Discussion

Solanum melongena L. or eggplant is an economically important and beneficial crop and is grown widely in tropical countries (Taha and Tijan, 2002). The plant was cultivated in the middle ages, mainly for medical reasons, in the area of South and East Asia (Kluza *et al.*, 2000). It is a very well appreciated vegetable all over India as well as in the state of West Bengal. It grows in almost all the districts of West Bengal. 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 disease (Rizza *et al.*, 2002). Proper disease management is an important aspect of successful cultivation of any crop. Like many other vegetables brinjal is also subject to attack by many fungi, bacteria, viruses and nematodes. 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 was undertaken and it is likely that the result will broaden the scientific base upon which total control of a fungal disease of brinjal may be established through an integrated disease management programme.

At the onset of the study, several fungi were isolated from infected parts (mainly leaves) of brinjal plants from the field at Barobisha (Latitude- 26°30' North and Longitude- 89°45' East). These were *Phomopsis vexans, Colletotrichum gloeosporioides, Alternaria solani, Fusarium solani, Rhizoctonia* sp. and *Sclerotium rolfsii* etc. *C. gloeosporioides* and *P. vexans* severely damaged the plants in young as well as mature stage. These pathogens last throughout the life cycle of the plant and produces anthracnose and fruit rot. *C. gloeosporioides* is the causal organism of anthracnose in brinjal (Wijesekara *et al.*, 2005; Fernandes *et al.*, 2002; Madeira and Reifschneider, 1987). *Phomopsis vexans* produces the symptoms like damping off, leaf blight, stem canker, collar rot and fruit rot of brinjal (Panwar *et al.*, 1970 and Singh, 1992). Verticillium wilt caused by *Verticillium dahliae* Kleb. is one of the most destructive diseases of egg-plant causing an estimated yield reduction of up to 50% (Bletsos *et al.*,1997 and Bueno *et al.*, 2000). *Fusarium oxysporum* f. sp. *melongenae* induces vascular wilt disease in egg-plant and causes heavy yield losses in Asian countries (Kennet *et al.*, 1970; Kishi, 1974 and Stravato *et al.*, 1993).

C. gloeosporioides and *P. vexans* were associated in severe leaf and fruit damage of brinjal plants in this area during the year 2001-2006. Although much work has been done in *P. vexans* but no such study were done with *C. gloeosporioides*. *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; Manandhar *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 crops like olive and avocado.

In the area of the present study, climatic conditions were optimally favourable for *C. gloeosporioides* to infect host plant as rainfall and humidity were very high. Green (1998) while studying on the distribution and severity of foliar diseases of yam observed that anthracnose disease is severe under conditions of high rainfall and relative humidity. So it is of utmost importance to know the relationship of the fungal pathogen *C. gloeosporioides* with brinjal varieties and control strategies of the pathogen as well as the disease. *C. gloeosporioides* was isolated from infected brinjal leaves from the field at Barobisha area and after verification of Koch's postulations, the fungus was identified in the laboratory. The fungus was also sent to Indian Type Culture Collection, IARI, New Delhi for identification.

The degree of susceptibility or resistance of a particular variety to a pathogenic fungus is determined through its differential pathogenicity to different varieties. Similarly pathogenicity of different fungi to a particular plant variety gives us information about different infecting ability of different pathogen. Pathogenicity of the isolated fungus *C. gloeosporioides* was tested following two different techniques, viz. detached leaf inoculation and whole plant inoculation technique. Results obtained from both the techniques were in agreement with each other. Dickens and Cook (1989) also used these methods to detect resistance and susceptibility of *Camellia* plants against *Glomerella cingulata*. Pandey *et al.* (2002) evaluated 41 entries consisting of promising varieties, lines, hybrids and local cultivars collected from different sources under natural epiphytotic condition against phomopsis blight in brinjal using whole plant inoculation technique. Madeira and Reifschneider (1987)

suggested that sub epidermal injection of 0.1 ml 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 48 h in dark and high relative humidity.

From the pathogenicity results it was evident that Pusa purple long was the most susceptible and Shamala variety was most resistant among the 28 varieties tested. No variety showed complete resistance to the pathogen. Shamala variety, Lalguli variety, Preeti variety and Aam begun were resistant while ail other varieties were either susceptible or moderately susceptible. Pusa purple long, Pusa purple round, Pant brinjal-4, Orissa green and Green round were susceptible against *C. gloeosporioides*. Pandey *et al.* (2002) also observed that none of the varieties were found completely resistant to phomopsis blight of brinjal. Two varieties viz. Ramnagar giant and KS-233 showed moderate resistance while others showed susceptibility. Thus the results of the present study are in conformity with that of earlier workers. Therefore, the identity of some brinjal varieties may inform us about their resistance or susceptibility towards foliar fungal pathogens. Such information might be helpful in disease management specially in multiple pathogen attack.

Plant pathogen exhibit considerable variation in cultural as well as pathogenic characters mostly due to genetic recombination during sexual reproduction (Shaner *et al.*, 1992). A thorough knowledge on the morphological and physiological characteristics thus becomes necessary after a fungal pathogen is isolated and identified. It also forms the basis of further studies on understanding disease development, host-pathogen interaction and control of the disease caused by the pathogen. This basic knowledge is important for any work on defense responses of the plant to pathogen attack or control strategies of the disease caused by the pathogen. Therefore a thorough microscopic observation of the morphological characteristics of mycelia and spores along with studies on growth conditions and nutritional requirements of the pathogen were undertaken.

Microscopic study of the fungus revealed that mycelia and conidia of the fungus were light colored. The length and bredth of conidia of the fungal isolate ranged between 13-16 µm and 4-6 µm respectively. The mature conidia were light, one-celled and hyphae were septate, the diameter of the mature hyphae was 3 - 5

µm. Similar observation regarding conidial size and shape has been reported by several authors (Kuo, 1999 and Kumar *et al.*, 2002). While studying on *C. gloeosporioides* causing yam anthracnose, Abang *et al.* (2002) reported that the conidia were hyaline with rounded apices and measured 15-18 µm long and 4-6 µm wide.

