Chapter-2

LITERATURE REVIEW

Plants in general are unable to escape inauspicious changes in their environment as most of them are sessile organisms. Therefore, to defend themselves against harmful conditions in their surroundings, elaborate mechanisms have developed in plants to adapt to various stress conditions through fast and dynamic changes in their physiology. Plants combat microbial invasions by using a variety of defense systems that are either preformed or are inducible. This leads to an overall increase in plant resistance not only against the invader but also against other pathogens. A thorough understanding of the underlying mechanism of induced resistance at the physiological and molecular levels and the signals which triggers it will lead to a better understanding of the spectrum of such resistance in plants and permit sustainable and effective management of diseases in the field.

At the onset of the present study it was considered to review the vast body of literature that has been documented by the earlier workers. The findings of the previous workers with respect to the present line of investigation are being presented, in a selective manner, in the following paragraphs. The observations have been divided into several facets which are listed below-

- Diseases of bottle gourd
- Identification of fungal pathogens
- *Colletotrichum gloeosporioides*, the fruit and vegetable pathogen
- *Fusarium incarnatum*, the soil borne pathogen
- Induction of systemic resistance
- Inhibition of pathogenic microorganisms by biocontrol agents and botanical extracts

2.1 Diseases of bottle gourd

A literature survey on the diseases of bottle gourd revealed that the plant is attacked by a wide variety of pathogens including viruses, bacteria and fungi. It is often subjected to a strong parasitic and diseases pressure. The diseases may be seedborne, soilborne, airborne or transmitted by insect

vectors. Multiple pathogens are observed to cause considerable damage in terms of yield and quality in crop fields (Lepoivre and Semal, 1989). In this section some of the previous reports since 2008 are presented.

2.1.1 Diseases caused by fungi

Fungi constitute a major group of pathogenic agents which infects a wide variety of herbaceous plants. They are distributed world-wide, especially in tropical and subtropical regions. Bottle gourd plants are particularly susceptible to several fungal pathogens which attack leaves, fruit and roots of this plant. Choi and Shin (2008) observed typical symptoms of downy mildew caused by *Pseudoperonospora cubensis* in bottle gourd plants growing in a commercial field at Hoengsong, Korea. The symptoms appeared as yellowish or light green lesions on upper leaf surfaces. The lower surfaces showed dark grey fungal growth. The angular lesions were delimited by leaf veins. Sporangiophores (230–500 ×5–7·5 µm) were straight to substraight, hyaline, tree-like, monopodially branched 4–6 orders. Sporangia (20–35·8 ×15–23·8 µm) were olivaceous brown, operculate and ellipsoidal. On comparison of the sequence of internal transcribed spacer region of the pathogen (Acc. No. DQ409815) obtained by PCR amplification of the respective gene with other such sequences available in the GenBank database, revealed similarity to sequences of *Pseudoperonospora cubensis* found on *Cucurbita moschata* (Acc. No. AY608619) and on *Cucumis sativus* (Acc. No. AY608616).

Kousik *et al.* (2008) reported that Powdery mildew caused by *Podosphaera xanthii* is an important pathogen and can cause significant damage to cucurbit crops. The authors inoculated *L. siceraria* for testing their susceptibility to powdery mildew in two greenhouse tests. The young seedlings of bottle gourd plants showed moderate resistance to the disease. The authors suggested that the resistant lines of bottle gourd, which is used as rootstocks for grafting watermelon, can be a valuable source of germplasm in rootstock breeding programmes.

Ling *et al.* (2008) observed wilting and crown necrosis in bottle gourd used as rootstock of watermelon in Charleston, SC. A fungus with white mycelia and brown sclerotia were isolated from four wilted plants. A single PCR product of approximately 680 bp was obtained on PCR amplification of the ribosomal internal transcribed spacer (ITS) region using the primers ITS1 and ITS4. The PCR amplicon was cloned into the TOPO TA cloning vector (Invitrogen, Carlsbad, CA) and sequenced (Acc. No. EU338381). BLASTN analysis of the sequence in the NCBI databases revealed that the sequence shared 99% similarity to the ITS sequences of *Sclerotium rolfsii* and *Athelia rolfsii* which is the perfect stage of *S. rolfsii*. The pathogen was used to inoculate 10-week-old bottle gourd plants following which it was held at high humidity and 25°C. Symptoms of wilting developed after 4 to 5 days of inoculation and the plants wilted completely within 7 to 10 days. The disease was confirmed to be southern blight of bottle gourd after reisolation of the pathogen from the inoculated plants.

In a study on the seed borne fungi of bottle gourd Sultana and Ghaffar (2009) isolated 45 species of fungi belonging to 22 genera from affected seed samples in Pakistan. Of the isolated species, 35 were reported as new records from seeds of bottle gourd in Pakistan. The range of occurrence and the average percent incidence of fungi in tested samples revealed that the most frequently isolated fungi were *Lasiodiplodia theobromae, Fusarium semitectum*, *Macrophomina phaseolina* and *Fusarium oxysporum*.

Dervis *et al.* (2010) reported the incidence of fungi in seed samples of bottle gourd that were collected from different parts of Turkey. The most frequently isolated fungal species were *Macrophomina phaseolina, Fusarium oxysporum, Epicoccum purpurascens* and *Sordaria fimicola*.

Endo *et al.* (2012) studied the pathogen of the cucurbitaceous crops including bottle gourd which were often attacked by powdery mildew fungus which caused a lot of damage in Morocco. Altogether 85 samples were collected from different regions in the country. Results showed the

existence of *Sphaerotheca fuliginea* associated with the disease. There was no instance of *Erysiphe cichoracearum*, *E. polyphaga* or *Leveillula taurica*.

Jadhav (2012) observed that wilt disease of bottle gourd caused by *Fusarium moniliforme* occurred in 31.33% in farmer's field at Hashiware in Raigad district of Maharashtra, India. The symptoms appeared on collar region which caused rotting of basal stem portion. Shrinking was observed in the stem which turned brown to dark brown in the affected region. Partial drying of tender shoots was noted and finally, the plants collapsed in severe conditions. The pathogen was isolated from symptomatic plants. For testing pathogenicity of the isolate, seeds of bottle gourd were sown in soil inoculated with the pathogen in pots. Dark brown lesions were found to develop on stem and shriveling of the stem which extended above the collar was noted. The underground stem showed pinkish white fungal growth.

