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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Enhancement of soybean (*Glycine max*) seedling vigor by *Trichoderma harsianum* as a biofertilizer

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Abstract

Efficient use of microorganisms applied as the biofertilizers is important in crop production to maximize producer's economic returns, maintain soil and water quality. In order to investigate the effects of *Trichoderma harsianum* and different manures on improvement of growth and development in soybean [*Glycine max* (L.) Merril.], an experimental was conducted at research farm of Islamic Azad University of Ghaemshahr using a factorial based completely randomized design with three replications during 2009. The field experiment laid out as factorial based on completely randomized design with four replications. Inoculation of *Trichoderma harsianum* and without inoculation and three levels of phosphorus (consisting of 0, 50 and 100 Kg ha^{-1}) were considered as treatments. Results showed that application of *T. harzianum* was not affected by primary emergence of seedling and chlorophyll content (SPAD) compared the control. Nevertheless, this treatment increased terminal emergence of seedling root, shoot length and seedling vigor. Treatment of different phosphorus showed that in zero amounts of phosphorus decrees the primary and terminal emergence of seedling and chlorophyll content (SPAD), root and shoot length and seedling vigor. Furthermore, application of *T. harzianum* reduced P application by 50 Kg ha^{-1}, significantly increased root length and dry matter of seedling compared to other plots without the *T. harzianum.* Overall, inoculation of *T. harzianum* could improve emergence seedling of soybean compared to control.

Keywords: soybean; seedling; vigor; *trichoderma*; biofertilizer

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Introduction

The fungal genus *Trichoderma* is cosmopolitan in soils, and the ecological adaptability of *Trichoderma* species evidenced by their widespread distribution, including under different environmental conditions and on various substrates (Powlson et al., 2001; Yadav et al., 2009). Fungal biofertilizers help to enhance crop yield and promote sustainable agricultural production and are safe to the environment (Bulluck et al., 2002; Leck et al., 2008). Different species of *Trichoderma* have the potential to control soil borne plant pathogens more effectively than chemicals (Aneja et al., 2005; Bennet and Whipps, 2008; Ruano and Lopez, 2009; Rojan et al., 2010). Use of these fungi is not as harmful to the environment as chemical pesticides (Bal and Altintas, 2006). In most instances, the biological control agents attributed increased plant growth and yields to the reduction in plant disease.

Trichoderma spp are thought to promote plant growth by at least two different mechanisms: (i) controlling the population of pathogenic micro-organisms in the rhizosphere (Brunner et al., 2005; Perazzolli et al., 2011), and by influencing plant physiology through mineral solubilization or hormone secretion (Latinas and Bal, 2008; Ainhoa et al., 2011). In vitro studies have shown that micronutrients and insoluble phosphates became soluble and available,

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therefore useful to the roots interacting with *T. harzianum* in the root zone (Rojan et al., 2010: Bombiti et al., 2011). Species in the filamentous fungal genus *Trichoderma* are of great economic importance as sources of enzymes and antibiotics; plant growth promoters; degraders of xenobiotics, and most importantly, as commercial biofungicides (Rojan et al., 2010). In addition to the above, various species of *Trichoderma* were also effective in the promotion of growth and yield in various crops. Cucumber, bell pepper and strawberry yields were increased significantly whit application of *T. harzianum* in the root zone (Altintas and Bal, 2005; Bal and Altintas, 2006; Elad et al., 2006). Effective bio-inoculants should preferably penetrate the roots to not only directly antagonist root pathogens, but also benefit plant growth and vigor through various mechanisms such as nutrient mobilization and induction of host defenses (Rudresh et al., 2005). Seed-applied microorganisms have the potential to become establish in the rhizosphere of plants, as they may transfer onto the developing root as it emerges from the seed (Harman, 1991). It is equally important that the microorganisms remain viable, can colonies the developing roots and rhizosphere in order to continue improving plant growth, and potentially control disease (Kleifeld and Chet, 1992; Chacon et al., 2007). Even small improvements in plant growth and health can result in significant economic benefits.

They can 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, compete with soil microorganisms for nutrients and/or space (Latinas and Bal, 2005). Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi, to penetrate leaf surfaces. Different mechanisms have been suggest as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compound. *Trichoderma* ability to attack other fungi (plant pathogens) mycelia, *Trichoderma* hyphens growing surround of other fungi mycelia, penetrate and feed from them. The ability of *Trichoderma* to recognize and parasitize phytopathogenic fungi in the rhizosphere has been ascribed to several complex mechanisms, such as nutrient competition, antibiosis, mycoparasitism, induction of systemic resistance, and increased plant-nutrient availability (Harman, 2006; Bal and Altintas, 2006; Dubey et al., 2007).

