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Eco-Friendly Management of Red Rot of Sugarcane, Caused by *Colletotrichum falcatum* Went.

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Abstract Sugarcane is an economically valuable crop which is vegetatively propagated in the tropics and sub tropics of the world. Red rot caused by *Colletotrichum falcatum*, is one of the most destructive diseases of sugarcane. To combat such diseases we are using synthetic chemicals at a very high rate. But use of these conventional synthetic chemicals in plant disease management has raised many problems like pollution, development of resistant strains of pathogens and slow bio-degradation of these chemicals. In the present study the focus of the experiments was on the eco-friendly management of the disease. The experiments were conducted *in vitro* to evaluate the effect of different plant extracts, essential oils and different strains of biocontrol agent (*Trichoderma harzianum*) on the growth of *C. falcatum*. Among the different plant extracts (viz. Aloevera, Eucalyptus, Marigold, Neem, Ocimum and Parthenium) tested, Parthenium at all concentrations, 5%, 10% and 15%, was highly effective in inhibiting the mycelial growth of the fungus up to 80% but marigold was found to enhance the growth of pathogen. After evaluating the efficacy of five essential oils (Citronella, Lemon grass, Lemon tulsi, Mentha and Peppermint oil), Peppermint, Mentha and Lemon tulsi were found most effective against the pathogen showing complete inhibition at 6 µl and 10 µl concentrations. Among nine strains of *Trichoderma harzianum* (TCMS-4, TCMS-16, Th-9, Th-34, Th-36, Th-37, Th-52, Th-53, Th-55) evaluated *in vitro*, maximum percent inhibition in mycelial growth was recorded in Th-53 (88.88%).

Keywords *Colletotrichum falcatum*, Red rot, Botanicals, Essential oils.

Introduction

Sugarcane (*Saccharum* spp.) is one of the most important sugar producing crops in the world [1]. It is a multi-product crop and has immense potential for diversification. It also plays very crucial role in the economy of the country. Red rot is one of the most devastating diseases of sugarcane caused by *Colletotrichum falcatum* Went. About 240 sugarcane diseases caused by various plant pathogenic organisms have been reported from the world [2]. Annual loss of revenues by *C. falcatum* infection in India is estimated to be between 500 and 1000 million USD

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Table 1. Effect of botanicals on the growth of *C. falcatum*.

Botanicals	Colony diameter (mm) 5%	10%	15%	% inhibition 5%	10%	15%
Aloevera	61.00	58.00	51.66	32.22	35.55	42.66
Eucalyptus	63.33	54.66	43.00	29.66	39.26	52.22
Marigold	73.16	75.66	77.66	18.71	15.93	13.71
Neem	75.66	58.00	40.00	16.00	35.50	55.55
Basil	50.66	46.33	42.33	43.77	48.55	53.00
Parthenium	20.66	20.00	18.00	71.11	77.77	80.00
Control	90.00	90.00	90.00	0.00	0.00	0.00
	Dose (A)		Treatment (B)		A×B	
CD at 5%	0.93		0.76		1.87	
CV	1.86					

[3]. The sugar requirement in India for 2030 is estimated to be 36 million tonnes for which the sugar recovery is to be 11% and average cane yield is to be 100 t/ha which can be fulfilled either by increasing the acreage or productivity [4].

Red rot disease of sugarcane is not only responsible for decreasing the yield, but also affect the quality of the cane and sugar content. So to overcome the problem of red rot of sugarcane different management strategies are being used. Use of different fungicides and anti-fungal compound are in practice but these chemicals shows the negative impact on the environment and are supposed to be harmful in long run. So the need of hour is to use such practices which are eco-friendly and at the same time are effective in controlling the disease. Keeping in view the devastating nature of disease and its impact on the economy of country and its eco-friendly management, the present experimentations were carried out in order to evaluate the efficacy of different plant extracts, essential oils and bio-control agent against red rot pathogen so that disease could be controlled effectively without showing harmful effect on the environment.

Materials and Methods

The red rot pathogen used in the experiments was isolated from infected canes collected from Sugarcane Pathology Block, Crop Research Center (CRC), Pantnagar.

Screening of plant extracts against *C. falcatum*

The leaf extracts of Aloevera, Eucalyptus, Marigold, Neem, Ocimum and Parthenium were prepared by using cold water extraction method [5]. The filtered extracts were taken in the study as 100% (stock solution). The appropriate amount of plant extract was mixed in sterilized distilled water to make the desired concentration (v/v) for experiments. For bioassay, double strength concentration of plant extracts were prepared by dissolving 10, 20 and 30 ml of plant extract in 90, 80 and 70 ml of sterilized distilled water, respectively to get the final concentrations of 5, 10 and 15%. Poisoned food technique [6] (plant extract amended Oat Meal Agar medium) was used to screen different plant extracts *in vitro*. 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 28±1°C for 7 days. Radial growth of test fungus was measured after 7 days of inoculation.

