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IN VITRO EVALUATION OF BIOCONTROL AGENTS AND FUNGICIDES ON WOOD DECAY FUNGI-*GANODERMA* ASSOCIATED WITH MORTALITY OF TREE LEGUMES

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Abstract

An experiment was conducted to isolate a number of biocontrol agent- *Trichoderma* spp. from infected spawn packets of oyster mushroom at National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh. These bio-control agents were used as antagonist against four wild wood decay fungi of *Ganoderma*, *viz., G. lucidum*-1*, G. lucidum*-2*, G. lucidum*-3, *G. applanatum* and two cultivated *G. lucidum*-4*, G.lucidum*-6 under *in vitro* condition. An *in vitro* trial of *Trichoderma* spp. against *Ganoderma* were performed by dual culture, by treating with volatile, non-volatile and naturally untreated metabolites of bio-control agents. In dual culture, all the *Trichoderma* species showed 70-100% mycelia inhibition of *G. lucidum*-1 and *G. lucidum*-2, 55.6-100% inhibition of *G. lucidum*-3, 20-66.7% of *G. applanatum,* 100% of *G. lucidum*-5, 75-100% of *G. lucidum*-6. Effects of heat killed extracts of *Trichoderma* spp. on growth of *G. lucidum*-2 (wild) and *G. lucidum*-6 (cultivated) were also evaluated. Fungicides Bavistin and Dithane M-45 were also used to investigate the mycelial growth inhibition of *Ganoderma* spp.

Keywords: Biocontrol, *Ganoderma,* Green mould, *Trichoderma*

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Introduction

Tree legumes are important throughout the tropics as sources of forage, firewood, charcoal, green manure and timber (Hughes and Styles, 1989). *Ganoderma* spp. are important wood-decaying fungi, occurring on conifers and hardwoods across the world. They are known as white-rot fungi which able to decay lignin as well as cellulose (Adaskaveg and Gilbertson, 1994). *Ganoderma* species caused the root and stem rot diseases result in losses of crops and trees in worldwide (Miller *et al*., 1994). Seven year-old trees had 10-15% mortality at moist sites due to *Ganoderma lucidum* (Pathak, 1986). Stressed and damaged Canary Island date palms often become inflicted by *Ganoderma applanatum*. Large numbers of trees have been known to kill in ten-year-old plantations due to *Ganoderma* spp. in Peninsular Malaysia (Lee, 2000). Tree mortality generally increases with time in areas where the *Ganoderma* disease is already present. Control of root rot diseases is difficult as the pathogens survive on woody material in the soil. Green mould disease caused by *Trichoderma* spp. one of the serious problem of oyster mushroom and white button mushroom. It causes large economic losses to the mushroom growers (Hatvani *et al*., 2007). Present investigation was carried out to evaluate the potential of fungi as biological control agents (BCA) and fungicides against pathogenic *Ganoderma* to tree legumes.

Materials and Methods

On the basis of symptomatological study, four species of *Trichoderma* namely *Trichoderma harzianum* Rifai, *T. koningii* Oudem, *T. viride* (green strain) Pers., and *T. viride* (yellow strain) Pers. were collected from spent (infected) mushroom spawn packets of *Pleurotus ostreatus* (Jacquin*ex*fr.) Kummer at National Mushroom Development and Extension Center, Savar, Dhaka, Bangladesh. *Ganoderma* species namely; *Ganoderma lucidum*-1 (Curtis) P. Karst., *Ganoderma lucidum*-2 (Curtis) P. Karst., *Ganoderma lucidum*-3 (Curtis) P. Karst., and *Ganoderma applanatum* (Pers.) Pat., were isolated from *Ganoderma* infected *Acacia auriculiormis* L. and *Albizia lebbeck* (L.) Benth trees of Jahangirnagar University Campus, Bangladesh. Two cultivated *Ganoderma* species were also collected from National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka namely; *Ganoderma lucidum*-5 (Curtis) P. Karst., and *Ganoderma lucidum*-6 (Curtis) P. Karst. The morphological characterization of *Trichoderma* spp. isolated from oyster mushroom growing substrates was

