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SOME NATURAL ALTERNATIVES FOR CONTROLLING *Meloidogyne incognita* (KOFOID AND WHITE) CHITWOOD UNDER LABORATORY CONDITIONS

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ABSTRACT

Four untraditional materials were tested to control *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 juveniles under laboratory conditions, *i.e.* magnetic iron, tourmaline, phosphate granules and saponins extract compared to traditional nematicide fenamifos (Nemacur® 40% EC). These materials were tested at the concentrations of 500, 1000 and 2000 ppm directly against *M. incognita* juveniles in Petri dishes and the results were obtained after 24 , 72 and 144 hr. All tested materials showed nematicidal effect on nematode juveniles and their mortality increased with increasing concentration and exposure period. Fenamifos was the most effective one on juvenile activity followed by saponins extract, phosphate granules, tourmaline, and finally magnetic iron. Numbers of active juveniles were 50.00, 90.34, 260.67, 280.34 and 460.67 juveniles/1 ml, respectively compared to control which valued 850.11 juveniles/1 ml at the concentration of 2000 ppm after 144 hours of treatment. On the other hand, fenamifos was the most effective one in inhibiting egg hatching followed by saponins extract, phosphate granules, tourmaline and magnetic iron in the concentrations 500,1000 and 2000 ppm after 144 hours of exposure. Generally, the tested eco-friendly materials showed nematicidal effect on juveniles and eggs of *M. incognita* under laboratory conditions and these materials should be tested under field or greenhouse conditions to show the effect on plant growth and nematode population.

Key words: *Meloidogyne incognita*, saponins extract, magnetic iron, tourmaline, phosphate granules and control.

INTRODUCTION

Plant parasitic nematodes constitute one of the most important pests infesting economic crops and cause a lot of losses, especially in developing countries (Sultana *et al.,* 2011). Root knot nematodes *M. incognita* occur of a large part of annual yield losses attributed to nematodes (Trudgill and Blok, 2001).

Nematicides in principle are usually used to control nematodes infesting fruits and vegetables cultivated areas in Egypt. Chemical nematicides has expensive cost added to agricultural costs moreover. On the other hand, these chemicals have environmental and human toxic effect (Barker and Koenning, 1998). There are many alternative materials for controlling root-knot nematodes, these materials can decrease nematicides risks among these materials, *i.e.* magnetic iron (Ismail *et al.,* 2010 and El-Sherif *et al.,* 2014), saponins extract (D'Addabbo *et al.,* 2011; Ibrahim and Srour, 2013; Ibrahim *et al.,* 2014; Yang *et al.,* 2015) and tourmaline which is used as a soil amendment to remediation alkaline soil (Wang *et al.,* 2014).

All the tested materials are inexpensive and widespread allover Egypt. Some of these materials are natural stones (tourmaline and phosphate granules) and the others are the results of some industrial processes (saponins and magnetic iron). The nematicide fenamifos is very effective nematicide used in fruit orchards for controlling nematodes.

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MATERIALS AND METHODS

Preparing of Nematodes Suspension and Egg Masses

Second stage juveniles of *M. incognita* were collected from an identified culture of root knot nematode that propagated on eggplant seedlings (*Solanum melongena* var. Black) planted in the greenhouse, Faculty of Agriculture, Zagazig University, Egypt. The galled roots were soaked in tap water for one hour to remove adhering soil particles. Egg masses needed for experiments were hand picked with fine forceps from small galls. The collected egg masses were surface sterilized in 0.5% sodium hypochlorite (Chlorex) for 3 minutes and quickly washed several times with distilled water to remove residues of NaOCL by passing the suspension through a 200-mesh (75 µm) sieve. The collected egg masses were then refrigerated overnight at 5°C and used in the next day for assay (Hussey and Barker, 1973). To obtain a second stage juvenile, egg masses were incubated in distilled water for 5 days at 25± 2°C. The juvenile suspension contained the freshly hatched juveniles was separated daily using a micropipette after sedimentation of egg masses.

