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Efficacy of plant extract and organic amendments against *Alternaria porri* (Ellis) Cifferi causing Purple Blotch of onion

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Purple blotch of onion caused by *Alternaria porri* (Ellis) Cifferi is one of the most destructive diseases causing heavy losses under field condition in warm and humid region.The aim of this study was to control the Alternaria leaf spot disease through botanicals and organic amendments. Application of neem leaf extract showed maximum inhibition of mycelial growth (76.31%)at 15% concentration followed by Mint (71.58%), *Aloe vera* (67.96%), Ginger (65.04%), *Lantana* (42.90%) and Rosemary (40.74%). In field evaluation, application of the aqueous extract of Neem at 15% concentration produced the maximum reduction in per cent disease index comprising 57.59%,followed by Mint (58.83), *Aloe vera* (62.39%), Ginger (64.45%), Lantana (66.10%) and Rosemary (67.54%) after 105 days of transplanting.The results of organic amendments indicated that application of cow urine produced maximum inhibition of mycelial growth (64.90%) at 15% concentration followed by neem cake (57.77%), Panchgavya (51.64%), ground nut cake (51.16%), Butter milk (12.11%) and Cow dung (6.78%), respectively. Both in *in vitro* and *in vivo* condition neem leaf extract at 15% concentration has been found effective for the control of *Alternaria porri*.

Keywords : *Alternaria porri*, onion, organic amendments. plant extract, per cent disease index

INTRODUCTION

Onion(*Allium cepa*), is a herbaceous biennial plant that belongs to genus *Allium*, family Alliaceae.It is *Kharif* crop sown during June- July in hilly region and the edible bulb is used as vegetable, spice and has many therapeutic properties and rightly called asthe"Queen of kitchen". Onion is believed to be originated in South-western Asia, being the centre ofdomestication and variability from where it was spread first across the world and has been cultivated for over 4700 years as annuals for bulb production **(**Etana *et al.* 2019).

According to NHB (2017-2018) report, the cultivable area of onionin Uttarakhand is approximately 294.83 thousand ha with a total production of 1712.90 MT.Plant grows best in sunny sheltered position in a rich light with well-drained soil, while cool weather is required at seedling stage and hot dry summer for its bulbs's ripening stage. Long days favor bulb growth (Ulhaq and Sanam, 2015).Onionhas strong, pungent flavor due to the presence of sulphur containing compound, *i.e*., "Allylpropyldisulphide" responsible for distinctive smell and pungency. The important principle components in onion are allicin, ajoene andallixin.

The presence of thiosulfates and sulphites make onion a potent herb. These components help to fight against cancer, high blood cholesterol and sugar, liver problems, rheumatism and intestinal problems if consumed regularly. It also has the pesticidal and fungicidal properties(Mahajan *et al*.2018). Onion contains thiosulphate, a compound that is effective in killing common bacteria *viz., Pseudomonas aeruginosa* (Kumar *et al.* 2010).

Onion crop is infected by several disease-causing pathogens including fungal diseases such as purple blotch, downy mildew, damping off, rust, smut, bulb canker, anthracnose, *Stemphylium*

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blight, neck rot, white rot, basal rot and storage rots; among them purple blotch of onion caused by *Alternaria porri* is one of the most destructive diseases causing heavy loss under field conditions. Nolla (1927) proposed the name "Purple blotch" and he named the causal organism as *Alternaria alli* which was later renamed as *Alternaria porri*. The pathogen belongs to phylum Ascomycotina, Class Dothidiomycetes and Order Pleosporales (Kirk *et al.*2008).The fungal spores germinate on onion leaves and produce small, water- soaked spots that turn brown. The elliptical lesion enlarges, becomes zonate and purplish. The margins may be reddish to purple in colour and surrounded by yellow zone. Mycelial growth of the fungus occurs over a temperature range of 6 to 34°C (optimum 25 to 27°C) at a relative humidity of 90 per cent (Mishra *et al.* 2014).

