



Comparative efficacy of arbuscular mycorrhizal fungi and abamectin-based pesticide to manage the root-knot nematode infestation on tomato plants

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Abbreviations

AMF – arbuscular mycorrhizal fungi
F% – Mycorrhizal colonization frequency
J2 – second-stage juveniles
MR – multiplication rate
MycF1 – Myco up attack formulated with clay
MycF2 – Myco up attack
RKN – Root Knot Nematodes

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Abstract

Background and purpose: Root Knot Nematodes (RKN) hamper plant growth and productivity. They are basically managed by chemicals arising environmental and health concerns.

Material and methods: Pots and greenhouse experiments were conducted to test the bio-control effect of Trivago SC20 (based on abamectin derived from *Streptomyces avermitilis*) and Myco-up attack (based on *Glomus iranicum* var *tenuihypharum* mycorrhizae compared with the two nematicides [Vydate® (oxamyl) for pot assay and Mocap® (ethoprophos) for greenhouse assay] in controlling *Meloidogyne javanica* infesting tomato. Gall index, gall number /g root, egg-masses/g root, RKN/ g root, multiplication rate, *M. javanica* density, nematodes groups frequency, hatched eggs/ egg masses, agronomic and production traits were studied to determine the efficiency of arbuscular mycorrhizal fungi (AMF) and abamectin compared with the fungicide application.

Results: The first experimental results showed that high nematode suppression was provided by *G. iranicum* and abamectin treatments. Both bio-nematicides were also effective in the control of *M. javanica* naturally infested tomato plants under greenhouse conditions. Besides, the *G. iranicum* and abamectin had a positive effect on plant growth because of stimulation of plant vegetation parameters, such as the shoot weight and root length. Furthermore, the AMF satisfactorily colonized the tomato roots.

Conclusions: Hence, tested abamectin and mycorrhizae-based products may be used for *M. javanica* management programs as an alternative and eco-friendly approach to fungicides, while preserving the environment.

INTRODUCTION

Meloidogyne spp. (root-knot nematode, RKNs) are among the most important plant-parasitic nematodes affecting tomato production (1). These nematodes are destructive to tomatoes and prominent throughout their crop production (2). The effect of synthetic chemical pesticides, commonly used for plant parasitic nematodes management, towards the environment and toxicity to non-target organisms and human health have increased the concern in developing natural origins pesticides (3).

Biological control using beneficial microorganisms viz., bacteria and fungi, was carried out for plant parasitic nematodes management (4).

The improvement of soil antagonistic potential by biological control provides a cost-effective, eco-friendly and sustainable alternative method to improve plant resilience for plant parasitic nematode infection (5).

Several bacteria, including *Streptomyces* species, were reported antagonistic to root-knot nematodes (6). *Streptomyces avermitilis* was discovered by Merck Sharp and Dohme Research Laboratories (Merck & Co., Inc.) in 1975 and demonstrated a nematicide potential (7). Avermectins are a class of macrocyclic lactones produced as secondary metabolites by this soil-borne actinomycete. Several avermectin formulations are available to control insects and mites infecting plants (8). Abamectin is a blend of 80% of avermectin B1a and 20% of avermectin B1b compounds. A wide range of commercial formulations suppress nematodes when applied as direct contact with plant-parasitic nematodes (8,9) and as a seed treatment (10,11).

Successful bio-control effect of arbuscular mycorrhizal fungi (AMF) has been observed against many phytopathogens (12). Additionally, AMF, obligate root symbionts, are one of the suggested alternative bio-rational tools used in the plant-parasitic nematodes management programs. The protective effect of AMF included the suppression of root-knot nematodes *Meloidogyne* spp. infecting tomato (13).

Given the growing interest in using safe and eco-friendly products for the root-knot nematode control, additional data are needed to determine their potential efficiency. The objectives of this study were to evaluate the biocontrol activity of two biological nematicides based on beneficial microorganisms, *Streptomyces avermitilis* and *Glomus iranicum*, towards plant-parasitic nematode *Meloidogyne javanica* on tomato.

