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In vitro and *in vivo* evaluation of fungicides against *Alternaria brassicae* of mustard

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Abstract
The study focused on a disease observed on the leaves of certain exotic Indian mustard varieties. The research aimed to assess the effectiveness of nine different fungal toxicants, including mancozeb (0.05%), azoxystrobin (0.05%), propiconazole (0.05%), thiram (0.05%), tebuconazole (0.05%), copper oxychloride (0.05%), hexaconazole (0.05%), captan (0.05%), and trifloxystrobin (0.05%), as well as a control group. Two methods were used for evaluation: under *in vitro* conditions using the Poison Food Technique (PFT), and under *in vivo* conditions through foliar application. This assessment was conducted to determine their effectiveness in controlling Alternaria blight during the Rabi season of 2022-23. The findings from the *in vivo* study indicated that among the fungicide treatments, tebuconazole (0.05%) exhibited the strongest efficacy in controlling Alternaria Blight of Mustard under field conditions. Additionally, Propiconazole (0.05%) also displayed notable effectiveness in controlling the disease. Conversely, copper oxychloride (0.05%) and captan (0.05%) were found to be the least effective among the tested fungicides. In the *in vitro* study, tebuconazole (0.05%) demonstrated the highest inhibition percentage over the control, achieving a substantial inhibition rate of 98.12%. On the other hand, copper oxychloride (0.05%) exhibited the lowest inhibition percentage over the control, with a significantly lower rate of 9.5%.

Keywords: Fungicides, *Alternaria brassicae*, mustard

Introduction
Mustard (*Brassica* spp.) is an important oilseed crop cultivated worldwide for its culinary, medicinal, and industrial applications. The Brassica genus includes various species, such as B. juncea (Indian mustard), *B. napus* (rapeseed or canola), and B. Rapa (Chinese cabbage), among others. Mustard plants are known for their adaptability to diverse climates and their ability to grow in various soil types. They are valued for their high oil content, with the seeds being a rich source of edible oil used in cooking and food processing. Furthermore, mustard leaves, stems, and flowers are utilized as nutritious vegetables and condiments, adding flavor to various dishes (Singh *et al*., 2017) [7].

Due to its versatility and economic significance, mustard cultivation plays a vital role in food security and agricultural economies. However, like all crops, mustard is susceptible to various biotic and abiotic stresses, including diseases caused by pathogens such as bacteria, fungi, and viruses. One of the most prevalent and damaging diseases affecting mustard crops is Alternaria blight, caused by the fungal pathogen *Alternaria brassicae.*

Alternaria Blight of Mustard
Alternaria blight, also known as black spot or leaf blight, is a widespread and destructive disease that impacts mustard crops worldwide. The disease is caused by the necrotrophic fungal pathogen *Alternaria brassicae*, which primarily targets the foliage of mustard plants. *Alternaria brassicae* exhibits a complex life cycle, with its survival stages encompassing both soil and crop debris. During conducive environmental conditions, the pathogen produces vast numbers of asexual spores (conidia), which can disperse over long distances through wind and rain splash, facilitating widespread infection (Meena *et al*., 2019) [3].

Symptoms of Alternaria blight in mustard initially appear as small, dark brown to blackish lesions with a characteristic yellow halo. As the disease progresses, these lesions enlarge and coalesce, leading to extensive defoliation, reduced photosynthetic capacity, and significant yield losses. In severe cases, the pathogen can also infect other plant parts, such as stems and pods, further impacting the overall crop quality (Sharma *et al*., 2018) [5].

Fungicides for Alternaria Blight Management

Chemical fungicides have been widely employed to control Alternaria blight due to their rapid action and proven effectiveness. Fungicides belonging to different classes, such as triazoles, strobilurins, and benzimidazoles, have demonstrated efficacy against *Alternaria brassicae* (Agarwal *et al*., 2021) [1]. When applied preventatively or at the early stages of disease development, fungicides can effectively reduce disease incidence and severity, leading to improved crop health and yield.

