



IN VITRO ANTAGONISM OF *Trichoderma* spp. AGAINST SIX SOIL BORNE FUNGAL PATHOGENS

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Abstract

The concept of biological control is getting more importance nowadays than the chemicals because of its ecofriendly nature. The most popular bio-control agent Trichoderma spp. is known for its antagonistic property against various soil borne fungal pathogens. By taking these into consideration an in vitro study was conducted with twenty native isolates of Trichoderma spp. isolated from soil samples collected from different locations of Kerala and important soil borne fungal pathogens viz., Pythium aphanidermatum, Phytophthora capsici, Fusarium oxysporum, Sclerotium rolfsii, Ganoderma lucidum and Rhizoctonia solani. The antagonistic efficiency of these isolates was tested by taking two released isolates of Trichoderma spp. viz., T. viride and T. harzianum of Kerala Agricultural University, as reference. The results revealed that all the twenty tested native isolates of Trichoderma spp. were efficient in controlling the pathogens, viz., P. aphanidermatum, G. lucidum, S. rolfsii and F. oxysporum than that of reference cultures. The isolates Tr9, Tr 48 and Tr 52 showed cent per cent inhibition over the pathogen, P. aphanidermatum. All the native isolates of Trichoderma spp. recorded 83.33-100 per cent inhibition on the growth of the pathogen, P. capsici. Except 3 isolates viz., Tr 28 (44.85%), Tr 76 (57.78%) and Tr 114 (58.18%), all showed higher antagonistic efficiency to G. lucidum than that of reference cultures. The native isolates recorded < 50 per cent inhibition over R. solani which was as same as that of reference isolates. All native isolates of Trichoderma spp. showed > 50 per cent inhibition on the growth of S. rolfsii whereas T. viride and T. harzianum showed a per cent inhibition of 28.11 and 35.22 respectively. All except Tr 41, Tr 78, Tr 81 and Tr 118 recorded cent per cent inhibition over F. oxysporum. Among the twenty native isolates of Trichoderma spp. tested, twelve isolates which were able to show higher antagonistic potential than the reference cultures of KAU, can be used for further use.

Keywords – Trichoderma, antagonism, in vitro, biocontrol, per cent inhibition, phytopathogens

I. INTRODUCTION

Biological control has emerged as an important alternative in managing soil borne plant diseases in recent years, as it is more eco friendly. Fungi in the genus, *Trichoderma* spp. are free-living and are common in soil and root ecosystems [1]. It has been known since at least the 1920s for their ability to act as biocontrol agents against plant pathogens. The fungus, *Trichoderma* spp. controls ascomycetous, deuteromycetous and basidiomycetous fungi, which are mainly soil-borne but also airborne pathogens [2]. The present study was conducted in Department of Plant Pathology, College of Horticulture, Kerala Agricultural University, Vellanikkara. For this study, a purposive sampling survey was conducted in northern, central and southern regions of Kerala and collected 51 soil samples from different locations.

Preliminary screening of 128 isolates of *Trichoderma* spp. (named as Tr1 to Tr 128) isolated from those soil samples yielded in the selection of 41 isolates of *Trichoderma* spp. from which 20 native isolates of *Trichoderma* spp. were taken for this study. The *in vitro* antagonistic performance of these isolates against six soil borne fungal pathogens viz., *Pythium aphanidermatum*, *Phytophthora capsici*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Ganoderma lucidum* and *Rhizoctonia solani* was tested by taking two isolates of *Trichoderma* spp. viz., *T. viride* and *T. harzianum* released and recommended by Kerala Agricultural University as reference.

II. MATERIALS AND METHODS

2.1. Isolation of pathogens

The soil borne fungal pathogens viz., *P. aphanidermatum*, *P. capsici*, *G. lucidum*, *R. solani*, *S. rolfsii* and *F. oxysporum* were isolated from the host plants ginger, pepper, coconut, rice, pepper and banana respectively by following the tissue segment method. The individual pure cultures of all the six phytopathogens were maintained on PDA slants and stored at 4°C for further use.

2.2. Isolation and maintenance of *Trichoderma* spp.

The isolation of *Trichoderma* spp. was carried out from the collected soil samples on three medias viz., PDA, Rose Bengal Agar and *Trichoderma* selective medium (TSM) using dilution plate technique. The colony counts of *Trichoderma* spp. in two dilutions ie. 10^{-3} and 10^{-4} on all the three mediums were estimated. The isolates were purified by single spore method; pure cultures of isolates of *Trichoderma* spp. were maintained on PDA slants and stored at 4°C for further use.

