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Evaluation of *Glomus mosseae* as Biocontrol Agents against *Rhizoctonia solani* on Tomato

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Abstract
R. solani is one of the phytopathogens that attack tomato cultivated causing seed decay, root and crown rot. The ability of arbuscular mycorrhiza (AM) to suppress root diseases caused by soilborne pathogens has been intensively studied in the last thirty years. The efficacy of AM (*Glomus mosseae*) as biological agents was assayed against *R. solani* on tomato seeds and seedling in greenhouse and field. The results appeared ability of AM (*Glomus mosseae*) for the suppression of *R. solani* at transplant time (day 30) in greenhouse experiment (76%) and field experiment results appeared significant differences at disease index and disease severity of tomato plants in mycorrhiza treatment (21%, 1 respectively). The mycorrhiza treatment results was positive influenced on plant growth and health indicators too (tomato seeds germination, fresh weight, dry weight, roots volume and fruit weight).
Keywords: *Rhizoctonia solani, Glomus mosseae,* Biological agent, Tomato.

Introduction

Tomato (*Lycopersicon esculentum*) is one of the most important vegetables in the world, including Iraq. *Rhizoctonia solani* is a species complex composed of several anastomosis groups (AGs) (Carling *et al.*, 2002). *R. solani* is one of the phytopathogens that attack tomato cultivated causing seed decay, root and crown rot (Montealegre *et al.,* 2010). There is a growing realization that biological control can be successfully exploited as an agricultural method for soil-borne pathogens (Papavizas and Lumsden, 1980).

In 1885 Frank was the first to describe the symbiosis between a fungus and the roots of trees and he released the word mycorrhiza (fungal root). Today we know that mycorrhizas are the most widespread associations between microorganisms and higher plants, occurring on roots of more than 80% of all plants (Frey-Klett *et al*., 2005).

The AM fungi were formerly included in the order Glomales in the Zygomycota (Redecker *et al*., 2000), but they have recently been moved to a new phylum Glomeromycota (Schu¨ßler *et al*., 2001).

The mycorrhizal fungi colonize the root cortex, then develop an external mycelium which is a bridge connecting the root with the surrounding soil microhabitats to change many key aspects of plant physiology. These include the supply of mineral nutrients to the plant, particularly those whose ionic forms have poor mobility or those present in low concentrations in the soil solution. This mainly applies to phosphate, ammonium, zinc, and copper (Jeffries *et al*.,2003).

Mycorrhizal symbioses can be found in almost all ecosystems worldwide to improve plant health and soil quality through key ecological processes. The AM fungi can benefit different crops, such as cereals and legumes, vegetable crops, fruit trees, shrubs, tropical plantation crops, ornamentals, and spices (Azco´n-Aguilar and Barea, 1997).

The AM association also improves plant health through increased protection against biotic and abiotic stresses, with possible applications in biocontrol of plant soil-borne microbial pathogens, and in bioremediation of polluted soils (Barea *et al*. 2005).

The ability of AM to suppress root diseases caused by soilborne pathogens has been intensively studied in the last thirty years. Fungal root pathogens are inhibited by mycorrhiza inoculation in the cases of *Verticillium dahlia* (Matsubara *et al*.,1995), *Fusarium oxysporum* on different crops (Filion *et al.*, 1999; Stephan *et al*.,1999), various *Phytophthora* species (Trotta *et al.*, 1996), *Rhizoctonia solani* (Stephan *et al*.,1999; Matloob and Juber,2013) and *Pythium ultimum* (Calvet *et al.*, 1993). Bacterial diseases are also reduced by mycorrhiza establishment on the root (Dehne, 1982). The suppression of nematode penetration and development following AM inoculation (Stephan *et al*.,1999;Diedhiou *et al.*, 2003).

The establishment of AM fungi in plant roots has been shown to reduce damage caused by soil-borne plant pathogens with an enhancement of plant resistance/tolerance in mycorrhizal plants. In any case, the effectiveness of the AM biocontrol is dependent on the AM fungus involved, as well as the substrate and the host plant (Whipps, 2004).

Different mechanisms have been suggested to account for this effect of AM fungi (Elmer, 2002).

- Increased nutrient uptake results in higher resistance of the plant to pathogen invasion.
- Competition for space.
- Plant morphological changes and barrier formation.

- Changes in biochemical compounds related with plant defense.
- Increased percentage of microbial antagonists in the rhizosphere.

Vesicular arbuscular mycorrhiza (VAM) have been shown to be an important tool in the biological control of soil-borne pathogens including Aphanomyces, Cylindrocladium, Fusarium, Macrophomina, Phytophthora, Pythium, Rhizoctonia, Verticillium, and Thielaviopsis as well as various nematodes not only in sustainable but also in organic agriculture (Harrier and Watson, 2004).

