Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www.jppp.journals.ekb.eg

Effect of Different Control Agents on *Meloidogyne incognita* (Kofoid and White) Chitwood Infecting Cucumber Plants

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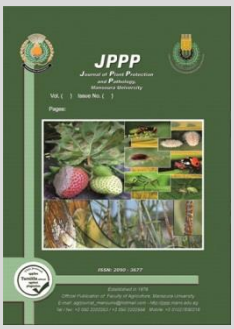

ABSTRACT

The stock solution 10% and half stock solution 5% of three oils namely; mineral oil (Diver oil 97 % EC), plant oils marjoram oil (*Origanum majorana* L.) and orange oil (*Citrus aurantium* L.), as well as two species of bacteria *Bacillus megaterium* and BTS1 (*Bacillus poylmyxa*) at two concentrations showed nematicidal activity against egg-mass and second stage juveniles of *Meloidogyne incognita* (Kofoid and White) Chitwood *in vitro* experiments. Egg hatching and juvenile mortality were significantly ($P \leq 0.05$) influenced by tested materials, concentration and exposure time. *B. megaterium* , diver oil and marjoram oil gave a higher effect, while *B. poylmyxa* was the lowest effective one. In case of juvenile mortality, *B. poylmyxa* , diver oil and marjoram oil gave the highest percentage of juvenile mortality (75.14, 73.00 and 61.85 %) after exposure to stock solutions (10%) and (53.00, 67.42 and 52.57 %) when exposed to half stock solution (5%) after seven days of treatment, respectively. Under greenhouse conditions**,** results indicated that, oxamyl, dry leaf powder of moringa (*Moringa oleifera*) and *B. megaterium* were the most effective in suppressing root galling of *M. incognita* infecting cucumber plants. Minimum number and gall size particularly ≥ 4mm was recorded in cucumber plants treated by oxamyl, moringa and *B. megaterium*. Data showed that moringa and *B. megaterium* could be used to increase crop yield of cucumber plants and for controlling root – knot nematode, *M. incognita.*

Keywords: *Meloidogyne incognita*, oxamyl, moringa, bacteria, plant oils, mineral oil, egg hatching

INTRODUCTION

Phytoparasitic nematodes are among the most difficult crop pests to control (Chitwood, 2002). Among of phytonematodes, *Meloidogyne* genus particularly, *Meloidogyne javanica*, *M. incognita*, *M. arenaria*, and *M. hapla*, are of major agronomic importance and caused least 90% of all damage caused by these nematodes (Castagnone -Sereno, 2002).

Root-knot nematodes, *M. incognita* (Kofoid and White) Chitwood (Tylenchida: Meloidogynidae) are the most damaged nematode species especially in the tropical and subtropical regions like Egypt, threatening the quality and quantity of infected crop yield and widespread throughout the world and are responsible for considerable yield losses of a wide range of cultivated crops causing an estimated $ 100 billion loss/year worldwide (Oka *et al.* , 2002). As result of wide host range, *M. incognita* may be the plant pathogen responsible for the greatest losses in food production throughout the world (Trudgill and Block, 2001). Infected plants show typical symptoms including root galling, stunting and nutrient deficiency, particularly nitrogen deficiency. In Egypt, this species is considered one of the most common important plant–parasitic nematodes which cause considerable damage to majority of economic crops especially in new reclaimed areas with sandy soils (Ibrahim, 1985).

Various ways have been used to control problems created by *Meliodogyne* species varied greatly by using different methods such as cultural practice , resistant cultivars and synthetic chemical nematicides which play the main role recommended for the control of root– knot nematodes (RKN) and targeted by 48% of globally use across crops (Coyne *et al.* , 2009). In developing countries, although chemical nematicides brought instant relative and prove to be efficient efficacy, the expansive cost of chemical control with most rural farmers not easy for them to accessible for the resource-poor farmers.

The nematicides have been applied widely to control this plant - parasitic nematodes with fast - acting and considerable results. On the other hand, these nematicides are unfriendly methods, costly and such as aldicarb residues which exceeded the reference dose in orange fruit at Sharkia Governorate, Egypt (Tchnouneou *et al.* 2002). They are very toxic, non-biodegradable and posed hazards to farmers and non-target groups (Chitwood, 2002; Oka *et al*., 2014; Bello *et al*., 2019). Also, using resistant cultivars are not available for many crops and repeated use of theses cultivars can select virulent biotypes that break resistance (Whitehead, 1988).

In the recent years, much attention had been given to the control of RKN infestation by biological agents (such as bacteria, fungi, actinomycetes and viruses) that reduce the number of disease producing activities of the pathogens (Symondson *et al*., 2002). Natural substances of certain plants which are harmless to mammals, easily obtainable, are environment friendly and can be integrated with other control procedures. Furthermore, many authors reported the nematicidal effect of orange oil (Shawky *et al.* , 2010). Plant extracts of chinaberry, datura, marigold and oleander increased biomass of infected pepper plants and decreased reproduction of *M.incognita* (Ali *et al*.,2018). Also, plant extracts of *Origanum majarana* containing some substances

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DOI:10.21608/jppp.2021.66215.1019

reduce the nematode populations. (Saravanapriya *et al.*, 2004, Shawky *et al.* , 2010).

The main view of the present study was to test eco-friendly materials belong to plant oils , mineral oil and bacteria species as compared to chemical nematicides , oxamyl on hatching of egg masses and free eggs as well as juvenile mortality (J2) of *M.incognita in vitro* to determine whether these oils and bacterial species are nematicidal or nematostatic (disorienting, immobilizing), cause inhibition of hatching or kill the eggs, reduce the invasion and check the development of nematodes after invasion.

Moreover, evaluate their effect on reproduction of *M.incognita* infecting cucumber plants under greenhouse conditions.

MATERIALS AND METHODS

Culturing of the root knot nematode, *M. incognita* and preparation of nematode inoculum for use in *in vivo* and *in vitro* tests:

Pure culture of *M. incognita*, was maintained in the greenhouse on the tomato susceptible cultivar Super Strain B for using as source of inoculum. A single egg mass was used to establish a nematode population. Species identification was based on juvenile measurements and examination of perineal pattern system of adult females according to Eisenback *et al*., (1981) . Infected tomato roots were cut into pieces of 2cm long and placed in a 600 ml flask with 200 ml of 0.5% sodium hypochlorite (180 ml water + 20 ml Clorox). The tightly capped flack was shaken for 3 minutes. The shaking partially dissolved the gelatinous matrix and thus freeing eggs from egg-masses (Hussey and Barker, 1973). The liquid suspension of eggs was poured through a 200-mesh sieve nested upon a 500-mesh sieve. Eggs collected on the 500 mesh sieve were immediately washed free of residual sodium hypochlorite solution under a slow stream of tap water and incubated in Petri dishes at 24±2°C until hatching. Newly hatched juveniles were collected by using a micropipette (El-Ashry *et al*., 2019).

Egg-masses of equal size needed to study the effect of the tested extracts on egg hatching of *M.incognita* were hand-picked with fine forceps from small galls on the infected tomato roots obtained from previously maintained pure culture. The collected egg-masses were surface sterilized in 1:500(v/v) aqueous solution of sodium hypochlorite (Clorox) for 5min (Hasseb *et al*.,2005).

Bacteria, seed oils and mineral oil used in the experiments:

The experiments were conducted with three oils, namely; mineral oil (Diver oil 97 % EC), plant oil (marjoram oil (*Origanum majorana* L.) and orange oil (*Citrus aurantium* L.)) which obtained from National Research Center, Cairo, Egypt.

