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DIRECT & INDIRECT EFFECT OF SOME BIOCONTROL AGENTS AGAINST ROOT-KNOT NEMATODES ON TOMATO

Galaal, Neveen M.[2]; Ayoub, Shymaa S. ; Mousa, E.M[1]. and Mahdy, M.E[1].

[1]Dept. of Agric. Botany, Fac. of Agric., Menoufia Univ., Shebin El-Kom, Egypt.

[2] Dept. of Nematology, Plant Pathology Research Institute, Agric. Res. Centre, Giza, Egypt.

ABSTRACT

In this research three different biocontrol agents namely : *Bacillus megaterium , Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) were tested as a biocontrol agents against root-knot nematodes , *Meloidogyne* spp. on tomato plants under greenhouse conditions by either direct or indirect effect. **Results of direct effect** revealed that the effective treatment with (*B. megaterium* + *T.harzianum* + AMF 4% + Nematode) reduced all related nematode parameters i.e. number of galls, egg masses, females and number of juveniles / 250 g soil, in comparing with plants treated with nematode alone. The highest significant reduction in galls, egg masses, females and J2 / 250g soil were 97, 97, 97 and 95%, respectively, followed by the treatment of *B. megaterium* + AMF 4% + Nematode by 95, 96 , 95 and 93% ,respectively. **Results of indirect effect** confirmed that the treatment of *B. megaterium* +*T.harzianum* + AMF 4% + Nematode reduced all related nematode parameters comparing with nematode alone by 93, 94, 93 and 95% , respectively , followed by *B. megaterium* + AMF 4% + Nematode by 92, 92 , 92 and 92%, respectively. Results found significantly enhancement in all vegetative plant growth parameters i.e. fresh shoot and root weights (g), dry shoot weight (g), plant height and root length (cm). Results also showed that all treatments significantly encourged antioxidant enzymes activity i.e. peroxidase and phenoloxidase. Membrane leakage% showed also highly significant decrease with all treatment compared with the plant treated with nematode alone.

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Key Words : **Root-knot nematodes *Meloidogyne* spp. ; *Bacillus megaterium* , *Trichoderma harzianum* , Arbuscular Mycorrhizal Fungi (AMF) ; Tomato (*Lycopersicon esculentum* Mill).**

INTRODUCTION

Root-knot nematodes *Meloidogyne* spp. are obligate endo-parasites and very damaging plant pests which are considered to be limited factor in crop production and agricultural productivity **(Bakr *et al*., 2011 and Ibrahim, 2011)**. Most cultivated plant species are susceptible to root-knot nematode infection **(Sasser and Carter, 1985)**. Root-knot nematodes attack more than 2000 species of plants and almost all cultivated plants such as vegetables, fruit trees or namentals in Egypt. It is are becoming serious pests to most vegetable crops, especially tomato plants and cause severe yield losses in new reclaimed soil.

Chemical nematicides are considered the most effective method in suppressing and controlling root-knot nematodes, but its means environmental pollution and very expensive in price **(Adegbite and Adesiyan, 2001; Abd-Elgawad, 2008).** During the last decades, nematologists world wide searched for a cheaper, safer and eco-friendly alternatives methods i.e. biological and cultural methods to control the plant parasitic nematodes.

Encouraging results were obtained with the use of (*Bacillus megaterium* , *Trichoderma harzianum* and Arbuscular Mycorrhizae Fungi) as a biocontrol agents of nematodes on different crops . Fungal strains grouped in the genus Trichoderma possess a wide spectrum of evolutionary responses that range from very effective soil colonization with high bio degradation potential, to non-strict plant symbiosis by strains colonizing the rhizosphere. Addition, some groups of strains with this conglomerate of biotypes are able to antagonize phytopathogenic fungi by using substrate colonization , antibiosis and mycoparasitism as the main mechanisms(**Hijeljord and Tronsmo ,1998).**

Trichoderma spp. have been described as biocontrol agents against plant parasitic nematodes . Several reports showed that *Trichoderma* spp. are

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able to suppress *Meloidogyne* spp. populations one of the most economical important group of plant parasitic nematodes world-wide and increase crop yield **(Sharon *et al.*,2001).** In addition to arbuscular mycorrhizas are characterized by the formation of unique structures , arbuscules and vesicles by fungi of the phylum Glomero mycota, and help plants to capture nutrients such as phosphorus , sulfur , nitrogen and micronutrients from the soil . It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonization of land by plants and in the evolution of the vascular plants. Mycorrhizal fungi colonized and protect the roots against nematode juveniles invasion. Therefore, the present investigation was planned to study some safety alternative methods to control plant parasitic nematodes using bacterial and fungal as a bio-control agents i.e. (*Bacillus megaterium* , *Trichoderma harzianum* and mycorrhizal fungi) either direct or indirect effect to manage root-knot nematodes *Meloidogyne* spp.

MATERIALS AND METHODS

In our research the experiment was carried out under greenhouse conditions at the farm of Faculty of Agriculture , Menoufia University , Shebin El-Kom to evaluate the effective bacteria isolate *Bacillus megaterium* and fungus isolate *Trichoderma harzianum* ,as well as arbuscular mycorrhizal fungi at the rate of 4% on *Meloidogyne* spp.

Direct effect :

Three weeks old tomato seedlings cv. Beto- 86 were transplanted into pots (15 cm in diam.) one seedling / pot . Each plastic pot containing 2 kg unsterilized sandy / clay mixture soil (2: 1 , v/v). Each plant inoculated with 3ooo eggs of *Meloidogyne* spp. Around roots one week after transplanting bacteria and fungi inocula were applied as a soil drench by receiving 10 ml for each plant and 80 g of mycorrhizal fungi (4%) each g contain 2500 spores . Plants watered daily and fertilized once a week with nutrient solution obtained from the international Egypt company for Agricultural and industrial Developing with 5 ml of 2 g/l (N: P : K , 20: 20 : 20). Two months from nematode inoculation plants were uprooted and their roots were carefully washed under running tap water . Nematode parameters i.e . number of galls ; egg masses ; females / root system and second stage

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juveniles / 250 g soil , final population (PF) as well as reproduction factor were determined .

Final nematode population (PF) was assessed according to the equation: PF = (No. of egg-masses × No. of eggs/ egg-mass) + No. of females + No. of developmental stages + No. of juveniles in soil. The reproduction factor of root-knot nematode (RF) was also recorded according to **Norton (1978).**

Egg masses were counted by staining the roots with phloxine- B (0.15g / L tap water) for 20 minutes as described by **Daykin and Hussey (1985) .** Females / root system were evaluated by submerging the roots in a beaker full of tap water at room temperature until they became softened . The roots were then washed through 250 and 500 μ sieves to separate the females and from the root debris , counted under a stereo-microscope (**Mahdy , 2002**) . Soil nematode juveniles (J_2s) were extracted and counted by using the traymodification of Baermann funnel as described by **Barker (1985)** . Plant growth parameters i.e. fresh shoot and root weights (g) and lengths (cm) were determined . Chemical composition i . e. antioxidant enzymes activity (phenoloxidase and peroxidase) as well as membrane leakage were also determined. Root infection with mycorrhizal fungi was also calculated according to **Phillips and Haynan (1970).** The percentage of root infection with mycorrhizal fungi was determined in 10 root sections from each mycorrhizal fungi treatment.

