Minufiya J. Agric. Res. Vol.37 No. 4(1): 773 - 780 (2012) **"http://www.mujar.net"**

تاثير بعض الكائنات الحية الدقيقة على إصابة نباتات الطماطم بنيماتودا تعقد الجذور ميلودوجينا انكوجنيتا

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الملخص العربى

تم تقييم إستخدام كل من البكتيريا *Azotobacter chroococcum* و *Bacillus megaterium* var. *phosphaticum* و كذلك مخلوط منهما ضد نيماتودا تعقد الجذور من جنس (ميلودوجينا انكوجنيتا) على نباتات الطماطم من نوع (بيتو٨٦) تحت ظروف الصوبة. وأثبتت الدراسة أن البكتريا *A. chroococcum* هى الأكثر تاثيرا فى تقليل أعداد نيماتودا تعقد الجذور بنسبة ٧٤.٦%، يلى ذلك بكتيريا *B. megaterium* var. *phosphaticum* بنسبة ٧١.٢% و أخيرا المخلوط من كل من نوعى البكتيريا السابقة بنسبة ٦٤% مقارنة بالمبيد الكيميائى (كمعاملة كيميائية مقارنة) بنسبة ٨٢%. و قد إستخدام جميع معاملات البكتيريا السابقة ضد نيماتودا تعقد الجذور إلى زيادة القياسات الخضرية مقارنة بالمعاملة الكيميائية. فعلى سبيل المثال، فان نوع البكتيريا *B. megaterium var. phosphaticum* أثبتت تفوقها على جميع المعاملات فى زيادة نمو الساق بنسبة ٩٦.٤٥% مقارنة بالمعاملة النيماتودية المقارنة.

EFFICACY OF SOME RHIZOSPHERE MICROORGANISMS ON ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* INFICTED TOMATO PLANTS

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(Received: Jun. 3, 2012)

ABSTRACT: *Azotobacter chroococcum, Bacillus megaterium var. phosphaticum and a mixture of the two previously microorganisms, were evaluated against root-knot nematode, Meloidogyne incognita on tomato plants cv. Beto86 under greenhouse conditions. Azotobacter chroococcum was the most effective microorganism in reducing the population of M. incognita (74.6%), followed by B. megaterium var. phosphaticum (71.2%), while the mixture of the two previously microorganisms gave only (64%), On the other hand "Nemacur10%G" gave (82%) reduction. Also, all the treatments of tested materials caused remarkable significant increase in plant growth parameters. For example, B. megaterium var. phozphaticum surpassed the other treatments even that of non-infected check one, causing (96.45%) increase of shoot weight.*

Key words: *Azotobacter chroococcum, Bacillus megaterium var. phosphaticum, root-knot nematode, Meloidogyne incognita, tomato cv. Beto86, "Nemacur10%G"*

