EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND SOME PLANT GROWTH PROMOTING RHIZOBACTERIA IN CONTROLLING ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) ON TOMATO UNDER GREENHOUSE CONDITIONS

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A greenhouse experiment was conducted to assess the influence of some plant growth-promoting bacteria such as *Azospirillum lipoferum, Azotobacter chroorcoccum and Rhizobium leguminosarum* alone or mixed with Arbuscular mycorrhizal fungi as bio fertilizer and bio control agents to enhance the growth of tomato (*Lycopersicon esculentum* cv. super strain P), suppress root knot nematode *Meloidogyne incognita* and microbial community composition. They improved plant growth through increased nutrient uptake in exchange for photosynthetic carbon from their host. Successful biocontrol has also in suppressed root knot nematode *Meloidogyne incognita.* In particular, combinations of AM fungi and PGPF may provide protection for plants at different times, under different conditions. Results indicated that all tested treatments significantly reduced root-knot nematode numbers and remarkable increase in the growth parameters as compared to the untreated inoculated plants. Both oxamyl and mixing of plant growth-promoting bacteria with arbascular mycorrhizae were the most effective treatments in decreasing the final nematode population in soil and roots, number of galls and rate of nematode buildup.

Keywords: *Lycopersicon esculentum*, *Meloidogyne incognita*, *Azotobacter chroorcoccum*, *Azospirillum lipoferum*, *Rhizobium leguminosarum*

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Nematodes form a highly diverse group comprising free-living nematodes as well as plant and animal parasites that can be found worldwide in various habitats (Ferraz and Brown, 2002). Many species of plant-parasitic nematodes (PPN) can act as pests on a wide range of important agricultural crops. They mostly live in the soil. Root-knot nematodes, which are considered to be the most damaging pests of agricultural crops worldwide (Jones et al., 2013 and Bartlem et al., 2014). The sedentary endoparasitic *Meloidogyne* spp. such as *M. incognita* and *M. javanica*, can result in complete crop losses in tobacco and tomato or sunflower and pepper, respectively (Wesemael et al., 2011). Tomato (*Solanum lycopersicum*) belongs to the Solanaceae family. Tomato is a rich source of micronutrients such as minerals, vitamins and antioxidants for a well-balanced human diet. It also contains high levels of lycopene, an antioxidant that reduces the risks associated with several cancers and neurodegenerative disease (Giovannucci, 1999). There are many pests and diseases damaging both the quality and quantity of tomato production. Plant-parasitic nematodes are one of them. They represent an important constraint on the delivery of global food security. Damage caused by plant-parasitic nematodes has been estimated at US$ 80 billion per year (Nicol et al., 2011).

The use of nematicides is being limited, given the increasing concern for human health as well as the environment, which has led to their ban. Alternative nematicides are being sought (Oka and Mizukubo, 2009 and Wesemael et al., 2011). Scientists are also looking for other nematode management strategies that fit into the recently launched framework of the Integrated Pest Management (IPM) directive of the European Union (EU directive 2009/128/EC), stating that member states have to implement IPM from 2014 onward, with the aim to reduce pesticide use and to promote non-chemical management practices as much as possible. One of the proposed environmentally friendly options to manage PPN is the use of biological control organisms, such as arbuscular mycorrhizal fungi (AMF).

Arbuscular mycorrhizal fungi are obligate root symbionts, estimated to colonize more than 80% of all land plant species. They improve plant growth through increased nutrient uptake in exchange for photosynthetic carbon from their host (Smith et al., 2010). Also, they can alleviate plant stress caused by abiotic as well as biotic factors, including PPN (Gianinazzi et al., 2010; Singh et al., 2011 and Vos et al., 2012) The biocontrol effect of AMF has been observed in a wide range of plant species and against many pathogens, most of them soil-borne fungal pathogens causing root rot or wilting, though successful biocontrol has also been observed against aboveground pathogens such as *Alternaria solani* in tomato (Harrier and Watson, 2004; Whipps, 2004; Fritz et al., 2006; Pozo and Azcón-Aguilar, 2007 and Jung et al., 2012). Both necrotrophic and biotrophic pathogens

have been reported to be suppressed by AMF, either directly or indirectly (Veresoglou and Rillig, 2012).

