

INTEGRATED MANAGEMENT OF ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* INFECTING COWPEA PLANTS

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Received: May 14, 2022

Accepted: Jun. 4, 2022

ABSTRACT: This experiment was conducted to evaluate the efficacy of saponin extract, algae, *Chlorella vulgaris*, and a bio-nematicide BioNematon (commercial formulation of the *Paecilomyces lilacinus*, half dose) and the possibility of creating an integrated control program on root-knot nematodes infected cowpea under field conditions compared to chemical nematicide, Carbofuran, as well as the effect of these treatments on the vegetative characteristics of cowpea plants. All treatments caused reduction percentages in root-knot nematode *Meloidogyne incognita* juveniles, to take into the triple treatment (saponin extract + algae + BioNematon half dose) which gave the highest reduction percentage (82.6%), followed by the chemical nematicide Carbofuran (80.3%), then the BioNematon half dose, algae and saponin extract solely by achievement (67.3, 66.3 and 58.4%), respectively, compared with control. Tribble treatment (saponin extract + algae + BioNematon) caused significant decrease in mature females, egg masses and root galls (82.9, 85.8, 80%) and it was (81.1, 82.8 and 80.0%) for Carbofuran treatment, respectively. As well as, triple treatment also improved the vegetative properties of cowpea plants, caused an increase in plant height, shoot weight and root weight by percentages (75.3, 78.8 and 96.3%) while contrariwise occurred by appliance of Carbofuran (46.9, 38.7 and 83.7%), respectively, compared to the control. This study succinct recommended that, the use of triple treatment (saponin extract + algae + BioNematon half dose) as an integrated control program for *M. incognita* which infected cowpea plants practically considerable by its safety, inexpensive, harmless, in addition it also reduced root-knot nematode, *M. incognita* juveniles, mature females, root galls, and improved the vegetative properties of cowpea plants in greater proportions than the chemical nematicide Carbofuran.

Key words: *Meloidogyne*, *Chlorella vulgaris*, BioNematon, Saponin, Control, Cowpea.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp is one of the most famous type of legume crops in the world, characterized by its high nutritional value because it contains many important nutritional compounds essentially for the body such as fiber, proteins, minerals (iron, copper, potassium and manganese), as well as containing vitamin B and low calories (Kamara *et al.*, 2016 and Kapravelou *et al.*, 2020). Kidney beans are among the best sources of vegetable protein, rich in healthy fibers, which control blood sugar levels, promote colon health, contain dietary fibers, which stimulate the digestion process and maintain the health of the digestive system (Prince *et al.*, 2020).

Root-knot nematodes *Meloidogyne incognita* infect cowpea plants, causing root galls,

weakening the roots, which leads to yellowing and falling of leaves and consequently caused great economic losses (Metwally *et al.*, 2019).

Chemical nematicides are widely used to control parasitic nematodes on vegetables, fruits and ornamental plants in Egypt, in spite of its expensiveness costs of other agricultural operations, not safety for humans, animals and plants (Barker and Koenning, 1998). There are many safety and alternative materials that can be used to control nematodes, such as saponin extract which can be applicator instead chemical nematicides (Yang *et al.*, 2015). Saponin extract was evaluated by El-marzoky and El-ashry, 2016 in Egypt and found to be effective against plant nematodes.

The green algae, *Chlorella vulgaris* contains proteins, amino acids, antioxidants, and major

and minor elements, therefore, it is considered one of the important growth factors for the plant and also caused aptly incensement in plant immunity in resisting pathogens as plant parasitic nematodes (Bileva, 2013).

BioNematon (a commercial formulation of the *Paecilomyces lilacinus*) bio-nematicide that attacks and kills eggs, mature females and reduces the population density of parasitic nematodes, in addition it can be release a leucino toxin, which has an exterminating effectiveness on plant parasitic nematodes (Ibrahim *et al.*, 2019).

The present study aims to use safety, alternative materials substituent chemical nematicides and to establish an integrated control program for root knot nematodes that infect cowpea plants.

MATERIALS AND METHODS

Potted experiment was conducted at the research Farm of the Faculty of Agriculture, Menoufia University, Shebin El-Kom , Egypt, to study the effect of saponin extract, algae *Chlorella vulgaris* and the bio-nematicide BioNematon (half dose) in comparison to the chemical nematicide Carbofuran (full dose) on root-knot nematodes *Meloidogyne incognita* which infected cowpea plants, as well as the possibility of establishing an integrated control program by these treatments and its effectiveness on the vegetative characteristics of cowpea plants under open field conditions at $25\pm 2^{\circ}\text{C}$.

