ABSTRACT

The present study deals with “Studies on some leaf and fruit diseases of *Lagenaria siceraria* (Molina) Standl. and their management” *Lagenaria siceraria* (bottle gourd) is an important member of the Cucurbitaceae family. It is a warm-season vegetable which is cultivated throughout India for its tasty and healthy fruit. It is popular in India not only as a food item but also for its medicinal properties. Bottle gourd has been used in Ayurveda and other folk medicines traditionally for treatment of diseases like hypertension, diabetes mellitus, ulcer, liver diseases, congestive cardiac failure (CCF) and piles. The fruit is rich with choline level-A lipotropic factor, a healer of mental disorders. Large areas of sub-Himalayan West Bengal is under bottle gourd cultivation. However chronic problems are persistent due to occurrences of diseases which limit the production. Pathogens of bottle gourd pose severe threat to its cultivation. The farmers of this region largely depend on the synthetic chemicals for disease management. Use of chemicals not only allows the development of resistance in the pathogens but also disturbs the normal soil microflora as well as create health hazard to human beings. Therefore, an eco-friendly approach is essential to control the fungal diseases in a more cost-effective and efficient manner.

At the beginning of the present study, bottle gourd fields of five districts of sub-Himalayan West Bengal *viz.* Darjeeling, Jalpaiguri, Coochbehar, Alipurduar and Uttar Dinajpur were surveyed for occurrence of diseases. 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. The major aspect of the study was identifying the cause of the diseases of this region and designing a stable plant protection strategy for efficient disease management. The main objectives of the study were: (i) Isolation of major pathogens responsible for foliar diseases and fruit rots in *Lagenaria*

siceraria; (ii) Pathogenicity test of isolated fungi (*Fusarium incarnatum* and *Colletotrichum gloeosporoides*) and assessment of diseases; (iii) Morphological and physiological characterization of the isolated pathogenic fungi; (iv) Molecular identification of the isolated pathogenic fungi and their phylogenetic analysis; (v) Induction of defense related enzymes by some abiotic inducers; (vi) Studies on PAL gene expression in *Lagenaria siceraria* after induction with abiotic inducers using semiquantitative RT-PCR (vii) Control of the pathoges using bio-control agents and botanicals.

During the survey it was found that 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. Colletotrichum* sp. isolate F/A/1 and *Fusarium* sp. isolate F/A/2 were consistent throughout the present study area.

Studies on morphological characters of the two pathogenic fungal isolates revealed that *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. Seven different media *viz.* PDA, OMA, CDA, RA, YEMA, MEA and LDA were used to study the growth of *C. gloeosporioides* and *F. incarnatum*. Among the media tested, LDA was best medium for vegetative growth while OMA was recorded as excellent medium for sporulation for both the fungi. Studies on

the mycelial growth at different pH in PDB medium 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.

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% nucleotide 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*.

After characterizing the two major pathogens associated with bottle gourd fruit rot disease, three different abiotic inducers [(Benzothiadiazole (BTH), β-aminobutyric acid (BABA) and γ-aminobutyric acid (GABA)] were used to activate defense signaling in bottle gourd and induce defense related enzymes to elevate host resistance. 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 to disease 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.

In addition to studies on activities of PR enzymes, 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.

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 almost 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*.

Ten different plants were selected 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. *Datura metel* and *Piper betle* showed growth inhibition of *C. gloeosporioides*, whereas, *Holarrhena antidysenterica* and *Murraya koenigii* showed growth inhibition of *F. incarnatum*. Other tested phytoextracts did not show significant growth inhibition in either of the pathogens.

The present study reports for the first time, the isolation of two pathogenic fungi, *C. gloeosporioides* and *F. incarnatum* from diseased bottle gourd plants. The major findings of the study suggest an eco-friendly, cost-effective means of fungal disease control. The use of these chemical inducers and microbial strains as bioinoculants provides an alternative for culminating the use of harmful pesticides and providing an efficacious control of fungal diseases.