Journal of Agricultural Technology 2010 Vol.6(1): 11-18
Available online http://www.ijat-rmutto.com
ISSN 1686-9141

Potential of the root endophytic fungus *Piriformospora indica; Sebacina vermifera* and *Trichoderma* species in biocontrol of take-all disease of wheat *Gaeumannomyces graminis* var. *tritici in vitro*

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Ghahfarokhi, R.M. and Goltapeh, M.E. (2010). Potential of the root endophytic fungus *Piriformospora indica; Sebacina vermifera* and *Trichoderma* species in biocontrol of take-all disease of wheat *Gaeumannomyces graminis* var. *tritici in vitro*, in Iran. Journal of Agricultural Technology 6(1): 11-18.

Gaeumannomyces graminis var. *tritici* is the causal agent of take-all disease of wheat, the most important damaging root disease is very important in North, Central and Southwest Provinces of Iran and worldwide. *Piriformospora indica* and *Sebacina vermifera*, which are a newly discovered arbuscular mycorrhiza-like fungus. They are a facultative symbiont and unlike arbuscular mycorrhizal fungi, can be cultured *in vitro*. *Trichoderma harzianum* and *T. viride* are efficient biocontrol agent that is commercially produced to prevent development of several soilborne pathogenic fungi. Interactions between *P. indica*, *S. vermifera*, *T. harzianum* strain 100, *T. viride* and soilborne fungi of *Gaeumannomyces graminis* var. *tritici* were investigated separately and in combination on PDA and Kafer medium. Opposing (Dual culture) cultures as well as colonization studies showed that the species of *P. indica*, *S. vermifera*, T100, *T. viride* with could produce a good zone of inhibition. Volatile metabolites test between *Trichoderma* species and *Ggt* inhibitory effects on growth *Ggt* mycilium. These fungal species are the most potent agents for the biocontrol of soilborn plant pathogen.

Key Words: *Gaeumannomyces graminis* var. *tritici*, *Piriformospora indica*, *Sebacina vermifera*, *Trichoderma*, biological control

Introduction

Take-all disease is an economically significant and damaging root disease of cereals and grasses worldwide. It is very important in temperate regions where wheat and grass culture is intensive (Bryan *et al*., 1995; Cook, 2003). *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var tritici Walker, a soilborne ascomycete, is the causal agent of take-all disease of cereal and

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grasses (Walker, 1972). *Piriformospora indica* (Verma *et al.*, 1998*)* and *Sebacina vermifera* (Warcup and Talbot, 1967), (Basidiomycota, Sebacinales) are a root endophytic fungus with a broad host spectrum and a new plant growth promoter. *P. indica* and *S. vermifera* colonizes the cortex of roots of a wide variety of plant species and promotes their growth, and induces resistance against soilborn fungal pathogens in a manner similar to arbuscular mycorrhizal fungi. It is characterized by the formation of typical pyriform chlamydospore; The fungus are a member of Basidiomycota (Verma *et al*., 1999; Blechert *et al*., 1999; Kumari *et al*., 2003; Pham *et al*., 2003; Singh *et al.*, 2003a, b). Biological control of take-all disease has been investigated intensively, largely because of a lack of commercially available alternatives, especially in reduced-tillage cropping systems that aggravate the disease but are increasingly encouraged to promote soil conservation (Cook and Weller, 1987). *Trichoderma* is a ubiquitous fungus found in air, soil, plant materials and other substrates. (Kligman, 1950) *Trichoderma harzianum* is an efficient biocontrol agent that is commercially produced to prevent development of several soilborn pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds (Haram *et al*., 1996; Zimand *et al*., 1996). One method of biocontrol of disease is using a mycorrhizal fungus (Dehne, 1982; Singh *et al.*, 2000; ST-Arnaud and Vajaronic, 2006; Khaosaad *et al*., 2007) and *Trichoderma* species (Chet *et al*., 1997; Yedidia *et al.*, 1999; Harman, 2004; Kucuk and Kivanc, 2003; Brozova, 2004). The aim of this study was biocontrol of take-all disease *in vitro* by using mycorrhizal-like fungus and *Trichoderma* species.

Materials and methods

Ggt cultures

Three Ggt strains were used: T16, T12 and T47. Cultures were maintained at 25±1 °C on potato dextrose agar (PDA).

Mycorrhizal cultures

Piriformospora indica and *S. vermifera* were grown on aspergillus broth and agar medium (Pham *et al*., 2003) at 30 °C for 7 d also grown on PDA medium too.

