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In-vitro Evaluation of Fungicides Against Radial Growth of *A. brassicae*

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ABSTRACT

Eight commercially available fungicides were evaluated *in-vitro* for their efficacy to inhibit radial growth of *Alternaria brassicae* using poison food technique. All the fungicides evaluated were found to be significantly effective at all their tested concentrations, however, Propiconazole and Hexaconazole were the most effective to exhibit cent percent inhibition in radial growth of *A. brassicae,* even at their lowest tested concentration (50 ppm). Complete inhibition of mycelial growth of test pathogen was also recorded in case of Vitavax and Flusilazole but at their relatively higher concentration (150 ppm). Azoxystrobin + Difenoconazole and Propineb were the next best fungicides in terms of mycelial inhibition at all the tested concentrations *i.e.,* 50, 100, 150 and 200 ppm. Efficacy of fungicides like Propiconazole, Hexaconazole, Azoxystrobin + Difenoconazole and Propineb remained constant with 100% inhibition at their higher concentrations, i.e., 200 ppm. Mancozeb was found to be least effective with maximum mycelial inhibition of 86.08% at 200 ppm, however, the lower concentrations like 50, 100 and 150 ppm exhibited 53.33%, 54.74% and 64.61% radial growth inhibitions, respectively.

Key Words: Chemical, Fungicide, Growth, Mustard.

INTRODUCTION

Indian mustard (*Brassica juncea*) is considered to be a leading oil seed crop in tropical and temperate regions, both in terms of global acreage as well as production. Among all the pests and pathogens reported on this crop, *Alternaria brassicae* (Berk.) causing Alternaria leaf blight or black leaf spot is one of the most serious threat which is known to cause severe losses both in terms of yield and oil content at all the growth stages (Kohl *et al,* 2010). This disease is reported to cause an average yield loss of 30-45% worldwide (Ahmad and Ashraf, 2016). The pathogen may cause damage to crop in various ways *i.e.,* shattering of pods, decreased seed quality, hampered weight and oil content seed produced. The infected seeds also exhibit a significant reduction in vigor and germination. Besides this, the well-developed infected leaves come up with reduced photosynthetic ability of the plants which ultimately affects seed weight and oil content. The incidence of this serious disease in mild to severe form have been reported from all the continents of the world, including India.

In India, the occurrence of this disease is a common problem in every mustard field of almost all the parts of the country, however, severe incidence has been reported from major mustard growing states namely, Himachal Pradesh, Haryana, Rajasthan, Uttar Pradesh, Uttarakhand, Bihar and Madhya Pradesh. There are no any reliable management practices available against this pathogen, but chemical tools are found to be promising up to some extent. In the view of its devastating nature and significance of the crop, it is necessary to figure out effective fungicides and their appropriate dosage in order to suppress the colonization of fungi in mustard crop. Therefore, in this experiment eight commercially available fungicides were evaluated *in-vitro* for their effective concentrations in inhibiting the radial growth of *A.brassicae*.

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Table1. *In-vitro* effect of different concentrations of chemical fungicides on radial growth of *A. brassicae* at 7 days.

Treatment	50 ppm		100 ppm		150 ppm		200 ppm	
	Radial Growth (mm)	Inhibition (%)	Radial Growth (mm)	Inhibition (%)	Radial Growth (mm)	Inhibition (%)	Radial Growth (mm)	Inhibition (%)
Control	90[a]	0[g]	90[a]	0[g]	90[a]	0[f]	90[a]	0[f]
Azoxystrobin + Difenoconazole	15.43[e]	82.85[c]	10.3[d]	88.55[d]	7.43[e]	91.74[b]	1.06[e]	98.81[b]
Flusilazole	15.13[e]	83.18[c]	9.46[e]	89.48[c]	0[f]	100[a]	0[f]	100[a]
Hexaconazole	0[g]	100[a]	0[g]	100[a]	0[f]	100[a]	0[f]	100[a]
Mancozeb	42.26[b]	53.03[f]	39.56[b]	56.03[f]	30.36[b]	66.25[e]	11.26[b]	87.48[e]
Metalaxyl + Mancozeb	35.3[c]	60.77[e]	28.08[c]	68.81[e]	25.06[c]	72.14[d]	9.5[c]	89.44[d]
Propiconazole	0[g]	100[a]	0[g]	100[a]	0[f]	100[a]	0[f]	100[a]
Propineb	19.8[d]	78[d]	10.06[d]	88.81[d]	8.43[d]	90.62[c]	7.13[d]	92.07[c]
Vitavax	6.3[f]	93[b]	5.3[f]	94.11[b]	0[f]	100[a]	0[f]	100[a]
SED	0.19	0.21	0.09	0.10	0.04	0.05	0.03	0.04
LSD(P<0.05)	0.43	0.48	0.21	0.23	0.11	0.12	0.08	0.09