Indirect effect (split- root system):

Tomato seedlings were grown in a growth chamber at 25 ± 3ºC with 16 hrs diurnal light , 60- 70 % relative humidity , plants were daily watered and fertilized once a week with 5 ml of 2 g/l (N: P : K , 20 :20 :20). Three weeks later , or when the seedlings were about 25- 30 cm in height , all roots were removed from the plants by slicing them off from the shoot 5 cm above the basal end with a sterile knife . The basal part of the stems were then longitudinally split approximately 5 cm upwards into two

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halves and each half was then placed in the planting substrate of separate pots (Fig.1) . Each pot was filled with 2 kg of a mixture of sandy loamy soil (2: 1 , v/ v) and the pots maintained at 25 ± 2º C in the glasshouse . One week after splitting the stems of the tomato plants , the adventitious roots in one pot (designated "inducer side ") of the twin – pot set up were treated with mycorrhizal fungi at 4% , *B. megaterium* and *T. harzianum* as well as seedlings received distilled water served as a control . The other side of the twin – pot was termed the (responder side) and inoculated with 3000 eggs of *Meloidogyne* spp. one week after treating the inducer side of the split – root plants with the bioagents . Each treatment was replicated three times and the plants were arranged in a completely randomized block design . The non-treated plants served as absolute control. After 60 days from nematode inoculation, roots uprooted and carefully washed under running tap water to remove soil particles . Nematode related parameters i.e . number of galls , eggmasses, females / root system , J2 / 250 g soil , final population and reproduction factor was calculated according to **Hussey and Barker (1973)** . Growth parameters i.e. fresh shoot and root weights (g) and dry shoot weight (g) , plant height and root length (cm) were recorded . Chemical analysis were determined as mentioned before .The percentage of root infection with mycorrhizal fungi was determind in 10 root sections from each mycorrhizal fungi treatment .

Maintenance of mycorrhizal fungi culture

Cultures of mycorrhizal fungi were obtained from Agriculture Research centre , Giza , Egypt and raised on sorghum seeds , which were surface sterilized with Clorox solution 0,01% and sown (5 seeds/pot) in clay pot (25cm in diam.) containing sterilized steam sand soil . Fifty spores of mycorrhizal fungi / pot were layered at 2-6 cm depths in 50 clay pots. Thinning was done to maintain one seedling /pot . After 120 days, the plants were uprooted and the spores were extracted by wet sieving and decanting method from the pot soil and the roots were stained and examined for mycohrrizal colonization . The roots colonized by mycorrhizal fungi were mixed with sand soil containing mixed spores of

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genera; *Glomus* spp. , *Gigaspora* spp. and *Acaulospora* spp. as inoculum . The inoculum added to the soil spread as a layer at the rate of 100 g /pot at a depth of 3-5 cm at the same time of transplanting.

Extraction and counting of mycorrhizal fungi spores.

Mycorrhizal fungi spores were extracted from rhizosphere soil (250g) around the root system of each sorgum plants . The soil was taken and diluted with 1 liter tap water , suspended and then sieved using wet sieving and decanting technique according to **(Jackson, 1958).** Four sieves (400, 250, 150 and 75 Mm) were used in this experiment . The 250, 150 and 75 Mm fractions were transferred into a glass bottle and diluted with water to give between 40-50 spores / ml. The number of spores were estimated by spreading certain volume of mycorrhizal spore suspension onto a grided filter paper or petri-dish which was divided into squares from its base . The number was recorded using a binocular microscope (30 - 50X) according to **(Daft and Hograph ,1983).**

Estimation of root infection with mycorrhizal fungi :

The root samples were washed several times with tap water to remove remaining soil particles . Each sample was cut into small pieces (1cm long) and covered with 10% KOH in test tubes . Test tubes were autoclaved at pressure “1” and temperature 121ºC for about 20 minutes according the modified method by **Kormanik *et al .,* (1980**).Depending on the age and size of the root to remove the host cytoplasm and most of the cell nucleic acid to allow stains penetration . the roots were then washed with tap water and acidified with 1% HCL for 1-2 min ., then the acid was poured off . Roots were submerged in the typan blue stain (0,05%) in lactic acid and heated in water bath at 80-90 C for 10-15 min., according to **Phillips and hayman (1970).** The percentage of root infection with mycorrhizal fungi was determind in 10 root sections from each mycorrhizal fungi treatment.

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Physiological and biochemical parameters were assessed as follow:-

ı. Membrane leakage :

To follow the Physiological changes on roots tissue membrane permeability were washed carefully . The membrane leakage (%) was estimated following the method of **Leopold *et al* ., (1981)** using the following equation: Membrane leakage (M1 %) = T1/ T2 x 100

Where : T1 = Initial absorbance of bathing medium T2 = Final absorbance of bathing medium after the boiling for 15 min .

II . Antioxidant enzymes activity : Perioxidase and polyphenoloxidase activities were measured according to the method described by **Fehrman and Dimond (1967)** and **Broesh (1954)** respectively.

Statistical Analysis:

Data were statistical analyzed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics, Crop, Rockville, MD).

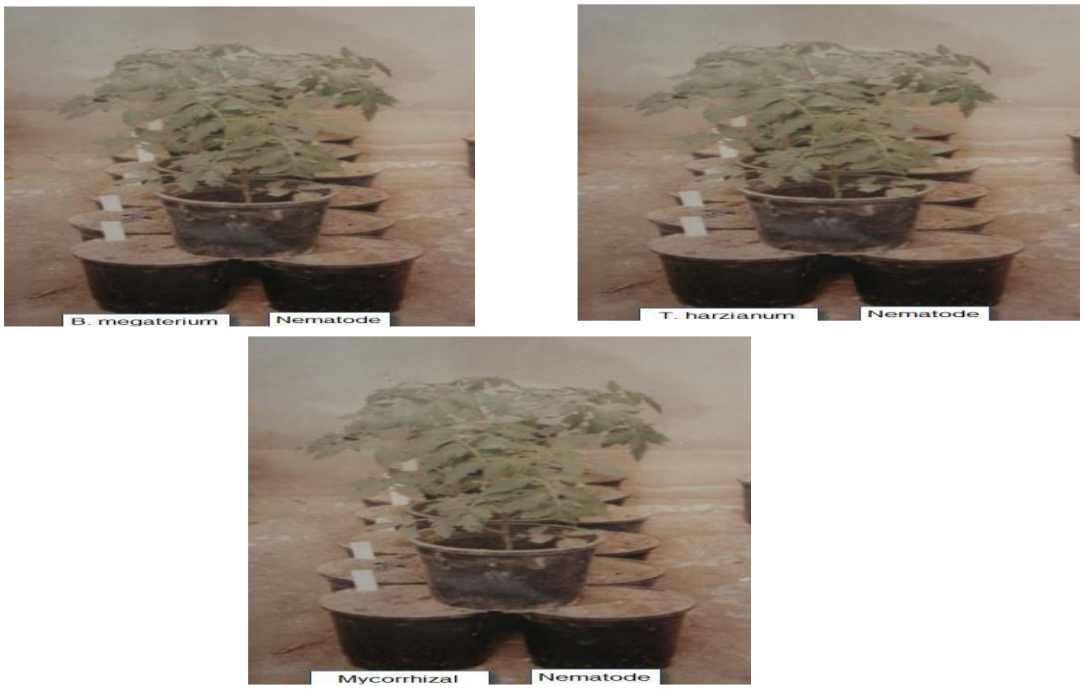

Fig.(1): Split root system technique as a method to control *Meloidogyne* spp.

