



Comparative Efficacy of Newly Registered Fungicides for the Management of Alternaria Leaf Spot of Cauliflower in Nepal

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ABSTRACT

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One of the major factor contributing to the decreased yield of cauliflower in Nepal is incorrect fungicide selection and dosages. Alternaria leaf spot (ALS) caused by *Alternaria brassicicola*, is a devastating disease that significantly reduces the quantity and quality of cauliflower. In vitro evaluation of seven different fungicides was done in a completely randomized design with five replications at different doses i.e., 50 ppm, 100 ppm, 150 ppm, and 200 ppm. All the tested fungicides significantly reduced ($P \leq 0.001$) mycelial growth of the pathogen in the poisoned food technique. The greatest reduction in mycelium growth was observed with hexaconazole and azoxystrobin + tebuconazole at the lowest tested concentration (50 ppm). Maximum inhibition of *A. brassicicola* growth was demonstrated by azoxystrobin + propiconazole at 200 ppm, followed by azoxystrobin + difenoconazole and copper oxychloride. The fungicides that were found effective in inhibiting mycelial growth should be tested under field conditions with multi-location and multi-strains pathogens to ensure that they meet specific requirements related to host and environment interaction. This will help to confirm their efficacy and determine the best application doses.

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Introduction

Cauliflower (*Brassica oleracea* L. var. *botrytis*) which belongs to the family Brassicaceae is one of the most important winter vegetable crops cultivated throughout the world. It is an annual herbaceous for growing vegetables and a biennial for growing seeds. In Nepal, fresh vegetable is cultivated in 289,839 ha with the production of 4,153,157 t where cauliflower is cultivated in almost all areas with an average yield of 16 t ha⁻¹ (MoALD, 2023). Minerals including iron, magnesium, phosphorus, potassium, and salt are abundant in it, along with vitamins A and B1 (Jaipaul et al., 2014). Although domestic vegetable output, particularly that of cauliflower is rising, their productivity is low, necessitating considerable imports to satisfy domestic demand (Ojha, 2016).

Leaf spot, downy mildew, damping off, club root, powdery mildew, white rust, black rot, bacterial soft rot, and cauliflower mosaic virus are the major diseases of cauliflower. Alternaria leaf spot (ALS), a devastating disease of cauliflower is caused by numerous species of *Alternaria*. *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schweinitz) Wiltshire are two species that are the main culprits in cauliflower. *Alternaria brassicicola*, a necrotrophic plant-pathogenic fungus, produces toxic secondary metabolites and proteins that cause cell death by toxin synthesis or direct cell damage (Ohm et al., 2012,

Paudel et al., 2023). ALS caused by *A. brassicicola*, are initially small, dark spots that rapidly grow into circular lesions that are up to 1 cm in diameter (Singh, 1998). The disease may result in a 20 to 80 percent loss in yield and a 59 percent loss in seed (Valvi et al., 2019). Cauliflower seed production may decrease by about 47.8% because of *Alternaria* rot (Hossain & Hossain 2010). The diseases can spread from these inoculum sources to nearby farms and persist in agricultural residues for some years (Humpherson-Jones, 1992). *Alternaria* may endure in residues after they become established, and occasionally they produce resting spores that help them endure in the soil (Thomma, 2003). Although the leaf spot of cauliflower is one of the main diseases affecting the crop caused by *A. brassicicola*, research on these issues is minimal.

Host resistance, particularly through the deployment of resistant varieties, is considered a sustainable and eco-friendly approach for long-term agriculture. However, challenges arise with the durability of host resistance, prompting the need for integrated approaches (Pathania et al., 2021). Cultural techniques frequently fail to manage the disease effectively. The disease is poorly tolerable by available cultivars; therefore introducing new types may not have a lasting effect. Finding a suitable, effective, and

financially viable approach to chemical control is therefore necessary. In most developing nations, where resistant cultivars are not easily accessible, fungicides are utilized to manage disease due to their immediate action in reducing the severity of the infection, convenience of administration, and widespread availability in the market. Due to its quick-acting mechanisms compared to biological and botanical fungicides, the administration of an efficient fungicide is necessary for the control of this disease (Corkley et al., 2022).

