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IN VITRO SCREENING OF BIO EFFICACY OF DIFFERENT ISOLATES OF *Trichoderma viride* AND *Pseudomonas fluorescens* ON THE MYCELIAL GROWTH OF *Sclerotium rolfsii* INCITANT OF GROUND NUT STEM ROT

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ABSTRACT

The antagonistic effect of three isolates Tr-DM, Tr-SNG and Tr-RGP of *Trichoderma viride* and three isolates Pf-DM, Pf-SNG and Pf-RGP of *Pseudomonas fluorescens* against *Sclerotium rolfsii* were assessed by dual culture technique *in vitro*. The isolates of fungal agent *T. viride* were more effective in controlling the mycelial growth of *S. rolfsii* when compared with isolates of bacterial bio control agent *P. fluorescens.* Among the fungal bio-control agents maximum inhibition (87.18%) of mycelial growth of *S. rolfsii* was observed with the DM-Tr isolate of *T. viride* and among bacterial isolates maximum inhibition (28.25%) of mycelial growth of *S. rolfsii* was observed with the Pf-DM isolate of *P. fluorescens*.

KEYWORDS: Bio agents, dual culture, inhibition, *Trichoderma viride* and *Pseudomonas fluorescens*, *Sclerotium rolfsii*.

INTRODUCTION:

Groundnut (Arachis hypogaea L.) is an important oil seed crop suitable for cultivation in tropical areas of the world. It is regarded as "King of oilseed crops" on account of its diversified uses. Groundnut is third largest oil seed crop grown in world and second in India. Groundnut seeds are rich in oil (43-55%) as well as protein (25-28%) and also contains 18 percent carbohydrates. It can supply about 5.6 and 5.8 calories pergram of kernel in the raw and roasted forms respectively. It is also very good source of minerals (calcium, magnesium and iron) and vitamins (B1,B2 and Niacin). Groundnut being a legume crop, it fixes a large amount of nitrogen and improves the fertility status of the soil. Groundnut cake is used as animal feed and the shell sometimes used as fodder.

S. rolfsii was first reported on tomato by Rolfs (1892) later the pathogen was named as *Sclerotium rolfsii* by Saccardo (1911). Higgins (1927) 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 subtropical regions of the world where bean, lima bean, onion, garden bean, pepper, potato, temperatures prevail. The fungus has a wide host rang potato, omato and water melon (Aycock, 1966). 500 speciesin about 100 families including ground.

Accoding to West (1961) the fungus belonging to the group of non-spore producing fungi and production of small round sclerotia is an important morphological characteristic feature of the organism. Initially the fungal mycelium is silky white in pure culture but gradually looses its luster and becomes somewhat dull in appearance. The mycelium is radiating with abundant aerial hyphae. The mycelum completely disappears over a period of three months leaving only sclerotial bodies. According to (Subramanian 1964 and Mehan *et al.,* 1995) sclerotial are at first white, becomes light brown to dark brown at maturity.

Stem rot of groundnut has become one of the major constraints in recent years. Management of stem rot disease is difficult because of soil borne nature and the chemical methods are very expensive and will not have good effect against the pathogen. In view of unsatisfactory management of this disease, a considerable attention has been given on the other non-chemical means of plant disease control *i.e.,* the integration of biological methods which include the use of eco-friendly bio control agents. Virupaksha *et al.,* (1997) reported the antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride*against *Sclerotium rolfsii* and found to be effective in inhibiting the mycelial growth and reducing production of sclerotial bodies irrespective of inoculation periods.

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against *Sclerotium rolfsii* and found to be effective in inhibiting the mycelial growth and reducing production of sclerotial bodies irrespective of inoculation periods.

