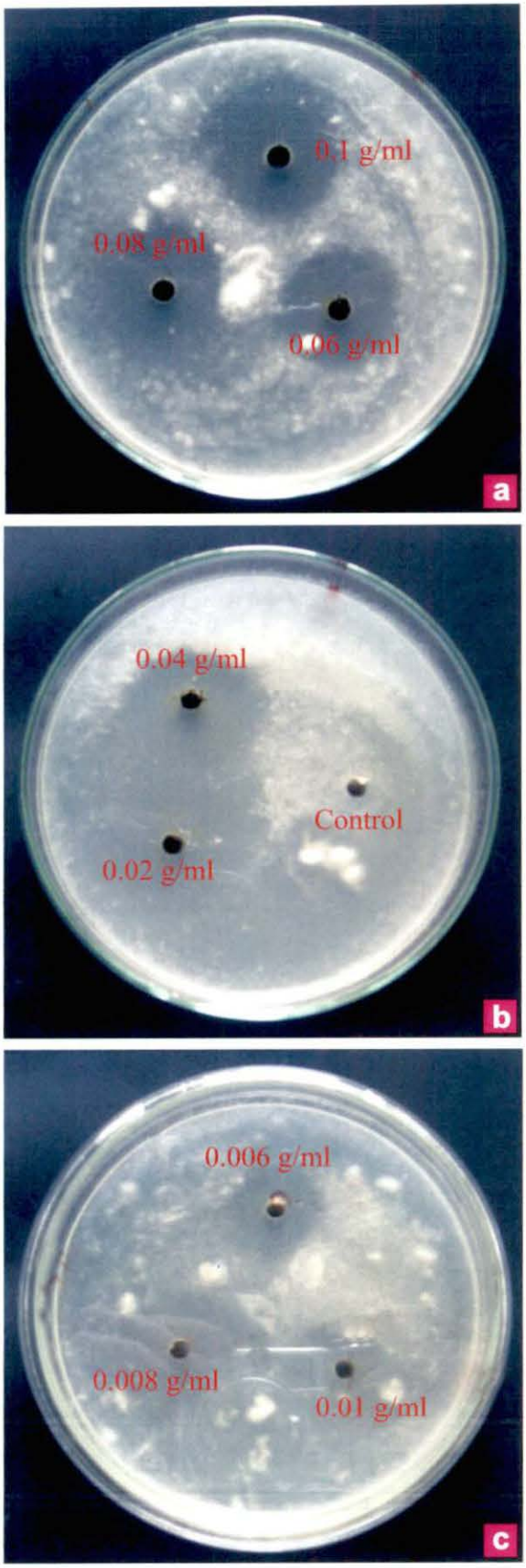

Plate 12: fig. a,b &c. Showing inhibition of growth of *Colletotrichum gloeosporioides* in presence of crude benzene extract of *Xanthium strumarium* in different concentrations poured in wells.

Six different concentrations (0.1, 0.08, 0.06, 0.04, 0.02 and 0.01 gm/ml) of the *Eucalyptus globosus* leaf extract were prepared by serial dilution of the stock. All the concentrations were tested for their MIC values. The MIC values of the *Eucalyptus globosus* leaf extract was 0.02gm/ml against *Fusarium equiseti.*

Table 4.1.9: Agar cup bioassay of *Eucalyptus globosus* leaf extract (extracted in benzene in soxhlet) against mycelia growth of *Fusarium equiseti*

Plant extract	Extract Concentration (g/ml)	Inhibition zone diameter(mm)
Benzene soxhlet extract of: *Eucalyptus globosus*	0.1	15
	0.08	12
	0.06	12
	00.04	11
	0.02	08
	0.01	00
	control	00

Mitracarpus verticillatus leaves were also extracted in benzene and were tested for their antifungal efficacy against mycelia growth of *Fusarium equiseti.* From the results (table 4.1.10) the minimum inhibitory concentration was found to be 0.01 gm/ml against mycelia growth of the *Fusarium equiseti in vitro.*

Table 4.1.10: Agar cup bioassay of leaf extract *Mitracarpus verticillatus* (extracted in benzene in soxhlet) against mycelia growth of *Fusarium equiseti*

Plant extract	Extract Concentration (g/ml)	Inhibition zone diameter (mm)
Benzene soxhlet extract of: *Mitracarpus verticillatus*	0.1	13
	0.08	12
	0.06	8
	0.04	8
	0.01	7
	0.005	00
	control	00

Similarly, different concentrations of benzene extract of *Borreria alata* leaves were tested for their antifungal activity against mycelia growth of *Fusarium equiseti.* The antifungal activity of *B. alata* has been shown in plate-13. Results (table 4.1.11) indicated that the minimum inhibitory concentration was 0.006 gm/ml among the tested concentrations (0. 1, 0.08, 0.06, 0.04, 0.02, 0.01, 0.008 and 0.006gm/ml).

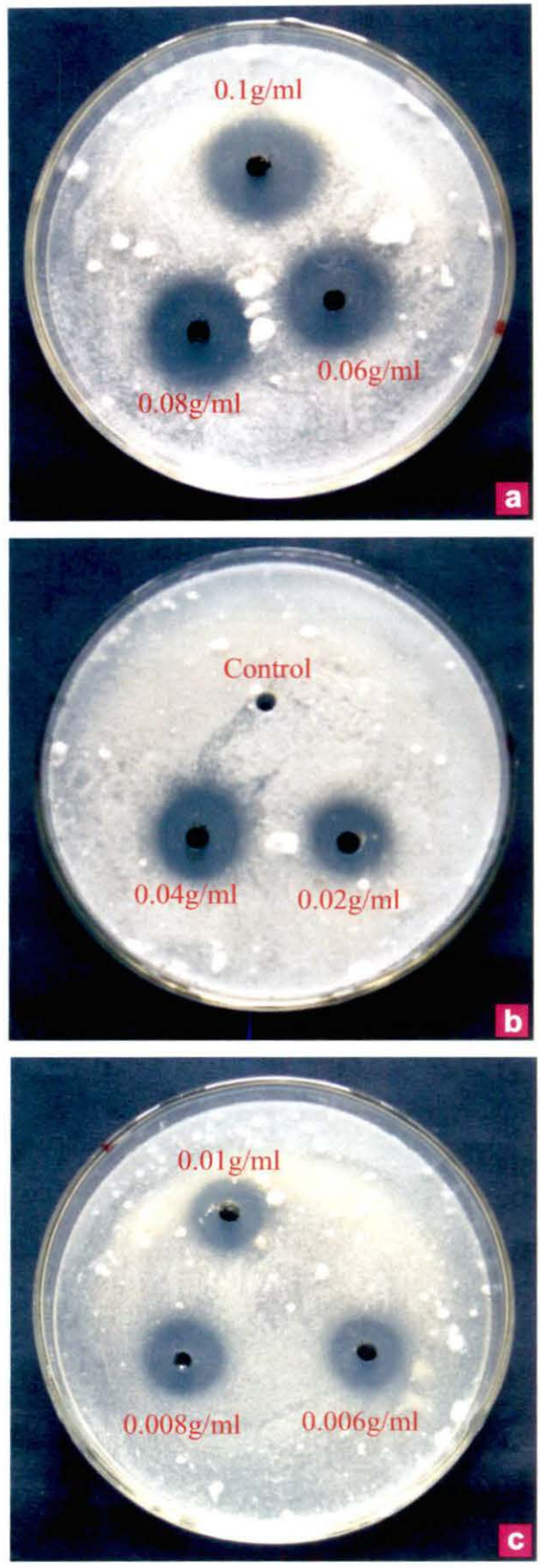

Plate 13: Antifungal activity of leaf extracts of *Borreria alata* **fig. a**, **b** and **c** showing inhibition of growth of *Fusarium equiseti* in PDA in presence of different concentrations of benzene extract of *Borreria alata*.

Table 4.1.11: Agar cup bioassay of *Borreria alata* leaf extract (extracted in benzene in soxhlet) against mycelia growth of *Fusarium equiseti*

Plant extract	Extract Concentration (g/ml)	Inhibition zone diameter (mm)
Benzene extract of:	0.1	20
	0.08	18
Borreria alata	0.06	17
	0.04	13
(Extracted in soxhlet)	0.02	12
	0.01	11
	0.006	9
	0.008	8
	0.009	00
	Control	00

Like the above mentioned three plants three other plants (*Datura stramonium Melastoma malabathricum, Vitex negundo*) which showed promising activity in spore germination bioassay against *Colletotrichum gloeosporioides* were selected for further experiments. Two plants (*D. stramonium* and *M. malabathricum*) were extracted in benzene but the third plant (*V. negundo*) was extracted in chloroform. These three plant extracts were tested for their antifungal efficacy *in vitro* following agar cup bioassay technique.

Results (table-4.1.12) indicated that mycelia growth of *Colletotrichum gloeosporioides* could be checked at 0.01gm/ml concentration of *Datura stramonium* leaf extract (benzene extract). Mininimum inhibitory concentration thus found to be 0.01gm/ml.

Table 4.1.12: Agar cup bioassay of *Datura stramonium* leaf extract (extracted in benzene in soxhlet) against mycelia growth of *Coletrotrichum gloeosporioides.*

Plant extract	Extract Concentration (gm /ml)	Inhibition zone diameter(mm)
Benzene soxhlet extract of:	0.1	15
	0.08	12
	0.06	9
	0.04	8
	0.01	7
Datura stramonium	0.008	0
	Control	0

Melastoma malabathricum leaves were also extracted in benzene in soxhlet. Six different concentrations were prepared by serial dilution and were tested following agar cup bioassay technique. Results of the experiment have been presented in table 4.1.13. Minimun concentration of benzene extract which could check the growth of *C. gloeosporioides* was 0.06 gm/ml. Thus MIC of benzene extract of *M. malabathricum* was determined as 0.06 gm/ml.