Nine different media viz. PDA, OMA, LEA, CDA, RA, YEMA, MEA, PCA and NA were used to study the growth of *C. gloeosporioides* in the present study. Among the media tested, Leaf extract agar (LEA) and Potato dextrose agar (PDA) were best growth medium for the fungus as they showed 70 mm radial growth (maximum measurable growth in 70 mm petridish) after 5 d of inoculation. Richard's agar also showed good growth. In case of spore formation, huge masses of pinkish acervuli were found in oat meal agar and yeast extract mannitol agar. Thakare and Patil (1995) while studying on leaf blight of *Chrysanthemum* caused by *C. gloeosporioides* also showed that Bean meal agar, potato dextrose agar and Richard's agar supported good growth. While studying on *Colletotrichum* isolates from *Hevea brasiliensis,* Kumar *et al.* (2002) reported that the isolates produced morphologically uniform conidia on potato dextrose agar (PDA). Mendoza *et al.* (2005) observed that the composition of the culture media and the pH appear to influence the growth, sporulation and morphology of the conidia of *S. schenckii.*

Growth study in liquid media showed that maximum growth was recorded in PDB after 20 days of incubation. After 20 days, mycelial dry weight declined due to autolysis and depletion of the media. OMB and RA also showed similar growth pattern. Sandhya Rani and Murthy (2004) used different solid and liquid media to study the growth of *C. gloeosporioides* isolated from cashew anthracnose. They observed that Richard's agar and potato dextrose agar supported good growth and sporulation. Among the liquid media, Richard's solution and potato dextrose broth supported good growth.

Nutritional requirement of the pathogen was studied and it was concluded that mannitol was the best carbon source for optimum growth and sporulation of *C. gloeosporioides.* Sorbitol produced mycelial growth and sporulation next to mannitol. Lactose showed minimum growth among the carbon sources tested. When organic nitrogen sources were tested, *C. gloeosporioides* showed highest growth and sporulation in peptone. Beef extract and yeast extract also showed satisfactory growth and sporulation after 12 d of incubation. Among the inorganic nitrogen sources tested, ammonium sulphate and ammonium phosphate showed medium growth without

any sporulation. Similar observation was reported by Jadhav *et al.* (2002) while studying on influences of different nitrogen and carbon sources on growth and sporulation of *C. gloeosporioides*. They observed that highest mycelial growth and sporulation was recorded when mannitol was used as carbon source and peptone was used as nitrogen source. Several workers (Jamaluddin, 1977; Devdath and Padmanavan, 1977; Jash *et al.* 2003) studied on the influence of various carbon and nitrogen sources on fungal metabolism. Jamaluddin (1977) reported that potassium nitrate is found to be satisfactory nitrogen source for the growth of *Aspergillus flavus*. Wu and Wu (2003) observed that *Alternaria protenta,* a pathogen of sunflower showed abundant sporulation on glucose peptone agar and leonien agar but not on dextrose nitrate agar.

Studies on the mycelial growth at different pH showed that the mycelial dry weight of C. *gloeosporioides* was maximum at pH 6.0 and lowest at pH 8.0. Mycelial dry weight of C. *gloeosporioides* was maximum at 28 °C. At 8 °C no mycelial growth was recorded. The result showed similarity with the results obtained by previous workers (Thakare and Patil, 1995; Dinh *et al.*, 2003; Gock *et al.*, 2003; Amborabé *et al.*, 2005; Lin and Sung, 2006 and Winder, 2006). Thakare and Patil (1995) observed that the optimum pH for growth of *C. gloeosporioides* was 4.1-6.8. Kang *et al.* (2003) also observed that optimum growth of the phytopathogenic fungus *C. gloeosporioides* was around the pH 6.0. Amborabé *et al.* (2005) while studying the influence of temperature and nutritional requirements for mycelial growth of *Eutypa lata*, a vineyard pathogenic fungus observed that the isolated strain of *E. lata* was able to grow in a large temperature range (2–30 °C). However, a higher temperature (35 °C) presented inhibitory effects on mycelial growth. Gock *et al.* (2003) studied on the influence of temperature, water activity and pH on growth of some xerophilic fungi and observed that the optimum growth occurred at 25 °C for *P. roqueforti* and *W. sebi*, at 30 °C for *Eurotium* species, *A. penicillioides* and *X. bisporus* and at 37 °C for *C. xerophilum*. These fungi all grew faster under acidic than neutral pH conditions.

Host-parasite interaction is an important aspect during the early stage of disease initiation, which includes spore germination, appressorium formation, penetration and early colonization (Kuo, 1999). Egley (1994) reported that germination, germ tube elongation, preparation for penetration and penetration are considered crucial for disease initiation by *C. gloeosporioides*. Several studies were undertaken

during the present work to evaluate the influence of environmental factors like incubation periods, pH, temperature etc. on spore germination, germ tube elongation and appressorium formation of *C. gloeosporioides in vitro.* Spore germination, germ tube elongation and appressoria formation were studied after different periods of incubation in two different ways. Spore suspensions prepared with sterile distilled water was used in one set while brinjal leaf decoction was added to sterile distilled water in another set. When sterile distilled water was used in spore suspension, germination of spores started after 10-12 hours of incubation. Spore germination, germ tube elongation and percent appressoria were recorded as 87.17%, 97.33 µm and 64.67% respectively after 48 h of incubation. Germination of spores started after 2 hours of incubation when brinjal leaf extract was added in spore suspension and nearly all the spores were germinated within 8 h of incubation. Percent germination of spores, germ tube length and percent appressoria formed were 98.30%, 142.50 µm and 62.50% respectively after 8 h of incubation. Similar experiment was done by Kuo (1999) using a two-step method to study the conidial germination and appressorium development in the mango anthracnose fungus *C. gloeosporioides* during a nine hour period and observed similar results. In their study Saha and Chakraborty (1990) also observed that spore germination of *Bipolaris carbonum* begun within 2-4 hour *in vitro.*