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RESULTS

A- Direct Effect :

Data presented in table (1) and Fig.(2,3&4 ; A&B) showed that application the different treatments with soil pots significantly reduced the nematode parameters i.e. number of galls, egg masses, females / root system and number of J2 /250 g soil compared to nematode treated plants alone. The greatest reduction in nematode parameters was recorded with the treatment of all bioagents i.e *B. megaterium* +*T.harzianum* + AMF 4% + Nematode by 97, 97, 97 and 95% ,respectively, followed by the treatment of *B. megaterium* + AMF 4% + Nematode by 95, 96 , 95 and 93% ,respectively and (*T.harzianum* + AMF 4% + Nematode) by 94 , 94 , 94 and 90 % ,respectively . The lowest value of nematode reproduction noticed with *B. megaterium* + *T.harzianum* + AMF 4% + Nematode, followed by *B. megaterium* + AMF 4% + Nematode and *T.harzianum* + AMF 4% + Nematode . The greatest values in nematode reproduction were found by *T. harzianum* + Nematode , followed by *B. megaterium* + Nematode compared to plants treated with nematode alone. *Bacillus megaterium* + *T.harzianum* + AMF 4% + Nematode encouraged the reduction percentage of galls and egg masses by (97 & 97%) , respectively ,followed by *B. megaterium* + AMF 4% + Nematode by (95&96%), respectively as shown in Fig.(2 ,A&B). Figure (3,A&B) showed that *B. megaterium* +*T.harzianum* + AMF 4% + Nematode appeared highest effectiveness of reduction in females / root system by 97% and number of J2 /250 g soil by 95% , followed by *B. megaterium*+AMF4%+Nematode. *Bacillus megaterium* +*T.harzianum* + AMF 4% + Nematode recorded the highest reduction % in final population and reproduction factor , followed by *B. megaterium* + AMF 4% + Nematode as shown in Figure (4).

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Table (1) : Direct effect of different biocontrol agents on nematode parameters of tomato plants infected with *Meloidogyne* spp.

Treatment	Galls/ root system	**Reduction %**	Eggmasses/ root system	**Reduction %**	Females/ root system	**Reduction %**	J_2/250g soil	**Reduction %**	Final population (PF)	Reproduction factor (RF)
B.megaterium +N	18c	**86**	17.3c	**86**	17.7c	**31**	500c	**76**	553	0.18
T.harzianum+ N	25b	**80**	23b	**81**	23.3b	**81**	650b	**69**	721	0.24
AMF4% +N	10de	**92**	9de	**93**	9.7de	**92**	276.7e	**87**	305	0.102
B.megaterium + AMF4% +N	6ef	**95**	5.3ef	**96**	5.7fg	**95**	150fg	**93**	167	0.06
T.harzianum + AMF4% +N	8ef	**94**	7ef	**94**	7.7ef	**94**	210ef	**90**	233	0.08
B.megaterium +*T.harzianum* + AMF4% +N	4f	**97**	3.3fg	**97**	4g	**97**	105g	**95**	116	0.04
B.megaterium + *T.harzianum* +N	13.3d	**89**	11.3d	**91**	11.7d	**91**	393.3d	**81**	430	0.14
Nematode alone (C^+)	124.7a	-	123a	-	123.7a	-	2100a	-	2471	0.82

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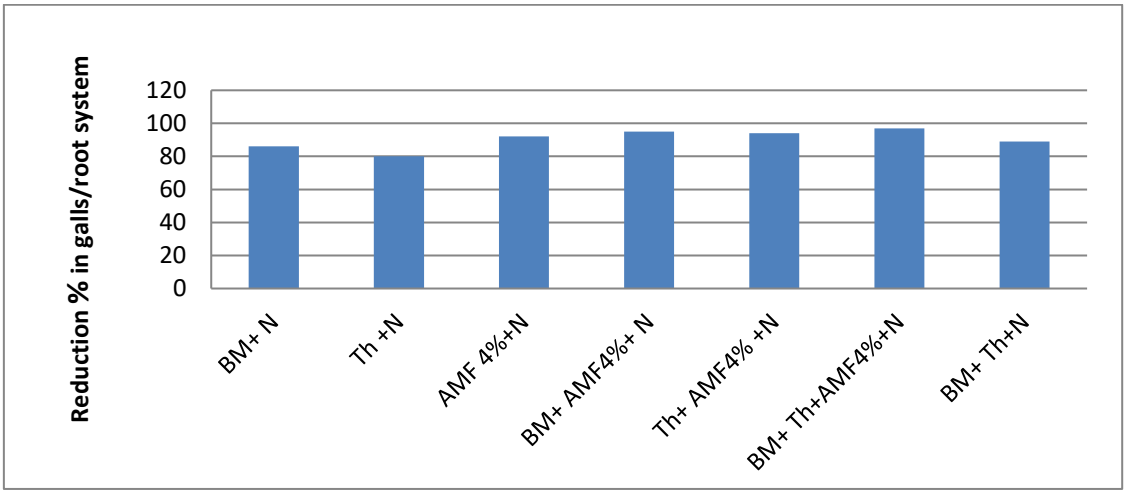
(A)



(B)

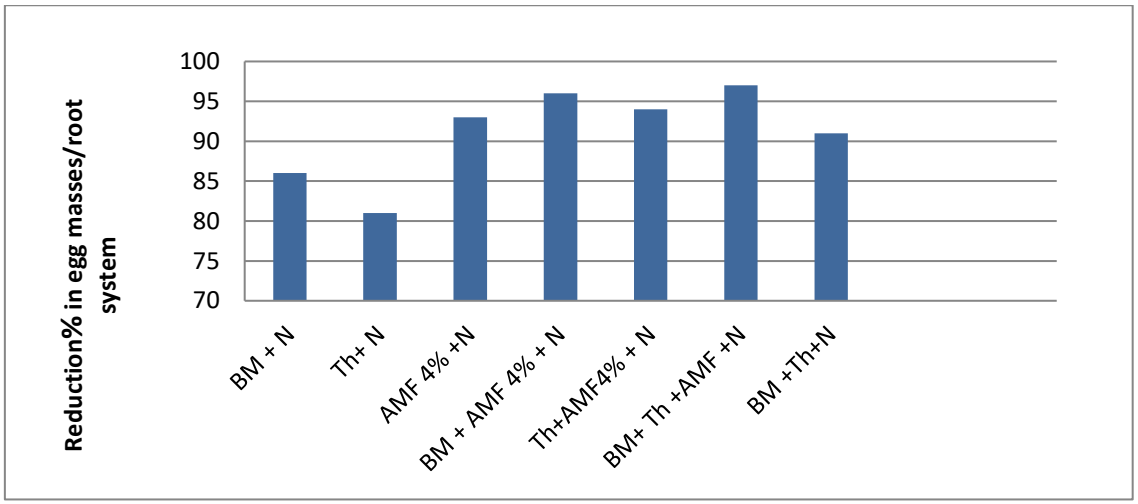


Fig. 2 (A&B): Integrated control of *Meloidogyne* spp. on tomato plants.

BM = *Bacillus megaterium*

Th = *Trichoderma harzianum*

AMF = Arbuscular Mycorrhizal Fungi

N= Nematode

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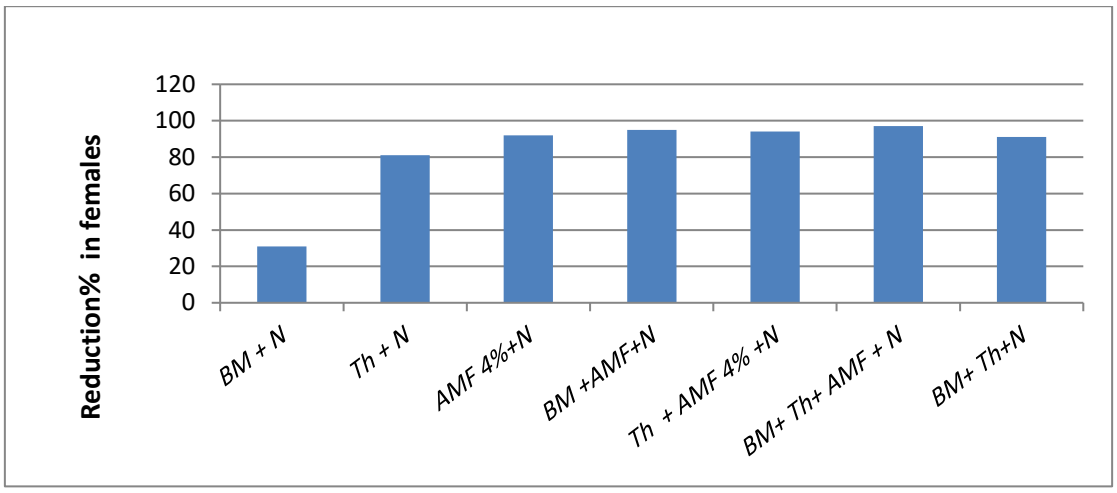
(A)



