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Studies on antifungal properties of some plant extracts agaisnt anthracnose of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsic*

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SUMMARY

To study the verious antifungal properties of ten medicinal plant extracts against anthracnose of chilli caused by *Colletotrichum capsici* . The antifungal properties of plant species *viz.*, *Cassia fistula, Callistemon lanceolatus, Duranta* spp., *Delonix regia, Lantana camera, Pongamia pinnata, Nerium indicum, Tagetus erecta, Eucalyptus citriodora, Azadirachta indica* was tested by poisoned food technique . Out of above ten plant extracts, *Callistemon lanceolatus* was found most effective in inhibiting mycelial growth of *C. capsic* under *in-vitro* condition. From the pot culture experiment, the antifungal properties of *Callistemon lanceolatus* were tested after extracting in 10 per cent concentration of five solvents *viz.*, acetic acid, acetone, ethanol, petroleum ether and chloroform alongwith distilled water as sixth solvent. Among the six solvents used for extraction of antifungal properties of *Callistemon lanceolatus* at 1:100 dilutions acetic acid showed minimum disease incidence and intensity of anthracnose of chilli under greenhouse condition.

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Anthracnose of chilli caused by *Colletotrichum capsici* , a coleomycetous fungus has been reported to be the most serious and destructive disease in the chilli growing areas of the country thereby causing substantial quantitative and qualitative losses. Keeping in mind, the economic importance of the disease, this disease can be controlled by using chemical fungicides. However, the indiscriminate use of chemicals is hazardous to microbial population, living beings and it would also lead to a serious soil and water pollution .Chilli is also used for direct consumption. Spraying of fungicides will cause residual effects. Hence, to find out alternative mean to chemical fungicides, botanical pesticides or biological agents should be used. With a view to identify effective plant extracts against *Colletotrichum capsici*, the causal organism of Anthracnose of chilli, present investigations were undertaken during *Kharif*, 2005 in laboratory with objective, screening of plant extracts for antifungal properties *in vitro* and effect of dilution of plant extracts for antifungal properties under greenhouse condition.

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MATERIALS AND METHODS

The pure culture of the pathogen isolated from ripened diseased fruits showing typical symptoms of anthracnose like circular, sunken with black margin spot covered with a pinkish mass of fungal spores and concentric markings with dark fructifications representing the fungal acervuli on common laboratory culture medium potato dextrose agar (PDA). Isolated and purified pathogen was sub-cultured on P.D.A. slants and kept at 28 $\pm$ 1^0 C for seven to eight days for good growth. Such slants were preserved in the refrigeration at 5 to 10^0C and the isolate was sub-cultured once in a month and also used for *in vitro* studies. The crude leaf extracts of *Cassia fistula, Callistemon lanceolatus, Duranta* spp., *Delonix regia, Lantana camera, Pongamia pinnata, Nerium indicum, Tagetus erecta, Eucalyptus citriodora, Azadirachta indica* were prepared by crude extraction method and used for screening. In this method, ten g of the plants were weighed and thoroughly washed. The plant material was then crushed in the mortar and pestle by adding 10 ml of sterilized water. After that the crushed material was strained through double layered muslin cloth and filter paper (Whatman No. 1) and the filtrate obtained was used in

the experiment. The crude extracts of ten plants were tried against the test fungus, by applying the poisoned food tests. The crude plant extracts were supplemented to sterilized PDA in 1:2 proportion and the poisoned medium was poured in Petriplates of 9 cm diameter. A 5 mm mycelial disc of test fungus taken from the periphery of a 7-10 days old culture with the help of sterilized cork borer and transferred in the center of poisoned PDA medium. The control sets were run side by side using sterilized distilled water instead of plant extracts. The Petriplates were incubated at room temperature (26-28^{0}C). On the 8th day diameter of mycelial growth was recorded. The per cent inhibition of the mycelial growth was calculated by the formula of Vincent (1927) as :

$$I = \frac{(C - T)}{C} \times 100$$

where,

I = Inhibition of mycelial growth.
C = Mycelial growth in control.
T = Mycelial growth in treatment

