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In vitro and *In-vivo* Evaluation of Different Fungicides against *Alternaria brassicae* Causing Alternaria Blight Disease of Mustard

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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

Mustard is the second most important oilseed crop in India after groundnut and contributes about 35% in the total vegetable oil production. Mustard crop is sensitive to numerous abiotic and biotic stresses, which affects the production and productivity drastically. Alternaria blight disease of

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mustard is one the most destructive disease which affects all growth stages and cause huge economical loss. Considering the severity and significance of damage caused by this disease, an *in vitro* and *in vivo* evaluation of eight fungicide was done against *A. brassicae*. It was found that Propiconazole 25 EC showed complete mycelial growth inhibition (100 %) at all the three concentrations i.e., 100, 200 and 300 ppm whereas, Tebuconazole 25.9 EC recorded complete mycelial growth inhibition at 200 and 300 ppm. Among the tested fungicides, the minimum percent disease severity and AUDPC on leaves was observed in crops sprayed with Azoxystrobin 12.5% + Tebuconazole 12.5% SC followed by Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 235 SC at 40, 55 and 70 days after spraying. Further, maximum seed yield was recorded in Azoxystrobin 12.5% + Tebuconazole 12.5% SC at 1345.49Kg/ha followed by Azoxystrobin 12.5% at 1265.63kg/ha.

Keywords: Alternaria blight disease; fungicide; poisoned food technique; AUDPC; correlation coefficient; disease severity.

1. INTRODUCTION

Mustard is an important oilseed crop and ranks third after soybean and palm oil in the world. In India, it is grown in an area of 6.23 mha with production and productivity of 9.34 mt and 1499 kg/ha respectively. Out of all the Rapeseed-mustard crops cultivated in India, mustard occupies about 75-80% of the total area occupied by Rapeseed-mustard (Annual report ICAR-DRMR, 2018-19). It is an important source of oil in India and contributes about 35% in the total vegetable oil production. It is the second most essential oilseed crop after groundnut and was significant contributor of the “Yellow Revolution” launched in 1986-1987 which aimed to increase edible oil production. Mustard is sensitive to numerous biotic and abiotic stresses which further widens the existing gap between potential yield and yield realized at the farmer’s field.

Among the major diseases affecting mustard, alternaria blight is one of the most destructive disease which occurs frequently throughout the world and infects all growth stages causing huge economical loss. An average yield loss of 35-38% has been reported from India (Kolte, 1985a,b; Kolte et al. 1987; Chattopadhyay, 2009). The alternaria blight infected seeds of rapeseed-mustard are reported to suffer a loss of 14.6-36% in oil content over the seeds of healthy plants (Ansari et al. 1988). In addition to direct yield loss, it also affects the quality and germination of seed by reducing the size of seed, colour and oil content (Kaushik et al.1984). A multi-pronged, farm level strategy combining genomic-assisted breeding, QTL mapping, integrated diseases management practices, weather based advisory services and forecasting is necessary to address yield loss due to alternaria blight (Jyoti et al. 2021).

Alternaria species are either saprophyte on organic substrate or parasites on living plants (Singh et al. 2017). This dual life cycle leads to a persistent pathogen population even when not actively infecting plant making it difficult to manage and eradicate the disease and can result in both pre and post-harvest losses. Symptoms of alternaria blight of mustard is characterized by formation of spots on leaves, stem and pods. Symptom in seedlings appears as small light brown lesion on cotyledon and hypocotyls and dark stem lesions appear immediately after germination of seedling which often results in damping-off or stunted seedlings**.** On adult plants, symptoms are first visible on lower leaves, which appear as black points that later enlarges into prominent, round concentric rings that are grey in colour. On siliqua and stem, brownish-black spots appear with grey centre. The primary infection starts from either diseased stubbles or neighbouring volunteer crops whereas, secondary infection takes place through conidia formed on diseased leaves or pods (Kumar et al. 2016) which help in persistence, recurrence, and spread of alternaria blight disease. The alternaria blight infection on leaves and siliqua reduces the photosynthetic area drastically (Meena et al. 2010). Infection on siliqua affects the normal seed development, its weight, colour and percent oil content and seed quality.

