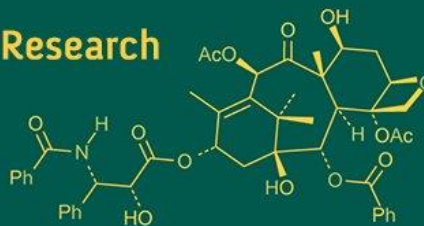


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Henia Soyam
School of Agriculture,
Uttaranchal University,
Dehradun, Uttarakhand, India

Sandeep Kumar
School of Agriculture,
Uttaranchal University,
Dehradun, Uttarakhand, India

Sharad Pandey
School of Agriculture,
Uttaranchal University,
Dehradun, Uttarakhand, India

Rajendra Prasad
School of Agriculture,
Uttaranchal University,
Dehradun, Uttarakhand, India

Lourembam Abinash Singh
School of Agriculture,
Uttaranchal University,
Dehradun, Uttarakhand, India

Albana Teja G Momin
School of Agriculture,
Uttaranchal University,
Dehradun, Uttarakhand, India

Role of bioagents and plant extracts in the management of *Alternaria* blight of Indian mustard caused by *Alternaria brassicae*: A comprehensive review

Henia Soyam, Sandeep Kumar, Sharad Pandey, Rajendra Prasad, Lourembam Abinash Singh and Albana Teja G Momin

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Abstract

Alternaria blight, caused by *Alternaria brassicae* (Berk.) Sacc., is among the most devastating foliar diseases of Indian mustard (*Brassica juncea* L.), causing yield losses ranging from 10-70% under favorable epidemic conditions. The indiscriminate use of synthetic fungicides for disease management has raised serious concerns over environmental safety, the development of fungicide resistance, and human health hazards. The present review consolidates and critically analyzes existing and recent research on the biological control potential of fungal and bacterial bioagents - *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis*—as well as the antifungal efficacy of botanicals derived from neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), ginger (*Zingiber officinale*), garlic (*Allium sativum*), and turmeric (*Curcuma longa*) against *A. brassicae*. Mechanisms of biocontrol, including mycoparasitism, antibiosis, competition, induced systemic resistance (ISR), and production of cell-wall-degrading enzymes are discussed. The phytochemical basis of botanical antifungal activity, comprising azadirachtin, eugenol, allicin, gingerol, and curcumin, is elaborated. The review also covers integrated disease management approaches, seed treatment protocols, field efficacy trials, and future perspectives for the commercialization of bioagents and botanicals.

Keywords: *Alternaria brassicae*, *Alternaria* blight, biological control, plant extracts, integrated disease management

1. Introduction

Indian mustard (*L. Czern. & Coss.*) is one of the most economically significant oilseed crops in India, occupying approximately 5.9 million hectares with a production of about 8.0-9.5 million tonnes annually (DRMR, 2023) [4]. India ranks among the top three producers of rapeseed-mustard globally, and the crop contributes nearly 28-30% of the country's total edible oil pool. Mustard is cultivated extensively across the Indo-Gangetic plains, particularly in Rajasthan, Uttar Pradesh, Haryana, Madhya Pradesh, and West Bengal. Despite its agricultural significance, mustard production is severely constrained by various biotic stresses, among which *Alternaria* blight is considered one of the most devastating foliar diseases. The disease manifests as dark brown to black, concentric-ringed necrotic lesions on leaves, stems, siliques, and seeds, ultimately reducing photosynthetic efficiency, seed weight, and oil content (Meena *et al.*, 2022) [17]. Under warm humid conditions (temperature 25-30°C, relative humidity >85%), the disease can cause yield losses of 10-70% and seed oil reduction of 5-15% (Gupta *et al.*, 2020) [5].

Historically, disease management has relied heavily on synthetic fungicides such as mancozeb, iprodione, carbendazim, and propiconazole. However, their repeated use has raised concerns regarding pathogen resistance, phytotoxicity, ecological imbalance, residue toxicity in food and environment, and the cost burden on small-scale farmers. Under these circumstances, biocontrol-based and botanical approaches offer promising, sustainable, and economically viable alternatives (Sharma & Saharan, 2021) [29].

Biological control exploits naturally occurring microorganisms, primarily *Trichoderma* spp. and fluorescent *Pseudomonas*, as antagonists against soil-borne and foliar pathogens.

Corresponding Author:
Henia Soyam
School of Agriculture,
Uttaranchal University,
Dehradun, Uttarakhand, India

Botanical fungicides, derived from plant extracts rich in secondary metabolites, provide a broad-spectrum, biodegradable, and locally accessible disease management option. The present review aims to comprehensively synthesize and critically evaluate research on the use of these bioagents and plant extracts for the management of *Alternaria* blight in Indian mustard.

