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Q1 ARTICLE TYPE

Evaluation of some fungicides and botanicals against leaf blight disease of mustard

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Abstract

The leaf blight disease of mustard caused by *Alternaria brassicae* is among the most destructive diseases which cause high losses in mustard production. In present study the efficacy of fungicides as well as some botanicals was evaluated *in vitro* at three concentrations *viz.*, 100, 150 and 200 ppm against mycelial growth of *Alternaria brassicae*. Among these fungicides it was observed that propiconazole 25% EC, propineb 70% WP and carbendazim 12% combined withmancozeb 63% WP was got hundred percent mycelial growth inhibition of *Alternaria brassicae in-vitro* at all three concentrations with comparison to control. The botanicals were also evaluated as crude extract in 20% concentration against *A. brassicae* under *In-vitro* conditions. Among these botanicals it was observed that *A. sativum* completely inhibited the growth of mycelium (0mm). *Azadirachta indica* and *Eucalyptus amyladin* also inhibit the growth of Alternaria blight significantly (11.33mm, 15.67mm respectively). The other plant extracts also showed blight growth inhibition significantly as compared to control which recorded maximum growth of mycelium (85.33mm) after seven days of inoculation.

Keywords: *Alternaria brassicae*, botanicals, fungicides, leaf blight, mustard.

Introduction

Present study was made with objectives to prevent the problem of leaf blight disease in significant oilseed crop *Brassica juncea* L. In this study fungicides were used in different concentrations and formulations which will provide authentic informations to farmers and applicators. In this study some medicinal plant extracts were also used as herbal fungicides, which is an ecofriendly approach to control leaf blight problem of mustard production. In India, *Brassica juncea* L. is the most important oilseed crop variety which contributes about 85% of oil seed production. India is the third largest oilseed economy after Canada and China in the world. In India mainly seven oilseed crops are grown among which rapeseed-mustard accounts nearly one-third of total oilseed production in India (Singh and Bansal, 2020) and ranks second after groundnut. In India the major production of mustard is found in Rajasthan, Madhya Pradesh, U. P., Haryana, Punjab, and Gujarat states. The oil of mustard has good nutritional value as, carbohydrates (4.50g), sugar (1.40g), dietary fiber (2.0g), fat (0.50g) and protein (2.60g) per 100g of seeds (Chowdhury *et al.* 2010). The disease incidence of fungi and attack of other pests affects the plant growth from seedling to fruit stage and yield loss. There are about thirty fungal diseases affecting crucifers as alternaria blight, white rust, sclerotinia stem rot and powdery mildew which have been reported and considered to be most devastating (Kumar *et al.* 2017). Among these diseases, Alternaria leaf blight also known as Alternaria dark spot disease which later enlarges and develops into prominent round spots with concentric rings. It is the most destructive disease of oilseed brassica crops in various continents. Alternaria leaf blight caused significant yield losses, 15 to 71 percent in production and 14 to 36 per cent also in oil content (Meena *et al*. 2010).

The Alternaria leaf blight pathogen is seedborne, soilborne and spread through air also. The incidence of leaf blight disease of mustard mainly accelerated by climatic conditions. The maximum occurrence of blight disease

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reported in moist climatic conditions and region of relatively high rainfall. The number of fungal spores, stage of host crop, period of rain and moisture on leaves influence the severity of this disease (Kumar *et al,* 2014). The chemical control of Alternaria blight requires subsequent fungicidal sprays. Its management is being done by two ways; first by crop rotation, seed treatment, field soil treatment, use of nutritional element and destruction of old crop debris. Second and most approachable way is application of chemical and herbal fungicides. The present study evaluated some significant botanicals (Onion, Garlic, Neem, Tulsi, Eucalyptus, Dhatura, Zinger, Lemon, and Turmeric) as crude extract against *A. brassicae* under *In-vitro* conditions. These medicinal plants have been established their potential against various diseases of plants, Inveiw of this these botanicals used in this study as herbal fungicides against leaf blight (*A. brassicae*) of mustard.