INTRODUCTION

Tomato (*Lycopersicon esculentum*, Mill.), is an important vegetable crop which heavily attacked by root-knot nematode, *Meloidogyne* spp. Management of root knot nematodes using chemical nematicides was predominant till 1982, but because of their high cost, toxic effect on beneficial soil bore microorganism and carcinogenic effect on human beings alternative approaches are practiced mainly through eco-friendly means like biological control agents, organic amendments, etc. (Singh and Sitaramaiah, 1966; Akhtar and Malik 2000; Briar *et al*., 2007). Moussa and Hague (1988) in their studies on the influence of *Fusarium oxysporium* f. sp. *glycines* on the invasion and development of *Meloidogyne incognita* on soybean reported that the invasion of root was not affected, but giant cell were invaded by the fungus and destroyed. Under glasshouse conditions aqueous cell suspension of the strains *Pseudomonas huorescens* CHAO (antibiotics-deficient) or CHAO/pME3424 (antibiotics overproducing) at various inocula levels, significantly reduced root-knot nematode development; Siddiqui and Shaukat (2003). Relatively, few studies on the tomato cultivar "Hildares", applications of the beneficial microorganisms reduced the number of galls and egg-sacs. However, combination of *Glomys intraradices* and *Trichoderma viride* did not result effect,synergism Masadeh *et al*., (2004). Desaeger *et al*., (2005) reported that, shoot dry weights showed negative and positive relationships with root-knot nematode infection (gall index) and number of effective rhizobium nodules, respectively. In fact, of the sedentary endoparasites, numbers of root-knot nematodes were reduced more by mycorrhizal infection than were those of cyst nematodes. The reduction in nematode damage by arbuscular mycorrhizal fungi (AMF) was not different for root-knot or cyst nematode infested plants Gera-Hola and Cooka (2005). Sun *et al.,* (2006) selected seventeen fungal isolates and four actinomycete isolates with high pathogenicity (*in vitro)* to test biocontrol efficacy in the greenhouse. And found that tomato root gall index were reduced by 13.4-58.9% compared to the no treatment control. Additionally, the beneficial effect of adding cyanobacterial powder of *Oscillatoria chlorina* into infested potted field soil increased exponentially with concentration up to 0.8%. Root galling and nematode population decreased by 68.9% and 97.6%, respectively at the highest dose (1%) of cyanobacterial powder compared with the untreated control Khan *et al*., (2007). The spore /crystalproteins of isolated *Bacillus thuriniensis* (Bt) showed that, the highest nematicidal activities of the root-knot nematode (*Meloidogyne incognita*), with the mortality range of 86-100%, Mohammed *et al*., (2008). Also, Sahebani and Hadavi (2008) in their results showed that, different concentrations (10^2–10^8 spores/ml) of *T. harzianum* decreased nematode infection and other parameters significantly, compared with control. *Trichoderm harzianum* was able to penetrate nematode egg mass matrix and significantly decreased nematode egg hatching level. In the green house trials, *Bacillus firmus*, commercial WP formulation (BioNem) applied at 8 g/pot (1200 cc soil) planted with tomato seedlings reduced gall formation by 91%, final nematode populations by 76% and the number of eggs by 45%. Consequently, plant height and biomass was increased by 71% and 50%, respectively, compared to the untreated control, 50- days after treatment application (Anastasiadisa *et al*., 2008; Terefe *et al*., 2009).From the previous review, the purpose of this study was to determine the effect of some microorganisms on root-knot nematode infecting tomato plants.

MATRIALS AND METHODS

Preparation of pure nematode cultures:

Eggs of *Meloidogyne incognita* were extracted from graived females in the tomato (*Lycopersicon esculentum* cv. Castle Rock) roots infected with the nematode using sodium hypochlorite solution (Hussey and Barker 1973). Second-stage juveniles (J2) were collected daily from eggs and stored at 15°C. The juveniles used in the experiments were less than 5 days old.

Preparation of the microorganisms inocula:

1- *Azotobacter chroococcum* isolate was grown on Jensen medium for 3 days. A loop of bacterial culture was inoculated into 100ml Jensen medium for 48h at 28C°±1. The bacterial inoculum was applied as a soil treatment at the rate of 5ml of the bacterial suspension (1×108cfu/ml) per plant.

2- *Bacillus megaterium* var. *phosphaticum* isolate was grown on Nutrient Broth medium for 3 days. A loop of the bacterial culture was inoculated into 100ml Nutrient Broth medium for 48h at 25C°±1 on a rotary shaker. The bacterial inoculum was applied as a soil treatment at the rate of 5ml of the bacterial suspension (1×10^8cfu/ml) per plant.

3- The mixture of *A. chroococcum* and *B. megaterium var. phosphaticum* was applied as a soil treatment at the rate of 2.5 ml for both of them per plant.

Plant material:

Seeds of tomato cv. Beto86 susceptible to *M. incognita* (Siddique and Ehteshamul-Haque, 2001), were surface sterilized with 1% Ca (Col) 2 and washed three times with sterile MgSO4 (0.1M) and dried under a laminar flow hood. The seeds were planted in 30 cm diameter earthen pots containing mixture of 1:1 steam-sterilized peat and sand. Six weeks old seedlings were then transplanted for use in the experiments.