2. The Pathogen: *Alternaria brassicae*

2.1 Taxonomy and Morphology

Alternaria brassicae (Berk.) Sacc belongs to the Kingdom Fungi, Phylum Ascomycota, Class Dothideomycetes, Order Pleosporales, and Family Pleosporaceae. Colonies of *A. brassicae* on potato dextrose agar (PDA) are initially greyish-white to pale olivaceous, becoming dark olivaceous-grey to black with age. Mycelium is septate, branched, and dark brown. Conidiophores are simple or branched, septate, pale to mid-brown, arising singly or in small groups. Conidia are produced in long chains of 4-12 spores, are large, obclavate to oblong-ellipsoidal, with a long beak, measuring $75\text{--}350 \times 15\text{--}22 \mu\text{m}$ (excluding beak), muriform with 6-13 transverse septa and 0-4 longitudinal septa (Simmons, 1992; Meena *et al.*, 2016) [31, 14, 15, 16]. The large conidia with long beaks are a key morphological feature distinguishing *A. brassicae* from *A. raphani* and *A. alternata*, which also infect cruciferous crops.

2. Area of Production and Economic Importance

Mustard is grown across diverse geographical regions with India being the world's largest producer, consumer, and exporter of mustard seed. According to recent agricultural statistics, mustard cultivation in India covers approximately 7.5-8.5 million hectares annually with production around 11-12 million metric tons. Major mustard-producing states include Rajasthan (contributing ~25% of national production), Madhya Pradesh, Odisha, Uttar Pradesh, and Haryana (Ministry of Agriculture, 2022).

Globally, the primary mustard producers are India (contributing approximately 11 million tons), Canada (3-4 million tons), China (3 million tons), and the European Union countries. Mustard production serves multiple purposes: edible oil production (main use), spice and condiment preparation, animal feed through cake residue, and increasingly in industrial applications. The crop's economic value in India exceeds Rs. 35, 000-40, 000 crores annually, making it a crucial component of the agricultural economy, particularly for smallholder and marginal farmers in semi-arid regions.

The yield potential of mustard ranges from 1.0-2.0 tons/hectare, but actual productivity remains suboptimal due to biotic stresses, particularly fungal diseases. *Alternaria brassicae*-induced losses reduce productivity by approximately 20-40% in non-endemic areas and up to 90% in areas with high disease pressure. Given the economic significance and dietary importance of mustard, implementing effective disease management strategies is crucial for maintaining stable production and ensuring food security.

2.2 Disease Cycle and Epidemiology

The pathogen primarily survives on infected crop debris, seeds, alternate brassica hosts, and volunteer plants. Conidia germinate in the presence of free moisture and penetrate host tissue directly through the cuticle or via stomata. The

disease cycle involves initial infection (primary inoculum from seed or debris), lesion development, sporulation (secondary inoculum), and airborne dispersal. The incubation period ranges from 3 to 7 days depending on environmental conditions. Environmental conditions such as temperatures between 18-27°C and relative humidity above 80% favor rapid disease development. The necrotrophic nature of the pathogen makes disease management particularly difficult because the fungus benefits from host cell death during infection.

The pathogen overwinters in infected plant residues, soil, and seeds, serving as primary sources of inoculum for the subsequent season. Crop debris contamination and survival of fungal propagules in soil can persist for extended periods, complicating disease management in the field.

3. Biological Control: Mechanisms and Overview

Biological control of plant diseases involves the use of living organisms or their metabolites to suppress pathogen populations and disease incidence. The main mechanisms employed by biocontrol agents (BCAs) include: (i) mycoparasitism—direct hyphal penetration and lysis of the pathogen; (ii) antibiosis—production of volatile and non-volatile antibiotics, cell wall-degrading enzymes (CWDEs), and secondary metabolites toxic to pathogens; (iii) competition for nutrients and space; (iv) induction of systemic resistance (ISR or SAR) in host plants; and (v) plant growth promotion through phytohormone production, phosphate solubilization, and siderophore secretion (Harman *et al.*, 2019) [7]. Biocontrol agents are increasingly being formulated as wettable powders (WP), talc-based formulations, liquid biofungicides, and seed coat products for field application.

3.1 Role of *Trichoderma viride* in Disease Management

Trichoderma viride is one of the most widely used fungal bioagents for the management of soil-borne and foliar plant pathogens. The fungus suppresses pathogens through several mechanisms including mycoparasitism, competition for nutrients and space, production of antibiotics, and induction of host resistance. Studies have shown that *T. viride* effectively inhibits mycelial growth and sporulation of *A. brassicae* under *in vitro* conditions.

The antagonistic activity of *T. viride* is mainly attributed to its ability to produce hydrolytic enzymes such as chitinases, glucanases, and proteases that degrade fungal cell walls. In addition, the bioagent secretes secondary metabolites with antifungal properties that suppress pathogen growth. Application of *T. viride* as seed treatment or foliar spray has resulted in reduced disease severity and enhanced plant vigor in mustard crops. Moreover, the fungus improves nutrient availability and root growth, thereby promoting overall plant health and tolerance against pathogen attack.

Studies have also reported that volatile organic compounds released by *T. viride* significantly reduce conidial germination and germ tube elongation of *A. brassicae*, thereby limiting early infection establishment. Furthermore, its rapid colonization ability enables it to outcompete the pathogen for nutrients, making it an effective preventive biocontrol strategy under both laboratory and field conditions.

