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Original article

# The effect of abamectin seeds treatment on plant growth and the infection of root-knot nematode *Meloidogyne incognita* (Kofoid and White) chitwood

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ABSTRACT

In this study, three concentrations (250, 500, and 1000 ppm) of abamectin 2% suspension concentration (SC) were used as cucumber seeds treatment. The seeds were treated with abamectin to reduce nematodes reproduction and their ability to penetrate the roots, then seed germination and plant growth were observed. All the concentrations didn't negatively affect seeds germination wherever the germination percent reached 80% at the concentration (1000 ppm) after 20 days of sowing. The effect of abamectin on root-knot nematode was studied by recording numbers of nematodes in 100 g/soil, numbers of the galls, egg mass on the root, and the nematode reproduction factor. All concentrations significantly affected the nematode reproduction parameters compared to control. Abamectin at (500 ppm) was the most effective concentration on reducing nematodes parameters, i.e., 26.57, 38.83, 47.40 %, and 3.15 for the above-mentioned parameters, respectively at the end of experimint. No significant difference between 500 ppm and 1000 ppm. We recommended using the abamectin in (500 ppm) concentration as a seed application to control *Meloidogyne incognita* in cucumber plants under greenhouse conditions to reduce its environmental toxic effect.



## 1. Introduction

Plant parasitic nematodes are the most widespread pathogens in the world and badly affect plant production, this problem attracts researchers to focus on these pests (El-Saadony et al., 2021). There are many genera and species of plant parasitic nematodes among them *Meloidogyne incognita,* which are called root-knot nematode, the danger of this nematode was due to its host wide range of about 200 hosts (Khalil, 2013). Moreover, the root-knot nematodes are responsible for damage of about 90% of all the damages caused in plants (Castagnone-Sereno, 2002). Obviously, after the nematode's infection, the plants become more susceptible to infection by the other pathogenic microorganisms (Fischer et al., 2008).

When focusing on economic plants, cucumber plants *Cucumis sativus* are the most important greenhouse plant in Egypt. The local production of this crop was 364,571 tons in 2019, with an estimated harvested area of about 16,104 ha (FAO, 2019).

Nematicides are commonly used to manage nematodes infesting fruit and vegetables in Egypt. Chemical nematicides increase agriculture costs, on the other hand, the use of non-chemical nematicides may be less expensive in addition to their environmental safety. The chemical treatment of the seeds is only active in the rhizosphere of the surrounding ground the root system of young plants and as a result, reduced the danger of the systemic effect of these materials additionally. The amount of active ingredi-

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ents necessary to treat a seed is lower than whether liquid or granular formulations are applied (Cabrera et al., 2009). Abamectin is a suitable compound for seed treatment as it can be stocked for several months with maintaining nematicidal properties. In addition, didn't accumulate on seeds or absorbed by plants at high concentrations (Wislocki, et al. 1989; Monfort, et al. 2006; Faske and Starr, 2007).

Abamectin is an efficient pesticide that has been used as an insecticide, acaricide, and nematicide in vegetables, fruits, and crop fields. Abamectin is part of the avermectin group, which is itself composed of lactone macrocyclic metabolites produced during the natural fermentation process of the bacterium *Streptomyces avermitilis* this bacterium is known to contain about 80 percent avermectin B1a and 20 percent avermectin B1b (Khalil, 2013). This study aims to focus on the use of abamectin as a seed treatment to reduce the environmental hazards of this pesticide when controlling root-knot nematodes and concerning the effect of this pesticide on plant growth.

## 2. Materials and methods

### 2.1. Experimental design

A healthy seeds of a cucumber plant *C. sativus* var. Hayel produced by Seminis company were separated and divided into four groups. Each group contains ten seeds that form the experimental treatments. Abamectin concentrations of 250, 500, and 1000 ppm were prepared from commercial products (Tervigo® 2 %SC) produced by Syngenta Agrochemical Company. The seeds were washed gently with tap water and soaked in each concentration separately for 15 min. and then removed from the pesticide. Each group of the seeds was dried by left on filter paper for 24 h in the lab, the control treatment (untreated seeds) was seeds soaked in the tap water and let dry for the next day.