The present isolate of *C. gloeosporioides* showed highest germination percentage at pH 6.0 which sharply declined at pH 7.25 and no germination was found at pH 9.00. Highest percent of appressoria formation (65.67%) were also observed at pH 6.0. Germ tube length (142.33 µm) was highest at pH 6.75 where no appressoria were formed. Saha and Chakraborty (1990) observed that pH 6.75 was best for germination of spores of *B. carbonum* while pH 7.2 was best for germ tube elongation. Callaghan (1974) observed that more than 97% of conidia in *Basidiobolus ranarum* germinate between pH 7-9. While Studying the effect of different culture media, pH and carbon sources on growth and sporulation of *Alternaria zinniae* causing leaf and flower blight of marigold, Jash *et al.* (2003) observed that the optimum pH for growth of the pathogenic fungus was found in the range of pH 6.0-6.5. During the present work, spore germination study at different temperature revealed that spore germination was optimum at 28 °C. No germination took place at 10 °C or below and 40 °C or above. The prevailing temperature in the areas covered during the present study and also in the adjoining areas does not reach above 40 °C. Hence spores get their optimum temperature throughout the year.

A successful disease manifestation requires compatible host pathogen interaction in suitable environmental conditions. The compatibility is determined by several factors contributed by both host and pathogen. Several workers have noticed that there is a unique serological similarity between pathogen and compatible host involving one or more antigenic determinants. In plants, the susceptibility towards a pathogen seems to increase with increase in similarity between the antigens. These antigens cross react with each other in experimental antigen-antisera reactions and produce precipitin bands. The present study was undertaken to determine the presence and the level of cross reactive antigens (CRA) between the 28 different brinjal varieties included in this study and the pathogen, *C. gloeosporioides*. Basic immunological techniques that are in use today are radial immunodiffusion, immunoelectrophoresis and agar gel double diffusion. These techniques were successfully utilized by several workers in demonstrating cross-reactive antigens (Alba and DeVay, 1985; Purkayastha and Banerjee, 1990; Chakraborty and Saha, 1994; Ghosh and Purkayastha, 2003; Dasgupta *et al.*, 2005).

For immunodiffusion studies, leaf antigens of susceptible and resistant brinjal varieties were cross reacted separately with antisera of *C. gloeosporioides*. The mycelial antigen of *C. gloeosporioides* was also cross-reacted with antisera of susceptible and resistant brinjal varieties. Mycelial antigen of *A. porri*, a non pathogen of brinjal was also used. From the results, serological comparison was done. No common antigen could be detected in agar gel double diffusion plates when antigens of resistant brinjal varieties were cross reacted with the antisera of *C. gloeosporioides*. When susceptible brinjal varieties were cross reacted with the antisera of *C. gloeosporioides,* common antigens were detected in the form of precipitin arcs. When antisera of both the resistant and susceptible brinjal varieties and *C. gloeosporioides* were reacted with antigen of *A. porri* (non-pathogen of brinjal) no precipitin arcs were detected.

Similar results were obtained by Purkayastha and Banerjee (1990) when they conducted immunodiffusion between antigen and antisera of soybean cultivars and the pathogen causing anthracnose (*Colletotrichum dematium* var. *truncata*). They too were able to detect precipitin bands in cross reaction between the pathogen, antisera and the antigen of susceptible host only and vice versa which indicated presence of CRA between these combinations only and not between resistant host and pathogen. Dasgupta *et al.* (2005) performed similar studies in tea varieties and cross reacted their antigen preparations with antisera of the leaf pathogen *Curvularia*

eragrostidis. They were also able to detect CRA only between susceptible varieties and the pathogen. Several other authors also obtained similar results in different host parasite combinations viz. jute and *Colletotrichum corchori* (Bhattacharya and Purkayastha, 1985), soybean and *Myrothecium roridum* (Ghosh and Purkayastha, 1990) and tea and *Bipolaris carbonum* (Chakraborty and Saha, 1994). Therefore, the results of the present study is in conformity with those obtained by previous workers.

In immunoelectrophoretic studies, antigen of *C. gloeosporioides* shared three precipitin bands with antisera of *C. gloeosporioides* (CgA). Antigens of susceptible varieties (Pusa purple long, Pusa purple round, Pant brinjal-4, and PK-123) shared four precipitin bands each in all the cases when reacted with antisera of Pusa purple long (PplA). Antigens of susceptible varieties (Pusa purple long, Pusa purple round, Pant brinjal-4, Orissa green and Green round) shared one precipitin band each in all the cases when reacted with antisera of *C. gloeosporioides* (CgA) while antigens of resistant varieties (Shamala variety, Lalguli variety, Preeti variety, Lalguli variety and Kuroi variety) showed no precipitin band. In reciprocal cross reaction, antigen of *C. gloeosporioides* shared one precipitin band with antisera of pusa purple long (susceptible variety) but no precipitin band with antisera of Shamala variety (resistant variety). No common antigenic relationship was noticed between the host plant (*Solanum melongena*) and non pathogen (*A. porri*).

Immunoelectrophoretic studies further confirmed the results of immunodiffusion. The advantage of immunoelectrophoresis over immunodiffusion is that complex antigenic mixture is separated because of the additional resolving power of the electrophoretic step. Purkayastha and Banerjee (1990) observed that the antibiotic cloxacillin when used as an elicitor of the host defense altered the antigenic patterns of soybean cultivars such that one specific precipitin band was found to be absent in immunoelectrophoretic studies between antigen of the treated leaves and untreated leaf antisera when compared with homologous reaction between antigen and antisera of untreated control. In another study by Ghosh and Purkayastha (2003) that involved ginger cultivars and *Pythium aphanidermatum* as host and pathogen respectively, both immunoelectrophoresis and cross immuno-electrophoresis (CIE) confirmed that cross reactive antigens were absent between antigens of infected rhizome or non pathogen and antiserum of avirulent strains of *P. aphanidermatum* SR 2, but CRA was easily noted when antigens of heavily infected ginger (cv. Mahima) were cross reacted with antiserum of the pathogen.

Ala-El- Dein and El-Kady (1985) used CIE techniques to resolve similarities and dissimilarities between the antigens present in *Botrytis cinerea* isolates and between antigens present in different species of *Botrytis*. From the results, they observed that each isolate was serologically different from the other and had species-specific antigens.

