

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 annuum*) 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.

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

Commented [DHN1]: Very brief. Rewrite it, mentioning the reasons for undertaking the study, the goal, and some simple details about the work methods and results

Commented [DHN2]: Very brief, rewrite

Commented [DHN3]: 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 the second position in production after China. Assam, Bihar, Rajasthan, Haryana, Uttar Pradesh, Orissa, Punjab, and West Bengal are majorly growing 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 a mixture of other crops with low water requirements (Jha *et al.*, 2013). In India, alternaria blight of mustard caused by *Alternaria brassicae* and *Alternaria brassicicola* is the most widespread and destructive disease of rapeseed-mustard, causing major yield losses that may range from 15 to 71 percent in productivity and 14 to 36 percent 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 a plant belonging to the family Brassicaceae and identified it as *Macrosporium brassicae* Berk., later renamed *A. brassicae* (Berk.) Sacc. by Saccardo (1886). Symptoms of the disease are characterised by the formation of spots on leaves, stems, and siliqua (Prasad and Lallu, 2006; Nayyar *et al.*, 2014). Infection of seeds by the pathogen may result in poor germination and can also deteriorate both the quality and quantity of oil content (Kolte, 1985; Gagandeep *et al.*, 2020). Foliar infection by the pathogen can be identified by greyish-centre brown to black spots with concentric rings, with differences in symptoms according to the host and environmental conditions.

Commented [DHN4]: on

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 brassicae* under *in vitro* conditions. To find out the efficacy of various fungicides and plants extracts viz., Carbendazim, Hexaconazole, Carbendazim + Mancozeb, Mancozeb, Chilli (*Capsicum annuum*) 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 $25\pm 2^{\circ}\text{C}$ 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*

Commented [DHN5]: State the country

Commented [DHN6]: Delete

Commented [DHN7]: 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 sterilised distilled water and subsequent surface sterilisation by immersion in a 1% NaOCl solution for 1 minute, followed by further rinsing with sterilised water 3-4 times. Excess moisture was eliminated by placing these pieces between two folds of sterilised blotting paper under aseptic conditions within the inoculation chamber. The surface-sterilised leaf spot pieces were then aseptically transferred into 9-cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at $25\pm 2^{\circ}\text{C}$ 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.

Commented [DHN8]: All work methods must have references

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.

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°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-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.

Commented [DHN9]: 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±10 C at incubator. The Plates were incubated for 168 hours and colony diameters were recorded.

Commented [DHN10]: 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.

Commented [DHN11]: You should discuss each section separately
Because the title was Results and Discussion (integrated, not separated).

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.

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%.

Commented [DHN12]: 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.E_m(±)	0.273	
	C.D. (5%)	0.828	

*Average of three replications

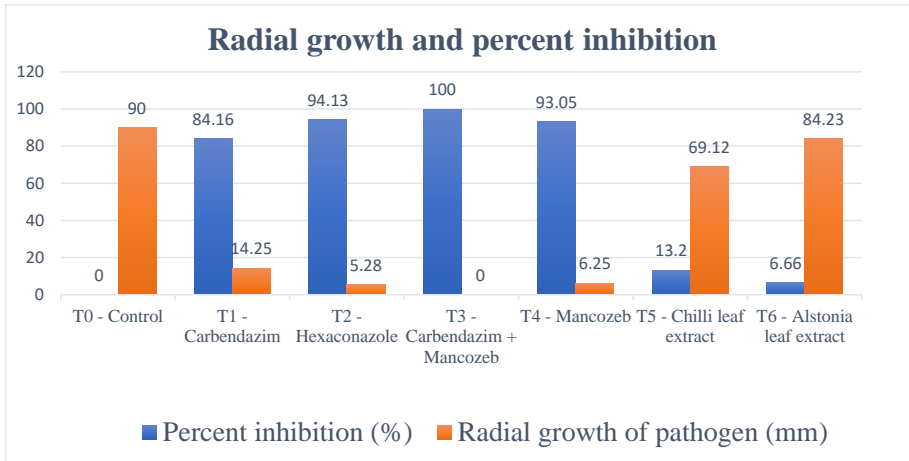


Fig. 1. Effect of fungicides and plant extracts on *Alternaria brassicae* on percent inhibition at 168 hours