Koffi *et al.* (2013) identified several fungal genera infecting *L. siceraria* from leaf samples with necrosis and discoloration symptoms from various locations of Côte d'Ivoire. Fungi were isolated from 750 samples with 7 distinct types of symptoms. Fungal genera found in all of the localities were *Aspergillus, Botryosphaeria, Cochliobolus, Colletotrichum, Fusarium, Lasiodiplodia, Phoma* and *Pestalotiopsis*. An ANOVA test revealed significant differences between fungal genera in terms of isolation frequency. Principal components analysis showed that fungus distribution in each locality was correlated with climatic factors. In another study (Koffi *et al.*, 2014), the authors identified several fungal genera infecting *L. siceraria* by PCR amplification of the nuclear ribosomal DNA region (ITS1, 5,8S and ITS2) of fungal strains with the universal primers, ITS1 and ITS4. The reaction generated only one 600 bp fragment. The sequence data obtained of the PCR products were aligned and compared with other sequences in the GenBank database. Homologous sequences of fungi were compared and several fungi were identified *viz. Botryosphaeria rhodina*, *Cochliobolus kusanoi*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*,

Phomopsis glabrae, *Fusarium solani*, *Diaporthe helianthi*, *Pestalotiopsis* sp. and *Rhizoctonia bataticola*.

Maheshwari *et al.* (2013) observed the incidence of Alternaria blight caused by *Alternaria cucumarina* and Cercospora leaf spot caused by *Cercospora* spp. in bottle gourd during the rainy season in 4 districts of Rajasthan, India. The pathogens were isolated from the symptomatic plants. Altogether 17 bottle gourd genotypes were inoculated by the *Alternaria* blight pathogen in field trials that were conducted during rainy season of 2011 and 2012 (Maheshwari and Choudhary, 2015). Of the tested plants, 6 varieties showed moderate resistance and 10 varieties showed moderate susceptibility. One germplasm 'Chomu Local' was found to be susceptible to this disease.

A leaf spot disease was observed on leaves of bottle ground in Lahore and Shiekhupura regions of Pakistan by Mukhtar *et al.* (2013). The causal organism was isolated, and on the basis of pathology and morphological characteristics, was identified as *Cercospora citrullina*. The disease was characterized by brown spots with distinct dark brown border that occurred on both sides of the leaves. The fungal conidiophores were simple, 2-30 in a divergent fascicle and multiseptate. They were mainly golden brown in colour that turned paler to subhyaline at the apex. The shape was straight to slightly curved (3-5×50-300 µm), geniculate and unbranched. Conidia were filiform, solitary and hyaline. They contained 1-16 septa and remained non-constricted at the septa (2.5-4×20–270 µm).

Shah *et al.* (2014) observed wilting in bottle gourd plants in Pakistan. The authors isolated the fungus *Fusarium oxysporum* from infected seed and wilted plant samples of bottle gourd and conducted pathogenicity test with the isolate. Data were collected in terms of plant growth (root, shoot and total plant length and weight), percentage of seed germination and leaf infection percentage. The intensity of *Fusarium* wilt was found to increase with increase in inoculum density when plant growth and mortality was computed. The percentage of seed germination also followed a similar pattern.

In a study on the symptomatology and epidemiology of the disease black fruit rot of bottle gourd caused by *Alternaria alternata*, Pawar *et al.* (2014) observed that, *A. alternata* grew most favourably at 26-28^{0}C although it was able to grow at a wide range of temperatures that ranged between 15-30^{0}C. High relative humidity (>90%) was necessary for optimum growth and sporulation of the pathogen. The mycelia of the pathogen were hyaline, septate and irregularly branched. Hyphae were of an average width of 3.50 µm (range 1.50-7.30 µm). Conidiophores arose singly or in groups short or long (2-6). Conidia (48×14 µm) bearing 2-10 septa occurred as long chains of 10 or more. Typical symptoms of water soaked lesions appeared on inoculated healthy fruits of bottle gourd which turned brown within 2-3 days.

Yan *et al.* (2016) reported the occurrence of root and collar rot caused by *Plectosphaerella cucumerina* in bottle gourd that was used as watermelon rootstock in China. The pathogen produced buff to salmon pink, slimy, appressed colonies with sparse aerial mycelia in potato dextrose agar plates. Microscopic examination of the hyphal coils showed that they were hyaline, branched, and septate. Conidiogenous cells were observed to be phialidic, 1-septate at the base. The phialide apex was broadest at base which gradually tapered towards the apex. Conidia (mean dimension 6.31±0.60×2.00±0.17 µm) were found to be guttulate, ellipsoidal and septate. PCR amplification of the ribosomal internal transcribed spacer region using the universal primers ITS1 and ITS4 produced a 526-bp fragment which was sequenced (Acc. No. KT826571). BLAST analysis revealed 99% identity with *P. cucumerina* previously isolated from melon (*Cucumis melo* L.) (Acc. No. HQ238997). Bottle gourd seedlings were inoculated with a conidial suspension (106 conidia/ml) and maintained at 25°C with a 12 h photoperiod. Root rot was observed in the inoculated plants after 7 days of inoculation. The pathogen was reisolated from the symptomatic roots, thereby confirming Koch's postulates. The authors suggested that since the disease was severe in China, there was a need for bottle gourd rootstocks that were more resistant to root and collar rot for grafting of watermelon.

Bottle gourd plants with powdery mildew symptoms were observed on *L. siceraria* in different fields of the Odisha state (India) in December 2014 (Nayak and Babu, 2017). The symptoms were circular white patches (1-2 mm in diameter) on the upper surface of the leaves. The patches coalesced and developed into larger irregular or circular spots on both sides of the leaf surfaces. Microscopic examination revealed that the conidiophores (110-220×11-13.5 µm) produced 3 to 5 immature conidia in chains. Foot cells (40-75 µm) were long and cylindrical and appeared slightly constricted at the basal septum. Conidia were hyaline, (25-40×17-22 µm) and displayed fibrosin bodies. PCR amplification of the internal transcribed spacer region of rDNA from conidia with primers ITS 1/ ITS 4 and sequencing of the resulting amplicon yielded a 182 bp sequence (Acc. No. KU376473). The sequence was analysed by BLAST homology search with GenBank database. The results revealed 100% similarity with *Podosphaera xanthii* (Acc. Nos. KX061106, KR779870). The authors determined pathogenicity by inoculating conidial suspension onto young leaves of five healthy potted *L. siceraria* Symptoms similar to that observed in field developed after 5-7 days of inoculation from which *P. xanthii* was reisolated.

