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Research Article

In vitro sensitivity of *Sclerotium rolfsii* towards some fungicides and botanicals

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Abstract: An *in-vitro* experiment was conducted to determine the effect of different fungicides, plant oils and plant extracts on radial colony growth of *Sclerotium rolfsii* Sacc. following poison food technique in PDA medium. One systemic (Carbendazim 50% WP as Bavistin @ 0.1%), three contact (Mancozeb 75% WP as Indofil M-45 @ 0.25%, Copper oxy chloride 50% WP as Blitox @ 0.4%, Chlorothalonil 75% WP as Kavach @ 0.2%) and three combination of systemic and contact fungicides (Carboxin 37.5% + Thiram 37.5% WP as Vitavax Power @ 0.2%), Metalaxyl 8% +Mancozeb 64% WP @ 0.25% as Krilaxyl Gold, Cymoxanil 8% + Mancozeb 64% WP as Curzet @ 0.25%) were evaluated against *S. rolfsii* in laboratory. Besides, four plant oils (Karanja oil from *Pongamia pinnata* L., Mahua oil from *Madhuca indica*, Neem oil from *Azadirechta indica*, Chaulmoogra oil from *Hydnocarpus wightiana* Blume) @ 5% (v/v) and two plant extract (Kamini – *Murriya exotica* L. and Nayantara – *Vinca rosea* L.) @ 10% (v/v) were also evaluated in laboratory. Viavax Power (95%) was the best fungicide to restrict the fungal growth effectively followed by Krilaxyl Gold (94.04%), Kavach (93.55%), Indofil M-45 (90.66%) and Curzet (87.77%). Blitox had negligible effect (20.17%) on reduction of mycelial growth. Among the plant oils and plant extracts, Karanja oil (88.49%) and *Murraya exotica* leaf extract (86.15%) were found effective in reducing the growth of *S. rolfsii*. This information may be helpful for developing suitable management practices against the foot rot causing soil borne, polyphagous destructive pathogen.
Keywords: Foot rot, *Sclerotium rolfsii*, management, fungicide, plant oil, plant extract, *in vitro* study

INTRODUCTION

Sclerotium rolfsii Sacc. is a soil borne polyphagous fungal pathogen distributed in tropical and sub-tropical regions of the world where high or warm temperature prevails. It attacks more than 500 species of plants in about 100 families [1-5]. The fungus is characterized by white fluffy, branched, septate mycelium, and spherical or irregular shaped brown sclerotia, which range from 0.5-2.0 mm in diameter and at maturity, resemble mustard seed. The mycelium of *S. rolfsii* survives best in sandy soils, whereas, the sclerotia survive best in moist, aerobic conditions found at the soil surface [6-7]. Management of the pathogen is quite difficult due to its polyphagus nature, off-seasonal survivality and wide adaptability. Present investigation was made to screen out the effective fungicides, plant oils and plant leaf extract for developing suitable management strategy by integration of both chemicals and non-chemicals against the pathogen.

MATERIALS AND METHODS

An *in vitro* trial was conducted at Department of Plant Protection, Palli-Siksha Bhavana, Visva-Bharati to determine the effect of different fungicides, plant oils and plant extracts on radial colony growth of *S. rolfsii* following poison food technique. One systemic [Carbendazim 50% WP as Bavistin (BASF) @ 0.1%], three contact [Mancozeb 75% WP as Indofil M-45 (Indofil) @ 0.25%, Copper oxy chloride 50% WP as Blitox (Rallis) @ 0.4%, Chlorothalonil 75% WP as Kavach (Syngenta) @ 0.2%] and three combination of systemic and contact fungicides [Carboxin 37.5% + Thiram 37.5% WP as Vitavax Power (Dhanuka) @ 0.2%, Metalaxyl 8% +Mancozeb 64% WP as Krilaxyl Gold (Krishi Rasayan) @ 0.25%, Cymoxanil 8% + Mancozeb 64% WP as Curzet (DoPont) @ 0.25%] were evaluated against *S. rolfsii* in laboratory. Besides, four plant oils viz. Karanja oil (*Pongamia pinnata*) Mahua oil (*Madhuca indica*) Neem oil (*Azadirechta indica*), Chaulmoogra oil (*Hydnocarpus wightiana*) and two plant leaf extract viz. Kamini (*Murriya exotica*) and Nayantara (*Vinca rosea*) were also evaluated in laboratory. After collection, the leaves were washed in distilled water clearly and surface sterilized by rubbing with absolute ethyl alcohol (95%) soaked sterilized absorbent cotton. Then it washed thoroughly with

sterilized water. Stock solutions of the materials were prepared by blending 100g of leaves in 100 ml of sterilized water.