conducted based on morphology such as colonies, hyphae, conidiophores, phialides and conidia. *Trichoderma harzianum* was characterized according to Choi *et al*. (2003) and Barnet (1960). Others isolates of *Trichoderma* were characterized as described by Barnet (1960). During present study, *Ganoderma* spp. were classified on based of morphological characteristics of fruit body such as size and color, and stripe attachment patterns (Corner, 1983). The cultural and microscopic characteristics of *Ganoderma lucidum* was determined as according to Schwarze and Ferner (2003) and Fernando (2008). The efficacy of *Trichoderma* isolates were evaluated against *Ganoderma* (4 wild, 2 cultivated) by dual culture technique as described by Dennis and Webster (1971). The pathogens inoculated by the pre-colonized agar plate method as described by Forley and Deacon (1985). The effect of released volatile metabolites of *Trichoderma* isolates on the mycelial growth of the *Ganoderma* spp. were evaluated as method described by Dennis and Webster (1971). The effect of non-volatile metabolites on tested fungi were evaluated as according to Kaur *et al*. (2006). Effects of natural untreated metabolites by dipping culture disc method was performed as mentioned by Ashrafuzzaman and Aminur (1992). There are different concentrations (30, 50 and 70 ppm) of fungicides, namely Bavistin and Dithane M-45 were used to see the mycelial growth inhibition of *Ganoderma* spp. on PDA medium using food poison technique. All of the inoculated and non-inoculated plates were incubated at 28±2ºC and percent of mycelia inhibition was calculated as the formula given by Kaur *et al*. (2006).

$$\text{Mycelial inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where,

C=Radial growth of control plates
T = Radial growth of treated plates

Results and Discussion

Inhibition of Ganoderma spp. by bio-control agents

In vitro dual culture tests against wild *Ganoderma* spp. revealed that percent of inhibition range of *Ganoderma lucidum*-1, 2, 3 and *G. applanatum* were: 85-100%, 70-100%, 55.6-100%, 55-67.7% due to *T. harzianum*, *T. koningii*, *T. viride* (green strain), *T. viride* (yellow strain), respectively (Table 1). During present study, *Trichoderma* showed the overgrowth on pathogens in some case that indicates the mycoparasitic nature of *Trichoderma* spp. *Trichoderma viride* effectively inhibited the growth of *G. lucidum* under *in vitro* condition. Cultivated *G. lucidum*-4 and 6 were inhibited 75-100% by *Trichoderma* spp. at 7 days after incubation during present study (Table 1). In our study, *Trichoderma* showed overgrowth on pathogens, which indicates the mycoparasitic nature of *Trichoderma* spp. Similarly, in dual culture technique, the maximum suppression of *Ganoderma applanatum* (72%) and *G. lucidum* (75%) over control was noted with *Trichoderma harzianum* (Srinivasulu and Raghava, 2009). Idris *et al*. (2008) also recognized *Trichoderma* spp. as well-known antagonists to many plant pathogenic *Ganoderma* spp. in oil palm. *Trichoderma viride* effectively inhibited the growth of *G. lucidum* under *in vitro* condition (Lingan *et al*., 2007). *Trichoderma atroviride* was also consistently and highly competitive against most wood decay fungi (Schubert *et al*., 2008). Red root disease of rubber (*Ganoderma psuedoferreum*) was inhibited by *Trichoderma* spp. (Ogbebor *et al*., 2010). The mycelial growth of *G. lucidum* was inhibited successfully by *T. viride, T. harzianum* and *T. virens* with 66.55%, 63.99% and 62.12%, respectively after 96 hrs of incubation (Chakrabarty *et al*., 2013). It has been revealed that *Trichoderma* spp. coiled round the hyphae of *Ganoderma* spp. both sparsely and intensely which was followed by penetration of *Trichoderma* spp. into the hyphae of *Ganoderma* spp., finally, lysis of the host mycelium was noticed (Srinivasulu and Raghava, 2009).