2.1.2. Diseases caused by bacteria, viruses and other agents

Bacterial diseases are not common but can occur during persistent warm and humid conditions. The main bacterial diseases found on bottle gourd are angular leaf spot caused by *Pseudomonas syringae* and bacterial wilt caused by *Erwinia tracheiphila*. However, bottle gourd plant is very sensitive to viral infection and major losses of production are reported owing to viral diseases. The common viruses that infect this plant are *Cucumber mosaic virus* (CMV), *Cucumber green mottle mosaic virus*, *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Pumpkin yellow vein mosaic virus* and *Papaya ringspot virus* (PRSV). Symptomatic observation cannot reveal the identity of the causal virus because of the similarity of the symptoms caused by different cucurbit viruses.

Bottle gourd crop was found to be infected by bacterial spot caused by *Xanthomonas cucurbitae* in Himachal Pradesh of India (Jarial *et al.*, 2015). A survey was conducted at 64 locations of five districts of to assess the spread of the disease. Disease severity varied from 12.50 to 78.33% leading to 10.07 to 70.61% loss in yield at different locations. The symptoms in the form of small marginal chlorotic spots appeared initially on leaves, which increased in size towards the centre of the leaf. At a later stage, necrotic areas developed in the chlorotic zone. Tendrils stem/vine and floral parts showed water soaked areas which ultimately turned into necrotic spots. In severe disease conditions, amber coloured ooze was noticed. Ultimately, the vine bacame totally necrotic and died. The symptoms on young fruits appeared in the form of water soaked spots which finally caused total rotting of the fruit. In case of mature fruits, small faded spots appeared on fruit peel which cracked as the disease progressed and similar ooze was visible on the spots. Fruit deformation was noted in severe cases and the entire fruit surface was filled with cracks that finally led to fruit rot. The pathogenicity of the isolated bacterium was confirmed. The bacterium was able to infect other crops such as pumpkin, cucumber and summer squash. Artificial inoculation of leaves and fruits was performed with bacterial suspension (10^4 cfu/ml) and incubated for 3–6 days for leaves and 5–8 days for fruits.

Takeshita *et al.* (2001) reported that bottle gourd plants infected with an isolate of *Cucumber mosaic virus* (CMV-KM) developed severe chronic mosaic symptoms with stunting. The virus induced enlarged chlorotic spots and rapidly spread over the inoculated cotyledons. Ito *et al.* (2008) reported the occurrence of *Tomato leaf curl New Delhi virus* (ToLCNDV) which is a *Begomovirus* of the family *Geminiviridae* in cucumber, bottle gourd and muskmelon from Thailand. The authors reported the complete nucleotide sequence of the pathogen which was found on plants showing severe yellow leaf disease symptoms. The virus was found to have a bipartite genome composed of DNA-A and DNA-B. The same virus causing chlorotic curly stunt disease in bottle gourd has been reported to occur in the vegetable growing areas of Delhi and adjoining state of Haryana

(Shohrab *et al.*, 2010). The infected plants were observed to be severely revealed that the virus was transmitted to many other cucurbits by the whitefly, *Bemisia tabaci*. Coat protein gene sequence analysis showed 100% sequence identity with all the isolates of ToLCNDV and two isolates of *Squash leaf curl China virus* (SLCCV). The full length amino acid sequence of the coat protein and replication initiator protein genes had 100% similarity with ToLCNDV-Svr and -Luffa isolates. In another report from India, mosaic mottling, chlorosis and yellowing of leaves has been observed in bottle gourd from the fields of Tamil Nadu (Nagendran *et al.*, 2016). The virus was identified by coat protein gene sequence analysis. The sequences had maximum identity of 94% towards the ToLCNDV reported from Spain and 98% towards the ToLCNDV reported from Asian countries.

Fidan *et al.* (2016) reported the screening of bottle gourd genotypes for *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV) and *Watermelon mosaic virus* (WMV) resistance under open field conditions. The results showed that 55% of the genotypes were affected by ZYMV, 21% with WMV and 12% with CMV, in single or mixed infections, and the rest of the accessions were virus-free. The authors further observed that ZYMV was transmitted by 3.19% of the seeds from infected fruits.

2.2. Identification of fungal pathogens

The correct identification of fungal plant pathogens is necessary for nearly all aspect of phytopathology. This includes basic research on pathogen biology as well as for the control of the diseases they cause. Traditionally, identifying the fungal pathogens associated with plant diseases depends on the explanation of visual symptoms along with the isolation, culturing and microscopic observations of the pathogen. The precision of these methods are limited by the expertise and knowledge of the person making the diagnosis. Diagnoses based on culturing the pathogen is often time consuming and can be impractical in case when quick results are needed (McCartney *et al.*, 2003). The most accepted method which is increasingly being used for diagnosis of phytopathogens is

the use of polymerase chain reaction (PCR) for amplification of specific nucleic acid sequences (Atkins and Clark, 2004). The internal transcribed spacer (ITS) regions of ribosomal RNA genes have been found to be particularly useful for separation of fungal taxa at the genus and also at the species level. This is possible because in these regions, the rate of accumulation of mutations often approximates to the rate of speciation (Bruns *et al.*, 1991).

Phylogenetic taxonomic studies of pathogens from a variety of sources is now most successfully and easily performed with the help of molecular techniques using the internal transcribed spacer region of ribosomal RNA gene (Huang *et al.*, 2012; Jeewon *et al.*, 2013). However, very little research has so far focused on characterization of fungal pathogens of bottle gourd by PCR based methods.

Identification of fungal pathogens of bottle gourd based on ribosomal RNA genes have been reported from Korea (Choi and Shin, 2008) North America (Ling *et al.*, 2008), Côte d'Ivoire (Koffi *et al.*, 2013) and China (Yan *et al.*, 2016). Recently, Nayak and Babu (2017) isolated the powdery mildew pathogen *Podosphaera xanthii* from affected bottle gourd plants growing in different fields of the state of Odisha in India. The authors confirmed the identity of the pathogen by analysing the rRNA gene sequences after amplifying them by PCR using ITS1 and ITS4 universal primers.

2.3. *Colletotrichum gloeosporioides*, the fruit and vegetable pathogen

The genus *Colletotrichum* is a well known pathogen of crop plants primarily in the tropical and subtropical regions, although there are some species that affects temperate crops. *Colletotrichum* species are able to inhabit the plants as a pathogen, an endophyte, an epiphyte, or as a saprobe (Hyde *et al.*, 2009; Tao *et al.*, 2013). *Colletotrichum*, as an asexual fungal genus, was incorporated in morphological classifications of the Ascomycota as its sexual genus *Glomerella*. Members of this genus include a number of plant pathogens of key importance, which causes diseases of a wide variety of herbaceous and woody plants. The pathogens mainly affect fruits and

vegetables leading to serious loss in production. High value crops such as strawberry, mango, citrus and avocado are often affected along with staple crops such as banana. *Colletotrichum* sp. infection in coffee berries in Africa left devastating impacts. The fungi also affect different cereals including sorghum, maize and sugar cane (Cannon *et al.*, 2012). The genus was voted the eighth most important group of phytopathogenic fungi in the world, on the basis of its economic importance and scientific significance (Dean *et al.*, 2012). Nine clades of the genus *Colletotrichum* has so far been recognized which includes acutatum, graminicola, spaethianum, destructivum, dematium, gloeosporioides, boninense, truncatum and orbiculare (Cannon *et al.*, 2012). Apart from these, other independent species have also been recognized to belong to the genus *Colletotrichum*.