2. MATERIALS AND METHODS

2.1 Isolation of the Pathogen

Mustard leaf samples infected with Alternaria blight were collected from Bihar Agricultural

University farm, Sabour, Bhagalpur. Infected leaf samples showing typical characteristic symptom of Alternaria blight were used for isolation of *A. brassicae*. Leaf samples were first washed under running tap water and then blot dried. Small bits of leaf with a healthy plus diseased portion were taken and sterilized with 4% sodium hypochloride for 1 min followed by three times washing in distilled water. The pieces were blot dried using sterilized blotting paper and transferred on solidified RRSA (Radish root sucrose agar) media and incubated for 7 days at 20 ± 2 °C. Sub-culturing was done using culture portion showing typical *A. brassicae* colony characteristics. Pure culture was obtained through single spore isolation.

2.2 Pathogenicity Test

Pathogenecity test was conducted in laboratory using susceptible *Brassica juncea* variety Varuna. A spore suspension of 10^4 spores/ml was used for inoculation on 25 day old plant (Kumar et al. 2014). The fungus was re-isolated from the infected leaves showing typical alternaria blight characteristics and maintained in RRSA media. The re-isolated fungus was similar to the original isolate of the pathogen in morphology. Thus, Koch's postulate was satisfied.

2.3 *In vitro* Evaluation of different Fungicides Against *Alternaria brassicae*

Evaluation of eight fungicide belonging to different groups and showing different mode of action was taken and tested viz., Mancozeb 75% WP, Propiconazole 25 EC, Tebuconazole 25.9% EC, Azoxystrobin 23% SC, Metalaxyl 8% + Mancozeb 64% WP, Tebuconazole 10% + Sulphur 65% WG, Azoxystrobin 12.5% + Tebuconazole 12.5%, Tebuconazole 50% + Trifloxystrobin 25% WG at three different concentration of 100 ppm, 200 ppm and 300 ppm. Different treatments were tested against the pathogen under *in vitro* condition to find out the relative efficacy in inhibiting the growth of culture by the "Poisoned Food Technique". A stock solution of 10000 ppm of each fungicide (treatment) was prepared by dissolving the required amount of it in a measured volume of sterilized distilled water. Then, the calculated amount of stock solution was added into warm RRSA media so as to obtain the required fungicide concentrations. It was then poured into sterilized petriplates and allowed to solidify. With the help of a sterilized cork borer, 5 mm bits of 10 days old *Alternaria* culture were taken. It was then inoculated at the center of poisoned media in three replications of each concentration. The fungal bits were inversely placed so that it comes in direct contact with the media. The inoculated Petri plates were kept in BOD incubator at 20 ± 2°C. Control was maintained without mixing the media with any fungicide and just inoculating it with test pathogen.

The efficacy of different chemicals was observed by measuring the colony diameter in millimeters (mm). Percent inhibition over control was assessed using the following formula (Vincent, 1947).

$$R = [(C-T) / C] * 100$$

Where,

R = Percent inhibition (%)
C = Radial growth of pathogen colony in control (mm)
T = Radial growth of pathogen colony in treatment (mm)

Table 1. List of fungicides and their concentrations evaluated under field condition for the management of alternaria blight disease

Treatment	Fungicides	Dosage
T1	Mancozeb 75% WP	2.5 gm/lit
T2	Propiconazole 25 EC	1ml/lit
T3	Tebuconazole 25.9% EC	1ml/lit
T4	Azoxystrobin 23% SC	2.5ml/lit
T5	Metalaxyl 8% + Mancozeb 64% WP	2gm/lit
T6	Tebuconazole 10% + Sulphur 65% WG	1gm/lit
T7	Azoxystrobin 12.5% + Tebuconazole 12.5% SC	3gm/lit
T8	Tebuconazole 50% + Trifloxystrobin 25% WG	1gm/lit
T9	Control	