Preparation of Saponin extract:

It is an extract of the leaves and stems of the *Quillaja saponaria* plant, obtained from the National Research Center, Dokki, Cairo. The tested solution prepared by adding 2 ml of the extract to the beaker and mixed with a small amount of distilled water and left for two days to extract all ingredients and then completed the volume to 100 ml with distilled water. It was added at a rate of 20 ml / plant according to (El-marzoky and El-ashry, 2016).

The green algae *Chlorella vulgaris*:

The green algae *Chlorella vulgaris*, obtained from the National Institute of Marine Sciences

and Fisheries. The trials included applying of 2.0 g algae per plant/pot dissolved in 100 ml water. The plants were watered once with this solution according to Bileva, 2013 .

Bio-Nematon:

Bio- Nematon 1 % WP contains 1×10^4 cfu/mg of fungus (commercial product of *Paecilomyces lilacinus*) was used according to the recommended dose (4kg/ fed) at the rate of 7 g per pot as half dose according to Ibrahim *et al.*, 2019. It was obtained from Nematode Research Department, Plant Pathology Institute, Agric. Research Center, Giza.

Carbofuran:

Common name is Carbofuran, mole Formula is $\text{C}_{12}\text{H}_{15}\text{NO}_3$, chemical Name is: 2, 3-di hydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate, Formulation is 10% G with trade name (Carburan). It was applied as soil treatment at the rate of 20 g per pot (6 kg / feddan). It was applied with nematode inoculation according to Abo-Korah, 2017.

Nematode culture:

The root-knot nematode *M. incognita* juveniles (J_{2s}) were collected from pure culture reared on cotton *Gossypium barbadense* (Gallini) variety (Delta Pin61) at 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 it 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 $25 \pm 1^{\circ}\text{C}$ for 3 to 4 days to obtain second stage juveniles (J_2) with modified Baermann funnel method (Gray, 1984) and used in the experimental infection procedure according to 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, sterilized clay-sand mixed soil (1:1, v/v) and a size of 25 cm. After sterilization, the seeds of the cowpea variety (Kafr El Sheikh) were planted. Two weeks after planting, root-knot nematode *M. incognita* infection was added at a rate of 1000 juveniles per cowpea seed and added by pipette into three holes around seeds, and each treatment replicated three replicates. Experiment layout was randomized complete block design. At the end of the experiment, vegetative measurements were taken.

Nematode Extraction and Enumeration:

Soil samples were taken after planting at 30, 60, 90 and 120 days. Three replicates, each one of 100 g soil and roots were taken from each treatment to extract nematodes, nematode extraction occurred by modified Baermann funnels for 72 hours, where it was counted and identified according to methods described by Southey, 1970. At the end of the experiment roots were carefully washed, and the nematode galls were counted and indexed according to Southey (1970) (0 galls / root = 0 index , 1-2 galls= 1 , 3-10 galls = 3 , 11-30 galls = 3 , 31-100 galls = 4 , more than 100 galls/ root = 5

index). 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). Also, one gram per root was stained by acid fuchsine lactophenol to count root knot nematode stages inside the roots with the aid of a dissecting microscope.

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).

$$\text{Increase or decrease \%} = \frac{\text{treatment} - \text{Control}}{\text{Control}} \times 100.$$

RESULTS AND DISCUSSION

Results in Table (1) revealed that, there are significant differences between all treatments under study which caused reduction in root-knot nematode *Meloidogyne incognita* juveniles that infected cowpea plants.

Table (1): Effect of certain treatments on reduction percentage and average numbers of *Meloidogyne incognita* juveniles infecting cowpea plants.

Treatments	Aver. no. of <i>Meloidogyne incognita</i> juveniles/ 100 g soil					Reduction %				
	Days post-treatments					30 Day	60 Day	90 Day	120 Day	Over all mean
	30 Day	60 Day	90 Day	120 Day	Overall mean					
Saponin extract	827.9 b	684.0 b	594.3 b	406.3 b	628.1 b	39.0	54.2	63.0	77.2	58.4
Algae <i>C. vulgaris</i>	712.9 d	591.3 c	431.3 c	264.0 c	499.9 c	47.5	60.4	73.1	85.2	66.6
Bio-Nematon (half dose)	749.0 c	536.0 d	409.0 d	251.0 d	486.3 d	44.8	64.1	74.5	85.9	67.3
Saponin+Algae +Bio-Nematon	467.0 f	284.0 f	198.9 f	71.0 f	255.2 f	65.6	81.0	87.6	96.0	82.6
Carbofuran (full dose)	507.0 e	331.9 e	217.3 e	97.9 e	288.5 e	62.6	77.8	86.5	94.5	80.3
Control	1357.9 a	1495.3 a	1607.3 a	1784.0 a	1561.1 a	-	-	-	-	-
LSD 5%	8.8	5.3	7.1	8.9	8.8	-	-	-	-	-

means in each column followed by the same letter (s) are not significant differences at 5% level

