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Effectiveness of certain safety commercial nematicides compare with a chemical nematicide Carbofuran for controlling *M. incognita* infected pomegranate plants

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ABSTRACT

With the growing environmental awareness, the identification of the dangers of chemical nematicides and the tendency of scientists to use safety nematicides, this study was determined to compare the effectiveness of three safety nematicides : Bio-arc (a commercial formulation *Bacillus megaterium*), Mycorrhizae (a commercial formulation of the *Glomus mosseae*) and Nemastrol (a commercial formulation of active ingredients) singly or in combination and the nematicide Carbofuran on root-knot nematodes *Meloidogyne incognita* infected pomegranate seedlings under open field conditions. In addition to its effect on the vegetative characteristics of pomegranate , and the rates of both peroxidase (PO) and polyphenol oxidase (PPO) enzymes in the roots of the pomegranate plants. Results show that, there are significant differences among all treatments. Nematicide Carbofuran gave the highest reduction percentages of *M. incognita* juvenile (83.0%), superior to that of safety commercial nematicides singly recording 66.4%, 64.3% and 60.3% for Nemastrol , Mycorrhizae and Bio-arc, respectively , compared to the control. Binary treatment Mycorrhizae + Nemastrol gave the highest reduction in *M. incognita* juvenile (83.7%), slightly more than the effect of the chemical nematicide Carbofuran. It also reduced the mature females, egg masses and root galls by (83.1, 86.1 and 80.0%) respectively, compared to Carbofuran (81.1, 85.0 and 80.0%) respectively. Binary treatments also improved the vegetative characteristics of pomegranate seedlings compared with Carbofuran, as it led to an increase in plant height, shoot weight and root weight by percentages 56.8, 40.2 and 87.2%, while it increased by Carbofuran treatment as 52.6, 40.0 and 80.0%, respectively compared with control. As well as binary treatments led to increase peroxidase and polyphenol oxidase enzyme rates by (10.3 and 9.5%) respectively, compared with control. Therefore, it could be recommend use these safety agents in binary treatments in integrated control programs for controlling root-knot nematodes that infect pomegranate plants because their greater effect than individually treatment producing better results than chemical nematicide.

Keywords: *Meloidogyne incognita*, Bio-arc, Mycorrhizae, Nemastrol, Control, Pomegranate.

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INTRODUCTION

Pomegranate, *Punica granatum* L. is one of the oldest fruits originating from Persia, and carries many nutritional benefits for human health, as it rich in compounds and vitamins that make it a healthy food to prevent many diseases, as well as it contains antioxidants, especially polyphenols, and vitamin C which plays a role in promoting immunity and maintaining the health of the digestive system (**Nour El-Deen, *et al.*, 2016**). The antioxidants in its juice with high concentration are believed to slow the progression of Alzheimer's disease and protect memory, also pomegranate juice is one of the most heart-healthy; improves blood flow; prevents arteries from becoming thick, and also slows the buildup of cholesterol in the arteries (**Wang, *et al.*, 2018**). Pomegranate is infected with plant parasitic nematodes, especially *Meloidogyne incognita*, which infected its roots and causes root knots, consequently the roots cannot absorb water & salts, and this causes general plant weakness which due to lack of fruit production, and death of the tree (**Nasira, *et al.*, 2011**). Biological control and the use of safety alternative nematicides to control plant-parasitic nematodes have attracted great attention to scientists because they are eco-friendly; safety for humans, animals and plants (**Sweelam, *et al.*, 2021**). Bio-arc (*Bacillus megaterium*) is a bio-nematicide that has an effective and powerful role against root knot nematodes, *M. incognita* and improve the characteristics of the plants (**Metwally, *et al.*, 2019**). *Bacillus megaterium* dissolves phosphorous and converts it from an unavailable compound to a compound that is easily absorbed by the plant (**Mostafa, *et al.*, 2018**). Nemastrol (mixture of active ingredients) is considered one of the safety and effective nematicides against root-knot nematode, *M. incognita* that infects sugar beet and stimulates plant resistance against root-knot nematodes (**Mostafa, *et al.*, 2014**). Mycorrhizae, *Glomus mosseae*, lives with the roots of the plant as a symbiotic life, hence it facilitates the absorption of water; salts and nutrients by the plant, and thus increases its resistance to root knot nematodes, as well as creating insufficient conditions for the development and reproduction of nematodes inside the roots (**Abo-Korah, 2017**).

This study aims to compare the efficacy of safety nematicides alone or in combination with a chemical nematicide on root-knot nematode, *M. incognita* infecting pomegranate plants.

