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ORIGINAL ARTICLE **OPEN ACCESS**

Testing The Effect of pH and Dextrose Levels in The Media on *Sclerotium rolfsii* Under The Presence of Antagonist and Testing The Efficacy of *Trichoderma* spp. NON Volatile Compounds and Neem Oil Against *Sclerotium rolfsii* Sacc.

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ABSTRACT
In the present investigation efforts were made to test the efficacy of *Trichoderma* spp. under different PH conditions of the PDA media and different levels of dextrose against *Sclerotium rolfsii invitro* by dual culture technique. Efforts were also made to test the efficacy of different concentrations of *Trichoderma* culture filtrate and neem oil on the growth of *S. rolfsii* using poisoned food technique. Under the neutral PH conditions and higher dextrose levels (10 gm), more inhibition percentage was observed. Similarly, Higher concentration (30%) of culture filtrate and neem oil shown more efficacy against *S. rolfsii* than the lower concentrations (20 and 10%) and all the treatments were significantly differed with each other.
Key words: *Scerotium rolfsii*, *Trichoderma* spp, Dual culture, Culture filtrate and Neem oil

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INTRODUCTION

S. rolfsii was first reported on tomato by [13] later the pathogen was named as *Sclerotium rolfsii* by [15]. Higgins [5] worked in detail on physiology and parasitism of *S. rolfsii*. This was the first detailed and comprehensive study in USA. It is distributed in tropical and sub tropical regions of the world where high temperatures prevail. The fungus has a wide host range of 500 species in about 100 families including groundnut, green bean, lima bean, onion, garden bean, pepper, potato, sweet potato, tomato and water melon [1].

S. rolfsii is a soil borne pathogen. Numerous chemicals inhibited the sclerotial germination and mycelia growth of *S. rolfsii* and efficiently controlled the disease caused by the pathogen on various crops. Carboxin was effective to inhibit *S. rolfsii* (Punja., 1985). The complete mycelia growth inhibition of *S. rolfsii* was reported with saff, tebuconazole, captan, calixin, ril F-004, tilt, idofil M-45, contaf, mancozeb, hinosan, thiram, antracol., benlate and manzate [12]. For the soil-borne pathogens, use of fungicides alone is not practical due to exorbitant cost and environmental hazards involved. Hence, integrated management of the disease using bio-control agents and chemicals is the best alternative.

Use of microorganisms beneficial to agriculture started more than 60 years ago because their capacity to convert unavailable and nutritionally important elements in to available ones, and as an alternate to increase plant resistance to adverse environment [9, 18]. These microorganisms species benefit plant development by owing to the fact that they increase nitrogen absorption, phytohormone synthesis, mineral solubulization and iron chelates. Some can also induce resistance against pests and inhibits soil pathogens through the production of antimicrobial metabolites. Furthermore, they improve soil structure and reduce soil erosion. Biological control methods involving use of natural antagonists of plant pathogens have been suggested as a safe alternative to chemical methods by several workers [11].

Biological control of soil borne plant pathogens by species of *Trichoderma* is a vital area of plant pathological research all over the world in these days [7]. A recent list of mechanisms are viz. competition

for nutrients or space, production of diffusible and or volatile antibiotics (antibiosis) like trichodermin, dermadin, trichoviridin and sesquiterpene hepatic acid [8] and hydrolytic enzymes like chitinase and β 1,3 glucanase these hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (mycoparasitism) [4].

Gour *et al*. [3] and Zape *et al*. [20] conducted studies on the effect of different PH conditions of the media on the *S. rolfsii* . Hussain *et al*. [6] used 13.5 gm dextrose, 12.5 gm sucrose and 12.5 gm starch for observing the radial growth of *S. rolfsii* on the corn meal agar. Results revealed that maximum growth observed on the dextrose 13.5 gm medium which is readily absorbed by the pathogen. Swathi *et al*. [17] tested the effect of non volatile compounds on *Sclerotium rolfsii* non volatile metabolites of Th4 *Trichoderma* isolate were more effective against *S. rolfsii* with 100% growth inhibition at 60 and 80% concentration compared to 69.3% inhibition at 80%concentration of Tv5 culture filtrate.

