

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

ABSTRACT

Mustard (*Brassica juncea*) is one among the major oil seed crops in India grown during Rabi season. *Alternaria* blight of mustard caused by *Alternaria brassicae* 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. An experiment was conducted at the laboratory of the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India. 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 brassicae* 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. Mancozeb + carbendazim were found most effective in inhibition of mycelial growth in *in vitro*. This study aimed to control it using a range of cost-effective and commercially available chemical fungicides. Therefore, an attempt was made to assess the effectiveness of these fungicides against *Alternaria* blight in mustard *in vitro*.

Keywords: *Alternaria* blight, *Alternaria brassicae*, botanicals, fungicides, *in vitro*, mustard

1. INTRODUCTION

Indian mustard (*Brassica juncea*) is a perennial herb within the Brassicaceae family, globally ranking third in oilseed crop importance for both production and productivity [6]. Cultivated across tropical and subtropical regions worldwide, India leads in mustard cultivation area and stands second in production, following China. Key mustard-growing states in India encompass Assam, Bihar, Rajasthan, Haryana, Uttar Pradesh, Odisha, Punjab, and West Bengal [13]. Given its extensive cultivation, mustard serves as an essential source of edible oil. Its preference for monoculture arises from its straightforward planting, harvesting, and marketing procedures compared to mixed cropping systems, coupled with its minimal water requirements [4].

In India, *Alternaria* blight of mustard, attributed to *Alternaria brassicae* and *Alternaria brassicicola*, stands out as the most prevalent and damaging disease affecting rapeseed-mustard crops, leading to significant yield reductions ranging from 15 to 71 percent in productivity and 14 to 36 percent in oil content [15]. Among the diseases affecting mustard, *Alternaria* blight, caused by *Alternaria brassicae* (Berk.) Sacc., has a global distribution. Berkeley first observed the fungal infection on plants within the Brassicaceae family in 1836, initially identifying it as *Macrosporium brassicae* Berk. Later, [12,18,19,20] renamed it as *A. brassicae* (Berk.) Sacc. in 1886.

Symptoms of the disease manifest as the formation of spots on leaves, stems, and siliqua [11, 10]. When seeds are infected by the pathogen, it often results in poor germination and a decline in both the quality and quantity of oil content [5, 3]. Foliar infection by the pathogen is identifiable by greyish-centered brown to black spots with concentric rings, with variations in symptoms observed 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* in mustard *in vitro*.

2. 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, India (Year 2022-23) to test the efficacy of different treatments with *Alternaria brassicae* 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.

Preparation of aqueous extract of plant

The fresh plant leaves were washed in distilled water and were separately homogenized with sterile distilled water at 1:1 w/v, (100g fresh leaves/seed with 100 ml of sterile distilled water) in a pestle and mortar separately of each plant and seed and filtered through a muslin cloth followed by sterilized Whatman No.1 Filter paper [7]. This formed 100 percent plant extract solution. The plant extracts so prepared were heated at 40°C for 10 minutes to avoid contamination. Aqueous extract of 10 % was prepared according to the treatment by mixing 10 ml of aqueous extract with 90 ml PDA respectively in separate conical flask. The media in conical flask were sterilized in an autoclave at temperature of 121°C for 20 minutes. The botanicals were evaluated *in vitro* through poison food technique [10]. The 20 ml sterilized media with botanical extract was poured into 90mm Petriplates under aseptic conditions in laminar air flow. After solidification of media 5mm disc of 7 days old subculture of *Alternaria brassicae* were placed in the centre of the Petriplates and one control plate which has only the PDA medium inoculated with culture disc and used as check. Three replicates were maintained for each test and those plates were incubated at 27±1 °C in incubator. The radial growth of mycelium was measured at 168 hrs. The radial growth of mycelium of each plate was measured by taking average of the two diameters taken at right angles for each colony.

Preparation of fungicidal treatments

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 brassicae* 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.

Percent inhibition in growth was calculated in relation to growth in control using the formula of Vincent [16]. The experiment was conducted in completely randomized block design (CRD) with three replications in each treatment. The variance ratio test at the 5% level of probability was used to determine the significance of treatment differences.