Table 4.1.13: Agar cup bioassay of *Melastoma malabathricum* leaf extract (extracted in benzene in soxhlet) against mycelia growth of *Colletrotrichum gloeosporioides*

Plant extract	Extract Concentration(gm /ml)	Inhibition zone diameter (mm)
Benzene soxhlet extract of: *Melastoma malabathricum*	0.3	10
	0.2	8
	0.1	8
	0.08	7
	0.06	6
	0.04	0
	control	0

On the basis of some preliminary results (results not shown) the leaves of *Vitex negundo* was extracted in chloroform and finally tested for their antifungal efficacy against *C. gloeosporioides.* Five different concentrations of the extract were made by serial dilution and were subjected to agar cup bioassay. Chloroform extract of 0.06 gm/ml concentration could check the growth of the fungus. Hence, minimum inhibitory concentration of the plant extract was 0.06 gm/ml (table 4.1.14).

Table 4.1.14: Agar cup bioassay of *Vitex negundo* leaf extract (extracted in benzene in soxhlet) against mycelia growth of *Colletotrichum gloeosporioides.*

Plant extract	Extract Concentration (gm /ml)	Inhibition zone diameter (mm)
Chloroform soxhlet extract of: *Vitex negundo*	0.3	12
	0.2	9
	0.1	8
	0.06	7
	0.08	0
	Control	0

4.1.7: Extraction of terpenoid fraction of *D. stramonium* and *C. mucronata* and bioassay of the fractions against *F.equiseti and C. gloeosporioides* (following disc diffusion bioassay).

D. stramonium and *C. mucronata* were selected for fractionation of terpenoid and other compounds. Details of the isolation procedures have been discussed in the materials and methods section 3.8.6 and table 3.3. Some of the fractions were subjected to disc diffusion bioassay. Details of the procedure of disc diffusion bioassay have been presented in section 3.12 of materials and methods. MIC values of terpenoid fractions of the two plants were determined against *F. equiseti* and *C. gloeosporioides.*

From the results (table 4.1.15 and plate 14) it is evident that terpenoid fraction of *D. stramonium* leaf extract could inhibit the growth of *C. gloeosporioides* and *F. equiseti.* MIC values of *D. stramonium* leaf extract (terpenoid fraction) against *F. equiseti* and *C. gloeosporioides* were also determined. MIC values from the results were determined to be 0.05 mg/ml and 0.1 mg/ml respectively against *F. equiseti* and *C. gloeosporioides.*

Similarly, *C. mucronata* leaf extract (terpenoid fraction) was tested against *F. equiseti.* The MIC value of *C. mucronata* was found to be 0.01 mg/ml.

Table 4.1.15: Bioassay of terpenoid fraction of leaf extracts (of *D. stramonium* and *C. mucronata* against mycelia growth of *F. equiseti* and *C. gloeosporioides* (following disc diffusion method).

Terpenoid fraction of leaf extract of:	*Fusarium equiseti*		*Colletotrichum gloeosporioides*	
	Extract Concentration (mg/ml)	Inhibition* zone diameter (mm)	Extract Concentration (mg /ml)	Inhibition zone diameter (mm)
D. stramonium	0.5	14	5	22
	0.2	12	2	20
	0.1	10	1	16
	0.05	09	0.5	12
	0.01	00	0.1	11
	-	-	0.05	00
C. mucronata	1.0	12	-	-
	0.5	11	-	-
	0.2	11	-	-
	0.05	10	-	-
	0.01	09	-	-
	Control (SDW)	00	00	00

*Data after 48 hours, – indicates not performed.

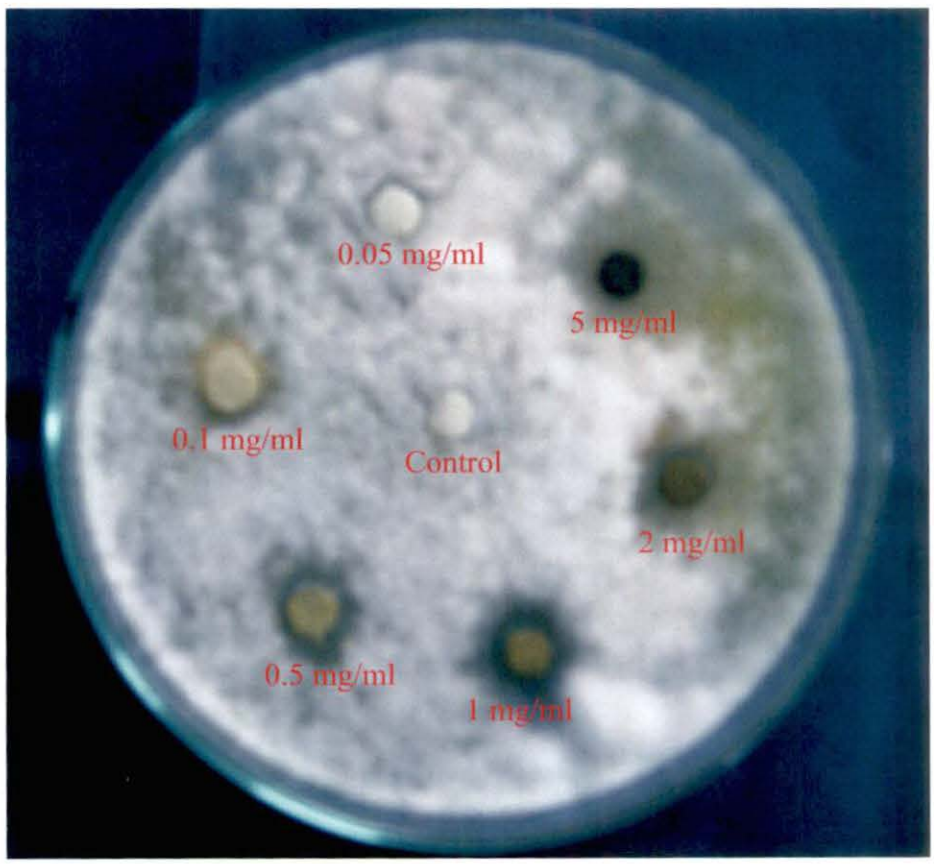

Plate 14: Antifungal activity of terpenoid fraction of *Datura stramonium* against *C.gloeosporioides* (following disc diffusion method)

4.1.8: Fractionation of crude leaf extract of *D. stramonium* and evaluation of different fractions for antifungal activity against *C. gloeosporioides* (following spore germination bioassay and disc diffusion bioassay).

Purification of antifungal principles and their characterization is an important task. The separation of antifungal properties of *D. stramonium* was done by column chromatography. The details of the chromatography technique have been discussed in section 3.17.1 of materials and methods. Each fraction was of 20 ml. Fractions were combined in some groups on the basis of their solvent type. First five fractions were of petroleum ether and the subsequent fractions were of mixture of ethyl acetate and hexane at different proportions. From the table 4.1.16 it is evident that the antifungal activity was present in the ethyl acetate and hexane mixture. Fractions 6-20 could check the germination of the fungus. The subsequent fractions after fraction-20 showed gradual decrease in the antifungal activity.

Fractions 6 - 20 were mixed and evaporated to dryness in a vacuum rotary evaporator at 40^0 C. The dry powder was dissolved in water and different concentrations were made by serial dilution of the stock solution and were kept in eppendorf tubes. Sterile filter paper blocks soaked in different concentrations were placed on surface of PDA medium pre-supplemented with spore suspension of *C. gloeosporioides*. Results (table 4.1.17 & plate 15) revealed that up to 0.2mg/ml concentration of the partially purified leaf extract could check spore germination.

4.1.16. Column fractions of *D. stramonium* and their antifungal efficacy against *C. gloeosporioides* after 24 hours.

Name of the solvent	Volume(ml)	Fraction no.	Percent Inhibition of spore germination of *C. gloeosporioides*
Petroleum ether	100	1-5	-
5% ethyl acetate +Hexane	100	6-10	100
10% ethyl acetate +Hexane	100	11-15	100
15% ethyl acetate +Hexane	100	16-20	100
20% ethyl acetate +Hexane	100	21-25	76±2.6
25% ethyl acetate +Hexane	100	26-30	45±2.1
30% ethyl acetate +Hexane	100	31-35	20±3.2
35% ethyl acetate +Hexane	100	36-40	5±2.4

