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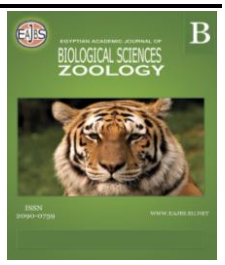

Effects of Roots-Applied Resistance Inducers on Penetration and Development of Root-Knot Nematode in Sugar-Beet

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ABSTRACT

This study is a part of an extended investigation that aims to clarify the nemastatic activity of two commercial formulations of resistance inducers; Bio-arc and Nemastrol against *Meloidogyne incognita* infected sugar-beet in vitro and in vivo. Generally, data indicate that the ability of all individuals of the stage juveniles to penetrate the roots and develop into the next stage with a shortage of the duration of the life cycle in sandy soil compared to clayey soil. The results showed that Nemastrol caused a significant reduction in the percentage of penetration of *M. incognita* at the second-stage juveniles. Bio-arc ranked the second one comparing to control in clayey and sandy soil. The duration of developmental four juvenile stages, as well as egg-laying females, have a clear reduction with Nemastrol in clayey and sandy soil more than Bio-arc comparing to control. The length of the life cycle varies, as treatments by Nemastrol were longer than Bio-arc in clayey and sandy soil (2-24 and 9-24 days), respectively. Besides, Nemastrol showed a poor formation of irregular giant cells devoided from the cytoplasm and contained less number of nuclei.

INTRODUCTION

Sugar-beet (*Beta vulgaris* L.) is considered the first alternative source of sugar to sugar-cane in Egypt, especially in the current period, with constant encouragement from the government to increase the area cultivated from alternative crops for sugar-cane. Where the total area planted with sugar beet was about 520 thousand feddans with average productivity of 18 tons / feddan for cultivation season, 2020 (Sugar Crop Board, Ministry of Agriculture and Land Reclamation, Egypt). Phytonematodes causing great damage equal to the damage caused by fungal diseases combined (Cooke, 1993). Globally, crop losses induced by phytonematodes have been evaluated at 80 $US billion annually (Nicol *et al.*, 2011). In Egypt, the phytonematodes infecting sugar-beet are numerous, as it was estimated to 18 genera, root-knot nematodes (RKN) *Meloidogyne* spp. were ranked at first in terms of presence and size of damage (El-Sherif *et al.*, 2010; El-Sagheer, 2020). In addition to the direct damage of RKN, penetration of nematodes in the roots causes an entry point for

infection by root-knot nematodes other pathogens (Khan, 1993 and Back *et al*., 2002). The root-knot nematode Meloidogyne incognita is one of the most damaging nematode infecting sugar-beet (El-Nagdi *et al*., 2004). Efforts to protect sugar-beet against root-knot nematode infection are crucial. Based on the slight of plant resistance to root-knot nematode and the environmental standards on chemical treatments use for management plant-parasitic nematodes, recently biological control as eco-friendly control standards have gained increasing attention (Williamson & Hussey, 1996 and Chitwood, 2002). Normally, plants have a different way of defense responses against various types of biotic and abiotic stresses, so a new strategy for adjusting plant-parasitic nematodes is based on the activation of the plant's defense system. Currently, many resistance inducers against various pests and pathogens are produced commercially, which are used as an effective alternative to traditional control methods (Burketova *et al*., 2015). The most important of these products are; plant growth rhizobacterium (PGPR) which belonging to *Bacillus* spp (Family: Bacillaceae) which almost linked with defense mechanisms by expression of the various accumulation of enzymes and hormones in addition to some changes in the chemicals inside the plants' roots (Ibrahim, 1991; Meena *et al*., 2000 and Verma *et al*., 2018).); A mixture of some active ingredients (glycosynolates, chitinase and tannins) it is considered one of the most promising materials for nematode control where has a wide spectrum of methods of inducing plant resistance (Mercer 1992; Cóndor, 2019), which did not receive enough study for their nematicidal effects (Sikandar *et al*., 2020). Therefore, the present study aims to examine the association between using a commercial formulation of resistance inducers; Bio-arc and Nemastrol against *Meloidogyne incognita* infected sugar-beet *in vitro* and *in vivo*.

MATERIALS AND METHODS

Preparation of *Meloidogyne incognita* Inoculum:

Coleus (*Coleus blumei*) root systems with heavily egg-masses have been carefully cleaned off soil by running tap water. Three egg masses were crushed to get the adult females that were used to confirm the species *Meloidogyne incognita*. Which was done using Taylor and Netscher (1974) protocol. Then the coleus roots were cut into small pieces and put in a glass container with enough solution of 1.0% NaOCl (Hussey, 1973) and shacked vigorously for 60 seconds. Solutions were passed quickly through two nested sieves (60 and 500 mesh) and thoroughly the collected eggs were washed with tap water to remove the bleach. The number of eggs per unit volume of water was counted. The newly hatched second-stage juveniles (J2s) of the root-knot nematodes were obtained from a pure culture maintained on coleus roots. At room temperature roots were incubated for 5-7 days by Baermann's technique for hatching.