Plant growth-promoting rhizobacteria (PGPR) are free living and may impart beneficial effects on plants. PGPRs are a specific group of soil bacteria that aggressively colonize the rhizosphere and rhizoplane, and substantially improve plant growth and productivity through various mechanisms like N2 fixation, solubilization of minerals phosphates and other essential nutrients, and biological control agents of phytopathogenic microorganisms (Bhattacharya and Jha, 2012). In addition, PGPR can protect plants from detrimental effects of environmental stress like flooding, drought, salinity and heavy metals

Authors have frequently described as PGPRs certain strains of *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Enterobacter* and *Serratia* (Kloepper and Beauchamp 1992), since they share common habitats, i.e., the root surface, and common functions, the AMF and PGPR have to interact during their processes of root colonization or functioning as root-associated microorganisms. Soil microorganisms, particularly PGPR, can influence AM formation and function and consequently, mycorrhizae can affect PGPR populations in the rhizosphere (Barea, 2000) Relationships between both types of microbes are under high specificity rules (Azcón, 1989).

Recently, numerous researches have focused on biological control agents with the objective of controlling the plant parasitic nematodes and to overcome the nematode damage by using mycorrhizal fungus (Duponnois and Plenchette, 2003 and Serfoji et al., 2010) or bacteria such as *Azospirillum brasilense* (Shamseldin et al., 2010), *Rhizobium leguminosarum* (Ashoub and Amara, 2010) and *Azotobacter chroorcoccum* (Siddiqui and Futai, 2009).

MATERIALS AND METHODS

The experiment was conducted at the experimental greenhouse of Nematodes Research Department, Plant Protection Dept., Desert Research Center (DRC), El-Matareya, Cairo, Egypt and at the Experimental Laboratory of Fertility and soil Microbiology Dep. Desert Research Center DRC), El-Matareya, Cairo, Egypt

1. Nematode Stock Culture and Propagation

To initiate and propagate pure stock culture of the root –knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, galled roots of highly infected eggplants were collected, single egg-masse was used to inoculate eggplants grown under greenhouse conditions. The identification of females perennial pattern was done according to Taylor et

al. (1955). Two months later re-inoculation on new eggplants continuously was done for preparing pure egg mass culture of *Meloidogyne incognita*.

2. Arbuscular Mycorrhizal Fungus

The spores of VAM were collected from different Egyptian governorates by the wet sieving technique, described by Gerdemann and Nicolson (1963). The collected VA Mycorrbizal spores were propagated in soil and the roots of barley plants were infected with VAM. These collected spores can be used as inoculum with rate (500 spores per plant).

3. Bacterial Inoculum Preparation

Azotobacter chroococcum and *Azospirillum lipoferum* were isolated from the rhizosphere of barley plant and *Rhizobium leguminosarum* isolated from nodules of faba bean located in Sahle El Tina, North Sinai. *Rhizobium leguminosarum* used from this study was grown on yeast-extract mannitol broth for 24 h at 28°C (Jordan, 1984). *A. chroococcum* was grown on modified Ashby's medium (Abd El-Malek and Ishac, 1968) for 5 days at 28±2°C and *A. lipoferum* was grown on Dobereiner media (Dobereiner et al., 1980), for 5 days at 28±2°C. The active PGPR was inoculated in 250 ml conical flasks that contained 100 ml specific media then enriched in the same medium and incubated to reach 10^8 cfu / ml. The bacterial inocula were applied as a soil treatment at the rate of 30 ml of bacterial suspension (10^8 cfu/ml) per plant and the mixture of them was applied as a soil treatment at the rate of 10 ml for the three of them per plant.

4. Evaluation of Bacterial Strains

4.1. Nitrogen fixation ability

Determination of nitrogen fixing activity in pure culture of *Rhizobium leguminosarum, Azotobacter chroococcum* and *Azospirillum lipoferum* was conducted. Bacterial isolates were grown on their specific media for three days, no inoculated media served as control. Afterwards, the concentration of nitrogen in each liquid culture was measured by digestion and subsequent measurement by the Kjeldahl method (Bremner and Mulvaney, 1982).

