5. Discussion

The most important plantation crop of north Bengal is tea. Other than tea, the region is also famous for cultivation of solanaceous plants like brinjal and tomato. All the three crops are affected by a number of fungal pathogens. The present practice of disease management is by chemical fungicides. However, organic farming has become necessary to address the consumer resistance towards the use of harmful synthetic chemicals (Pretorius *et al.* 2002). Thus, the search for natural products as herbicides, fungicides or pesticides has gained priority (Duke *et al.* 1995; Ushiki *et al.*1996).

Ayurveda is a type of curative skill practiced in India. Ayurvedic practitioners largely depend on plant extracts; both 'pure' single plant preparations and/or mixed formulations. Ayurvedic preparations have antimicrobial activity against *Aspergillus* spp. (Dhuley, 1998) and *Propionibacterium acnes* (Paranjpe and Kulkarni, 1995). Several other workers also reported about the antifungal properties of the medicinal plants (Buwa and Staden, 2006; Dabur et al 2004a; Jayaraman *et al.* 2008; Ravindra and Kumar, 2007)

Initial screening of plants and chemical analysis of plant components are essential before application of plant extracts in agricultural fields. The present work was undertaken with an objective to find some potential plant extracts (from the geographical area of north Bengal) for field application as biofungicides. Two pathogens of brinjal, one pathogen of tomato and one pathogen of tea were used to evaluate the efficacy of various plant extracts.

At the onset of the present study, four different pathogens were isolated from infected plant parts of brinjal, tomato and tea as pure cultures. All the fungal isolates were subjected to verification of Koch's postulates.

During the present study *F. equiseti* was isolated as a pathogen of brinjal. To our knowledge this is a first report of *F. equiseti* causing disease in brinjal. Although several members of the genus *Fusarium* are well known pathogens, reports on *F. equiseti* as a pathogen are comparatively less. *F. equiseti* has been isolated by Baird and Carling (1998) from intact senescent cotton primary roots from Georgia. Gilbert *et al.* (2003)

isolated *F. equiseti* as the predominant organism in *F.* head blight affected wheat plant. Around 8.1% of leaf sections showed presence of *F. equiseti* during 1998-2000. Some other authors reported *F. equiseti* as saprophytic and storage fungi (McAllister et al. 1997; Hamman *et al.* 1995). On the other hand some reports suggest *F. equiseti* as a biocontrol agent (Horinouchi *et al.* 2007; Nitao *et al.* 2001).

Colletotrichum gloeosporioides was isolated from leaves of brinjal showing anthracnose symptom producing pathogen in brinjal from several locations in the region of the present study. It was first isolated from brinjal fields in Khoribari (Phansidewa) and later from Rajganj, Belakoba and Itahar. *C. gleosorioides,* is the causal organism of anthracnose in brinjal (Wijesekara *et al.* 2005; Fernandes *et al.* 2002; Madeira and Reifschneider, 1987). *C. gloeosporioides* has a wide host range in different vegetables and horticultural crops that help it to survive throughout the year (Jeffries *et al.* 1990; Denoyes and Bandry, 1995; Manadhar *et al.* 1995; Zulfiqar *et al.*1996; Pandey *et al.* 1997; Gaikwad *et al.* 2005). Gaikwad *et al.* (2005) showed that the diseased plant parts such as fruits, twigs, seed and stem pith and bark of stem helped the pathogen to survive up to next crop season. Similar results were also reported earlier by Cocciola *et al.* (1996) and Karunaratne *et al.* (1999) who observed perpetuation of *C. gloeosporioides* to succeeding crop season in dispersed plant parts like twigs, fruits, stems, etc. in olive and avocado. Thus our results agree to the findings of these authors.

Alternaria alternata was originally isolated from tomato collected from Haldibari (Cooch Behar), a place situated in north Bengal, renowned for the cultivation of tomato. Later the pathogen was also isolated from Raiganj (Uttar Dinajpur), Balurghat (Dakshin Dinajpur) and Falakata (Jalpaiguri). *A. alternata* was previously reported in Pakistan as a saprophytic pathogen of tomato causing post harvest losses in high frequency (Akhtar *et al.* 1994). But in 2004, the authors reported that one distinct pathotype out of 35 *A. alternata* isolates (collected from fruits from fields and market) produced leaf blight symptoms (Akhtar *et al.* 2004). Raja and Reddy (2007) reported *Alternaria* spp. as a pathogen of brinjal. Several authors have reported *A. alternata* as a foliar pathogen in basil (Taba *et al.* 2009), sugar beet (Hudec and Rohacik, 2002), sunflower (Godika *et al.* 2000), tomato (Morris *et al.2002*), pepper and egg plant (Ouf *et al.* 1998) and aloe

(Kamalakannan *et al.* 2008). Thus our observation that *A. alternata* is a pathogen of tomato is in agreement with others.