Novel mechanisms of action in fungicides can be evaluated to improve long-term disease management, lessen selection pressure, and prevent the emergence of resistance. Applying different fungicides as foliar sprays or seed treatments has been shown to be successful in controlling ALS. Examining novel fungicide compounds and their combinations with distinct mechanisms of action is therefore essential to overcoming these obstacles (Loona et al., 2025). The goal of this research was to identify novel and efficient fungicides to combat the leaf spot caused by *A. brassicicola*.

Materials and Methods

Experimental Site and Study Design

The experiment was carried out in the Plant Protection Laboratory under the Ministry of Industry, Agriculture and Cooperatives, Directorate of Agriculture Development, Koshi province which is located in Jhumka, Sunsari district of Nepal. Seven chemical fungicides were evaluated at four different concentrations i.e. 50 ppm, 100 ppm, 150 ppm & 200 ppm under complete randomized design (CRD) each replicating 5 times. The details of all the seven treatments used are mentioned in Table 1. These fungicides were selected due to their availability on the market, novelty and legally registered in the country.

Isolation, Purification and Pathogenicity Test of The Pathogen

Alternaria brassicicola was isolated from an infected leaf of cauliflower collected from the field of Plant

Protection laboratory Jhumka, Sunsari. The infected leaves were air-dried, placed in a paper bag, and refrigerated at 4°C upon arrival in the lab for pathogen isolation. Spores were teased from the diseased area and examined under a microscope to detect pathogenic fungi. Once *Alternaria brassicicola* was identified, leaves were cut with a sterile blade into tiny pieces ranging from 1 to 1.5 cm. The pieces were disinfected with 1% sodium hypochlorite (NaOCl) solution for two minutes, and then washed three times with distilled water. Extra moisture was removed with sterile blotting paper. The leaf sections were put on a sterile petri plate with three layers of wet blotting paper to create a moist chamber for fungal sporulation at 25±2°C in the biological oxygen demand (BOD) incubator for 24 hours. The single spore isolation approach was used to pick up the spore with a thin flattened needle under the stereomicroscope (Olympus/SZX16) and deposit it aseptically on the water agar (20 g agar/l distilled water). After 24 hours of spore germination, a single spore was aseptically transferred from water agar to separate culture tubes of potato dextrose agar (PDA) slants (200 g potato infusion, 20 g dextrose, 20 g agar for 1 lt) under a laminar flow chamber using a stereomicroscope and inoculating needle. To suppress bacteria development, 50 ppm of streptomycin sulfate was added to the PDA. To get pure mono-conidial isolate, the tubes were cultured in an incubator at 25±2°C for 12 days. For short-term preservation, PDA slants were stored at 4°C in the refrigerator as a pure stock culture for future research (Deep & Sharma, 2012).

Five plants were sprayed with a spore suspension (10⁴ spores/ml) as the part of pathogenicity test. To ensure there was enough humidity, the plants were covered with plastic bags for 72 hours following inoculation. By substituting sterile water for the fungal suspension, a different pot of plants was maintained as a control. These plants were inspected on a regular basis to document symptoms ten days following inoculation. After being re-isolated from the infected leaves of inoculation plants, the causal fungus were compared to the pathogens that were initially identified (Patel et al., 2023).

Table 1. List of fungicides used for in-vitro study at Plant Protection Laboratory Jhumka, Sunsari.