MATERIALS AND METHODS

Pot experiment

A pot experiment was conducted in an unheated greenhouse at Chott Meriem (Sousse, Tunisia), using 12 cm diam. Pots containing 1000 ml of soil mixture composed by sand, soil and peat (1: 1: 1; v/v). The *Meloidogyne javanica* population was initially isolated from tomato at Chott Meriem and maintained on tomato plants cv. Rio-grande. A pure culture was initiated at the greenhouse (25°C), starting from a single egg mass. Inocula for the experiments were obtained after two months by egg masses extraction from infected tomato roots followed by 6-days incubation to obtain freshly hatched second-stage juveniles (J2). Freshly emerging juveniles were collected and used for the experiment (14). Fifteen days old seedlings were inoculated by the nematodes at 1000 larvae/pot.

Two bio-pesticides based on two beneficial microorganisms viz., *Streptomyces avermitilis* and *Glomus irani-*

cum var *tenuihypharum* were tested. The Myco-up attack was based on *Glomus iranicum* var *tenuihypharum*. Two solid formulations of mycorrhizae were used: one was formulated with clay (MycF1: Myco up attack) and the second without it (MycF2: Myco up attack). The recommended application rates were 0.1 g and 0.2 g/plant, respectively. Soil treatment of both formulations was done at tomato transplantation. The abamectin product was based on *Streptomyces avermitilis*. At fermentation stage, the latter produced the avermectine, the active molecule which is a macrocyclic lactone (15). Tervigo, a suspension concentrate (SC) of abamectin (Syngenta) was applied at the recommended dose of 5 l/ha. Consequently, the recommended application rate was 0.16 ml per plant. The pot experiment was conducted with an oxamyl treatment to compare two biological nematicides. The commercial nematicide Vydate®, with active chemical substance oxamyl (DuPont de Nemours South Africa (Pty) Ltd.; 10% oxamyl) was applied at the recommended dose of 20 Kg/ha. The trial included 6 treatments [MycF1: Myco up attack; MycF2: Myco up attack; Oxamyl; Tervigo; RKN and negative control (plants treated only with sterilized distilled water)] arranged in a completely randomized block design with ten replicates per treatment. All plants were maintained in the greenhouse at 25±3°C with 65±5% relative humidity. Irrigation and fertilization were homogeneously supplied according to plants need (16). After 60 days of nematode inoculation, plant height, fresh root and shoot weights were recorded.

Greenhouse experiment

The Greenhouse assay was located at Higher Agonomic Institute of Chott Meriem, Tunisia. The experiment was conducted with an ethoprophos (Bayer Crop-Science, AG) treatment to compare two biological nematicides. Mocap (10% ethoprophos; BAYER Crop-science, Rhone-Poulenc, Inc.) was applied at the recommended application rate of 50 kg/ha. Biological products-treated soil was evaluated based on four treatments, which included: i) MycF1 - solid clay formulation of *Glomus iranicum* var *tenuihypharum*; ii) abamectin; iii) ethoprophos; iv) RKN: naturally infested soil with *Meloidogyne javanica*. Treatments were arranged in a randomized complete block design and replicated four times. Plots consisted of four rows, 35-cm wide, and 12-m long. Blocks were separated by 3-m alley. Plant height, fresh shoot weight, fresh root weight and collar diameter were determined from all treatments at five months after tomato plantation.

Nematode reproduction assessment

Root galling rating was evaluated using a 0-5 scale (Gall index) whereby, 0 = no visible galling, 1 = 10% of the root system galled and trace infection with a few small galls, 2 = ≤ 25% of the roots galled, 3 = 26 to 50%; 4 = 51 to 75%; and 5 = >75% roots galled (17). Root galls and

egg masses were counted, and the results were expressed per gram of roots and per whole root system. Eggs from galled tomato root tissue were collected with NaOCl 1% (18) and counted. The root and soil nematodes were extracted from 5 g roots by centrifugal blender method (19) and 500 cm³ soil by flotation-centrifugation technique according to the modified method by De Grisse (20) and counted under a stereomicroscope. Then, the multiplication rate (MR) was calculated by dividing final nematode inoculums to initial population.