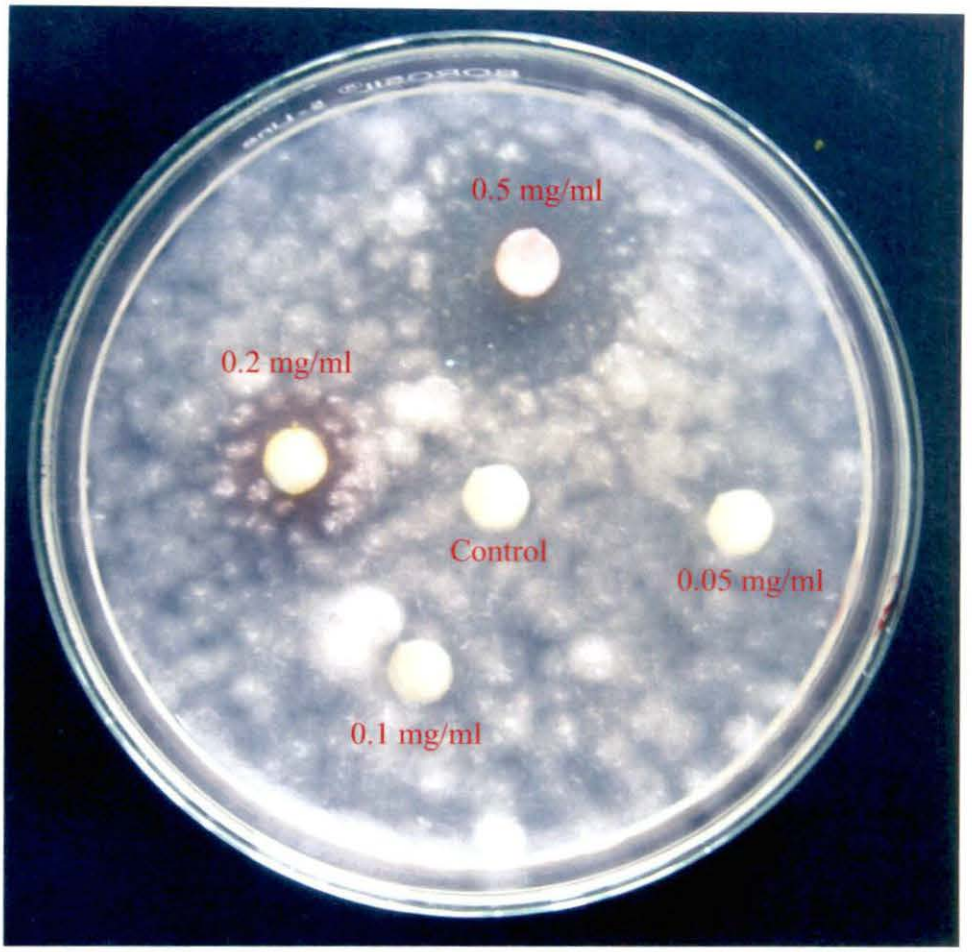

Plate 15: Antifungal activity of combined column fractions (fraction 6-20) of *Datura stramonium* against *C.gloeosporioides.*

Table 4.1.17: Inhibition of growth of *C. gloeosporioides* by different concentrations of *D. stramonium* active leaf fractions (following disc diffusion method).

Plant extract (by the column-separation method)	*C. gloeosporioides*	
	Extract Concentration (mg/ml)	Inhibition zone diameter (mm)
D. stramonium (Combined fractions of 6 to 20)	0.5	13
	0.2	12
	0.1	0
	0.05	0
	Control	0

4.1.9: Fractionation of crude leaf extract of *Datura stramonium* and evaluation of different fractions for antifungal activity against *F. equiseti* (following spore germination bioassay and disc diffusion bioassay)

D. stramonium was also fractionated in a different set of solvents. This was done to isolate antifungal properties of the plant against *F. equiseti*. Details of the solvent system, volume of the solvent used and fraction numbers have been mentioned in the table 4.1.18. Each fraction was of 20 ml and the first five fractions were of petroleum ether and the subsequent fractions were of mixture of dichloromethane and petroleum ether at different proportions. Finally dichloromethane was mixed with ethyl acetate fraction at different concentrations. From the table 4.1.16 it is evident that the antifungal activity was present in the ethyl acetate and dichloromethane mixture. Fractions 26 - 35 could check the spore germination of the fungus. Fractions 1- 25 could not check the germination of the fungus.

Fractions 26 - 35 were mixed and evaporated to dryness in a vacuum rotary evaporator at 40^0 C. The dry powder was dissolved in water and different concentrations were made by serial dilution of the stock solution and were kept in eppendorf tubes. Sterile filter paper blocks soaked in different concentrations were placed on surface of PDA medium pre-supplemented with spore suspension of *F. equiseti*. Results (table 4.1.18 and plate-16) revealed that up to 0.08mg/ml concentration of the partially purified leaf extract could check spore germination. The combined fractions 26-30 were also tested following disc diffusion method. From the results of the disc diffusion experiments the minimum

concentration of the combined fraction (fractions 26 – 35) which could inhibit the growth of the fungus was found to be 0.08mg/ml (Table 4.1.19).

4.1.18. Column fractions of *D. stramonium* and their antifungal efficacy against *F.equiseti* after 24 hours.

Solvent used	Volume (ml)	Fraction No.	Percent Inhibition of spore germination of *F. equiseti*
Petroleum ether	100	1-5	0
25% Dichloro methane+ Petroleum ether	100	6-10	0
50% Dichloro methane + Petroleum ether	100	11-15	0
75% Dichloro methane + Petroleum ether	100	16-20	0
Dichloro methane	100	21-25	0
25% ethyl acetate+ Dichloro methane	100	26-30	100
50% ethyl acetate+ Dichloro methane	100	31-35	100
75% ethyl acetate+ Dichloro methane	100	36-40	75±3.9

Table 4.1.19: Inhibition of growth of by different concentrations of *D. stramarium* (following disc diffusion method).

Plant extract (by the column separation method)	*F. equiseti*	
	Extract Concentration (mg/ml)	Inhibition zone diameter (mm)
D. stramarium (Combined fractions of 26 to 35)	0.5	12
	0.4	10
	0.2	9
	0.1	8
	0.08	7
	Control	0

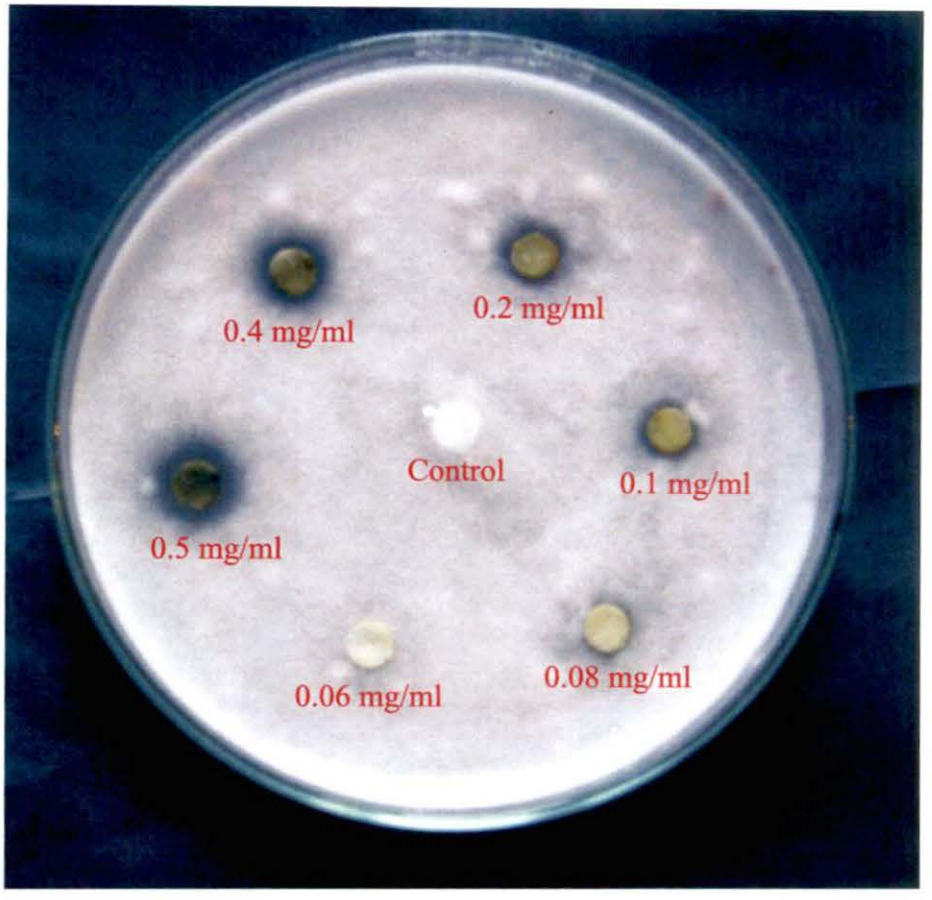

Plate 16: Antifungal activity of combined column fractions (fraction 26-35) of *Datura stramonium* against *F.equiseti.*

4.1.10: Antifungal sensitivity assay of three common fungicides

Three commonly used fungicides were tested for their efficacy against the two fungal pathogens. Bioassay was done taking three to four different concentrations of three different fungicides nysyatin, captaf and 'bavistin. The concentrations were selected on the basis of some preliminary experiments. It was also considered worthwhile to determine the MIC values of the fungicides tested against the two pathogens and to compare with the botanicals found to be potential in the present study. The three different concentrations of nystatin and four different concentrations of captaf and bavistin were selected for determination of minimum inhibitory concentrations against the two fungi. Minimum inhibitory concentrations (MIC) were measured following standard techniques (Suleman *et al.* 2002) as described in the materials and methods (section -3.13). The fungal pathogens showed growth inhibition zones around agar cups containing effective concentrations of the fungicides. The lowest concentration which could inhibit growth of a fungi, were considered as MIC of that fungicide against the fungi tested.

Different concentrations of the fungicides were prepared by serial dilution of the fungicide solutions. Separate PDA plates, after solidification, were inoculated with 1 ml of spore suspension (either of *F. equiseti* or *C. gloeosporioides*). Spore suspension was spreaded uniformly on the agar surface by a glass spreader. Three to four agar cups or wells (of 5 mm diameter) were made on the inoculated plates aseptically and 50 µl fungicides of different concentrations were poured in the wells. The wells were marked and the plates were incubated at 28 $\pm 1^0$ C in an incubator. The plates were observed after one and two days of inoculation and the least concentration in which the pathogenic fungi could not grow were considered as minimum inhibitory concentration. The results of agar cup bioassay against fungal pathogens have been presented in table 4.1.20. The antifungal activity of the three fungicides have also shown in the plates-17 &18.

