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A Review of eco-friendly Management of *Alternaria* Species

C. Jayapradha* . I. Yesu Raja

Department of Plant Pathology, Tamil Nadu Agricultural University, Madurai

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ABSTRACT

Alternaria diseases on crop plants cause huge yield losses and reduce the economic value of the crop plants in conventional production system and are very difficult to manage. Chemical fungicides like dithane M-45, antrocol, captan, difolaton, blitox-50 gave satisfactory control but are dangerous to the ecosystem. Other methods of managing Alternaria pathogens like use of bio- control agents, medicinal plants, various plant products etc. have some potential. This review is an effort to through some light on the different eco-friendly strategies for the management of Alternaria species under different agro- climatic conditions.

1. Introduction

Control of plant disease is a pressing need for Indian agriculture given the growing human population and reducing land availability. The increasing demand for sustainable food supply is met through higher inputs including chemical applications in the form of fertilizers and pesticides. However, the repeated use of chemicals result in the environmental pollution and in the pathological purview, may also lead to development of resistance in the target organism. As the country is now advocating natural farming and for organic farming, it is important to address plant disease management also using eco-friendly approaches, for maintaining ecosystem health and human nutrition through balanced food chain in agro-ecosystems.

Eco-friendly methods, using biocontrol agents and many plant products to suppress plant disease, offers a powerful alternative tool to synthetic chemicals with similar targets. The rich diversity of microbial population and the availability of numerous medicinal plants provides a seemingly endless resource for this purpose. Many of the fungal pathogens causing foliar diseases in plant species *viz*. leaf spots, leaf blights and leaf blotch. Among these pathogens, This mini review is an attempt to collate available information on eco-friendly management practices of *Alternaria* species for enhancing the agricultural productivity in Indian soils under different agro-climatic conditions. *Alternaria* species are potential cosmopolitan fungi under the division of Ascomycota and can be found in soil, plant, food, feed and indoor air (Nayyar *et al.,* 2014). It is an opportunistic pathogen on numerous hosts causing at least 20% of agricultural spoilage, most severe losses may reach upto 80% of the yield. (Nowicki, Marcin *et al.,* 2012).

2. Occurrence of Alternaria and Symptomatology

The genus *Alternaria* has more than 50 species. The typical character of this fungi is spores (conidia) that are produced in chains, multi-celled and pigmented (Anuj Mamgain *et al.,* 2013). The genus is characterized by the formation of polymorphous conidia either singly or in short or longer chains and provided with cross, longitudinal, as well as oblique septa and having longer or short beaks. The spores of these polyphagus fungi occur commonly in the atmosphere and also in the soil. The teliomorphs (sexual stage) are known in a very few species and placed in the genus *Pleospora* of Loculoascomycetes (under Sub-division: Ascomycotina), in which muriform ascospores are produced in bitunicate asci (Verma and Verma, 2010). A great number of species were recorded for the genus *Alternaria* infecting different crops causing world-wide economic loss (Kirk *et al*., 2008). Among the different diseases caused by the genus *Alternaria*, blight disease is one of the most dominant one that causes average yield loss in the range of 32-57 per cent (Conn and Tewari, 1990).

*Corresponding author: jpradha910@gmail.com

Sharma *et al.* (2005) reported that the pathogen *Alternaria alternata* causing leaf spot on apple is characterized by the appearance of 1.3 mm diameter reddish brown necrotic spots on young apple leaves, later coalescing to form bigger blotches and giving blighted appearance to the foliage followed by defoliation. Dark brown to black elongated and mid ribs in early part of the season causing severe yellowing and dropping of the infected leaves. The leaf spot diseases of *Aloe vera* caused by *Alternaria alternata* (Fr.) Keissler agg. is has been reported later by Kamalakannan *et al.* (2008). Vikas *et al.* (2010) found that the symptoms of *Alternaria* leaf blight in sunflower appeared in the form of characteristic small circular, brown patches on the surface of leaves. As the disease progressed, these brownish patches grew in size and finally coalesced to cover the entire surface of leaves producing blight symptoms. The blighted leaves finally get curled and became dark black in colour. Zhang *et al*. (2011) reported that the symptoms of *A. alternata* in *Euphorbia lathyris* appeared in early summer on stems as circular or irregularly shaped, small, brown to black spots or/and on shriveled leaf apices. The lesions rapidly expanded around the stems or along the leaf blades in the rainy season. Shokooh Farhood and Shervin Hadian (2012) reported that the symptoms of *A. alternata* of gerbera at the initial stage of the infection were brown, small, scattered spots on the leaves that gradually become round or irregular. Spots coalesce to affect large areas of leaves and cause defoliation**.**

3. Pathogenicity of the pathogen

Kamalakannan *et al.* (2008) confirmed the pathogenicity of *A. alternata* on *Aloe vera* by inoculation of spore suspension in pinpricked leaf pieces. Manjunath Hubballi *et al*. (2011) reported that the disease intensity of noni had significantly varied with that of different methods of inoculation, amongst which, the pin prick with spore suspension spray method was the best, which recorded 58.60 PDI, followed by the pin prick with mycelial inoculation method (52.23 PDI). The lowest PDI was however recorded in the mycelial inoculation method (16.37 PDI).