Materials And Methods

Survey and Collection

In the year 2022-23 mustard cropping fields were surveyed and black spot leaves, stem, pods of mustard were identified and selected randomly from study area of district Meerut (U.P.). On the basis of symptoms produced by the pathogen, the samples of *Alternaria brassicae* was collected as infected leaves of plants.

Isolation and Purification of *Alternaria Brassicae*

The collected leaf samples were washed in fresh distilled water. The leaf samples were chopped and grind to filter between sterile filter papers. The filtered extracts were aseptically put as single spot on Potato Dextrose Agar media plates and incubated at 25±2°C for 7 days. After incubation obtained mycelium were transferred to fresh PDA media slant. Purification of the isolated fungus was carried out by using hyphal tip techniques given by Dhingra and Sinclair (1985).

Identification of Alternaria Brassicae

The first report of Genus *Alternaria* was given by Nees (1816) with *A. tenuis* as the type species and only single member of the genus, which later described as *Alternaria alternata* as type species. On the basis of common characteristics of conidia length, width and septation the genus *Alternaria* could be organized into six groups and each group designated by a typical species (Elliot 1917). After that the characterization of genus was expanded into 14 species groups on the basis of characteristics of conidia and catenulation, Simmons (2007).

Pathogenicity Test

Pathogenicity of purified isolates of *Alternaria brassicae* was tested on seedlings growing in plastic pots. Three weeks seedlings were inoculated with pure cultured fungal conidia. After inoculation seedlings were kept in moist chamber for 72 hrs. The observations were noted after seven days of inoculation. After two weeks, the Alternaria blight was re-isolated from the leaves showing symptoms. The culture examined under microscope and again inoculated on PDA media plates to obtained test culture for *in-vitro* study.

Collection and Preparation of Botanical Extract

Ten plant extracts and products (Onion, Garlic, Neem, Tulsi, Eucalyptus, Dhatura, Zinger, Lemon, and Turmeric) were collected from Ch. Charan Singh University, campus, Meerut and local market. The crude extract was prepared from leaves/rhizome/seeds after washing with water. The material of selected plants was chopped, grind and filter in 100 mesh sieve and autoclave solution is ready for *in-vitro* study. The crude extract was used in ratio of 20ml solution of each botanical separately mixed with 80 ml PDA media and pinch of streptomycin sulphate (antibiotic) was mixed just before pouring. The other plates inoculated with mycelium disc of *A. brassicae* without plant extracts maintained as control. The radial growth of *A. brassicae* colony was measured from 3DAI, 5DAI and 7DAI.

Preparation and application of fungicides dose

In present *in-vitro* study, eight fungicides were selected in following formulations as; Carboxin 37.5%WP+Thiram 37.5%WP, Propineb 70%WP, Hexaconazole 5%SC, Carbendazim 50%WP, Mancozeb 63%WP, Propiconazole 25%EC, Carbendazim 12%+Mancozeb 63%WP and Tebuconazole 50%+Trifloxystrobin 25%WG and prepared in three concentrations as, 100, 150 and 200 ppm. The application of fungicides dose was done by the poisoned food techniques of Nene and Thapliyal (1979). The prepared dose of all the fungicides was mixed in 20 ml of PDA media and sterilized in autoclave. This aliquot was poured into pre-sterilized petri plates. A 7mm mycelium disc of pathogenic fungi was placed on prepared media plates. These plates were kept as upside down position to prevent from moisture contamination and maintain the growth. The other plates inoculated with mycelium disc of *A. brassicae* without fungicides maintained as control. The radial growth of *A. brassicae* colony was measured after 7DAI in all three contrations.