2.4 *In vivo* Evaluation of Fungicides Against *Alternaria brassicae*

The experiment was conducted at BAU Research farm, Sabour (coordinates: 25° 13' 36.7" N and 87°03' 00.7" E) during Rabi season, 2020-2021 with susceptible mustard variety-Rajendra sufalam to evaluate eight different fungicide for the management of Alternaria blight disease (Table 1). The fungicides were sprayed twice, first spray was given at the onset of disease incidence and the second spray was given 15 days after 1st spray under field condition with the help of Knapsack sprayer.

2.5 Disease Severity on Leaves

Disease severity (%) was calculated on the basis of 0-5 rating scale (Conn et al.1990). The details of rating scale are given in the Table 2. For observation, sixth leaf from the top of ten randomly selected and tagged plants were taken plant. Disease severity was recorded at 40, 55 and 70 DAS at 15 days interval.

On the basis of 0-5 rating scale (Table 2), disease severity (%) was calculated by using the following formula by Mckinney, 1923.

$$\text{Disease index (\%)} = \frac{\text{Sum of all numerical rating}}{\text{Number of leaves examined} \times \text{Maximum grade}} \times 100$$

Table 2. Detail of evaluation system for accessing Alternaria blight disease

Grade	Extent of infection
0	No symptom
1	1-10% leaf area infection
2	11-25% leaf area infection
3	26-50% leaf area infection
4	51-75% leaf area infection
5	>75% leaf area infection

2.6 AUDPC (Area under Disease Progress Curve)

Area under disease progress curve (AUDPC) of alternaria blight disease on leaves was calculated on the basis of disease index using the formula given by Shaner and Finney (1977).

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2][X_{i+1} - X_i]$$

Where.,

Y_i = disease index (per unit) at ith observation
X_i = time (day)
N= total number of observation

2.7 Yield (kg/ha)

Yield of each genotype was recorded from each plot and yield in kg/ha was calculated after harvesting of mustard seeds.

2.8 Correlation between Weather Parameter and Disease Severity of Alternaria Blight on Leaves

The data on the weather parameters (maximum and minimum temperature, maximum and minimum RH) prevailing during the period of investigation were collected from meteorological observatory of BAU, Sabour. Correlation analysis between weather factors and disease severity on leaves of different treatment was done using OPSTAT software.

3. RESULTS

3.1 Effect of different Fungicides on Mycelial Growth of *Alternaria brassicae*

The effect of fungicide on mycelial growth of *A.brassicae* was evaluated at 7 and 14 DAI at 100, 200 and 300 ppm. The percent mycelial growth inhibition was significant for all the concentrations. Tebuconazole 25.9 EC at 7 DAI showed 100 % inhibition of mycelial growth over control at all concentration while at 14 DAI 100% mycelial growth inhibition was observed only at 200 and 300 ppm. Propiconazole 25 EC showed 100 percent inhibition in both the observations (7 and 14 DAI) at all the concentrations i.e., 100, 200 and 300 ppm. The minimum percent inhibition was observed in Azoxystrobin 23% SC (24.04) followed by Metalaxyl 8% + Mancozeb 64% WP (26.33%) and Mancozeb 75% WP (28.62). At 14 DAI, the maximum percent mycelial growth inhibition over control at 200ppm was observed in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (90.80%) followed by Tebuconazole 50% + Trifloxystrobin 25% WG (90.45%) and Tebuconazole 10% + Sulphur 65% WG (78.25%) whereas, the minimum percent inhibition was observed in Azoxystrobin 23% SC (30.15%) followed by Mancozeb 75% WP (32.82%) and Metalaxyl 8% + Mancozeb 64% WP (35.86%). It was observed that Azoxystrobin

12.5% + Tebuconazole 12.5% SC and Tebuconazole 50% + Trifloxystrobin 25% WG were statistically at par (Table 3, Fig. 1).

