Chapter-5

DISCUSSION

Fresh Fruits are a source of different types of minerals, vitamins and essential nutritional components. Fruits also contain huge amount of water. Hence, fruits are very much prone to fungal attack. Worldwide the cultivation of fruits is a major challenge due to presence of high water, minerals and nutrients. Some of such fruits are bottle gourd, tomato, watermelon, grapes and cucumber.

In the present study, bottle gourd [*Lagenaria siceraria* (Molina) Standl. (synonym *Lagenaria vulgaris* Ser.)] fields of five districts of sub-Himalayan West Bengal *viz.* Darjeeling, Jalpaiguri, Coochbehar, Alipurduar and Uttar Dinajpur were surveyed. Disease incidence varied from month to month and place to place. Severity of the diseases was found at an alarming percentage of around 40%, in the plains of Darjeeling and Jalpaiguri district. Lesions on fruits began as irregular, brown water soaked spots that enlarged, coalesced and spread around the fruit within 4-5 days. Fungal mycelia appeared outside the fruit and finally the whole fruit was covered with fungus. As the disease progressed, portions of the fruits were distorted and dried and growth was reduced. Lesions on leaves were less severe. It began as small brown spots near the margins which enlarged and progressed inward. In advance stage the lesions turned dark brown and more than 50% of the leaves were affected. Altogether 13 fungal cultures (KBG-01, KBG-02, KBG-03, KBG-04, F/A/1, L/A/1, N/F/1, N/L/1, K/F/1, K/L/1, F/A/2, MBF-03 and MBL-01) were obtained in PDA. But pathogenicity tests further confirmed that two pathogens *viz.*, *Fusarium* sp. and *Colletotrichum* sp. were consistent throughout the present study area.

Several fungal diseases of bottle gourd have been identified so far from India and abroad. Black fruit rot by *Alternaria alternata* (Pawar *et al.* 2014), powdery mildew by *Podosphaera xanthii* (Nayak and Babu, 2017) are some examples of bottle gourd diseases in India. Several other fungal pathogens were also reported worldwide which were *Cercospora citrullina* (Mukhtar *et al.* 2013), *Fusarium oxysporum* (Shah *et al.* 2014), *Fusarium moniliforme* (Jadhav, 2012), *Sclerotium rolfsii* (Ling *et al.* 2008). In 2009

Sultana and Ghaffar reported the presence of variety of seed borne fungi like *Lasiodiplodia theobromae, Fusarium semitectum, Macrophomina phaseolina* and *Fusarium oxysporum* in bottle gourd. Koffi *et al.* (2013) isolated and identified seven different fungal genera *viz.*, *Aspergillus, Botryosphaeria, Cochliobolus, Colletotrichum, Fusarium, Lasiodiplodia* and *Phoma* causing necrosis and discoloration of leaves of bottle gourd. *Cercospora citrulina* and *Alternaria cucumerina* were reported to cause major problems by producing leaf spot and leaf blight disease and reducing in fruit yield (Maheshwari *et al.* 2013).

In the present study *Colletotrichum* sp. showed simple appresoria and 18.4±1.2 µm × 4.9±0.7 µm straight, elliptical conidia with broadly rounded ends under light microscope. In case of *Fusarium* sp. both macro- and micro- conidia were observed. Macroconidia were 29±1.9 µm × 3±1.7 µm, 3-5 septate and slightly curved with tapered ends, whereas, microconidia were 11±1.6 µm × 3±1.9 µm, single celled, nonseptate and ovoid. Straight, elliptical conidia with broadly rounded ends is the characteristic feature of *Colletotrichum* sp. (Gangadevi and Muthumary 2008; Lima *et al.* 2011; Sawant *et al.* 2012; Liu *et al.* 2013), whereas, *Fusarium* sp. can be identified through the presence of septate macroconidia and single celled microconidia (Ramdial *et al.* 2016).