Finally, at the end of the experiment, collected egg-masses from tomato roots of each treatment were incubated for egg hatching test. Three egg masses were laid on a micro-sieve (40 µm pore), placed on 5 cm diameter Petri plates and submerged with distilled water. Petri plates were maintained at 25±3°C in darkness. The number of hatched juveniles was counted after 72 h and 168 h. Each treatment was repeated three times, and the test was carried out twice.

Mycorrhizal root colonization

Mycorrhizal colonization was determined by staining the tomato roots according to Phillips and Hayman (21). Roots were mounted on slides and observed with a light microscope (40X magnification). Mycorrhizal colonization frequency (F%) was assessed according to Trouvelot *et al.* (22).

Statistical analysis

The experiments were repeated twice, and data from the pot and greenhouse experiments were subjected to Tukey's multiple comparison tests using IBM SPSS Statistics for Windows, Version 20.0 (Released 2011. IBM Corp, Armonk, NY). The level of significance was set at $P < 0.05$ in all analyses.

RESULTS

Effect on tomato plant growth

The plant growth parameters of tomato seedlings grown in pots are presented in Table 1. It was evident from the results that *M. javanica* inoculation caused a significant decrease in major plant growth parameters such as the dried shoot weight, plant height and root length as compared with healthy tomato seedlings. However, the root weight of root-knot-nematode infested seedlings increased, and this was maybe due to the formation of the galls. The fresh shoot weight showed insignificant variations with treatments. All applied treatments enhanced the tomato plant growth in comparison with *M. javanica* treatment. Abamectin and the clay formulation of *G. iranicum* (MycF1) showed the best growth improvement (Table 1).

In greenhouse experiment, MycF1 and abamectin soil treatments increased the shoot height and collar diameter considerably compared with naturally infested tomato. The shoot weight and root length increased insignificantly with all treatments (Table 2). In addition, a significant increase was reported with all treatments. The MycF1 showed the high yield followed by abamectin and ethoprophos comparing with infested plants with RKN (Figure 1).

Plant disease symptoms and nematode reproduction

The plant symptoms disease in terms of Gall index, galls and egg masses number and nematode reproduction decreased on all pot grown tomato. Chemical treatment (oxamyl), applied to tomato plants grown in *M. javanica* inoculated artificially, showed the best reduction on the soil nematode levels in comparison to untreated conditions and other treatments (Table 3). The best effects for the overall reduction in nematodes and attack symptoms, comparing the two formulations of *G. iranicum*, were demonstrated

Table 1. Effect of soil treatment by two *Glomus iranicum* formulations MycF1 and MycF2 and abamectin-based product on tomato growth, 60 days after *Meloidogyne javanica* inoculation under pots experiment.

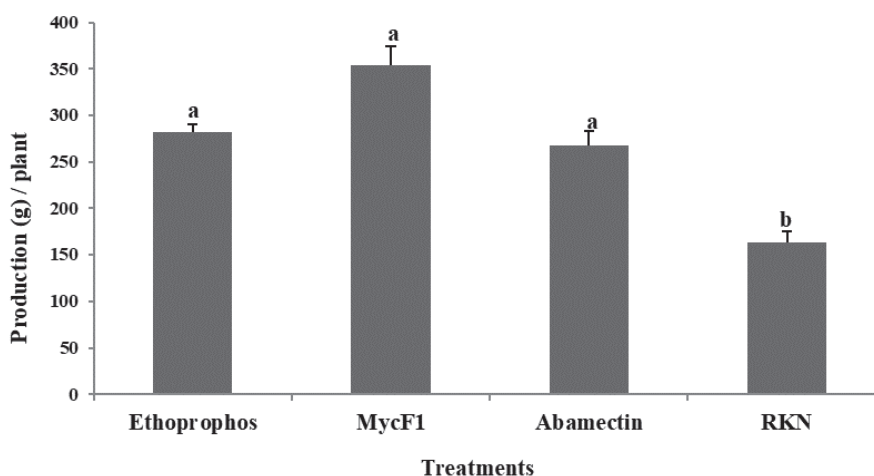
Treatments	Shoot			Root	
	Height	Fresh weight	Dried weight	Length	Weight
Oxamyl	5555±320 ab	2190±159 a	313±015 a	1966±225 ab	2283±821 ab
Abamectin	6944±815 a	2207±290 a	275±036 ab	1988±385 ab	1501±811 bc
MycF1	5266±45 b	2013±152 a	271±026 ab	2224±344 ab	2900±869 a
MycF2	5533±961 ab	1972±615 a	207±083 c	1977±479 ab	1557±469 bc
RKN	5533±474 ab	2268±138 a	286±041 ab	2516±324 a	3021±868 a
Control	6844±1063 a	1872±269 a	230±031 bc	1655±408 b	523±345 c
P-value	<001	≥005	<001	<001	<001

