Summary

1. The present study deals with "Screening, extraction and application of botanical fungicides against some important fungal pathogens of economically important crops of north Bengal".
2. After a short introduction to the work, a brief review of literature related to use of botanicals in the economically important crops have been presented. This section deals with observations of the previous workers in concord with the present line of investigation. The review is selective manner rather than comprehensive. This study involves investigations on three different crops of major economic importance. For convenience, the review has been grouped separately under subheadings like diseases of tomato, diseases of tea, diseases of brinjal, disease control by botanicals and disease control by selected natural chemicals.
3. The objective of the study were i) Identification of potential plants for the presence of different antifungal compounds, effective against major phyto-pathogens. ii) Screening of plants for potential antifungal properties. iii) Solvent Extraction of plant parts for partial isolation of the active principles. iv) Isolation and identification of major pathogen(s). v) Bio-assay of the plant extracts. vi) Comparison of different extraction processes for obtaining the antifungal compounds from their sources. vii) Application of selected bio-products in the plants as anti-fungal agents to control the pathogen(s) and assessment of disease reduction.
4. A detailed description of different experimental procedures and techniques used during the present study has been given in different sections of materials and methods.
5. The work was carried out after thorough survey of different diseases found in the different fields of North Bengal. During the survey several pathogens were found from the diseased plants. The pathogens were isolated, selected and identified. The crops selected for the present study were brinjal, tomato and tea which are of

substantial importance in the area of the present study. Altogether four organisms viz. *Fusarium equiseti*, *Colletotrichum gloeosporioides*, *Alternaria alternata* and *Pestalotiopsis theae* were found to cause severe damages to those crops and hence selected for this study.

6. Pathogenicity test of four fungal pathogens were performed separately in different varieties of the respective host plants. Susceptible plants were identified and the *in vivo* studies were performed in the susceptible plants.
7. For growth and sporulation all the fungal cultures except *C. gloeosporioides*, were maintained in PDA. *C. gloeosporioides* was grown in OMA for sporulation.
8. Scanning electron microscopy (SEM) were performed to understand the details of the surface morphology of the fungal pathogens as well as to get accurate measurement of the spore and hyphae.
9. At the beginning of the study on disease control using botanicals ethanol (50%) extracts of eighty different species of plants were screened for their potential antifungal properties against the four studied pathogens following spore germination bio-assay technique. Extracts of *Xanthium strumarium*, *Crotalaria mucronata* and *Datura stramonium* showed promising antifungal activity in spore germination bioassay against *F. equiseti* and *C. gloeosporioides*. Leaf extracts (50% Ethanolic) of *Datura innoxia*, *Datura metel*, *X. strumarium* and *D. stramonium* could check spore germination of *A. alternata* completely. Similarly, *Polyalthia longifolia*, *X. strumarium* and *D. stramonium* leaf extracts could also inhibit spore germination of *P. theae* completely.
10. 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. Extracts 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.

Chloroform extract 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.

11. The minimum inhibitory concentrations of selected plant extracts were determined with the soxhlet extracts against the test pathogens following agar cup bioassay method. Lowest MIC (2mg/ml) was produced by *Datura stramonium* extracts against *C. gloeosporioides*. Other effective extracts exhibiting low MIC value were *Borreria alata* (0.006gm/ml), *Ocimum gratissimum* (0.008gm/ml) and *Xanthium strumarium* (0.008gm/ml).
12. Control of the fungal pathogens by three commercial fungicides was also undertaken for comparison with that of plant extracts. From the results it was evident that all the fungicides tested were effective.
13. Leaves of two plants (*D.stramonium* and *Crotalaria mucronata*) were chemically fractionated and each fraction was tested for antifungal properties by spore germination bioassay. The terpenoid fraction of both plant extracts showed significant antifungal activity against *F. equiseti*, *C. gloeosporioides* and *A. alternata*. MIC values of the terpenoid fraction of *Datura stramonium* was 0.05 mg/ml against *F. equiseti*, 0.1 mg/ml against *C. gloeosporioides* and 0.5mg/ml against *A. alternata*. Similarly, the MIC of terpenoid fraction of *C. mucronata* leaf extract was 0.01 mg/ml against *F. equiseti*.
14. 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. PL 4, AS 1 - AS 4 and DS 1 fractions were most effective against *P. theae*. All these fractions showed MIC value of 0.1 mg/ml.
15. TLC was performed to separate different chemical components of the soxhlet extracts using two different solvent systems [chloroform–methanol (9:1) and hexane-ethyl acetate- methanol (60:40:1)]. The developed chromatograms were subsequently subjected to TLC plate bioassay. The R_f of the antimicrobial zones on TLC plates

were recorded. *X. strumarium* leaf extract (in benzene) showed a single antifungal zone at R_f 0.85 against *F. equiseti, C. gloeosporioides* and *A. alternata.* Single antifungal zones were also noticed with extracts of *B. alata* (R_f 0.38) and *P. longifolia* (Rf 0.6). *D. stramonium* showed multiple antifungal zones whose R_f differed when tested against different pathogens. *Clerodendrum viscosum, Allium sativum* and *Crotalaria mucronata* also produced multiple inhibition zones indicating presence of more than one antifungal component in their extracts.

16. Apart from bioassay, thin layer chromatograms of soxhlet extracts were subjected to chromogenic spray with four different reagents. The colour developments of the zones (corresponding to antifungal zones on bioassay plates) were recorded and were compared with the characteristic indicative colour. The antifungal component in leaf extract of *X. strumarium, P. longifolia* and *C. viscosum* appears to be bitter principles. The antifungal compound at R_f 0.24 of *D. stramonium* was identified as triterpene while that R_f 0.5 was identified as monoterpene alcohol. The antifungal component of *C. mucronata* and *B. alata* was also found to be triterpenes.
17. One plant extract *(D. stramonium*) was subjected to column separation. The separated fractions were tested for antifungal activity against *F. equiseti* and *C. gloeosporioides* following spore germination bioassay. Potential fractions (fraction 6-20 & fraction 26-35) were further tested by disc diffusion bioassay and MIC were determined.
18. For studying the effect of plant extracts *in vivo*, susceptible plant varieties of brinjal, tomato and tea were treated with selected plant extracts separately. Following the treatment, the plants were subjected to challenge inoculation by the respective pathogens. *D. stramonium* and *X. strumarium* extracts (0.5g/ml in distilled water supplemented with 0.05% tween 20) reduced the occurrence of diseases caused by *C. gloeosporioides* or *F. equiseti* in the susceptible inoculated plants in comparison to control. *C. mucronata* and *B. alata* were also found to be effective in reducing disease caused by *F. equiseti* in brinjal. In case of tomato plants, *X. strumarium* and *D. stramonium* extracts significantly inhibited *Alternaria* blight disease caused by *A. alternata* in the Karan variety. Grey blight of tea caused by *P. theae* was best controlled by *A. sativum* bulb extract followed by leaf extracts of *P. longifolia* and *D.*

stramonium. All these plant extracts are bio-products which may be used for field application.

19. The findings of the present study have been discussed in detail and compared with the results of other prominent works.
20. In conclusion, it may be said that several plant extracts were found to be effective in controlling diseases of economically important crops like tea, tomato, and brinjal. Our study on botanicals and their application will broaden the knowledge base on which a total control of these diseases may be established.