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ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS *IN VITRO* AGAINST *COLLETOTRICHUM FALCATUM* CAUSING RED ROT OF SUGARCANE

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ABSTRACT

The red rot disease of sugarcane caused by the fungi *Colletotrichum falcatum* is a serious threat for the sugar industry. Synthetic fungicides are used to control the fungi usually which are harmful for human health and the environment. Keeping in view the potential of some bioactive indigenous plant a study was carried out on their efficacy against the fungi *Colletotrichum falcatum.* Extracts of five indigenous plants (neem, *Azadirachta indica*; turmeric, *Curcuma longa*; kuth, *Saussurea lappa;* garlic, *Allium sativum* and *Gliricidia sepium*) were tested in three concentrations (5.0, 10.0 and 15.0%) against *Colletotrichum falcatum* to evaluate the antifungal activity *in vitro*. All the botanicals evaluated, suppressed the mycelial growth to some extent. The maximum inhibition in mycelial growth was observed at 15 percent of *Azadirachta indica* (93.25%), followed by *Saussurea lappa* (83.64%), *Curcuma longa* (79.23%) and *Gliricidia sepium* (59.23%).

Key-words:

INTRODUCTION

Sugarcane is an economically important crops, grown at 1132 thousand hectares during 2015-16, with the production of 65.5 million tonne (Pakistan Economic Survey 2015-16). Sugarcane production sometimes deteriorated due to various stresses, pests and diseases. The worldwide loss in cane yield and sugar recovery is approximately 5-10 % per annum (Viswanathan and Samiyappan, 1999). Fungi are responsible for numerous diseases of plants resulting in substantial loss in crops yield. The red rot disease of sugarcane is caused by the fungal pathogen *Colletotrichum falcatum* Went. It is one of the oldest diseases of sugarcane and is of great economic importance and a serious threat to the sugar industry particularly in sub-tropical countries (Alexander and Viswanathan, 2002; Viswanathan and Samiyappan, 1999; Guerber and Correll, 2001; Armstrong-Choa and Banniza, 2006). During the recent years, the greatest loss to sugarcane industry in Pakistan was due to red rot. The loss in cane weight was recorded to be about 29.07 % and resulted in 30.8 % loss in sugar recovery (Hussnain and Afghan, 2006) and in other countries such as USA, Bangladesh, India, Australia and Thailand (Viswanathan and Samiyappan, 1999). Usually commercial fungicides are being used for the control of red rot disease which is harmful for human and agroecosystem. There has been a rising concern on the research plant extracts for control of pest and diseases in agriculture which are less harmful to the human health and environment (Costa *et al.,* 2000, Duru *et al.,* 2003, Nasir *et al.,* 2014). Awareness in medicinal plants has been rapidly growing for their commercial and socio-economic importance. The bioactive derivatives of plants have a strong basis for stimulating its conservation (Coley *et al.,* 2003). Many plants have antifungal effects and can be used to control certain fungal diseases of crops. Pakistan has history on the folk use of plants. Although there are more than 6,000 species of higher plants, only 12% are documented for medicinal use (Shinwari, 2010). In this regard effect of extracts of five indigenous plants (neem, *Azadirachta indica* A. Juss; turmeric, *Curcuma longa* L.; kuth, *Saussurea lappa* Decne; garlic, *Allium sativum* L. and *Gliricidia sepium* Jacq.) were tested in three concentrations (5.0, 10.0 and 15.0%) against *Colletotrichum falcatum* to evaluate inhibitory effect on the growth of the fungi in *vitro*.

MATERIAL AND METHODS

Efficacy of different botanicals, essential oils and fungicides at different concentrations was evaluated on radial growth of test fungus by Poisoned Food Technique.

Screening of botanicals against the test pathogen:

The plant materials were grounded to fine powder of 60 meshes. The plant extracts were prepared by cold water extraction method described by Shekhawat and Prasad (1971)**.** The samples were washed separately in tap water and

finally three times in distilled water. They were crushed in mortar and pestle by adding distilled water @ 1 mL/g fresh weight. The extracts were clarified by passing through two layers of cheese cloth and finally through Whitman No. 1 filter paper. The filtered extracts used for study as 100 % extract. The suitable quantity of plant extracts were mixed in sterilized distilled water for making the concentrations (v/v) for the trials. For bioassay, double strength concentrations of botanicals were prepared by dissolving 10, 20 and 30mL of plant extract in 90, 80 and 70 mL of sterilized distilled water, respectively to get the final concentrations of 5, 10 and 15 %.

Treatment Method:

The culture of fungus, *C. falcatum* was used to study the antifungal activity of plant extracts. Poisoned food technique (plant extract amended Oat Meal Agar medium) was used to screen different plant extracts *in vitro* (Nene and Thapliyal, 2000), different concentrations (5, 10, and 15 %) of plant extracts were combined in Oat Meal Agar medium for inoculation of the test pathogen in sterilized Petri plates. The isolated pathogen grown on Oat Meal Agar medium was placed at the center of Petri plates containing different concentration of the poisoned medium and incubated at 25±1ºC for 7 days. All trials were replicated three times, under the same temperature and humidity. Mycelial growth of test fungus was measured after 7 days. Percent inhibition in growth was determined with the help of mean colony diameter and calculated by using the following formula (McKinney, 1923):

$$\text{Inhibition} = \frac{\text{Colony Growth in Control} - \text{Colony Growth in treatment}}{\text{Colony Growth in control}} \times 100$$

Statistical analysis

The results were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range test and Fisher's least significant difference (LSD) test at P=0.05 was also performed (Zar, 2009).