In the present study, for further characterization of the pathogens ITS regions of both the fungi were amplified through PCR and the sequences were submitted in the GenBank, where *Colletotrichum* sp. was identified as *C. gloeosporioides* isolate F/A/1 (GenBank Accession No. KC355249) and *Fusarium* sp. was identified as *Fusarium* sp. isolate F/A/2 (GenBank Accession No. KR263845). During the phylogenetic analysis *C. gloeosporioides* formed a single cluster including the sequence of the present study. Different *Colletotrichum* species showed different clusters in the phylogenetic tree. Further, different south-east Asian *C. gloeosporioides* isolates were clustered together indicating that *C. gloeosporioides* isolates from south-east Asia share a common ancestral origin. In case of *Fusarium* sp., the present isolate showed 99% nt identity with *F. incarnatum* from

China infecting *Morchella importuna* and clustered together with *F. incarnatum* from different areas, whereas, different *Fusarium* species formed separate clusters. So, from the study the present *Fusarium* isolate can be identified as *F. incarnatum*.

In India *Colletotrichum capsici* the casual fungus of die back/fruit rot, causes heavy losses (upto 60%) in chilli (*Capsicum annum* L.) production (Kaur *et al.* 2011). In Serbia, four different species of *Colletotrichum* i.e *Colletotrichum gloeosporioides, Colletotrichum acutatum, Colletotrichum coccodes,* and *Colletotrichum dematium* were reported as the causal pathogens responsible the occurrence of anthracnose on tomato (Zivkovic *et al.* 2010). In Brazil various fruits like mango, guava, papaya, avocado etc. were reported to be susceptible to anthracnose caused by *Colletotrichum gloeosporioides.* In case of subtropical crops such as apple, grape, peach etc. the disease is caused by *C. acutatum* (Peres *et al.* 2002). In 2009 Masayahit *et al.* reported the occurrence of anthracnose on dragon fruit in Malysia caused by *Colletotrichum gloeosporioides.* In 2008 Miles and Schilder reported that *Colletotrichum acutatum* causes anthracnose fruit rot disease in blueberries which is the most common and widespread disease of blueberries in the United States. They also reported pre-harvest fruit losses of 10 to 20 percent and post-harvest losses of up to 100 percent. Zhang *et al.* in 2014 reported the occurance of anthracnose disease on *Trichosanthes kirilowii* in China, caused by *Colletotrichum gloeosporioides.* Chowdappa *et al.* (2009) reported of occurrence diverse molecular groups among *C. gloeosporioidies* and *C. acutatum* populations associated with grape anthracnose in India. It was reported that all three morpho groups of *Colletotrichum i.e. C. gloeosporioidies* (Type-I), *C. acutatum* (Type-II and III) can be distinguished based on ITS-RFLP profile as generated by restriction enzyme, among which *C. gloeosporioidies* has been established as dominant pathogen with very limited occurrence of *C. acutatum*. In the dendrogram analysis, it was observed that genetic similarity between isolates derived from sequences of ITS region of rRNA indicated that *C. gloeosporioidies* and *C. acutatum* isolates were clustered as separate groups

and two molecular sub groups were established within each species. Sanabria *et al.* (2010) reported anthracnose in mango and citrus by the species *C. gloeosporioides*. They also reported about the two molecular sub groups of *C. gloeosporioidies* and *C. acutatum*. Chowdappa and Kumar (2012) further reported 79 isolates of *C. gloeosporioides*, categorized into two groups based on cultural and morphological criteria from all mango growing areas of India. Phylogenetic analysis also corresponded to the two subgroups previously identified by morphological and restriction digestion patterns of ITS region. Again 25 isolates of *C. gloeosporioides* were found to be associated with anthracnose of orchids in Sikkim and characterized through PCR of ITS region (Chowdappa *et al.* 2012). For last few years, *C. gloeosporioides* was found to be associated with anthracnose disease of several plants like, walnut (Zhu *et al.* 2014); cashew and mango (Souza *et al.* 2011; Zakaria *et al.* 2015); papaya (Stracieri *et al.* 2016). Several other *C. gloeosporioides* infections were also reported from India and abroad like leaf spot of *Boehravia diffusa* (Gautam *et al.* 2012); anthracnose of mango (Kumari *et al.* 2017), tulip (Choi *et al.* 2012), stonecrop (Jeon and Kwak, 2016), chilli (Than *et al.* 2008), tea (Wang *et al.* 2016), *Actinidia arguta* (Deng *et al.* 2017), papaya, guava, custard apple and pomegranate (Sharma and Kulshrestha, 2015).