Results

In-vitro mycelial growth inhibition by Fungicides

In-vitro mycelial growth inhibition **o**bserved that some fungicides showed complete inhibition in low concentration and some of them check the growth in low to high concentrations. Propiconazole 25%EC, propineb 70%WP and carbendazim 12% + mancozeb 63%WP showed maximum (hundred percent) inhibition, at 100 ppm concentration, followed by hexaconazole 5%SC (84.00%), carboxin 37.5% + thiram 37.5%WP (84.26%) and tebuconazole 50% +

trifloxystrobin 25%WG (69.96%). The Least percent inhibition was observed by carbendazim 50% WP (52.48%) followed by mancozeb 75% WP (65.04%).

At 150ppm, carboxin 37.5%WP + thiram 37.7%WP, propineb 70%WP, hexaconazole 5%EC, propiconazole 25%EC and carbendazim 12% + mancozeb 63%WP showed hundred percent inhibition of mycelial growth of *A. brassicae* followed by tebuconazole 50% + trifloxystrobin 25%WG (80.78%) and least percent inhibition was recorded by carbendazim 50%WP (65.07%) followed by mancozeb 75% WP (75.30%).

At 200ppm, carboxin 37.5% + thiram 37.5%WP, propineb 70%WP, hexaconazole 5%EC, propiconazole 25%EC and carbendazim 12% + mancozeb 63%WP, tebuconazole 50% + trifloxystrobin 25%WG and mancozeb 75%WP showed complete inhibition(100%) of mycelial growth of *A. brassicae.* Least percent inhibition was recorded by carbendazim 50%WP (72.96%) (Table 1 & Fig. 1).

In-vitro mycelial growth inhibition by Botanicals

The observations on *in-vitro* mycelial growth inhibition of *A. brassicae* by ten botanicals at three time intervals as 3 DAI, 5 DAI and 7 DAI showed that all the botanicals were significantly superior in comparison to control. *A. sativum* completely control the mycelium growth (0mm) at all the time 3-7 DAI followed by *Azadirachta indica* 0.00, 7.75 and 11.40mm and *Lantana Camera* 0.00, 11.30 and 21.70mm at 3, 5, 7 DAI respectively. *Eucalyptus amyladin* 8.30, 9.70, 15.70mm at 3,5,7 DAI respectively, *Ocimum sanctum* (13.70, 12.30, 20.50mm) and followed by *Datura stramonium*, , *Zingiber officinale*, *Allium cepa*, *Curcuma longa* and with least *Citrus aurantifolia* (12.30, 29.30, 61.70mm at 3,5,7 DAI respectively), in comparison to maximum growth (85.30mm) recorded in control (Table 2 & Fig. 2).

