Fungicide Sensitivity of *Alternariabrassicicola*: An *In vitro* Study for Managing Alternaria Blight in Brassica juncea

Comment [sk1]: In vitro study on fungicide sensitivity of *Alternariabrassicola* for managing blight in Mustard, *Brassica juncea*

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Abstract

Alternariabrassicicola, a phytopathogenic fungus, incites *Alternaria* leaf blight in mustard, leading to substantial yield and quality losses. The current study aimed to investigate the efficacy of seven different fungicides against *A. brassicicolain vitro*.Seven fungicides were evaluated at different concentrations of 50, 100, 250, and 500 ppm by adopting poisoned food technique in Completely Randomized Design (CRD). Among the different fungicides tested, Tebuconazole + Trifloxystrobin and Tebuconazoleexhibited cent per cent growth inhibition at 100-500 ppm while Mancozeb + Metalaxyl and Mancozeb exhibited cent per cent growth inhibition at 250-500 ppm. Chlorothalonilshowed 83.33% growth inhibition at 500 ppm and Thiophanate methyl showed the least efficacy (48.00% growth inhibition) at 50 ppm.Thefindings from the study proved that Tebuconazole-based fungicides are highly effective against *A. brassicicola*. Lower concentrations (50-100 ppm) of certain fungicides also exhibited promising results.

Key Words:*Alternariabrassicicola*, *Alternaria* leaf blight, mustard, chemical fungicides, *in vitro* evaluation.

Introduction

Mustard (*Brassica juncea*) is a prominent *rabi* oilseed crop in India, ranking second in importance next to groundnut in terms of area and production. It is extensively cultivated as a pure crop and also as an intercrop in marginal and sub-marginal soils across eastern, northern, and north-eastern states. The cool and moist climate during winter months facilitates luxuriant growth and productivity of mustard in these regions.Mustard seeds are known by various local names, including sarson, rai, raya, torai, and lahi. However, the crop is prone to numerous bacterial, fungal, and viral diseases. Among these, *Alternaria* blight, caused by *A.brassicicola*, is a globally widespread and economically significant disease.*Alternaria* blight caused by *A. brassicicola*has been reported to inflict heavy yield losses to the tune of 35-60% (Kolte*et al*., 1987) in mustard crop. Typical symptoms of *Alternaria* blight disease are the formation of spots on leaves, stems and siliquae. The concentric black spots produced were usually grey coloured

and their characters varied with host and alsoenvironmental factors. Initially symptoms appeared on the lower leaves as black points, later which enlarged to develop into prominent, round, concentric spots of various sizes. As disease progressed, the lower leaves will defoliate and disease appears on middle and upper leaves. At the later stage of the plant growth, spots appear on siliquae and stem. The spots were round black and quite conspicuous and the siliquae turn completely black. On the stem, black, elongated spots become visible in the form of black streaks with or without necrotic gray centres. Considering economic importance of the mustard crop and destructive nature of *Alternaria*blight incited by *A.Brassicicola*in mustard,

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Materials and Methods

The experiment was carried out in in Completely Randomized Design using poisoned food technique. Seven chemical fungicides i.e. Mancozeb, Mancozeb + Metalaxyl, Tebuconazole + Trifloxystrobin, Thiophanate methyl, Tebuconazole, Chlorothalanil and Carbendazim were evaluated at four different concentrations such as 50, 100, 250 and 500 ppm respectively. Each of the treatment was replicated three times.

Isolation, purification and maintenance of pure culture

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Survey was collected and leaf samples were collected from the mustard field. Spores were teased from infected portion for microscopic examination to check the presence of pathogenic fungus. After confirming the presence of *Alternaria brassicicola*, leaves were cut into small pieces (1-1.5cm) with sterile blade. These pieces were disinfected with 0.5% sodium hypochlorite (NaOCl) solution for two minutes followed by three washings with distilled water and excessive moisture was removed using sterile blotting paper. The sterilized leaf pieces were placed on PDA medium using sterilized forceps and incubated at 27 ± 1 °C for 7 days. On the basis of morphological characters of conidia as described by Yu (2015) pathogen was identified as *Alternaria brassicicola*. Then the culture was purified by transferring small piece of agar containing spore to another Petri plate containing media and incubated at 27 ± 1 °C for 7 days. The pathogen was sub cultured three times to obtain pure culture and pure culture thus obtained was preserved in PDA slant at 4 °C.

