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Isolation of antagonistic fungi from rhizospheres and its biocontrol activity against different isolates of soil borne fungal pathogens infected legumes

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Abstract. *Yusnawan E, Inayati A, Baliadi Y. 2019. Isolation of antagonistic fungi from rhizospheres and its biocontrol activity against different isolates of soil borne fungal pathogens infected legumes. Biodiversitas 20: 2048-2054.* Soilborne diseases caused by *Rhizoctonia solani* and *Fusarium* sp. are biotic limits for legume production. Biological controls offer environmental friendly control for these pathogens. This study aimed to isolate and screen *Trichoderma* from different rhizospheres and to obtain effective *Trichoderma* isolates to suppress in vitro growth of the soil borne pathogens. The antagonistic inhibitory activity was performed by dual culture method. Seven out of forty indigenous *Trichoderma* isolates collected from East Java, Indonesia effectively suppressed the growth of different fungal isolates, namely *Rhizoctonia solani* (R.s1), *R. solani* (R.s2) as well as *Fusarium* sp. which infected soybean and mung bean. In vitro study showed different suppression of the pathogens on dual culture tests. The seven isolates inhibited the growth of *R. solani* (R.s1), *R.solani* (R.s2) and *Fusarium* sp. ranging from 90.0 to 99.6%, 72.8 to 82.4%, and 67.9 to 90.8%, respectively. Isolate origin and genetic variability of *Trichoderma* played an important role in the antagonistic activity. The fast-growing of selected *Trichoderma* showed their abilities for space occupation and nutrition competition, which involved in the antagonistic activity. The mycelial growth of *Trichoderma* over pathogens showed hyperparasitism mechanism. In addition, coiling of *Trichoderma* over hyphal pathogens was observed during microscopic observation. The seven *Trichoderma* isolates, therefore, are promising as biological control agents against the soil borne fungi infected legumes.

Keywords: Antagonistic activity, dual culture, inhibition, isolation, soilborne fungi

INTRODUCTION

Wilt diseases caused by soil borne fungi are biotic obstacles to maintain yield production of legume crops. Soil borne pathogens infect many crops from seedling to generative stage. Aggressive growth of *R. solani*, for example, was able to devastate soybeans in tidal swamp area in Barito Kuala, Kalimantan Indonesia with disease severity of 50-75% (Rahayu 2014). Another isolate of *R. solani* in combination with *Fusarium* sp. infected around 25 % of the population of mung bean germplasm collections in Indonesia. An in-vitro study showed that *R. solani* infection in mung bean caused the reduction of survival seedling growth and wilting. *Fusarium* sp. acted as secondary pathogens following infection by primary pathogens, *R. solani*. High severity of wilt disease occurred in all susceptible mung bean genotypes. Wilt disease appeared from one week after planting (Setyorini and Yusnawan 2014), so precautions to control these pathogens can be carried out earlier before the emerging plant.

Management of these fungal soilborne pathogens is challenging since the fungal propagules exist in the soil, causing difficulties in controlling them. Synthetic fungicide applications such as triazole, carbendazim, carboxin, and thiram have been used to suppress the growth of *R. solani* (Akhter et al. 2015; Datta et al. 2016). However, continuous application of fungicides, particularly in an endemic area is considered unfriendly environmental control, that may affect other living organisms such as beneficial soil microbes, and also leading to strain resistance to certain active compounds of the fungicides (Sundaramoorthy and Balabaskar 2013). The use of biocontrol agents for controlling soilborne pathogens either fungi or bacteria such as *Trichoderma*, *Streptomyces*, *Bacillus*, and *Pseudomonas* as an alternative to chemical fungicides is rapidly demanding to minimize such negative effects (Woo et al. 2014).

Trichoderma spp. are well known as biocontrol agents in agriculture against a wide range of fungal pathogens such as *Botrytis cinerea*, *Fusarium oxysporum*, *Sclerotium rolfsii*, and *Rhizoctonia solani* (Mayo et al. 2015; John et al. 2015; You et al. 2016; Mahmoud and Abdalla 2018). *Trichoderma* is soil inhabitant fungi, which have several modes of action to inhibit fungal pathogens, such as mycoparasitism, enzyme secretion particularly chitinase and glucanase for degrading cell wall of pathogenic fungi (Küçük and Kývanç 2008). *Trichoderma* also produces volatile and non-volatile organic compounds which act as antibiotics (Dubey et al. 2007; Ajith and Lakshmidevi 2010).