As well as, two species of bacteria Bectogrow roots (*Bacillus megaterium*) obtained from SCAD Company bio-fertilizer – Egypt and BTS_1 (*Bacillus poylmyxa*) 5 L. / 200 L. obtained from El Sadat City – Egypt.

Preparing of oils and bacteria used in the experiments:

The experiments were conducted with three oils of mineral oil (diver oil), plant oils (orange oil and marjoram oil) as well as, two commercial bacteria products (*B. megaterium* and *B. poylmyxa*).

To prepare standard solutions (S), required amounts of these oils (10 ml/liter) were dissolved in a small quantity of water and 2 drops of the surfactant. Then, Tween 80 0.5% v/v were added and then volume was made up 1liter with distilled water only. With these arbitrarily standard solutions, half concentration of oils (S/2) was prepared by diluting with distilled water for different experiments. In bacteria treatments, the standard concentration of 10^{8} cfu/ ml and half standard concentration (S/2), 10^{4} cfu/ ml were used.

***In vitro* bioassay:**

Effect of bacterial spp., plant oils and mineral oil on immobility and mortality of *M. incognita* juveniles.

The assessment was carried out in a 9-cm diameter Petri dishes containing total volume of 10 ml of different tested materials and their two concentrations (S and S/2).

The experimental concentrations, S and S/2 in three tested oils and 10^{8} & 10^{4} cfu/ ml, were prepared from the stock by adding the requisite amount of distilled water (DW) including 0.1 ml of nematode suspension, carrying 100 freshly hatched juveniles. Petri dishes were covered with lids, randomized in a moist chamber and incubated at 25 ± 3 °C and numbers of emerged or dead juveniles were calculated daily using a research microscope (100 x magnifications) , but tables contained only data of 1 ,3 ,5 ,7 and 10 days after treatment. Distilled water (DW) was used as the control. Each treatment replicates five times.

About 50 juveniles were observed under a stereo dissecting microscope at 35. Then treated nematodes were washed off on a 20-mm polyethylene sieve. After washing, a 5-ml nematode suspension was transferred to a clean dish and left at 25 ± 3 °C. After 24 h the nematodes were again monitored at 35x. Nematodes were considered immobile if they failed to respond to stimulation with a bristle. Up to 10 nematodes which still remained inactive were handpicked and stained in New Blue R. to differentiate between live and dead nematodes (Shepherd, 1962).

All the nematodes which did not recover in water after 24 h were stained dark blue indicating that they were dead. Care was taken that during evaluation; there should be shallow layer of extract, so that enough oxygen should be available to nematodes. At various time intervals, the numbers of immobile nematodes in each treatment were recorded. Evaluation of egg immobility was made three times consisting of four plates per experimental unit, repeated on two occasions and analyzed independently.

***In vitro* evaluation of ovicidal and nematicidal effect of plant oils, mineral oil and bacteria species on *M.incognita*:**

A-Effect on free eggs and J2 of *M.incognita*:

The two concentrations (10^{8} &10^{4} cfu/ ml) of bacteria species (*B. megaterium* and *B. poylmyxa*) and three oils of diver oil, orange oil and marjoram oil (stock solution (S) and half stock solution (S/2)] were evaluated *in vitro* by adding 10 ml from each of them in Petri dishes (5 cm diameter). While control treatment supplanted by 10 ml of distilled water only. *In vitro* tests of evaluation, 0.1 ml containing of 200 free eggs of *Meloidogyne incognita* or 100 infective juveniles (J2) were added to each Petri dish to test efficacy of bacterial species and oils as ovicidal or larvicidal. All treatments were incubated at 25± 3º C and numbers of emerged or dead juveniles were calculated daily using a research microscope (100 x magnifications) , but tables contained only data of 1 ,3 ,5 ,7 and 10 days after

treatment. Percentage of hatching inhibition or dead J_2 in comparison with negative treatment (control) was calculated according to according to Abbott (1925) formula:

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

$$\text{Mortality (\%)} = (\text{No. of dead juveniles/Total number of juveniles}) \times 100$$

B-Effect on *M. incognita* egg-masses:

A healthy and uniform five egg- masses of *M. incognita* were added to 10 ml of prepared concentrations of three tested oils and two bacteria species.

To evaluate the ovicidal and larvicidal of tested oils and bacteria, 10 cm diameter of Petri dishes was provided with 10 ml of prepared concentrations of bacteria and botanical oils were added to each petri dish. Petri dishes in control treatment contained only distilled water. The same protocol as in free eggs or J2 was used to calculate daily number of J2 emerged from treated egg- masses by incubating dishes at 25 ±3º C by using a research microscope (100 X magnifications) and table contained data of 1 ,3 ,5 , 7 and 10 days after treatment. Moreover, the following equation was used to calculated percentages of hatching inhibition.

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

Greenhouse Experiments:

Seeds plants, *Cucumis sativus* L. cv.012 seedlings were sown in 15 cm diameter plastic pots filled with 1500 g mixture of sterilized sandy soil (77.5% sand , 11.5% clay , 7.1% silt , 65 g peat moss , and 3 mg urea fertilizer per kg of soil). After four weeks of sowing, seedlings were thinned to three plants per pot. Inoculum of *M. incognita* were adjusted to 1000 J2/ml and one ml contains 1000 IJs of newly hatched juveniles were used to each cucumber plant. IJs were poured into three holes around root system of each plant and directly covered with moist soil after inoculation.

Effectiveness of bacteria, oils and dry leaf powder of moringa and water hyacinth on plant growth of infested cucumber plants:

The greenhouse experiments were conducted in Plant Protection Department, Faculty of Agriculture , Zagazig University , Egypt on the cucumber plants , *Cucumis sativus* L. cv. 012 as a sensitive host plants.

The investigated treatments included: untreated plants without J2 infection (as negative control), infected plants with J2 (as positive control) as well as, treatments received 10 g of grinded moringa and water hyacinth. 25 ml stock solution of diver oil , orange oil and marjoram oil /plant ; 25 ml of standard solution (10^8 cfu/ ml) of two bacteria species compared with treatment of 10 ml volume of, oxamyl nematicide (0.2 ml formulation/plant) diluted in 10 ml water and poured near the stem.

Ten grams of dry leaf powder of moringa and water hyacinth were incorporated with the upper 5 cm of soil around each plant as described by (Refaat *et al.*, 2020).

All plants were inoculated with 1000 newly hatched infective juveniles of *M. incognita*. 25 ml of prepared stock solution of tested oils and two tested bacteria species and 10 ml volume of oxamyl (0.2 ml formulation/plant) was applied to cucumber seedlings. Plants of positive control treatment were inoculated with *M. incognita* alone besides healthy plant (without nematode inoculum or other tested materials). Each treatment was replicated five times. All pots were arranged in a randomized complete design in the greenhouse at 27±3ºC. , and received similar horticultural treatments.

The cucumber growth responses and RKN pathogenesis-related parameters were investigated after 60 days. Plant growth parameters were shoot and root fresh weight and leaves number/plant were assessed. Whereas, the nematode parameters included number of galls, egg masses, and IJs /250 g soil. Also, root gall and egg mass indices (RGI and EI) and reproduction factor (RF) were evaluated.