Soybean suffers from various fungal diseases in its entire growing period from germination of seeds to the mature plant stage. Diseases caused by fungal pathogens account for approximately 50% of all soybean disease losses around the world (Wrather and Koenning, 2006). In addition, Seedling emergence is an important trait that can limit commercialization of seeds (Shoresh and Harman, 2008). A rate of seedling emergence and leaf appearance is important in developing a corn crop with earlier canopy closure and better seasonal light interception. Therefore, this study was designed to find out the effect *Trichoderma harzianum* and different phosphorus on emergence and seedling parameters of soybean under field conditions.

Materials and Methods

In order to investigation the effect of *trichoderma harzianum* and different phosphorus on growth and development of soybean [*Glycine max* (L.) Merril.], an experimental was conducted at research farm of Islamic Azad University of Ghaemshahr using a factorial based completely randomized design with three replications during 2009. Inoculation of *Trichoderma harsianum* and without inoculation and three levels of phosphorus (consisting of 0, 50 and 100 Kg ha^{-1}) considered as treatments. Control plants were also available. Briefly, this involved storing agar plugs taken from the periphery of an actively growing colony in a 10% (v: v) aqueous solution of glycerol in straw ampoules. Fungi recovered from this system were subsequently grown on potato dextrose agar (PDA; Merck) (chacon et al., 2007) slopes for storage for 7 days at 30°C in a thermostatic incubator chamber with air circulation. This period showed to be sufficient for fungi sporulation. The substrates used were wheat bran. Wheat bran showed to be the most suitable substrate to produce *Trichoderma* spores for all strains that were evaluated (Cavalcante et al., 2008). The substrates were sterilized at 121°C for 15 min in a Phoenix autoclave model AV 50 and cooled down to room temperature before the inoculation, which was done until 24 h after sterilization. Availability of water in the soil plays an important role in facilitating establishment and effectiveness of *Trichoderma* in the soil. Irrigation was done on a regular basis. Primary and terminal emergence of seedling recorded on the 10th day after sowing, shoot length and total weight recorded. In addition, Seedling vigor index was recorded after 10 days. Seeds considered germinated when the radical extended through the seed coat.

Vigor index for each treatment was determined using this formula (Seedling Vigor = [root length + shoot length] × percentages of germination) developed by Abdul-Baki and Anderson (1973). The plants was removed 10 day after sowing, and roots were washed using slow running water to remove soil particles and organic debris. The dry mass of shoot and root samples, root length and shoot length was determined after drying in an oven at 60°C with forced air. Ten normal seedlings selected at random from each treatment of the germination test on ten day (final count day) and used for measuring root length. The root length measured from the tip of primary root to the base of the hypocotyls and the mean root length expressed in centimeters. The shoot length measured from the base of the primary leaf to the base of the hypocotyls and the mean shoot length expressed in centimeters. Data were subjected to ANOVA using the SAS statistical software package using GLM (SAS Institute, 2000) and Duncan's multiple range tests was performed to compare the treatment means. The level of statistical significant was accepted as P<0.05 (Steel and Tore 1960).

Results and Discussion

Effect of *T. harzianum* and different rate of phosphorus on emergence and early growth of soybean is show in Table 1. Statistical analysis showed significant differences in treatments at $P \leq 0.05$ levels. Application *T. harzianum* was significant on terminal emergence, root length, shoot length, dry weight and seedling vigor. Treatment of different amount of P (0, 50 and 100 Kg ha^{-1}), were affected on seedling growth (Table 1). Application of *T. harzianum* and reduce P application by 50 Kg ha^{-1}, increased dry matter of seedling and root length (14.3% and 21.1%) compared to other plots without the *T. harzianum.* but had no markedly effect on primary and terminal emergence, shoot length, SPAD and seedling vigor (Table 1, Fig. 1 & 2).

Treatment of different phosphorus showed that, in zero amounts of phosphorus decrees the primary and terminal emergence of seedling and chlorophyll content (SPAD), root and shoot length and seedling vigor. Bulluck et al (2002) found greater fungal species diversity under organic cultivation than under conventional cultivation with inorganic fertilizer. Enhanced plant vigor has also observed following application of *Trichoderma* species to other crops. Results showed that application of *T. harzianum* was not affected of primary emergence of seedling and chlorophyll content (SPAD) compared the control (Table 1). Nevertheless, this treatment increased terminal emergence of seedling, root and shoot length and seedling vigor.

Positive effects of *Trichoderma spp.* are not limited to the above, many species of *Trichoderma* promoted growth and development of seedlings of crops (Rabeendran et al., 2000; Hanson, 2000; Bal and Altintas, 2006). Studies have been confirmed in case of *T. harzianum* to enhanced seed germination root and shoot length (Dubey et al., 2007) as well as increasing the frequency of healthy plants and boosting yield (Rojoa et al., 2007). Root colonization by *Trichoderma* spp therefore induces significant changes in the plant metabolic machinery (Rojan et al., 2010: Bombiti et al., 2011). Root colonization by these fungi, also frequently enhances root growth and development, crop productivity, resistance to a biotic stresses and the uptake and use of nutrients (Harman, 2005).