Effect of microorganisms inocula level on nematode life cycle:

Seedlings of six weeks old, tomato plants were transplanted in 30 cm diameter plastic pots containing a mixture of 1:2 sterilized clay/sand soil. Twenty four pots were inoculated with *M. incognita* juveniles at the rate of 1,000 (J2) per pot at the planting time. Seven days later, 12 pots were treated with 5 ml of previously concentrations of bacterial emulsions1, 2, and mixed with the previously concentrations 1&2. Tomato seedlings inoculated with nematode without bacterial inoculation (inoculated with sterile distilled water) were used as control. The remaining four pots served as untreated controls without nematode, and also previous numbers were treated by chemical control 2g "Nemacur 10% G" per pot. Microorganism's emulsions were added to the soil by a pipette in a hole in soil then irrigated by water. The treated plants were left after treatment for forty five days. Weight and length of root and shoot were determined. Nematode population in soil and developing stages in roots were counted. The data was statistically analyzed.

RESULTS

Azotobacter chroococcum and *Bacillus megaterium* var. *phosphaticum* and the nematicide "Nemacur10%G**"** were applied to test their suppressive effect on the root-knot nematode, *Meloidogyne incognita* infecting tomato plants cv. Beto86.

Efficacy of some rhizosphere microorganisms on the inhibition of the development and reproduction of *M. incognita*:

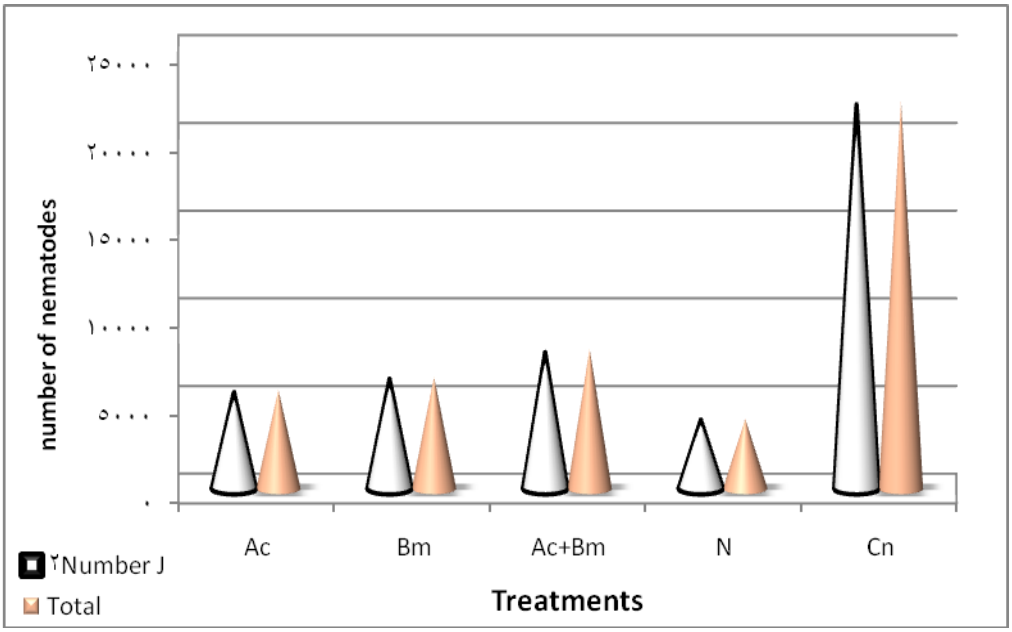
In vivo studies (Table 1 & Fig. 1) reflected that the applications of *Azotobacter chroococcum*, *Bacillus megaterium* var. *phosphaticum* and the mixure of the two bacteria in comparing with the nematicides i.e., "Nemacur10%G". All the treatments gave significant reduction in the average number of galls, immature stages, females and egg-laying females in tomato roots. The same result was noticed on the average number of juveniles in soil and total population of nematodes as well as the rate of build-up compared to those of control (untreated-infected plants). Also, results indicate that *A. chroococcum*, *B. megaterium* var. *phosphaticum* as well as the treatment of the nematicide "Nemacur10%G" were significantly suppressed in comparison with that of the check (control with nematode).

Generally, it could be concluded that the treatment of *A. chroococcum* was the most effective one in reducing the population of *M. incognita* (74.6%) reduction, followed by *B. megaterium* var. *phosphaticum* giving (71.2%), the treatment of the mixture was (64%), in comparison with the nematicide, "Nemacur10%G" which caused (82%) reduction (Fig. 2).

Table (1): Effect of some rhizosphere microorganisms on root-knot nematode, *M. incognita* stages infected tomato plants Beto 86.