The triple treatment (saponin extract + algae *C. vulgaris* + BioNematon) resulted the highest reduction percentage in *M. incognita* juveniles (82.6%), followed by Carbofuran (80.3%), then BioNematon, algae and saponin extract solely gave reduction percentages of (67.3, 66.6 and 58.4%) respectively compared to control treatment.

These results are coincident with those obtained by Pelah *et al.*, 2002 who recognized that, the saponin extract from the *Quillaja saponaria* plant has a significant role in controlling the development of insects and plant parasitic nematodes. D'Addabbo *et al.*, 2011 recorded that, saponin extract has a significant effect on reducing the numbers of plant parasitic nematodes, such as *Xiphinema index*, *Meloidogyne incognita* and *Globodera rostochiensis*. (El-marzoky and El-ashry, 2016) showed that, the effect of saponin on parasitic nematodes is due to its chemical composition where saponin are steroid or triterpenoid glycosides and can use as natural nematicide.

Data in Table (2) showed that, tri treatment (saponin extract + algae *C. vulgaris* + BioNematon half dose), outperformed the rest of treatments as it reduced the mature females, egg masses and root galls by (82.9, 85.8, 80.0%), followed by that occurred in appliance of the chemical nematicide Carbofuran (81.1, 82.8 and 80.0%), respectively, in comparison with control. These results confirm that, that of triple

treatment is more efficient than the chemical nematicide Carbofuran in controlling root knot nematodes *M. incognita* that infected cowpea plants.

The obtained results are in agreement with those obtained by Narasimhamurthy *et al.* (2017) who found that, *Paecilomyces lilacinus*, attack nematode eggs and eliminate root knots nematodes, as well as improving the vegetative characteristics of the plant. Azam *et al.* (2013) recorded that, *P. lilacinus* causes the formation of colonies of fungi on the roots of the plant and attacks the egg masses, as the shell wall decomposes by secreting the chitinase enzyme and feeds on its contents, then attacks and kills the whole female. Metwally *et al.* (2019) indicated that, BioNematon is an effective bio-nematicide against root-knot nematodes *M. incognita* that infect cowpea plants, as it reduced root-galls by (60.3%) and egg masses by (26.2%).

Data presented in Table (3) summarized that, the triple treatment (saponin extract + algae *C. vulgaris* + BioNematon half dose) led to improvement in the vegetative properties of cowpea plants, caused an increments in plant height, shoot weight and root weight with percentages (75.3, 78.8 and 97.6%) superior of both Carbofuran which gave (46.9, 38.7 and 83.7%) respectively, and each of the rest solely treatments compared with control.

Table (2): Effect of different treatments on mature females, egg-masses and root gall index associated with cowpea plants.

Treatments	Mature females /5groot	No of egg-masses	root gall index	Decrease %		
				Mature females	Egg-masses	root gall index
Saponin extract	17.1 b	15.0 b	3.0 b	36.6	35.6	40.0
Algae <i>C. vulgaris</i>	12.3 c	9.3 d	2.0 c	54.4	60.1	60.0
BioNematon (half dose)	14.0 c	11.9 c	2.0 c	48.1	48.9	60.0
Saponin+ Algae +BioNematon	4.6 d	3.3 e	1.0 d	82.9	85.8	80.0
Carbofuran (full dose)	5.1 d	4.0 e	1.0 d	81.1	82.8	80.0
Control	27.0 a	23.3 a	5.0 a	-	-	-
LSD 5%	1.7	0.8	0.2	-	-	-

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

Table (3): Influence of some cowpea plant growth parameters by tested treatments.