Tested Resistance Inducers:

Bio-arc :

A local formulation as a commercial product of phosphorus soluble bacterium, *Bacillus megaterium* (25×10^6 cfu/g) at 2.5g/ L of distilled water, enrolled by the Egyptian Ministry of Agriculture and Land Reclamation with registration number 1087.

Nemastrol:

The mixture of some active ingredients (chitinase, glycosynolates & tannins) as a commercial product, with the recommendation rate 5L/ Feddan, was obtained from Royal company for agricultural development, Egypt.

***In vitro* Experiments:**

The experiment was designed to evaluate the impact of previous products on the hatchability and mortality of *M. incognita* in lab conditions (23+2°C).

Nemastrol was applied at three rates; 0.25, 0.5, and 1 ml/ petri dish, and Bio-arc was used at

four rates; 5, 10, 15, and 20ml/ petri dish. One milliliter of tested products was added to 4 ml of *M. incognita* suspension containing 100 eggs, and 100 J2s in a petri dish (5 cm diameter) in a single treatment for each rate (Thorne,1961). The suspension in distilled water was used as control. Each treatment was replicated three times.

The dishes were screening under a binocular microscope after 24, 48, and 72 hours (Southey,1986) and surviving and dead larvae were counted as well as the numbers of hatched juveniles were recorded after 10 days. Mortality percentages and egg hatching inhibition were calculated using the following formula:

$$\text{Mortality \%} = \left(\frac{\text{Number of survived larvae in control} - \text{Number of survived larvae in treated}}{100 - \text{Number of survived larvae in control}}\right)$$

$$\text{Hatching inhibition \%} = \left(\frac{\text{Number of juveniles hatched in control} - \text{Number of juveniles hatched in treated}}{\text{Number of juveniles hatched in control}}\right) \text{x}100$$

Greenhouse Experiments:

A greenhouse experiment was conducted using sandy and clayey soil to estimate the impact of Bio-arc and Nemastrol on the penetration ability of *Meloidogyne incognita* individuals to infect the sugar-beet roots cv. Nejama as a susceptible cultivar. For each soil type, 48 pots filled with 300g of sterilized soil were planted with three seeds of sugar-beet and irrigation water was added when the plant is needed. After one month from germination, plants were thinned to one seedling/pot. Simultaneously, plants were treated with bio-arc at 20ml/ pot, Nemastrol at 0.25ml/ pot. Both bio-agents were applied as a soil drench in three holes around the plant. After Fifteen days, seedlings were inoculated with 300 larvae of *M. incognita*. Also, three non-inoculated served as a control for each soil type. In a greenhouse (27 ± 3ºC with 12 h photoperiod) the pots were arranged in a randomized complete block design.

Penetration and Development:

The effect of tested resistance inducers on the penetration of *M. incognita* J2s into sugar-beet roots was investigated using penetration inhibition test described by Southey (1970) and Bybd *et al*., (1983). For all treatments and control, sugar-beet plants were uprooted after 1st, 2nd, 3rd, 6th, 9th, 13th, 16th, 18th, 21, and 24 days from nematode inoculation. Roots were stained with acid fuchsin. Where the infected roots were placed in 1.5% sodium hypochlorite (NaClO) solution for four minutes. then removed from these solutions and placed and boiling for 30 s in staining that contains 30 mL water and 1 mL stain (750 mL distilled water, 250 mL acetic acid and 3.5 g acid fuchsin,). The roots were washed carefully in running water and removed to glycerine (acidified), and heated to the boiling, and then cooled to lab temperature. The stained developmental stages inside the roots were examined and counted under a stereoscopic microscope.

Histological Study:

The selected portions of *M. incognita* infected sugar-beet roots from all treatments were carefully washed from soil. Roots cut into 3-5 mm long sections. Then fixed in FAA solution (2.4 parts formalin as 37% formaldehyde, 1.6 parts acetic acid, 60 parts ethanol 95 % and 80 parts of distilled water) (Johansen, 1940 and Southey 1986). The root sections were dehydrated in tertiary butyl alcohol (TBA) depending on the protocol described by Johansen (1940) and Goodey (1949). After the dehydration process, the solution was replaced with a mixture of butyl alcohol and paraffin oil (PO) at (1:1) for one hour or more, according to the thickness of the roots. The root segments were picked up from the previous mixture, and placed on the surface of the consolidated PO solution and placed disclosed in the oven at a bit above the liquefying point of the paraffin. After two hours, the BA- PO mixture was poured down and replaced with pure melted paraffin wax and kept in the oven for two hours (Finley,

1981).