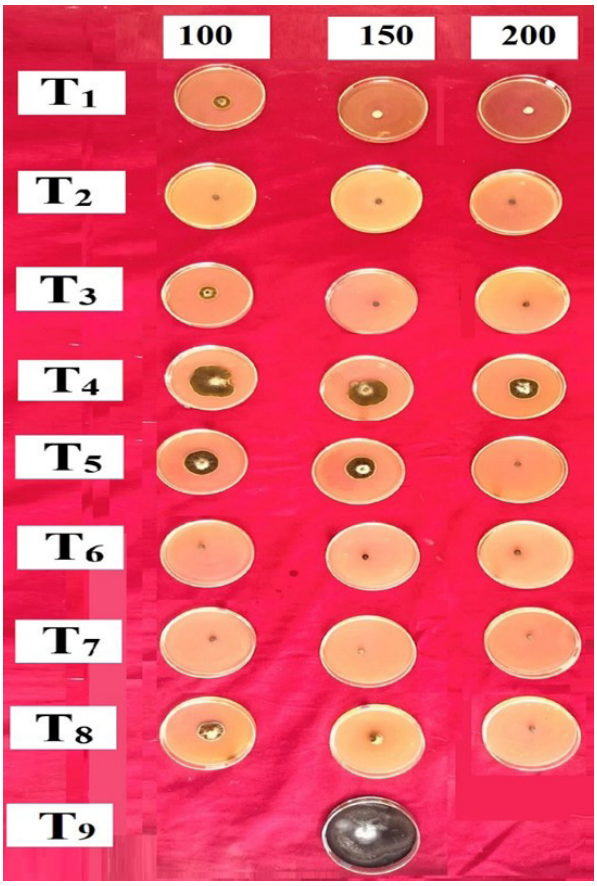
Discussion

The observations of this study revealed that Propineb 70%WP, Propiconazole 25%EC and Carbendazim 12% + Mancozeb 63%WP completely inhibited the mycelial growth of the pathogen followed by hexaconazole 5%SC, carboxin 37.5% + thiram 37.5%WP and tebuconazole 50% + trifloxystrobin 25%WG. These results are in conformity with the findings of Kiran *et al.* (2018). Pun *et al.* (2020) reported that carbendazim + mancozeb (Saaf) recorded 100 per cent

Table 1: *In-vitro* effects of different fungicides formulations in three concentrations on percent mycelial growth inhibition of fungus (*A. brassicae*)

Treatments	*Formulations*	*Percent mycelial growth inhibition*			
		100 ppm	*150 ppm*	*200 ppm*	*Mean*
T_1	Carboxin 37.5% + Thiram 37.5% WP	84.26 (66.66)	94.44 (76.41)	98.15 (82.27)	92.28 (75.11)
T_2	Propineb 70% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_3	Hexaconazole 5% SC	84.00 (66.45)	93.89 (75.70)	97.00 (80.03)	91.63 (74.06)
T_4	Carbendazim 50% WP	52.48 (46.42)	65.07 (53.77)	72.96 (58.68)	63.51 (52.96)
T_5	Mancozeb 63% WP	65.04 (53.75)	75.30 (60.20)	100.00 (90.00)	80.11 (67.98)
T_6	Propiconazole 25% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_7	Carbendazim 12% + Mancozeb 63% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_8	Tebuconazole 50%+ Tri-floxystrobin 25%WG	69.96 (56.77)	80.78 (64.00)	93.70 (75.48)	81.48 (65.42)
T_9	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Concentrations Mean		72.86 (62.23)	78.83 (66.68)	84.65 (72.94)	78.78 (67.28)
		S Em±	CD at 5%	CV (%)	
Fungicides		0.29 (0.24)	0.82 (0.69)		
Concentrations		0.15 (0.13)	0.43 (0.36)		
F X C		0.56 (0.45)	1.58 (1.27)	1.22 (1.16)	

*Average of three replications, **Figures in parentheses are Arcsine transformed values.

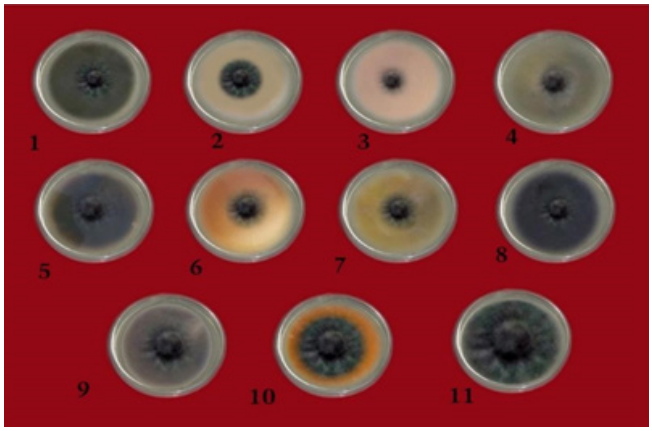


(Here, $T_{1=}$ Carboxin37.5%WP+Thiram 37.5%WP, $T_{2=}$ Propineb 70%WP, $T_{3=}$ Hexaconazole 5% SC, $T_{4=}$ Carbendazim 50% WP, $T_{5=}$ Mancozeb 63% WP, $T_{6=}$ Propiconazole 25% EC, $T_{7=}$ Carbendazim 12% WP + Mancozeb 63% WP, $T_{8=}$Tebuconazole 50% +Trifloxystrobin 25% WG, $T_{9=}$Control)

Fig. 1: *In vitro* evaluation of fungicides against *Alternaria brassicae* mycelial growth inhibition at different dose concentrations.

