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Evaluation of botanicals against the *Colletotrichum capsici* causing anthracnose of Chilli (*Capsicum annum* L.) under lab and field condition

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Abstract
An investigation was carried out at experimental block for chilli crops, Vegetable Research Centre, Pantnagar to evaluate seven botanicals under *in vitro* and best of three botanicals among them were tested under *in vivo* conditions against anthracnose of chilli (*Colletotrichum capsici*).The experiment was conducted during rabi season of 2018 – 2019. Results revealed that among the seven botanicals tested for their efficacy against *C. capsici* under lab condition at 5 per cent concentration neem was found to be superior with maximum growth inhibition of 27.77 per cent of the test pathogen followed by Tulsi (21.87%) as compared to other botanicals whereas minimum growth inhibition of 3.70 per cent was observed in Parthenium. Among the seven botanicals, at 10 per cent concentration Neem (32.96%) with maximum growth inhibition was again found to be superior followed by Tulsi (27.26%) as compared to other botanicals whereas Parthenium (6.30%) showed minimum growth inhibition of the test pathogen. Among the seven botanicals, at 15 per cent concentration Neem (44.21%) was found to be the best followed by Tulsi (42.93%) with maximum growth inhibition whereas drumstick (9.25%) showed minimum growth inhibition. Among the 10 treatments under field condition Neem @ 15 per cent showed minimum PDI (48.88%) followed by Neem @10% (55.28%) and were found to be effective as compare to other treatments. Neem @15 per cent gave maximum yield (13.39q/ha) followed by Neem@10 per cent (13.23q/ha) as compared to other treatments and minimum yield (10.86 q/ha) was recorded in check.

Keywords: Anthracnose, Chilli, PDI and Yield

Introduction
Chilli *(Capsicum annum* L.) is an important spice as well as vegetable crops which are grown globally. It is believed to have originated in Southern American tropics and was first cultivated in Peru at around 7500 BC (MacNeish, 1964) [10]. The introduction of chilli in India goes to Columbus, who brought the seeds from Spain, which subsequently spreading in Europe, Africa and Asia (Heiser, 1976) [6]. It is nutritionally rich in vitamins C and A. It contains l.29 mg protein, l1mg calcium, 870 l.U. vitamin A, l7.5 mg ascorbic acid, 0.06 mg thiamin, 0.03 mg riboflavin and 0.55 mg niacin per 100 g edible fruit (Joshi and Singh, 1975) [7]. It possesses two important chemical compound groups i.e. capsaicinoid alkaloids i. e. capsaicin and carotenoids. India is the largest producer, consumer and exporter of chilli in the world. In India, total cultivated area and production have been estimated 309 Mha and 3592 MT respectively (NHB 2017-18) [12]. In Uttarakhand, the total area and production have been estimated 2762.08 ha and 9158.16 Metric tons respectively (Anonymous, 2018) [2]. Though there is scope for enhancing the production of chilli in the state, biotic factors like diseases hamper their successful cultivation. The major diseases affecting the crop include anthracnose and fruit rot, bacterial wilt, chilli mosaic, mottle and leaf curl are the most serious destructive diseases of chilli (Issac, 1992 and Anand *et al.*, 2010) [1]. Anthracnose of chilli caused by *Colletotrichum capsici* (Syd.) Butler and Bisby has been considered as a most notorious pathogens worldwide causing economically important disease anthracnose (die-back, ripe fruit rot and leaf spot) in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits (Bailey and Jeger, 1992; Dean *et al.* 2012 and Freeman *et al.*1998) [3, 4, 5]. The disease intensity in Uttarakhand has been found to be increasing due to changing environmental conditions and has become a concern for the farmers. Since the use of plant botanicals is the most efficient, non-hazardous, environmentally safe and economical way to manage plant diseases, the present study was undertaken to evaluate the different kinds of

botanicals against anthracnose disease of chilli under both *in vitro* and *in vivo* conditions as a management point of view.