Mycelial inhibition = (Radial growth in control – Radial growth in treatment) / (Radial growth in control) × 100

3. RESULTS

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

Fungicides and plant extracts were assessed for their impact on pathogen growth using the poison food technique. The outcomes regarding the influence of various treatments, including fungicides and plant extracts, on the mycelial growth and percent inhibition of *Alternaria brassicae* are outlined in Table-1, Figure-1 and Plate-1

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

The data presented in table-1, depicted in figure-1 and Plate-1 represents that the radial growth was found minimum in (T₃)- Carbendazim + Mancozeb (0mm), followed by (T₂)- Hexaconazole (5.28mm), T₁ - Carbendazim @ 0.1% (14.25mm), T₅ - Chilli leaf extract (69.12mm), T₆ - Alstonia leaf extract (84.23mm) as compared to treated check (T₄)-Mancozeb (6.25mm) and (T₀)- 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 (T₃) – Carbendazim + Mancozeb @ 0.2% (100%), followed by (T₂)- Hexaconazole @ 0.05% (94.13%), (T₁)- Carbendazim (84.16%), (T₅)- Chilli leaf extract @ 10% (13.2%), (T₆)- Alstonia leaf extract @ 10% (6.66%) as compared to treated check (T₄)- Mancozeb @ 0.2%, (93.05%) and (T₀)-Control (0%) untreated check. Statistically, all the treatments were significant over Control.

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

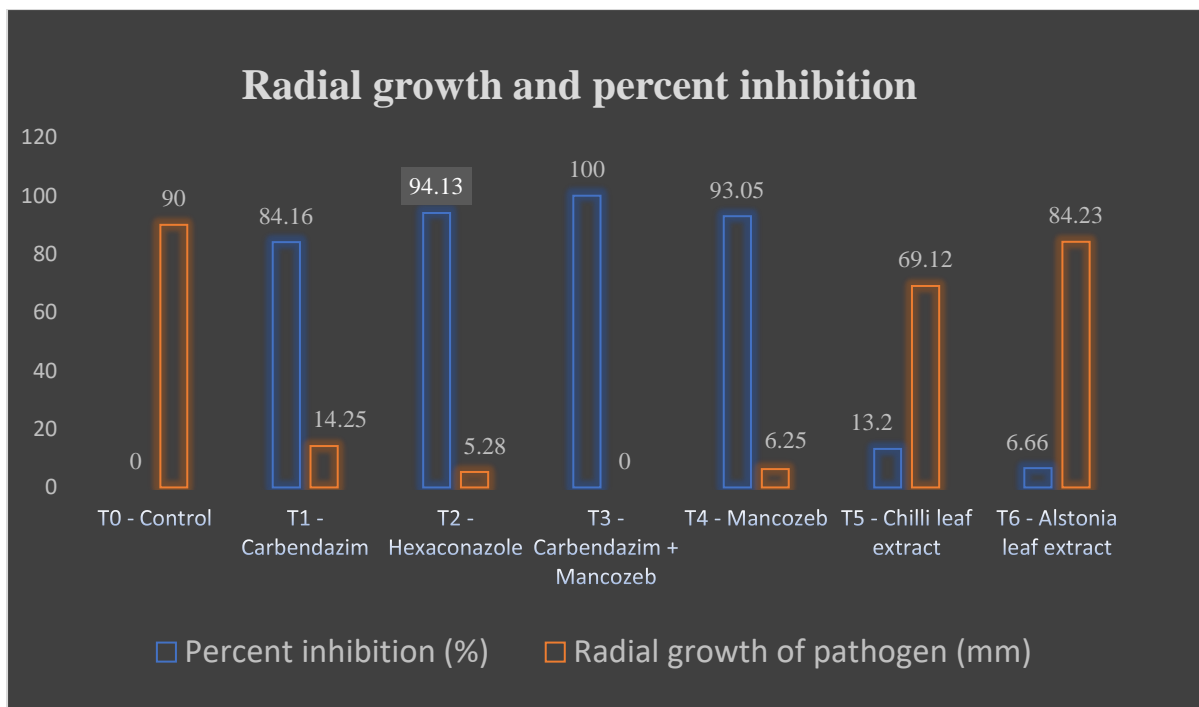


Fig. 1. Effect of fungicides and plant extracts on *Alternaria brassicae* on percent inhibition at 168 hours

