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Antifungal properties of plant extracts against anthracnose of chilli caused by *Colletotrichum capsici*

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SUMMARY

To study the antifungal properties of two plant extracts at different dilutions against anthracnose of chilli caused by *Colletotrichum capsici* under *in vitro* condition. The antifungal properties of plant species *viz.*, *Callistemon lanceolatus* and *Pongamia pinnata* were tested after extracting in 10 per cent concentration of five solvents *viz.*, acetic acid, acetone, ethanol, petroleum ether and chloroform along with distilled water as sixth solvent. Among the six solvents used for extraction of antifungal properties of *Callistemon lanceolatus, Pongamia pinnata* separately and combination of both *C. lanceolatus* and *P. pinnata* at 1:10, 1:100 and 1:1000 dilutions acetic acid showed complete inhibition of mycelial growth of *Colletotrichum capsici.*

Key words : Plant extracts, Anthracnose, Chilli

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 to chemical fungicides, botanical pesticides or biological agents should be used. With a view to identify effective plant extracts against *Colletotrichum capsici*, present investigations were undertaken during *Kharif*, 2005 in laboratory with the objective, to study effect of plant extracts for antifungal properties.

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^{0}C and the isolate was sub-cultured once in a month and also used for *in vitro* studies. The leaf extracts of *Callistemon lanceolatus* and *Pongamia pinnata* 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 washed by sterilized water and crushed in mortar and pestle after addition of ten ml of either diluted solvent or distilled water. 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 evaporation of solvent. As the acetone was evaporated, acetone free extract was 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 and extract of Karanj mixed in above 10% solvents at 1:10, 1:100 and 1:1000 dilutions were made. Also instead of using solvents sterilized water mixed in extracts of bottle brush and extract of Karanj at 1:10, 1:100 and 1:1000

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dilutions were carried out by adoption poison food test. A mycelial bit was inoculated at the center of each poisoned medium and observations were recorded when mycelial growth in control set touched to the edges of Petriplates. The combination of these two plant extracts were mixed in the experiment *i.e.* same quantity (0.5 ml) of each extracts in five different solvents and at 1:10,1:100 and 1:1000 dilution were taken. Further same procedure described earlier was adopted.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been presented under following heads :

***Callistemon lanceolatus* :**

The results regarding , the effect of different solvents *i.e.* acetic acid, acetone, ethanol, petroleum ether, chloroform and distilled water used for extraction of extract of *C. lanceolatus* at 1:10 dilution and their antifungal effect on inhibition of mycelial growth of test fungus was studied. From the results presented in Table 1 it was revealed that *C. lanceolatus* extracted in acetic acid, acetone, ethanol and chloroform inhibited the mycelial growth of test fungus up to 94.44 per cent over control and these treatments were at par with each other followed by petroleum ether (91.11 %) and distilled water (60%). The control set showed maximum mycelial growth of test fungus. At 1:100 dilution indicated that the extract of *C. lanceolatus* extracted in acetic acid showed maximum inhibition of mycelial growth over control of test fungus (94.44 %). However, the extract of *C. lanceolatus* extracted in acetone, ethanol, petroleum ether, chloroform and distilled water showed 54.44, 63.33, 61.88, 57.77 and 54.77 per cent inhibition, respectively. These treatments were at par with each other. At 1:1000 dilution, it was seen that the extract of *C. lanceolatus* extracted in acetic acid at 1:1000 dilution showed maximum per cent inhibition of mycelial growth of *C. lanceolatus* over control (94.44%). The per cent inhibition of mycelial growth recorded in other solvents used for extraction of *C. lanceolatus* as per descending order were ethanol (52.22%), petroleum ether (48.88%), chloroform (38.55%), distilled water (27.77%) and acetone (26.66%). Acetone and distilled water showed same effect in inhibiting the mycelial growth of *C. Capsici*

***Pongamia pinnata*:**

The effect of different solvents used for extraction of extract of *Pongamia pinnata* at 1:10 dilution and their antifungal effect on inhibition of mycelial growth of test fungus was studied. From the results presented in Table 2 it was revealed that , the per cent inhibition of mycelial growth of test fungus by extract of *P. pinnata* extracted in acetic acid ,acetone, petroleum ether, chloroform, ethanol and distilled water, was 94.44, 78.22, 75.22, 67.11, 58.55 and 44.44 per cent, respectively. All treatment showed significant differences except acetone and petroleum ether which were at par with each other. Acetic acid used for extraction of *P. pinnata* showed maximum inhibition of mycelial growth of test fungus . At 1:100 dilution showed per cent inhibition of mycelial growth by acetic acid (94.44%), ethanol (43.33%), petroleum ether (66.66%), chloroform (56.66%), acetone (39.33%) and distilled water (36.00%). Among them extract of *P. pinnata* extracted in acetic acid showed maximum per cent inhibition. All treatments showed statistically significant differences . At 1:1000 dilution , the extract of *P. pinnata* in acetic acid showed maximum per cent inhibition (94.44%) which was followed by chloroform

Table 1 : Effect of different solvents use for extraction of *C. lanceolatus* at different dilutions on mycelial growth of *C. capsici* (on solid medium)