In Serbia, *Fusarium oxysporum* has been reported as the most important species of pathogen causing fruit rot and also wilt in tomato by rendering root and basal stem deterioration (Ignjatov *et al.* 2012). Some species of *Fusarium* i.e. *Fusarium semitectum*, *Fusarium oxysporium, Fusarium moniliforme* (Hawa *et al.* 2010) and *Fusarium solani* (Rita *et al.* 2013) were also found to cause wilt and stem rot in dragon fruit. Identification of *F. incarnatum* through PCR of ITS region was also reported in black spot disease of chinese jujube from China (Guo *et al.* 2016). Divakara *et al.* (2014) characterized several *Fusarium* species from sorghum through sequencing of *tef-1a* gene, where *F. incarnatum*, *F. verticillioides* and *F. thapsina* were grouped in three subgroups. *F. incarnatum* was also reported to be associated with several diseases like,

canker of walnut (Seta *et al.* 2004; Singh *et al.* 2011); fruit disease of bell pepper (Ramdial *et al.* 2016); stalk rot on maize (Gai *et al.* 2016); root rot of *Morus alba* (Chen *et al.* 2017). However, to the best of our knowledge, the present study was the first report on the fruit and leaf disease of bottle gourd caused by *F. incarnatum* and C. *gloeosporioides*.

Microscopic study of the fungus revealed that conidia of *C. gloeosporioides* were aseptate, straight and elliptical with broadly rounded ends. *F. incarnatum* produced both macro- and micro-conidia; macroconidia were septate and slightly curved with tapered ends, whereas, microconidia were single celled, aseptate and ovoid. Similar observation regarding conidial size and shape has been reported by several authors (Seta *et al.* 2004; Choi *et al.* 2012; Gai *et al.* 2016; Ramdial *et al.* 2016). While studying on *C. gloeosporioides* causing anthracnose of the hard kiwi fruit (*Actinidia arguta*) in China, Deng *et al.* (2017) reported that the conidia were hyaline with rounded apices conidia were one-celled, cylindrical, aseptate and hyaline. In a study on canker in walnut in Jammu and Kashmir, India, caused by *F. incarnatum*, Singh *et al.* (2011) also observed that the macroconidia were four to eight septate and either straight or slightly curved. While studying *F. incarnatum* causing disease in mulberry in China, Chen *et al.* (2017) observed that macroconidia were spindle and slightly curved, 3 to 4 septate and the apical cell was uniformly tapering, while single-celled microconidia were ovoid.

Seven different media *viz.* PDA, OMA, CDA, RA, YEMA, MEA and LDA were used to study the growth of *C. gloeosporioides* and *F. incarnatum* in the present study. Among the media tested, LDA was best medium for vegetative growth while OMA was recorded as excellent medium for sporulation for both the fungi. 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). 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. Several authors (Singh *et al.* 2011; Gai *et al.* 2016; Ramdial *et al.* 2016) have used carnation leaf agar to study the growth and morphology of *F. incarnatum.*

Studies on the mycelial growth at different pH showed that the mycelial dry weight of C*. gloeosporioides* was maximum at pH 6.0 and that of *F. incarnatum* was maximum at pH 6.5. Least growth was recorded at pH 8.0 for both fungi. The optimum temperature for the growth of both the pathogens was 28^0C. Kang *et al.* (2003) also observed that optimum growth of the phytopathogenic fungus *C. gloeosporioides* was around the pH 6.0. Thakare and Patil (1995) observed that the optimum pH for growth of *C. gloeosporioides* was 4.1-6.8. Sharma and Kulshrestha (2015) observed that the optimum temperature for growth of *C. gloeosporioides* is 25-28°C, and pH 5.8-6.5. However, Jayalakshmi (2010) reported that the highest radial growth and sporulation of the fungus was 30^0C. In a study on *Fusarium* species isolated from maize, Marin *et al.* (1995) observed that the optimum range of temperature for growth of the fungi was 25-30^0C and the optimum pH ranged from 5.5 to 7. In the present study, while observing the growth in liquid media under varying conditions it was found that maximum growth was recorded in PDB after 20 days of incubation. After 20 days, mycelial dry weight declined possibly due to autolysis and depletion of the media.

