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## Potential of mycorrhiza-like fungi and *Trichoderma* species in biocontrol of Take-all Disease of wheat under greenhouse condition

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Ghahfarokhy, M.R., Goltapeh, E.M., Purjam, E., Pakdaman, B.S., Modarres Sanavy, S.A.M. and Varma, A. (2011). Potential of mycorrhiza-like fungi and *Trichoderma* species in biocontrol of Take-all Disease of wheat under greenhouse condition. *Journal of Agricultural Technology* 7(1): 185-195.

*Gaeumannomyces graminis* var. *tritici* (Ggt) the causal pathogen of wheat (*Triticum aestivum* L.) take-all disease is very destructive in north, central and southwest provinces in Iran as well as in the world. The endophytic fungi *Piriformospora indica* and *Sebacina vermifera* are newly discovered arbuscular mycorrhiza-like fungi that could be found in close association with various plant species. But compared to mycorrhizal fungi, these are amenable to axenic cultivation. These fungi tremendously improve the growth and overall biomass production of different plants like herbaceous monocots, dicots, trees, including medicinal plants and several economically important crops. *Trichoderma harzianum* and *T. viride* are efficient biocontrol agents that are commercially applied to prevent development of several soilborne pathogenic fungi. The experimental design was a completely randomized design with three replication. Average of two repeated experiments. A factorial experiment was conducted based on completely randomized block design with 3 replications. Analysis of variance using SAS software (SAS Institute, 1988) was carried out on plant growth measurements. Treatment means were compared with Duncan test at the 5% level of probability. The effect of the interactions among mycorrhiza-like fungi, *Trichoderma* species and Ggt on root of wheat in greenhouse were investigated and demonstrated that these fungi could inhibit progressive take-all disease in roots of wheat plants.

**Key words:** *Gaeumannomyces graminis* var. *tritici*, *Piriformospora indica*, *Sebacina vermifera*, *Trichoderma* species, biocontrol, greenhouse, Iran

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## Introduction

Take-all disease is an economically significant and damaging root rot 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 grasses (Walker, 1972). *Piriformospora indica*, an endophytic fungus has been isolated from Thar desert, India in 1997 (Verma *et al.*, 1998), and also *Sebacina vermifera*, an endophytic fungus has been isolated from a desert in Germany (Warcup and Talbot, 1967). These fungi are members of Sebacinaceae, sebacinales, Agaricomycotina, Basidiomycota. *P. indica* and *S. vermifera* have enormous potential for plant growth promotion through colonization of their roots (Franken *et al.*, 1998; Blechert *et al.*, 1999; Varma *et al.*, 1999; Singh *et al.*, 2000a; Rai *et al.*, 2001; Malla *et al.*, 2002, Rai and Varma, 2002). These sebacineous fungi are characterized by the formation of typical pyriform chlamydospores. *P. indica* and *S. vermifera* are similar to arbuscular mycorrhizal fungi in many respects (Varma *et al.*, 1999; Singh *et al.*, 2000b; Varma *et al.*, 2001). But unlike arbuscular mycorrhizal fungi, these can be cultured in artificial medium. These fungi enter the root cortex and form inter- and intra-cellular hyphae. Within the cortical cells, the fungi often form dense hyphal coils or branched structures intra-cellular. These also form spore- or vesicle-like structures within or between the cortical cells. Like AMF, hyphae multiply within the host cortical tissues and never traverse through the endodermis. Likewise, they do not invade the aerial portion of the plant (stem and leaves). Interestingly, the host spectrum of *P. indica* and *S. vermifera* is very similar to those of AMF (Varma *et al.*, 1999). The filamentous fungus *T. harzianum* is one of the most potent agents for the biocontrol of soil borne plant pathogens (Elad, 2000). *Trichoderma* spp. is free-living ubiquitous fungi that are highly interactive in root, soil and foliar environments. It has been known for many years that they produce a wide range of antibiotic substances and that they parasitize other fungi (Sivasithamparam and Ghisalberti, 1998). They also compete with other microorganisms; for example, they compete for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil and more generally, they compete with soil microorganisms for nutrients and/or space (Howell, 2002; Elad, 1996). Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi (Zimand *et al.*, 1996).

