



## IN VITRO SCREENING OF ANTAGONISTIC *Trichoderma* spp. ISOLATES AGAINST *Macropomina phaseolina*

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ABSTRACT

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Six isolates of *Trichoderma* spp. were tested for their antagonist potential against four isolates of *M. phaseolina*, incitant of groundnut dry root rot by following dual culture technique *in vitro*. Among all the isolates of *Trichoderma*, GRT5 shown highest (59.48%) mean inhibition followed by GRT2 (59.38%), GRT1 (59.27%) and GRT4 (58.96%). From the above study top four isolates of *Trichoderma* spp. which showed maximum mean inhibition per cent when dual cultured with four isolates of *M. phaseolina* viz., GRT5, GRT2, GRT1 and GRT4 were considered as effective *Trichoderma* isolates and one isolate of pathogen i.e., SmMp was considered as a more virulent pathogen which exhibited lowest mean percent inhibition (56.81%) in dual culture.

**KEYWORDS:** *Trichoderma* spp. *M. Phaseolina*, Dual culture, Inhibition percent and Dry root rot.

### INTRODUCTION

Groundnut is a major oil seed crop in India covering an area of 4.59 M ha with a production of 6.73 M t averaging a productivity of 1.46 t ha<sup>-1</sup>. In Andhra Pradesh, it is grown over an area of 0.77 M ha with a production of 0.80 M t and productivity of 1.03 t ha<sup>-1</sup> (Commodity Market of India, 2016). In Andhra Pradesh, majority of groundnut crop is cultivated during *kharif* in Anantapuramu, Chittoor, Kurnool, Kadapa districts occupying 97.42 per cent area of the total groundnut growing area in Andhra Pradesh (Anonymous, 2014-15).

Several factors such as water stress, pests, and diseases are responsible for the low productivity of groundnut in A.P (1.03 t ha<sup>-1</sup>) compared with national average (1.46 t ha<sup>-1</sup>). Diseases such as late leaf spot (*Phaeoisariopsis personata*), collar rot (*Aspergillus niger*), stem rot (*Sclerotium rolfsii*) and dry root rot (*M. phaseolina*) are of major concern among fungal diseases.

In groundnut, *M. phaseolina* is known to cause root, stem, peg and pod rots and leaf spots on seedlings and on older plants. It also causes seedling blight, root rot and charcoal rot diseases on more than 500 plant species from more than 100 families distributed worldwide. The disease appears in hot and dry weather when soil temperature is 80-95°F (27-35°C) for 2-3 weeks. The disease control is inefficient or difficult by using the chemical fungicides.

The fungus is a facultative parasite capable of living saprophytically on dead organic tissue, particularly on many of its natural hosts producing sclerotial bodies. The fungus is mainly a soil dweller and spreads from plant to plant through irrigation water and implements and cultural operation. The sclerotia and pycniospore may also become air borne and cause further spread of the pathogen (Rangaswami and Mahadevan, 2008).

Biological control seems to offer a practicable approach. As biological control has several advantages (when applied either alone or in combination with other management practices) like ecofriendly, effective against soilborne diseases and having growth promoting activity which cannot be possible by chemicals.

Biocontrol agents like *Pseudomonas fluorescens* and *Trichoderma* spp. have been assessed for their efficacy against *M. phaseolina* and *S. rolfsii* (Ganasen and Sekar, 2012; Monali *et al.*, 2016).

Application of two different biocontrol agents or two strains of the same biocontrol agent with different mechanisms of action gives the advantage of complementing each other in nullifying the deleterious effect of plant pathogens (Mishra *et al.*, 2013; Rajasekhar *et al.*, 2016).

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## MATERIALS AND METHODS

Screening of *Trichoderma* isolates for their antagonistic potential against *M. phaseolina* was done using dual culture method (Morton and Straube, 1955).

