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

ABSTRACT

Among the five different fungicides and three types of plant bio-extract assessed against *Alternaria alternata*, causing alterneria 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 inMeerutandminimum incidence was recorded(10.9 %)inLakhaoti,Bulandshahrsurvey and metrological data presented in the table.Theincidence of the disease varied from 10.9 to 37.8 percent due to difference inenvironmentalcondition. 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 (Thaiophinate Methyl 70%WP and Carbendazim 50%WP) and Neem give the better results compared to others. Maximum 100% inhibition was recorded in Thaiophinate Methyl 70%WP and 94.45% was recorded Carbendazim 50%WP at 2000ppm 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.

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

1. INTRODUCTION

Brinjal (*Solanum melongena* L.) is an importance 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). 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, lover 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).

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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~~aphrodisiac, ~~cardotonic~~cardiotonic, laxative of inflammation and as an excellent ~~cholestrol~~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 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.)

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Nutritional Value and Composition of Brinjal Fruits			
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. MATERIALAND METHODS

The experiment was done under *In-Vitro*conditions, Department of Plant PathologyLaboratory 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).

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2.1 Collectionof diseases 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 envelops and brought to the laboratory for culturing of pathogen 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 ± 2^0Cfor 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, 20-25conidia 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 ± 2^0C 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

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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 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 25^0C to 28^0C.

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2.4Efficacy of different fungicides and plant bio-extracts

Relative efficacy of Five selected fungicides (Chlorothalonil, Azoxystrobin, Propineb, Carbendazim,Thiophinate 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. 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 25^0C to 28^0C 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 laboratoryevaluation were employed further in two ways, namely seed dressers as well as spray fungicides.

2.5Statistical 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 collectingnatural specimen's severity of the disease on Leaf spot of Brinjal in field during themonth of October to November, 2021-22 at different 4 district of UP. Disease samplewere collected for isolation and further used in different studies. The results of surveyarepresentinTable-1, revealed that the Leaf spot of Brinjalprevalent in all localities of U.P. which are surveyed. The maximum incidence (37.8 %) was recorded, Rajpura inMeerutandlowest(10.9 %)incidencewas found inLakhaoti,Bulandshaher. The highestincidencewasrecord at 28± 2^0Ctemperature with 80-85% Relative Humidityin the months of August- September.Theincidence of the disease varied from 10.9 to 37.8 percent due to difference inenvironmentalconditionandthevarietygrown. The incidence of the disease in the university research area was estimated between 1.0 to 40 %,respectively.

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

Sr.No.	DistrictArea		Averagepercentageof Diseaseincidence%
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

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

Metrological observation during the year-2021							
Months	Rainfall (mm)	Relative humidity (%)			Temperature (^{0}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

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.

3.4Identification and Morphological character

Mycelium, septate, branched, hyaline mycelium later turning to pale olive buff, smooth,3.0-5.3µmwide. Conidiophorewereshortorlong,darkbrown color,bearingsinglebutsometimetwoconidiain chainatthetip and30-132×5.2-7.9µminsizewith 1-8septa. Conidiawerelightdarkbrown,solitarysome timesinshortchain(5-11)inacropetal manner. In shape, the conidia are obclavate but few were oval or pyriformwitharathershort, brodlyroundedbasewith3-8transverseseptaandseverallongitudinalseptawithvariablesizeandshape.Thesizes of conidia were 136-46.3µm×4.6-14.12µm(Av.32.45×11.36µm).

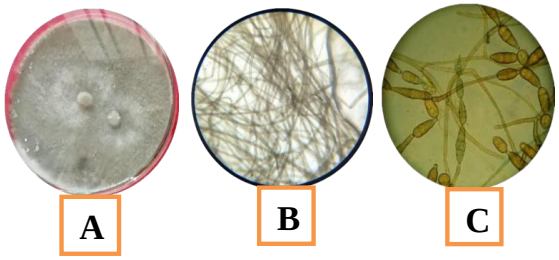
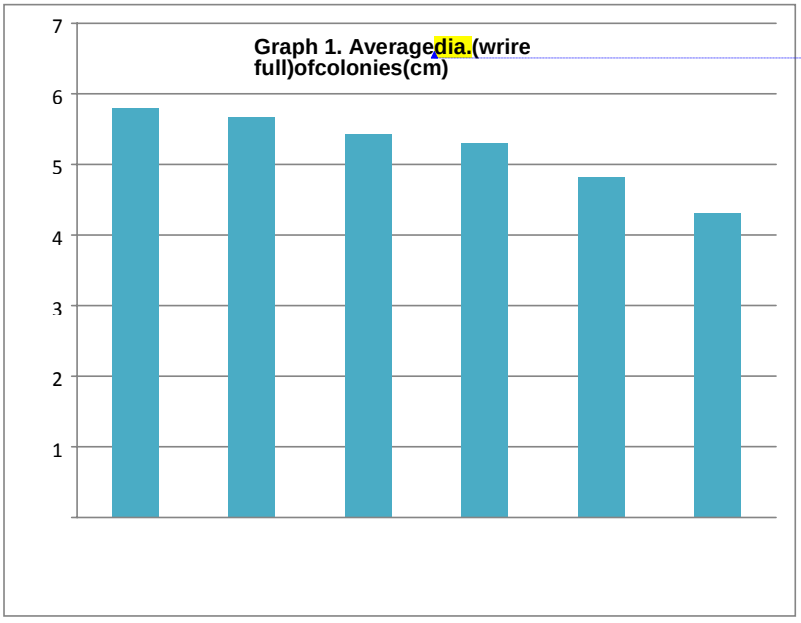


Fig.2 (A) Pure culture, (B) Mycelium microscopic structure, (C) Conidia with Conidiophore.

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**	



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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 daysmaximum inhibition of mycelial growth over control was recorded in Thaiophinate 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%.

Table 3. Inhibitory effect of different fungicides on Mycelial growth of *Alternaria alternata*in –vitro after 8 days inoculation at 28±1^{0}c.

Sr. No.	Treatment	Percent inhibition of mycelium growth Concentration (ppm)			Ave. Dimension of fungal growth	Mean % Inhibition
		500	1000	2000		
1.	Thaiophinate 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 mycilial growth with maximum 100 percent mycilial growth inhibition was recorded in Thaiophinate Methyl 70%WP significant superior over control at 500,1000,2000 ppm concentration and other non-systemic fungicides Propinabe 70% WP, 94.45 percent mycilial growth inhibition was recorded and Neem extract is giving bettrer results then other plants extracts.

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