Nitrogenase activity was determined by acetylene reduction technique using gas chromatography (Hewlett Packard chromatography model HP 6890 GC) fitted with dual flame detector and 150 cm x 0.4 cm diameter stainless still column fitted with propak x R100-120 mesh according to methods of Hardy et al. (1973) and Somasegaran and Hoben (1985). The results were presented as n. mole C_2H_4 /ml culture /h.

4.2. Phosphate solubilization

Pikovskaya's medium (glucose 10 g/L, $Ca_3(PO_4)_2$ 5 g/L, $MgSO_4.7H_2O$ 0.1 g/L, NaCl 0.2 g/L, $(NH_4)_2SO_4$ 0.5 g/L, $FeSO_4.7H_2O$

0.002g/L, KCl 0.2 g/L, yeast extract 0.5 g/L, MnS O_4 0.002 g/L, agar 20 g/L, pH7.0) with 2.4 mg/ml bromothymol blue was used for phosphate solubilization. The media inoculated with the isolates were incubated for 48 h and observed for yellow color change as positive for solubilizing phosphate (Pikovskaya, 1948).

4.3. Indole acetic Acid (IAA) production

Indole acetic acid (IAA) production was estimated in all bacterial cultures by using spectrophotometer as described by Ehmann (1977). For this purpose, 50 ml of each specific medium supplemented with 1 g/L of L-tryptophan as a precursor of IAA was inoculated with selected bacterial isolate, incubated at 30°C for 48 h and centrifuged at 6000 rpm for 30 min to collect supernatant. Then, supernatant and Salkowski reagent (2.0 ml of 0.5M $FeCl_3$ + 98.0 ml of 35% $HClO_4$) were mixed in test tubes at the ratio of 1: 2 and the contents were allowed to stand for half an hour for color development. The intensity of color was measured at 520 nm by using spectrophotometer and compared with standard curve of IAA.

5. Greenhouse Experiment

Greenhouse experiment was conducted to study the effect of mycorrhizal fungi (*Glomus mosseae*) alone or as mixture with some plant growth-promoting bacteria (PGPB) (*Azospirillum lipoferum*, *Azotobacter chroococcum* and *Rhizobium leguminosarum* on nematode population of *Meloidogyne incognita*. One-month old tomato seedlings (*Solanum lycopersicum* cv. super strain P) with uniform size were transplanted singly in 15 cm clay pots filled with mixture of sterilized clay and sand soil (1: 2, v/v). After ten days, the tomato plants were treated with mycorrhizal fungi (*Glomus mosseae*) and some PGPB; *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Rhizobia* spp. After two weeks, nematode inoculation was added with 2000 newly hatched second stage larvae of *Meloidogyne incognita* by making 3 holes at different depths (2-3 cm) around the roots and immediately after inoculation the roots were covered with soil. Then the previously microbial inoculants were re-added at the same rate, after 15 days of infestation. The experiment was divided to eleven treatments. Every treatment consists of three replicates. The treatments were as follows:

1. AM fungi
2. AM fungi + *Azospirillum lipoferum*
3. AM fungi + *Azotobacter chroorcoccum*
4. AM fungi + *Rizobium leguminosarum*
5. AM fungi + *Azospirillum lipoferum* + *Azotobacter chroococcum* + *Rizobium leguminosarum*
6. *Azospirillum lipoferum*
7. *Azotobacter chroococcum*
8. *Rizobium leguminosarum*

9. Control without nematode
10. Chemical control "oxamyl 24% EC" was applied at the rate of 0.03 ml/pot
11- Control with nematode

All treatments were arranged in a completely randomized design under greenhouse condition at temperature degree 35±2ºC. All plants received 1 g NPK (19-19-19) a slow release fertilizer at planting every two weeks. Pots were watered periodically every three days. The plants were harvested after 60 days from inoculation time.

