Summary

After a short introduction to the work a brief review of the literature related to the present lines of investigation has been presented. The review mainly deals with diseases of brinjal plants, growth and physiology of the pathogen, common antigenic relationship, plant disease alteration by chemical treatment and disease control by fungicides, antagonistic organisms and botanicals.

A detailed description of different experimental procedures and techniques used during the present study has been given in the materials and methods section.

The present work started with a thorough survey of fungal diseases of brinjal at Barobisha and other parts of North Bengal. During the survey, several pathogens were isolated from different brinjal plants. These were *Phomopsis vexans, Colletotrichum gloeosporioides, Alternaria solani, Fusarium solani, Sclerotium rolfsii* etc. Among the isolated fungi, *C. gloeosporioides* was consistently found to produce anthracnose on leaves and fruits in North Bengal.

Pathogenicity of *C. gloeosporioides* was determined on 28 different brinjal varieties following detached leaf inoculation technique and whole plant inoculation technique. From the results of pathogenicity test, Pusa purple long (Ppl) variety was found most susceptible while Shamala variety (Shav) was observed as most resistant against the pathogen.

The morphology of the pathogen was observed in PDA, OMA and PDB. In PDA and PDB, the mycelia of the fungus was found to be white, which gradually turned pale yellowish and further darker to gray. Huge mass of pinkish acervuli were produced in OMA. The mature hyphae was septate and was 3 - 5 µm in diameter. The mature conidia were one-celled whose length and breadth ranged between 13 - 16 µm and 4 - 6 µm respectively.

Growth of the fungus in different solid and liquid media, in different periods of incubation at different temperature and different pH was estimated. LEA and PDA were the best medium for growth while sporulation was best in OMA and YEMA where huge masses of pinkish acervuli were observed. Among the liquid media tested, the fungus showed maximum growth in PDB after 20 days of incubation. Highest mycelial dry weight was recorded at 28 °C and pH 6.0 among different temperature and pH tested. Mannitol was the best carbon source among the different carbon sources tested. Highest growth was recorded in peptone among different nitrogen sources tested. Beef extract and yeast extract also showed satisfactory growth.

Germination of spores, appressoria formation as well as germ tube elongation of the fungus was studied at different periods of incubation, different pH and at different temperatures. Germination of spores normally started after 10 h in sterile distilled water. However when brinjal leaf extract was mixed with sterile distilled water at very low concentration germination began only after 2 h. The fungus showed highest germination at pH 6.0 but no germination was found at pH 9.00. Appressoria formation was high at pH 6.0 but germ tube growth was more at pH 6.75. Spore germination was optimum at 28 °C. No germination took place at 10 °C or below and 40 °C or above.

For detection of the presence of cross reactive antigens (CRA) between the susceptible varieties of brinjal and the pathogen, immunoserological techniques like immunodiffusion and immunoelectrophoresis was performed. In agar gel double diffusion test, antisera raised against the susceptible (Pusa purple long) and resistant (Shamala variety) varieties of brinjal and the pathogen (*C. gloeosporioides*) were allowed to react with antigen preparation of some susceptible and resistant brinjal varieties, pathogen as well as non-pathogen (*A. porri*). Common antigenic relationship were present in homologous reactions as well as in cross reactions between antisera of *C. gloeosporioides* and antigens prepared from leaves of susceptible varieties (Pusa purple long, Pusa purple round, Pant brinjal 4, Orissa green, Green round). No precipitation bands were observed when antigens of resistant varieties (Shamala variety, Lalguli variety, Preeti variety, Aam begun and Kuroi variety) and non-pathogen (*Alternaria porri*) were reacted with antisera of *C. gloeosporioides*. Common precipitation bands were also found in reciprocal cross reactions between antisera against Pusa purple long and antigens of *C. gloeosporioides*. Precipitin band was absent in reaction between the antisera of Pusa purple long and antigen of non-pathogen. Immunoelectrophoretic study revealed that the antigen of *C. gloeosporioides* shared one precipitin arc when treated with the antisera of susceptible variety (Pusa purple long). Antigens of susceptible varieties shared one precipitin band in each case when reacted with antisera of *C. gloeosporioides* while antigens of resistant varieties showed no precipitin band. Antigen of *C. gloeosporioides* shared one precipitin band with antisera of susceptible variety although it shared no precipitin band with antisera of resistant variety.

For detecting the level of common antigens, between different brinjal varieties and the pathogen, indirect ELISA was performed using leaf antigens of all the

28 brinjal varieties tested and mycelial antigen of the pathogen (*C. gloeosporioides*). Besides, antigen of a non-pathogen was also included. Antisera raised against two brinjal varieties, one susceptible and the other resistant (as stated earlier) and antisera raised against pathogen was used for the antigen antisera reactions. Higher ELISA values in cross reactions indicated susceptibility while lower ELISA values indicated resistance. Pusa purple long (susceptible variety) showed maximum ELISA value (0.532) while Shamala variety (resistant variety) showed minimum ELISA value (0.185) when reacted with antlsera of *C. gloeosporioides* at 20 μg ml^{-1} concentration. In reciprocal reactions, the antigen of the pathogen showed higher absorbance value when tested with the antisera of susceptible variety than when tested (in three different concentrations) with antisera of resistant variety. This clearly indicated that cross-reactivity was higher between pathogen and susceptible variety than between pathogen and resistant variety .