Different other methods have been reported to determine the pathogenicity. A few of them have been discussed below.

(i) Farhood *et al.* (2012) prepared the conidial suspension from 10 days old culture of *A. alternata* and adjusted the conidial concentration to 2×10^5 per ml using haemocytometer. The gerbera plants were cultivated in pots with sterile soil mixture and they were spray inoculated with 100 ml of the spore suspension per pot. Inoculated plants were placed in a humid chamber with 100% relative humidity at 25°C for 24 hours. The plants were covered tightly with a transparent nylon foil, after 24 hours plants were uncovered and constant humidity was maintained by spraying tap water. The plants were grown in a greenhouse (25–30°C) for 2 weeks for the symptom development. After 2 weeks typical symptoms were produced on the inoculated leaves. The pathogen from the infected leaf was re-isolated on PDA medium as described above. The morphological and cultural characteristics of the re-isolated organism were compared with the original pathogen and they were the same.

(ii) Ramjegathesh *et al.* (2012) prepared the conidial suspension ($5x10^5$ spores ml^{-1}) in sterile distilled water from 9 days old PDA culture of the different isolates of *A. alternata.* These spore suspension were sprayed on the 30 days old onion plants. Twelve days after inoculation the symptoms first appeared in the form of small whitish flecks on the leaf tip. The size was increased and became sunken lesions. The lesions gradually exhibited a grayish tint at the centre surrounded by yellow hallow.

(iii) Suman Lata Gupta *et al*. (2013) carried out the pathogenicity test on cotyledon stage at 15 days and 75 days old plants of linseed variety Chambal. Some leaves and buds were inoculated by the pathogen through spraying. Ten plants per pot were raised and three replications were taken for the study. During the pathogenicity test of leaf spot and black bud disease, pathogen (*A. lini*) found to paralyze the leaves, floral organ and buds.

4. Variability of the pathogen

Conidial characters of Alternaria spp.

Anand (2002) observed that the isolates of *A. alternata* fruit rot of chilli varied in morphological character. The colour of the conidia ranged from brown to dark brown. The number of cells per conidium (1-6) as well as length (10-50 µm) and width (5-20 µm) of the conidia varied among the isolates. Kamalakannan *et al.* (2008) reported that the conidiophores of *A. alternata* causing leaf spot on *Aloe barbadensis* were branched, straight, golden brown in colour, measuring 15 mm long and 2-6 mm thick. The conidia were golden brown in colour, produced in long branched chains, obclavate in shape. Dhiraj Singh *et al*. (2009) reported that mycelial radial growth of *A. brassicae* varied from 34.6-81.1 mm with creamish, light brown to dark brown in colour and compressed to fluffy mycelial growth.

The average conidial length ranged from 117.0 to 192.0 µm and breadth from 14.0 to 24.0 µm. The conidial beak length varied from 42.0 to 116.0 µm, number of horizontal/longitudinal septa ranged from 6 to 9 and vertical / transverse septa ranged from 1 to 3.Shiv Shakthi
et al. (2013) reported the variation in radial growth, colour of colony, conidial septation, and the distance between the septum of mustard *Alternaria* blight. Savitha *et al*. (2013) reported that the variability for cultural characters of 14 isolates of *Alternaria* sp. such as colony diameter, colony colour, type of margin and sporulation in sesame *Alternaria* blight.

Virulence of the pathogen

Kamalalakshmi (1996) reported that among six isolates of *A. alternata* on jasmine, isolate from Madurai district was the most virulent and less virulent isolate was from Checkanoorani. Virulence is the measure or degree of pathogenicity of an isolate or race in the host (Singh 2002). Thrall *et al*. (2005) found significant differences in the lesion size produced by the *A. brassicicola* isolates on *Cakile maritima*. Among the two isolates of *A. carthami* one produced more severe leaf spots than the other when inoculated on safflower cultivars (Rai and Kumari, 2009). Deivendran (2009) reported that among the ten isolates of *A. palandui* on onion, isolate from Chempatti was the most virulent followed by Usilampatti. The conidiophore of *A. macrospora* causing leaf blight of Bt-cotton was solitary, simple, straight and pale brown in colour with narrow reddish brown beak (Ramegowda and Naik 2008).

The colony colour may varied from light grey to blackish colour. The conidia of *A. alternata* leaf blight of Bt-cotton reportedly formed in chains of upto seven and were pale brown in colour with several transverse and longitudinal septa. Manjunath Hubballi *et al*. (2011) reported that different isolates of *A. alternata* causing leaf blight of noni varied with respect to virulence. The maximum PDI was recorded in AA1 (61.00 PDI) followed by AA10 (60.33 PDI), AA11 (56.34 PDI) and AA2 (54.07 PDI). The minimum disease intensity was recorded in AA6 which showed 10.33 PDI. The results clearly indicated that the isolate AA1 was highly virulent and isolate AA6 was least virulent. Variation in the virulence may be attributed to the genetic make up of the isolate. Sofi *et al*. (2013) reported that the *A. mali* isolate (Am-1) of apple was highly virulent as compared to other isolates with the highest total lesion area of 122.4 mm.

