Journal of Scientific Research and Reports

Volume 30, Issue 7, Page 246-251, 2024; Article no.JSRR.118171
ISSN: 2320-0227

In vitro Evaluation of Fungicides and Plant Derived Extracts on Mycelial Growth of *Alternaria brassicae*

Faijuddin Ahammad [a++*], Pankaj N. Mapari [a++], Rohit Kumar [a++], Shivani Datre [a++], Jagriti Thakur [a++], Prafull Patil [a++] and Sunil Zacharia [a#]

[a] Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj, U.P.-211007, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jsrr/2024/v30i72141

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/118171

Original Research Article

Received: 08/04/2024
Accepted: 11/06/2024
Published: 20/06/2024

ABSTRACT

Mustard (*Brassica juncea*) is one among the major oilseed crops in India grown during *Rabi* season. Alternaria blight of mustard caused by *Alternaria brassicae* is most widespread and destructive disease of 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*

[++] *M.Sc. Research Scholar;*
[#] *Associate Professor;*
**Corresponding author: E-mail: faijuddin.book25@gmail.com;*

Cite as: *Ahammad, Faijuddin, Pankaj N. Mapari, Rohit Kumar, Shivani Datre, Jagriti Thakur, Prafull Patil, and Sunil Zacharia. 2024. "In Vitro Evaluation of Fungicides and Plant Derived Extracts on Mycelial Growth of Alternaria Brassicae". Journal of Scientific Research and Reports 30 (7):246-51. https://doi.org/10.9734/jsrr/2024/v30i72141.*

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 (5.28 mm), Mancozeb (6.25 mm), Carbendazim (14.25 mm), Chilli leaf extract (69.12 mm) and Alstonia leaf extract (84.23 mm) as compared to Control (90 mm) untreated check. Carbendazim + Mancozeb 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 brassicae; Alstonia scholaris; Brassica juncea; Capsicum annum; fungicides; In vitro.

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 [1]. 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 [2]. 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 [3].

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 [4]. Among the diseases affecting mustard, Alternaria blight, caused by *Alternaria brassicae* (Berk.) Sacc, has a global distribution [5]. First observed the fungal infection on plants within the Brassicaceae family in 1836, initially identifying it as *Macrosporium brassicae* Berk. Later, [6] renamed it as *A. brassicae* (Berk.) Sacc. in 1886.

Symptoms of the disease manifest as the formation of spots on leaves, stems, and siliqua [7,8]. 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 [9,10]. 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 AND 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 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.

2.1 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 [11]. This formed 100 percent plant extract solution. The plant extracts so prepared were heated at 40ºC 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 [12]. The 20 ml sterilized media

with botanical extract was poured into 90 mm Petri plates under aseptic conditions in laminar air flow. After solidification of media 5 mm disc of 7 days old subculture of *Alternaria 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 check. Three replicates were maintained for each test and those plates were incubated at 27±1 ºC 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.

2.2 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 brassica* served as control. Three replications were maintained for all the treatments and plates were incubated in BOD incubator at a temperature of 25 ± 1ºC. 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 [13]. 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

3.1 *In vitro* Effect of Fungicides and Botanicals on Radial Growth (mm) and Per Cent Inhibition of *Alternaria brassicae* at 10 % Concentration at 168 hrs

As shown in Table -1, depicted in Figure -1 and plate -1 revels that at 10 % concentration after 168 hrs incubation, the minimum radial growth of *Alternaria brassicae* was observed in (T_3)- Carbendazim + Mancozeb (0 mm), followed by (T_2)- Hexaconazole (5.28 mm), (T_1)- Carbendazim (14.25 mm), (T_5)- Chilli leaf extract (69.12 mm), (T_6)- Alstonia leaf extract (84.23 mm) as compared to treated check (T_4)- Mancozeb (6.25 mm) and (T_0)- Control (90 mm) untreated check. Statistically, all the treatments were significant over Control.