T	Commercial Name	Chemical Name	Active Ingredient	SS	Chemical Group*	FRAC-MoA Group Names*
1	Titan	Hexaconazole	5 % EC	S	Triazole	Demethylation inhibitors (DMI)
2	Apollo	Azoxystrobin + Propiconazole	7.1 + 11.9 % SE	S	Methoxyacrylates + Triazole	Quinone outside inhibitors (QoI) and Demethylation inhibitors (DMI)
3	Godiwa super	Azoxystrobin + Difenconazole	18.2 + 11.4% SC	S	Methoxyacrylates + Triazole	Quinone outside inhibitors (QoI) and Demethylation inhibitors (DMI)
4	Raptor	Azoxystrobin + Tebuconazole	11 + 18.3% SC	S	Methoxyacrylates + Triazole	Quinone outside inhibitors (QoI) and Demethylation inhibitors (DMI)
5	Indofil Z-78	Zineb	75 % WP	NS	Dithiocarbamates and relatives	Dithiocarbamates and relatives (electrophiles)
6	Nagcoper	Copper oxychloride	50% WP	NS	Inorganic	Inorganic
7	Indofil M-45	Mancozeb	75% WP	NS	Dithiocarbamates and relatives	Dithiocarbamates and relatives

T: Treatment; SS: Systemic/non-Systemic; S: Systemic; NS: Non- systemic, *FRAC Code List. (2024)

***In vitro* Assay of Different Chemical Fungicides**

In vitro assays of seven different fungicides were performed against *Alternaria brassicicola* using "poison food techniques" (Schmitz, 1930) at four different concentrations (50, 100, 150, and 200 ppm). A 10,000-ppm stock solution on the primary active component of each fungicide was prepared, and the necessary amount of each chemical was aseptically added to 100 ml of sterilized PDA medium to provide the appropriate concentrations of each fungicide. To avoid bacterial contamination, a micropipette was used to apply 50 ppm of streptomycin sulfate to the PDA medium at 45-50°C. A sterile cork-borer was used to cut a 5 mm diameter mycelial disc off the edge of a 7-day-old fungal culture, and the disc was then injected onto the plates after having solidified. Simultaneously, the fungus was cultured on a chemical-free PDA medium as a check and incubated at 25±2°C. The colony diameter of each fungus was measured until the petri plates (90 mm diameter) under control were completely filled with fungus mycelial growth. The measuring scale was used to calculate the mean values of the fungal colonies' longitudinal growth from two distinct angles in millimeters (mm).

Assessment of Percentage Growth Inhibition (PGI)

The formula for percentage inhibition of mycelial growth was calculated as (Abbott, 1925).

$$PGI = (C-T)/C \times 100$$

Where,

C = Growth of mycelium in control (mm)

T = Growth of mycelium in treatment (mm)

Statistical Analysis

The data from the experiment were entered and saved in Microsoft Excel. To correctly interpret the data, analysis of variance (ANOVA), a post-hoc test using DMRT, and graphs were constructed using R and its packages, agricolae, and ggplot2 at a 5% level of significance.

Results

Identification and Pathogenicity Test of The Pathogen *Alternaria Brassicicola*

After pathogenicity test, the earliest symptoms were little yellow pecks on the oldest leaves. The spots were tan to dark brown, darker, and grow to a circle. The spot appeared large, with concentric rings of light and dark surrounded by a yellow halo. Isolation and identification of fungus was done from the diseased leaves (Patel et al., 2023).

Conidia was straight, narrowly to broadly ovoid, tapering slightly towards the apex, pale to dark olivaceous brown, often lacking longitudinal septa in small conidia or few longitudinal septa in one or more cells in larger conidia, slightly to distinctly constricted at transverse septa, apical cell rounded and true apical beak is absent. This morphological structure is also supported by Corlett & MacLachy (1996) and Patel et al. (2023).