On the next day, the seeds were sawed in sterilized 15 cm diameter plastic pots contain formaldehyde sterilized soil (50% sand +50% peat moss) and left in the greenhouse in the Faculty of Agriculture, Zagazig University, Egypt. The temperature and humidity were obtained which were 25 ± 3 ℃ and 70%, respectively. The pots were randomly arranged in the greenhouse and have agricultural transactions regularly. The pots were monitored until germination and then the plants were thinned to two plants in each pot, data were recorded after 10, 15, and 20 days of the first seed germinated. The most important vegetative features of the cucumber plants were recorded. The recorded data were percent of germination, shoot length, and root length. The percent of germination was calculated by Eq. (1):

$$\text{Percent of germination}(\%) = \frac{\text{Number of emerged plants}}{\text{Total number of sawed seeds}} \times 100 \quad (1)$$

The data was recorded after 10, 15, and 20 days of seed planting.

The shoot length was measured by using a centimeter's ruler, the measurement was from the highest top of the plant to the beginning of the root. The root length was measured in the same way from the root beginning to the highest point of the root. Measurements were recorded after 10, 15, and 20 after first seed germination. All cucumber seedlings were measured and returned immediately to their pots to maintain their vitality for the inoculation experiment. To determine the effect of abamectin on seeds and plant characters the reduction percentage in shoot and root length was calculated by Eq. (2):

$$\text{Percentage of reduction}(\%) = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100 \quad (2)$$

### 2.2. Preparing the inoculum of Root-knot nematodes M. Incognita

The pure culture of *M. incognita* was maintained through the greenhouse onto the tomato *Lycopersicon esculentum* susceptible cultivar Super Strain B used as a source of inoculum. Infective second-stage juveniles were collected from a single egg-masses by separating eggs from their gelatinous matrix, infected tomato roots were cut into pieces each one was about 2 cm and put on a 500 ml flask contained 200 ml sodium hypochlorite 0.5 % concentration (180 ml water + 20 ml commercial Clorox). These pieces of roots were shaken gently to separate eggs (Hussey and Barker,1973). The suspension containing the eggs was poured through a 200 mesh sieve nested upon a 500 mesh sieve, the eggs were collected immediately from the 500 mesh sieve and washed with a slow stream of tap water to release the residual of the sodium hypochlorite. The collected eggs were transferred with light tape water to 100 ml beaker and then distributed in Petri dishes and incubated at 25 ± 1 °C until they hatched. The newly hatched juveniles were collected each other day and quantified in 1 ml of the suspension for use in infection treatment. After 10, 15, and 20 days of a plant emerging it used about 1000 active second-stage juveniles for each pot to determine the progression of the infection and nematicide effectivity, number of nematode juveniles in 100 g soil, number of galls on the root, and number of egg masses on the root were recorded after three weeks of inoculation for each group separatly (Li et al., 2020). The reduction in nematode reproduction parameters was calculated in equation (2).

The nematode reproduction factor (RF) was calculated for each treatment separately for determining which concentration reduced nematode reproduction the calculation was by Eq. (3):

$$\text{Reproduction Factor (RF)} = \frac{\text{Final population (FP)}}{\text{Initial Population (IP)}} \quad (3)$$

The initial population (IP) was determined by the number of nematodes added to the experimental pot (1000 juveniles) and it was attributed to soil weight in the pot (1500 g soil) and calculate nematode numbers in 100 g soil.

### 2.3. Statistical analysis

This experiment was carried out in a completely randomized block design with 5 replications for each treatment and each replicate consisted of two plants, and the mean numbers were compared by Duncan's multiple range test at $p \leq 0.05$ level of probability by using Mstat software version 4 (MSTAT VERSION 4, 1987).

## 3. Results

### 3.1. The effect of abamectin on plant characters

This study aims to investigate the effect of seed treatment with nematicide as an alternative method for direct application of those materials to the soil, by this way we can avoid the negative effect in the soil ecosystem especially the bad effect on natural enemies and free-living soil fauna. In the same way, these nematicides must not affect plant growth and reproduction. Data in Tables 1-3 showed the effect of the cucumber seeds treatment with the nematicide abamectin in concentrations 250,500, and 1000 ppm on the percent of germination after 10,15, and 20 days of sawing, and this effect was completed by recording the main vegetative

**Table 1**
The effect of abamectin concentrations on cucumber seed's vitality and plant productive characters after ten days of planting and germination.

| Treatment | 10-days after planting | 10-days after germination | |
|---|---|---|---|
| | Percent of germination (%) | Shoot length (cm) | Root length (cm) |
| Control (Untreated seeds) | 40 (0.00) | 3.5a (0.00) | 2.8a (0.00) |
| 250 ppm | 40 (0.00) | 3.2a (8.50) | 2.3a (17.85) |
| 500 ppm | 20 (50.00) | 3.1b (12.90) | 1.9b (32.14) |
| 1000 ppm | 0 (100.00) | 1.9c (45.71) | 0.9c (67.85) |

*Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.
*Values between brackets refer to the percent of reduction (%) in vegetative characters.