MATERIAL AND METHODS

Preparation of modified PDA

For preparation of one litre modified PDA media, 250 gm fresh potato slices were boiled till they became soft then the extract was filtered through the muslin cloth. 20 gm of agar was poured in to the 500 ml of water then heated for 5 min in microwave oven and to the extract, agar solution added. To this 2, 5, 10 gm dextrose was added separately then heated in microwave oven till the media became transparent. Finally the media poured into the 250 ml conical flasks, each conical flask containing the 150 ml media and autoclaved at 121^{o}C for 15 min.

Similarly**,** for preparations of one litre modified PDA media, 250 gm fresh potato slices were boiled still they become soft then the extract was filtered through the muslin cloth. 20 gm of agar was poured in to the 500 ml of water then heated for 5 min in microwave oven and to the extract, agar solution added. To this 20 gm dextrose was added then heated in microwave oven till the media became transparent. Then to decrease the PH of the media 0.1N Hcl and to increase the PH 0.1N NaoH was used. Six, seven, eight PH levels containing PDA media was prepared. Then finally the media poured into the 250 ml conical flasks, each conical flask containing the 150 ml media and autoclaved at 121^{o}C for 15 min.

To test the efficacy of *Trichoderma* spp. against *S. rolfsii* under different PH conditions of the media such as 6, 7, 8 and under different dextrose levels of the media such as 2, 5, 10 gm dual culture technique was used [2].

Twenty ml of sterilized modified PDA was poured in to the each Petri plate of 9 cm diameter aseptically. Mycelial discs measuring 6 mm diameter from four day old cultures of both fungal antagonist and the test pathogen were inoculated 7 cm apart (leaving 1 cm from periphery). Plates were incubated at 28±1^{o}C. Observations were recorded on mycelial growth of *S. rolfsii* and per cent inhibition in growth of *S. rolfsii* was calculated using the following formula [19].

$$I = \frac{C - T}{C} X100$$

Where,

I = Per cent reduction in growth of test pathogen.
C = Radial growth (mm) in monocultured check.
T = Radial growth (mm) in dual cultured plates.

Preparation of culture filtrate of *Trichoderma* spp:

In 250 ml conical flask 150 ml PDB poured and sterilized under autoclave. To this prepared PDB, actively growing three days old culture of 4mm *Trichoderma* disc was inoculated and allowed to grow for one week at 28±1^{o}C. After one week mycelia mat removed and the culture filtrate filtered twice with whatman filter paper 1 and to eliminate the bacterial contamination, the culture filtrate was filter sterilized with bacterial proof filter under vaccuam. This sterilized culture filtrate was used for poisoned the food.

Poisoned Food Technique:

Poisoned food technique was used to assess the efficacy of non volatile compounds of *Trichoderna* spp. and neem oil against *S. rolfsii*. The procedure followed was as described by [10] for fungicidal assay.

Three different conc. i.e., 10, 20, 30 percent of *Trichoderma* culture filtrate and neem oil separately used in the present investigation by mixing appropriate quantity in equivalent mass of double strength PDA.

Such prepared PDA plates were inoculated with 4 mm culture disc of (2 day old) of *S. rolfsii* for poisoned food technique under aseptic conditions.

Plates were incubated at 28±1^{o}C and observed for mycelia growth. Pathogen check was maintained for comparison. Each treatment replicated thrice.

RESULTS AND DISCUSSION

In the present investigation efforts were made to test the efficacy of *Trichoderma* spp. under different PH conditions of the PDA media and different levels of dextrose against *Sclerotium rolfsii in vitro* by dual culture technique. Observations on radial growth and percentage inhibition were recorded on fourth day when control occupies full plate. The results were analysed using CRD and data was presented in Fig 1 and 2.

Dual culture was done by inoculating *S. rolsii* and *Trichoderma* spp. test isolate at 8 cm distance leaving 1 cm from the periphery of 9 cm diameter sterile PDA plate. Monocultured check also maintained.

