

# In-vitro efficacy of different chemical fungicides and plant extracts against *Alternaria alternata* causing Alternaria leaf spot disease in brinjal

## ABSTRACT

Five different chemical fungicides and three plant extracts were evaluated against *Alternaria alternata* causing Alternaria leaf spot disease in brinjal. The experiment was carried out in the laboratory of plant pathology at Chaudhary Charan Singh University Meerut, during kharif season 2021-22. During survey the maximum disease incidence was recorded (37.8 %) Rajpura in Meerut and minimum (10.9 %) incidence was recorded in Lakhaoti, survey and metrological data presented in the (table 2 & 3). The incidence of the disease varied from 10.9 to 37.8 percent due to difference in environmental condition. The concentration of all fungicides and Plant-extracts was kept at 500, 1000 and 2000 ppm. Mycelial growth was measured to continue zero to ten days. Mainly four chemical fungicides (Thiophanate Methyl 70%WP, Propineb 70% WP, Carbendazim 50%WP and Chlorothalonil 75% WP) and Neem extract significantly inhibited the mycelial growth of the pathogen (*Alternaria alternata*). Among all those, four fungicides and Neem extract giving the better results. Maximum 100% mycelial growth inhibition was recorded in Thiophanate Methyl 70%WP, 94.45% mycelial growth inhibition was recorded in Propineb 70% WP, 86.09% mycelial growth inhibition was recorded in Carbendazim 50%WP and 84.80 growth inhibition was recorded in Chlorothalonil 75% WP and in plant extract 18.53% inhibition was observed in Neem and 14.33% inhibition was observed in garlic. However, Azoxystrobin 23% SC and Onion also had some effect, which is mentioned in the result of the paper.

Keywords: Efficacy, Fungicides, Plant extract, *Alternaria alternata*.

## 1. INTRODUCTION

*Solanum melongena* L. commonly known as brinjal, is an important solanaceous crop of tropical and sub-tropical regions in India and world. It is also known as eggplant and also be considered as a "king of vegetable". The brinjal or eggplant or Aubergine (French name) has originated in India sub-continent and china" (Thomopson and Kelly, 1957). "Alternaria leaf spot disease of brinjal caused by *Alternaria alternata* is an important disease in the major crop growing area of the state. Described the symptom of *Alternaria altarnata* dark brown concentric rings appeared on leave and turned yellowing indicating loss of chlorophyll from infected leaves" by Bhatt *et al.*, (2013) "Heavy damage of the plants due to Alternaria leaf spot occur especially occur during cool and humid weather, coupled with cloudiness which favors the occurrence and spread of the disease. When humid conditions prevailing at ground level, lower leaves are first attacked and infection spreads to the upper leaves and fruits. The spots are mostly irregular and collapse to cover a large leaf area. Considering the seriousness of the problem, the present investigation was carried out. Significant variations in Mycelial growth, color of mycelium and length of conidia, pattern of conidiophores emergence, shape of conidia and their germination" Singh and Singh (2003).

"Asia has the largest eggplant area that comprises more than 90% of the world production. India is considered to be the Centre of Origin of cultivated eggplant, from where it spread to the other parts of the world. It is used as an excellent remedy for those suffering from liver complaints. Eggplant fruit is used in ayurvedic medicine for curing the diabetes. It is also used as good aphorodisiac, cardotonic, laxative of inflammation and as an excellent cholestrol regulator with a good source of ascorbic acid and some phenolics, which are powerful antioxidants. The flavonoid nasunin, an antioxidant and free radical scavenger present, protects the cell membrane from damage. Eggplant is also considered as a very good source of dietary fibre, which lowers risk of coronary heart disease. For processing purposes, the fruit should have high dry matter content and a low level of phenolics. Bitterness in eggplant is due to the presence of glycoalkaloids. The glycoalkaloid contents in the Indian commercial cultivars of eggplant vary from 0.37 to 4.83 mg/100 gm fresh weight" (Bajaj *et al.*, 1981). "In addition it

also contains trace elements and alkaloid called solanin which has medicinal properties” (Ayokroyd, 1963).

Table: 1. Composition of brinjal (*Solanum melongena* L.)M.Y Naeem *et al.*, 2019.