MATERIALS AND METHODS

Potted experiment was conducted at the research farm of the Faculty of Agriculture, Menoufia University, Shibin El-Kom , Egypt, to reveal a comparison between the effect of

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three safety nematicides (Bio-arc: Mycorrhizae and Nemastrol) singly or in combination against root knot nematode, *Meloidogyne incognita* infected pomegranate (*Punica granatum* L.) seedlings compared with the chemical nematicide Carbofuran, in addition to its effectiveness on vegetative traits; the activity of peroxidase (PO) and polyphenol oxidase (PPO) enzymes under open field conditions.

Preparation of treatments:

Bio-arc: It is a bio-nematicide and its commercial formula is soluble phosphorous in addition to bacteria *Bacillus megaterium* (25×106 cfu/g) @ 2.5 g/L., which applied with nematode infection at a rate of 20 ml per seedling, according to (**Mostafa, *et al.*, 2018**). It was obtained from the Agricultural Research Institute, Giza, Egypt.

Mycorrhizae: Vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus mosseae*), it is a bio-nematicide, contain 300 propagules/gram. Each pot received endomycorrhiza inoculated with (3g) infested soil and (0.5 g) of the onion roots colonized by *Glomus mosseae*, according to (**Sweelam, *et al.*, 2021**) its appliance with nematode infection.

Nemastrol: A native commercial formulation of active ingredients containing glycosynolates (12%), chitinase (12 × 105 IU), cytokinins (200 ppm), flavonoids (5%) and β 1–3, Glucanase (2 × 105 IU) at the rate of 5 L/feddan. It was applied with nematode infection at a rate of 0.25 ml per seedling according to (**Mostafa, *et al.*, 2018**).

Carbofuran: It is a nematicide, with trade name (Carburan), common name is Carbofuran, mole Formula is $C_{12}H_{15}NO_3$, chemical Name is 2, 3-di hydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate. Formulation is 10% G and its application as soil treatment at the rate of 6 kg / feddan (20 g per pot). It was applied with nematode infection according to (**Abo-Korah, 2017**).

Nematode culture:

The root-knot nematode, *M. incognita* juvenile (J_2) were collected from pure culture on cotton *Gossypium barbadense* (Gallini) variety (Delta Pin61) in nematode laboratory of the Entomology and Zoology Department, Faculty of Agriculture , Menoufia University; cotton roots were washed with the egg masses in the water to clean them from the soil sticking to the roots, then the roots cutting to pieces of (1 cm) long and stirred in sodium hypochlorite (0.5%) for 3 minutes and shake well in sterile water (**Kerry and Bourne, 2002**). Nematode eggs were incubated at 25 ± 1°C for 3 to 4 days to obtain second stage

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juveniles (J_2) with modified Baermann funnel method (**Gray, 1984**) and used in the experimental infection procedure (**Chuixu *et al.*, 2013**).

Experimental preparation and design:

The experiment was conducted at the research Farm of the Faculty of Agriculture, Menoufia University, under open field conditions in plastic pots with a capacity of 4 kg, of sterilized clay-sand mixed soil (1:1, v/v) and a size of 25 cm. After sterilization, the seedlings of the pomegranate variety (Wonderful) were planted at the old of one and a half years. Two weeks after planting, root-knot nematode *M. incognita* infection was added at a rate of 1000 juvenile per pomegranate seedling and added by pipette into three holes around seedling, and each treatment replicated three replicates. Experiment layout was randomized complete block design. At the end of the experiment, vegetative measurements were taken and the activity of peroxidase (PO) and polyphenol oxidase (PPO) enzymes present in the roots were analyzed.

Nematode Extraction and Enumeration:

Soil samples were taken after planting at 60, 120 and 240 days. Three replicates, each one of 100 g soil and 1 g roots were taken from each treatment to extract nematodes, by modified Baermann funnels for 72 hours and counted . At the end of the experiment roots were carefully washed, and the nematode galls were counted and rated as mentioned in Table 1 (**Southey, 1970**), as well as egg masses were assessed by staining the roots with Phloxin-B solution (0.15 g/l tap water) for 20 minutes according to (**Daykin and Hussey, 1985**).

One gram of root was stained by acid fuchsine lactophenol to count root knot nematode stages inside the roots with the aid of a dissecting microscope.

Table (1): Rating scale levels of galls numbers

number of galls/ root system	Gall index
0	0
1-2	1
3-10	2
11-30	3
31-100	4
>100	5

Activity of plant enzymes:

Peroxidase (PO) and polyphenol oxidase (PPO) were determined in dried root tissues (0.5 g) according to the methods of (**Amako *et al.*, 1994**) and (**Coseteng and Lee 1987**) . The analyzes were carried out at the National Research Center in Giza, Egypt.

