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Field Application of *Trichoderma* spp. for Controlling the Root- Knot Nematode, *Meloidogyne javanica* in Peanut Plants

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ABSTRACT

A field experiment was conducted to study the effect of three *Trichoderma* species, *T. harzianum*, *T. viride* and *T. virens* on root- knot nematode, *Meloidogyne javanica* infecting peanut plants, compared to commercial product Bio-Nematon® (*Purpureocillium lilacinum*) and chemical nematicide of Oxamyl®. The averages of total microbial soil community, i.e. aerobic bacteria, spore-forming bacteria and fungi in peanut rhizosphere as well as the frequency % of common fungi with different treatments, during growing season, were recorded. Treatments significantly (P=0.05) suppressed J_2 in soil and J_2, females, galls and egg masses in roots and improved peanut plant growth and yield parameters. At mid-season, *Trichoderma* spp. had more effective nematicidal effects in reducing nematode parameters than Bio-Nematon® in some cases. The highest percentages reduction in J_2 in soil (being 81%) was recorded with *T. viride* followed *T. harzianum* (77%) and *T. virens* (73%), compared to untreated control. At harvest time, *T. viride* recorded the highest nematode reduction in J_2 in soil (68%), but *T. harzianum* recorded the highest reduction in total developmental stages of nematode (68%), galls (84%) and egg masses (84%), compared to *T. viride*, *T. virens* and Bio-Nematon®. The treated soil rhizosphere differed in total microbial counts as well as frequency of common fungi according to the tested treatment that increased total microbial counts. The treatments highly increased the growth and yield criteria of peanut as indicated by fresh & dry weights of plants as well as number & weight of pods and weight of 100 seeds of peanut plants.

***Keywords*:** Biological control, Field application, *Trichoderma* spp., *Meloidogyne javanica*, Peanut

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is very substantial annual herb legumes in tropical and subtropical areas of the world. The peanut is very important food and oil seed crop and well known as a monetary source in the semi-arid tropics. It is rich in energy and contains health benefit nutrients, minerals, antioxidants and vitamins which are considered an essential for optimum health (Settaluri et al., 2012). In Egypt, peanut is grown in light soils as well as in recently reclaimed sandy areas, where the cultivated area reached to being 190.000 hectare for the season 2017 (Anonymous, 2017). The production of peanut is negatively affected by soil borne fungi as well as plant-

parasitic nematodes (PPNs), where root-knot nematodes, *Meloidogyne* spp., are among the major nematodes species that attack peanut plants (Abd-Elgawad, 2014). The most destructive soil-borne fungi, *Rhizoctonia solani* and *Fusarium oxysporum*, which attack peanut causing quantitative and qualitative losses of peanut yield (Ziedan, 2000 and Abd-El-Khair et al., 2016).

The PPNs cause great damage to agricultural production on various crops all over the world. Because of the toxic effect of chemical nematicides, it is necessary to develop non-chemicals and eco-friendly control strategies for controlling nematodes. Therefore, *Trichoderma* spp. may play important roles in biological control of soil borne pathogens or PPNs (Papavizas, 1985 and Bastakoti et al., 2017). *T.harzianum* and *T. lignorum* could decrease the root-galling index and the number of eggs on roots and improve the growth parameters of nematode-infected plants in short-term experiments. In a long-term experiment, the treatments could improve the growth and yield in nematode-infected plants (Spiegel and Chet, 1998). *T. harzianum* also could control the root galling of root-knot nematode (*Meloidogyne javanica*) and increased the fresh weight in nematode-infected tomatoes in greenhouse conditions (Sharon et al., 2001). Shawky et al. (2010) mentioned that *T. harzianum* highly increased the juvenile mortality of *M. javanica*, at all the exposure periods, especially after 72 hrs *in vitro* tests. Under greenhouse conditions, *T. harzianum* increased the total fresh weights of shoots and roots, numbers of branches & pods, plant height and pods weight/plant of peanut. *T. harzianum* and *T. viride*, singly or mixture, increased the percentages of juvenile mortality and inhibited the egg hatching of *M. javanica in vitro* tests and field applications (Metwaly et al., 2015). Also, they could suppress the reproduction of nematodes and reduce the root galling as well as increase the tomato plants growth in greenhouse experiment. The antagonistic effect of both fungi was increased with increasing densities of their inocula. Results revealed that *T. harzianum* was better efficacy, than *T. viride* (Al- Javeed, 2016).