Most studies focus on the antifungal activity of *Trichoderma* spp. against specific plant pathogens, such as an application of *Trichoderma* to control *Rigidoporus microporus* (Setyawan et al. 2014), the use of *T.*

asperellum against *F. oxysporum* f. sp. *cucumerinum* (Saravanakumar et al. 2016), and *T. asperellum* to manage vascular streak dieback disease of cocoa in Indonesia (Rosmana et al. 2015). Research on antifungal properties of *Trichoderma* to control several pathogens either from different species or isolates or strains of the same species has not been yet extensively explored, particularly in legumes. Suppression of *F. oxysporum* and *Alternaria alternate*, two fungal pathogenic species causing vascular wilt and blight diseases, was achieved through systemic resistance induction by *T. viridae* on leguminous crops of *Cajanus cajan*, *Vigna radiata*, and *Vigna mungo* (Rao et al. 2015). Agamani et al. (2017) reported that *Trichoderma* spp. were effective for suppressing *Rhizoctonia baticola*, *F. oxysporum* f.sp.*ciceri*, and *S. rolfsii* on infected chickpea.

There is a need to explore indigenous potential antagonistic fungi of *Trichoderma* isolated from different rhizospheres to suppress the incidence of soilborne diseases, particularly infected soybean and mung bean in Indonesia. Hence, this study aimed to isolate and screen *Trichoderma* from different rhizospheres as well as to obtain effective strains of *Trichoderma* in suppressing in vitro growth of soilborne pathogens of soybean and mung bean.

MATERIALS AND METHODS

Root and soil collection and isolation media

Twenty six samples of soil and roots from different crops were collected from around East Java, Indonesia, during the cultivation seasons of 2015 and 2016. The samples were packed into separate plastic bags and transferred to Mycology Laboratory of Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Malang, East Java. Potato dextrose agar (PDA) supplemented with chloramphenicol was used to isolate antagonistic fungi (Abo-Elyousr et al. 2014).

Antagonistic fungal isolation

Roots were washed and disinfected with 0.5% NaOCl by dipping for 2-3 minutes, followed by washing in sterile water then air dried on sterile filter paper inside laminar air flow. The roots were placed inside a Petri dish containing PDA enriched with chloramphenicol (Abo-Elyousr et al. 2014). Soil samples collected around root crops (10 g) were diluted in 100 mL of sterile water to obtain 10^{-1} dilution, and the soil suspension was spread on PDA containing chloramphenicol.

Further dilutions of soil suspension (10^{-2} and 10^{-3}) were carried out to isolate soil fungi. After three to five days of incubation, *Trichoderma* grown on the PDA were re-cultured on the PDA slant. The same procedure to identify and characterize the fungi was conducted as above.

Morphological identification of antagonistic fungi

Fungi grown and identified as *Trichoderma* were re-cultured on PDA slant. Morphological identification was carried out according to Gams and Bissett (1998). The individual fungus was cultured on PDA. Radial growth, time, and the abundance of sporulation were recorded for five days.

Pathogen isolation and identification

Soil borne pathogens were obtained from the fungal culture collection of the Mycology Laboratory, ILETRI. The soil borne pathogens were isolated from soybean infected by *Rhizoctonia solani* [denoted as *R. solani* (R.s1)] (Rahayu 2014) as well as mung bean infected by *Rhizoctonia solani* [denoted as *R. solani* (R.s2)] and *Fusarium* sp. (Setyorini and Yusnawan 2014). Brief general procedure for fungal isolation as follows: infected tissues were surface sterilized with 0.5% NaOCl, followed by rinsing with sterile water. After air dried in the laminar air flow, the infected tissue was placed inside Petri dish containing PDA amended with chloramphenicol. After five to seven days of incubation, fungi grown on the PDA was transferred to the PDA slant. The pure cultures were identified as described by Rahayu (2014), Setyorini and Yusnawan (2014).