Table 1. An *in vitro* mycelial growth inhibition (%) of *Ganoderma* spp. by four *Trichoderma* spp. in dual culture technique at 32±2ºC temperature.

Antagonists	Mycelial growth inhibition (%) of *Ganoderma* spp.					
	Wild				Cultivated	
	G1	G2	G3	G4	G5	G6
T. harzianum	85 b	100 a	55.6 c	62ab	100 a	75 b
T. koningii	80 c	100 a	80 a	60bc	100 a	100 a
T. viride (Green strain)	80 c	70 b	77.8 a	67.7 a	100 a	75 b
T. viride (Yellow strain)	100 a	100 c	66.7 b	55 c	100 a	100 a

Data recorded after 7 days of incubation; Data represent as mean value of three replications; Column having the same letters do not differ significantly at 5% level of significance; G1 = *Ganoderma lucidum*-1, G2 = *G. lucidum*-2, G3 = *G. lucidum*-3, G4 = *G. applanatum*, G5 = *G. lucidum*-5 (cultivated), G6 = *G. lucidum*-6 (cultivated).

Effect of volatile, non-volatile and natural untreated metabolites

The current study confirmed that the volatile metabolites had a fungistatic rather than a fungicidal effect. Volatile metabolites secreted by *Trichoderma* spp. showed significant effect in controlling *Ganoderma* spp. The range of inhibition of *Ganoderma lucidum*-1, 2, 3 and *G.*

applanatum was found 68.85%, 41.2-53%, 55.6-72.2%, 75-85%, respectively by *Trichoderma* spp. (Table 2). Volatile metabolites of *T. viride* showed the maximum inhibition than other isolates. In the present study, the average inhibition was recorded as 0-33.3% in *Ganoderma* spp. by non-volatile compound and *T. viride* was found more effective than others (Table 2). Present results are supported by earlier workers. *Trichoderma viride, T. hamatum* and *T. harzianum* were reported to be very effective in producing volatile and non-volatile metabolites against *Ganoderma lucidum* and *G. applanatum* (Srinivasulu and Raghava, 2009). Bruce *et al*. (2000) cited that volatile metabolites of *T. viride* having significant effect on wood decay fungi. Idris *et al*. (2008) reported 318 isolates of *Trichoderma* and tested against pathogenic *Ganoderma*. Effect of natural untreated metabolites of *Trichoderma* spp. showed variable inhibitory effects on studied organisms. *T. viride* (green strain) showed the maximum inhibition in test fungus except *G. applanatum* (Table 2). There is lack of information regarding the effect of natural untreated metabolites on *Ganoderma* spp.

Heat killed extract trial among GI-2 (wild) and GI-6 (cultivated)

Effect of heat killed extract of *Trichoderma* spp. on *G. lucidum*-2 and *G. lucidum*-6 showed significant difference in comparison to control at three different temperatures. Table 3 depicted that *G. lucidum*-2 (G2) and *G. lucidum*-6 (G-6) was largely inhibited 69% and 81% by *T. koningii* at 28±2ºC and 32±2ºC temperature, respectively. *T. viride* (green strain) showed better performance to control *G. lucidum*-6 at both28±2ºC and 32±2ºC temperature. At temperature 35ºC, *G. lucidum*-2 was inhibited (40%) due to *T. viride* (yellow) and lowest by *T koningii* (3.3%). *Ganoderma lucidum*-6 was completely inhibited by all of the antagonists. There is no literature available in this regard by previous workers. During present investigation, the aggressiveness of *Trichoderma* spp. studied varied more or less to previous mentioned workers. This might be due to difference in site of isolation. In literature, *Trichoderma* spp. were collected from soil rhizosphere but in the present study isolates were collected from spent mushroom compost.

Table 2. An *in vitro* mycelial growth inhibition (%) of *Ganoderma* spp. by four *Trichoderma* spp. at 28±2ºC temperature due to volatile, non-volatile and naturally untreated metabolites.