The root tissues were placed in molds and added the liquid paraffin until rises above the tissues. When the paraffin began to coherence plunged into the freezer until they solidified. The blocks were dropped from the molds and sectioned 10-12 µm thickness by rotatory microtome. Then, the paraffin strips were placed on glass slides and kept in the incubator at 40 ^{o}C until the water was evaporated. The paraffin wax was removed by placed the slides at 60 °C for about one hour until they melted the wax. The staining process was done by using the safranin and fast green according to the protocol described by Johansen (1940) and Sass (1951). On the slides, the mounting medium was applied and was covered carefully. The slides were placed in the incubator at 60°C to 24 for drying of the mounting medium (Bybd *et al.*, 1983). The final slides were examined and photographed using Carson digital microscope, model zPix MM-940, USA.

Data Analysis:

The data were subjected to analysis of variance (ANOVA) in a completely randomized design with three replicates and the means were compared by L.S.D. test at 0.05 levels, using Costat software (Costat Statistical Software, 1990).

RESULTS

Impact of Resistance Inducers on Egg Hatching and Juveniles Survival of *Meloidogyne incognita In Vitro*:

The impact of Bio-arc at four tested rates (5, 10, 15, and 20 ml) and Nemastrol (0.25, 0.5, and 1.0 ml) on egg hatching and juvenile mortality of *M. incognita* were studded in laboratory conditions. Data in Table (1) revealed that all tested treatments caused a significant reduction ($P \leq 0.05$) in the number of second-stage juveniles hatched from egg and hatching inhibition percentage at all treatments, compared to the non-inoculated control. Among all treatments, Bio-arc at 20 ml better results than did those of 5 to 15 ml. However, a positive correlation was achieved among tested rates. Meanwhile, the higher the rates the greater inhibition in egg hatching was recovered. Nemastrol (99.0 %) at 1.0 ml sustained the highest and significant inhibition in hatching rate followed by Bio-arc at 20 ml (96.0 %). However, the least inhibition hatching rate was recorded with Bio-arc at 5 ml (21.0 %). The previously mentioned treatments showed nematicidal activity against newly hatched juveniles of *M. incognita* survival after three times of exposure. A positive correlation among bio-arc treatment at different concentrations after 24, 48, and 72h were revealed. Herein, Nemastrol exceeded other treatments of bio-arc with different rates at three exposure periods. The highest percentage of *M. incognita* juvenile mortality (100.0%) was significantly recorded with the highest concentration of bio-arc. However, the least percentage of juvenile mortality was recorded with bio-arc at 5 ml after 24h. On the other hand, moderate results in nematode survival were recorded with all treatments at 48 h (Table 1).

Impact of Resistance Inducers on The Penetration of *M. incognita* to Roots of Sugar-Beet Under Greenhouse Conditions:

The influence of Bio-arc at (20 ml) and Nemastrol at (1.0 ml) on penetration and lifecycle of *M. incognita* on sugar-beet plant var. Nejama and grown in clayey and sandy, were shown in Table (2). All investigations indicate that the sandy soil showed a remarkable increase in development and penetration of *M. incognita* to roots of sugar-beet with significant levels. Also, based on soil type all investigations showed that the life cycle of *Meloidogyne incognita* in the sandy soil faster and more developed than the clayey soil (Table 2). Where Bio-arc in clayey soil showed % of penetration with the second stage (3.0%) after the third day of the infection followed by third stage (3.0%) after six days then fourth stage (7.0%) after nine days of infection. Meanwhile, adult females developed in roots

after thirteen days of infection. Herein, egg masses appeared on roots (1.0%) at sixteen days of the infection. Moreover, developmental stages of *M. incognita* made feeding sides with giant cells which shaped galls on roots cells and appeared at nine days (3.0%) of the infection. Also, data showed that Nemastrol at 1.0 ml caused a significant reduction in % of penetration of *M. incognita* at the J2s after three days (5.0%) comparing to control. Meanwhile, the third stage (1.0%) and the fourth stage (1.0%) have appeared after nine days of infection. Adult females (16.0%) developed in roots after eighteen days of infection with no appearance for egg masses (0.0%) during the studied life cycle. Giant cells appeared at nine days (1.0%) of the infection comparing to control. On the other hand, in sandy soil, Nemastrol resulted in a pronounced suppression of the penetration of J2s with % (3.0%) which appeared on the second day followed by (1.0 %) for the third stage on the sixth day and fourth stage (7.0 %) on a ninth day. Further, adult females (3.0%) developed in roots after sixteen days of infection. Moreover, egg masses were suppressed (2.0%) after the 21st day. Giant cells appeared after nine days (6.0%) of the infection comparing to control. Bio-arc was the second rank after Nemastrol too in the suppression of penetration for all the developmental stages i.e. second stage (5.0 %) on the second day, the third stage, and galls formation (3.0%) on the sixth day, the fourth stage (7.0%) on a ninth day, adult females and egg masses appearances (3.0%) on thirteen days (Table 2). In contrast, the chemical nematicide Oxamyl showed significant suppression of the penetration of the second stage (2.0; 3.0%) on the third day, the third stage (1.0; 1.0%) on the sixth day, the fourth stage (1.0; 5.0%) and galls formation (2.0; 3.0%) on the ninth and thirteen days, adult females (2.0; 3.0 %) on eighteen and sixteen days, and egg masses appearances (0.0; 2.0) on the 21st day on the in clayey and sandy soil, respectively (Table 2).