The leaf extracts of *Callistemon lanceolatus* were prepared by solvent extraction method and used for screening. In this method, ten grams weighed plant material was surface sterilized by 1% $HgCl_2$ and at last washed by sterilized water and crushed in mortar and pestle. The pulp was taken in conical flask and to it acetone (extra pure) was added in 1:4 proportion (w/v). A cork with a refluxing glass tube (1 mm diameter and 50 cm height) was fitted to the flask and they were made airtight with plaster of paris. These flasks were held in water bath at 60^{0}C temperature for one hour for refluxing. As the acetone was evaporated acetone free extract were filtered through filter paper (Whatman No.1). This filterate was used in food poisoned tests.By employing the same methodology the ethanol, acetic acid, chloroform and petroleum ether extracts were obtained. Five solvents *i.e.* acetone, acetic acid, chloroform, ethanol and petroleum ether at 10% concentration along with distilled water as sixth solvent were used. Separate sets of extracts of bottle brush mixed in above 10% solvents at 1:100 dilutions were made. Also instead of using solvents sterilized water mixed in extracts of bottle brush at 1:100 dilutions were made for pot culture experiment. The pots under experiment were observed daily for reading the first incidence of anthracnose under glasshouse condition. Immediately after appearance of disease, spraying of extract of *Callistemon lanceolatus* extracted in six different solvents at 1:100 dilution was carried out. Subsequent two sprays were carried out at an interval of 15 days.

For disease incidence number of plants infects was recorded periodically and the percentage disease incidence was calculated by following formula:

$$\text{Percentage disease incidence} = \frac{\text{Number of infected leaves/plant}}{\text{Total number of leaves/plant}} \times 100$$

The first observation was recorded on the same day on the appearance of disease symptions and subsequent three observation were recorded at an interval of 15 days. Three branches of each plant, one each from top, middle and bottom were tagged for recording observations. Each branch having six leaves were examined individually and their intensity of disease was recorded separately.

Percentage of disease intensity was carried out for each plant and then for each treatment.

Percentage disease intensity (P.D.I) was calculated on the basis of formula adopted by Horsfall and Barratt (1945).

$$PDI = \frac{\text{Total numerical scale}}{\text{Number of leaves examiend x Maximum scale}} \times 100$$

RESULTS AND DISCUSSION

The results regarding , effect of antifungal property from crude extracts of ten plants on mycelial growth of *C. capsici* are given in Table 1. From result it was observed that amongst the ten extracts, the extracts of

Table 1 : Effect of crude extracts of ten plants on mycelial growth of the test fungus, *C. capsici* (On solid media)

Sr. No.	Plant extracts used	Mycelial growth of *C. capsici* on solid media	
		Diameter in cm	Per cent inhibition over control
1.	*Cassia fistula*	6.5	27.77
2.	*Callistemon lanceolatus*	0.6	93.33
3.	*Duranta spp.*	5.4	40.00
4.	*Delonix regia*	4.6	48.88
5.	*Lantana camera*	6.4	28.88
6.	*Pongamia pinnata*	3.6	60.00
7.	*Nerium indicum*	6.9	23.33
8.	*Tagetus erecta*	6.0	33.33
9.	*Eucalyptus citriodora*	5.6	37.77
10.	*Azadirachta indica*	4.5	50.00
11.	Control	9.0	0.00
	S.E. $\pm$	0.1134	
	C.D. (P=0.05)	0.3320	

C. lanceolatus was found to be the most effective by recording inhibition of mycelial growth up to 93.33 % which was followed by *P. pinnata* (60%). The per cent inhibition of mycelial growth recorded in other extracts as per descending order were *Azadirachta indica*(50%) and *Delonix regia* (48.88%) which were at par with each other. The extracts of *Eucalyptus citriodora* (37.77%), *Tagetus erecta*.(33.33%), *Lantana camera* (28.88%) and *Cassia fistula* (27.77%) were at par with each other. The maximum mycelial growth of test fungus was observed on control treatment .

The results regarding incidence are given in Table 2. From results it was observed that the disease incidence of chilli antracnose before spraying of extracts of *C. lanceolatus* was more or less same in all treatments ranging from 26.64 to 26.85 per cent and were found statistically non-significant among all the treatments. Among, the solvents used for extraction of plant extracts, acetic acid showed minimum incidence of disease as compared to others. Ethanol and petroleum ether used for extraction of *C. lanceolatus* were at par with each other at Ist, IInd and IIIrd sprays of both plant extracts. Also acetone and chloroform extracts of *C. lanceolatus* were at par with each other. Untreated plants showed maximum incidence of disease with increasing rate at every subsequent observation. After every spray of plant extracts disease incidence was found to be decreasing.

