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PATHOGENICITY OF *MELOIDOGYNE INCOGNITA* RACE 1 ON TOMATO AS INFLUENCED BY DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGI AND BIOFORMULATED *PAECILOMYCES LILACINUS* IN A DYSTERIC CAMBISOL SOIL

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Abstract: A greenhouse experiment was conducted to investigate the single and combined effects of different arbuscular mycorrhizal fungi (AMF) and bioformulated *Paecilomyces lilacinus* against *M. incognita* race 1 on tomato. Dysteric Cambisol soil was used. The experiment took place in Calabar, Cross River State, Nigeria. The experiment was laid out as a 3x6 factorial in a completely randomized design (CRD) with three replications. Three applications of the bionematicide were combined with five species of AMF plus an uninoculated control. The results indicated that AMF species differed significantly ($p \leq 0.05$) in their efficacy of gall and egg mass inhibition, tomato root colonization rate as well as growth and fresh fruit yield enhancement. *Glomus etunicatum* and *G. deserticola* were the most efficient species. Two applications of the bionematicide more significantly ($p \leq 0.05$) reduced galling and egg production than a single application. Individual combinations of two AMF (*G. etunicatum* and *G. deserticola*) with a double application of the bionematicide, resulted in the greatest gall and egg mass inhibition and consequently the greatest growth and fresh fruit yield enhancement.

Key words: arbuscular mycorrhizal fungi, biocontrol, dysteric cambisol, *Meloidogyne incognita*, *Paecilomyces lilacinus*, tomato

INTRODUCTION

The commercially produced tomato is one of the most highly cherished fruit vegetables in Nigeria. The total area under tomato production in tropical Africa is about 300,000 ha with an estimated annual production of 2.3 million tonnes (Van der Vossen *et al*. 2004). Nigeria is the largest producer in tropical Africa with 26,000 ha yielding 879,000 tonnes of fresh fruits annually (FAO 2004). The fruit is rich in vitamins A, C, thiamine, riboflavin, and niacin as well as some minerals like potassium and sodium (Janes 1994).

Tomatoes can grow in many soil types, ranging from sandy loam to clay-loam. These soil types must be rich in organic matter with an optimum pH range of 6–7 (Van der Vossen *et al*. 2004). Poor tomato yield in Nigeria has been attributed, in part, to nematode diseases (Olabiyi 2005; Udo *et al*. 2008; Ogwulumba *et al*. 2011). Tomato plants infested with root-knot nematodes usually have galled roots and are very vulnerable to rot and wilt-inducing pathogens (Sasser 1980; Wiliamson and Gleason 2003). The most effective method of nematode disease control is the use of synthetic chemical nematicides. However, health hazards, and the attendant adverse effects of these chemicals on the beneficial non-target organisms and the environment are serious constraints. Of late, alternative nematode management options have been sought by many researchers to reverse this ugly trend. The exploitation of biocontrol methods for the integrated management of plant parasitic nematodes using ubiquitous antagonistic organisms, is fast gaining wide acceptance in many developed and developing countries (Oyekanmi *et al*. 2007; Shreenivasa *et al*. 2007; Zhang *et al.* 2008; Oclarit and Cumagun 2009; Hashem and Abo-Elyours 2011). The fungus, *Paecilomyces lilacinus* (Thom.) Samson has been reported as a potential biocontrol agent for root-knot nematodes and other plant parasitic nematodes (Jatala 1979; Cabanillas and Barker 1989; Oclarit and Cumagun 2009). This fungus parasitizes the egg of root-knot nematode and its other life stages. Similarly, many researchers have reported the effectiveness of arbuscular mycorrhizal fungi (AMF) in the control of root-knot nematodes and other nematodes in many crops (Diederichs 1987; Shreenivasa *et al*. 2007; Zhang *et al*. 2008; Odeyemi *et al*. 2010). The mechanism involved in nematode suppression by AMF is still topical (Gera Hol and Cook 2005). There has been induced systemic resistance/tolerance due to improved host nutrition, changes in the root morphology, histopathological and biochemical changes (Singh *et al*. 1990; Morandi 1996; Masadeh *et al*. 2004). The use of biological formulations that contain a mixture of biocontrol agents has been widely acclaimed

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by many researchers in the management of plant parasitic nematodes (Meyer and Roberts 2002; Oyekanmi *et al*. 2007; Akhtar and Siddique 2008). Different results have been obtained where *P. lilacinus* and AMF were combined to be used in the control of root-knot disease (Al-Raddad 1995; Sharma and Trivedi 1997; Rao *et al*. 1998; Rumbos *et al*. 2006; Hashem and Abo-Ehyousr 2011). The disparity between results was attributed to many factors including; incompatibility of biocontrol agents, edaphic factors, plant genotype, and nematode species. This trial was conducted with the objective of assessing the effects of different AMF in combination with bioformulated *P. lilacinus* in the management of *Meloidogyne incognita* on tomato in a Dysteric Cambisol soil, in Southeastern Nigeria.