Although through immunoelectrophoresis number of precipitin arcs present were determined but quantification of common antigens could not be done. With a view to quantify the common antigens and to make a gradient of common antigenic similarity it was decided to perform indirect ELISA which on the basis of certain distinct values gives us a clear picture of similarity and disparity among the host and pathogen. The gradient of similarity or disparity is the indicator of susceptibility and resistance respectively as shown by Dasgupta *et al.* (2005).

Indirect ELISA is one of the most specific and rapid methods for detecting CRA at a very low concentration and identifying fungal diseases (Purkayastha and Banerjee, 1990; Sundaram *et al.*, 1991; Chakraborty and Saha, 1994; Kratka *et al.*, 2002; Ghosh and Purkayastha, 2003; Musetti *et al.*, 2005; Dasgupta *et al.*, 2005). Eibel *et al.* (2005) developed a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) by raising polyclonal antibodies against *Ustilago nuda* and barley plant. Dasgupta *et al.* (2005) performed ELISA between tea varieties and *Curvularia eragrostidis*, which revealed the presence of a certain minimum level of antigens for compatible host-pathogen interaction. Several workers have used ELISA for early detection of pathogens (Chakraborty *et al.*, 1996 and Ghosh and Purkayastha, 2003).

In the present study, cross-reactive antigens (CRA) were detected in indirect ELISA using very low concentration of antigens and antisera. In this study, the higher ELISA values in cross reactions revealed the presence of more CRA, which indicated the susceptibility of the variety. Similarly, lower ELISA values revealed lower amount of CRA that indicated resistance. The results obtained by indirect ELISA values i.e. the degree of susceptibility and resistance was in conformity with the results of pathogenicity tests. The three concentrations of the antigens of *C. gloeosporioides* showed higher absorbance values when tested with antisera of susceptible variety (Pusa purple long) than when tested with antisera of resistant variety(Shamala). Higher absorbance values were also observed in reciprocal test of this combination i.e. in case of the antigens of susceptible varieties (Pusa purple long, Pusa purple

round, BE-706, Orissa green etc.) tested with the antisera of *C. gloeosporioides* than in case of the antigens of resistant varieties (Shamala, Preeti, Laiguli, Aam begun etc.) tested with the same antisera. The results thus clearly indicated the presence of maximum cross reactivity between susceptible varieties (Pusa purple long, Pusa purple round, BE-706 etc.) and *C. gloeosporioides*. ELISA values were higher in homologous reactions in all combinations.

Purkayastha and Banerjee (1990) detected cross reactive antigens using indirect ELISA technique between susceptible soybean cultivars and the virulent strain of *C. dematium* var. *truncata* at a very low concentration. Dasgupta *et al.* (2005) also detected CRA while studying the pathogenicity of *Curvularia eragrostidis* against tea varieties by analyzing the antigenic patterns of host and pathogen. They used indirect ELISA which revealed the presence of low level of common antigens between all combinations. They observed that a certain minimum level of antigens was present for compatible host-pathogen interactions that was significantly higher than incompatible interactions. CRA was also detected in other host pathogen combinations like *Phytophthora infestans* and potato (Alba and DeVay, 1985) and *Phytophthora fragariae* and strawberries (Mohan, 1988) by indirect ELISA.

In the present study, although immunodiffusion and immunoelectrophoresis could not detect CRA between resistant host and pathogen, ELISA showed presence of common antigens in cross reactions between antisera of the pathogen and antigens prepared from all brinjal varieties, both susceptible and resistant. The levels of common antigens between resistant varieties and the pathogen were however significantly lower. ELISA values, in cross reactions, revealed a direct correlation with results of pathogenicity test and established the degree of susceptibility or resistance of a particular variety. Thus ELISA may be used to determine the pathogenicity of a strain in different cultivars accurately. This would help in selecting resistant varieties for cultivation and contribute towards long term disease control.

Immunocytolocalization technique has been frequently used in several studies for determining cellular location of a variety of molecules. This is a powerful tool which detects and locates specific molecules with great accuracy by utilizing an antisera as probe. In the present study this technique has used to determine cellular locations of CRA in leaf sections of brinjal varieties and mycelial cells and spores of the fungal pathogen *C. gloeosporioides*. Polyclonal antibodies (raised against the pathogen and the susceptible and resistant host variety) were used as antisera

probes. For visualization, these were indirectly labelled with colloidal gold and subsequently enhanced with metallic silver. Several authors have used immunofluorescence technique for cellular location of CRA (Chakraborty and Saha, 1994; Chakraborty *et al.*, 1997; Wakeham and White, 1996; Kratka *et al.*, 2002; Dasgupta *et al.*, 2005).

Although immunofluorescence is often used in localization studies, natural autofluorescence sometimes causes hindrance in locating CRA. Several workers (Kuo, 1999; Lee *et al.*, 2000; Nahalkova *et al.*, 2001; Trillas *et al.*, 2000; Kang and Buchenauer, 2002; Wang *et al.*, 2003) have used immunogold labelling for cellular location studies in electron microscope. However, in the present study, the immunogold labelling followed by silver enhancement was done specifically to study in the light microscope, which is a new approach for studying cellular location of CRA.

When leaf sections were treated with homologous antisera, maximum precipitation was observed in the epidermal regions, mesophyll tissues and vascular bundle elements of the leaves. When leaf section of susceptible variety (Pusa purple long) was treated with antisera of *C. gloeosporioides* and labelled with immunogold particles enhanced by silver precipitation, CRA was observed mainly in the epidermal regions. Mesophyll tissues and vascular bundle elements also showed marginal darkening which indicate presence of CRA in these areas also. When leaf section of resistant variety (Shamala) was treated with the antisera of pathogen, no such precipitation was observed. Similar results were obtained by Dasgupta *et al.* (2005) when they studied immunolocalization of CRA between tea and *Curvularia eragrostridis* by using fluorescence labeling techniques. DeVay *et al.* (1981) studied innunolocalization of CRA in roots of succeptible young cotton plants treated with antiserum of *Fusarium oxysporum* f. sp. *vasinfectum* and observed that CRA was located at the epidermal and cortical cells as well as in the endodermis and xylem tissues.