3.2 Role of *Trichoderma harzianum* in Disease Management: *Trichoderma harzianum* is another important

fungal antagonist extensively studied for biological control of plant diseases. Similar to *T. viride*, it exhibits multiple mechanisms of pathogen suppression including antibiosis, mycoparasitism, and induced systemic resistance. The fungus colonizes the rhizosphere effectively and competes aggressively with plant pathogens for nutrients and ecological niches. In dual culture assays, *Trichoderma harzianum* consistently exhibits strong mycoparasitic behaviour, characterized by coiling of its hyphae around the pathogen hyphae, followed by penetration and subsequent lysis of the host cells. This antagonism is further supported by the production of both volatile and non-volatile inhibitory metabolites (Benhamou & Chet, 1993; Harman *et al.*, 2004; Poveda, 2021) [1, 6, 25].

Antagonistic Mechanisms Against *A. brassicae*

Several studies have reported that *T. harzianum* significantly inhibits radial growth and sporulation of *A. brassicae*. The bioagent produces volatile and non-volatile metabolites that adversely affect fungal development. Furthermore, *T. harzianum* stimulates host defense mechanisms by activating defense-related enzymes and strengthening structural barriers against infection. Its ability to survive under diverse environmental conditions and compatibility with organic farming practices make it a valuable component of sustainable disease management programs (Harman *et al.*, 2004) [6].

3.3 Role of *Bacillus subtilis* in Disease Management

Bacillus subtilis is a beneficial rhizobacterium known for its strong antagonistic activity against numerous plant pathogens. The bacterium suppresses fungal pathogens through production of antibiotics, lipopeptides, siderophores, and hydrolytic enzymes. In the case of *Alternaria* blight, *B. subtilis* has shown remarkable ability to inhibit conidial germination and mycelial growth of *A. brassicae*.

Yadav *et al.* (2015) [41] reported that culture filtrate of *B. subtilis* at 10% concentration inhibited mycelial growth by 68.7% and conidial germination by 72.4%. Tiwari and Tiwari (2017) [35] demonstrated that seed treatment with *B. subtilis* (10^9 cfu/mL) significantly reduced seed-borne infection by 71.3% and improved seedling vigour index. Foliar spray of *B. subtilis* wettable powder (10^8 cfu/mL) reduced disease incidence by 54.8% and severity by 49.6% under field conditions. Combination of *B. subtilis* with *T. harzianum* demonstrated synergistic inhibition of the pathogen, with 84.6% mycelial growth inhibition (Meena *et al.*, 2016) [14, 15, 16].

The bacterium produces antimicrobial compounds such as surfactin, iturin, and fengycin, which disrupt fungal cell membranes and inhibit fungal development. In addition to direct antagonism, *B. subtilis* induces systemic resistance in plants by activating defense-related pathways involving salicylic acid and jasmonic acid signaling. Seed treatment and foliar application of *B. subtilis* formulations have resulted in reduced disease incidence and enhanced plant growth in mustard crops (Verma *et al.*, 2014). Due to its rapid multiplication and environmental adaptability, *B. subtilis* is considered an effective biological control agent for integrated disease management.

3.4 Role of *Pseudomonas fluorescens* in Disease Management: *Pseudomonas fluorescens* is a plant growth-

promoting rhizobacterium widely recognized for its biocontrol potential against fungal diseases. The bacterium suppresses pathogens through production of antibiotics, hydrogen cyanide, siderophores, and various antifungal metabolites. Several studies have demonstrated that *P. fluorescens* effectively reduces the growth and sporulation of *A. brassicae*.

One of the important mechanisms of *P. fluorescens* is the induction of systemic resistance in host plants. The bacterium enhances plant defense responses by stimulating the production of defense enzymes such as peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase. These defense responses strengthen plant resistance against pathogen invasion. In addition to disease suppression, *P. fluorescens* promotes plant growth through improved nutrient uptake and phytohormone production. Its dual role as a growth promoter and disease suppressor makes it highly valuable for sustainable agriculture.

4. Role of Plant Extracts (Botanical Fungicides)

Plant-derived extracts (botanicals) represent an ancient and increasingly validated approach to plant disease management. They contain complex mixtures of secondary metabolites—alkaloids, terpenes, phenolics, flavonoids, essential oils, and glycosides, that collectively or individually exert antifungal effects through multiple mechanisms including disruption of cell membrane integrity, inhibition of spore germination, interference with metabolic pathways, and induction of host resistance (Doughari, 2015; Patel & Ingle, 2022) [3, 23]. Their broad-spectrum bioactivity, biodegradability, low mammalian toxicity, and local availability make them highly attractive for disease management in resource-limited farming systems.

4.1 Neem (*Azadirachta indica*)

Active Constituents and Mechanism

Neem (Family: Meliaceae) is among the most extensively studied botanicals for the management of *Alternaria* blight. Neem leaf and seed kernel extracts contain bioactive compounds such as azadirachtin, nimbolide, nimbin, and salannin. These compounds interfere with fungal ergosterol synthesis (analogous to sterol demethylation inhibitors), inhibit spore germination and germ tube elongation, and disrupt cell membrane permeability (Mishra & Singh, 2020) [18]. Commercial neem-based formulations, including neem oil emulsions and aqueous neem extracts, are readily available and relatively affordable. The main limitations include variability in bioactive compound concentrations depending on plant part used, extraction method, and storage conditions. Standardization of neem extracts regarding azadirachtin content and development of stabilized formulations remain ongoing research priorities.