Therefore, this present study was conducted to study the effect of *Trichoderma* spp., i.e. *T. harzianum*, *T. viride* and *T.virens* in comparison to the commercial product, Bio-Nematon® (*Purpureocillium lilacinum* formally known *as Paecilomyces lilacinus*) and chemical nematicide of Oxamyl® against root- knot nematode, *M. javanica* and their effect on soil bacterial and fungal communities in peanut plants, grown in field naturally infested with nematode.

MATERIALS AND METHODS

1. Initial soil sample collection

Soil samples were randomly collected, from each experimental plot, naturally infested with root-knot nematode (*Meloidogyne javanica*) to determine its initial second juvenile stages (J_2s) according to the method described by Barker (1985). Therefore, five sub-samples (each of it 250g) were collected from each plot at a depth of 15-30 cm one week prior to planting time. Then, soil sub-samples of each plot were mixed well together and then an aliquot of composite soil sample (about 250g) was used for nematode extraction.

2. Source of peanut seeds

Seeds of peanut cv. Giza 6, which is susceptible to *M. javanica*, were obtained from Department of Horticulture, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

3. *Trichoderma* spp. isolation and preparation

Three of *Trichoderma* species i.e. *T. harzianum*, *T. viride* and *T.virens* were isolated and identified in Plant Pathology Department, National Research Centre, Egypt. Sorghum: Sand: Water (2:2:1, V: V: V) medium in plastic bags were prepared. Then, medium bags were sterilized at 121ºC for 30 min. Each medium bag was separately inoculated with each *Trichoderma* spp. and then incubated at 30 °C±2 for 14 days. Each inoculum of *Trichoderma* spp. was adjusted to 1 X 10^8 propagules and applied at 200g per m^2. The commercial product of Bio-Nematon® (*P.lilacinum*, 10^8unit /ml) and chemical nematicide of Oxamyl® at 3 litre per Feddan were applied for comparison.

4. Field experiment

The experiment were conducted, during growing season of 2019,in a clay loam soilnaturally infested with *M. javanica* under a spraying irrigation system by overhead sprinklers in Mansoria Village, Giza Governorate, Egypt. The experiment consisted of 21 plots each (3 x 2m^2) in area. Each plot composed of three rows with 10 holes per row. Each row was 3 m in length, 20 cm in height and 40 cm in width. *Trichoderma* spp. isolates were applied, as soil treatment before sowing, at the rate of 200 g/m^2. Sorghum medium only was applied for comparison control. Bio-Nematon® and chemical nematicide of Oxamyl® were applied at recommended rates as mentioned previously. After application, surface disinfected peanut seeds (cv. Giza 6) were sown at the first week of Mayof growing season in all treatments at the rate of two seeds /hole. Three plots were used as replicates for each treatment as well as the untreated controls. Irrigation, recommended fertilizer levels and agronomical practices were followed as usual in the reclaimed sandy soils without adding chemicals. The applied treatments were as follows: 1-*T. harzianum*, 2- *T. viride*, 3-*T. virens*, 4-Bio-Nematon®, 5-Oxamyl®, 6-Sorghum medium only and 7-untreated control.

4.1. Determination of the effect of *Trichoderma* spp. on:

4.1.1. Root-knot nematode, *Meloidogyne javanica*

Effects of *Trichoderma* spp., Bio-Nematon® and Oxamyl® on population of *M. javanica* were determined at midgrowing season as well as at harvest time of peanut plants. Five sub-samples (plant rhizosphere soil and roots) were collected from each treated plot as well as the untreated controls. The soil sub-samples were thoroughly mixed and the composite sample of each plot was processed. Finally soil nematode population was extracted by method described by Barker (1985) and expressed as J_2 in 250g soil. The infested roots were gently taken off from soil, washed with tap water to free of debris, cut into approximately 1-2 -cm pieces and mixed with water and the numbers of the nematode stages inside 5g roots (J_2, females and egg), were extracted by electric blender, galls and egg masses on plant roots (5g) were also determined by using incubation method described by Young (1954). All numbers of nematode parameters were counted using a light microscope.

The reduction percentages of J_2 nematode in soil were determined according to Henderson and Tilton formula (Puntener, 1981) as follows:

Nematode reduction % = {1- (PTA/ PTB x PCB / PCA)} x 100

where; PTA= population in treated plot after application; BTB= population in the treated plot before application; PCB= population in the check plot before application and PCA= population in the check plot after application.

The percentages of nematode reduction in total nematode stages inside the roots (J_2, females, eggs), galls and egg masses on roots per 5g were calculated with respect to untreated control (nematode only) as follows.