**Table 2**
The effect of abamectin concentrations on cucumber seed's vitality and plant productive characters after fifteen days of planting and germination.

| Treatment | 15-days after planting | 15-days after germination | |
|---|---|---|---|
| | Percent of germination (%) | Shoot length (cm) | Root length (cm) |
| Control (Untreated seeds) | 80 (0.00) | 4.8a (0.00) | 3.5a (0.00) |
| 250 ppm | 60 (25.00) | 4.1a (14.58) | 2.9a (17.14) |
| 500 ppm | 40 (50.00) | 3.9ab (18.75) | 2.6ab (25.71) |
| 1000 ppm | 40 (50.00) | 2.8c (41.66) | 1.5c (57.14) |

*Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.
*Values between brackets refer to the percent of reduction (%) in vegetative characters.

**Table 3**
The effect of abamectin concentrations on cucumber seed's vitality and plant productive characters after twenty days of planting and germination.

| Treatment | 20-days after planting | 20-days after germination | |
|---|---|---|---|
| | Percent of germination (%) | Shoot length (cm) | Root length (cm) |
| Control (Untreated seeds) | 100 (0.00) | 5.9a (0.00) | 4.2a (0.00) |
| 250 ppm | 100 (0.00) | 5.0a (15.25) | 3.9b (7.14) |
| 500 ppm | 80 (20.00) | 4.6b (22.03) | 3.8c (9.52) |
| 1000 ppm | 80 (20.00) | 3.0c (49.15) | 1.9d (54.76) |

*Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.
*Values between brackets refer to the percent of reduction (%) in vegetative characters.

characters (shoot and root lengths) after 10, 15, and 20 days of a plant emerging. After 10 days of sowing, the seeds germination was reduced by 50–100% when treated with abamectin (500 and 1000 ppm), respectively; however, the germination decreased to 20 % after 20 days of sowing in the same treatment. On the other hand, the seed treated with the concentration of 250 ppm germinated in the same percent compare to untreated seeds (control) after 10 days of planting, this percent was reduced after 15 days of planting which was 25% compared to control. The highest reduction in germination (100%) was in the seeds treated by 1000 ppm after 10 days of planting this delay in germination may be due to the temporary effect of abamectin on the germs.

Regarding the effect on the shoot and root length, there are no significant differences between the concentration 250 ppm and the control at the three different periods after germination. The most effective concentration was 1000 ppm which reduced the shoot length by 49.15% after 20 days and reduced the root length by 67.85 % after 10 days of germination. The concentration of 500 ppm was mild in its effect on the shoot length the lowest effect was 12.90% after 10 days and 9.52 % after 20 days for the root length. The variations between abamectin concentrations on shoots and roots may depend on the local effect of abamectin in the rhizosphere and the degradation of the nematicide by the time. The results showed that no negative effect of abamectin seeds treatment on seeds germination and plant growth parameters.

### *3.2. The effect of abamectin on M. Incognita infection and development in cucumber*

Data in Tables 4-6 showed that the effect of the treatment of cucumber seed with the nematicide abamectin on root-knot nematode infection. All the recorded data had marked a positive effect of abamectin treatments in all concentrations. In low concentration, the nematode population reduced by 2.85, 9.39, and 16.08 % after 10, 15, 20 days of seed germination, respectively. While the 500 ppm concentration was the most effective one in reducing the soil nematode population on the seedlings 15 days old (28.57%), there is no significant differences between this concentration and the highest concentration at the seedlings 15 and 20 days old. These results pushed to use the concentration 500 ppm in seed coating.

This reduction effect was increased in galls and egg masses numbers on the root which reduced by 36.61 and 45.32% respectively in 15 days old seedlings and the positive effect was increasing as the plant growing up which reached 38.83 and 47.40% for the medium concentration 500 ppm, in 20 days old seedlings. The most reduction in root galling and egg mass was due to 500 ppm concentration which recorded 38.83 and 47.40%, respectively on the day 20th of plant age. The positive effect of the abamectin was recorded firstly in the lowest concentration (250 ppm) which was recorded 25.72 and 21.76 % on the 20 days old seedlings, these values may be unsatisfactory for reducing nematode population so it could be recommended to use the upper concentrations.