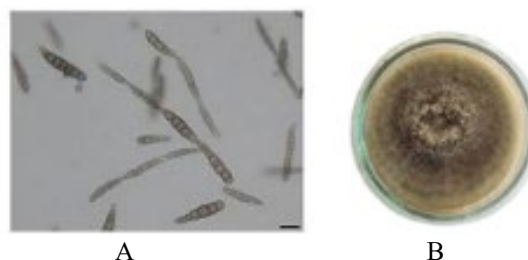


Fig 1. A) Conidia B) Pale brown mycelial colony of *Alternaria brassicicola*

Mycelial Growth (MG) in Diameter

The efficacy of seven chemical fungicides was evaluated against *A. brassicicola* by employing the poison food technique. All tested chemical fungicide had a substantial impact ($P < 0.001$) on preventing the growth of pathogens compared to the control. Mycelium growth (MG) ranged between 5 and 64.2 mm, with control plates having the greatest value (90 mm). The results showed that the most efficient fungicides for controlling *Alternaria brassicicola* mycelium growth were hexaconazole alone and in combination with azoxystrobin + tebuconazole at all concentrations tested (50 ppm, 100 ppm, 150 ppm, and 200 ppm) that was found to be 5mm. The same result was also found in azoxystrobin + propiconazole at 200 ppm. The highest mycelial growth was observed in zineb 50ppm, 100ppm, 150ppm & 200ppm i.e. 64.2 mm, 64 mm, 61.6 mm & 59.5 mm respectively. This is represented in the Figure 2.

Percentage inhibition (PI) of mycelial growth

Among the fungicides, hexaconazole and azoxystrobin + tebuconazole in all the concentrations and azoxystrobin + propiconazole of 200 ppm were found to inhibit mycelial growth (94.44%) throughout the whole experiment. The lowest inhibition percentage was observed at 50 ppm of zineb (28.67 %). While the inhibitory effect of mancozeb, copper oxychloride, azoxystrobin + difenoconazole increased with an increase in concentration but gradually decreased with time. The data on inhibition percentage on mycelial growth is presented in Figure 3.

Discussion

One of the main obstacles in cauliflower production is the leaf spot caused by *Alternaria brassicicola*. Because of the pathogen's diversity, quick dissemination, and resistance strains, managing this disease is challenging. The purpose of the study was to assess the effectiveness of various commercially available synthetic fungicides at varying concentrations to determine the right dosage of chemicals to use. All the tested fungicides exhibited control over the mycelial growth of the pathogen in varying degrees and they were significantly different from control at different concentrations (50 ppm, 100 ppm, 150 ppm, and 200 ppm). The results showed that the most efficient fungicides for preventing *Alternaria brassicicola* mycelium growth were hexaconazole alone and in combination with azoxystrobin + tebuconazole at all concentrations tested (50 ppm, 100 ppm, 150 ppm, and 200 ppm). Azoxystrobin + propiconazole at 200 ppm was also found effective against *A. brassicicola*.