Trichoderma cultures

Trichoderma species were used: *T. harzianum* strain 100, *T. viride*. Cultures were maintained at 25±1 °C on PDA.

Dual culture tests

Interactions between antagonistic fungi and pathogenic fungi were determined by the method described by Kucuk and Kivanc (2003), with slight modifications. This study was carried out in four phases: In the first phase, 5 mm mycelial discs of *P. indica* and *S. vermifera* were placed on one side of PDA plates and incubated at 28 ± 1 ºC for 3-4 days before placing 5 mm discs of *Ggt* mycelium taken from the margins of 4 days old cultures on the other side of the plates. The colonies were examined for a zone of inhibition between the *Ggt* mycelium and *P. indica* and *S. vermifera*.

In the second phase, 5 mm mycelial discs of *P. indica* and *S. vermifera* were placed on one side of a petri dish containing PDA and Kafer, while 5 mm mycelial disks of *Ggt* were placed on the opposite side of the plate and incubated at 28± 1°C.

In the third phase, 5 mm mycelial discs of *T. viride* and T100 were placed on one side of a petri dish containing PDA, while 5 mm mycelial discs of *Ggt* were placed on the opposite side of the plate and incubated at 28± 1°C.

In the fourth phase 5 mm mycelial discs of *P. indica* and *S. vermifera* were placed on one side of a petri dish containing PDA, while 5 mm mycelial discs of *T. viride* and T100 were placed on the opposite side of the plate and 5 mm mycelial discs of *Ggt* were placed on the center of the plate in opposite side antagonistic fungi. The overgrowth of colonies of the test fungi by the antagonist was determined.

Volatile metabolites

The effect of volatile metabolites produced by the antagonistic fungi following the method described by Dennis and Webster (1971) and Goyal *et al*. (1994) with slight modifications. 5 mm mycelial discs Ggt were placed on the center of the petri dish containing PDA after 4 day, when some mycelium growth, the bottom petri dish (*Ggt*) was removed and placed on another plate containing PDA and 5 mm mycelial discs of *Trichoderma* spp. and taped together by adhesive tape. In the control, *Ggt* petri dish were removed and placed over another PDA petri dish without *Trichoderma* spp. All of the plates were incubated at 25 ± 1 ºC for 7 days and percent inhibition was recorded

daily (every 24 hours) by comparing growth of *Ggt* mycelium controls with treatment growth using the following equation (Vincent, 1947):

$$\text{Percentage inhibition} = \frac{\text{Colony growth rate in plates (control) - colony growth rates in each treatment}}{\text{Colony growth rates in plates (control)}} \times 100$$

Comparision of antagonistic fungi in colonization of Ggt mycelium

This study was carried out in sex phases using method described by Mohammadi Goltapeh and Danesh (2006), with slight modifications. In the first phase 5 mm discs of *P. indica* and *S. vermifera* were placed on PDA plates and incubated at 28 ± 1 ºC for 3-4 days before placing 5 mm discs of *Ggt* mycelium taken from 4 days old cultures on center of the plates. In the second phase, 5 mm discs of *Ggt* mycelium were placed on PDA plates and incubated at 26 ± 1 ºC for 3-4 days before placing 5 mm discs of *P. indica* and *S. vermifera* mycelium taken from 4 days old cultures on center of the plates. In the third phase, 5 mm discs of *Ggt* mycelium were placed on PDA plates and incubated at 26 ± 1 ºC for 10-12 days before placing 5 mm discs of *P. indica* and *S. vermifera* mycelium on center of the petri dish. In the fourth phase, 5 mm discs of *T. viride* and T100 were placed on center of petri dish containing PDA and concordant 5 mm discs of *Ggt* mycelium were placed on. In the fifth phase, 5 mm discs of *Ggt* mycelium were placed on PDA plates and incubated at 26 ± 1 ºC for 3-4 days before placing 5 mm discs of *T. viride* and T100 mycelium taken from 4 day old cultures on center of the plates. In the sixth phase, 5 mm discs of *Ggt* mycelium were placed on PDA plates and and incubated at 26 ± 1 ºC for 10-12 day before placing 5 mm discs of *T. viride* and T100 mycelium on center of the petri dish.