5.1. The reduction percentage in nematode enumeration and plant growth parameters determination

Soil of each pot was processed for nematode extraction by sieving and Baerman–pan technique (Southey, 1970). A count of second stage juveniles (J2) in soil of each pot was determined by means of Hawksley counting slide and stereoscopic microscope. Also, average numbers of eggs/egg masses were determined by rinsing four randomizely selected egg masses per root system of each replicate in 1% sodium hypochlorite to release eggs from egg matrix. Then, the released eggs were suspended in water and counted under stereoscopic microscope. Collected juveniles were counted. Galls and egg-masses and their indices were rated. The reduction percentage in galls formation, egg-masses production, also female's numbers were counted and juveniles' number were calculated according to the following formula: (Control-Infected)/ Control) ×100

The final population and nematode buildup were calculated for all treatments.

Final Population (F.P.) included number of juveniles in soil+ egg-masses+ females.

Rate of buildup (PF/PI) =

Final nematode population PF/ Initial nematode population PI (Norton, 1978).

Plant growth response based on shoot length fresh and dry shoot weights as well as root fresh weight and length were determined and calculated for all treatments. At harvest, the following microbiological measurements were conducted:

5.2 Fungal spore enumeration

The Spores from lower sieve were then washed onto a 9 girded filter paper disc. The filter paper was transferred clean Petri dish lids and enumerated. AM fungal spore enumeration included both dead and viable spores, although every attempt was made to count only healthy-looking spores. Spores were recorded as representatives of AM fungal species present in 100 g of sample (Smith and Dickson, 1997). This was done using a dissecting microscope (Leica S4E).

5.3. Dehydrogenase activity

Soil dehydrogenase activity (µg TPF/g dry soil/24 h) was analyzed by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) as described by Friedel et al. (1994).

6. Statistical Analysis

Statistical analysis was carried out according to the procedure "Anova" recorded by Snedecor and Cochran (1980). Means of treatments were compared by Dancann's Multiple Range Test at 5% level of probability. These steps were accomplished using SPSS Program version 16.

RESULTS

Through a greenhouse experiment, application of mycorrhizal fungi (*Glomus mosseae*) and some PGPB; *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Rhizobium leguminosarum* alone and as mixed genera with arbascular mycorrhizae (AM) fungi on nematode population of *Meloidogyne incognita*, in compare with the nematicide oxamyl (24% EC) as in table (1) showed that, all treatments significantly affected *Meloidogyne* sp. and could arrest its reproduction rate compared with control treatment, concerning reduction percentage of juveniles number, galls, females, egg-masses, eggs per egg-mass, the final population and nematode buildup were diminished that occurred with all treatments. So, a successfully gradual scale for the effectiveness of used application can be done as follows: mixed all PGPB with mycorrhizal fungi> *Azospirillum lipoferum*+ mycorrhizal fungi> *Rizobium leguminosarum* + mycorrhizal fungi> *Azotobacter chroococcum* + mycorrhizal fungi> *Azospirillum*> *Rhizobia* spp> mycorrhizal fungi> *Azotobacter chroococcum.* This scale was related with the final population reduction and nematode buildup when compared with untreated control

The highest reduction percentage of juveniles was obtained from mixed all PGPB with mycorrhizal fungi (86.88%) and *Azospirillum lipoferum* + mycorrhizal fungi (80.6%), while the lowest was obtained from *Azotobacter chroococcum* (57.2%) and mycorrhizal fungi (56.35%). Also, treatments of mixed PGPB with mycorrhizal fungi and *Azospirillum lipoferum* still gave the highest effect in galls and females reduction where they could increase this reduction until reaching 94.1%, 92.1%, 95.8% and 90.5%, respectively. While, *Azospirillum lipoferum* and *Azotobacter chroococcum* recorded the lowest effect in galls (72.3% and 67.4%, respectively). While the lowest females' reduction percentages were registered by *Rizobium leguminosarum* (69.8%) and *Azotobacter chroococcum* (56.4%). Also, production of egg masses and inhabitation of

Table (1). Effect of arbuscular mycorrhizal fungi and some plant growth-promoting bacteria on development and multiplication of *Meloidogyne incognita* infesting tomato.