However, the overuse and reliance on chemical fungicides can lead to the development of resistant pathogen strains, rendering these treatments less effective over time (Nagaraja *et al*., 2020) [4]. Additionally, the environmental impact and potential harm to non-target organisms are concerns associated with fungicide usage.

Materials and Methods

The laboratory work took place within the Department of Plant Pathology at Lovely Professional University. For the field experiment, the Student Research Farm at Lovely Professional University was utilized during the 2022-23 Rabi season. The primary objective was to assess the effectiveness of various fungicides against Alternaria blight in Indian mustard under natural conditions.

The experimental setup involved the utilization of a plot size measuring 5x3 meters, with a plant spacing arrangement of 30x10 centimeters. The experimental design employed was a Randomized Block Design, and the highly susceptible Indian mustard variety 'Varuna' was selected for the study. To support the growth of the plants, a combination of nitrogen, phosphorus, and potassium fertilizers (NPK) was administered. Specifically, the application rates were set at 120 kg/ha for nitrogen, 60 kg/ha for phosphorus, and 60 kg/ha for potassium.

The fertilization process was divided into two stages. Initially, half of the prescribed nitrogen dosage, the full phosphorus dosage, and the full potassium dosage were applied as a basal treatment. Subsequently, during the first irrigation cycle, the remaining half of the nitrogen dose was administered as a top dressing.

The field experiment was replicated three times, ensuring the reliability of the results and the robustness of the conclusions drawn. Through this comprehensive approach, the research aimed to provide valuable insights into the efficacy of various fungicides in combatting Alternaria blight in Indian mustard while operating within the framework of natural environmental conditions.

Isolation and maintenance of pure culture

Infected mustard leaves were collected from the campus of Lovely Professional University in Jalandhar, India. The goal was to confirm the presence of the pathogenic fungus *Alternaria brassicae.* To achieve this, spores of the fungus were gently extracted from the infected leaf segments and carefully examined under a microscope.

To prepare for further analysis, the mustard leaves were meticulously sliced into small pieces, measuring 1-1.5cm each. These fragments were then treated with a 0.5% solution of Sodium Hypochlorite (NaOCl) for two minutes, effectively eliminating potential surface contaminants. Following this, the disinfected fragments underwent three rinses with distilled water to remove any remaining traces of the disinfectant.

To remove excess moisture, sterile blotting paper was employed to gently dry the disinfected leaf fragments. These treated fragments were subsequently placed onto Potato Dextrose Agar (PDA) medium using sterile forceps. The PDA medium provided a conducive environment for the fungus to grow.

The petri dishes containing the leaf fragments and PDA medium were placed under controlled conditions in an incubator set at a temperature of 27 ± 1 °C. This environment facilitated the growth of the fungus over a span of seven days.

The identification of the pathogenic species, *Alternaria brassicae*, was achieved by carefully observing its distinct morphological features, particularly its conidia (spores). The identification process adhered to the criteria outlined by Yu (2015) as well as Corlett and MacLatchy (1996a, 1996b).

To maintain a pure culture, a segment of agar containing spores was judiciously transferred to a fresh petri dish with growth medium. This process effectively eliminated any potential contaminants, resulting in a pristine culture. This purified culture underwent three consecutive sub-culturing cycles to uphold its purity and vitality.

For long-term preservation, the final uncontaminated culture was stored on a slant of PDA at a temperature of 4°C. This controlled environment ensured the culture's viability over an extended period of time.

In summary, this systematic approach serves the purpose of isolating, identifying, purifying, and preserving the fungal pathogen *Alternaria brassicae* from infected mustard leaves. Researchers and plant pathologists specializing in *Alternaria brassicae* infections in mustard plants can find practical value in employing this methodology.