For finding the potential fungal antagonist against test pathogen, 5 mm mycelial disc of fungal antagonist was placed at 1 cm away from the periphery of 9 cm sterile Petri plate containing 20 ml of sterile PDA. Then, 5mm mycelial disc of the test pathogen was placed opposite to the mycelial disc of fungal antagonist at 1cm away from the periphery of Petri plate. The plates were kept in incubator at  $25 \pm 2^\circ\text{C}$  for incubation. Readings were recorded when the pathogen in the monoculture grown fully.

Per cent inhibition of mycelial growth of test pathogen over control was calculated by the formula given by Vincent (1927).

$$I = \frac{C-T}{C} \times 100$$

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.

## RESULTS AND DISCUSSION

Six isolates of *Trichoderma* spp. were tested for their antagonist potential against four isolates of *M. phaseolina*, incitant of groundnut dry root rot following dual culture technique *in vitro*. The results were analysed using two factorial CRD and were represented in Table 3. 1.

Among the six isolates of *Trichoderma* spp. tested against the four isolates of *M. phaseolina*, maximum mean inhibition per cent (59.48%) was observed with the GRT5 isolate followed by GRT2 (59.38%), GRT1 (59.27%), GRT4 (58.96%) and GRT6 (58.13%). The isolate GRT5 significantly differed with GRT6 and GRT3 isolates and on par with GRT2, GRT1 and GRT4 isolates. Lowest mean inhibition per cent (58.02%) was observed with the GRT3 isolate, which significantly differed with the GRT5, GRT2 and GRT1 isolates, insignificant with the GRT4 and GRT6 isolates.

Interaction effects between RgMp isolate of pathogen dual cultured with the all the six isolates of *Trichoderma* spp. revealed that maximum inhibition per cent (67.92%) was observed with the GRT5 isolate followed by GRT2 (62.92%), GRT4 (61.67%), GRT3 (60.42%), GRT1(58.75%) and GRT6 (57.50%) isolates. The isolate GRT5 significantly differed with the all the remaining isolates. Lowest inhibition per cent (57.50%) was observed with GRT6 isolate, which significantly differed with all the remaining isolates of *Trichoderma* spp. except isolate GRT1.

When TpMp isolate of pathogen dual cultured with all the six isolates of *Trichoderma* spp. maximum inhibition per cent (61.25%) was observed with the isolate GRT6 followed by GRT1 (58.75%), GRT5 (58.33%), GRT4 (57.92%) and GRT3 (56.67%) isolates. The isolate GRT6 significantly differed with all the remaining isolates of *Trichoderma* spp. The lowest inhibition per cent (55.42%) was observed with the isolate GRT2, which significantly differed with the other remaining isolates except isolate GRT3.

Interaction effects between SgMp and all the six isolates of *Trichoderma* spp. revealed that maximum inhibition per cent (60.42%) was observed with GRT1, GRT2 and GRT4 isolates of *Trichoderma* which insignificantly differed among them and also with other remaining isolates of *Trichoderma* spp. except GRT5 (55.83%). Lowest inhibition per cent (55.83%) was observed with the isolate GRT5, which significantly differed with the all the remaining isolates of *Trichoderma* spp.

Results from dual culture interaction studies in case of SmMp isolate and six isolates of *Trichoderma* spp., revealed that maximum inhibition per cent (59.17%) was observed with isolate GRT1 followed by GRT2 (58.75%), GRT3 (56.67%), GRT4 (55.83%) and GRT5 (55.83%). Lowest inhibition per cent (54.58%) was observed with isolate GRT6.

From the above study top four isolates of *Trichoderma* spp. which showed maximum mean inhibition per cent when dual cultured with four isolates of *M. phaseolina viz.*, GRT5, GRT2, GRT1 and GRT4 were considered as effective *Trichoderma* isolates. Besides the one isolate of pathogen which showed lowest mean per cent inhibition (56.81%) in dual culture i.e., SmMp was considered as virulent pathogen.