Efficacy Studies

At 10-15% concentration, aqueous NLAE inhibits mycelial growth by 65-80% *in vitro* (Chaudhary & Sinha, 2021) [2]. Commercially formulated neem oil (0.5-1.0%) reduces conidial germination by 75-90% within 24 h. In field trials in Rajasthan, three foliar sprays of NKE (5%) at pre-flowering, early pod, and pod-filling stages reduced *Alternaria* blight severity by 52-64% compared to unsprayed controls, with yield improvement of 15-22% (Patel *et al.*, 2022; Yadav & Singh, 2021) [24, 39].

Shukla *et al.* (2022) found that neem oil (5%) and neem leaf extract effectively reduced disease severity and improved growth and yield parameters in mustard. Field trials conducted by Meena *et al.* (2016) demonstrated that three sprays of NSKE (5%) at 15-day intervals during crop growth significantly reduced disease severity index from 58.3% (untreated) to 29.6% (NSKE-treated), with no phytotoxic effects. Neem oil (3%) was found to be superior to neem leaf extract, with Yadav *et al.* (2018) reporting 80.5% inhibition of mycelial growth under *in vitro* conditions. The broad-spectrum activity and ISR-inducing capability of neem extracts make them especially valuable in integrated disease management.

Neem oil (3-5% emulsion) and leaf extract (10-20%) significantly reduce *A. brassicae* sporulation and spore germination *in vitro*. Field trials demonstrate 35-70% disease reduction, with effectiveness increasing with higher concentrations and frequent applications. The broad-spectrum nature of neem provides additional benefits against insect pests and other fungal pathogens (Ponomarenko *et al.*, 2011).

4.2 Ginger (*Zingiber officinale*)

Active Constituents and Mechanism

Ginger rhizomes (Family: Zingiberaceae) contain bioactive phenylpropanoids, gingerols (6-, 8-, 10-gingerol), shogaols, zingerone, paradol, and essential oil components (zingiberene, β -bisabolene). 6-gingerol and its dehydration product 6-shogaol are particularly effective antifungal agents acting by disrupting fungal cell membranes and inhibiting ergosterol biosynthesis (Mohammadi *et al.*, 2021) [20]. These compounds also exhibit anti-sporulation activity.

Efficacy Studies

Ginger rhizome extract (10-20%) inhibits mycelial growth by 48-65% *in vitro*. At 15% concentration, spore germination inhibition reaches 70-80%. Acetone extract of ginger at 2000 μ g/mL demonstrated 78% inhibition of conidial germination (Jain *et al.*, 2022) [9]. When combined with neem extract in field sprays, the mixture showed synergistic effects with 68-72% disease suppression.

Singh *et al.* (2013) [34] reported that aqueous ginger extract at 10% and 20% concentrations inhibited mycelial growth of *A. brassicae* by 52.6% and 68.4%, respectively, under *in vitro* conditions. Conidial germination was suppressed by 60.2% at 20% concentration. Tiwari and Tiwari (2017) [35] found that ethanolic ginger extract at 15% concentration caused 72.8% inhibition of mycelial growth. Foliar application of ginger extract (10%) reduced *Alternaria* blight incidence by 41.3% in field trials. The anti-inflammatory and antioxidant properties of gingerols may also contribute to induced plant defence responses.

4.3 Garlic (*Allium sativum*)

Active Constituents and Mechanism

Linn. (Family: Amaryllidaceae) contains alliin which is converted to allicin (diallyl thiosulfinate) by the enzyme alliinase when it is crushed. Allicin is a broad-spectrum antifungal compound. The organosulfur compounds such as allicin, diallyl disulfide (DADS), diallyl trisulfide (DATS), and ajoene, which are found in garlic, have been used in traditional medicine for a long time. The antimicrobial activity of these compounds is strong against bacteria and fungi and other microorganisms. Allicin, which is produced

by the enzymatic reaction of alliin during the damage of garlic tissue, has a short time period of action and its primary effect is a rapid inhibition of the synthesis of fungal cell wall and the disruption of the cellular respiration of pathogenic fungi. Another bioactive antifungal component of garlic oil is Ajoene, Diallyl disulfide (DADS), and Diallyl trisulfide (DATS).

However, garlic extracts are susceptible to degradation following exposure to light and heat, necessitating storage under controlled conditions. Development of stabilized formulations of organosulfur compounds represents an important research direction for improving practical applicability and consistency of garlic-based biocontrol products.

Efficacy Studies

Garlic bulb aqueous extract (5-20%) inhibits mycelial growth by 70-88% *in vitro*, making it one of the most potent botanical extracts evaluated against this pathogen (Sharma & Dubey, 2021) [28]. Conidial germination inhibition exceeds 90% at 15% extract concentration. In field trials, three sprays of garlic extract (10%) at 10-day intervals from disease initiation onwards reduced disease severity by 60-72% and improved mustard seed yield by 18-24% over untreated plots (Meena *et al.*, 2022; Singh & Yadav, 2020) [17, 32].