Table 1. Impact of Bio-arc and Nemastrol on mortality rate and egg hatching inhibition of *Meloidogyne incognita*

Treatments	Rate (ml/Petri)	Exposure period			
		24 h	48 h	72h	10 days
		Mortality%	Mortality %	Mortality%	Egg Hatching Inhibition%
Bio-arc	5	29.0^{h}	44.0 ^{f}	93.0 ^{c}	21.0^{d}
	10	38.0^{g}	52.0 ^{e}	97.0 ^{b}	45.0^{c}
	15	44.0 ^{f}	73.0^{d}	97.0^{b}	52.0^{b}
	20	50.0^{e}	75.0 ^{d}	100.0 ^{a}	96.0^{a}
LSD 0.05		2.00			2.12
*F		327.53			1958
P		.0000			.0000
Nemastrol	0.25	46.0^{f}	83.0^{c}	100.0^{a}	97.0^{b}
	0.5	98.0^{b}	98.0^{b}	100.0^{a}	98.0^{ab}
	1.0	100.0^{a}	100.0^{a}	100.0^{a}	99.0^{a}
LSD 0.05		2.23			1.18
*F		350.85			4.45
P		.0000			ns
Control		3.0^{j}	10.0^{i}	16.0 ^{i}	0.0^{k}

*Each value presented the mean of three replicates. *M. incognita* (100 J2s / eggs).
According to Duncan`s multiple range tests, means in each column followed by the same letter(s) did not differ at $P \leq 0.05$.
*F & P values presented the interaction between treatment concentrations * Exposure period as 2 Way Completely Randomized test.

Table 2. Impact of Bio-arc and Nemastrol on the penetration and development of *Meloidogyne incognita* stages on the root of sugar-beet cv. Nejma in two soil types.

