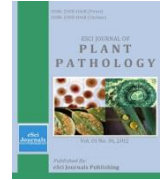




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THE POTENTIAL OF FIVE ECO-BIORATIONAL PRODUCTS ON THE REPRODUCTION OF ROOT-KNOT NEMATODE AND PLANT GROWTH

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ABSTRACT

This work aimed to select potentially useful eco-biorational product that could be used to reduce the reproduction of root-knot nematode. The experiment was carried out in pots under net house. The results revealed that the bio-product Dipel® (*Bacillus thuringiensis*) proved to be the most effective treatment that reduced the root galls and egg masses by 71.60 and 77.78%, respectively. Also, Dipel® (*B. thuringiensis*) & Bio-nematon® (*Paecilomyces lilacinus*) showed their superiority between all treatments on the shoot, root length and root weight.

Keywords: *Meloidogyne* spp., antagonistic fungi, antagonistic bacteria, fosthiazate and nematodes management.

INTRODUCTION

Tomato plant (*Solanum lycopersicum*) is an important vegetable crop for nutritive sources such as carbohydrates, minerals and vitamins in Egypt (Howeedy *et al.*, 2003). The most serious problems that threaten cultivated tomatoes are pests and diseases. The plant parasitic nematodes (PPN) have been found to be the most common and destructive diseases in the last two decades, and one of the most difficult plant diseases to control. The latest statistics showed that the estimated losses induced by PPN were \$118 billion worldwide (Atkinson *et al.*, 2012).

There are thousands of nematodes genuses, but the most destructive genus around the world is the root-knot nematodes (*Meloidogyne* spp.). *Meloidogyne* spp can parasite on more than 2000 host species including vegetables, fruit trees, oil crops, fiber crops, grains crops and leguminous crops, next to weeds which is considered secondary host to nematodes (Khalil, 2013a). The most well-known species of root-knot nematode are *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, which are responsible for high economic losses to varied crops. A number of methods for the root-knot nematodes management have been applied, and different levels of successes were displayed on crop

protection (Randhawa *et al.*, 2001 & Sakhuja and Jain 2001). It was necessary to find alternatives and / or new approaches to manage and eliminate the plant nematodes diseases. The soil-inhabiting fungus *Paecilomyces lilacinus* (Thom) Samson (Eurotiales: Trichocomaceae) is capable of parasitizing nematode eggs, juveniles and females resulting in reduced soil population densities of plant parasitic nematodes (Atkins *et al.*, 2005 and Khalil *et al.*, 2012b). Furthermore, *Trichoderma* spp. plays major roles in controlling the plant diseases in roots, soil and foliar environments (Thangavelu and Mustafa, 2012).

Also, the bacterium *Bacillus thuringiensis* Berliner produces parasporal crystalline proteinaceous inclusions. Most of these crystal proteins or δ -endotoxins are toxic to larvae of lepidopteran, dipteran or coleopteran insects (Knowles and Dow, 1993), pathogenic protozoa, mites and nematodes (Fettelson *et al.*, 1992). Meanwhile, it was reported that some strains of *Bacillus subtilis* have exhibited enormous potential as biocontrol agents in the management of root-knot nematodes (Karanja *et al.*, 2007). Therefore, the objective of this study was to testify the efficiency of the commercial products as an alternative nematicides.

MATERIALS AND METHODS

The tested products:

The following tested eco-biorational products against the RKN (root knot nematode) were used:

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- Nemathorin® 10% G (fosthiazate), [RS-S-sec-butyl O-ethyl 2-oxo-1, 3-thiazolidin-3-yl phosphonothioate;(RS)-3-[sec-butylthio(ethoxy)phosphinoyl]-1,3-thiazolidin-2-one.
- Bio-Nematon® 1.15% WP, contains 1*10⁸ cfu/g of fungus (*Paecilomyces lilacinus*).
- Bio Zeid® 2.5% WP, contains 1 x 10⁷ cell / g of fungus (*Trichoderma album*).
- Stanes sting® contains 1*10⁹ cell/ml of bacterium (*Bacillus subtilis*).
- Bio Arc® 6% WP, contains 25 x 10⁶ cell / g of bacterium (*Bacillus megaterium*).
- Dipel® 6.4% WG, containing 32,000 clu /mg of bacterium (*Bacillus thuringiensis* var. *kurstaki*).