Materials and Methods

Collection, isolation, identification, purification, maintenance and pathogenicity of the pathogen

Freshly infected fruits of chilli exhibiting typical symptoms of anthracnose (fruit rot) were collected from Vegetable Research Centre in a paper bag and brought to the laboratory for microscopic examination, isolation and further studies. Infected diseased samples were cut transversally into small pieces of 2-3 mm size with the help of sterilized sharp blade. The small pieces were sterilized with one per cent of sodium hypochlorite solution for 30 seconds and thoroughly washed three times in sterilized distilled water. Later on the pieces were transferred on to sterilized blotter paper to remove excess moisture from the samples. These pieces were then transferred to petriplates containing PDA medium under aseptic conditions followed by incubation at 28±2ºC.The isolated fungus was identified as *Colletotrichum capsici* on the basis of morphological characteristic such as presence of acervuli, setae and conidia. Slide of the diseased sample were also prepared in cotton blue and lacto phenol and examined under compound microscope (10X, 40X and 100X) and photomicrograph was taken. The pure culture of the pathogen was obtained by single hyphal tip isolation technique. Pure cultures were maintained on PDA slants at 4ºC in refrigerator and sub cultured on petri plates containing potato dextrose agar medium for further experiments. Sufficient care should be taken to maintain genetic purity of isolates throughout the study. The pathogenicity of the pathogen was determined by Pin Prick Method described by Singh and Kaur (1990) [16] under *in vitro* condition. Susceptible cultivar (Pant C-1) was selected for pathogenicity test. The spore suspensions of the pathogen were obtained by adding 10 ml of sterilized distilled water to 10 day old culture grown on PDA in 30 ml test tube, maintaining the spore concentration of $1X10^6$ spores / ml. Three chilli fruits collected from the field were surface sterilized with 1 per cent sodium hypochlorite solution for 30 seconds and washed thrice with sterile distilled water. These chilli fruits were air dried by placing on sterilized blotting paper and subsequently inoculated with spore suspension of isolated fungus. In Pinprick method, the sterilized needles were used and two pricks were given on the fruit prior to the inoculation through drop of spore suspension of the pathogen. The inoculated fruits were placed in moist chambers and incubated at 25±2°C. The disease development was recorded by measuring lesion length of the diseased portion on the chilli fruit after 8 days of inoculation. Re-isolation was made from infected fruit parts and compared with original cultures thus confirming koch's postulates.

Preparation of aqueous extract

Seven botanicals i.e. Neem, Marigold, Tulsi, Congress grass, Lantana, Eucalyptus and Moringa were used against the test pathogen for evaluating their efficacy. Leaves of these seven botanicals were collected from Pantnagar and three concentrations 5, 10 and 15 per cent were used for their evaluation against the *C. capsici* both *in vitro* and *in vivo*. Healthy and fresh leaves of selected botanicals were taken and washed with tap water followed by sterile distilled water and then dried in room temperature. After removal of water these are chopped into small bits with sterilized sharp knife. Then each leaf sample was separately grinded and homogenized in mechanical grinder with equal quantity of sterile distilled water (1:1 w/v). The obtained homogenate was strained through double layered sterilized muslin cloth followed by filtration through Whatman filter paper No.l using volumetric flasks (50 ml capacity). The obtained clear leaf extracts serve as the standard/stock solution of 100 per cent and subsequently 5, 10 and 15 per cent were formed.

In vitro evaluation of botanicals against *C. capsici*

In vitro evaluation of botanicals was done through Poisoned Food Technique with Completely Randomized Design as described by Nene and Thapliyal (1993) [13]. The details of treatment for *in vitro* evaluation of botanicals were listed in table2. An appropriate quantity of each leaf extract was mixed separately in the molten and cooled PDA medium in conical flask (250 ml capacity) to get desired concentrations (5, 10 and 15 per cent) of each extract. On solidification of PDA in petri plates, all the plates were inoculated aseptically by placing 5.0 mm mycelial disc of 7 days old culture of *C. capsici* in the centre. Petri plates containing PDA without plant extract serve as a control. Each set of treatment were replicated thrice and inoculated plates were incubated at 28 ± 2°C in BOD incubator till the control plates fully covered with mycelial growth of the test pathogen. Observations on radial mycelial growth of the pathogen were recorded in each treatment and per cent growth inhibition of the test pathogen over control was calculated by following formula (Vincent, 1947) [18].

$$\text{Per cent inhibition (PI)} = \frac{C-T}{T} \times 100$$

Where
PI= % of inhibition over control,
C= diameter of fungal growth in control plate
T= diameter of fungal growth in test plate.

