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REVIEW

Alternaria pathogenicity and its strategic controls

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ABSTRACT

The Deuteromycetes fungal genus *Alternaria* comprises of different saprophytic as well as endophytic species and is well known for its notoriously destructive plant pathogen members. It has been found to have a drastic effect on the members belonging to the plant families such as Cucurbitaceae, Brassicaceae, Solanaceae which are having nutritional as well as economical food value. Majority of the members of *Alternaria* lack sexuality altogether, although few species have been found to have sexual stage in their life cycles. Several types of genes ranging from protein encoding genes to those involved in signal transduction cascades are found to be responsible for the pathogenesis. Production of host-specific toxins (HSTs) is found to be an affirming factor of pathogenesis. Most fungal host-specific toxins are metabolites although toxic substances including despipeptides and fucicoccin-like compounds. Genes encoding the biosynthesis of these HSTs are often contained on mostly conditionally dispensable chromosomes. The necrotrophic nature of *Alternaria* species typically leads to extensive damage of the plant and harvest product, with seedlings seldom surviving an attack. Apart from the role of toxins in *Alternaria* pathogenesis, few genes and /or gene products have been found to have a propounding effect as a pre-requisite for pathogenicity. For controlling the diseases, numbers of new chemicals are evaluated along with various biological control agents including bacteria, actinomycetes and fungi. Some plants and plant products are also found to be useful in controlling *Alternaria* infection.

Key Words: *Alternaria*, crop pathology, disease management, pathogenicity factors, toxins.
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1. Introduction

Mustard (*Brassica juncea*) forms an important part of the total oilseed production in India. As far as the statistical figures are concerned, out of 75.55 million tonnes of estimated rapeseed (*Brassica napus*) and mustard production over 30.51 m ha across the Globe, India produces 7.36 m tonnes from 6.18 m ha with 1190 kg/ha productivity (GOI, 2009; Meena *et al.*, 2010). As far as the Indian perspective of the disease is concerned, the losses caused by the disease is estimated to be 47% of the yield loss (Kolte, 1985) with no established source of transferable resistance in any of the hosts. Average yield losses in the range of 32-57% due to *Alternaria* blight have been reported by several workers (Conn and Tewari, 1990).Therefore, studies on the effective control of diseases caused by *Alternaria* is of utmost importance.

The focused pathovars belonging to the genus *Alternaria* affect most Cruciferous crops, including broccoli and cauliflower (*Brassica oleracea* L. var. *botrytis* L.), field mustard and turnip (*B. rapa* L. (synonym: *B. campestris* L.), Chinese mustard or leaf mustard (*B. juncea*), Chinese or celery cabbage (*B. pekinensis*), cabbage (*B. oleracea* var. *capitata*), rape (*B. campestris*) and radish (*Raphanus sativus*).

The genus *Alternaria* belongs to the Phylum: Ascomycota, Subdivision: Pezizomycotina, Class: Dothediomycetes, Order: Pleosporales and Family: Pleosporaceae. *Alternaria* belongs to the division Deuteromycota with several species. Its multicellular pigmented spores are produced in chains or in branching fashions. The spores are broadest near the base and taper gradually to an elongate beak. When *Alternaria* attacks the host leaf, morphologically it produces a series of concentric rings around the initial site of attack. This gives a "target spot" effect that is associated with early blight. Species of the genus are cosmopolitan and can survive as saprophytes as well as weak parasites. 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 telomorphs (sexual stage) are known in a very few species and placed in the genus *Pleospora* of Loculoascomycetes (under Sub-division: Ascomycotina), in which sleeper-shaped, 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, 2008). Works pertaining to the collection, isolation and characterization of other *Alternaria* species are also being carried out for its elaborate studies. *Alternaria alternate* caused early blight of potato, leaf spot disease in *Withania somnifera* (Pati *et al.*, 2008) and can infest many other plants. It also causes upper respiratory infections in AIDS patients, asthma in people with sensitivity and has been implicated in chronic rhinosinusitis. *Alternaria arborescens* (causes stem canker of tomato), *Alternaria arbusti* (causes leaf lesions on Asian pear), *Alternaria blumeae* (causes lesions on *Blumea aurita*), *Alternaria brassicae* (infests many vegetables and roses), *Alternaria brassicicola* (grows on cole crops), *Alternaria brunsii* (causing cumin bloosem blight), *Alternaria carotiincultae* (causes leaf blight on carrot), *Alternaria carthami*, *Alternaria cinerariae*, *Alternaria citri*, *Alternaria conjuncta* (grows on parsnip), *Alternaria dauci* (grows on carrot), *Alternaria dianthi*, *Alternaria dianthicola*, *Alternaria euphorbiicola* (infests cole crops), *Alternaria gaisen* (causes ringspot disease of pears), *Alternaria helianthicola*, *Alternaria hungarica*, *Alternaria infectoria* (infests wheat), *Alternaria japonica* (infests cole crops), *Alternaria limicola* (earliest diverging lineage of Section Porri), *Alternaria linicola*, *Alternaria longipes* (infects tobacco), *Alternaria molesta* (may cause skin lesions on porpoises), *Alternaria panax* (causes ginseng blight), *Alternaria petroselini* (causes parsley leaf blight), *Alternaria radicina* (causes