Pestalotiosis theae is one of the major foliar pathogens of tea and cause grey blight disease. Grey blight disease is very common in the present study area which causes severe damage to tea leaves (Chakraborty *et al.* 1995, 1996). Sometimes, the pathogen also damages the pluckable leaves which are used for production of tea and thus hamper the quality and quantity of the production. The present strain has been isolated from the tea leaves collected from Garubathan. *P. theae* has been reported to occur in other plant hosts also. One of the hosts of *P. theae* is sweet persimmon (Chang *et al.*1998). *Pestalotiopsis* species have been found to be associated with podocarpaceae, theaceae and taxaceae in south china (Wei *et al.* 2007).

The four fungal pathogens isolated in the present study were maintained in the laboratory in suitable media for sporulation and growth. In general, PDA was used as the media for growth. For sporulation, *C. gloeosporioides* was grown in OMA while the other three fungi sporulated well in PDA. The fungi were studied for their surface morphology and surface contour through scanning electron microscopic observations.

Fusarium equiseti produced reddish brown colour in tubes and plates (Plate 8 & 9). Comma-shaped micro conidia in aerial mycelium were evident. Macro conidia were strongly septate and sickle shaped (plate 9). The basal cell was distinctly foot shaped. Length and breadth of mature macro conidia were 15-27 micrometer and 3-5 micrometer respectively. Our study is in agreement with that of de-Hoog *et al.* (2000). The authors suggested that macroscopic and microscopic features, such as, colour of the colony, length and shape of the macroconidia are important features for the differentiation of *Fusarium* species. Rhoobunjongde *et al.* (1991) also observed almost similar features in *Fusarium moniliforme*. They found that fungal hyphae were hyaline. Macro conidia were septate (3-4 septa) slightly sickle to cigar shaped. Micro-conidia, with 0-1 septa, were variable in size and shape. While studying fungal growth of *F. moniliforme* MRC-826, Harrison *et al.* (1990) reported that the fungal culture contained pinkish-orange colonies with a tannish-brown underside. Balali and Iranpoor (2005) reported that isolates of *F. oxysporum* from different hosts produced white colonies on PDA with aerial mycelium but the lower

surfaces of the colonies were pink or light to dark violet. They also found canoe-shaped macroconidia with 3-5 septa and ovoid to ellipsoid uni or bi-cellular, microconidia.

Studies on culture morphology of the isolated *C. gloeosporioides* strain revealed that mycelia were white in colour, which gradually turned pale yellow and further darker to gray (plate 10). Huge masses of pinkish acervuli were produced in OMA, which were much less when grown in PDA. Mycelia and conidia of the fungus were light colored. The length and breadth of the mature conidia were 12-15 micrometer and 3.5-5.5 micrometer respectively. Sangeetha and Rawal (2009) studied eight isolates of *C. gloeosporioides.* Length of spores of the isolates was marginally different from our study however breadth of the spores was similar.

Microscopic observation of the isolated *A. alternata* strain revealed that immature mycelia were hyaline but on maturity it became gray in colour. Conidia of the fungus were obclavate to beaked and brownish to olive in colour having transverse and longitudinal septa. Conidia were produced from simple septate conidiophores in simple or branched acropetal chains. The length and breadth of mature conidia were 18-36 µm and 5-10 µm respectively. The diameter of the mature hyphae ranged between 3-5 µm. Our findings are similar to that reported by Maiti *et al.* (2007) who also found the diameter of mature hyphae to be 3-5 µm. Slavov *et al.* (2004) reported that the colour of fungal colonies (*Alternaria alternata* tobacco pathotype isolate 0-268) was usually dark brown to dark olive-green-brown, but quite often lighter and almost white colonies sometimes appear under the same conditions with the same medium. These results were also in agreement to the findings of the present study. However, in natural habitats the conidia of *A. alternata* are larger, have longer beaks, and are more uniform in size than those produced *in vitro* on common agar media (Misaghi *et al.* 1978).

