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Full Length Research Paper

Effect of media on growth and antagonistic activity of selected *Trichoderma* strains against *Ganoderma*

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This study was conducted to determine the effect of culture media on the growth rate and antagonistic activity of *Trichoderma harzianum* strain 1132 and *Trichoderma virens* strain 128 in *Ganoderma* sp. The two growth media evaluated were potato sucrose agar (PSA) and potato dextrose agar (PDA). Growth rate was determined by the rate of spore density production. Significantly higher spore production was observed on PSA for *T. virens* strain 128 (9.12×10^{10}) and *T. harzianum* strain 1132 (8.58×10^{11}) compared to PDA with values of 6.82×10^{10} and 5.62×10^{9} spores, respectively. Both strains showed the antagonistic activity against the wood decay fungus *Ganoderma*. However, the antagonistic activity higher in *Trichoderma* cultured on PSA media with antagonistic activity of 66% in *T. virens* strain 128 and 62% in *T. harzianum* 1132, respectively. These results suggest the significant potential for the use of PSA media as a growth and activity enhancer of *Trichoderma* strains used in biocontrol programs.

Key words: Antagonist activity, culture media, *Trichoderma* sp., G*anoderma.*

INTRODUCTION

Trichoderma sp. as a biocontrol agent of plant diseases has been recognized since the early 1930s (Weindling, 1934). Weindling described the mycoparasitic action of *Trichoderma* on *Rhizoctonia* and *Sclerotinia* and its beneficial effects on plant disease control. Subsequently, many studies have shown that *Trichoderma* sp. is effective biocontrol agents for managing plant disease (Schubert et al., 2008; Howell, 2003). *Trichoderma* sp. has been proven to be highly effective for controlling *Ganoderma boninense* infection in oil palms (Izzati and Abdullah, 2008; Sundram et al., 2008; Sariah et al., 2005; Susanto et al., 2005; Abdullah et al., 2003). Kashem et al. (2011) also reported that *Trichoderma* spp. control the foot and foot rot of lentil (*Lens culinaris* Medik) caused by *Fusarium oxysporum*. *Trichoderma* can be found in nearly all agricultural soils. Most species grow rapidly, produce abundant conidia, and have a wide range of enzymes including cellulases. They have the hallmarks of ruderals species (Mathivanan et al., 2000). *Trichoderma* is able to grow in soils having a pH range of 2.5 to 9.5, although most prefer a slight to moderately acidic environment. The species that prefer the more acidic soils are usually regarded as having a more stress-tolerant growth habit and are less aggressive. All species can produce colonies which have either white to yellow to green mature fruiting areas. Colonies can have floccose and elliptical conidia, or tufted non-floccose globulose conidia.

Different species of *Trichoderma* have shown antagonistic activity on other species of fungi via several processes. These include production of soluble

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antibiotics (peptides), volatile and non-volatile antibiotics, or by direct parasitism. This is achieved when they coil around the hyphae of other fungi in a process called mycoparasitsm limits the growth and activity of plant pathogenic fungi (Chet et.al., 1998). However, the biocontrol activity depends upon biomass production (Singh et al., 2007). Currently, there is a limited knowledge available with respect to biomass production of *Trichoderma* sp. on different media in *in vitro* studies. Hence, the present study was conducted to evaluate growth rate and antagonistic activity of *T. harzianum* (T1132*) and T. virens* (T128) on potato sucrose agar (PSA) and potato dextrose agar (PDA) media. The growth rate was evaluated based on spore counts and the antagonistic activity was determined by PIRG analysis (percentage inhibition radial growth).

MATERIALS AND METHODS

Preparation of PDA and PSA media

Potato dextrose agar (PDA) media was prepared by adding 19 g of PDA to 500 ml distilled water and the mixture was homogenized in the microwave and autoclaved at 121°C for 15 min. The Potato sucrose agar (PSA) media was prepared by conventional method. Briefly, 300 g peeled potato was cut into small pieces and then boiled to produce a homogenate. The homogenate was then sieved using a cloth net. The potato broth was poured into 1000 ml of distilled water containing 21.3 g agar and 20 g sugar. The mixture was autoclaved at 121°C for 15 min.