carrot decay), *Alternaria raphani*, *Alternaria saponariae*, *Alternaria selini* (causes parsley crown decay), *Alternaria senecionis*, *Alternaria solani* (causes early blight in potatoes and tomatoes), *Alternaria smyrnii* (infests alexanders and parsleys), *Alternaria tenuissima*, *Alternaria triticina*, *Alternaria zinniae*

2. Disease symptoms caused by *Alternaria*

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% (Conn and Tewari, 1990). Symptoms of this disease include presence of irregular, often circular brown to dark brown colour leaf spots on the leaves with concentric lines inside the spots. Often the circular spots coalesce to form large patches resulting in the leaf blight. In several cases, small dark coloured spots are also formed on pods and tender twigs (Valkonen and Koponen, 1990). For *Alternaria* blight management, early sowing (Meena *et al.*, 2002) of properly stored clean certified seeds after deep ploughing along with clean cultivation, timely weeding and maintenance of optimum plant population , avoidance of irrigation at flowering and pod formation stages are some of the steps to be followed for an efficient management of the disease.

3. Biology of *Alternaria*

Conidiophores of majority of the species of *Alternaria* produce asexual spores (conidia) measuring between 160-200 µm long. Under *in vitro* conditions, sporulation occurs at a temperature range of 8-24 ºC, where mature spores occur after 14-24 h. Optimum temperatures are between 16 and 24 ºC where sporulation time ranges from 12 to 14 h. Moisture in the presence of rain, dew or high humidity are essential for infection and a minimum of 9-18 h are required for majority of the species (Humperson-Jones and Phelps, 1989). Continuous moisture of 24 h or longer practically guarantees infection (Rangel, 1945; Chupp and Sherf, 1960). Relative humidity of 91.5% (at 20 ºC) or higher will result in the production of large numbers of mature spores in 24 h (Humpherson-Jones and Phelps, 1989).

4. Epidemiology

The major sources of transport of these pathogens are the infected seeds with spores on the seed coat or the presence of mycelium under the seed coat. The dissemination of spores occurs by wind, water, tools and animals. The fungus can survive in susceptible weeds or perennial crops (Rangel, 1945; Chupp and Sherf, 1960; Maude and Humpherson-Jones, 1980a, b). Presence of infected crops left on the ground after harvest also serves as a source of infection for majority of the *Alternaria* species. In one study, infected leaves of oilseed rape and cabbage placed outdoors on soil produced viable spores for as long as leaf tissues remained intact. For oilseed rape, this was up to 8 weeks and for cabbage up to 12 weeks (Humpherson-Jones, 1989). This type of spread is likely to occur in seedling beds as well, and seedlings from infected seed beds can carry the inoculum to the field (Rangel, 1945).