Biocontrol in-vitro test

Dual culture in-vitro test of *Trichoderma* against soil borne fungi was performed according to John et al. (2010) with minor modification. Five-day old of *Trichoderma* and 7-day old of soil borne fungi were cut from advancing edge. A 5 mm diameter of *Trichoderma* and soil borne fungi disks were placed on the opposite with a distance of 3 mm and incubated at room temperature. The soil borne fungi grown without *Trichoderma* were used as a negative control. Radial growth inhibition of soil borne fungi and overlapping growth by *Trichoderma* spp. were recorded and compared with the negative control. The percentage of inhibition was calculated as follows: $\% I = [(C - T)/C] \times 100$, where I = growth inhibition, C = radial growth of pathogens on negative control, T = radial growth of pathogens challenged with *Trichoderma* (Abo-Elyousr et al. 2014). The treatment was arranged in a Completely Randomized Design with three replicates.

RESULTS AND DISCUSSION

Isolation of antagonistic fungi

Antagonistic fungi offer environmentally friendly controls to suppress the incidence and severity of wilt caused by pathogens transmitted through the soil. Forty antagonist fungi obtained from different plant rhizospheres and soil were isolated and cultured on PDA during exploration around East Java, Indonesia (Table 1). All antagonistic fungi were identified as *Trichoderma* spp based on microscopic identification (Gams and Bissett 1998). This genus is easily isolated from soil with high organic compounds and plant roots as rhizosphere competence and endophytic fungi (Brotman et al. 2013; Cai et al. 2015) as was done in this study.

Radial growth of all isolates varied greatly (Table 2). The growth of the antagonistic fungi ranged from 0.1 to 0.8 cm on the first day of incubation on PDA. Three isolates of T9A, T21D, and T22E showed the slowest growth (± 0.1 cm), whereas T4B, TPa1, and T15B were the fastest growing isolates (± 0.8 cm). *Trichoderma* was categorized as fast growing fungi on general media such as potato dextrose-based media (Mishra et al. 2011; Tapwal et al. 2011; Jahan et al. 2013).

On the second day after inoculation, the radial growth of all isolates ranged from 0.6 to 3.1 cm. The fastest growth isolate (± 3.1 cm) was about five times faster than the slowest growth isolate (± 0.6 cm). Most isolates fully covered the PDA three days after incubation. Fast growing isolates had the potential as antagonistic fungi since they were able to compete for the available space and nutrition which may inhibit the growth of plant pathogens (Brotman et al. 2010; Borrero et al. 2012; Abo-Elyousr et al. 2014).

Time of sporulation of all isolates was not significantly different ($p < 0.05$) (Table 2). The isolates needed 2.5 to 4 days for sporulation after being cultured on PDA. Conidia produced by all *Trichoderma* isolates were qualitatively different as indicated by the different intensity of green colored mature conidia on PDA. Isolates of T 7A, T 17 C, T Pa1, and T Vi1 produced more conidia than other isolates. Isolates which produce abundant conidia are potential to be utilized as biocontrol agents, especially if conidia are encapsulated on seeds as seed treatments (Rao et al. 2015; Fiorentino et al. 2018).

Table 1. *Trichoderma* isolated from the rhizosphere of several plant species collected from different regions in East Java

Rhizosphere	Isolate origin	Code
Sugar cane	Gending, Probolinggo	T 4, T 4A, T 4B
Onion	Gending, Probolinggo	T7A
Taro	Gending, Probolinggo	T 8B, T 8C
Chili	Alasrejo, Banyuwangi	T 9A
Mungbean	Genteng, Banyuwangi	T 14A
Mungbean (wilt)	Genteng, Banyuwangi	T 15A, T 15B, T 15C
Soybean (wilt)	Genteng, Banyuwangi	T 16A
Taro	Genteng, Banyuwangi	T 17A, T 17B, T 17C, T 17D
Sweet potato	Genteng, Banyuwangi	T 18A
Paddy	Genteng, Banyuwangi	T 19A
Mungbean	Genteng, Banyuwangi	T 20A, T 20B
Mungbean	Genteng, Banyuwangi	T 21A, T 21B, T 21C, T 21D
Mungbean (wilt)	Genteng, Banyuwangi	T 22A, T 22C, T 22E
Taro	Genteng, Banyuwangi	T 24A
Paddy	Rogojampi, Banyuwangi	T 25A
Dioscorea	Genteng, Banyuwangi	T 26A
Cassava	Paiton, Probolinggo	T 36
Soybean	Besuki, Situbondo	T 37
Soybean	Genteng, Banyuwangi	T Au1
Soybean	Genteng, Banyuwangi	T At1
Peanut	Genteng, Banyuwangi	T Ci1
Soybean	Genteng, Banyuwangi	T Ko1
Peanut	Muneng, Probolinggo	T M6
Peanut	Muneng, Probolinggo	T M20
Soybean	Genteng, Banyuwangi	T Pa1
Peanut	Genteng, Banyuwangi	T Vi1