Collection of *Ganoderma* and *Trichoderma* spp.

Ganoderma mycelium was isolated from the innermost contextual tissues of *Ganoderma,* also called fruiting body of *Ganoderma*. Pieces of tissues measuring 3 × 3 × 2 mm each were cut out from the contextual layer and surface sterilized in 3% NaOCl (Clorox) for 1 min and again shaken with sterilized distill water for 15 s to remove the Clorox. A single piece was picked with a sterilized scalpel and placed in the centre of a Potato Dextrose Agar (PDA) culture plate. The agar media had earlier been steam- sterilized at 121°C for 15 min, following treatment of media with sterilized 0.5 g streptomycin. The samples were seeded into the Petri dishes for 4 days to grow the mycelia. Next, *Ganoderma* was cultured on slides and the morphology with clam connection which is a characteristic of *Ganoderma* was observed under the light microscope. After ensuring the selection of *Ganoderma*, then, fresh mycelium was sub-culture in PSA and PDA media and mycelia growth was observed. Mycelia growth in PSA media observed to be faster than in the PDA media. The mycelia were again sub-cultured on PSA contained plate for the wood decay test with the isolated *Ganoderma* sp. *T. harzianum* (strain 1132) and *T. virens* (strain 128) were obtained from the slant stock culture of the Mycology Laboratory, Department of Biology, Universiti Putra Malaysia (UPM).

Wood decay test of isolated *Ganoderma*

Wood decay test of *Ganoderma* was performed using rubber wood blocks. Freshly cut rubber wood blocks (2.5 × 2.5 × 5 cm^3) were used to prepare Gano-wood block inocula as described in (Naher et al., 2011). Briefly, the blocks were soaked in distilled water for overnight. After overnight socking, the blocks were placed in autoclavable polypropylene plastic bags (10 × 32 cm) and autoclaved at 121°C for 45 min. After that, 100 ml of PSA medium was transferred into each block, and the blocks were again autoclaved at 121°C for 45 min. Subsequently, the sterilized rubber wood blocks were cooled in a laminar flow and turned every 2 min to ensure that they absorbed the PSA medium properly. Seven-day old *Ganoderma* sp. cultures were cut into small pieces and transferred into the plastic bags containing the sterilized blocks; 100 ml of PSA medium were added, and the blocks were incubated for 10 to 12 weeks at room temperature (Abdullah et al., 2001). The control blocks were prepared without *Ganoderma* inoculation. Similar weights of wood blocks were selected for control and Gano-wood block preparation. The control and Gano-wood blocks were weighed every month by using an electric balance for three months to determine whether *Ganoderma* was consuming or decaying the tissue.

Growth and antagonistic activity of *Trichoderma* spp. strains cultured on different media

The growth of *T. harzianum* strain 1132 and *T. virens* strain 128 were determined by measuring spore counts of these two fungi using haemocytometer under the microscope. The following formula was used for calculating the production of *Trichoderma* spores:

$$\text{Spore numbers per ml of sample} = \frac{No.of\ spores}{No.of\ counting\ boxes} \times Total\ boxes \times \frac{1}{0.00025 mm^3} \times 10^3$$

