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Review

Alternaria blight of oilseed Brassicas: A comprehensive review

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Oilseed brassicas also known as rapeseed-mustard is an important group of oilseed crop in the world. These crops are susceptible to a number of diseases caused by biotic and mesobiotic pathogens. Among various diseases, Alternaria leaf blight also known as Alternaria dark spot is the most destructive disease of oilseed brassicas species in all the continents. This disease is known to be incited by *Alternaria brassicae*, *Alternaria brassicicola* and *Alternaria raphani* singly or by mixed infection. Alternaria leaf spot pathogens are necrotrophs and produces lesions surrounded by chlorotic areas on leaves, stems and siliquae causing reduction in the photosynthetic areas, defoliation, and early induction of senescence. Alternaria blight causes considerable reduction in quantity and quality of harvested brassica products. The Alternaria leaf blight pathogens are seedborne, soilborne and airborne. The pathogens are greatly influenced by weather with the highest disease incidence reported in wet seasons and in areas with relatively high rainfall. The concentration of conidia, age of the host plants, and wetness period on leaves also influence the severity of the disease. This paper reviews the research and development of Alternaria blight in the oilseed brassicas (rapeseed-mustard) during the past years in relation to pathogen taxonomy, biology, epidemiology, host pathogen interaction and management through chemicals, botanicals, biological, cultural, and biotechnological approaches. The paper also attempts to present future outlook and strategy for Alternaria blight of rapeseed-mustard research.

Key words: Alternaria blight, rapeseed-mustard, symptoms, variability, pathogen, survival, management.

INTRODUCTION

Oilseed brassicas often called rapeseed-mustard is the third most important oilseed commodity in the world after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq.) in world agriculture and India is the third largest producer with global contribution of 28.3% acreage and 19.8% production (Shekhawat et al., 2012; Bandopadhyay et al., 2013). Among the oilseed brassicas, mustard (*Brassica juncea*), yellow sarson (*Brassica campestris* var. yellow *sarson*), brown sarson (*Brassica campestris* var. brown *sarson*), toria (*Brassica campestris* var. toria), oilseed rape (*Brassica napus*), and Karan rai (*Brassica carinata*) are grown for edible oil, whereas black mustard

(*Brassica nigra*) is used as a condiment and for pickle making. The leaves of the young plants are used in the human diet as a green vegetable. The oilseed brassicas usually contain 38-57% of erucic acid, 4.7-13% of linolenic acid and 27% of oleic and linoleic acids, which are of high nutritive value required for human health. Oilseed brassicas is gaining importance globally due to its advantage over other oilseeds namely: high yield potential, low moisture requirement, higher return at low cost production, and wider adaptability for various farming conditions which hold promise towards having the next yellow revolution (Kumar, 2012).

Oilseed brassicas are exposed to various pathogens, which infect and disturb the normal physiological functions during growth and development. Among the diseases that hampered the productivity of oilseed brassicas, Alternaria blight is most recognized disease worldwide. The disease is also known as Alternaria black spot or dark spot disease in Europe and Canada (Degenhardt et al., 1974). The Alternaria leaf spot disease incited by *A. brassicae* is more destructive and occurs more frequently than the one caused by other two species namely *Alternaria brassicicola* and *Alternaria raphani*. The disease occurs in Canada (Petrie, 1973, 1974; McDonald, 1959), England (Loof and Appleqvist, 1972; Evans 1983), France (Loof and Appleqvist, 1972), Germany (Borg, 1952), Holand (Flik and Saaltink, 1950), India (Dey, 1949; Mukadam and Despandey, 1977; Kolte and Tiwari, 1978; Vasudeva, 1958), Poland (Francel, 1983), SriLanka (Bond, 1947), Spain (Romero and Jimenez Diaz, 1980), Sweden (Loof and Appleqvist, 1972), Australia (Sivapalan and Browning, 1992), USA (Babadoost and Gabrielson, 1979) and Trinidad (Fajardo and Palo, 1934).

The disease appears as brown or greyish spots on leaves, stems, and on siliquae during ripening stage. Alternaria blight causes substantial yield losses as a result of several factors including reduced photosynthetic potential, early defoliation, flower-bud abortion, premature ripening, siliquae dehiscence, seed shriveling (Seidle et al., 1995), and reduced seed size and impairs seed color and oil content (Kaushik et al., 1984).This review describes the pathogens of Alternaria blight of oilseed brassicas, epidemiology, host pathogen interaction and management through various approaches.

THE PATHOGEN

Alternaria leaf blight of oilseed brassicas is known to be incited by three species namely *Alternaria brassicae* (Berk.) Sacc., *Alternaria brassicicola* (Schw.) Wiltshire., and *Alternaria raphani* Groves and Skolko (Jasalavich et al., 1995; Saharan and Mehta, 2002). The genus Alternaria belongs to the phylum Ascomycota which consists of both saprophytic and pathogenic species. Alternaria belongs to the class Dothideomycetes, order Pleosporales, and family Pleosporaceae. *Alternaria* spp. is characterized by formation of polymorphous conidia either singly or in short or longer chains with longitudinal and transverse septa with long or short beaks. Among these species, *A. brassicae* is the most destructive and occurs more frequently in many parts of world. The *A. brassicicola* is also cosmopolitan in distribution and may cause the infection simultaneously with *A. brassicae* on the same plants. *A. raphani* is less destructive than the other two species but it is the most common in Canada. *A. raphani* has been also reported from Denmark, Egypt, Greece, India, Japan, Netherlands and USA. (Saharan and Mehta, 2002). Khan et al. (1998) reported 26.5% infection by *A. brassicicola* and 22.6% by *A. brassicae* whereas the rest 50.9% is accounted for concomitant infection of *A. brassicae* and *A. brassicicola*. The taxonomy of Alternaria is based primarily on the morphology and development of conidia and conidiophore, and to a lesser degree on host plant association and colony morphology (Elliott, 1917; Wiltshire, 1933; Simmons, 1967). The morphological, cultural and physiological characteristics, genetic diversity and virulence-associated genes of these three species are as follows:

Alternaria brassicae

The mycelium of *A. brassicae* is septate, brown to brownish grey in colour. The conidiophores are dark, septate, arise in fascicles, measuring 14-74 × 4-8 µm. Conidia are brownish black, obclavate, borne singly or sparingly in chains of 2-4, muriform with long beak and the overall conidial size ranges between 148-184 × 17-24 µm with 10-11 transverse and 0-6 longitudinal septa. This species represent slow and rudimentary growth in media and forms chlamydospores in less frequency (Kolte, 1985). Sporulation occurs between the temperatures of 8-24°C but optimum temperatures range between 16-24°C. *A. brassicae* germinates over a wide range of temperature, however, germination occurs most quickly, when the temperature is between 21-28°C. As the temperature decreases, the time period it takes for germination increases (Degenhardt et al., 1982).Sharma et al. (2013) studied the 32 Indian isolates of *A. brassicae* and found that colony of the isolates on PDA varied between light olive gray to olivaceous black whereas mycelia colour varied between brown to golden. Most of the conidia were long obpyriform in shape with long beak and colour was found golden or brown with mostly smooth surface. Sporulation of each isolates on the different

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media was found almost similar. All isolates studied by Sharma et al. (2013) were pathogenic in nature but not directly related to the cultural and the morphological characteristics. Pramila et al. (2014) studied the 10 Indian isolates of *A. brassicae* and grouped the isolates based on cultural characteristics in three groups. Group 1 isolates produces circular white colonies with a fluffy appearance with smooth colony margins. Group 2 isolates have off white colony with a feathery appearance and is circular with all type of margins. Group 3 isolates having light brown colony with cottony appearance and colony are circular in shape with wavy and rough margins. Pramila et al. (2014) also reported that different isolates of *A. brassicae* showed variable pathogenic response on host *B. juncea* cultivar Divya.

A temperature range of 25 to 30°C and 15 to 35°C was found optimum for mycelial growth and sporulation of *A. brassicae*, respectively. Mycelial growth was most favoured by 100% relative humidity with a gradual reduction in growth and sporulation till 70% RH and a decrease in growth and sporulation at 60 and 50% RH (Meena et al., 2012). The most favourable temperature for sporulation of *A. brassicae* had earlier worked out as 23-25°C by Kadian and Saharan (1984) and Ansari et al. (1989) which indicates the adaptability of this fungus to varied environmental conditions (Meena et al., 2012). Polymorphism within an *Alternaria* species by RAPD molecular marker has been described by many workers (Sharma and Tewari, 1995; Sharma and Tewari, 1998; Kumar et al., 2008). Sharma and Tewari (1995) observed polymorphism among *A. brassicae* isolates from different geographical regions of the world. However, Sharma and Tewari (1998) found low intra-regional variation among Indian and Canadian isolates of *A. brassicae* with 75% similarity among them. Sharma et al. (2013) molecularly characterized the 32 Indian isolates by using transcribed spacer region primers ITS1- 5' TCC GTA GGT GAA CCT GCG 3' and ITS4- 5' TCC TCC GCT TAT TCA TAT GC 3' and found the PCR products of amplicons of ~550 to 600 bp. Analysis of ITS revealed *A. brassicae* isolates were 56% similar to each other and 99% similar to the *A. brassicae* isolates in NCBI database. Pramila et al. (2014) revealed high degree of genetic variability among ten isolates of *A. brassicae* of Pantnagar region of Uttarakhand, India by 26 RAPD primers indicating the existence of different strains of pathogen. Various studies indicates high genetic divergence among isolates of *A. brassicae*. Although, the genus *Alternaria* is known as an imperfect fungus, it shows genetic variability within a species and this variability might be due to the existence of mutation, somatic hybridization, heterokaryosis, uniform host selection, extensive dispersal or of a cryptic sexual stage. This could be the probable possible reason behind extreme and different disease reaction of germplasm at Pantnagar from observations at most of other locations. In order to provide a better picture of the pathogenic as well as genetic divergence among *A. brassicae* populations of India, there is need to conduct similar holistic investigation among higher number of *A. brassicae* isolates which could be helpful to generate resistant material against *Alternaria* blight in oilseed Brassicas (Pramila et al., 2014).

A. brassicicola

Mycelium of necrotrophic fungus *A. brassicicola* is septate, olive grey to greyish black in colour. The conidiophores are olivaceous, septate, branched, measuring 35-45 µm in length and 5-8 µm in width. Conidia are dark, cylindrical to oblong, muriform without beak measuring 44-55 µm in length and 11-16 µm in width with 5-8 transverse and 0-4 transverse septa. The fungus grows faster in media with high sporulation and appears as well developed black sooty colony with distinct zonations (Kolte, 1985). *A. brassicicola* sporulates at a temperature range of 8 to 30°C. Optimum temperatures for sporulation is between 18 and 30°C where the average sporulation time is 13 h. *A. brassicicola* germinates at higher temperatures. *A. brassicicola* begins to germinate 98% of its spores at 15°C after 10 h of incubation whereas 98% germination occurs after 3 h at 31°C (Degenhardt et al., 1982). Plants inoculated with *A. brassicicola* develop symptoms most quickly at 25°C, while seedlings from infected seeds develop symptoms most quickly at 30°C. No germination occurs at 3°C for all three pathogens namely *A. brassicicola, A. brassicae and A. raphani* (Bassey and Gabrielson, 1983).