Eggplants are richest source of copious nutrients and their contents (mentioned in table1). It is the complete set of minerals, vitamins, nutritional fiber, protein, anti- oxidants, along with phytochemical that having scavenging activities (Noda *et al.*, 2000, Whitaker and Stommel).

<b>Nutritional Value and Composition of Brinjal Fruits</b>			
Moisture	92.7%	Iron	0.9 mg.
Protein	1.4 g.	Sodium	3.0 mg
Fat	0.3 g.	Potassium	200 mg.
Minerals	0.3 g.	Copper	0.17 mg.
Fibre	1.3 g.	Sulphur	44.0 mg
Carbohydrate	4.0 g.	Chlorine	52.0 mg
Calories	2.4 kcl	Vitamin A	124.0 I.U.
Calcium	18.0 mg.	Thiamine	0.04 mg
Oxalic acid	18.0 mg.	Riboflavin acid	0.11 mg.
Phosphorus	47.0 mg.	Vitamin C	12.0 mg

## 2. MATERIAL AND METHODS

The experiment was done under in-vitro conditions, Department of Plant Pathology Laboratory at Chaudhary Charan Singh University Meerut (U.P.) in India. Efficacy of different fungicides and some plant extracts against *Alternaria alternata* causing Alternaria leaf spot of brinjal during kharif season (2021-22).

### 2.1 Collection of diseased plant sample

During survey sample was collected from infected plant leaves. The infected leaves were carefully collected in envelopes and taken to the laboratory for culturing of pathogens and further study.

### 2.2 Isolation of Pathogen (*Alternaria alternata*)

First of all, the surface of the infected leaf was treated with spirit. Then cut the infected part of the leaf along with the some healthy part of the leaf. “Each small pieces dip in 0.1 % mercuric chloride solution for sterilization followed by three washing with sterilized distilled water. These bits were transferred aseptically to 2 percent Potato Dextrose Agar in Petri-dishes separately. Incubation was done  $25 \pm 2^{\circ}\text{C}$  for 1 week” (Krishnamurthy *et al.*, 2019). Sub-culture from uncontaminated peripheral growth was made on PDA slants. Single spore technique was used for the purification of the fungus. After sporulation, conidial suspension was made in sterile water and the dilution was adjusted such that in one loop full, 20-25conidia could be controlled under low power objective of microscope. After 12 hours of incubation at  $25 \pm 2^{\circ}\text{C}$  the single germinating conidium was cut with the help of dummy

objective and transferred to PDA slants. They were subsequently allowed to grow and sporulate. Monoconidial culture established in this way was maintained by periodical transfer on PDA slants. After transferring to slant, microscopic examination of the growth was also done to ensure the growth of *Alternaria alternata*.

### 2.3 Identification and characterization of pathogenic fungi

“The pathogen was identified on the basis of its morphological and cultural character, as well as pathogenic behavior towards the host. The morphological characters Viz., shape, size, septation and colour of conidia were recorded. To study the morphological characters particularly asexual organ of the fungus, the temporary slides, were prepared in cotton blue from one week old culture. The morphological character was recorded after growing it on 2% Potato Dextrose Agar medium in Petridis. The inoculated Petri-plates were incubated for 7 days at room temperature 25°C to 28°C”(Simmons 2007).

### 2.4 Efficacy of different fungicides and plant-extracts

“Relative efficacy of Five selected chemical fungicides (Chlorothalonil, Azoxystrobin, Propineb, Carbendazim, Thiophanate methyl ) and three botanical extract (Neem, Garlic and Onion) were tested at 500,1000 and 2000ppm concentration only their inhibition were recorded effect of the growth of the pathogen on 2% Potato dextrose agar medium. The requisite quantities of the above fungicides were thoroughly mixed in 2% sterilized warm unsolidified potato dextrose agar medium and shaken well to make it homogenous. Five mm circular discs from 10-day-old culture of the pathogen were cut by sterilized cork borer and placed in the centre of each fungicide in Petridis's in such a way that the fungus came in direct contact with the medium. A separate check having no fungicide was also maintained. The inoculated plates were incubated for 8 days at 25°C to 28°C for the growth of mycelium. The efficacy of various fungicides was assessed by measuring the radial growth of the fungus colony. The fungicides which were found effective in laboratory evaluation were employed further in two ways, namely seed dressers as well as spray fungicides” (Chaudhari *et al.*,2021).