The pathogen is seed borne and causes 60% loss in bulb yield and the extent of loss depends on crop growth stage and infection time (John *et al.* 2018).

Yield losses occur primarily through loss of leaf tissue and subsequent reduction in the rate of bulb development. Purple blotch is the most important disease in Northern India, which causes considerable loss in seed crop as well as bulb crops. It can cause severe damage and cause losses of about 80 to 85% on the crop by affecting leaves and seed stalk, as obtained from national sample survey 2009-10 (Mishra and Gupta, 2012).Management of disease through chemicals is not always effective and desirable.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 to be 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 (Panchal *et al*. 2009). We are using botanicals and organic amendments which are easily available and have active ingredients for the control of *Alternaria porri*. Our aim is to control *Alternaria porri* through botanicals and organic amendments.

MATERIALS AND METHODS

The present investigation on "Management of purple blotch of onion caused by *Alternaria porri* (Ellis) Cifferi through botanicals and organic amendments was conducted in the Department of Plant Pathology Laboratory and organic block, College of Horticulture (VCSGUUHF), Bharsar, Pauri Garhwal, Uttarakhand during *Kharif* Season 2019. The details of Experimental material used and the methodology adopted are described below.

The entire experiment has two phases- first as *in vitro* and second as *in vivo*.The lab experiment comprises of 6 different plant extracts, T-1 Control,T—2 Neem, T—3 *Aloe vera*, T-4 Rosemary,T-5 Mint,T-6 *Lantana*,T-7 Ginger and secondly six organic amendments- T 1 Control,T-2 Cow urine,T-3 Cow dung,T-4 Panchgavya,T-5 Neem Cake,T-6 Ground nut cake and T-7 Milk, which were in triplicates.

Preparation of aqueous extract: Healthy and fresh leaves of each plant material Viz. Neem, *Aloe vera*, Rosemary,Mint, *Lantana* and Ginger were washed thoroughly in cold running tap water and then air dried separately. Knownweight of Plant material were grouped using Mortar and pestle by adding equal amount of distilled water.The materials were homogenized for five minutes, filtered through double layer muslin cloth followed by Whatman 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.

In-vitro bioassay of botanicals and organic amendments

In-vitro evaluation of botanicals and organic amendments against test fungus was determined by poisoned food technique (Nene and Thapliyal, 1993). For bioassay, the 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 to get the final concentration of 5, 10 and 15% each botanical separately. On the basis of previous study we

have we have selected these concentration. Botanicals were sterilized in autoclave at 121^{0}C for 15 min **(**Wahyu *et al* 2020).For organic amendments bioassay, concentrations were prepared by dissolving 4, 7 and 10 ml of stock solution of organic amendments in 96, 93, and 90 ml of sterilized PDA respectively to get the final concentration of 4, 7 and 10% each separately. Thereafter, the flasks were shaken gently to ensure proper mixing of botanicals and organic amendments in PDA, followed by mixing 20 ml of molten PDA in each Petri plate. After solidification of media, mycelial disc of 5 mm diameter were cut from three-week-old culture and inoculated with the help of sterilized cork borer. Petri plates were inoculated at 25±2^{0}C. Suitable controls were kept in which the culture disc weregrown under similar conditions on PDA without any treatment. The radial colony growth of the fungi was measured after 7 days of incubation. The efficacy of botanicals was expressed as per cent inhibition of mycelial growth over control, calculated by the formula suggested by Vincent (1947).

In-vivo bioassay of botanicals

The concentration of botanicals were prepared by dissolving 150 ml of stock solution of plant extract in 850 ml of distilled water to make the final concentration of 15% for conducting the experiments. Three sprayings of all the treatments were undertaken at an interval of 15 days, starting first spraying at 60 days after transplanting. The experiment was laid in a completely randomized block design with three replications for each treatment during *Kharif* season. One plot per replication was maintained as unsprayed control. The variety used in the research study was African dark red.

