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Management of Alternaria Leaf Spot of Brinjal (*Solanum melongena*) Caused by *Alternaria alternata* (Fr.) Keissler Through Botanicals (*In vitro*)

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ABSTRACT

The present investigation entitled "Management of Alternaria Leaf Spot of Brinjal (*Solanum melongena*) caused by *Alternaria alternata* (Fr.) Keissler Through Botanicals" was carried out at Veer Chandra Singh Garhwali, Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal during year 2021 – 22. Brinjal is a highly productive crop and usually finds its place as poor man's crop. Fruits are an excellent source of vitamin A, especially those in the 'B' group. Brinjal crop suffers from several diseases caused by various plant pathogens. Alternaria leaf spot caused by *Alternaria alternata* (Keissler) is an imported disease of brinjal crop in India. The result obtained in the present research study maximum mycelium growth of pathogen was observed on Potato dextrose agar media (88.75 mm) at 7th day and among the botanicals, maximum inhibition was recorded in Garlic (51.58 mm) followed by Turmeric (51.03 mm) at 15% of concentration.

Keywords *Alternaria alternata*, Mycelial, PDA, Garlic.

INTRODUCTION

Brinjal (*Solanum melongena* L.), a nightshade crop belonging to the family Solanaceae. It is also known as Egg plant in English and Aubergine in French. Brinjal is said to have originated in India and is the world's second-largest producer, after China. In India Brinjal is cultivated on around 669 thousand hectares, with a yield of 12400 thousand metric tonnes and a productivity of 18.54 metric tonnes per hectare. Major Brinjal producing states are West Bengal, Orissa, Karnataka, Andhra Pradesh, Maharasthra, Bihar and Madhya Pradesh (Kumar *et al*. 2021). In Uttarakhand the area and production of Brinjal is 1685 ha, 26.356 Tonnes respectively. Nutritionally Brinjal have low caloric value, high phenolic contents, vitamins and bioactive compounds. Minerals including K, Ca, Mg, P, Na, Fe, Cu and Zn are also part of brinjal beneficial for human health (Ayaz *et al*. 2015). Brinjal has high moisture contents low caloric value and source of antioxidants like Flavonoids, Anthocyanin, Ascorbic acid, Proteins Polyphenylene Peroxidases (PPO). The brinjal plant includes an alkaloid known as "so-

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lanine," which is present in the roots and leaves and is used in ayurvedic therapies for diabetic patients.

Brinjal crop suffers from several diseases caused by various plant pathogens. Alternaria leaf spot caused by *Alternaria alternata* (Keissler) is an imported disease of brinjal crop in India. This disease is severe and appearing causing heavy loss in fruit yield. *Alternaria alternata* a fungal pathogen responsible of developing small, circular, concentric, brownish dark necrotic spots that extend and cause leaf senescence (El-Gali and ZI 2015). Report of yield losses up-to 25% in Jaipur district due to Alternaria leaf spot and fruit rot of brinjal (Ahir and Basad 2013). For the management of foliage diseases such leaf spot caused by *Alternaria* spp., several effective chemicals/fungicides have been described (Kumar *et al*. 2013). However, due to their high cost, environmental safety, residual dangers, and the development of pesticide resistance in plant diseases, chemicals are not regarded a long-term option. Phytoextracts of higher plant species with proven antifungal action have also been reported efficient against numerous *Alternaria* spp. As a result, finding safe, effective, and environmentally friendly disease management strategies is a top priority in today's intensive agriculture (Sadana and Didwania 2015).

MATERIALS AND METHODS

The present investigation on Study on "Management of Alternaria leaf spot of Brinjal (*Solanum melongena*) caused by *Alternaria alternata* (Fr.) Keissler" was conducted in the Department of Plant Pathology laboratory of College of Horticulture, VCSG, UUHF, Bharsar, Pauri Garhwal, Uttarakhand during March of 2022. The details of the experimental material used and the methodology adopted are described below.