AFLP found reliable tool for identifying *A. brassicicola* because of clear polymorphism both within and between species. The analysis of eighteen isolates of *A. brassocicola* raveled moderate levels of genetic diversity within species. The number of polymorphic loci and the percentage-shared bands indicated genetic differences between species. Within the *A. brassicicola* group, 16.7-27.9% of the loci were polymorphic, while the remainder were monomorphic. The genetic distance between the isolates of *A. brassicicola* ranged from 0.00 -0.04 suggesting that they are closely related (Bock et al., 2002). Teleomorphs of most of the *Alternaria* spp. do not exist or remain unidentified (Simmons, 1978). Many Alternarias are therefore likely to be haploid fungi existing in a vegetative phase, reproducing asexually, and would be expected to have a high level of clonality (Vogler et al., 1991).

During host infection, *A. brassicicola* is exposed to high levels of defense compounds, such as phytoalexins and glucosinolate breakdown products, and the ability to overcome these antimicrobial metabolites is a key factor in determining fungal virulence (Pochon et al., 2013). The *A. brassicicola* genome size is approximately 31.9 Mb. (Ohm et al., 2012). Several genes in *A. brassicicola* linked to the pathogenesis have been reported (Cho et al., 2007, 2009a; Craven et al., 2008; Kim et al., 2007,

2009; Oide et al., 2006; Srivastava et al., 2012). One of the genes of major interest has been a transcription factor, *AbSte 12* that is controlled by *Amk1* in *A. brassicicola* (Cho et al., 2007). The deletion mutant for *AbSte 12* gene showed pleiotropic phenotypes, including the inability to produce mature conidia, slight reduction of vegetative growth rates and complete loss of pathogenicity. Two novel virulence factors encoding a transcription factor *AbPro1* and two component histidine kinase gene *AbNIK1* were discovered. Deletion of *AbPro1* resulted in a 70% reduction in virulence and a 25% reduction in vegetative growth rates *in vitro*. Deletion of *AbNIK1* resulted in a near complete loss of virulence, increased sensitivity to osmotic stress and no changes in vegetative growth rates *in vitro* (Cho et al., 2009b). Cho et al. (2012) reported transcription factor gene, *Amr1*, which negatively regulates a subset of these genes during late-stage pathogenesis and positively regulates melanin biosynthesis during conidiogenesis. *Amr1* is a homolog of *Cmr1*, a transcription factor that regulates melanin biosynthesis in several fungi. The deletion ∆*amr1* mutants used pectin as a carbon source more efficiently than the wild type *A. brassicicola,* where melanin is deficient and more sensitive to UV light and glucanase digestion. RNA-seq analysis revealed that three genes in the melanin biosynthesis pathway, along with the deleted *Amr1* gene, were expressed at low levels in the mutants. In contrast, many hydrolytic enzyme-coding genes were expressed at higher levels in the mutants than in the wild type during pathogenesis. The increase in virulence of the deletion mutants of *amr1* suggested that the loss of the gene was beneficial to pathogenesis. The results of this study suggested that a gene important for survival in nature negatively affected virulence, probably by a less efficient use of plant cell-wall materials. Cho et al*.,* (2012) speculated that the functions of the *Amr1* gene are important for the success of *A. brassicicola* as a competitive saprophyte and plant parasite.

Dehydrins belong to the late embryogenesis-abundant (LEA) protein family believed to play a role in the protection against cold- and dehydration-related stresses. Pochon et al. (2013) studied the role of fungal dyhydrin like proteins in pathogenicity and protection against environmental stresses in *A. brassicicola*. Three dehydrin protein encoding genes called AbDhn1, AbDhn2 and AbDhn3 were identified in the *A. brassicicola* genome. The expression of these dehydrin gene was induced in response to various stresses and found to be regulated by the AbHog1 MAP Kinase pathway. They showed that dehydrin-like proteins have an impact mainly on oxidative stress tolerance and on conidial survival upon exposure to high and freezing temperatures. They also revealed that the double deletion mutant ∆∆*abdhn1-abdhn2* was highly compromised in its pathogenicity. By comparison to the wild-type, this mutant exhibited lower aggressiveness on *Brassica oleracea* leaves. The double mutant was also affected with respect to conidiation, another crucial step in the epidemiology of the disease.

A. raphani

Mycelium is cottony whitish to greenish grey, which become dark olive because of ageing. The conidiophores are septate, olive brown, single or branched, 29-160×4-8 µm in size. Conidia are muriform with poorly developed or no beak, olive brown to dark, obclavate and less uniform in shape in comparison with *A. brassicae* and *A. brassicicola*. Conidia are more or less pointed at each end and appear singly or in chains of upto six spores. The overall length of conidia ranges between 60-83 µm in length and 13-21 µm in width with 6-9 transverse and 3-6 longitudinal septa. The fungus appears as cottony colonies on the media with less sporulation. The fungus produces olive brownish chlamydospores in culture as well as on the partially decayed affected plant part (Kolte, 1985). Conidia of *A. raphani* germinate at a temperature range of 7 to 31°C. The optimum temperature for *A. raphani* is 23°C or greater, where, 98% of the spores germinate after 6 h of incubation. The lowest temperature where 98% of the spores germinate is at 13°C, which requires 10 h of incubation for germination (Degenhardt et al., 1982).