Preparation of Tested Material Solutions

The solution of each material was prepared by adding 0.5, 1 and 2 g of the tested powder materials (phosphate granules, tourmaline and magnetic iron) to a beaker and soaked with a small amount of distilled water to about 2 days in the room temperature and then completed the volume for 1000 ml with the distilled water. The saponins extract was obtained from non-commercial product and the tested solution prepared by adding 0.5, 1 and 2 ml of the extract to the beaker and mixed with a small amount of distilled water and left for 2 days to extract all ingredients and then completed the volume to 1000 ml with distilled water. The nematicide fenamifos (Nemacur® 40% EC) was prepared by adding the recommended dose to a beaker and completed the volume to 1000 ml, the nematicide was tested at one concentration only which is the recommended dose.

The Nematicidal Effect of the Tested Materials on Nematode Juveniles

Ten ml of nematode suspension containing about 1000 juveniles in each one was added to five ml of concentrations (500, 1000 and 2000 ppm) in 9 cm diam. Petri dishes. The control treatment was prepared using distilled water without any added material. Each treatment was replicated three times. All treatments were left under room temperature (28±3 ˚C) to determine the effect of these materials on juvenile mortality. All treatments were kept for 24, 72 and 144 hours and the dead juveniles (not active) were counted in 1 ml under 100 X magnification after these periods. The mortality percentages were calculated from the following equation:

$$\text{Mortality (\%)} = \frac{\text{Dead juveniles}}{\text{Total number of juveniles}} \times 100$$

Effect of the Tested Materials on Eggs Hatching

Five egg masses of uniform size were added to ten ml of each concentration (500, 1000 and 2000 ppm) in 9 cm diam. Petri dishes. The control treatment was prepared using distilled water without any of the tested materials. Each treatment was replicated three times. All treatments were left under room temperature 28±3˚C. Numbers of hatched juveniles were counted using a research microscope (100X magnification) after 24, 72 and 144 hours of exposure. Percentage of hatching inhibition was calculated in comparison with the control treatment, according to the following equation:

$$\text{Egg hatching inhibition (\%)} = \frac{\text{Control - treatment}}{\text{Control}} \times 100$$

All data were statistically analyzed using SPSS 10 Computer Program. General means were compared with Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of the Tested Materials on Nematode Juveniles Activity

Data in Tables 1, 2 and 3 and Figs. 1, 2 and 3 showed the effect of the concentrations 500, 1000 and 2000 ppm, respectively of the tested

Table 1. Number of active *M. incognita* juveniles after 24, 72 and 144 hours at 500 ppm concentration of the tested materials

Exposure time (hour)	Number of active juveniles in 1 ml					
	Fenamifos	Magnetic iron	Saponins extract	Phosphate granules	Tourmaline	Control
24	230d	900a	660.67c	820b	830.67b	900a
72	50e	830.34a	560.34d	770.34c	800.34b	850.67a
144	50e	770.34b	410.34d	720.67c	760.34b	810.67a

* Means followed by the same letter in rows are not significantly different at P≤0.05 according to Duncan's multiple range test.

Table 2. Number of active *M. incognita* juveniles after 24, 72 and 144 hours at 1000 ppm concentration of the tested materials

Exposure time (hour)	Number of active juveniles in 1 ml					
	Fenamifos	Magnetic iron	Saponins extract	Phosphate granules	Tourmaline	Control
24	230f	810.67b	430.34e	660d	710c	900a
72	50f	760.67b	240.34e	520.34d	610c	840.67a
144	50f	560.67b	130.34e	380.34d	510.67c	820.67a

* Means followed by the same letter in rows are not significantly different at P≤0.05 according to Duncan's multiple range test.