Studies on the present isolate of *P. theae* revealed that immature mycelia were hyaline which on maturity became gray in colour. Conidia of the fungus were septate with three setae and one tail. Three distinct cells were in the middle. The innermost cell of the spore was dark than the other two cells. The setae and tail were hyaline. Conidia were produced from simple septate conidiophores. The length and breadth of mature conidia were 16-24 µm and 5-7 µm respectively. The diameter of the mature hyphae ranged

between 3-5 µm. Murugan *et al.* (2001, 2007) studied the morphology and ultrastructure of *Pestalotiopsis rhododendri* and *Pestalotiopsis maculans.* They found that the size of the conidia of *P. rhododendri* was 22-26 µm x 6-8 µm through light and transmission electron microscopic studies. Thus our results are in agreement to their findings.

Shaner *et al.* (1992) stated that plant pathogens exhibit considerable variation in cultural as well as in pathogenic characters. Hence, after the isolation of the fungi from diseased plants, pathogenicity test were carried out with the isolates on whole plants or detached leaves. Results obtained by following the detached leaf technique as well as whole plant inoculation technique revealed that different varieties of a host possessed differential level of susceptibility towards the pathogen. Thus the susceptible and resistant varieties of a host could be selected for the purpose of experiments, where susceptible varieties were used to test the efficacy of the antifungal extracts.

From the pathogenicity results it was evident that Green round variety of brinjal was the most susceptible and Muktakeshi variety was most resistant among six varieties tested against both the pathogens of brinjal (*C. gloeosporioides* and *F. equiseti*). Pandey *et al.* (2002) observed that none of the tested varieties of brinjal were completely resistant to *Phomopsis* blight. Two varieties (Ramnagar giant and KS-233) showed moderate resistance but the others showed susceptibility. Madeira and Reifschneider (1987) suggested that sub epidermal injection of 0.1ml of conidial suspension of *C. gloeosporioides* utilizing a hypodermic syringe was the most effective inoculation method. Fransisco-Neto *et al.* (1995) observed that the infection of the leaves of *Passiflora alata* and *P. edulis* f. sp. *flavicarpa* by two isolates of *C. gloeosporioides* was more severe when the inoculated leaves or plants were incubated during 48h in dark and high relative humidity. Thus the results of the present study are in conformity with earlier report. Stravato and Cappelli (2000) tested twelve wild Solanum accessions at seedling stage for resistance to four isolates of *Fusarium oxysporum* f. sp. *melongenae.* They found two highly susceptible and three highly resistant plants against the *Fusarium* isolates tested. Thus from the above studies, the level of resistance or susceptibility of the tested brinjal varieties to *C. gloeosporioides* and *F. equiseti* was distinct.

Among the tested tomato varieties, Karan variety showed maximum disease development against *Alternaria alternata*. Besides Karan, other varieties like Romeo, Pasuja, Trishul, Mahyco and Abinash-2 were also considered as susceptible. On the other hand US1080 variety was considered as the most resistant as it showed no disease symptoms. Raja and Reddy (2007) showed disease index of some solanaceus plants by conventional pathogenicity test with *Alternaria* spp.

Detached leaf inoculation technique was followed to determine pathogenicity of *P. theae*. TV-9 was considered as the most susceptible as it showed maximum disease development among the six tea varieties tested against the pathogen. Varieties like TV-18 and TV-22 were also considered as susceptible as they also showed significantly higher disease development than the other three varieties. On the other hand three varieties TV-25, TV-26 and TV-30 were resistant as they produced minimum visible disease development against *P. theae*. Yanase and Takeda (1987) also detected the resistance of tea varieties to grey blight disease of tea caused by *Pestalotiopsis longiseta* in laboratory condition in Japan. Hu-Shu Xia (1996) reported the pathogenicity of *P. theae* in 18 tea cultivars in Anhui province, China and found two cultivars as highly resistant. Pathogenicity test of *P. theae* in tea was also performed by Chakraborty *et al.* (1995, 1996). They found Teenali 17/1/54 as most susceptible but TV 9 was not included in their test. In the present experiment TV-9 was found to be most susceptible among different varieties which did not include Teenali 17/1/54. The experimental results and design were in conformity with that of Chakraborty *et al.* (1995, 1996).