Evaluation of fungicides against Alternaria blight *In-Vivo*

The experiment was conducted in a field with plots measuring 5 x 3 meters and a spacing of 30x10 centimeters. A Randomized Block Design was employed with three replications. The chosen Indian mustard variety, 'Varuna', which is highly susceptible to the disease, was sown during the Rabi season in the first week of November.

To nourish the plants, fertilizers containing nitrogen (N), phosphorus (P), and potassium (K) were applied at rates of 120 kg/ha of N, 60 kg/ha of P, and 60 kg/ha of K. The nitrogen was split into two doses, with half of it being applied as a top dressing during the first irrigation.

For the disease management experiment, the required amount of each fungicide was calculated, and a spray solution was prepared using water. The fungicides were dissolved in a small

Quantity of water and then diluted to the desired volume. Application was carried out using a high-volume knapsack sprayer with a capacity of 10 liters. Each fungicide was sprayed three times: first when the disease was observed, and subsequently, two more sprays were administered at intervals of 15 days.

The severity of the disease was evaluated one week after the final spray using a scale ranging from 0 to 6. The scale was as follows:

0: No disease
1: Up to 5% leaf area infected
2: > 5% to 10% leaf area infected
3: > 10% to 20% leaf area infected
4: > 20% to 30% leaf area infected

5: > 30% to 50% leaf area infected
6: > 50% leaf area infected

Using this assessment, the percent disease intensity (PDI) was calculated for each treatment. Additionally, the yield from each plot was recorded for each treatment. This allowed for the observation of differences in yield among the treatments, and yield per hectare (ha-1) was computed accordingly.

$$\text{Percent disease control} = \frac{Disease\ intensity\ in\ control - Disease\ intensity\ in\ treatments}{Disease\ intensity\ in\ control} \times 100$$

In vitro evaluation of chemical Fungicides

To evaluate the efficacy of chemical fungicides, a precisely calculated quantity of a concentrated solution was blended with sterilized Potato Dextrose Agar (PDA). This resulted in final concentrations of 50ppm, 100ppm, and 200ppm. Each 80mm sterilized Petri dish was then filled with twenty milliliters of the modified PDA solution and allowed to solidify. In parallel, a control setup was maintained, consisting solely of PDA without any plant extracts or chemical fungicides.

Using a sterile corn borer, a circular disc measuring 7mm in diameter was excised from a 9-day-old culture of *Alternaria brassicae*. This disc was subsequently placed at the center of both the solidified modified PDA and the control medium. This experimental arrangement was replicated across three individual Petri dishes for each treatment. Following this, the Petri dishes were carefully transferred to an incubator set at a constant temperature of 27 ± 1 °C, and they remained there for a duration of seven days.

Growth inhibition test

After a week of incubation, the extent of mycelial growth was assessed in each treatment using a vernier caliper scale. The percentage of mycelial growth inhibition, compared to the control, was computed utilizing the formula provided by Vincent (1947, as referenced in Kantwa *et al*., 2014; Roopa *et al*., 2014) [9].

$$PGI = \frac{(C-T)}{C} \times 100$$

Where, PGI = Percent growth inhibition, C = Growth of hyphae in control (mm) and T= Growth of hyphae in treatment (mm).