Table 3. Number of active *M. incognita* juveniles after 24, 72 and 144 hours at 2000 ppm concentration of the tested materials

Exposure time (hour)	Number of active juveniles in 1 ml					
	Fenamifos	Magnetic iron	Saponins extract	Phosphate granules	Tourmaline	Control
24	230f	660.67b	130.34e	460.67d	580.34c	900a
72	50f	610.67b	140.34e	330.34d	500c	830.67a
144	50e	460.67b	90.34d	260.67c	280.34c	810.67a

* Means followed by the same letter in rows are not significantly different at P≤0.05 according to Duncan's multiple range test.

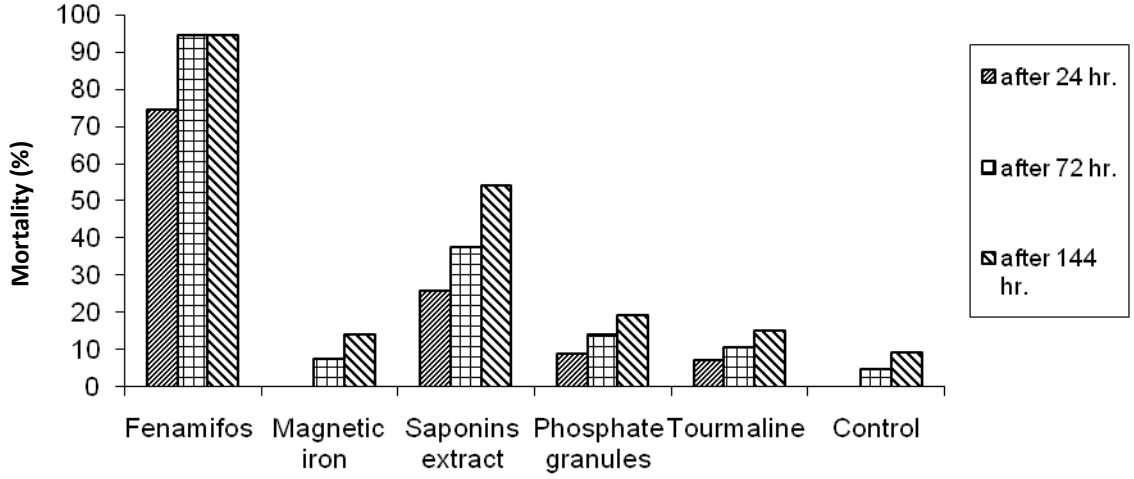

Fig. 1. Effect of 500 ppm concentration of the tested materials on *M. incognita* juveniles mortality

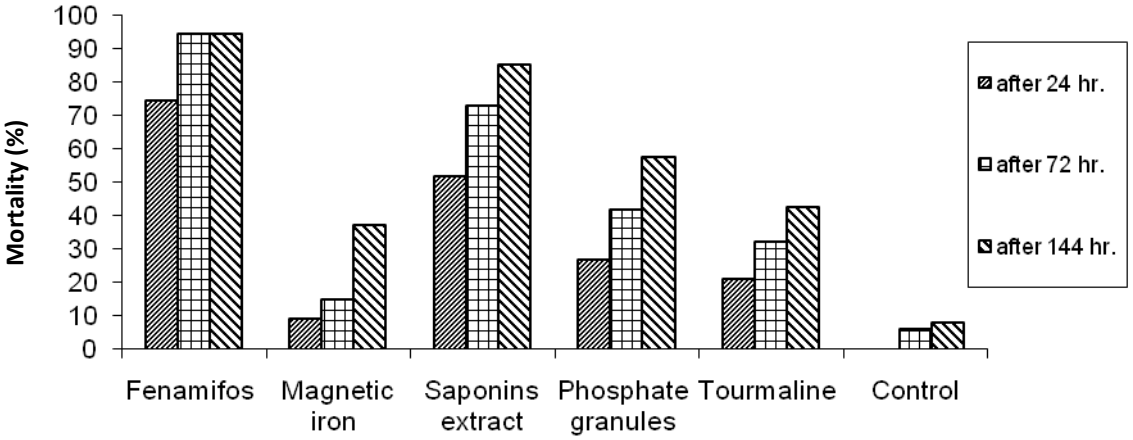

Fig. 2. Effect of 1000 ppm concentration of the tested materials on *M. incognita* juveniles mortality