Antagonistic activity of *Trichoderma* against *R. solani* infected soybean

Rahayu (2014) reported that *R. solani* (R.s1) caused damage on soybean in tidal swamp areas. In vitro antagonistic study of *Trichoderma* against *R. solani* (R.s1) showed that *Trichoderma* had promising inhibition (Figure 1). Several *Trichoderma* isolates inhibited about 50% against *R. solani* (R.s1) after the third days of dual culture test, namely T 7A (51.8%), T 8B (55.7%), T 8C (56.3%), and T M20 (54.4%). Eight potential isolates inhibited *R. solani* (R.s1) up to 95-100%, *i.e*. T 4A, T 22A, T At1, T 16A, T 15C, T 20B, T4, and T 15A. Microscopic observations of T 4 and T 15A isolates showed hyphal coiling of *Trichoderma* spp. over *R. solani* (R.s1) (Figure 2).

Tabel 2. The growth, sporulation time and conidia abundance of *Trichoderma* on Potato Dextrose Agar

Isolate	Radial growth (cm)		Green sporulation (dai)	Conidia abundance
	Day 1	Day 2		
T 4	0.25 opq	2.30 fghi	3.0 a	+
T 4A	0.45 ijkl	2.59 bcde	3.0 a	+
T 4B	0.78 ab	2.70 b	3.0 a	+
T 7A	0.69 bc	2.93 a	3.0 a	+++
T 8B	0.30 nop	2.33 fgh	3.0 a	++
T 8C	0.65 cde	2.65 bcd	3.0 a	++
T 9A	0.10 r	1.60 m	3.5 a	++
T 14A	0.50 fghij	2.39 efg	2.5 a	++
T 15A	0.20 pqr	2.13 hijk	3.0 a	++
T 15B	0.85 a	3.03 a	3.0 a	++
T 15C	0.25 opq	2.01 jkl	3.0 a	++
T 16A	0.65 cde	2.65 bcd	2.5 a	++
T 17A	0.40 jklmn	1.50 mn	3.0 a	++
T 17B	0.25 opq	1.88 l	4.0 a	+
T 17C	0.49 ghijk	2.50 bcdef	3.0 a	+++
T 17D	0.59 cdefg	2.45 cdef	2.5 a	++
T 18A	0.68 bc	2.55 bcde	3.0 a	++
T 19A	0.59 cdefg	2.56 bcde	3.0 a	++
T 20A	0.66 cd	2.55 bcde	3.0 a	++
T 20B	0.48 hijk	2.05 jkl	3.0 a	++
T 21A	0.18 qr	1.34 n	3.5 a	++
T 21B	0.40 jklmn	2.63 bcd	4.0 a	+
T 21C	0.23 pq	2.19 ghij	3.0 a	+
T 21D	0.10 r	0.78 o	3.5 a	++
T 22A	0.60 cdef	2.66 bc	3.0 a	+
T 22C	0.30 nop	2.03 jkl	4.0 a	+
T 22E	0.10 r	0.56 o	4.0 a	++
T 24A	0.15 qr	1.85 l	4.0 a	+
T 25A	0.19 qr	2.10 ijk	3.0 a	++
T 26A	0.34 mno	2.44 def	3.0 a	+
T 36	0.20 pqr	1.50 mn	3.5 a	++
T 37	0.55 efghi	1.29 n	4.0 a	+
T M20	0.44 defgh	2.38 efg	2.5 a	++
T M6	0.68 fghij	3.06 a	3.0 a	++
T At1	0.36 bc	2.95 a	2.5 a	++
T Au1	0.61 jklm	2.03 jkl	3.0 a	+
T Ci1	0.50 lmn	2.03 jkl	3.0 a	++
T Ko1	0.56 cde	2.94 a	3.0 a	++
T Pa1	0.83 a	2.96 a	2.5 a	+++
T Vi1	0.39 klmn	1.95 kl	3.0 a	+++

