**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 desipitepides and fusicoccin-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 cabbages (*B. pekinensis*), cabbages (*B. oleracea var. capitata*), rape (*B. campestris*) and radish (*Raphanus sativus*).

The genus *Alternaria* belongs to the Phylum: Ascomycota, Subdivision: Pezizomycotina, Class: Dothidiomycetes, 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. 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. This gives a "target spot" effect that is associated with early blight 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 brassicicola* (grows on cole crops), *Alternaria carotinica* (causing cumin bloosom blight), *Alternaria carthami*, *Alternaria cinerae*, *Alternaria cirtii*, *Alternaria conjuncta* (grows on parsnip), *Alternaria dauci* (grows on carrot), *Alternaria dianthus*, *Alternaria diantiola*, *Alternaria euphorbciola* (infests cole crops), *Alternaria gaisen* (causes ringspot disease of pears), *Alternaria helianthica*, *Alternaria hungarica*, *Alternaria infectoria* (infests wheat), *Alternaria japonica* (infests cole crops), *Alternaria limicola* (earliest diverging lineage of Section Porri), *Alternaria limicola*, *Alternaria longipes* (infests tobacco), *Alternaria moesta* (may cause skin lesions on porpoises), *Alternaria panax* (causes ginseng blight), *Alternaria petroselini* (causes parsley leaf blight), *Alternaria radicina* (causes...
pathogenicity is the ability to cause disease. The toxins produced by the different pathotypes of Alternaria are sensitive to the toxin. Such correlations between HST production and pathogenicity in the pathogens and between toxin and 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^{-7} 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 desipeptides- 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).

Alternaria species also produce types of toxins that are non-host specific. In addition to AB- toxin, other toxic substances including desipeptides and fusicoccin- 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 suites 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; Kohimoto 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 Phelp, 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 °C (Humpherson-Jones and Phelp, 1989; Rotem, 1994). Some weedy cruciferous plants such as A. thaliana, C. sativa and C. barua-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. cuumerina* causing leaf blight of watermelon. It was followed by Bavistin, Dithane Z-78, Difolatan, Bitlox 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* (Choulaw *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* (Datkar, 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, Iksstein 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). Sidiuaskiene *et al.* (2003) found that Amistar was very effective in controlling *Alternaria* leaf spot in cucumber, cabbage and tomato and 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, Foltal, Bayenlot, Baycor and Contaf 5 EC, in terms of additional yield. They advocated three sprays of Dithane M-45 (0.25%), Kavach (0.1%) or Foltal (0.25%) at 10 days interval for adoption by the farmers for controlling *A. brassicicola* on cabbage (Verma and Verma, 2010). The sulfinilamide 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. cuumerina* (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. cuumerina*. 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. Arkal Alok, Arka Abha, Arka meghali, Arka Saurab, IHR-305, IHR-308, IHR-2266, IHR-2285 and IHR-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 seedborne *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 ciliaris, Mentha piperita, Prospopsis spicigera, Allium cepa, A. sativum, Lawsonia inermis, Argemone mexicana, Datura stramonium and Clerodendron inerme completely inhibited the spore germination of A. brassicaceae 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 ≥ 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.

**References**


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