5. Cultural characteristics

Culture media for the growth of Alternaria spp

Hashem Abeer *et al*. (2014) found that the best media for radial growth and sporulation of *A. alternata* of *Avicennia marina* was potato dextrose agar. Manjunath Hubballi *et al*. (2010) observed the maximum growth of *A. alternata* causing leaf blight of *Morinda citrifolia* in host leaf extract medium, followed by potato dextrose agar (PDA). Parul Trivedi Mishra and Versha Mishra (2012) found that the best growth of the pathogen *A. alternata* of cotton was observed on PDA medium, followed by Richards agar, Czapek's agar, Coon's agar and leaf decoction agar. Dipak *et al*. (2013) found that the mycelial growth and conidial production of A. *alternata* causing leaf blight of gerbera was excellent in nonsynthetic media like oat meal agar and PDA. The best media for growth and sporulation of *A. lini* was potato dextrose agar followed by oat meal and Richard's agar media (Suman Lata Gupta *et al*., 2013). Munde *et al*. (2013) found that the maximum growth of *A. solani* was obtained on yeast extract glucose agar medium followed by soil extract agar medium, oat meal agar and Sabourd's agar

Carbon and nitrogen source for the growth of Alternaria sp.

Singh (2000) reported sucrose as the best carbon source for the growth of *A. porri*. Nallathambi and Thakore (2004) reported that maltose and sucrose favoured highest mycelial growth of *A. alternata*. Ramjegathesh and Ebenezar (2012) reported that the maximum growth of *A. alternata* was recorded in maltose, followed by glucose and sucrose. Rajmane and Korekar (2012) reported that fructose was the best carbon source followed by sucrose for *A. alternata* causing post-harvest disease of papaya fruits. Lactose supported the best growth of *A. porri* followed by galactose and dextrose (Madhavi *et al*., 2012). Ramjegathesh and Ebenezar (2012) reported that maximum growth of *A. alternata* was recorded in potassium nitrate, followed by sodium nitrate and ammonium molybdate, while thiourea had minimum mean mycelial growth. Rajmane and Korekar (2012) reported that sodium nitrate was the best nitrogen source followed by calcium nitrate for fungi *A. alternata* causing post-harvest disease of mango fruits. The fungus *A. porri* exhibited good growth in Czapek's Dox broth with urea as nitrogen source followed by magnesium nitrate (Madhavi *et al*., 2012).

Growth on solid media

Babu (1994) reported that potato dextrose agar medium was the best culture medium for *A. solani* causing leaf blight of tomato, followed by oat meal agar and Czapek Dox agar medium. Potato dextrose agar was the best for *A. alternata* causing leaf blight of tomato and jasmine (Kamalalakshmi 1996). Czapek Dox agar was the best medium for the growth and sporulation of *A. alternata* (Pandey and Vishwakarma, 1998). Jagtap *et al*. (2012) reported that potato dextrose agar was found suitable and encouraged the maximum radial mycelial growth (88.18mm) of the *Alternaria* leaf blight of cotton. Savitha *et al*. (2013) reported that the best media for growth and sporulation of *A. sesami* is potato dextrose Agar (87.33mm). Suman Lata Gupta *et al*. (2013) reported that the maximum mycelial growth of *A. lini* was observed on potato dextrose agar medium, followed by oat meal agar and Richard's agar.

Growth on liquid media

Mohapatra *et al*. (1977) recorded the maximum growth of *A. solani* on potato dextrose broth, followed by Richard's medium, Czapek's Dox and oat meal medium. Babu (1994) found that potato dextrose broth was the best for the growth of *A. solani* inciting tomato leaf blight followed by czapek Dox broth. Kamalalakshmi (1996) reported that potato dextrose broth was the best for the growth of *A. alternata* causing leaf blight of jathimalli. Suman Lata Gupta *et al*. (2013) reported that the maximum mycelial dry weight of *A.lini* was observed on potato dextrose agar medium, followed by oat meal broth and Richard's broth.

Utilization of carbon sources

Goyal (1977) reported that the maltose was found more effective for the mycelial growth of *A. alternata.* Nallathambi and Thakore (2004) reported that when maltose was used as a carbon source in the basal medium, it favored significantly highest mycelial growth of *A. alternata* and the second best was sucrose. Thaware *et al.* (2010) reported that maltose produced the maximum mean mycelial growth of *A. alternata*. Ramjegathesh (2012), recorded that the maximum mycelial growth recorded in maltose (8.82cm), followed by glucose (8.67cm), sucrose (8.29cm) and fructose (7.83cm), while the carboxymethyl cellulose had the minimum mycelial growth (7.39 cm) compared to control respectively.