Where, 0.0025 mm^3 = total volume of haematocytometer, and 10^3 = spores in ml

The antagonistic activity of *T. harzianum* strain 1132 and *T. virens* strain 128 against *Ganoderma* sp. was investigated in a dual culture experiment. A 6 mm diameter agar disc was taken from the edge of an actively growing pure culture of *Ganoderma* and placed 1 cm inside the edge of the two different Petri dishes containing PSA and PDA, respectively. The samples were allowed to grow for 3 days, by which time the colony had grown to ~2 cm in diameter. Next, a 6 mm diameter disc was taken from the *T. harzianum* 1132 or *T. virens* 128 culture and placed on the opposite side of the Petridish containing the *Ganoderma* sp. The control plates contained only *Ganoderma*. The experiment was run in triplicate. *Trichoderma* is a faster growing fungus, and hence three days later *Trichoderma* was placed on the *Ganoderma* culture in the Petri dishes. The attached time of *T. virens* 128 or *T. harzianum* 1132 with *Ganoderma* was approximately 76 to 78 h, in the PSA media and 83 to 86 h in the PDA media (Figure 2). The experiment was extending for another 2 days. At this time the percentage inhibition of radial growth (PIRG) of *Ganoderma* caused by *T. harzianum* 1132 and *T. virens* T128 respectively, was calculated as follows (Figure 2):

$$PIRG = \frac{R1 - R2}{R1} \times 100$$

Where, PIRG= percentage inhibition of radial growth, R1= the radial growth of the *Ganoderma* colony in the absence of the *T. harzianum* 1132 or *T. virens* T128, and R2= the radial growth of the *Ganoderma* colony in the presence of the *T. harzianum* 1132 or *T. virens* T128.

RESULTS AND DISCUSSION

Trichoderma sp. are generated well recognized biocontrol

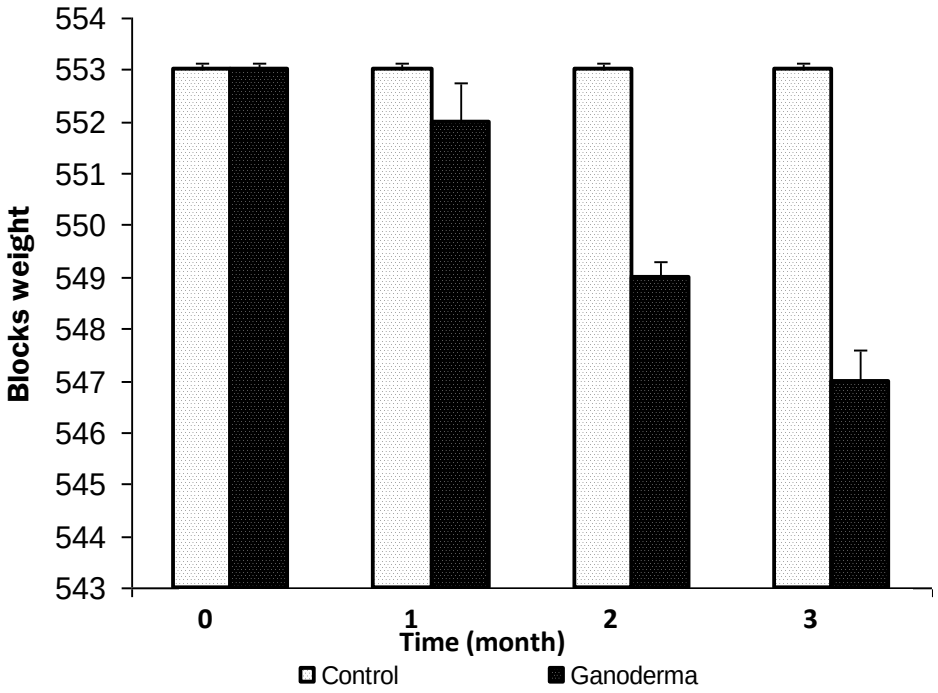

Figure 1. Wood decay test of *Ganoderma boninense* (the test was carried out over three months).