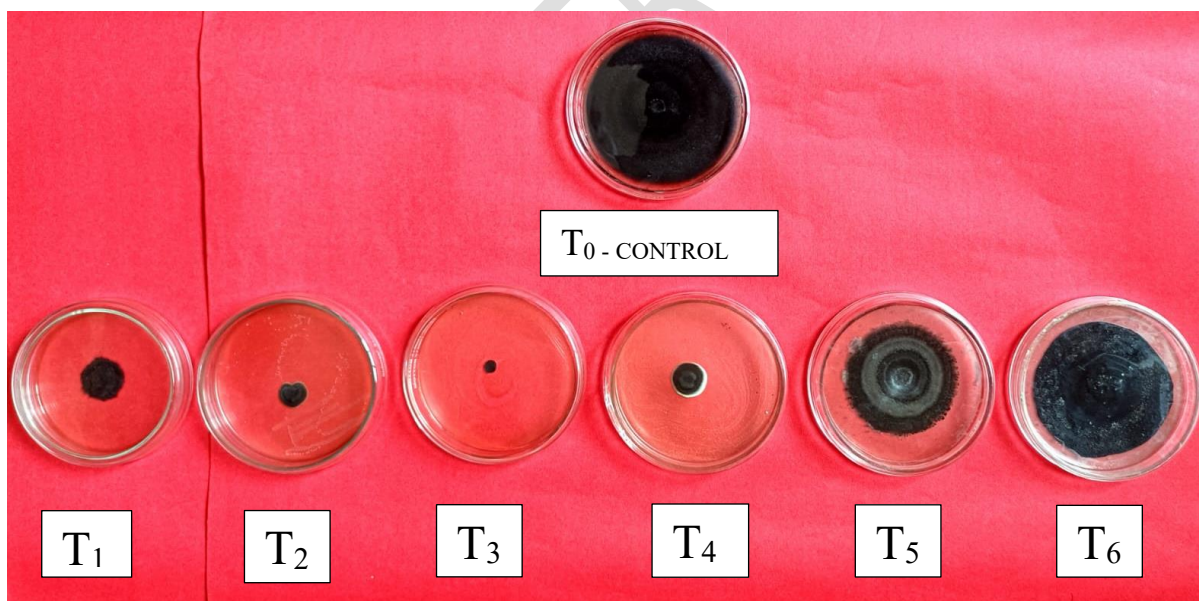


Plate-1. Response of fungicides and plant extracts against *Alternaria brassicae* on mycelial growth

4. DISCUSSION:

Four fungicides and two plant extracts of namely Carbendazim, Hexaconazol, Carbendazim + Mancozeb, Mancozeb, Chilli (*Capsicum annum*) and Alstonia (*Alstonia scholaris*) were tested at 10 per cent concentrations and significantly inhibited mycelial growth of *Alternaria brassicae* in *in vitro*. In laboratory, out of four fungicides and two plant extract tested by

Poisoned Food Technique, carbendazim + mancozeb was found highly effective followed by Hexaconazole, Mancozeb, Carbendazim, Chilli leaf extract and Alstonia leaf extract in inhibiting mycelial growth of *Alternaria brassicae*. The importance of chemicals cannot be denied in disease management.

Similar findings were reported by [8], who found that the combination of Carbendazim + Mancozeb was most effective in inhibiting mycelial growth *in vitro*. Among the fungicides tested, Carbendazim + Mancozeb was significantly superior, achieving 100% inhibition of mycelial growth at concentrations of 100 and 150 ppm. These results are supported by the work of [2] who reported that hexaconazole was highly effective in inhibiting mycelial growth, achieving a 95.56% inhibition rate. [14] evaluated that mancozeb was found to be the best fungicide in reducing the radial growth and germination of conidia in laboratory. Furthermore, [17] evaluated extracts from 54 plants against *Alternaria brassicae* and found that chilli extract inhibited mycelial growth by 56.5% at a 10% concentration. [8] also evaluated various leaf extracts and discovered that Alstonia inhibited the mycelial growth of the fungus by 62.30% at a 10% concentration.

5. CONCLUSION

The research conducted herein presents a comprehensive evaluation of various fungicidal treatments and plant extracts against the mycelial growth of *Alternaria brassicae*, a notorious pathogen responsible for causing Alternaria blight of mustard, a significant threat to crop yields worldwide. Through meticulous experimentation utilizing the poison food technique under controlled *in vitro* conditions, the efficacy of different treatments was systematically assessed. Notably, among the tested treatments, Carbendazim + Mancozeb emerged as the most promising candidate, exhibiting the highest level of radial growth inhibition compared to the control group. This compelling finding underscores the potent fungicidal activity of Carbendazim + Mancozeb against *Alternaria brassicae*, thereby positioning it as a superior option for the management and mitigation of Alternaria blight in mustard crops. The robustness of these results, supported by rigorous experimental methodology and statistical analysis, lends credence to the recommendation of Carbendazim + Mancozeb as a preferred strategy for combating the devastating impact of *Alternaria brassicae* on agricultural productivity.

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