(B)

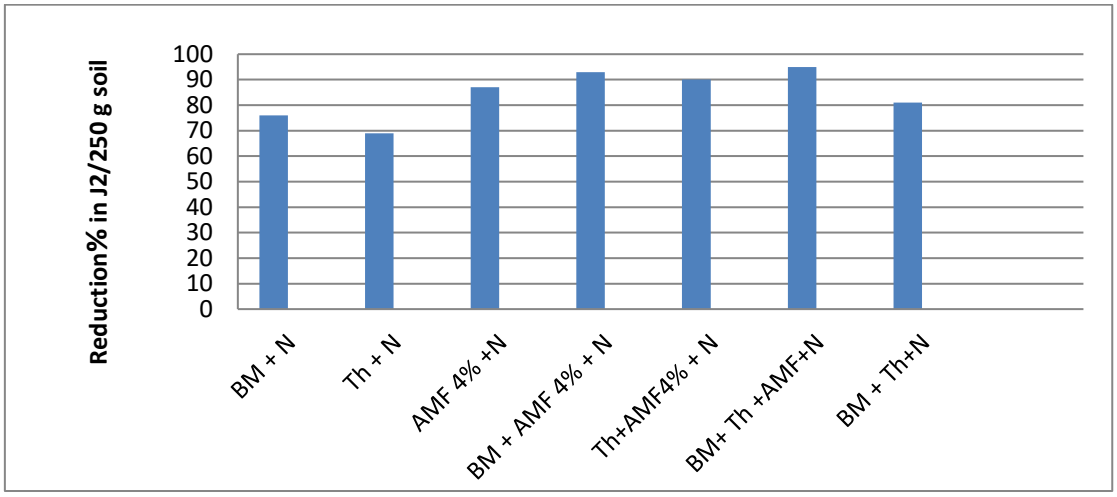


Fig.3 (A&B): Integrated control of *Meloidogyne* spp. on tomato plants.

BM = *Bacillus megaterium*

Th = *Trichoderma harzianum*

AMF = Arbuscular Mycorrhizal Fungi

N= Nematode

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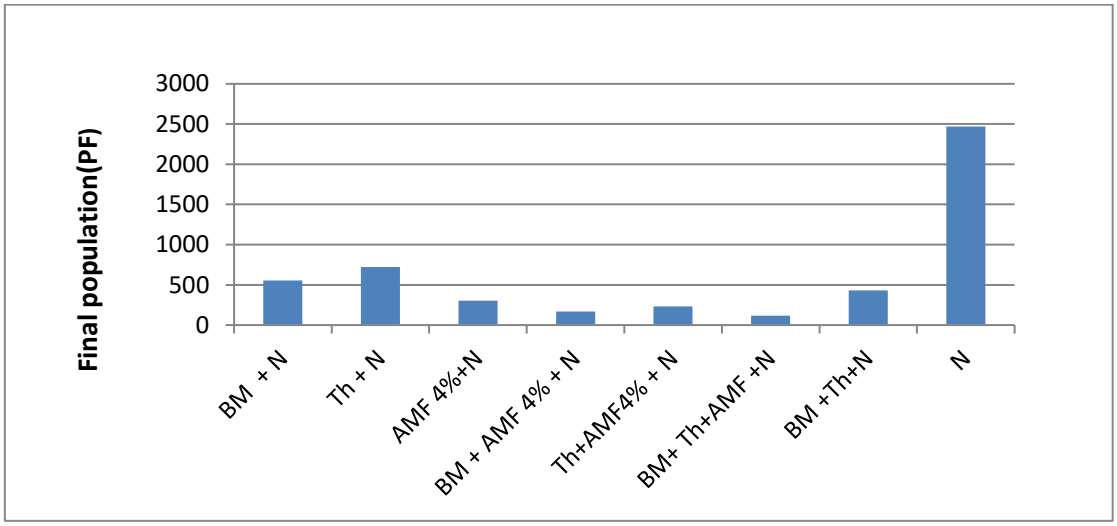
(A)



(B)

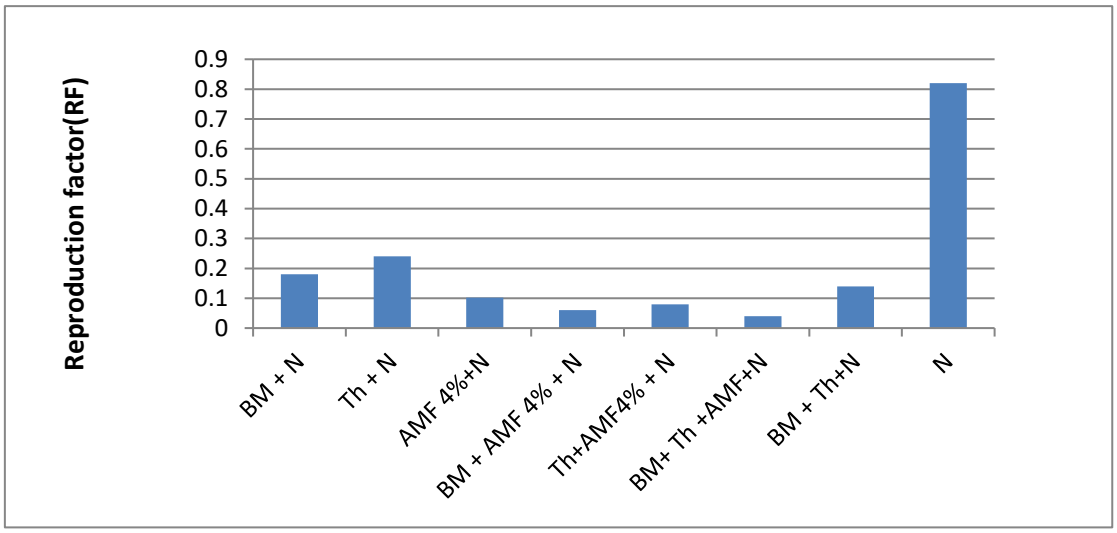


Fig.4(A&B) : Integrated control of *Meloidogyne* spp. on tomato plants.

BM = *Bacillus megaterium*

Th = *Trichoderma harzianum*

AMF = Arbuscular Mycorrhizal Fungi

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N= Nematode

Results showed that inoculation the plants with *Meloidogyne* spp. enhanced the roots infection with mycorrhizal fungi at 4% of soil weight after 60 days from nematode inoculation as shown in table (2) . The highest enhancement of mycorrhizal infection observed after 60 days from nematode inoculation.

The number of mycorrhizal fungi spores in soil increased and reached to maximum level (6750 spores / 100 g soil) with the nematode treated plants combined with *B. megaterium* plus *T. harzianum* , while the minimum level (5000 spores / 100 g soil), was achived with nematode combined with *B. megaterium* alone (Table 2).

Table (2) : Direct effect of different biocontrol agents and nematodes on mycorrhizal fungi infection in tomato roots .