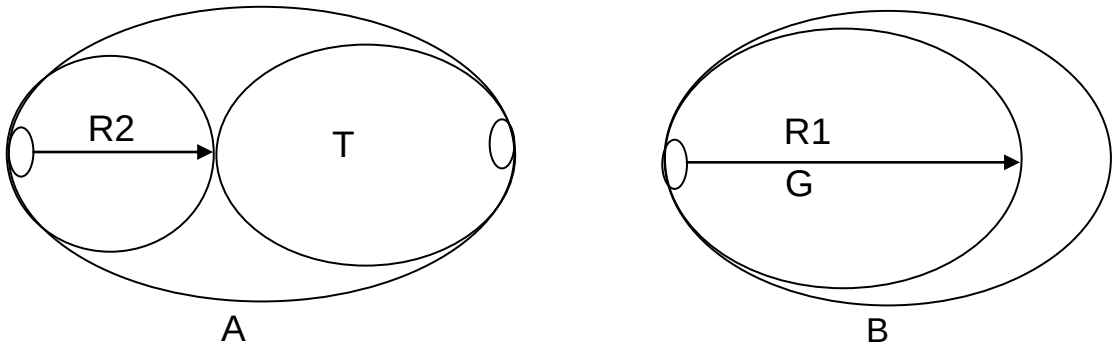

Figure 2. Measurement of percentage inhibition of radial growth (PIRG) of *Ganoderma*. Figure 2A shows the interaction *Ganoderma* (G) and *Trichoderma* (T) either strains 1132 or 128 within 76-86 h, while B shows the control plate of *Ganoderma* culture at 5 days.

agents in the management of plant diseases (Vinale et al., 2008; Verma et al., 2007) and they have been previously shown to have a high efficacy for controlling *Ganoderma* (Abdullah et al., 2003). The present study was designed to evaluate the biocontrol activity of *Trichoderma* sp. cultured on two different medium against the wood decay fungus *Ganoderma*. A wood decay test was also carried out to determine the decaying ability of the isolated *Ganoderma*. The results showed that *Ganoderma* completely colonized the wood within three months with weight losses of around 6 gm, which was significant ($p< 0.05$) compared to the control blocks (Figure 1). The findings indicated that this was a strong wood decaying fungus. Hence this *Ganoderma* sp. was then used in subsequent experiments.

The growth rates of *T. harzianum* (strain 1132) and *T. virens* (128) as determined based on spore production showed that the growth rates of *T. harzianum* (1132) and *T. virens* (128) cultures were significantly different in the two media. The growth rates of *T. harzianum (*1132) on PDA and PSA media were 5.62×10^9 and 8.58×10^{11}, respectively, while the growth rates of *T. virens* (128) on PDA and PSA media were 6.82×10^{10} and 9.12×10^{10}, respectively. A dynamic spore density was observed