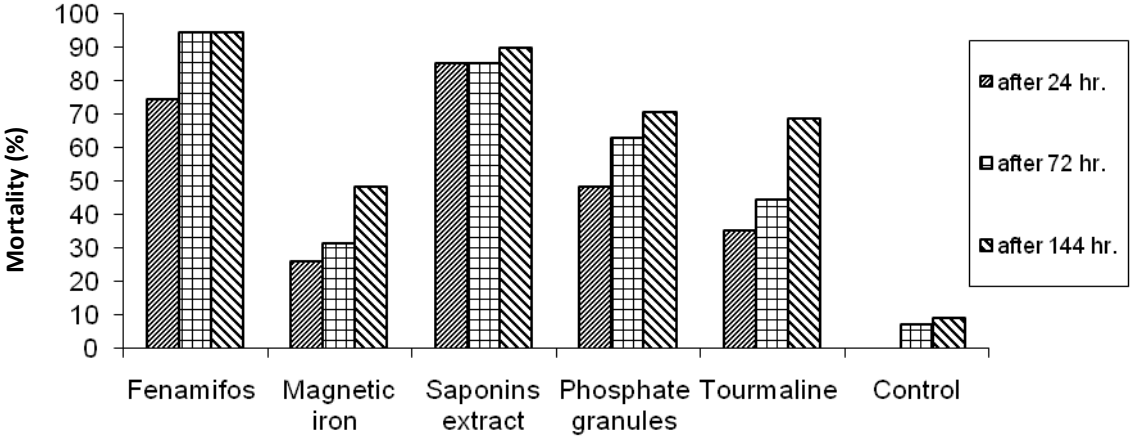

Fig. 3. Effect of 2000 ppm concentration of the tested materials on *M. incognita* juveniles mortality

materials on numbers of active *M. incognita* juveniles. Data showed that, the magnetic iron was the least effective one on juveniles activity at the concentration 500 ppm. Since, the numbers of active juveniles were 900, 830.34 and 770.34 juveniles per 1ml after 24, 72 and 144 hour, consecutively with significant difference compared to the control in the case of the first and third exposure times, followed by tourmaline, phosphate granules and saponins extract with numbers (830.67, 800.34 and 760.34 juveniles / 1 ml), (820, 770.34 and 720.67 juveniles / 1 ml) and (660.67, 560.34 and 410.34 juveniles / 1 ml), respectively. There is no significant differences between magnetic iron and control after 24 and 72 hours, and between tourmaline and phosphate granules after 24 hours at this concentration.

The most effective material was the nematicide fenamifos. The numbers of motile juveniles were 230, 50 and 50 juveniles /1 ml after 24, 72 and 144 hours, respectively, compared to control 900, 850.67 and 810.67 juveniles /1 ml after the same periods of exposure.

At the concentration 1000 ppm the numbers of active juveniles were decreased by increasing exposure time and the same result was obtained. The toxic effect in ascending order was as follows magnetic iron, tourmaline, phosphate granules, saponins extract and fenamifos. The numbers of active juveniles after 144 hours of exposure were 560.67, 510.67, 380.34, 130.34 and 50 juveniles/1 ml, consecutively compared to the number in control treatment 820.67 juveniles /1 ml, there are significant differences between all treatments in different exposure periods.

The highest concentration 2000 ppm was the most effective one after 24 hours of exposure. The active juveniles numbers in magnetic iron were (660.67 juveniles/ 1 ml) followed by tourmaline (580.34 juveniles/ 1 ml), phosphate granules (460.67 juveniles/1 ml), saponins extract (130.34 juveniles/ 1 ml) and fenamifos (230 juveniles/ 1 ml) compared to control (900 juveniles/ 1 ml). The efficacy of the tested materials was increased after 144 hours of treatment with numbers 460.67, 280.34, 260.67 , 90.34 and 50 juveniles/ 1 ml of magnetic iron, tourmaline, phosphate granules, saponins extract and fenamifos, respectively in comparison to control (810.67 juveniles/ 1 ml).

