Assesment of native *Trichoderma* species against *Rigidoporus* and *Fusarium* isolates pathogenic to jak trees

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Introduction

The soil is a reservoir of inocula of soil borne plant pathogens and they cause a wide array of soil borne diseases in a range of economically important crops, while silently hidden in the rhizosphere. Majority of soil-borne plant pathogens are widely distributed in agricultural soils. The fungal pathogens responsible for root rot and collar rot of jak were revealed as *Rigidoporus microporus, Fusarium oxysporum* and *Fusarium solani* in a previous study. *R. microporus* is the causal agent of white root disease which is a serious disease in rubber plantations in Sri Lanka (Jayasuriya and Wettasinghe, 2004). *F. solani* and *F. oxysporum* have been confirmed to pathogenicity of several other perennial trees suffering from root and stem rots (Javid *et al*., 2005).

Biological control through rhizosphere inhabiting microorganisms has been reported as a promising component in integrated management of root diseases of perennial trees. Among a wide range of potential bio-control agents of soil borne pathogens, *Trichoderma* spp. are in the forefront. Wider availability in many root ecosystems, free-living nature and ability to control pathogenic microbes through a wide range of modes of antagonism are favorable attributes that *Trichoderma* spp. possess, as biological control agents. They are considered as promising biological control agents against numerous phytopathogenic fungi since they are capable of inhibiting phytopathogenic fungi either by including resistance and plant defense reaction or by direct antagonism through mycoparasitism and competition or by producing antibiotics. Considering the multi-functional antagonistic ability of *Trichoderma* spp., the isolated species were tested in the present study to determine their efficiency in controlling *R.microporus, F.oxysporum* and *F. solani* the root rot/collar rot causing pathogens of jak tree. Through this *in-vitro* assay, it was intended to observe the mode of antagonism, especially focusing on competition, antibiosis and mycoparasitism.

***** Short Communication***

Materials and methodology

Six isolates of *Trichoderma* (*T. hamatum, T. harzianum, T. viride, T. koningiopsis, T. gamsii* and *T. erinaceum*) were tested against *R. microporus* (*R. microporus* SEG and *R. Microporus* PM52) and *Fusarium* isolates (*oxysporum* and *solani*) which were isolated and molecularly confirmed in a previous study as the root rot and collar rot causal agents of jak. It was done by dual culture plate technique using PDA as the culture medium. PDA was used as the culture medium based on the results of the previous experiments on colony growth of fungal isolates on different culture media. Dual culture plate technique as described by Jayasuriya and Thennakoon (2007) was followed to check the antagonism of different *Trichoderma* isolates against the fungal pathogens, *in-vitro*. Briefly, an isolate of the fungal pathogens was inoculated on to PDA plate and three days later, an isolate of *Trichoderma* was inoculated to same plate with a distance of 3.5 cm. For six *Trichoderma* isolates, six separate experiments were carried out according to a complete randomized design with 10 replicates. The dual culture plates were incubated at room temperature under natural light conditions and pH of the medium was adjusted 5.5 which favors the growth of both organisms. For each experiment, controls were maintained by culturing the fungal isolate without the influence of *Trichoderma* isolate. Radius of the colony growth under the influence of *Trichoderma* and without the influence of *Trichoderma* was measured for each fungal isolate. Percentage colony growth inhibition of a given fungal pathogen was calculated using the following equation. Data was analyzed using SAS statistical software version 9.1 and significance of the effect of each *Trichoderma* isolate on colony growth inhibition was determined by ANOVA and mean separation was done by Duncan's Multiple Range Test.

R1 – radius of the pathogen away from the antagonist (*Trichoderma* spp.) (cm)

R2 – Radius of the pathogen towards to the antagonist (*Trichoderma* spp.) (cm)

$$\text{Percentage colony growth inhibition of the pathogen} = \frac{R1 - R2 \times 100\%}{R1}$$

Results and discussion

Percentage colony growth inhibition of the fungal pathogens by the six *Trichoderma* spp. is summarized and depicted by Table 1.

Table 1. Percentage colony growth inhibition of *R. Microporus* and *Fusarium* isolates by *Trichoderma* species in dual cultures

Trichoderma spp.	% of growth inhibition of root and collar rot pathogens			
	Rmj1	Rmj8	Fo	Fs
T. hamatum	81[a]	24[a]	45[ba]	28[cb]
T. viride	42[b]	34[a]	70[a]	51[ba]
T. koningiopsis	26[b]	20[a]	50[ba]	50[abc]
T. gamsii	30[b]	21[a]	25[b]	25[c]
T. harzianum	25[b]	33[a]	67[a]	67[a]
T. erinaceum	24[b]	20[a]	53[a]	48[abc]
CV%	2.44	2.44	2.44	2.44

Means with the same letter along given a column are not significantly different at P = 0.05

Rmj1 and Rmj8 were denoted as *microporus* two isolates which were molecularly equal to *microporus* SEG and *R. microporus* PM52 respectively. Fo and Fs were denoted as *F. oxysporum* and *F. solani* respectively.

