

A Review: Management of *Alternaria* and its Mycotoxins in Crops

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Article Info

Article history:

Received Sep 18, 2014
Revised Nov 12, 2014
Accepted Nov 25, 2014

Keyword:

Alternaria
Mycotoxin Management

ABSTRACT

The Deuteromycetes fungal genus *Alternaria* comprises of different saprophytic as well as endophytic species which are very common and worldwide in their occurrence. Important host plants include several important crops. *Alternaria* spp. generally attacks the aerial parts of its host and early blight diseases caused by these fungi inflict serious damage to these crops. There is a growing concern of *Alternaria* spp. due to their ability to produce mycotoxins with different toxicological properties, which are harmful for human and animal health. Accurate identification of *Alternaria* spp. and their metabolites is a crucial phase in the implementation of preventive measures and controls in the system from farm to fork. Considering the importance of *Alternaria* spp. occurrence on crops and *Alternaria* toxins risk assessment, additional studies in this area are indispensable.

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1. INTRODUCTION

Alternaria spp. are cosmopolitan mould fungi and can be found in soils, plants, food, feed and indoor air. The genus *Alternaria* includes both saprobes and plant pathogens which have been reported worldwide infecting crops in the field and causing post harvest decay of many plant products (Thomma, 2003). *Alternaria* species are frequently found on small grains, causing yield losses in production and processing. Due to their growth even at low temperature, *Alternaria* spp. are well known post harvest pathogens, responsible for spoilage of food during refrigerated transport and storage (Ostry, 2008).

1.1. The Genus *Alternaria*

Alternaria Nees, ex Fr. belongs to the sub-division Deuteromycotina, class Hyphomycetes, family Dematiaceae. Species of the genus are cosmopolitan, surviving both 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 soil. The telomorphs (sexual stage) are known in a very few species and placed in the genus *Pleospora* of class Loculoascomycetes of sub-division Ascomycotina, in which sleeper-shaped, muriform ascospores are produced in bitunicate asci. The genus *Alternaria* was first recognised by Nees in 1817 (Nees, 1817). In 1836, Berkeley (1836) identified the causal fungus on plants belonging to family Brassicaceae as *Macrosporium brassicae* Berk, which was later renamed as *Alternaria brassicae* (Berk.) Sacc (1886). Thereafter, Elliot studied the taxonomy of *Alternaria* in detail (Elliot, 1917). Wiltshire pioneered the basic studies of this group of hyphomycetes. His descriptive literature was fundamental to the prevailing concepts of *Alternaria*, *Macrosporium* and *Stemphylium*

(Wiltshire, 1933, 1938). Later, Neergaard made an extensive study on the taxonomy, parasitism and economic significance of this genus (Neergaard, 1945). The morphological variations of *Alternaria* species were described by Joly (1959) and later he divided these in three sections and proposed a simple key for identification and determination of the most common species (Joly, 1964). The characteristic features of a number of *Alternaria* species are described in “Dematiaceous Hyphomycetes” (Ellis, 1971) and “More Dematiaceous Hyphomycetes” (Ellis, 1976).

1.2. 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 °C, where mature spores occur after 14-24 h. Optimum temperatures are between 16 and 24 °C 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 (Humpherson-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 °C) or higher will result in the production of large numbers of mature spores in 24 h (Humpherson-Jones and Phelps, 1989).

2. 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).

2.1. Symptoms

Alternaria generally attacks the aerial parts of its host plants. On leaves, symptoms of *Alternaria* infection typically start as a small, circular, dark spot. As the disease progress, the circular spots may grow up to 1 cm or more in diameter and are usually grey, grey-tan or near black in color. This pathogen does not have a uniform growth rate, thus spots develop in a target pattern of concentric rings. Apart from the target pattern, the lesion is also often covered with fine, black, fuzzy growth. This growth is the *Alternaria* fungus sporulating on the dying host tissue. Many *Alternaria* species also produce toxins that diffuse into the host tissue ahead of the fungus. Therefore, it is not uncommon to see a yellow halo that fades into the healthy host tissues that surround the target spot. On the other hand, dark and sunken lesions are usually the expression of *Alternaria* infections on roots, tubers, stem, and fruits. The fungus may sporulate in these cankers, causing a fine, black, velvety growth of fungus and spores to cover the affected area.