Nematode reduction (%) =	Untreated control-Treated / Untreated control	X 100

4.1.2. Root nodules numbers, plant growth and yield parameters

Effects of *Trichoderma* spp., Bio-Nematon® and Oxamyl® on number of roots bacterial nodules /plant, growth parameters of peanut plants viz. Fresh & dry weights of plant (g) as well as the peanut yield parameters viz. no. of pods / plant, weight of pods per plant (g) and weight of 100 peanut seeds were recorded. Five peanut plants from each treated plot and untreated control, at mid grown season as well as at harvest time were obtained. The increases percentages in growth as well as yield parameters of plant in each treatment were calculated according to the following formula:

Increase (%) =	Treated - Untreated control / Untreated control	X 100

4.1.3. Total microbial counts

Effects of *Trichoderma* spp. on total count of aerobic bacteria, spore-forming bacteria and fungi in the rhizosphere of peanut plants were determined at one month after sowing as well as at mid and end of growing season by dilution method using plate count technique (Ghini et al., 2007) on suitable medium (Bridson, 1995). Five-soil samples (200-g-soil) were taken from each plot at a depth of 15-30 cm and then the composite sample of each plot was processed as follows. Ten grams of each composite soil sample were separately shaken in 90 ml of sterilized distilled water in a 250 ml flask to give a dilution of 10^{-1}. Then, serial dilutions of each fresh soil sample suspension were prepared up to 10^{-7}, by transferring 1 ml of sample suspension to 9 ml sterilized distilled water in a test tube under sterile conditions. Four plates were prepared as replicates for each dilution of soil sample. Aliquots 1.0 ml of 10^{-5} - 10^{-7} dilution were transferred separately into sterilized Petri plates filled with nutrient glucose 2% agar (NA) medium (Peptone 5 g, Beef extract 3 g, Glucose 20g , Agar 15 g, Distilled water 1L, pH 7) for determining the aerobic bacterial count. After 2 days of incubation at 30ºC ± 2, the resulted bacteria were recorded as a number of colony-forming units (CFU) per 10 g of soil. The sample dilution of 10^{-1} was pasteurized, at 80 °C for 20 min, to determine the spore-forming bacteria count. Aliquots of 1.0 ml of 10^{-3}-10^{-5} dilutions was transferred separately into sterilized Petri plates filled with NA. Then, the plates were incubated for 2 days at 30 ºC±2. The resulted spore-forming bacteria were recorded as CFU/10 g of soil. Aliquots of 1.0 ml of each 10^{-3} and 10^{-4} dilution were transferred separately into sterilized Petri plates

filled with Martin medium (Glucose 10g, Peptone 5g, KH_2PO_4 1g, $MgSO_4$ 0.5g, Rose Bengal 30µg, streptomycine 0.03g, Agar 15g, Distilled water 1 L) to count the total fungi. The inoculated plates were incubated at 30ºC ±2 for 7 days and then the count of the resulting fungi was recorded. Then, the total microbial counts, as Log_{10} CFU/10g soil, were recorded as averages of microbial count atthree above sampling periods (Hammam et al., 2019).

4.1.4. Frequency % of common fungi

Effect of tested treatments on the frequency percentages of common fungi in the peanut rhizospheres was determined as CFU per 10 g of soil on Martin medium as mentioned before (Ghini et al., 2007). Five replicated plates were applied for each dilution per soil sample. Then, the plates were incubated at 30ºC±2 for 7 days. The resulted fungi were identified to genera and species level according to the key of morphological and cultural characters described by Ellis (1971) and Barnett & Hunter (1972). Each isolated fungus was counted and its frequency percentage was calculated as averages of counts of three above sampling periods, according to the following formula:

Frequency of common fungus (%) = (Fungus no. / Total fungi no.) X 100

5. Statistical analysis

Data of nematode reproduction and plant growth parameters were subjected to analysis of variance (ANOVA) using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co., USA. The means were compared with Duncan's multiple range tests (Snedecor and Cochran, 1999).

RESULTS

1-Effect of *Trichoderma* spp. on root-knot nematode parameters

Effects of the tested species of *Trichoderma*, in comparison with Bio-Nematon® and Oxamyl® on nematode parameters of *M. javanica*, at mid grown season and at harvest time, are summarized in Tables (1 & 2). The obtained data showed that all treatments had the potential activity to reduce the root–knot nematode reproduction, compared to untreated control. At mid-season, the nematicidal effects of applied *Trichoderma* spp. were more effective in reducing J_2 in soil, than Bio-Nematon® . The highest percentages reduction in J2 in soil was recorded with *T.viride* (being 81%), followed by *T. harzianum* (77%), *T. virens* (73%) and Bio-Nematon® (54%), compared to untreated control. The results showed that Bio-Nematon® gave the highest reduction in total developmental stages (63%) and galls (70%), followed by *T. harzianum* (60, 63%), *T. viride* (54, 59%) and *T. virens* (45, 56%), compared to untreated control, respectively. Bio-Nematon® also highly reduced the number of egg masses (67%), followed by *T. virens* (60%), *T. harzianum* (53%) and *T. viride* (53%), compared to untreated control (Table 1).