Except for Rmj8 isolate, percentage colony growth inhibition of other fungal pathogens varied significantly under the influence of a given *Trichoderma* speices used for antagonistic assay (Table 1). For example, Rmj1isolate had the higher colony growth inhibition by *T. hamatum.* The rest of the five *Trichoderma* speices showed a significantly lower colony growth inhibition of Rmj1 than that by *T. hamatum*, and there were no significant differences of the inhibition ability among those five species (Table 1). With reference to *F. oxysporum*, the higher percentage colony growth inhibition was shown by *T. viride* and the lowest was shown by *T. gamsii, T. harzianum* and *T. gamsii* gave the highest and lowest colony growth inhibition of *F. solani* respectively.

It was observed that some of the *Trichoderma* species, namely *T. hamatum, T. harzianum* and *T. erinaceum* developed inhibition zones against Rmj1 and Rmj8 isolates and the colony growth of the two pathogens was inhibited (Table 2). No such inhibition zones were observed with the other *Trichoderma* species with the four fungal pathogens or the three *Trichoderma* species given in Table 2 with *Fusarium* isolates.

Table 2. Inhibition zones developed by *Trichoderma* species with *R. Microporus* isolates

Trichoderma species	Radius of the inhibition zone (cm) with Rmj1	Radius of the inhibition zone (cm) with Rmj8
T. hamatum	0.5	0.7
T. harzianum	0.9	1.2
T. erinaceum	0.9	1

Therefore, based on the observations of Table 1 and 2 the mode of antagonism of the six *Trichoderma* species used, can be summarized as follows (Table 3).

Table 3. Different modes of action of *Trichoderma* spp. Against fungal pathogens

Trichoderma species	Rmj1	Rmj8	Fo	Fs
T. hamatum	Competition and antibiosis	Competition and antibiosis	Competition	Competition
T. harzianum	Competition and antibiosis	Competition and antibiosis	Competition	Competition
T. erinaceum	Competition and antibiosis	Competition and antibiosis	Competition	Competition
T. viride	Competition	Competition	Competition	Competition
T. koningiopsis	Competition	Competition	Competition	Competition
T. gamsii	Competition	Competition	Competition	Competition

Jayasuriya and Tennakoon (2007) have explained the possibility of introducing biological control agents, particularly *Trichoderma* spp. to management of white root rot pathogen of rubber. Isolation of *T. harzianum* from rubber growing soils in Sri Lanka and its high efficiency in controlling *R. microporus* has been reported by Jayasuriya and Tennakoon (2007). The results showed the availability of a wide range of *Trichoderma* spp. in the rhizosphere of homegardens and hence potential of using them for the biological control of root and collar rot diseases of jak. *In-vitro* efficiency of the six *Trichoderma* spp. used in this study to control the four fungal isolates, it was also observed the mode of antagonism exhibited by the *Trichoderma* spp. Findings of the peresent study revealed the ability of some *Trichoderma* spp. (i.e. *T. viride, T. harzianum, T. gamsii, T. hamatum*) to exhibit competiton and the ability of some *Trichoderma* spp. (i.e. *T. erenacium, T. harzianum*) to show antibiosis against the four fungal isolates. Our observations are in par with Kaewchai and Soytong (2010), who have reported similar observations. According to Kaewchai and Soytong (2010), *T. hamatum* STN07 and *T. harzianum* STN07 have shown more than 50% colony growth inhibition of *R. microsporus* through competition by rapidly growing over the colony of the pathogen. Bastakoti *et al*. (2017), have reported the bio-control ability of *Trichoderma* isolate TS215 against *Fusarium solani* has shown inhibition of the fungal pathogen (*F. solani*), and it has been suggested the antagonistic ability of the *Trichoderma* could be due to release of antibiotic or antibiotic-like substances. However, our findings revealed that a given isolate of a pathogen species show variation in colony growth inhibition by different *Trichoderma* spp. Moreover, different isolates of a given pathogen species showed variation in colony growth inhibition when subjected to the control by the six *Trichoderma* spp. Such biological variations are possible when biological control

is practiced. Therefore, use of all potential *Trichoderma* spp. identified in the present study, as a mixture would be a more effective, as we have to target on several root rot and collar rot pathogens.

Conclusion

All six *Trichoderma* spp. were capable of inhibiting colony growth of the four fungal pathogens under *in-vitro* conditions at varying degrees. Except for *R. microporus* (Rmj8) isolate, percentage colony growth inhibition of other fungal pathogens varied significantly under the influence of a given *Trichoderma* speices used for antagonistic assay. Competition and antibiosis were identified as the modes of antagonism of the *Trichoderma* spp. used for the study.

References

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