

Efficacy of different fungicides and plant bio-Extracts against *Alternaria alternata* causing *Alternaria* leaf spot of brinjal under in-vitro condition

Comment [DS1]: Correct it as:
Plant extracts

ABSTRACT

Among the five different fungicides and three types of plant bio-extract assessed against *Alternaria alternata*, causing *alternaria* leaf spot of brinjal under in-vitro condition. The experiment was undertaken in the laboratory of plant pathology at Chaudhary Charan Singh University Meerut during kharif season 2021-22. The maximum disease incidence was recorded (37.8 %) Rajpura in Meerut and minimum incidence was recorded (10.9 %) in Lakhaoti, Bulandshahr survey and metrological data presented in the table. 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 bio-extract was kept at 500, 1000 and 2000 ppm. Mycelial growth was measured to continue zero to fifteen days. Total fungicides and two plant bio-extract significantly inhibited the mycelial growth of the pathogen (*Alternaria alternata*). Among all those two fungicides (Thiophanate Methyl 70% WP and Carbendazim 50% WP) and Neem give the better results compared to other. Maximum 100% inhibition was recorded in Thiophanate Methyl 70% WP and 94.45% was recorded Carbendazim 50% WP at 2000 ppm concentration and 18.53% inhibition was observed in Neem and 14.33% inhibition was observed in garlic. However, other fungicides and Plant Bio-extract also had some effect, which is mentioned in the result of the paper.

Comment [DS2]: Not clear; Rephrase it for better understanding

Comment [DS3]: Correct it as:
Carried out

Comment [DS4]: Correct as:
Bulandshahr, according to the survey

Comment [DS5]: maintained

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Comment [DS8]: Correct as:
Maximum inhibition (100%)

Comment [DS9]: Correct as:
followed by 14.33% inhibition with in garlic extract.

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Besides these, the study recorded the antifungal activities with other fungicides and Plant extracts.

Keywords: Efficacy, Fungicides, Plant bio-Extract, *Alternaria alternata*.

1. INTRODUCTION

Brinjal (*Solanum melongena* L.) 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 alternata* dark brown concentric rings appeared on leaf and turned yellowing indicating loss of chlorophyll from infected leaves by Bhatt *et al.*, (2013). *Alternaria* leaf spot heavily damage the plants, 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).

Comment [DS11]: Important

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Heavy damage of the plants due to *Alternaria* leaf spot occur especially occur during cool...

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 aphrodisiac, cardotonic, laxative of inflammation and as an excellent cholesterol 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

Comment [DS13]: Reference?

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).

List 1. Composition of brinjal (*Solanum melongena* L.)(?)

Comment [DS14]: Reference?

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 kcal	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 bio-extract against *Alternaria alternata* causing Alternaria leaf spot of brinjal during kharif season (2021-22).

Comment [DS15]: *in vitro*

Comment [DS16]: Correct it

2.1 Collection of diseased sample

A sample was collected during survey from an infected plant leaves from agricultural experimental research farm at CCS University Meerut and referenced district. Infected leaves, fruits and stem were collected in envelopes and brought to the laboratory for culturing of pathogen and further study.

Comment [DS17]: Correct as: Collection of diseased plant samples

Comment [DS18]: Not clear; needs rephrasing

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. 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, 20-25 conidia could be controlled under low power objective of microscope. One such loop full was mixed with 25 ml melted with sterilized agar (2%) and poured in sterile petri dishes. 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

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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 Morphological characters

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.

Comment [DS20]: Correct as: Identification and characterization of pathogenic fungi

2.4 Efficacy of different fungicides and plant bio-extracts

Relative efficacy of Five selected fungicides (Chlorothalonil, Azoxystrobin, Propineb, Carbendazim, Thiophanate methyl) and three botanical extracts (Neem, Garlic and Onion) were tested at 500, 1000 and 2000 ppm 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. Medium containing different fungicides was then poured in 90 mm diameters sterilized Petridis with four replication of each Petridis and allowed to solidify. 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.

Comment [DS21]: Correct it

Comment [DS22]: Correct as: Selected chemical fungicides

Comment [DS23]: Text presentation is not scientific. Needs rephrasing

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.

13.0 Results and Discussion

13.1 Prevalence of disease according to survey

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 samples were collected for isolation and further used in different studies. The results of survey are present in Table-1, 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. The incidence of the disease in the university research area was estimated between 1.0 to 40 %, respectively.

Comment [DS24]: 13.0?

Comment [DS25]: Correct as: Prevalence of the disease

Comment [DS26]: Text presentation is not scientific; needs rephrasing

Table 1.A.Occurrence of Leaf spot of Brinjal in different district of U.P during 2021-22

Sr.No.	DistrictArea	AveragepercentageofDiseaseincidence%	
1.	Meerut	Modipuram	17.2
		Jaani	22.5
		Rajpura	37.8
		Pahasu	12.5
2.	Bulandshahr	Lakhaoti	10.9
		Khurja	21.3
		Sikandrabad	32.4
3.	Hapur	Hapur	20.8
		Garhmukteshwar	27.7
		Simbhaully	25.4
4.	Shamli	Shamli	23.1
		Kairana	33.9
		Kandhla	28.5

Comment [DS27]: Correct as:
Disease incidence (%)

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Table 1.B. Metrological observation at growth stage of the plant during the year 2021-22

Comment [DS28]: Correct as: Meteorological

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

Comment [DS29]: Meteorological

3.3 Symptoms:

This disease manifests as small, dark brown to black spots on leaves, which gradually enlarge and merge, leading to extensive necrosis. As the infection progresses, the spots develop a concentric ring pattern, characteristic of *Alternaria spp.* The fungus primarily infects older leaves but can spread to younger foliage under conducive condition, such as high humidity and optimum temperatures in severe cases, the leaves may exhibit chlorosis, wilting, and premature defoliation, compromising the plants photosynthetic capacity and overall health.