Result and Discussions

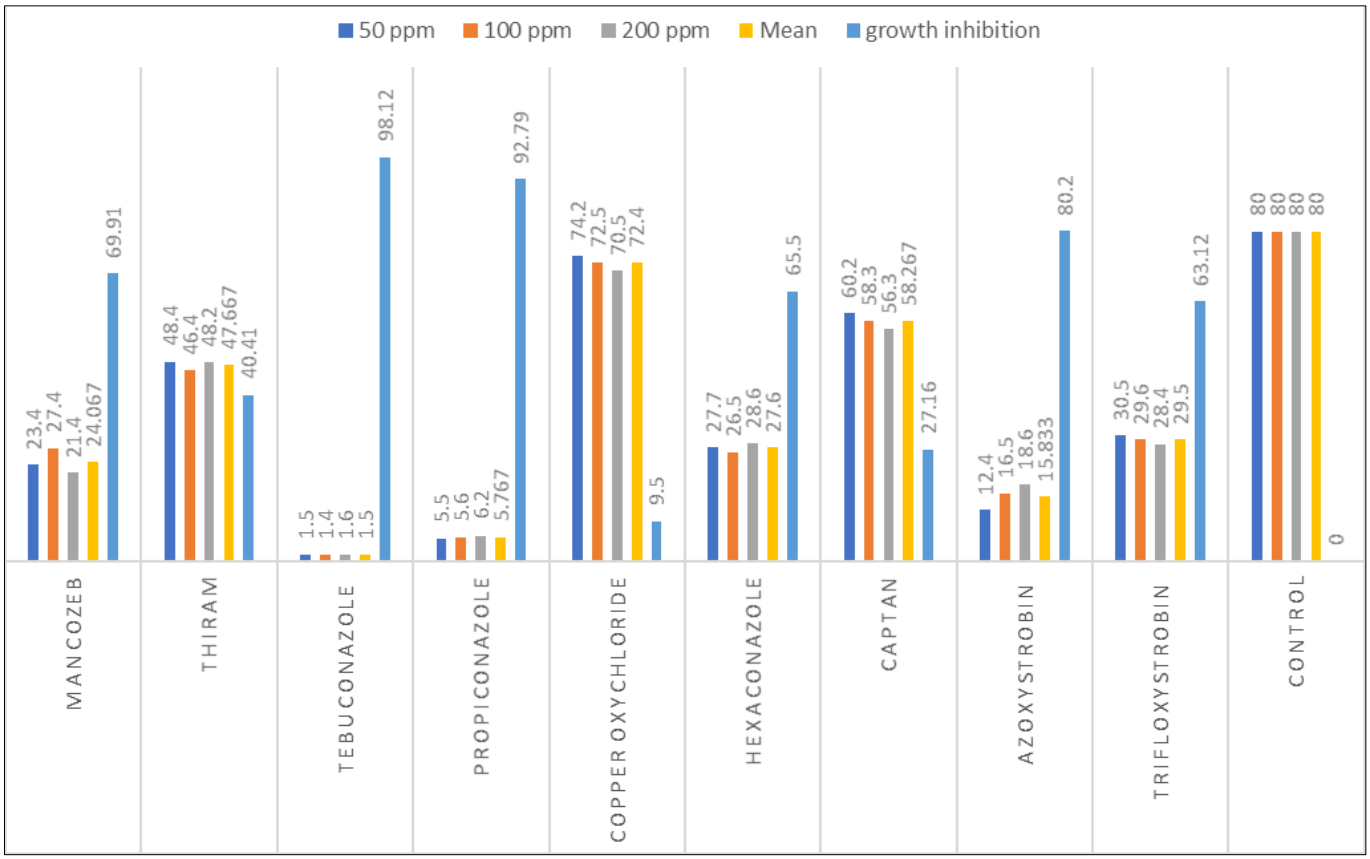
Evaluation of fungicides against *Alternaria brassicae* In-vitro

The conducted fungicide tests exhibited noteworthy efficacy in restraining the growth of the pathogen. The outcomes, as depicted in Table 1, underscore the effectiveness of these fungicides under *in vitro* conditions. The results clearly demonstrate the capacity of all the fungicides to impede the growth of the test fungus when compared to the control group.

Among the array of nine fungicides examined in the laboratory, tebuconazole and propiconazole emerged as the most potent. They demonstrated a remarkable ability to entirely suppress the fungus's growth, achieving inhibition rates of 98.12% and 92.79%, respectively, in contrast to the control group. On the other hand, the remaining seven fungicides displayed varying degrees of efficacy in inhibiting the fungus's growth. However, these fell short of achieving complete inhibition, thus categorizing them as partially effective.