Tukey's Multiple Comparison Test, values followed by various superscripts differ significantly at $P \leq 0.05$. Probabilities associated with individual F tests. Means± standard error. MycF1: solid formulation of *Glomus iranicum* with clay; MycF2: solid formulation of *Glomus iranicum* without clay; RKN: infested seedlings with the root-knot nematode *M. javanica*; Control: healthy seedlings.

Table 2. Effect of soil treatment by *Glomus iranicum* MycF1 and abamectin-based product on tomato growth at five months' post-transplantation under greenhouse experiment.

Treatments	Shoot		Root		Collar Diam
	Height	Weight	Length	Weight	
Ethoprophos	8100±632 a	25302±1138 a	221±395 a	1511±119 b	297±017 ab
MycF1	7595±426 a	24873±1061 a	2395±419 a	2307±169 ab	313±029 a
Abamectin	7615±378 a	23166±1265 a	2375±565 a	3161±644 ab	308±031 a
RKN	578±299 b	19389±1528 a	2165±462 a	5503±583 a	282±040 b
P-value	<001	≥005	≥005	<001	<001

Tukey's Multiple Comparison Test, values followed by various superscripts differ significantly at $P \leq 0.05$. Probabilities associated with individual F tests. Means± standard error. MycF1: solid formulation of *Glomus iranicum* with clay; RKN: naturally infested soil with the root-knot nematode *M. javanica*.

**Figure 1.** Tomato yield variation depending on soil treatment with abamectin and *Glomus iranicum* at five months post-transplantation. MycF1: solid formulation of *Glomus iranicum* with clay; RKN: naturally infested soil with the root-knot nematode *M. javanica* under greenhouse experiment.

with the clay formulations of mycorrhiza (MycF1). Besides, the observation of hyphal structures, arbuscules and vesicles inside the stained roots confirmed the well-established mycorrhizal colonization. The mycorrhizal colonization assessment showed that fungal colonization of *G. iranicum* (F%) rated 62% of analyzed roots (Table 3).

Subsequently to the previous experiment, MycF1 was chosen for greenhouse experiment. Gall index, galls and

egg masses numbers and *M. javanica* root population were decreased with all applied treatments. MycF1 was reported the best in nematode development reduction in tomato roots (Table 4). Along with the greenhouse experiment, the time course variation of root RKN population was assessed. The highest decrease in root nematode density was reported with abamectin and ethoprophos treatments (Figure 2). The egg hatching test showed that

Table 3. Effect of soil treatment by two *Glomus iranicum* formulations MycF1 and MycF2 and abamectin-based product on plant disease symptoms and nematode reproduction in tomato seedlings, infested with *Meloidogyne javanica*, 60 days after inoculation under pots experiment.