Jasalavich et al. (1995) studied the nuclear 18s rRNA, 5.8s rRNA and the internal transcribed spacers (ITS1 and ITS2) sequence of *A. raphani, A. brassicae*, *A. brassicicola, A. alternata* and *Pleospora herbarum*. The 5.8s rDNA sequences from the *A. raphani, A. brassicae*, *A. brassicicola, A. alternata* were identical and differed at only one base pair from that of *P. herbarum*. The internal transcribed spacer sequences, especially ITS1, were very variable in both base composition and length. The 18s rDNA sequences were highly conserved, but enough variability was present to distinguish genera clearly. Phylogenetic analysis of the sequence data sets by both parsimony and maximum likelihood methods clearly separated genera and species. All the *Alternaria* species were closely related. Pleospora also appeared to be more closely related to Alternaria than to leptosphaeria. Based on the taxonomy, Wiltshire (1947) considered *A. raphani* and *A. brassicae* to be closely related. Jasalavich et al. (1995) found that *A. brassicicola* is actually more closely related to *A. raphani* than to *A.brassicae* based on the rDNA sequences data. *A. alternata*, *A. brassicae*, *A. brassicicola* and *A. raphani* formed clade of very closely related sister taxa. The 18s rDNA resolved two subclades of species within Alternaria at a level of confidence of 98%, based on five informative sites contained within the 300 bp at the 3'-end of the sequences alignment. Complete resolution of the species of Alternaria was achieved with the ITS sequence data which were much variable and contained more phylogenetically informative sites than the 18s rDNA. The study indicates that *A. raphani, A. brassicae*, *A. brassicicola,*

A. alternate encompasses less genetic variability.

Chou and Wu (2002) studied the phylogenetic analysis of internal transcribed spacer regions (ITS 1and ITS 2) of different fungi and they got positioned filament beaked Alternaria as a monophyletic group discrete from the other members of genus Alternaria. Filament beaked *Alternaria* spp. formed a well supported group. The second group consisted of small spored *A. brassicicola and A. raphani* and the large spored *A. brassicae*. This study also indicates less genetic diversity in Alternaria species causing Alternaria leaf blight on rapeseed-mustard.

HISTORY OF *ALTERNARIA* AND ALTERNARIA LEAF SPOT

Nees first described the genus *Alternaria* in 1817 with type species *Alternaria tenuis*, which was later renamed as *A. alternata*. Berkeley (1836) noticed fungal infection on plant belonging to the family Brassicaceae and identified this fungus as *Macrosporium brassicae* Berk., which was later renamed as *A. brassicae* (Berk.) Sacc by Saccardo (1886). In India, the Alternaria blight was first observed on sarson from Tirhoot in 1901 (Butler, 1918) but the fungus was thought to be new and described as *Sporodochium brassicae* Mass. Later, Mason (1928) first observed the *Alternaria* spp. from a herbarium material of sarson from Pusa (Bihar) India. Elliott (1917) and Wiltshire (1933) studied the taxonomy of *Alternaria* in detail. Later, Neergaard (1945) made a detailed study of the taxonomy, parasitism and economic significance of *Alternaria* genus. Joly (1959) described the morphological variations in *Alternaria* spp. and later he divided these into three sections and gave a key for identification of most common species of *Alternaria* genus (Joly, 1964). Subramanian (1971) described the Indian species of *Alternaria* in detail. The morphological characteristics of various *Alternaria* spp. are described in Dematiaceous Hyphomycetes (Ellis, 1971) and in more Dematiaceous Hyphomycetes (Ellis, 1976). Simmons (2007) compiled his lifetime work in his book *Alternaria*: An Identification Manual in which he described the taxonomy, nomenclature and classification to facilitate accurate identification of species of *Alternaria*.

YIELD LOSSES

Alternaria blight occurs every year in all the rapeseed-mustard growing areas of the world. This disease causes an average yield loss of 46-47% in yellow *sarson* and 35-38% in mustard (Kolte, 1985a, b; Kolte et al., 1987, 2002; Chattopadhyay, 2008) and even up to 70% in *Brassicas* species. In Canada, 20 to 30% yield losses were recorded due to this disease (McDonald, 1959; Conn et al., 1990). In India, losses of 15 to 71% were reported by different workers (Kadian and Saharan, 1984; Singh and Bhowmik, 1985; Kumar, 1986; Ram and Chauhan, 1998). Kolte et al. (1987) reported that the disease causes losses in 1000-seed weight (g) of yellow sarson and mustard of 23 and 24%, respectively. In addition to quantitative loss, seed quality in terms of seed size, seed color and oil contents are also reduced due to the fungus infection (Kaushik et al., 1984; Kumar, 1997). Reduction in oil content up to 4.8% have been reported by Degenhardt et al.(1974) but Ansari et al.(1988) reported the reduction in oil content of rapeseed cultivars between 14.58 and 35.97% and 14.12 - 29.07% in mustard cultivars in India. Rotem (1994) stated that Alternaria black spot could be a devastating disease resulting in 25-50% yield reductions in crops such as canola or rape.

DISEASE SYMPTOM

A. brassicae, *A. brassicicola*, and *A. raphani* cause more or less similar symptoms on leaves, stem and siliquae of oilseed brassicas. Spots produced by *A. brassicae* appear to be usually grey in color when compared with black sooty velvety spots produced by *A. brassicicola*. Spots induced in response to *A. raphni* showed distinct yellow hallow around them. However, the symptoms may vary with the host and environment (Meena et al., 2010). Symptoms are first visible with appearance of black points. Later, these spots enlarge and develop in to prominent round spots with concentric rings showing target board characteristic of the spot. Many spots coalesce to form large patches and causing blighting and defoliation of the leaves. In some Brassicas species, formation of constricting rings in the lesion and zone of yellow halo around the lesions are very prominent (Saharan and Mehta, 2002). Disease symptoms often occurs on the older leaves, since they are closer to the soil and are more readily infected as a consequence of windblown or rain-splashed spores. At the later stage of plant growth, symptom of the disease also develops elongated spots without concentric rings on stem and siliquaeas. Deep lesions on the siliquae cause infection in the seeds. Alternaria spot on leaves and siliquae reduces the photosynthetic area drastically and cause the formation of the small, discoloured and shrivelled seeds. Alternaria blight adversely affects the oil content in seed and quality of the seed (Meena et al., 2010) (Figure 1).