At harvest time, *T. viride* (68%), recorded the highest nematode reduction in J2 in soil, followed by *T. harzianum* (58%), *T. virens* (47%) and Bio-Nematon® (40%), compared to untreated control (Table 2).

Table 1: Effect of *Trichoderma* spp. on *Meloidogyne javanica* parameters in rhizosphere and roots of peanut plants, under field conditions, at mid growing season.

Treatments	Nematode parameters											
	J_2 in soil (250g)			Developmental stages					Galls		Egg masses	
				J_2 in roots (5g)	Females	Eggs	Total					
	Initial	Mid - season no.	Red. %				No.	Red. %	No.	Red.%	No.	Red. %
T. harzianum	111[a]	201[d]	77	120[c]	120[b]	240[c]	480[d]	60	10[d]	63	7[c]	53
T.viride	98[ab]	143[c]	81	110[c]	110[b]	330[b]	550[c]	54	11[cd]	59	7[c]	53
T.virens	95[b]	196[d]	73	110[c]	110[b]	440[a]	660[b]	45	12[c]	56	6[cd]	60
Bio-Nematon®	89[b]	315[c]	54	90[d]	90[c]	270[c]	450[d]	63	8[e]	70	5[d]	67
Oxamyl®	93[b]	121[f]	83	80[d]	80[c]	160[d]	320[e]	73	7[e]	74	6[cd]	60
Sorghum	86[b]	428[b]	35	240[b]	240[a]	160[d]	640[b]	47	16[b]	41	11[b]	27
Untreated	90[b]	698[a]	-	480[a]	240[a]	480[a]	1200[a]	-	27[a]	-	15[a]	-

* Means in each column, followed by the same small letter are not significantly different according to Duncan`multiple range test (P = 0.05).

Table 2: Effect of *Trichoderma* spp. on *Meloidogyne javanica* parameters in rhizosphere and roots of peanut plants, under field conditions, at harvest time.

Treatments	Nematode parameters											
	J_2 in soil (250g)			Developmental stages					Galls		Egg masses	
				J_2 in roots (5g)	Females	Eggs	Total					
	Iinitial	No.	Red.%				No.	Red.%	No.	Red.%	No.	Red.%
T. harzianum	111[a]	900[e]	58	70[c]	140[c]	280[d]	490[g]	68	16[e]	84	11[d]	84
T.viride	98[ab]	600[f]	68	140[b]	210b[c]	350[c]	700[d]	54	19[d]	81	13[d]	81
T.virens	95[b]	975[d]	47	80[c]	240[b]	240[d]	560[f]	63	24[c]	77	18[c]	73
Bio-Nematon®	89[b]	1011[c]	41	60[c]	180[bc]	360[c]	600[e]	60	20[d]	80	12[d]	83
Oxamyl®	93[b]	391[g]	78	50[c]	270[b]	450[b]	770[b]	49	18[de]	82	14[cd]	80
Sorghum	86[b]	1244[b]	25	100[bc]	250[b]	375[c]	725[c]	52	30[b]	71	25[b]	64
Untreated	90[b]	1743[a]	-	300[a]	550[a]	660a	1510[a]	-	102[a]	-	70[a]	-

* Means in each column, followed by the same small letter are not significantly different according to Duncan`s multiple range test (P = 0.05).

On the other hand, *T. harzianum* recorded the highest reduction in total stages of nematode (being 68%), followed by *T. virens* (63%), Bio-Nematon® (60%) and *T. viride* (54%) compared to untreated control. *T. harzianum* (84%) also recorded the highest reduction in number of galls, followed by *T. viride* (81%) and Bio-Nematon® (80%) and *T. virens* (77%), compared to untreated control. The highest reduction in egg masses was recorded with *T. harzianum* (84.3%), followed by Bio-Nematon® (83%), *T. viride* (81%) and *T. virens* (73%), compared to untreated control. Results showed that Oxamyl® had the highest reduction against previous nematode criteria in some cases at mid growing season or harvest time. Sorghum medium gave the least nematode reduction, compared to untreated control (Table 2).

2-Effect of *Trichoderma* spp. on number of root nodules

At mid growing season, Bio-Nematon® significantly increased the number of root bacterial nodules being 100%, followed by *T. virens* (90%), *T. harzianum* (80%) and *T. viride* (40%), compared to untreated control. At harvest time, Bio-Nematon® also highly increased the number of root nodules being 40%, followed by *T. virens* (33%), *T. harzianum* (27%) and *T. viride* (20%), compared to untreated control. The reduction effects of Oxamyl® or Sorghum medium only are listed in Table (3).