Results and discussion

Experimental results suggest that the possibility of using mycorrhizal-like fungus *P. indica*; *S. vermifera* and *Trichoderma* spp. to control root disease of wheat (take-all disease by *Ggt*). *Piriformospora indica*, *S. vermifera* and *Trichoderma* species in opposing culture and colonization culture test inhibition of growth T12, T16 and T47 isolates of Ggt as well as volatile metabolites of *Trichoderma* species could inhibit growth and activity of the *Ggt* isolates. Hyphal investigation showed that *P. indica*, *S. vermifera* and *Trichoderma* spesies could coiled around *Ggt* mycelium and penetrate inter their hyphae and inhibition of activity, growth and progressive growth in mycelium.

Trichoderma Pers. ex Fr., a genus has gained immense importance since last few decades due to its biological control ability against several plant

pathogens (Agrios, 1997). The filamentous fungus *T. harzianum* and *T. viride* are one of the most potent agents for the biocontrol of soilborn plant pathogens (Cook and Vesth, 1991). Kucuk and Kivanc (2004) demonstrated *Trichoderma* species inhibition the growth of soilborn plant pathogens include *Gaeumannomyces graminis* var. *tritici*, *Fusarium culmorum* and *F. moniliforme* by volatile metabolites *in vitro* and all isolates of *T. harzianum* grew considerably faster on PDA than did the pathogens, in the same conditions of culture. *Trichoderma* species are known to produce a number of antibiotics, such as trichodermin, trichodermol, harzianum-A and harzianolide (Simon *et al.*, 1988; Schirmbock *et al*., 1994; Dennis and Webester, 1971). Shalini and Kotasthane (2007) showed that all strains including *T. harzianum, T. viride* and *T. aureoviride* were inhibited the growth of *Rhizoctonia solani*. Our study also demonstrated the effect of *Trichoderma* volatile metabolites have high potential to control the *Ggt* and could inhibit the growth of *Ggt* mycelium. Antifungal activity of tested strains of *T. harzianum and T. viride* on pathogens Ggt *in vitro* shown in Table 1 and Fig.1. Opposing cultures test of *P. indica; S. vermifera* and *Trichoderma* species with *Ggt* mycelium produced a good zone of inhibition around the *Ggt* mycelium (Fig. 2). Our result in agreement with Mohammadi Goltapeh and Danesh (2006) their showed that the coiling and penetration hyphae of *Trichoderma* species around *Agaricus bisporus* mycelial. Also hyphae of *P. indica*; *S. vermifera* and *Trichoderma* species coiled around the *Ggt* mycelium, as well as penetrating them in the same way (Fig. 3). Colonization of *Ggt* mycelium with *P. indica, S. vermifera*, T-100 and *T. viride* to inhibit their growth. Antagonistic and mycorrhizal-like fungia are the most potent agents for biocontrol of root plant`s pathogens (Ghisaleberti *et al*., 1990; Lorito *et al*., 1994; Singh and Faull, 1990; Varma and Schuepp, 1995).

Table 1. Effect of volatile metabolites produced by *T. harzianum* and *T. viride* on Ggt mycelia growth (mm).*

Take - all	**Trichoderma**	
	T-100	***T.viride***
T 47	13.67	10
T 16	14	14.33
T 12	13	12.67

*(mean represent in the fourth day)

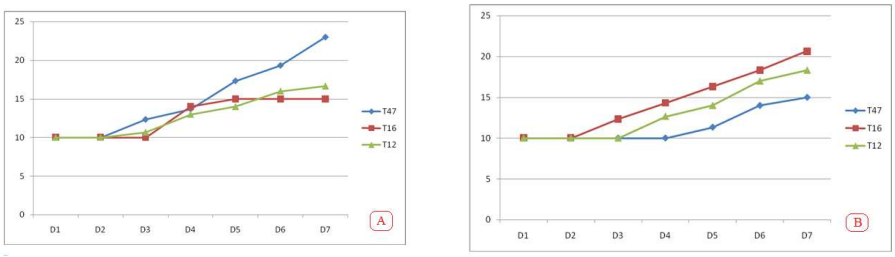

Fig.1. Antifungal activity of tested strains of T-100 *and T. viride* on pathogens on *Ggt* mycelia growth in seven days (A, B) respectively.