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Statistical analysis:

The obtained data were subjected to analysis of variance (ANOVA) using CoStat Software, Version 6.4 (2008). The mean differences were compared by Least Significant Difference (L.S.D. 5%).

Reduction percentages were counted according to (**Abbott formula 1925**).

Increase or decrease % = treatment – control / control x 100

RESULTS AND DISCUSSION

Data presented in Table (2) revealed the superiority of the chemical nematicide Carbofuran over three safety nematicides when applied individually recording the highest reduction in *M. incognita* juveniles by 83.0% , followed by Nemastrol , Mycorrhizae and Bio-arc (66.4 , 64.3 and 60.3%), respectively. However, when safety agents were applied in combination, they gave a higher reduction in *M. incognita* juveniles than when applied individually. Nemastrol + Mycorrhizae treatment gave the highest reduction in *M. incognita* juveniles by 83.7% , superior to the chemical nematicide Carbofuran (83.0%) .

Table (2): Effect of certain commercial nematicides on reduction and average numbers of *M. incognita* juveniles infecting pomegranate seedlings.

Treatments	Aver. no. of *Meloidogyne incognita* juveniles/ 100 g soil				Reduction %			
	Days post-treatments							
	60 Days	120 Days	240 Days	Overall mean	60 Days	120 Days	240 Days	overall mean
Bio-arc	849.0 b	609.0 b	461.0 b	639.7 b	40.9	64.3	75.7	60.3
Mycorrhizae	784.0 c	569.0 d	367.0 c	573.3 c	45.5	66.7	80.7	64.3
Nemastrol	714.0 d	581.0 c	321.0 d	538.7 d	50.4	65.9	83.0	66.4
Bio-arc + Mycorrhizae	637.0 e	358.0 f	146.0 f	380.3 e	55.7	79.0	92.3	75.7
Bio-arc + Nemastrol	612.0 f	374.0 e	168.0 e	384.7 e	57.4	78.1	91.1	75.3
Mycorrhizae + Nemastrol	497.0 h	191.0 h	57.0 g	248.3 g	65.4	88.8	97.0	83.7
Carbofuran	514.0 g	203.0 g	63.0 g	260.0 f	64.3	88.1	96.7	83.0
Control	1438.0a	1708.0a	1897.0a	1681.0a	-	-	-	-
LSD 5%	5.2	8.6	6.9	8.7	-	-	-	-

Means in each column followed by the same letter are not significant differences at 5% level

The obtained results were in agreement with **Mostafa, *et al.*, 2014** who reported that, Bio-arc and Nemastrol are safety nematicides which lead to high reduction in *M. incognita* population that infected sugar beet, and considered as stimulators of systemic resistance to the plant. Also, **Sikora *et al.*, 2007** found that *Bacillus megaterium* has an active role in attacking

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root-knot nematodes, and its effectiveness was increased by adding organic materials. Furthermore, **Oka *et al.*, 1999** reported that acquired immunity to the plant can be achieved by applying biotic or abiotic inducers.

Data in Table (3) revealed that application of safety agents in binary treatments gave better decrease percentages than singles. Nemastrol reduced mature females, egg masses and root galls by 61.7, 53.6 and 40% , respectively, followed by Mycorrhizae and finally Bio-arc. The bi-combined treatment of Mycorrhizae + Nemastrol reduced mature females, egg masses and root galls by 83.1, 86.1 and 80% , comparing to the chemical nematicide, Carbofuran 81.8, 85.0 and 80% , respectively.

These results are coincident with those obtained by **Mostafa, *et al.*, 2018** who reported that Nemastrol reduced root-knot nematode egg masses due to presence of chitinase and glucanase enzymes, which works to break down the chitin component of the eggshell. **El Deriny, 2016** recorded that Nemesitrol has an effective role in increasing plant resistance to soil pathogens, specially parasitic nematodes. **Mostafa, *et al.*, 2014** recorded that Nemastrol has a greater effect than Bio-arc on root-knot nematode, *M. incognita* infection, as it leads to a reduction in whole females, egg masses, and root galls in greater proportions than Bio-arc, while the binary treatment was the best.

Table (3) Impact of certain commercial nematicides *M. incognita* mature females; egg-masses and root gall index associated with pomegranate plants.