MATERIALS AND METHODS

Source of experimental materials

A starter culture of five indigenous species of arbuscular mycorrhizal fungus inoculum: *Glomus etunicatum* (Becker and Gerdemann), *G. mosseae* (Nicolson and Gerd.) Gerd. and Trappe., *G. clarum* (Nicolson and Schenk), *G. deserticola* (Trappe, Bloss and Menge) and *Gigaspora gigantea* (Nicolson and Gerd.) Gerd. and Trappe. were obtained from the Soil Microbiology Unit of the Department of Agronomy, University of Ibadan, Ibadan, Oyo State, Nigeria. A bioformulation containing *P. lilacinus* as the active ingredient, with a trade name PL Gold TM, was obtained from the Biological Control Products, South Africa (Pty) Ltd. The registration number is L7698 Act No. 36/1947. According to the manufacturer, it is a wettable powder spore concentrate of *P. lilacinus*, a fungal nematicide with an active ingredient of $4x10^9$ spores/gram used with a Gold Starter (fungal spore activator). Seeds of the test plant, tomato CV. Roma VF were obtained from the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. The tomato cultivar is highly susceptible to *M. incognita* and *M. javanica* (Udo *et al*. 2008; Ogwulumba *et al*. 2011).

Multiplication of starter culture of AMF and *M. incognita* inoculum.

The starter culture of each AMF is made up of chopped roots of the trapping plants, spores, chlamydospores and soil. This culture was multiplied in a steam-sterilized soil, planted with maize, and irrigated with Hogland's Solution (half-strength low in phosphorus) for three months. The spore density of all the AMF inoculum ranged from 43–51 spores/10 g of soil as estimated by the method of Gerdemann and Nicolson (1963). An indigenous population of *M. incognita* race 1, maintained on begonia (*Begonia rex-cultorum*), served as the inoculum source. This population was multiplied on Cock's comb (*Celosia argentea* Linn.) and planted in a steam-sterilized soil-peat mixture in the greenhouse of the Faculty of Agriculture, University of Calabar, Cross River State, Nigeria.

Preparation of Nematode and *P. lilacinus* Inocula

Heavily galled roots of *C. argentea* were uprooted and gently washed with tap water to remove the adhering soil particles. The galled roots were cut into 1–2 cm segments for egg extraction, using the method of Hussey and Barker (1973). This method involved shaking the galled root segments in 0.50% sodium hypochlorite solution in a 500 ml conical flask covered tightly with a rubber bung for 4 min. The obtained egg suspension was then passed through a 200-mesh sieve nested over a 500-mesh sieve. The eggs trapped in the 500-mesh sieve were then rinsed under a gentle stream of cool tap water. The eggs were transferred into a beaker with the help of a wash bottle and the inoculum density was adjusted as desired under a Stereoscopic microscope. The number of eggs in 1ml of the egg suspension was ascertained to be, on average, 500. Three counts were done using a multiple tally counter in a nematode counting dish. Fifty grams of the spore powder of bionematicide (PL Gold TM) was weighed out and mixed with 50 ml of the spore activator (mixture ratio of 1:1). The mixture was allowed to stand for an hour before being diluted further with 30 litres of distilled water.

Nursery/inoculation of tomato seedlings with AMF

The tomato plants were inoculated with the respective AMF species. Sandy soil mixed with poultry manure at a ratio of 3:1 by volume, was steam-sterilized. Four kilograms of the steam-sterilized soil mixture was used to fill a plastic basket. Then, 250 g of the arbuscular mycorrhizal fungus inoculum was added to the top 5 cm layer (Oyekanmi *et al*. 2007). Seeds of Roma VF tomato were surface sterilized with a 0.50% concentration of sodium hypochlorite solution for five minutes. The seeds were rinsed immediately with three changes of distilled water. The seeds were then drilled in each basket. After emergence, seedlings were thinned to 40 per basket. Seedlings raised in baskets without AMF served as the control. The seedlings were watered appropriately.