5. *Alternaria* toxins

A number of plant pathogenic fungi including *Alternaria* produce toxins that can damage plant tissues. Toxins are often classified as host selective (host specific) or nonspecific. Host-selective toxins (HSTs) are toxic only to host plants of the fungus that produces the toxin. In contrast, nonspecific toxins can affect many plants regardless of whether they are a host or non-host of the producing pathogen. Yoder (1980) classified toxins of plant pathogens as a pathogenicity factor or a virulence factor by considering the possible involvement of toxins in pathogenesis: pathogenicity is the ability to cause disease (a qualitative term), whereas virulence refers to the extent or severity of the disease caused (a quantitative term). Most HSTs are considered to be pathogenicity factors, which the fungi producing them require to invade tissue and induce disease (Wolpert *et al.*, 2002; Howlett, 2006). All isolates of the pathogen that produce an HST are pathogenic to the specific host; all isolates that fail to produce HSTs lose pathogenicity to the host plants. Plants that are susceptible to the pathogen are sensitive to the toxin. Such correlations between HST production and pathogenicity in the pathogens and between toxin sensitivity and disease susceptibility in plants provide persuasive evidence that HSTs can be responsible for host-selective infection and disease development. On the other hand, the exact roles of nonspecific toxins in pathogenesis are largely unknown, but some are thought to contribute to features of virulence, such as symptom development and in plant-pathogen propagation.

The *Alternaria* HSTs involve a diverse group of low-molecular-weight substances, and most were found in culture filtrates as families of closely related compounds. The Alternaria HSTs cause necrosis on leaves of susceptible cultivars at concentrations as low as 10^{-8} to 10^{-9} M and no necrosis on leaves of resistant cultivars even at higher concentrations (Otani *et al.*, 1995). Several different types of genes have been found to be responsible for the pathogenicity of the fungus. Genes encoding for different physiological parameters such as cell wall degrading enzymes, toxins and transporter proteins involved in signal transduction cascades such as mitogen activated proteins (MAP) kinases are some of the different types of genes responsible for the pathogenicity. The toxins produced by the different pathotypes of *Alternaria* are mainly low molecular weight secondary metabolites. Some of the types of toxins are reported to have a sphingolipid like molecular structure (Wang *et al.*, 1996; Gilchrist, 1997). Other types of toxins include some desipeptide- based molecules (Johnson *et al.*, 2000). Most fungal toxins are metabolites but in some cases a toxic peptide has been found to be a major virulence factor such as in the case of wheat pathogen *Pyrenophora triticirepentis* (Ballance *et al.*, 1989; Tomas *et al.*, 1990; Tuori *et al.*, 1995). Likewise, proteinaceous toxin (AB- toxin) is produced by *A. brassicola* and is produced only on host plants (Otani *et al.*, 1998).

A*lternaria* species also produce types of toxins that are non-host specific. In addition to AB- toxin, other toxic substances including desipeptides and fucicoccin- like compounds are also being produced by different pathotypes of *Alternaria* (McKenzie *et al.*, 1988; Cooke *et al.*, 1997; MacKinnon *et al.*, 1999). Although different structurally diverse suits of toxic substances are being produced by *Alternaria* species, some pathotypes of the species share common toxin biosynthetic building blocks (Nakashima *et al.*, 1985; Nakatsuka *et al.*, 1986, 1990; Feng *et al.*, 1990; Kohmoto *et al.*, 1993). With the DNA sequences corresponding to the toxin biosynthetic genes becoming available, two characteristics became evident: 1) these genes were part of larger gene clusters responsible for toxin production; 2) these toxin biosynthetic clusters

were localized to the small chromosomes noted previously (Akamatsu *et al.*, 1997). Studies on different *Alternaria* pathotypes reveal that the fungi bearing the additional chromosomes could be cured of them or lose them through repeated sub-culturing, suggesting that they might be not required for normal saprophytic growth implying that genes located on these elements might confer selective advantages in certain situations or ecological niches (Johnson *et al.*, 2000). In light of this fact, it has been found by Masunaka *et al*. (2005) that there is a strong possibility of an occurrence of a genetic hybrid.