Three different concentrations of fungicide (nystatin) were assessed for their efficacy against *F. equiseti* and *C. gloeosporioides*. Minimum inhibitory concentration of *F. equiseti* was found to be 5mg/ml and of *C. gloeosporioides* it was 0.5 mg/ml (table 4.1.20). Similarly, fungicides captaf was also used to control the two pathogens and the MIC values of captaf were 3mg/ml and 0.5 mg/ml against *F. equiseti* and *C. gloeosporioides* respectively. Bavistin was also used to control the two pathogens. Bioassay studies of

bavistin against *F. equiseti* and *C. gloeosporioides* showed 0.2 mg/ml as minimum inhibitory concentration.

Table 4.1.20: Inhibition of growth of *F. equiseti* and *C. gloeosporoides* by different concentrations of fungicides (following agar cup method).

Fungicide	*F. equiseti*		*C. gloeosporoides*	
	Concentration (mg/ml)	Diameter of inhibition zone (mm)	Concentration (mg /ml)	Diameter of inhibition zone (mm)
Nystatin	10	12	2	13
	5	4	1	6
	1	0	0.5	6
	-	-	0.2	00
Captaf	10	12	1	10
	5	10	0.5	7
	3	8	0.1	00
	1	0	-	-
Bavistin	10	10	5	17
	5	8	1	10
	1	7	0.4	8
	0.2	6	0.2	7
	0.1	00	0.1	00
Control	-	0	-	0

4.1.11: Separation of plant extracts on TLC plates developed in suitable solvent and bioassay of the plates for antifungal properties against the two pathogens of brinjal.

On the chromatogram bioassay of potential plant extracts and determination of R_f value(s) of the antifungal zones detected on the silica gel plates are of great importance. Zones corresponding to antifungal zones if sprayed with specific chromogenic reagent produce significant identifiable colours, which may give us opportunity to identify a chemical to a certain extent. In the present study these properties have been exploited to identify the antifungal compounds up to a certain group (such as triterpene, terpene, monoterpene alcohols, bitter principle etc.).

The selected extracts were run on previously activated TLC plates and the plates were developed separately in two solvents [either (Hexane:Ethylacetate:Methanol::60:40:1) or (Chloroform:Methanol::9:1)]. The plates were dried in the air at room temperature (28^0 C). Spores of a test fungus (either of *F. equiseti* or of *C. gloeosporioides*) were mixed with Richard's solution and finally the mixed suspension was sprayed on the silica gel coated

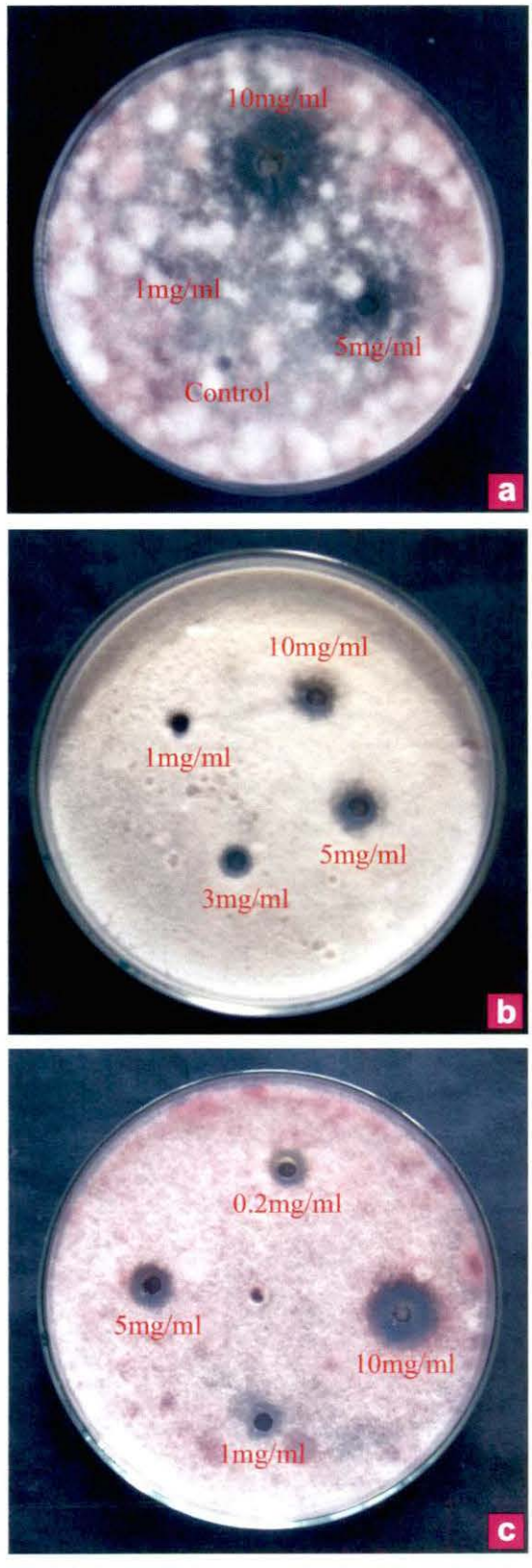

Plate 17: Fungicidal activity against *F.equiseti* **fig.a.** showing inhibition of growth of fungus at different concentrations of nystatin **fig.b.** showing inhibition of growth of fungus at different concentrations of captaf **fig.c.** showing inhibition of growth of fungus at different concentrations of bavistin.

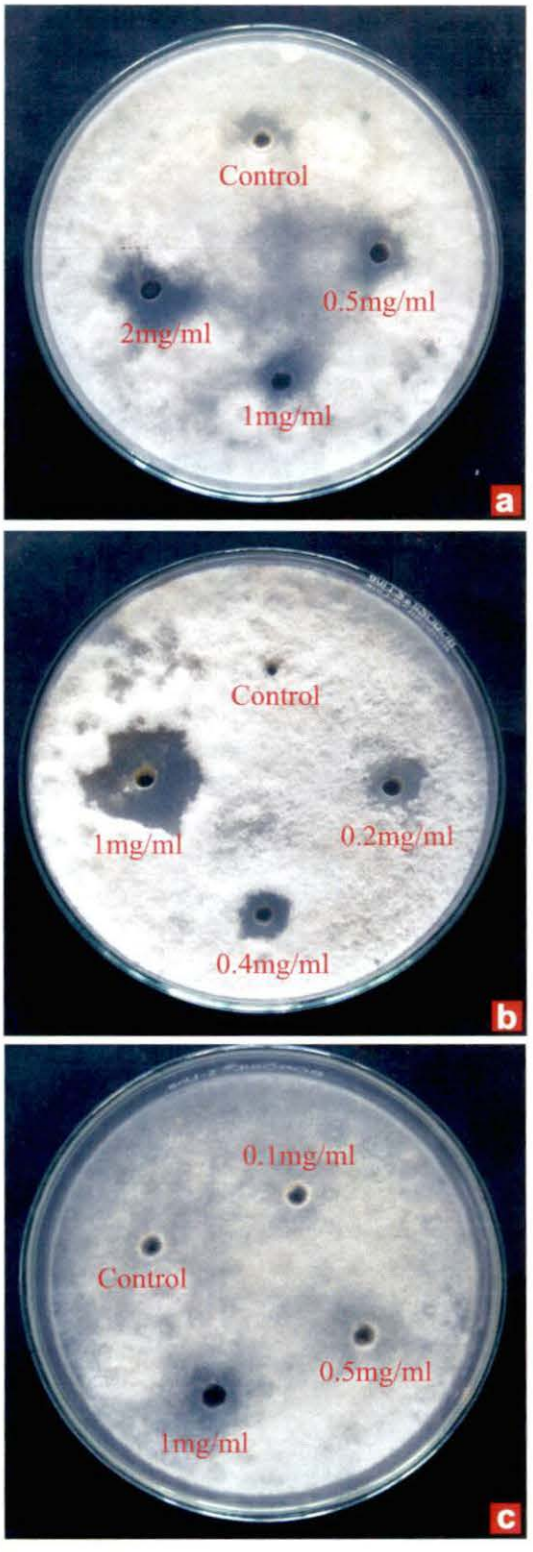

Plate 18: Fungicidal activity against *C.gloeosporioides*
fig. a. Nystatin used as fungicide at different concentrations
fig. b. Bavistin used as fungicide at different concentrations
fig. c. Captaf used as fungicide at different concentrations.

plates. The plates were placed in a humid chamber at 25±1^{0}C for 2-3 days. Details of the techniques have been presented under materials methods (section-3.15.1.). After 3 days, the inhibition zones were observed and the diameter of the inhibition zones was measured. The R_f of the inhibition zones were calculated and have been presented in the tables 4.1.21. and 4.1.23. Photographs of TLC plate bioassay against *C. gloeosporioides* have been presented in plates-19 & 20.

The antifungal zones against *C. gloeosporioides* have been presented in table 4.1.21. The *X. strumarium* plant extract tested against the pathogen have shown one distinct antifungal zone (plate-19) at R_f 0.85. The diameter of the antifungal zone was 20mm. The *D. stramonium* plant extract tested against the pathogen have shown three antifungal zones (plate-20) at R_f 0.24, 0.50 and 0.70. The diameter of the antifungal zones was 10, 14 and 8 mm respectively.

Similarly, the antifungal zones against *Fusarium equiseti* have been presented in table 4.1.22. The *Xanthium strumarium* plant leaf extract tested against the pathogen have shown one distinct antifungal zone (plate 21) at R_f 0.85. The diameter of the antifungal zone was 20mm. The *C. mucronata* plant extract tested against the pathogen has shown three antifungal zones (plate-22) at R_f 0.15, 0.38 and 0.70. The diameter of the antifungal zones was 8, 10 and 20mm respectively. The third plant (*B. alata*) leaf extract showed one distinct antifungal zone of 30mm diameter at R_f 0.38. The photographs of the TLC plate bioassay against *F. equiseti* have been presented in the plates-21, 22 & 23.