Treatments	Number of galls	Immature stages	Females	Egg-laying females	Number of Juveniles	Total*	Rate** of build-up
Ac	61.5 bc	22.5 c	42.75 ab	71 ab	5484 b	5620.25 b	5.6 b
Bm	56 bc	40 b	36.25 b	53 bc	6237.5 b	6366.75 b	6.4 b
Ac+ Bm	81 ab	46.5 ab	41.25 ab	99.25 ab	7760 b	7947 b	7.9 b
Nemacur 10%G	25 c	15.25 c	25 b	24 c	3912 b	3976.25 b	3.9 b
Control with nematode	104 a	59.25 a	65 a	85 ab	21884 a	22093.25 a	22.1 a

Means followed by the same letter(s) within each column are not significantly different ($P \le 0.05$) according to Duncan’s multiple range test.
*Total population including immature stages + females + egg-laying females + numbers of juveniles in soil.
**Rate of build-up = pf (final population /initial population) Norton, 1978.



Note: Ac= *Azotobacter chroococcum*, Bm= *Bacillus megaterium* var. *phosphaticum*, Ac+Bm= Mixture of the bacteria; N: Nemacur 10%G; Cn= Control with nematode.

Fig. (1): Efficacy of some rhizosphere microorganisms on juvenile numbers in soil and total numbers of root-knot nematode infecting tomato plants cv. Beto86.

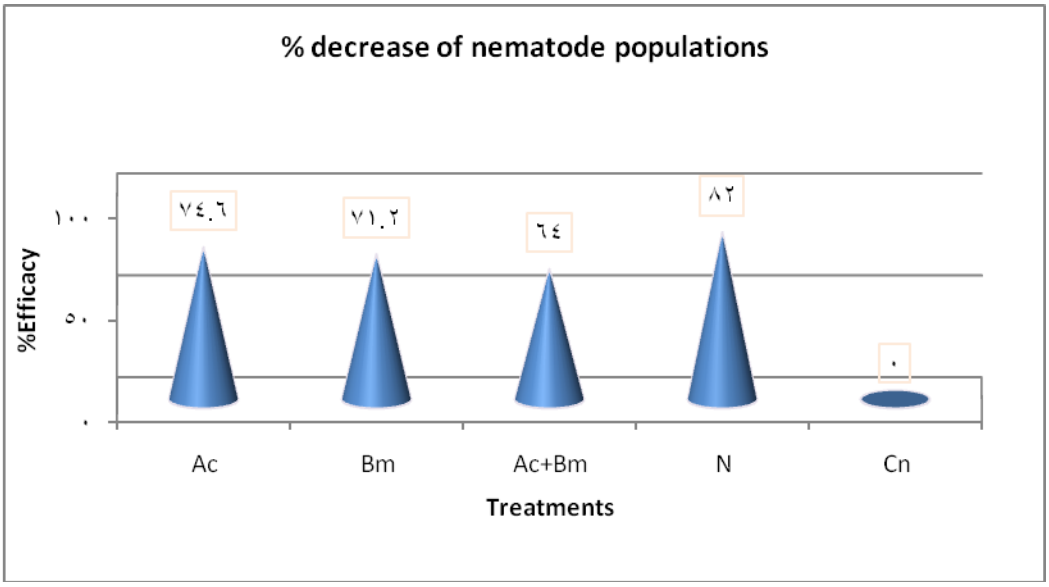


Fig. (2): Relative efficacy of some microorganism's additives applied to tomato plants cv. Beto86 for controlling *M. incognita*.

Efficacy of some rhizosphere microorganisms on improving growth tomato plants cv. Beto86 infected by *M. incognita*:

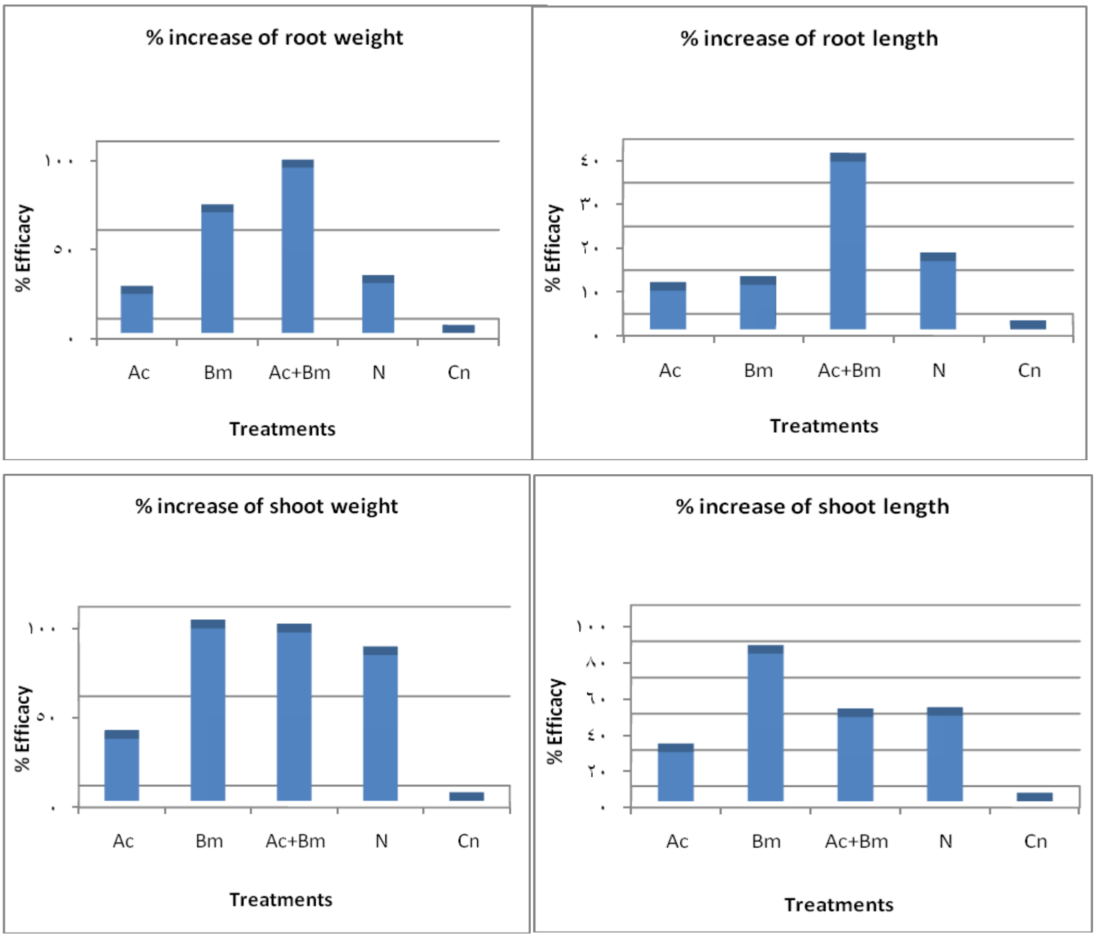
Data on growth criteria based on weight and length of shoot and roots shown in Table 2 & Fig. 3 clear that all tested treatments successfully improved the growth criteria. For instance, shoot weight of the treated plants were much higher than those of the check with nematode. *Bacillas megaterium* var. *phosphaticum* surpassed the other treatments even that of non-nematizied check one giving (96.45%)increase percentage., registering the highest percentage of increment, followed the treatment of by *A. chroococcum* plus *B. megaterium* var. *phosphaticum* and *A. chroococcum* treatments resulting (94.2 and 34.74%), respectively; while the treatment of the nematicide "Nemacur10%G" increased shoot weight ranged between (27.14 - 81.43%). Also, noticeable effect on root weight was obtained by applying the used materials. The percentages of increase of such parameters were ranged between (21.95 - 93.1%), as well as the treatment of the nematicide "Nemacur10%G" gave (28.05%)increase. On the other hand, less effect on root length was obtained by applying the used materials, which ranged between (8.81 - 38.39%) as well as "Nemacur10%G" (15.58%).

In general, all treatments as well as the nematicide "Nemacure 10% G" caused remarkable increae in the plant growth parameters.

Table (2): Effect of some rhizosphere microorganisms on tomato plants inoculated with root-knot nematode.