Application of treatments

Surface soil (0–15 cm) was collected from Obubra Local Government Area, Cross River State, Nigeria and transported to the greenhouse of the Faculty of Agriculture, University of Calabar. The soil is classified as Dysteric Cambisol (FAO/UNESCO, 1974). It was analyzed for its physicochemical properties, pre-plant nematode density and AMF spore density using the methods of Tel and Rao (1982), Coyne *et al*. (2007) and Gerdemann and Nicolson (1963), respectively. Fifty-four plastic pots perforated at the bottom were each filled with 3 kg of unsterilized top soil. Four-week-old tomato seedlings biologically enhanced with the different species of AMF were transplanted to each pot. Each seedling was inoculated with 5,000 eggs of *M. incognita* by pouring 10 ml of the prepared nematode inoculum into three 5 cm deep holes made around each stand. The seedlings were irrigated lightly with tap water and then inoculated with 30 ml of the spore mixture (*i.e*. 0.05 g spore powder/plant ≡ $2x10^8$ spores/plant). The spores were flushed down the 15 cm depth of the root zone with excess irrigation water. Seedlings where bionematicide was not applied, served as the control. The application was repeated two weeks after the first application for those treatments that required two applications. The seedlings were randomly arranged on

the greenhouse benches. Plants were grown at mean day and night temperatures of 28°C and 20°C, respectively for 65 days. The pots were watered once daily (300 ml).

Experimental design and data collection

The experiment was laid out as a 3x6 factorial in a completely randomized design (CRD) with 3 replications. The frequency of bionematicide application (no application, applied once at transplanting and applied twice *i.e.* at transplanting and two weeks later) was combined in a factorial fashion with the five species of AMF plus the uninoculated control, to give 18 treatment combinations. At plant maturity, the following data were collected: number of galls and egg masses per root system, fresh weight of root per plant, shoot length, mycorrhizal root colonization and total fresh fruit weight per plant. For egg mass count, fresh root was stained with phloxine B (0.15 g/l) for 15 minutes (Daykin and Hussey 1985). The root gall index was determined on a 0–5 scale rating according to Taylor and Sasser (1978); 0 = 0, 1 = 1–2, 2 = 3–10; 3 = 11–30; 4 = 31–100, and 5 = more than 100 galls per root system. The proportion of root colonized by AMF was determined by a grid-line intersecting method of Giovannetti and Mosse (1980) after clearing the roots with KOH (Phillips and Hayman 1970) and staining the roots in 0.05% trypan blue-lactophenol.

Data Analysis

A two-way analysis of variance (ANOVA) was used to test the significance of the treatments. Significant treatment means were separated using Fisher's Least Significant Difference (F-LSD) at a 5% level of probability. All statistical analyses were performed with MINITAB 15 Statistical Software.

RESULTS

The results of the physicochemical properties of Obubra soils are presented in table 1. The soil is sandy loam in texture, slightly acidic and low in total N, available P, exchangeable K, and Na but high in organic matter content, exchangeable Mg and percentage base saturation. The nematode density and mycorrhizal spore density were 214/200 g of soil and 243/100 g of soil, respectively.

Inoculation of AMF significantly ($p \leq 0.05$) reduced the severity of root galling compared with the control, with the exception of *G. gigantea* (Table 2). *G. etunicatum* was the most efficient species in gall reduction. Application of bioformulated *P. lilacinus* significantly reduced ($p \leq 0.05$) root galling compared with no application. Double application significantly reduced ($p \leq 0.05$) root galling more than the single application. Interaction between the two factors was significant. The least galling was obtained when *G. etunicatum* and *G. deserticola* were combined with double application of PL Gold™. Gall index (GI) was reduced from 4.22 in non-mycorrhizal plants to less than 4 in mycorrhizal plants (Table 2). The lowest GI of 3 was obtained when seedlings were inoculated with AMF and double treated with *P. lilacinus*. Egg-mass production significantly ($p \leq 0.05$) declined in mycorrhizal plants compared with non-mycorrhizal plants (Table 3).