After characterizing the two major pathogens associated with bottle gourd fruit rot disease, three different abiotic inducers were used in the present study to activate defense signaling in bottle gourd and induce defense related enzymes to elevate host resistance. Benzothiadiazole (BTH) is a salicylic acid (SA) analogue that induce systemic acquired resistance (SAR) signaling in plants and were reported time to time in conferring resistance against several bacterial and fungal pathogens (Friedrich *et al.* 1996; Görlach *et al.* 1996; Dann *et al.* 1998; Beckers and Conrath, 2007; Cao *et al.* 2011; Azami-Sardooei *et al.* 2013; Zhu *et al.* 2016). β-Aminobutyric acid (BABA) is a non-protein amino acid, which induces resistance in plants against a fungi and bacteria through callose

deposition, hypersensitive response and trailing necroses formation (Hong *et al.* 1999; Cohen *et al.* 1994; Zimmerli *et al.* 2000; Cohen, 2001; Jakab *et al.* 2001; Cohen, 2002; Porat *et al.* 2003; Baysal *et al.* 2005; Ranjini *et al.* 2016; Zeighaminejad *et al.* 2016; Shaw *et al.* 2017). γ-aminobutyric acid (GABA) is also a non-protein amino acid that is synthesized via decarboxylation of glutamate by L-glutamate decarboxylase (Forde and Lea, 2007). In vertebrates, GABA act as inhibitory neurotransmitter in central nervous system (Schousboe and Waagepetersen, 2007). For last few years GABA has been reported as an abiotic inducer of plant defense by increasing the defense related enzymes activity during salinity, osmotic and biotic stress (Kim *et al.* 2009; Scholz *et al.* 2009; AL-Quraan *et al.* 2015; Ramesh *et al.* 2015; Li *et al.* 2017).

For the experimental design plants were grouped into ten different sets, i.e., one set of untreated-uninoculated (control), two sets of untreated-inoculated (one set for *C. gloeosporioides* and one set for *F. incarnatum*), three sets of treated-uninoculated (BTH-treated, BABA-treated, GABA-treated) and six sets of treated-inoculated (BTH treated-*C. gloeosporioides* inoculated, BABA treated-*C. gloeosporioides* inoculated, GABA treated-*C. gloeosporioides* inoculated, BTH treated-*F. incarnatum* inoculated, BABA treated-*F. incarnatum* inoculated, GABA treated-*F. incarnatum* inoculated). In each set disease index and four defense related enzymes levels (peroxidase, β-1,3-glucanase, chitinase and phenylalanine ammonia lyase) were measured to correlate the effect of these inducers on host defense. From the results it was found that BTH and BABA induced plants were more tolerant than GABA induced one. However, when the enzyme levels were calculated, all the enzymes were found to be elevated on the 4th day post treatment/inoculation. Peroxidase level was higher in GABA induced plants followed by BTH and BABA induced one. β-1,3-glucanase level was higher in GABA induced plants than BTH and BABA induced one when challenge inoculated with *C. gloeosporioides*, whereas, it was higher in BTH induced plants followed by GABA and BABA induced one when challenge inoculated with *F. incarnatum*. Chitinase levels were higher in BABA

induced plants followed by GABA and BTH induced one. Phenylalanine ammonia lyase activity was higher in BTH-treated plants followed by BABA and GABA treated plants.

According to Azami-Sardooei *et al.* (2013), foliar applications of BTH (50 mg/l on tomato, 250 mg/l on bean and cucumber) reduced the susceptibility against Botrytis cinerea. Although, higher concentration of BTH under pathogen-free conditions showed considerable negative effect on vegetative and generative growth like- plant height, flower and fruit numbers. Two or four applications of BTH on soybean cultivars Elgin 87 and Williams 82 *in vivo* reduced white mold infection (caused by *Sclerotinia sclerotiorum*) by 20-60% and also increase the yield in comparison to control (Dann *et al.* 1998). During the study on role of BTH in resistance against powdery mildew infection Gorlach *et al.* (1996) reported that BTH not only induce acquired resistance in plants but also induce the expression of wheat chemically induced genes like- lipoxygenase and sulfur-rich protein coding genes. According to them BTH was more effective than 2,6-dichloroisonicotinic acid and salicylic acid. According to Zhu *et al.* (2016), BTH treatment not only delayed fruit ripening but also helped in maintaining fruit cell structure integrity, fruit color and fruit quality. Further, BTH also reduced the disease susceptibility through increasing the activity of defense related enzymes (i.e., chitinase, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase), antioxidant properties (amount of hydrogen peroxide) and reducing malondialdehyde activity.