Treatments	Shoot				Root			
	weight (g)	% increasing	length (cm)	% increasing	weight (g)	% increasing	length (cm)	% increasing
Ac	6.05bc	34.74	22.25bc	27.14	1.92c	21.95	20.13ab	8.81
Bm	11.45a	96.45	31.75a	81.43	4.13ab	67.89	20.38ab	10.16
Ac+ Bm	8.72ab	94.2	25.63ab	46.48	4.75a	93.1	25.63a	38.39
Nemacur 10%G	8.15ab	81.51	25.75ab	47.14	3.15a-c	28.05	21.38ab	15.58
Control with nematode	4.49c	0	17.5c	0	2.46bc	0	18.5b	0
Control without nematode	11.31a	-	23bc	-	4.08ab	-	21ab	-

Means followed by the same letter(s) within each column are not significantly different (P≤0.05) according to Duncan's multiple range test.



Note: : Ac= *Azotobacter chroococcum*, Bm= *Bacillus megaterium* var. *phosphaticum*, Ac+Bm= Mixture of the bacteria; N: Nemacur 10%G; Cn= Control with nematode.

Fig. (3): Relative increase in growth of tomato cv. Beto86 due to applications of some rhizosphere microorganisms additives to root-knot nematode suppressive.

DISCUSSION

Obtained results indicate that *Azotobacter chroococcum*, *Bacillus megaterium* var. *phosphaticum* and their combination have significant potential as biocontrol agent against the root-knot nematode, *Meloidogyne incognita* under greenhouse conditions. Tested microorganisms can significantly reduced the population of nematode and disease severity. Suitable rate of *A. chroococcum*, *B. megaterium* var. *phosphaticum* and their combination for suppressing nematode action activities such as number of galls, immature stages, females, egg-laying females and number of juveniles was observed in 5ml of the each bacterial suspension (1×10^{8}cfu/ml) concentration per plant. Theses results are in agreement with those reported by Siddiqui and Shaukat, 2003; Masadeh *et al*., 2004; Gera-Hola and Cooka 2005; Sun *et al*., 2006; Anastasiadisa *et al*., 2008 and Terefe *et al*., 2009. In the present study, application of all treatments at sowing time improved plant growth in the nursery. For example, application of *B. megaterium* var. *phosphaticum* at previously concentration increased shoot weight by 96.45% and length by 88.43%. Terefe *et al*., (2009) also reported that, *Bacillus firmus* reduced gall formation by 91%, final nematode populations by 76% and the number of eggs by 45%. Consequently, plant height and biomass was increased by 71% and 50%, respectively, compared to the untreated control, 50- days after treatment application. The shorter length of seedling of untreated control might be due to stunting action of *M. incognita*. This type of stunting or reduced lengths caused by root-knot nematode has been reported by Sahebani and Hadavi 2008; Anastasiadisa *et al*., 2008.

AKNOLEDGMENT

The authors gratefully thanks Dr. Wahby M. Hassany for assist in the statistical analysis. Also, thanks Mr. Tamer M. Mosalamy for kind help in this work.

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تم تقييم إستخدام كل من البكتيريا ***Azotobacter chroococcum*** و ***Bacillus megaterium*** var. ***phosphaticum*** و كذلك مخلوط منهما ضد نيماتودا تعقد الجذور من جنس (ميلودوجينا انكوجنيتا) على نباتات الطماطم من نوع (بيتو٨٦) تحت ظروف الصوبة. وأثبتت الدراسة أن البكتريا ***A. chroococcum*** هى الأكثر تاثيرا فى تقليل أعداد نيماتودا تعقد الجذور بنسبة ٧٤.٦%، يلى ذلك بكتيريا ***B. megaterium*** var. ***phosphaticum*** بنسبة ٧١.٢% و أخيرا المخلوط من كل من نوعى البكتيريا السابقة بنسبة ٦٤% مقارنة بالمبيد الكيميائى (كمعاملة كيميائية مقارنة) بنسبة ٨٢%. و قد إستخدام جميع معاملات البكتيريا السابقة ضد نيماتودا تعقد الجذور إلى زيادة القياسات الخضرية مقارنة بالمعاملة الكيميائية. فعلى سبيل المثال، فان نوع البكتيريا ***B. megaterium var. phosphaticum*** أثبتت تفوقها على جميع المعاملات فى زيادة نمو الساق بنسبة ٩٦.٤٥% مقارنة بالمعاملة النيماتودية المقارنة.