Mycelia and spores of *C. gloeosporioides* which were grayish in normal condition, turned dense blackish when treated with antisera of *C. gloeosporioides* (i.e. homologous treatment) followed by immunogold labelling and subsequent silver enhancement. When treated with antisera of susceptible host (Pusa purple long) followed by immunogold labelling and silver enhancement, dense blackish colour was observed mainly in the hyphal tips indicating presence of CRA. Similar treatment

when done with antisera of resistant variety (Shamala), no darkening was observed indicating disparity in the antigens. Chakraborty and Saha (1994) also observed CRA in the hyphai tips and in patch like areas on conidia and mycelium by FITC labeiling of the antisera.

Presence of common antigens between susceptible host and pathogen was further confirmed by immunolocalization studies. These common antigens may be involved densely in the invasion of pathogen and its growth and proliferation in host tissues. On the other hand, it may act as immunosuppressor and performed indirectly by not allowing the host defense machinary to successfully inhibit pathogen attack. More research is required to clearly define the exact role of CRA in host pathogen interaction. More knowledge on susceptibility factors of the host and virulence factors of the pathogen should be able to throw light on how CRA performs in compatible interactions and further disease establishment.

Development of a disease requires a susceptible host plant, a virulent pathogen and a suitable environment (Agrios, 1997). Changes in any of these three factors may result in less or no disease. Plants have evolved a number of inducible defense mechanisms to respond to both biotic and abiotic stress. Plants protect themselves from disease by defense mechanisms in which the signal molecules like salicylic acid, jasmonic acid and ethylene often play crucial roles (Pieterse *et al.*, 2002). When plants are necrotized by biotic or abiotic agents they systemically develop resistance to diseases, known as systemic acquired resistance (SAR) (Sticher *et al.*, 1997; Mauch-Mani and Metraux, 1998). The phenomenon of SAR suggests that there is a signal that originates at the site of elicitor (biotic and abiotic) application and moves throughout the plant. The activation of SAR turns the compatible plant-pathogen interactions into an incompatible one (Uknes *et al.*, 1992). Local or systemic resistance is triggered in the majority of the plants by pathogen attack or other kind of physical damage, as well as certain chemical treatment and presence of some biological control agents (Segarra *et al.*, 2006; Harman *et al.*, 2004). Rapid and localized death of a few plant cells at the site of attempted penetration by the pathogen is the early macroscopic event of hypersensitive response (HR). The systemic acquired resistance (SAR) is correlated with the accumulation of pathogenesis related (PR) proteins, generally assumed to be marker of defense response (Ward *et al.*, 1991; Ryals *et al.*, 1996; Delaney, 2004). The early cell death has often been emphasized as a mechanism of inhibition of pathogen development. In addition, the dying cells may also secrete signals that induce systemic resistance in the whole plant (Wang *et al.*, 2003).

During the present study, an attempt was made to induce resistance in experimental brinjal plants against *C. gloeosporioides* by application of chemicals which are well known as elicitors of defense response. From the results of preliminary screening by treatment with eleven chemicals and five plant extracts, Jasmonic acid (10^{-3} M), 2,1,3-benzothiadiazole (10^{-4} M), 4-amino butyric acid (10^{-4} M), sodium azide (10^{-4} M), salicylic acid (10^{-4} M) and leaf extracts of *Jasminum jasminoides* and *Azadirachta indica* were effective in inducing disease resistance. Mean disease index/plant were much lower in the treatments mentioned above after 6, 9 and 12 days of inoculation. Those chemicals and extracts were selected for furthur studies on both susceptible (Pusa purple long) and resistant variety (Shamala). Spraying of the elicitors caused reduction of the disease occurrence was observed in both susceptible and resistant varieties. Amount of orthodihydroxy phenol and total phenol was found to be increased in both the resistant and susceptible varieties after application of the chemicals and extracts.

Several authors have induced defense response by using different chemical elicitors that activated SAR (Chakraborty and purkayastha, 1987; Leroux, 1996; Reuveni *et al.*, 1997; Pieterse *et al.*, 1998 and Siegrist and Buchenauer, 2002). Meena *et al.* (2001) reported that salicylic acid induces resistance in groundnut against the late leaf spot caused by *Cercosporidium personatum*. A foliar application of SA at the concentration of 1 mM significantly reduced late leaf spot disease intensity and increased the pod yield under greenhouse condition. Siegrist and Buchenauer (2002) reported that K_2HPO_4 was effective in inducing a high level of systemic protection in cucumber plants against anthracnose caused by *Colletotrichum lagenarium*. Using sodium azide (100 µg ml^{-1}), Chakraborty and Purkayastha (1987) induced resistance in the susceptible soybean cultivar (Soymax) against *Macrophomina phaseolina*, a pathogen of charcoal rot disease. Purkayastha and Banerjee (1990) used cloxacillin for induction of resistance in susceptible soybean variety against *Colletotrichum dematium* var. *truncata*. SAR was induced by exogenous application of salicylic acid or synthetic compounds such as CGA-245704 (a benzothiadiazole derivative) and CGA-41396 (2,6-dichloroisonicotinic acid) by Kessmann *et al.* (1994) and Lawton *et al.* (1996). Jasmonic acid and its methyl esters were used as inducer of resistance in potato and tomato plants against *Phytophthora infestans* (Cohen *et al.*, 1993). Kato *et al.* (1984) used probenazole as a systemic compound to induce resistance against *Pyricularia oryzae* in rice plant.

Some plant extracts have also been used to enhance in plant defense reaction. Some of them are leaf extract of *Azadirachta indica* in barley (Paul and Sharma, 2002) and *Acalypha indica* in ginger (Ghosh and Purkayastha, 2003). However, there are no reports of extracts of *Jasminum jasminoides* being effective as an elicitor for inducing plant defense response. In a study, Farmer and Ryan (1992) had reported that *Artemisia tridentale,* a plant possessing methyl jasmonates in leaves can induce resistance to tomato plants when grown together in chamber.