3.2 Effect of Fungicide on Alternaria Blight Disease Severity, AUDPC and Yield

The percent disease severity on leaf increased progressively with age of plant and was observed in all the treatments. Minimum disease severity was observed in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (5.67), Azoxystrobin 23% SC and Tebuconazole 50 % + Trifloxystrobin 25% WG (6.00). All the treatments showed significant difference at 55 and 70 DAS. At 55 and 70 DAS, the minimum disease severity was observed in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (8.67 and 9.33) respectively. It was followed by Tebuconazole 50 % + Trifloxystrobin 25% WG (9.0 and 11.33) and Azoxystrobin 23% SC (10.67 and 13.33) at 55 and 70 DAS respectively. Azoxystrobin 12.5% + Tebuconazole 12.5% SC and Tebuconazole 50 % + Trifloxystrobin 25% WG and Azoxystrobin 23% SC were found statistically at par at 55 DAS while at 70 DAS, Azoxystrobin 12.5% + Tebuconazole 12.5% SC was statistically different with Azoxystrobin 23% SC. At 55 DAS and 70 DAS, the maximum percent severity was observed in control (28.67 and 45.33) respectively. Maximum reduction over control and minimum average disease severity was observed in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (71.13 and 7.89) (Table 4).

The area under disease progress curve (AUDPC) was calculated for all the treatments and AUDPC was found lower in all the treatments as compared to control. The minimum AUDPC was recorded in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (242.55) followed by Tebuconazole 50 % + Trifloxystrobin 25% WG (264.97) and Azoxystrobin 23% SC (305.02). Whereas, maximum AUDPC was calculated in control (830.02) followed by Metalaxyl 8% + Mancozeb 64% WP (539.99).

The seed yield (kg/ha) was found to be statistically significant among all the treatments (Table 4). All the treatment recorded comparatively more yield than control (881.94 kg/ha). The maximum seed yield was recorded in Azoxystrobin 12.5% + Tebuconazole 12.5% SC followed by Azoxystrobin 23% SC and Tebuconazole 50% + Trifloxystrobin 25% WG i.e., 1345.49, 1265.63 and 1260.42 Kg/ha respectively.

3.3 Correlation Co-efficient (R) between Weather Parameters and Percent Disease Severity on Leaves of Alternaria Blight Disease

The correlation between disease intensity on leaves and weather parameters i.e., maximum and minimum temperature, maximum and minimum RH were calculated. It was observed that maximum (24.3-28°C) and minimum temperature (11.6-14.7°C) showed a non-significant positive correlation with disease severity while maximum (89.1%-86.3%) and minimum relative humidity (59.9%-68.9%) recorded a non-significant negative correlation with disease severity on leaves (Fig. 2).

4. DISCUSSION

It can be concluded that Propiconazole is superior among all the treatments *in-vitro* followed by Tebuconazole 25.9 EC, Azoxystrobin 12.5 % + Tebuconazole 12.5 % and Tebuconazole 50 % + Trifloxystrobin 25 % WG. The fungicide Propiconazole 25 EC and Tebuconazole 25.9 EC found superior in the evaluation belongs to Triazole group of fungicide and are excellent systemic fungicide against ascomycetes. Their effectiveness against *A. brassicae* could be attributed to their mode of action. These fungicides interfere with normal ergosterol biosynthesis mechanism in the fungus cell and causes demethylation of C-14 which leads to accumulation of C-14 methyl sterols. Ergosterol plays a vital role in the fungus cell wall formation, and is for crucial for its proper structure and functioning. Lack of normal sterol slows down and checks the growth of fungus.