Reproduction of RKN as (RF) the equation of [RF $=P_{intial}/ P_{final}$] was estimated in 100 g soil and nematode population included (number of J2 in soil) by using a combination of sieving and Baermann trays extraction technique (Hooper, 1990). Gall diameter intervals were assessed according to (El -Ashry *et al.*, 2020) whereas root-knot index (RKNI) was evaluated using the scale proposed by (Tylor and Sasser, 1978). To prevent all uprooted cucumber plants from drying, tissue papers were used to cover plants until the end of recording the various measurements. The parameters changing (%), increase or reduction assigned to negative or positive control values, and the current equations were used.

$$\text{Reduction (\%)} = ((\text{Control} - \text{Treated})/\text{Control}) \times 100$$

and $$\text{Increase(\%)} = ((\text{Treated} - \text{Control})/\text{Control}) \times 100$$

Moreover, a parallel experiment was conducted to evaluate effectiveness of the three botanical oils and two bacteria species application on the development of *M. incognita* population when a single egg mass was placed into amended soil prior to planting of cucumber. Greenhouse temperature was 25±3°C and all plants received similar horticultural treatments. The following protocol was used to estimate development of *M. incognita* population as follows:

Soil was amended with 40 ml of standard concentration of three botanical oils and 40 ml (10^8 cfu/ ml) of two bacteria species as described before. Sterilized plastic pots with formalin solution (5%) filled with 1500 g steam-sterilized soil (2:1 v/v sandy soil: clay soil) and kept at water-saturated capillary mat. Three days after treatment of tested materials, a single egg mass of *M. incognita* was placed in the center of each pot. Soil without amendment served as control treatment. Each treatment was replicated five times. After one week, 25-day-old cucumber plants were transplanted individually into treated pots and then each pot was placed in a plastic plate to avoid cross contamination. After two months of cucumber planting, plants were gently removed from pots and soaked in distilled water for one hour. The roots were stained in phloxin B and total root gall numbers and egg masses index (RGI and EI) were calculated (Bridge and Page, 1980).

Statistical analysis

Data were subjected to oneway analysis of variance (ANOVA) using CoStat V 6.451. Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability

RESULTS AND DISCUSSION

Effect of tested eco-friendly materials on **hatching of M. incognita free eggs:**

Tested half stock solution of *B. megaterium*, *B. poylmyxa* and diver oil significantly inhibited hatching ($P \leq 0{:}05$) from the control, whereas orange oil had a small stimulating effect

on hatch (Fig. 2). By increasing concentration of tested materials, hatching was inhibited accordingly (Fig. 2).

When egg masses were replaced into water, hatching resumed (Fig. 1a&2a). Number of emerged J2 increased by increased of time exposure intervals in control treatment to reach the maximum number (1071.6) of *M.incognita* J2 after 10 days of treatment . Percent of egg immobility was increased by time of exposure and tested concentrations (Fig. 1b&2b) and s concentration was more effective than S/2 with all tested materials. On the other hand, percentages of dead eggs reached to 25.6 , 19.9 and 16.8 % after 5 days of exposure to S concentration in Petri dishes treated with of *B. megaterium*, *B. poylmyxa* and diver oil, respectively. The parallel values with S/2 concentration were 21.36, 17.39 and 13.28% in Petri dishes treated with *B. megaterium*, *B. poylmyxa* and diver oil, respectively.

After 7 and 10 days, the hatching increased significantly ($P \leq 0{:}05$) even for egg masses which were exposed to higher concentration of *B. megaterium* (39.6%), *B. poylmyxa* (31.70 %)and diver oil (25.80%) whereas, orange oil (20.40%) was the least effective one after exposure to s concentration (Fig.1 c). percentages of dead eggs in Petri dishes treated with S/2 concentration were 34.25 , 28.60 , 22.40 and 18.80 % for *B. megaterium*, *B. poylmyxa* , diver oil and orange oil, respectively. On the other hand, hatched juveniles within tested bacteria or botanical oils remained inactive for some time depending on the concentration and then revived even in the presence of tested eco-friendly materials or when transferred to fresh distilled water. A completely survive juveniles was obtained when put in water.

Ovicidal activity of tested bacteria and seed oils on hatching of* M. incognita *egg masses:

The egg hatching of *M. incognita* was significantly ($P \leq 0.05$) inhibited by the exposure of egg – masses to the tested concentrations (10 ٪ and 5 ٪) used to judge the ovicidal effect of the tested materials (Table 1). The inhibitory effect varied according to material type, concentration and exposure time. One day after exposure, percentages of egg hatching inhibitory were 88.12, 83.11, 84.69, 19.78 and 39.31% with *B. megaterium* , *B. poylmyxa* , diver oil , orange and marjoram oil, respectively. After 3 and 5 days, the highest ovicidal effect was detected with marjoram oil followed by *Bacillus poylmyxa* , *B. megaterium* and Diver oil, at the tested stock solution recording 89.63&91.93, 84.14&87.96 and 86.58&89.16 9.00% respectively. After 7 and 10 days of treatment, the inhibition effect of the tested material was expanded to reach a relatively higher values at stock solution (10%) i.e., 88.15 &78.10 , 84.78&71.10 , 88.53& 77.11 , 55.20 &35.19 and 50.51 &51.43% with *B. megaterium*, *B. poylmyxa* , , diver, orange and marjoram oil, consequently. Generally, the rate of egg hatching inhibition was inversely proportion to the dilution of the tested materials. As the dilution decreased to half (5%), the same trend was found at the tested materials. The distilled water (control) gave significantly ($P \leq 0.05$) highest number of eggs that hatched compared to other treatments recording 75.80 , 295.20 , 734.40 , 938.40 and 1071.6 after 1, 3,5, 7 and 10 days, respectively.

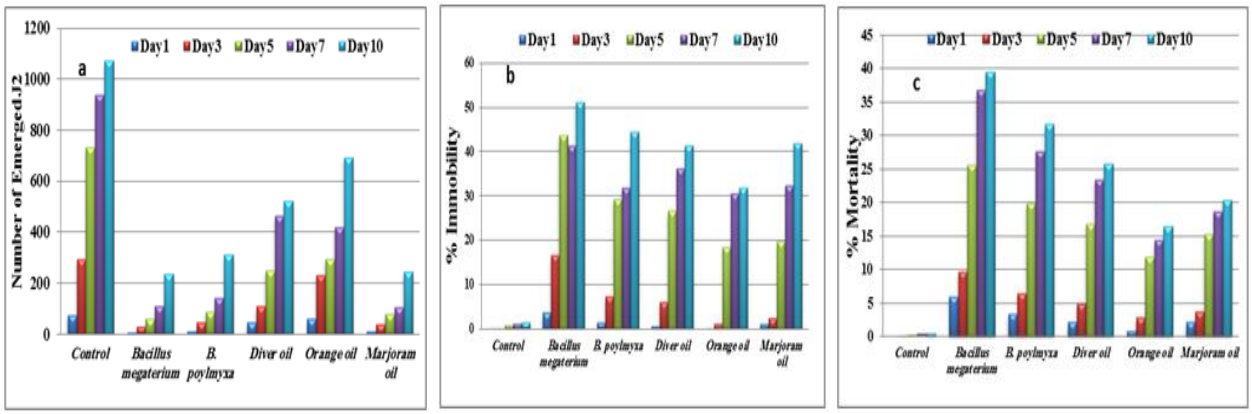

Fig. 1. The exposure effect of different tested materials at recommended concentration (S) on number of emerged (a), immobility (b) and mortality (c) of *M.incognita.*J2 in free eggs.