After getting the information of bioassay, the bioassay guided fractions were subjected to chromogenic spray with four different reagents separately. The details of the chromogenic analysis have been presented in table 4.1.23 and 4.1.24 respectively for *C. gloeosporioides* and *F. equiseti.* Details of the reagent preparation and application along with the table of analysis (Table 3.4) have been presented in the materials and methods section 3.16.

The antifungal zone shown by *X. strumarium* against both the pathogens seems to be bitter principles because dark-green colour was observed after anisaldehyde reagent spray. The same zone in separate plate when sprayed with Vanillin-sulphuric acid mixture became light green which also indicated the presence of bitter principle (Plate-24).

Three different antifungal zones were found in case of leaf extract of *D. stramonium*. The antifungal zone İ (R_f 0.24) was identified as triterpene due development of red-violate colour with 'anisaldehyde reagent'. The same R_f when sprayed with 'Vanillin- sulphuric acid' produced blue-green colour and indicated the presence bitter principle. 'Anisaldehyde reagent' produced light-violate colour at R_f 0.50 and indicated the presence of terpenes. The same R_f when sprayed with 'Vanillin-sulphuric acid' produced blue colour which indicated the presence of monoterpene alcohols. However, R_f 0.70, the third antifungal zone, could not be identified due to insignificant colour development in both the cases of spray shown in the referred table 4.1.22 and in the plate-25. Two other spray reagents (10% KOH and Dragendorff reagent) were also sprayed but they could not produce any significant colours for identification of the compounds.

The *C. mucronata* plant extract tested against the *F. equiseti* have shown three antifungal zones at Rf 0.15, 0.38 and 0.70. All the antifungal zones were compared with TLC plates sprayed with different chromogenic spray reagents (plate-26). All the three zones of *C. mucronata* were found to be triterpenes. The *B.alata* leaf extract were also subjected to chromogenic analysis (Plate-27). From the colour reactions the antifungal zone was also found to be triterpene. Two other spray reagents (10% KOH and Dragendorff reagent) were also sprayed but they could not produce any significant colours for identification of the compounds.

Table 4.1.21: TLC plate bioassay of crude leaf extracts of two different plants against *C. gloeosporioides*.

Extracts of the plants	No. of inhibition Zones	*C. gloeosporioides*	
		Diameter of Inhibition zones (mm)	R_f
X. strumarium	Zone 1	20	0.85
D. stramonium	Zone 1	10	0.24
	Zone 2	14	0.5
	Zone 3	8	0.70

*Crude leaf extract =100 µg/ml.

TLC plates developed in solvent = Hexane:Ethylacetate:Methanol :: 60:40:1

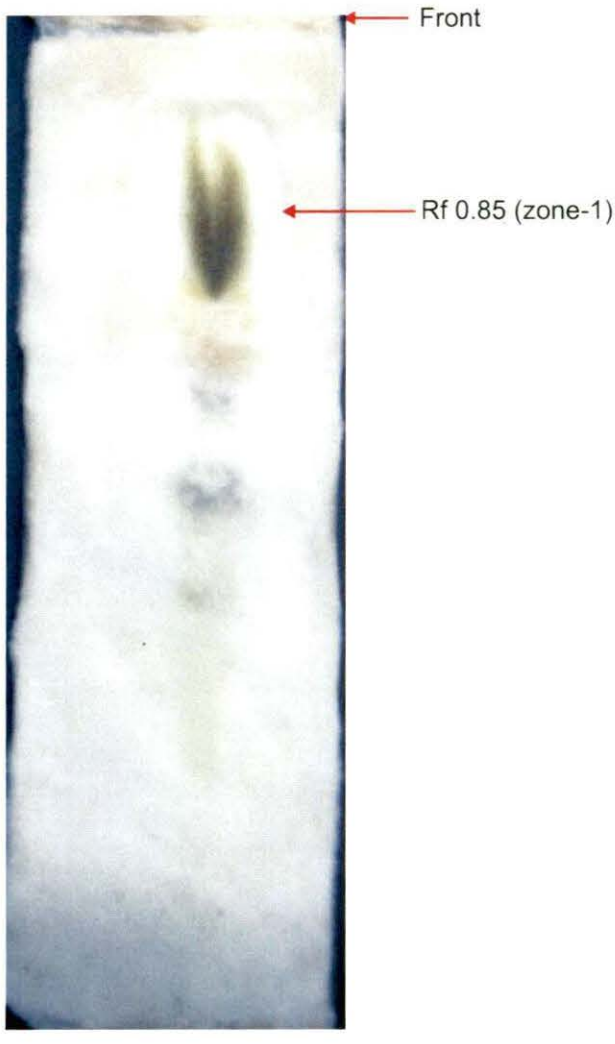

Plate 19: Bioassay of crude leaf extract of *Xanthium strumarium* developed in hexane: ethyl acetate: methanol (60:40:1) and sprayed with Richard's solution supplemented with spore suspension of *Colletotrichum gloeosporioides*. Number of antifungal zone in parentheses along with R_f value has been presented.

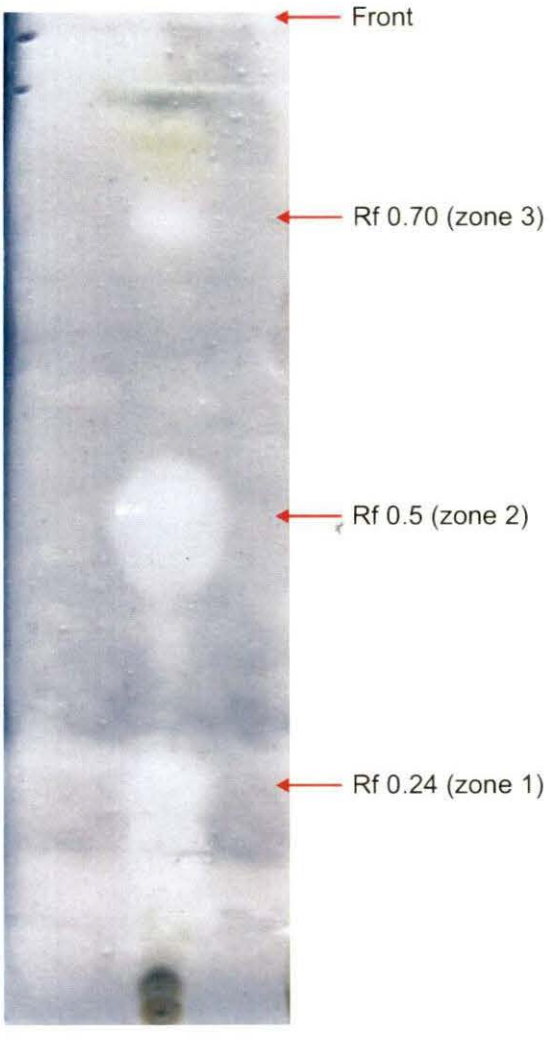

Plate 20: Bioassay of crude leaf extract of *Datura stramonium* developed in hexane: ethyl acetate: methanol 60:40:1) and sprayed with Richard's solution supplemented with spore suspension of *Colletotrichum gloeosporioides*. Number of antifungal zones in parentheses along with R_f values have been presented.

Table 4.1.22: TLC plate bioassay of crude leaf extracts of three different plants against *Fusarium equiseti.*

Extracts of the plants	No. of inhibition Zone on TLC plates	*F. equiseti*	
		Diameter of Inhibition zones (mm)	R_f
Xanthium strumarium	Zone 1	30	0.85
Crotalaria mucronata	Zone 1	8	0.15
	Zone 2	10	0.38
	Zone 3	20	0.70
Borreria alata	Zone 1	30	0.38

*Crude leaf extract =100 µg/ml.
TLC plates developed in solvent = Hexane:Ethylacetate:Methanol :: 60:40:1

Table 4.1.23: Chromogenic analysis of crude leaf extracts of *two* different plants against *Colletotrichum gloeosporioides.*

Extracts of the plants	TLC plate bioassay guided antifungal zones and their Rf in parentheses	*Spray reagent*			
		Anisaldehyde		*Vanillin H_2SO_4*	
		Colour	Probable compound	Colour	Probable compound
Xanthium strumarium	Zone 1 (0.85)	Dark green	Bitter principle	Blue-green	Bitter principle
Datura stramonium	Zone 1 (0.24)	Red-violate	Triterpene	Blue-green	Bitter principle
	Zone 2 (0.5)	Light violate	Terpenes	Blue	Monoterpene alcohol
	Zone 3 (0.70)	Light yellow	ND	Dark yellow	ND

*Crude leaf extract =100 µg/ml.; ND = Not defined
TLC plates developed in solvent = Hexane:Ethylacetate:Methanol :: 60:40:1