Treatments	%Mycorrhizal fungi infection	No. of spores / 100 g soil
B .megaterium +N	00	00
T .harzianum+ N	00	00
AMF 4% + N	100	5300
B .megaterium+ AMF 4% +N	100	5000
T. harzianum + AMF 4% +N	100	5100
B .megaterium + T .harzianum + AMF 4% +N	100	6750
B.megaterium+T.harzianum+N	00	00
Nematode alone (C^+)	00	00

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Data presented in table (3) showed that application the different treatments of biocontrol agents with soil pots significantly enhanced all vegetative plant growth characters i.e. fresh shoot and root weights (g) , dry shoot weight (g), shoot and root lengths (cm) compared to plants treated with nematode alone . The greatest enhancement was recorded with the treatment of *B.megaterium +T.harzianum* + AMF 4% + Nematode by 347 , 240, 669, 72 and 192 %, respectively, followed by *B .megaterium* + AMF 4% + Nematode by 304 , 198 , 515, 61 and 151% ,respectively and *T. harzianum* + AMF 4% + Nematode by 262 , 155 , 385 , 115 and 47% , respectively . The lowest effect recorded with (*T. harzianum* + Nematode) by 85 , 43 , 154, 22 and 37% , respectively.

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Table (3) : Direct effect of different biocontrol agents on plant growth parameters of tomato plants infected with *Meloidogyne* spp.

Treatment	Fresh shoot weight(g)	**Efficacy %**	Fresh root weight (g)	**Efficacy %**	Dry shoot weight(g)	**Efficacy %**	Shoot length (cm)	**Efficacy %**	Root length (cm)	**Efficacy %**
B.megaterium +N	10f	**113**	7.7ef	**64**	3.7ef	**185**	62.7cd	**27**	11de	**51**
T.harzianum+ N	8.7fg	**85**	6.7fg	**43**	3.3ef	**154**	60.3d	**22**	10de	**37**
AMF4% +N	15d	**219**	10d	**113**	5.6cd	**331**	70.3bc	**43**	12.7d	**74**
B.megaterium + AMF4% +N	19b	**219**	14b	**198**	8b	**515**	79.3a	**61**	18.3b	**151**
T.harzianum + AMF4% +N	17c	**262**	12c	**155**	6.3c	**385**	72.6b	**47**	15.7c	**115**
B.megaterium +*T.harzianum* + AMF4% +N	21a	**304**	16a	**240**	10a	**669**	85a	**72**	21.3a	**192**
B.megaterium + *T.harzianum* +N	12,7e	**262**	8.7de	**85**	4.6de	**254**	70bc	**42**	12.3d	**96**
Control (C-)	7g	**49**	5.7gh	**21**	2.7f	**11**	55de	**12**	8.7ef	**19**
Nematode alone (C+)	7g	**347**	5.7gh	**21**	2.7f	**11**	55de	**12**	8.7ef	**19**

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Data presented in table (4) showed that all treatments significantly decreased of membrane leakage of root cells compared with plants treated with nematode alone. Application of *B. megaterium* + *T. harzianum* + AMF 4% +N combined gave the highest reduction in membrane leakage, followed by the treatment of *B. megaterium* + AMF 4% +N ,whereas the least effect obtained with the treatment of *T.harzianum* + N .

Data showed also that application *B. megaterium* + *T. harzianum* + AMF 4% +N combined was the effective one in increasing the antioxidant enzymes activities i.e (peroxidase and phenoloxidase) , whereas the lowest one recorded with the treatment of *T.harzianum*+ N when compared with plants treated with nematode alone .

Table (4) : Direct effect of different biocontrol agents on chemical components of tomato plants infected with *Meloidogyne* spp.

Treatments	Membrane leakage %	Peroxidase (O.D. /g f. wt/ min	Polyphenol oxidase (O.D. /g f. wt/ min
B.megaterium +N	73	0.63f	1.2e
T.harzianum+ N	78	0.43g	0.8f
AMF 4% + N	64	1.2d	1.43cd
B.megaterium + AMF 4% +N	58	1.6b	1.63ab
T.harzianum + AMF 4% +N	63	1.3c	1.53bc
B.megaterium + *T.harzianum*+ AMF 4% +N	56	1.8a	1.8 a
B.megaterium + *T.harzianum* +N	70	0.93e	1.3d
Control(C^-)	80	0.43g	0.6g

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test (P≤0.05).

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B- Indirect Effect (split- root system):-

Data presented in table (5) and illustrated in Fig.(5,6&7 ; A & B) showed that application of the different treatments with soil pots significantly reduced the nematode parameters i.e. number of galls , egg masses (Fig. 5), females / root system (Fig. 6) and number of J2 /250 g soil compared to the control (plants treated with nematode alone). The greatest reduction was also recorded with the treatment of *B. megaterium+T. harzianum* + AMF 4% + Nematode by 93, 94, 93 and 95% , respectively , followed by *B. megaterium* + AMF 4% + Nematode by 92, 92 , 92 and 92%,respectively and *T .harzianum* + AMF 4% + Nematode by 90 , 91 , 90 and 90 % respectively .

The lowest value of nematode reproduction was noticed with the treatment of *B. megaterium + T. harzianum* + AMF 4% + Nematode , followed by the treatment of *B.megaterium* + AMF 4% + Nematode and *T.harzianum* + AMF 4% + Nematode . The greatest values were found by the treatment *T.harzianum* + Nematode , followed by *B.megaterium* + Nematode as shown in Fig.(7).

Generally it can be concluded that all applied treatments markedly reduced nematode population and reproduction factor compared to plants treated with nematode alone .

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Table (5): Effect of biotic inducer as indirect effect for management of *Meloidogyne* spp. on tomato plants.

Treatment	Galls/ root system	**Reduction %**	Egg masses/ root system	**Reduction %**	Females/ root system	**Reduction %**	J_2/250g soil	**Reduction %**	Final population (PF)	Reproduction factor (RF)
B.megaterium +N	23c	82	22c	83	23c	82	510 c	76	578	0.19
T.harzianum+ N	30b	77	28b	78	28b	78	660 b	69	746	0.25
AMF 4% +N	15de	88	14de	89	15de	89	287 e	87	330	0.11
B.megaterium + AMF 4% +N	13ef	92	10ef	92	11fg	92	169 fg	92	194	0.06
T.harzianum + AMF 4% +N	11ef	90	12ef	91	13ef	90	220 ef	90	256	0.08
B.megaterium +*T.harzianum* + AMF 4% +N	9f	93	8fg	94	9g	93	115 g	95	141	0.05
B.megaterium + *T.harzianum* +N	18d	86	16d	87	17d	87	403 d	81	455	0.15
Nematode alone (C^+)	130a	-	128a	-	129a	-	2110 a	-	2497	0.83

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test (P≤0.05).

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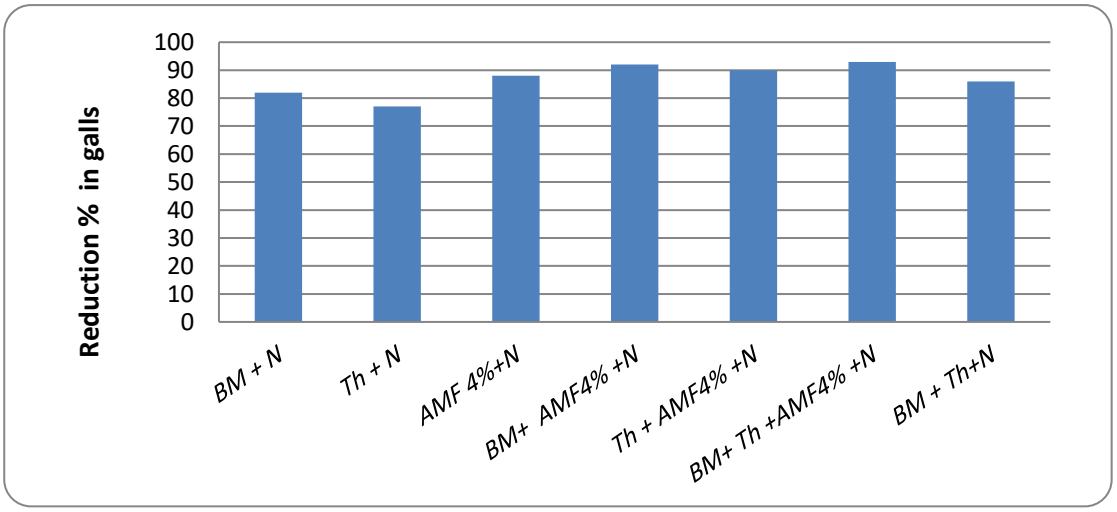
(A)