MATERIALS AND METHODS

The infected plant material was visually examined and collected randomly from mustard crop cultivated in experimental fields of Department of Plant Protection, Aligarh Muslim University, India. The plants with characteristic symptoms were identified and infected leaves and siliquae with small, circular, brown, necrotic leaf spots, with characteristic spots were collected and kept in clean poly bags. The specimens were preserved for future use and further studies. The brought samples were subjected to washing under tap water before preliminary microscopic studies. The infected leaves and siliquae were also kept into moist chamber to observe the sporulation on naturally infected parts. For further studies, the associated pathogen was isolated on potato dextrose agar (PDA, HiMedia®, India) by the tissue segment method, as described by Rangaswami (1958). The washed leaves with typical symptoms of *A. blight* were cut into small pieces of 1.0 to 1.5 cm (bearing diseased tissue along with healthy tissues). The pieces were further surface sterilized with 0.1% sodium hypochlorite (NaOCl) for 1 minute followed by three consecutive washing with sterile distilled water. These surface sterilized pieces were then kept between two layers of sterilized blotting papers to remove excess moisture, and thereafter placed in the Petri plates containing solidified PDA medium, which were later incubated at 25±1°C for seven days. The fungus was sub-cultured by the single spore isolation technique (Ricker and Ricker, 1936) followed by multiple sub-culturing. The pure cultures were further stored and maintained on PDA slants for further studies.

The isolated fungus, cultured on PDA was further subjected to cultural and morphological studies and identified as *A. brassicae*. The morphological characters were noted by observing the microscopic slides prepared from the fungal culture, the slides were stained by lactophenol/ cotton blue and covered with glass cover slip. The morphological observations in terms of mycelial structure and conidial structure (i.e., shape, size, septation, beak length) were taken at 10X, 40X and 100X (Awasthi and Kolte, 1989; Simmons, 2007).

The effect of eight fungicides *viz.,* Propineb, Flusilazole, Propiconazole, Hexaconazole, Vitavax, Azoxystrobin + Difenoconazole, Metalaxyl+Mancozeb and Mancozeb was tested at four concentrations *i.e.,* 50, 100, 150 and 200 ppm. The efficacy of these fungicides and concentrations were evaluated in terms of per cent radial growth inhibition of *A. brassicae* on potato dextrose agar medium using poison food technique (Dubey and Patel, 2001). To prepare the poisoned media the fungicides were weighted according to their required concentrations and mixed with 100 ml of autoclaved Potato Dextrose Agar and then poured into 90mm sterilized petri plates (20 ml each). After solidification of the medium each plate was centrally inoculated with 5mm disc taken from a week-old culture of *A. brassicae.*

Each treatment, alongwith control (Petri plates with no fungicides) was maintained in triplicate. The inoculated Petriplates were incubated at 25±2°C, until the control Petri plates are completely covered with the growth of the test pathogen. The efficacy of each treatment was figured out by calculating the percent inhibition of the radial growth by using formula proposed by Vincent (1947)

RESULTS AND DISCUSSION

This *in-vitro* study was conducted to ascertain the potential of commercially available fungicides and to determine their effective concentration against *A. brassicae.* The observations were recorded in terms of radial growth and percent growth inhibition and are presented in Table 1 and Plate 1. The results revealed that all the fungicides tested were superior to the control and significantly inhibited growth of the *A. brassicae* at all their tested concentrations. It was noted that percent radial growth inhibition of the pathogen was directly proportional to the increasing concentration of all the tested fungicides. Significant per cent inhibition was recorded in Azoxystrobin + Difenoconazole as compared to control at 7 days after incubation. Complete suppression of *A. brassicae* radial growth (100% inhibition) was recorded with Propiconazole and Hexaconazole at 50, 100, 150 and 200 ppm. However, Flusilazole and Vitavax caused 100% inhibition in the fungus colonization at 150 and 200 ppm. The inhibition was 89.48% and 94.11% due to the treatment of Flusilazole and Vitavax, respectively at 100 ppm concentration and 83.18 and 93.0%, respectively at 50 ppm. Results indicated that Metalaxyl+Mancozeb and Mancozeb at 150 and 200 ppm can control the pathogen better at initial stage of infection. However, Propiconazole and Hexaconazole can manage the pathogen completely with 100% inhibition even at 50 ppm.

Present results were supported by Valvi *et al* (2019) due to effective inhibition percent recorded in Propiconazole, Difenoconazole, Azoxystrobin and Mancozeb treatments. Rajesha *et al* (2020) identified effective 100% inhibition rate of *A. brassicae* in case of Difenoconazole and Propineb at 500 ppm and Azoxystrobin and Propiconazole at 750 ppm. The observations from investigation of Prasad *et al* (2009) and Meena *et al* (2004) regarding efficacy of combination of chemical fungicide, who observed that application of fungicide in combination is most effective to reduce the growth of *A. brassicae*. Results reported by Yadav (2003) and Godikand Pathak, (2002) on the efficacy of systemic and non-systemic fungicides against Alternaria leaf blight of mustard are in confirmation. However, radial growth obtained in present study in Metalaxyl+Mancozeb and mancozeb treatment were recorded in contrary with only 26.00 mm and 28 mm radial growth at 125 ppm in study conducted by Singh et al (2017). Study also exhibited the significant effect of Mancozeb in controlling the growth of pathogen. Effect of fungicide varies from one isolate to another isolate of pathogen; this could be the reason for slight variation in the result obtained.