Mortality percentages of the tested materials were calculated depending on the previous equation. All materials had a significant nematicidal effect on juveniles. Figure 1 shows the effect of 500 ppm concentration on *M. incognita* juveniles mortality. The most effective material after 24 hours was the nematicide fenamifos (74.44%) followed by saponins extract (25.92%), phosphate granules (8.88%), tourmaline (7.03%) and the least effective one was magnetic iron (0%). The mortality percentages after 72 and 144 hours in fenamifos at this concentration was (94.44%) and in saponins extract the mortality percentage increased from 37.4% after 72 hr., to 54.06% after 144 hr., other treatments have low percentages which ranged from 7.04% to 14.06%.

The mortality percentages increased at the highest concentration 1000 ppm. For instance, saponins extract was the second effective one after fenamifos 24 , 72 and 144 hours after treatment showing the mortality of 51.84, 72.95 and 85.17%, respectively followed by phosphate granules 26.66, 41.84 and 57.4%. The tourmaline recorded percentages valued 21.11, 32.22 and 42.58% after 24, 72 and 144 hr., respectively. The lowest one was magnetic iron with percentages values 9.25, 14.81 and 37.03% in the same periods of exposure, respectively. The eco-friendly material effect on this concentration was increased compared with the first concentration (500 ppm) in the same periods of exposure (Fig. 2).

The most effective concentration was 2000 ppm for all the tested materials causing high mortality of nematode juveniles. The mortality percentages were 85.17, 85.22 and 89.62% in saponins extract treatment and the lowest effect was in magnetic iron with percentages amounted 25.92, 31.47 and 48.14% after 24, 72 and 144 hours of exposure, respectively (Fig.3).

The Inhibition Effect of the Tested Materials on *M. incognita* Eggs Hatching

Data in Table 4 shows the percentage inhibition in eggs hatching of *M. incognita* at the concentration 500 ppm after 24, 72 and 144 hours of exposure. The nematicide fenamifos

Table 4. Percentage inhibition of the tested materials on *M. incognita* eggs hatching after 24, 72 and 144 hours at 500 ppm concentration

Exposure time (hour)	Inhibition (%) in eggs hatching				
	Fenamifos	Magnetic iron	Saponins extract	Phosphate granules	Tourmaline
24	73.19	0	24.67	7.63	5.78
72	93.19	6.15	36.15	12.81	9.48
144	94.20	12,81	52.81	18.0	13.92

was the superior one over all treatments, the inhibition percentages were 73.19, 93.19 and 94.20% after 24, 72 and 144 hours, respectively at the recommended dose of the nematicide followed by saponins extract, phosphate granules, tourmaline and magnetic iron with percentages of inhibition valued as much as 52.81, 18.0, 13.92 and 12.81% after 144 hours of treatment at this concentration.

Data in Table 5 shows the same trend of the tested materials at the concentration 1000 ppm, since fenamifos was the most effective one in the inhibition of eggs hatching, while the saponins extract was the second most effective with percentages 50.59, 71.7 and 83.92% after 24, 72 and 144 hours, respectively. On the other hand magnetic iron was the least one with inhibition percentages valued 8.0, 13.56 and 35.78% after the same exposure times, respectively.

The most effective results of the tested materials were at the concentration 2000 ppm, data in the Table 6 shows that the saponins extract recorded high percentages 63.92, 75.32 and 88.37%, nearly similar to those in respect to fenamifos 73.19, 93.19 and 94.20% after 24, 72 and 144 hours of treatment. The other tested natural materials had inhibition percentages estimated with 69.11, 67.26 and 46.89% for phosphate granules, tourmaline and magnetic iron, respectively after 144 hours of exposure.