The positive effects of mycorrhizal fungi on plant development are well known. In a study on the favorable effects of mycorrhizal varieties on *R. solani* in 2 different potato varieties it was reported that disease due to *R. solani* was reduced in shoots and crowns by 60%-71.2% in plants inoculated with *Glomus etunicatum* (Yao *et al.,* 2002). In-vitro studies showed that strawberry plant roots inoculated with *G. etunicatum* and *G. monosporum* reduced *Phytophthora fragariae* sporulation by 67% and 64% respectively in 48 h (Norman and Hooker, 2000).

Arbuscular mycorrhizal, *G. intraradices* significantly reduced the *R. solani* disease incidence and severity in bean plants under different conditions (Matloob and Juber, 2013).

We have been focusing in this study on the possibility using mycorrhizal for disease prevention in tomato grown in College of Agriculture – Baghdad university – Iraq, against *R. solani* (isolate IQ-45, accession number KF372650). To the study effects of mycorrhizal fungi on the disease severity of *R.solani* and the role of mycorrhizal fungi on tomato plants development.

Material and Methods

Preparation of *R. solani* Inocula

Isolate IQ-45 (*R. solani* accession number: KF372650) was maintained on potato sucrose agar (PSA). Wheat grains (200 g) were placed in a 1 liter Erlenmeyer flask with 600 ml tap water containing 250 µg/ml chloramphenicol. The water was heated to boiling and the flask was removed from the heater and left to settle for 10 min. The grains were then washed three times with tap water and the excess water was carefully strained to prevent the grains from sticking together in a clump during autoclaving. The grains were autoclaved (121 °C and 1.5 bar) for 1 h on each of 2 consecutive days. The sterile grains were separately placed in Petri dishes and inoculated with mycelial discs from a 2-3 day old taken from the margins of *R. solani* IQ-45 cultures growing on PSA. Cultures were incubated 7-10 days at 25± 1°C in the darkness. During this period, the grains were shaken daily to prevent them from sticking together due to hyphal growth. *R. solani* inoculum was stored at 5°C until use (Sneh *et al*., 2004).

Mycorrhiza Experiment in Greenhouse

Experiment carried out in the greenhouse of plant protection department, College of agriculture, university of Baghdad - Iraq.

After sterile soil in autoclaved (121 °C and 1.5 bar) for 1 h on each of 2 consecutive days were distributing on 15 cm diameter plastic pots and inoculated with grains colonized by *R. solani* isolate IQ-45 (rate 0.1% volume) and incubated in humidity for four days before inoculum with 20g / pot from *Glomus mosseae* (supplier from Emirates Bio Fertilizer Factory) and it is let for 10 days before planting under greenhouse condition.

After 14 days, five Tomato seeds (*Lycopersicon esculentum*) (Dalal F1, Royal Crown seeds-Holland) were sown per pot with three replicates for each treatment. The measurements of seedlings emergence were done 13, 23and 30 days after sowing. Data on seedling fresh and dry weights were determined. Tomato seeds treatments were conducted as follows:

- Control.
- My + R.s.IQ-45 [Mycorrhiza (*Glomus mosseae*) + *R. solani* IQ-45].
- My [Mycorrhiza (*Glomus mosseae*)].
- R.s.IQ-45 (*R. solani* IQ-45).

The results of experiment were analyzed statistically using complete randomized design (CRD).

Mycorrhiza Experiment in the Field

Field experiment carried out in the fields of Plant Protection Department, College of Agriculture, University of Baghdad during the spring season 2013.

The field ground was tilling twice and then leveling and divided into three plots with two rows (width = 50 cm and length = 10 m) and planted with 10 Tomato seedling, placed on 35cm between them. Installed drip irrigation tape on each plot for irrigated.

Tomato seeds planted in the greenhouse of the Department of Plant protection in plastic trays (50 cells) consisted a peat moss and left to six weeks until become the seedling age of 4-5 true leaves.

R.solani isolate IQ-45 inoculum (0.1% volume / hole) and *Glomus mosseae* inoculum (20 g / hole) were inoculated in soil before 10 days from Tomato seedling transfer.

The experiment treatments were conducted as follows:

- Control.
- My + R.s.IQ-45 [Mycorrhiza (*Glomus mosseae*) + *R. solani* IQ-45].
- My [Mycorrhiza (*Glomus mosseae*)].
- R.s.IQ-45 (*R. solani* IQ-45).

The results for experiment were taken after 100 days from planted. Data were obtained on Tomato fresh weight, dry weight, fruit weight, disease severity and disease index was assessed and scored on Tomato plant roots using a disease index scale from 0-5, where 0= 121 – 150g (Tomato plant roots weight); 1= 91 – 120g; 2= 61- 90g; 3= 31 – 60g; 4= 1 -30g; 5= Dead plant. Experimental design was a Randomized Complete Block Design (RCBD) with three replicate for each treatment.