Utilization of nitrogen sources

Karlatti *et al.* (1990) observed that ammonium nitrate was the best source of nitrogen for the growth of *A. zinnae.* Thaware (2010) reported that ammonium nitrate was relatively more effective for mycelial growth of *A. alternata*. Ramjegathesh (2012) reported that potassium nitrate produced the maximum mean mycelial growth (9.00 cm) followed by sodium nitrate (7.32cm) and ammonium molybdate (6.18cm), while thiourea had minimum mean mycelial growth (2.63cm) in *A. alternata.*

6. Physiological studies

Growth on pH

Awadhiya (1991) found that the spore germination of *A. carthami* causing leaf blight disease of safflower varied at different pH levels and the maximum germination (68%) occurred at pH 6, followed by at 7 (56%). Deivendran (2009) found that the low pH (4.5) was ideal for the growth of *A. palaundi*, while the minimum growth was recorded in pH 8.5 irrespective of the isolates. Patil *et al.* (1995) reported that the optimum pH range for *A. tenussima* was between pH 5.4 and 7.8. Kamalalakshmi (1996) observed the maximum mycelial growth of all the six isolates of *A. alternata* causing leaf blight of Jathimalli was great at pH 5.5 followed by pH 5.0, while the growth was the minimum at pH 8.5. Nallathambi and Thakore (2004) reported that pH 6.5 favoured the maximum growth of *A. alternata.* Parul Trivedi Mishra (2011), observed the maximum fungal growth (493.0 mg) at pH 6.5 followed by 7.0 and 6.0 pH weighing 461.50 and 435.20 mg, respectively. The least growth weighing 72.80 and 54.0 mg were recorded at pH 2.50 and 12.0 which suggested that high alkaline and acidic behaviour did not favour the growth of the pathogen. Neelakanth *et al*. (2012) reported that among the different pH tested, in pH 6.0 mycelial growth was more. Ramjegathesh and Ebenezar (2012) reported that pH 4.5 was favoured to be ideal and produced the maximum mean mycelial growth (8.87 cm), followed by 4.0 (8.66cm), while the minimum growth was observed in pH 9.0. Hiremani *et al.* (2012) reported that pH 6.0 favoured the maximum growth of *A. ricini*. Suman lata gupta *et al*. (2013) found that pH range 6.5 was the best, followed by 7.5 and good at the range 5.5, then by 8.5. It was also observed that pH 4.5 was poor, followed by 3.5 and 9.0.

Thermal death point

Kamalalakshmi (1996) reported that thermal death point for *A. alternata* causing leaf blight of Jathimalli was 48^{o}C**.**

Deivendran (2009) reported that the thermal death point for *A. palandui* was 47^0C. Kannan and Mohan (2010) reported that the conidia of *A. alternata* germinate to a temperature of 45°C, but they failed to germinate at 46°C. Since, this pathogen is reported recently on *Aloe vera*, the variability of the isolates of this pathogen in thermal death point has not been reported earlier. However the same was reported in respect of other species of *Alternaria* affecting other crops. Kamalalakshmi (1996) reported that thermal death point for *Alternaria alternata* causing leaf blight of Jathimalli was 48°C**.** Deivendran (2009) reported that the conidia of all isolates of *A. palandui* (onion leaf blight) were germinated at 46°C. The isolates I_6 and I_8 were failed to germinate at 47 °C.

7. Management of Alternaria infestation Biological control

Efficacy of Phylloplane microflora against Alternaria spp.

Ahmed and Saleh (1989) isolated *Fusarium solani* from tomato phylloplane which inhibited the mycelial growth of *A. solani*. Stretch (1989) isolated *Pseudomonas cepacia*, *Aerobasidium pullulans* and several unidentified isolate from blue berry leaf which showed inhibitory effect against the tomato fruit rot pathogen (*A. alternata*). Tyagi *et al*. (1990) isolated C*ladosporium herbarum*, P*enicillium* sp*., Aeurobasidium pullulans* from the phylloplane of onion and among these *C. herbarum* was the most effective in reducing the mycelial growth of onion leaf blight pathogen *A. porri*. Babu (1994) isolated *Aspergillus* sp*., Fusarium* sp., and a gram positive bacterium from the tomato phylloplane, of which, *Aspergillus* sp. recorded the maximum inhibition of the mycelial growth of *A. solani* causing tomato leaf blight *in vitro.* Kamalalakshmi (1996) reported that phylloplane microflora of jasmine such as *Aspergillus flavus, A. niger, Trichoderma* sp*., Penicillium* sp. and a gram positive bacterium were effectively reduced the mycelial growth of the jasmine leaf blight pathogen, *A. alternata.*

Bernardo *et al*. (2008) found that the two antagonist ic bacteria *viz. Bacillus cereus, Novosphingobium capsulatum* were isolated from the phylloplane region of tomato leaves and are effective in controlling tomato late blight (*Phytophthora infestans*). Sowndhararajan *et al*. (2012) found that the effectiveness of the phylloplane isolates of Bacillus *subtilis* in controlling black rot disease of tea. Gokil Prasad Gangwar (2013) found that spraying of phylloplane isolates of *Pseudomonas sp*. from paddy were effective in reducing disease severity of bacterial leaf blight.

Effect of Bacterial antagonists on Alternaria sp.