Desired concentration of fungicides/plant oils/plant extracts were added to the sterilized melted PDA medium separately and mixed thoroughly by gently stirring just before pouring into the petriplate. PDA medium was amended with individual plant extract 10% (v/v), plant oil 5% (v/v) emulsified with 0.1% oil emulsifier (Tween 80) in total volume, and fungicides in above mentioned dose. After thorough mixing of each product separately with PDA medium approximately 20 ml of that of each was poured into separate petriplate. After solidification, the plates were inoculated by placing 5 mm mycelial disc of *S. rolfsi* from the periphery of 10 days old culture and sclerotia from one month culture. The inoculums were placed at the centre of the petriplates and incubated at 28±1ºC. Suitable checks (without any addition of chemical) were kept for comparison.

The plates were arranged in Completely Randomized Design (CRD) with three replications. There were five petriplate per replication per treatment. Mycelial Growth of the fungus was measured by taking the diameter in two directions and the average was recorded at an interval of 24 hours. Final growth reading was recorded when the growth of the fungus in control plate was full. Inhibition of radial growth was computed based on colony diameter on control plate using the specified formula [8].

Average radial growth of the fungus (cm) after 96 hours was computed from the data recorded at 24, 48, 72 and 96 hour and per cent inhibition of radial growth over control was calculated using the average value.

The data were subjected to statistical analysis following CRD[9]. Necessary transformations were made whenever required. SAS 9.2 software was used for calculation of the data.

RESULTS AND DISCUSSION

In vitro evaluation of fungicides

Efficacy of seven fungicides was tested at recommended concentrations by poisoned food technique. The results revealed that there was significant difference in mean radial growth of the fungus (cm) after 24 hours of observation among all the treatments with untreated control. Maximun mean radial growth (in cm) of the fungus were observed in untreated control (0.87) followed by Blitox (0.24) and Bavistin (0.16). No growth was recorded in the treatments viz. Indofil M-45, Viavax Power, Kavach, Curzet and Krilaxyl Gold, these were significantly differed from other treatments. After 48 hours of incubation, maximum mean radial growth was recorded in untreated control (5.26) followed by Blitox (3.81). All the treatment differed significantly from Blitox and control. Negligible radial growth was observed in Viavax Power (0.08) and it differed significantly from Bavistin (0.57) and Blitox. No significant difference among the treatment viz. Viavax Power, Krilaxyl Gold (0.10), Kavach (0.13), Indofil M-45 (0.14) and Curzet (0.19) were observed. Significant difference in respect to mean radial growth was recorded in untreated control (7.58) from all other treatments at 72 hours of observation. Minimum mean radial growth of the fungus was observed in Viavax Power (0.31) that was differed significantly from Curzet (0.96), Bavistin (1.70) and Blitox (5.39). Whereas no significant difference was recorded among the chemicals viz. Viavax Power (0.31), Krilaxyl Gold (0.42), Kavach (0.45) and Indofil M-45 (0.62) used in the experiment. After 96 hours of observation it was found that all the treatments differed significantly regarding mean radial growth (in cm) from Bavistin (4.11), Blitox (8.23) and untreated control (8.98). Minimum radial growth was observed in Viavax Power (0.71) followed by Krilaxyl Gold (0.79) and Kavach (0.84). No significant difference was found among the treatments Viavax Power, Krilaxyl, Kavach and Indofil M-45 (1.29) and also Curzet (1.55) and Indofil M-45. Blitox and untreated control were at par to each other (Table 1).

Table-1: Efficacy of fungicides on radial growth of *S. rolfsii*

Treatment	**Dose**	**Mean radial growth of the fungus (cm) after**			
	(%)	24 Hour	48 Hour	72 Hour	96 Hour
Indofil M-45	0.25	0.0 (0.71)*^{c}	0.14 (0.80)^{d}	0.62 (1.05)^{de}	1.29 (1.33)^{cd}
Bavistin	0.1	0.16 (0.81)^{b}	0.57 (1.03)^{c}	1.70 (1.47)^{c}	4.11 (2.13)^{b}
Blitox	0.4	0.24 (0.86)^{b}	3.81 (2.07)^{b}	5.39 (2.42)^{b}	8.23 (2.95)^{a}
Viavax Power	0.2	0.0 (0.71)^{c}	0.08 (0.76)^{d}	0.31 (0.90)^{e}	0.71 (1.10)^{d}
Kavach	0.2	0.0 (0.71)^{c}	0.13 (0.79)^{d}	0.45 (0.98)^{de}	0.84 (1.15)^{d}
Curzet	0.25	0.0 (0.71)^{c}	0.19 (0.83)^{d}	0.96 (1.21)^{d}	1.55 (1.43)^{c}
Krilaxyl Gold	0.25	0.0 (0.71)^{c}	0.10 (0.77)^{d}	0.42 (0.95)^{e}	0.79 (1.13)^{d}
Control	-	0.87 (1.17)^{a}	5.26 (2.40)^{a}	7.58 (2.85)^{a}	8.98 (3.08)^{a}
SEm(±)		0.04	0.08	0.11	0.12
CD (p=0.01)		0.07	0.17	0.22	0.25
CV		5.06	8.51	8.72	7.99