Table 1. Physicochemical properties of the soil

Physical and chemical properties	
Sand [%]	68.00
Silt [%]	12.00
Clay [%]	20.00
Texture	sandy loam
pH [H_2O]	6.50
Total N [%]	0.17
Available P [mg/kg]	2.25
Organic carbon [%]	2.03
Organic matter [%]	3.52
Exchangeable K [cmol/kg]	0.18
Exchangeable Ca [cmol/kg]	9.40
Exchangeable Mg [cmol/kg]	3.80
Exchangeable Na [cmol/kg]	0.10
Exchangeable Acidity [cmol/kg]	0.70
Effective Cation Exchange Capacity (ECEC) (cmol/kg)	14.00
% base saturation	96.00

However, inhibition of egg production was highest with *G. etunicatum* and *G. deserticola*. A double application of the bionematicide was more effective in inhibiting egg production than a single application. The least number of egg-masses was produced when bionematicide was applied twice and in a combination with *G. deserticola* or *G. gigantea* or *G. etunicatum*. Mycorrhizal plants had significantly ($p \leq 0.05$) greater fresh root weights than non-mycorrhizal plants (Table 3). *G. deserticola* was the most efficient species. The highest increase in fresh root weight was obtained when plants were inoculated with AMF and treated twice with the bionematicide. There were significant differences among the species of AMF in their root colonization rates (Table 4). *G. etunicatum* and *G. deserticola* had the highest colonization of more than 80%. Uninoculated plants were lightly colonized. Application of *P. lilacinus* had no significant ($p > 0.05$) effect on tomato root colonization by AMF. Arbuscular mycorrhizal fungi species differed in their ability to enhance tomato growth (Table 4). The tallest plants were found in *G. deserticola* and *G. mosseae* inoculated plants. Growth enhancement was greater with the double application of the bionematicide combined with all the AMF species compared with only one application. The effects of AMF and *P. lilacinus* inoculation on tomato shoot dry matter accumulation and fresh fruit yield are presented in table 5. Mycorrhizal plants significantly accumulated ($p \leq 0.05$) more dry matter than their non-mycorrhizal counterparts. *G. deserticola* inoculated plants had the highest shoot dry weight. Application of the bionematicide twice, in combination with the AMF, significantly increased shoot dry matter accumulation compared with no application. There was a significant ($p \leq 0.05$) increase in fresh fruit weight with AMF inoculation relative to the uninoculated. *G. deserticola* inoculated plants had the highest yield. *P. lilacinus* inoculation significantly ($p < 0.05$) enhanced fruit yield. *G. etunicatum* and *G. deserticola* in combination with a double application of PL Gold™ produced the significantly highest fruit yield.

Table 2. Effects of arbuscular mycorrhizal fungus and *P. lilacinus* on the number of galls per root system and gall index (GI)* of tomato inoculated with *M. incognita*

Mycorrhizal fungus	No. of galls/root system			Mean
		P. lilacinus		
	control	applied once	applied twice	
Control	116.67	55.00	37.67	69.78
G. etunicatum	41.33	25.00	17.67	28.00
G. mosseae	65.00	45.00	24.33	44.78
G. clarum	113.33	27.33	21.33	54.00
G. gigantea	92.33	71.67	27.33	63.78
G. deserticola	65.00	33.00	15.67	37.89
Mean	82.28	42.83	24.00	
	Gall Index (GI)			
Control	4.67	4.00	4.00	4.22
G. etunicatum	4.00	3.33	3.00	3.44
G. mosseae	4.00	3.67	3.00	3.56
G. clarum	5.00	3.33	3.00	3.78
G. gigantea	4.00	4.00	3.00	3.67
G. deserticola	4.00	4.00	3.00	3.67
Mean	4.28	3.72	3.17	

		No. Galls	Gall Index
LSD (0.05) for *P. lilacinus* (F) means	=	5.86	0.18
LSD (0.05) for mycorrhizal fungus (M) means	=	8.24	0.26
LSD (0.05) for (FxM) interaction means	=	14.27	0.45

* 0 – Immune, 1 – Highly resistant, 2 – Resistant, 3 – Moderately susceptible
4 – Susceptible, 5 – Highly Susceptible

Table 3. Effects of arbuscular mycorrhizal fungus and *P. lilacinus* on number of egg masses per root system and root fresh weight [g /plant] of tomato inoculated with *M. incognita*