HOST AND PATHOGEN INTERACTION

Fungi infect their hosts in a sequence of events including adhesion to the host surface, followed by penetration and growth into the host tissue. Alternaria leaf spots start to initiate after landing conidia of *A. brassicae*, *A. brassicicola* and *A. raphani* on the host surface. After adhesion on the host surface, conidium germinates in the presence of moisture readily by giving rise to a germ tube

Figure 1. a: Symptoms of leaf spot caused by *Alternaria brassicae* on mustard; b: Symptoms of leaf spot caused by *Alternaria brassicicola* on mustard; c: Symptoms of leaf spot caused by *Alternaria raphani*on mustard; d: Large necrotic patches on mustard leaf after merging of spots; e: Symptoms of *Alternaria* spp. on the pods of *Brassica juncea*; f: Symptoms of *Alternaria* spp. on the pods of *B. campestris* var. yellow *sarson*; g: Concentric ring type of spot of *Alternaria* spp. on pod of *B. campestris* var. yellow *sarson;* h: Dark black symptoms of *Alternaria* leaf blight pathogens on stem of mustard; i: Concentric ring type of symptoms of Alternaria leaf spot on the stem of *B. campestris* var. yellow *sarson*; j: Conidia of *Alternaria brassicae* (600×); k: Conidia of *Alternaria brassicicola* (1050×); l: Conidia of *Alternaria raphani* (1000×).

and appressorium develops from the end of a fungal germ tube and grow into the underlying epidermal cell using a penetration peg. Pathogens undergo morphological changes during the initial stages of pathogenesis leading to the formation of infection structures accompanied by physiological changes to support the

penetration process (Cho et al., 2009). Morphogenesis and physiological alteration are often triggered by signals provided by the host plant (Yang et al., 2005). After growth of the fungal hyphae in the host plant, a typical necrotic lesion develops on the surface of the host leaves. Eventually, aerial conidiophores differentiate from hyphae within the lesions, releasing thousands of new conidia. Since, Alternaria leaf spot is a polycyclic plant disease, the conidia servers as secondary inoculum for the next infection cycles. Alternaria lesions often occurs on the older leaves, since they are closer to the soil and are more readily infected as consequence of rain splash or windblown rain. Since *Alternaria* is a necrotroph, it must kill the host plant's cell to survive. This is accomplished by secretion of toxins consisting of secondary metabolites and proteins. These toxins are host specific and non-host specific in nature which imparts the ability to the pathogen to infect wide range of hosts (Kohmato et al., 1995; Nishimura and Kohmoto, 1983; Agrios, 2005).

TOXINS OF *A. BRASSICAE* AND *A. BRASSICICOLA*

Host-pathogen factors play a crucial role in the initiation and severity of the diseases. Toxins released by the pathogens during pathogenesis are one of such factors. Many pathogenic species of *Alternaria* are prolific toxin producers which facilitate their necrotrophic life style as two step process: Killing host cell directly (Necrosis) or inducing programmed cell death with toxins, then decomposing host tissue with cell wall degrading enzymes (Cho et al., 2012). *A. brassicae* is known to produce four phytotoxins, which are low molecular weight cyclodepsipeptides named destruxins (Agarwal et al., 1994; Ayer and Pena-Rodriguez, 1987; Bains and Tewari, 1987; Buchwaldt and Jensen, 1991; Tewari and Bains, 1997). Destruxin B is the major phytotoxin produced by the pathogen in liquid culture, and other three namely homodestruxin B, destruxin B_2, and desmethyl destruxin are produced in less amount (Tewari and Bains, 1997). Bains and Tewari (1987) reported that destruxin B is toxic to Brassica but not to non-host and they classified destruxin B as host specific toxin. In contrast, Buchwaldt and Green (1992) observed the non-hostspecific toxicity of destruxin B. Parada et al. (2007) studied the host specificity of destruxin B and indicated its non-host specific nature supporting the result of Buchwaldt and Green (1992). Bains et al. (1993) worked on the host specificity of homodestruxin B and observed the symptom of different severities in leaves of various non-host plants and suggested that homodestruxin B is non-host specific toxin. Host selective toxins are toxic only to the host plant, and have an important role in pathogenesis as primary determinants of virulence or pathogenicity. The majority of the host specific toxins are low molecular weight secondary metabolites belonging to various classes of chemical compounds and have been extracted from liquid cultures. However, production of toxins by germinating spores on host plants suggests the very early participation of toxins at the site of initial contact of pathogen on plant surface (Nishimura and Kohmoto, 1979, 1983). In search of host specificity of *Alternaria* toxin on brassicas plants, Otani et al. (1998) reported the proteinaceous AB toxin in spore germination fluids of *A. brassicicola* on brassica plants. In addition to AB toxin, *A. brassicicola* produces other toxic substances including despipeptides and fucicoccin like compounds (Cooke et al., 1997; MacKinnon et al*.,* 1999; Mckenzie et al., 1988). Parada et al. (2008) observed that *A. brassicae* produces a new host specific toxin named ABR toxin in spore germination fluids on host plants and induce water soaked symptoms followed by chlorosis only in Brassica leaves. Unlike other toxins reported to be produced by *A. brassicae*, ABR toxin appeared to be a protein. ABR toxin at the concentration of 0.5 to 1.0 µg/ml produced water soaked symptoms followed by chlorosis on brassicas leaves while non- host leaves were not affected even at 50 µg/ml indicating the host specific toxicity of ABR toxin. These results indicated the ABR toxin from *A. brassicae* fits the criteria of host specific toxin and plays a key role as a disease determinant of *A. brassicae*. *A. brassicicola* and *A.brassicae* pathogenic to brassicas have a similar host range and AB and ABR toxin have similar host specificity but these toxins are different in their molecular weight. In recent studies, Brassicicolin A emerged as most selective phytotoxic metabolite produced in liquid culture of *A. brassicicola* (Pedras et al., 2009). Toxic metabolites released by the *A. brassicae* and *A. brassicicola* are most important factor in interaction between host and pathogen. Homodestruxin B and Destruxin B caused chlorosis and necrosis similar to that caused by *A. brassicae* and phytotoxicity of these toxins are similar (Buchwaldt and Green, 1992). Homodestruxin B may play a minor role in black spot disease when compared with that of destruxin B, because it is produced in much smaller quantities (Ayer and Pena-Rodriguez, 1987; Buchwaldt and Jensen, 1991). AB and ABR toxin cause water soaked symptoms followed by chlorosis only in Brassicas leaves indicating the importance of these toxins. The role of the host specific toxins in initial colonization is not to kill host cells prior to penetration but to predispose the host cell beneath germinating spores to accept penetration. Toxin also induces accessibility of the host plant to fungal invasion (Parada et al., 2008). There is no report so far on toxin production by *A. raphani.*