Treatments	Galls/ root	% R	In soil	% R	Egg masses/ root	% R	Eggs/ egg mass	% R	Females	R %	FP	% R	RB
AMF + Mixure of bacteria	35h	0.194	408f	86.8	11g	96.4	90e	83.9	15g	95.81	434g	88.5	0.22
AMF	126e	78.8	1353b	56.4	84d	72.5	184c	67.1	89d	75.1	1526c	59.4	0.76
AMF + *Azospirillum*	48g	91.9	630e	79.7	34f	88.9	122d	78.2	39f	89.1	717f	80.9	0.36
AMF + *Azotobacter*	68f	88.5	853d	72.5	51e	83.3	243b	56.6	58e	83.8	962e	74.4	0.46
AMF + *Rhizobia*	42gh	92.9	600e	80.6	28f	90.8	127d	77.3	34f	90.5	663f	82.4	0.33
Azospirillum	144d	75.8	1122c	63.8	100c	67.3	192c	65.7	108c	69.8	1330d	64.7	0.67
Azotobacter	194b	67.4	1327b	57.2	138b	.954	203c	63.8	156b	56.4	1621b	56.9	0.81
Rhizobium	165c	0.372	817d	73.6	92cd	69.9	233b	58.4	101c	71.8	1012e	73.1	0.51
Oxamyl (24% EC)	33h	0.594	200 g	93.5	15g	95.1	88e	84.3	20g	94.4	235h	93.8	0.12
Control	595a	------	3100a	----	306a	----	560a	-----	358 a	-----	3763 a	-----	0.91
LSD at 0.05	12.5	-------	40.5	------	11.1	----	21.6	-----	9.0		54.0	-----	-----

*In each column, means followed by the same letter are not differ significantly at $p \leq 0.05$ according to Duncan's multiple range test.
*R= Reduction = (Control-Infected)/ Control) ×100
*FP = Final population included number of juveniles in soil+ egg-masses + females.
*RB =Rate of buildup (PF/PI) = Final population PF/ Initial population PI (Norton, 1978).

egg production was more pronounced in treatments were adversely affected by the mixed PGPB with mycorrhizal fungi.

The effect of mycorrhizal fungi (*Glomus mosseae*) and some PGPB; *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Rhizobium leguminosarum* alone and mixed genera of AM fungi on growth of tomato infected with *Meloidogyne incognita.*

In comparison with the nematicide oxamyl (24% EC) recorded in table (2), all treatments succeeded in improving the plant growth parameters as compared to check with nematode. For instance, the treatment of mixed PGPB with mycorrhizal fungi gave the best result in increment the fresh, dry weight, shoot length and fruit weight with averages 109.8%, 98.7%, 62.0% and 440.0%, followed by the treatment of *Azospirillum lipoferum* + mycorrhizal fungi (*Glomus mosseae*), which gave 84.0%, 76.3%, 48.9%, 343%, respectively, as compared with check treated with nematode. On the other hand, *Rhizobium leguminosarum* recorded less averages of 4.6%, 13.2%, 2.6% and 77.0% in fresh, dry weight, the shoot length and fruit weight, respectively, followed by the treatment of *Azotobacter chroococcum* that recorded 4.6%, 13.2%, 21.8% and 67%.

The growth rate of root lengths was recorded increasing at all treatments, which ranged between 51.7% and 11.9% and root weight, which ranged between 44.0% and 4.4% as compared with check treated with nematode. In general, all treatments of tested materials as well as oxamyl (24% EC) caused remarkable increase in the plant growth parameters.

Nitrogenase enzyme is considered as an indication of the ability of free-living bacteria to fix atmospheric nitrogen. According to data illustrated in table (3), both *Azotobacter chroococcum* and *Azospirillum lipoferum* recorded high nitrogenase activity (77.40, 81.539 n. mole C_2H_4/ml/h), while *Rhizobium leguminosaru* fixed nitrogen in nodules. *Azotobacter* and *Azospirillum* isolates exhibited nitrogenase activities ranged from 15.4 to 96.1 n. mole C_2H_4 /ml/h. All strains were subjected for dissolving phosphate in growing medium, and recorded 32, 27 and 29 ppm, respectively. Microorganisms belonging to the genera *Pseudomonas, Bacillus, Rhizobium, Azotobacter* and *Azospirillum* spp. frequently have the ability to solubilize phosphorus. On the other hand, *Azospirillum lipoferum* produced the highest amount of IAA (160.31 µg/ml), followed by isolates *Rizobium leguminosarum* and *Azotobacter chroococcum,* which gave 140.21 and 90.25 µg/ml) respectively. Nitrogen fixers achieved different activities of IAA ranging from 31 to 168 µg/ml and gibberellins from 2.8 to 29.4 µg/ml. It was clear from table (4) that, the count of spores was increased by inoculation with VAM. The inoculation with PGPR mixture + AMF + N recorded the highest value (384 spores/g dry soil), followed by AMF with single bacterial inoculation while AMF + N recorded the least one (208 spores/g dry soil).