Mycorrhizal fungus	No. of egg masses /root system			Mean
		P. lilacinus		
	control	applied once	applied twice	
Control	96.67	23.33	12.00	44.00
G. etunicatum	27.67	10.67	8.67	15.67
G. mosseae	30.00	18.67	11.00	19.89
G. clarum	70.00	12.33	8.33	30.22
G. gigantea	62.67	30.00	8.00	33.56
G. deserticola	26.67	10.33	7.00	14.67
Mean	52.28	17.56	9.17	
	Root fresh weight [g/plant]			
Control	8.29	11.66	13.02	10.99
G. etunicatum	12.70	14.20	15.11	14.01
G. mosseae	12.13	13.89	14.39	13.47
G. clarum	10.76	15.47	17.42	14.55
G. gigantea	11.78	13.27	15.97	13.67
G. deserticola	14.15	16.37	18.66	16.39
Mean	11.63	14.14	15.76	

		No. of egg masses	Root fresh wt.
LSD (0.05) for *P. lilacinus* (F) means	=	1.52	0.49
LSD (0.05) for mycorrhizal fungus (M) means	=	2.15	0.69
LSD (0.05) for (FxM) interaction means	=	3.72	1.20

Table 4. Effects of arbuscular mycorrhizal fungus and *P. lilacinus* on percentage of mycorrhizal colonization and shoot length [cm/plant] of tomato inoculated with *M. incognita*

Mycorrhizal fungus	Mycorrhizal colonization [%]			Mean
	P. lilacinus			
	control	applied once	applied twice	
Control	16.00	17.33	17.33	16.89
G. etunicatum	84.00	79.67	83.67	82.45
G. mosseae	76.33	76.00	74.67	75.67
G. clarum	63.33	63.33	62.33	63.00
G. gigantea	70.00	70.33	70.33	70.22
G. deserticola	84.00	84.00	87.00	85.00
Mean	65.61	65.11	65.89	
	Shoot length [cm/plant]			
Control	42.33	57.33	66.33	55.33
G. etunicatum	60.00	67.33	72.00	66.44
G. mosseae	62.00	67.67	71.67	67.11
G. clarum	60.33	65.00	71.67	65.67
G. gigantea	61.33	65.67	71.33	66.11
G. deserticola	62.00	70.00	74.33	68.78
Mean	58.00	65.50	71.22	

		Mycorrhizal	Shoot length colonization
LSD (0.05) for *P. lilacinus* (F) means	=	N.S	1.71
LSD (0.05) for mycorrhizal fungus (M) means	=	1.67	2.42
LSD (0.05) for (FxM) interaction means	=	NS	4.19

Table 5. Effects of arbuscular mycorrhizal fungus and *P. lilacinus* on shoot dry weight and total fresh weight of fruit of tomato inoculated with *M. incognita*

Mycorrhizal fungus	Shoot dry weight [g/plant]			Mean
	P. lilacinus			
	control	applied once	applied twice	
Control	9.26	13.50	14.70	12.49
G. etunicatum	14.26	15.42	16.41	15.36
G. mosseae	14.97	16.85	17.65	16.49
G. clarum	12.64	15.62	18.36	15.54
G. gigantea	14.09	15.09	18.08	15.75
G. deserticola	15.76	15.73	20.48	17.32
Mean	13.50	15.37	17.61	
	Total fresh weight [g] of [fruit/plant]			
Control	30.80	52.35	58.29	47.15
G. etunicatum	39.97	61.19	77.15	59.44
G. mosseae	43.50	58.12	69.82	57.15
G. clarum	36.22	52.82	65.65	51.56
G. gigantea	45.80	55.06	63.10	54.65
G. deserticola	51.48	65.60	75.77	64.28
Mean	41.30	57.52	68.30	

		Shoot dry weight	Total fresh wt.
LSD (0.05) for *P. lilacinus* (F) means	=	0.59	2.32
LSD (0.05) for mycorrhizal fungus (M) means	=	0.84	3.28
LSD (0.05) for (FxM) interaction means	=	1.47	5.68

