Research Article

The Potential of Milbemectin on the Formation of Galls and Egg Masses of *Meloidogyne javanica* on Eggplants

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Abstract | The root-knot nematode (*Meloidogyne javanica*) is one of the most destructive pests to vegetable crops worldwide. Non-fumigant and/or fumigant nematicides, as well as biological agents are the most utilized tools to manage the root-knot nematodes (RKNs). Environmental and health defects were notice with using synthetic nematicides. Thus, in this work an attempt was conducted to provide alternative approach could be listed in control measures of RKNs. Two pot experiments were conducted to assess the efficacy of the milbemectin on galls and egg masses formation of *M. javanica* in roots of eggplants. Milbemectin (Milbeknock 1% EC) was applied at 0.1% (1 ml/l) and 0.5% (5 ml/l) in compared with the non-fumigant nematicide fenamiphos (40% EC). Results revealed that all tested doses of milbemectin suppressed galls (ranged from 50.59 to 75.50%, of both experiment) and egg masses (ranged from 66.67 to 87.01%, of both experiment) significantly, while fenamiphos recorded moderate reductions. Also, the measured plant indices were augmented with all applied treatments. Therefore, from this study it can prove that milbemectin possess efficacy on galls and egg masses of root –knot nematode (*M. javanica*).

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Introduction

Root-knot nematodes (*Meloidogyne* spp.), are sedentary endoparasites that considered the most threats to vegetable crops production around the world (Ibrahim, 2011; Forghani and Hajihassani, 2020). The current management practices are not enough to manage *Meloidogyne* spp., when the most reciprocal strategy in Egypt or African countries is using synthetic nematicides (Khalil and Alqadasi, 2019). There are only six active ingredients of non-fumigant nematicides that authorized as for use in vegetable crops; oxamyl, ethoprophos, fenamiphos, fosthiazate, abamectin and fluopyram. The frequent use of these nematicides could exert high selective pressure on root-knot nematodes (RKNs) and soil microbiota. Thus, scientists are search for new effective nematicides with different modes of action (Talavera-Rubia *et al*., 2020).

Milbemectin belongs to milbemycin group which is an antibiotic isolated from the soil bacterium *Streptomyces hygroscopicus* subsp. *Aureolacrimosus*. Milbemectin was recorded acaricidal efficacy against spider mites, as well as nematicidal activity against *Bursaphelenchus xylophilus* and *Hirschmanniella diversa* (Yang *et al*., 2013; Bi *et al*., 2015; Takagi *et al*., 2020). Furthermore, milbemectin is a macrocyclic lactone with similar molecular structure to other members of the family used as biopesticides against RKN, as

abamectin and emamectin benzoate (Rehman *et al.*, 2009; Saad *et al.*, 2017; Khalil and Darwesh, 2018; Radwan *et al.*, 2019). Therefore, this investigation aimed to estimate the influence of milbemectin as soil application against galls and egg masses formation of *M. javanica*, on roots of eggplants.

Materials and Methods

Nematode inoculum

The population of root-knot nematode (*Meloidogyne javanica*) was obtained from EL-Bossaily area, Rashid region, Behera governorate, Egypt. The species of RKN was identified as *M. javanica*, by using female perineal patterns method according to Taylor and Netscher (1974). The eggs of root knot nematode (*M. javanica*) were extracted by the sodium hypochlorite method from infested roots (Hussey and Barker, 1973). Meanwhile, to count the egg masses they were stained by using aqueous solution of phloxine B (0.15 g/L water) for 15 minutes according to the method of Holbrook *et al.* (1983).

Pot experiments

Two pot experiments were carried out to evaluate the potential of milbemectin against *M. javanica* on eggplants. The used pots of 15 cm diameter were filled with 1 kg of sterilized sandy loam soil. Each pot was transplanted with one eggplant seedling (*Solanum melongena* L., cv. Black Beauty) of 40 days-old. Each pot was inoculated with 5000 nematode eggs after two days from transplanting time by pouring the nematode suspension into holes made 2-4 cm below the soil surface around the base of the plants.