The *C. gloeosporioides* species complex has been studied extensively by several workers (Cannon *et al.*, 2012; Liu *et al.*, 2015; De Silva *et al.*, 2017). It exhibits a wide range of variability in terms of morphology and pathogenicity in numerous hosts and includes a number of important plant pathogens. The pathogen primarily affects the leaves and fruits but can also infect other parts of the plant. The fungus has been associated with at least 1,972 different host-pathogen combinations which includes a wide range of fruit crops (Phoulivong *et al.*, 2010; Rampersad, 2014). Due to this immense diversity, it has been established that *C. gloeosporioides* is a species complex that includes 22 species and one subspecies within the *C. gloeosporioides* complex. These are *C. asianum, C. cordylinicola, C. fructicola, C. gloeosporioides, C. horii, C. kahawae* subsp. *kahawae, C. musae, C. nupharicola, C. psidii, C. siamense, C. theobromicola, C. tropicale*, and *C. xanthorrhoeae, C. aenigma, C. aeschynomenes, C. alatae, C. alienum, C. aotearoa, C. clidemiae, C. kahawae* subsp. *ciggaro*, *C. salsolae*, *C. ti* and. *C. queensl*and*icum* (Weir *et al.*, 2012).

A literature review on the diseases of *C. gloeosporioides* reveals that the fungus has a wide host range. It is reported to cause anthracnose on a variety of fruits, including mango, banana, apple, guava, papaya, strawberry, citrus and grapes. It also causes substantial loss to large

number of crops such as cereals, coffee and legumes etc. *C. gloeosporioides* causes infection by the hemibiotrophic mode. Necrotrophic phase and the biotrophic phase are found to occur sequentially (Sharma and Kulshrestha, 2015). Ratanacherdchai *et al.* (2007) performed RAPD analysis of 18 isolates that were obtained from three chilli varieties, chilli pepper (*Capsicum annuum*), bird's eye chilli (*C. frutescens*) and long cayenne pepper (*C. annuum var acuminatum*). Analysis of the RAPD dendrograms using UPGMA showed that *C. gloeosporioides* and *C. capsici* were clearly distinct and *C. gloeosporioides* isolates were less closely related than *C. capsici* isolates. In a similar study on anthracnose in chilli, Than *et al.* (2008) isolated *C. acutatum*, *C. capsici* and *C. gloeosporioides* from chilli fruits. DNA sequence data obtained by sequencing the PCR amplified ITS rDNA and β-tubulin (*tub* 2) gene regions were subjected to phylogenetic analyses. The results revealed three major clusters that represented these species. Direct correlation was observed between the phylogenetic groupings and morphological characters such as colony growth rate and conidium shape in culture. Pathogenicity tests were conducted by inoculating the susceptible Thai elite cultivar *Capsicum annuum* cv. Bangchang by the three isolated species. Results revealed that all the species were able to cause chilli anthracnose.

Masyahit *et al.* (2009) studied the occurrence of anthracnose on dragon fruit (*Hylocereus* spp.) plantations in Peninsular Malaysia. The infected fruit and stem showed reddish-brown lesions along with symptoms of chlorotic halo. The lesions had brown centers which grew bigger in severe cases and then coalesced to eventually rot. The isolated fungal pathogen produced whitish-orange colony in cultures. Microscopic examination revealed capsule-like conidia and septate hypae. The pathogenicity test was done to confirm the virulent character. The pathogen was identified as *Colletotrichum gloeosporioides*.

Prihastuti *et al.* (2009) studied on *Colletotrichum* species associated with *Coffea* (coffee). The *Colletotrichum* species isolated from diseased coffee berries in northern Thailand were compared to species reported to cause

coffee berry disease elsewhere based on their morphological, cultural, biochemical and pathogenic characters. Along with these, DNA sequence analyses as done based on combined datasets of partial actin, β-tubulin (tub2), calmodulin, glutamine synthetase, glyceraldehyde-3-phosphate dehydrogenase genes and the complete rDNA ITS1-5.8S-ITS2 regions. Results showed that the isolates clustered into three species, *viz. Colletotrichum asianum* sp. nov., *C. fructicola* sp. nov. and *C. siamense* sp. nov. The data from phylogenetic analysis matched with groupings based on morphological characters. The authors observed that the biochemical and DNA sequence data could differentiate between *C. kahawae*, *C. gloeosporioides* and the new *Colletotrichum* species. However, all of the new genera reported in this paper have later been included in the *C. gloeosporioides* species complex (Weir *et al.*, 2012).

In a study on pomegranate anthracnose affecting leaves and fruits in the Bagalkot, Koppal, Bijapur Gadag and Raichur districts of Karnataka, Jayalakshmi (2010) identified the causative agent as *C. gloeosporioides*. Maximum growth of the pathogen was noted after 12 days of incubation on Potato dextrose broth at 27±1^{0}C. On the other hand, maximum sporulation was recorded at 30^{0}C with alternate 12 hours light and dark period. The cultivars Ganesh, Araktha and Kesar showed susceptible reaction while other 16 genotypes showed moderately susceptible reaction under detached leaf technique. The pathogen also inhibited root and shoot elongation and seed germination of sorghum seeds and induced phytotoxic symptoms on tomato seedlings.

Choi *et al.* (2012) observed the anthracnose disease on the leaves of tulip trees (*Liriodendron chinense*) in Korea. The causative pathogen was isolated from infected leaves which grew on PDA as whitish mycelia that turned dark gray and finally formed salmon-coloured conidial masses. The cylindrical and ovoid conidia measured 10-18×3-5 µm. Appressoria (6-20×4-12 µm) observed on water agar appeared as pale brown, one-celled, ellipsoidal or clavate and thick-walled. Pathogenicity was confirmed by artificial inoculation of tulip leaves. The internal transcribed spacer region

was amplified by PCR using universal ITS1 and ITS4 primers. Based on phylogenetic analysis of the sequence of the amplicon, the pathogen was identified as *C. gloeosporioides*. Gautam *et al.* (2012) also observed *C. gloeosporioides* induced leaf spots in *Boehravia diffusa* plants from various regions of Bilaspur in Himachal Pradesh, India. The symptoms which appeared initially as a small circular spots enlarge gradually and finally caused drying of the leaves.