In vivo evaluation of botanicals against *C. capsici*

A field trail was conducted at Vegetable Research Centre in 2018-19 to evaluate the botanicals against *C. capsici* under *in vivo* condition. The experiment was laid down in Randomized Block Design with ten treatments along with three replications. Three best botanicals at all the three concentration (5, 10 and 15 per cent) were tested among the seven treatments which were tested under *in vitro* condition. A total of nine treatments along with check (water spray) were tested. Botanical extracts were prepared as described above at three concentration *viz*. 5, 10 and 15 per cent. First foliar spray was given just after appearance of the disease symptoms followed by second foliar sprays after 10 days. Observations were taken at 10 days after each spray. The severity of the diseases was recorded on randomly selected 5 plants from each plots with five fruits were selected from each plants. Further, Percent Disease Index was recorded on the basis of 0 to 9 disease rating scale in table1 (Mayee and Datar, 1986) [11]. After last spray, yield q/ha, per cent disease over control and per cent yield increase over control were calculated. Percent Disease Index (PDI) was calculated by formula described by (Wheeler, 1969) [19]. Data analysis was performed by STPR software.

$$\text{PDI} = \frac{\text{Sum of all disease rating}}{\text{Total no of fruits observed} \times \text{Maximum disease grade}} \times 100$$

Per cent disease control over check calculated by formula described by (Singh *et al.*, 2014) [17]

$$\text{Per cent disease control over check} = \frac{\text{Disease severity in check} - \text{Disease severity in treatment}}{\text{PDI in check}} \times 100$$

Per cent yield increase over check was calculated by using the following formula

$$\text{Per cent yield increase over check} = \frac{\text{Yield(Q/ha)in treatment} - \text{Yield(Q/ha)in check}}{\text{Yield(Q/ha)in treatment}} \times 100$$

Table1: Disease rating scale for anthracnose disease described by (Mayee and Datar, 1986) [11].

Category	Grade/ Numerical value/ Disease rating scale	Per cent fruit area covered (%)
I	0	No infection
II	1	Slightly -10
III	3	11-25
IV	5	26-50
V	7	51-75
VI	9	>75

Table 2: List of botanicals tested against the *C. capsici*

S. No.	Common name	Botanical name	Family
1	Neem	*Azadirachta indica*	Meliaceae
2	Marigold	*Tagets* spp.	Asteraceae
3	Congress grass	*Parthenium hysterophorus*	Asteraceae
4	Tulsi	*Ocimum sanctum*	Labiatae
5	Nilgiri	*Eucalypts*. Spp	Myrtaceae
6	Drumstick	*Moringa oleifera*	Moringaceae
7	Ghaneri	*Lantana camara*	Verbenaceae

Results and Discussion

The experimental results revealed that among the seven botanicals viz. Neem, Tulsi, Marigold, Parthenium, Eucalyptus, Drumstick and Lantana under *in vitro* condition (Table3, plate-1), Neem was found to be effective at all concentrations and showed growth inhibition percent at 15, 10 and 5 percent was 44.21, 32.96 and 27.77 percent respectively followed by Tulsi with growth inhibition per cent 42.93, 27.26 and 21.87 at 15, 10 and 5 per cent concentration respectively. The minimum growth inhibition was recorded in case of the Lantana with 10.75, 16.30 and 18.52 per cent at 5, 10 and 15 per cent conc. respectively. The result obtained in the present investigation are in accordance with the findings of Kumar *et al.* (2015) and that the plant extract of neem and garlic at 4 per cent showed completely inhibited the mycelial growth of *C capsici*. Rahman *et al.* (2011) reported that 100 percent inhibition of conidial germination and shortest germ tube formation of *C. capsici* in *Azadiracta indica* (leaf), *Ocimum sanctum* (leaf) and *Curcuma longa* (rhizome) extracts after 4 to 24 hours of incubation. Raj *et al.* (2013) reported that Tulsi at 10 per cent followed by Adathoda (*Adathoda vesica*) at 10 per cent were found to be effective and gave 100 per cent mycelial inhibition of *Colletotrichum capsici* at all the concentration. Neem plants contains chemical compounds such as azadirachtin, nimbecedine and nimbin which are active triterpenoid compound having antimicrobial properties.

Table 3: *In vitro* evaluation of botanicals against *C. capsici*

S. No.	Treatments	Radial growth (mm)			Growth inhibition (%)		
		5%	10%	15%	5%	10%	15%
1	Neem	65.01	60.33	50.21	27.77	32.96	44.21
2	Tulsi	70.31	65.47	51.36	21.87	27.26	42.93
3	Marigold	79.66	70.52	62.33	11.48	21.64	30.74
4	Parthenium	86.67	84.33	73.31	3.70	6.30	18.54
5	Eucalyptus	79.50	74.34	73.32	11.67	17.40	18.53
6	Drumstick	85.12	83.34	81.67	5.42	7.40	9.25
7	Lantana	80.32	75.33	73.33	10.75	16.30	18.52
8	Check	90.00	90.00	90.00	0.00	0.00	0.00
	Botanical (B)	Concentration (C)			B*C		
S.E.m±	0.065	0.0395			0.111		
CD at 5%	0.183	0.112			0.318		
CV	2.56						