4.4 Turmeric (*Curcuma longa*)

Active Constituents and Mechanism

Linn. (Family: Zingiberaceae) rhizomes are rich in curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin), volatile essential oils (turmerone, zingiberene, ar-turmerone) and polysaccharides. Curcumin has been found to inhibit the synthesis of ergosterol, a component of the cell membrane of fungi, and the germination of fungal spores. The main yellow polyphenol curcumin displays antifungal activity by creating oxidative stress in fungal cells, inhibiting mitochondrial dehydrogenase, cell wall synthesis and interfering with fungal virulence factors, protease & lipase (Tran *et al.*, 2020) [36].

The main limitation of curcumin-based products is low bioavailability due to poor aqueous solubility and rapid degradation. Development of nano-formulations and use of bioavailability enhancers such as piperine (from black pepper) have been explored to improve efficacy and practical applicability of curcumin-based fungicidal preparations.

Efficacy Studies

The rhizome powder extract of turmeric was found to be effective in inhibiting *in vitro* by 50-68%. Conidial germination and germ tube growth were shown to be significantly inhibited by curcumin at 200-500 μ g/mL. The aqueous extract of turmeric was applied as a field spray at 5% concentration and the incidence of *Alternaria* blight was reduced by 42-55% when tested in different locations of Uttar Pradesh at fortnightly intervals (Rani *et al.*, 2021). When combined with garlic extract, the phytochemical mixture showed complementary antifungal activity (Sharma *et al.*, 2023). Karthikeyan *et al.* (2022) reported that turmeric extract at 15% concentration recorded 74.26% inhibition of mycelial growth of *A. brassicae* under *in vitro* conditions.

4.5 Tulsi (*Ocimum sanctum* / *Ocimum tenuiflorum*)

Active Constituents and Mechanism

The Tulsi leaf extract contains eugenol (70 - 80% of the essential oil), eugenol oxide, methyl eugenol, β -caryophyllene, ursolic acid, rosmarinic acid and flavonoids like orientin and vicenin. The antifungal activity stems from the ability of eugenol to inhibit branching of hyphae, inhibit mycelial growth, and cause mitochondrial dysfunction at minimal inhibitory concentrations (MIC) of 0.5-2.0 mg/mL (Rao *et al.*, 2020) [27].

Efficacy Studies

In vitro, the leaf aqueous extracts of tulsi have been seen to inhibit the mycelial growth by 55-72% at 10-20% concentration. The essential oil (500 μ g/mL) inhibited conidial germination by 88% (Kumar *et al.*, 2023) [11]. Mustard 10% leaf extract application at fortnightly intervals in the field significantly reduced the disease incidence and severity by 45-58% in Madhya Pradesh (Pandey & Dixit, 2021) [22].

4.6 Other Botanicals with Reported Activity

In addition to the major botanicals discussed above, several other plant-derived extracts have exhibited considerable antifungal potential against *Alternaria brassicae*, although they have received comparatively less research attention. These include *Lantana camara* leaf extract (40-62% inhibition at 10% concentration), *Calotropis procera* latex (55-70%), *Eucalyptus globulus* leaf extract (45-60%), *Withania somnifera* root extract (48-55%), *Aegle marmelos* leaf extract (42-58%), *Datura stramonium* leaf extract (50-65%), and *Pongamia pinnata* seed extract (40-55%). The inhibitory effects of these botanicals indicate their potential as eco-friendly alternatives for the management of *Alternaria* blight in mustard. These botanicals warrant further field-level validation. Extracts at 10-20% concentration have shown 50-65% inhibition of *A. brassicae* growth *in vitro*. Chilli pepper (*Capsicum annuum*) extracts, rich in capsaicinoids, exhibit antifungal properties. Capsicums contain capsaicin and related compounds with pronounced antifungal activity. Extracts at 30-50% concentration inhibit *A. brassicae* conidial germination and mycelial growth by 60-80%. The aqueous and alcoholic extracts are both effective, though the alcoholic extracts are often more potent. An application frequency of 7-10 days provides good disease control under field conditions.

5. Formulation and Application Strategies

5.1 Plant Extract Formulations

Plant extracts can be formulated as aqueous, alcoholic, or oil-based preparations. Aqueous extracts are economical and suitable for spray application but have limited persistence. Stabilization using emulsifiers, surfactants, or adhesive agents improves spray retention and efficacy. Encapsulation of volatile compounds in nano-particles enhances stability and controlled release. Standardization of extract concentrations to ensure consistent active compound levels is essential for reproducible results and regulatory approval.

5.2 Bioagent Formulations

The commercial bioagent formulations are powder, granular, and liquid (aqueous or oil-based). Talc-based formulations have a variable spore viability, are inexpensive, and have good shelf-life. Liquid formulations

are more concentrated and give more viable cells and spray coverage, but must be refrigerated and stored. Formulations that include biochar, clay minerals, and polymers are new formulations that extend shelf-life without compromising viability. Reduce the amount of the products used by applying bioagents to the seed (biopriming) to protect the crop in early season. A combination of complementary bioagents in a multi-strain formulation provides greater coverage of pathogens and minimizes risk of resistance development.