Methodology

In laminar air flow cabinet isolation and cultural studies were carried under aseptic conditions using spirit and flame for sterilizing inoculating needles and forceps tips. The working surface of laminar air flow cabinet was surface sterilized by swabbing with 2% formaldehyde solution or ethanol or spirit. Sterilization of all the glass wares were done in an electronic oven at 180°C for 3 hrs. Sterilization of media was done in an electric vertical autoclave which uses moist heat or steam for sterilization at 121°C with a pressure of 15 lbs for 20 minutes.

Preparation of medium

Potato dextrose agar (PDA) medium was prepared by peeled potato, dextrose and Agar dissolving in 1000 ml of water. The solution was heated completely to dissolve the solid components of the medium and then sterilized by autoclaving.

Collection, isolation and identification of the pathogen

Infected fruit of Brinjal with fruit spot were collected from experimental site, Vegetable Research and demonstration block, College of Horticulture VCSG, UUHF Bharsar, Pauri Garhwal, Uttarakhand and used for isolation of the pathogen. The sample were transferred to laboratory to isolate and identify the pathogen. The sample collected were washed with tap water and air dried and infected lesions with healthy part were cut into small pieces of about 2 cm each and then surface sterilized by immersing in 0.1% sodium Hypochloride solution for 30 seconds. These pieces were thoroughly washed in at least three changes of sterilized distilled water to remove the residue of Sodium Hypochloride and then aseptically the leaf pieces are transferred to Petri plates containing sterile Potato Dextrose Agar medium. After that incubation is done at 25±2°C temperature and observed daily for mycelial growth of the fungus. After one week profuse growth of the fungus was observed.

The morphological characters of the fungus were studied to confirm the identity of the isolated pathogen. The slides were made in water and cotton blue stain, after that fungal growth of the pathogen was examined through the compound microscope at 40X magnification.

Pathogenicity test

The pathogenicity test was conducted in Department of Plant Pathology, laboratory according to Koch's postulates. Healthy host plants were selected and

thoroughly cleaned with sterilized distilled water. The conidia of the test pathogens were taken from freshly prepared ten days old culture and then it were suspended in sterilized water to obtain 10 conidia per ml. Different inoculation methods were applied on potted plants grown in sterilized soil. Within 7-8 days of inoculation leaves showed typical *Alternaria* symptoms. After that re-isolation was done from diseased tissues of artificially infected plants using PDA plate technique. Then the isolate obtained was compared with the original culture for confirmation of same pathogenic isolates which were inoculated.

Preparation of aqueous extract

Healthy and fresh leaves of each plant material i.e., Onion, Turmeric, Aloe vera, Garlic, Datura, Neem and Mint were washed thoroughly in cold running tap water and then air dried separately. Known weight of plant materials were ground using mortar and pestle by adding equal amount of distilled water 1:1 (W/V). The material were homogenized for 5 minutes, filtered through double layered muslin cloth followed by Whatman's No. 1 filter paper and filtrates were considered as standard extract (100%) or stock solution. The appropriate amount of plant extract was mixed in sterilized molten PDA to make desired concentration (V/V) for experiment.

To study growth of pathogen on different media

The variation in cultural characters of *Alternaria alternata* were studied on the following solid media for studying fungal growth. The growth characters of the fungus were studied on five different solid media. All the media were sterilized at 121ºC on 15 pounds pressure for 15 minutes. 20 ml of each of the medium were poured in to 90 mm diameter Petri dishes.

Such plates were inoculated with five mm disc of fungal growth and incubated at 25ºC. Each treatment were replicated four times. The fungal colony measured after 7 days.

In vitro* evaluation of different botanicals against *Alternaria alternata

Seven different botanicals namely - Onion, Turmeric, Aloe vera, Garlic, Datura, Neem, Mint with a control were tested on different per cent concentrations like 5, 10 and 15% by Food Poison Technique and radial growth were recorded at different concentration at 7 days of interval.