(B)

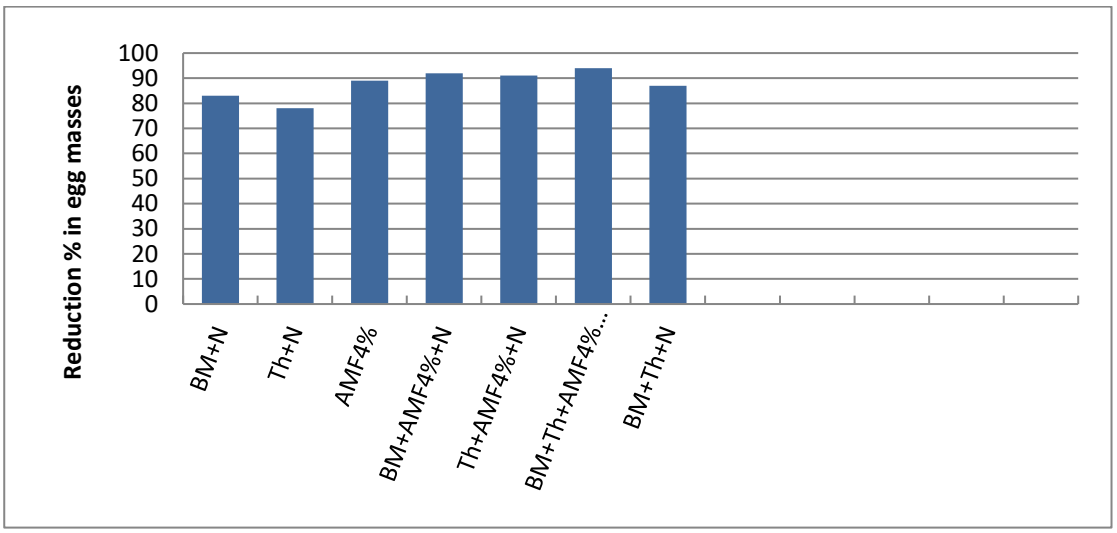


Fig.5 (A&B) : Indirect Effect of the different biocontrol agents against *Meloidogyne* spp. as a split root system.

BM = *Bacillus megaterium*

Th = *Trichoderma harzianum*

AMF = Arbuscular Mycorrhizal Fungi

N= Nematode

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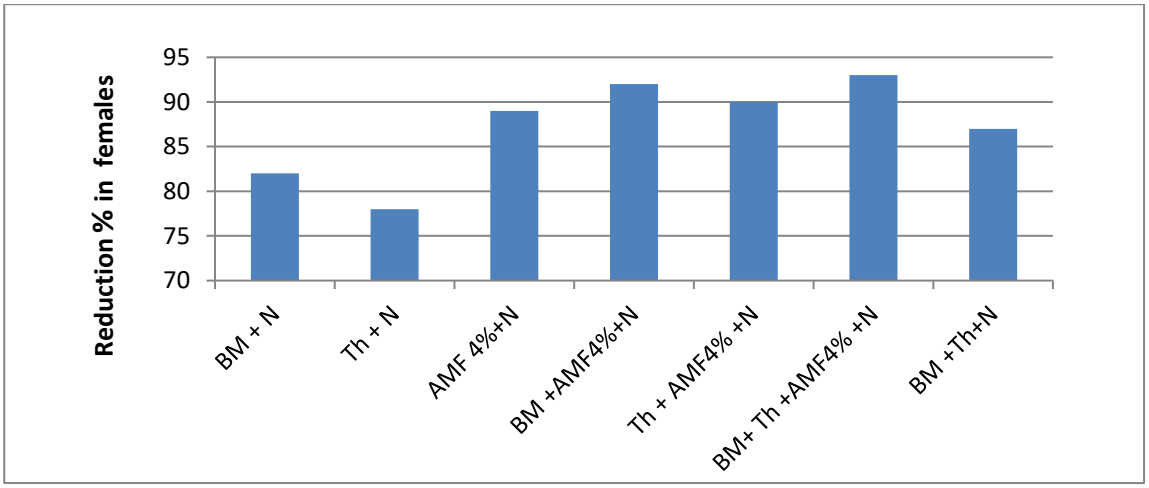
(A)



(B)

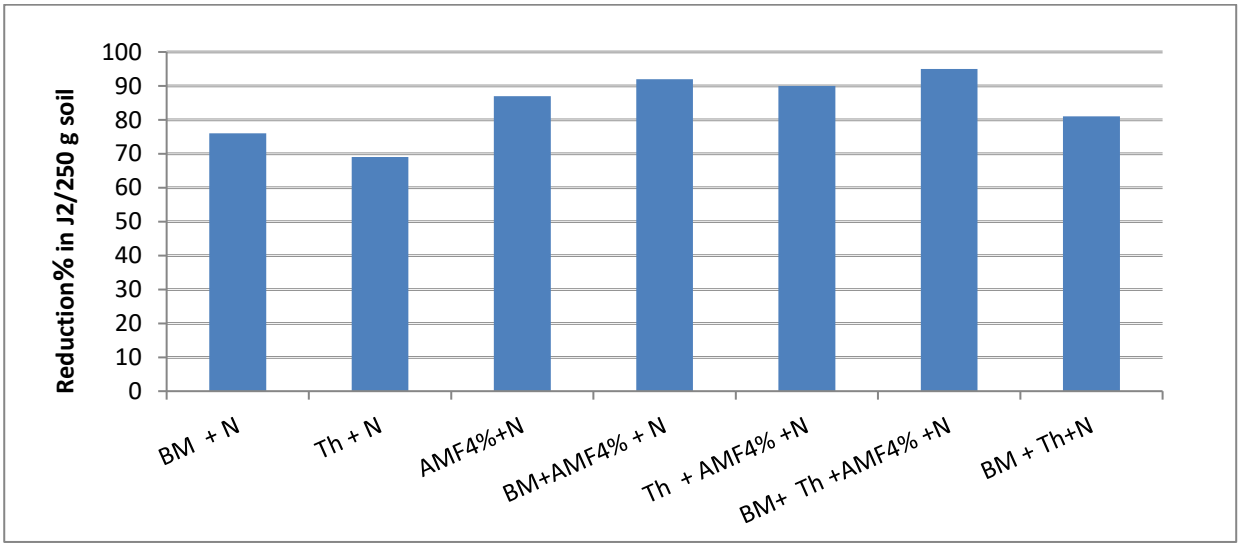


Fig.6(A&B) : Indirect Effect of the different biocontrol agents against *Meloidogyne* spp. as a split root system .