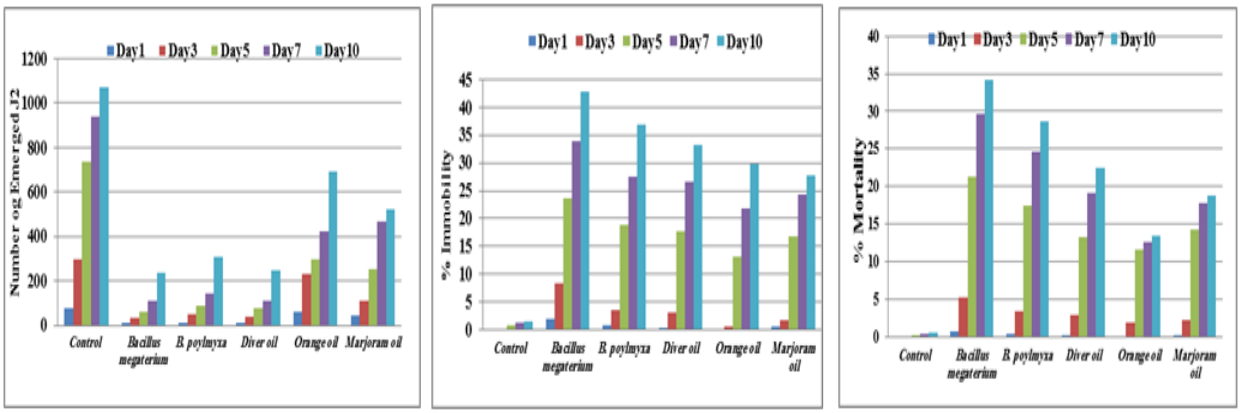

Fig. 2. The exposure effect of different tested materials at half recommended concentration (S/2) on number of emerged (a) , immobility (b) and mortality (c) of *M.incognita.*J2 in egg masses.

Table 1. Eggs hatching inhibition percentages of *M. incognita* after exposure to stock and half-stock solution of tested bacteria species and oils on eggs *in vitro*.

Concentration (v/v)	Exposure time (Day)	Distilled water (DW)	*Bacillus megaterium*	*B. poylmyxa*	Diver oil	Orange oil	Marjoram oil
Stock solution	1	75.80a	9.00 d (88.12)	12.80 d (83.11)	11.60 d (84.69)	60.80 b (19.78)	46.00 c (39.31)
	3	295.20 a	30.60 d (89.63)	46.80 d (84.14)	39.60 d (86.58)	231.60 b (21.54)	109.80 c (62.80)
	5	734.40 a	59.20 e (91.93)	88.40 d (87.96)	79.60 de (89.16)	295.60 b (59.74)	250.60 c (65.87)
	7	938.40 a	111.20 e (88.15)	142.80 d (84.78)	107.60 e (88.53)	420.40 c (55.20)	464.40 b (50.51)
	10	1071.6 a	234.60 e (78.10)	309.60 d (71.10)	245.20 e (77.11)	694.40 b (35.19)	520.40 c (51.43)
Half-Stock solution	1	75.80 a	27.20 e (64.11)	39.20 d (48.28)	33.60 de (55.67)	61.60 b (18.73)	54.00 c (28.75)
	3	295.20 a	100.80 d (65.85)	190.00 c (35.63)	106.40 d (63.95)	231.20 b (21.68)	263.80 ab (10.63)
	5	734.40 a	231.20 d (68.51)	353.60 c (51.85)	238.80 d (67.48)	449.20 b (38.83)	373.80 c (49.10)
	7	938.40 a	309.20 c (67.05)	571.20 b (39.13)	326.00 c (65.26)	583.20 b (37.85)	564.40 b (18.96)
	10	1071.6 a	568.00 e (46.99)	664.40 d (37.99)	617.20 de (42.40)	856.00 b (20.11)	760.40 c (29.04)

****Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water according to the following formula:**

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

*****Different letters in the same row indicate significant differences ($P \leq 0.05$) according to Duncan`s multiple range test.**

Larvicidal Activity of tested materials on second stage juveniles of *M. incognita in vitro*:

Likewise, the tested materials were found to be significantly ($P \leq 0.05$) effective against juveniles of *M. incognita*. Among which *B. megaterium* and diver were the most effective materials followed by marjoram oil then orange oil then, while *B. poylmyxa* was the lowest toxic one. As exposure time increased from 1 to 10 days after treatment, larvicidal effect of the tested materials was increased at both tested concentrations (Table 2). The highest value of juvenile mortality was detected with Diver oil at stock solution after 10 days of exposure (89.85 %) followed by *B. megaterium* (89.57%), marjoram oil (84.14%), orange oil (37.85%), while *B.polmyxa* (25.85%) was the lowest toxic one at the same concentration and exposure time. The respective values for these tested materials at half stock solution (5%) after the same period were 84.42 %, 72.85 % 69.28 %, 33.28% and 3.71% , with diver oil, marjoram oil, *B. megaterium*, orang oil and *B. poylmyxa*, consequently. Generally, the nematicidal effect of the tested materials on *M. incognita* juveniles were directly proportioned to concentration and exposure time. As the concentration and exposure time increased, percentage mortality was increased also. After 7 days , standard dilution of *B.megaterium* , diver oil , marjoram oil, orange oil and *B. poylmyxa* caused 75.14 % , 73.00 % , 61.85 % , 26.14 % and 23.00 % , while half stock solution (5%) after the same period gave 67.42 % , 53.00 % , 52.57 % , 19.57 % and 19.82 % in case of *B.megaterium* , diver oil , marjoram oil, orange oil and *B. poylmyxa*, respectively.

Table 2. Mortality percentage of *M. incognita* juveniles exposed to stock and half solution of tested material at different exposure periods *in vitro*.

Concentration (v/v)	Exposure time (Day)	Control	*Bacillus megaterium*	*B. poylmyxa*	Diver oil	Orange oil	Marjoram oil
Stock solution	1	0.14 f	26.57 a	2.85 e	24.71 b	11.42 d	20.00 c
	3	2.57 e	84.14 a	10.42 e	84.14 a	34.42 c	73.42 b
	5	1.14 f	55.00 a	20.28 e	47.42 b	19.57 d	41.00 c
	7	1.85 f	75.14 a	23.00 d	73.00 b	26.14 d	61.85 c
	10	3.71 e	89.57 a	25.85 d	89.85 a	37.85 c	84.14 b
Half-Stock solution	1	0.14 f	16.42 a	1.71 d	16.00 a	4.28 c	11.85 b
	3	2.57 e	62.42 b	7.14 d	75.85 a	26.14 c	62.57 b
	5	1.14 f	40.42 a	16.57 d	38.85a	10.57 c	33.00 b
	7	1.85 f	53.00 b	19.85 d	67.42 a	19.57 c	52.57 b
	10	3.71 e	69.28 c	21.85 e	84.42a	33.28 d	72.85 b

*****Different letters in the same row indicate significant differences ($P \leq 0.05$) according to Duncan`s multiple range test.**

Effect of bacteria, oils and dry leaf powder of moringa and water hyacinth on plant growth of infested cucumber plants

The obtained results presented in Table (3) revealed that the effect of curative treatments of abovementioned materials on growth of cucumber plants was indicated by fresh root weight and shoot fresh weight , it was clear that all tested treatments ameliorated growth of the sponge gourd to certain extend as compared to plants inoculated with *M. incognita* alone. Generally *B.megaterium* , Diver oil , orange oil , marjoram oil , moringa and *B. poylmyxa* Surpassed water hyacinth in improving plant growth parameters percent increase in root fresh weight and shoot fresh weight in treatments of *B.megaterium* , Diver , orange , marjoram , moringa , *B. poylmyxa* And water hyacinth were 16.91 (25.36) , 7.30 (19.12) , 5.63 (13.38) , 1.04 (7.92) , 40.08 (7.10) , 4.80 (9.10) , 1.25 (2.55) % , respectively. The parallel values in oxamyl treatment were 37.16 and 43.26 %, respectively. For number of leaves /plant, it was clear that of the eight tested materials oxamyl significantly improved number of leaves/plant (76.92) compared to control (48.00). Regarding to nematode parameters, results in Table (3) showed that oxamyl significantly had high effect which recorded 78.82 % reduction in root galling followed by moringa (63.21%) and *B.megaterium* (41.40%). The least percentage reduction of root galling was recorded with water hyacinth treated plants (10.19%).