DISCUSSION

Arbuscular mycorrhizal fungi inoculation inhibited gall formation and egg-mass production by *M. incognita* race 1 on tomato. There was a change in the general susceptibility of the tomato plants with AMF inoculation. *G. etunicatum* and *G. deserticola* were the most efficient species in gall and egg production inhibition. This result corroborates the findings of Diederichs (1987), Shreenivasa *et al*. (2007) and Zhang *et al*. (2008) who observed significant differences among different AMF species in their ability to inhibit root galling and egg production by root-knot nematodes. Physiological and biochemical changes leading to increased production of phytoalexins, phenols, lignin, phenylanine, serine chitinase, and leading to reduced leakage of carbohydrate from root cells, could possibly explain the apparent resistance/tolerance induced in mycorrhizal plants as observed by earlier researchers (Dehne 1982; Umesh *et al*. 1988; Morandi 1996; Graham 2001). Root galling was very severe in the control plants. This could be attributed in part to the conducive soil environment which was dominated by sand as well as the high pre-plant nematode density. This finding validates the report of earlier investigators (Windham and Barker 1986; Olowe 2005) who observed increased penetration, greater damage, and reproduction by root-knot nematodes in sandy soil than in clay soil.

Application of bioformulated *P. lilacinus* significantly reduced root galling and egg production by the nematode species. This result validates the report of Oclarit and Cumagun (2009) and Khalil *et al*. (2012) that *P. lilacinus* is an effective biocontrol agent of *M. incognita* on tomato. The efficacy of the fungus was greater with a double application than with a single application. This finding confirms the report by Cabanillas and Barker (1989) and Rumbos *et al.* (2006) that pre-plant application and repeated applications of *P. lilacinus* at planting and the later growth stage of the crop were more effective in reducing the damage and reproductive potential of root-knot nematodes than a single application. *P. lilacinus* has been considered to have the greatest potential for application as a biocontrol agent in sub-tropical and tropical agricultural soils (Morgan-Jones *et al*. 1984). The vegetative hyphae of the fungus penetrate the egg and infect the first stage juvenile. Adult females could also be penetrated through the vulva or anus. Penetration is enhanced through the production of lytic enzymes, serine protease and chitinase (Khan *et al*. 2004). Park *et al*. (2004) reported the production of leucinotoxin and other nematicidal compounds by *P. lilacinus*. The overall effect was the decrease in population and pathogenicity of the nematode species.

The application of both biocontrol agents was more effective in gall and egg production inhibition than the single application. This effect was very conspicuous with *G. clarum*. Galling and egg production was very high when plants were inoculated only with *G. clarum* but when combined with *P. lilacinus*, galling and egg production was greatly reduced. This validates the synergistic interaction between the two biocontrol agents as reported earlier by some authors (Al-Raddad 1995; Rao *et al*. 1998; Bhat and Mahmood 2000; Sharma and Trivedi 1997). It is possible that the growth and activity of the arbuscular mycorrhizal fungus could have been boosted by the spore activator used in the inoculum preparation of *P. lilacinus*. The activator is quite rich in nutrients needed for growth by microorganisms. The compatibility of both organisms is further illustrated by non-inhibition of root colonization by AMF with *P. lilacinus* inoculation. However, the compatibility of AMF with the egg parasitic fungus differed amongst the different species of the mycosymbiont. The greatest gall and egg mass inhibition occurred when *G. etunicatum* and *G. deserticola* were individually combined with a double application of bionematicide. In this study, it is possible that the two beneficial microbes could have deployed various mechanisms mentioned previously to suppress the growth and infectivity of *M. incognita* on tomato. Growth, dry matter accumulation, and fruit yield were enhanced with the application of one or both of the control agents. Root galling by *M. incognita* is reported to impair water and nutrient uptake, translocation, photosynthesis, and more (Wiliamson and Gleason 2003). Reduction in root galling and nematode reproduction by *P. lilacinus*, and induction of systemic resistance/tolerance through an improved host nutrition or modification of mycorrhizosphere by AMF, could possibly account for the growth and yield enhancement. The highest fresh fruit yield was obtained when *G. etunicatum* and *G. deserticola* were separately combined with *P. lilacinus*. Coincidentally, these two species of AMF had the highest rate of root colonization (> 80%). In conclusion, the trial showed that the efficacy of AMF in controlling root-knot disease and growth enhancement in tomato, differed. The combined application of the two biocontrol agents was more effective than the individual application. The best combination was between the two AMF (*G. etunicatum* and *G. deserticola*) and double application of the bionematicide. Thus, combined application of both AMF and PL Gold^{TM} at the manufacturer's rate could be used to manage the pest in a more sustainable and eco-friendly way.

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