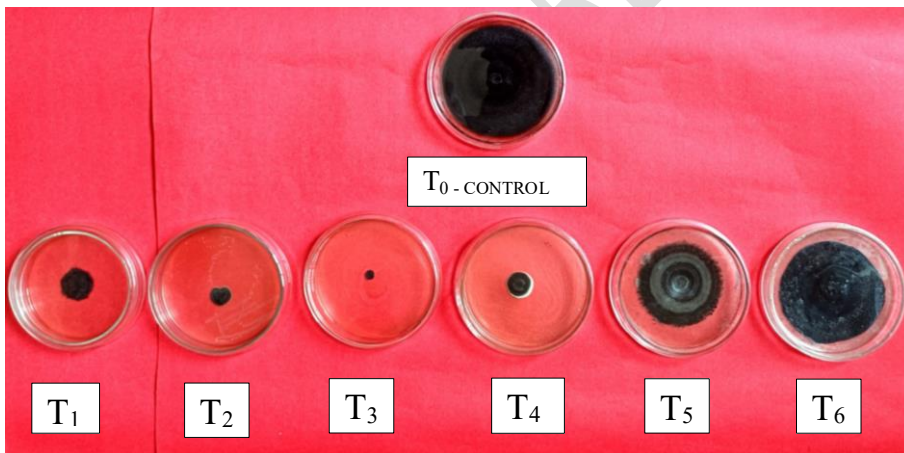


Plate-3. Response of fungicides and plant extracts against *Alternaria brassicae* on mycelial growth

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

Commented [DHN13]: Rewrite

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.

REFERENCES

- Berkeley, M. J. (1836).** *Macrosporium brassicae* spp. *Smith England Flora*. 5: 339.
- Chouhdhary, S., Ghasolia, R. P., Shivran, M., Yadav, R. and Bairwa, V. (2020).** Management of *Alternaria* Leaf Spot of Lehsua through Plant Extracts and Fungicides. *International Journal of Current Microbiology and Applied Science*. 9(2): 2573-2580.
- Gagandeep, S. S., Amanpreet, S. and Bahaderjeet, S. (2020).** Symptoms and Management of *Alternaria* blight of Indian Mustard (*Brassica juncea*). *Journal of Plant Development Sciences*. 12(3): 165-170.
- Gomez, K. A. and Gomez, A. A. (1984).** Statistical procedures for Agricultural research (2nd ed.). John Wiley and sons, New York, P: 680.
- Jha, P., Kumar, M., Meena, P. D. and Lal, H. C. (2013).** Dynamics and management of *Alternaria* blight disease of Indian mustard (*Brassica juncea*) in relation to weather parameters. *Journal of Oilseed Brassica*. 4(2): 66-74.
- Kolte, S. J. (1985).** Diseases of Annual Edible Oilseed Crops, Rapeseed-Mustard and Sesame Diseases, *CRC Press Inc. Boca Raton, Florida*. 2: 135.
- Kumar, D., Maurya, N., Bharati, Y. K., Kumar, A., Kumar, K., Srivastava, K., Chand, G., Kushwaha, C., Singh, S. K., Mishra, R. K. and Kumar, A. (2014).** *Alternaria* blight of oilseed Brassicas: A comprehensive review. *African Journal of Microbiology Research*. 8(30): 2816-2829.
- Kumar, R., Bhaskar, R., Mapari, P. N., Datre, S., Ahammad, F., Patil, P. and Simon, S. (2023).** Compatibility analysis of mushroom with different botanicals in in-vitro condition. *International Journal of Environment and Climate Change*. 13(12): 1017-1023.
- Mahapatra, S. and Das, S. (2013).** Bio efficacy of botanicals against *Alternaria* Leaf Blight of Mustard under Field Condition. *The Bioscan*. 8(2): 675-679.
- Meena, S., Godika, S., Ghasolia, R. P., Sumitra., Nitharwal, N. and Kardam, V. K. (2022).** Management of *Alternaria* blight disease (*Alternaria brassicae*) of mustard through plant extracts and fungicides. *The Pharma Innovation Journal*. 11(1): 58-67.
- Nayyar, B. G., Akhund, S. and Akram, A. (2014).** Management of *Alternaria* and its mycotoxins in crops. *Scholarly Journal of Agricultural science*. 4(7): 432-437.
- Prasad, R. and Lallu. (2006).** Management of *Alternaria* blight of mustard with combination of chemicals and botanicals. *Annals of Plant Protection Science*. 14(2): 313-320.
- Saccardo, P. A. (1886).** *Alternaria brassicae* (Berk) Sacc. *Michelia*: 2: 172.

- Sharma, B., Kumari, P., Kumari, R., Tripathi, S. K., Kumar, A. and Dhara, P. K. (2020).** Impact of Multistorey System on Yield and Soil Quality of Mustard in Alfisols of Eastern India. *ADRRI Journal of Agriculture and Food Sciences*. 4: 27-46.
- Singh, J., Kumhar, D. R., Kumari, S. and Kumar, R. (2022).** Isolation, purification and management of *Alternaria brassicae* through cow by product in vitro. *The Pharma Innovation Journal*. 11(4): 700-703.
- Yadav, J. K., Singh, H. K., Singh, S. K., Kavita. and Singh, S. (2019).** Efficacy of plant extracts against *Alternaria brassicae* under *in vitro* condition. *Journal of Pharmacognosy and Phytochemistry*. 8(1): 528-532.

UNDER PEER REVIEW