Table 3: Effect of *Trichoderma* spp. on root nodules in peanut plants naturally infected with *Meloidogyne javanica*, under field conditions, at mid growing season and at harvest time.

Treatments	Root nodules			
	At mid-season		At harvest- season	
	No.	Increase %	No.	Increase %
T. harzianum	18^{bc}	80	38^{bc}	34
T. viride	14^{d}	40	36^{cd}	20
T. virens	19^{ab}	90	40^{ab}	33
Bio-Nematon®	20^{a}	100	42^{a}	40
Oxamyl®	15^{d}	50	40^{ab}	33
Sorghum	17^{c}	70	34^{d}	13
Untreated	10^{e}	-	30^{e}	-

* Means in each column, followed by the same small letter are not significantly different according to Duncan`s multiple range test (P = 0.05).

3-Effect of *Trichoderma* spp. on growth and yield parameters

At mid growing season, *T. viride* highly increased the peanut plant fresh weight being 147%, followed by *T. virens* (143%), *T.harzianum* (113%) and Bio-Nematon® (93%), compared to untreated control. significantly increased *T.harzianum* the plant dry weight being 183%, followed by *T. virens* (141%), *T. viride* (86%) and Bio-Nematon® (28%), compared to untreated control. Results cleared that Oxamyl® could increase the above plant growth parameters being 149 and 225%, while increases were 24 and 61%, with Sorghum medium only, respectively (Table, 4). Concerning to the peanut yield at harvest time, *T. virens* significantly increased the percentages of number of peanut pods/plant being 213%, followed by Bio-Nematon® (147%), *T. harzianum* (140%) and *T. viride* (133%), compared to untreated controls. *T. viride* significantly increased the percentages of weight of pod /plant being 169%, followed.

Table 4: Effect of *Trichoderma* spp. on growth and yield parameters of peanut plants naturally infected with *Meloidogyne javanica*, under field conditions, at mid of growing season and harvest time

Treatments	Growth and yield parameters									
	At mid season				At harvest time					
	Plant Fresh weight (g)		Plant Dry weight (g)		No. of pods/ plant		Weight of pods/plant		Weight of 100 seeds (g)	
	G	Inc. %	G	Inc. %	No.	Inc. %	G	Inc. %	G	Inc. %
T. harzianum	129.3^{b*}	113	18.1^{b}	183	36.0^{bc}	140	372.1^{c}	157	240.3^{d}	166
T. viride	150.1^{a}	147	11.9^{c}	86	35.0^{bc}	133	390.3^{b}	169	310.2^{b}	244
T. virens	147.1^{a}	142	15.4^{ab}	141	47.0^{a}	213	306.4^{d}	111	240.4^{cd}	167
Bio-Nematon®	117.5^{b}	93	8.2^{d}	28	37.0^{bc}	147	300.1^{d}	107	240.0^{c}	166
Oxamyl®	151.4^{a}	149	20.8^{a}	225	57.0^{a}	280	432.7^{a}	199	440.4^{a}	388
Sorghum	75.2^{c}	24	10.3^{cd}	61	31.0^{c}	107	294.2^{d}	103	230.6^{d}	156
Untreated	60.8^{c}	-	6.4^{e}	-	15.0^{e}	-	144.9^{e}	-	90.2^{e}	-

* Means in each column, followed by the same small letter are not significantly different according to Duncan`s multiple range test (P = 0.05).

by *T. harzianum* (157%), *T. virens* (111%) and Bio-Nematon® (107%), compared to untreated control *T. viride* significantly increased the percentages of weight of 100 peanut seeds being 244%, followed by *T. virens* (167%), *T. harzianum* (166%) and Bio-Nematon® (166%), compared to untreated control. Oxamyl, and significantly increased percentages of above yield parameters (weight of pod /plant and the weight of 100 peanut seeds) being 280, 199 and 388%, while Sorghum medium only improved the percentages of yields by 107, 103 and 156, respectively (Table 4),