Table 2: *In-vitro* mycelial growth inhibition of *Alternaria brassicae* by different botanicals at different time intervals.

Botanicals	*Mycelial growth (mm)*		
	3 DAI	*5 DAI*	*7 DAI*
Allium sativum	0.00	0.00	0.00
Allium cepa	8.40	16.40	41.70
Lantana camera	0.00	11.30	21.70
Datura stramonium	7.30	13.30	20.40
Azadirachta indica	0.00	7.75	11.40
Citrus aurantifolia	12.30	29.30	61.70
Zingiberofficinale	8.30	13.70	30.40
Curcuma longa	15.00	27.30	51.70
Eucalyptus amyladin	8.30	9.70	15.70
Ocimum sanctum	13.70	12.30	20.50
Control	30.30	50.30	85.30
CV			
CD 5%	0.11	0.20	0.40



(Here, **1=** *Azadirachta indica,* **2=** *Allium cepa,* **3=** *Allium sativum,* **4=** *Lantana camera,* **5=** *Datura stramonium,* **6=** *Zingiberofficinale,* **7=** *Citrus aurantifolia,* **8=** *Ocimum sanctum,* **9=** *Eucalyptus amyladin,* **10=** *Curcuma longa,* **11=** Control)

Fig. 2: *In vitro* evaluation of botanical against *Alternaria brassicae* mycelial growth after seven days of inoculation

growth inhibition whereas; 50 ppm carbendazim was least effective in reducing fungal growth. Gusain and Ravi (2021) observed that propiconazole 25% EC and hexaconazole 5%SC inhibited maximum mycelium growth at (100, 250, 500, 750 and 1000 ppm) under *in vitro* study. Rajvanshi *et al*. (2020) reported that minimum growth of pathogen was recorded in iprodione 25% + carbendazim 25% followed by propineb 70% WP. Singh *et al.* (2022) reported that the percent mycelial growth inhibition by mancozeb alone and mancozeb+carbendazim found same (0.15, 0.20 and 0.25 percent) and mancozeb+azoxystrobin formulation caused (0.25, 0.05 and 0.1 percent). Propineb 70%WP also significantly reduced the mycelial growth of *Alternaria brassicae* at all three concentrations (100, 150, 200ppm) of fungicides. Jackson and Kumar (2019) reported that azoxystrobin and propiconazole were found to be the most efficient fungicide to inhibit mycelium growth of *Alternaria* sp. However, the formulation of tebuconazole + trifloxystrobin also found effective (93.70%) at 200ppm followed by hexaconazole, agreed with study of Yadav (2016). The efficacy of some botanicals against Alternaria blight of mustard also observed in earlier time by Patni *et al.* (2005) and Patni and Kolte (2006). Antifungal activity of plant extracts to inhibit mycelial growth of leaf blight of mustard *in-vitro* evaluated by Meena and Sharma (2012).

Conclusion

In present *In-vitro* study the three fungicides propiconazole 25% EC, propineb 70% WP and carbendazim 12% combined with mancozeb 63% WP were found highly efficacious against leaf blight fungus (*Alternaria brassicae*) caused hundred percent mycelial growth inhibition at the minimum dose 100 ppm in comparison to highest dose 200 ppm of other fungicides. The botanicals were also caused significant

management of *Alternaria brassicae*. *A. sativum* checked hundred percent mycelium growth followed by *Azadirachta indica*, *Lantana camera, Eucalyptus amyladin* and *Ocimum sanctum*.

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