Copper oxychloride and captan exhibited the least efficacy among the tested fungicides, with inhibition percentages of merely 9.5% and 27.16%, respectively, compared to the control group. Conversely, other substances like mancozeb, thiram, hexaconazole, azoxystrobin, and trifloxystrobin demonstrated commendable inhibition against the fungus. Mancozeb recorded a growth inhibition of 69.91%, thiram exhibited 40.41% inhibition, hexaconazole displayed 65.5% inhibition, azoxystrobin showcased 80.2% inhibition, and trifloxystrobin registered a growth inhibition of 63.12% when compared to the control group.

S. No	Treatments	50 ppm	100 ppm	200 ppm	Mean	Growth Inhibition (%)
T1	Mancozeb	23.4	27.4	21.4	24.067	69.91
T2	Thiram	48.4	46.4	48.2	47.667	40.41
T3	Tebuconazole	1.5	1.4	1.6	1.5	98.12
T4	Propiconazole	5.5	5.6	6.2	5.767	92.79
T5	Copper oxychloride	74.2	72.5	70.5	72.4	9.5
T6	Hexaconazole	27.7	26.5	28.6	27.6	65.5
T7	Captan	60.2	58.3	56.3	58.267	27.16
T8	Azoxystrobin	12.4	16.5	18.6	15.833	80.2
T9	Trifloxystrobin	30.5	29.6	28.4	29.5	63.12
T10	Control	80	80	80	80	0
	C.D, SE(m), SE(d), C.V.	2.976, 1.002, 1.416, 4.784				



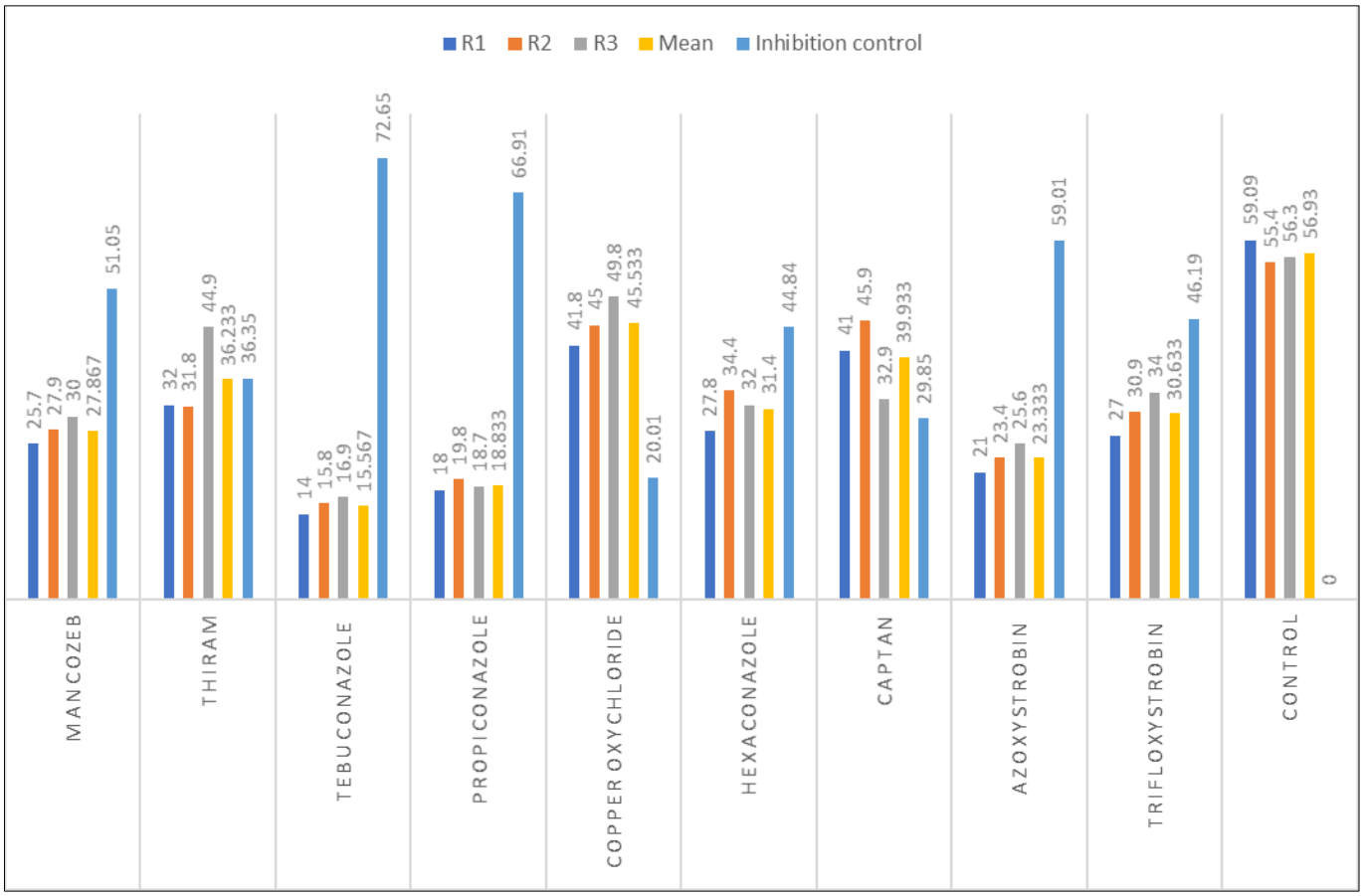
Evaluation of fungicides against *Alternaria brassicae In vivo*