Poisoned food technique

The poisoned food technique was adopted for *in vitro* evaluation of botanicals against the test fungus. For bioassay, concentrations of botanicals were prepared by dissolving 5, 10 and 15 ml of stock solution of plant extract in 95, 90, and 85 ml of sterilized PDA respectively to get the final concentration of 5, 10 and 15% each botanical separately. After that the flasks were shaken gently to ensure proper mixing of botanicals in PDA (Nene and Thapliyal 1979).

Then 20 ml of molten PDA was poured in each Petri plate. After solidification of media, mycelial disc of 5 mm diameter were cut from three weeks old culture and inoculated with the help of sterilized cork borer. Incubation of Petri plates was done at 25±2°C. Suitable control were kept in which the culture disc were grown under same condition on PDA without botanicals. The radial colony growth of the fungi was measured at 7 day of incubation. The efficacy of botanicals was expressed as per cent inhibition of mycelial growth over control, calculated by using formula suggested by (Vincent 1947).

Observations recorded

Size of fungal colony (mm) the size fungal colony was observed by the radial growth of fungal colony with the help of measuring scale from two different directions and the mean of the observation considered as a radial growth of colony.

Per cent mycelial inhibition Per cent inhibition in growth was calculated in relation to growth in control using the following formula of (Vincent 1947).

$$PGI = \frac{C - T}{C} \times 100$$

Where
PGI = Per cent inhibition

C = Radial growth of fungus in control
T = Radial growth of fungus in treatment.

RESULTS AND DISCUSSION

Isolation, identification and pathogenicity test of the pathogen

In Table 1 Isolation was made from infected portion of plant isolation was done by tissue isolation technique. Microscopic observation showed that light brown color conidia produced in two to five spores in chain or singly. The pathogenicity of *Alternaria alternata* was confirmed by artificially inoculating the healthy plants. In initial symptoms of Alternaria leaf spot was recorded seven to eight days after inoculation on inoculated leaves and fruits (Devi *et al*. 2016 and Kumar 2017).

Growth of *Alternaria alternata* on different solid media

In Table 2 revealed distinct variation among five different solid media for the growth of the pathogen. On 7th day of incubation maximum growth was observed on Potato dextrose agar (72.62 mm) followed by Oat meal agar (71.25 mm), Czapek's dox agar (70.62 mm), Malt extract agar media (69.12 mm) while minimum radial growth was recorded in Richard's synthetic agar media (67.12 mm) Ginoya and Gobel (2015), Devappa and Thejakumar (2016).

Effects of botanicals on radial growth of pathogen

Table 1. Effect of different inoculation methods on symptoms development of *A. alternata.*

Treatments details	Incubation period (days) ±SE (m)	Types of symptoms
T_1 (Control)	00.00±00.00	No symptoms
T_2 (Soil inoculation)	8.00*±0.40	Brown lesions
T_3 (Foliar spray inoculation)	2.25*±0.47	Brown lesions
T_4 (Syringe inoculation)	5.75*±0.47	Brown lesions
T_5 (Wound inoculation)	6.50*±0.28	Brown lesions
SE (d)	0.53	
$CD_{(0.05)}$	1.14	

Significant at 5% level of significance as compared with control.

Table 2. Effect of different media on mycelial growth (mm) of the pathogen.

Treatments	7 day± SE (m)
T_1 (Potato dextrose agar)	72.62±0.82
T_2 (Richard's synthetic agar)	67.12±0.23
T_3 (Czapek's dox agar)	70.62±0.42
T_4 (Oat meal agar)	71.25±0.32
T_5 (Malt extract agar)	69.12±0.55
SE (d)	0.73
$CD_{(0.05)}$	1.57

*Significant at 5% level of significance as compared with control.