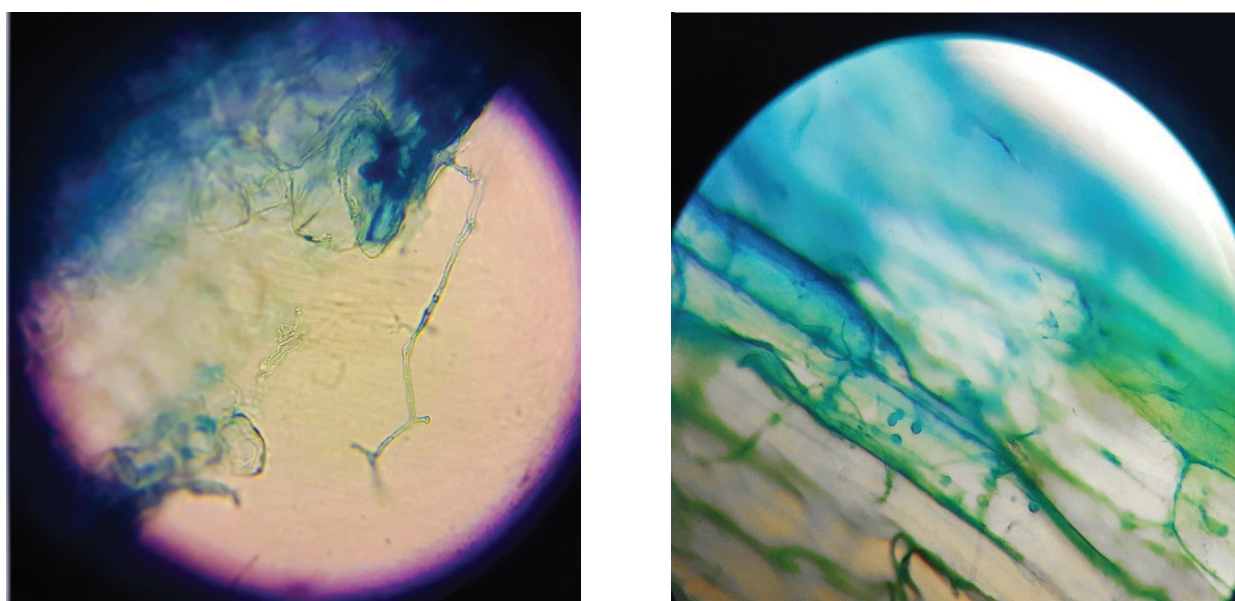
Treatments	Gall Index	Gall Number /g root	Egg-Masses /g root	MR
Oxamyl	100±000 c	2111±124 b	1055±719 c	87±037 b
Abamectin	55±005 c	1433±194 b	433±759 c	93±033 b
MycF1	222±009 b	8022±217 a	4088±957 ab	147±051 b
MycF2	222±008 b	9011±586 a	2244±283 bc	151±064 b
RKN	333±007 a	12400±482 a	4600±375 a	608±056 a
P-value	<001	<001	<001	<001

Tukey's Multiple Comparison Test, values followed by various superscripts differ significantly at $P \leq 0.05$. Probabilities associated with individual F tests. Means± standard error. MycF1: solid formulation of *Glomus iranicum* with clay; MycF2: solid formulation of *Glomus iranicum* without clay; RKN: infested seedlings with root-knot nematode *M. javanica*; Control: healthy seedlings; MR: multiplication rate: Pff/Pi.

Table 4. Plant disease symptoms and nematode reproduction in roots of tomato plants, infested with *Meloidogyne javanica* and treated with abamectin and *Glomus iranicum* at five months' post-transplantation under greenhouse experiment.

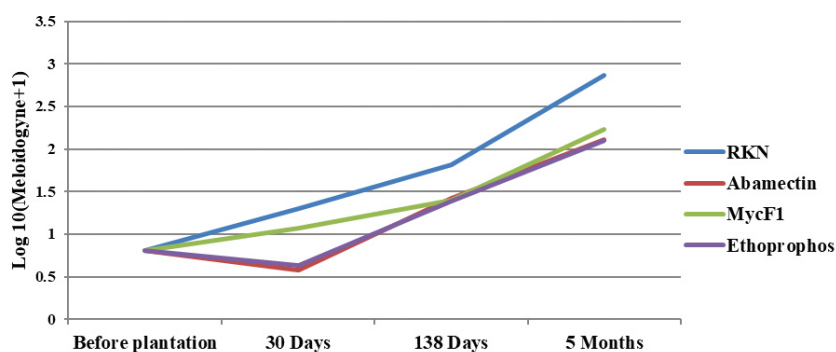
Treatments	Gall Index	Gall Number /g root	Egg-Masses/ g root	RKN/ g root
Ethoprophos	24±1.99 b	2325±1062 b	4675±1424 ab	24699±1494 b
MycF1	11±1.11 b	1575±1163 b	3050±1568 b	32134±1890 ab
Abamectin	215±10.8 a	1475±1147 b	5075±1837 ab	30595±1729 ab
RKN	26±1.08 b	3650±858 a	8975±1036 a	46164±1707 a
P-value	<001	<001	<001	<001