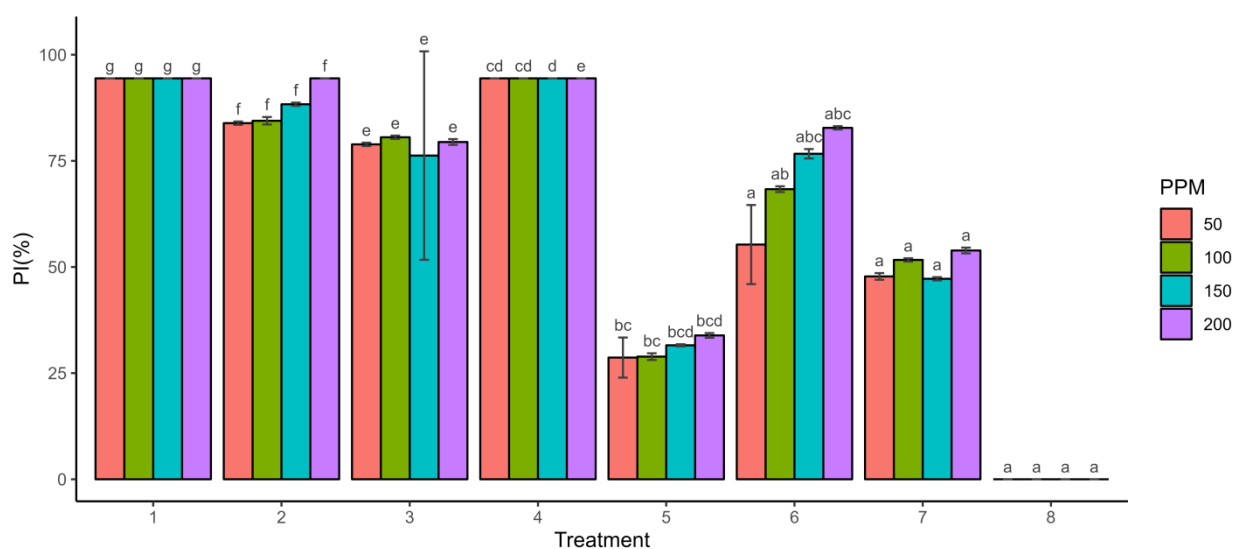


Figure 2. Effect of fungicide (different concentration) in the diameter growth of mycelium of *Alternaria brassicicola* by poisoned food technique (at 12 days).

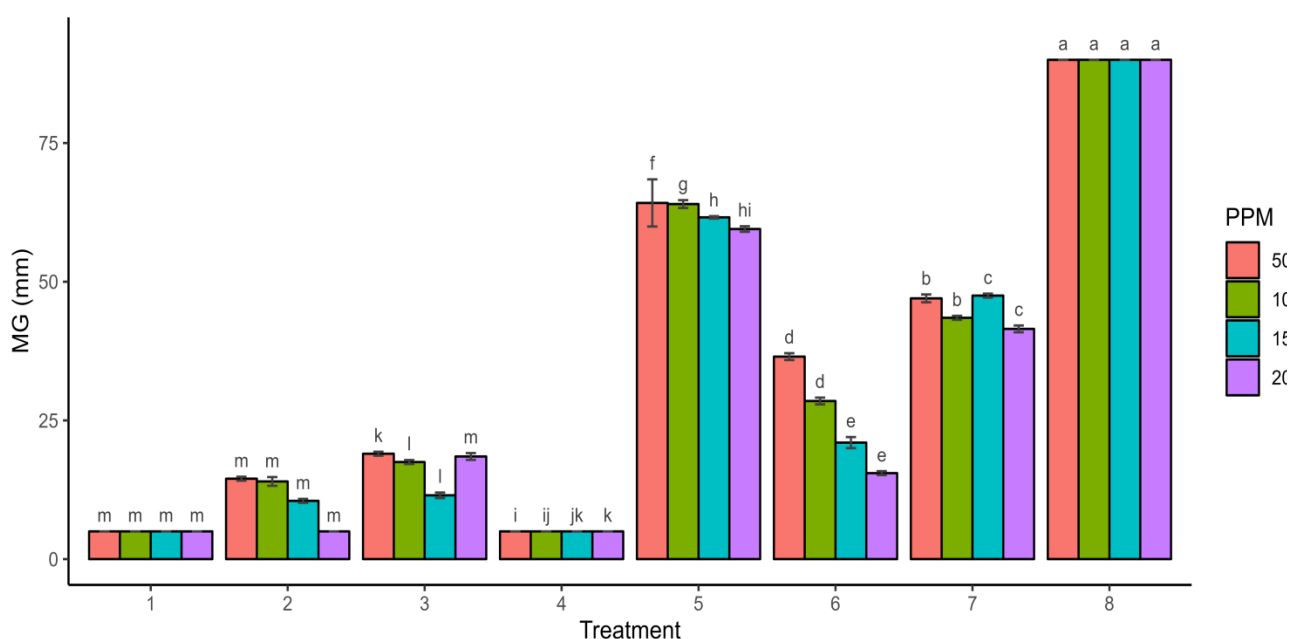


Figure 3. Percentage inhibition in colony growth of *A. brassicicola* over control by poisoned food technique (at 12 days).

