

Eco friendly management of *Alternaria* blight disease: A Review

Abstract:

Control of plant disease is a pressing need for Indian agriculture because of the growing human population and reducing land availability. The increasing demand for sustainable food supply is fulfilled through higher inputs including chemical applications in the form of fertilizers and pesticides. *Alternaria* leaf spot of crucifers is one of the most devastating disease worldwide causing around 20-80% yield losses. Different chemical fungicides have historically provided good control, but because of their toxic nature, ecosystem health has suffered drastically. So new approaches for controlling *Alternaria* diseases is the demand of current scenario. These approaches include the use of bio-control agents, medicinal plants, diverse plant-derived products, resistant varieties, etc. These tactics support sustainable agricultural methods in addition to reducing the environmental hazards connected to chemical fungicides. In order to battle *Alternaria* species across a range of agroclimatic conditions, this review aims to shed light on a variety of environmentally friendly tactics. It emphasizes the significance of using comprehensive and environmentally conscious management strategies in crop protection. This fungal disease, caused by various *Alternaria* species, poses a challenge to sustainable agriculture, necessitating eco-friendly management strategies. Recent studies highlight the potential of botanical extracts, biocontrol agents, and innovative technologies as viable alternatives to chemical fungicides, which often harm the environment and human health. Greener control methods of *Alternaria* fungi can increase agricultural production, improving the economy and global health.

Keyword: Alternaria, Bioagent, Plant extract, ecofriendly, fungicides

1. INTRODUCTION:

With the increasing population and degradation of available land, plant disease control is becoming increasingly important for Indian agriculture. Pesticides and fertilizers are two examples of the increased inputs needed to meet the growing demand for a sustainable food supply. However, frequent chemical use pollutes the environment and might cause pathological conditions. It can also cause the target organism to become resistant to the chemicals. Since the nation is currently supporting organic and natural farming, it is crucial to treat plant diseases using environmentally friendly methods in order to preserve ecosystem health and human nutrition through a balanced food chain in agro-ecosystems. For many sensitive regions of the world, the occurrence and severity of plant disease outbreaks are increasing, posing serious and expanding concerns to primary productivity, global food security, and biodiversity loss. Both yield and ecological losses result from these disease outbreaks. This has a direct effect on food security, local economies, and other related socioeconomic factors. Therefore, to create agricultural and natural ecosystems that are resilient to climate change, a better understanding of how climate change affects the molecular, epidemiological, and ecological interactions between diseases, plants, and the microbial communities that are associated with them is required.

Alternaria black spot of cruciferous vegetables, caused by different species of *Alternaria*, remains an increasing threat to *Brassicaceae* crops throughout the world [1]. Under the Ascomycota division, *Alternaria* species are potentially global fungi that are found in soil, plants, food, feed, and indoor air [2]. The opportunistic pathogen affects a wide range of hosts, accounting for at least 20% of agricultural spoilage, with the most severe losses potentially amounting to 80% of the crop [3]. *Alternaria* blight the most common disease caused by the genus *Alternaria* is responsible for about 32-57% loss in yield [4]. These pathogens are responsible for significant seed yield loss in oleraceous brassicas, the most significant part of economic importance [5].

Although the use of resistant cultivars is an ideal solution to the disease, crucifer cultivars resistant to black leaf spot are currently scarce. The use of fungicides is still the most popular method for control of black leaf spot of crucifers. Dithiocarbamate based fungicides are being used to control this disease. Mancozeb belonging to ethylene bis-dithiocarbamate (EBDC) group of fungicides can effectively control this disease [6]. Ethylene thiourea (ETU) the decomposed product of EBDC is reported to be carcinogenic [7]. Excess use of sprays and higher volumes of fungicides leads to development of residues in the consumable part of the vegetables and also develops resistance [8]. This necessitates the need to look for safer, eco friendly and effective management strategies of the disease. Biocontrol agent, phytoextracts different cultural practices are safe and cheaper that are equally effective as compared to fungicides.