One method of biocontrol of diseases is to use mycorrhizal fungi ( Dehne, 1982., Singh *et al.*, 2000b; St-Arnaud and Vajaronic, 2007; Khaosaad *et al.*, 2007) and *Trichoderma* species (Chet *et al.*, 1997; Yedidia *et al.*, 1999; Harman

*et al.*, 2004; Kucuk and Kivanc, 2004; Brozova, 2004). The aim of this study was to study the possibility of biocontrol of take-all disease using mycorrhiza-like fungi and *Trichoderma* species under greenhouse conditions.

## **Materials and methods**

### ***Ggt* cultures**

Three *Ggt* strain was used: T16 was collected from Markazi province. Cultures were maintained at 25±1°C on potato dextrose agar (PDA).

### ***Mycorrhiza-like* fungi cultures**

*Piriformospora indica* and *S. vermifera* were grown on aspergillus broth and agar medium (Kaefer, 1977) 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.

### ***Pot culture experiment***

Two wheat cultivars, Shahriar and Alvand were used in greenhouse experiments. The isolate of take all pathogen applied in these studies was T16. The study included fifty treatments each of three replicates for each wheat cultivar and considered all feasible interactions among *Ggt*, mycorrhiza-like fungi and *Trichoderma* species. The soil applied in greenhouse cultures was a mixture of soil: peat mass: perlite prepared at a ratio of 1: 1: 1, sterilized three times in three successive days at 121 °C under pressure of 1.5 atm for 100 minutes. The inoculum of *Ggt* was prepared through inoculation of wheat grains that had first been boiled for 30 minutes, then washed with sterilized cool water and distributed into 500 ml glass milk bottle and twice sterilized at 120 °C at 1.5 atm for 30 minutes in two successive days. For this purpose, five plugs of 5mm disks of 4 day old potato dextrose cultures of *Ggt* T16 strain cultures were used per flask under completely sterile conditions. Inoculate of *Trichoderma* were also prepared following the above-mentioned procedure. To prepare inoculate of *P. indica*, and *S. vermifera*, the Kaefer broth was made in flasks and sterilized through autoclaving under the conditions mentioned above and left to cool, then separately inoculated with five plugs of 5 mm disks of 4

day old cultures of *P. indica*, and *S. vermifera* grown on solid Kaefer media (Pham *et al*, 2003., Kaefer, 1977). The inoculated flasks of Kaefer broth were incubated on a rotary shaker adjusted to 100 rpm for 10 days. The pots were inoculated three times using the prepared inoculate and in all situations considered to make inoculations with pathogen, mycorrhiza-like fungi and *Trichoderma* isolate. After 4-5 days of fungal establishment, wheat grains were planted. After establishment of grains, other post-planting states of inoculation with pathogen, mycorrhiza-like fungi and *Trichoderma* isolate were made. All symptoms and impacts of take-all disease on plant growth, inter-node distances, and number of ears were recorded during plant development. Plants were grown under traditional greenhouse conditions with a temperature about 22 °C. At harvest time, other parameters such as the dry weight of culms, roots, and ears were registered. The roots were stained according to the method suggested by Phillip and Hayman (1970). The roots were washed with tap water, were kept in 10% potassium hydroxide (KOH) aqueous solution over a night, then washed in sterilized distilled water for 3-5 times. Later, the root samples were kept in 1% chloric acid (HCl) for 3-4 minutes. Eventually, root segments were stained with trypan blue (5%) and observed under a microscope with the magnifications of 100-400 ×.

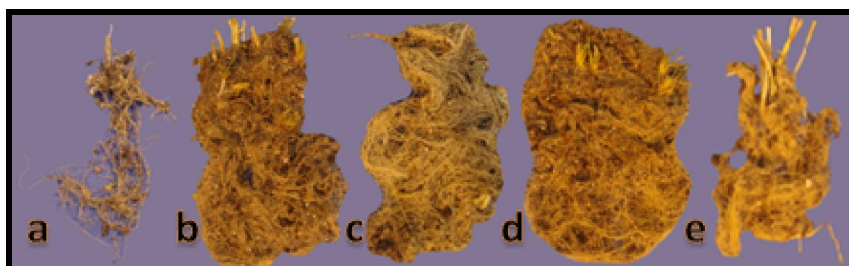
### ***Statistical analysis***

The data were statistically analyzed using general SAS software (SAS Institute, 1988) was carried out on plant growth measurements. Means comparing performed using Duncan multiple range test.