Catska (1987) reported that *Azospirillum brasilense, Pseudomonas putida, Agrobacterium radiobacter* inhibited the growth of *A. alternata in vitro*. Casida and Lukezic (1992) observed that application of *Pseudomonas* strain 679-2 on tomato reduced the leaf spot incited by *A. solani*. Kamalalakshmi (1996) observed that *Pseudomonas fluorescens* recorded the maximum growth inhibition of Jathimalli leaf blight pathogen *A. alternate,* followed by *Bacillus subtilis in vitro*. Babu *et al.* (2000) found that spraying of tomato, plants with *P. fluorescens* strains reduced 15-38 % leaf blight incidenced caused by *A. solani*. Babu *et al.* (2000) reported that the effective inhibition of mycelial growth of *A. solani* causing leaf blight of tomato by *B. subtilis.* Adul Hafeez *et al.* (2001) reported that *B. subtilis* effectively inhibited the growth of *A. solani.* Mohan *et al.* (2002) observed that *P. fluorescens* was antagonistic to *A. palandui*. Karthikeyan *et al.* (2005) reported that *P. fluorescens* (Pf_1) was found to inhibit the growth of the pathogen *A. palandui* causing leaf blight of onion. Akbari and Parakhia (2007) observed good antagonism of *B. subtilis* with *A. alternata*. Vihol *et al*. (2009) reported that *P. fluorescens* and *B. subtilis* found to inhibit the mycelial growth of *A. bumsii* by 45.3 and 26.7 per cent, respectively under *in vitro* condition.

Arjunan Muthukumar *et al.* (2013) reported that the *P. fluorescens* isolated from ribbon plant rhizosphere and phylloplane was found to be highly effective in inhibiting the mycelial growth of *A. alternata* causing leaf blight of ribbon plants. Yadav *et al.* (2013) reported that the *P. fluorescens* was found economical and effective for the management of *A. porri* causing purple blotch of onion followed by *B. subtilis.* Angayarkanni *et al.* (2014) reported that *P. fluorescens* isolate AUPF6 showed broad-spectrum protection against stevia leaf spot pathogen *A. Alternata.* Angayarkanni *et al*. (2014) reported that *P. fluorescens* isolate AUPF6 showed broad-spectrum protection against stevia leaf spot pathogen *A. alternata***.**

8. Mode of action of bacterial antagonists

Hydrogen cyanide

Ahl *et al*. (1986) detected the production of cyanic acid by *P. fluorescens* on cotton roots which was inhibitory to *Theilaviopsis basicola.* The involvement of HCN in the suppression of plant pathogens was reported by several workers in various hosts (Voisard *et al.* 1989; Weststeijn, 1990). HCN produced by *P. fluorescens* strain CHA0 contributed to the suppression of black root-rot of tobacco caused by *T.basicola* (Defago *et al.,* 1990). Wei *et al*. (1991) reported the production of HCN by *P.*

fluorescens G8-4, *P. aureofaciens* 28-9 and 36-5 and *P. putida* 34-13 *in vitro*. The production of volatile cyanide was very common among the rhizosphere *Pseudomonads* (Dowling and O'Gara, 1994).

Five cotton rhizobacteria (CRb) *viz*. antagonistic to race 32 of *Xanthomonas axonopodis* pv. *malvacearum* (Xam R-32), *Pseudomonas alcaligenes* (CRb-9 and CRb-14), *P. fluorescens* (CRb-26 and CRb-39) and *P. putida* (CRb-17), the produced antimicrobial secondary metabolites including HCN and siderophores (Mondal *et al*., 2000). HCN from *P. fluorescens* strain CHAO played a significant role in disease suppression of *Fusarium oxysporium* f. sp., *lycopersici* in tomato (Duffy and Raaijmakers 2003). Kumar *et al*. (2009) demonstrated that *Pseudomonas aeruginosa* LES4, an isolate of tomato rhizosphere was found to have positive plant growth promoting attributes like production of indole acetic acid, HCN and siderophore, solubilization of inorganic phosphate along with urease, chitinase and ß-1, 3 glucanase activities. In addition, it showed strong antagonistic effect against *M. phaseolina* and *F. oxysporum.* Muthukumar *et al.* (2010) found that the *P. fluorescens* isolate EBS 20 produced higher levels of extracellular metabolites like HCN when compared with other isolates which was highly effective in inhibiting the growth of *Pythium aphanidermatum* inciting chilli damping-off.

Siderophores

Siderophores are low molecular weight compounds that are produced under iron limiting condition, chelate the ferric ion (Fe^{3+}) with a high specific activity, and serve as vehicles for the transport of Fe (III) into a microbial cell (Neilands, 1981). Most of them are either hydroxymate or catecholate groups that are involved in iron (III) chelation (Neilands, 1981). Misaghi *et al*. (1982) reported for the first time that fluorescent pigment produced by *P. fluorescens* exhibited fungistatic property by inhibiting the growth of *Geotrichum candidum.* Siderophore produced by *Pseudomonas* sp. and the other similar rhizobacterial organisms (*Bacillus, Enterobacter*) have been used in the biological control of damping off of cotton caused by *Pythium ultimum* (Laha *et al*., 1992). Siderophores called pyoveridines have been implicated in ISR induced by Fluorescent Pseudomonads (Duijff *et al.,* 1993; Maurhofer *et al.,* 1994). Pseudomonads also produced two other siderophores, pyochelin and its precursor salicylic acid and pyochelin which was thought to contribute to the protection of tomato plants from *Pythium*, *P. aeruginosa* (Buysens *et al*., 1996). The catechol siderophore biosynthesis gene in *S. marcescens* associated with ISR was reported to be responsible for disease suppression (Press *et al.,* 2001). Kavitha (2004) reported that the production of siderophores by *B. subtilis* isolate M3 and salicylate type of siderophore by the isolate CBE4 of *B. subtilis.* Rajkumar (2006) reported the production of siderophores by *P. fluorescens* isolate 1 and salicylate type of siderophore by the isolate of *P. fluorescens*. Umamaheswari *et al*. (2008) reported the production of siderophores from two strains of *P. fluorescens*. Muthukumar *et al.* (2010) found that the *P. fluorescens* isolate EBS 20 produced higher levels of extracellular metabolites like siderophore when compared with other isolates which was highly effective in inhibiting the growth of *Pythium aphanidermatum* inciting chilli damping-off.