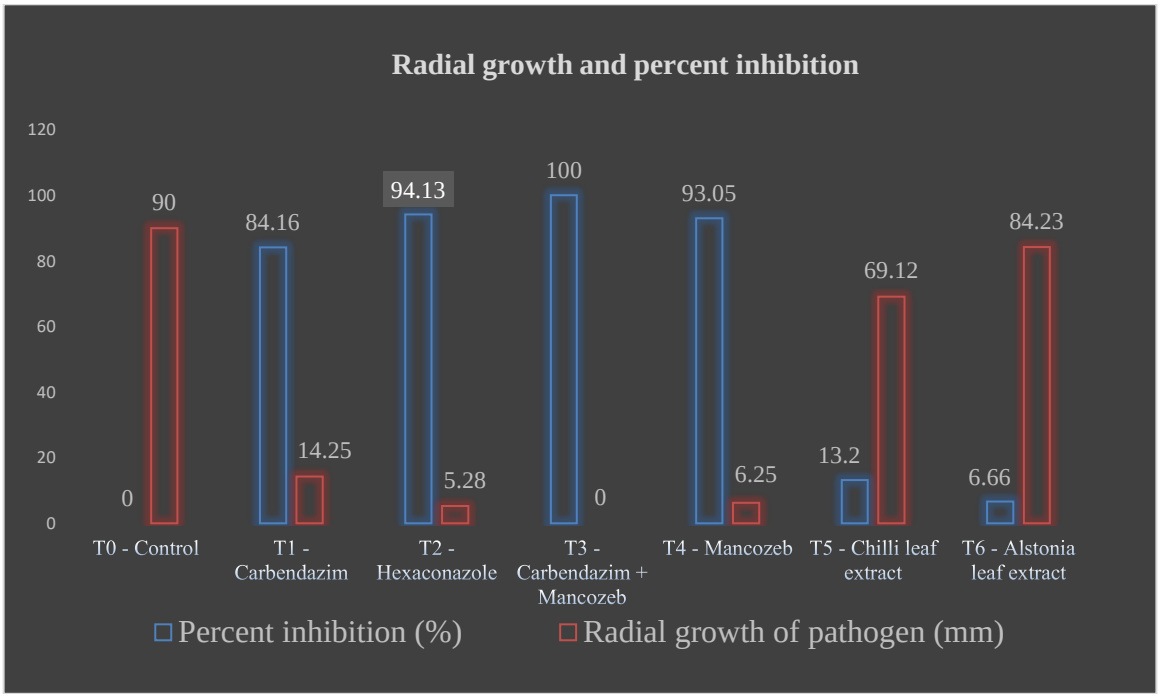

Fig. 1. Effect of fungicides and plant extracts on *Alternaria brassicae* on percent inhibition at 168 hours

Table 1. Effect of fungicides and plant extracts on *Alternaria brassicae* by poison food technique

S. No.	Treatments	Mycelial 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*

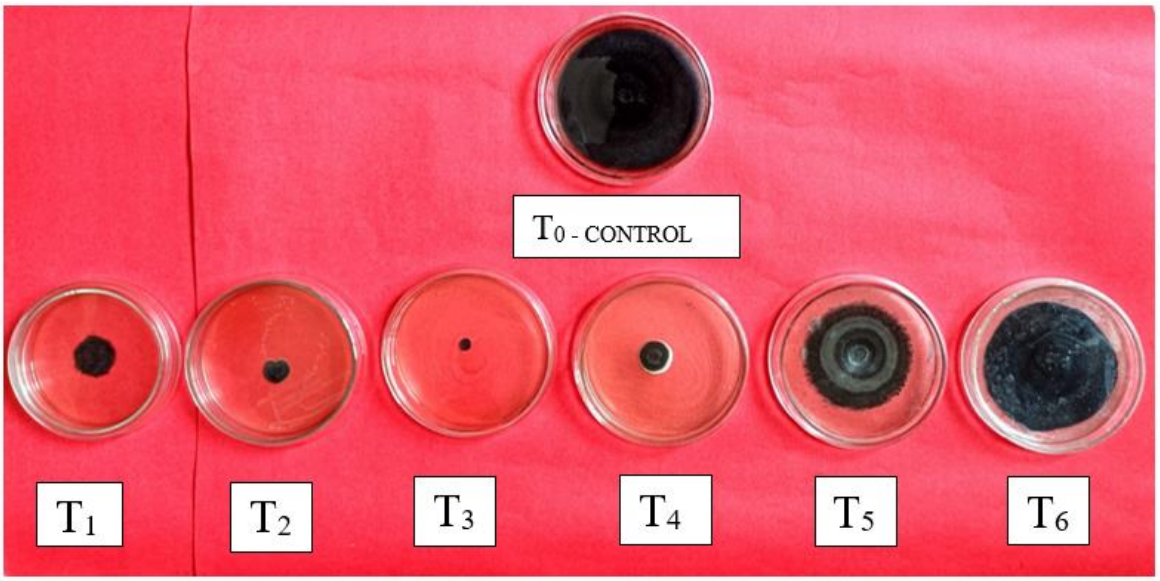

Plate 1. Response of fungicides and plant extracts against *Alternaria brassicae* on mycelial growth

The result showed that maximum percentage of inhibition was observed in (T_3)- Carbendazim + Mancozeb (100 %), followed by (T_2)- Hexaconazole (94.13 %), (T_1)- Carbendazim (84.16 %), (T_5)- Chilli (*Capsicum annum*) leaf extract (13.2 %), (T_6)- Alstonia (*Alstonia scholaris*) leaf extract (6.66 %) as compared to treated check (T_4)- Mancozeb (93.05 %) and (T_0)- Control (0 %) untreated check. Statistically, all the treatments were significant over Control.