Phenolic compounds accumulate in numerous plant species following infection with plant pathogens (Bazzalo *et al.*, 1985; Bajaj, 1988; Baker *et al.*, 1989; Borkar and Verma, 1991; Mahadevan, 1991; Baysal, *et al.*, 2005 and Conceicao *et al.*, 2006). There is often a greater increase in phenolic biosynthesis in resistant host species than in susceptible host and it is sometimes postulated that the increase in phenolic compounds is part of the resistance mechanism. Some of these compounds are toxic to pathogenic and nonpathogenic fungi and have been considered to play an important role in disease resistance (Vidyasekharan, 1988). During the present study, some chemicals and extracts were able to induce resistance in susceptible varieties of brinjal. This induction of resistance was correlated with an increase of the levels of phenolic compounds. Thus the results are in conformity with the findings of the previous workers. Hence these chemicals can be used to elicit defense response against anthracnose in brinjal.

In spite of the emergence of several alternative ways of disease control, chemical control is still important and is being continuously used in integrated disease management protocols. This is a cheap, quick and easy method to check diseases because of its direct antagonistic mode of action against the pathogen. It either kills the pathogen instantly or minimizes the pathogens growth and proliferation immediately. Due to its superior efficacy, fungicide application studies were included in the present work. Results revealed that bavistin was the most effective and produced minimum MIC (minimum inhibitory concentration) value among the six fungicides tested against the pathogen. While evaluating the effect of different chemical and alternative compounds, Benato *et al.* (2002) reported that procholaraz and imazalil was the most effective product on the control of yellow passion fruit rots caused by *Colletotrichum gloeosporioides.*

Fernandes *et al.* (2001) observed *in vitro* sensitivity to benomyl, a commonly recommended fungicide against anthracnose, among isolates of *C. gloeosporioides*

obtained from sweet paper *(Capsicum annum)*, Garden egg (*Solanum glio*) and egg-plant (*Solanum melongena*). Most of the isolates coming from sweet pepper fruits showed sensitivity to benomyl while isolates obtained from garden egg and egg plant fruits did not show sensitivity. Carbendazim (Chemical name for bavistin) is reported to be very useful fungicide by several workers. It has been successfully utilized either alone or in combination with other fungicides for the control of several plant diseases. Cromey *et al.* (2001) reported the control of *Fusarium* head blight (FHB) by carbendazim, tebuconazole and azoxystrobin. Yadav and Majumdar (2005) reported complete inhibition of *Lasiodiplodia thaeobromae* causing die back of guava by carbendazim. Maharshi and Ahir (2005) studied on the collar rot management in peanut with fungicidal seed treatment and observed that combined application of systemic fungicides like carbendazim or thiophanate methyl with thirum provided efficient control. There are also some reports on resistance of *C. gloeosporioides* to fungicides (Farungsang and Farungsang, 1992). However, the present isolate was susceptible to most of the tested fungicides *in vitro* and may be used to control anthracnose in brinjal.

Although chemical fungicides are very effective, their indiscriminate use is harmful to humans and adversely affect the microbial population present in the ecosystem. Therefore an eco-friendly approach to control the plant diseases is necessary. Biological control provides an alternative where a micro-organism that is non pathogenic to the plant but antagonistic towards plant pathogens is used.

Among fungal antagonists, *Trichoderma* spp. are most commonly used mainly due to their high efficacy in controlling several diseases. Several authors have reported the successful use of different isolates of *Trichoderma* for controlling many plant diseases (Maity and Sen, 1985; Latunda Dada 1993; Prasad *et al.*, 1999; Biswas, 1999; Jadeja, 2003; Saravanan *et al.*, 2003; Roberts *et al.*, 2005). Among bacterial antagonist *Pseudomonas* sp. is of special interest because of their strong antifungal activity which is attributed to several extracellular secretory factors like many antifungal lytic enzymes, several different antibiotics and siderophores (Bonsall *et al.*, 1997; Ongena *et al.*, 1999; Yang and Crowley, 2000 and Cornells and Matthijs, 2002).

Although biological control of many pathogens are reported in literature, no such work has been done to control the anthracnose fungus *C. gloeosporioides* causing disease in brinjal. In the present study, *Pseudomonas* sp. (Isolate-I) showed

maximum inhibition in dual culture technique. *T. virens* (Isolate-II) showed maximum growth inhibition among the fungal antagonists followed by *T. harzianum* and *T viride*. Cell-free culture filtrates of fungal antagonist of *T. virens* (Isolate- II) showed maximum growth inhibition followed by *T. harzianum*. It clearly indicated the presence of inhibitory activity in the extra cellular fluid. When crude culture filtrates containing spores were used, *T viride, T. harzianum, T. virens* (Isolate-I) and *T. virens* (Isolate-II) totally checked the growth of *C. gloeosporioides in vitro*. When crude culture filtrates of *Trichoderma* spp. were sprayed exogenously, *T. virens* (Isolate- II) showed maximum disease control efficacy. All tested antagonists showed significant control of the disease.

Trichoderma spp. and pseudomonads has been shown to control diseases of brinjal. Hundoo and Dwivedi (1993) found *Trichoderma* spp. to be antagonistic against *Fusarium solani* causing root disease in eggplant. Martins-corder and Melo (1997) reported that seed germination and the vigor of brinjal plant was enhanced by applying isolates of *Trichoderma viride* and *T. koningii*. It was verified that in natural soil the *Trichoderma* spp. isolates were effective in enhancing the seed germination. In another study, damping off of eggplant caused by *Fusarium* sp., *Pythium* sp. and *Rhizoctonia* sp. was prevented by fluorescent pseudomonads and *Trichoderma* spp.(Bucki *et al.*, 1998). *Phomopsis vexans,* a pathogen of brinjal was effectively controlled by bacterial antagonosts *Bacillus* sp. and *Pseudomonas* sp. (Meena *et al.*, 2000). Mycelial growth and pycnidial formation was inhibited by *T. koningii* (Jadeja, 2003). *Trichoderma* sp has been shown to control *C. gloeosporioides* by Gupta *et al.* (2005). Both *in vitro* and *in vivo* studies showed that *T. viride* was effective in controlling anthracnose of French bean caused by *C. gloeosporioides. In vitro* experiments on *Sclerotium rolfsii,* the causal agent of collar rot of brinjal showed that *T. viride* was most effective among several biocontrol agents tested (Jadon *et al.*, 2005b). While studying on biological control of wilt of brinjal caused by *Fusarium oxysporum* with some fungal antagonists, Wani (2005) reported that biocontrol agents, *T. viride* and *T. harzianum* brought about significant reduction in the pathogenic effect of *F. oxysporum* in brinjal.

