

EVALUATION OF SOME FUNGICIDES, BOTANICALS AND ESSENTIAL OILS AGAINST THE FUNGUS COLLETOTRICHUM FALCATUM CAUSING RED ROT OF SUGARCANE

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ABSTRACT

Different botanicals, fungicides and essential oils were evaluated *in vitro* against *Colletotrichum falcatum*. Amongst botanicals evaluated, it was found that at 15 per cent concentration, maximum inhibition in mycelial growth (92.59%) was recorded in *Ocimum* followed by Turmeric (79.25%), Ginger (75.92%), Onion (72.41) and minimum inhibition in mycelial growth (64.44%) was recorded in Garlic. Among five essential oils at 10 μ L concentration, complete inhibition in mycelial growth was recorded by Peppermint oil and Mentha oil, followed by Geranium oil (75.83%), Patchouli oil (70.00%) while minimum inhibition in mycelial growth was recorded in Palmaroza oil (63.89%). Out of four fungicides, at 20 ppm concentration, Bavistin showed complete inhibition of mycelial growth of the test fungus followed by Folicur (88.89 %), Contaf (81.89%) while least inhibition was by Tilt (80.78%).

INTRODUCTION

Sugarcane is one of the major commercial crop playing pivotal role in agriculture and industrial economy of the country. India is known as original home of sugarcane and sugar. Red rot is one of the important and threatening disease of sugarcane caused by *Colletotrichum falcatum* Went. Vishwanathan and Samiyappan (1999a) by their studies conducted at SBI, Coimbatore revealed that pathogen infection has drastically reduced brix, sucrose %, purity and CCS % in the diseased canes. The affected canes recorded 25-75 % reduced sucrose content than the healthy canes. Satyavir *et al.*, (2002) revealed that red rot infection reduces 7.1-32.5 % in extraction, 7.4-38.7 % in polarity, 0.5-8.3 % in purity co-efficient, 7.8-39 % in CCS and increase of 19.2-40.95 % in reducing sugars. 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 (Premachandran, 2012). Sunil kumar and Yadav (2007) has also reported the efficacy of plant extracts of *Azadirachta indica* and *Allium sativum* against the *Colletotrichum* sp. The fungicidal spectrum of leaf extract of Garlic and Onion has already been investigated by Shekhawat and Prasad 1971; Misra and Dixit, 1976 and Tariq and Magee, 1990. Present studies are in accordance with those of Kzl, S *et al.* (2005), who reported effect 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 μ l. Among several reasons incidence of the diseases is most important. Red rot is one of the major diseases which is not only responsible for decreasing the yield but also the quality of the cane and sugar content. Keeping in view the importance of sugarcane and its economic value and visualizing the seriousness of disease the present investigations were carried out in order to evaluate the efficacy of different fungicides, botanicals, essential oils so that disease could be controlled effectively.

MATERIALS 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 leaf and bulb extracts of Ginger, Garlic, Turmeric, Onion and, *Ocimum* (Table 1) were prepared by cold water extraction method described by Shekhawat and Prasad (1971a). 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 Whatmann No. 1 filter paper. These filtered extracts were taken in the study as 100 % extract. 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 concentrations of botanicals 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 %. 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 incorporated 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 centre of Petri plates containing different concentration of the poisoned medium and incubated at $25 \pm 1^\circ\text{C}$ for 7 days. Radial growth of test fungus was measured after inoculation till 7 days at an interval of 24 hrs.

Per cent inhibition in growth was determined with the help of mean colony diameter and calculated by using the following formula (McKinney, 1923):

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

Where,

X = colony diameter in check

Y = colony diameter on amended medium

Screening of essential oils against the test pathogen

During the study five essential oils *i.e.* Peppermint oil (*Mentha piperata*), Patchouli oil (*Pogostimon patchouli*), Mentha oil (*Mentha citrata*), Palmaroza oil (*Cymbopogon martini*) and Geranium oil (*Pelargonium graveolens*) were used. Five concentrations *i.e.* 2 μL tr, 4 μL tr, 6 μL tr 8 μL tr and 10 μL tr were used (Table 2). Firstly the discs were sterilised by autoclaving them and the disc was put into sterilized petriplate and with the help of micropipette different conc. of oil was put on the disc *i.e.* 2 μL tr, 4 μL tr 6 μL tr 8 μL tr and 10 μL tr and fungal disc of 5mm were put in the Petri plate having Oat Meal Agar media 4cm apart from each other. Then the Petri plates in replication of three were incubated at $25 \pm 1^\circ\text{C}$ for 7 days. The growth of pathogen was measured after seven days of incubation .

