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In vitro Study on Fungicide Sensitivity of *Alternaria brassicicola* for Managing Leaf Blight in Mustard (*Brassica juncea*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Alternaria brassicicola, a phytopathogenic fungus, incites *Alternaria* leaf blight in mustard, leading to substantial yield and quality losses. This study aimed to investigate the efficacy of seven chemical fungicides against *A. brassicicola in vitro*. Seven chemical fungicides were assessed at 50, 100, 250, and 500 ppm concentrations using the poisoned food technique and Completely Randomized Design (CRD). Tebuconazole + Trifloxystrobin and Tebuconazole exhibited

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100 per cent growth inhibition at 100-500 ppm. Mancozeb + Metalaxyl and Mancozeb demonstrated 100% growth inhibition at 250-500 ppm. Chlorothalanil displayed 83.33% growth inhibition at 500 ppm, while Thiophanate methyl showed the least efficacy (48.00% growth inhibition) at 50 ppm. The findings suggest that Tebuconazole-based fungicides are highly effective against *A. brassicicola*. Lower concentrations (50-100 ppm) of certain fungicides also exhibited promising results.

Keywords: Alternaria brassicicola; Alternaria leaf blight; mustard; chemical fungicides; In vitro evaluation.

1. INTRODUCTION

Mustard *(Brassica juncea*) is an important Rabi oilseed crop in India, ranking second in area and production after groundnut. It is widely grown both as a sole crop and as an intercrop in the marginal and sub-marginal soils of the eastern, northern, and north-eastern states. The cool and moist winter climate in these regions promotes the healthy growth and high productivity of mustard. The seeds of mustard are known by various local names, such as sarson, rai, raya, torai, and lahi. However, the crop is highly susceptible to a range of bacterial, fungal, and viral diseases. One of the most significant diseases affecting mustard is *Alternaria* blight, caused by the fungus *Alternaria brassicicola*. This disease is widely distributed across the globe and has a severe economic impact. *Alternaria* blight has been reported to cause yield losses of 35-60% in mustard crops (Kolte et al., 1987).

The disease is characterized by the formation of distinct spots on leaves, stems, and siliquae. These spots are typically grey with concentric black rings, though their appearance can vary depending on the host and environmental factors. Initial symptoms appear as small black dots on the lower leaves, which enlarge over time to form prominent, round, concentric spots of varying sizes. As the disease progresses, the lower leaves begin to defoliate, and the disease spreads to the middle and upper leaves. In the later stages of plant growth, the spots also appear on siliquae and stems. On the siliquae, the spots become round and black, eventually causing the entire siliquae to turn black. On the stems, black, elongated streaks with or without necrotic grey centers become visible.

Given the economic importance of mustard and the destructive nature of *Alternaria* blight caused by *Alternaria brassicicola*, the present study focuses on the *in vitro* evaluation of fungicides to combat this pathogen.

2. MATERIALS AND METHODS

The experiment was conducted using a Completely Randomized Design (CRD) with the poisoned food technique. Seven chemical fungicides were evaluated, including Mancozeb, Mancozeb + Metalaxyl, Tebuconazole + Trifloxystrobin, Thiophanate methyl, Tebuconazole, Chlorothalonil, and Carbendazim. These fungicides were tested at four different concentrations: 50, 100, 250, and 500 ppm. Each treatment was replicated three times.

2.1 Isolation, Purification and Maintenance of Pure Culture of the Pathogen

The pathogen *Alternaria brassicicola* was isolated from infected mustard leaves collected from the field. To isolate the test fungus, spores were extracted from the infected areas for microscopic examination to confirm the presence of the pathogenic fungus. Once the presence of *A. brassicicola* was confirmed, the leaves were cut into small pieces (1–1.5 cm) using a sterile blade. These pieces were then disinfected by immersing them in 0.5% sodium hypochlorite (NaOCl) solution for 2 minutes, followed by three washes with distilled water. Excess moisture was removed using sterile blotting paper. The sterilized leaf pieces were placed on potato dextrose agar (PDA) medium using sterile forceps and incubated at 27 ± 1°C for 7 days. Based on the morphological characteristics of the conidia, as described by Tu (2015), the pathogen was identified as *A. brassicicola*. To purify the culture, small pieces of agar containing spores were transferred to a fresh Petri plate with new media and incubated at 27 ± 1°C for 7 days. The pathogen was sub cultured three times to obtain a pure culture, which was then preserved on a PDA slant at 4°C.

2.2 *In-vitro* Evaluation of Fungicides

For the evaluation of chemical fungicides, the appropriate volume of stock solution was mixed with sterilized PDA to achieve final concentrations of 50 ppm, 100 ppm, 250 ppm, and 500 ppm. Twenty millilitres of the amended PDA were dispensed into each 90 mm sterilized Petri plate and allowed to solidify. A control treatment, where no fungicide was added, was also prepared. Using a sterilized cork borer, a 7 mm diameter disc from a 9-day-old culture of *A. brassicicola* was transferred to the center of the solidified media in both the amended and control plates. Each treatment was repeated three times. The Petri plates were then incubated at 27 ± 1°C for a duration of seven days.