According to Zimmerli *et al.* (2000), BABA activated callose deposition, hypersensitive response and formation of trailing necroses in *Arabidopis* and provided resistance against *Peronospora parasitica*. Though, BABA did not induce the accumulation SAR-associated mRNAs like- *PR-1* and the ethylene or jasmonic acid dependent *PDF1.2* mRNA, it promoted the accumulation of *PR-1* mRNA and papilla formation in *Arabidopis* after attack by *P. parasitica*. BABA (20mM) induced resistance in grape peel tissues against *Penicillium digitatum* at and nearby vicinity of BABA application. Upto 100mM BABA concentration also showed

antifungal activity by inhibiting spore germination and germ tube elongation. Along with BABA application also elevated chitinase and phenylalanine ammonia lyase (PAL) gene expression (Porat *et al.* 2003). Foliar application of BABA on tomato increased phenylalanine ammonia-lyase, peroxidase and H_2O_2 concentration leading to significant suppression of bacterial canker disease caused by *Clavibacter michiganensis ssp. michiganensis* (Baysal *et al.* 2005).

According to Scholz *et al.* (2015) in *gad1/2* double mutant and *gad1/2×pop2-5* tripple mutant *Arabidopsis thaliana* plants GABA played direct and systemic defense response against herbivory, where GABA activity was not dependent on defense related phytohormones or jasmonates and vice versa. According to Ramesh *et al.* (2015) GABA inhibited the aluminium-activated malate transporter leading to altered pollen tube and root growth, tolerance to alkaline/acidic pH and high aluminium ion concentration. AL-Quraan *et al.* (2015) reported that synthetic 1,2,3-thiadiazole compounds (I, II and III) increased the GABA and malondialdehyde levels and reduced carbohydrate, protein levels along with reduced fresh and dry weight in Jordan 1 and 2 cultivar of lentil. Li *et al.* (2017) reported that under low light stress GABA increased chlorophyll content, net photosynthetic rate, stomatal conductance, quantum yield and photochemical efficiency of PSII, electron transport rates and photochemical quenching coefficient in pepper. In GABA primed plants under low light stress increased activities of antioxidant defense related enzymes (i.e., superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, ascorbate and glutathione) resulted in low malondialdehyde content, superoxide anion radical and hydrogen peroxide production and high level of photochemical efficiency. From the discussion it was observed that PAL was affected mostly by these abiotic inducers (i.e., BTH and BABA).

An interesting observation from the present study was observed during the estimation of β-1,3-glucanase from untreated-uninoculated

(control), treated-uninoculated, untreated-inoculated and treated-inoculated plants. From the results it was observed that β-1,3-glucanase content decreased in untreated-inoculated plants in comparison to control, whereas, in treated-uninoculated plants highest β-1,3-glucanase level was observed. Though, the enzyme level increased in treated-inoculated plants, the enzyme levels was lower than the treated-uninoculated one.

According to Rose *et al.* (2002) β-1,3-glucanase played important role in plant defense through the degradation of β-1,3/ β-1,6-glucans of pathogen. However, some pathogens like- *Phytophthora sojae* (Rose *et al.* 2002) and *Fusarium verticillioides* (Sánchez-Rodríguez *et al.* 2017) were reported to secrete endoglucanase inhibitor during disease establishment. Xyloglucan specific endoglucanase inhibitors were also identified from *Nicotiana benthamiana*, (similar to tomato and tobacco) that inhibited *Colletotrichum destructivum* and *Colletotrichum orbiculare* and *Pseudomonas syringae pv. tabaci* population (Xie *et al.* 2008). Thus, from the above results it can be said that the two pathogenic fungi such as *C. gloeosporioides* and *F. incarnatum* might possibly secreted some kind of endoglucanase inhibitor(s) that was responsible for reduced β-1,3-glucanase level in those plants of the present study.

However, in the present study it was found that *C. gloeosporioides* was the major pathogen of bottle gourd. Disease index were found to be lower in BTH and BABA induced plants than GABA induced plants. All the enzyme levels were higher on the 4th day post inoculation and from the previously mentioned discussion it was observed that PAL was mostly affected by both the abiotic inducers (i.e., BTH and BABA). Thus, phenylalanine ammonia lyase (PAL) transcript accumulation on 4th day after BTH and BABA induction were calculated through semi-quantitative RT-PCR after challenge inoculation with the major fungal pathogen, *C. gloeosporioides*. From the results it was found that, treated, inoculated and treated-inoculated plants showed elevated transcript accumulation in comparison to control. However, in only inducer treated plants higher transcript accumulation was observed in case of BABA-treated one. But,

when the induced plants were challenge inoculated with *C. gloeosporioides* higher accumulation of PAL transcript was observed in case of BTH-treated and *C. gloeosporioides* inoculated one. Nevertheless, in both the cases reduced disease index was observed.