A number of plant species have been reported to possess some natural substances in their leaves and bulb which are toxic to many phytopathogenic fungi. Several authors have used plant extracts with antifungal activity to control plant diseases (Park *et al.* 2008; Ajibesin *et al.* 2008; Khalil *et al.* Al-Howiriny *et al.* 2005; Saxena *et al.*, 2003; Sharma *et al.*, 2002; Mittal *et al.*, 2002; Ali *et al.*, 2001; Bhandary *et al.* 2000; Deena and Thopil, 2000). Natural fungi toxic substances are less harmful than chemical fungicides. The plant kingdom therefore has a vast potential for providing antifungal chemicals as only very few compounds have so far been classified and the majority remains to be explored. Sub-Himalayan West Bengal has a thick forest cover over large areas and is extremely rich in diverse flora. The region has been declared as hot-spot zone with respect to bio-diversity

(Rai and Das, 2002). Therefore plant species with known bioactivity and several unexploited species from this region have been included in this study.

Initially 80 plant extracts (50% ethanolic) were screened *in vitro* for their antifungal properties against the four pathogens (*F. equiseti, C. gloeosporioides, A. alternata* and *P. theae*) following spore germination technique. Spore germination is a determining factor for the pathogen during the early phase of host colonization (Egley, 1994). Use of alcoholic extracts for screening of antifungal properties has been reported by several authors (Shalini and Srivastava, 2009; Veljic *et al.* 2008; Vukovic *et al.* 2007; Singh and Karnwal, 2006). In the present study, 50% ethanolic extract of *Xanthium strumarium* and *Datura stramonium* were effective in controlling spore germination (100% control) of *C. gloeosporioides* and *F. equiseti*. About seventeen other plants have also shown antifungal potentiality (>80% inhibition) against these two pathogens. Leaf extract of *Datura metel, X. strumarium* and *D. stramonium* exhibited 100% of inhibition of spore germination of *A. alternata*. Spore germination of *P. theae* was completely inhibited by leaf extract of *Polyalthia longifolia, X. strumarium* and *D. stramonium.* Many authors have reported the antifungal potentiality of several plant extracts through spore germination bioassay (Prusky *et al.* 1982; Rahmani *et al.* 2004; Abou-Jowdah *et al.* 2002). Kim *et al.* (2002) showed that crude extracts of *Xanthium strumarium* inhibited zoospore germination of *Phytophthora drechsleri*, the causal agent of Atractylis rot, *in vitro*.

Following the spore germination bioassay poisoned food technique was utilized to additionally test five plants, *Crotalaria mucronata, Datura stramanium, Mitracarpus verticillatus, Piper betle* and *Xanthium strumarium,* selected on the basis of antifungal efficacy against *F. equiseti* and *C. gloeosporioides.* Another set of five plants (*Allium sativum Clerodendrum viscosum, D. stramonium, P. longifolia* and *X. strumarium*) were similarly tested against *A. alternata.* Leaves were extracted in three different solvents, water, ethanol and ethyl acetate. Radial growth inhibition was studied and was presented as percent inhibition of mycelia growth. From the results it was evident that aqueous and ethanolic leaf extracts showed less activity than ethyl acetate extracts. Damayanti *et al.,* (1996) treated pineapple fruits infested with *C. paradoxa* by *X. strumarium* extract and found reduced disease severity. Several other studies supported that *X. strumarium* leaf extracts have antifungal activity. Numerous studies have been documented about the