Biological control is essentially a natural phenomenon that safegaurds the plant kingdom from diseases. But in cultivations where higher production is all the matters, diseases are often catastrophic and require intense management planning to control them. Leaf diseases are controlled easily by spraying exogenous fungicides

but due to awareness on harmful effect of fungicides on environment as well as humans, it is essential to use ecofriendly measures. *Trichoderma* and pseudomonads are ubiquitous soil organisms with antifungal activity. Though biocontrol organisms is mostly used to control root diseases, its culture filtrate can be used as foliar sprays to control leaf diseases also (Elad *et al.*, 1995; Elad, 2000; Perello *et al.*, 2003, 2006). This method has been used in the present study for *in vivo* experiments and the results were encouraging. The present study clearly indicated that the tested microorganisms can be successfully used to control anthracnose of brinjal.

Plants have been shown to possess natural fungitoxic substances which 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 exploited. Sub Himalayan West Bengal, which is the area of the present study, has thick forest cover over large areas and is extremely rich in diverse flora. This region has been declared as hot spot zone with respect to biodiversity (Rai and Das, 2002). Therefore along with plant species with known bioactivity, several unexploited species from this region have been included in this study. Initially 84 plant extracts (both aqueous and 50% ethanolic) were screened *in vitro* for their antifungal properties against the pathogen *C. gloeosporioides* by spore germination bioassay technique. Spore germination is a determining factor for the pathogen during the early phase of host colonization (Egley, 1994). During the preliminary studies, 23 plant extracts showed antifungal activity against the pathogen. Among these *Datura metel, Cannabis sativa and Allium sativum* showed 100% inhibition of spore germination. Aqueous and 50% ethanilic extract of *P. longifolia* also showed strong inhibition. Among others, aqueous extracts of *Solanum torvum, Aegle marmelos, Solanum khasianum, Melia dubia, Murraya koeningii, Vitex negando* and *Zingiber officinale* and ethanol extracts (50%) of *Azadirachta indica, Syzygium cumini, A. marmelos, M. dubia, Vitex negando, S. torvum* and *S. khasianum* showed significant inhibition. Average germ tube length was found smaller than control when the spores were allowed to germinate in the presence of these extracts. Maximum inhibition of germ tube length was found in both aqueous and ethanolic extract (50%) of *P. longifolia.*

On the basis of the results of the above study, some extracts were selected for agar cup bioassay. *A. sativum* developed a large inhibition zone with the diameter of 40 mm. *D. metel* and *P. longifolia* also produced big inhibition zones. Other extracts

which showed significant inhibition zones were *Psidium guajava*, *V. negando*, *Z. officinale*, *Z. chrysanthum*, *M. dubia*, *C. sativa* and *A. indica*.

In order to seperate the antifungal compound in the potential plant extracts, these extracts were further subjected to "on the chromatogram" bioassay on thin layer chromatography (TLC) plates. This is a very quick and easy method for screening antifungal compounds in phytoextracts (Hostettmann *et al.*, 2001, Guleiria and Kumar, 2006b). Several workers (Chakraborty and Saha, 1994; Chakraborty *et al.*, 1995; Kagale *et al.*, 2004 and Saha *et al.*, 2005b) have utilized this method to isolate natural products of various chemical structures. In the present study, extracts from five different plants (*D. metel, A. sativum*, *P. longifolia, C. sativa* and *A. indica*) showed inhibition zones on TLC plates. *D. metel* showed three distinct antifungal zones on the chromatogram (R_f: 0.25, 0.65 and 0.97) while *P. longifolia* developed two such antifungal zones (R_f: 0.33 and 0.96). Bulb extracts of *A. sativum* showed a large single inhibition zone (R_f: 0.95) having a diameter of 24 mm which was biggest among the tested extracts. *C. sativa* developed two inhibition zones (R_f: 0.17 and 0.23) while *A. indica* showed two very small inhibition zones (R_f: 0.48 and 0.67). In a study for screening phytoextracts for controlling gray blight in tea caused by *Pestalotiopsis theae*, Saha *et al.* (2005b) observed that *P. longifolia* produced a single large inhibition zone (R_f : 0.56) in bioautography on TLC plates. This may be because these authors have used different solvent mixtures to develop the chromatogram.

Finally, poisoned food assay was performed with these five selected aqueous plant extracts to further confirm their fungitoxic characters *in vitro*. *A. sativum* bulb extracts totally checked the growth of the pathogen *C. gloeosporioides*. All other extracts showed significant control of the pathogen in PDA and antifungal assay agar media used for assay.

The results of *in vitro* studies were further confirmed by *in vivo* application of the selected extracts in brinjal plants. Crude aqueous extracts of five plants were applied on a susceptible brinjal variety (Pusa purple long) to test their ability as disease control agents against anthracnose. *A. sativum* bulb extracts showed maximum control of the disease followed by *D. metel* after 12 days of inoculation with *C. gloeosporioides*. *P. longifolia, C. sativa* and *A. indica* extracts also showed significant reduction in disease occurrence.