Jeon and Kwak (2016) observed anthracnose disease symptoms on stem of stonecrop (*Sedum kamtschaticum*) in open fields in South Korea. The symptoms appeared as black irregular smudged spot (2-5 cm). The putative pathogen was isolated from the lesion and was identified as *C. gloeosporioides* through studies on morphological characteristics and phylogenetic analyses of ITS region sequence of ribosomal RNA gene. On artificial inoculation of healthy stonecrop plants with the isolated pathogen, disease symptoms similar to the original field symptoms appeared on the plants. The pathogen, which was re-isolated from the lesions of the inoculated plants, showed the same characters when compared to the original isolate.

In a study on leaf anthracnose on tea (*Camellia sinensis*), Wang *et al.* (2016) collected 106 *Colletotrichum* isolates from 15 tea production provinces in China. For identifying the isolates, the authors used morphological studies along with multi-locus phylogenetic analysis that included ITS, actin, glyceraldehyde-3-phosphate dehydrogenase, calmodulin, partial sequences of the chitin synthase 1, beta-tubulin, and glutamine synthetase gene sequences. In addition, the isolates that were found to belong to the *C. gloeosporioides* species complex were further analysed using the glutamine synthetase and the Apn2-Mat1-2 intergenic spacer region genes. Results revealed that the isolates belonged to 6 known species *viz. C. fructicola*, *C. siamense*, *C. camelliae*, *C. fioriniae*, *C. cliviae* and *C. karstii.* Moreover 3 species (*C. aenigma*, *C. endophytica*, and *C. truncatum*) were reported as new records. Further, a novel species, *C. wuxiense* was also reported. One particular strain could not be

distinguished and therefore described as *Colletotrichum* sp. Pathogenicity tests conducted by leaf inoculation with six different species *viz. C. aenigma*, *Colletotrichum* sp., *C. camelliae*, *C. endophytica*, *C. siamense*, and *C. wuxiense* showed that *C. camelliae, C. endophytica* and *C. aenigma* were more pathogenic than the other tested isolates.

Deng *et al.* (2017) observed anthracnose symptoms on young and mature leaves of the hard kiwi fruit (*Actinidia arguta*) in China. The disease symptoms appeared as watery lesions which then turned into black or brown spots. At an advanced stage, the lesions turned gray at the centers with dark brown edges. Lesions showing black acervuli were found to be scattered on adaxial leaf surfaces. Leaves with lesions fell under dry conditions. The putative pathogen was isolated on potato dextrose agar and incubated at 25°C with an alternate 12-h light and dark cycle. The colonies showed white mycelia with pinkish-orange conidial masses which after 5 days became gray. Microscopic examination revealed that the conidia (11.31-16.22×3.22-5.51 µm) were one-celled, cylindrical, aseptate and hyaline. Setae were dark brown (80.23-110.52 µm) and the conidial appressoria (5.46-10.43×4.25-8.36 µm) were light brown with smooth edges and slightly irregular to circular in shape. Phylogenetic analysis was done for three isolates using gene sequenes of ITS, glyceraldehyde-3-phosphate dehydrogenase, β-tubulin and the partial mating type locus *MAT1-2*. Results showed that the isolates were *C. gloeosporioides*. Artificial inoculation of the isolates to healthy hard kiwi seedlings in the greenhouse produced symptoms similar to the original field symptoms.

2.4. *Fusarium incarnatum*, the soil borne pathogen

Fusarium species are ubiquitous in soil, and have a wide host range which can cause diseases in plants, humans, and domesticated animals (Agrios, 2005). They are considered economically important pathogens that can infect almost all major agricultural crops. They are reported to invade all vegetative and reproductive parts of the plant producing diseases such as wilts, rots or blights. In fact, members of this genus have been isolated from the soils in every continent except Antarctica (Windels, 1992). There

has been a dramatic increase in the knowledge on diversity of *Fusarium* species along with their evolutionary relationships because of the application of multilocus molecular phylogenetics and genealogical concordance phylogenetic species recognition over the last decade. Currently it is estimated that the genus *Fusarium* comprises at least 300 genealogically exclusive phylogenetic species although less than half have been described formally (Aoki *et al.,* 2014).

*Fusarium incarnatum***,** also known by the synonyms *F. pallidoroseum* and *F. semitectum*, is often considered as an important colonizer of plant tissues and causes several plant diseases. The fungus was found to be associated with canker disease in walnut (*Juglans regia*) that reduced its production in Argentina (Seta *et al.*, 2004). Symptoms on fruits appeared as brown necrotic spots (20 mm diameter and depth 5 mm). Affected fruits were found to abscise prematurely. The putative fungal pathogen was isolated on potato dextrose agar and identified as *F. incarnatum* on the bases of its cultural features and micromorphology. Artificial inoculation of wounded branches of healthy one year old walnut plants by spraying with conidial suspension produced dieback after 30 days of inoculation. Cankers were observed 60 days post inoculation. *F. incarnatum* was reisolated from all the inoculated plants thereby confirming Koch's postulates. In a similar study, Singh *et al.* (2011) also observed canker in walnut in Jammu and Kashmir, India. The authors found cankerous growth on seedling stems which later extended to lateral branches. The putative fungal pathogen was isolated on potato dextrose agar and cultural and morphological studies were done using carnation leaf agar. Powdery white to rosy fungal colonies that was floccose in appearance when kept for 7 days at 25^{0}C was noted. Macroconidia (30-35×3.5-5.7 µm) were four to eight septate and either straight or slightly curved. The pathogen was identified as *F. incarnatum.* For pathogenicity test, bruised branches of one-year-old walnut plants were sprayed with conidial suspension and maintained at 85% relative humidity and 20±2^{0}C for 48 h. Branch dieback followed by canker symptoms were produced on inoculated plants after 50 days of inoculation.

Abdul-Aziz *et al.* (2012) collected 15 seed samples of local alfalfa (*Medicago sativa* L.) from open fields in Saudi Arabia and screened them for seed-borne mycoflora. Altogether, 24 genera and 35 species of fungi were isolated. The authors also studied the syndromes of seed discoloration which revealed that seed discoloration adversely affected seed germination. The most common pathogenic fungi observed on discolored seeds were *Stemphylium botryosum* and *Fusarium incarnatum*.