In vitro screening of fungicides against the pathogen

In vitro, efficacy of different fungicides against *C. falcatum* was studied by poisoned food technique (Sharville, 1960). The fungicides *viz.*, Propiconazole, Hexaconazole, Carbendazim and Tebuconazole were evaluated against the test fungus at the concentration of 5, 10, 15, 20ppm. The colony diameter was measured. Ten mL stock solution of 10000 ppm concentration of each fungicide was prepared in the distilled water in test tube. Required amount of the solution was added into 250 mL flask containing 60 mL of the sterilized melted Oat Meal Agar, so as to get final required concentrations of 5, 10, 15 and 20 ppm. The medium was mixed thoroughly before plating. Each media toxicated with fungicide was poured in three Petri plates. Non toxicated media was poured into Petri plates kept as a check. After solidification of media, a 5 mm mycelia disc of 6 days old culture of the test pathogen was cut with sterile cork borer and placed in centre of each

Petri plate. The Petri plates were incubated at $25 \pm 1^\circ\text{C}$. After 7 days of incubation the radial growth was measured. The per cent inhibition in growth was determined with the help of mean colony diameter and calculated by using the formula given by McKinney (1923).

RESULTS AND DISCUSSION

Effect of essential oils on growth of the test fungus

Five essential oils *viz.*, Patchouli oil (*Pogostimon patchouli*), Geranium oil (*Pelargonium graveolens*), Mentha oil (*Mentha citrata*), Palmaroza oil (*Cymbopogon martini*) and Peppermint oil (*Mentha pepperata*) were screened using poisoned food technique to check the efficacy against the test fungus. Inhibition of mycelial growth varied significantly with different essential oil at different concentrations *viz.*, 2 μL , 4 μL , 6 μL , 8 μL and 10 μL . The data (Table 1) revealed that at 2 μL concentration, maximum inhibition of mycelial growth were recorded in Peppermint oil (89.25%) followed by Mentha oil (82.78%), Geranium oil (27.78%), Patchouli oil (25.00%) while minimum inhibition in mycelial growth was recorded in Palmaroza oil (20.22%). At 4 μL concentration, maximum inhibition of mycelial growth was recorded in Peppermint oil (92.59%) followed by Mentha oil (89.44%), Geranium oil (40.22%), Patchouli oil (36.67%) while minimum inhibition in mycelial growth was recorded in Palmaroza oil (29.17%). At 6 μL concentration, complete inhibition in mycelial growth was recorded by Peppermint oil and Mentha oil, followed by Geranium oil (53.89%), Patchouli oil (47.41%) while minimum inhibition in mycelial growth was recorded in Palmaroza oil (42.97%). At 8 μL concentration, complete inhibition in mycelial growth was recorded by Peppermint oil and Mentha oil, followed by Geranium oil (65.00%), Patchouli oil (60.00%) while minimum inhibition in mycelial growth was recorded in Palmaroza oil (52.03%). At 10 μL concentration, complete inhibition in mycelial growth was recorded by Peppermint oil and Mentha oil, followed by Geranium oil (75.83%), Patchouli oil (70.00%), while minimum inhibition in mycelial growth was recorded in Palmaroza oil (63.89%). Thus the above results indicate that the Peppermint oil and Mentha oil are the most effective against the test fungus showing complete inhibition above 4 μL concentration. 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. Higher concentration of some essential oils inhibits the mycelial growth of various fungi reported by Kzl, *et al.* (2005). The work is in accordance with Bisht *et al.* (2013), they reported that maximum inhibition of fungal pathogen was in higher concentration of peppermint oil.

Effect of botanicals on growth of the test fungus

Present investigation was carried out to find out the efficacy of botanicals against the test fungus. Five plant extracts *viz.*, Ginger, Garlic, Turmeric, Onion and Ocimum were evaluated using poisoned food technique to check the efficacy of botanicals against *C. falcatum*. Inhibition of mycelial growth varied significantly with different botanicals at different concentrations *viz.*, 5.0 per cent, 10.0 per cent and 15.0 per cent. The data (Table 2) revealed that at 5 per cent concentration, maximum inhibition in mycelial growth

Table 1: Efficacy of different Essential oils on the growth of *C. falcatum* at 2, 4, 6, 8 and 10 μ L concentration

EssentialOils	Radial growth of fungus (mm)					Growth inhibition (%)				
	2 μ L	4 μ L	6 μ L	8 μ L	10 μ L	2 μ L	4 μ L	6 μ L	8 μ L	10 μ L
Geranium	65.00	53.80	41.50	31.50	21.75	27.78	40.22	53.89	65.00	75.83
Patchouli	67.50	57.00	47.33	36.00	27.00	25.00	36.67	47.41	60.00	70.00
Mentha	15.50	9.50	0.00	0.00	0.00	82.78	89.44	100.0	100.0	100.0
Peppermint	9.67	6.67	0.00	0.00	0.00	89.25	92.59	100.0	100.0	100.0
Palmaroza	71.80	63.75	51.33	43.17	32.50	20.22	29.17	42.97	52.03	63.89
Control	90.0	90.0	90.0	90.0	90.0	00.00	00.00	00.00	00.00	00.00
CD at 5%	Dose (A)		Treatment (B)		A \times B					
	0.36	0.36	0.81							