antifungal properties of plant extracts (Suhr and Nielson, 2003; Mishra and Dubey, 1994). Hassanein *et al.,* (2008) studied leaf extracts of neem (*Azadirachta indica*) and chinaberry (*Melia azedirach*) against two tomato pathogenic fungi *Alternaria solani* and *Fusarium oxysporum,* the causal agents of early blight and wilt diseases of tomato plant respectively. Park *et al.* (2008) tested *in vivo* fungicidal activity of medicinal plant extracts (at concentrations of 0.5, 1 and 2 mg/ml) against six phytopathogenic fungi. Nariman (2009) analyzed twenty plant extracts following disc diffusion method. Usha *et al.* (2009) reported that floral malformation caused by *Fusarium mangiferae* is a serious threat to mango cultivation in various countries. Methanol-water (70/30 v/v) extracts of *D. stramonium* showed strong antifungal activity against the said pathogen. Numerous studies have documented about the antifungal effect of plant essential oils (Suhr and Nielson, 2003; Mishra and Dubey, 1994). Screening of indigenous local herbs and plant materials for antifungal properties have been reported in several articles from Australia (Cox *et al.* 1998), Argentina (Penna *et al.*, 2001), Finland (Rauha *et al.*, 2000) and India (Ahmad and Beg, 2001). Jha and Sharma (2008) screened leaf extracts of 83 plant species (aqueous and autoclaved) for antifungal efficacy *in vitro* against *Rhizoctonia bataticola*. They found *Rannunculus scleratus*, *X. strumarium*, *Ipomoea carnea*, *Ocimum basilicum* and *Eclipta alba* to be effective. Extract of *R. scleretus* was highly effective while extracts of *X. strumarium*, *O. basilicum* and *I. carnea*, had their effects on sclerotia. Khanzada, (2006) showed the effectiveness of various extracts against *C. paradoxa.* Kanauchi *et al.* (1999) showed that the extract of cocklebur (*X. strumarium*) contains xanthatin, an antimicrobial substance. MICs of xanthatin were 25-100 µg/ml against *Candida* sp., *Pichia* sp., *Saccharomycopsis* sp. and *Torulaspora* sp.

Selected plants found to be effective were subjected to further study. The potential plants were exhaustively extracted in a soxhlet using three different solvents, viz. benzene, chloroform and hexane. All such extracts were tested by agar cup bioassay and a comparison was drawn among the efficacy of the three solvents. Leaves of eleven plants (*X. strumarium*, *Borreria alata, Clerodendrum viscosum, Annonus squamosa, Vitex negundo, Eucalyptus globosus, Tectona grandis, Piper betle, Crotalaria mucronata, Mitracarpus verticillatus* and *Datura stramonium*) were tested against C. *gloeosporioides* and *F. equiseti* and results showed that benzene was the best solvent because the benzene

extract showed highest antifungal activity. Leaves of only one plant (*Melastoma malabathricum*) extracted in chloroform showed slightly higher antifungal activity than its benzene extract. Similar studies were conducted against the pathogen, *A. alternata* and *P. theae* with respective selected plant extracts. In all cases benzene extract showed highest antifungal activity.

Minimum inhibitory concentration of the soxhlet extracts were determined by agar cup technique. Lowest MIC was recorded by extracts of *D. stramonium* against *C. gloeosporioides*. *X. strumarium* leaf extract exhibited a low MIC value of 0.008g/ml when tested with *C. gloeosporioides*. However, the same extract recorded a much higher value (0.06g/ml) when tested against *A. alternata*. On the other hand, *B. alata* maintained sufficiently low MIC 0.006g/ml & 0.006g/ml respectively against both *F. equiseti* and *A. alternata*. Other extracts considered effective were *E. globosus* (0.02g/ml) and *M. verticillatus* (0.01g/ml) against *F. equiseti*, *M. melabathricum* (0.06g/ml) against C. *gloeosporioides*, *A. squamosa* (0.06g/ml) and *O. gratissimum* (0.008g/ml) against *A. alternata*.

Kanauchi *et al.*, (1999) showed that the extract of cocklebur (*X. strumarium*) contains xanthatin, an antibacterial substance. Minimum inhibitory concentrations (MICs) of xanthatin were 12.5-100μg/ml against *Bacillus* sp. MICs of xanthatin were 25-100 μg/ml against *Candida* sp., *Pichia* sp., *Saccharomycopsis* sp. and *Torulaspora* sp. They also suggested that xanthatin from cocklebur leaf extract against *Bacillus* sp. and some film-forming yeast may be used to prevent contamination in koji and during production of alcoholic beverages. Compound from *Terminalia arjuna* were found to be effective against *Fusarium equiseti* and other two pathogens by Digrak et al. (1999). The leaf extract of *Clerodendrum viscosum* completely checked the radial growth of the test fungus *Curvularia lunata*. The leaf extract 1:10 dialution was the most successful for the inhibition of the test fungus in term of its growth (Parimelazhagan and Franchis, (1999)