Percent inhibition in growth was determined with the help of mean colony diameter and calculated by using the following formula:

$$\text{Percent inhibition} = \frac{X - Y}{X} \times 100$$

Where, X=colony diameter in check, Y =colony diameter in amended medium.

Screening of essential oils against the *C. falcatum*

During the study five essential oils i.e. Citronella oil, Lemon grass oil, Lemon tulsi oil, Mentha oil and Peppermint oil, were used. Five concentrations i.e. 2 µl, 4µl, 6 µl, 8 µl and 10 µl were tested during the experiment. The Whatmann paper discs were sterilized by autoclaving them and were put into sterilized petri plate containing Oat Meal Agar media with the help of clean forceps. Later with the help of micropipette different concentration of oil was poured on the disc

Table 2. Efficacy of different essential oils on the growth of *C. falcatum* at 2, 4, 6, 8 and 10 µl concentration.

Essential oils	Radial growth of fungus (mm)					Growth inhibition (%)				
	2 µl	4 µl	6 µl	8 µl	10 µl	2 µl	4 µl	6 µl	8 µl	10 µl
Peppermint	18.00	10.00	0.00	0.00	0.00	80.00	88.88	100	100	100
Citronella	49.00	37.66	31.00	21.00	0.00	45.50	58.20	65.50	76.60	100
Mentha	20.00	12.00	0.00	0.00	0.00	77.77	86.80	100	100	100
Lemon grass	40.00	37.00	19.00	0.00	0.00	55.55	58.88	78.88	100	100
Lemon tulsi	47.00	27.00	0.00	0.00	0.00	47.70	70.00	100	100	100
Control	90.00	90.00	90.00	90.00	90.00	0.00	0.00	0.00	0.00	0.00
	Dose (A)		Treatment (B)		A × B					
CD at 5%	0.69		0.75		1.69					
CV	3.81									

i.e. 2 µl, 4 µl, 6 µl, 8 µl and 10 µl and then fungal dosc of 5 mm were put 6 cm apart from the oil disc. The petri plates were then incubated at 28±1°C for 7 days. Each treatment was replicated thrice. After 7th day of incubation the growth of pathogen was measured.

Screening of *Trichoderma* strains for *in vitro* antagonism against *C. falcatum*

The *Trichoderma* strains used in the experiment were procured from Bio-Control Laboratory, Department of Plant Pathology, G.B.P.U.A and T, Pantnagar. *In vitro* evaluation of *Trichoderma* strains for antagonism against the test pathogen were carried out on Potato Dextrose Agar medium using dual culture method [7]. Twenty milliliters of sterilized melted media was aseptically poured in a sterilized 90 mm diameter petri plate and allowed to solidify. Mycelial disc (5 mm in diameter) of both *Colletotrichum falcatum* and different strains of *Trichoderma* were cut with the help of sterilized cork borer from the edge of four days old culture plates and were placed on solidified PDA in such a manner that both the disc lie just opposite to each other (approximately 6 cm apart from each other). Inoculated petri plates were incubated at 28±1°C. Periodic observation on the growth of bio-control agent (*Trichoderma* strains) and the ability of bio-control agent to colonize the pathogen was recorded. The percent inhibition in growth of pathogen was calculated by the following formula described by McKinney [8].

$$\text{Percent inhibition} = \frac{X - Y}{X} \times 100$$

Where, X=Diameter of fungal growth in check plate, Y=Diameter of fungal growth in treatment.

Results and Discussion

Effect of botanicals on the growth of *C. falcatum*

Inhibition of mycelial growth varied significantly with different botanicals at different concentrations (Table 1). The results obtained revealed that at 5% concentration, maximum inhibition in mycelial growth (71.11%) was recorded in Parthenium, followed by Ocimum (43.77%), Aloevera (32.22%), Eucalyptus (29.66%) and minimum inhibition (16.00%) in mycelia growth was recorded in neem.At 10% concentration, maximum inhibition in mycelial growth (77%) was recorded in Parthenium, followed by Ocimum (48.55%), Eucalyptus (39.26%), Aloevera (35.55%) and Neem (35.50%). At 15% concentration, maximum inhibition in mycelial growth was recorded in Parthenium (80%), followed by Neem (55.55%), Ocimum (53.00%), Eucalyptus (52.22%) and Aloevera (42.66%). From the above data it can be summarized that Parthenium, at all concentrations, i.e. at 5%, 10% and 15%, was comparatively highly effective in inhibiting the mycelial growth. Other botanicals also showed inhibitory action but were less effective as compared to Parthenium. In case of marigold results were totally

Table 3. Effect of different strains of *Trichoderma* on growth of *C. falcatum*.