To find out tissue and cellular location of CRA shared by the pathogen and brinjal leaves, immunogold labelling studies followed by silver enhancement were performed. Leaf sections (cut through midrib) of susceptible and resistant brinjal varieties and mycelia and spores of *C. gloeosporioides* were used as antigens. Both leaf sections and fungal mycelia were treated with antisera and subsequently subjected to immunogold labelling and silver enhancement. When the leaf sections of susceptible variety (Pusa purple long) were treated with antisera of *C. gloeosporioides* followed by immunogold-silver precipitation, CRA was observed in the epidermal regions, mesophyll tissues and vascular bundle elements of the leaves. When leaf sections of resistant variety (Shamala variety) were treated with the antisera of the pathogen no precipitation was observed. Spores and mycelia, when treated with antisera of susceptible variety, turned dense black due to precipitation indicating the presence of CRA. The density of the black colour was markedly less when treated with antisera of resistant variety.

After morphological, physiological and virulence studies on the pathogen *C. gloeosporioides* further work was undertaken to device specific control measures of the disease caused by the pathogen in brinjal plants. Disease control may be achieved either by enhancing the innate defense response of the host or by inhibiting the proliferation of the pathogen. For induction of systemic acquired resistance eleven different chemicals and five aqueous plant extracts were sprayed on susceptible brinjal plants before they were inoculated with the pathogen. Out of eleven chemicals

tested, five (2,1,3-benzothiadiazole, 4-amino butyric acid, sodium azide, salicylic acid and jasmonic acid) were significantly effective in reducing the disease occurrence. Out of five leaf extracts tested, *Jasminum jasminoides* showed excellent control of the disease followed by *Azadirachta indica*. Hence these chemicals and botanicals were selected and sprayed on two selected varieties of brinjal plants (Pusa purple long and Shamala variety). All the chemicals and extracts showed significant reduction in disease occurrence susceptible varietiy (Pusa purple long). The concentrations of both orthodihydroxy phenol and total phenol were always found to be higher in the leaves of resistant variety compared to susceptible one.

In another approach, control of disease using fungicides was undertaken. Bioassay of six different fungicides were performed *in vitro* using the fungi *C. gloeosporioides*. Minimum inhibitory concentration (MIC) values of the test fungicides were determined following poisoned food technique. Bavistin showed complete inhibition at the concentration of 250 µg ml^{-1} and produced the lowest MIC value (200 µg ml^{-1}).

For biological control of the disease, antagonistic potentialities of some known biocontrol agents (*Trichoderma viride*, *T. koningii*, *T. harzianum*, *T. virens* -I, *T. virens*-II and bacterial antagonist *Pseudomonas* sp.) on the growth of *C. gloeosporioides* were tested through different experiments. Among the pseudomonads, *Pseudomonas* sp. (Isolate-I) showed maximum inhibition. Among the fungal antagonists *T. virens* (Isolate-II) showed maximum growth inhibition. *In vivo* application of spore suspension of *T. virens* (Isolate-II) showed maximum reduction of disease followed by *T. harzianum*.

Several plants were screened for their potential antifungal activity. Aqueous and 50% ethanol extracts of twenty three species of plants were short listed. Both the aqueous and 50% ethanol extracts of *Datura metel*, *Cannabis sativa* and *Allium sativum* showed 100% inhibition of germination. Leaf extract of *Polyalthia longifolia* inhibited 90.07% (aqueous) and 80.07% (ethanolic) spore germination respectively. Among other extracts *Solanum torvum*, *Aegle marmelos*, *Azadirachta indica*, *Solanum khasianum*, *Melia dubia*, *Murayya koeningii*, *Vitex negando*, *Psidium guajava*, *Solanum xanthocarpum*, *Syzygium cumini*, *Dioscorea alata*, *Vitex negando* and *Zingiber officinale* showed inhibition of spore germination significantly. In agar cup bioassay *A. sativum* developed a large inhibition zone with a diameter of

40 mm. *D. metel* and *P. longifolia* showed inhibition zones of 18.17 mm and 13.20 mm respectively.

In TLC plate bioassay five plant extracts showed distinct inhibition zones. They were *D. metel, A. sativum*, *P. longifolia, C. sativa* and *A. indica. In vitro* bioassay of these five selected aqueous plant extracts following poisoned food technique showed that *A. sativum* bulb extract completely inhibited the growth of the pathogen. Antagonistic activities of aqueous extracts of these five plants were tested *in vivo* on susceptible brinjal variety. *A. sativum* bulb extracts showed maximum control of the disease followed by *D. metel. P. longifolia, C. sativa* and *A. indica* showed reduction in disease occurrence.

Implications of the results have also been discussed in the discussion section.