**Table 4**
The abamectin effect on nematode and its ability to penetrate plant roots and reproduction in 10-days old seedlings of seeds treatment.

| Treatments | No. of nematodes in 100 g/ soil | No. of the galls on the root | No. of the egg mass on the root |
|---|---|---|---|
| Control (Untreated seedlings + nematodes) | 210a (0.00) | 15.3a (0.00) | 30.6a (0.00) |
| 250 ppm | 204a (2.85) | 12.6b (17.64) | 25.3b (17.32) |
| 500 ppm | 196ab (6.66) | 11.3c (26.14) | 18.6c (39.21) |
| 1000 ppm | 190ab (9.52) | 11.6c (14.18) | 17.3c (43.46) |

*Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.
*Values between brackets refer to the percent of reduction (%) in nematode reproduction parameters.

**Table 5**
The abamectin effect on nematode and its ability to penetrate plant roots and reproduction in 15-days old seedlings of seeds treatment.

| Treatments | No. of nematodes in 100 g/ soil | No. of the galls on the root | No. of the egg mass on the root |
|---|---|---|---|
| Control (Untreated seedlings + nematodes) | 266a (0.00) | 18.3 (0.00) | 35.3a (0.00) |
| 250 ppm | 241ab (9.39) | 13.6b (25.68) | 27.3b (22.66) |
| 500 ppm | 190b (28.57) | 11.6c (36.61) | 19.3c (45.32) |
| 1000 ppm | 196b (26.31) | 11.6c (36.61) | 19.6c (44.47) |

*Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.
*Values between brackets refer to the percent of reduction (%) in nematode reproduction parameters.

**Table 6**
The abamectin effect on nematode and its ability to penetrate plant roots and reproduction in 20-days old seedlings of seeds treatment.

| Treatments | No. of nematodes in 100 g/ soil | No. of the galls on the root | No. of the egg mass on the root |
|---|---|---|---|
| Control (Untreated seedlings + nematodes) | 286a (0.00) | 20.6a (0.00) | 38.6a (0.00) |
| 250 ppm | 240ab (16.08) | 15.3b (25.72) | 30.3b (21.76) |
| 500 ppm | 210b (26.57) | 12.6c (38.83) | 20.3c (47.40) |
| 1000 ppm | 216b (24.47) | 13.3 cd (35.43) | 20.6c (46.63) |

*Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.
*Values between brackets refer to the percent of reduction (%) in nematode reproduction parameters.

On the other hand, there are no significant differences between the concentration of 500 and 1000 ppm on the number of galls and the egg mass in the 15th and 20th days of plant age. These results may pouch in a recommendation to use the effective and low concentration (500 ppm) as an alternative for the high concentration (1000 ppm) whereas the last one is more toxic and more expensive.

The nematode reproduction factor (RF) was described in Fig. 1 it was varied, and it increases with the plant's progress in age. The highest RF was 3.61 in the 250-ppm concentration after 15 days of seeds germination compared to the control which was 3.99, while the lowest (RF) was in the 500 ppm treatment in15 days old seedlings which was 2.85. The (RF) were 3.06, 2.94, and 2.85 in the treatments 250, 500, and 1000 ppm respectively in 10-days old seedlings compared to control which was 3.15 in the same plant age. On the other hand, the (RF) was the greatest in the plant 20$^{th}$ day old which were recorded 3.60, 3.15, and 3.24 in 250, 500, and 1000 ppm compared to control 4.29. These increases in nematode reproduction may be due to increases in plant growth and root growth complete and the decreases in the nematicide efficacy over time by natural degradation but these results don't mean that the negative effect was increased by the increase in nematode reproduction and this point need for future studies.

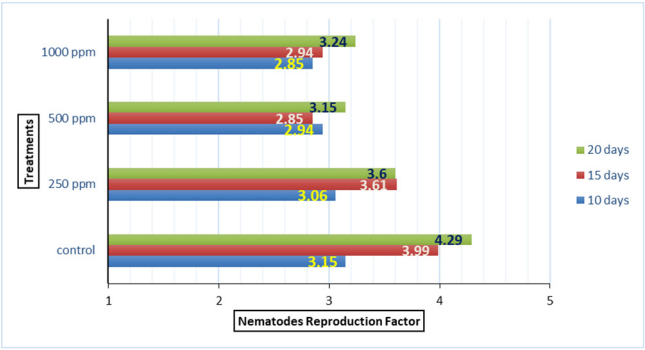


**Fig. 1.** The effect of seed treatment with abamectin in concentrations 250, 500, and 500 ppm on the reproduction factor (RF) of the *Meloidogyne incognita* after 10, 15, and 20 days of seeds germinationns.