The findings extracted from Table-2 and the corresponding histograms (depicted in Figures 4 and 5) underscore the significant effectiveness of all the tested fungicides in managing the disease when compared to the control group. This efficacy was not limited to laboratory conditions alone, as demonstrated by the field experiments.

Among the fungicides tested, tebuconazole and propiconazole stood out with impressive percentages of disease control in field conditions. They exhibited disease control rates of 72.65% and 66.91%, respectively, when compared to the control group. In contrast, copper oxychloride and captan displayed the least efficacy in disease control, manifesting disease percentages of 20.01% and 29.85% over the control group, respectively.

Moreover, the other chemical fungicides also showcased notable disease control percentages in relation to the control. Mancozeb exhibited a disease control percentage of 51.05%, thiram demonstrated 36.35% disease control, hexaconazole displayed 44.84% disease control, azoxystrobin presented 59.01% disease control, and trifloxystrobin registered a disease control percentage of 46.19% over the control group.

S. No.	Treatment	R1	R2	R3	Mean	Inhibition control
T1	Mancozeb	25.7	27.9	30	27.867	51.05
T2	Thiram	32	31.8	44.9	36.233	36.35
T3	Tebuconazole	14	15.8	16.9	15.567	72.65
T4	Propiconazole	18	19.8	18.7	18.833	66.91
T5	Copper Oxychloride	41.8	45	49.8	45.533	20.01
T6	Hexaconazole	27.8	34.4	32	31.4	44.84
T7	Captan	41	45.9	32.9	39.933	29.85
T8	Azoxystrobin	21	23.4	25.6	23.333	59.01
T9	Trifloxystrobin	27	30.9	34	30.633	46.19
T10	Control	59.09	55.4	56.3	56.93	0
	C.D, SE(m), SE(d), C.V.	6.47, 2.161, 3.056, 11.472				



Conclusion

Alternaria leaf blight is a global concern for the mustard crop, causing substantial economic losses. Various chemical fungicides are presently accessible in the market to manage this disease. The findings of this research demonstrate a noteworthy capacity of all examined chemical fungicides and to suppress the disease compared to the control group. However, the widespread and unchecked utilization of chemical fungicides has led to numerous health risks and adverse environmental consequences. Consequently, employing these potent chemicals at lower, effective concentrations could offer a safer approach to mitigate health hazards and reduce environmental pollution.

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