Means with the same letter are not significantly different, *Figures in parentheses are the corresponding angular transformed values

The per cent inhibition of average radial mycelial growth over control after 96 hours of observation was found highest in the treatment Viavax Power (95.00%) followed by Krilaxyl Gold (94.04%), Kavach (93.55%) and Indofil M-45 (90.66%). No significant difference was recorded among the treatments Viavax Power, Krilaxyl Gold, Kavach, Indofil M-45 and Curzet (87.77%). Bavistin was found less effective (70.44%) but negligible effect was recorded in Blitox (20.17%). All the treatments were differed significantly from Blitox. From the above discussion it was found that Viavax Power is the best fungicide to restrict the fungal growth effectively whereas Blitox has negligible effect (Table 1). Viswakarma and Basu [10] reported that seed treatment with Vitavax (Carboxin) was the most effective against the root disease of *Cicer arietinum* caused by *S. rolfsii.* Vrany *et al.* [11] and Virupaksha Prabhu [12] reported the effectiveness of Daconil 2785-W-75 and Chlorothalonil against the pathogen. Complete growth inhibition of *S. rolfsii* through Dithane M-45 and Agallol was recorded by Harlapur and Srikant Kulkarni [13] and Virupaksha Prabhu *et al.* [14]. Palaiah [15] observed sensitivity of Thiram and other chemicals to various isolates of *S. rolfsii*. No inhibitory effect of Carbendazim and Copper oxy-chloride was recorded by Johnson and Subramanyam [16]. The present findings were also corroborated with the earlier report made by Madhavi and Bhattiprolu [5].

Table-2: Efficacy of plant oils and plant extracts on radial growth of *S. rolfsii*

Treatment	Dose (% v/v)	Mean radial growth of the fungus (cm) after			
		24 Hour	48 Hour	72 Hour	96 Hour
Plant oil					
Karanja	5	0.04 (0.74)*^{c}	0.33 (0.91)^{d}	0.81 (1.14)^{c}	1.37 (1.36)^{d}
Mahua	5	0.04 (0.74)^{c}	0.34 (0.92)^{d}	1.16 (1.28)^{bc}	3.07 (1.88)^{b}
Neem	5	0.12 (0.79)^{bc}	1.08 (1.26)^{b}	1.34 (1.35)^{bc}	2.26 (1.65)^{bcd}
Chaulmoogra	5	0.26 (0.87)^{b}	0.64 (1.06)^{cd}	1.18 (1.29)^{bc}	2.02 (1.57)^{bcd}
Leaf extract					
Muraiya exatica	10	0.00 (0.71)^{c}	0.38 (0.93)^{d}	1.05 (1.24)^{bc}	1.64 (1.45)^{cd}
Vinca rosea	10	0.21 (0.84)^{b}	0.69 (1.09)^{c}	1.39 (1.37)^{b}	2.70 (1.78)^{bc}
Control	-	1.08 (1.26)^{a}	4.40 (2.21)^{a}	7.14 (2.76)^{a}	8.90 (3.06)^{a}
SEm(±)		0.04	0.07	0.09	0.17
CD (p=0.01)		0.08	0.15	0.20	0.36
CV		5.39	7.01	7.74	11.28

Means with the same letter are not significantly different, *Figures in parentheses are the corresponding angular transformed values

In vitro evaluation of plant oils and plant extracts

Efficacy of four plant oils and two leaf extract was tested by poisoned food technique. In all the cases a significant differences were recorded in untreated control from other treatments. The results depicted that there was no significant difference in mean radial growth (cm) of the fungus after 24 hours of observation among the treatments *Murraya exotica* leaf extract, Mahua oil, Karanja oil and Neem oil except Chaulmoogra oil (0.26), *Vinca rosea* leaf extract (0.21) and control (1.08). No mean radial growth of the fungus was observed in leaf extract of *Murraya exotica* whereas negligible growth was recorded in Mahua oil and Karanja oil. Neem oil, Chaulmoogra oil and leaf extract of *Vinca rosea* showed similar trend. After 48 hours of incubation, maximum mean radial growth was recorded in untreated control (4.40) followed by Neem oil (1.08). All the treatment differed significantly from control whereas no significant difference was recorded among *Murraya exotica* leaf extract (0.38), Mahua oil (0.34), Karanja oil (0.33), Chaulmoogra oil (0.64) and *Vinca rosea* leaf extract (0.69). No significant difference in respect of mean radial growth was recorded among all the treatments except untreated control (7.14) at 72 hours of observation. Minimum mean radial growth of the fungus was observed in the treatment Karanja oil (0.81) followed by *Murraya exotica* leaf extract (1.05), Mahua oil (1.16) and Chaulmoogra oil (1.18). After 96 hours of observation it was recorded that there were no significant differences regarding mean radial growth among all the treatments except Mahua oil (3.07) and untreated control (8.90). Minimum radial growth was observed in Karanja oil (1.37) followed by leaf extract of *Murraya exotica* (1.64). Karanja oil found most effective to restrict the fungal growth *in vitro* (Table 2).