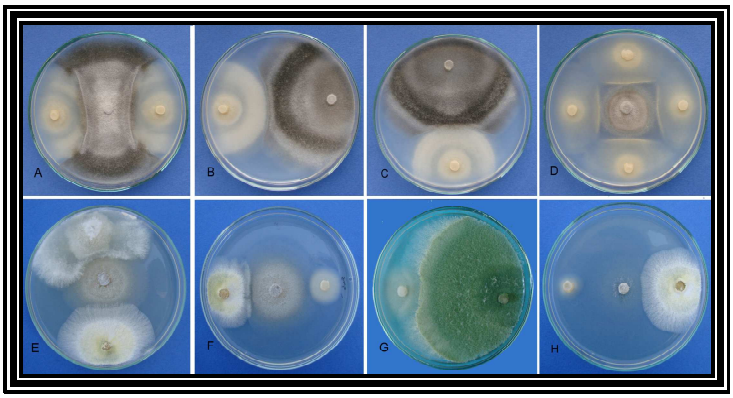

Fig. 2. Opposing culture of root endophytic fungus and *Trichoderma* species. (A; B; C and D) interaction between *P. indica*, *S. vermifera* with isolate *Ggt* that produced a good zone of inhibition (E; F; G and H) interaction between *Trichoderma* species and isolate of *Ggt* showing zone of inhibit.

Acknowledgment

This study was supported by the Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modarres University.

References

Agrios, G.N. (1997). Plant pathology. Academic Press, New York, USA. Blechert, O.G., Kost, A., Hassel, K.H., Rexer, and Varma, A. (1999). First remarks on the symbiotic interaction between *Piriformospora indica* and terrestrial orchids. *In* Varma, A. and Hock, B. (eds.), Mycorrhiza: Structure, Function, Molecular biology and Biotechnology, 2nd edn, p. 683-688. Springer-Verlag, Germany.

Brozova, J. (2004). Mycoparasitic fungi *Trichoderma* spp. in plant protection. Plant Protect. Sci, 40: 63-74.

Bryan, G.T., Daniels, M.J. and Osbourn, A.E. (1995). Comparison of fungi within the *Gaeumannomyces-Phialophora* complex by analysis of ribosomal DNA sequences. Appl. Environ. Microbiol. 61: 681-689.

Chet, I., Inbar, J. and Hadar, I. (1997). Fungal antagonists and mycoparasites. In: Wicklow DT, Söderström B (eds) The Mycota IV: Environmental and microbial relationships. Springer-Verlag, Berlin., pp 165-184.

Cook, R.J. and Vesth, R.J. (1991) .Wheat Health management. American Phytopathological Society, St. Paul, MN.

Cook, R.J. (2003). Take-all of wheat. Physiology Molecular Plant Phytopathology. 62: 73-86.

Cook, R. J. and Weller, D.M. (1987). Management of take-all in consecutive crops of wheat or barley. Pages 41-76 in: Innovative Approaches to Plant Disease Control. I. Chet, ed. John Wiley & Sons, New York.

Dehne, H.W. (1982). Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72: 1115-1119.

Dennis, C. and Webster, J. (1971). Antagonistic properties of species groups of Trichoderma III. Hyphal Interaction. Trans. British Mycological Society 57: 363-369.

Ghisalberti, E.L., Narbey, M.J., Dewan, M. and Sivasin, K. (1990). Varability among strain of *Trichoderma harizanum* in their ability to reduce take-all and to produce pyrones. Plant Soil 121: 287-291.

Goyal, S.P., Jandaik, C.L. and Sharma, V.P. (1994). Effect of weed fungi metabolites on the mycelial growth of *A. bisporus* (Lang.) Imbach. Mushroom Research 3: 69-74.

Haram, S., Schickler, H., Oppenheim, A. and Chet, I. (1996). Differential expression of *Trichoderma harzianum* chitinases during mycoparasitism. Phytopathology 86: 980-985.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. Nature Reviews. 2: 43-56.

Khaosaad, T., Garcı–Garrido, J.M., Steinkellner, S. and Vierheili, H. (2007). Take-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil and Biochemistry 39: 727-734.

Kligman, A.M. (1950). Handbook of Mushroom Culture. Keneth Square, Pennsylvania. Kucuk, C. and Kivanc, M. (2003). Isolation of *Trichoderma* spp. and their antifungal, biochemical and physiological features. Turk J Bio. l27: 247-253.

Kucuk, C. and Kivanc, M. (2004). In vitro antifungal activity of strains of *Trichoderma harzianum*. Turk J Biol. 28: 111-115.

Kumari, R., Yadav, H.K., Bhoon, Y.K. and Varma A. (2003). Colonization of cruciferous plants by *Piriformospora indica*. Current Science (in Press).