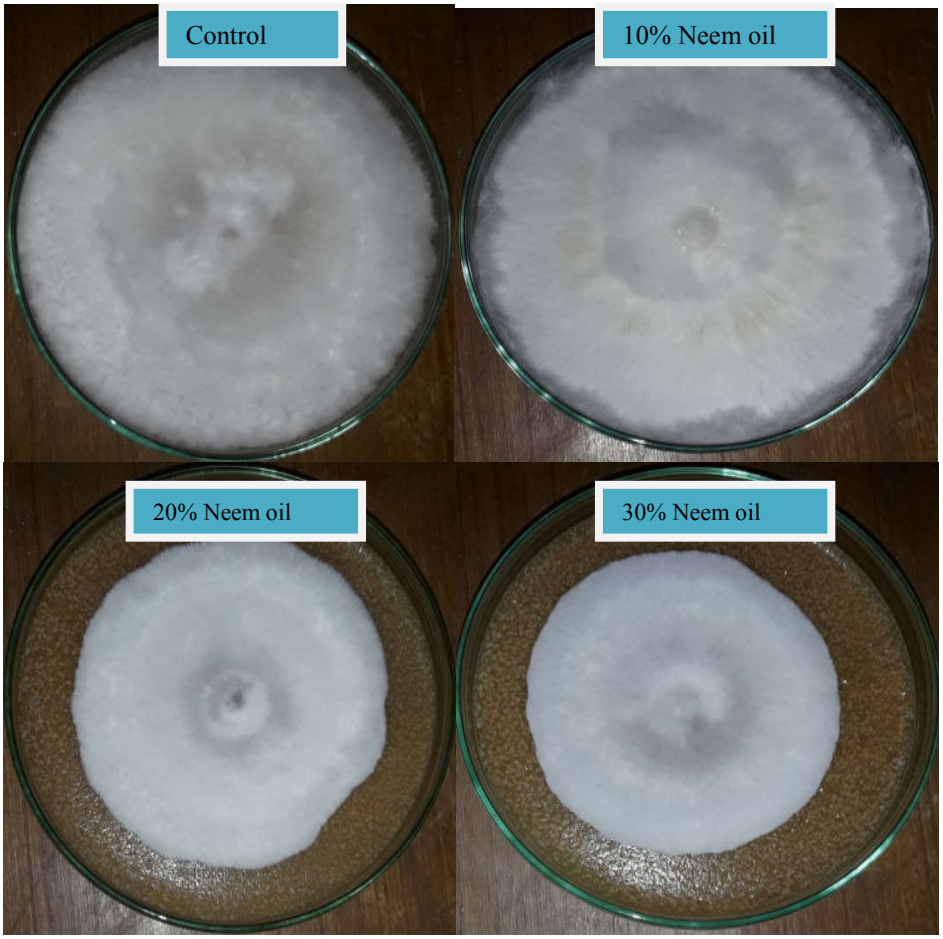

Plate 1. Mycelia growth inhibition of *Sclerotium rolfsii* in poison food technique with different concentrations of neem oil

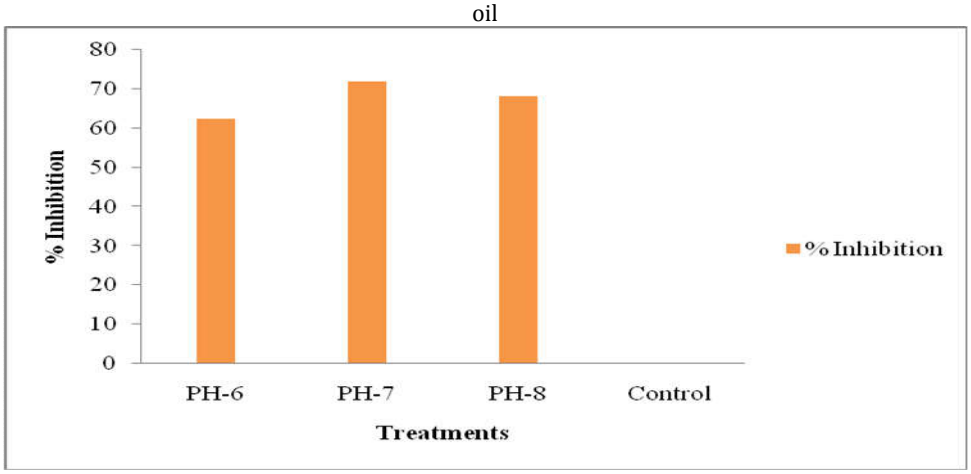

Fig 1. Percent inhibition of *Sclerotium rolfsi* when dual cultured with Trichoderma spp. under different PH conditions of the PDA media.