Phenylalanine ammonia lyase (PAL) is the key enzyme of phenylpropanoid pathway that converts phenylalanine to trans-cinnamic acid. Different types of secondary metabolites flavonoids, flavons, isoflavonos, lignins, anthocyanins and coumarins are synthesized from this pathway. The phenylpropanoid intermediates play important roles in host resistance during pathogen attack and elevated phenylalanine ammonia lyase activity helps in the synthesis of these secondary metabolites (Bevan *et al.* 1989; Dixon and Paiva, 1995; La Camera *et al.* 2004; Huang *et al.* 2010; Vogt, 2010).

Transcriptional regulation of genes encoding different enzymes of phenylpropanoid pathway occurs in response to some stimuli (Kim and Huang, 2014). Transcripts of PAL genes have been found to accumulate in response to elicitors as well as in many different incompatible host-pathogen combinations (Mauch-Mani and Slusarenko, 1996). According to Friedrich *et al.* (1996), BTH activated salicylic acid accumulation at the site or downstream of SAR signaling pathway and regulated expression of *nahG* gene in tobacco. However, cyclohexamide subdued *PR-la* promoter in tobacco that was induced by BTH and salicylic acid using *cis-acting* element. BTH (0.2 g/l) treatment showed higher anthocyanin level and elevated activity of glucose-6-phosphate dehydrogenase (G6PDH), shikimate dehydrogenase (SKDH), tyrosine ammonia lyase (TAL), phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H) and dihydroflavonol 4-reductase (DFR) in strawberries during 10 days of storage at 1˚C (Cao *et al.* 2011). BABA treatment in pearl millet and challenge inoculation with *Sclerospora graminicola* upregulated the resistance gene analogue- RGPM213 that encode resistance proteins having ser-thr kinase domain (Ranjini *et al.* 2016). BABA (4 mM) treatment conferred resistance against cucurbit powdery mildew and increased

guaiacol peroxidase and PAL activity in squash (Zeighaminejad *et al.* 2016). Shaw *et al.* (2017) analyzed differentially expressed transcripts of maize plants using suppression substractive hybridization and reported that BTH, BABA and GABA might be effective in combating against *C. gloeosporioides*, where BTH and BABA served a better role.

In the present study, four known antagonistic bacterial isolates (*Serratia marcescens, Pseudomonas putida, Bacillus amyloliquefaciens, Rhizobium radiobacter*) were used *in vitro* to control the growth of *Colletotrichum gloeosporioides* and *Fusarium incarnatum*. From the results it was observed that *B. amyloliquefaciens* and *R. radiobacter* were more effective in inhibiting the growth of either of the fungi. *S. marcescens* and *P. putida* also showed more or less similar result against *C. gloeosporioides*. However, *P. putida* was moderately effective against *F. incarnatum*, whereas, *S. marcescens* was least effective in controlling the growth of *F. incarnatum*.

All the above mentioned bacteria are known to have antagonistic effects against certain bacteria and fungi. *Serratia marcescens* was reported to have antagonistic effect on rice sheath blight causing *Rhizoctonia solani* (Someya *et al.* 2005). In another study, *Escherichia coli, Klebsiella ozaenae, Pseudomonas maltophila, Bacillus circulans, Bacillus sphaericus, Bacillus coagulans, Serratia marcescens* and *Streptococcus* spp. were effective against some pathogenic fungi (*Alternaria porri, Fusarium oxysporum, Sclerotium rolfsii* and *Botryodiplodia theobromae*), where *S. marcescens* completely inhibited spore germination of *S. rolfsii* (Sivanantham *et al.* 2013). According to Karimi *et al.* (2012) *Pseudomonas putida* isolate P9 and P10 were proved to be antagonists against *Fusarium oxysporum f. sp. ciceri* in dual-culture assay. Antifungal activity of cell suspension and cell-free supernatant of *Bacillus amyloliquefaciens* SYBC H47 culture against *Aspergillus niger, Mucor racemosus, Fusarium oxysporum, Penicillium citrinum* and *Candida albicans* was reported by Li *et al.* (2016). They also reported that *B. amyloliquefaciens* inhibited the conidia germination and mycelia growth of *Botryosphaeria dothidea*.