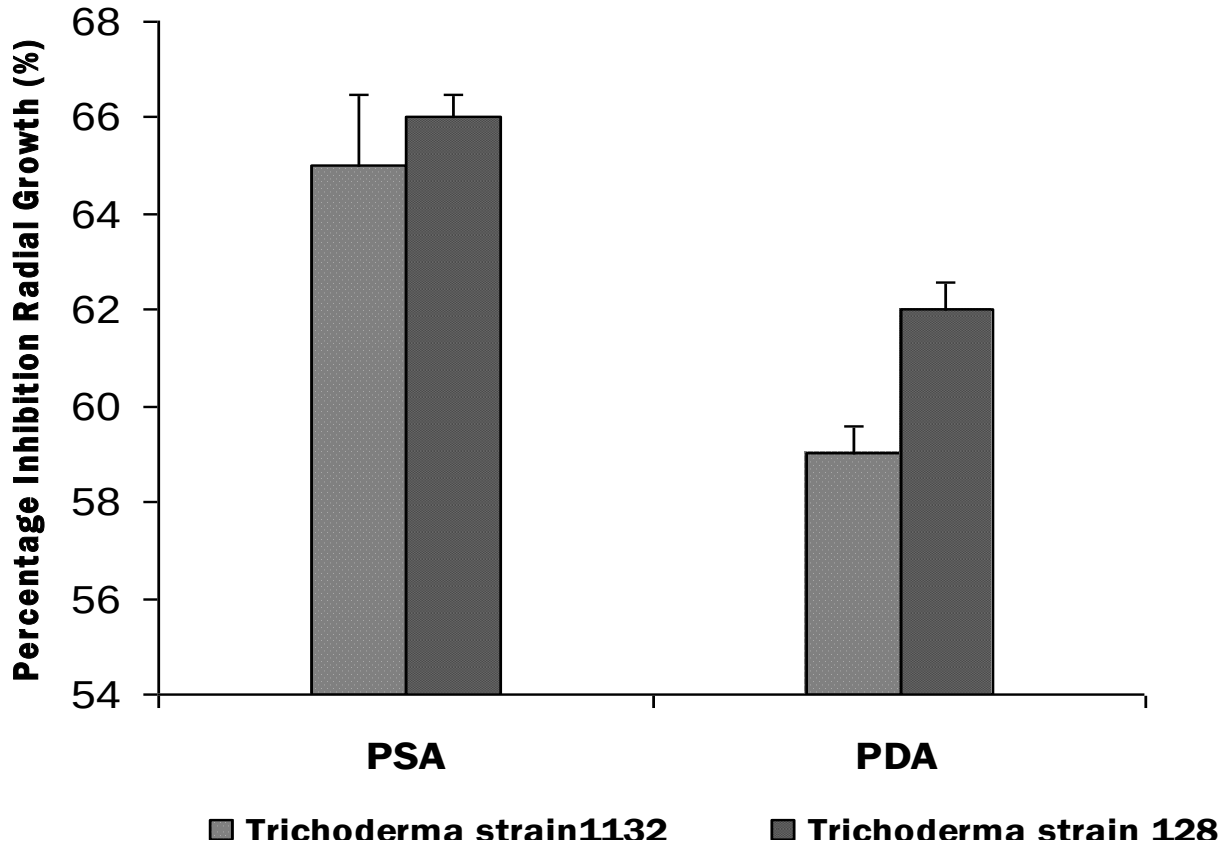

Figure 3. PIRG analysis on *Ganoderma* using *Trichoderma* spp. cultured on PSA and PDA media.

on PSA media for both tested fungi, but the growth rate was higher in *T. virens* strain 128 compared to *T. harzianum* strain 1132. The resulting data indicated that *Trichoderma* sp. growth was higher in PSA media for both tested fungi, which also showed that *Trichoderma* sp. cultivation was better with sucrose than dextrose or glucose augmentation. A previous study had however shown that growth of *Trichoderma* sp. growth was better in glucose media (Rodrigues et al., 2009). It was not clear that why this study data is not similar than other study, whereby it might be that the sucrose and potato were better carbon sources for growth of *Trichoderma* sp. in the PSA medium compared to glucose alone in the PDA medium.

Antagonistic activity based on the dual culture experiment showed that *T. harzianum* strongly inhibited the growth of *Ganoderma* (Figure 3). The PIRG rate of *Ganoderma* was found to be 59% with the *T. harzianum* strain 1132 on PDA and 65% on PSA, while with the *T. virens* strain T128 PIRG was 62% on PDA and 66% on PSA contained Petri dish (Figure 3). Perello et al. (2003) reported that *Trichoderma* sp. inhibited *Drechslera triticirepentis* colony growth with PIRGs of 50% and 74% in dual culture experiments. Sariah et al. (2005) had also described *T. harzianum* as a good biocontrol agent for controlling *Ganoderma*. However, the present study demonstrated the significant potential of PSA as an enhancing media for more effective control of *Ganoderma*.

In conclusion the results of this study data indicated better growth of *T. virens* and *T. harzianum* on PSA medium, which also resulted in higher inhibition of *Ganoderma*. Thus, PSA might therefore serve a beneficial carrier medium for culturing *Trichoderma* sp. used in the biocontrol of *Ganoderma* species and possibly other pathogens as well.

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