2.2. *Alternaria* Toxins

In addition to losses in food and feed production, many *Alternaria* species are mycotoxin producers with different toxicological properties. The most important *Alternaria* toxins are alternariol (AOH), alternariol monomethyl ether (AME), altenuen (ALT), tenuazoic acid (TEA) and altertoxins (ATX-I, II, III) (Logrieco et al., 2009). The occurrence of *Alternaria* toxins in small grains and cereal-based products is a global issue of high concern, due to their potential health risks for humans and/or livestock. It has been reported that some *Alternaria* toxins might have even the carcinogenic effect (Liu et al., 1992). Based on the requests from the European Commission (EC) in order to highlight the need for possible follow up actions, the European Food Safety Authority (EFSA) provided a scientific opinion on the risks for animal and human health related to the presence of *Alternaria* toxins in food and feed (EFSA, 2011). Moreover, *Alternaria* spores are considered to be one of the most prolific fungal allergens, which has been associated with respiratory allergies and skin infections (Corden et al., 2003; Kilic et al., 2010; Pavon et al., 2010). Since the great importance of the genus *Alternaria* related to food safety and quality of small grains and cereal-based products, this paper presents the extensive overview of *Alternaria* species on crops with focus on mycotoxin risks in food chain.

The problem of mould damage and the hazard of consuming damaged grains have been recognized since historical times, but mycotoxins have attracted considerable attention especially over the last three decades (Bhaat and Miller, 1991). Mycotoxins are secondary metabolites, produced by a range of fungal

species. Generally, mycotoxins are chemically and thermally stable compounds, surviving storage and the most food processing conditions and therefore, persist to the final products (Matić et al., 2008). Mycotoxins in cereal-based foods and feeds are a global issue of high concern, due to their potential health risks for humans and/or livestock (Köppen et al., 2010). The *Alternaria* genus produces more than 70 mycotoxins and phytotoxins but only few occur naturally in foodstuffs or are of major toxicological significance. *A. alternata* is considered as the most important toxin producing species (Battilani et al., 2009).

The most important *Alternaria* toxins are divided into three main structural classes according to Ostry (2008), Logrieco et al. (2009) and Battilani et al. (2009):

a) Dibenzofuran derivatives: alternariol (AOH), alternariol monomethyl ether (AME), altenuen (ALT), altenuisol (AS);

b) Tetramic acid derivatives: tenuazonic acid (TEA)

c) Perylene derivatives: altertoxins I, II, III (ATX-I,-II,-III)

d) *Alternaria* toxins have been detected in wide range of cereal grains and small-grains based products such as bread and rolls, musli, fine bakery wares, pasta etc. (EFSA, 2011).

There are reports of AOH, AME and TEA in “black point wheat” on German market (Siegel et al., 2009, Asam, 2011), AOH, AME and ALT in Slovakian (Maškova et al., 2012) and Czech grains (Malachova et al., 2011), AOH and AME in small cereal grains in Poland (Grabarkiewicz Szczesna et al., 1989) and AOH was detected in Estonian grains (Kütt et al., 2010). Li & Yoshizawa (2000) analyzed wheat kernels in China which were significantly invaded by *Alternaria* spp., mostly *A. alternata*, with an average infection frequency of 87.3%. AOH was detected in 20 of 22 tested samples between 116-731 µg/kg and AME at a mean level of 443 µg/kg (range= 51-1426 µg/kg) in 21 samples. The presence of TEA, as major *Alternaria* toxin in terms of quantity, was detected with an average level of 2419 µg/kg and with a maximum quantity of 6432 µg/kg. The toxigenic potential of *Alternaria* strains isolated from Argentinean wheat, showed that TEA was the toxin produced at the highest concentration, but in lower frequency (72%), compared to AOH (87%) and AME (91%) (Patriarca et al., 2007).

3. 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 (Mamgain et al., 2013).

By Ground Preparation: The residues from the previous crop should be incorporated. Apart from this, balanced crop nutrition especially of potassium should be provided (Mamgain et al., 2013).

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*, *Prosopis 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.

4. 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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