Inhibition of mycelial growth of S. rolfsii by fungicides, plant oils and plant extracts after 96 hours

Among all the treatments including chemicals, plant oils and plant's leaf extract regarding per cent inhibition of radial mycelial growth over control after 96 hours of observation was found highest in the treatment Viavax Power (95.00%) followed by Krilaxyl Gold (94.04%), Kavach (93.55%) and Indofil M-45 (90.66%), Karanja oil (88.49%), Curzet (87.77%), *Murraya exotica* leaf extract (86.15%) and Chaulmoogra oil (81.45%). No significant difference was recorded among the treatments Viavax Power, Krilaxyl Gold, Kavach, Indofil M-45, Karanja oil,

Curzet, *Murraya exotica* leaf extract. Treatments viz. Chaulmoogra oil (81.45%), Mahua oil (79.15%), Neem oil (78.31%), leaf extract of *Vinca rosea* (77.39%) and Bavistin (70.44%) showed similar trend without showing significant difference among them. But negligible effect was recorded in Blitox (20.17%). All the treatments were differed significantly from Blitox. It was found from the experiment that Viavax Power is the best fungicide to restrict the fungal growth effectively whereas Blitox showed negligible effect. Mahua oil (79.20%), Neem oil (78.30%) and leaf extract of *Vinca rosea* (77.40%) were effective than Bavistin (70.52%) and Blitox (20.07%). Karanja oil and leaf extract of *Murraya exotica* can be used to check the fungal growth effectively than the other plant oils or plant extract (Table 3).

Table-3: Inhibition of mycelial growth of *S. rolfsii* using different fungicides and botanicals

Treatment	Average radial growth of the fungus (cm) after 96 hours	Per cent inhibition of radial growth over control after 96 hours
Chemicals		
Indofil M-45	0.52 (1.00)*^{hg}	90.66 (72.27)^{ab}
Bavistin	1.63 (1.45)^{c}	70.44 (57.30)^{d}
Blitox	4.42 (2.21)^{b}	20.17 (25.56)^{e}
Viavax Power	0.28 (0.88)^{h}	95.00 (77.20)^{a}
Kavach	0.36 (0.92)^{hg}	93.55 (75.35)^{a}
Curzet	0.68 (1.08)^{efgh}	87.77 (69.58)^{abc}
Krilaxyl Gold	0.33 (0.91)^{h}	94.04 (76.06)^{a}
Plant oils		
Karanja oil	0.64 (1.06)^{fgh}	88.49 (70.31)^{abc}
Mahua oil	1.15 (1.28)^{cde}	79.15 (62.95)^{cd}
Neem oil	1.20 (1.30)^{cd}	78.31 (62.41)^{cd}
Chaulmoogra oil	1.03 (1.23)^{def}	81.45 (64.71)^{bcd}
Leaf extracts		
Muraiya exatica	0.77 (1.12)^{defg}	86.15 (68.37)^{abc}
Vinca rosea	1.25 (1.32)^{cd}	77.39 (61.77)^{cd}
Control	5.39 (2.42)^{a}	-
Control	5.67(2.49)^{a}	-
SEm(±)	0.09	3.86
CD (p=0.01)	0.18	7.93
CV	7.97	7.28

Means with the same letter are not significantly different, *Figures in parentheses are the corresponding angular transformed values

Banerjee *et al.* [17] observed that Neem, Citronella and Karanja oil strongly inhibited sclerotial germination of *Sclerotium rolfsii* infecting rice under *in vitro* condition whereas, Mahua oil was non-effective. But, in the present investigation it was revealed that Neem oil is less effective than the Karanja, Chaulmoogra and Mahua oil. Calpouzos[18] reported different plant oils and their action regarding the plant disease control.

CONCLUSION:

In-vitro bioassay study revealed that Viavax Power is the best fungicide to restrict the fungal growth effectively followed by Krilaxyl Gold, Kavach, Indofil M-45 and Curzet. Blitox had negligible effect on mycelial growth. Among the plant oils and plant extracts, Karanja oil and *Murraya exotica* leaf extract recorded efficacious than Chaulmoogra oil, Mahua oil, Neem oil and leaf extract of *Vinca rosea.* To develop a holistic management strategy more studies are required. The alkaloids present in plant oils and plant leaf extracts that actually effective in reducing the growth of *Sclerotium rolfsii* have to be identified.

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