Percentages of reduction in egg – masses could be arranged in ascending order as follows : water hyacinth (17.23%) , orange (25.54%) , *B. poylmyxa* (27.28%) , marjoram (30.67%) , Diver (30.93%) , *B.megaterium* (46.08%) , moringa (46.86%) and oxamyl (74.93%). Also , number of galls (diameter ≥ 4mm) significantly decreased to reach 17.00 , 21.00 , 24.80 , 26.40, 29.40 , 30.20 and 33.20 with moringa , *B.megaterium* , Diver, orange , marjoram , *B. poylmyxa* and water hyacinth , respectively. However no galls (≥ 4mm) were recorded with oxamyl. Maximum percentages of reduction in soil juvenile number per 100 g soil were recoded when plants treated with oxamyl (79.47%) followed by moringa (46.82%) and *B.megaterium* (36.84%).

Table 3. Changes in Plant parameters, nematode parameters and soil parameters of cucumber **Plants, *Cucumis sativus* L. cv. 012 infected with J_2 of *Meloidogyne incognita* after treatment with bacteria, botanical oils, dry leaf powder of moringa and water hyacinth in comparison with oxamyl under greenhouse conditions.**

Treatments	Plant parameters			Nematode parameters			Soil parameters
	Fresh root weight (% Increase)	Fresh shoot weight (% Increase)	Number of leaves/ plant	No. of galls (% Decrease)	No. gall ≥4 mm	No. egg masses (% Decrease)	No. IJs /100 g soil (% Decrease)
untreated plants	7.36 a (34.91)	16.79 a (34.60)	15.00 a (48.00)	0.00 h	0.00 f	0.00 f	0.00 g
Positive control infected with RNK *M. incognita*	4.79 e	10.98 f	7.80 g	125.60 a	34.80 a	153.20 a	418.00 a
M.incognita + *B. megaterium*	5.60 c (16.91)	13.34 c (25.36)	11.80 c (51.28)	73.60 f (41.40)	21.00 d	82.60 d (46.08)	264.00 d (36.84)
M.incognita + *B. poylmyxa*	5.02 de (4.80)	11.98 e (9.10)	9.00 e (15.38)	98.00 cd (21.97)	30.20 abc	111.40 b (27.28)	315.40 b (24.54)
M.incognita + Diver oil	5.14 d (7.30)	13.09 cd (19.21)	10.60 d (35.89)	104.20 d (17.03)	24.80 c	105.80 c (30.93)	308.00 c (26.31)
M.incognita + Orange oil	5.06 de (5.63)	12.45 d (13.38)	9.40 e (20.51)	110.40 bc (12.10)	26.40 cd	114.20 b (25.54)	324.80 b (22.29)
M.incognita + Marjoram oil	4.84 de (1.04)	11.85ef (7.92)	8.60 ef (10.25)	108.60 bc (13.53)	29.40 bc	106.20 ab (30.67)	337.80 b (19.16)
M.incognita + Moringa	6.57 b (37.16)	11.76 ef (7.10)	13.20 b (69.23)	46.20 e (63.21)	17.00 e	81.40 d (46.86)	222.20 e (46.84)
M.incognita + Water hyacinth	4.85 de (1.25)	11.26ef (2.55)	8.20 f (5.12)	112.80 b (10.19)	33.20 ab	126.80 b (17.23)	311.80 b (25.40)
M.incognita + Oxamyl	6.71 ab (40.08)	15.73 b (43.26)	13.80 b (76.92)	26.60 g (78.82)	0.00 f	38.40 e (74.93)	85.80 f (79.47)

The same letter (s) in columns indicates no significant differences at $P \leq 0.05$ according to Duncan's multiple range test.

$$\text{Reduction } (\%) = \frac{\text{Control - Treated}}{\text{Control}} \times 100$$

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

The obtained results revealed that all treatments significantly ($p \leq 0.05$) reduced galling (as indicated by number of galls) and reproduction (as indicated by number of egg – masses on roots and number of juveniles in soil) compared to plants inoculated with *M. incognita* alone. Data showed that oxamyl proved to be the most suppressive on root knot nematode , *M. incognita* infesting cucumber which recorded (26.60) followed by moringa (46.20) then *B.megaterium* (73.60). Gall ratings were lower for chemical pesticide (oxamyl), *B.megaterium*, moringa, *B. poylmyxa* with root galling 3.0, 4.0, 4.0 , and 4.0 , respectively (Table 4).Concerning egg masses index , data revealed that , oxamyl of all tested materials gave good results as compared to other tested materials recording the lowest value (3.0). Maximum percentage of reduction in soil juvenile number per 100 g soil was recorded when plants treated with oxamyl (79.47%) and therefore the lowest reproduction factor (0.686) was detected with oxamyl.

Table 4. Efficacy of bacterial species, **oils, dry leaf powder of moringa and water hyacinth in comparison with oxamyl on galling and reproduction of *M.incognita* infecting** cucumber plants**, *C. sativus* under greenhouse conditions.**

Treatments	Root parameters			Soil and root parameters	
	No. of galls	Root Gall Index	Egg masses Index	Nematode population density (J_2) 100g Soil /plant/Pot	
		EI	RGI	Final population (% Reduction)	Reproduction factor (RF=Pf/Pi)
Positive control infected with RNK *M. incognita*	125.60 a	5	5	418.00 a	3.344
M.incognita + *B. megaterium*	73.60 f	4	4	264.00 d (36.84)	1.698
M.incognita + *B. poylmyxa*	98.00 cd	4	5	315.40 b (24.45)	2.523
M.incognita + Diver oil	104.20 d	5	5	308.00 c (26.31)	1.630
M.incognita + Orange oil	110.40 bc	5	5	324.80 b (22.29)	2.598
M.incognita + Marjoram oil	108.60 bc	5	5	337.80 b (19.18)	2.702
M.incognita + *Moringa*	46.20 e	4	4	222.20 e (46.84)	1.778
M.incognita + Water hyacinth	112.80 b	5	5	311.80 b (25.40)	2.494
M.incognita + Oxamyl	26.60 g	3	3	85.80 f (79.47)	0.686

****The same letter (s) in columns indicates no significant differences at $P \leq 0.05$ according to Duncan's multiple range test.**

$$\text{Reduction} \quad (\%) = \frac{\text{Control} \quad - \quad \text{Treated}}{\text{Control}} \times 100$$

Number of galls and reproduction of *M.incognita* on cucumber plants infected with eggs treated with stock concentration of bacteria species and botanical oils for one week showed moderate effect on ability of emerged juveniles on invasion of cucumber roots (Table 5). Results showed gradually decrease in number of galls and egg- masses between pots infected by fresh extracted eggs and other pots infected with eggs treated for 7 days by bacteria and botanical oils. Number of galls and egg-masses were 120.60 &135.8 , 71.40 & 98.40 , 92.20 &106.0 , 85.60 & 98.40 , 105.40 & 121.80 and 77.50 & 81.60 in treatments infected with fresh extracted eggs *M.incognita* and other eggs treated with *B. megaterium, B. poylmyxa* , diver oil, orange oil and marjoram oil ,respectively. *B. megaterium,* diver oil and marjoram oil were the most effective than orange oil and *B. poylmyxa.* RF factors reduced as compared to plants infected with fresh extracted eggs of *M.incognita* . Moreover, *B. megaterium* and marjoram oil were the most effective with RF 3.66 and 3.84, respectively (Table 5).

