In vitro evaluation of fungicides and plant derived extracts on mycelial growth of *Alternaria brassicae*

ABSTRACT

An experiment was conducted at the laboratory of the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh. To test the efficacy of different treatments *viz*., Carbendazim @ 0.1%, Hexaconazole @ 0.05%, Carbendazim + Mancozeb @ 0.2%, Mancozeb @ 0.2%, Chilli (*Capsicum annum*) and Alstonia (*Alstonia scholaris*) @ 10% against *Alternaria brassice* under *in vitro* condition. Fungicides and botanicals were tested through the poisoned food technique at 10 % concentrations and 168 hours of incubation. The Minimum radial growth was observed in Carbendazim + Mancozeb (0 mm) followed by Hexaconazole (5.28mm), Mancozeb (6.25mm), Carbendazim (14.25mm), Chilli leaf extract (69.12mm) and Alstonia leaf extract (84.23mm) as compared to Control (90mm) untreated check.

Keywords*: Alternaria brassicae,* botanicals, fungicides, *in vitro*.

INTRODUCTION

Indian mustard (*Brassica juncea*) is a perennial herb of the Brassicaceae family and is the third most important oilseed crop in the world in terms of production and productivity (**Kumar *et al*., 2014**). It is grown both in tropical and subtropical regions of the world. India holds the first position in area and second position in production after China. The Assam, Bihar, Rajasthan, Haryana, Uttar Pradesh, Orissa, Punjab and West Bengal are majorly growing of mustard in India **(Sharma *et al*., 2020)**. It is a source of edible oil, which cannot be replaced. It is convenient as monoculture because one crop is easier to plant, harvest and market than mixture of other crop with low water requirement (**Jha *et al*., 2013**). In India, alternaria blight of mustard caused by *Alternaria brassicae* and *Alternaria brassicicola* is most widespread and destructive disease of rapeseed-mustard causing major yield losses that may range from 15 to 71 per cent in productivity and 14 to 36 per cent in oil content **(Singh *et al*., 2022)**. Among the diseases of mustard, alternaria blight, caused by *Alternaria brassicae* (Berk) Sacc. is found to be distributed worldwide and **Berkely (1836)** noticed the fungal infection on plant belonging to the family Brassicaceae and identified as *Macrosporium brassicae* Berk. later renamed as *A. brassicae* (Berk.) Sacc. by **Saccardo (1886)**. Symptoms of the disease are characterized by the formation of spots on leaves, stem and siliqua (**Prasad and Lallu, 2006 and Nayyar *et al*., 2014**). Infection of seeds by the pathogen may result in poor germination and can also deteriorate both quality and quantity of oil content (**Kolte, 1985 and Gagandeep *et al*., 2020**). Foliar infection by the pathogen can be identified by greyish centre brown to black spots with concentric rings with difference in the symptoms according to the host and environmental conditions.

Given the significance of the disease, this study aimed to control it using a range of cost-effective and commercially available chemical fungicides and botanicals. Therefore, an attempt was made to assess the effectiveness of these fungicides and botanicals against Alternaria blight of mustard *in vitro*.

MATERIALS METHODS

The present investigations were carried out in the laboratory, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh (Year 2022-23) to test the efficacy of different treatments with *Alternaria brassice* under *in vitro* conditions. To find out the efficacy of various fungicides and plants extracts *viz.*, Carbendazim, Hexaconazole, Carbendazim + Mancozeb, Mancozeb, Chilli (*Capsicum annum*) and Alstonia (*Alstonia scholaris*) leaf extract against *Alternaria brassicae* were used.

Isolation of pathogen

The leaf spots and lesions, displaying the initial and prominent characteristic symptoms of alternaria blight, were chosen for the isolation of the pathogen. These selected infected spots underwent thorough washing 3-4 times in sterilized distilled water and subsequent surface sterilization by immersion in a 1% NaOCl solution for 1 minute, followed by further rinsing with sterilized water 3-4 times. Excess moisture was eliminated by placing these pieces between two folds of sterilized blotting paper under aseptic conditions within the inoculation chamber. The surface-sterilized leaf spot pieces were then aseptically transferred into 9 cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25±2°C for 7 days. After 3 days, mycelial growth was observed around the leaf bits. From this colony growth, a portion from the periphery specifically, a single hyphal tip was isolated and transferred to another medium. The culture of *Alternaria brassicae* was purified using the hyphal tip method and maintained through periodic sub-culturing on PDA Petri plates and slants.