Sr. No.	Solvents used for extraction	Mycelial growth on solid medium					
		1:10 dilution		1:100 dilution		1:1000 dilution	
		Diameter in cm	Per cent inhibition over control	Diameter in cm	Per cent inhibition over control	Diameter in cm	Per cent inhibition over control
1.	Acetic acid	0.5	94.44	0.5	94.44	0.5	94.44
2.	Aceton	0.5	94.44	4.1	54.44	6.6	26.66
3.	Ethanol	0.5	94.44	3.3	63.33	4.3	52.22
4.	P. ether	0.8	91.11	3.4	61.88	4.6	48.88
5.	Chloroform	0.5	94.44	3.8	57.77	5.5	38.55
6.	Distilled water	3.6	60.00	4.0	54.77	6.5	27.77
7.	Control	9.0	0.00	9.0	0.00	9.0	0.00
	S.E. ±	0.0471		0.6165		0.0617	
	C.D.(P=0.05)	0.1428		1.865		0.1815	

Table 2 : Effect of different solvents use for extraction of *P. pinnata* at different dilutions, on mycelial growth of *C. capsici* (on solid medium)

Sr. No.	Solvents used for extraction	Mycelial growth on solid medium					
		1:10 dilution		1:100 dilution		1:1000 dilution	
		Diameter in cm	Per cent inhibition over control	Diameter in cm	Per cent inhibition over control	Diameter in cm	Per cent inhibition over control
1.	Acetic acid	0.5	94.44	0.5	94.44	0.5	94.44
2.	Aceton	1.96	78.22	5.5	39.33	6.46	28.22
3.	Ethanol	3.7	58.55	5.1	43.33	5.2	43.22
4.	P. ether	2.2	75.22	3.0	66.66	4.6	48.88
5.	Chloroform	2.96	67.11	3.9	56.66	4.5	50.00
6.	Distilled water	5.0	44.44	5.8	36.00	6.5	27.77
7.	Control	9.0	0.00	9.0	0.00	9.0	0.00
	S.E. $\pm$	0.082		0.09		0.113	
	C.D. (P=0.05)	0.25		0.275		0.344	

(50.00%), petrolium ether (48.88%), ethanol (43.22), acetone (28.22%). Treatment 6 (distilled water) and treatment 2 (acetone) were at par with each other.

Combination of *Callistemon lanceolatus* and *Pongamia pinnata:*

The effect of different solvents used for extraction of extract of combination of *Callistemon lanceolatus* and *Pongamia pinnata* at 1:10 dilution and their antifungal effect on inhibition of mycelial growth of test fungus was studied. From the results presented in Table 3, it was revealed that , acetic acid and ethanol used for extraction of combination *Callistemon lanceoatus* and *P. pinnata* showed 94.44 per cent inhibition of mycelial growth of test fungus which was followed by chloroform (90.00%), actone (63.33%). petroleum ether and distilled water (57.77%) which were at par with each other . At 1:100 dilution , among the six solvents, acetic acid showed maximum per cent inhibition of mycelial growth of test fungus (94.44%), other solvents petroleum ether (70.00%) and chloroform (68.88%) were at par with each other. Rest of treatments in order of merit were acetone (61.11%) , ethanol (54.44%) and distilled water (37.11%). At 1 :1000 dilution , acetic acid showed the highest per cent inhibition of mycelial growth of *C. capsici* (94.44%) while other solvents in order of their efficiency were ethanol (38.88%), petroleum ether (37.11%), acetone and chloroform (30.44%), and distilled water (25.25%). Treatments 2 (acetone) and 5 (chloroform) were at par with each other. Also treatment 3 (ethanol) and 4 (petroleum ether) were found at par with each other.

Thus from overall results it was observed that acetic acid used for extraction of *C. lanceolatus* and *P. pinnata* and combination of both *C. lanceolatus* and *P. pinnata* at 1:10 on growth of test fungus were more useful. The results obtained for efficacy of these plant extracts against

Table 3 : Effect of different dilutions of combination of extracts of *C. lanceolatus* and *P. pinnata* in different solvents for their antifungal properties on inhibition of mycelial growth of test fungus, *C. capsici*

Sr. No.	Solvents used for extraction	Mycelial growth on solid medium					
		1:10 dilution		1:100 dilution		1:1000 dilution	
		Diameter in cm	Per cent inhibition over control	Diameter in cm	Per cent inhibition over control	Diameter in cm	Per cent inhibition over control
1.	Acetic acid	0.5	94.44	0.5	94.44	0.5	94.44
2.	Aceton	3.3	63.3	3.5	61.11	6.3	30.4
3.	Ethanol	0.5	94.44	4.1	54.44	5.5	38.88
4.	P. ether	3.8	57.77	2.7	70.00	5.7	37.11
5.	Chloroform	0.9	90.00	2.8	68.88	6.3	30.44
6.	Distilled water	3.8	57.77	5.7	37.11	6.7	25.25
7.	Control	9.0	0.00	9.0	0.00	9.0	0.00
	S.E. $\pm$	0.422		0.235		0.125	
	C.D. (P=0.05)	0.128		0.712		0.379	

C. capsici in present study are in consonance with the earlier finding reported by the several investigators (Bairwa *et al*., 2002; Devis *et al*., 2003; George *et al*., 2003; Rangarajula *et al*., 2003; Swamy and Kulkarni, 2003; Bagri *et al*., 2004; Shinde and Patel, 2004.)

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