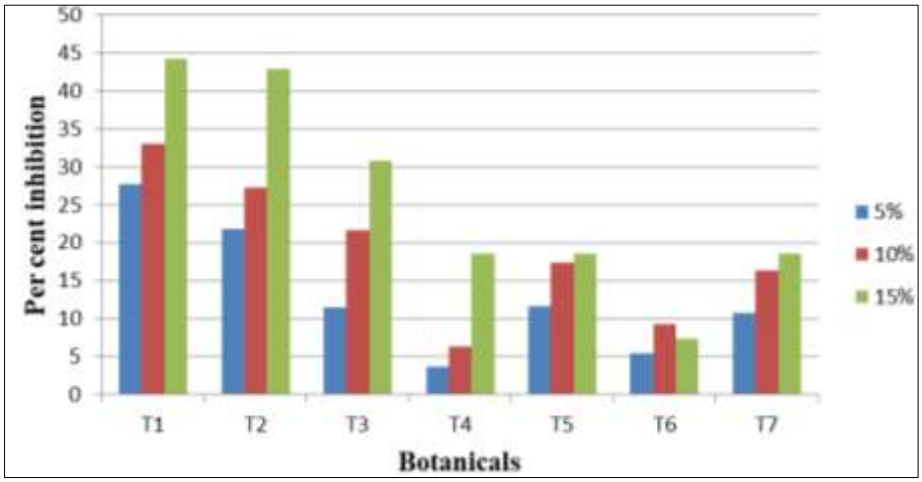


Fig 1: *In vitro* evaluation of botanicals against *C. capsici*

The data presented in (Table4, plate2, figure2) revealed that at 5% concentration Neem showed PDI of 39.79 per cent followed by Marigold with PDI of 38.40 per cent and Tulsi showed PDI of 40.45 per cent after first spray. Neem exhibited 64.90 per cent of PDI followed by Marigold with PDI of 65.81. Tulsi showed disease PDI of 69.13 at same concentration after second spray. Maximum per cent disease control of 13.12 per cent was recorded in case of Neem followed by Marigold with 12.03 and Tulsi showed per cent disease control of 7.58 per cent. Maximum yield of 12.13 q/ha was recorded in Neem followed by Marigold with 11.89 q/ha and Tulsi with 11.78 q/ha. Per cent yield increase over check of 10.19 per cent was recorded in case of Neem which was maximum among 5 per cent concentration followed by Marigold with 8.08 per cent and Tulsi with 3.38 per cent only. At 10% concentration, Neem exhibited PDI of 25.39 per cent followed by Marigold with PDI of 40.09 per cent and Tulsi showed PDI of 23.65 per cent after 1st spray. PDI of 55.28 per cent was recorded in Neem followed by Marigold with PDI of 61.39. Tulsi showed PDI of 64.06 at same concentration after second spray. 19.40 per cent disease control was recorded in Neem followed by Marigold with 17.48 and Tulsi with 14.37 per cent. 13.23 q/ha yield was recorded in Neem followed by Marigold with 12.73 q/ha and Tulsi with 11.78 q/ha. 23.84 per cent yield increased over check was recorded in Neem followed by Marigold with 17.13 per cent and Tulsi with 11.75 per cent. At 15 per cent concentration Neem showed minimum PDI of 21.73 per cent followed by Marigold with PDI of 29.41 per cent and Tulsi showed PDI of 40.26 per cent after 1st spray. Minimum PDI of 48.88 per cent was recorded in Neem followed by Marigold with 60.50 per cent PDI. Tulsi showed PDI of 63.38 at same concentration after second spray. Maximum per cent disease control of 23.51 per cent was recorded in Neem followed by Marigold with 19.13 and Tulsi showed 15.27 per cent disease control. Maximum yield of 13.39 q/ha was recorded in Neem followed by Marigold with 12.99 q/ha and Tulsi with 12.65 q/ha. Yield increase over check was recorded in Neem (29.68 %) which was maximum followed by Marigold with 21.52 per cent and Tulsi with 15.46 per cent.