CONCLUSION

It has been concluded from the present investigation that propiconazole and hexaconazole were effective in showing100% growth inhibition of *A.brassicae* at 50,100,150 and 200 ppm at 7-days

Each value is an average of 3 replicates. Value within a column followed by same letter are not significantly different at (P≤0.05) according to Tukey test.

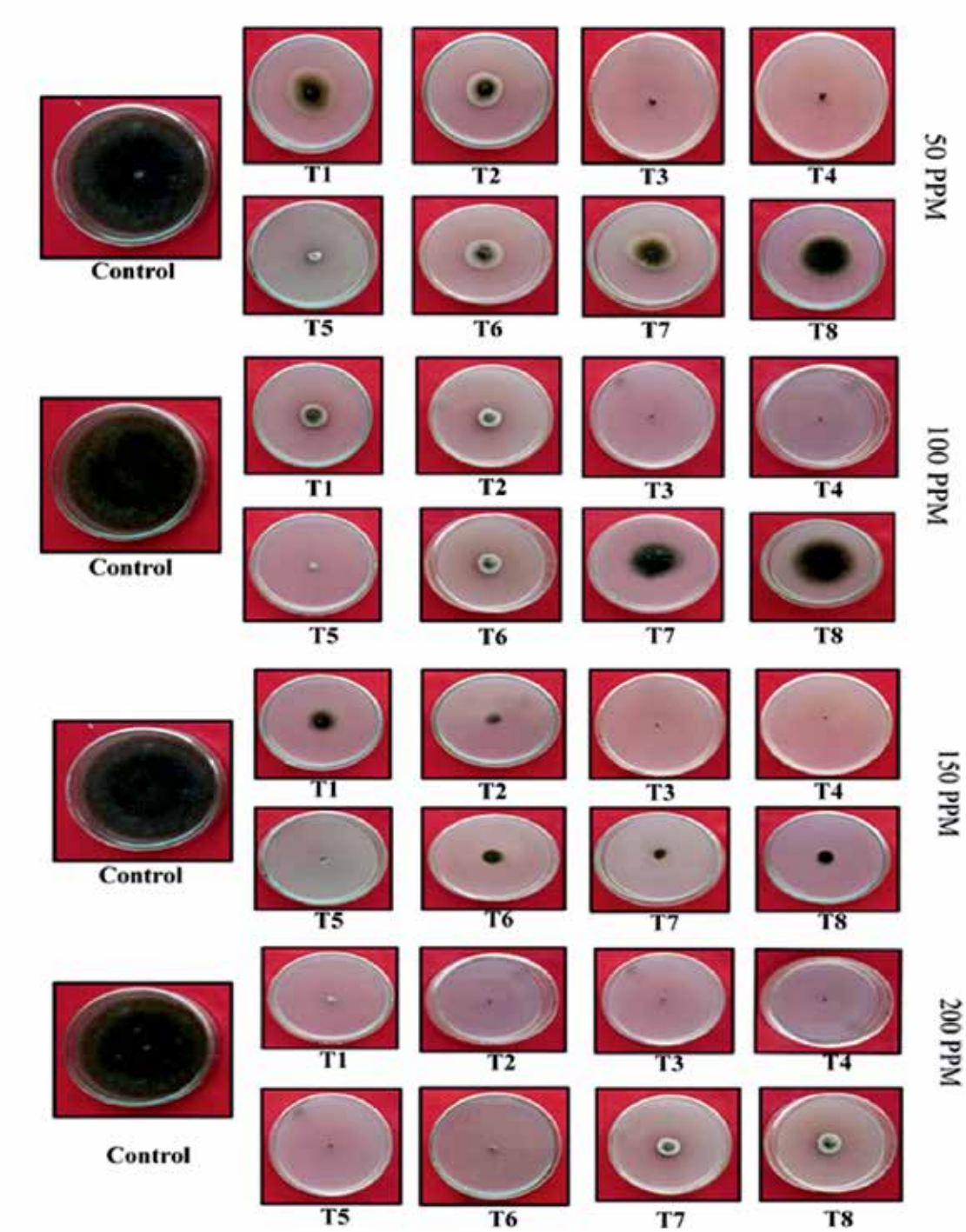

Plate 1: Mycelial growth *A. brassicae* on PDA media poisoned with fungicide

T1= Azoxystrobin + Difenoconazole, **T2=** Flusilazole, **T3=** Hexaconazole, **T4=** Mancozeb, **T5=** Metalaxyl + Mancozeb, **T6=** Propiconazole, **T7=** Propineb, **T8=** Vitavax

after inoculation. Although, vitavax and flusilazole have also exhibited 100% growth inhibition at 150 and 200 ppm at 7 days after inoculation. These fungicides need to be tested further under field conditions against *A. brassicae* in order to achieve the confirmation.

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