6. The *Alternaria* pathosystem

Brassicaceae, the crucifer plant family, consists of approximately 3,500 species in 350 distinct genera. The important crop species keeping in view the economic perspective falling in the genus *Brassica* include *B. oleracea* (vegetables), *B. rapa* (vegetables, oilseeds, and forages), *B. juncea* (vegetables and seed mustard), and *B. napus* (oilseeds) (Westman *et al.*, 1999). Black spot disease caused by *Alternaria* brassicola is of worldwide economic importance (Humpherson-Jones and Maude, 1982a, b; Humpherson-Jones, 1983, 1985, 1989; Humpherson-Jones and Phelps, 1989; Rotem, 1994; Sigareva and Earle, 1999a). The black spot can be a devastating disease resulting in 20-50% yield reductions in crops such as canola or rape (Rotem, 1994). *A. brassicicola*, however, is not limited to infection of leaves, and can infect all parts of the plant including pods, seeds, and stems, and is of particular importance as a post-harvest disease (Rimmer, 1995). The necrotrophic nature of the *Alternaria* species leads to extensive damage of the plant and harvest product (Humpherson-Jones, 1985; Rimmer, 1995). Spread of the disease is mainly by the rain and wind dislodged spores. The optimum conditions for sporulation and infection include a minimum wet period of 13 h and ambient temperatures of 20-30 ºC (Humpherson-Jones and Phelps, 1989; Rotem, 1994). Some weedy cruciferous plants such as *A. thaliana*, *C. sativa* and *C. bursa-pastoris* have been found to have immunity against the pathogen but no satisfactory source of resistance has been identified among cultivated *Brassica* species (Conn *et al.*, 1988; Sigareva and Earle, 1999a, b; Westman *et al.*, 1999).The genetic basis for the resistance have been found to involve additive and dominant gene action (King, 1994).

7. Identification of pathogenicity factors

The work done by Yao and Koller (1994, 1995), Berto *et al.* (1999) and Cho *et al.* (2006) reveal the functional redundancy of lipases in regards to pathogenicity. Interestingly, one of the factors responsible for the pathogenicity has been predicted to be secondary metabolite production. Recently a non-ribosomal peptide synthase gene (*NPS6*) in *Cochliobolus heterostrophus* and *A. brassicicola* was found to direct the biosynthesis of a siderophore metabolite important for oxidative stress tolerance and pathogenicity (Oide *et al.*, 2006). The secondary metabolite corresponding to or synthesized via *AbNPS2* has yet to be characterized. Clearly more research is needed to further characterize secondary metabolite biosynthetic genes and their role in pathogenicity and fungal development. Another important area of investigation in the *Alternaria*-Brassicaceae pathosystem is the fungal signal transduction. For example, disruption of the Fus3/Kss1 MAP kinase homolog (Amk1) in *A. brassicicola* resulted in a complete loss of pathogenicity as observed in other fungi (Cho *et al.*, 2006, 2007). Interestingly, in the latter study it was shown that addition of long polypeptide nutrients partially restored pathogenicity to the mutants. In addition, two novel virulence factors by Cho *et al*. (2008) were predicted to encode a transcription factor (AbPro1) and a two-component histidine kinase gene (AbNIK1). Both of these kinases are pathogenicity factors in phytopathogenic fungi. Slt2 was found to be associated with cell wall integrity and HOG with oxidative stress tolerance (Xu, 2000). Another major work pertaining to the studies related to the identification of virulence factors was the disruption of Aso-1, a gene required for hyphal fusion (anastomosis) which was also found to be required for pathogenicity in *Alternaria* species (Craven *et al.*, 2008). Eventually, over a hundred genes have been functionally analyzed through various techniques like gene knockout and overexpression experiments making *A. brassicicola* the species of choice for functional genomics research to define conserved virulence mechanisms for this important genus of fungi (Oide *et al.*, 2006; Cho *et al.*, 2006, 2007; Kim *et al.*, 2007; Cho, 2008).