BM = *Bacillus megaterium*

Th = *Trichoderma harzianum*

AMF = Arbuscular Mycorrhizal Fungi

N= Nematode

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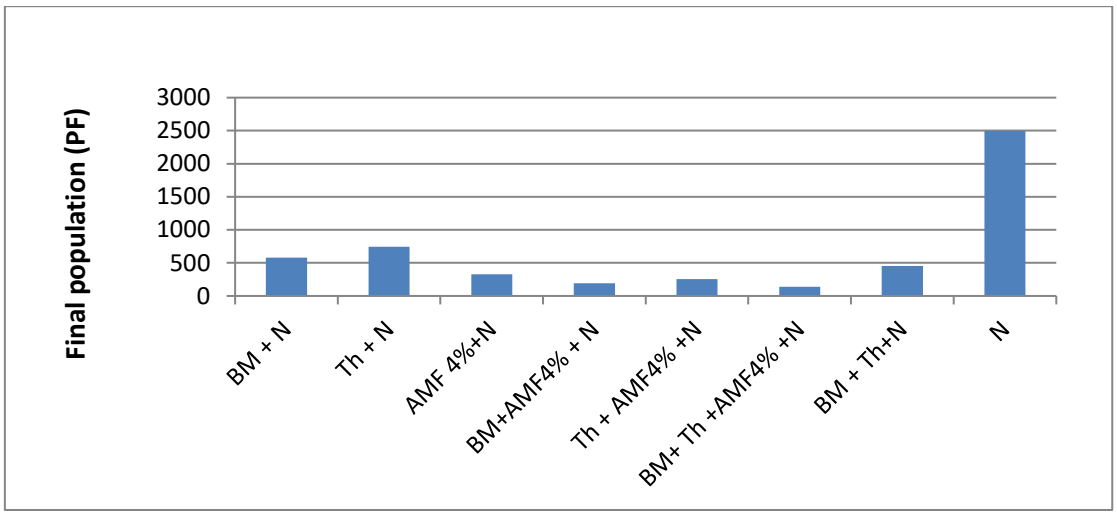
(A)



(B)

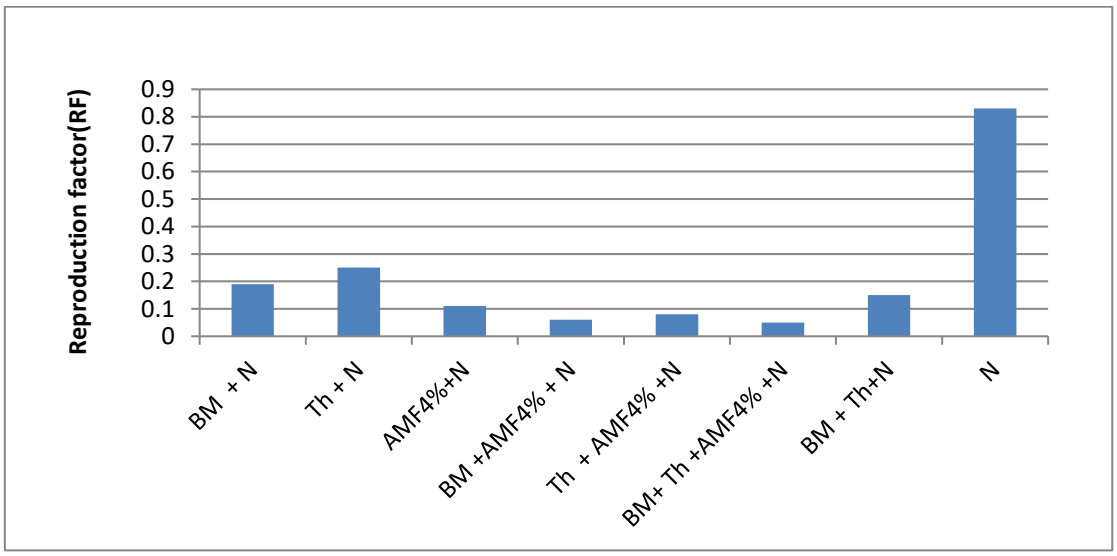


Fig.7 (A&B) : Indirect Effect of the different biocontrol agents against *Meloidogyne* spp. as a split – root system.

BM = *Bacillus megaterium*

Th = *Trichoderma harzianum*

AMF = Arbuscular Mycorrhizal Fungi

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N= Nematode

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Results showed that inoculation the plants with *Meloidogyne* spp. enhanced the roots infection with mycorrhizal fungi after 60 days of nematode inoculation compared with plants treated with nematode alone as shown in Table (6) . The highest enhancement of mycorrhizal infection observed in all treated plants with mycorrhizae. The number of mycorrhizal fungi spores in soil increased and reached to maximum level (6550 spores / 100 g soil) with the treatment of all bioagents combined with nematode .

Table (6) : Indirect effect of different biocontrol agents on mycorrhizal fungi infection in tomato roots infected with *Meloidogyne* spp.

Treatments	% Mycorrhizal fungi infection	No. of spores / 100 g soil
B.megaterium +N	00	00
T.harzianum + N	00	00
AMF 4% + N	100	5200
B.megaterium + AMF 4% +N	100	4700
T.harzianum+ AMF 4% +N	100	5000
B.megaterium + T.harzianum + AMF 4% +N	100	6550
B.megaterium + T.harzianum +N	00	00
Nematode alone (C^{+})	00	00

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Data presented in Table (7) showed that application of different treatments with soil pots significantly enhanced all vegetative plant growth characters i.e. fresh shoot and root weights (g) ,dry shoot weight (g), shoot and root lengths (cm) compared to plants treated with nematode alone. The greatest encourgement recorded with the treatment of *B. megaterium* + *T.harzianum* + AMF 4% + Nematode by 163 , 160, 150, 66 and 117%, respectively, followed by the treatment of *B. megaterium* + AMF 4% + Nematode by 138 , 140 , 117, 55 and 92% , respectively. The lowest effect recorded with the treatment of *T.harzianum* + Nematode by 50 , 40, 33, 20 and 25% respectively, compared with plants treated with nematode alone as shown in Table (7).

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Table (7): Effect of biotic inducer as indirect effect for management *Meloidogyne* spp. on tomato plants.

Treatment	Fresh shoot weight (g)	**Efficacy %**	Fresh root weight (g)	**Efficacy %**	Dry shoot weight (g)	**Efficacy %**	Shoot length (cm)	**Efficacy %**	Root length (cm)	**Efficacy %**
B.megaterium +N	13ef	**63**	15f	**50**	9ef	**50**	68cd	**26**	16de	**33**
T.harzianum + N	12fg	**50**	14fg	**40**	8ef	**33**	65d	**20**	15de	**25**
AMF 4% +N	15d	**88**	20d	**100**	11cd	**67**	73bc	**35**	18d	**50**
B.megaterium + AMF 4% +N	19b	**138**	24b	**140**	13b	**117**	84a	**55**	23b	**92**
T.harzianum + AMF 4% +N	17c	**113**	22c	**120**	11c	**83**	78b	**44**	21c	**75**
B.megaterium +*T.harzianum* + AMF 4% +N	21a	**163**	26a	**160**	15a	**150**	90a	**66**	26a	**117**
B.megaterium + *T.harzianum* +N	14de	**75**	18e	**80**	10de	**67**	75bc	**39**	17d	**41**
Control(C^-)	9cd	**13**	14d	**40**	9d	**50**	60cd	**11**	17cd	**42**
Nematode alone(C^+)	8h	-	10h	-	6g	-	54e	-	12f	-

Columns followed by different letters are significantly different according to Duncan,s Multiple Range Test ($P \leq 0.05$).

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Data presented in table (8) showed that all treatments at all application methods significantly decreased of membrane leakage compared with plants treated with nematode alone . *(B. megaterium* + *T. harzianum* + AMF 4% +N) gave the highest reduction in membrane leakage followed by (*B. megaterium* + AMF 4% +N) ,whereas the least effect observed with (*T.harzianum*+ N) .

Data showed also that *(B. megaterium* + *T. harzianum* + AMF 4% +N) was the effective one in increasing the antioxidant enzyme activities i.e (peroxidase and phenoloxidase) , whereas the lowest one recorded with (*T.harzianum*+ N) when compared with plants treated with nematode alone

Table (8) : Indirect effect of different biocontrol agents on chemical components of tomato plants infected with *Meloidogyne* spp.