Penetration of nematode stages %											
Treatments	**Nematode stages**	**1**	**2**	**3**	**6**	**9**	**13**	**16**	**18**	**21**	**24**
Clayey soil											
Bio-Arc	Second stage	0.0r	0.0r	3.0op	2.0pq	6.0lm	1.0qr	1.0qr	1.0qr	0.0r	0.0r
	Third stage	0.0r	0.0r	0.0r	3.0op	2.0pq	3.0op	1.0qr	1.0qr	1.0qr	0.0r
	Fourth stage	0.0r	0.0r	0.0r	0.0r	7.0kl	7.0kl	2.0pq	2.0pq	0.0r	0.0r
	Adult stage	0.0r	0.0r	0.0r	0.0r	0.0r	1.0qr	1.0qr	8.0jk	6.0lm	14.0fg
	Egg laying female	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	1.0qr	1.0qr	6.0lm	13.0gh
	Galls	0.0r	0.0r	0.0r	0.0r	3.0op	6.0lm	9.0ij	14.0fg	16.0e	18.0d
Nemastrol	Second stage	0.0r	0.0r	5.0mn	2.0pq	2.0pq	0.0r	0.0r	0.0r	0.0r	0.0r
	Third stage	0.0r	0.0r	0.0r	0.0r	1.0qr	1.0qr	4.0no	1.0qr	0.0r	0.0r
	Fourth stage	0.0r	0.0r	0.0r	0.0r	1.0qr	1.0qr	1.0qr	0.0r	0.0r	0.0r
	Adult stage	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	16.0e	18.0d	21.0c
	Egg laying female	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r
	Galls	0.0r	0.0r	0.0r	0.0r	1.0qr	2.0pq	5.0mn	10.0i	10.0i	10.0i
Oxamyl	Second stage	0.0r	0.0r	2.0pq	1.0qr	2.0pq	1.0qr	2.0pq	0.0r	0.0r	0.0r
	Third stage	0.0r	0.0r	0.0r	1.0qr	1.0qr	1.0qr	1.0qr	1.0qr	1.0qr	1.0qr
	Fourth stage	0.0r	0.0r	0.0r	0.0r	0.0r	1.0qr	1.0qr	2.0pq	2.0pq	2.0pq
	Adult stage	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	2.0pq	2.0pq	2.0pq
	Egg laying female	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r	0.0r
	Galls	0.0r	0.0r	0.0r	0.0r	0.0r	2.0pq	3.0op	3.0op	5.0mn	5.0mn
Control	Second stage	0.0r	0.0r	8.0jk	4.0no	4.0no	3.0op	3.0op	3.0op	2.0pq	2.0pq
	Third stage	0.0r	0.0r	0.0r	1.0qr	7.0kl	7.0kl	3.0op	2.0pq	1.0qr	1.0qr
	Fourth stage	0.0r	0.0r	0.0r	1.0qr	9.0ij	12.0h	8.0jk	5.0mn	13.0gh	18.0d
	Adult stage	0.0r	0.0r	0.0r	0.0r	0.0r	1.0qr	2.0pq	3.0op	13.0gh	16.0e
	Egg laying female	0.0r	0.0r	0.0r	0.0r	0.0r	6.0lm	6.0lm	10.0i	15.0ef	26.0a
	Galls	0.0r	0.0r	0.0r	1.0qr	6.0lm	13.0gh	15.0ef	19.0d	23.0b	27.0a
LSD 0.05		1.287									
*F		32.77									
P		0.0000									
Penetration of nematode stages %											
Treatments	Nematode stages	1	2	3	6	9	13	16	18	21	24
Sandy soil											
Bio-Arc	Second stage	0.0x	5.0st	5.0st	7.0qr	3.0uv	1.0wx	0.0x	0.0x	0.0x	0.0x
	Third stage	0.0x	0.0x	0.0x	3.0	5.0st	2.0vw	1.0wx	1.0wx	0.0x	0.0x
	Fourth stage	0.0x	0.0x	0.0x	0.0x	7.0qr	7.0qr	2.0vw	2.0vw	0.0x	0.0x
	Adult stage	0.0x	0.0x	0.0x	0.0x	0.0x	3.0uv	5.0st	11.0mn	19.0ef	21.0d
	Egg laying female	0.0x	0.0x	0.0x	0.0x	3.0uv	9.0op	11.0mn	11.0mn	13.0kl	16.0hi
	Galls	0.0x	0.0x	0.0x	3.0uv	4.0tu	11.0mn	13.0kl	14.0jk	16.0hi	20.0de
Nemastrol	Second stage	0.0x	3.0uv	3.0uv	5.0st	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x
	Third stage	0.0x	0.0x	0.0x	1.0wx	1.0wx	1.0wx	1.0wx	0.0x	0.0x	0.0x
	Fourth stage	0.0x	0.0x	0.0x	0.0x	7.0qr	3.0uv	1.0wx	1.0wx	1.0wx	1.0wx
	Adult stage	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x	3.0uv	6.0rs	8.0pq	10.0no
	Egg laying female	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x	2.0vw	3.0uv
	Galls	0.0x	0.0x	0.0x	0.0x	6.0rs	6.0rs	8.0pq	8.0pq	12.0lm	14.0jk
Oxamyl	Second stage	0.0x	0.0x	3.0uv	3.0uv	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x
	Third stage	0.0x	0.0x	0.0x	1.0wx	3.0uv	1.0wx	1.0wx	0.0x	0.0x	0.0x
	Fourth stage	0.0x	0.0x	0.0x	0.0x	5.0st	3.0uv	1.0wx	1.0wx	1.0wx	1.0wx
	Adult stage	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x	3.0uv	6.0rs	6.0rs	6.0rs
	Egg laying female	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x	0.0x	2.0vw	2.0vw
	Galls	0.0x	0.0x	0.0x	0.0x	3.0uv	3.0uv	5.0st	7.0qr	7.0qr	7.0qr
Control	Second stage	0.0x	8.0pq	8.0pq	11.0mn	18.0fg	4.0tu	4.0tu	3.0uv	3.0uv	3.0uv
	Third stage	0.0x	0.0x	0.0x	4.0tu	16.0hi	4.0tu	2.0vw	1.0wx	1.0wx	1.0wx
	Fourth stage	0.0x	0.0x	0.0x	3.0uv	13.0kl	15.0ij	7.0qr	5.0st	5.0st	5.0st
	Adult stage	0.0x	0.0x	0.0x	0.0x	2.0vw	12.0lm	17.0gh	20.0de	24.0c	29.0b
	Egg laying female	0.0x	0.0x	0.0x	0.0x	7.0qr	14.0jk	17.0gh	19.0ef	21.0d	25.0c
	Galls	0.0x	0.0x	0.0x	2.0vw	10.0no	18.0fg	18.0fg	21.0d	28.0b	31.0a
LSD 0.05		1.371									
*F		31.22									
P		0.0000									

*Each value presented the mean of five replicates. N = *M. incognita* (100 J2s/ plant).

According to Duncan`s multiple range tests, means in each column followed by the same letter(s) did not differ at $P \leq 0.05$.