## 4. Discussion

The previous results strongly reinforce the efficacy of using abamectin as the seeds treatment in decreasing the nematode reproduction without significant negative effect on seed germination and plant growth, and many authors agreed with these results e.g., Cabrera et al. (2009) studied the effect of abamectin at concentrations 0.00, 0.03, 0.1, 0.30, 0.60, and 1.0 mg/seed of maize, sugar beet, and cotton seedlings and found that at these concentrations there is no negative effect on root and shoot weight. Muzhandu et al. (2014) indicated that tobacco seeds treated with abamectin were not significantly affected in germination and survival when compared with the control, and they decided that the abamectin had no phytotoxic effect on tobacco seedless in all tested concentrations.

da Silva et al. (2017) decided that maize seed treatments with abamectin significantly improved most root development variables and abamectin had a large positive effect on seedling health. Rodrigues et al. (2017) reported that the quality of the seeds of the watermelon is not affected by the concentration of abamectin when used in 0.75 g up to 600 g/ ai (active ingredients) per 1000 seeds. Hnoosh and Aljuaifari (2020) showed that the okra seeds when treated with abamectin (*Streptomyces avermitilis*) were significantly improved compared to non-treated seeds. There was no negative effect on plant growth and development after thirty days of planting this including the number of leaves for each plant and weight of the root length and at the end of the season, there is no negative effect on okra plant production.

The nematicidal effect of abamectin, when used as a seed coating, was described by the following authors whose results agree with the results obtained. El-Nagdi and Youssef (2004) tested the effect of soaking faba bean seed in some bi-agents for controlling *M. incognita* among of those materials was abamectin. They attributed the reduction effect of abamectin to the effect of the abamectin on nematodes movement and infective behavior in the parasites. Cabrera *et.al.* (2009) demonstrated that seed treatment with abamectin is effective in reducing early root infection of *Pratylenchus zeae* on maize plants, *Meloidogyne incognita* in cotton plants, and *Heterodeta schachtii* in sugar beet plants.

Bessi et al. (2010) studied the effect of cotton seed treatment with abamectin in *Meloidogyne incognita* penetration after 3, 9, and 15 days of germination and they found that the abamectin can lower nematodes penetration in the roots and can decrease the colonization and reproduction of *M. incognita*, and they suggested that using abamectin as a complementary method to reduce the amount of nematicide applied to the soil. Zasada et al. (2010)

tested the effect of oxamyl, abamectin, and methomyl on seeds treatment in cucumber *C. sativus* and cotton *Gossypium* spp. They found that a high dose of methomyl can decrease *M. incognita* infection similar to abamectin, and they notice that the nematode infection increased with the time after seed treatment. da Silva et al. (2017) provided that abamectin in combination with fungicidal seed treatment can significantly improve the protection of the maize root system against *Pratylenchus penetrans* infection and can maintain the seedlings in a healthy state. Hnoosh and Aljuaifari (2020) found that the treatment of okra seed with *Streptomyces avermitilis* as an active ingredient of abamectin effect on *Meloidogyne* spp. stages and reduced the average of all nematode stages (juveniles and eggs) in all concentrations compared to control treatment.

Hawk and Faske (2020) indicated that cotton and soybean seeds treatment with abamectin and fluopyram suppressed *M. incognita* root penetration that contributes to forming root galls and nematode reproduction in cotton and soybean plants, and suggested that these nematicides at low concentration can inhibit nematode motility for penetrating the roots.

## 5. Conclusion

The application of abamectin as SC formulation at concentrations 250, 500, and 1000 ppm for cucumber *C. sativus* seeds doesn't affect seeds germination and plant growth under greenhouse conditions after 10, 15, and 20 days of planting, when looking at the nematicidal effect of abamectin it was significantly affected at all tested concentrations and with mention to there is no significant effect between the concentrations 500 and 1000 ppm. The nematode reproduction factor (RF) for the root knot nematode *Meloidogyne incognita* was decreased at all concentrations compared to the control treatment.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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