The nematode inoculation: The tomato plants were infected with root-knot nematode eggs which isolated from the infested roots of the eggplant (*Solanum melongena* L.) that obtained from Rashid region, Behera Governorate, Egypt. Sodium hypochlorite (NaOCl) was utilized for isolation of nematode eggs from root galls according to Hussey and Barker (1973). Moreover, the roots were stained for 15 minutes in an aqueous solution of Phloxine B stain to detect the presence of nematode egg masses (Holbrook et al., 1983).

The Pots experiment: The pot experiment was carried out using tomato plants cv. super strain B, the Pots were 15 cm in diameter and 20 cm in depth and each pot filled with 1kg of autoclaved artificial mixture soil {1clay: 2 sand (v/v)}. The isolated eggs of root-knot nematode were applied at the rate of 5000 eggs / pot. Six treatments were applied, next to untreated check and each treatment was replicated five times, and each replicate contains one plantlet. Fifty days after planting, the seedlings were uprooted and root systems were assessed for galling (number of galls/root system), and egg masses/root system, in addition to the shoot length, shoot weight, root length and root weight.

Application of eco-biorational products: The tested products were applied to soil as one-time drench according to the recommended dose as following: Bio Zeid® applied at the rate of 40 kg / fed; Bio Arc® utilized at 40 kg / fed; Stanes sting® at the rate of 1L /100L water; Bio-Nematon® at the rate of 1.2 kg / 100L water and Nemathorin® at the rate of 12.5 kg / fed. While, the

suggested dose of Dipel® was 3 kg / fed. All treatments were applied two days after infection. The tomato plants were fertilized by (N: P: K 18:18:18 + TE).

Statistical analysis: Data of the present study were analyzed using variance test (ANOVA). The experimental design was a complete randomized design. The least significant differences (LSD) at the 5% level of probability were determined using a computer program Costat software (1988).

RESULTS

The impact of certain eco-biorational products on galls and egg masses formation were recorded in Table (1) and Fig. (1). The obtained results revealed that all treatments reduce the galls without any significant differences. *B. thuringiensis* reduced the gall formation by 71.60%, followed by *B.subtilis*, *P. lilacinus*, *B. megaterium*, *T. album* and fosthiazate that recorded 60.94, 58.58, 57.98, 52.65 and 51.50 % reduction, respectively.

On the other hand, *B. thuringiensis* proved to be the most effective treatment which minified the egg masses by 77.78%, followed by *P. lilacinus*, *T. album*, *B. subtilis*, fosthiazate and *B. megaterium* which recorded 65.18, 63.33, 62.96, 59.27 and 57.04% reduction, consecutively. According to obtained data it was found that all treatments increased the shoot system growth significantly as compared with untreated check as shown in table 2. There were no significant differences on shoot height among all treatments in comparison with untreated check.

Table 1. The effect of treatments on mean numbers of galls and egg masses.

Treatments	Mean no. of galls / root system	Mean no. of egg masses/ root system
<i>P. lilacinus</i>	23.33 b	15.67 b
<i>T. album</i>	23.67 b	19.33 b
<i>B. megaterium</i>	26.67 b	16.50 b
<i>B. subtilis</i>	22.00 b	16.67 b
<i>B. thuringiensis</i>	16.00 b	10.00 c
fosthiazate	27.33 b	18.33 b
Untreated check	56.33 a	45.00 a

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05.