### **Results and discussion**

Results demonstrate that *P. indica* and *S.vermifera* and *Trichoderma* species colonizes the roots of wheat and inhibited from growth, development and progressive Ggt (the causal agent of take-all disease of wheat). Comparison of root colonized with Ggt, mycorrhiza-like fungi and *Trichoderma* species showed that colonization of root with Ggt less growth than colonization of root with mycorrhiza-like fungi and *Trichoderma* species (Fig. 1), and also growth stem, leaf and shoots in wheat of colonized with mycorrhiza-like fungi and *Trichoderma* species better than wheat of colonized with Ggt (Fig. 2). Hyphal investigation showed that *P. indica*, *S. vermifera* and *Trichoderma* species could coiled around Ggt mycelium on roots and penetrate inter their hyphae and cells of roots and inhibition of activity, growth and progressive growth in mycelium (Fig. 3). Plants incubated increase growth and biomass roots. The dry weight of shoots, roots and stems of wheat inoculated plants was higher than

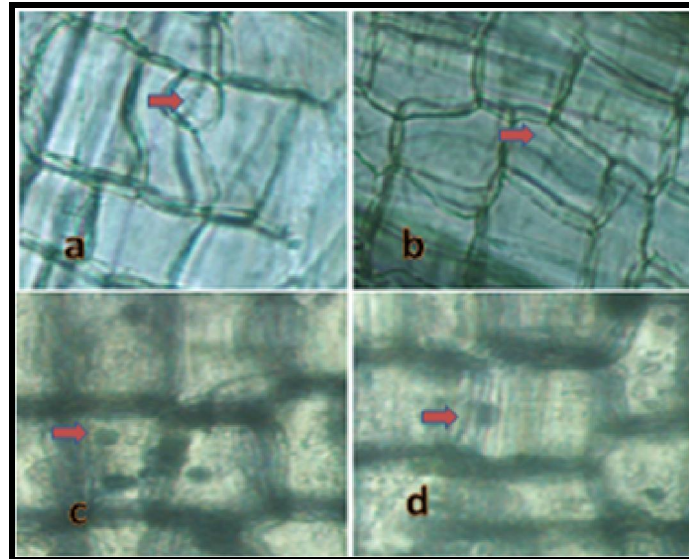
that of the corresponding controls. Weight of root in wheat colonization with mycorrhiza-like fungi and *Trichoderma* species very further than wheat colonization with Ggt. In plants inoculated with pathogen and antagonists, treatments 17, 36 and 37 had the highest efficiency and treatments 2, 3 and 4 had the lowest efficiency in pathogen (T16). We concluded that interactions between pathogen and antagonists led to increase root biomass and overall growth of plants (Fig. 4).



**Fig. 1.** Comparison of growth colonized root with pathogen, mycorrhiza-like fungi and *Trichoderma* species. (a). Colonized root with Ggt. (b,c,d,e) colonized root with mycorrhiza-like fungi and *Trichoderma* species respectively.