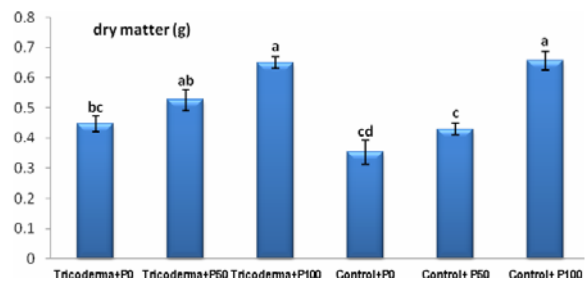

Fig. 1: Effect of *trichoderma harzianum* and different phosphorus on dry matter in soybean

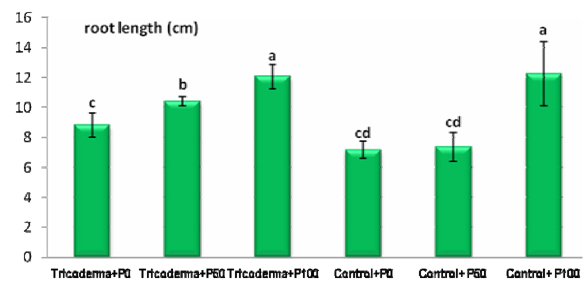

Fig. 2: Effect of *trichoderma harzianum* and different phosphorus on root length in soybean

Seed vigor, an important agronomic trait defined as the potential to produce vigorous seedlings. This characteristic of seed is a measure of the quality of seed, and involves the viability of the seed, the germination percentage, germination rate and the strength of the seedlings produced (Leck et al., 2008). In theory, seed vigor may influence crop yield through both indirect and direct effects. The indirect effects include those on percentage emergence and time from sowing to emergence. These influence yields by altering plant population density, spatial arrangement, and crop duration. Furthermore, according to results application of *T. harzianum* and reduced P application by 50 Kg ha^{-1}, significantly increased root length and dry matter of seedling compared to other plots without the *T. harzianum* (Fig. 2).

Promotion of growth by *Trichoderma spp.* is a result of increased root area allowing the roots to explore larger volumes of soil to access nutrients, and increased solubility of insoluble compounds as well as increased availability of micronutrients (Hancon, 2000; Leck et al., 2008). According to Altintas and Bal (2008) results, the application of *Trichoderma spp* increased

Table1: Effect of *T. harzianum* and different rate of phosphorus on emergence and early growth of soybean

Source variation	Primary Emergence	Terminal Emergence	Root length	Shoot length	Dry Weight	SPAD	Seedling vigor
T.harsianum							
Inoculation	26.5[a]	67.5[a]	10.09[a]	13.81[a]	0.54[a]	38.17[a]	26.37[a]
No-inoculation	27.1[a]	58.7[b]	8.25[b]	12.80[b]	0.48[b]	36.92[a]	19.61[b]
Phosphorus							
0 Kg ha^{-1}	22.00[b]	53.83[c]	6.99[c]	11.34[c]	0.400[c]	36.33[b]	13.63[c]
50 Kg ha^{-1}	27.83[a]	62.83[b]	8.38[b]	12.63[b]	0.478[b]	38.94[a]	20.84[b]
100 Kg ha^{-1}	31.00[a]	72.83[c]	12.13[a]	15.94[a]	0.653[a]	37.37[ab]	34.50[a]
Significant							
T. harzianum (A)	16.05 ns	346.7**	15.29**	4.60*	0.016**	2.57 ns	205.3**
Phosphorus (B)	125.05**	542.0**	42.47**	33.7**	0.100**	5.08 ns	427.2**
A * B	15.38 ns	27.55 ns	6.65*	1.63 ns	0.005*	2.97 ns	20.6 ns
Error	17.33	21.88	1.22	0.81	0.0007	4.47	6.14
CV	15.45	7.40	12.4	6.79	5.44	5.47	10.77

Levels of significant:* P< %5, ** P<%1, NS = not significant

the growth of seedling corn with induced resistance, changes in the microfloral composition on roots, enhanced nutrient uptake, including but not limited to nitrogen, enhanced solubilization of soil nutrients, enhanced root development, increased root hair formation and deeper rooting.

Conclusions

The present study concludes that *Trichoderma harzianum* have potential to enhance the seedling emergence in soybean which can be useful to enhance the emergence of soybean seeds besides reducing loses due to delayed germination. *Trichoderma harzianum* promoted the growth of soybean plant and increased seedling vigor.

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