MATERIAL AND METHODS:

List of different isolates of bio agents used in the study

S . NO	Isolates	
	Trichoderma viride	*Pseudomonas fluorescens*
1	Tr-SNG	Pf-SNG
2	Tr-DM	Pf-DM
3	Tr-RGP	Pf-RGP

Evaluation of efficacy of *Trichoderma* isolates

Three isolates of *T. Viride* available at Plant Pathology lab, S.V.Agricultural College, Tirupati were evaluated in *in vitro* against *S. rolfsii* by dual culture method (Dennis and Webster, 1971). Twenty ml of sterilized PDA was poured in to 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). Four replications were maintained for each treatment. Radial growth of the mycelium was recorded and percent inhibition over control was calculated. The data were analysed statistically using Completely Randomized Design (CRD)

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

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

Evaluation of efficacy of *P. fluorescens* isolates

Evaluation of efficacy of P. *fluorescens* available at Plant Pathology lab, S.V.Agricultural College, Tirupati were evaluated *in vitro* against *S. rolfsii* by dual culture method (Dennis and Webster, 1971). *S. rolfsii* was nocultated at ithe center of PDA plate. Test bacterial cultures were str -eaked individually on both the sides of the *S. rolfsii* at 2.5 cm distance leaving 2.0 cm periphery. Plates inoculated with *S. rolfsii* alone were maintained as checks. Inoculated plates were incubated at 28 ± 2^{o}C. Four replications were maintained for each treatment. Observations were recorded considering zone of inhibition up to four days when *S. rolfsii* completely occupied the plate in monoculture check. Per cent inhibition of mycelia growth over control was calculated using the formula given. In the data was analyzed statistically using CRD design.

RESULTS AND DISCUSSION

Effect of different isolates of *Trichoderma viride* and *Pseudomonas fluorescens* were presented in Table 1 and Fig 1.

Significant differences were observed among the three isolates of *T. viride* in inhibiting the mycelial growth of *S. rolfsii.* Maximum percent inhibition (87.18%) mycelial growth was observed in Tr-DM isolate followed by Tr-SNG isolate (79.13%). Tr-RGP isolate proved to be least effective (75.93%) among the three isolates.

Among the three isolates of *P. fluorescens,* maximum percent inhibition (28.25%) of mycelial growth of *S. rolfsii* was observed with Pf-DM isolate followed by Pf-SNG isolate (9.40%). Pf-RGP isolate did not show any effect in inhibiting the mycelial growth of *S. rolfsii.*

Among the three *T. viride* fungal bio agents and three bacterial *P. fluorescens* bio agents, fungal bio agents proved to be significantly highly effective on mycelia growth of *S. rolfsii* compared to bacterial bio agents.

Antagonistic activity of *T. harzianum* and *T. viride* against *S. rolfsii* was found to be effective in inhibiting the mycelial growth and reducing production of sclerotial bodies was reported by Virupaksha *et al.,* (1997) and Rajalakshmi (2002), Salvi *et al.,* (2017) screened three bio-agents *in vitro* namely *T. viride, T. harzianum* and *P. fluorescens* and found that *T. viride* showed maximum growth inhibition (83.33%) against *S. rolfsii.* Ganesan and Gnanamanikyam (1987) and Wokocha *et al.,* (1986) reported that, the native strain of *P. fluorescens* restricted the growth of *S. rolfsii* causing stem rot of groundnut. Chanutsa *et al.,* (2014) reported 100 per cent inhibition in growth of *S. rolfsii with* culture filtrate of *P. fluorescens.*

SUMMARY AND CONCLUSION:

The antagonistic effect of three isolates Tr-DM, Tr-SNG and Tr-RGP of *Trichoderma viride* and Pf-DM, Pf-SNG and Pf-RGP of *Pseudomonas fluorescens* against *S. rolfsii* were assessed by dual culture technique *in vitro.* The isolates of fungal biocontrol agent *T. viride* were more effective in controlling the mycelial growth of *S. rolfsii* when compared with isolates of bacterial biocontrol agent *P. fluorescens*. Among the fungal biocontrol agents maximum inhibition (87.18%) of mycelial growth of *S. rolfsii* was observed with the Tr-DM isolate of *T. viride* and among bacterial isolates maximum inhibition (28.25%) of mycelial growth of *S. rolfsii* was observed with the Pf-DM isolate of *P. fluorescens* and they were significantly different from each other.