ENVIRONMENTAL INFLUENCE ON THE PATHOGEN BIOLOGY AND ALTERNARIA LEAF SPOT

The ideal weather condition required for adhesion, germination, penetration and establishment of pathogen in host plays a major role in the development of disease. Other factors such as density of airborne conidia (Humpherson-Jones and Ainsworth, 1982), plant age

(Awasthi and Kolte, 1994), temperature, humidity and wetness period (Hong and Fitt, 1995) are reported to influence the severity of Alternaria leaf blight. The greater density of pathogen inoculum within or near fields of host plants reaches the hosts and increases the chances of an epidemic greatly. Moisture not only promotes new succulent and susceptible growth in the host, but, more importantly, it increases sporulation of pathogenic fungi. The most common effect of temperature on epidemics, however, is its effect on the pathogen during the different stages of pathogenesis, that is, spore germination, host penetration, pathogen growth or reproduction, invasion of the host, and sporulation. Plants change in their reaction to disease with age. The change of resistance with age is known as ontogenic resistance (Agrios, 2005). The susceptibility in oilseed brassicas increase with the age of the host plant (Saharan and Mehta, 2002)

For infection, a minimum period of 4 h of leaf wetness is required to cause infection in the host plant. Increase in the period of leaf wetness at 25°C increases infection and spread of the disease rapidly (Saharan and Mehta, 2002). Degenhardt et al. (1982) reported that *A. brassicae* germinates more quickly at 21-28°C. Saharan and Mehta (2002) reported congenial factor for germination of Alternaria spores are darkness or low light intensity, 25°C temperature and more than 90% relative humidity. *A. raphani* and *A. brassicicola* germinate at a range of 7-31°C but the optimum temperature for *A. raphnai* is 23°C or greater. At 13°C, 98% conidia germinate after 10 h of incubation. *A. brassicicola* conidia begin to germinate 98% at 15°C after 10 h of incubation whereas the conidia take only 3 h for germination at 31°C. Conidia of all three species are unable to germinate at 35°C. Kadian and Saharan (1984) reported that darkness or low light intensity with 25°C temperature and 90% humidity is optimum for conidial germination of *A. brassicae*. Continuous moisture of 24 h or longer practically guarantees infection (Chupp and Sherf, 1960; Rangel, 1945). Spread of the disease is mainly by the rain and wind dislodged spores. Optimum conditions for sporulation and infection include a minimum wet period of 13 h and ambient temperatures between 20-30°C (Humpherson-Jones and Phelps, 1989; Rotem,1994). Relative humidity of 91.5% (at 20°C) or higher will result in the production of large number of mature spores in 24 h. Sporulation of *A. brassicae* has been reported to be favoured by darkness. Moisture in the presence of rain, dew, or high humidity is essential for infection, and a minimum of 9 -18 h is required for *A. brassicae* and *A.brassicicola* in oil rape and cabbage (Humpherson-Jones and Phelps, 1989).

SOURCES OF INOCULUM

Seed

Siliquae are also infected by the Alternaria blight pathogens at late stage of plant growth. Hence, infected seeds with spores on their coat or mycelium under their seed coats could be the main source of transport for these pathogens (Shrestha et al., 2000). *A. brassicicola* survives as dormant mycelium or conidia in or out of the seed coat (Richardson, 1970; Petrie, 1974; Neergaard, 1977; Knox-Davies, 1979). Seed borne inoculum plays a role in disease cycle in temperate climate whereas it fails to survive in tropical regions (Awasthi and Kolte, 1994). *A. brassicae* predominantly found in the seed coat and rarely in embryos of rapeseed-mustard and causes lesion developments on the cotyledonary leaves and then in the first true leaves (Shrestha et al., 2000). Shrestha et al. (2003) reported the survival of *A. brassicae* in seeds stored in room temperatures (11-25°C) for 10 and 6 months at 30°C. Some workers reported that the inoculum present on the seed get eliminated in hot period and do not serve as a primary source of inoculum in plains. Shrestha et al. (2003) studied the importance of survival of the fungus in the seeds at low temperature and concluded that infected seeds act as a source of primary inoculum in Nepal.

Crop residue

Infected crops left on the soil after harvest also serves as a source of inoculum for *A. brassicae* and *A. brassicicola.* Humpherson-Jones (1989) observed that infected leaves of oilseed rape placed outdoors on soil produced viable spores for up to 8 weeks, as leaf tissues remained intact. On leaves exposed in November and January, spore concentration decreased with the time but on leaves exposed between April and June, spores concentrations increased up to 9 fold in the first 4-6 weeks and then declined. On stem section of seeds plants of oilseed rape and cabbage similarly placed on the soil, the fungi produced viable spores for up to 23 weeks with spore concentration increasing up to 11 fold in the first 6-8 weeks after harvest. Humpherson-Jones (1989) concluded that infected debris of brassicas crop remaining in the soil after harvest may provide a source of inoculum for Alternaria leaf spot infection which may implicate the spread of the disease within and between crops.