The non-fumigant nematicide, fenamiphos (fenamiphos 40% EC) was applied to the soil at the recommended dose, while milbemectin (Milbeknock 1% EC) was applied at the suggested concentrations (0.1 and 0.5%) to the soil after a day of inoculation time. All pots were replicated four times and arranged in a complete randomized design on a bench in a greenhouse. The irrigation and fertilization were applied when appropriate.

After 65 days from inoculation time, plants were uprooted and the roots were washed free of adhering soil. Meanwhile, the plant height and fresh weights of shoot and root, as well as, the number of galls and egg masses /root system were estimated. The reductions (%) in root galls and egg masses were calculated as follow:

$$\text{Reduction (\%)} = \frac{\text{control} - \text{treatment}}{\text{control}} \times 100$$

Furthermore, the increases (%) in plant indices were calculated by the next formula:

$$\text{Increase (\%)} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

Statistical analysis

The obtained data were statistically analyzed according to CoStat Software (2005) Version: 6.303. Results of the present work were subjected to the analysis of variance test (ANOVA) as complete randomized design (CRD). Comparison among means was made via the least significant difference (LSD) test at the 5% level of probability.

Results and Discussion

Based on root galls, results indicated that milbemectin at 0.5% was the superior treatment which recorded 70.29% reduction, followed by fenamiphos and milbemectin at 0.1% with 61.76 and 50.59%, respectively, during the 1st experiment (Table 1). In the 2nd experiment, the same trend was noticed where milbemectin at 0.5% was the highest treatment followed by fenamiphos and milbemectin at 0.1% with suppression in root galls estimated by 75.50, 67.38 and 60.54%, consecutively.

On the other hand, the egg masses formation was affected by the applied treatments significantly. In the 1st experiment, results revealed that milbemectin at 0.5% recorded the highest potential by 75.00% reduction, while milbemectin at 0.1% and fenamiphos recorded 67.50 and 53.75%, respectively. Furthermore, during the 2nd experiment the application of milbemectin at 0.5% gave the highest decreasing (87.01%), followed by milbemectin at 0.1% (66.67%) and fenamiphos (62.34%). There weren't significant differences between both milbemectin concentrations in galls and egg masses formation, except egg masses in the first experiment.

Only a single study was published recently showed that the efficacy of milbemectin against the second stage juveniles in soil (omit). Talavera-Rubia *et al.* (2020) found that using milbemectin at 1.5 and 2.5L/ha reduced soil population by 52 and 59%, respectively. Milbeknock® found to minimized the egg masses

of *Meloidogyne* spp., significantly by 99% in tomato roots compared to untreated nematode-infected plants (Talavera-Rubia *et al*., 2020). Also, in the same study, milbemectin decreased the egg hatching, soil population and tomato root infection. The mode of action of milbemectin against *Meloidogyne* species is mostly looks like other members of macrocyclic lactones (abamectin and emamectin benzoate). It was suggested that milbemectin is effect on the δ-aminobutyric acid (GABA) receptors and glutamate-gated chloride channels, which activate the influx of chloride ions causing hyperpolarizing in nerve and muscle cells, and disturbing the neuromuscular transmission leading to death (Martin *et al*., 2002).

Fenamiphos has efficacy to reduce gall index, egg-masses in carnation and population of J2 in the soil significantly (Kimenju *et al*., 2014). Moreover, Maggie *et al*. (2016) mentioned that fenamiphos reduced galls (90.64%) and egg masses (93.25%). In a study, it was recorded that application of fenamiphos and abamectin were minimized the formation of galls and egg masses by 91.73 and 90.80%, and 66.69 and 66.31%, respectively (Saad *et al*., 2017). The efficacy of fenamiphos against RKN is depending on the inhibition in acetylcholinesterase (AChE) at cholinergic synapses in the nematode nervous system (Opperman and Chang, 1990).

The effect of applied treatments on growth indices such as shoot and root height and weight of eggplants were recorded in the termination of two experiments (Table 2). During the 1st experiment the applied treatments increased shoot height at range of 12.99 to 29.87%, and shoot weight from 10.35 to 77.76%. Besides, the root length was augmented at range of 2.60 to 33.77%, while root weight was increased at range of 3.89 to 40.68%. Fenamiphos was the most effective treatment and there weren't significant differences between milbemectin concentrations except in root system length.