Table 5. Galling and reproduction of *M.incognita* on cucumber plants infected with eggs treated with stock concentration of bacteria species and botanical oils for one week.

Treatments	*M. incognita* galls and reproduction:				
	No. of galls	No. of Egg-masses	RGI	Nematode population density (J_2) 100g Soil /plant/Pot	Reproduction factor (RF=Pf/Pi)
Cucumber plants infected with fresh extracted eggs of RNK *M. incognita*	120.60 a	135.8 a	5	395.40 a	5.93
Eggs treated with stock concentration (S) for 7 days					
M.incognita + *B. megaterium*	71.40 de	98.4 d	4	244.00 d	3.66
M.incognita + *B. poylmyxa*	92..20 bc	106.6 c	5	305.40 b	4.58
M.incognita + Diver oil	85.60 d	98.4 d	4	285.00 c	4.27
M.incognita + Orange oil	105.40 b	121.8 ad	5	304.80 b	4.57
M.incognita + Marjoram oil	77.50de	81.6 de	4	256.00 d	3.84

***Each value is a mean of five replicates.**

The same letter (s) in columns indicates no significant differences at $P \leq 0.05$ according to Duncan's multiple range test.

Discussion

Botanical oils have nematicidal properties on eggs and J2 of RKN particularly *M.incognita* as a result of egg hatching inhibition and suppression of nematode population (Ali *et al*., 2018) Many factors affect on toxicity of botanical extractssuch as solvents used in extracts may cause phytotoxicity. As showed by (Al-Saba *et al*., 2001 and Babaali *et al*., 2017), variation between solvent extraction and nematicidal effect determined by with different plant extracts.

As result of alteration in population of other microorganisms in treated soils , nematicidal effect of some compounds have ability to effect on survival of nematode eggs and J2 mortality (Khan *et al*.,2008). For example, plant extracts derived from *Brassica napus, Lantana camara, Tagetes erecta* and *Azadirachta indica* inhibited eggs hatching of RKN (*Meloidogyne incognita*), leading to immobilization and later death of second stage juveniles. Under greenhouse conditions, application of *Lantana camara* and *Trichoderma harzianum* decreased the incidence and reproduction of root knot nematodes, their populations, egg masses and the number of galls in tomato(Singh and Siddiqui, 2010).

The active compounds in the plant extracts also affect root knot nematodes through paralysis, reducing infectivity potential and death of the infective juveniles (Oka, *et al.*, 2012). Plant extracts and essential oils have also been reported to cause mortality for J2 of RKN (Singh *et al*., 2001).

Some of the phytochemicals (Total alkaloids, flavonoids, phenolic, saponins and tannins) were found in botanical extracts are lipophilic enabling them to easily dissolve the cytoplasmic membrane of nematodes thereby interfering with protein structures responsible for growth, development and survival (Pavaraj *et al*.,2012).

As well as, The inhibition of egg hatching, motility and mortality of second stage juveniles of root knot nematodes is attributed to presence of phytochemicals such as alkaloids, tannins and glycosides in plants (Akyazi, 2014 and Asif *et al*., 2017).

Moreover, under field conditions, Ahmad *et al.*, 1991 mentioned to vary in sensitivity of phytonematode species to the same plant extract as shown in *M. incognita* and *H. indicus* compared to *Tylenchorhynchus vulgaris* populations to growing *Tagetes erecta* and *T. patula.* Some botanical extracts inhibited egg hatchability of *M. incognita* such as *Melia azedarach* leaf extracts inhibited which delay embryonic development and killed J2 (Lee,1987). More factors effect on toxicity against eggs and J2 of RKN. High concentration, plant species and exposure time (Ramakrishnan *et al*., 1999; Devi, 2008; Chaudhary *et al*.,2013; Ganaie and Khan, 2016) were the most effective in egg hatchability and causing a reduced effect/mortality of *M. incognita* juveniles (Sharma, 1996).

Use commercial *Bacillus* spp. against RKN, *M.incognita* as biological control tool have various advantages, particularly *Bacillus* spp. able to survive for extended periods under negative conditions (Singh and Siddiqui, 2010). Various mechanisms employed by rhizobacteria to reduce nematode damage and reproduction in plants have been suggested and include regulating nematode behaviour, interfering with nematode-host recognition, competition for nutrients, plant growth promotion, induced systemic resistance (Ongena and Jacques, 2008; Siahpoush *et al*., 2011; Adam *et al*., 2014), and production of exudates that inhibit egg hatching, reduce juvenile survival and/or kill nematodes directly (Lian *et al*., 2007; Peng *et al*., 2011; Zhang *et al*., 2012; Oliveira *et al*., 2014).

Nevertheless, utilization of bacterial under field conditions performed rarely (El-Ashry *et al*.,2020) because of costs (reduces profit margins) especially for low value field crops (Sikora and Pocasangre, 2008), variations in product distribution, applied directly to the root zone of treated plants to manage phytonematodes mainly with root knot nematodes.

Numerous bacterial isolates, among them *Pseudomonas* spp. have been found to have nematicidal properties against root knot nematodes (Siddiqui *et al*., 2009; Timper *et al*., 2009; Singh and Siddiqui, 2010; Bagheri *et*

al., 2014). A number of *Bacillus* isolates have also been screened and found to have nematicidal properties against *M. javanica in vitro* (Ashoub and Amara, 2010) and *in vivo* (Wei *et al*., 2014; Xiong *et al*., 2015).

Adam *et al.* (2014) found that *Bacillus subtilis* isolates repelled root knot juveniles and also induced systemic resistance in tomato plants, thereby reducing nematode reproduction. Also, suppress plant diseases in addition to root knot nematodes and hence provide new possibilities for plant disease management. In concerning of *Bacillus megaterium*, Padgham and Sikora (2007) assessed the ability of mentioned bacteria species to reduce J2 penetration and migration of *M. graminicola* to the rice root zone. El-Ashry *et al*., 2020 assessed the ability of rhizobacteria to manage RKN, *M.incognita* as eco-friendly tool under Egyptian field conditions. Alijani *et al*,2015 and Babaali *et al*.,2017)

Bacillus isolates have been found to produce proteolytic enzymes, which are responsible for nematode mortality (Chantawannakul *et al*., 2002; Tian *et al*., 2007; Mohammed *et al*., 2008). *Bacillus* isolates ideal biocontrol agents for RKN management (Chinheya *et al*., 2017).

Incorporation of leaf powder of the tested botanicals into the soil significantly suppressed galling and reproduction of *M.incognita* and consequently enhanced the growth of cucumber plants. Pots amended with 10g leaf powder of the moringa gained better growth of cucumber in than water hyacinth appears to be due to suppression of galling and reproductions of *M.incognita*. Moreover, leaf powders of moringa and water hyacinth have effect as manural effect and increase the tolerance and resistance of cucumber plants against *M.incognita*. This result is in agreement with finding of Elsaedy *et al*., (2015) ; Mostafa *et al*., (2017) and Refaat *et al*.,2020.