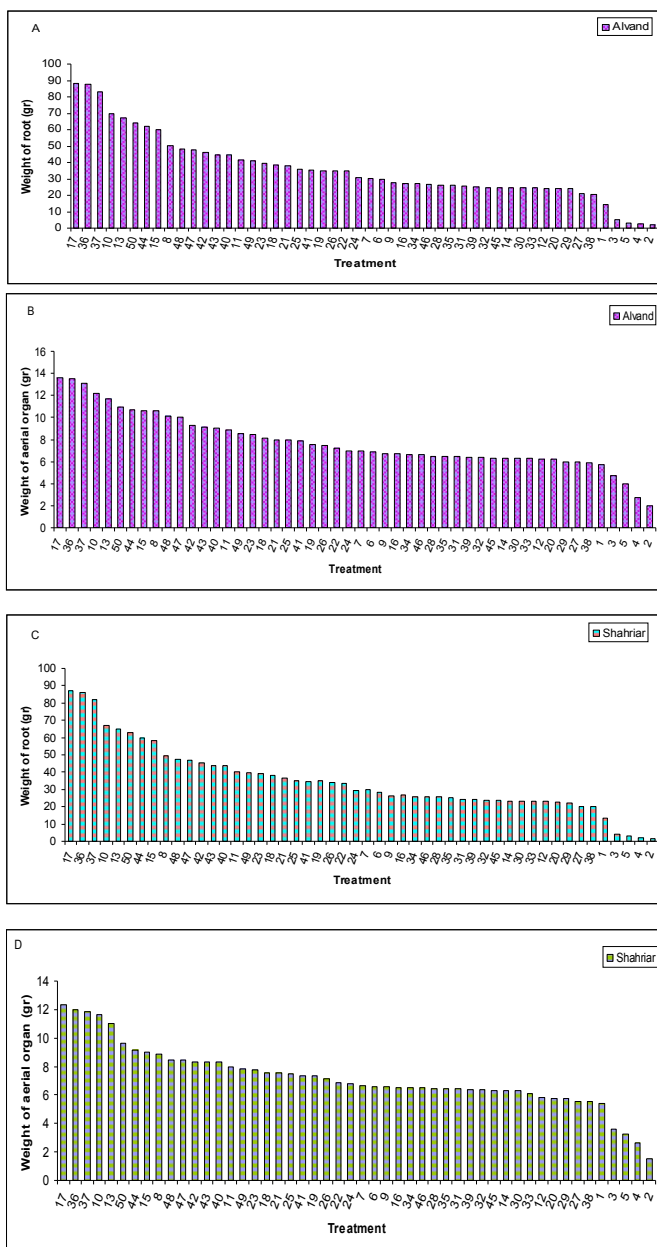


**Fig 2.** Comparison of growth colonized plant with Ggt, mycorrhiza-like fungi and *Trichoderma* species. (a) Colonization of plant with Ggt. (b) plant (control). (c,d) Colonization of plant with mycorrhiza-like fungi and (e, f) colonization of plant with *Trichoderma* species.



**Fig 3.** Formation of clamydospores in mycorrhiza-like fungi and hyphal penetrate inter cells of wheat root colonization with mycorrhiza-like fungi and *Trichoderma* species. (a, b) hyphal penetrate inter cells of wheat root colonized and (c, d) Formation of clamydospores in mycorrhiza-like fungi inter cells of wheat root.

*P. indica* colonizes the roots of host plants as diverse as *Zea mays* L., *Nicotiana tabacum* L., *Petroselinum crispum* L., *Populus tremula* L., *Setaria italica* L., *Oryza sativa* L., *Sorghum vulgare* L., *Triticum sativum* L., *Glycine max* L. Merr., *Cicer arietinum* L., *Solanum melongena* L., *Artemissia annua* L. and *Bacopa monniera* L. Wett. Also like AMF, *P. indica* does not colonize the members of Brassicaceae and the myc-mutants of *Glycine max* and *Pisum sativum* (Varma *et al.*, 1999; Varma *et al.*, 2000). *P. indica* has an even wider host range than individual AM species, and the benefits for the host are comparable with that of AMF, although they result from an interaction involving only one fungus and the host. *P. indica* exerts several positive effects on colonized host plants when grown in pot cultures (Singh *et al.*, 2000a). These fungi similar to mycorrhizal fungi were controlled take-all disease in barley plant (Khaosaad *et al.*, 2007). The fungus enhances the growth of *Adhatoda vasica* (Rai and Varma, 2005). The fungus are able to associate with the roots of various plant species in a manner similar to arbuscular mycorrhizal fungi and promotes plant growth (Varma *et al.* 1999, 2001, Pham *et al.*, 2003, Singh *et al.*, 2003, Shahollari *et al.*, 2005). Rai *et al.*, (2001) reported growth increase in *Withania somnifera* and *Spilanthes calva* that colonized by *P. indica*. There was a remarkable enhancement in the growth rate of the plant inoculated with *P. indica* (Rai and varma, 2005).



**Fig 4.** Comparison weight of root and aerial organ in tow type of wheat (Shahriar and Alvand). (A, B) Weight of root and aerial organ in Alvand respectively. (C, D) Weight of root and aerial organ in Shariar respectively (mean represent).