Sustainable techniques, such as the use of biocontrol agents and various plant products to reduce plant disease, provide a potent substitute for synthetic chemicals that have comparable goals. A seemingly limitless resource is available for this purpose due to the abundance of medicinal plants and the enormous diversity of the microbial population. Numerous foliar diseases in plant species, such as leaf spots, leaf blights, and leaf blotches, are caused by fungus pathogens. Among these diseases, this brief study aims to compile knowledge on environmentally friendly *Alternaria* species management techniques for future need.

2. Biology:

Most *Alternaria* species generate asexual spores, or conidia, that are between 160 and 200 μm length, which are produced by their conidiophores. In vitro, sporulation takes place between 8 and 24 $^{\circ}\text{C}$, and mature spores appear after 14 to 24 hours. The ideal temperature range for sporulation is 16–24 $^{\circ}\text{C}$, with a 14-hour time frame. For the majority of species, moisture in the form of rain, dew, or high humidity is necessary for infection and must be present for at least 9–18 hours [9]. Infection is guaranteed when there is constant wetness present for 24 hours or more [10, 11]. Large numbers of ripe spores will be produced in less than 24 hours with a relative humidity of 91.5% (at 20 $^{\circ}\text{C}$) or above [9].

3. Symptomatology:

Aneja and Agnihotri reported in brassica crop that the symptoms of *alternaria* initially starts as tiny brown to black dots on the lower leaves, which quickly multiply and enlarge to form noticeable round spots. These spots are associated with concentric rings that vary in size, color, shape, and intensity depending on the host plant and causal species under various environmental conditions. The disease eventually causes the entire leaf to become infected and defoliate. The infection then appears as tiny spots on the middle and top leaves. During the advanced phases of plant development, the dots typically manifest as black stripes on the stem and siliquae, which eventually merge to turn the siliquae black [12]. In tomato that the disease causes oval to angular lesions that are 1-2 mm in diameter and form concentric rings surrounded by a yellow chlorotic halo. The symptoms are brown necrotic lesions that are visible on older leaves and advance upwards as the plants age. The severe phase of the disease results in premature defoliation and drying of the plant [13].



Fig: Typical leaf spot caused by *A. brassicicola* in cauliflower

4. Epidemiology:

Humpherson-Jones and Phelps reported that the pathogens are greatly influenced by weather with the highest disease incidence reported in wet seasons and in areas with relatively high rainfall. Humidities equal to or higher than 91.5% and 87% were required for the sporulation in *A. brassicae* and *A. brassicicola* on naturally-infected leaf discs of oilseed rape and cabbage respectively. The optimum temperatures for sporulation were 20–30°C for *A. brassicicola* and 18–24°C for *A. brassicae*. At these temperatures both fungi produced spores in 12–14 h. Sporulation in *A. brassicae* was inhibited above 24°C [9]. Biswas, M. K. conducted an experiment to study how different weather parameters affect the development of Alternaria leaf spot of mustard under the agro-ecological conditions of red and lateritic belt of West Bengal. He found that a maximum temperature of 23.83 to 29.63°C was most favoured for the disease development. Also Maximum relative humidity (80.33 to 90.55 %), minimum relative humidity (52 to 58 %) and average sunshine of 7 to 8 hours per day favoured the disease development. He further concluded that different meteorological factors viz., maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and daily sunshine hours were collectively contributed to 53.7 - 96.9 % for the development of the lesion size caused by *Alternaria brassicae* in mustard field [14]. Manjhi *et al.*, examined the weather parameter of Alternaria blight of mustard and observed that rainfall and relative humidity were more correlated with disease intensity than maximum temperature. A relative humidity of $\geq 70\%$ coupled with warm weather ($\geq 28^\circ\text{C}$) and intermittent rains favoured the disease development [15].