In the present study, three different fungicides have been tested to determine their efficacy against the four pathogens, *F. equiseti*, *C. gloeosporioides*, *A. alternata* and *P.theae*. Minimum inhibitory concentration (MIC) of Nystatin, Captaf and Bavistin were determined against the fungal pathogens. The objective of use of fungicides in the present

study is to compare the efficacy of fungicides with that of botanicals. Among the fungicides tested, Captaf showed MIC of 3 mg/ml, 0.5mg/ml, 5 mg/ml and 2 mg/ml respectively against *F. equiseti, C. gloeosporioides, A.alternata* and *P. theae.* Comparatively higher MIC was found in case of Nystatin and Bavistin except when bavistin used against *C. gloeosporioides P. theae.* The antifungal activity of the plant extracts which recorded a lowest MIC of 2mg/ml compared favorably with that of synthetic fungicides. The fractionated extracts were more effective with an MIC value of 0.05 mg/ml (*D. stramonium*) against *F. equiseti.* Raghavendra (2009) compared the antifungal activity of alkaloid extract of *Prosopis juliflora* (Sw.) DC. (Mimosaceae) against *A. alternata* with synthetic fungicides viz., blitox, captan, dithane M-45 and thiram. They found that alkaloid extract was highly effective even at the dosage lesser than the synthetic fungicides.

Leaves of two plants, *D. stramonium* and *C. mucronata* were chemically fractionated following a scheme presented in table-3.3. The fractions were alkaloids, quarternary alkaloids, terpenoids/ phenolics, polysaccharides and fat/wax/neutral compounds. The terpenoid fraction of both plants showed significant antifungal activity against *F. equiseti, C. gloeosporioides* and *A. alternata* when tested by spore germination method. MIC values of terpenoid fraction of *D. stramonium* extract were 0.05mg/ml against *F. equiseti,* 0.1mg/ml against *C. gloeosporioides* and 0.5mg/ml against *A. alternata* when tested by disc diffusion method. Similarly, the MIC of *C. mucronata* leaf extract against *F. equiseti* was found to be 0.01 mg /ml. The MIC value of *D. stramonium* leaf extract (terpenoid fraction) was 0.5mg/ml against *A. alternata.* Several other authors have also observed that the antifungal components in many plants are terpenoids (Shafi *et al.* 2004; Barrero *et al.* 2000; Barre *et al.*1997).

In order to control *P. theae* causing grey blight in tea, three most potential plant extracts (*Polyalthia longifolia, Datura stramonium* and *Allium sativum*) were chemically fractionated and all fractions were screened for antifungal activity. *Polyalthia longifolia* fraction PL3 and PL 4, all *Allium sativum* fractions (AS 1 - AS 4) and *Datura stramonium fraction* DS 1 were most effective against *P. theae.* All these fractions showed MIC value of 0.1 mg/ml. Literature reports indicate that *P. longifolia* bark extracts are antifungal due to the presence of 16-oxocleroda-3, 13E-dien-15-oic acid, kovavenic acid and

16β–hydroxycleroda-3,13-dien-15,16-olide (Rashid *et al.*, 1996). Annapurna *et al.* (1983) evaluated leaf extracts of *P. longifolia* with different solvents of increasing polarity for antagonism against some pathogenic fungi and bacteria. Murthy *et al.* (2005) isolated diterpenoids from the hexane extract of the seeds of *P. longifolia* that showed significant antibacterial and antifungal activities.