Lorito, M.C.K., Peterbauer, C.K., Hayes, C. and Herman, G.E. (1994). Synergistic intraction between fungal cell-wall degrading enzymes and different antifungal compound on spore germination. Microbiology. 140: 623-629.

Mohammadi Goltapeh, E. and Danesh, Y.R. (2006). Pathogenic interactions between *Trichoderma* species and *Agaricus bisporus*. Journal of Agricultural Technology 2(1): 29-37.

Pham, G.H., Singh, An., Malla, R., Kumari, R., Saxena, A.K., Rexer, K-H., Kost, G., Luis, P., Kaldorf, M., Buscot, F., Herrmann, S., Peskan, T., Oelmuller, R., Mittag, M., Declerck, S., Hehl, S. and Varma, A. (2003). Interaction of *Piriformospora indica* with diverse microorganisms and plants. *In* Varma, A., Abbott, L., Werner, D. and Hampp, R. (eds.), Plant Surface Microbiology. Springer- Verlag, Germany (in press).

Poromarto, S.H., Nelson, B.D. and Freeman, T.P. (1998). Association of binucleate *Rhizoctonia* with soybean and mechanism of biocontrol of *Rhizoctonia solani*. Phytopathol. 88: 1056-1067.

Schirmbock, M., Lorito, M. and Wang, Y.L. (1994). Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. Appl Environ Microbiol. 60: 4344-4370.

Shalini, S. and Kotasthane, A.S. (2007). Parasitism of *Rhizoctonia solani* by strain of *Trichoderma* spp. EJEAFChe. 1579-4377.

Simon, C. and Sivasithamparam, M. (1988). Interactions among *G. graminis* var *tritici*, *Trichoderma koningii* and soil bacteria. Can. J. Microbiol. 34: 871-876.

Singh, An., Singh, Ar., Kumari, M., Rai, M.K. and Varma, A. (2003a). Biotechnology importance of *Piriformospora indica*-A novel symbiotic mycorrhiza-like Fungus: An Overview. Plant Biotechnology- the Special Issue. Indian Journal of Biotechnology 2: 65-75.

Singh, An., Singh, Ar., Kumari, M., Rai, M.K.,Varma, A., Kumar, S. and Sharma, A.P. (2003b). AMF-like-fungus: *Piriformospora indica* – a boon for plant industry. p. 101-124. *In* B.N. Prasad(ed.), Biotechnology in Sustainable Biodiversity and Food Security. Science Publishers Inc., Enfield, New Hampshire, USA.

Singh, J., and Faull, J.L. (1990). Hyperparasitism and Biological Control. CRC Press. 167-179.

Singh, R., Adholeya, A. and Mukerji, K.G. (2000). Mycorrhiza in control of soil-borne pathogens. In: Mukerji, K.G., Chamola, B.P. and Singh, J (Eds.), Mycorrhizal Biology. Kluwer Academic/Plenum Publishers New York, USA., pp. 173–196.

St-Arnaud, M. and Vujanovic, V . (2006). Effect of the arbuscular mycorrhizal symbiosis on plant diseases and pests. In: Hamel, C. and Plenchette, C. (Eds.), Mycorrhizae in crop production: applying knowledge. Haworth Press, Binghampton, NY, USA (in press).

Varma, A. and Schuepp, H. (1995). Mycorrhization of commercially important micropropagated plant. Crit. Rev. Biotecnology. 15: 313-328.

Varma, A., Verma, S., Sudha, N., Sahay, S., Butehorn, B. and Franken, P. (1999). *Piriformospora indica*, a cultivable plant growth promoting root endophyte. Applied and Environmental Microbiology 65: 2741-2744.

Verma, S., Varma, A., Rexer, K.H., Kost, G., SarbhoY, A., Bisen, P., Butehorn, B. and Franken, P. (1998). *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia 95: 896-903.

Vincent, J.H. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. Nature 15: 850.

Walker, J. (1972). Type studies on *Gaeumannomyces graminis* and related fungi. Trans. Br. Mycol. Soc. 58: 427-457.

Warcup, J.H., and Talbot, P.H.B. (1967). Perfect states of Rhizoctonias associated with orchids. New Phytol. 66: 631-641.

Yedidia, I., Benhamou, N. and Chet, I. (1999). Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol. 65: 1061-1070.

Zimand, G., Elad, Y. and Chet, I. (1996). Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. Phytopathology 86: 1255-1260.

(Received 25 February 2009 accepted 13 September 2009)