4 -Effect of *Trichoderma* spp. on microbial counts

Total microbial counts of aerobic bacteria, spore-forming bacteria and fungi in peanut rhizosphere, with different treatments, as averages of microbial count at different sampling times during growing season, are listed in Table (5). The total aerobic bacterial counts were in the ranges of 6.06 to 6.24 log10 CFU/10g soil with *Trichoderma* spp., higher than 5.88, 5.95, 5.91 and 5.89 log10 CFU/10g soil with Bio-Nematon®, Oxamyl®, Sorghum medium only and untreated control, respectively. The highest bacterial count was recorded with *T. harzianum*, followed by *T. viride* and *T. virens*, respectively. The spore-forming bacteria counts were in the ranges of 4.65 to 4.82 log10 CFU/10g soil with *Trichoderma* spp., where the highest count was recorded with *T. harzianum* , followed by *T. virens* and *T. viride* , higher than 44.53 , 4.43 , 4.56 and 4.32 log10 CFU/10g soil with Bio-Nematon®, Oxamyl®, Sorghum medium and untreated control, respectively. The total fungi counts were ranged from 4.91 to 4.97 log10 CFU/10g soil with *Trichoderma* spp., higher than 4.82 ,4.85, 4.85 and 4.80 log10 CFU/10g soil with treatments of Bio-Nematon®, Oxamyl®, Sorghum medium and untreated control, respectively. No significant differences were recorded among treatments. The highest fungal count was recorded with *T. viride*, followed by *T. virens* and *T. harzianum* (Table 5).

Table 5: Effect of *Trichoderma* spp. on total microbial counts (as Log10 CFU/10g soil) in rhizosphere of peanut plants naturally infected with *Meloidogyne javanica*, under field conditions.

Treatments	Averages of $\log_{10}$ of total microbial counts (CFU/10g soil)		
	Aerobic bacteria (10^5)	Spore-forming bacteria (10^4)	Total fungi (10^4)
T.harzianum	6.24[a]	4.82[a]	4.91[a]
T.viride	6.09[b]	4.65[b]	4.97[a]
T. virens	6.06[b]	4.69[ab]	4.95[a]
Bio-Nematon®	5.88[c]	4.53[bc]	4.82[a]
Oxamyl®	5.95[c]	4.43[cd]	4.85[a]
Sorghum	5.91[c]	4.56[bc]	4.85[a]
Untreated	5.89[c]	4.32[d]	4.80[a]

* Means in each column, followed by the same small letter are not significantly different according to Duncan`s multiple range test (P = 0.05).

5- Effect of *Trichoderma* spp. on frequency % of common fungi

Results revealed that the fungi of *Aspergillus* spp., *A. niger, Fusarium* spp., *Penicillium* spp., *Rhizopus nigricans*, *Trichoderma* spp. and others (Unidentified

Table 6: Effect of *Trichoderma* spp. on the frequency (%) of common fungi in the rhizosphere of peanut plants naturally infected with *Meloidogyne javanica*, under field conditions.

Treatments	Frequency % of common fungi						
	Aspergillus spp.	*Aspergillus niger*	*Fusarium* spp.	*Penicillium* spp.	*Rhizopus nigricans*	*Trichoderma* spp.	Others
T.harzianum	19.3^{ab}	10.0^{bc}	10.4^{bc}	25.8^{a}	1.9^{c}	28.8^{a}	3.8^{c}
T.viride	23.3^{a}	9.3^{b}	6.3^{b}	22.3^{a}	2.2^{b}	31.6^{a}	5.0^{b}
T. virens	17.5^{b}	13.9^{c}	6.3^{d}	18.4^{b}	2.5^{e}	36.5^{a}	4.9^{d}
Bio-Nematon®	28.8^{a}	11.6^{b}	10.0^{b}	31.9^{a}	4.2^{b}	6.9^{b}	6.6^{b}
Oxamyl®	30.7^{a}	11.4^{b}	11.4^{b}	28.0^{a}	2.8^{c}	10.6^{b}	5.1^{c}
Sorghum	23.9^{a}	19.4^{aS}	8.1^{b}	22.5^{a}	1.7^{b}	18.7^{a}	5.7^{b}
Untreated	27.9^{a}	13.8^{b}	13.1^{bc}	29.3^{a}	3.6^{d}	7.9^{bc}	4.4^{cd}

*Means in each column, followed by the same small letter are not significantly different according to Duncan`s multiple range test (P = 0.05).

fungi) were the common fungi in the rhizosphere of peanut plants under field conditions. Details of the frequencies % of common fungi are listed in Table (6). The treatments of *Trichoderma* spp. increased the frequency of above fungi were in the ranges of 17.5 – 23.3%, 9.3 – 13.9%, 6.3 – 10.4%, 18.4 – 25.8%, 1.9 – 2.5%, 28.8 – 36.5 % and 3.8- 5.0%, respectively. On the other hand, in the treatments of Bio-Nematon®, Oxamyl® and Sorghum medium the frequencies of the same fungi were in the ranges of 23.9-30.7%, 11.4-19.4%, 8.1-11.4%, 22.5-31.9%, 1.7-4.2%, 6.9-18.7% and 5.1- 6.6%, respectively. In untreated control, the common fungi frequencies % were 27.9, 13.8, 13.1, 29.3, 3.6, 7.9 and 4.4%, respectively (Table 6).