5. Conidial Characters:

As reported by Devi *et al.*, among 8 isolates of *Alternaria brassicae*, maximum conidial length was 21.7 μ and minimum 15.6 μ . The average conidial length were varied from 15.6 μ to 21.7 μ with a range of conidial length from 14 μ to 26 μ . They found that maximum conidial breadth was 2.7 μ and minimum conidial breadth was 1.5 μ . The average conidial width varied from 1.8 μ to 2.7 μ with a range from 1.5 μ to 3 μ . Horizontal and vertical septation in conidia also showed variation in different isolates. The average number of horizontal septa was maximum in Ab6 (13.8) with a range of 8-14 and minimum in Ab7 (6.8) with a range of 5-8 were observed. The average number of vertical septa was highest in Ab8 (4.2) with a range from 1-5 and lowest in Ab5 (1.5) with a range from 1-2. The average number of beak length was highest in Ab4 (9.8 μ) with a range from 5-10 μ and lowest in Ab8 (6.5 μ) with a range from 5-9 μ . Finally it was revealed that smallest size of conidia in isolates Ab8 and longest size of conidia in isolates Ab4 were observed [16]. Mehra *et al.*, reported significant morphological variability in respect of conidial length, conidial width, conidial beak length, number of septa and number of cells of 20 different isolates of *Alternaria brassicae* [17].

6. Culture Media for Growth of *Alternaria* spp.

According to Abeer *et al.*, potato dextrose agar was the most effective medium for *A. alternata* of *Avicennia marina*'s radial growth and sporulation [18]. Hubballi *et al.* found that host leaf extract medium was the best medium for *A. alternata* causing leaf blight on *Morinda citrifolia* followed by potato dextrose agar (PDA) media [19]. Mishra & Mishra discovered that PDA medium exhibited the highest growth of the cotton pathogen *A. alternata*, with Richards agar, Czapek's agar, Coon's agar, and leaf decoction agar following that [20]. In nonsynthetic media such as oat meal agar and PDA, *A. alternata*, the pathogen that causes gerbera leaf blight, exhibited outstanding mycelial growth and conidial production [21]. Richard's agar medium and potato dextrose agar were the next best media for *A. lini* growth and sporulation [22]. Munde *et al.* found that maximum growth of *A. solani* was obtained on yeast extract glucose agar medium followed by soil extract agar medium, oat meal agar and Sabour's agar medium [23]. Aneja & Agnihotri reported in Alternaria Blight of Brassica that the shape of colonies has been seen to vary; the diameter ranges from 32 to 68 mm, the surface texture is velvety to wooly, and the color is olive green to dark green, with sporulation ranging from minimal to intense [12]. Kiran *et al.*, reported that Cultural characters of the seven isolates were differ in average growth rate, growth pattern and colony colour. The colony colour of those isolates were initially white

but later turns to brown to light brown and to grey brown. Colony characters of *A. brassicicola* were described as olive to grey brown in colour and showed velvety growth [24]. The mycelium was septate, branched and produced conidiophores [25]. Rahimloo & Ghosta stated that the colony colour of 38 isolates of *A. brassicicola* were grey to brown and average growth rate was 0.78 cm /day [26].

7. Pathogenicity:

Pathogenicity of *Alternaria alternata* was confirmed by Sarkar *et. al.*, in which initial symptoms of *Alternaria* leaf spot was recorded 7-9 days after inoculation on leaves with a small, circular necrotic spot with development of concentric rings [27]. Pathogenicity test of seven isolates of *A. brassicicola* was confirmed that was obtained during the survey by artificial inoculation on healthy plants [24]. Aboomer *et. al.*, confirmed the pathogenicity of *Alternaria brassicicola* in cabbage by injecting spore suspension in healthy plants. Infection was appeared in healthy plants within approximately 10-15 days [28]. Thambi *et al.*, confirmed the pathogenicity of *Alternaria* spp. collected from the diseased leaves of broccoli by detached leaf method inoculated with 10^4 spores/ml . Results showed the appearance of brown spots on the tested healthy broccoli leaves within 72 h of incubation at 25 ± 2 °C [29]. Deep *et. al.*, conducted pathogenicity test for 32 isolates of *Alternaria brassicicola* in susceptible variety of cauliflower. They found all the isolates pathogenic in nature. Out of which nine were highly pathogenic (spot size more than 1cm), eight isolates were moderately pathogenic (spot size were 0.6-1cm) and rest fifteen isolates were lowest pathogenic (spot size were 0.2-0.5cm) [30].