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Table 1: Efficacy of different isolates of *T. viride* and *P. fluorescens* on mycelial growth of *S.rolfsii* after 4 days of incubation.

Bioagent *T. viride*	Mycelial growth(cm)	Inhibition (%)
Tr-SNG	1.57	79.13 (62.83**)
Tr-DM	1.02	87.18 (69.01)
Tr-RGP	1.92	75.93 (60.60)
P. fluorescens		
Pf-SNG	4.07	9.40 (17.75)
Pf-DM	3.22	28.25 (32.09)
Control	4.50	
C.D.		1.84
SE(m)		0.62
SE(d)		0.88
C.V.		3.59

**** Figures in parentheses are angular transformed values**

Tr-DM - Damaramadugu isolate
Tr-SNG - Sangam isolate
Tr-RGP - Rangampet isolate

Pf-DM - Damaramadugu isolate
Pf-SNG - Sangam isolate
Pf-RGP - Rangampet isolate

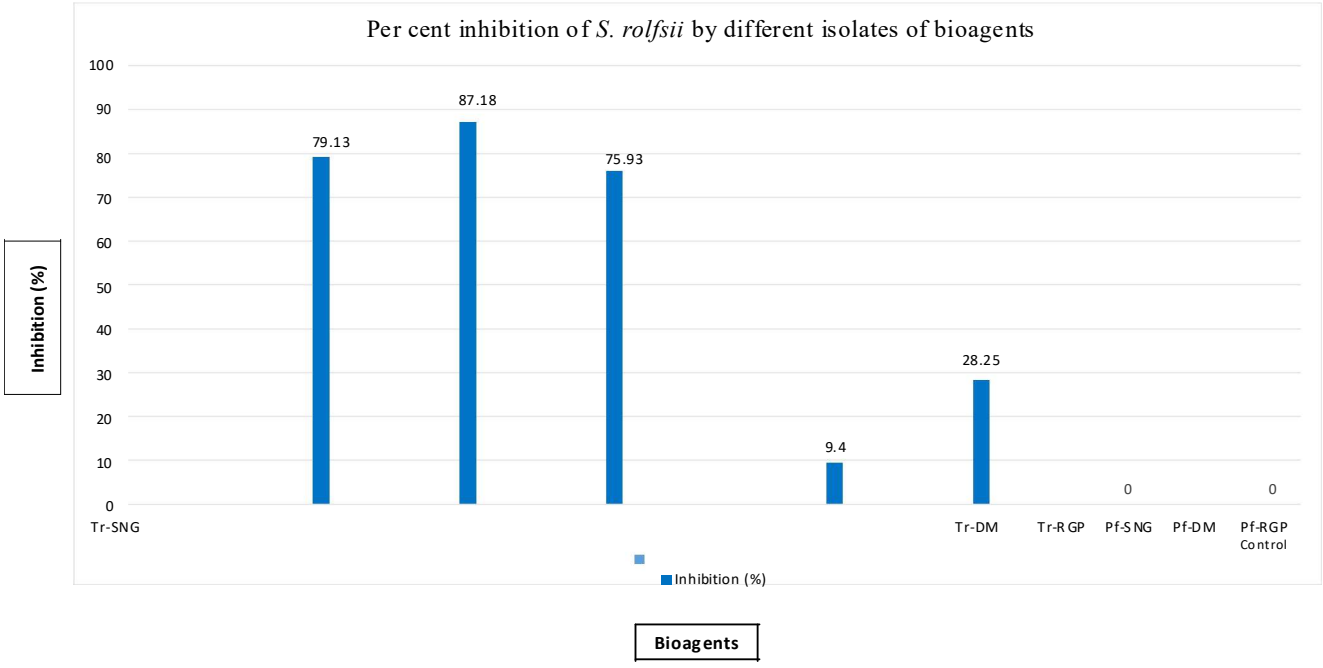

Fig. 1. Efficacy of different isolates of *T. viride* and *P. fluorescens* on mycelial growth of *S.rolfsii* after 4 days of incubation.