Note: sporulation: + = few, ++ = medium, +++ = plenty, dai = day after inoculation on PDA. Numbers in the same column followed by the same letter are not significantly different based on the LSD test at $\alpha = 0.05$

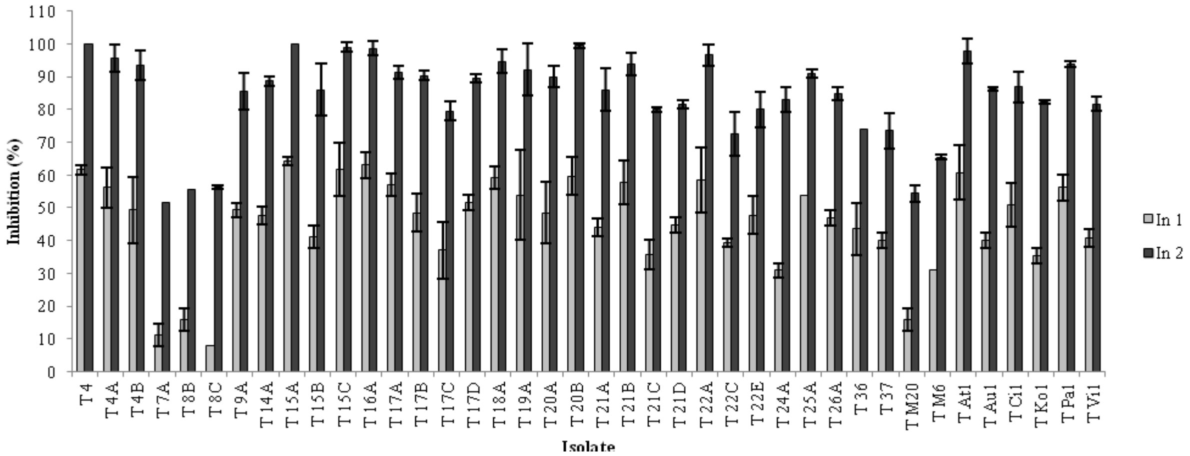

Figure 1. In vitro antagonism of *Trichoderma* against *R. solani* (Rs1). In 1 = inhibition on day 2, In 2 = inhibition on day 3 on dual culture test. Error bars indicate standard deviations

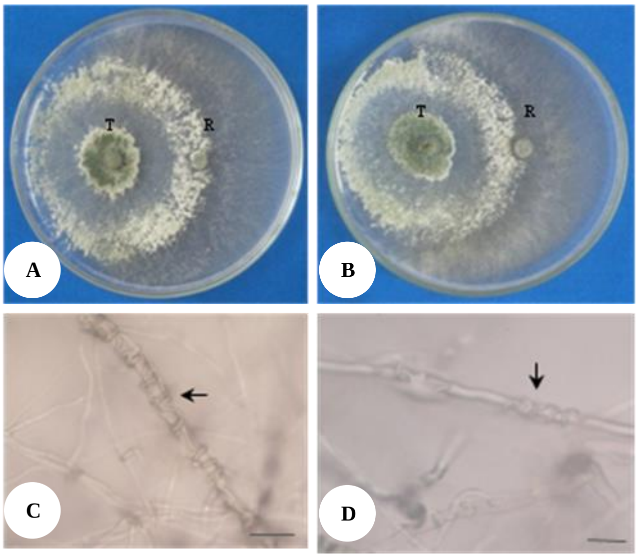

Figure 2. In vitro antagonism of T 4 and T 15A isolates against *R. solani* (R.s1) (a and b), and microscopic observation of T 4 and T 15A isolates against *R. solani* (R.s1) (c and d). T = *Trichoderma* sp., R = *R. solani* (R.s1). The arrow shows hyphal coiling of *Trichoderma* over *R. solani* (R.s1)