1=Plant, 2=Pathogen, 3=Infection after germination, 4= Infection 15 day after germination, 5= Infection 30 day after germination, 6= *P. indica*, 7= *S. vermifera*, 8= *P. indica*+ *S. vermifera*, 9= *T. viride*, 10= *T100*, 11= *T. viride*+ *T100*, 12= *T100*+*P. indica*, 13= *T100*+*S.vermifera*, 14= *T. viride*+*P. indica*, 15= *T. viride*+*S. vermifera*, 16= *T. viride*+ *T100*+ *P. indica*, 17= *T. viride*+ *T100*+ *S. vermifera*, 18= *T100*+ *S. vermifera*+ *P. indica*, 19= *T. viride*+ *S. vermifera*+ *P. indica*, 20= *T. viride*+*T100*+ *S. vermifera*+ *P. indica*, 21= *P. indica*+ Pathogen, 22= *S. vermifera*+ Pathogen, 23= *S. vermifera*+ *P. indica*+ Pathogen, 24= *T. viride*+ Pathogen, 25= *T100*+ Pathogen, 26= *T. viride*+ *T100*+ Pathogen, 27= *T. viride*+*P. indice*+ Pathogen, 28= *T. viride*+ *S. vermifera*+ Pathogen, 29= *T100*+ *P. indica*+ Pathogen, 30= *T100*+ *S. vermifera*+ Pathogen, 31= *T. viride*+ *T100*+ *P. indica*+ Pathogen, 32= *T. viride*+*T100*+ *S. vermifera*+ Pathogen, 33= *T100*+ *S. vermifera*+ *P. indica*+ Pathogen, 34= *T. viride*+ *S. vermifera*+ *P. indica*+ Pathogen, 35= *T. viride*+*T100*+ *S. vermifera*+ *P. indica*+ Pathogen, 36= Pathogen+ *P. indica*, 37= Pathogen+ *S. vermifera*, 38= Pathogen+ *P. indica*+ *S. vermifera*, 39= Pathogen+ *T. viride*, 40= Pathogen+ *T100*, 41= Pathogen+*T. viride*+ *T100*, 42= Pathogen+ *T100*+*P. indica*, 43= Pathogen+ *T100*+*S. vermifera*, 44= Pathogen+*T. viride*+*P. indice*, 45= Pathogen+ *T. viride*+*S. vermifera*, 46= Pathogen+ *T. viride*+ *T100*+ *P. indica*, 47= Pathogen+ *T. viride*+ *T100*+ *S. vermifera*, 48= Pathogen+ *T100*+ *S. vermifera*+ *P. indica*, 49= Pathogen+ *T. viride*+ *S. vermifera*+ *P. indica*, 50= Pathogen+ *T. viride*+*T100*+ *S. vermifera*+ *P. indica*.

*P. indica* has been reported to be involved in the improvement of growth and biomass production in a range of hosts (2001) such as monocots and dicots, shrubs and trees, medicinal plants (Kumari *et al.*, 2004) and several economically important crops (Rai *et al.*, 2001; Varma *et al.*, 1999, 2000). Also, it has been proven that *P. indica* has an inductive effect on the growth of terrestrial orchids (Blechert *et al.*, 1999; Kaldorf *et al.*, 2005). Survey showed that *S. vermifera* has the better performance than *P. indica* that is the same findings Barazani *et al.* (2005). Serfling *et al.* (2007) were demonstrated *P. indica* reduce significantly negative effect severity of typical leaf (*Blumeria graminis* f. sp. *tritici*), stem base (*Pseudocercospora herpotrichoides*), and root (*Fusarium culmorum*) pathogen on Wheat and increase growth, development and biomass root. *P. indica* was increased disease resistance, salt-stress tolerance and higher yield in barley (Waller *et al.*, 2005). Our findings are in agreement with those of the above mentioned authors.

*Trichoderma* is able to biologically control multiple number of plant pathogens (Agrios, 1997). Lee *et al.* (2006) proved that *Trichoderma* species could control *Botrytis cinerea*. El-Katatny and coworker (2000) indicated the suppressive impact of *T. harizanum* on the activity of *Sclerotium rolfsii*. The mycoparasitic activity of *T. viride* on the mycelia of *Ceratocystis paradoxa* has been proven (Eziashi *et al.*, 2007). Harman and Taylor (1988) demonstrated that it was feasible to increase biological control activity and competitive potential of *Trichoderma* strains through acidification of soil and rhizosphere. Acidic condition may increase the conidia production and germination (Chet and Baker, 1980; Danielson and Davey, 1973; Schüepp and Frei, 1969). Mycelial growth (Hadar *et al.*, 1984), and production and activity of antimicrobial compounds such as antibiotics and lytic enzymes (Chet and Baker, 1980; Dennis and Webster, 1971). Alkaline soil decreases conidial germination of *Trichoderma* spp. And lead to a decreased biocontrol activity of *T. harizanum* (Papavizas, 1985). Findings are in agreement with those of the above mentioned authors.

### **Acknowledgment**

This study was supported by the Department of Plant Pathology, Tarbiat Modarres University in Tehran.



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(Received 17 Febuary 2010; accepted 10 October 2010)