Results

Greenhouse experiment

The number of healthy tomato seedlings were not different among treatments 12 days after sowing, except for My + R.s.IQ-45 treatment, which differed from all the rest. In 22 days after sowing, the numbers of healthy tomato seedlings were not different among all treatments. Control treatment in this date was found significant differed from all the rest, except My treatment was not differ. More differences among treatments were detected on day 30. Treatments with My+R.s.IQ-45 and My did not differ from tomato seedling in Control treatment, which they were significant differ from R.s.IQ-45 treatment (Table 1).

Table 1. Mean number of healthy Tomato seedling after infested with *R. solani* in greenhouse.

Treatment	% Healthy tomato seedlings Days after sowing		
	12 days	22 days	30 days
Control	88 a	92 a	92 a
My + R.s.IQ-45	76 b	76 b	76 a
My	80 ab	84 ab	80 a
R.s.IQ-45	84 ab	80 b	48 b

The numbers with the same latter are not significantly different.

Tomato seedlings fresh and dry weights data are summarized in Table 2. The My + R.s.IQ-45 and My treatments were improved seedlings growth, as demonstrated by mean seedlings fresh and dry weights. Minimum fresh and dry weights were obtained by sowing in both R.s.IQ-45 and Control treatments. While the maximum fresh and dry weights were obtained by sowing in both My + R.s.IQ-45 and My treatments, which they were significant differed on another treatments.

Table 2. Tomato seedlings fresh and dry weights in the greenhouse at day 30 after infested with *R. solani*.

Treatment	Mean Tomato seedlings weight (g)	
	Fresh weight	Dry weight
Control	1.210 c	0.434 c
My + R.s.IQ-45	1.428 b	0.528 b
My	1.988 a	0.736 a
R.s.IQ-45	1.028 d	0.378 d

The numbers with the same latter are not significantly different.

Field experiment

The analysis of field experiment results appeared in Table 3, significant differences in disease index and disease severity of tomato plants appeared among treatments.

Table 3. Determination of disease index percentage and disease severity for Tomato plants infected by *R. solani* in the field.

Treatment	Tomato plants after 100 days from sowing	
	Disease index %	Disease severity
Control	17 d	0.80 b
My + R.s.IQ-45	21 b	1.00 b
My	19 c	0.93 b
R.s.IQ-45	81 a	4.00 a

The numbers with the same latter are not significantly different.

Disease index scale from 0-5, where 0= 121 – 150g (Tomato plant roots weight); 1= 91 – 120g; 2= 61- 90g; 3= 31 – 60g; 4= 1 -30g; 5= Dead plant.

The rate of disease index and disease severity in R.s.IQ-45 treatment were statistically higher than the other treatments and it is significant differed from all.

Tomato seedlings roots volume, fresh weights, dry weights and tomato fruits weights data are appeared in Table 4, revealed that all treatments reduced the occurrence of the diseases. Regarding the morphogenesis of survival tomato plants are represented by the roots volume, fresh weights, dry weights and tomato fruits weights. This data reveal that these records increased in the percentage roots volume, fresh weights, dry weights and tomato fruits weights in all treatment compared with treatment of R.s.IQ-45and these was significant differed from all treatments.

Table 4. Effect of treatments on Tomato plants growth in the field infested with *R. solani*.

Treatment	Tomato plants growth indicated			
	Roots volume (cm^3)	Fresh weight (g)	Dry weight (g)	Tomato fruits weight (g)
Control	85.6 a	407.5 a	82.0 a	3341 b
My + R.s.IQ-45	67.3 b	340.3 a	70.0 b	3980 a
My	73.9 ab	351.9 a	70.4 b	2388 c
R.s.IQ-45	38.6 c	213.6 b	41.9 c	847 d

The numbers with the same latter are not significantly different.

Discussion

In this research, we were using mycorrhizal for disease prevention in tomato grown and study effects of mycorrhizal fungi on the disease severity of *R.solani* and the role of mycorrhizal fungi on tomato plants development.

However, from greenhouse experiment results appeared more differences among treatments were detected on day 30. Treatments with My+R.s.IQ-45 and My did not differ from tomato seedling in Control treatment, which they were significant differ from R.s.IQ-45 treatment. Thus, from field experiment results showed the rate of disease index and disease severity in R.s.IQ-45 treatment were statistically higher than the other treatments and it is significant differed from all the rest. So the mycorrhiza treatment results was reflected on plant growth and health indicators too (measured as tomato seeds germination, fresh weight, dry weight, and roots volume) and control the pathogen at greenhouse and field experiments. There are many reports on the successful use of Mycorrhizal as biological control agents (Calvet *et al.*, 1993; Trotta *et al.*, 1996; Filion *et al.*, 1999; Yao *et al.*, 2002; Harrier *et al*, 2004; Whipps, 2004; Barea *et al*. 2005; Matloob and Juber,2013).

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