Plate- 1. Isolation of pathogen

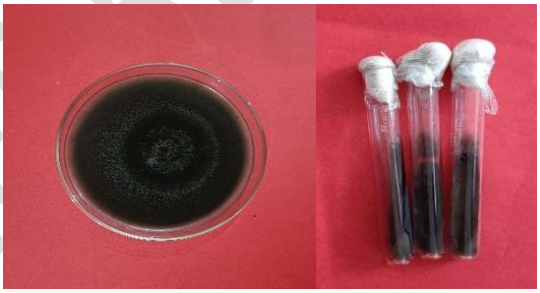

Plate- 2. Pure culture of *Alternaria brassicae*

Preparation of aqueous extract of plant

The botanical extract was prepared following the standard procedure outlined by **Mahapatra and Das (2013).** Mature leaves were gathered and sterilized with distilled water. Subsequently, the leaves were homogenized in a pre-chilled pestle and mortar using chilled, sterilized distilled water. An aqueous extract of this botanical (1:1 w/v) was then prepared by combining 100 grams of fresh leaves with 100 ml of sterile distilled water and crushing in a blending device. The resulting extract was filtered by double layers of moistened muslin cloth followed by sterilized Whatman No.1 filter paper **(kumar *et al.*, 2023)**. The filtrate obtained was deemed as 100% plant extract. Aqueous extracts (10%) were prepared according to the treatment and mixed with 90 ml of PDA each, respectively, in separate 250 ml conical flasks. The media in the conical flasks were sterilized in an autoclave at a temperature of 121°C for 20 minutes. Following autoclaving, approximately 20 ml of the medium was dispensed into each 90 ml sterilized Petri dish.

In vitro* evaluation of fungicides and plant extracts against *A. brassicae

Carbendazim, Hexaconazole, Carbendazim + Mancozeb and Mancozeb @ 0.2% were dissolved in 100 ml of sterilized melted PDA prior to inoculation of *Alternaria brassicae*. PDA plates without chemical but inoculated with *Alternaria brassica* served as control. Three replications were maintained for all the treatments and plates were incubated in BOD incubator at a temperature of 25±1°C. The colony diameter of the fungus was measured on 7th day of incubation and compared with the colony growth of the fungus in control.

Twenty ml of sterilized molten PDA were carefully poured into sterilized Petri dishes and left to solidify. Upon solidification of PDA, all the treatments and control plates were aseptically inoculated by placing a 5 mm mycelial disc in the centre obtained from a week old actively growing pure culture of *A. brassicae* were placed in the centre of the Petri plates and one control plate which has only the PDA medium inoculated with culture disc and used as control. Each treatment and control were repeated three times to make three replications. Replicates were maintained for each test and those plates were incubated at 25 ± 1^0 C at incubator. The Plates were incubated for 168 hours and colony diameters were recorded.

The radial growth of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony. The experiment was conducted in completely randomized design (CRD) with three replications in each treatment given by **Gomez and Gomez, 1984**.

The inhibition percentage was calculated using the formula:

$$I = \frac{C-T}{C} \times 100$$

Where,

I = Percent inhibition
C = Growth (mm) of test fungus in untreated control plates (average of both diagonals),
T = Growth (mm) of test fungus in treated plates (average of both diagonals).

RESULTS AND DISCUSSION

In vitro evaluation of fungicides and plant extracts on *Alternaria brassicae*

Fungicides and Plant extracts were evaluated on the growth of pathogens by poison food technique. The results of the effect of different treatments *i.e.*, fungicides and plant extracts on mycelial growth and percent inhibition of *Alternaria brassicae* are presented in Table- 1, Figure- 1 and Plate -3.