Salicylic acid production (SA)

Meyar *et al.* (1992) demonstrated that production of SA by *P. aeruginosa* strain 7NSK2 was essential for induction of resistance in bean against *Botrytis cinerea* whereas SA deficient mutants were not able to induce resistance. Muthukumar *et al.* (2010) found that the *P. fluorescens* isolate EBS 20 produced higher levels of extracellular metabolites like salicylic acid when compared with other isolates, which was highly effective in inhibiting the growth of *Pythium aphanidermatum* inciting chilli damping-off. Savitha *et al.* (2011) reported that salicylic acid at 1% was effective in suppressing the pathogen *A. sesami* causing leaf blight of sesame and resulted in higher vigour index.

9. Organic amendments in disease management

Effect of oil cakes on Alternaria spp.

Khan *et al*. (1973) reported that the neem cake reduced the *A. tenuis*. Kannan and Mohan (2010) reported that the minimum diameter of mycelial growth (2.71cm) and the maximum percentage of inhibition (68.74%) were recorded in mahua cake, followed by neem seed kernel extract (3.33 cm and 61.59%), pungam cake extract (3.43cm and 60.43%) and neem cake extract (4.27 cm and 50.74%) in *A. alternata* causing leaf blight of *Aloe vera*.

Effect of plant oils on Alternaria spp.

Kamalalakshmi (1996) found that palmarosa oil (0.025%) caused total inhibition of both the mycelial growth and spore germination of the pathogen *A. alternata* causing leaf blight of Jathimalli followed by neem oil (3%), neem cake extract (10%), neem seed kernel extract (5%). Neem oil at three per cent controlled the leaf blight incited by *A. solani* and reduced the disease upto 53.0 per cent in field condition (Babu *et al*.,

2000). Izabela *et al*. (2004) reported out the active compound carotol from carrot seed oil inhibited the growth of *A. alternata* of carrot by 65 per cent. Karthikeyan *et al.* (2005) reported that the neem oil reduced the leaf blight disease of onion incited by *A. palandui*. Nehal *et al.* (2009) observed highest reduction in disease incidence of *A. solani* causing early blight of potato and yield increase in treatments with per cent of carnation, caraway, and thyme oils. Kannan and Mohan (2010) reported that eucalyptus oil (2%) was recorded highest reduction of mycelial growth (70.00%) of the pathogen *A. alternata* causing leaf blight of *Aloe vera*. Ramjegathesh *et al.* (2011) found that spraying of neem oil (3%) was effective against the *A. alternata* causing leaf blight of onion. Chethana *et al.* (2012) reported that the neem oil and pongamia oil (2.0%) recorded 76.94 and 69.94 per cent inhibition respectively in *A. porri* causing purple blotch disease of onion. Sutha Rani (2013) reported that mahua oil (3%) registered the maximum mycelial growth inhibition of *R. solani,* followed by neem oil (3%).

Effect of plant extracts on Alternaria spp.

Shekhawat and Prasad (1971) found that garlic and onion extract gave good inhibition to spore germination of *Alternaria tenuis, Helminthosporium sativum* and *Curvularia penniseti* due to presence of allicin in garlic and protocatechuic acid and catechol in onion, which were responsible for bursting the young hyphae of fungus. Misra *et al*. (1974) tested leaf extracts of *Allium sativum* and *Ranunculus clematis* on spore germination of *Alternaria alternata, Helmithosporium gramineum* and *Curvularia lunata* and reported that plant extracts showed antifungal activity against all these test fungi. Antifungal activity of some leaf extracts of medicinal plants on *C. lunata* was reported by Bhowmick and Vardhan (1981). Abraham and Prakasan (2001) reported that the 10 per cent concentration of leaf extract of *Azadirachta indica, Ocimum sanctum* and *Vitex negundo* proved inhibitory against *C. lunata.* Karthikeyan Muthuswamy *et al.* (2007) reported that the leaf extract of zimmu exhibited strong antifungal activity against *A. flavus, F. moniliforme, C. lunata* and *A. alternata* and caused *in vitro* fungal growth inhibition of 73, 71.1, 70.0 and 74.4% respectively. Pawar and Kolhe (2010) reported that the maximum growth inhibition of *Aspergillus flavus* and *C. lunata* were caused by *Adhatoda vasica* where as *Alternaria alternata*, *Fusarium roseum* and *Trichoderma viride* were retarded by *Azadirachta indica* at 10 per cent aqueous leaf extract. Akinbode (2010) showed that *Gliricidia sepium, Tithonia diversifolia, Phyllanthus amarus* and *Morinda lucida* @ 100 per cent of each plant extract incorporated into the growth medium separately was found to be more effective in inhibiting the growth of *C. lunata* when compared to the 75 and 25 per cent.