Results of current experiment are in line with the work of many researchers who also used same fungicides and reported that it showed significant results against *A. brassicicola*. Different chemicals were investigated in vitro in which it was recorded that hexaconazole was the most effective fungicide, exhibiting 100% total inhibition. It was followed in order of effectiveness by carbendazim + mancozeb (93.81%) and mancozeb (89.05%), while the least effective fungicides were carbendazim (27.83%) and copper oxychloride (72.05%) (Pun et al., 2020). Four systemic fungicides were tested for their ability to control *Alternaria brassicicola* at three different concentrations: 0.05%, 0.1%, and 0.2%. The findings showed that at 0.05%, 0.1%, and 0.2% doses, all three triazoles (propiconazole, tebuconazole, and hexaconazole) completely inhibited the growth of the pathogen, with the least amount of inhibition caused by azoxystrobin (27.7%, 31.1%, and 33.3%) (Kiran et al., 2018). Similarly, different

chemicals were investigated in vitro in which it was recorded that complete inhibition of *A. brassicicola* by hexaconazole, mancozeb at 250, 500 and 1000 ppm and by metalaxyl + mancozeb at 500 and 1000 ppm and least inhibition at carbendazim (Tu, 2015). The mycelial growth was reduced by 70.64 percent by picoxystrobin, but 99.98 percent by systemic fungicides such as propiconazole, difenoconazole, and azoxystrobin (Ladumor et al., 2019).

By focusing on particular cell organelles and impairing their cellular processes, each chemical fungicide or active ingredient inhibits the growth of fungal infections through a variety of target sites and mechanisms of action (Hermann & Stenzel, 2019; Vielba-Fernandez et al., 2020; Hu & Chen, 2021). For instance, propiconazole, a systemic fungicide and a demethylation inhibitor (DMI) of sterol biosynthesis, damages fungal cell membranes through deketalization, which causes the dioxolane moiety in fungi to disappear, and enzymatic oxidation at the side chain

connected to the dioxolane ring. This prevents demethylation (Li et al., 2020). Azoxystrobin inhibits the production of ATP by forming a strong bond with the Qo site of Complex III of the mitochondrial electron transport chain. These fungicides eventually prevent fungal pathogens from growing their spores or mycelial growth (Rodriguez-Morelos et al., 2021; Andrade et al., 2022).

It was reported that the growth of *Alternaria brassicicola* is completely inhibited by the fungicides propiconazole and tebuconazole (Tu & Somasekhara, 2015). It was found that the fungicide hexaconazole shown 100% inhibition of the pathogen *Alternaria alternata*, the incitant of alternaria blight of tomato (Singh & Singh, 2006). Synthetic fungicides effectively suppress infections by either damaging their cell membrane or its permeability or by obstructing their metabolic activities (Kakraliya et al., 2018). All of these results corroborate the conclusions of this study. Based on the explanation above, it can be said that fungicides are an effective way to control ALS of cauliflower.

Conclusion

A serious disease that influences brassica plants all around the world is *Alternaria* leaf spot. Various chemical fungicides are available in the market to manage this disease. From the aforementioned experiment, it can be concluded that all fungicides used against *Alternaria brassicicola* demonstrated an inhibitory impact but some fungicides are more effective than others are. Therefore, depending on fungicide availability, the most effective fungicides can be used to control this disease. While all fungicides are effective, hexaconazole and azoxystrobin + tebuconazole have the greatest effectiveness at varying concentrations (50 ppm, 100 ppm, 150 ppm, and 200 ppm). In laboratory settings, azoxystrobin + propiconazole at 200 ppm is also very effective against *A. brassicicola*. Determining the degree of pathogen control in in-vivo situations will require further validation of these results using these treatments on infected host plants under field conditions. Long-term usage of chemical fungicides can harm farmers' health as well as the quality of the soil in their fields. The best ways to use chemical fungicides as little as possible are through the farmer's careful selection of fungicides and minimal treatment dose. It is necessary to apply fungicides in the field at a 10% higher concentration than in vitro to effectively control the disease. Various field research and multi-location trails can also be conducted for this purpose.

Declarations

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Conflict of Interest

The authors have declared no conflict of interest regarding the publication of this manuscript.

Ethics Approval and Consent

Any opinions, results, conclusions, or recommendations expressed in this publication are solely

those of the authors and do not necessarily represent the views of the institutions with which they are associated.

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