From the results presented in Table 3 it was seen that before spraying the disease intensity percentage was more or less same in all the treatments, ranging from 7.71 to 9.98 per cent and was found statistically non-significant among the treatments.

From the data, it was observed that extract of *C. lanceolatus* extracted in acetic acid was significantly superior over all the treatments in checking the spread of disease and had shown minimum disease intensity *i.e.* 10.14, 9.1 and 7.71 per cent observed after IInd, IIIrd and IVth, respectively. The maximum rate of increase in disease intensity from 9.98 to 19.32 per cent was noticed in untreated pots. In IInd and IIIrd observation extracts of *C. lanceolatus* in acetone, ethanol, petroleum ether and chloroform showed same effect in checking the spread

Table 2 : Effect of different solvents used for extraction of antifungal property from *C. lanceolatus* at 1:100 dilution on disease incidence (%) of chilli anthracnose under glasshouse condition

Sr. No.	Solvents used	Ist observation	IInd observation	IIIrd observation	IVth observation
1.	Acetic acid	20.13 (26.64)	18.86 (25.77)	17.60 (24.80)	16.70 (24.12)
2.	Acetone	20.13 (26.64)	24.90 (29.93)	23.30 (28.86)	22.90 (28.59)
3.	Ethanol	20.33 (26.78)	19.06 (25.92)	17.80 (24.95)	16.23 (23.73)
4.	P. ether	20.33 (26.78)	19.46 (26.21)	18.00 (25.10)	17.30 (24.58)
5.	Chloroform	20.40 (26.85)	31.10 (28.73)	23.01 (28.73)	21.23 (27.42)
6.	Distilled water	20.13 (26.64)	25.04 (30.26)	25.26 (30.20)	24.23 (29.47)
7.	Control	20.27 (26.78)	32.83 (34.94)	38.09 (38.50)	49.00 (44.43)
	S.E. $\pm$	NS	0.33	0.58	0.50
	C.D. (P=0.05)	-	1.0	1.75	1.436

NS=Non-significant

Table 3 : Effect of different solvents used for extraction of antifungal property from *C. lanceolatus* at 1:100 dilution on disease intensity (%) of chilli anthracnose under glasshouse condition

Sr. No.	Solvents used for condition of *C. lanceolatus*	Ist observation	IInd observation	IIIrd observation	IVth observation
1.	Acetic acid	1.85 (7.71)	3.10 (10.14)	2.50 (9.10)	1.85 (7.71)
2.	Acetone	1.85 (7.71)	4.30 (11.97)	3.70 (11.09)	3.90 (11.39)
3.	Ethanol	2.50 (9.10)	3.70 (11.09)	3.10 (10.14)	2.50 (9.10)
4.	P. ether	2.50 (9.10)	4.30 (11.97)	3.10 (10.14)	2.50 (9.10)
5.	Chloroform	2.50 (9.10)	3.70 (11.09)	3.10 (10.14)	2.50 (9.10)
6.	Distilled water	2.50 (9.10)	3.70 (11.09)	4.30 (11.97)	3.70 (11.09)
7.	Control	3.08 (9.98)	7.50 (15.89)	9.90 (18.34)	11.12 (19.32)
	S.E. $\pm$	NS	0.89	0.93	0.93
	C.D. (P=0.05)	-	2.40	2.82	2.80

NS=Non-significant

of disease *i.e.* they were at par with each other, while in IVth observation extracts of ethanol, petroleum ether and chloroform were found at par with each other.

The pot culture studies revealed that spraying of plant extracts significantly reduced the disease incidence and intensity of anthracnose of chilli. Among the solvent used acetic acid extracts showed minimum disease incidence and intensity of anthracnose of chilli. Variable disease reactions and varied degree of disease incidence in chillies against *C. capsici* were reported earlier by several research workers. (Mah, 1985; Palarpawar and Ghurde, 1989; Kaur and Singh, 1990; Opina, 1994; Basak 1997; Jayalakshmi and Seetharaman, 1998.)

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