10. Induction of systemic resistance

The defense enzymes include Peroxidase (PO), Polyphenol Oxidase (PPO), Phenylalanine Ammonia Lyase (PAL) which catalyses the formation of lignin, (Bashan *et al.,* 1985) and also they were involved in phytoalexin and phenolics biosynthesis. Loganathan (2002) reported that induction of defense related proteins *viz.* phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, phenol, chitinase, and β – 1-3- glucanase were found to be in higher levels in treatments involving bioformulation mixture containing *Pseudomonas fluorescens* (Pf_1) against fungal pathogen and root knot nematode in cabbage and cauliflower. Kamalakannan (2004) reported that soil application of bio control agents such as *Trichoderma* species and *P. fluorescens* induced coleus plants to synthesize more amount of peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and total phenols. Saikia *et al.* (2004) reported that the *Pseudomonas fluorescens* has different mechanisms to reduce plant diseases such as accumulation of phenolic compounds, increasing activity of PAL, PR-proteins and lysis of the fungal pathogen cell wall by secretion of extra cellular lytic enzymes. Karthikeyan *et al.* (2005) reported that the Native PAGE of PO showed induction of PO_2 isoform in both the resistance and susceptible cultivar against the leaf blight pathogen *A. palandui* of onion, Isoform analysis of PPO also exhibited induction of pathogen in Pf_1 treated plants challenged with pathogen. Similarly, the activity of β–1-3-glucanase was greatly induced in Pf_1 treated plants challenged with pathogen as compared to controls. Thus, the *P. fluorescens* treated onion plants showed significant increase in the levels of PO, PPO and β-1-I-gulcanase enzymes in comparison to the plants, challenged with the pathogen. Delivery of *P. chlororaphis* and *B. subtilis* as seed treatment (Nakkeeran *et al*., 2006), seedling dip and soil application controlled damping-off of hot pepper by inducing the defense gene products such as peroxidase, polyphenol oxidase, phenylalanine ammonia - lyase, chitinase and glucanase. Anand *et al.* (2009) studied the induction of defence compounds and enzymes involved in the Phenylpropanoid pathway ripe and green chilli fruits inoculated with *C. capsici* and *A. alternata*. Total phenol and activity of PAL, PO, PPO, and catalase increased in the inoculated ripe and green chilli fruits compared to the corresponding healthy fruits. Malini (2011) revealed that seedling root dipping in 0.4 per cent *Streptomyces exfoliatus* plus foliar application of 0.2 per cent *S. exfoliatus* 15, 30 and 45 DAP and the another treatment comprising seedling root dipping in 0.4 per cent *S.*

violaceusniger plus FA of *S. violaceusniger* @ 0.2 per cent 15, 30 and 45 DAP recorded higher activity of PO, PPO and PAL enzymes in leaves against *Helminthosporium oryzae.*

Peroxidase (PO)

Bruce and West (1989) reported that the PO is a key enzyme in the biosynthesis of lignin. Peroxidase is one of the key enzymes involved in the phenyl propanoid pathway and it is involved in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross linking, IAA oxidation, cross linking of extension monomer, oxidation of hydroxy – cinnamoyl alcohols into free radicals intermediates and wound healing (Vidhyasekaran *et al.,* 1997a). Ramamoorthy *et al.* (2002) reported that the high-level expression of PO in *P. fluorescens* (Pf1) treated tomato plants challenged with *F. oxysporum* f. sp. *lycopersici.* Rajkumar (2006) reported that the peroxidase activity was significantly increased from second day after inoculation and it was maximum on five days after inoculation in banana but an increase in the activity was recorded upto seven days after inoculation with the consortia formulation of BPf1 + BBs1, which was challenged with *E. caratovora* sp. *caratovora*. Ashok Kumar Meena and Godara (2011) reported that the specific activity of peroxidase remained higher in diseased leaves of susceptible as compared to moderately resistant and resistant varieties of clusterbean.

Angayarkanni *et al.* (2014) reported that in stevia plants, the expression of PO isoforms, PO1and PO2 was greater in *P. fluorescens* pretreated plants challenged with the pathogen and also plants inoculated with the pathogen alone than in untreated plants or plants treated with *P. fluorescens* alone. Angayarkanni *et al.* (2014) reported that the higher PO activity was noticed in cucumber roots treated with *P. corrugate* challenged with *Pythium aphanidermatum*.