The result corroborates the findings of Jackson and Kumar (2019) where Propiconazole showed 100 % inhibition of *A. brassicae* at much lower concentration i.e., 10, 25, 50, 75 and 100 ppm. Tebuconazole 250 EC + Trifloxystrobin WG 75, Propiconazole 25 EC, Tebuconazole 250 EC and Difenconazole 25 EC were highly effective in inhibiting the growth of *A.brassicae* (100 %) at 0.05 %, 0.1 % and 0.2 % concentration (Rajvanshi et al. 2020). Fungicides Propiconazole, Tebuconazole, Copper oxychloride and Hexaconazole are effective against *A. Brassicicola* at 0.05 %, 0.1 % and 0.2 % concentration (Kiran et al. 2018). In-vitro evaluation of six fungicide against *A. solani* showed that Propiconazole 25 EC showed maximum mycelial growth inhibition at 50 and 100 ppm whereas, it showed complete inhibition at 250 ppm followed by Carbendazim 50 WP (Husain et al. 2020). Six systemic fungicides were evaluated against *A. porri* at 0.5, 1, 10, 25, 100 and 200 µg/ml concentration, among the fungicides complete mycelial growth inhibition was recorded by Tebuconazole 25 EC at 25 µg/ml whereas, Tebuconazole 50 % + Trifloxystrobin 25 % WG and Propiconazole 25 EC showed complete mycelial growth inhibition at 100 and 200 µg/ml concentration respectively (Yadav et al. 2017).

All the fungicides significantly reduced alternaria blight severity on leaves over untreated control. The result revealed that the maximum reduction of alternaria blight severity on leaves was recorded in Azoxystrobin 12.5% + Tebuconazole 12.5% SC @ 3 g/ml followed by Tebuconazole 50% + Trifloxystrobin 25% WG @ 1ml/lit and Azoxystrobin 23 %SC @ 2.5ml/lit. The minimum AUDPC was also recorded in Azoxystrobin 12.5% + Tebuconazole 12.5% followed by Tebuconazole 50% + Trifloxystrobin 25% WG. The fungicides, Azoxystrobin and Trifloxystrobin belongs to Strobilurins group which are effective against most fungal diseases. They are systemic in nature and interferes with fungal cell respiration by blocking electron transfer at the quinol oxidation site in the cytochrome bc1 complex, thereby hampering ATP production (Agrios, 2005). Tebuconazole on the other hand belongs to Triazole group which inhibits ergosterol biosynthesis and disrupts normal sterol formation required for the structure and functioning of cell wall. These chemicals prevents further infection, invasion of host tissue and thus controls severe infection. Seed treatment with Apron @ 6g/kg and then fungicide application of Nativo @ 0.05 % was found most effective for the management of alternaria blight disease followed by Difenconazole @ 0.05 % and iprodione @ 0.2 % (Singh et al. 2018). The result corroborates with the findings of Pattanaik and Priyadarshini, (2020) who reported that plants treated with Azoxystrobin 11% + Tebuconazole 18.3 % was found most effective on early blight of tomato. The foliar spray of Azoxystrobin 11 % + Tebuconazole 18.3 % recorded least incidence of early blight disease (Palaiah et al. 2020). Combination of Propiconazole 13.9% + Difenoconazole 13.9% EC (22.67±1.76) was found to be superior among seven treatment for the control of alternaria leaf and pod blight in radish (Adhikari et al., 2023).

Table 3. Effect of fungicide on percent mycelial growth inhibition of *Alternaria brassicae*