These results agree with D'Addabbo *et al.* (2011) who showed the nematicidal effect of saponins extracted from alfalfa *Medicago sativa* and their effect on some species of plant parasitic nematodes like *Xiphinema index*, *Meloidogyne incognita* and *Globodera rostochiensis*. They found the chemical profile of saponins consisted of three major chemical groups steroid glycosides, steroid alkaloid glycosides and triterpene glycosides, which include the largest number of structures. The results showed that exposure for 16 or 24 hr. at the concentration of 500 µg 1 ml of the saponins extract induced 90–100% mortality of *X. index* and *M. incognita*.

Ibrahim and Srour (2013) indicated the lethal effect of saponins extracted from *M. sativa*. They prepared 100% concentration stock solution and diluted to 75%, 50% and 25% and they referred this nematode lethal effect to the contents of chemical compounds like oleanolic acid, 2β-hydroxyoleanoic acid, bayogrnin, hederagenin, 2β3β-dihydroxy- 23oxo-olean-12en-28oic acid, medicagenic acid, zanhic acid and soyasapogenol B, and these data indicated that the nematicidal activity of saponins from *M. sativa* could be attributed to their ability to inhibit cholesterol accumulation in *M. incognita* eggs and larvae.

Ibrahim *et al.* (2014) estimated the nematicidal efficacy of saponins extract from some plants against the second stage juveniles of *Meloidogyne* spp. They found that in the concentration 10 ppm the inhibition percent of second stage juveniles was 22.2%, the concentration 100 ppm was 33.4 %, the concentration 1000 ppm was 43% and in the concentration 10000 ppm the inhibition percent was 53.1%, and they found the inhibition percentage of the juveniles to penetrate the root of eggplant was 100% in the concentration 10000 ppm under greenhouse conditions. On the same side, Yang *et al.* (2015) studied the suppression effect of saponins extracted from *Camellia* seed cake on juveniles mortality and

Table 5. Percentage inhibition of the tested materials on *M. incognita* eggs hatching after 24, 72 and 144 hours at 1000 ppm concentration

Exposure time (hour)	Inhibition (%) in eggs hatching				
	Fenamifos	Magnetic iron	Saponins extract	Phosphate granules	Tourmaline
24	73.19	8.0	50.59	25.41	19.86
72	93.19	13.56	71.7	40.59	30.97
144	94.20	35.78	83.92	56.15	41.33

Table 6. Percentage inhibition of the tested materials on *M. incognita* eggs hatching after 24, 72 and 144 hours at 2000 ppm concentration

Exposure time (hour)	Inhibition (%) in eggs hatching				
	Fenamifos	Magnetic iron	Saponins extract	Phosphate granules	Tourmaline
24	73.19	24.67	63.92	46.89	33.92
72	93.19	30.22	75.32	61.70	43.19
144	94.20	46.89	88.37	69.11	67.26

eggs hatching of *M. javanica*, and they used an aqueous extract of the seed cake in many concentrations. The results indicated that saponins extracts have nematicidal activity against nematode juveniles in 100 g/l nearly 100% and this effect declined gradually to 50 g/ l and the effect on eggs hatching was in concentration 5g/l after 72 hours, the authors refered the effect of saponins nematicidal effect to their chemical structure where saponins are steroid or triterpenoid glycosides and can use as natural nematicide.