Tukey's Multiple Comparison Test, values followed by various superscripts differ significantly at $P \leq 0.05$. Means± standard error. Probabilities associated with individual F tests. MycF1: solid formulation of *Glomus iranicum* with clay; RKN: naturally infested soil with root-knot nematode *M. javanica*.

**Figure 2.** Observation of hyphal structures: (A) arbuscules and vesicles inside tomato roots; (B) treated by *Glomus iranicum* formulation at five months post-transplantation.

RKN females' fecundity, collected from treated roots with abamectin, were the most affected and showed the less egg hatching rate. The number of hatched juveniles treated with ethoprophos was higher (Figures 3 and 4).

The variation of soil nematode communities for each treatment is presented in Table 5. The lower root knot-nematode population was recorded with MycF1 treatment followed by abamectin and chemical treatment.

**Figure 3.** Time course variation of *Meloidogyne javanica* population on tomato root under greenhouse, depending on soil treatment with abamectin and *Glomus iranicum*. MycF1: solid formulation of *Glomus iranicum* with clay; RKN: naturally infested soil with the root-knot nematode *M. javanica*.

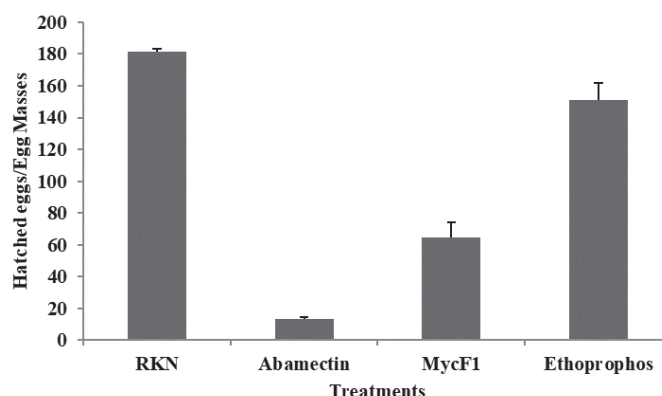


Figure 4. Egg hatching of *Meloidogyne javanica* post-treated with abamectin and *Glomus iranicum*. MycF1: solid formulation of *Glomus iranicum* with clay; RKN: naturally infested soil with the root-knot nematode *M. javanica*.

Table 5. Soil nematode communities' prevalence depending on abamectin and *Glomus iranicum* treatments, five months' post-transplantation under greenhouse experiment.

Treatments	Nematodes groups frequency %			
	Free Living	Fungivores	Meloidogyne	PPN
RKN	3011±87 c	316±27 c	6073±108 a	598±69 b
Abamectin	5168±92 a	417±61 b	3432±95 c	981±77 a
MycF1	5535±74 a	553±82 b	2970±44 c	940±63 ab
Ethoprophos	3896±59 b	962±88 a	4102±70 b	1038±55 a
<i>P-value</i> ^b	<001	<001	<001	<001

Tukey's Multiple Comparison Test, values followed by various superscripts differ significantly at $P \leq 0.05$. Probabilities associated with individual F tests. Means± standard error. MycF1: solid formulation of *Glomus iranicum* with clay; RKN: naturally infested soil with the root-knot nematode *M. javanica*, PPN: other plant parasitic nematodes.

Apart from *M. javanica* decrease, the soil nematode composition showed an increase of free-living nematodes in comparison with untreated and naturally infested soil with nematodes (Table 5).