Treatments	Percent growth inhibition					
	100 ppm		200 ppm		300 ppm	
	7 DAI	14 DAI	7 DAI	14 DAI	7 DAI	14 DAI
Mancozeb 75% WP	51.43 (45.80)*	28.62 (32.33)	54.71 (47.68)	32.82 (34.94)	57.58 (49.34)	36.63 (37.23)
Propiconazole 25 EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Tebuconazole 25.9EC	100.00 (90.00)	91.22 (72.74)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Azoxystrobin 23% SC	45.49 (42.39)	24.04 (29.34)	47.94 (43.80)	30.15 (33.29)	51.84 (46.04)	38.16 (38.14)
Metalaxyl 8% + Mancozeb 64%WP	52.05 (46.15)	26.33 (30.86)	55.12 (47.92)	35.86 (36.77)	58.60 (49.93)	39.69 (39.03)
Tebuconazole 10% + Sulphur 65% WG	74.59 (59.71)	47.70 (43.67)	84.63 (66.93)	78.25 (62.19)	90.16 (71.71)	88.92 (70.57)
Azoxystrobin 12.5% + Tebuconazole 12.5% SC	90.78 (72.31)	90.07 (71.61)	91.39 (72.92)	90.80 (72.31)	100.00 (90.00)	91.60 (73.13)
Tebuconazole 50% + Trifloxystrobin 25% WG	89.75 (71.31)	88.17 (69.86)	90.78 (72.30)	90.45 (71.98)	92.00 (73.55)	91.21 (72.74)
Control	0.00	0.00	0.00	0.00	0.00	0.00
C.D.at 1%	1.12 (0.92)	1.18 (0.87)	1.54 (1.19)	2.06 (1.34)	1.57 (1.08)	1.97 (1.46)
S.Em.±	0.37 (0.31)	0.39 (0.29)	0.51 (0.40)	0.69 (0.45)	0.53 (0.36)	0.66 (0.49)
C.V.%	0.96 (0.92)	1.24 (1.03)	1.28 (1.16)	1.92 (1.42)	1.26 (1.00)	1.75 (1.49)

**Data in the parenthesis are angular transformed values*

Table 4. Effect of fungicide on alternaria blight disease severity, AUDPC and yield

Treatment	Dosage	Percent disease severity			Mean	Reduction over control (%)	AUDPC	Yield (Kg/ha)
		40 DAS	55 DAS	70 DAS				
Mancozeb 75% WP	2g/lit	8.00 (16.42)*	16.00 (23.54)	30.67 (33.61)	18.22 (24.54)	33.33	530.02	1046.88
Propiconazole 25 EC	1ml/lit	8.67 (17.09)	13.33 (21.32)	24.67 (29.76)	15.56 (22.76)	43.07	450.00	1133.68
Tebuconazole 25.9% EC	1ml/lit	6.33 (14.56)	11.33 (19.65)	15.33 (23.03)	11.00 (19.09)	59.75	332.40	1203.13
Azoxystrobin 23% SC	2.5ml/lit	6.00 (14.14)	10.67 (19.04)	13.33 (21.40)	10.00 (18.21)	63.41	305.02	1265.63
Metalaxyl 8% + Mancozeb 64% WP	2gm/lit	7.33 (15.67)	18.00 (25.07)	28.67 (32.36)	18.00 (24.39)	34.14	539.99	1071.18
Tebuconazole 10% + Sulphur 65% WG	1gm/lit	7.67 (16.02)	12.00 (20.26)	14.00 (21.93)	11.22 (19.43)	58.95	342.52	1227.43
Azoxystrobin 12.5% + Tebuconazole 12.5% SC	3gm/lit	5.67 (13.72)	8.67 (17.09)	9.33 (17.76)	7.89 (16.22)	71.13	242.55	1345.49
Tebuconazole 50% + Trifloxystrobin 25% WG	1gm/lit	6.00 (14.14)	9.00 (18.43)	11.33 (19.65)	8.78 (17.10)	67.87	264.97	1260.42
Control	-	8.00 (16.34)	28.67 (32.36)	45.33 (42.31)	27.33 (30.36)	-	830.02	881.94
S.Em.±	-	0.71 (0.79)	0.95 (0.79)	0.68 (0.51)	-	-	-	0.17
C.D. at 5%	-	NS	2.85 (2.39)	2.03 (1.55)	-	-	-	0.51
C.V.(%)	-	17.29 (8.90)	11.50 (6.27)	5.47 (3.31)	-	-	-	13.31