A wide spectrum of secondary metabolites such as phenols, flavonoids, quinones, tannins, essential oils, terpenoids, alkaloids, saponins and sterols which showed antimicrobial activity have been reported from higher plants. Barrero *et al.*, (2000) investigated antifungal efficacy of sesquiterpene lactones isolated from the six *Centaurea* species (*C. bombycina* Boiss ex D.C., *C. granatensis* Boiss, *C. monticola* Boiss, *C. incana* Desf., *C. maroccana* Ball. and *C. sulphurea* Willd.) against the fungus *Cunninghamella echinulata*. They reported that the two compounds costunolide and dehydrocostunolide, were responsible for the antifungal activity. In another study involving other species of *Centaurea,* Skaltsa *et al.*, (2004) reported the presence of several antifungal compounds. Marthanda *et al.* (2005) reported antifungal activity of the diterpenoids, 16α-hydroxy-cleroda-3,13-(14)-*Z*-diene-15,16-olide and 16-oxo-cleroda-3,13-(14)-*E*-diene-15-oic acid isolated from the hexane extract of the seeds of *Polyalthia longifolia.* Adou *et al.*, (2005) fractionated methanol and ethyl acetate extracts of two lianas from the genus *Casimirella* collected from Surinam rainforest. They isolated five new diterpenoids which are humirianthone, 1-hydroxy-humirianthone, 15*R*humirianthol, patagonol and patagonal. Scher *et al.* (2004) prepared a dichloromethane and a methanol extract of the liverwort *Bazzania trilobata* (L.) S.F. Gray (Lepidoziaceae) and showed their antifungal activity against the phytopathogenic fungi *Botrytis cinerea*, *Cladosporium cucumerinum*, *Phythophthora infestans*, *Pyricularia oryzae* and *Septoria tritici.* The authors observed that the antifungal activity was due to the presence of terpenoids. From these extracts, they isolated six antifungal sesquiterpenes: 5- and 7-hydroxycalamenene, drimenol, drimenal, viridiflorol, gymnomitrol and chloroisopiagiochin. Fujita *et al.*, (2005) isolated a sesquiterpene from *Polygonum punctatum* Elliot. (Polygonaceae) and found fungicidal activity against a food spoilage yeast, *Zygosaccharomyces bailii.* Ahmed *et al.*, (2005) screened antifungal compounds (terpenoids) from the roots of the wild carrot, *Daucus carota* L. ssp. *carota* (Apiaceae) and it was found to contain a range of antifungal activity

against *Fusarium oxysporum* and *Aspergillus niger*. Singh *et al.* (1980) showed that some essential oils (extracted from three *Cymbopogon species* and *T. ammi*) were more active than some of the prevalent synthetic fungicides and thus they suggested exploitation of natural fungicides following successful infield trials. Christian and Susana, (2008) showed that the essential oils of 18 plants possess antifungal properties against three common pathogens: *Penicillium*, *Fusarium*, and *Pythium*. Five oils [cinnamon (*Cinnamomum zeylanicum* Blume), clove (*Eugenia caryophyllata* Thunb.), oregano (*Origanum minutiflorum* O. Schwarz and P.H. Davis), savory (*Satureja montana* L.), and thyme (*Thymus vulgaris* L.)] completely controlled all three pathogens *in vitro*.

Thin layer chromatography (TLC) plate bioassay is considered as an important step towards discovery of new fungicides (Hostettmann *et al.* 2000). The method is one of the very quick and easy methods for screening antifungal compounds in phytoextracts (Guleria and Kumar 2000). The simple and visual nature of the technique makes it easy to identify the number of antifungal compounds in plant extracts and eliminate the unnecessary compounds. Several workers (Kagale *et al.* 2004, Saha, 1993, Chakraborty *et al.* 1995) have utilized the method to isolate natural products of various chemical compounds.

In this study TLC plate bioassay were performed to confirm the antifungal activity by visualizing the number of antifungal compounds in the extracts. The subsequent chemical analysis based on chromogenic spray reagents revealed the nature of antifungal compounds that may be responsible for the inhibition of the fungal pathogens. During the present study benzene extract of *X. strumarium* leaves showed single antifungal zone at R_f at 0.85 against *F. equiseti*, *C. gloeosporioides* and *A. alternata*. Chromogenic sprays revealed that this zone contained bitter principles. Other plants such as *B. alata* also showed a single antifungal zone against *F. equiseti* which was identified as triterpene. *D. stramonium* extracts produced three distinct antifungal zones against *C. gloeosporioides*. The antifungal compound at R_f 0.24 was identified as triterpene while that at R_f 0.5 was identified as monoterpene alcohol. The third antifungal compound could not be identified with any of the reagents used in the study. *C. mucronata* extracts also showed three antifungal zones on TLC plates all of which were detected as triterpenes after chromogenic sprays. *C. viscosum* showed two antifungal zones against *A. alternata* which were

identified as bitter principles. *P. longifolia* extracts rested against P. theae produced a single large inhibition zone at Rf 0.6, which was identified as bitter principles. *A. sativum* extracts showed two antifungal zones against *P. theae*. 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 the species.