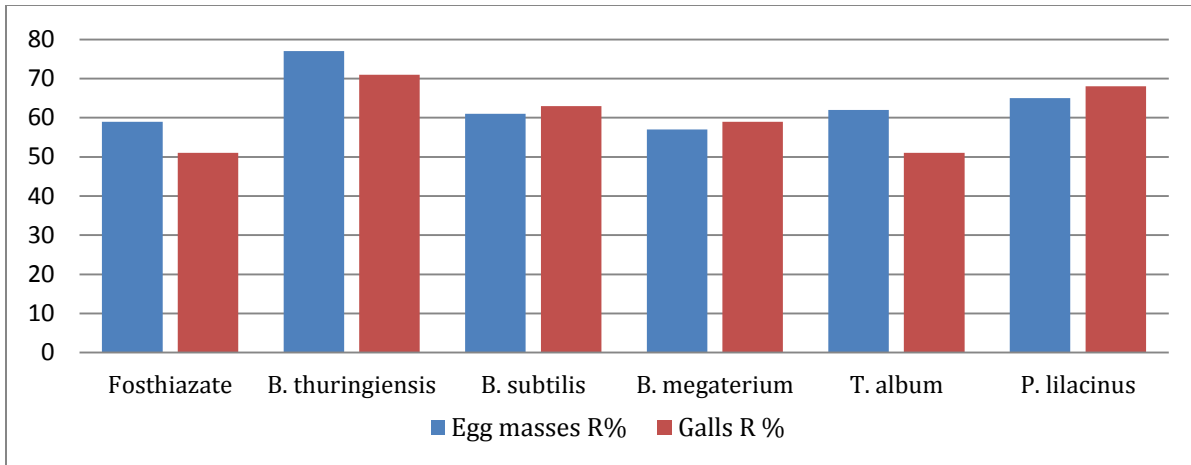


Fig. (1): the reduction percentage (R%) of bio-products on galls and egg masses.

B.thuringiensis was the best treatment which recorded 90.44% augmentation, followed by *P. lilacinus*, fosthiazate, *T. album*, *B. megaterium* and *B. subtilis* achieving 89.60, 89.21, 86.99, 77.25 and 75.96% increasing, respectively. Moreover, *B. thuringiensis* increased shoot weight by 53.10%, followed by *P. lilacinus*, *B. megaterium*, *T. album*, *B. subtilis*, next to fosthiazate with increasing values 35.03, 35.03, 25.92 and 23.03%, respectively.

In respect to the effects of bio-products on the root system growth it were also recorded in table (3). *P.*

lilacinus and *B. thuringiensis* showed the largest increase in root length with 59.49 and 43.14%, consecutively. Meanwhile, *B. megaterium*, *B. subtilis* and fosthiazate increased the root length by 35.96, 33.33 and 25.5%, respectively.

The antagonistic bacteria *B. thuringiensis* and *B. megaterium* were .increased the root weight by 57.78 and 50%, consecutively, followed by *T. album*, *B. subtilis* and *P. lilacinus* with 28.61, 7.22 and 2.78% increasing, respectively. While fosthiazate was the minimal treatment which reduced root weight by 2.78%.

Table 2. The effect of bio-products on plant shoot growth characteristics.

Treatments	Shoot height (cm)	I %	Shoot weight (g)	I %
<i>P. lilacinus</i>	48.67a#	89.60*	16.48ab	37.80
<i>T. album</i>	48.00a	86.99	16.15b	35.03
<i>B. megaterium</i>	45.50a	77.25	16.15b	35.03
<i>B. subtilis</i>	45.17a	75.96	15.06b	25.92
<i>B. thuringiensis</i>	49.00a	90.44	18.31a	53.10
fosthiazate	48.57a	89.21	14.72b	23.03
Untreated check	25.67b	-	11.96c	-

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05,

* Increasing percentages.

Table 3. The effect of bio-products on plant roots' growth indices.

Treatments	Root length (cm)	I %	Root weight (g)	I %
<i>P. lilacinus</i>	40.67a#	59.49*	3.69cd	2.78
<i>T. album</i>	28.17cd	10.50	4.63bc	28.61
<i>B. megaterium</i>	34.67b	35.96	5.40ab	50.00
<i>B. subtilis</i>	34.00b	33.33	3.86cd	7.22
<i>B. thuringiensis</i>	36.5ab	43.14	5.68a	57.78
fosthiazate	32.00bc	25.5	3.50d	-2.78
Untreated check	25.50d	-	3.60d	-

#Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05

* Increasing percentages.