CONCLUSION

From results of current paper, effectiveness of botanical oils or grinded powders and bacteria depends on the plant species, methods of extract and plant parts while, efficacy of bacteria depends on used species, strains, and isolates. To ensure their efficiency after application, mixtures of eco-friendly agents (bacteria and plant extracts) must be used in organic farms or with medicinal and aromatic plants. They can be used as soil treatments against *M.incognita* which will delay the invasion of cucumber roots by southern RKN and delay their development. Moreover, acceptable control was obtained from eco-friendly bioagents against RKN *in vitro* and greenhouse conditions when compared with standard chemical nematicde, oxamyl.

REFERENCES

Abbott, W. S.(1925). A method of computing the effectiveness of an insecticide. J. of Economic Entomol. 18: 265-267

Adam, M.; Heuer, H.and Hallmann, J. (2014). Bacterial antagonists of fungal pathogens also control root-knot nematodes by induced systemic resistance of tomato plants. PLoS ONE 9, 1–8.

Ahmad, R.;Tariq, M.;Javed, N. and Inam-ul-Haq, M.(1991). Effect of extract of various plants on egg hatching and larval mortality of root-knot nematode, *Meloidogyne incognita*. Journal of Phytopathology,38–42.

Akyazi, F.(2014).Effect of some plant methanol extracts on egg hatching and juvenile mortality of root-knot nematode *Meloidogyne incognita*, Am. J. Exp. Agric.,11:1471–1479.

Ali, A. A.I.; El-Ashry, R. M. and Ramadan, M. M. (2018). Phytochemical Analysis of Some Aqueous Leaf Extracts and Their Nematicidal Activity Against *Meloidogyne incognita* on Pepper. Egypt. Acad. J. Biolog. Sci., 10(2): 133– 151. https://doi.org/10.21608/eajbsf.2018.123512.

Alijani, Z.; Olia, M.; Sharifnabi, B. and Jaimand, K. (2015). Effect of different inoculum densities of root-knot nematode, *Meloidogyne javanica* on progress of disease and production of secondary compounds in pot marigold, *Calendula officinalis*. Journal of Plant Pathology,51:215-228.

Al-Saba, R.F.; Ammi, S.N. and Al-Zarry, A.J.B.(2001). Effect of *Datura stramonium* extracts on root-knot nematodes *M. javanica* in tomato plant (research note). [Arabic]. Dirasat: *Agricultural Sciences*, 28, 219–226.

Asif, M.;Tariq, M.; Khan,A. and Siddiqui, M.A. (2017).Biocidal and antinemic properties of aqueous extracts of *Ageratum* and *Coccinia* against root-knot nematode, *Meloidogyne incognita in vitro*, J. Agric. Sci.,2:108–122.

Bagheri, N.;Ahmadzadeh, M.and Heydari, R.(2014). Effects of *Pseudomonas fluorescens* strain UTPF5 on the mobility, mortality and hatching of root-knot nematode *Meloidogyne javanica*. Arch. Phyto. Plant Prot. 47, 744–752.

Babaali, D.; Roeb, J.; Hammache, M. and Hallmann, J. (2017). Nematicidal potential of aqueous and ethanol extracts gained from *Datura stramonium*, *D. innoxia* and *D. tatula* on *Meloidogyne incognita*. Journal of Plant Diseases and Protection, 124, 339–348.

Bello, Oluwasesan M.; Ogbesejana, Abiodun B.; Adetunji, Charles Oluwaseun; Oguntoye, Stephen O. (2019). Flavonoids isolated from *Vitex grandifolia*: an underutilized vegetable, exert Monoamine A & B inhibitory and anti-inflammatory effects and structure activity relationship (SAR). Turk. J. Pharm. Sci., 16 (4): 437–443.

Bridge, J. and Page, S.L.J. (1980). Estimation of root-knot nematode infestation levels on roots using a rating chart. Trop. Pest Manage, 26:296–298.

Castagnone-Sereno, P. (2002). Genetic variability in parthenogenetic rootknot nematodes, *Meloidogyne* spp., and their ability to overcome plant resistance genes. Nematologica, 4:605–608.

Chaudhary, k.k. and R.K. Kaul(2013). Efficacy of *Pasteuria penetrans* and various oil seed cakes in management of *Meloidogyne incognita* in chilli pepper (*Capsicum annuum* L.). J. Agric. Sci. and Technol. , 15 (3) : 617 – 626.

Chantawannakul, P.;Oncharoen, A.; Klanbut, K.; Chukeatirote, E. and Lumyong, S. (2002). Characterization of proteases of Bacillus subtilis Strain 38 isolated from traditionally fermented soybean in Northern Thailand. Sci. Asia, 28: 241–245.

Chinheya , C. C.; Kwasi S. Yobo and Mark D. Laing (2017). Biological control of the root knot nematode, *Meloidogyne javanica* (Chitwood) using *Bacillus* isolates, on soybean. Biological Control ,109 : 37-41.

Chitwood, D.J. (2002). Phytochemical based strategies for nematode control. Annu. Rev. Phytopatol., 40: 221–249.

Coyne, D.L., H.H.Fourie and M.Moens(2009). Current and future management strategies in resource-poor farming. In : Root- knot nematodes , Eds : Perry R.N. , Moens M. , Starr J.L.Wallingford, UK:CAB Int. 444 - 475.

Devi, L.J.(2008). Evaluation of chloroform methanol extracts of different medicinal plants on egg hatching and larval mortality of *Meloidogyne incognita*. Journal of Nematology, 38:168–171.

Eisenback, J. D.; Hirschmann, H.; Sasser,J.N. and Triantaphyllou, A.S. (1981). A guide to the four most common species of root-knot nematodes (*Meloidogyne* species), with a pictorial key. Raleigh, North Carolina State University and U.S. Agency for International Development , 48pp.

El-Ashry, R.M.;Ali, A.A.I.and Awad, S.E.(2020). Enhancing application Efficiency of *Pseudomonas* SPP. and *Serratia marcescens* isolates against *Meloidogyne incognita* in tomato plants. Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control 12,127–145. https://doi.org/10.21608/eajbsf.2020.116752.

El-Ashry, R. M.; Ali, A. A. I. and Elsobki, A. E. A. M. (2019). Nematicidal properties of three adjuvants for management of southern root-knot nematode, *Meloidogyne incognita in vitro* and under greenhouse conditions. Journal of Plant Protection and Pathology, 10 (11):511-519.

El-Saedy, M. A. M.; El-Sayed, M. E. A. and Hammad, S. E. (2015). Efficacy of boron, silicon, jojoba and four bio-products on controlling *Meloidogyne incognita* infecting Thompson seedless grapevines. American Eurasian Journal Agricultural & Environmental Science, 15(9): 1710-1720.

Ganaie, M.A. and Khan, T.A.(2016). In vitro studies of some root extracts on egg hatching and larval mortality of *Meloidogyne incognita*. Trends in Biosciences,9:578–583.

Haseeb A.; Sharma A. and Shukla P.K.(2005).Studies on the management of root-knot nematode, *Meloidogyne incognita*-wilt fungus, *Fusarium oxysporum* disease complex of green gram, *Vigna radiata* cv ML-1108. J Zhejiang Univ. Sci. B. 6(8):736-742.

Hooper, D.J.(1990). Extraction and processing of plant and soil nematodes, in: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford, UK, pp. 45–68.

Hussey, R.S. and K.R. Barker (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. , including a new technique. Plant Dis. Rep., 57 : 1025 – 1028.