Treatments	Mature females per5g/root	No of egg-masses	root gall index	Decrease %		
				Mature females	Egg-masses production	root gall index
Bio-arc	17.9 b	19.0 b	4.0 b	42.8	33.8	20.0
Mycorrhizae	14.3 c	14.0 c	3.0 c	54.3	51.2	40.0
Nemastrol	12.0 d	13.3 d	3.0 c	61.7	53.6	40.0
Bio-arc + Mycorrhizae	9.3 e	10.3 e	2.0 d	70.3	64.1	60.0
Bio-arc + Nemastrol	8.9 e	9.9 e	2.0 d	71.6	65.5	60.0
Mycorrhizae + Nemastrol	5.3 f	4.0 f	1.0 e	83.1	86.1	80.0
Carbofuran	5.7 f	4.3 f	1.0 e	81.8	85.0	80.0
Control	31.3 a	28.7 a	5.0 a	-	-	-
LSD 5%	1.1	0.7	0.5	-	-	-

Means in each column followed by the same letter are not significant differences at 5% level

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Data in Table (4) indicated that all treatments cause considerable improvement in the vegetative characteristics of pomegranate seedlings. Binary treatment (Mycorrhizae + Nemastrol) was superior and exquisitely treatment that improved the vegetative properties of pomegranate seedlings, as it results an considerable increase in plant height , shoot weight and root weight by 56.8, 40.2 and 87.2% , respectively, followed by Carbofuran 52.6, 40.0 and 80.0% with slightly differences, respectively.

Table (4): Responsiveness of some pomegranate plants growth parameters influenced by adopted treatments.

Treatments	plant height (cm)	shoot weight (g)	root weight (g)	Increase %		
				plant height	shoot weight	root weight
Bio-arc	91.0 f	68.9 f	6.3 d	16.7	15.0	14.5
Mycorrhizae	98.3 e	71.0 e	7.9 c	26.0	18.5	43.6
Nemastrol	106.0 d	74.3 d	8.3 c	35.9	24.0	50.9
Bio-arc + Mycorrhizae	112.9 c	77.0 c	9.0 b	44.7	28.5	63.6
Bio-arc + Nemastrol	114.0 c	80.3 b	9.3 b	46.2	34.0	69.1
Mycorrhizae + Nemastrol	122.3 a	84.0 a	10.3 a	56.8	40.2	87.2
Carbofuran	119.0 b	83.9 a	9.9 a	52.6	40.0	80.0
Control	78.0 g	59.9 g	5.5 e	-	-	-
LSD 5%	1.7	1.9	0.5	-	-	-

Means in each column followed by the same letter are not significant differences at 5% level

The obtained results are in harmonic with **Abo-Korah, 2017** who found that the application of mycorrhizae facilitates the absorption of nutrients by plant roots and consequently improves plant vegetative characteristics, producing considerable increase in length of plant and shoot weight. Moreover, **Ahmed *et al.*, 2009** recorded that Mycorrhizae increases plant resistance and stimulates plant roots to produce amino acids as serine and phenylalanine which have an exterminating effectiveness on parasitic nematodes.

Data in Table (5) show that all treatments increased the activity of peroxidase (PO) and polyphenol oxidase (PPO) enzymes, except for the chemical nematicide Carbofuran, which reduced its activities by 2.7 and 3.8%, respectively, compared with control. Binary treatment of Mycorrhizae + Nemastrol caused the highest rates of peroxidase (PO) and

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polyphenol oxidase (PPO) enzymes with percentages by 10.3 and 9.5%, respectively, compared to the control treatment.

Also, **Govindappa *et al.*, 2010** recorded that, increasing the activity of defense–related enzymes peroxidase and polyphenol oxidase in plant roots has an indicator of increasing plant resistance to pathogens, especially parasitic nematodes. In addition, **Passardi *et al.*, 2004** found that, peroxidase and polyphenol oxidase enzymes increased the vitality and thickening of plant cell walls (lignification and suberization) increasing plant resistant to pathogens. Recently, **Mostafa, *et al.*, 2018** recorded that the application of Nemastrol plus Bio-arc increased the rates of peroxidase and polyphenol oxidase enzymes in the sugar beet roots.

Table (5). Impact of adopted commercial nematicides on the activities of peroxidase (PO) and polyphenol oxidase (PPO) enzymes in pomegranate roots.