Antagonistic activity of *Trichoderma* against *R. solani* infected mung bean

Another isolate of *R. solani* (R.s2) which infected mung bean (Setyorini and Yusnawan 2014) was also challenged with *Trichoderma*. The highest suppression of *Trichoderma* against *R. solani* (R.s2) was less than 90% at the end of observation (Figure 3). The highest growth inhibition (87%) against *R. solani* (R.s2) was obtained from T 24A and T vi1 isolates, while other isolates inhibited <87%. Isolates of T 4 and T 15A which showed the most effective inhibition (100%) against *R. solani* (R.s1), were only able to inhibit the growth of *R. solani* (R.s2) of 64.7% and 61.5%, respectively. The results suggested that the same *Trichoderma* isolates had different antagonistic activity against different isolates of pathogens, although the pathogens were from the same species. Similar mechanism of *Trichoderma* coiling over *R.solani* (R.s2) was also observed in this study.

Antagonistic activity of *Trichoderma* against *Fusarium*

Fusarium sp. associated with wilt disease on mung bean as a secondary pathogen (Setyorini and Yusnawan 2014) was also challenged with *Trichoderma* spp. The results showed that no *Trichoderma* isolates were able to inhibit 100% the growth of *Fusarium* sp. (Figure 4). T 20A had the highest inhibition (90.8%) against *Fusarium* sp. followed by isolates of T 15C and T 16A (77.1%), while isolates of T4 and T 15A were only suppressed 55.7% and 57.2% the growth of *Fusarium*. Hypha of *Trichoderma* isolate grew over *Fusarium* on the last day of observation (Figure 5a). Microscopic observation showed that *Fusarium* was overlaid by *Trichoderma* hypha (Figure 5).

Antagonistic activity of *Trichoderma* spp. against three isolates of the soil borne fungi

Seven *Trichoderma* isolates had good inhibition against three isolates of soil borne fungi with the range of inhibition as follows: *R. solani* (R.s1) (90.0-99.6%), *R. solani* (R.s2) (72.8-82.4%), and *Fusarium* sp. (67.9-90.8%) (Table 3). Radial growth of *Trichoderma* itself on PDA varied from 2.0 to 2.9 cm with the sporulation time varied from 2.5-3.0 days (Table 2). Although having different speed of radial growth when growing individually, the antagonistic properties of these *Trichoderma* spp. were not much different against each soil borne pathogen (Table 3), except for T 20A in inhibiting *Fusarium* sp.

Trichoderma has been widely used as a biological control against soil borne pathogens since several species show antagonistic properties both in vitro and in vivo (Morsy et al. 2009; Akrami et al. 2009). Current in vitro study showed that different *Trichoderma* isolated from different rhizospheres had different antagonistic activities against different isolates of phytopathogenic fungi. This finding suggested that the genetic variability of antagonistic isolates played an important role in biocontrol activity. The results of this study were in agreement with a study conducted by Moosa et al. (2017) in which the variability of antagonistic activity depends on the isolate genetic potential and its origin.

It was demonstrated that seven *Trichoderma* isolates had an antagonistic activity to suppress the growth of three soil borne pathogens with different levels of inhibition. The results showed that one isolate could inhibit the growth of several soil borne pathogens. The highest antagonistic activity in this present study was greater than that of *Trichoderma* T021 isolate (72.77% inhibition) in suppressing the growth of *R. solani* infected *Phaseolus vulgaris* as reported by Mayo et al. (2015). Nevertheless, when compared with inhibition percentage of of *Trichoderma* isolates of Tv-2, Th-7, and Th-5 (79.2 – 82.8%) against *F. oxysporum* f.sp. *lentis* (Sharfuddin and Mohanka 2012), inhibition of the seven *Trichoderma* isolates (67.9 – 70.9%) against *Fusarium* sp. in the current study showed lower inhibition values. The study conducted by Sharfuddin and Mohanka (2012), however, only used one fitopathogen species to test *Trichoderma* antagonistic activity.