On the other hand, the nematicidal effect of magnetic iron was indicated by many authors, such as Fahmy (2007) who showed the plant amended effect of magnetic iron, he indicated that suitable magnetic treatment increased the absorption and assimilation of nutrients and ameliorated photosynthetic activities. In the study, the author showed the effects of pre-sowing magnetic treatments on the growth and yield of the tested plants, under field conditions the treatments led to a significant increase in root length, fresh and dry root weight, stem length, fresh and dry stem weight, leaf area and foliate dry weight, this effect can make the plant most tolerant to nematode infection. Ismail *et al.* (2010) tested the effect of magnetic iron on management root knot nematode *M. incognita* under open grapevine field conditions. The effects on yield, shoot length and chemical properties of the grapevine berries were studied, recorded data showed significant reduction in *M. incognita* populations either in the soil or in roots in two different grapevine cultivars through two seasons. El-Sherif *et al.* (2014) studied the effect of magnetic iron, *Bacillus thuringiensis* and dry leaf powder of moringa comparing with oxamyl on management *M. incognita* infecting eggplant under greenhouse conditions. Results showed that all treatments reduce the final number of nematode population, number of galls and egg masses, magnetic iron was the least effective one in their effect with percent reduction 87.4, 78.2 and 85.4%, respectively. The triple treatment of the tested materials had the greatest effect on nematode parameters. There was no clear data about the nematicidal properties of tourmaline and

phosphate granules on *Meloidogyne* spp. Wang *et al*. (2014) showed that tourmaline had an effect on soil pH and can use to repaired alkaline soil and could increase indigenous microbial populations and increase Ca, Mg and K ions content. Data showed also the tourmaline increase in the ion content of water-soluble nutrient elements, such as Ca and Mg, in soils where tourmaline was added indicates that an ion exchange mechanism is involved, providing nutrients for plant growth.

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بعض البدائل الطبيعية لمكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* تحت الظروف المعملية

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أُختبرت أربعة مواد غير تقليدية في مكافحة نيماتودا تعقد الجذور *M. incognita* تحت ظروف المعمل و كانت تلك المواد هي الحديد المغناطيسي والتورمالين والفوسفات الحبيبي ومستخلص الصابونين مقارنة بالمبيد النيماتودي الفينـاميفوس (نيماكور٤٠% مركز قابل للاستحلاب)، أُستخدمت هذه المواد بتركيزات ٥٠٠ ، ١٠٠٠و٢٠٠٠ جزء في المليون مباشرة على الطور اليرقي الثاني لنيماتودا تعقد الجذور في أطباق بتري وأخذت النتائج بعد ٢٤ و٧٢ و١٤٤ ساعة من المعاملة لمختلف التركيزات، أوضحت النتائج أن كل المواد المختبرة أثرت علي النيماتودا و أن نسبة الموت في الطور اليرقي الثاني قد زادت بزيادة التركيز وبزيادة مدة التعرض لهذه المواد و كان المبيد النيماتودى الفينـاميفوس الأكثر تـأثيراً على اليرقات تلاه مستخلص الصابونين ثم الفوسفات الحبيبي والتورمالين وأخيراً الحديد المغناطيسي حيث بلغت أعداد الأفراد المتحركة من النيماتودا صفر ،٥٠٠ ، ٩٠,٣٤ ، ٢٦٠,٦٧ ، ٢٨٠,٣٤ و ٤٦٠,٦٧ يرقة / ملل على التوالي مقارنة بالمعاملة الضابطة ٨٥٠.١١ يرقة / ملل وذلك عند التركيز ٢٠٠٠ جزئ في المليون وبعد ١٤٤ ساعة من المعاملة، وفي نفس السياق كان التـأثير الأكبر في تثبيط فقس البيض من نصيب الفينـاميفوس تلاه في ذلك مستخلص الصابونين والفوسفات الحبيبي والتورمالين وأخيراً الحديد المغناطيسي وذلك في التركيزات ٥٠٠ ، ١٠٠٠ و٢٠٠٠ جزء في المليون، وبعد ٢٤ ساعة من المعاملة، وتشير النتائج في هذا البحث إلى أن استخدام المواد الصديقة للبيئة قد يكون لها تـأثير قاتل للطور اليرقي الثاني لنيماتودا تعقد الجذور تحت ظروف المعمل وتحتاج هذه المواد لدراسات في المستقبل تحت ظروف الحقل أو الصوبة لمعرفة تأثيرها على نمو النبات وتعداد النيماتودا المصاحبة له.

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