Table 4: *In vivo* evaluation of botanicals against anthracnose disease of chilli

Treatment	Per cent Disease Index		Percent Disease Control	Yield (q/ha)	Percent Yield Increase
	10 days after I spray	10 days after II spray			
Neem @5%	39.79 (39.10)*	64.90 (53.68)*	13.12	12.13	10.19
Tulsi @5%	40.45 (39.47)	69.13 (56.29)	7.58	11.78	3.38
Marigold @5%	38.40 (38.28)	65.81 (54.27)	12.03	11.89	8.08
Neem @10%	25.39) (30.23)	55.28 (48.04)	19.4	13.23	23.84
Tulsi@10%	23.65 (29.08)	64.06 (53.19)	14.37	12.35	11.75
Marigold@10%	40.09 (39.27)	61.39 (51.61)	17.48	12.73	17.13
Neem@15%	21.73 (27.76)	48.88 (44.35)	23.51	13.39	29.68
Tulsi@15%	40.26 (39.38)	63.38 (52.84)	15.27	12.65	15.46
Marigold@15%	29.41 (32.78)	60.50 (51.08)	19.13	12.99	21.52
Check	50.01 (44.99)	77.14 (61.50)	-	10.86	-
SEm±	1.75	3.24	-	1.17	-
CD at 5 %	5.13	9.51	-	3.43	-
CV	8.10	8.59	-	15.49	-

***** Value shown in parenthesis represents angular transformed values.

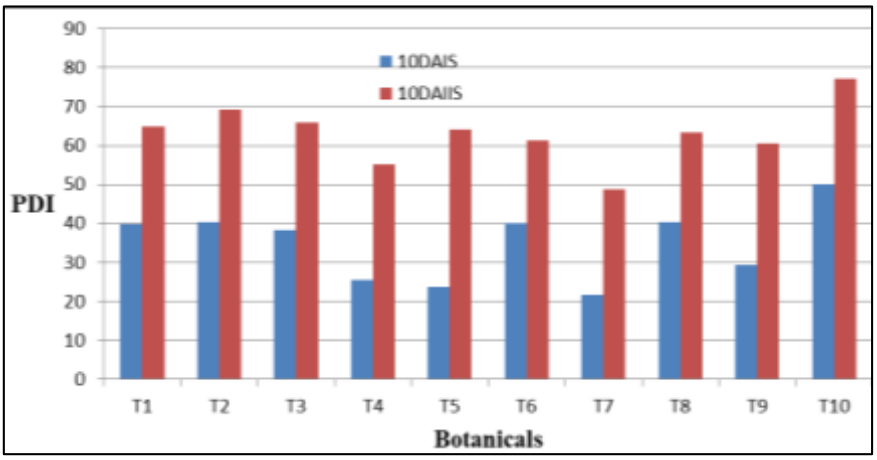
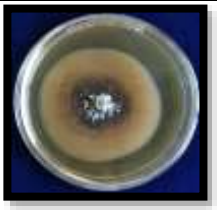
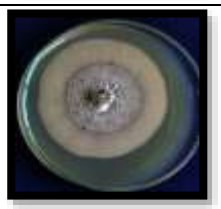


Fig 2: *In vivo* evaluation of botanicals against anthracnose disease of chilli showing Per cent Disease index (PDI)

	Concentration			
Name of botanicals	**5%**	**10%**	**15%**	**Control**
Neem				
Tulsi				
Marigold				
Parthenium				
Eucalyptus				
Lantana				

Drumstick				

Plate 1: *In vitro* evaluation of botanicals against *Colletotrichum capsici*

				
Field plots	**Die-back symptoms**	**Symptoms on fruits**	**Check plots**	**Treated plots**

Plate 2: *In vivo* evaluation of botanicals against *Colletotrichum capsici*.

Summary and conclusion

Thus, it can be concluded that among the 10 treatments under field condition Neem @ 15 per cent showed minimum PDI (48.88%) followed by Neem @10% (55.28%) and were found to be superior as compare to other treatments. Neem @15 per cent gave maximum yield (13.39q/ha) followed by Neem@10 per cent (13.23q/ha) as compared to other treatments and minimum yield (10.86 q/ha) was recorded in check. Neem extracts are good alternative to synthetic pesticides because of their easily available, environmently safe, cost effective, low to moderate mammalian toxicity. Though Neem based products have been successfully used for pest control in agriculture since long, the registered neem products for control of pathogens or disease vectors affecting human, still need to be explored. It is suggested that the further researches on neem and Tulsi as a potent botanical for the chilli anthracnose should be focused on its active molecule identification, quantification and also its biochemical effect on the pathogen is required.

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