Treatments	Absorbance units/mg protein		Increase or decrease %	
	Peroxidase (PO) activity	Polyphenol oxidase (PPO) activity	(PO) activity	(PPO) activity
Bio-arc	0.293 f	0.389 f	+1.3	+0.8
Mycorrhizae	0.298 e	0.392 e	+3.1	+1.5
Nemastrol	0.304 d	0.396 d	+5.1	+2.5
Bio-arc + Mycorrhizae	0.309 c	0.407 c	+6.9	+5.4
Bio-arc + Nemastrol	0.312 b	0.411 b	+7.9	+6.4
Mycorrhizae + Nemastrol	0.319 a	0.423 a	+10.3	+9.5
Carbofuran	0.281 h	0.371 h	-2.7	-3.8
Control	0.289 g	0.386 g	-	-
LSD 5%	0.001	0.002	-	-

Means in each column followed by the same letter are not significant differences at 5% level

This study recommend that, the combination use of safety nematicides in integrated control programs against plant-parasitic nematodes effectively eliminates root-knot nematode, *M. incognita* population that infect the pomegranate and improves its vegetative

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characteristics by a higher percentage than ones , In addition this binary treatment overcome the chemical nematicide Carbofuran.

In conclusion, application of safety and inexpensive commercial nematicides in binary treatments (Mycorrhizae + Nemastrol) gave a higher reduction percentage of *M. incognita* infected pomegranate plants than the chemical nematicide Carbofuran, such findings consider as an excellent model for nematode control to take into several improvement of pomegranate vegetative properties and thus could be effectively used with other cultural, biological and chemical nematode management strategies.

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تأثير بعض مبيدات النيماتودا التجارية والآمنة مقارنة مع المبيد الكيميائي الكاربوفيوران في مكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* التي تصيب الرمان

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الملخص العربي

بتقدم الوعي بالظروف البيئية المحيطة وبالتعرف على مخاطر المبيدات الكيميائية ، إتجه العلماء فى مجال مكافحة النيماتودا إلى إستخدام مبيدات نيماتودية آمنة. وتهدف هذة الدراسة إلى مقارنة تأثير ثلاثة مبيدات نيماتودية تجارية آمنة وهي البيوارك (مستحضر تجاري لبكتريا باسلس ميجاتيريم) ، الميكورهيزا (مستحضر تجاري لفطر جلومس موسيس) والنيماسيترول (مستحضر تجاري لمواد فعالة) بتطبيقها منفردة أو مجمعة مع المبيد الكيميائي الكاربوفوران فى مكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* التي تصيب شتلات الرمان تحت ظروف الحقل. كما تم دراسة تأثيرها على صفات شتلات الرمان الخضرية وتقييم النشاط الإنزيمي لأنزيمات البيروكسيديز والبولي فينول اوكسيديز في جذور نبات الرمان.

أظهرت النتائج وجود فروق معنوية بين جميع المعاملات التي تم تطبيقها وسجل المبيد الكيميائي الكاربوفيوران أعلى نسبة موت في يرقات نيماتودا تعقد الجذور (83.0٪) متفوقاً على المبيدات التجارية والآمنة عندما تم تطبيقها بصورة فردية حيث حقق النيماسيترول نسبة موت (66.4٪) يليه الميكورهيزا (64.3٪) ثم البيوراك (60.3٪) وذلك مقارنة بالكنترول. وعند تطبيق المبيدات التجارية الآمنة في صورة ثنائية أعطت المعاملة (الميكورهيزا+ النيماسيترول) أعلى نسبة موت في يرقات نيماتودا تعقد الجذور وصلت إلى (83.7٪) متفوقة على المبيد الكيميائي الكاربوفيوران. كما أنها قللت من نسبة الإناث الناضجة ، كتل البيض والعقد الجذرية بنسب (83.1 ، 86.1 ،80.0٪) على التوالي مقارنة بتأثير المبيد الكيميائي الذي أعطى نسب أقل (81.1 ،85.0 ، 80.0٪) على التوالي. كما أدت هذة المعاملة الثنائية إلى تحسين الصفات الخضرية لشتلات الرمان حيث أدت إلى زيادة طول النبات ، وزن المجموع الخضري ، ووزن الجذور بنسب (56.8 , 40.2 و87.2٪) على التوالي ، بينما إنخفضت هذة النسب مع المبيد الكيميائي إلى (52.6 , 40.0 و 80.0٪) على التوالي مقارنة بالكنترول. علاوة على ذلك أثرت المعاملة الثنائيةعلى زيادة تركيز إنزيمات البيروكسيديز والبولي فينول اوكسيديز بنسب وصلت إلى (10.3 و 9.5٪) مقارنة بالكنترول.

لذا ، توصي هذة الدراسة إلى إستخدام المبيدات التجارية الآمنة بصورة ثنائية في برامج المكافحة المتكاملة لنيماتودا تعقد الجذور التي تصيب الرمان حيث انها أعطت نتائج أفضل من تطبيقها في صورة فردية وأفضل من المبيد النيماتودى الكيميائي.

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