Table 1: Rating scale of 0-9 points

Category	Grade/ numeric value	Infected plant part
I	0	Disease free
II	1	1-10
III	3	11-25
IV	5	26-50
V	7	51-75
VI	9	>75

Observations on per cent disease index were recorded at 60, 75, 90 and 105 days after transplanting. Five plants per treatments per replication were selected randomly, tagged and rating of each leaveswas done by using a 0-9 rating scale (Table 1) given by Mayee and Datar (1986).

Per cent disease index was calculated with the help of following formula and the scoring table given by Wheeler, 1969.

$$PDI = \frac{\text{Summation of all numerical rating}}{\text{No. of Plant observed x maximum grade value 9}} x100$$

Statistical analysis

The data obtained for different season during laboratory investigation was analyzed by using standard statistical procedure in the completely Randomized design. The mean value of data were subjected to analysis of variance as described by Gomez and Gomez (1984) by using MS excel and OPSTATE.The data obtained for different season during the field investigation were analysed by using standard statistical procedure in the completely randomized block design(RCBD).The statistical analysis is carried out for each observed character under the study using MS-Excel and OPSTATE.

RESULTS AND DISCUSSION

The effect of certain botanicals on per cent mycelium inhibition of *Alternaria porri* under *in vitro* condition is tabulated in Table 2. The observations were taken after 7 days of inoculation. Per cent mycelium inhibition varied significantly with different botanicals and their concentrations. The results indicated that maximum per cent mycelial growth inhibition over control was observed in T_2 (Neem) at 15% (76.31%) followed by T_5 (Mint) (71.58%), T_3(*Aloe vera*) (67.96%), T_7 (Ginger) (65.04%), T_6 (*Lantana*) (42.90%) and T_4 (Rosemary) (40.74%). All the treatments were found significant as compared to control. This experiment revealed that Neem exhibited positive influence in reducing the growth of the pathogen, followed by Mint. The results obtained are in agreement with the earlier studies of Bhandekar *et al.* (2019); Jhala *et al.* (2017) and Waghe *et al.* (2015) where it was reported that Neem was

Table 1: Effect of botanicals on per cent mycelium inhibition of pathogen at different concentrations

T. No	Treatments	5% ± S.E.(m)	10% ± S.E.(m)	15% ± S.E.(m)
T_1	Control	0.00±0.00	0.00±0.00	0.00±0.00
		(0.00)	(0.00)	(0.00)
T_2	Neem	47.98*±0.87	63.79*±1.85	76.31*±0.53
		(43.82)	(52.99)	(60.85)
T_3	*Aloevera*	39.83*±1.73	54.64*±0.96	67.96*±1.11
		(39.11)	(47.64)	(55.51)
T_4	Rosemary	9.23*±0.59	23.07*±2.22	40.74*±1.25
		(17.66)	(28.64)	(39.64)
T_5	Mint	41.99*±1.90	59.07*±0.80	71.58*±1.46
		(40.37)	(50.20)	(57.78)
T_6	*Lantana*	11.81*±1.55	24.13*±2.56	42.90*±1.87
		(20.01)	(29.34)	(40.89)
T_7	Ginger	36.87*±0.51	53.07*±0.79	65.04*±0.53
		(37.37)	(46.74)	(53.73)
SE(d)		1.72 (1.19)	2.21 (1.45)	1.60 (0.96)
$C.D._{(0.05)}$		3.74 (2.57)	4.79 (3.14)	3.48 (2.09)

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

Table 2. Effect of different botanicals on per cent disease index (PDI) after different days of transplanting (DAT)