**Data in the parenthesis are angular transformed values*

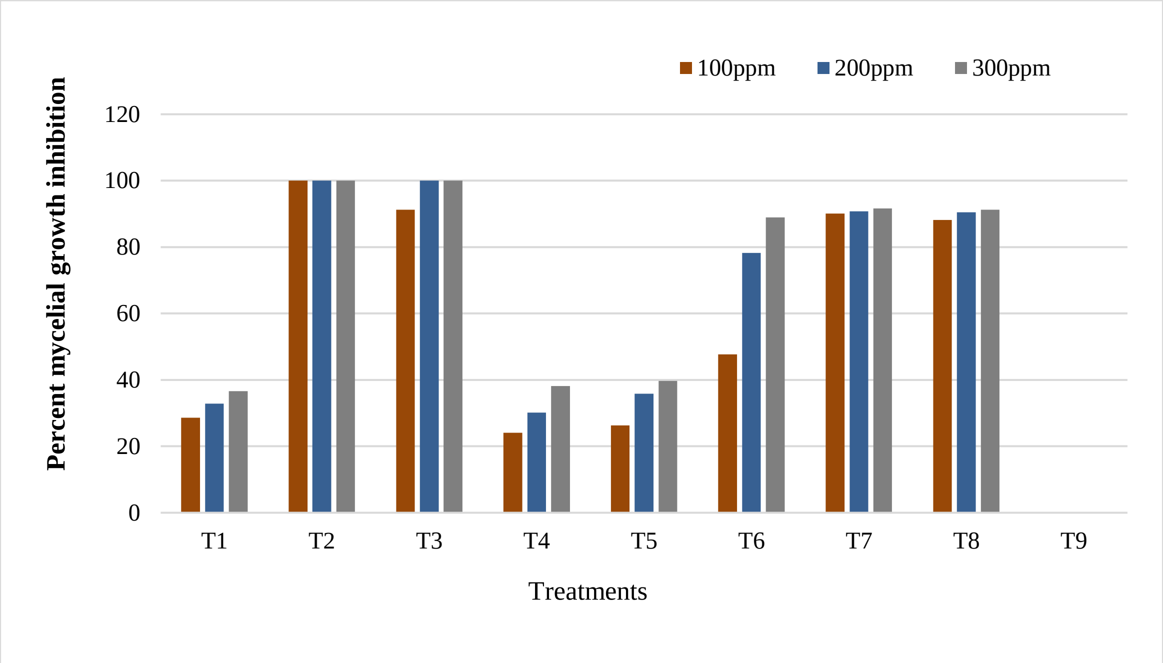

Fig. 1. ***In vitro*** **effect of different fungicides on mycelial growth inhibition of** ***Alternaria brassicae*** **pathogen at14 days after inoculation (DAI)**