6. Mechanisms of Bioagents

Bioagents control *A. brassicae* through multiple interrelated mechanisms. (i) Mycoparasitism involves the direct attack on pathogen hyphae and spores via coiling, penetration, and subsequent cell wall degradation. (ii) Antibiosis refers to the synthesis of both volatile and non-volatile secondary metabolites that suppress pathogen growth. (iii) Competition occurs through rapid colonization of the phyllosphere and rhizosphere, thereby limiting access of the pathogen to nutrients and space. (iv) Induced Systemic Resistance (ISR) enhances the plant's innate defense system by activating signaling pathways mediated by salicylic acid (SA) and jasmonic acid/ethylene (JA/ET). (v) Enzymatic activity includes the production of cell wall-degrading enzymes such as chitinases, β -1, 3-glucanases, and proteases that disrupt pathogen structural integrity (Meena *et al.*, 2016; Singh *et al.*, 2014) [14, 15, 16, 33].

7. Integrated Disease Management (IDM)

The integration of bioagents with plant-derived extracts offers an effective IDM strategy by targeting pathogens through multiple mechanisms at the same time, minimizing the likelihood of resistance development, and delivering a wider range of complementary activities against the pathogen. Studies have demonstrated significantly enhanced disease suppression when seed treatment was combined with garlic or neem foliar sprays, reducing *Alternaria* blight severity by 70-78% compared to 50-65% with either treatment alone (Rana & Sood, 2022) [26].

Integrated disease management strategies involving seed treatment with bioagents followed by foliar application of botanical extracts have demonstrated considerable reduction in disease incidence and improvement in yield parameters. Furthermore, integration of these biological approaches with cultural practices such as crop rotation, timely sowing, and field sanitation can provide long-term and sustainable disease management. Monitoring of disease development through regular crop inspection and use of weather-based disease prediction models enables targeted interventions at appropriate times, maximizing efficacy while minimizing input costs and environmental impact. Farmer field schools and participatory research approaches have proven effective in promoting adoption of IPM practices among smallholder mustard growers.

8. Recent Advances and Future Perspectives

The development of biotechnology and microbiological research has opened up new possibilities of enhancing the efficiency of plant extracts and bioagents in disease management. Nanotechnology-based formulations, microbial consortia, and encapsulated bioagents are being developed to improve stability and field performance. Molecular research into host-pathogen interactions and

microbial antagonism is also aiding in the discovery of novel antifungal agents and mechanisms of disease suppression (Mukherjee *et al.*, 2019) [21].

The latest advances in the field are: (i) nano-bioformulations with chitosan or silica nanoparticles for improved delivery and longevity of *Trichoderma* and *Bacillus*; (ii) genomic/transcriptomic characterization of biocontrol gene clusters specific for *Alternaria* suppression; (iii) enhancement of biocontrol traits of *Trichoderma* using CRISPR technology; (vi) nano-emulsified botanical formulations with enhanced rainfastness and photostability; (iv) the selection of elite biocontrol strains based on metabolomic profiling of 2, 4-diacetylphloroglucinol (2, 4-

DAPG), lipopeptides, and volatile organic compounds associated with rainfastness and photostability; (v) the development of synergistic biocontrol consortia comprising *Trichoderma*, *Bacillus*, and *Pseudomonas* for multi-target disease suppression; (vi) formulation of nano-emulsified botanical products with improved rainfastness and photostability. The ability to integrate precision agriculture tools (disease forecasting models, remote sensing-based disease detection) with the timing of bioagent and botanical application should greatly enhance the efficacy of a field and reduce resource use efficiency (Verma *et al.*, 2024; Kumar *et al.*, 2024) [37, 12].

Table 1: Summary of Bioagents Used Against *Alternaria brassicae*

Bioagent	Type	Mode of Action	Disease Reduction (%)
<i>Trichoderma</i> spp.	Fungal	Mycoparasitism, Antibiotics	45-65
<i>Bacillus subtilis</i>	Bacterial	Lipopeptides, Resistance	50-70
<i>Pseudomonas fluorescens</i>	Bacterial	Phenazines, Resistance	55-75
<i>Streptomyces</i> spp.	Actinobacterial	Multiple antibiotics	60-85

Table 2: Summary of Plant Extracts Used Against *Alternaria brassicae*

Plant Source	Active Compounds	Optimal Concentration	Disease Reduction (%)
Neem	Limonoids, Azadirachtin	5-10%	60-75
Garlic	Allicin, Sulfur compounds	20-40%	50-70
Chili pepper	Capsaicin	30-50%	60-80
Turmeric	Curcumin, Phenolics	2-4%	60-75
Tulsi	Eugenol, Rosmarinic acid	5-10%	45-70