T. No.	Treatments	Dose	Per cent disease index			
		(%)	60 DAT	75 DAT	90DAT	105 DAT
T_1	Control	15	28.46*±0.67	38.42*±0.77	54.52*±0.64	69.27*±0.47
			(32.22)	(38.28)	(47.57)	(56.31)
T_2	Neem	15	18.18*±1.03	27.19*±0.46	41.95*±0.83	57.59*±0.77
			(25.21)	(31.41)	(40.35)	(49.34)
T_3	Aloevera	15	21.03*±0.74	32.06*±1.23	46.96*±1.61	62.39*±0.81
			(27.27)	(34.46)	(43.24)	(52.15)
T_4	Rosemary	15	28.01±0.47	36.51*±0.58	50.60*±0.92	67.54±0.54
			(31.94)	(37.16)	(45.32)	(55.24)
T_5	Mint	15	20.39*±0.67	29.85*±0.51	43.55*±0.22	58.83*±1.04
			(26.83)	(33.10)	(41.27)	(50.06)
T_6	Lantana	15	26.42*±0.24	36.05*±0.04	50.22*±1.02	66.10*±1.08
			(30.91)	(36.88)	(45.11)	(54.37)
T_7	Ginger	15	23.35*±1.02	33.77*±1.00	46.02*±0.61	64.45*±0.53
			(28.87)	(35.51)	(42.70)	(53.38)
SE(d)			0.66 (0.47)	0.70 (0.42)	1.18 (0.67)	0.98 (0.58)
$C.D._{(0.05)}$			1.46 (1.03)	1.55 (0.93)	2.60 (1.49)	2.16 (1.29)

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

Table 3 : Effect of organic amendments on per cent mycelium inhibition of pathogen at different concentrations

T. No	Treatments	% Inhibition of mycelia growth mycelial growth at different concentration of treatments		
		4%	7%	10%
T_1	Control	0.00±0.00(0.00)	0.00±0.00(0.00)	0.00±0.00(0.00)
T_2	Cow urine	43.98*±0.63(41.52)	49.56*±1.55(44.73)	64.90*±0.83(53.65)
T_3	Cow dung	0.85*±0.23(5.17)	4.27*±0.54(11.87)	6.78*±2.02(14.71)
T_4	Panchgavya	41.55*±0.48(40.12)	47.71*±0.74(43.67)	51.64*±0.40(45.92)
T_5	Neem cake	43.23*±1.74(41.08)	50.46*±1.75(45.25)	57.77*±1.28(49.45)
T_6	Groundnut cake	29.78*±2.14(33.03)	41.22*±0.86(39.92)	51.16*±1.07(45.65)
T_7	Butter milk	4.06*±1.36(11.20)	6.72*±1.22(14.90)	12.11*±0.45(20.35)
SE(d)		1.70 (1.58)	1.56 (1.18)	1.50 (1.45)
$C.D._{(0.05)}$		3.70 (3.42)	3.39 (2.55)	3.26 (3.14)

Value in parenthesis are angular transformed *Significant at 5% level of significance as compared control ± = S.E.(m)

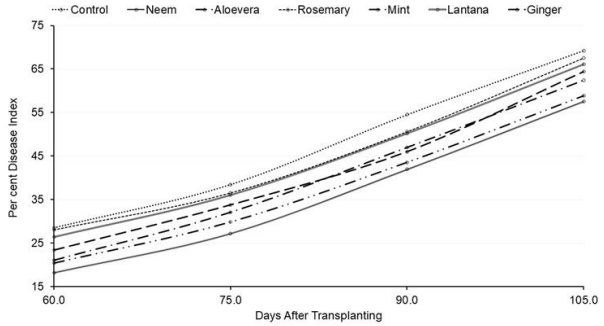

Fig. 1. Per cent disease index as a function of elapsed time

found to be most effective against *Alternaria porri,* followed by Mint. Falake *et al.* (2015) reported that *Azadirachta indica* was found most effective in inhibiting the growth of *Alternaria porri*. Abdul-Hafez *et al*. (2014) also reported that Neem showed maximum mycelial growth inhibition among all treatments against *Alternaria solani.*The most important active constitute is Azadiractine and the others are nimboline, nimbin, nimbidin, which are effective for the control of purple blotch of onion. Mohamad *et al.*(2016). reported that neem and its chemicals play role in scavenging of free radiation generation and prevention of disease pathogenesis.