Poly Phenol Oxidase (PPO)

Nakamura and Oku (1960) reported increased activity of PPO in both ripe and green chilli fruits inoculated with *C. capsici* and *A. alternata.* Chen *et al.* (2000) reported that PPO was stimulated by PGPR or by the pathogen, but the wounds on split roots did not influence PPO activity compared to intact control in 13 days. Gadre *et al.* (2002) reported that the results showed that polyphenol content was high in resistant, medium in moderately resistant and low in susceptible cultivars. The content was reduced due to disease infection but the reduction per cent was more in susceptible cultivar. Umamaheswari *et al.* (2009) reported that application of *B. subtilis* (Bsw1) two days prior to the pathogen *A. alternata* (causing leaf blight of watermelon) induced a two fold increase in PPO activity on six and nine days after inoculation compared to plants sprayed with the pathogen alone. Ashok Kumar Meena and Godara (2011) reported that the Polyphenol oxidase activity was found higher in moderately resistant varieties, followed by susceptible and resistant varieties of clusterbean. Therefore, Poly Phenol Oxidase enzymes play an important role in defence mechanism against *Alternaria* blight in clusterbean.

Phenylalanine Ammonia Lyase (PAL)

Chen *et al.* (2000) reported that the plants treated with *Pseudomonads* strains initially showed higher levels of PAL as compared to control. Ramamoorthy *et al.* (2002) reported that the increased activity of PAL was in *P. fluorescens* isolate Pf1 treated tomato and pepper seedlings challenged with the *F. oxysporum* f. sp. *lycopersi*ci and *Colletotrichum capsici*. Kavino *et al*. (2008) and Sullivan (2009) reported that the PAL activity could be induced during plant pathogen interactions. Radja Commare *et al.* (2004) reported that seedling dip with talc based formulation of *P. fluorescens* induced the activity of PAL in finger millet leaves against blast disease. Pf4-99 treatment was highly effective (Vinod Kumar *et al.,* 2007) in enhancement of PAL activity in chickpea against the root rot pathogen *Macrophomina phaseolina*. Umamaheswari *et al.* (2009) observed that activity of PAL in watermelon pre- treated with biocontrol agent was induced upon challenge inoculation with *A. alternata*. The PAL activity was significantly higher in plants pre- treated with *B. subtilis* (Bsw_1) with subsequent pathogen inoculation. The plants treated with pathogen alone have also enhanced PAL activity compared to healthy control. Recently, Angayarkanni *et al.* (2014) reported that invasion of root tissues by the pathogen might have resulted in decreased activity of PAL whereas earlier an increased activity of PAL due to *P. fluorescens* isolate AUPF6 treatment might have prevented fungal invasion and thus the activity was maintained at the higher levels.

Total Phenols

M'Piga *et al*. (1997) reported that the total phenol content increased in ripe as well as green chilli fruits in response to inoculation with *C. capsici* or *A. alternata.* The increase was very rapid and much more conspicuous in green chilli fruits than in ripe chilli fruits. One of the immediate host responses to infection is the accumulation of phenolics around the infection site.

Renuka *et al.* (2007) reported that the biochemical analysis of chrysanthemum plant infected with leaf blight *A. chalmydospora* revealed that the quantity of total sugars, reducing sugars, non reducing sugars and phenols were less in top, middle and bottom healthy leaves than in the diseased leaves. Higher content of sugars and phenols were recorded only in bottom infected leaves when compared to top and middle leaves.

Vinod Kumar *et al.* (2007) reported higher accumulation of phenols content in chickpea in prior application of Pf4-94 challenged with *Macrophomina phaseolina.* Angayarkanni *et al.* (2014) reported that leaf spot disease caused by *A.alternata* of *Stevia rebaudiana.* Bertoni has maximum phenolic content was observed in *P. fluorescens* pretreated plants challenge inoculated with the inoculation of pathogen and the higher amounts of phenolics were noticed upto 5th day after the pathogen challenge and remained at higher level. In plants inoculated with the pathogen alone the phenolic content declined to the initial level on the seventh day after inoculation. Plants treated with *P. fluorescens* alone had also increased content of phenolics compared to untreated plants.

Conclusion

The above review clearly indicates that *Alternaria* is a destructive pathogen causing widespread destruction in vegetables and economically important crops. But with the utilization of advanced knowledge, tools and techniques in plant disease management it becomes easier to control this cosmopolitan fungus. Knowledge at molecular level, studying the role of phytotoxic secondary metabolites, utilization of various biotechnological tools like gene alteration- disruption will help in developing of resistant varieties against *Alternaria* species. From the perspective of hill agriculture, the incidence and severity of foliar diseases caused by *Alternaria* species was more in major crop plants of Himachal Pradesh, Uttrakhand, Arunachal Pradesh, Jammu and Kashmir in the Himalayas and in Maharashtra, Tamil Nadu and Kerala in the Western Ghats. The high incidence levels of *A. alternata* observed, suggest that it may be determine, amongst other mycotoxins, if *Alternaria* occur in these commodities. Cultural practices, chemical control, integrated disease control are the common practices followed in hilly areas that could possibly influence the management of *Alternaria*. Current practices for controlling plant diseases are however based largely on genetic resistance in the host plant, crop management and its environment, and use of synthetic pesticides. Application of fungicide is the common practice for the management of *Alternaria* species.

But keeping health hazards in view, alternate and eco-friendly methods of disease control *viz*. use of bio-control agents, plant and natural products, growing disease resistant varieties and alteration in the agronomic practices are advisable to ensure sustainability in agricultural production and that also can help maintain the ecology of the agro-ecosystem *per se.*

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