8. Management of *Alternaria* spp. :

Since a number of *Alternaria* species infect crops of economic importance, there is a strong need for effective control of this pathogen. Several research works focusing different management practices are going on. Some of them are discussed below with special reference to environmental friendly nature. The extensive use of chemical fungicides has to be curbed immediately because of the possible harm they could do to the environment and public health.

8.1 Cultural Management:

The planting of susceptible varieties in field should be avoided with infected residues from a previous crop retained on the surface. Apart from this, balanced crop nutrition especially of potassium should be provided [31]. Various cultural practices *viz.* covering the cauliflower nursery with nylon net, growing nursery under poly cover, use of hessian cloth (shelter belt) in the field and removal of diseased foliage is an effective mean of eco friendly management of *Alternaria* Blight and Black rot of Cauliflower [32].

8.2 Nano particle based management:

Using nanoparticles as a management strategy for *Alternaria* leaf blight is a possible substitute for conventional fungicides. Nanoparticles provide a number of benefits for managing diseases, including a high surface area to volume ratio that improves their interaction with fungal pathogens and a small size that facilitates effective penetration of plant tissues. Furthermore, compositions based on nanoparticles can offer long-lasting defense against *Alternaria*, negating the need for repeated applications. This novel method presents a safer and more effective substitute for traditional fungicides, with considerable promise for long-term disease control in agriculture. Taha *et al.*, studied nano-selenium (nano-Se) and nano-silica (nano-SiO₂) against the leaf spot disease caused by *Alternaria alternata* in common bean (*Phaseolus vulgaris* L.). The in vitro study showed that 100 ppm nano-Se had an efficacy rate of 85.1% on *A. alternata* mycelial growth, followed by the combined applications (Se + SiO₂ at half doses) with an efficacy rate of 77.8%. The field study showed that nano-Se and the combined application of nano-Se and nano-SiO₂ significantly decreased the disease severity of *A. alternata* [33]. Significant antifungal potential of Mycogenic copper oxide nanoparticles (M-CuO NPs) was recorded by Gaba *et al.*, as it inhibited the growth of *A. brassicae* up to 92.9% and 80.3% in supplemented media with C-CuO NPs at 200 ppm dose [34].

8.3 Essential oil and Biopolymers:

Biofungicides derived from essential oils and biopolymers are being utilized as eco-friendly solutions for combating plant-infecting fungi [35]. Essential oils stand out as a promising option for controlling diseases spread by these fungi, provided it should have efficiency, biodegradability, and non-toxic to human health and environment [36]. Recently, numerous research findings have shown the effectiveness of essential oils. For instance, Feng *et al.*, found that essential oils from thyme (*Thymus vulgaris*), eucalyptus (*Eucalyptus globulus* Labill), cassia (*Cassia didymobotrya*), sage (*Salvia officinalis*), and nutmeg (*Myristica Fragans Houtt*) had a suppressive impact on *A. alternata*, responsible for forming necrotic lesions [37]. Zaker *et al.*, showed that eucalyptus (*Eucalyptus globulus*), peppermint (*Mentha piperita*), lavender (*Lavandula angustifolia*), and alcoholic solutions of datura were effective against *Alternaria alternata*. [38].

Conversely, biopolymers derived from carbohydrates like alginate and chitosan are highly appealing for shielding plants from fungal infections because of their antifungal characteristics and their ability to degrade naturally [39]. Applying chitosan, which is a modified form of chitin discovered in the exoskeleton of crustaceans like shrimp, crab, lobster, and shellfish, as a protective layer for crops against fungal diseases is extensively researched [40].