DISCUSSION

According to this study, the efficiency of tested eco-biorational products which can reduce the reproduction of root-knot nematode were seen by the suppressing of the galls and egg masses formation and enhancement of plant growth. *B. subtilis* and *B. thuringiensis* are considered the most well-studied bacteria against plant parasitic nematodes (Crickmore *et al.*, 1998; Dawar *et al.*, 2008; Radnedge *et al.*, 2003; Radwan, 2007; Siddiqui and Mahmood, 1999).

Ashoub and Amara (2010) investigated certain isolates of *B. thuringiensis* and *P. fluorescens* *in vivo* and *in vitro* against *Meloidogyne incognita*, and their results indicated that all *B. thuringiensis* isolates reduced galls formation by 81.8 and 94.6%, and egg masses by 87.7 and 93.9%, respectively, *in vivo*. Also, Prakob *et al.* (2009) found that *B. subtilis*, *P. aeruginosa* and *P. lilacinus* decreased nematode population densities, suppressed nematode infection and galls on lettuce roots and increased the weight of lettuce plants. In addition, Khalil *et al.* (2012b) found that formulated *B. subtilis* caused reduction for both galls and egg masses by 53.64 and 71.76 %, respectively.

Several reports clarified that the basic mechanisms of *B. subtilis* included direct parasitism, production of extracellular antibiotics metabolites or catabolic enzymes (e.g. proteases, chitinases and glucanases), stimulation of host defenses, incensement of plant growth, induced systemic resistance in plants, suppression of the plant diseases and secreting volatile nematicidal substances (Huang *et al.*, 2009; Huang *et al.*, 2005b; Ji *et al.*, 2006; Kloepper and Ryu, 2006; Lahlali *et al.*, 2013; Siddiqui and Mahmood 1999; St Leger, 1995).

On the other hand, Mena *et al.* (1996) recorded that the *B. thuringiensis* var. *kurstaki* controlled *M. incognita* and *Radopholus similis* Cobb on *Cucurbita pepo*. While, Radwan (1999) observed that the shoot and root length and fresh weight of tomato plants were increased in the presence of *B. thuringiensis* var. *kurstaki* and Oxamyl.

B. thuringiensis (Bt) produces one or more parasporal crystal inclusions (Cry or d-endotoxins). These toxins are known to be toxic to a wide range of insect species (Feitelson *et al.*, 1992). Some Cry proteins are also toxic to nematodes (Feitelson *et al.*, 1992). To date, five Cry proteins (Cry5B, Cry6A, Cry13, Cry14A, Cry21A) known to be toxic to larvae of a number of free-living or parasitic nematodes (Crickmore *et al.*, 1998; Marroquin

et al., 2000 and Wei *et al.*, 2003). Additionally, a number of studies have reported direct antagonistic effects of other bacteria to pathogenic nematodes belonging to the genera *Heterodera* and *Meloidogyne*, included *B. amyloliquefaciens*, *B. cereus*, *B. licheniformis*, *B. megaterium* and *B. thuringiensis*.

In surveys have been conducted worldwide to detect fungal parasites of *Meloidogyne* spp. was found that there are more than 30 genera and 80 species of fungi such as *Arthrobotrys* spp., *Monacrosporium* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *P. lilacinus* and *Verticillium chlamydosporium*, (Chen *et al.*, 1996a; Godoy *et al.*, 1983; Li *et al.*, 1994; Rocuzzo *et al.*, 1993 and Wang *et al.*, 2001). However, in China, the predominant fungal species which collected from plant roots and infested soil was *P. lilacinus* that represented 49.3% of the isolates during a survey (Sun *et al.*, 2006).