DISCUSSION

Plant-parasitic nematodes cause serious damages to many organically grown vegetables and crops, where *Meloidogyne* spp. (root-knot nematodes) is one of the most destructive plant-parasitic nematodes. Application of chemicals becomes widely used, but application of biological approach has a better solution, through applications of bio-agents for reducing the population of pests infecting crops. Especially, biological products were developed and with more time become marketable worldwide. Our results showed that *Trichoderma* spp. had suppressive effects on the parameters of *M. javanica* viz. J_2 in soil and J_2, females, galls and egg masses in rhizosphere or roots of peanuts under naturally infestation conditions, where the tested *Trichoderma* spp. subsequently increased the plant growth parameters of peanuts viz. Fresh & dry weights of plant as well as the peanut yield parameters viz. no. of pods /plant, weight of pods per plant and weight of 100 seeds. These results are agreement with those obtained by many workers as follows; *T. harzianum* could suppress the soil nematode population and root galls of root-knot nematode in some soybean varieties as well as increased the plant height, the number of branches and yield of soybean (Izuogu and Abir, 2015). *T. harzianum* could inhibit the egg-hatching and immobilize the juveniles of *M. incognita in vitro* tests. The fungus also could manage the root-knot nematode in tomato, under greenhouse conditions, by reducing incidence, population, reproduction rate as well as number of galls and egg masses per plant (Feyisa et al., 2015). El-Nagdi and Abd-El-Khair (2017) cleared that *T. harzianum* highly reduced numbers of J_2 in soils or roots and galls or egg masses of *M. incognita*, followed by *T. virens* and *T. viride*. The treatments could increase the growth parameters of cowpea viz., length of shoot, weight of fresh and dry shoot and number of leaves. Abd-El-Khair et al. (2018) showed that *T. harzianum* and *T. virens* combined reduced the *M. incognita* parameters in soil & roots of eggplants in pots experiment. The treatments could increase the growth parameters of eggplants (Abd-El-Khair et al., 2018). *T. harzianum* either singly or combined with *Saccharomyces cerevisiae* showed the most nematode suppressive effect on *M. javanica*, as well as improved the peanut yield production, plant growth parameters and seed nutrient contents of peanut plants (Osman et al., 2020).

Our results revealed that *Trichoderma* spp. could increase the soil microbial community as total counts or frequency of fungi, comparing with other treatments as well as untreated control. Results cleared that all treatments highly reduced frequencies% of *Fusarium* spp., where *T.viride* and *T.virens* resulted in the highest reduction, compared to untreated control. The frequencies of *Aspergillu*s spp. or *Penicillium* spp. were highly increased in the rhizosphere treated with Bio-Nematon®, Oxamyl® and Sorghum, more than *Trichodema* spp. treatments. These obtained results in agreement with those recorded by Korayem et al. (2019). They documented

that *Aspergillus* spp., *A. niger*, *Fusarium* spp., *Penicillium* spp. as well as *Trichoderma* spp. commonly occurred in the rhizosphere of wheat grown in different governorates of Egypt. *T. hamatum* and *T. album* could significantly reduce the occurrence of *R. solani* and *F. solani*. The treatments could increase the frequency of *Aspergillus* spp., then *A. niger*, *Penicillium* spp. and *Trichoderm*a spp. comparing to the controls (Abd-El-Khair and El-Nagdi, 2014).

Trichoderma spp., as biocontrol agents against many plant pathogens, is able to infect nematode eggs and juveniles of *M, javanica* according to greenhouse and laboratory studies. *T. harzianum* could significantly decrease the infection of nematode and others parameters. It was able to penetrate the nematode egg mass matrix as well as could significantly decrease the nematode egg-hatching levels by activating of specific resistance-related enzymes viz. POX (peroxidase), (PPO) polyphenol oxidase and (PAL) phenylalanine ammonia lyase in inoculated plants (Sahebani and Hadavi, 2008 and Hyder et al., 2017). For example, *T. harzianum* reduced the incidence or pathogenicity of *M. javanica* in tomatoes (Naserinasab et al., 2011). *Trichoderma* spp. are widely applied in agriculture may due to their well control mechanisms. The usage of these microbial inoculants in *Trichoderma*-based products could attract the attention of many researchers to discover more on others benefit of *Trichoderma* spp. Through research works worldwide, *Trichoderma* spp. successes in controlling of plant diseases, improving plant growth as well as decomposition or bioremediation processes. Their secondary metabolites production play an important role in agro ecosystem and could be applied as–environmentally friendly practices (Zin and Badaluddin, 2020).