Table (2). Plant growth parameters of tomato affected by *Meloidogyne incognita* and treated by arbuscular mycorrhizal fungi and some plant growth-promoting bacteria under greenhouse conditions.

Treatments	Shoot							Root			
	Length (cm)	% Increase	Fresh Weight (g)	% Increase	Dry Weight (g)	% Increase	Fruit Weight	% Increase	Length (cm)	% Increase	Fresh Weight (g)	% Increase
AMF+Mixure of bacteria	103.70a	62.0	103.9a	109.8	15.1a	98.7	54.0a	440	21.7a	51.7	13.1a	44.0
AMF	90.3bc	41.0	83.4d	68.5	11.5c	51.3	37.0c	270	19.3ab	20.6	9.6de	5.4
AMF+ *Azospirillum*	93.7bc	46.4	87.1c	76.0	12.6bc	65.8	42.0b	320	19.7ab	35.0	13.0ab	42.9
AMF +*Azotobacter*	81.0d	26.5	55.3f	11.7	9.6 d e	26.3	34.3c	243	16.0de	11.9	12.9ab	41.8
AMF + *Rhizobium*	95.3b	9.48	91.1b	84.0	13.4b	76.3	44.7b	343	19.7ab	35.0	12.9ab	41.8
Azospirillum	65.7e	2.6	51.5gh	4.6	8.6ef	13.2	17.7e	77	16.0de	11.9	9.4de	4.4
Azotobacter	78.0d	21.8	51.8fgh	4.6	8.6ef	13.2	16.7e	67	16.3cde	14.0	9.4de	4.4
Rhizobium	91.7bc	43.2	86.0cd	73.7	12.7bc	65.8	18.3de	83	18.7bc	30.8	12.0bc	31.9
Oxamyl (24% EC)	88.0c	34.5	54.0fg	9.1	9.2de	21.0	20.0de	100	16.7cd	30.8	10.2de	12.1
Untreated plants	90.7bc	41.7	60.3e	21.8	10.1d	32.9	21.7d	117	18.3bcd	28.0	11.0cd	20.9
Control	64.0e	----	49.5h	----	7.6f	------	10.0f	----	14.3e	----	0.1e9	---
LSD at 0.05	5.89		3.6079		1.28		3.38		2.4128		1.5693	

*In each column, means followed by the same letter are not differ significantly at $p \leq 0.05$ according to Duncan's multiple range test.

The value of dehydrogenase activity was increased by inoculation with mixed PGPR + VAM + nematode, it recorded 390 µg TPF/g dry soil/24 h, followed by single inoculation with AMF, compared to control (untreated plants). Inoculation with *Azospirillum* gave the highest result among the other bacterial treatment and plant with active nematode gave the least result.

Table (3). Count of vesicular arbuscular mycorrhiza (VAM) and dehydrogenase activity in the rhizosphere of tomato plant at the harvest.

Treatment	VAM count (Spores /200 g dry soil)	Dehaydrogenase (µg TPF/g dry soil/24 h
AMF + Mixure of bacteria	384A	390A
AMF	208E	237F
AMF + *Azospirillum*	288B	290B
AMF +*Azotobacter*	224D	221G
AMF + *Rhizobium*	240C	257D
Azospirillum	Nil	262C
Azotobacter	Nil	243E
Rhizobium	Nil	241F
N + oxamyl (24% EC)	Nil	Nil
Untreated plants	Nil	173
Control	Nil	191H
LSD at 0.05	2.8881	3.0348

*In each colum, means followed by the same letter are not differ significantly at ($p \leq 0.05$) according to Duncan's multiple range test.