In Table 3 among the seven botanicals the minimum radial growth of *Alternaria alternata* was recorded in T_5, Garlic (34.77 mm) followed by T_3, Turmeric (35.17 mm) T_7, Neem (36.90 mm). The maximum radial growth of fungus was recorded in T_4, Aloe vera (57.35 mm). All the botanicals tested were significant over control Ul-Haq *et al*. (2014) and Jadav and Kadvari *et al*. (2019).

Per cent mycelium inhibition of *A. alternata*

In Table 4 The observations were recorded at 7 days of inoculation. Among seven botanicals the maximum per cent mycelial growth inhibition over control was observed in T_5, Garlic (51.58 mm), followed by T_3, Turmeric (51.03 mm) T_7, Neem (48.62 mm). The minimum mycelium inhibition was recorded in T_4, Aloe vera (20.16 mm) at 15% concentration. All the

Table 3. Effects of botanicals on radial growth (mm) of pathogen at different concentrations (%).

T.No.	Treatments	5%±SE (m)	10%±SE (m)	15%±SE (m)
T_1	Control	71.84±0.34	71.84±0.34	71.84±0.34
T_2	Onion	57.10*±0.20	48.72*±0.08	45.80*±0.12
T_3	Turmeric	50.57*±0.25	48.35*±0.43	35.17*±0.18
T_4	Aloe vera	61.12*±0.18	60.12*±0.28	57.35*±0.42
T_5	Garlic	45.20*±0.50	37.20*±0.12	34.77*±0.12
T_6	Datura	52.15*±0.30	46.07*±0.25	39.92*±0.25
T_7	Neem	49.02*±0.21	45.12*±0.36	36.90*±0.18
T_8	Mint	58.72*±0.26	58.12*±0.08	55.92*±0.15
	SE (d)	0.42	0.39	0.35
	$CD_{(0.05)}$	0.88	0.81	0.72

*Significant at 5% level of significance as compared with control.

Table 4. Effects of botanicals on per cent mycelium inhibition of pathogen at different concentration (%).

Treatments	5%±SE (m)	10%±SE (m)	15%±SE (m)
T_1 (Control)	0.00±0.00	0.00±0.00	0.00±0.00
	0	0	0
T_2 (Onion)	20.51*±0.46	32.16*±0.42	36.24*±0.15
	(26.91)	(34.53)	(37)
T_3 (Turmeric)	29.60*±0.33	32.69*±0.75	51.03*±0.49
	(32.94)	(34.85)	(45.57)
T_4 (Aloe vera)	14.90*±0.54	16.55*±0.31	20.16*±0.53
	(22.69)	(23.99)	(26.66)
T_5 (Garlic)	37.08*±0.67	48.21*±0.41	51.58*±0.25
	(37.49)	(43.95)	(45.89)
T_6 (Datura)	27.40*±0.59	37.00*±1.01	44.42*±0.42
	(31.54)	(37.44)	(41.78)
T_7 (Neem)	31.75*±0.43	37.18*±0.56	48.62*±0.41
	(34.28)	(37.55)	(44.19)
T_8 (Mint)	18.43*±0.65	19.08*±0.48	22.15*±0.34
	(25.4)	(25.88)	(28.06)
SE (d)	0.71 (0.48)	0.80 (0.49)	0.52 (0.32)
$CD_{(0.05)}$	1.48 (1.01)	1.67 (1.03)	1.81 (0.67)

*Significant at 5% level of significance as compared with control
( ) Value in parenthesis are angular transformed.

botanicals were found significant as compared with control.

In the initial stage of infection small, scattered dark brown colored necrotic spots with concentric rings having yellow margin appeared on older leaves. Finally, the spots coallapse which later on resulted in withering and shredding of leaves. The morphological characters of the fungus *A. alternata* were studied regarding its mycelium, conidiophores, conidia from the culture grown on Potato dextrose agar obtained after 7 days of inoculation. The conidiophores of the fungus were light brown and separate measuring 60 - 90 ~m in length and 4-8 ~m in breadth. Conidia were muriform with 1-8 transverse septa, with short beak. Pathogenicity test of the isolated pathogen were carried out and maximum incubation period of symptoms development was observed in Soil inoculation (8.00) and the minimum incubation period was observed in foliar spray inoculation (2.25 days). *In-vitro*, Potato dextrose agar showed maximum growth (72.62 mm) of the pathogen while minimum growth was observed in Richard's synthetic agar (67.12 mm). Among the botanicals, maximum inhibition of mycelial growth was recorded in Garlic (51.58 mm) followed by Turmeric (51.03 mm) at 15% concentration.