The mycelial growth of the selected *Trichoderma* over the mycelial of soil borne pathogens showed hyperparasitism phenomenon that responsible for the antagonistic mechanism (Doley and Jite 2012) as shown in this study. In addition to hyperparasitism properties, *Trichoderma* is also able to grow very fast so that it can occupy space and compete in the use of nutrients (Devi et al. 2012). Microscopic observations showed hyphal coiling of the pathogens by the indigenous *Trichoderma* (Figure 2 and 5). Several mechanisms occurred during coiling, including producing enzymes of the antagonistic fungi which degrade the cell wall of the pathogens such as chitinase, cellulose, β-1,3-glucanase, β-1,6-glucanase, and protease. These enzymes play important roles during mycoparasitism as well as hyphal colonization (Küçük and Kývanç 2008; Jayalakshmi et al. 2009).

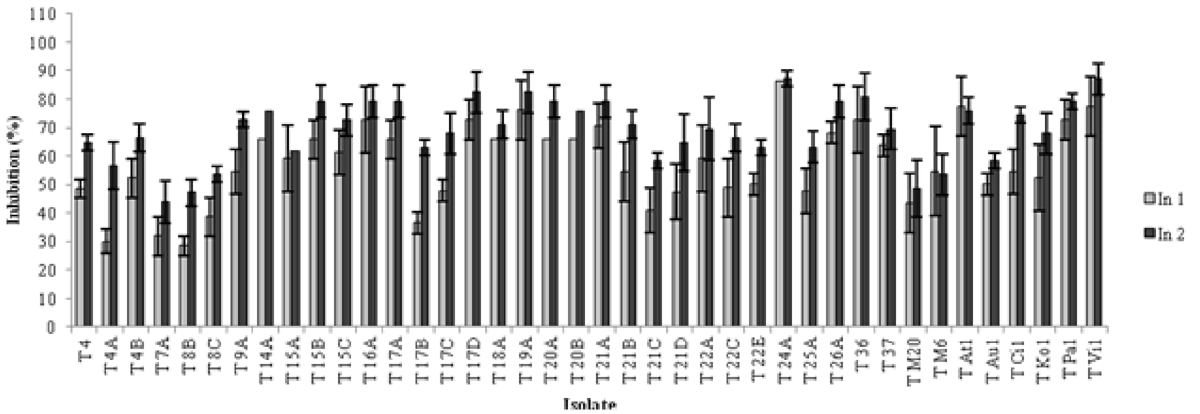

Figure 3. In vitro antagonism of *Trichoderma* against *R. solani* (Rs2). In 1 = inhibition on day 3; In 2 = inhibition on day 4 on dual culture test. Error bars indicate standard deviations

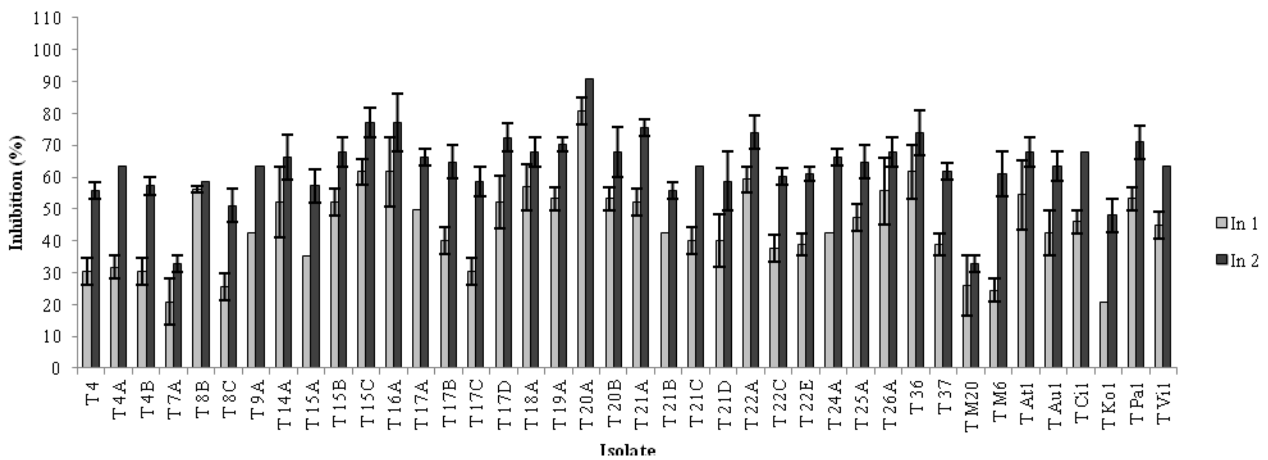

Figure 4. In vitro antagonism of *Trichoderma* against *Fusarium* sp. In 1 = inhibition on day 3, In 2 = inhibition on day 4 on dual culture test. Error bars show standard deviations.