F & P values presented the interaction between treatment *RKN stages * No. of observation as 3 Way Completely Randomized test

Effect of Resistance Inducers on Histopathological Modifications Induced by Root-Knot Nematode in Tissues of Sugar-Beet Roots:

This part of the study aimed to correlate the penetration of *Meloidogyne incognita* under previous treatments with the histological modification in sugar-beet roots under greenhouse conditions. Generally, all treatments did not prevent *M. incognita* from penetrating the roots but differed in the time required to reach the vascular cylinder and to develop giant cells and develop to the next stage of life. The transverse section in sugar-beet roots (cv. Nejama) infected with *Meloidogyne incognita* showed that the existence of giant cells and cell wall shows a specific, clear and core spindle and ranges in a single cell, the cytoplasm dense and dark, the presence of a large food site, shows the female inside the cell clearly, as well as showing clearly the death of cells (Figs. 1C & 2C). Where in the transverse sections showed in sugar-beet roots after 24 hours of penetration in clayey soil, the root-knot nematode, *M. incognita* induced pronounced alterations in cells of cortical and stellar regions in roots of sugar-beet. In clayey soil, infected roots of sugar-beet had well-defined and rounded or oval giant cells in the stele regions with a number ranging from 2 to 4. Also, the existence of giant cells and cell wall shows a specific, clear and core spindle and ranges from 3-9 in a single cell, the cytoplasm dense and dark, the presence of a large food site, shows the female inside the cell clearly, as well as showing clearly the death of cells. There may be in some giant cell cytoplasm focused on the cell wall, we note that some giant cells, which have the female nematodes, are free from the cytoplasm. And Hypertrophied nuclei were scattered or aggregated in the cytoplasm with numbers ranging from 5-8. In some instances, dense cytoplasm was found to encounter small vacuoles. The xylem cells exhibited abnormalities in the structure near the giant cells. (Fig.1C). In contrast, sugar-beet roots after 24 hours of penetration in sandy soil, giant cells were found prolonged in vascular parenchyma cells with different shapes from circular to irregular shape. Clusters of giant cells ranging from 3 to 4 were observed occupying a considerable area in the vascular tissues inducing compressed cells and disruption in xylem and cortex layers, as a result of the presence of giant cells and the growth of the nematode females. Giant cells were characterized with thickened walls, dense granular cytoplasm, containing several hypertrophied nuclei scattered in the cytoplasm and ranged from 5 to 13. Nuclei were spindle or circular. In a few instances, a large vacuole was observed surrounded by dense cytoplasm (Fig. 2C). As for the histological modifications in sugar-beet roots infected with *M. incognita* and treated with Bio-arc, poorly formed giant cells in the central cylinder ranged from 2-5 were detected in the stellar region with limited hypertrophy or hyperplasia. For both soil types, giant cells contained less or free of cytoplasm, fewer numbers of nuclei, vacuolated in most instances, and were smaller than those of untreated infected roots in clayey or sandy soil, respectively (Fig. 1A& Fig. 2A). The xylem cells exhibited small abnormalities in the structure. Infected sugar-beet roots infected with *M. incognita* and treated with Nemastrol showed a poor formation of irregular giant cells divided from the cytoplasm and contained less number of nuclei (Fig. 1B&2B). However, infected roots and treated with Oxamyl, some giant cells were found to be collapsed whether in clayey or sandy soil, moreover, there are new healthy cells formed to vessels (Figs.3 A&B).

Impact of Bio-arc and Nemastrol on Development Duration and Life Cycle of *Meloidogyne incognita* on Sugar-Beet Roots in Two Soil Types:

Generally, data indicate that the ability of all individuals of the second-stage juveniles to penetrate the roots and develop into the next stage (Table 3 and Figs.1,2, 3). Where in the clayey soil the use of Nemastrol was delayed the ability and beginning of second-stage juveniles to penetration of roots, with about 6 days late for other treatments and control. Which led to the shortage of the duration of the second-stage juveniles took (9-18 days) and increases the duration required to develop to the third stage by a couple of days

compared with nematode alone as control (3-16), Bio-arc (3-16) and chemical control oxamyl (3-16). This is supported by the size of the larva in the transverse section of the root (Figs. 1and 3). These observations were similar to the third and fourth larval stages for all treatments. On the contrary to what is expected, the Egg-laying females' stage decreased in all treatments compared to the control (16-24) as it did not appear or noticed until the root-knot formed and appeared on the roots in the best result in decreasing of galls number of Nemastrol treatment with equal by Oxamyl treatment (13-24). As for sandy soils, the period of all stages, in general, has decreased compared to clayey soil. Where, the second stage larva appeared inside the root tissues in all treatments (2 days) (Fig. 2) except for the chemical treatment (3 days). Then it turned to the following stages in fewer time periods compared to clay soil (Table 3).