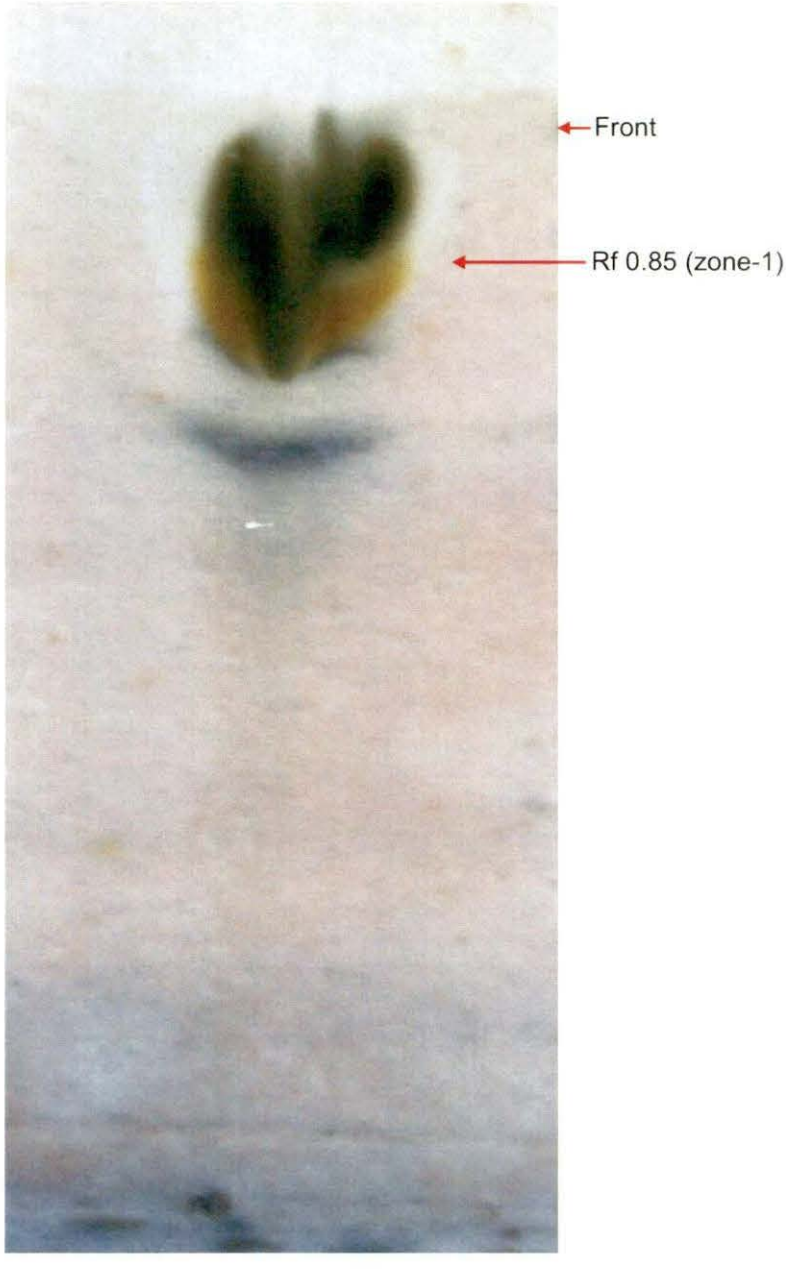

Plate 21: Bioassay of crude leaf extract of *Xanthium strumarium* developed in hexane: ethyl acetate: methanol(9:1) and sprayed with Richard's solution supplemented with spore suspension of *Fusarium equiseti*. Number of antifungal zone in parentheses along with R_f value has been presented.

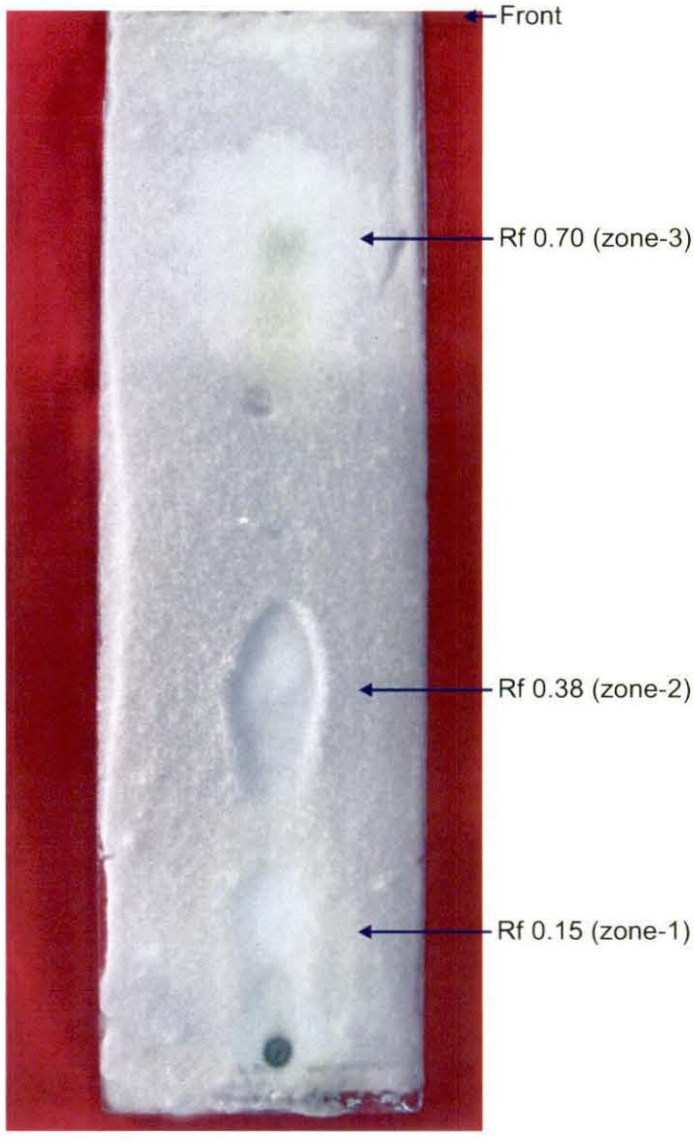

Plate 22: Bioassay of crude leaf extract of *Cratolaria nucronata* developed in hexane: ethyl acetate: methanol (60:40:1) and sprayed with Richard's solution supplemented with spore suspension of *Fusarium equiseti*. Number of antifungal zones in parentheses along with R_f values have been presented.

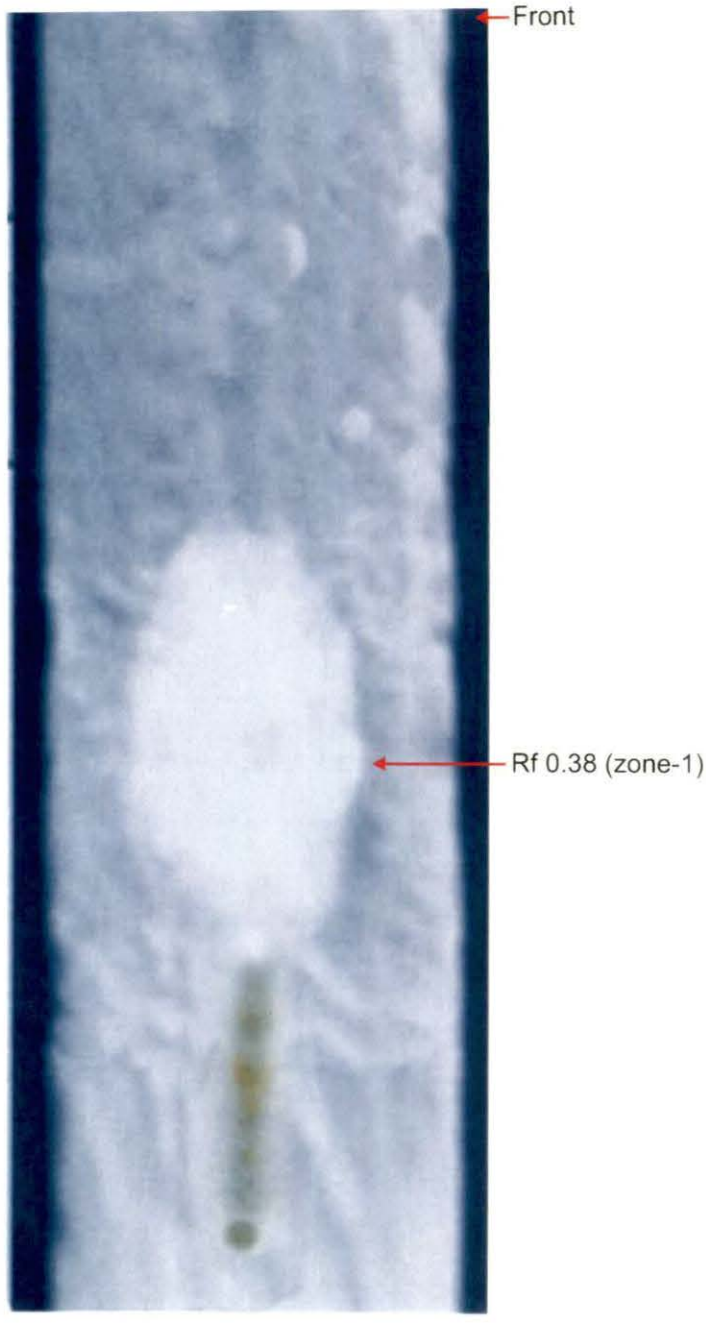

Plate 23: Bioassay of crude leaf extract of *Borreria alata* developed in hexane: ethyl acetate: methanol (60:40:1) and sprayed with Richard's solution supplemented with spore suspension of *Fusarium equiseti*. Number of antifungal zone in parentheses along with R_f value has been presented.

Table 4.1.24: TLC plate bioassay of crude leaf extracts of three different plants against *Fusarium equiseti.*

Extracts of the plants	TLC plate bioassay guided antifungal zones and their Rf in parentheses	*Spray reagent*			
		Anisaldehyde		*Vanillin H_2SO_4*	
		Colour	Probable compound	Colour	Probable compound
Xanthium strumarium	Zone 1 (0.85)	Dark green	Bitter principle	Blue-green	Bitter principle
Crotalaria mucronata	Zone 1 (0.15)	Red-violate	Triterpene	No colour	-
	Zone 2 (0.38)	Red-violate	Triterpene	No colour	-
	Zone 3 (0.70)	Red-violate	Triterpene	Dark yellow	ND
Borreria alata	Zone 1(0.38)	Red-violate	Triterpene	No colour	-

*Crude leaf extract =100 µg/ml.; ND = Not defined;
TLC plates developed in solvent = Hexane:Ethylacetate:Methanol :: 60:40:1

4.1.12: *In vivo* control of foliar pathogens of brinjal by application of plant extracts.