Radial growth of *Alternaria brassicae* after 168 hrs. of incubation

The data presented in table-1, depicted in figure-1 and Plate-1represents that the radial growth was found minimum in (T_3)- Carbendazim + Mancozeb (0mm), followed by (T_2)- Hexaconazole (5.28mm), T_1 - Carbendazim @ 0.1% (14.25mm), T_5 - Chilli leaf extract (69.12mm), T_6 - Alstonia leaf extract (84.23mm) as compared to treated check (T_4)-Mancozeb (6.25mm) and (T_0)- control (90mm) untreated check. Statistically, all the treatments were significant over Control.

Percent inhibition of *Alternaria brassicae* after 168 hrs of incubation:

The data presented in table-1, depicted in figure-1 and Plate-1 represents that the percent inhibition was found maximum in (T_3) – Carbendazim + Mancozeb @ 0.2% (100%), followed by (T_2)- Hexaconazole @ 0.05% (94.13%), (T_1)- Carbendazim (84.16%), (T_5)- Chilli leaf extract @ 10% (13.2%), (T_6)- Alstonia leaf extract @ 10% (6.66%) as compared to treated check (T_4)- Mancozeb @ 0.2%, (93.05%) and (T_0)-Control (0%) untreated check. Statistically, all the treatments were significant over Control.

Similar findings were reported by **Meena *et al.* (2022)** reported that Carbendazim + Mancozeb was found most effective in inhibition of mycelial growth in *in vitro*. **Meena *et al.* (2022)** among the leaf extract evaluated Alstonia inhibited the mycelial growth of fungus (62.30) at 10% concentration, among the fungicide Carbendazim + Mancozeb was found significantly superior at 100 and 150 ppm with cent per cent inhibition of mycelial growth. This work draws support from the findings of **Choudhary *et al*. (2020)** reported that hexaconazole was effective in inhibiting mycelial growth up to (95.56%) and Alstonia (42.41%) was found least effecting in inhibiting mycelial growth. **Yadav *et al*. (2019)** evaluated extracts of 54 plants against *Alternaria brassicae* among which mycelial inhibition by chilli *in vitro* at 10% concentration was found to be 56.5%.

Table 1.

S. No.	Treatments	Radial growth(mm)	Per cent inhibition (%)
T_0	Control (untreated check)	90	0
T_1	Carbendazim	14.25	84.16
T_2	Hexaconazole	5.28	94.13
T_3	Carbendazim + Mancozeb	0	100
T_4	Mancozeb (treated check)	6.25	93.05
T_5	Chilli leaf extract	69.12	13.2
T_6	Alstonia leaf extract	84.23	6.66
	S.Em(±)	0.273	
	C.D. (5%)	0.828	