4. DISCUSSION

Four fungicides and two plant extracts of namely Carbendazim, Hexaconazole, Carbendazim + Mancozeb, Mancozeb, Chilli (*Capsicum annum*) leaf extract and Alstonia (*Alstonia scholaris*) leaf extract 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 Meena et al. [14], 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 Choudhary et al. [15] who reported that

hexaconazole was highly effective in inhibiting mycelial growth, achieving a 95.56% inhibition rate. Singh et al. [16] evaluated that mancozeb was found to be the best fungicide in reducing the radial growth and germination of conidia in laboratory. Furthermore, Yadav et al. [17] evaluated extracts from 54 plants against *Alternaria brassicae* and found that chilli leaf extract inhibited mycelial growth by 56.5 % at a 10 % concentration. Meena et al. [14] also evaluated various leaf extracts and discovered that Alstonia leaf extract inhibited the mycelial growth of the fungus by 62.30% at a 10 % concentration.

5. CONCLUSION

The research presented here offers a thorough evaluation of various fungicidal treatments and plant extracts against the mycelial growth of *Alternaria brassicae*, a notorious pathogen responsible for causing Alternaria blight in mustard, which significantly threatens crop yields worldwide. Through meticulous experimentation using the poison food technique under controlled in vitro conditions, the efficacy of different treatments was systematically assessed. Notably, among the treatments tested, 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 managing and mitigating 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar D, Maurya N, Bharati YK, Kumar A, Kumar K, Srivastava K, Chand G, Kushwaha C, Singh SK, Mishra RK, Kumar A. Alternaria blight of oilseed Brassicas: A comprehensive review. African Journal of Microbiology Research. 2014;8(30):2816-2829.
2. Sharma B, Kumari P, Kumari R, Tripathi SK, Kumar A, Dhara PK. Impact of multistorey system on yield and soil quality of mustard in alfisols of Eastern India. ADRRI Journal of Agriculture and Food Sciences. 2020;4:27-46.
3. Jha P, Kumar M, Meena PD, Lal HC. Dynamics and management of Alternaria blight disease of Indian mustard (*Brassica juncea*) in relation to weather parameters. Journal of Oilseed Brassica. 2013;4(2):66-74.
4. Singh J, Kumhar DR, Kumari S, Kumar R. Isolation, purification and management of *Alternaria brassicae* through cow by product *In vitro*. The Pharma Innovation Journal. 2022;11(4):700-703.
5. Berkeley MJ. *Macrosporium brassicae* spp. Smith England Flora. 1836;5:339.
6. Saccardo PA. *Alternaria brassicae* (Berk) Sacc. Michelia. 1886;2:172.
7. Prasad R, Lallu. Management of alternaria blight of mustard with combination of chemicals and botanicals. Annals of Plant Protection Science. 2006;14(2):313-320.
8. Nayyar BG, Akhund S, Akram A. Management of alternaria and its mycotoxins in crops. Scholarly Journal of Agricultural science. 2014;4(7):432-437.
9. Kolte SJ. Diseases of annual edible oilseed crops, rapeseed-mustard and sesame diseases, CRC Press Inc. Boca Raton, Florida. 1985;2:135.
10. Gagandeep SS, Amanpreet S, Bahaderjeet S. Symptoms and management of alternaria blight of Indian mustard (*Brassica juncea*). Journal of Plant Development Sciences. 2020;12(3):165-170.
11. Kumar R, Bhaskar R, Mapari PN, Datre S, Ahammad F, Patil P, Simon S. Compatibility analysis of mushroom with different botanicals in in-vitro condition. International Journal of Environment and Climate Change. 2023;13(12):1017-1023.
12. Nene YL, T Thapliyal PN. Fungicides in plant disease control. Oxford and IBH Publishing Company. 2000;(12):531-533.

13. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947;159:850.
14. Meena S, Godika S, Ghasolia RP, Sumitra, Nitharwal N, Kardam VK. Management of alternaria blight disease (*Alternaria brassicae*) of mustard through plant extracts and fungicides. The Pharma Innovation Journal. 2022;11(1):58-67.
15. Choudhary S, Ghasolia RP, Shivran M, Yadav R, Bairwa V. Management of alternaria leaf spot of lehsua through plant extracts and fungicides. International Journal of Current Microbiology and Applied Science. 2020;9(2):2573-2580.
16. Singh A, Tomar DS, Nayak MK, Srivastava AK. Screening of systemic and non-systemic fungicides against Alternaria blight of mustard (*Brassica juncea*). Annals of Plant and Soil Research. 2017; 19(3):266-270.
17. Yadav JK, Singh HK, Singh SK, Kavita, Singh S. Efficacy of plant extracts against *Alternaria brassicae* under *In vitro* condition. Journal of Pharmacognosy and Phytochemistry. 2019;8(1): 528-532.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/118171