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In-vitro evaluation of fungicides

For the evaluation of chemical fungicides, calculated amount of stock solution was mixed in sterilized PDA to make final concentration of 50ppm, 100ppm, 250ppm and 500ppm. Twenty ml of amended PDA was poured in each 90mm sterilized petri plate and allowed to solidify A check was also maintained where no fungicide was added in the medium. A circular disc of 7mm diameter from 9 days old culture of *Alternaria brassicicola* was cut with sterilized cork borer and inoculated in the centre of solidified amended as well as control media. Each treatment was replicated in three petri plates. Then the petriplates were incubated at 27 ± 1 °C for seven days.

Growth inhibition test

The observation on mycelial growth was recorded after 7 days of incubation in each treatment using Vernier calliper scale. The percent growth inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947).

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

Where,

C = Diameter of the colony in control (mm)

T = Diameter of the colony in treatments (mm)

Results and Discussion

In Vitro Evaluation of Fungicides

The efficacy of different fungicides was shown in the table showing 7 days of incubation. The efficacy of different chemical fungicides against test fungus was evaluated in vitro using poisoned food technique. The data on inhibition percent is presented in table. An insight into data reveals that all the tested chemical fungicides showed significant effect against pathogen growth over control (78.00 mm). The extent of mycelial growth inhibition increased with

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increase in their concentration. The tablesshow the percentmycelial growth and percentmycelial growth inhibition at different concentrations at seventh days after incubation. The data showed (Table 1) that increase in concentration of the fungicides caused increased inhibition of mycelial growth of the fungal pathogen (*Alternariabrassicicola*). Among those, T3(Tebuconazole 50% + Trifloxystrobin 25%), showed highest inhibition per cent (87.82, 100, 100and 100) followed by T5Tebuconazole (86.53, 100,100 and 100%) ,T2Mancozeb 64% + Metalaxyl 8%(75.00, 89.74, 100 and 100%), and T1Mancozeb (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 T4Thiophanate methyl (38.46, 57.05, 64.14 and 66.02%) followed by T7Carbendazim (41.02, 60.89, 63.46 and 69.23%) and T6Chlorothalanil(53.84, 69.23, 78.20 and 83.33%) at different 50, 100 , 250 and 500 ppm concentrations.

Our results are in accordance with the findings ofMeena*et al.* (2020), who tested different fungicides, *viz.*, Mancozeb, Metalaxyl MZ, Copper oxy-chloride, Copper hydroxyl- chloride, Carbendazim, Azoxystrobin, Tebuconazol, Nativo and leaf extracts of *A. indica*, *P. pinnata* and *M. alliacea* was performed following the "poisoned food technique". Among the tested fungicides Tebuconazole + Trifloxystrobin and Tebuconazol(0.1%) were found most effective to inhibit the mycelium growth up to 89 per cent.Hussain*et al*. (2018), observed that the Topsin-M and TopasFungicides did not proved better as compare to Nativo and Cabriotop fungicides.Similar resultwas recorded in findings of Panwar et al., (2013) who reported complete growth inhibition of Alternaria in tebuconazole followed by mancozeb and least inhibition in carbendazim. Similarly, Tu (2015) recorded complete inhibition of *A. brassicae* by tebuconazole, mancozeb at 250, 500 and 1000 ppm and by metalaxyl + mancozeb at 500 and 1000 ppm and least inhibition at carbendazim. Kumar and Kumar (2022), Biswas and Ghosh (2018) and Kantwaet al.,(2014) obtained significant growth inhibition effect of *Alternaria*sp. in mancozeb. Synthetic fungicides bring about the inhibition of pathogens either by destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogen and hence are effective (Kakraliyaet al., 2018). Higher inhibition effect of tebuconazole is due to inhibition of ergo sterol biosynthesis, controlling the growth and reproduction of fungal pathogen (Muhamadet al., 2010).

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Table 1: *In vitro* evaluation of fungicides on mycelial growth inhibition of *Alternariabrassicicola*

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				

Conclusion

Alternarialeaf blight is a worldwide disease of economic importance in mustard crop. Different chemical fungicides are commercially available in market to control this disease. This study revealed significant inhibition effect of all the tested chemical fungicides over control. Tebuconazole + Trifloxystrobin and Tebuconazole proved to be the most effective chemical fungicide recording 100% growth inhibition at 100ppm, 250ppm and 500ppm concentrations whereas, Thiophanate methyl was least effective in reducing fungal growth. Indiscriminate application of chemical fungicides have resulted several health hazards, negative impacts in environment so, the use of effective chemical at possible lower concentrations could be safer way to minimize health hazards and environmental pollutions. The effectiveness of certain chemical fungicides, even at lower concentrations (50 ppm and 100 ppm), in controlling

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Alternariabrassicicola. Utilizing these fungicides can minimize hazardous effects and provide a viable solution for managing *Alternaria* leaf blight in mustard crops.

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