With the objective of identification of *A. brassicola*, an attempt was made to examine the role of cutinase genes in *A. brassicicola* pathogenesis (Yao and Koller, 1994, 1995). In these studies, biolistic transformation was used to disrupt the *CUTAB1* gene. Disruption of *CUTAB1* affected saprophytic growth since cutin was no longer able to be utilized as a sole carbon source, but this disruption had no significant effect on *A. brassicicola* pathogenicity. An extracellular lipase was found to be produced by *A. brassicicola in vitro* (Berto *et al.*, 1999). In this study anti-lipase antibodies were found to significantly decrease of the ability of *A. brassicicola* to cause disease on cauliflower leaves. However, disruption of four predicted *A. brassicicola* lipase genes expressed during plant infection did not result in reduced virulence on cabbage (Cho *et al.*, 2006).

One area of interest regarding *A. brassicicola* pathogenicity lies in the area of secondary metabolite biosynthesis. Recently a non-ribosomal peptide synthase gene (*NPS6*) in *Cochliobolus heterostrophus* and *A. brassicicola* was found to direct the biosynthesis of a siderophore metabolite important for oxidative stress tolerance and pathogenicity (Oide *et al.*, 2006). In another study, a non-ribosomal peptide synthase gene (*AbNPS2*) was found to be important for cell wall integrity, conidial viability, and virulence of aged spores of *A. brassicicola* (Kim *et al.*, 2007). The secondary metabolite corresponding to or synthesized via *AbNPS2* has yet to be characterized. Clearly more research is needed to further characterize secondary metabolite biosynthetic genes and their role in pathogenicity and fungal development.

Another area ripe for exploration in the *A. brassicicola*-Brassicaceae pathosystem is fungal signal transduction mechanisms. Disruption of the Fus3/Kss1 MAP kinase homolog (*Amk1*) in *A. brassicicola* resulted in a complete loss of pathogenicity as observed in other fungi. Interestingly, in the latter study it was shown that addition of long polypeptide nutrients partially restored pathogenicity to the mutants (Cho *et al.*, 2006, 2007).

8. Disease management

Since a number of *Alternaria* species infect crops of economic importance, there is a strong need to effectively control for this pathogen. There are different methods which are therefore needed for its control.

By Planning
The planting of susceptible varieties in field should be avoided with infected residues from a previous crop retained on the surface.

By Ground Preparation
The residues from the previous crop should be incorporated. Apart from this, balanced crop nutrition especially of potassium should be provided.