Table 1: *The influence of milbemectin against galls and egg masses formation of root-knot nematode (M. javanica) in roots of eggplants during two pot experiments.*

Treatments	1st Experiment				2nd Experiment			
	Galls / root system		Egg masses / root system		Galls / root system		Egg masses / root system	
	Mean	Decrease (%)	Mean	Decrease (%)	Mean	Decrease (%)	Mean	Decrease (%)
Milbemectin 0.1%	42.00b	50.59	19.50bc	67.50	69.25b	60.54	38.50b	66.67
Milbemectin 0.5%	25.25c	70.29	15.00c	75.00	43.00c	75.50	15.00c	87.01
Fenamiphos	32.50bc	61.76	27.75b	53.75	57.25bc	67.38	43.50b	62.34
Untreated check	85.00a	----	60.00a	----	175.50a	----	115.50a	----

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05, Means are the average of four replicates.

Table 2: *The effect of milbemectin on the growth indices of egg plants infected with root-knot nematode (M. javanica).*

Treatments	Shoot system				Root system			
	Height		Weight		length		Weight	
	Mean (cm)	I%	Mean (g)	I%	Mean (cm)	I%	Mean (g)	I%
1st Experiment								
Milbemectin 0.1%	22.13ab	14.94	4.29b	10.35	19.75b	2.60	2.39ab	12.50
Milbemectin 0.5%	21.75ab	12.99	5.04b	29.63	25.75a	33.77	2.53ab	19.10
Fenamiphos	25.00a	29.87	6.92a	77.76	23.00ab	19.48	2.98a	40.68
Uninfected untreated check	22.00ab	14.29	4.29b	10.35	20.25b	5.19	2.20b	3.89
Untreated check	19.25b	0.00	3.89b	0.00	19.25b	0.00	2.12b	0.00
2nd Experiment								
Milbemectin 0.1%	33.25ab	15.65	5.40bc	18.74	29.63b	3.04	2.74bc	25.24
Milbemectin 0.5%	32.63ab	13.48	7.09a	55.77	34.50ab	20.00	2.88ab	31.58
Fenamiphos	37.50a	30.43	6.75ab	48.35	36.38a	26.52	3.43a	56.62
Uninfected untreated check	33.00ab	14.78	5.20c	14.18	29.50b	2.61	2.53bc	15.66
Untreated check	28.75b	0.00	4.55c	0.00	28.75b	0.00	2.19c	0.00

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05, Means are the average of four replicates.

In respect to the 2nd experiment, the augmentation in shoot height and weight were ranged from 13.48 to 30.43% and 14.18 to 55.77% over control, respectively. Meanwhile, root length was increased at range of 2.61 to 26.52%, while root weight recorded increment ranged from 15.66 to 56.62%. Fenamiphos was the superior treatment which increased all plant indices. Also, there weren't any significant differences between milbemectin concentrations except in shoot system weight. The obtained results are in agreement with Maggie *et al.* (2016) who found that plant growth indices of tomato plants such as fresh weight, dry weight, root length and shoot height were enhanced. While, Al-Hazmi *et al.* (2017) indicated that fenamiphos as soil drench, seed dressing and seed dep increased the green beans fresh weight by 42.57, 59.40 and 48.52%, consecutively. Meanwhile, fenamiphos and abamectin increased the growth indices of tomato plants (Saad *et al.*, 2017).

Conclusions and Recommendations

Plant parasitic nematode (*Meloidogyne* sp.) is very devastating disease to a lot of vegetable crops. As has been mentioned above, the present study revealed that milbemectin which is an acaricide was effective in reducing galls an egg masses of *M. javanica* in eggplants. Also, the impacts on plant growth improved relatively in compared with untreated check. More studies are needed in open field.

Novelty Statement

Globally, this investigation is the 2nd study for the nematicidal activity of milbemectin (acaricide) against the root-knot nematode (*Meloidogyne javanica*). Activity was proved and confirmed.

Conflict of interest

The authors have declared no conflict of interest.

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