Fig-1. Initial symptoms of Alternaria leaf spot of Brinjal.

Comment [DS30]: Present it under Introduction section

3.4 Identification and Morphological character

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 136-46.3µm × 4.6-14.12µm (Av. 32.45 × 11.36µm).

Comment [DS31]: Correct as: Identification and characterization of pathogenic fungi

Comment [DS32]: Reference manual used for identification of fungi?

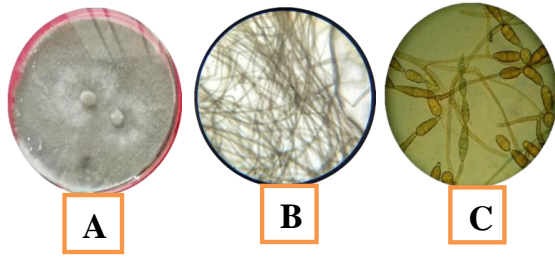
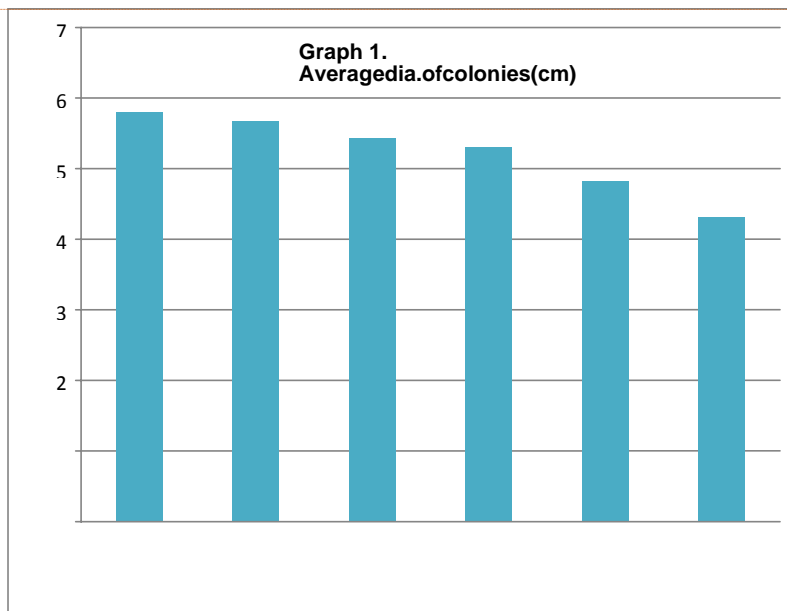


Fig.2 (A) Pure culture, (B) Mycelium microscopic structure, (C) Conidia with Conidiophore.

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 2.

Table 2. Fungaldryweightandsporulationof *Alternariaalternata*ondifferentsolid media after 8 daysof incubation.

S. N.	SolidMedia	Averagedia.ofcolonies(cm)	Sporulation
1	B.S. medium	5.80	Excellent
2	Leaf extractmedium	5.67	Excellent
3	Kirchhoff'smedium	5.43	Good
4	P.D.A.	5.30	Good
5	Oatmeal	4.83	Fairly
6	A.&H.medium	3.32	Poor
	C.D.at5%	1.34	



Comment [DS33]: Present the data using either table or graph; not both

3.5 In-Vitro evaluation of different fungicides and plant extracts

Mainly five fungicides and three plant extracts were evaluated in three replications at 500,1000 and 2000 ppm concentrations for the study of the effect against *Alternaria alternata* by poisoned food technique mentioned in material and methods, assessed result were presented in table-3. The results revealed that, effects of fungicides on the fungal growth were significant. After 10 days maximum inhibition of mycelial growth over control was recorded in Thiophanate Methyl 70%WP (100.0%) which was significantly superior and Propineb 70% WP (94.45%) good second inhibition mycelial growth over control was recorded. Carbendazim 50% WP (86.9%), Chlorothalonil 75% WP (84.80%), Azoxystrobin 23% SC (65.54%) was the next best fungicides in that order. In plant extract maximum inhibition was recorded in Neem extract 18.53% and second good garlic 14.33%, lowest inhibition was recorded in Onion 11.4%.

Comment [DS34]: Presentation of text is not scientific; needs rephrasing

Comment [DS35]: Present the data of any other research study for comparison and discussion.

Comment [DS36]: *in vitro*

Table 3. Inhibitory effect of different fungicides on Mycelial growth of *Alternaria alternata* –*in vitro* after 8 days inoculation at 28±1^oc.

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		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.	Control	-----	-----	-----	100.0	0.00
C.D 5% Level					1.812	



Fig.3 Poisoned food technique with different fungicides and plant extracts

CONCLUSION

On the basis of observation. It is concluded that the lowest mycelial growth with maximum 100 percent mycelial growth inhibition was recorded in Thiophanate Methyl 70%WP significant superior over control at 500,1000,2000 ppm concentration and other non-systemic fungicides Propinabe 70% WP, 94.45 percent mycelial growth inhibition was recorded and Neem extract is giving better results than other plants extracts.

Comment [DS37]: Text presentation is not clear; needs rephrasing for better understanding.

Reference

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Comment [DS38]: Should add 5-7 references of the recent years (<5 years). References should be presented adhering to the guidelines of the journal.

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