### 2.5 Statistical analysis of data

The laboratory experiment was conducted with C.R.D. design. The data with appropriate transformations, where ever required, were analysed with the help of analysis of variance techniques. The 'F' value was tested and critical difference (C.D.) was calculated at 5% level of significance for comparing treatment means.

## 3.0 Results and Discussion

### 3.1 Prevalence of the disease

In order to know the disease incidence survey were carried out for collecting natural specimen's severity of the disease on Leaf spot of Brinjal in field during the month of October to November, 2021-22 at different 4 district of UP. Disease sample were collected for isolation and further used in different studies. The results of survey are represent in Table-2, revealed that the Leaf spot of Brinjal prevalent in all localities of U.P. which are surveyed. The maximum incidence (37.8 %) was recorded, Rajpura in Meerut and lowest (10.9 %) incidence was found in Lakhaoti, Bulandshaher. The highest incidence was record at 28± 2°C temperature with 80-85% Relative Humidity in the months of August- September. The incidence of the disease varied from 10.9 to 37.8 percent due to difference in environmental condition and the variety grown.

**Table: 2 Occurrence of Leaf spot of Brinjal in different district of U.P during 2021-22**

Sr.No.	District	Area	AveragepercentageofDisease incidence%
1.	<b>Meerut</b>	Modipuram	17.2
		Jaani	22.5
		Rajpura	37.8
		Pahasu	12.5
2.	<b>Bulandshahr</b>	Lakhaoti	10.9
		Khurja	21.3
		Sikandrabad	32.4
3.	<b>Hapur</b>	Hapur	20.8
		Garhmukteshwar	27.7
		Simbhally	25.4
4.	<b>Shamli</b>	Shamli	23.1
		Kairana	33.9
		Kandhla	28.5

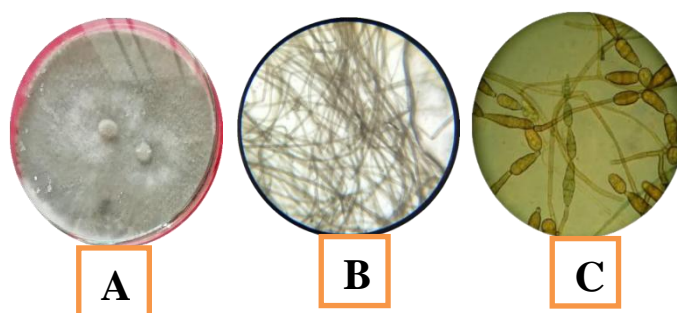
**Table: 3 Metrological observations at growth stage of the plant during the year 2021-22**

Metrological observation during the year-2021							
Months	Rainfall (mm)	Relative humidity (%)			Temperature (°C)		
		8:30 am	6:30 pm	Ave.	Max.	Min.	Ave.
<b>July</b>	345.8	82.0	73.1	77.55	37.1	32.0	34.55
<b>August</b>	382.1	85.2	73.0	80.1	31.5	26.1	28.8
<b>September</b>	482.0	91.0	83.0	87	32.5	26.7	29.6
<b>October</b>	231.4	79.9	74.0	76.95	32.0	21.3	26.65
<b>November</b>	Nil	80.3	64.0	72.15	26.5	12.4	19.45
<b>December</b>	Nil	88.4	71.0	79.7	22.4	11.2	16.8

### 3.2 Identification and characterization of pathogenic fungi

Mycelium, septate, branched, hyaline mycelium later turning to pale olive buff, smooth, 3.0-5.3µm wide. Conidiophore were short or long, dark brown color, bearing single but sometimes two conidia in chain at the tip and 30-132 × 5.2-7.9µm in size with 1-8 septa. Conidia were light dark brown, solitary some times in short chain (5-11) in acropetal manner. In shape, the conidia are obclavate but few were oval or pyriform with a rather short, broadly rounded base with 3-

8 transverse septa and several longitudinal septa with variable size and shape. The sizes of conidia were  $136-46.3\mu\text{m} \times 4.6-14.12\mu\text{m}$  (Av.  $32.45 \times 11.36\mu\text{m}$ ).



**Fig.1 (A) Pure culture, (B) Mycelium microscopic structure, (C) Conidia with Conidiophore.**

Fungi can be grown on different culture media; natural, synthetic and semi-synthetic media were used for the growth and sporulation of the fungus and results obtained presented in the table 1.