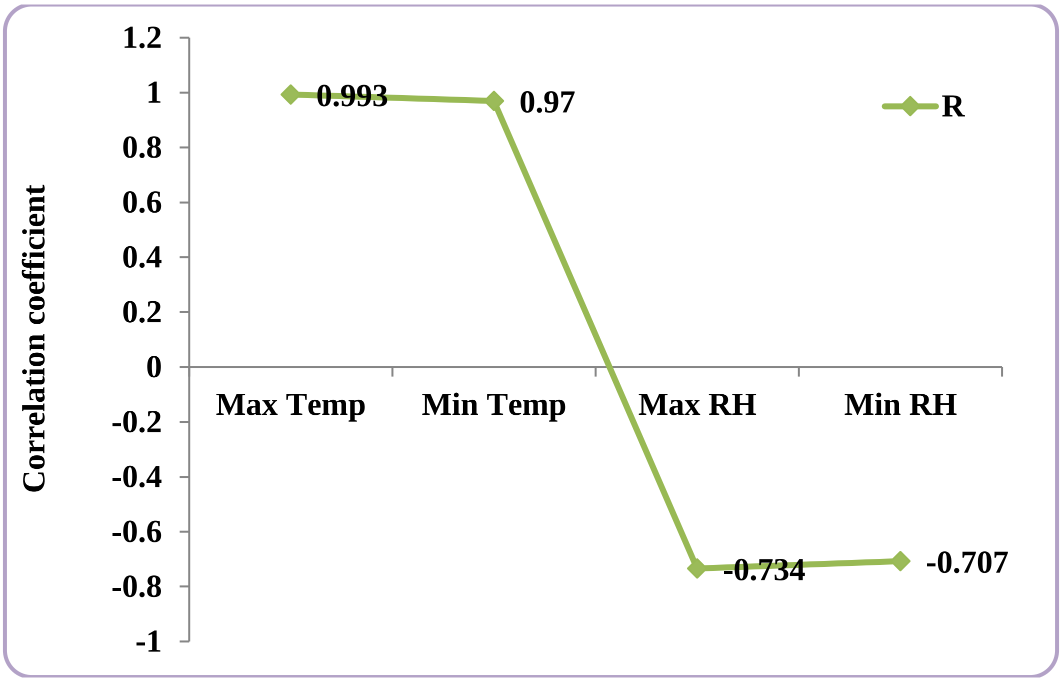

Fig. 2. Correlation between percent disease severity on leaves and weather parameters

Seed yield was found statistically significant among the treatments and all the treatments recorded comparatively more yield over control. The maximum seed yield was recorded in Azoxystrobin 12.5% + Tebuconazole 12.5% SC followed by Azoxystrobin 23 % SC and Tebuconazole 50 % + Trifloxystrobin 25% WG. The findings are in congruent with Singh et al. (2018) who reported that maximum seed yield and yield increase in rapeseed-mustard was observed in treatment Nativo (Tebuconazole + Trifloxystrobin) @ 0.05 % followed by

Difenconazole @ 0.05%. Thabrez et al., 2024 reported minimum alternaria blight disease severity and highest yield (1676.17kg/ha) in treatment with Mancozeb spray at 45 DAS followed by spray of Azoxystrobin at 60 DAS.

In the present study we found a positive correlation between disease severity on leaves and weather parameters (maximum and minimum temperature, maximum and minimum RH). Correlation analysis of disease severity on leaves and weather factors showed a positive correlation with maximum and minimum temperature while minimum and maximum RH showed a negative correlation (Sohi et al. 2020). Rapeseed-mustard sown on 5th December showed a positive correlation with disease severity and maximum and minimum temperature while minimum RH showed negative correlation with disease severity (Mahapatra and Das, 2015).

5. SUMMARY AND CONCLUSION

All the eight fungicides viz., Mancozeb 75% WP, Propiconazole 25 EC, Tebuconazole 25.9% EC, Azoxystrobin 23% SC, Metalaxyl 8% + Mancozeb 64% WP, Tebuconazole 10% + Sulphur 65% WG, Azoxystrobin 12.5% + Tebuconazole 12.5% SC, Tebuconazole 50% + Trifloxystrobin 25% WG significantly reduced mycelial growth of *A. brassicae* pathogen over control on RRSA media. Among all the eight fungicides evaluated *in vitro,* Propiconazole 25 EC showed complete mycelial growth inhibition at all the concentrations i.e., 100, 200 and 300 ppm at 7 and 14 Days after inoculation. Among the tested fungicides, the minimum mean percent disease severity on leaves, AUDPC and maximum yield was observed in crops sprayed with Azoxystrobin 12.5% + Tebuconazole 12.5% SC followed by Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 235 SC. These fungicides can be used as a preventive spray in mustard field for the control of alternaria blight of mustard. Although the incorporation of these fungicide as an integrated disease management component will be recommended for not only effectively managing the disease but also to promote ecological balance and sustainability. Further, long term studies should be conducted to study the effect of such fungicide on pathogen resistance and environment and to assess the best strategy for management.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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