RESULTS AND DISCUSSION

The study was carried out to know the effect of five indigenous plants (neem, *Azadirachta indica*; turmeric, *Curcuma longa*; kuth, *Saussurea lappa*; garlic, *Allium sativum* and gliricidia, *Gliricidia sepium*) against *Colletotrichum falcatum* by using poisoned food technique at three concentrations *viz.*, 5.0, 10.0 and 15.0 percent. The results are depicted in Table-1 revealed that all the plants extracts inhibited the growth of *Colletotrichum falcatum*. Two way ANOVA was also performed on the data of plants extracts under laboratory condition (Table 3). All the Plant extracts showed significant inhibitory effect ($p<0.001$) compared to controls. The concentrations effects were also found to be significant ($p<0.001$) compared to control. Plant extracts was also highly significant ($p<0.001$). The first and second order interactions were significant (p at the most 0.001). In general, all the plant extracts inhibited the growth of *Colletotrichum falcatum.*

Table 1. Mean and SE of clear zone diameter of *Colletotrichum falcatum* as influenced by different Plant Extracts

Conc. %	**Plant Extracts**				
	Azadirachta indica	*Saussurea lappa*	*Curcuma longa*	*Gliricidia sepium*	*Allium sativum*
0	5.9 ± 0.05	5.9 ± 0.03	5.8 ± 0.06	5.9 ± 0.15	5.9 ± 0.33
5	1.3 ± 0.1	2.3 ± 0.17	2.6 ± 0.05	4.5 ± 0.2	4.3 ± 0.1
10	0.8 ± 0.1	1.8 ± 0.5	1.4 ± 0.1	3.9 ± 0.05	2.7 ± 0.1
15	0.4 ± 0.1	1.3 ± 0.17	0.78 ± 0.06	3.3 ± 0.1	2.7 ± 0.1

It was discovered that the maximum inhibition in mycelial growth (93.25%) was observed at 15 percent concentration by *Azadirachta indica*, followed by *Saussurea lappa* (83.64%), *Curcuma longa* (79.23%) and *Gliricidia sepium* (59.23%) and *Allium sativum* (37.00%). At 10 percent concentration, maximum inhibition in mycelial growth (81.62%) was noted in *Azadirachta indica* followed by *Saussurea lappa* (73.53%), *Curcuma longa* (71.38%), *Gliricidia sepium* (51.67%) and *Allium sativum* (33.00%). At 5 percent concentration, maximum inhibition in mycelial growth (79.21%) was recorded again in *Azadirachta indica* followed by Turmeric (59.25%), *Saussurea lappa* (55.82%), *Gliricidia sepium* (32.47%) and *Allium sativum* (22.63%). Bhardwaj and Sahu (2014) recorded 79.25% inhibition in mycelial growth at 15 per cent concentration by Turmeric and minimum inhibition in mycelial growth (64.44%) was recorded in Garlic. These findings are similar to our findings. In our studies

minimum inhibition in mycelial growth (22.63%) was recorded by *Allium sativum* (at 5%) too. The data showed that neem, *Azadirachta indica* at all concentrations was highly effective for inhibiting the mycelial growth (Table 2).

Table 2. Percent inhibition in mycelial growth of *Colletotrichum falcatum* by test plants.

Conc. %	**Mycelial inhibition by the test plants (%)**				
	Azadirachta indica	*Curcuma longa*	*Saussurea lappa*	*Gliricidia sepium*	*Allium sativum*
5%	79.2	59.25	55.82	22.63	32.47
10%	81.62	71.38	73.53	33	51.67
15%	93.25	79.23	83.64	37	59.23

These findings are in line with the findings of Kumar and Yadav (2007) who also reported the efficacy of plant extracts of *Azadirachta indica* and *Allium sativum* against the *Colletotrichum* sp. *Allium sativum* was found least effective among others at all the concentrations contradictory to earlier studies by Tariq and Magee, (1990).

In Pakistan the red rot diseases has caused extensive damage in recent past and got the status of the most destructive and an important hazard in the cultivation of sugarcane (Chaudhry *et al.,* 1999). There is a dearth for research on the fungicidal activities of plants. Some studies are conducted on neem, turmeric, garlic and some other plants however no study is conducted on the fungicidal activity of *Saussurea* (kuth) and *Gliricidia.* This is a first hand report on the efficacy of these two plants as fungicide, but still it is a preliminary report which require further research to attest its validity.

Table 3. Results of two way analysis of variance (ANOVA) for plant extract on growth of *Colletotrichum falcatum.*

Source	**SS**	**df**	**MS**	**F**	**P**
Main Effect					
Concentration	160.2071	3	53.402	1144.336	0.001***
Plant Extract	43.5583	4	10.889	233.348	0.001***
Interaction					
Concentration X Plant extract	16.0736	12	1.339	28.7029	0.001***
Error	1.866	40	0.0466		
Total	221.705	59			

$Lsd_{0.05} = 0.159_{(Conc.)}$ $0.178_{(Plant\ ext.)}$

Table 3. (Cont.): Results of Duncan's multiple range test. Means followed by different letters are significantly different (P<0.05) for factor Concentration.

Rank	**Treatment**	**Mean**	**n**	**Non- significant range**
1	1	5.92	15	A
2	2	3.0266	15	B
3	3	2.16	15	C
4	4	1.726	15	D

Table 3 (Cont.): Results of Duncan's multiple range test. Means followed by different letters are significantly different (P<0.05) for factor Plant extract.

Rank	**Treatment**	**Mean**	**n**	**Non- significant range**
1	4	4.425	12	A
2	5	3.96	12	B
3	2	2.841	12	C
4	3	2.67	12	C
5	1	2.13	12	D

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