Song *et al.* (2014) isolated *Fusarium* cf. *incarnatum* from the stem of *Panax ginseng* plants with rot symptoms. Microscopic examination of PDA cultures revealed hyaline microconidia and falcate or slightly curved macroconidia with multiple septa. The features were similar to the typical mycological characteristics of *Fusarium***.** Artificial inoculation of root discs as well as roots of plants with conidial suspensions of the isolated fungus showed root rot symptoms. For identification of the pathogen, the translation elongation factor-1α gene (EF-1α) was amplified by PCR using EF1/EF2 primers and sequences obtained (Acc. No. KC478361) were subjected to phylogenetic analysis. Results showed that the generated sequences had 100% sequence identity to other *F.* cf. *incarnatum* strains (Acc. Nos. JF270205 and GQ339786).

In a study on stalk rot caused by *Fusarium* spp. in maize Gai *et al.* (2016) observed dark pith disintegration of the stalks in China. Pathogen was isolated from pith tissue after surface sterilization in streptomycin amended potato dextrose agar and grown on both potato dextrose agar and carnation leaf agar. White to yellowish brown cotton-like colony was observed in culture. Macroconidia (36.5±5.5 µm × 4.5±0.8 µm) were found to be slightly curved, 3 to 5 septate, with a foot-shaped basal cell and a tapering apical cell. Microconidia (22.8±5.0 µm × 3.6±0.6 µm) were 1 to 5 septate, fusoid; chlamydospores were absent. For phylogenetic analysis, the partial elongation factor-1 alpha (EF-1α) gene was amplified by PCR using EF-1H and EF-2T primers. The obtained sequence (Acc. No. KT313002) was found to be 99% similar to other sequences of *Fusarium incarnatum* deposited in GenBank. Pathogenicity tests were conducted on

maize seedlings in pot experiments. Stunted plants with light-colored leaves and fewer lateral roots were observed after 2 months of inoculation. The pathogen was reisolated from the infected mesocotyls which showed similar characteristics as the original isolates.

Ramdial *et al.* (2016) observed symptoms of fruit rot in bell peppers (*Capsicum annuum* L.) in North Trinidad. The symptoms which appeared as large, necrotic lesions (up to 40 mm in diameter), with signs of internal decay were seen on mature pepper fruit in 17 fields. Fungal pathogen was isolated from infected fruits in potato dextrose agar. Microconidia (10-12× 3-4 µm) were ovoid, nonseptate, single-celled and hyaline. Macroconidia (28-31×3-5 µm) were slightly curved, four- to five septate and tapered at the apex. PCR amplification of the translation elongation factor 1a (EF1a) was carried out using primers EF1 and EF2 and the obtained amplicons were sequenced. (Acc. No. KR003731). The sequence when subjected to BLAST search showed 99% identity to EF1a sequences of *F. incarnatum* in GenBank (Acc. Nos. JF270259 and KF993974). Additionally, the sequence showed 100% identity to the *Fusarium-incarnatum equiseti* species complex in the *Fusarium*-ID database. Pathogenicity tests were conducted on wounded and nonwounded healthy, mature pepper fruits. Results showed that wounded fruits developed necrotic lesions.

Marcenaro and Valkonen (2016) studied fungal pathogens associated with common bean (*Phaseolus vulgaris* L.) in Nicaragua. Analysis of the internal transcribed spacer sequences (ITS1 and ITS2) of the ribosomal RNA genes of the pathogenic isolates revealed the occurrence of three *Fusarium* genera including *F. incarnatum.* Guo *et al.* (2016) observed round, slightly concave severe dark brown to black spots (3-5 mm in diameter) on the fruit epicarp of Chinese jujube (*Ziziphus jujube* Mill.) in Xinjiang, China. The diseased fruit tasted bitter and were inedible. The pathogen was isolated from diseased fruits and was identified as *Fusarium*. The ITS region of rRNA gene and the β-tubulin gene was amplified by PCR. The ITS sequences (Acc. Nos. KP133058 and KP133059) were 99% identical to that of *F. incarnatum* (Acc. No. JN986779). The β-tubulin gene

sequences (Acc. Nos. KP133060 and KP133061) were also 99% identical to that *F. incarnatum* (AB587036). Pathogenicity was tested by inoculating fresh fruits with PDA plugs of the fungal isolate. Disease symptoms appeared on the fruits after 10 days. The pathogen was reisolated from symptomatic fruits.

Chen *et al.* (2017) observed symptoms of root rot in mulberry tree (*Morus alba* L.) in China. The infected stems and roots showed brown xylem which turned black. Cortex rot was noted in roots and leaves wilted. The pathogen was isolated from root tips of symptomatic plants on potato dextrose agar. Fungal colonies produced white to light beige aerial mycelia. Macroconidia (22.6-37.2 µm × 3.67-5.01 µm) were slightly curved, 3-4 septate and the apical cell was uniformly tapering. Microconidia (10-12 × 3-4 µm) were ovoid and single-celled. PCR amplification of partial translation elongation factor-1 alpha (EF-1α) and beta-tubulin (TUB2) genes was carried out and sequences were deposited in GenBank (Acc. Nos. KY509036 and KY509037 respectively). BLAST analysis with the EF-1α and TUB2 sequences revealed that they were respectively 100% and 99% identical to *F. incarnatum* sequences. Pathogenicity tests showed that root rot symptoms developed in plants inoculated with the isolated *F. incarnatum* pathogen.

2.5. Induction of systemic resistance

Plants utilize multiple layers of defense to resist pathogen attack. Such defenses include both preformed and inducible mechanisms (Spoel and Dong, 2012). In tissues infected by pathogen, the plant recognizes specific molecular patterns or PAMP/MAMP (pathogen/microbe associated molecular pattern) which is conserved among groups of microbes. This results in the activation of PTI (PAMP-triggered immunity), which restricts the level of pathogen growth. Contrary to PTI, ETI (effector-triggered immunity), which gets activated in plants in response to race-specific effectors released by the invading pathogen, has better impact on limiting pathogen growth (Fu and Dong, 2013; Shah and Zeier, 2013). The plant genome encodes several resistance (R) proteins that facilitate the plant to

identify avirulence (avr) factors produced by the pathogens. The interaction between R and avr in the infected tissue activates the synthesis of antimicrobial compounds, reactive oxygen species (ROS), and sometimes the hypersensitive response (HR) that directs programmed cell death. These responses constitute the innate immunity of the plant. In addition, a secondary resistance response gets induced in the healthy plant tissues which confer a long-lasting resistance to subsequent infections by a broad range of pathogens (Mou *et al.*, 2003).