CONCLUSION

The Potato dextrose Agar was observed effective for the growth and development of *Alternaria alternata* and the most effective to prevent against this pathogen was Garlic which showed maximum result in inhibition of *Alternaria alternata*. Hence, Ecofriendly management gave good results for the betterment of end users.

REFERENCES

Ahir R, Prasad B (2013) Survey and occurrence of leaf spot of brinjal caused by *Alternaria alternata* (Fr.) keissler in jaipur district. *Adv Life Sci* 2 (1): 71-72.

Ayaz FA, Colak N, Topuz M, Tarkowski P, Jaworek P, Seiler G, Inceer H (2015) Comparison of nutrient content in fruit of commercial cultivars of eggplant (*Solanum melongena* L.). *Polish J Food Nutrition Sci* (65): 251-259.

Devi PA, Mohan SM, Murugapriya M, Kalieswari M, Maharaja N (2016) Morphological and Cultural Characters in Determination of Virulence of *Alternaria helianthi* on Sunflower. *World J Agric Sci* 12 (2): 91-96.

El-Gali, Z I (2015) *Trichoderma harzianum* as a root treatment to control *Alternaria alternata* ceratoni leaf spot on *Ceratonia siliqua*. *Sky J Agricult Res* (4): 161-166.

Ginoya CM, Gohel NM (2015) Cultural and morphological variability among the isolates of *Alternaria alternata* (Fr.) Keissler, incitant of fruit rot of chilli. *Int J Pl Protection* 8(1): 118-125.

Jadav AH, Kadvani DL (2019) Efficacy of different phytoextracts against *Erysiphe cichoracearum* dc causing powdery mildew of okra. *J Pharmacog Phytochem* (8): 538-540.

Kumar M, Bhadauria V, Singh K, Singh C, Yadav AK (2013) Screening of chilli germplasm for resistance to Alternaria leaf spot disease. *Archives Phytopathology Pl Protection* (46): 463-469.

Kumar P (2017) Management of Alternaria leaf spot of brinjal (*Solanum melongena* L.) caused by *Alternaria alternata* (Fr.) Keissler. MSc thesis. SKN College of Agriculture, Jobner, SKRAU, Bikaner, pp 62.

Kumar S, Kansal S, Bhardwaj C, Kaushal S, Dangi R, Thakur A, Karn M (2021) Effect of abiotic factors on progression of Alternaria blight of brinjal. *The Pharma Innovation J* 10(6): 95-100.

Nene YL, Thipliyal PN (1979) Fungicides in Plant Disease Control. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi , pp

325.

Sadana D, Didwania N (2015) Bioefficacy of fungicides and plant extract against *Alternaria solani* causing early blight of tomato. International conference on plant marine and environmental science (1): 38-42.

Thejakumar MB, Devappa V (2016) Efficacy of different fungicides against *Alternaria alternata* and *Cercospora capsici* under in *vitro* conditions. *Int J Adv Biological Biomedical Res* 3(5): 126-129.

Ul-Haq S, Hasan SS, Dhar A, Mital V, Sahaf KA (2014) Antifungal Properties of Phytoextracts of certain medicinal plants against Leaf Spot Disease of Mulberry, *Morus* spp. *J Pl Pathology Microbiol* (5): 1-5.

Vincent JM (1947) The esters of 4-hydroxyl benzoic acid and related compound Methods for the study of their fungistatic properties. *Ind J Sci* (16): 749-755.