Trichoderma spp. includes a great number of strains which has potential producers of bioactive secondary metabolites against plant parasitic nematode *M. incognita*. *T.harzianum* and *T. viride* showed the highest reduction juveniles (J_2s) mortality and could inhibit egg-hatching of *M. incognita* (Khan et al., 2020). The penetration and colonization of the roots by *T. harzianum* enhanced the activity of pathogenesis related proteins up to 72 h post-inoculation. This effect includes the coiling of hyphal and formation of aspersoria. Application of *T. harzianum* (T-203) with cucumber roots highly activated chitinase, 1,3-glucanase, cellulase, and peroxidase, up to 72 h post-inoculation, while a chemical inducer treatment responses the plant defence through, 2,6-dichloroisonicotinic acid. The association of *Trichoderma* with roots reduces the root disease through activating the defense response of plants (Yedidia et al., 2000). *Trichoderma*, mycorrhizal or endophytic fungi are filamentous fungi successfully applied for biological controlling as agents against nematodes , as resistance inducers, for reducing the nematode damages by directly parasitism, antibiosis, paralysis or lytic enzymes production as well as minimizing the harms by spacing or resource-competition, by providing higher nutrients and water uptake to the plant, or by modifying the roots morphology. The bio-agent fungi could induce resistance on nematodes through activation of hormone-mediated viz. salicylic and jasmonic acid, strigolactones among others as plant-defense mechanisms. Altering the transport of chemical defense components through the plant or the synthesis of plant secondary metabolites and various enzymes can also contribute to enhancing plant defenses (Poveda et al., 2020).

CONCLUSION

In conclusion, the present study demonstrated that *Trichoderma* spp. and *Purpureocilliun lilacinum* can provide satisfactory control of root-knot nematode, *M.*

javanica in peanut. Biocontrol agent did not only have suppressive effects against nematodes, but also affect the total microbial counts of aerobic bacteria, spore-forming bacteria and fungi and increased the parameters of the growth and pod of peanuts.

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الملخص العربى

التطبيق الحقلي لفطر التريكوديرما في مكافحة نيماتودا تعقد الجذور *Meloidogyne javanica* فى نباتات الفول السودانى

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صممت تجربة حقلية لدراسة تأثير ثلاثة أنواع من الجنس *Trichoderma* وهم *T. harzianum*، *T. viride* و *T. virens* على نيماتودا تعقد الجذور(*Meloidogyne javanica*) التي تصيب نبات الفول السوداني ، مقارنة بالمنتج التجاري (*Purpureocillium lilacinum*) Bio-Nematon® و المبيد النيماتودا الكيميائي Oxamyl®. تم تسجيل متوسطات العدد الكلى الميكروبي للبكتيريا والبكتيريا المتجرثمة والفطريات في ريزوسفير الفول السوداني بالإضافة إلى نسب تكرار الفطريات الشائعة مع المعاملات المختلفة خلال موسم النمو. أدت المعاملات المعنوية (P=0.05) إلى تقليل أعداد الطؤر اليرقى المعدى الثانى في التربة و الطؤر اليرقى المعدى الثانى والإناث وكتلة البيض في الجذور، وحسن صفات النمو الخضرى والمحصول لنبات الفول السوداني. في منتصف الموسم امتلك *Trichoderma* spp. فاعلية عالية كمبيد نيماتودى أكثر من Bio-Nematon® في بعض الحالات. أعلى نسب مئوية للانخفاض في J_2 في التربة حوالى (81%) سجل مع *T. viride*، تليها *T. virens* (73٪) و *T. harzianum* (77٪) ، مقارنة بالمعاملات غير المعالجة. عند وقت الحصاد ، سجل *T. viride* أعلى إنخفاض لاعداد J_2 في التربة (68٪) ، لكن سجل *T. harzianum* أعلى إنخفاض في إجمالي مراحل تطور النيماتودا (68٪) ، عقد الجذور النيماتودية (84٪) وكتل البيض 84٪) مقارنة بـ *T. viride* و *T. virens* وBio-Nematon®. .إختلفت الريزوسفير المعامل في إجمالي عدد الميكروبات وكذلك تكرار الفطريات الشائعة وفقًا للمعاملات المختبرة التي زادت من إجمالي عدد الميكروبات. زادت المعاملات بشكل كبير من صفات النمو الخضرى والإنتاجية للفول السوداني كوزن طازج وجاف للنبات وكذلك عدد ووزن القرون ووزن 100 بذرة من نبات الفول السودانى.