Table (4). Plant growth promoting traits of rhizobacterial strain.

Test organisms	Nitrogenase activity (n. mole C_2H_4/ml h)	Phosphate dissolving activity (ppm)	IAA (µg/ml)
Azotobacter chroococcum	77.402	32	90.25
Azospirillum lipoferum	81.534	27	160.31
Rhizobium leguminosarum	-----	29	140.21

DISCUSSION

PGPR and AM fungi in tomato roots tested under greenhouse conditions had an effect on decreasing the final *M. incognita* population and the rate of nematode buildup. Physiological and biochemical changes caused by mycorrhizal fungi in the host plant generally reduce the severity of nematode population, that cause an inhibitory effect on nematode development through releasing organic acids, which are often accompanied with the release of other metabolites, mainly, phytohormones and lytic enzymes (Mukerji and Ciancio, 2007 and Akhtar and Siddiqui, 2008).

In addition, the increased phenolic compounds phytoalexins, lignin, phenols, sugars and amino acid phenylalanine and serine in mycorrhiza treated plants have been suggested to play an important role in the plant defense mechanism (Zhang et al., 2008). Also, PGPR strains *Azotobacter chroococcum* and *Azospirillum brasilense* inhibited egg hatching and killed juveniles by producing a wide variety of antibiotics, hydrolytic enzymes, organic compounds, HCN, phenol oxidation and protease (Insunza et al., 2002). *Azotobacter chroococcum*, *Azospirillum brasilense* and *Rhizobium leguminosarum* may also improve plant growth, nodulation parameters as well as chemical components by the production of biologically active substances of growth hormones (IAA, gibberellins and auxins) or by converting unavailable minerals and organic compounds into forms available to plants (Siddiqui and Mahmood, 1999 and Ashoub and Amara, 2010).

In addition, PGPR strains usually have been found to increase the root length and root biomass and this better developed root system may increases the mineral uptake in plants (Khalid et al., 2004; Siddiqui and Akhtar, 2007). The AM fungi and PGPR can stimulate each other and play a synergistic activity and function in improving plant growth and in reducing plant disease (Shreenivasa et al., 2007).

Reports about mycorrhiza and PGPR-induced resistance against plant-parasitic nematodes have been reviewed by Pinochet et al. (1996), Verhagen et al. (2004), Hol and Cook (2005), Timonen and Marschner (2006), Akhtar and Siddiqu (2008) and Pieterse et al. (2009).

Arbuscular mycorrhizal fungi are known to be able to increase the uptake of water and mineral nutrients for their host plant, such as phosphate and nitrogen (Parniske, 2008 and Baum et al., 2015), but probably also micro-elements such as zinc (Smith and Smith, 2011a, b). In return, they receive photosynthetic carbon from their host (Gianinazzi et al., 2010). Similar to the protection of the plant by AMF against various abiotic stress factors such as drought, cold or heavy metal toxicity (Singh et al., 2011).

AMF could also compensate for damage caused by pathogens. Although higher uptake of phosphate has been proposed as a mechanism for the AMF-mediated biocontrol, addition of phosphate to non-mycorrhizal plants did not result in a similar reduction of pathogen infection (Bodker et al., 1998). Fritz et al. (2006) reported that there is thus not always a positive correlation between increased phosphate uptake and plant growth promotion in mycorrhizal plants, as in some cases plant growth suppression resulted as a consequence of AMF colonization. Dehydrogenase is an oxidoreductase, which only present in viable cells and is maker of soil health and is a valid indicator of changes in total microbial load in soil management (Roldán et al., 2004).

Even when phosphate transport from the AMF to the host plant was taking place (Smith and Smith, 2011a), the results confirm the suitability of AMF inoculation to improve plant health of tomato infected with root knot nematode. The management of these symbionts represents a suitable biocontrol strategy against root knot nematode in this crop. The supplementary addition of other beneficial microorganisms such as PGPR can also be considered as a method of enhancing the AMF effect. However, due to the high specificity involved in these types of interactions, a previous screening to select the best microbe-host plant combination should be done in order to optimize results (Attia, 1999).