By Fungicides
One of the most effective measures to control the disease caused by *Alternaria* is the effective application of fungicides. Thiram (75%) proved as the most effective fungicide at 5000 ppm while complete inhibition of *Alternaria* was noticed at 10,000 ppm in the case of Thiram (TMTD 80%) and Arasan 50% (Sahni and Singh, 1967). Apart from this, work done by Fugro *et al.* revealed that Dithane M-45 was significantly superior to others against *A. cucumerina* causing leaf blight of watermelon. It was followed by Bavistin, Dithane Z-78, Difoltan, Blitox and Bordeaux mixture. Similarly for control of *Alternaria* blight of cauliflower, Captafol was found to be the best followed by Dithane M-45 to provide maximum yield (Sinha and Prasad, 1989) where as for *Alternaria* blight of radish seed crop, Dithane M-45 (0.25%) proved most effective, followed by 0.4% Bordeaux mixture (Hussaini and Singh, 1989). Mancozeb (0.2%) was found most effective for inhibiting the mycelial growth of *A. solani* (Choulwar *et al.*, 1989). The effectiveness of Mancozeb in controlling early blight of tomato was confirmed by Singh *et al.* (2001). Different hormones such as Indole-3-Butyric Acid or Naphthalic acid at 200 µg/lit concentrations for 30 min have been found to delay the fruit rot caused by *A. alternata* (Datar, 1996). In controlling *Alternaria* blight of potato, the combination of Emisan-6 with Indofil M-45 was found to be most effective followed by the combination of Emisan-6 with Indofil Z-78 (Singh *et al.*, 1997). Mancozeb followed by Thiram, Bavistin and Iprodione also proved effective as seed dresser. Among non-systemic fungicides Iprodione and Mancozeb and among systemic fungicides thiophanate methyl was found to be effective under in vitro conditions by Prasad and Naik (2003). Singh and Singh (2006) tested efficacy of seven fungicides viz., Chlorothalonil, Copper oxychloride, Azoxystrobin, Propineb, Copper hydroxide, Mancozeb at 2500, 2000, 1000, 500 and 250 ppm and Hexaconazole at 1000, 500, 200, 100 and 50 ppm against *A. alternata* causing blight of tomato. Their observations revealed that all the fungicides significantly reduced the radial growth of the fungus. However, hexaconazole was very effective as it caused 100% growth inhibition (Verma and Verma, 2010). The best control of *Alternaria* leaf spot disease of bottle gourd was obtained by spraying recommended @ 0.2% Indofil M-45 followed by Chlorothalonil, Cuman L, Ridomil, Indofil Z-78, Copper oxychloride, Jkstein and Topsin-M (Katiyar *et al.*, 2001). Indofil M-45, Indofil Z-78, Vitavax and Kavach were found to be most effective in reducing the mycelial growth of *A. alternata* infecting brinjal in vitro followed by Bavistin, Benlate and Thiram (Singh and Rai, 2003). Sidlauskiene *et al.* (2003) found that Amistar was very effective in controlling *Alternaria* leaf spot in cucumber, cabbage and tomato as it reduces the disease incidence by 88-93%; whereas Euparen plus Bion were found to increase biological efficiency (Verma and Verma, 2010). Singh and Singh (2002) reported that three sprays of 0.25% Dithane M-45 proved superior to other fungicides e. g., Kavach, Foltaf, Bayleton, Baycor and Contaf 5 EC, in terms of additional yield. They advocated three sprayings of Dithane M-45 (0.25%), Kavach (0.1%) or Foltof (0.25%) at 10 days interval for adoption by the farmers for controlling *A. brassicicola* on cabbage (Verma and Verma, 2010). The sulfanilamide derivatives of chitosan prepared by Mei *et al.* (2007) showed significant inhibiting effect on *A. solani* at 50 to 500 µg/ml concentrations. The potassium and sodium bicarbonate and Nerol (a commercial product of the citrus essential oil fractions) had great inhibitory effect against *A. solani* causing early blight of potato. Complete inhibition of fungus was obtained with potassium or sodium bicarbonate at 2% and Nerol at 0.5% (Abdel Kareem, 2007).

By seed treatment
This method is an effective measure in controlling *Alternaria* diseases as it helps in reducing primary inoculums. The hot water treatment of seeds at 50°C for 30 min to control *Alternaria* diseases in cabbage was recommended by Walker (1952) while Ellis (1968) recommended same temperature for 25 min to eliminate *Alternaria* infection from Brassicaceae seeds. Seed treatment with Thiram plus Captan (1:1) 0.3% and four sprays of Zineb (0.25%) were found quite effective to control this disease in chilli (Jharia *et al.*, 1977).

By disease resistant varieties
With the release of various disease resistant varieties, the in-built resistance is increased and it becomes economical for the farmers making it effective throughout the life. For example, *Cucumis melo* line MR-1 is resistant to *A. cucumerina* (Thomas *et al.*, 1990), whereas Mathur and Shekhawat (1992) found watermelon varieties Sel-1 and Sugarbaby to be resistant and Meetha, Durgapura, AY, WHY & WHY-4 to be highly susceptible and RW-177-3, RW-1, RW-187-2 and Milan as moderately susceptible against *Alternaria* leaf spot. Katiyar *et al.* (2001) found three varieties of bottle gourd namely, Azad Harit, 7002 and 7003 to be resistant against *A. cucumerina*. Two highly resistant chilli genotypes, CA 87-4 and CA 748 were identified against fruit rot caused by *Alternaria* (Sujatha Bai *et al.*, 1993), whereas tomato genotypes viz. Arka Alok, Arka Abha, Arka meghali, Arka Saurabh, IIHR-305, IIHR-308, IIHR-2266, IIHR-2285 and IIHR-2288 were found to be resistant against early blight (Matharu *et al.*, 2006). Similarly, workers across the world are working on the expression of various genes encoding for proteins vital for inducing resistance in various crops.