Table 3 and Fig.1 show the effect of botanicals on per cent disease index of *Alternaria porri* in *in-vivo* condition. It can be deduced from the data that per cent disease severity ranged from 57.59 to 69.27% after 105 days of transplanting. The lowest per cent disease severity was observed in treatment T_2 (57.59%) followed by T_5 (58.83%), T_3 (62.39), T_7 (64.45%), T_6 Lantana (66.10%) and T_4 (67.54%). The highest per cent disease severity was recorded in T_1 Control (69.27%). All the treatments under study were significant as compared to control, except for treatment T_4 (67.54%) which was found statistcally at par with control. In the present investigation, the maximum disease index was recorded in Neem followed by Mint. Neem showed more effectiveness against the pathogen and minimum per cent disease index was recorded in Neem extract sprayed plot. The results obtained are in conformity withAbdul-Hafez *et al.* (2014) who reported that *Azadirachta indica* showed maximum reduction in disease severity of *Alternaria porri* under greenhouse condition. Abdul- Hafez *et al*. (2013) also reported that Neem showed maximum reduction in per cent disease index. Similar results were found by Brahmane *et al.* (2015) reported that under *in vivo* conditions Neem seed kernal recorded mean reduction in per cent disease index over control followed by *Mentha arvensis* against *A. porri*. *Mentha arvensis* consist of menthol which is effective for the control of *Alternaria porri. Mentha arvensis* essential oils is antiinflamtory in LPS stimulating response *via* inhibitory ERK/NE signaling pathway and antiaroberenatis like effect in 2-4 Dithiocarbonate.Singh *et al*. (2021) reported that neem could be used as an excellent product in crop protection for agriculture.

The data of organic amendments on per cent mycelium inhibition of *Alternaria porri* in *in vitro*

condition is tabulated in Table 4. The observations were taken after 7 days of inoculation. The per cent mycelium inhibition varied significantly with different botanicals and their concentrations. The results indicated that maximum per cent mycelial growth inhibition over control was observed in T_2 (Cow urine) at 10% (64.90%), followed by T_5 (Neem cake) (57.77%), T_4 (Panchgavya) (51.64%), T_6 (Groundnut cake) (51.16%), T_7 (Butter milk) (12.11%) and T_3 (Cow dung) (6.78%). All the treatmentswerefound significant as compared with control. This experiment revealed that Cow urine exhibited positive influence in reducing the growth of the pathogen. The result obtained are in agreement with earlier studies of Manasa *et al.* (2014) who reported that Cow urine showed less colony diameter as compared to control in case of *Alternaria helianthi*.Prakash and Sinha (2017) reported that per cent inhibition increases with the increase in concentration of Cow urine. Sharma *et al*. (2010) also reported that Cow urine decreases the percentage of germination with increase in concentration.Komal *et al.*(2002) reported that Ayurveda recommended Panchgavya to treat disease condition ease of multiple system including severe condition.

Among botanicals *Azadirachta indica* plant extract @ 15% concentration showed maximum inhibition of mycelial growth and minimum per cent disease index.Among Organic amendments Cow urine @ 10% concentration showed maximum inhibition of mycelial growth of the pathogen. Botanicals and organic amendments gave good results and are safer alternative to chemical fungicides in managing the disease. But keeping health hazards in view, alternate and eco-friendly methods of disease reducing plant products are reported to be gaining importance in crop protection in view of their selective properties (as insecticides, fungicides and anti-viral) low cost and safely too.Toxic and hazardous to the environment, synthetic fungicides are used to manage crop diseases caused by fungal pathogens and now the trend is turning towards safer, safe and sound eco-friendly management of fungal pathogens. Botanicals and organic amendments are ecofriendly method for the control of *Alternaria porri.*

DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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