Induced resistance in plants is a condition that is triggered by biological or chemical inducers that protects the plants against future stress caused by biotic factors such as pathogenic microorganisms (Kuc, 1982). Induction of resistance in plants can be achieved in response to several factors including pathogen infection, insect herbivory, treatment with specific chemicals or colonization of the plant roots by specific beneficial microbes (Pieterse *et al.*, 2014). Under the condition of induced resistance, latent defense mechanisms of the plant are activated. Upon challenge from specific external biotic stress, the mechanisms are expressed both locally and systemically and an enhanced level of protection is achieved not only against the original stressor, but against a broad spectrum of attackers (Walters *et al.*, 2013). A well connected network of multiple signaling pathways regulates induced resistance where plant hormones play a major role (Pieterse *et al.*, 2014).

Induced resistance in plants can be broadly divided into two main types: induced systemic resistance (ISR) and systemic acquired resistance (SAR). ISR is mediated by a jasmonate and ethylene-sensitive pathway and can be induced by colonization of plant roots by specific strains of plant growth promoting rhizobacteria. SAR is mediated by a salicylic acid (SA)-dependent process which can develop upon treatment with various agents or elicitors that includes certain chemicals, botanical extracts, virulent or avirulent pathogens and beneficial microbes (Choudhary *et al.*, 2007; Spoel and Dong, 2012). Development of SAR requires a particular time period depending on the plant and elicitor, during which several defense related

genes are activated and expressed; and defense enzymes such as the pathogenesis related proteins get accumulated (Choudhary *et al.*, 2007). In addition to direct activation of defences, induction of resistance can cause priming of cells, which can lead to a stronger elicitation of these defences triggered by pathogen attack. SAR can even be passed on to progeny through epigenetic regulation (Fu and Dong, 2013). Induced resistance therefore occurs as a result of a combination of priming and direct induction (Goellner and Conrath, 2008; Ahmad *et al.*, 2010). In last fifteen years, extensive research work has been performed for the establishment of SAR by the application of a variety of biotic and abiotic inducers, and phyto-extracts (Yoshioka *et al.*, 2001; Meena *et al.*, 2001; Higa *et al.*, 2001; Kaur and Kolte, 2001; Paul and Sharma, 2002; Ghosh and Purkayastha, 2003; Nakashita *et al.*, 2003; Li *et al.*, 2008; Frías *et al.*, 2013; Gao *et al.*, 2014; Dewen *et al.*, 2017).

The term SAR was coined by Ross in the 1960s for the phenomenon in which, the tissues away from the site of infection develop resistance in response to an infection that has occurred elsewhere in the plant (Ross, 1961). SAR is characterized by enhanced levels of SA and studies have shown that for the establishment of SAR, signalling and accumulation of SA are essential (Pieterse *et al.*, 2014). On resistance induction, Local SA levels are reported to increase along with the formation of a mobile signal which is transported throughout the plant. This further leads to local SA production in leaves distant from the site of infection. Besides, a well co-ordinated activation of pathogenesis related (PR) genes that encode PR proteins occur simultaneously. Many of these proteins are antimicrobial in nature that defend the plant against invading pathogen. The set of antimicrobial protein/peptide genes that get induced includes PR-1, PR-2 (β-1,3-glucanase) and PR-5 (thaumatin-like protein) (Choudhary *et al.*, 2007, Pieterse *et al.*, 2014).

Although SA get accumulated in the phloem sap of plants that express SAR, it has been experimentally proved in grafted tobacco plants that SA itself is not translocated as signal to the distant tissues (Vernooij *et*

al., 1994). However, SA is required for SAR because experimental transgenic *Arabidopsis* plants that express the nahG gene encoding salicylate hydroxylase, an enzyme that converts SA to catechol, are incapable of inducing SAR (Gaffney *et al.*, 1993; Lawton *et al.,* 1995). Further studies at the biochemical and gene level revealed the involvement of several metabolites including azelaic acid, pipecolic acid, the diterpenoid dehydroabietinal, a glycerol-3-phosphate dependent factor and methyl salicylate in long-distance SAR signalling. Additionally the involvement of flavin dependent monooxygenase is necessary for SAR which functions in amplifying the long-distance signals from the original primary leaves (Pieterse *et al.*, 2014). The *Arabidopsis* NPR1 (nonexpresser of PR genes 1) protein is considered as the master regulator of SAR. The NPR1 adaptor proteins NPR3 and NPR4 are sites for binding SA which regulates their interactions with NPR1, and controls stability of the NPR1 protein. However, the process of interaction between TGA transcription factors and NPR1 that leads to expression of defense genes is still not well understood. In addition, several other factors including redox regulators, the mediator complex, WRKY transcription factors, endoplasmic reticulum–resident proteins, and DNA repair proteins play significant roles in SAR.

The PR proteins comprise altogether 17 families of induced proteins. These families are numbered in the order in which they were discovered. Many of these proteins are directly involved in limiting proliferation of the invading pathogen. PR-1 is the best characterized of these proteins and regarded as the marker of SAR (Ryals *et al.*, 1996; Van Loon, 2006). PR-2 family has been identified as β-1,3-glucanases and the PR-3, PR-4. PR-8 and PR-11 were identified as endochitinases all of which can act against fungi. This is because the cell walls of many fungi are the polysaccharides β-1,3-glucan and chitin which are substrates for β-1,3-glucanases and chitinases, respectively. These proteins have been shown to inhibit fungal growth *in vitro* and even functions synergistically to cause stronger inhibition (Sela-Buurlage *et al.*, 1993). Presence of chtinases in plants which themselves lack chitin have led to the conclusion that these hydrolytic proteins are part of the defense response involved in

systemically induced resistance (Van Loon, 2006). PR-6 is a proteinase inhibitor, which together with the chitinases could act against nematodes and herbivorous insects. PR-7 is an endoproteinase of the subtilisin-like proteinase family that is the most prominent in tomato and might function in dissoloution of the microbial cell wall (Jorda *et al.*, 2000). The PR-8 family possesses lysozyme activity which may target the pathogenic bacteria. PR-9 is a specific peroxidise that plays a role in reinforcement of plant cell wall by catalysing lignifications and provide improved protection against multiple pathogens (Passardi *et al.*, 2004). Members of PR-10 have been found to be homologous to ribonucleases, and some possess weak ribonuclease activity (Bufe *et al.*, 1996). These proteins are assumed to be functional against viral invaders. Members of the PR-12 (defensins) and PR-13 (thionins), both have broad spectrum antibacterial and antifungal activities (Epple *et al.*, 1997; Lay and Anderson, 2005). PR-14 proteins are lipid transfer proteins which also possess antifungal and antibacterial activities (Garcia-Olmedo *et al.*, 1995). Members of PR-15 are proteins of monocots and are germin-like oxalate oxidases. PR-16 also of monocots constitutes families of oxalate oxidase like proteins that possess superoxide dismutase activity. Members of PR-17 proteins resemble zinc-metalloproteinases and have been found in infected tobacco, wheat and barley (Christensen *et al.*, 2002). In general, there are considerable variations among the members of the same PR ptotein family (Van Loon *et al.*, 2006). Moreover, all the PR-proteins are not found in all the plant species.