Weed host and other alternative host

The fungus can survive in susceptible weeds or perennial crops (Chupp and Sherf, 1960; Rangel, 1945; Maude and Humpherson-Jones, 1980a, b) and these weed host plants help the Alternaria blight pathogen to propagate the infective entities which serve as the source of inoculum for the oilseed Brassicas. The weed hosts of Alternaria blight pathogens infecting oilseed brassicas are namely: *Convolvulus arvensis, Camelina sativa, Crambe maritime, Chenopodium album, Crambe abyssinica, Anagallis arvensis.*

MEASUREMENT OF THE DISEASE SEVERITY

An appropriate method of disease assessment is a prerequisite for the identification of resistance to Alternaria blight in oilseed brassicas. The appearance of disease is observed both on leaves and siliquae, which is responsible for yield losses. Therefore, disease is assessed both on leaves as well as on siliquae based upon visual assessment. A key for assessment of Alternaria blight on rapeseed and mustard has been proposed by Conn et al. (1990). The infected leaves defoliates after some time which poses some difficulty in assessment of Alternaria blight in mustard. The study of Alternaria blight progression in mustard indicated that the resistance is of slow disease development. Recently, AUDPC is being used to assess the level of Alternaria blight in mustard (Meena et al., 2011b). They also reported that development of the disease was influenced by the growth stages of the crop and delay in sowing results in an increase in disease severity and reduction in yield was observed (Howlider et al., 1989). Sowing of rapeseed–mustard between 30 September to 15 October was found most favourable for reducing leaf spot incidence and increasing yield (Sinha et al., 1992) in India.

MANAGEMENT OF ALTERNARIA BLIGHT

Structural and biochemical defense

Oilseed brassicas are continually exposed to a number of pathogens and, as a result, they have evolved intricate defense mechanisms to recognize and defend themselves against a wide array of these pathogens by structural defense (Meena et al., 2010) and by inducing a set of defense responses that can defeat the invading pathogens (Vishwanath et al., 1999). Structural defense against Alternaria blight is found to be associated with factors that discourages conidial retention on host surface like high deposits of epicuticular wax (Meena et al., 2010) that form a physical barrier as a hydrophobic coating to reduce deposition of water borne inoculum, reduce rate of conidial germination, and germ tube formation (Skoropad and Tiwari, 1977; Saharan,1992). The species *B. napus, B. carinata* and *B. alba* have relatively more epicuticular wax than *B. rapa* and *B. juncea,* and tend to be less sensitive to infection of Alternaria blight pathogens (Conn et al., 1984; Tewari, 1986). Biochemical defense against Alternaria leaf blight in mustard has been found to be associated with leaf enzymes associated with the phenolic pathway and higher leaf sugar contents (Singh et al., 1999). Resistance in *Camelina sativa* (wild allies of Brassicas also known as false flax) against *A. brassicae* has been found to be associated with the presence of chemical compounds, camalexins, somewhat similar to a fungicide available in the market (Browne et al., 1991). Resistance in *Camelina sativa* against *A. brassicae* due to production of the Phytoalexin camalexin has been also reported by Jejelowo et al. (1991) and Thomma et al. (1999). Camalexin also contributes to Alternaria resistance in an indirect way, as camalexin was found to inhibit production of the toxin destruxin B in *A. brassicae* (Pedras et al., 1998).

Search for resistant genotypes

Due to severe losses caused by Alternaria blight in oilseed brassicas, the objective of oilseed breeders is the development of resistant lines against Alternaria blight. Several attempts have been made in past to find out the sources of resistance against Alternaria blight, but resistant sources have not been reported in any cultivated *Brassica* species, but a high degree of resistance against the Alternaria blight has been reported in *B. alba* (Conn et al., 1988), *Eruca sativa* (Conn and Tewari, 1986; Tewari, 1991), and *Sinapis alba* (Kolte, 1985a; Brun et al., 1987; Ripley et al., 1992; Sharma and Singh, 1992; Hansen and Earle, 1995, 1997). The highest degree of resistance to *A. brassicae* was found in the wild relatives of Brassica outside the tribe Brassicaceae. These are false flax (*Camelina sativa*), Shepherd's purse (*Capsella bursa-pastoris*) (Conn et al., 1988), and *Neslia paniculata* (Tewari and Conn, 1993). In search of resistance sources for transfer of resistant genes, Sharma et al. (2002) evaluated thirty-eight species belonging to nine genera, including cultivated and wild allies of the genus *Brassica* under epiphytotic conditions for two years. Eight species namely *B. desnottesii, C. sativa, Coincya pseuderucastrum, Diplotaxis berthautii, Diplotaxis catholica, Diplotaxis cretacea, Diplotaxis erucoides,* and *Erucastrum gallicum* were found completely resistant, whereas others were classified as moderately resistant, susceptible and highly susceptible. A wide range of variation was also observed within the species of genus *Diplotaxis* but the genus *Diplotaxis* was found to be more resistant than the genus *Brassicas*. Sharma et al. (2002) concluded that source of resistance to *A. brassicae* are available within coeno species of *Brassicas* (Tribe) from which genes for resistance can be introgressed into cultivated *Brassica* species. The seven species other than *C. sativa* were identified as completely resistant to Alternaria leaf spot, belong to coeno species of *Brassica* (tribe Brassicaceae). Since resistance is unavailable within the cultivated species, these eight resistant wild species could be used as donor parents for introgressing resistance to leaf spot disease in Indian mustard.

Development of transgenics

Unavailability of resistance gene within the crossable germplasm of *Brassica* necessitates use of genetic

engineering strategies to develop genetic resistance against this pathogen. Mondal et al. (2007) got the integration and expression of the class 1 basic glucanase gene in mustard transgenic and observed that the transgenics arrested hyphal growth of *A. brassicae* by 15-54%.