Antagonists	Mycelial growth inhibition (%) of *Ganoderma* spp											
	Volatile				Non volatile				Naturally untreated			
	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
T. harzianum	68.8c	41.2b	72.2a	75.0b	0.0c	10.0a	22.2b	0.0c	6.3d	29.4b	0.0b	*
T. koningii	68.8c	53.0a	72.2a	75.0b	0.0c	0.0B	33.3a	50.0b	25.0b	29.4b	11.1a	*
T. viride (green strain)	81.3b	53.0a	55.6b	85.0a	18.8b	11.8a	33.3a	75.0a	62.5a	47.1a	11.1a	*
T. viride (yellow strain)	85.0a	53.0a	72.2a	75.0b	12.5a	0.0b	22.2b	0.0c	18.8c	29.4b	*	*

Data recorded after 7 days of incubation; Data represent as mean value of three replications; Column having the same letters do not differ significantly at 5% level of significance; G1 = *Ganoderma lucidum*-1, G2 = *G. lucidum*-2, G3 = *G. lucidum*-3, G4 = *G. applanatum;* Ganoderma* was not inhibited but enhanced.

Table 3. Effects of heat killed extracts of *Trichoderma* spp. on mycelia growth of *G.lucidum*-2 (wild) and *G. lucidum*-6 (cultivated) at three different temperatures.

Antagonists	Mycelial growth inhibition (%) of *G.lucidum*-2 (wild) and *G.lucidum*-6 (cultivated) at three different temperatures					
	28±2ºC		32±2ºC		35ºC	
	G-2	G-6	G-2	G-6	G-2	G-6
T. harzianum	40.5 c	63 b	29 c	41.4 c	80 a	100 a
T. koningii	69 a	81 a	60.4 b	79.3 b	3.3 d	100 a
T. viride(green strain)	59.5 b	81 a	79.2 a	96 a	20 c	100 a
T. viride (yellow strain)	16.7 d	48.2 c	23 c	48.3 c	40 b	100 a

Data recorded after 7 days of incubation; Data represent as mean value of three replications; Column having the same letters do not differ significantly at 5% level of significance; G-2= *Ganoderma lucidum*-2 (wild) and G-6= *G. lucidum*-6 (cultivated)

Effect of fungicides on Ganoderma spp.

In vitro fungicidal effects on studied organisms found very significant. Bavistin showed complete mycelial inhibition in case of all selected organisms at 30, 50 and 70 ppm concentrations (Table 4) while Dithane M-45 was not satisfactory as compared to Bavistin. Present results are in conformity with the previous findings. Donghua *et al*. (1999) reported the strongest mycelial inhibitory effect on *Ganoderma lucidum* by Bavistin and Dithane @ 0.500-0.667 g/L concentration had no inhibition or had a little promotion on *G. lucidum*. Chakrabarty *et al*. (2013) cited that Bavistin (03%) was able to inhibit mycelial growth (91.33%) of *G. lucidum* after 144 hrs of incubation.

Table 4. Effect of different concentration of Bavistin and Dithane M-45 on mycelial growth of *Ganoderma* spp. at 28±2ºC temperature.

Concentration of fungicides	Mycelial growth inhibition (%) of *Ganoderma* spp.							
	Bavistin				Dithane M-45			
	G-1	G-2	G-3	G-4	G-1	G-2	G-3	G-4
30 ppm	70.00 b	99.94 a	99.94a	34.80b	0.00c	54.97a	16.00a	21.80b
50 ppm	100 a	99.00 a	99.40a	34.80b	34.30b	60.00a	20.00a	21.00b
70 ppm	99.30 a	99.94 a	99.97a	56.50a	59.00a	10.00b	20.00a	39.00a

Data recorded after 7 days of incubation; Data represent as mean value of three replications; Column having the same letters do not differ significantly at 5% level of significance; G1 = *Ganoderma lucidum*-1, G2 = *G. lucidum*-2, G3 = *G. lucidum*-3, G4 = *G. applanatum.*

It can be concluded that both biocontrol agent-*Trichoderma* and Bavistin were found to be effective to control *Ganoderma* infection. Therefore, either utilization of *Trichoderma* or Bavistin is preferable to control stem and root rot of higher plant like tree legume.

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