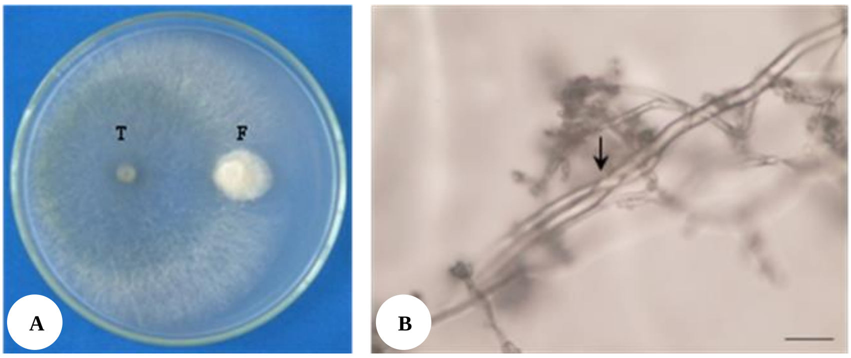

Figure 5. In vitro antagonism of *Trichoderma* isolate of T 20A against *Fusarium* sp. (A), and microscopic observation of T 14A isolate against *Fusarium* sp. (B). T = *Trichoderma* sp., F = *Fusarium* sp. The arrow shows coiling of *Trichoderma* over *Fusarium* sp.

Table 3. Growth inhibition of *Trichoderma* spp. against different isolates of soil borne pathogens

Trichoderma	**Inhibition (%)**		
	***R. solani* (R.s1)**	***R. solani* (R.s2)**	***Fusarium* sp.**
T 20A	90.0 c	79.2 ab	90.8 a
T 19A	92.2 bc	82.4 a	70.2 bc
T Pa1	93.9 abc	79.2 ab	70.9 bc
T At1	97.8 ab	76.0 ab	67.9 c
T 16A	98.7 ab	79.2 ab	77.1 b
T 15C	99.1 a	72.8 b	77.1 b
T 20B	99.6 a	76.0 ab	67.9 c

Note: numbers followed by the same letters in the same column are not significantly different based on the LSD test (α = 5 %)

Trichoderma isolated from crop rhizosphere as performed in this study might have rhizosphere competence ability. This interaction between *Trichoderma* and plants triggers plant growth and increases plant nutrient absorption (Harman et al. 2004). The ability of antagonistic fungi to increase plant health through the induction of systemic resistance to plant pathogens is reported in some species of *Trichoderma*. Gajera et al. (2015) reported that *T. viride* induced plant resistant by activating phenylpropanoid pathway to increase polyphenol oxidase and phenylalanine ammonia lyases. Another study by Christopher et al. (2010) showed that there is an increase in enzymes which belongs to the pathogenesis-related proteins such as the polyphenol oxidase and phenylalanine ammonium lyase activity. An increase in total phenolic contents after *T. virens* colonization was also reported (Christopher et al. 2010). Further study will be done to determine the benefits of the interaction between selected *Trichoderma* from this study and leguminous crops, and also the identification of *Trichoderma* species by molecular work.

In summary, seven isolates of *Trichoderma* (T At1, T 15C, T 16A, T 19A, T 20A, and T 20B) had potent antagonistic activity against *R. solani* (R.s1), *R. solani* (R.s2) and *Fusarium* sp in vitro. Competition of space and nutrition, as well as mycoparasitism, was performed by these selected *Trichoderma*. These antagonistic fungi are promising as biocontrol agents against multi-isolates of phytopathogens. The protective ability of the selected *Trichoderma* to plants and the role of these antagonists to induce systemic resistance need to be further studied.

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