Rhizobium radiobacter showed antagonistic activity against *Fusarium oxysporum, Fusarium solani* and *Panax herbarum* (Fan *et al.* 2016). However, according to Charest *et al.* (2005) humic substances extracted from de-inking paper sludge compost inhibited the natural antagonistic effect of *Rhizobium radiobacter* against *Pythium ultimum*.

Several phytoextracts were also used in the management of several bacteria and fungi by several workers. Ten different plants were selected for the present study to compare their antifungal effect against the two fungal pathogens *viz.*, *C. gloeosporioides* and *F. incarnatum* using poisoned food technique. From the results it was found that alcoholic extract of *Azadirachta indica* was effective against both the fungi i.e., *C. gloeosporioides* and *F. incarnatum*. Datura metel and Piper betle showed growth inhibition of *C. gloeosporioides*, whereas, *Holarrhena antidysenterica* and *Murraya koenigii* showed growth inhibition of *F. incarnatum*. Other phytoextracts used in the present study did not show significant growth inhibition in either of the pathogens.

Effect of different phytoextracts on different fungi and bacteria was reported by several workers. Inhibitory effect of neem (*Azadirachta indica*) extracts on *Aspergillus* and *Rhizopus* (Mondall *et al.* 2009), *Trichophyton mentagrophytes, Trichophytonrubrum, Microsporum canis* and *Epidermophyton floccosum* (Salazer *et al.* 2015) were reported. *Datura metel* extract was effective against *Trichoderma harzianum, Trichoderma viride, Fusarium oxysporum f. sp. melonis, Fusarium oxysporum f. sp. lycopersici* and *Fusarium oxysporum f. sp. tuberosi* (Rinez *et al.* 2013). *Piper betle* extract showed inhibitory effect on *Colletotrichum gloeosporioides, Rhizoctonia solani, Fusarium oxysporum* f. sp. *cubense, Sphaceloma ampelinum, Colletotrichum capsici, Alternaria brassicicola* and *Pyricularia oryzae* (Singburaudom, 2015). According to Balakumar *et al.* (2011) *Ocimum sanctum* extract was proved to be effective against *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum gypseum, Microsporum nanum* and *Epidermophyton flocossum*. Phytoextract of *Holarrhena antidysenterica* significantly inhibited the growth of *Penicillium expansum*

and *Aspergillus fumigatus* (Baviskar and Dekate, 2016). *Trichophyton mentagrophytes* and *Microsporum gypseum* were reported to be inhibited by *Murraya koenigii* extract (Jayaprakash and Ebenezer, 2012). Extract of *Leucas aspera* inhibited the growth of *Penicillium* sp. and *Candida tropicalis* (Babu *et al.* 2016). Inhibitory effect of *Mangifera indica* extract on *Candida glabrata* was reported by Jain and Nafis (2011). *Lagerstroemia speciosa* extract was effective against 12 bacterial and 3 fungal isolates *viz.*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella paratyphi*, *Vibrio mimicus*, *Vibrio parahemolyticus, Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae, Aspergillus niger, Sacharomyces cerevaceae* and *Candida albicans* (Nasrin *et al.* 2012). *Boerhavia diffusa* extract were proved significantly effective against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* (Das, 2012).

This study revealed some new facts of fundamental importance. The survey made in five districts of sub-Himalayan West Bengal revealed that the fungal pathogens *F. incarnatum* and *C. gloeosporoides* attack bottle gourd plants and severely damage fruits and leaves of the plants. To the best of our knowledge, this study is the first report on *F. incarnatum* and *C. gloeosporoides* as pathogen of *L. siceraria*. Induction of several pathogenesis related enzymes by abiotic elicitors have been observed. Differential expressions of PAL gene have been studied by semi-quantitative RT PCR method following induction of resistance. Successful priming was observed when plants were treated with BTH or GABA. Hence, these chemicals may be used to manage fruit and foliar diseases of bottle gourd infected by *F. incarnatum* and *C. gloeosporioides*. Additionally, the growth of the above mentioned two pathogens were also found to be inhibited by some antagonists and botanical in *in-vitro* studies. Some of them have potential to control the pathogens. Thus the present study significantly throws light towards significant management of bottle gourd plants.