Treatments	Membrane leakage %	Peroxidase (O.D. /g fr. wt/ min.	Polyphenol oxidase (O.D. /g fr. wt/ min.
B.megaterium +N	72	0.87f	1.1e
T.harzianum+ N	77	0.57 g	0.7 f
AMF 4%+N	63	1.3d	1.4c
B.megaterium + AMF 4%+N	57	1.5 b	1.6 b
T.harzianum + AMF 4%+N	62	1.4 c	1.5 bc
B.megaterium + *T.harzianum*+ AMF 4% +N	54	1.7 a	1.7 a
B.megaterium + *T.harzianum* +N	68	1.1e	1.23d
Control (C^-)	77	0.37 h	0.5 g
Nematode alone (C^+)	88	0.1 i	0.2h

Columns followed by different letters are significantly different according to Duncan,s Multiple Range Test ($P \leq 0.05$).

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DISCUSSION

Plant parasitic nematodes are among the most destructive major pest of crop plants. The common means of control for these pests mostly use chemicals like nematicides , which are environmentally unfriend and costly especially in large scale agricultural production system . However, recently there are other alternative strategies how development of resistant crop varieties and exploiting natural resistance gene of plants in conventional breeding programs . Moreover , molecular biotechnological application which are considered as an effective measure to control these pests . These are major different in the suitability of particular plants species and varieties as a host for each nematode **(Zebire 2017).** Inourstudy biofertilizers as biotic inducers and different safety bioagents i. e . *Bacillus* spp. , *Trichoderma* spp. and arbuscular mycorrhizal fungi were evaluated against root-knot nematodes *Meloidogyne* spp. as an alternative methods to the expensive and polluted chemical nematicides . Our results indicated that all treatments i. e *Bacillus* spp. and *Trichoderma* spp. were effective in reducing significantly all nematode parameters and significantly increased the plant growth which reflected a healthy plants compared to the untreated control .

Trichoderma spp. More effective which recorded highly significant reduction in nematode parameters . Fungal strains of Trichoderma possess a wide spectrum of evolutionary responses that range from very effective soil colonization , with high biodegradation potential , to non- strict plant symbiosis by strains colonizing the rhizosphere as reported by **Hjeljord and Tronsmo,(1998).**

Sharon *et al* ., (2011) mentioned that *Trichoderma* spp. is one of the fast growing fungi and is widely distributed in soil , it is different species have attracted much attention as biocontrol agents of nematode , besides their parasitic activites the different species of Trichoderma produce some nematicidal compounds , such as acetic acids .

Sharon *et al* ., (2001) reported that *Trichoderma* spp. have also been described as biocontrol agents against nematodes . This results due to mechanisms of this fungal activity against root –knot nematodes is limited , the

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ability of *Trichoderma* spp. to colonize eggs and infect second – stage juveniles *in vitro* has been demonstrated are able to suppress *Meloidogyne* spp. populations and increase crop yields .They found that also *Trichoderma harzianum* reduced root galling of root-knot nematode *M. javanica* on tomato plants**.**

Trichoderma harzianum found to be most effective when treated at 2 g / pot and enhanced all plant growth characters with reduction in the root-knot infestation as reported by **Usman and Siddiqui (2012).**

Rao *et al.,* (1996) confirmed that *Trichoderma* spp. have also been described as biocontrol agent against plant - parasitic nematodes . Several reports showed that *Trichoderma* spp. are able to suppress *Meloidogyne* spp. populations and increase crop yields.

Sharma and Pandey (2009) revealed that the fungal agents of *T. harzianum* and *Paecilomyces lilacinus* not only reduced penetration and development of *M. incognita* , but also increased plant growth.

Al-Hazmi *et al* ., (2010) found that soil application of antagonistic fungi would be able to control the nematodes development and also improve the yield of mulberry .

Tomato plants treated with Trichoderma isolates were less attacked by the root –knot nematodes and also shows significantly reduction in root gall / root system of tomato plants as reported by **Neog *et al* .,(2014) .**

Trichoderma has not only been prove to parasitize nematodes and inactivate pathogen enzymes but also help in tolerance to stress conditions by enhanced root development as reported by **Harman (2000)** .

Sharon *et al* ., (2001) recorded that the proteolytic and chitinolytic activities produced by different strains of trichoderma may be responsible for the biocontrol activity against nematodes . The level of proteolytic activity seems to correlate with nematode control abilities .

Bacillus spp. is very important bio- agent that has several benefits compared to other rhizobacteria . Biological control is free from residual

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and adverse environmental effects . Hence , biological control is gaining more importance in the recent decades as reported by **Mahesha *et al* ., (2017).**

Many species of Bacillus and Pseudomonas have been reported as plant growth promoting rhizobacteria (PGPR) producing iron-chelating siderophores antibiotics or hydrogen cyanide , and these compounds have been implicated in the reduction of deleterious and pathogenic rhizosphere microorganisms , creating an environment more favourable for root growth . So that the effort was made to identify the good antagonistic bacteria against suppression of nematode egg hatching as reported by **Siddiqui and Mahmood (2003)** .

El-Hadad *et al* .,(2011) revealed that *Bacillus megaterium* inoculation was the most efficient strain in reducing *M. incognita* and consequently enhanced tomato growth .

Mostafa *et al*.,(2018) found that *Bacillus megaterium* and other bio-agents controlled root –knot nematodes infecting sugar beet under field conditions. The results indicated that integration of two or more components of such bio agents gave better results in sugar beet growth parameters than did single ones.

Radwan *et al* .,(2012) reported that *Bacillus megaterium ,Trichoderma album , Trichoderma harzianum* and *Ascophyllum nodosum* were significantly superior over the untreated check in reducing the root galls and J_2 of the nematode in the soil expect *Trichoderma harzianum*at 10 and 25 ml/kg soil against J_2. *Bacillus megaterium* at 10 m l/kg soil achieved the highest significant reduction in the number of root galling .

Our results revealed that treating the plants with mycorrhizal fungi significantly reduced all nematode parameters i.e. number of galls , egg masses , females , J2 in soil , final population and reproduction factor compared with plants treated with nematode alone .

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These results may be referred to different mode of actions involved in nematode reduction such as :-

1- An early colonization of plant roots with mycorrhizal fungi can protect the plant against nematode invasion by parasitism , competition or physical enclosure through a mycelia mat according to **Diedhiou *et al* ., (2003)** .
2- Arbuscular mycorrhizal fungi colonized paranchyma cells of plant roots and are know to alter the root physiology and induce local and systemic resistance . Induced resistance can be associated with wall appositions reinforced by callose and the elicitation of thickening of root cell walls, consequently reduced the nematode invasion into roots as reported by **Lindermann (1994)** and **Cordier *et al* ., (1998) .**
3- The mycorrhizal fungi may be able to cause an alteration in root exudates that could affect the root attractiveness to nematodes as recorded by **Thomson Cason *et al* ., (1983)**.

4- Arbuscular mycorrhizal fungi are obligate biotrophs that colonize plant root endogenously and root surface as cited by **Salerno *et al* ., (2000) and Benhamou and Garand (2001) .**
5- The induction of resistance to internal root colonization by mycorrhizal fungi might also contribute to limit further development of *M. incognita* within roots and consequently reduced nematode infection as confirmed by **Thomson Cason *et al* ., (1983)** and **Diedhiou *et al* ., (2003)**.

6- The mycorrhizal fungi cause changes in the post – inflectional nematode host interaction by altering the nematode reproduction and / or development . Once J_2 larvae of *Meloidogyne* spp. intered a root , an alteration in the post – infectional relationship is evident and limit the nematode larval development inside the roots as observed **by Thomson Cason*et al* ., (1983).** The infection with mycorrhizal fungi on tomato roots as well as the number of mycorrhizae spores in soil were enhanced when inoculated at different doses with *Meloidogyne* spp. compared with the treated plants with nematode alone.

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 as reported by **Smith *et al*., (2010).**

Arbuscular mycorrhizal fungi are able to increase the uptake of water and mineral nutrients for their host plant, such as phosphate and nitrogen as reported by **Baum *et al*., (2015).**

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