2.3 Growth Inhibition Test

Mycelial growth was measured after 7 days of incubation for each treatment using a scale. The percentage of mycelial growth inhibition compared to the control was calculated using the formula provided by Vincent (1947).

$$\text{Per cent inhibition } = \frac{(\mathrm{C}-\mathrm{T})}{\mathrm{C}} \times 100$$

Where,
C = Diameter of the colony in control (mm) and
T = Diameter of the colony in treatment (mm)

3. RESULTS AND DISCUSSION

3.1 *In Vitro* Evaluation of Fungicides

The efficacy of various fungicides is presented in the Table 1, based on observations made after 7 days of incubation. The effectiveness of the chemical fungicides against the test fungus was evaluated *in vitro* using the poisoned food technique. The inhibition percentages are shown in Table 1. Analysis of the data reveals that all the chemical fungicides tested significantly reduced pathogen growth compared to the control (78.00 mm). The degree of mycelial growth inhibition increased with higher fungicide concentrations. Table 1 clearly illustrates the percent mycelial growth and corresponding percent inhibition at different concentrations, observed on the seventh day of incubation. The data showed (Table 1) that increase in concentration of the fungicides caused increased inhibition of mycelial growth of the fungal pathogen (*A. brassicicola*). Among the tested fungicides, T3 showed highest inhibition per cent (87.82, 100, 100and 100) followed by Tebuconazole (T5) (86.53, 100,100 and 100%), Mancozeb 64% + Metalaxyl 8% i.e (T2) (75.00, 89.74, 100 and 100%), and (T1) Mancozeb (71.79, 79.48, 85.89 and 100%) at 50, 100, 250 and 500 ppm concentrations, respectively.

Whereas minimum inhibition per cent was showed by (T4) Thiophanate methyl (38.46, 57.05, 64.14 and 66.02%) followed by Carbendazim (41.02, 60.89, 63.46 and 69.23%) and Chlorothalanil (53.84, 69.23, 78.20 and 83.33%) at different 50, 100, 250 and 500 ppm concentrations.

The present findings align with the results of Meena et al. (2020), who evaluated several fungicides, including Mancozeb, Metalaxyl MZ, Copper oxychloride, Copper hydroxyl-chloride, Carbendazim, Azoxystrobin, Tebuconazole, Nativo, and leaf extracts of *A. indica*, *P. pinnata*, and *M. alliacea* using the poisoned food technique. Among the tested fungicides, Tebuconazole + Trifloxystrobin and Tebuconazole (0.1%) were the most effective, achieving up to 89% inhibition of mycelial growth. Hussain et al. (2018) observed that Topsin-M and Topas fungicides were less effective compared to Nativo and Cabriotop. These findings are consistent with those of Panwar et al. (2013), who reported complete growth inhibition of *Alternaria* with Tebuconazole, followed by Mancozeb, and the least inhibition with Carbendazim. Similarly, Tu (2015) found complete inhibition of *A. brassicae* by Tebuconazole and Mancozeb at concentrations of 250, 500, and 1000 ppm, and by Metalaxyl + Mancozeb at 500 and 1000 ppm, with Carbendazim showing the least effect. Kumar & Kumar (2022), Biswas & Ghosh (2018), and Kantwa et al. (2014) also reported significant inhibition of *Alternaria* spp. by Mancozeb. Synthetic fungicides inhibit pathogens by disrupting cell membranes, altering permeability, or interfering with metabolic processes, thereby exerting their fungicidal effects (Kakraliya et al., 2018). The enhanced efficacy of Tebuconazole is primarily due to its inhibition of ergosterol biosynthesis, which disrupts the growth and reproduction of fungal pathogens (Muhamad et al., 2010).

Table 1. *In vitro* evaluation of fungicides on mycelial growth inhibition of *Alternaria brassicicola*

Treatments	Fungicides	Mean colony diameter (mm) at different concentrations (ppm)				Growth inhibition (%) at different concentrations (ppm)			
		50	100	250	500	50	100	250	500
T1	Mancozeb	22.0	16.0	11.0	0	71.79	79.48	85.89	100.00
T2	Mancozeb + Metalaxyl	19.5	8.0	0	0	75.00	89.74	100.00	100.00
T3	Tebuconazole + Trifloxystrobin	9.5	0	0	0	87.82	100.00	100.00	100.00
T4	Thiophanate methyl	48.0	33.5	28.0	26.5	38.46	57.05	64.10	66.02
T5	Tebuconazole	10.5	0	0	0	86.53	100.00	100.00	100.00
T6	Chlorothalanil	36.0	24.0	17.0	13.0	53.84	69.23	78.20	83.33
T7	Carbendazim	46.0	30.5	28.5	24.0	41.02	60.89	63.46	69.23
T8	Control	78.00	78.00	78.00	78.00				

4. CONCLUSION

Alternaria leaf blight is a globally significant disease affecting mustard crops, causing substantial economic losses. A variety of fungicides are commercially available to control this disease. The present study demonstrated a significant inhibitory effect of all the tested chemical fungicides compared to the control. Tebuconazole + Trifloxystrobin and Tebuconazole were identified as the most effective fungicides, achieving 100% growth inhibition at concentrations of 100 ppm, 250 ppm, and 500 ppm. In contrast, Thiophanate methyl was the least effective in reducing fungal growth. However, the indiscriminate use of chemical fungicides can pose health risks and negatively impact the environment. Therefore, employing effective fungicides at lower concentrations could offer a safer approach, reducing both health hazards and environmental pollution. The effectiveness of certain chemical fungicides, even at lower concentrations (50 ppm and 100 ppm), in controlling *Alternaria brassicicola*. Utilizing these fungicides can minimize hazardous effects and provide a viable solution for managing *Alternaria* leaf blight in mustard crops.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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