**Table 1. *In vitro* evaluation of efficacy of antagonistic *Trichoderma* spp. isolates against *M. phaseolina* in dual culture technique**

Treatments	RgMp			TpMp			SgMp			SmMp			Mean A
	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	
GRT1	3.30	58.75 (50.02)	3.30	58.75 (50.02)	3.17	60.42 (50.99)	3.27	59.17 (50.26)	3.26	59.27 <sup>abc</sup> (50.32)			
GRT2	2.97	62.92 (52.47)	3.57	55.42 (48.09)	3.17	60.42 (51.00)	3.30	58.75 (50.02)	3.25	59.38 <sup>ab</sup> (50.39)			
GRT3	3.17	60.42 (51.00)	3.47	56.67 (48.81)	3.33	58.33 (49.78)	3.47	56.67 (48.81)	3.36	58.02 <sup>de</sup> (49.60)			
GRT4	3.07	61.67 (51.73)	3.37	57.92 (49.54)	3.17	60.42 (50.99)	3.53	55.83 (48.33)	3.28	58.96 <sup>abcd</sup> (50.15)			
GRT5	2.57	67.92 (55.48)	3.33	58.33 (49.78)	3.53	55.83 (48.33)	3.53	55.83 (48.33)	3.24	59.48 <sup>a</sup> (50.48)			
GRT6	3.40	57.50 (49.30)	3.10	61.25 (51.48)	3.27	59.17 (50.27)	3.63	54.58 (47.61)	3.35	58.13 <sup>de</sup> (49.66)			
<b>Mean B</b>	3.08	61.53 <sup>a</sup> (51.67)	3.36	58.06 <sup>c</sup> (49.62)	3.27	59.10 <sup>b</sup> (50.23)	3.46	56.81 <sup>d</sup> (48.89)					
<i>M. phaseolina</i> monoculture	8.00	0.00											
<b>Factors</b>	<b>C.D (P=0.01)</b>	<b>C.D (P=0.01)</b>	<b>SEm±</b>	<b>SEm±</b>	<b>SEm±</b>	<b>SEm±</b>							
<i>Trichoderma</i> spp. Isolates	0.09	1.12	0.03	0.39									
<i>M. phaseolina</i> Isolates	0.07	0.91	0.03	0.32									
Interactions	0.18	2.24	0.06	0.78									

\*Values are the means of three replications; Values in the parenthesis are angular transformed values

The results were in agreement with the findings of Karthikeyan *et al.* (2006) who reported that *T. viride* (Tv1) and *T. harzianum* were most effective in reducing the mycelial growth and sclerotial formation of *M. phaseolina* causing dry root rot of groundnut. They also reported that volatiles of Tv1 had shown greater fungistatic effect on *M. phaseolina* under *in vitro* conditions.

Ramezani (2008) studied efficacy of four fungal bioagents *viz.*, *T. hamatum*, *T. harzianum*, *T. polysporum* and *T. viride* under *in vitro* conditions against the brinjal root rot pathogen, *M. phaseolina*. He reported that *T. harzianum* produced the maximum inhibition zone of 18.20 per cent compared to the minimum of 7.30 per cent by *T. hamatum*.

## SUMMARY AND CONCLUSION

Among the six *Trichoderma* isolates tested against four isolates of *M. phaseolina*, isolate GRT5 was superior with highest (59.48%) mean inhibition followed by GRT2 (59.38%), GRT1 (59.27%) and GRT4 (58.96%) isolates which were on par with each other. From the above results top four isolates of *Trichoderma* spp. which showed maximum mean inhibition per cent when dual cultured with four isolates of *M. phaseolina viz.*, GRT5, GRT2, GRT1 and GRT4 were considered as effective *Trichoderma* isolates. Besides this one isolate of pathogen *i.e.*, SmMp was considered as a more virulent pathogen which showed lowest mean percent inhibition (56.81%) in dual culture.

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