Antagonistic activity of aqueous extracts (0.5g / ml distilled water) of *D.stramonium* and *X.strumarium* were tested *in vivo* on a susceptible brinjal variety (green round). The plants were sprayed with aqueous extracts in separate sets. On control set, sterile distilled water was sprayed. After 24 hours, the plants in each set were inoculated with *C. gloeosporioides*. The details of the experimental techniques etc. have been presented in materials and methods (section-3.6.3 and 3.7.2). In control set, the plants were inoculated with spore suspension of *C. gloeosporioides*. Results of the experiment have been presented in the table 4.1.25. From the results it was evident that both the plant extracts tested showed significant reduction in mean disease index.

X.strumarium reduced anthracnose disease caused by *C. gloeosporioides* significantly. *X. strumarium* sprayed and inoculated plants showed disease index of 0.2 and 0.5 respectively after 8 days and 10 days. After the same period of incubation disease index

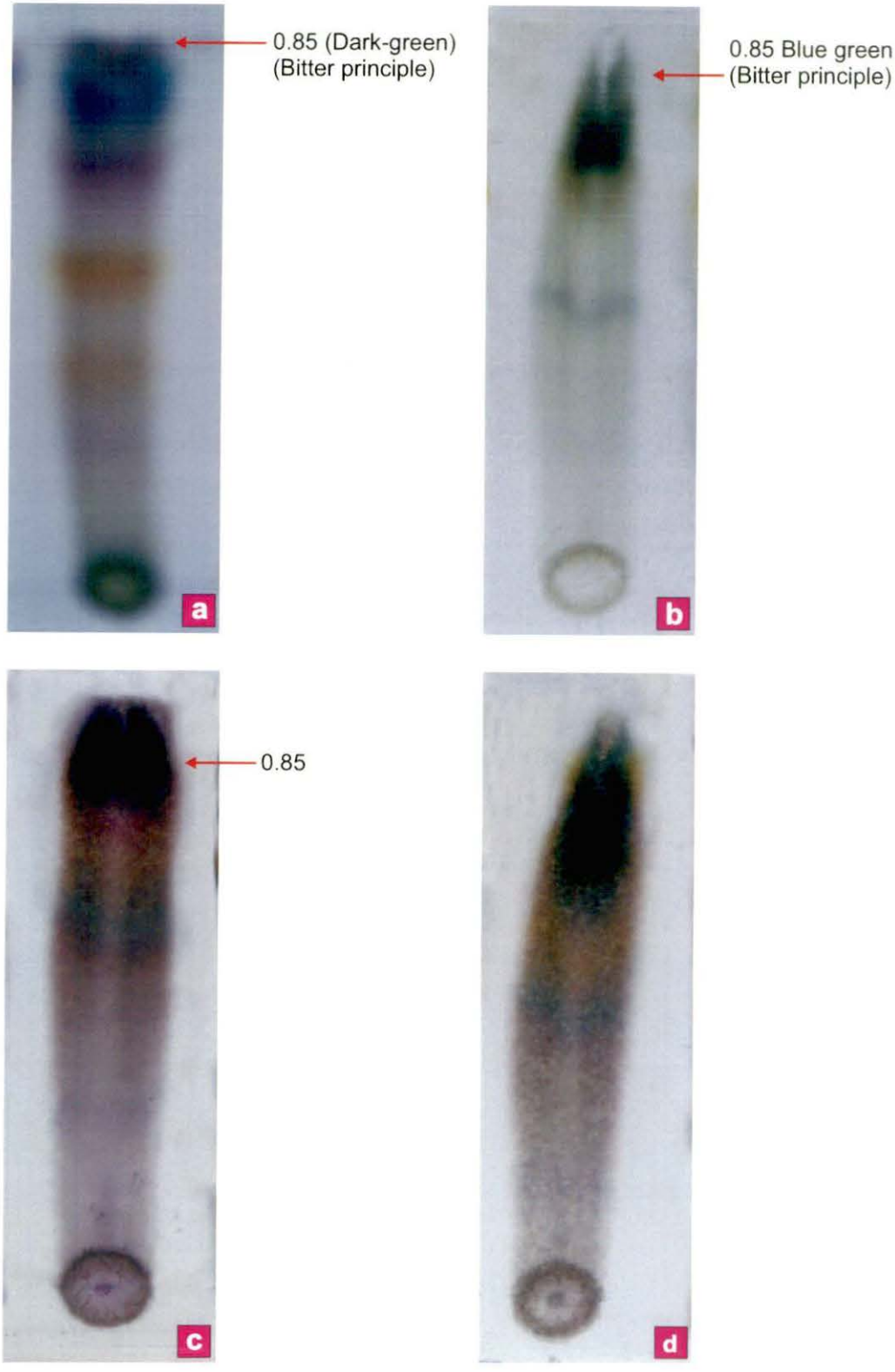

Plate 24: TLC plates

fig.a. *X.strumarium* leaf extract developed in hexane:ethyl acetate:methanol (60:40:1) and sprayed with Anisaldehyde reagent **fig.b.** *X.strumarium* developed in hexane:ethyl acetate:methanol (60:40:1) and sprayed with Vanillin-H_2SO_4 reagent **fig.c.** *X.strumarium* developed in chloroform:methanol (9:1) and sprayed with Anisaldehyde reagent **fig.d.** *X.strumarium* developed in chloroform:methanol (9:1) and sprayed with Vanillin-H_2SO_4 reagent.

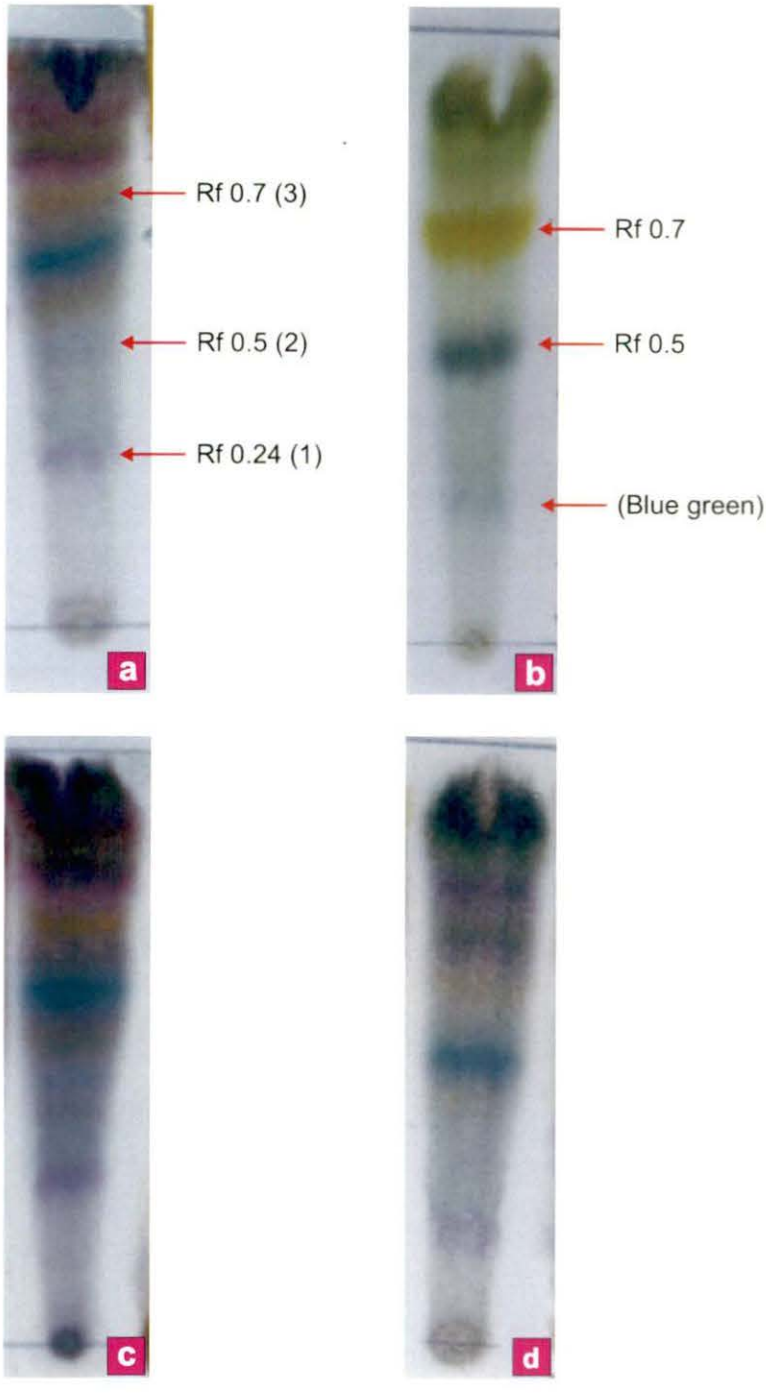

Plate 25: TLC plates

fig.a. *D.stramonium* leaf extract developed in hexane:ethyl acetate:methanol (60:40:1) and sprayed with Anisaldehyde reagent **fig.b.** *D.stramonium* developed in hexane:ethyl acetate:methanol (60:40:1) and sprayed with Vanillin-H_2SO_4 reagent **fig.c.** *D.stramonium* developed in chloroform:methanol (9:1) and sprayed with Anisaldehyde reagent **fig.d.** *D.stramonium* developed in chloroform:methanol (9:1) and sprayed with Vanillin-H_2SO_4 reagent.