8.4. Resistant Variety:

The introduction of different crop varieties resistant to diseases has boosted their inherent resistance, making them more cost-effective for farmers and ensuring their effectiveness over time. For instance, the Cucumero melo line MR-1 is immune to *A. cucumerina* [41], while Mathur & Shekhawat discovered that watermelon varieties Sel-1 and Sugarbaby possess resistance, and Meetha, Durgapura, AY, WHY & WHY-4 are highly vulnerable to *Alternaria* leaf spot [42]. Similarly, RW-177-3, RW-1, RW-187-2, and Milan are moderately resistant to the same disease. Katiyar *et al.*, identified three bottle gourd varieties, Azad Harit, 7002, and 7003, as resistant to *A. cucumerina* [43]. Additionally, two highly resistant chili varieties, CA 87-4 and CA 748, were found to be effective against fruit rot caused by *Alternaria* [44], and tomato varieties such as Arka Alok, Arka Abha, Arka Meghali, Arka Saurabh, IHR-305, IHR-308, IHR2266, IHR-2285, and IHR-2288 were shown to be resistant to early blight [45].

Arka Kalyan was found to be a resistant variety of onion against foliar blight caused by different species of *Alternaria* [46]. Different wild species of brassica was screened for resistance against *alternaria* leaf spot and complete resistance was found in *Capsella* while resistance in *Lepidium*, *Camelina* and *Biscutella* was observed [47]. Recent developments in the fight against *Alternaria* blight in India have highlighted the potential of Australian genotypes, particularly 'JM06014' and 'JM018'. These varieties have demonstrated significant field resistance to *Alternaria* blight, making them promising candidates for cultivation in regions affected by this fungal disease [48]. Likewise, researchers worldwide are focusing on the manipulation of genes that encode for essential proteins for inducing resistance in various crops are going on.

8.5 Bioagents:

Given the antagonistic characteristics of different bacteria and actinomycetes, there is a push for the use of bio-control agents. Their growing use is also largely due to the fact that they are environmentally beneficial. Zaker & Mosallanejad conducted in vitro evaluation of two fungal bio-agents viz., *Trichoderma viride*, *Trichoderma harzianum*, and two bacterial bio-agents *Pseudomonas fluorescens* and *Bacillus subtilis* against *A. alternata* for the antagonistic activity by dual culture technique. They found that all the antagonistic fungi and bacteria inhibited the growth of *A. alternata* ranging from 51.21 to 83.73 per cent. *T. harzianum* was found to be superior over all treatments with 83.73 per cent growth inhibition followed by *T. viride* (78.45%) and *P. fluorescens* (63.00%), while *B. subtilis* (51.21%) was found to be least effective [38]. Sarkar *et al.*, found *T. harzianum* to be the most effective antagonist in suppressing mycelia growth of *Alternaria alternate* (76.23%) [27]. Aboomer *et al.*, found similar results where mycelia growth of *Alternaria brassicicola* was

inhibited by *T. harzianum* upto 40% [28]. Mamgain *et al.*, conducted in vitro evaluation of four biocontrol agents viz., *Trichoderma viridae*, *Trichoderma harzianum*, *Trichoderma virens* and *Aspergillus niger* against *Alternaria brassicae*. Their findings showed that the antagonists either by demonstrating inhibitory zones or by overgrowing, greatly restrict the growth of *Alternaria brassicae*. *T. viridae* was shown to be the most successful biocontrol agent against *Alternaria brassicae*, showing an 80.68% inhibition rate compared to the other treatments. This was followed by *T. harzianum*, *T. virens* and finally *Aspergillus niger* with 78.4 percent, 75 percent and 55.68 percent inhibition respectively [31]. Singh & Abhimanyu studied the efficacy of *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. koningii* and *Pseudomonas fluorescens* in controlling *A. brassicae* causing Indian mustard blight under laboratory conditions. All the fungal antagonists found to inhibited the growth of *A. brassicae*, with *T. viride* recording the highest growth inhibition of the pathogen (67.9%) [50].

8.6 Plant Extract:

Out of six different plant extract tested against *Alternaria alternate* Neem leaf extract was found to be most effective inhibiting 55% of the pathogen, followed by garlic cloves (41.25%), Tulsi leaf extract (36.25%), nilgiri leaf extract (32.5%), mixture of onion and garlic leaf extract (30%) and parthenium(28.75%) [27]. Similar results were found by Aboomer *et al.*, where Neem leaf extract and Garlic showed better results in inhibition of mycelia growth of *Alternaria brassicicola* in vitro. [28]. Similar results were also reported by Habib *et. al.*, where Neem and Garlic showed better result in reduction of severity of the disease [51]. Guleria and Kumar showed that plants treated with neem leaf extract showed a considerable reduction in *Alternaria* leaf spot disease. The control group showed the highest level of disease severity (65%), while the plants treated with a 1:2 dilution of neem leaf extract only showed 10% of severity [51]. Crude extract from dried leaf tissues of *Agave americana* possessed excellent antifungal activity against *A. brassicae*, the casual agent of *Alternaria* blight of Indian mustard [52].