Table 3. Impact of Bio-arc and Nemastrol on development duration and life cycle of *Meloidogyne incognita* on sugar-beet roots in two soil types

Nematode stage / **Treatments**	**Duration of nematode stages (in days)**			
	Bio-arc	**Nemastrol**	**Oxamyl**	**Nematode alone**
Clayey soil				
Second stage larva	3-18	9-18	3-16	3-18
Third stag larva	6-24	9-18	6-24	6-24
Fourth stage larva	9-24	9-24	13-24	6-24
Adult female	13-24	16-24	18-24	13-24
Egg- laying female	0-0	0-0	0-0	16-24
Galls	6-24	13-24	13-24	6-24
Total life cycle	3-24	9-24	3-24	3-24
Sandy soil				
Second stage larva	2-6	2-6	3-13	2-9
Third stage larva	6-18	6-16	6-16	6-24
Fourth stage larvl	6-24	9-24	9-24	6-24
Adult female	13-24	16-24	16-24	9-24
Egg- laying female	16-24	21-24	21-24	16-25
Galls	6-24	9-24	9-24	6-24
Total life cycle	2-24	2-24	3-24	2-24

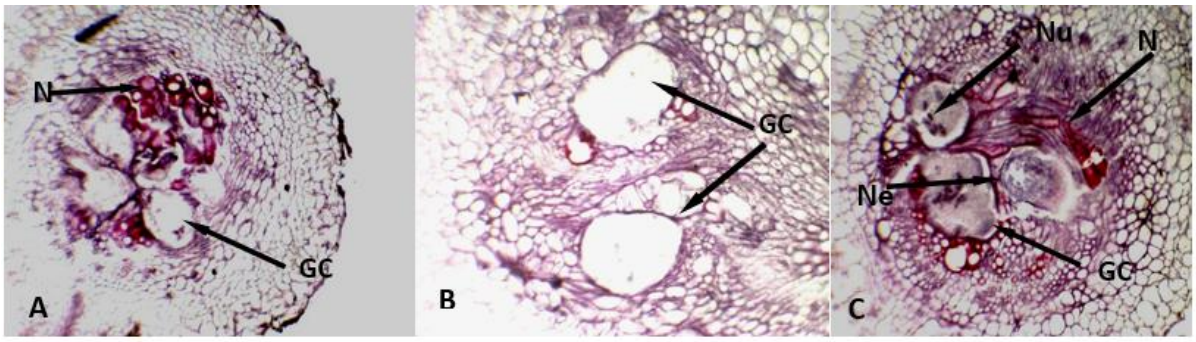

Fig. 1. Transverse section of sugar-beet infected with *M. incognita* after 24 days in clayey soil and treated by Bio-arc application (A); Nemastrol (B) and nematode alone.(C) (Gc) Giant cells; (N) Necrosis; (Nu) Nucleus; (Ne) Nematode.

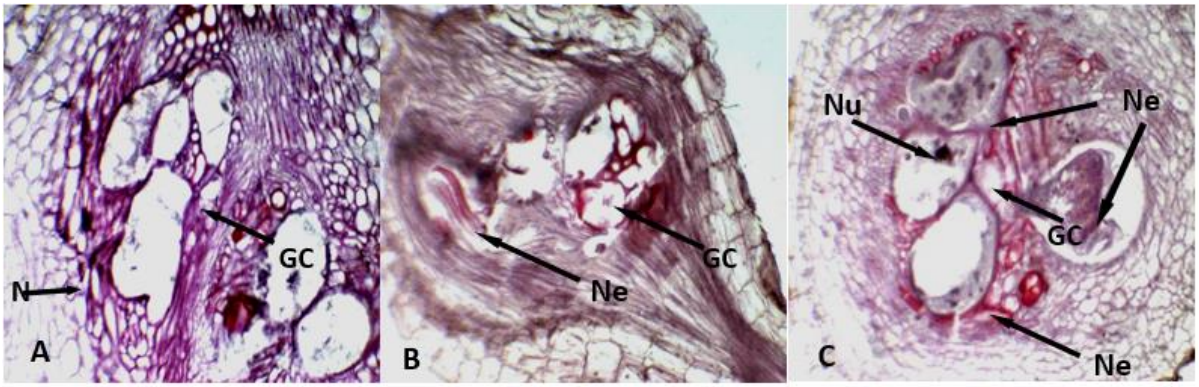

Fig. 2. Transverse section of sugar-beet roots infected with *M. incognita* after 24 days in sandy soil and treated by Bio-arc application (A); Nemastrol (B) and nematode alone (C).(Gc) Giant cells; (N) Necrosis; (Nu) Nucleus;(Ne)Nematode.