Several investigators have reported antifungal activity of different plant extracts (Singh *et al.*, 1995; Bhandari *et al.*, 2000; Deena and Thopil, 2000; Natrajan *et al.*, 2001; Ali *et al.*, 2001; Mittal *et al.*, 2002; Sharma *et al.*, 2002; Saxena *et al.*, 2003; Al-Howiriny *et al.*, 2005; Saha *et al.*, 2005a). Most of the studies are based on crude extracts prepared from the plants collected randomly or based on known ethnomedical use. Biological evaluation has been conducted on plants from different regions including India (Goel *et al.*, 2002; Perumal Samy, 2005; Lakshmi *et al.*, 2006), China (Hu *et al.*, 2001); Jordon (Alkofahi *et al.* 1996,1997); Mexico (Andrade-Cetto and Heinrich, 2005); New Zealand (Bloor, 1995); Malaysia (Wiart *et al.*, 2004); Pakistan (Ali *et al.*, 2001); Bangladesh (Rahaman *et al.*, 2001); Papua New Guinea (Rao, 1996), Indonesia (Kevin *et al.*, 1999); Egypt (Khafagi and Dewedar, 2000); Thailand (Chuakul, 2000); Ghana (Konning *et al.*, 2004); Tanzania (Boer *et al.*, 2005) and Turkey (Dulger and Gonuz, 2004). However very few work has been done based on native plants of the area of the present study.

A. sativum has been reported to posses antifungal activity by several workers (Jadeja, 2003; Curtis *et al.*, 2004 and Saha *et al.*, 2005b). The activity of *A. sativum* has been attributed to several compounds like allicin, E-ajoene, Z-ajoene, alliin, allitridin etc (Ankri and Mirelman, 1999; Yoshida *et al.*, 1999a,b; Miron *et al.*, 2002; Liu *et al.*, 2004; Hughes *et al.*, 2005 and Baghalian *et al.*, 2006). 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. The nature of the active compound is briefly discussed in view of the observed activity in methanol extracts. Murthy *et al.* (2005) isolated diterpenoids from the hexane extract of the seeds of *P. longifolia* that showed significant antibacterial and antifungal activities. Active components of *D. metel* includes atropine, hyoscyamine and scopolamine (Al-Humaid , 2004). *A. indica* (neem) is well known for its antifungal properties and has been used extensively in traditional Indian medicine. Its biocative chemicals are protomeliacins, limonoids, isomeldenin, nimonol etc. (Suresh *et al.*, 1997). *C. sativa* contains cannabidiol, cannabinol, tetrahydrocannabinol, which is responsible for its bioactivity (Izquierdo *et al.*, 1973). Several workers have studied the control of diseases caused by *C. gloeosporioides* in various plants (Athukoralage *et al.*, 2001; Bautista-Baños *et al.*, 2003 and Peraza-Sánchez *et al.*, 2005). Leaf extracts from *Citrus limon and Persea americana*

totally inhibited growth of *C. gloeosporioides* causing papaya and mango fruit rot after storage (Bautista-Banos *et al.*, 2002).

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. Both the ethanol and aqueous extracts of *Allium sativum, Datura metel, Dryopteris filix-mas, Zingiber officinale, Smilax zeylanica, Azadirachta indica and Curcuma longa* were used for control of four different pathogens (*Pestalotiopsis theae, Colletotrichum camelliae, Curvularia eragrostidis and Botriodiplodia theobromae*) of tea by Saha *et al.* (2005a). They reported complete inhibition of spore germination by using the botanicals.

Kagale *et al.* (2004) showed that leaf extracts of *Datura metel* significantly reduced the *in vitro* growth of *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Methanol extract exhibited the best control of the pathogens recording 10-35% more toxicity than aqueous extract. Foliar application of leaf extracts effectively reduced the incidence of sheath blight and bacterial blight diseases of rice under greenhouse condition. The methanol extract of *D. metel* when subjected to thin layer chromatography (TLC) showed a spot with relative front (R_f) value of 0.705.

Antimicrobial activity of different concentrations (50, 100, 200, 300 and 500 ml/ l) of essential oil extracts of three type of onions (green, yellow and red) and garlic against two bacteria, *Staphylococcus aureus*, *Salmomella enteritidis*, and three fungi, *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum*, was investigated by Benkeblia (2004). Wiart *et al.* (2004) screened 72 methanolic extracts obtained from the leaves, barks, and roots of 50 plant species from traditional medicine of Perak, Peninsular Malaysia and found broad spectrum activity of *Polyalthia lateriflora*, *Solanum torvum* and *Piper stylosum*. Perumal Samy (2005) studed antimicrobial activity of some medicinal plants from India and reported that methanol extracts of *Zingiber officinale*, *Asteracantha longifolia*, *Citrus acida*, *Salacia microsperma* and *Tinospora cordifolia* were effective. Singh and Singh (2005) evaluated antifungal activities of leaf extracts of 26 angiospermic plants and two most popular chemical fungicides sporgon and bavistin against pathogenic fungi *Mycogone perniciosa, Verticillium fungicola* var. *fungicola* and *Fusarium moniliformae* causing diseases in white button mushroom. They observed that leaf extracts from *Aegle marmelos, Berberis aristata, Cannabis sativa, Cleome viscosa, Erigeron karvinskianus* and *Leonotis nepetaefolia* were effective against tested pathogenic fungi.

The present study was initiated based on the need for eco-friendly fungicides to control anthracnose of brinjal..The results were encouraging since several plants showed remarkable antagonistic activity against the pathogen *C. gloeosporioides in vitro. In situ* studies also exhibited good results indicating that many plant extracts have a definite potential to control anthracnose in brinjal. Among the effective extracts, *Allium sativum* and *Datura metel* can be tested further in field experiments. They may be used singly or in combination to control anthracnose. Further these may be integrated with other biocontrol agents and may be used in fields as part of integrated disease management system.

All the investigations presented here has confirmed and also extended some of the findings of the earlier workers. During this study, certain new facts of fundamental importance have also been revealed. Pathogenicity of *C. gloeosporioides* have been tested in several brinjal varieties in two different ways. The significance of antigenic relationship with regard to compatible interaction between *C. gloeosporioides* and brinjal varieties has been demonstrated by various serological techniques. Correlation between pathogenicity test and different serological experiments was observed and was confirmed with indirect ELISA. Major cross-reactive antigens between the brinjal plant and the pathogen were detected in the cells of brinjal and pathogen *C. gloeosporioides* through immunogold labelling followed by silver enhancement using light microscope. Resistance was induced in susceptible brinjal varieties using some chemicals and plant extracts. Hence, this study has provided an insight to formulate a definite defence inducer against anthracnose disease caused by *C. gloeosporioides.* Present study designs the suitable control measures of the disease using biocontrol agents and botanicals.