8.7 Integrated Disease Management:

Numerous methodologies are currently employed for the management of *Alternaria* blight in Brassicas, specifically encompassing chemical, cultural, nutrient modification, and biological strategies. In light of heightened awareness regarding the potential hazards associated with fungicide application, considerable emphasis is being directed towards an integrated approach to pathogen management. The incineration of crop residues from the preceding year, adherence to timely sowing schedules, utilization of healthy certified seeds, regular weeding practices, application of balanced nutrient doses, maintenance of optimal plant population density, and the avoidance of irrigation during the crop's susceptible stages (45 and 75 days after sowing) may contribute to the reduction of disease incidence. The application of potash(K) at a rate of 40 kg/ha along with the soil application of minerals such as sulfur, borax, potash, and zinc, has been demonstrated to be effective in the management of *Alternaria* blight in mustard [54,55,56]. These minerals have been shown to enhance plant resistance. Kumar and Kumar discovered that plants were least affected by the disease at 45 cm between rows compared to when seeds were sown by broadcast, and that crops sown early and regularly weeded had lower disease incidence [57].

8.8 Molecular based control method:

Carrascal-Hernández *et al.* highlighted the application of 'omics' and gene editing through the CRISPR/Cas9 system and RNAi technologies focusing their effectiveness as emerging greener alternatives for controlling this phytopathogenic fungi. This work explores the transformative potential of CRISPR/Cas9 gene-editing technology in enhancing plant resistance against *Alternaria*, a significant fungal pathogen [58]. This innovative approach offers a promising alternative to traditional disease management practices, which often rely on chemical fungicides.

8.9 Host Resistance:

Source of resistance in different brassicaceous vegetables have been identified, which can be incorporated through conventional and biotechnological techniques under suitable agronomically sound yield and quality bases to be effective against *Alternaria* spp.. However, exploitation of this information to manage *Alternaria* disease under field conditions need much more emphasis [59].

9. Future work strategy:

Future opportunities for the environmentally friendly control of *Alternaria* blight appear bright and varied. First, more research into innovative biological control agents, such as microbial consortia and beneficial microorganisms, has the potential to improve disease suppression and advance soil health and biodiversity. Furthermore, potential for the production of genetically modified crops with improved resistance to *Alternaria* infections are presented by advances in molecular biology and biotechnology. By facilitating early diagnosis and focused application of control measures, the integration of precision agriculture tools, such as remote sensing and data analytics, can further optimize disease management tactics. Furthermore, resilient agroecosystems that are less prone to *Alternaria* blight and other diseases can be created with the adoption of sustainable farming methods such crop rotation, intercropping, and organic farming. In order to ensure the long-term sustainability of agricultural production systems, cooperative research projects and knowledge-sharing platforms will be crucial for hastening the adoption of eco-friendly management techniques among farmers globally.

10. Conclusion:

In conclusion, a viable path toward sustainable agriculture is provided by the environment friendly control of *Alternaria* blight. Considerable progress has been achieved in lowering the dependency on chemical fungicides with managing the disease through the investigation of alternative tactics such as biological control agents, plant extracts, essential oils, host resistance, nano particle-based formulations. In addition to being effective in managing *Alternaria* blight, these environmentally friendly methods also have the advantage of having less adverse effect on the environment, preventing resistance from developing, and enhancing the general health of the agroecosystem. To make these techniques more cost-effective and scalable, as well as to optimize them for a variety of crops and environmental circumstances, more research is necessary. Through sustained innovation and the incorporation of environmentally conscious management techniques into agricultural systems, we may work towards a future where crop protection is more resilient and sustainable.

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