**Table: 4 Fungal dry weight and sporulation of *Alternaria alternata* on different solid media after 8 days of incubation.**

S. N.	Solid Media	Average dia. of colonies (cm)	Sporulation
1	B.S. medium	5.80	Excellent
2	Leaf extract medium	5.67	Excellent
3	Kirchhoff's medium	5.43	Good
4	P.D.A.	5.30	Good
5	Oatmeal	4.83	Fairly
6	A.&H. medium	3.32	Poor
<b>C.D. at 5%</b>		<b>1.34</b>	

### 3.3 In-Vitro evaluation of different fungicides and plant extracts

Five chemical fungicides and three plant extracts were evaluated against *Alternaria alternata* at 500, 1000, 2000 ppm concentrations. The results revealed that, an effect of fungicides on the fungal growth was significant. Assessed results were presented in (table 4). Among all the treatments, Thiophanate Methyl 70% WP showed the (100%) mycelial growth inhibition and minimum growth inhibition was recorded in Onion (11.04%).

**Table: 5** Inhibitory effect of different fungicides on Mycelial growth of *Alternaria alternata* in vitro after 8 days inoculation at 28±1<sup>0</sup>c.

Sr. No.	Treatment		Percent inhibition of mycelium growth Concentration (ppm)			Ave. Dimension of fungal growth	Mean % Inhibition
			500	1000	2000		
1.	Thiophanate Methyl 70%WP	Methyl	100.0	100.0	100.00	0.00	100.0
2.	Propineb 70% WP		86.23	97.12	100.00	0.82	94.45
3.	Chlorothalonil 75% WP		73.79	86.56	94.05	1.35	84.80
4.	Carbendazim 50% WP		73.02	86.89	98.37	1.42	86.09
5.	Azoxystrobin 23% SC		56.45	67.11	73.07	1.49	65.54
6.	Neem		9.56	17.04	29.00	3.32	18.53
7.	Garlic		6.01	14.29	22.69	3.38	14.33
8.	Onion		5.79	9.89	17.46	3.56	11.04
9.	<b>Control</b>		-----	-----	-----	100.0	0.00
<b>C.D 5% Level</b>						<b>1.812</b>	



**Fig.2** Poisoned food technique with different fungicides and plant extracts.

Comprison with other research studies can provide valuable insights into the effectiveness of the tested fungicides and plant extract against *Alternaria alternata* a study by Sharma *et al.*,(2020) investigated the inhibitory effects of fungicides and plant extracts on *Alternaria alternata* in vitro. Their findings showed similar trends in fungicidal efficacy, with Thiophanate Methyl 70% WP exhibiting the highest inhibition (100.0%) of mycelial growth, consistent with our study. Additionally, Propineb 70%WP showed significant inhibition (94.45%), aligning with our results. Furthermore, a study by Patel *et al.*,(2019) evaluated the efficacy of plant extracts. Their result demonstrated comparable inhibition percentages with Neem extract showing the highest inhibition (19.0%), similar to our findings (18.53%). Garlic extract also exhibited notable inhibition (14.33%), consistent with both studies. However, contrasting results were observed in a study by Singh *et al.*,(2018), where Azoxystrobin 23%SC displayed higher inhibition (78.6%) compared to our study (65.54%). This disparity could be attributed to variations in experimental conditions, fungal strains or concentration used. Overall, the effectiveness of Thiophanate Methyl, Propineb, Neem and garlic extracts against

*Alternaria alternata* aligns with previous research, suggesting their potential as effective management options for controlling leaf spot disease on Brinjal plants.

## CONCLUSION

After observing the experiment, it was concluded that Thiophanate Methyl 70%WP had the lowest mycelial growth and showed a maximum of 100% mycelial growth inhibition. This was significantly better than the control at a concentration of 500, 1000, and 2000 ppm. Other non-systemic fungicides like Propinabe 70% WP had a mycelial growth inhibition of 94.45 percent. Additionally, it was found that Neem extract showed better results than other plant extracts such as garlic and onion.

## ACKNOWLEDGEMENT

The authors are grateful to the Department of Plant Protection, CCS University Meerut, for providing the best research platform. Authors are also grateful to Dr. Ajay Kumar (Assistant professor, department of Plant Pathology) Amar Singh college, Lakhawati, Bulandshahr, for best guidance and support.

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