Table 3: Comparative Efficacy of Bioagents and Plant Extracts

Agent/Extract	Application Rate	Disease Reduction (%)	Mode of Action	Key Advantage
<i>T. harzianum</i>	10 ⁷ -10 ⁸ spores/ml	40-65	Mycoparasitism, Antibiosis, ISR	Multiple action modes
<i>T. viride</i>	10 ⁷ -10 ⁸ spores/ml	35-60	VOC production, Enzyme synthesis	Tropical climate suited
<i>B. subtilis</i>	10 ⁸ CFU/ml	30-55	Antibiosis, Competition	Stable spore form
Neem	3-5% emulsion	35-70	Azadirachtin-mediated inhibition	Broad-spectrum activity
Ginger	10-20% extract	40-65	ROS generation, Membrane disruption	Locally available
Garlic	10-25% extract	45-70	Sulfur compound interference	Highest <i>in vitro</i> activity

9. Conclusion

Alternaria blight, caused by *Alternaria brassicae* continues to pose a serious threat to Indian mustard production. The bioagents and botanical extracts of neem, tulsi, ginger, garlic, and turmeric have demonstrated substantial potential for disease suppression through diverse and complementary mechanisms. *In vitro* studies consistently report 50-85% inhibition of pathogen growth, and field trials indicate 40-75% disease reduction with concomitant improvements in seed yield and quality. Integrated approaches combining bioagents, botanicals, reduced-dose fungicides, and cultural practices offer the most promising route to durable, sustainable, and economically viable disease management. Concerted efforts in standardization, large-scale production, farmer education, and policy support are essential to translate laboratory and field successes into widespread adoption by Indian mustard farmers.

Alternaria blight, caused by *Alternaria brassicae*, is a serious threat to Indian mustard production. Significant bioactive compounds have been identified in bioagents as well as in plant extracts of neem, tulsi, ginger, garlic, and turmeric, which suppress disease through diverse and complementary mechanisms. It has been consistently reported for 50 - 85% inhibition of pathogen growth in *in vitro* studies, while field trials have shown disease reduction ranging from 40 - 75% along with an increase in yield and

quality of seeds. A combination of bioagents, botanicals, reduced doses of fungicides, and cultural practices is best suited for achieving durable, sustainable, and economically successful disease management. A concerted effort towards standardization, large-scale production, farmer training, and policy intervention is required to translate laboratory and field results into practical applications for Indian mustard farmers.

References

- Benhamou N, Chet I. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: ultrastructure and gold cytochemistry of the mycoparasitic process. *Phytopathology*. 1993;83(10):1062-1071.
- Chaudhary R, Sinha AP. Antifungal activity of neem leaf extract against *Alternaria brassicae* causing leaf blight of Indian mustard. *Pesticide Research Journal*. 2021;33(1):98-105.
- Doughari JH. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In: *Phytochemicals, A Global Perspective of Their Role in Nutrition and Health*. InTech; 2015.
- ICAR-Directorate of Rapeseed-Mustard Research. Annual report 2022-23. DRMR; 2023.