Treatments	plant height (cm)	shoot weight (g)	root weight (g)	Increase %		
				plant height	shoot weight	root weight
Saponin extract	34.2 e	15.0 e	5.0 d	15.5	9.5	16.3
Algae <i>C. vulgaris</i>	39.7 c	17.5 c	6.1 c	34.1	27.7	41.8
BioNematon (half dose)	37.0 d	16.3 d	7.0 b	25.0	19.0	62.8
Saponin+ Algae +BioNematon	51.9 a	24.5 a	8.5 a	75.3	78.8	97.6
Carbofuran (full dose)	43.5 b	19.0 b	7.9 a	46.9	38.7	83.7
Control	29.6 f	13.7 f	4.3 e	-	-	-
LSD 5%	1.7	0.8	0.6	-	-	-

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

The obtained results are in harmony with those obtained by (Bileva, 2013) and (Bumandalai and Tserennadmid, 2019) who recognized that, the algae *Chlorella vulgaris* act as a good organic bio-fertilizer for the plant, thus increasing its resistance against plant parasitic nematodes and improves the vegetative properties of the plant. Veronico and Melillo (2021) recorded that, algae *Chlorella vulgaris* contain many vitamins, amino acids and natural growth regulators such as cytokinin and indole butyric acid, which due to retain of chlorophyll, prevent its decomposition, encourage both cell division and root growth, consequently it increasing plant resistance to pathogens specially plant parasitic nematode.

This study recommends the possibility of using the three treatments saponin extract, algae *C. vulgaris* and BioNematon (half dose) together in an integrated control program for root-knot nematodes *Meloidogyne incognita* that infected cowpea plants, as they are considered safety, cheap and available and have a greater effect than the chemical nematicide Carbofuran.

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المكافحة المتكاملة لنيماتودا تعقد الجذور *Meloidogyne incognita* التي تصيب نباتات اللوبيا

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الملخص العربي

أجريت هذه التجربة لتقييم كفاءة مستخلص الصابونين , الطحلب الأخضر *Chlorella vulgaris* ومبيد النيماتودا الحيوي بيونيماتون (مستخلص تجاري لفطر *Paecilomyces lilacinus*) نصف جرعة وإمكانية إنشاء برنامج مكافحة متكاملة منهم لنيماتودا تعقد الجذور التي تصيب نباتات اللوبيا وتأثيرهم على الصفات الخضرية لنباتات اللوبيا كذلك مقارنة تأثير هذه المعاملات بتأثير مبيد النيماتودا الكيميائي الكارببوفوران .

جميع المعاملات أدت إلى خفض أعداد يرقات نيماتودا تعقد الجذور *Meloidogyne incognita* مع الأخذ في الاعتبار أن المعاملة الثلاثية المكونة من (مستخلص الصابونين + الطحلب + بيونيماتون نصف جرعة) أعطت أعلى نسبة موت في يرقات نيماتودا تعقد الجذور (٨٢,٦٪) يليها مبيد النيماتودا الكيميائي كارببوفوران (٨٠,٣٪) ويليه بيونيماتون نصف جرعة , الطحلب و مستخلص الصابونين عند تطبيقهم بصورة فردية بنسب (٦٧,٣ , ٦٦,٣ و ٥٨,٤٪) على التوالي مقارنة بمعاملة الكنترول.

أدى تطبيق المعاملة الثلاثية (مستخلص الصابونين + الطحلب + نصف جرعة من بيونيماتون) إلى نقص واضح في عدد الإناث الكاملة , كتل البيض والعقد الجذرية بنسب (٨٢,٩ , ٨٥,٨ و ٨٠,٠٪) على التوالي متفوقة على مبيد النيماتودا الكيميائي الكارببوفوران بنسب (٨١,١ , ٨٢,٨ و ٨٠,٠٪) على التوالي كما أدت المعاملة الثلاثية إلى تحسين الصفات الخضرية لنباتات اللوبيا حيث أدت إلى زيادة طول النبات , وزن المجموع الخضري ووزن الجذور بنسب (٧٥,٣ , ٧٨,٨ و ٩٦,٣٪) بينما قلت هذه النسب عند تطبيق الكارببوفوران إلى (٤٦,٩ , ٣٨,٧ و ٨٣,٧٪) على التوالي مقارنة بالكنترول.

لذلك توصي هذه الدراسة بتطبيق المعاملة الثلاثية (مستخلص الصابونين + الطحلب + بيونيماتون نصف جرعة) واستخدامها كبرنامج مكافحة متكاملة لنيماتودا تعقد الجذور التي تصيب نباتات اللوبيا وذلك لأنها أكثر أمناً وأرخص ثمناً كما أنها قللت من يرقات نيماتودا تعقد الجذور والإناث الكاملة والعقد الجذرية وحسنت الصفات الخضرية لنباتات اللوبيا بنسب أفضل من مبيد النيماتودا الكيميائي الكارببوفوران.