Sl. No.	Strains of *T. harzianum*	Colony diameter of test pathogen strains (mm)	Percent inhibition (%)
1	TCMS-16	22.66	74.82
2	TCMS-4	19.33	78.52
3	Th-36	20.66	77.04
4	Th-9	20.66	77.04
5	Th-53	10.00	88.88
6	Th-37	24.33	72.96
7	Th-55	21.33	76.30
8	Th-34	19.33	78.52
9	Th-52	18.33	79.63
10	Control	90	00.00
CD at 5%	1.58		
CV	3.89		

opposite as compare to others. In marigold it was observed that as concentration of plant extract increased there was increase in mycelial growth of the pathogen. Hence it was found that marigold extract enhances the growth of *Colletotrichum falcatum* instead of showing inhibitory action on it.

Bhardwaj and Sahu [9] also reported the efficacy of plant extract of Ocimum against the *Colletotrichum falcatum*. Yadav et al. [10] and Husain [11] have also observed the effect of *Azadirachta indica* (neem) against *Colletotrichum falcatum*. It has been reported that the aqueous extract of leaves of neem (*Azadirachta indica*) at four different doses (5, 10, 15 and 20%) was able to manage *Alternaria* leaf blight of mustard under field conditions. Neem leaf extract @ 15% was most effective against the disease as well as increased the yield of mustard [12].

Effect of essential oils on the growth of *C. falcatum*

Efficacy of different essential oils against test pathogen varied significantly at different concentrations viz., 2 µl, 4 µl, 6 µl, 8 µl and 10 µl (Table 2). The data revealed that at 2 µl concentration, maximum inhibition of mycelial growth were recorded in Peppermint oil (80.00%), while minimum inhibition in mycelial growth was recorded in Citronella oil (45.50%). At 4 µl concentration, maximum inhibition of mycelial growth was recorded in Peppermint oil (88.88%), and least inhibition was recorded in Citronella oil (58.20%). At 6 µl concentration, complete inhibition was recorded by Peppermint oil, Mentha oil and Lemon-tulsi oil. At 8 µl concentration Peppermint oil, Mentha oil, Lemon grass oil and Lemon-tulsi oil were 100% effective against the pathogen. At 10 µl concentration all essential oils were 100% effective in checking the mycelial growth of the pathogen. Thus, the above results showed that the essential oils used in study were quite effective against the test fungus. The results indicate a need for more testing of these oils against the pathogen which can lead to a better alternative for the management of pathogen.

These studies are in accordance with the reports that among five essential oils tested against *C. falcatum*, complete inhibition in mycelial growth was recorded by Peppermint oil and Mentha oil [9]. Kzl et al. [13], also reported effect of essential oils of some medicinal plants viz., *Cuminum cyminum, Anethum graveolens, Coriandrum sativum, Pimpinella anisum, Mentha spicata, Hyssopus officinalis* and *Foeniculum vulgare* against four plant pathogens viz., *Clavibacter michiganensis* subsp. *michiganensis, Pseudomonas syringae* pv, *Xanthomonas campestris* pv *malvacearum* and *Macrophomina phaseoli* at concentrations of 5, 10 and 15 µl. Also it has been reported that among the two essential oils Lemon grass and Lemon tulsi oil, evaluated against *Staphylococcus aureus* (bacteria) and *Aspergillus niger* (fungi), maximum anti-microbial activity was shown by lemon grass oil than ocimum oil [14].

Effect of different strains of *Trichoderma* on the growth of *C. falcatum*

All the strains of *Trichoderma* used were quite effective in reducing the mycelial growth of test fungus (Table 3). Maximum percent inhibition in mycelia growth was recorded in Th-53 (88.88%), followed by Th-52 (79.63%), TCMS-4 and Th-34 (78.52%), Th-36 and Th-9 (77.04%), Th-55 (76.30%), TCMS-16 (74.82%)and minimum percent inhibition was shown by Th-37 (72.96%). The difference in percent inhibition in mycelial growth indicates the difference in their efficacy against the pathogen. These findings are

confirmatory with the findings of Srivastava et al. [15] who have reported the antagonistic effect of the *Trichoderma harzianum* against *C. falcatum*. This effectiveness of strains may be due to the mechanism of antibiosis of pathogen by *Trichoderma* strains which has been reported earlier by several workers. The result so obtained indicate that there is need for *in vitro* evaluation of other isolates of *Trichoderma* sp. against the pathogen which then could lead to the better eco-friendly management of the red rot of sugarcane.

Conclusion

On the basis of above experiments it could be concluded that those essential oils, plants extracts and *Trichoderma* strains which are showing effective results against *Colletotrichum falcatum*, can be used in future as a part of integrated disease management against red rot of sugarcane. This will lead to reduction in use of pesticides and will act asa boon for our clean and green environment.

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