Gene transfer for resistance in *Brassica* against Alternaria blight

Tuan and Garg (2001) did the gene transformation in *Brassica* sp. using particle bombardment. Cotyledon and hypocotyls of different species of *Brassica* have been used as target explants. Transient expression of uidA gene has been obtained when either been constructed with CaMV35S or Actin promoters. The uid4 gene encodes the 1, Beta-glucuronidase (GUS) enzyme. Its a reporter genesystem, particularly useful in plant molecular biology and microbiology. The highest expression was recorded between 10 to 15 h after bombardment. Plasmids pBI121, pBI221 and pDM803 were used to carry uidA gene. Further transformation events should be carried out to obtain highest transformation efficiency.

Use of fungicides

In the absence of resistant cultivars, fungicides provide the most reliable means of disease control (Vyas, 1993). Multiple applications of fungicides are required to achieve economic yield and acceptable quality in infected crops. Khan et al. (2007) sprayed three systemic fungicides Thiophanate methyl, Ridomil MZ (Mancozeb, 64%+ Metalaxyl, 8%), and Carbendazim alone and in combination with four non systemic fungicides Captan, Mancozeb, Zineb, and Thiram in the field at 0.2% $a.i.L^{-1}$. Ridomil MZ was most effective followed by the combination of Carbendazim + Captan. Singh and Singh (2006) reported that three consecutive sprays of Mancozeb resulted in maximum control of Alternaria leaf blight intensity followed by schedule with two consecutive sprays of Mancozeb (0.2%) and third of Rodomil MZ (0.25%). Foliar sprays of Mancozeb have been found most effective in disease management (Meena et al., 2004, 2011; Mondal et al., 2008).

Use of botanicals

Spray of Eucalyptus leaf extracts significantly reduced the number of spots/leaf, minimum size of spots, minimum disease index and highest yield followed by *Calotropis*, *Ocimum* and *Polyanthai* extracts spray (Patni et al., 2005; Patni and Kolte, 2006). Foliar sprays of aqueous bulb extract of *Allium sativum* (garlic) and *Eucalyptus globulus* (Eucalyptus) have been reported to effectively manage the Alternaria blight on leaves and pods and could be ecofriendly substitute for chemical fungicide mancozeb in management of mustard diseases (Meena et al., 2008, 2011a; Yadav, 2009).

Biological management

Some research findings indicate possibility of biological management of Alternaria blight of *Brassicas*. Foliar application of soil inhabitants isolates of *T. harzianum* (Patni et al., 2005; Meena et al., 2004, 2008, 2011a) and *P. fluorescens* (Patni et al., 2005; Meena et al., 2011a) were found effective in management of Alternaria blight.

Cross protection

Resistance in susceptible mustard cv. PR-15 against the highly and moderately virulent isolates of *A. brassicae* was induced using an avirulent *A. brassicae* isolate. The induction of resistance due to avirulent isolate against highly virulent and moderately virulent isolate of *A. brassicae* resulted in significant reduction in disease severity (Vishwanath et al., 1999).

Integrated management

There are many methods which are presently being used to manage Alternaria blight of *Brassicas*, that is, chemical, cultural, modification of nutrient, and biological. Due to increased awareness on the risks involved in use of fungicides, much attention is being focused on the integrated approach of pathogen management. Burning of crop debris of previous year, timely sowing, use of healthy certified seed, timely weeding, use of balance dose of nutrient, maintenance of optimum plant population, avoidance of irrigation at susceptible stage of crop (45 and 75 DAS) may help to minimize the disease incidence. Application of potash at 40 kg/ha (Sharma and Kolte, 1994; Godika et al., 2001), and soil application of minerals like sulphur, borax, potash and zinc are found effective in the management of Alternaria blight of mustard (Meena et al., 2011). These minerals were found to increase resistance in plants. Kumar and Kumar (2006) found minimum disease severity at 45 cm row spacing in comparison with broadcast method of sowing and found less disease severity in early sown, weeded crops.

FUTURE WORK STRATEGY

The following issues need to be addressed for Alternaria blight in oilseed *Brassicas*.

1. Relative dominance of *A. brassicae, A. brassicicola* and *A. raphani* during different stages of growth of oilseed

brassicas.
2. Development of molecular markers for identification of Alternaria leaf spot pathogens infecting oilseed brassicas.
3. Phylogeny of Alternaria blight pathogens of oilseed brassicas.
4. Gene expression in Alternaria bilght pathogens and host during infection of susceptible, tolerant and resistant varieties.
5. Search of resistant and moderately resistance sources against Alternaria blight in oilseed brassicas and wild relatives.
6. Search for alternative control methods of Alternaria leaf spot of oilseed brassicas.

CONCLUSION

There is no doubt that Alternaria leaf blight is the most destructive disease of oilseed brassicas across the world because of the cosmopolitan and damaging nature of pathogenic *Alternaria* species. Alternaria blight causes considerable reduction in the quality and quantity of harvested products of oilseed brassicas, and no proven source of resistance has been identified among cultivated oilseed brassica crop species. However, high level of resistance to *A. brassicae* have been reported in coenospecies (wild species) of *Brassicas* viz., *B. desnottesii, C. sativa, C. pseuderucastrum, D. berthautii, D. catholica, D. cretacea, D. erucoides* and *E. gallicum* which were found completely resistant. Due to lack of resistance in cultivated *Brassicas* against Alternaria leaf blight, other methods could be used for management of this disease.

One of the most commonly used methods is the use of fungicides. In spite of tremendous use of fungicides against pathogens, these fungicides cause serious health hazards to human beings and also they cause environmental pollution. Hence, nowadays more emphasis is made on other methods of disease management like growing moderately resistant varieties, use of plant and natural products, and bio-control agents and alterations in agronomic practices because they are more economical, eco-friendly and safe.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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