Since it was evident that SA is the endogenous signal for the induction of SAR, therefore characterisation of synthetic chemicals that are able to mimic SA in activating SAR became the focus of several studies. The compound 2,6-dichloroisonicotinic acid and its methyl ester (both referred to as INA) were the first synthetic compounds shown to activate SAR, thus providing resistance to a wide range of diseases (Métraux *et al.*, 1991; Ryals *et al.*, 1996). As some crops tolerate INA insufficiently, a novel benzothiadiazole (BTH, synonym 'acibenzolar-S-methyl) soon became very attractive compound in activating SAR (Friedrich *et al.*, 1996; Görlach *et*

al., 1996; Lawton *et al.*, 1995). Several reports suggest that BTH is capable of providing protection to various crops in the field against a broad spectrum of diseases (Beckers and Conrath, 2007). Thus BTH was popular for practical use in agriculture and was introduced as a 'plant activator' (Ruess *et al.*, 1996) with trade names such as Actigard1, Bion1, or Boost1. However, in most of the cases, BTH has not been economically viable due to the moderate activity which was in contrast to the excellent and strong action of standard fungicides.

Over the past decade, many natural and synthetic compounds have been reported to be capable of inducing defense activity in plants. This includes the nonprotein amino acid b-aminobutyric acid (BABA), which was shown to be associated with enhanced resistance to several pathogens, insects, nematodes as well as abiotic stresses such as drought and salt stress. Foliar application of BABA suppressed downy mildew in field-grown grape caused by *Plasmopara viticola*. BABA also protected potato and tomato plants in the field against *Phytophthora infestans* and inhibited wilt disease in melon induced by *Monosporascus cannonballus* (Beckers and Conrath, 2007). Moreover, BABA also exhibited synergistic interaction with certain fungicides or plant activators (Cohen, 2002). The mechanism of action of BABA differs from other plant activators (INA, BTH) which function via the SAR pathway of PR-proteins against all pathogens. BABA has been thought to defend the oomycetes independent of the SA pathway. However, it utilizes the SA pathway to develop protection against bacteria, TMV, and a necrotrophic fungus (Cohen, 2002).

Plants have developed multiple defence signalling pathways to combat pathogen attacks and adjust to adverse environmental conditions (Jones and Dangl, 2006). Phenylpropanoids are secondary metabolites of plants that are used for diverse end products. Transcriptional regulation of enzyme levels in the phenylpropanoid pathway exhibits the different types of controls exerted on the pathway (Huang *et al.*, 2010; Vogt, 2010). Phenylpropanoid pathway yields cell wall associated phenolics, lignin, flavonoid pigments, antimicrobial phenolics and UV protectants which are

synthesized in response to several factors including pathogen attack, mechanical and UV stress as well as normal developmental signals (Bevan *et al.*, 1989; Dixon and Paiva, 1995; La Camera *et al.*, 2004). Transcriptional regulation of genes encoding different enzymes of phenylpropanoid pathway occurs in response to these various stimuli. A primary enzyme considered to be involved in regulating this pathway is phenylalanine ammonia lyase (PAL, EC 4.3.1.5). PAL is an inducible enzyme that catalyses the conversion of phenylalanine to cinnamic acid, which is the precursor of all phenylpropanoids. This is the first step in the phenylpropanoid pathway, and is a crucial regulation point between primary and secondary metabolism (Kim and Hwang, 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). PAL functions in producing precursors for lignin biosynthesis and other phenolics that get accumulated in response to elicitor treatment or pathogen infection, including SA that has been shown to be essential SAR.

2.6. Inhibition of pathogenic microorganisms by biocontrol agents and botanical extracts

Chemical pesticides which are used to manage plant diseases have negative impacts on the environment and on human health. Besides, growing cost of pesticides (Gerhardson, 2002; Compant *et al.*, 2005), development of pathogen resistance (Van den Bosch and Gilligan, 2008) and ineffectivity of chemicals in fastidious cases (Compant *et al.*, 2005) have made pesticides unpopular. This has become a matter of increasing concern and there has been an all-round demand for healthy food that is free of pesticides residues. This awareness has compelled rapid development of environmental friendly disease management strategies. Over the last two decades, the significance of research on biocontrol agents and antimicrobial botanical extracts has increased immensely. But, inspite of this urgent need to find alternative arsenals to combat phytopathogens, not many biocontrol agents or phytoextracts have been registered till today

and those registered do not always produce predictable results (Fravel, 2005; Gerbore *et al.*, 2014).

The term biological control in plant protection studies refers to the use of organisms for restricting the growth of pests and pathogens. In plant pathology, it applies to the use of microbial agents with antagonistic activity against plant pathogens for the suppression of diseases. These antagonistic microbes use diverse mechanisms to restrict pathogen growth. These may directly cause harm to the pathogen or may act indirectly by enhancing host resistance. Direct methods include parasitism; antibiosis; degradation of pathogenicity factors; competition for nutrients, space or infection sites and production of cell wall degrading enzymes. Indirect methods include plant growth promotion and induction of systemic resistance (Pal and Gardener, 2006; Whipps, 2001). A wide spectrum of bacteria and fungi has been utilized to control or inhibit plant pathogens and stimulate plant growth. Some of the most reported genera includes *Trichoderma*, *Bacillus*, *Pseudomonas*, *Serratia*, *Streptomyces*, *Azospirillum*, *Burkholderia* , *Enterobacter* and *Rhizobium* (Shaikh *et al.,* 2016).

Plants naturally synthesize a wide range of secondary metabolites of diverse chemical groups which represent a significant source of antimicrobials, pesticides and many pharmaceutical drugs. In these natural sources, an array of molecules with antifungal activity against different types of fungus of specific importance has been reported. An estimate shows that 70-80% of total world population depends on traditional herbal medicines for primary health care needs (Hamayun *et al.* 2006). Plants are considered as useful sources of antifungal molecules that are benign to the environment. The use of botanical extracts for controlling diseases of plants have gained importance due to the recent global awareness on hazards of using chemical fungicides. These include accumulation of fungicides residues in food chain, high costs, development of resistance, associated resurgence in fungi and risk to human health (Van den Bosch and Gilligan, 2008). Using botanical pesticides is advantageous because these are safe to non-target organisms,

biodegradable, renewable and go well with the sustainability of local ecology and environment. These have made the natural products an attractive resource for novel plant protection strategies.