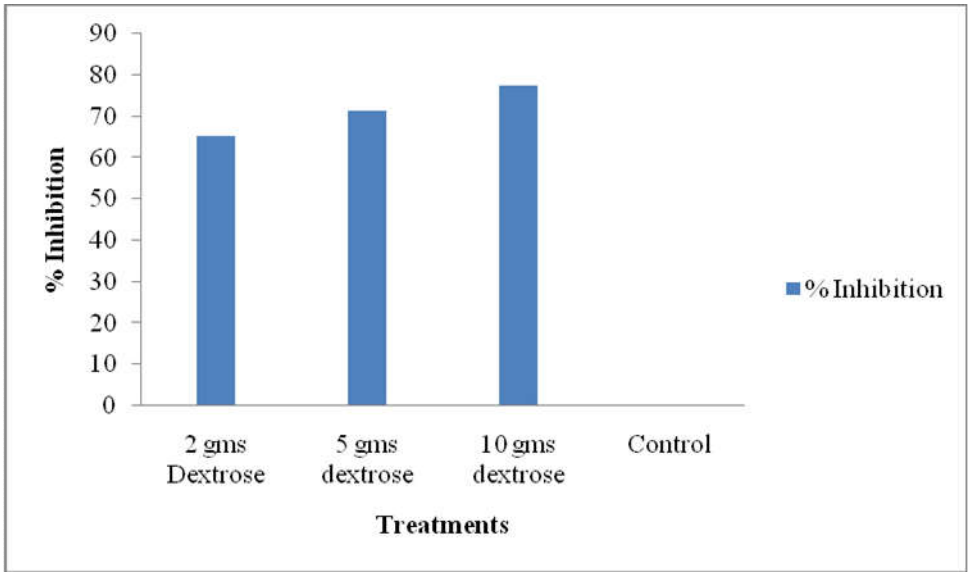

Fig 2. Percent inhibition of *Sclerotium rolfsii* when dual cultured with *Trichoderma* spp. under modified dextrose levels on PDA media.

S. No	Treatments	% Inhibition
1	10% Culture filtrate	0.00
2	20% Culture filtrate	17.04
3	30% Culture filtrate	34.81
4	Control	0.00
	C.D.	2.74
	SE(m)	0.82
	SE(d)	1.17
	C.V.	9.07

Table 1. Percent inhibition of *Sclerotium rolfsii* in poision food technique with different concentrations of *Trichoderma* spp. culture filtrate

S. No	Treatments	% Inhibition
1	10% Neem oil	0.00
2	20% Neem oil	24.44
3	30% Neem oil	38.52
4	Control	0.00
	C.D.	2.45
	SE(m)	0.74
	SE(d)	1.05
	C.V.	8.16

Table 2. Percent inhibition of *Sclerotium rolfsii* in poision food technique with different concentrations of neem oil

When the pathogen was dual cultured with the antagonist under modified PH conditions of the PDA media such as 6, 7, 8, maximum inhibition (72.08%) was observed with the PH-7, followed by PH-8 (68.33%). Least inhibition (62.50%) observed with PH-6 treatment. All the treatments significantly differed among them. Under PH-6 conditions *Trichoderma* spp. sporulation was very less compared with PH-7, PH-8 and control.

Under the neutral PH conditions more inhibition percentage was observed. These neutral conditions may favour the growth of *Trichoderma* spp. it may leads to production of higher amount of secondary metabolites by *Trichoderma* spp or this PH conditions may be less favourable for the growth of *S. rolfsii*.

Similar reports were obtained by Gour *et al*. [3] the pathogen, *S. rolfsii* grew on a wide range of pH from 4 to 9 but the maximum growth of the fungus was recorded on the medium having pH value as 6.0 (87.00 mm) followed by pH 5.0 (76.67 mm), lowest mycelial growth was obtained at pH 9.0 (28.67 mm) and pH 8.0 (40.33 mm). He concluded that optimum pH for best growth of *S. rolfsii* lies between pH 6 to 7.