The antagonistic fungus *P. lilacinus* proved its activity against root-knot nematodes on varied crops. Several reports clarified that using formulated *P. lilacinus* reduced the formation of galls and egg masses (Udo *et al.*, 2013). Meanwhile, Khalil *et al.* (2012b) confirmed that liquid Bio-Nematon® (*P. lilacinus*) and Dipel 2x® (*B.thuringiensis* var. *kurstaki*), were the most effective treatments which suppressed the galls by 66.67 and 60.15 %, respectively, while decreased the egg masses by 75.97% and 74.97%, consecutively.

Also, Kiewnick and Sikora (2006) recorded that the fungal biocontrol agent, *P. lilacinus* strain 251 (PL251) was potential to control the root-knot nematode *Meloidogyne incognita* on tomato. The pre-planting soil treatment reduced root galling by 66% and number of egg masses by 74%. *P. lilacinus* was effective against the root knot nematode and significantly reduced the galls number, egg masses and eggs per egg mass. Moreover, the enhancement of plant growth (Ganaie and Khan, 2010; Oclarit *et al.*, 2009; Prakob *et al.*, 2007 and Siddiqui *et al.*, 2001).

The action of *P. lilacinus* against plant parasitic nematodes was interpreted in multitude investigations. Khan *et al.* (2006b) and Khan *et al.* (2004) recorded the directed penetration of fungal hypha to the female cuticle of *M. javanica* by transmission electron microscopy. While, Park *et al.* (2004) reported that *P. lilacinus* could be produce leucino toxin and other nematicidal compounds. In the laboratory test this fungus infested eggs of *M. incognita* and destroys the embryos within 5 days because of simple penetration of

the egg cuticle by individual hypha aided by mechanical and/or enzymatic activities, in addition to killing juveniles and females of *M. incognita* and *Globodera pallida* (Jatala, 1986). It was mentioned that *P. lilacinus* caused substantial egg deformation in *M. incognita*, these deformed eggs never matured or hatched (Jatala *et al.*, 1985). The serine protease produced by *P. lilacinus* might play a role in penetration of the fungus through the egg shell of the nematode (Bonants *et al.*, 1995 and Khan *et al.*, 2004).

Also, it was reported that *T. viride* reduced galls formation and egg masses of *Meloidogyne incognita*, infecting Okra (Kumar *et al.*, 2012). Le *et al.* (2009) investigated the potential of *Fusarium* and *Trichoderma* isolates against *M. graminicola* in rice. The results showed that *Trichoderma* isolates reduced galls formation up to 38%, while *Fusarium* isolates reduced the galls by 29–42%. Furthermore, Krishnaveni and Subramanian (2004) and Sharma (1999) indicated that *T. harzianum*, *T. viride* and *P. fluorescens* were effective in controlling the plant parasitic nematodes. Kavitha *et al.* (2007) found that *P. fluorescens*, *B. subtilis* and *T. viride* showed a significant increase in the plant growth parameters. However, the phytonematodes are affecting the *Trichoderma spp.* through the production of volatile and nonvolatile toxic metabolites, antibiotics, viridin, viridian, gliovirin, glisoprenins, heptelidic acid and others (Vey *et al.*, 2001). Fosthiazate which belong to organophosphate group is inhibit the acetylcholine esterase (AChE) in various parts of the nervous system of nematodes and provides a highly performance as systemic nematicide. The results in this study are in agreement with those obtained by other researcher (Giannakou *et al.*, 2005; Pathan *et al.*, 2005; Russo, *et al.*, 2003; Saad *et al.*, 2011) who found that fosthiazate was effective against RKN.

Besides, Radwan *et al.*, (2012) confirmed that fosthiazate was the most effective treatment against the root galls formation in compared with four granular nematicides namely, cadusafos, carbofuran, ethoprop and oxamyl. Also, all treatments increased the plant growth indices. Whilst, Kesba (2011) found that nemathorin® 10% G (fosthiazate) was the superior treatment which reduced the galls and egg masses between all other treatments.

CONCLUSION:

It could be concluded that application of formulated eco-biorational products were effective against the

reproduction of RKN and proved the plant health.

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