One plant extract (*Datura*) was subjected to column separation. The separated fractions were tested against *F. equiseti* and *C. gloeosporioides* following spore germination bioassay. Potential fractions (fraction 6-20 & fraction 26-35) were also tested by disc diffusion bioassay. Combined column fractions (6- 20) could inhibit the growth of *C. gloeosporioides* at a concentration of 0.2mg/ml. Another column fraction (26–35) could check the spore germination of the *F. equiseti* at a concentration of 0.08mg/ml.

The most potential plant extracts were tested *in vivo* by application of the extracts in three different plants viz. brinjal, tomato and tea which were experimentally inoculated by respective pathogens. Antifungal extracts were dried and the dry compounds were dissolved in water. The water soluble extracts were finally mixed with tween-20 as a wetting agent. In case of brinjal, aqueous extracts (0.5g / ml distilled water) of *D. stramonium* and *X. strumarium* were tested on green round variety for the control of anthracnose caused by *C. gloeosporioides*. Similarly, control of disease caused by *F. equiseti* was tested by *X. strumarium, C. mucronata* and *B. alata* extracts. In both cases significant disease reduction was noted. *X. strumarium* and *D. stramonium* extracts were also effective (83% reduction in disease index) in controlling *Alternaria* blight of tomato in Karan variety. Grey blight in tea was controlled effectively by extracts of *A. sativum* and *P. longifolia*. Extracts from other two plants (Zingiber *officinale* and *Allium sativum*) were also active as evident from the results. Among these extracts, *A. sativum* (garlic) and *Z. officinale* (ginger) are well known and costly and their commercial exploitation may not be feasible for the purpose of disease management. But at acute diseased condition, use of the two extracts may be recommended without any cytotoxicity test because the plant products

are consumed by man. Leaf exract of *Polyalthia longifolia*) may be used after cytotoxicity test if used during leaf harvesting season. However, during the winter when the collection of leaves is suspended *P. longifolia* extracts may be used. 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 and all the tested extracts had a fungistatic rather than fungicidal effect. Chakraborty *et al.* (2009) showed significant reduction of wilt of brinjal caused by *F. solani* by preparations of *A. sativum* and *A. indica* along with soil solarization.

Jadeja (2003) observed that bulb extract of garlic was most effective against *Phomopsis vexans* casing diseases on brinjal. He found that leaf extracts of *Datura,* congress grass, neem and *Lantana* showed antifungal activity against the pathogen. Siva et al (2008) performed *in vivo* pot culture experiment employing water extract of six plant species. They showed reduction in disease symptoms of the brinjal plants caused by *F. oxysporum* f. sp. *melongenae.* Hassanein *et al.,* (2008) observed that tomato plants sprayed with 20% aqueous neem leaf extracts lowered the disease incidence to 42.54% in pathogenicity (*in vivo*) test of *Alternaria 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%. Hadizadeh *et al.* (2009) tested *in vivo* efficacy of various concentrations of oils of *Urtica dioica* and *Thymus vulgaris* against *A. alternata,* a pathogen of tomato fruit and found effective results.

The present study was initiated based on the need for eco-friendly fungicides to control diseases, of brinjal, tomato and tea, the three economically important horticultural/plantation crops of north Bengal. From the present study we may conclude that the bio-products may be used in the field for successful management of the specific diseases of brinjal (caused by *F. equiseti* and *C. gloeosporioides*), tomato (caused by *A. alternata*) and tea (caused by *P. theae*). The results of the study were encouraging since several plants showed remarkable antagonistic activity against the pathogens *in vitro. In situ* studies also exhibited good results indicating that many plant extracts have a definite potential to control the diseases of the crops. In the course of this investigation certain new

facts of fundamental importance have been revealed. All other results are almost in conformity with those obtained by previous workers. Thus in general this work have confirmed and extended some of the findings of the earlier workers. It dealt with isolation and identification of pathogens of the three major plants of north Bengal. Leaf extracts of several plants have shown potential antifungal activity both *in vitro* and *in vivo.* The potential phytoextracts may be integrated with other biocontrol agents and may be used in fields as part of integrated disease management system for control of the diseases of Brinjal, tomato and tea, the three economically important horticultural/plantation crops of north Bengal.