The best-studied mechanisms of bacterial plant growth promotion include providing plants with resources/nutrients that they lack such as fixed nitrogen, iron and phosphorus. Many agricultural soils lack a sufficient amount of one or more of these compounds, so that plant growth is suboptimal. To obviate this problem and obtain higher plant yields, farmers have become increasingly dependent on chemical sources of nitrogen and phosphorus Besides being costly.

The production of chemical fertilizers depletes nonrenewable resources, the oil and natural gas are used to produce these fertilizers, and poses human and environmental hazards.

It would obviously be advantageous if efficient biological means of providing nitrogen and phosphorus to plants could be used to substitute for at least a portion of the chemical nitrogen and phosphorus that is currently used. In addition to Rhizobia spp., a number of free-living bacteria, for example *Azospirillum s*pp., are also able to fix nitrogen and provide it to plants (Bashan and Levanony, 1990).

However, it is generally believed that free-living bacteria provide only a small amount of what the fixed nitrogen that the bacterially-associated host plant requires (James and Olivares, 1997). In this study, AMF inoculation increased phosphate-solubilizing (Rodríguez and Fraga, 1999). The inoculation process has a positive effect on the activity and abundance of microbial community in the rhizosphere, which appeared as

remarkable increase in the dehydrogenase activity of treated plant compared to control. Measurement of dehydrogenase activity by indigenous microorganisms in soil has the potential to serve as a useful indicator of the microbial activity to determine the relative effectiveness of the plant rhizosphere in soils (Mathew and Obbard, 2001 and Omer, 2017).

That cause an inhibitory effect on nematode development through releasing organic acids, which are often accompanied with the release of other metabolites, mainly phytohormones and lytic enzymes (Mukerji and Ciancio, 2007 and Akhtar and Siddiqui, 2008). In addition, the increased phenolic compounds phytoalexins, lignin, phenols, sugars and amino acid phenylalanine and serine in mycorrhiza treated plants have been suggested to play an important role in the plant defense mechanism (Zhang et al., 2008). Also, PGPR strains; *Azotobacter chroococcum* and *Azospirillum brasilense* inhibited egg hatching and killed juveniles by producing wide variety of antibiotics, hydrolytic enzymes, organic compounds, HCN, phenol oxidation and protease (Insunza and Eriksson, 2002).

CONCLUSION

Meloidogyne incognita on tomato can be controlled in order to improve growth characters and chemical components of infected plants by using some PGPB; *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Rhizobia* sp. alone and as mixed genera with AM fungi.

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دراسة تأثير فطر الميكروهيزا وبعض أنواع البكتريا المنشطة للنمو في مكافحة نيماتودا تعقد الجذور لنبات الطماطم تحت ظروف الصوبة

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تم إجراء تجربة تحت ظروف الصوبة لتقييم تأثير بعض أنواع البكتيريا المشجعة لنمو النبات مثل *Azotobacter chroorcoccum*, *Azospirillum lipoferum* و*Rhizobium leguminosarum* بمفردهما أو خلطها مع فطريات الميكروهيزا Arbuscular mycorrhizal fungi كسماد حيوي وعوامل مكافحة حيوية. وذلك لتحسين نمو نباتات الطماطم صنف سوبر ستريم بي وكذلك تثبيط نيماتودا تعقد الجذور التي تصيب جذور تلك النباتات وتم تحسين نمو النبات من خلال تشجيع نمو الكائنات الدقيقة وزيادة امتصاص العناصر المغذية. أوضحت النتائج أن جميع المعاملات التي تم اختبارها قللت بشكل كبير أعداد نيماتودا تعقد الجذور وأدت إلى زيادة ملحوظة في معدلات النمو مقارنة بالنباتات الغير معاملة. وقد أعطت المعاملة بخليط البكتريا مع الميكروهيزا بالإضافة إلى معاملة المبيد الكيماوي الأوكساميل (كمعاملة كميائية للمقارنة) أفضل النتائج في تقليل التعداد الكلي للنيماتودا في التربة وعلى الجذر وتقليل العقد الجذرية ومعدل التكاثر للنيماتودا.