Zape *et al*. [20] also found cultural studies of the test fungi on different pH levels revealed that pathogens had a wide range of pH. The maximum radial growth of *S. rolfsii* was observed at pH 6.5 followed by pH 6.0 and 7.0 and whereas, for formation of sclerotial, it was at pH 7.0.

When the *S. rolfsii* dual cultured with *Trichoderma* spp. under modified dextrose levels in the PDA media such as 2, 5, 10 gm, maximum mycelia growth inhibition (77.5%) observed with the 10 gm dextrose followed by 5 gm dextrose (71.25%). Least inhibition (65.41%) observed with the 2 gms dextrose. All the treatments significantly differed with each other.

Under high sugar conditions the growth of S. rolfsii inhibited more at the same time high dextrose levels may favours the growth of Trichoderma spp. it may leads to production of extra cellular metabolites.

Similar reports also obtained by Hussain *et al*. [6] they used 13.5 gm dextrose, 12.5 gm sucrose and 12.5 gm starch for observing the radial growth of *S. rolfsii* on the corn meal agar. Results revealed that maximum growth observed on the dextrose 13.5 gm medium which is readily absorbed by the pathogen.

In the present investigation efforts were also made to test the efficacy of different concentrations of *Trichoderma* culture filtrate and neem oil on the growth of *S. rolfsii* using poisoned food technique.

Ten, twenty, thirty percent concentration of *Trichoderma* culture filtrate and neem oil were used for assessment of growth of *S. rolfsii*. Observations on radial growth were recorded and results were analysed using CRD, data was presented in Table 1, 2 and Plate 1.

When the radial growth of *S. rolfsii* observed under the 10, 20, 30% concentration of culture filtrate of *Trichoderma* spp in poisoned food maximum mycelia growth inhibition (34.81%) was observed with 30% culture filtrate followed by 20% culture filtrate (17.04%). Least inhibition percentage (0.00%) observed with 10% culture filtrate. All the treatments were significantly differed with each other.

Higher concentration (30%) of culture filtrate show more efficacy against *S. rolfsii* than the lower concentrations. Non volatile compounds present in the culture filtrate play the role in suppression of growth of *S. rolfsii*.

Similar reports obtained by Swathi *et al*. 2015. non volatile metabolites of Th4 Trichoderma isolate were more effective against *S. rolfsii* with 100% growth inhibition at 60 and 80% concentration compared to 69.3% inhibition at 80%concentration of Tv5 culture filtrate.

When the radial growth of *S. rolfsii* observed under the 10, 20, 30% concentration of neem oil in poisoned food, maximum mycelia growth inhibition (38.52%) was observed with 30% neem oil followed by 20% neem oil (24.44%). Least inhibition percentage (0.00%) observed with 10% culture filtrate. All the treatments were significantly differed with each other.

Higher concentration of neem oil (30%) show more efficacy against *S. rolfsii* than the lower (20 and 10%) concentrations.

Similar results were obtained by Suryawanshi *et al*. [16]. Maximum inhibition of *S. rolfsii* observed with the neem oil (43.6%) followed by Eucalyptus oil in Poisoned food technique.

CONCLUSION

The present investigation concluded that different PH and different levels of dextrose conditions influenced the growth of the pathogen and antagonist and also affects the compatibility between them.

Neutral PH conditions may favour the growth of the antagonist in the presence of pathogen that may leads to suppression of the pathogen growth or this neutral conditions may be less favour the growth of pathogen in dual culture compared with PH-6 and 8. Lower (PH-6) may not favour the sporulation of *Trichoderma* under the presence of pathogen or less favourable.

Higher sugar levels may favour the growth of *Trichoderma* spp in dual culture it may leads to production of secondary metabolites ultimately affect the growth of pathogen compared with 2 and 5 gm dextrose or higher dextrose levels less favourable for the pathogen growth.

A higher concentration (30%) of culture filtrate was more effective towards the *S. rolfsii* than the lower concentrations such as 10 and 20% in dual culture. Secondary metabolites present in the culture filtrate (Non volatile compounds) may play the role in suppression of the growth of the pathogen.

Neem oil plays the important role in the suppression of growth of the pathogen. Higher concentration (30%) of neem oil showed the more efficacy against pathogen than the lower concentrations (20, 10%) comparatively.

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