2.3. *In vitro* screening of *Trichoderma* spp. against soil borne fungal pathogens

The antagonistic activity of the twenty isolates of *Trichoderma* spp. against six soil borne fungal pathogens viz., *P. aphanidermatum*, *P. capsici*, *G. lucidum*, *R. solani*, *S. rolfsii* and *F. oxysporum* was tested *in vitro* using dual culture experiment. Petri dishes containing PDA were inoculated with 8mm diameter mycelial disc of each pathogen and *Trichoderma* spp. on the opposite sides of the same Petri plate at a distance of 2cm from the periphery. The control for each dual culture was maintained without antagonist (only pathogen). Each treatment was replicated thrice. The plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$). The plates were evaluated for antagonistic activity, considering the ability of the microorganisms to reduce pathogen colony expansion. Per cent inhibition on radial growth of each pathogen was calculated and compared with that of reference cultures of KAU.

$$\text{Per cent inhibition of pathogen} = [(C-T)/C] \times 100$$

C- Growth of the pathogen in the control plate

T- Growth of the pathogen in the dual culture plate

III. RESULTS AND CONCLUSIONS

The results of the *in vitro* antagonistic study revealed that all the twenty native isolates of *Trichoderma* spp. were found efficient in inhibiting the growth of *P. aphanidermatum* with similar or better performance than that of reference cultures of *T. viride* and *T. harzianum* (Table.1). Among that isolates Tr9, Tr 48 and Tr 52 showed cent per cent inhibition over the same pathogen. All the native isolates of *Trichoderma* spp. recorded 83.33-100 per cent inhibition on the growth of the pathogen, *P. capsici*. Except 3 isolates viz., Tr 28 (44.85%), Tr 76 (57.78%) and Tr 114 (58.18%), all showed higher

antagonistic efficiency to *G. lucidum* than that of reference cultures. Among them the antagonistic potential of Tr 76 and Tr 114 was better than that of *T. harzianum* (52.12%) of KAU. The native isolates recorded < 50 per cent inhibition over *R. solani* which was as same as that of reference isolates. All native isolates of *Trichoderma* spp. except Tr 114 (49.67%) showed > 50 per cent inhibition on the growth of *S. rolfisii* whereas *T. viride* and *T. harzianum* showed a per cent inhibition of 28.11 and 35.22 respectively. All except Tr 41, Tr 78, Tr 81 and Tr 118 recorded cent per cent inhibition over *F. oxysporum*. Even then, those four isolates were able to show a per cent inhibition of > 68% to the same pathogen. At the same time reference cultures of *T. viride* and *T. harzianum* of KAU could only record a per cent inhibition of 61.56 and 33.33 respectively against the same pathogen which was lesser than that of native isolates of *Trichoderma* spp.

Table. 1. Comparison of antagonistic efficiency of native and reference cultures of *Trichoderma* spp. under *in vitro* condition

Isolates of <i>Trichoderma</i> spp.	Percentage of inhibition of pathogens					
	<i>P. aphanidermatum</i>	<i>P. capsici</i>	<i>G. lucidum</i>	<i>R. solani</i>	<i>S. rolfisii</i>	<i>F. oxysporum</i>
Tr 2	87.44	100	66.17	41.11	51.89	100
Tr 9	100	100	70.30	49.22	65.56	100
Tr 14	94.44	100	83.33	42.22	55.56	100
Tr 28	94.44	88.33	44.85	41.89	63.33	100
Tr 34	72.22	100	81.82	44.44	56.67	100
Tr 41	95.22	100	81.82	50.00	56.67	78.72
Tr 42	83.33	100	72.73	44.44	55.22	100
Tr 43	88.89	100	75.00	42.22	55.56	100
Tr 48	100	96.67	61.82	41.89	61.11	100
Tr 52	100	100	93.94	45.22	61.11	100
Tr 76	94.44	88.89	57.78	42.56	62.56	100
Tr 78	93.33	100	75.00	43.00	50.33	68.09
Tr 81	87.00	100	75.00	42.22	51.89	68.09
Tr 86	87.00	100	61.67	42.22	53.33	100
Tr 97	88.89	83.33	81.82	48.11	54.44	100
Tr 101	81.11	88.89	69.09	44.44	54.78	100
Tr 109	88.89	94.11	72.73	44.44	55.56	100
Tr 114	89.22	100	58.18	49.22	49.67	100
Tr 118	85.56	88.89	72.73	46.67	51.89	96.38
Tr 126	81.11	88.89	72.73	44.44	52.56	100
<i>T. harzianum</i>	81.89	100	52.12	41.11	35.22	33.33
<i>T. viride</i>	72.56	100	60.00	44.44	28.11	61.56

These results revealed that the *in vitro* antagonistic performance of all the tested native isolates of *Trichoderma* spp. was better than that of reference cultures of *T. viride* and *T. harzianum* with respect to one or other pathogen used for the experiment. The performance of native and reference cultures against *R. solani* and *P. capsici* was found to be similar. Native isolates controlled the pathogens, *P. aphanidermatum*, *G. lucidum*, *S. rolfisii* and *F. oxysporum* more efficiently compared to reference cultures of *Trichoderma* spp. Even though the isolates Tr 48, Tr 97, Tr 101, Tr 109, Tr 118 and Tr 126 could not show more antagonism against the pathogen, *P. capsici* than the reference cultures, they all showed better performance to all other tested pathogens. Among the twenty native isolates of *Trichoderma* spp. twelve isolates were efficient in showing better per cent of inhibition to all the tested pathogens than that of

reference cultures. Hence these isolates can become efficient biocontrol agents for future use. Further study is needed for the evaluation of their antagonistic efficiency under field condition.

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Reference

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