Table 2: Effect of plant extract on radial growth of *C. falcatum*

Plant extract	Radial growth of fungus (mm)			Growth inhibition (%)		
	5%	10%	15%	5%	10%	15%
Garlic	56.00	42.50	32.00	37.78	52.78	64.44
Onion	46.33	35.50	24.83	48.52	60.55	72.41
Ginger	42.17	32.33	21.67	53.14	64.08	75.92
Turmeric	32.50	24.50	18.67	63.69	72.78	79.25
Ocimum	22.75	12.83	6.67	74.72	85.74	92.59
Control	90.0	90.0	90.0	00.00	00.00	00.00
CD at 5%	Dose (A)		Treatment (B)		AxB	
	0.49	0.64	1.10			

Table 3: Efficacy of different Fungicides on the growth of *C. falcatum* at 5, 10, 15 and 20 ppm concentration

Chemical	Radial growth of fungus (mm)				Growth inhibition (%)			
	5 ppm	10 ppm	15 ppm	20 ppm	5 ppm	10 ppm	15 ppm	20 ppm
Tilt	37.3	30.3	24.3	16.3	58.56	66.33	73.00	81.89
Contaf	35.3	30.0	27.0	17.3	60.78	66.66	70.00	80.78
Bavistin	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0
Folicur	21.7	17.3	11.0	10.0	75.89	80.78	87.78	88.89
Control	90.0	90.0	90.0	90.0	00.00	00.00	00.00	00.00
CD at 5%	Dose (A)		Treatment (B)		A \times B			
	2.94	2.94	5.88					

(74.72%) was recorded in Ocimum followed by Turmeric (63.65%), Ginger (53.14%), Onion (48.52) and minimum inhibition in mycelial growth (37.78%) was recorded in Garlic. At 10 per cent concentration, maximum inhibition in mycelial growth (85.74%) was recorded in Ocimum followed by Turmeric (72.78%), Ginger (64.08%), Onion (60.55) and minimum inhibition in mycelial growth (52.78%) was recorded in Garlic. At 15 per cent concentration, maximum inhibition in mycelial growth (92.59%) was recorded in Ocimum followed by Turmeric (79.25%), Ginger (75.92%), Onion (72.41) and minimum inhibition in mycelial growth (64.44%) was recorded in Garlic. From the data it can be summarized that Ocimum at all concentrations *i.e.* at 5 per cent, 10 per cent and 15 per cent was highly effective in inhibiting the mycelial growth. Garlic also showed the inhibition but was found least effective among others at all the concentrations. The fungicidal spectrum of leaf extract of Garlic and Onion has already been investigated by Shekhawat and Prasada (1971a); Misra and Dixit, (1976) and Tariq and Magee, (1990). Sunil kumar and Yadav (2007) has also reported the efficacy of plant extracts of *Azadirachta indica* and *Allium sativum* against the *Colletotrichum* sp

Effect of fungicides on growth of the test fungus

Poisoned food technique were conducted to observe the effect

of four systemic fungicides on growth of *C. falcatum*. Fungicides *viz.*, Tilt (Propiconazole), Contaf (Hexaconazole), Bavistin (Carbedazim) and Folicur (Tebuconazole) in different concentrations *viz.*, 5, 10, 15 and 20 ppm were evaluated. The data (Table 3) revealed that at 5 ppm concentration, Bavistin showed complete inhibition of mycelial growth of the test fungus followed by Folicur (75.89 %), Contaf (60.78%), while least inhibition was by Tilt (58.56%). At 10 ppm concentration, Bavistin showed complete inhibition of mycelial growth of the test fungus followed by Folicur (80.78 %), Contaf (66.66%), while least inhibition was by Tilt (66.33%). At 15 ppm concentration, Bavistin showed complete inhibition of mycelial growth of the test fungus followed by Folicur (87.78 %), Contaf (73.00%), while least inhibition was by Tilt (70.00%). At 20 ppm concentration, Bavistin showed complete inhibition of mycelial growth of the test fungus followed by Folicur (88.89 %), Contaf (81.89%), while least inhibition was by Tilt (80.78%). It can be concluded that, Bavistin was found to be highly effective showing complete inhibition at all the concentrations. Folicur is next to Bavistin in inhibition, while, Contaf and Tilt gave near about same inhibition at all concentrations. These results are in accordance with Singh *et al.* (1980), Waraitch (1989) who reported that Carbendazim was most effective against *C. falcatum*. Subhani *et al.* (2008) also reported that fungicides Benomyl, Folicur, Ridomyl

completely inhibited growth of *C. falcatum* at 5, 10, 15 and 20 µg/mL while, Tilt did that at 20 and 50 µg/mL.

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