By bio-control agents
Keeping in view the antagonistic properties of various bacteria and actinomycetes, the use of various bio-control agents is being encouraged. Another important reason of their increased application is the fact that they are eco-friendly too. The antagonists like *Chaetomium globosum*, *Trichoderma harzianum*, *T. koningii* and *Fusarium* spp. effectively controlled seed-borne *A. raphani* and *A. brassicicola* in radish (Vananacci and Harman, 1987) Effective inhibition of mycelial growth of *A. solani* causing leaf blight of tomato by *Bacillus subtilis* and *Trichoderma viridae* has also been reported (Babu *et al.*,

2000). It was also found that *Bacillus* and *Pantoea* had strong antifungal activity both in *in vitro* as well as *in vivo* conditions, but *Curtobacterium* and *Sphingomonas* showed antifungal activities only in *in vitro* against *A. solani* isolated from tomato (Zhao *et al.*, 2008).

By herbal extracts and natural products

The use of various herbal extracts and natural products is being encouraged because these cause no health hazard or pollution. The extracts of *Canna indica*, *Convolvulus arvensis*, *Ipomoea palmata*, *Cenchrus catharticus*, *Mentha piperita*, *Prosopsis spicigera*, *Allium cepa*, *A. sativum*, *Lawsonia inermis*, *Argemone mexicana*, *Datura stramonium* and *Clerodendron inerme* completely inhibited the spore germination of *A. brassicae* isolated from leaves of cauliflower (Sheikh and Agnihotri, 1972). The inhibitory effect of garlic bulb extract on the mycelial growth of *A. tenuis* –causal organism of brinjal leaf spot was reported by Datar (1996). The strong inhibitory action of ethanol or methanol extract of speed weed (*Polygonum perfoliatum*) against conidial germination of *A. brassicicola* causing leaf spot of spoon cabbage was reported from Ching (2007). The neem leaf extract showed high efficacy to inhibit the radial growth of *A. solani* (43.3 and 26.7% at 0.1% and 0.01%, respectively) (Sharma *et al.*, 2007). Hence there are a number of herbal extracts and herbal products which are found effective in controlling diseases caused by *Alternaria* with no health hazards or pollution.

By other methods

Apart from the various methods mentioned above, several other methods can also be employed which would help in combating devastating effects caused by *Alternaria* species. Gomez-Rodriguez *et al.* (2003) found that intercropping of tomato with marigold (*Tagetes erecta* L.) induced a significant reduction in early blight caused by *A. solani*. This was achieved by means of three different mechanisms like:

(i) the allelopathic effect of marigold on *A. solani* conidial germination,

(ii) by altering the microclimatic conditions around the canopy, particularly by reducing the number of hours/day with relative humidity $\geq 92\%$, thus diminishing conidial development and

(iii) by providing a physical barrier against spreading the conidia.

In addition to this, incorporation of residues as soon as possible after harvest is another measure to reduce the harmful effects of *Alternaria*. Control of alternative weed hosts also help in the same.

Conclusions

From the above studies, it is concluded that *Alternaria* is a very destructive pathogen causing a widespread destruction in vegetables and other economically important crops. But with the utilization of advanced techniques, it becomes easier to control this cosmopolitan fungus. Substantial progress has been made in studying the molecular basis for the biosynthesis of phytotoxic secondary metabolites and their role in plant disease development. Utilization of various techniques like gene disruption will allow for an elaborate understanding of its various virulence factors and its physiology. As far as the control of *Alternaria* is concerned, application of fungicides is a common method for the same. But keeping in view, the various health hazards these cause to the human beings, emphasis is being laid on the other method of disease control like growing disease resistant varieties, use of plant and natural products, bio-control agents and alterations in agronomic practices etc. because they are more economical, eco-friendly and safe.

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