5. Gupta VK, Yadav V, Singh D, Kumar A. Epidemiology and yield losses associated with *Alternaria* blight of rapeseed-mustard in India: a review. *Indian Journal of Agricultural Sciences*. 2020;90(10):1845-1853.
6. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species are opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*. 2004;2(1):43-56.
7. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*. 2019;2(1):43-56.
8. ICAR-Directorate of Rapeseed-Mustard Research. Package of practices for rapeseed-mustard crop in India. DRMR; 2022.
9. Jain A, Tiwari AK, Sharma P. Antifungal activity of ginger (*Zingiber officinale*) rhizome extract against *Alternaria brassicae* affecting Indian mustard. *Agricultural Sciences*. 2022;13(2):89-98.
10. Karthikeyan R, Kumar S, Prasad R, Singh M. *In vitro* evaluation of fungicides and botanicals against *Alternaria brassicae* causing leaf blight of mustard. *International Journal of Plant Pathology and Microbiology*. 2022;1(1):16-19.
11. Kumar S, Rai R, Pathak R. Essential oil of tulsi (*Ocimum sanctum*) against *Alternaria brassicae*: chemical composition, antifungal efficacy, and field performance. *Industrial Crops and Products*. 2023;195:116423.
12. Kumar V, Singh RP, Verma A. Next generation biocontrol strategies for mustard diseases: from genomics to field deployment. *Plant Disease*. 2024;108(3):456-471.
13. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Journal of Agricultural Sciences*. 2008;4(S):839-843.
14. Meena M, Zehra A, Dubey MK, Aamir M, Gupta VK, Upadhyay RS. Comparative evaluation of biochemical changes in tomato infected by *Alternaria alternata* and its toxin. *Frontiers in Microbiology*. 2016;7:1408.
15. Meena PD, Gupta R, Awasthi RP, Kolte SJ, Reddy NEP, Kumar A, et al. *Alternaria* blight disease of oilseed Brassicas: biology, ecology and disease management. Springer; 2016.
16. Meena PD, Saharan GS, Mehta N, Gupta R. *Alternaria* diseases of crucifers: biology, ecology and disease management. Springer; 2016.
17. Meena PD, Awasthi RP, Chattopadhyay C, Kolte SJ, Kumar A. *Alternaria* blight: a chronic disease in rapeseed-mustard. *Journal of Oilseed Brassica*. 2022;1(2):1-11.
18. Mishra S, Singh R. Antifungal properties of neem (*Azadirachta indica*) seed kernel extract against *Alternaria brassicae*: biochemical and histochemical basis. *Journal of Plant Protection Research*. 2020;60(2):125-135.
19. Ministry of Agriculture and Farmers Welfare. Agricultural statistics at a glance 2021. Government of India; 2022.
20. Mohammadi S, Jafari M, Aliakbarlu J. Antifungal activity of ginger and its components: a review. *Phytochemistry Reviews*. 2021;20(4):921-937.
21. Mukherjee A, Sarkar B, Gupta S. Molecular insights into host-pathogen interaction in *Alternaria brassicae* pathosystem. *Physiological and Molecular Plant Pathology*. 2019;106:72-80.
22. Pandey S, Dixit VK. Field efficacy of *Ocimum sanctum* (tulsi) leaf extract in reducing *Alternaria* blight of mustard. *Indian Journal of Plant Protection*. 2021;49(3):210-218.
23. Patel H, Ingle Y. Botanical fungicides: a review of their role in plant disease management with special reference to Brassica diseases. *Phytopathologia Mediterranea*. 2022;61(1):5-28.
24. Patel M, Ramesh R, Singh A. Evaluation of neem-based products against *Alternaria* blight of mustard under field conditions in semi-arid Rajasthan. *Pesticide Research Journal*. 2022;34(2):178-185.
25. Poveda J. *Trichoderma* as a biological control agent against pests and pathogens: a review. *Biological Control*. 2021;159:104634.
26. Rana S, Sood SC. Integration of *Trichoderma harzianum* with garlic extract for management of *Alternaria* blight of mustard. *Annals of Plant Protection Sciences*. 2022;30(2):155-161.
27. Rao A, Sharma D, Srivastava M. Antifungal mechanisms of eugenol against *Alternaria brassicae*: membrane integrity, enzyme inhibition, and gene expression studies. *Postharvest Biology and Technology*. 2020;162:111093.
28. Sharma M, Dubey SK. Antifungal activity of garlic (*Allium sativum*) extract against major fungal pathogens of mustard. *Plant Disease Research*. 2021;36(2):89-96.
29. Sharma P, Saharan GS. Biological management of mustard diseases. In: *Diseases of Oilseed Crops*. 2nd ed. Scientific Publishers; 2021. p. 243-290.
30. Shukla AK, Rahul SN, Singh SK. Studies on antimicrobial effect of botanicals on leaf blight of Indian mustard caused by *Alternaria brassicae*. *International Journal of Plant & Soil Science*. 2022;34(23):642-648.
31. Simmons EG. *Alternaria* taxonomy: current status, viewpoint, challenge. In: Chelkowski J, Visconti A, editors. *Alternaria: Biology, plant diseases and metabolites*. Elsevier; 1992. p. 1-35.
32. Singh D, Yadav AK. Field management of *Alternaria* blight of mustard using garlic extract under semi-arid conditions. *Annals of Plant Protection Sciences*. 2020;28(2):182-187.
33. Singh D, Prasad R, Yadav SP. Characterization of *Alternaria brassicae* causing *Alternaria* blight of rapeseed-mustard (*Brassica juncea* L.) and its management. *The Bioscan*. 2014;9(Suppl 3):1207-1212.
34. Singh R, Yadav V, Tiwari A. Antifungal efficacy of *Allium sativum*, *Zingiber officinale*, and *Allium cepa* extracts against *Alternaria brassicae*. *Plant Pathology Journal*. 2013;12(3):136-143.
35. Tiwari RK, Tiwari M. Ethanolic extracts of *Zingiber officinale* and *Datura stramonium* as antifungal agents against *Alternaria brassicae* of mustard. *Journal of Applied and Natural Science*. 2017;9(2):876-881.
36. Tran TN, Tran TT, Nguyen TA, Pham TV. Antifungal activity of curcumin and its molecular mechanism: a review. *Molecules*. 2020;25(24):5990.
37. Verma J, Yadav B, Singh D. Nano-encapsulated botanicals for improved disease management in Indian

- mustard: current status and future directions. *Industrial Crops and Products*. 2024;212:118356.
38. Yadav MK, Prabha K, Chand R. *In vitro* efficacy of some plant extracts against seed-borne *Alternaria brassicae* of Indian mustard. *Annals of Plant Protection Sciences*. 2020;28(1):53-58.
39. Yadav S, Singh K. Comparative efficacy of neem-based products against *Alternaria* blight of Indian mustard under field conditions. *Indian Journal of Plant Protection*. 2021;49(2):112-119.
40. Yadav S, Ameta BL, Meena PD. Integrated management of *Alternaria* blight of mustard using bioagents and botanical extracts. *Journal of Oilseed Brassica*. 2018;9(1):45-54.
41. Yadav SV, Singh HV, Ameta BL. Evaluation of bioagents and plant extracts for management of *Alternaria* blight of mustard. *Indian Phytopathology*. 2015;68(4):385-390.
42. Yadav VK, Kumar A, Choudhary M. Seed treatment with *Trichoderma harzianum* formulations for management of *Alternaria* blight of mustard: dose standardization and mechanism study. *Biological Control*. 2023;180:105195.