***Average of three replications**

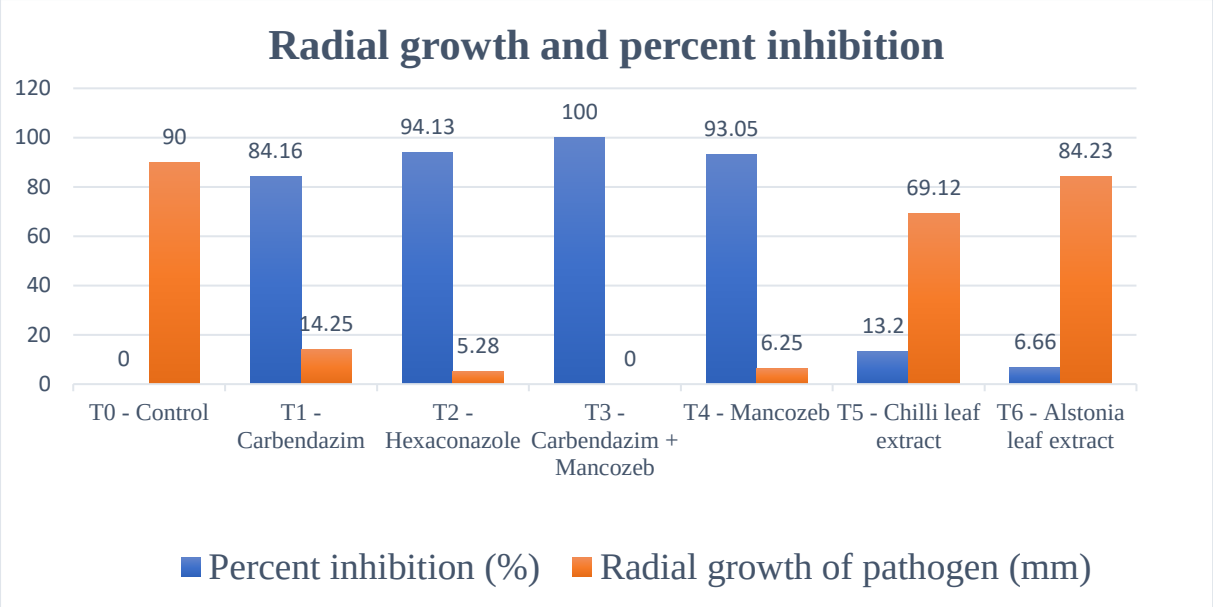

Fig. 1. Effect of fungicides and plant extracts on *Alternaria brassicae* on percent inhibition at 168 hours

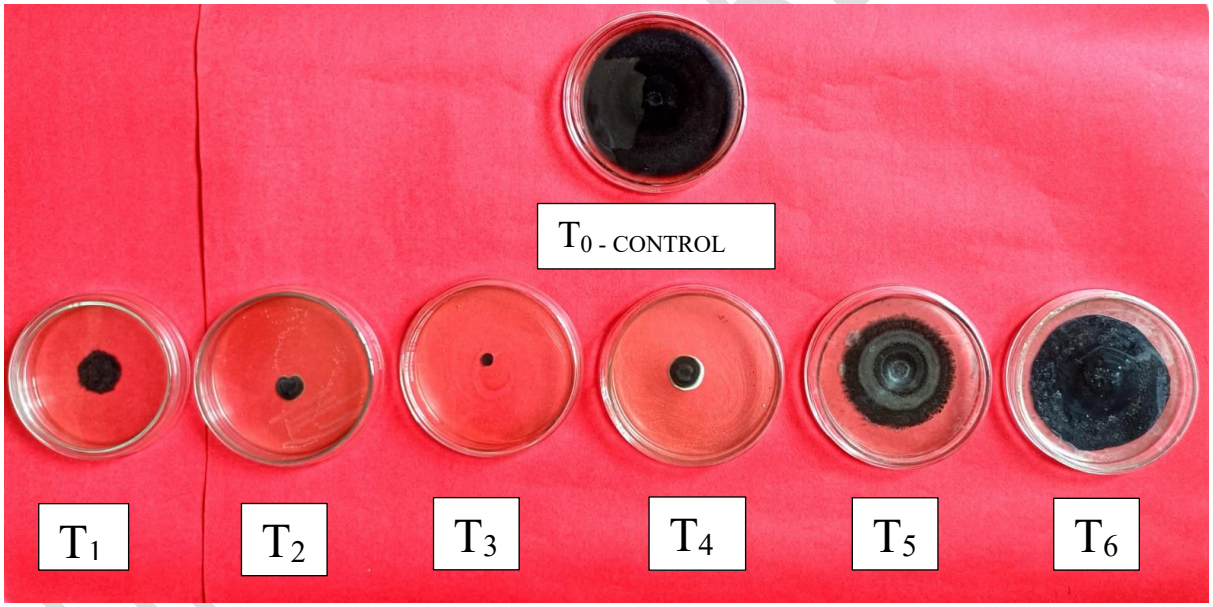

Plate-3. Response of fungicides and plant extracts against *Alternaria brassicae* on mycelial growth

CONCLUSION

Under *in vitro* conditions, the fungicides and plant extracts were assessed using the poison food technique. In this study, Carbendazim + Mancozeb exhibited the highest level of radial growth

inhibition compared to the control. Therefore, the in vitro evaluation indicates that Carbendazim + Mancozeb is the most effective fungicidal treatment against the mycelial growth of *Alternaria brassicae* among the tested substances. Based on these findings, it is recommended to use Carbendazim + Mancozeb to manage Alternaria blight of mustard disease.

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