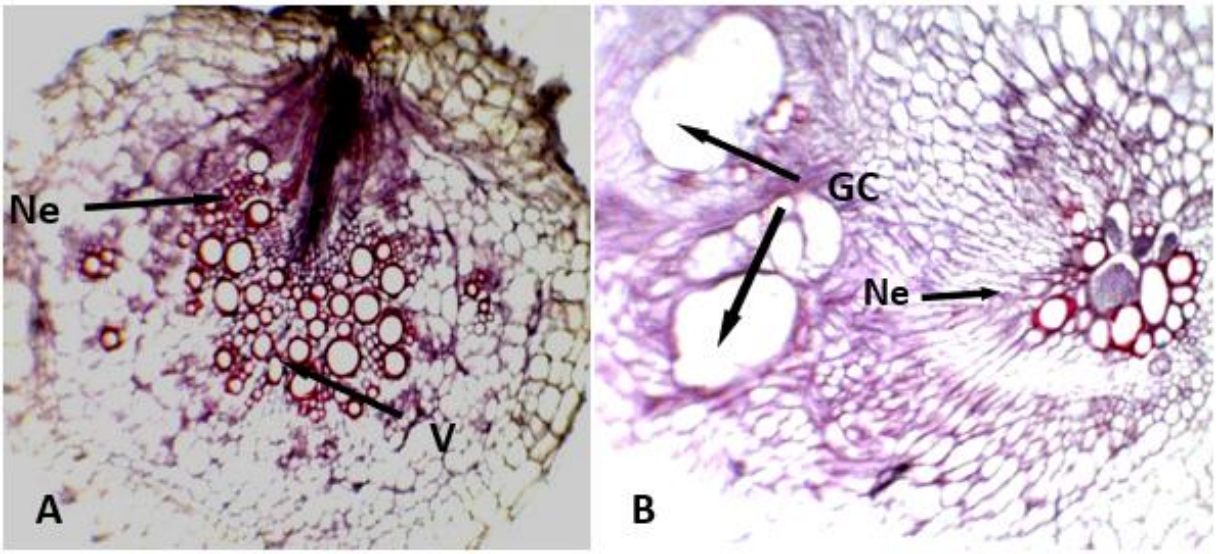

Fig. 3. Transverse section of sugar-beet roots infected with *M. incognita* and treated by Oxamyl application in clayey (A) and sandy soil (B). (Gc) Giant cells; (N) Necrosis; (V)Vessel; (Ne) Nematode.

DISCUSSION

Bacillus megaterium is a non-parasite bacterium has been estimated for its effects on the viability of root-knot nematodes (Ibrahim, 2010 and El- Hadad *et al*., 2011). Furthermore, *B. megaterium* can extensively colonize the rhizosphere and suppressed remarkably the sugar-beet cyst nematode infection under greenhouse (Neipp and Becker, 1999). Herein, total nematode population and root galling of *M. incognita* infecting sugar-beet were significantly suppressed by both treatments applied in clayey and sandy soil. El-Nagdi *et al*. (2013) reported the nematicidal activity of *Bacillus* spp. against *M. incognita*, and indicated that the possibility of using it to suppress plant-parasitic nematodes in organic farming systems as part of integrated pest management programs. These findings confirmed the current evidence that the total nematode population and root galling of *M. incognita* infecting sugar-beet were significantly suppressed by Nemastrol. Among concomitant treatments using two components, Bio-arc or Nemastrol gave synergistic activity and significantly suppressed root galling. However, antagonistic interaction in the total nematode population was recorded within such treatments. These results are in agreement with Radwan, *et al*. (2011) who found that the efficacy of bio-products (Bio-arc or Bio-zeid) against *M. incognita* was increased by the addition of oxamyl to the soil. *B. megaterium* as a phosphate-solubilizing bacterium is considered one of the microorganisms that capable of dissolving the unavailable phosphorus compounds in soil rendering them available for growing crops (Radwan, 1983). Increased phosphorus concentration may lead to a reduction in root-knot nematodes. on the other hand, when linking penetration potential with histological damage, the histopathological studies indicated that all treatments did not prevent the root-knot nematode from penetrating the roots and cause many changes in the composition of the root tissue structure was reported previously (Holtmann *et al*., 2000; Palomares-Rius *et al*., 2017 and Sato *et al*. 2019). Our results indicated that the ability of treatments with resistance inducers to reduce the ability of the root-knot nematode to cause more damage to root tissues, in line with what Pegard *et al*. (2005) found. With treatment by Bio-arc, the syncytia were found mostly in the central cylinder, with marked changes in the nature of the cellular structure of syncytia, the giant cell number ranged from 2-5 in the stellar region with limited hyperplasia with harmony with Nguyen *et al*. (2018). While in treatment by Nemastrol noted that a poor formation of irregular giant cells contained a smaller number of nuclei contrary to normal structure and (Siddique *et al*., 2018). These changes may be an expression of the plant's response to the tested treatments as a defense against penetration of root-knot nematode as reported by Tordable *et al*. (2010) and Pegard *et al*. (2005).

Conclusions:

The understanding of the nematicidal properties of resistance inducers in plants is promising tolls for eco-friendly integrated crop management strategies. Where it can be used appropriately to limit phytnematodes penetration into the plant below the critical point of infestation. Therefore, there is an urgent need to further study these materials to optimize the using it of management strategies.

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