DISCUSSION

Our study determined that using abamectin and mycorrhizae-based products as soil treatments are effective ways of reducing root and soil infestation by *M. javanica* and had positively affect tomato plant growth in both pot and greenhouse experiments.

These findings are in line with other results reporting the great biocontrol potential of abamectin soil treatment towards root-knot nematodes and their positive effect in plant growth promotion (23). Besides, Cabrera *et al.* (24) reported the efficiency of tomato seed treatment by abamectin against three species of *Meloidogyne*: *M. incognita*, *M. arenaria* and *M. javanica*. The seed treatment showed a significant reduction on the gall number, egg masses, females and the juveniles under field conditions. It promoted the plant growth parameters, including length and weight of shoots in comparison with untreated plants and

chitin treatment, as previously reported by Korayem *et al.* (25). Furthermore, abamectin treatment was effective also in controlling other plant-parasitic nematodes, including *Hoplolaimus* and *Tylenchorhynchus* as noted in previous studies of Blackburn *et al.* (26) and Jansson and Rabatin (27).

The findings of AMF biocontrol potential against plant parasitic nematodes are in accordance with the reports of Banito *et al.* (28) which indicated the excellent biocontrol potential of several *Glomus* species against root-knot nematodes infecting tomato crops. In addition, the combined use of indigenous *Glomus* species and organic amendment provided an efficient biocontrol potential against *Meloidogyne* spp. on tomato pathosystem (29). The protective effect of AMF was observed in a wide range of plant species and against many pathogens (30, 31). Furthermore, several reports found that biological control with arbuscular mycorrhizal fungi provided a growing issue about sustainable and environmental pest management tool (32–34). Similar plant growth promoting traits of AMF use have been reported by Smith *et al.* (35) and this effect was explained due to the increase of nutrient uptake in exchange for photosynthetic carbon

from their plant host. Moreover, the AMF have been reported to be mainly used as plant bio-fertilizers (36, 37).

Besides, the considerable colonization of galled tomato roots by tested mycorrhiza suggested that the direct competition for space between arbuscular mycorrhizal fungi and *M. javanica*, sedentary endo-parasitic nematode, could explain the high biocontrol potential of this microorganism. The AMF root colonization and the direct mechanism against nematodes had been reported different within plant parasitic nematodes (ecto-parasitic/endoparasitic, sedentary/migratory endoparasitic) and plant host species (38–40). The high fungal colonization, plant growth and yield boosting and the plant-parasitic nematode decrease suggested that several mechanisms could be involved in the *G. iranicum*-mediated biocontrol, including direct effect on the pathogen, involving competition for space or nutrients, or indirect, plant-mediated, effects by plant-growth promotion (41–43).

Mycorrhizal colonization of tomato plants can be challenging due to their lower mycotrophy compared to other species (44). The degree of mycorrhizal colonization is often estimated using indices that consider RKN proliferation and overall plant health. The enhanced growth of AMF-treated tomato plants may be attributed to acclimation, which can help hosts resist RKN infections for extended periods (44, 45). Additionally, AMF hyphae may replace portions of roots damaged by RKN, contributing to tomato tolerance. Furthermore, environmental stress can influence shoot development and photosynthetic activity, which may be positively affected by AMF colonization. This suggests that mycorrhized tomato plants can support multiple RKN reproductive cycles, while non-mycorrhized plants may succumb to infections due to poor root and shoot growth (45). Economically, AMF treatments may increase or stabilize tomato fruit production despite RKN proliferation (44). The varying responses of tomato plants to AMF bioprotection highlight the host-specific nature of this phenomenon (46).

CONCLUSION

To summarize, this study revealed that the application of soil microorganisms (fungi and bacteria) based-products could be a suitable option to mitigate the adverse effects produced by root-knot nematode. Furthermore, abamectin and arbuscular mycorrhizal fungi promoted the plant growth infected with root-knot nematodes. Both biological products could provide a sustainable environmental tool to protect vegetable crops from pathogens.

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