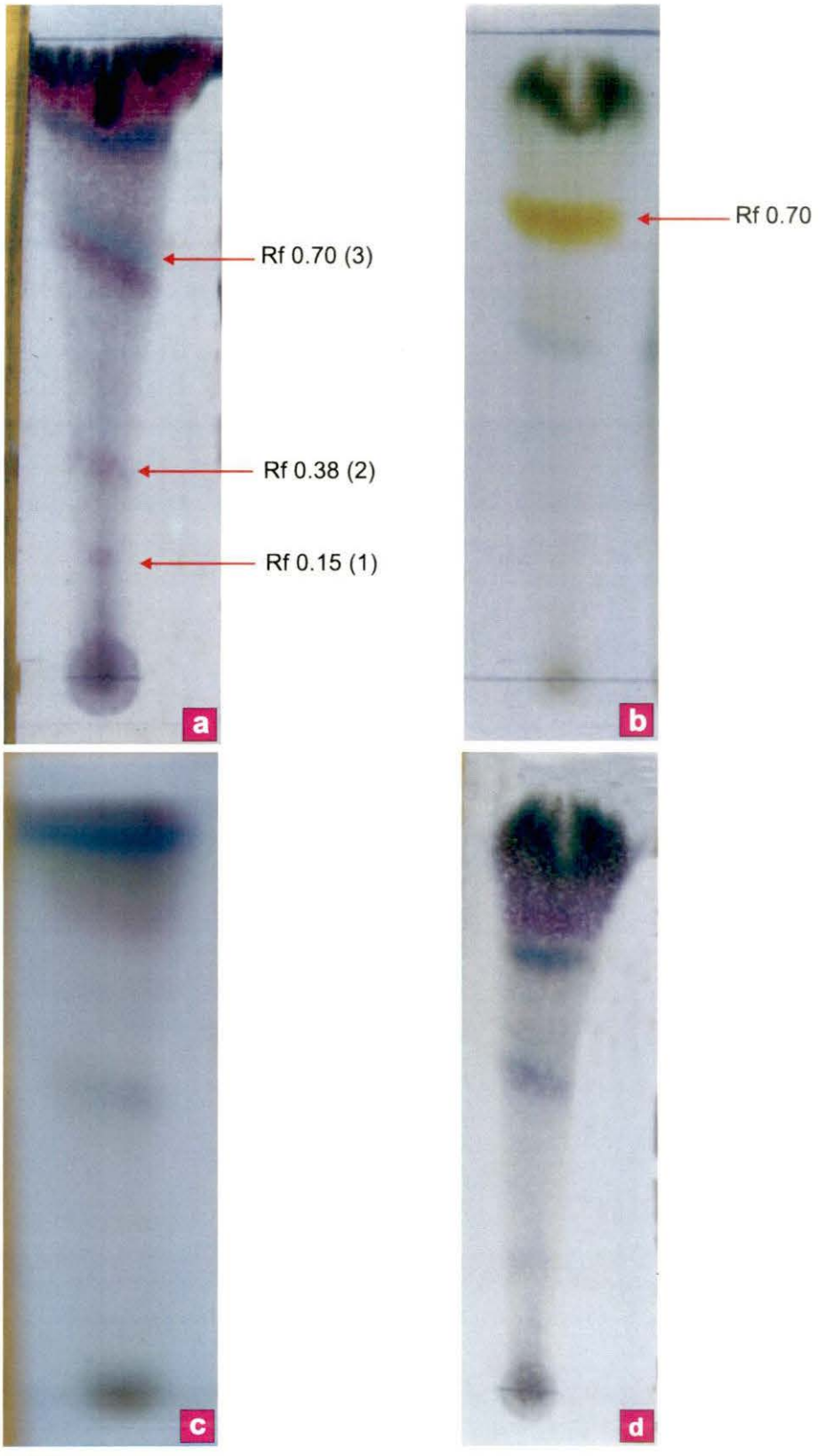

Plate 26: TLC plates

fig.a. *C.mucronata* leaf extract developed in hexane:ethyl acetate:methanol (60:40:1) and sprayed with Anisaldehyde reagent **fig.b.** *C.mucronata* developed in hexane:ethyl acetate:methanol (60:40:1) and sprayed with Vanillin-H_2SO_4 reagent **fig.c.** *C.mucronata* developed in chloroform:methanol (9:1) and sprayed with Anisaldehyde reagent **fig.d.** *C.mucronata* developed in chloroform:methanol (9:1) and sprayed with Vanillin-H_2SO_4 reagent.

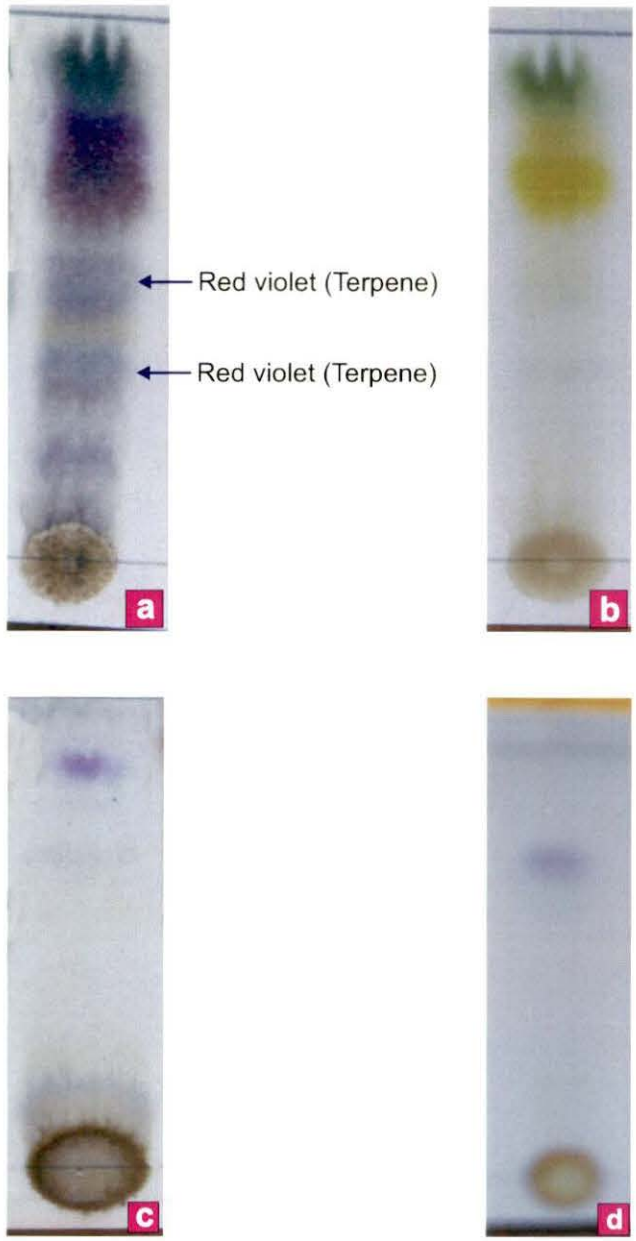

Plate 27: TLC plates

fig.a. *B.alata* leaf extract developed in hexane:ethyl acetate:methanol (60:40:1) and sprayed with Anisaldehyde reagent **fig.b.** *B.alata* developed in hexane:ethyl acetate:methanol (60:40:1) and sprayed with Vanillin-H_2SO_4 reagent **fig.c.** *B.alata* developed in chloroform:methanol (9:1) and sprayed with Anisaldehyde reagent **fig.d.** *B.alata* developed in chloroform:methanol (9:1) and sprayed with Vanillin-H_2SO_4 reagent.

of 10.74 and 13.24 was evident in control plants. When plants were pre-sprayed (by *D. stramonium)* and inoculated (by the *C.gloeosporioides*) disease index was 0.4 and 0.8 respectively after 8 days and 10 days of incubation.

Table 4.1.25: *In vivo* control of anthracnose of brinjal (cv Green round) caused by *C. gloeosporioides* by foliar application of plant extracts of *Datura stramonium and Xanthium strumarium*

Extracts of the plants	Mean disease index/plant*				
	Incubation periods(Days)				
	2	4	6	8	10
Datura stramonium	0	0	0	0.4±0.10	0.8±0.20
Xanthium strumarium	0	0	0	0.2±0.12	0.5±0.15
Control	1.6±0.36	3.08±0.27	7. 69±0.94	10.74±2.42	13.24±2.18
CD at 5%	-	-	-	-	2.7

*Mean of three replications. Data after ± represent standard error values. - = not performed.

Similar experiments were also performed to control *F. equiseti* with three different plant extracts (*X. strumarium*, *C.mucronata* and *B.alata*). Finally the results have been presented in the table-4.1.26.

F.equiseti caused stem rot disease was reduced by all the three plant extracts (*X. strumarium, C. mucronata* and *B. alata*) tested. *X. strumarium, C. mucronata* and *B. alata* sprayed and inoculated plants showed disease index of 0.63, 0.35 and 0.58 respectively after 10 days of inoculation. After the same period of incubation the untreated-inoculated plants showed disease index of 8.52.

Table 4.1.26: *In vivo* control of stem rot disease of brinjal (cv Green round) caused by *F. equiseti* by foliar application of plant extracts of *Xanthium strumarium, Crotalaria mucronata* and *Borreria alata*

Extracts of the plants	Mean disease index/plant*				
	Incubation periods(Days)				
	2	4	6	8	10
Xanthium strumarium	0	0	0	0	0.63±0.14
Crotalaria mucronata	0	0	0	0	0.35±0.11
Borreria alata	0	0	0	0	0.58±0.16
Control	0	1.1±0.30	4.1±0.62	6.3±1.92	8.52±1.26
CD at 5%	-	-	-	-	2.3

*Mean of three replications. Data after ± represent standard error values. - = not performed.