Ibrahim, L.K.A.(1985).The statuts of root – knot nematodes in the Middle East, region. VII of the Internatinal Meloidgyune project. Pp. 373 -378 , In : An Advanced Treatise on *Meloidogyne* Vol.I: Bilogy and Control, Eds: J.N. Sasser and C.C. Carter, Dept. Plant Path.,N.C.State Univ. and U.S. Agency Int. Dev. Raleigh, 422

Khan, A.; Sayed, M.; Shaukat, S.S. and Handoo, Z.A. (2008). Efficacy of four plant extracts on nematodes associated with papaya in Sindh, Pakistan, Nematol. Mediterr., 36 : 93–98.

Lee, S.Y.and Kim, K.Y. (2016). Antagonistic potential of Bacillus pumilus L1 against rootknot nematode, *Meloidogyne arenaria*. J. Phytopathol.,164: 29–39.

Lian, L.H.; Tian,B.Y.; Xiong, R.;Zhu, M.Z.; Xu, J. and Zhang,K.Q.(2007). Proteases from *Bacillus*: a new insight into the mechanism of action for rhizobacterial suppression of nematode populations. Lett. Appl. Microbiol.,45: 262–269.

Manar M. Refaat; Mostafa E. Mahrous; Ramadan M. El-Ashry and Amr M. ElMarzoky (2020).Nematicidal potential of some botanicals against *Meloidogyne Incognita in vitro* and *in vivo*. Bioscience Research, 17(1): 157-164.

Mohammed, S.H.; El Saedy, M.A.; Enan, M.R.;Ibrahim, N.E.;Ghareeb, A., Moustafa, S. A. (2008). Biocontrol efficiency of *Bacillus thuringiensis* toxins against root-knot nematode, *Meloidogyne incognita*. J. Cell Mol. Biol.,7: 57–66.

Mostafa, M.A. ; Mohamoud,W.A.; Anany, A.E.and El-Sagheer, A.M. (2017). Plant essential oils as eco-friendly management tools for root - knot nematode on cucumber plant. Journal of Zoology Studies, 4(1):1-5.

Oka, Y. ; Keltai, H. ; Bar- Eyal, M. ; Mor, M. ; Sharon, E. ; Chet. I and Spiegal, Y. (2002). New strategies for the control of plant parasitic nematodes. Pest Management Science, 56 :983 – 988.

Oka, Y.; Ben-Daniel,B. and Cohen,Y.(2012). Nematicidal activity of the leaf powder and extracts of *Myrtus communis* against the root-knot nematode *Meloidogyne javanica*, Plant Pathol.,61:1012–1020.

Oka, Y.; Shuker, S.;Tkachi, N.;Trabelcy, B.; Gerchman, Y.(2014). Nematicidal activity of *Ochradenus baccatus* against the root-knot nematode, *Meloidogyne javanica*. Plant Pathol., 63 (1): 221–231.

Oliveira, D.F.;Júnior, S.;Nunes, A.S.; Campos, V.P.; Pinho, R.S.D.and Gajo, G.C.(2014). Purification and identification of metabolites produced by Bacillus cereus and *B. subtilis* active against *Meloidogyne exigua*, and their in silico interaction with a putative phosphoribosyltransferase from *M. incognita*. Anais da Academia Brasileira de Ciências,86: 525–538.

Ongena, M.and Jacques, P.(2008). *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 16, 115–125.

Padgham, J.L. and Sikora, R.A. (2007). Biological control potential and modes of action of *Bacillus megaterium* against *Meloidogyne graminicola* on rice. Crop Prot. 26, 971-977.

Pavaraj, M. ; Bakavathiappan, G.;and Baskaran, S. (2012). Evaluation of some plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita*, J. Biopestic., 5 :106–110.

Peng, D.;Chai, L.;Wang, F.;Zhang, F.;Ruan, L. and Sun, M. (2011). Synergistic activity between *Bacillus thuringiensis* Cry6Aa and Cry55Aa toxins against *Meloidogyne incognita*. Microbial Biotechnol.,4:794–798.

Ramakrishnan, S.; Gunasekaran, C.R.;Vadivelu, S., and Viswanath, S.M.(1999). Effect of various aqueous leaf extracts on the larval mortality of *Meloidogyne incognita*, infesting tobacco. Tobacco Research,25: 37–39.

Ramezani Moghaddam, M.; Mahdikhani Moghaddam, E.; Baghaee Ravari, S.and Rouhani, H.(2014). The nematicidal potential of local *Bacillus* species against the root-knot nematode infecting greenhouse tomatoes. Biocontrol Sci. Technol.,24:279–290.

Saravanapriya , B. ; M. Sivakumar ; G. Rajendran and S. Kuttalam (2004). Effect of different plant products on the hatching *Meloidogyne incognita* eggs. Indian J. Nematol., 34: 690 – 697.

Sharma, G.C. (1996). Leaf extract and their action against *Meloidogyne incognita* (in vitro). Journal of Phytological Research, 9:155–157.

Shawky , Samaa , M. ; A.E. Khalil and Soliman , Manal , M. (2010). N on chemical control of root – knot nematode ; *Meloidogyne javanica* on peanut in Egypt. Zagazig J. Agric. Res.,37(1) : 185 – 206.

Shepherd, A.M. (1962). New Blue R, a stain that differentiate between living and dead nematodes. Nematologica ,8: 201–208.

Siahpoush, S.;Sahebani, N. and Aminian, H.(2011). Change of some defense compounds of cucumber treated with *Bacillus cereus* and salicylic acid against *Meloidogyne javanica*. Afr. J. Plant Sci.,5: 829–834.

Siddiqui, Z.A. ;Qureshi, A. and Akhtar, M.S.(2009). Biocontrol of root-knot nematode *Meloidogyne incognita* by Pseudomonas and *Bacillus* isolates on *Pisum sativum*. Arch. Phytopath. Plant Prot.,42: 1154–1164.

Sikora, R.A.; Pocasangre, L.; zum Felde, A. ; Niere, B. ; Vu, T.T. and Dababat, A.A., (2008). Mutualistic endophytic fungi and in-planta suppressiveness to plant parasitic nematodes. Biol. Control, 46:15–23.

Singh, P. and Siddiqui, Z.A.(2010). Biocontrol of root-knot nematode *Meloidogyne incognita* by the isolates of *Pseudomonas* on tomato. Arch. Phytopathol. Plant Prot., 43: 423–1434.

Singh, U.P.; Prithiviraj,B.; Sarma, B.K.; Singh, M. and Ray,A.B.(2001). Role of garlic (*Allium sativum* L.) in human and plant diseases, Indian J. Exp. Biol.,39:310–322.

Symondson, W. ; K. Sunderland ; M. Greenstone (2002). Can generalist predators be effective biocontrol agents? Annual Review of Entomolgy, 47 (1) : 561 - 594.

Tayor, A.L. and Sasser, J.N. (1978). Biology identification and control of root – knot nematodes (*Meloidogyne* spp.) Coop. Pub. Dept. Plant Pathol. North Carolina State Univ. and U.S. Agency Int. Dev. Raleigh , N.C. 111pp.

Tchnouneou, P.B.,Ashour, B.A.; Young,G.M.; Ragheb, D.A.;Romeh, A.A.; Goma, El- Sheik,S.E. ; Lidell, F.P.;Ibitayo, O.and Assad, J.C. (2002). Health risk assessment of pesticides usage in Menia El- Kamh Province of Sharkia Governorate in Egypt. Int. J. Mol. Sci, 3(10) : 1082-1094.

Tian, B.; Yang, J.and Zhang, K.Q.(2007). Bacteria used in the biological control of plantparasitic nematodes: populations, mechanisms of action, and future prospects. FEMS Microbiol. Ecol.,61: 197–213.

Timper, P.;Kone, D., Yin, J.; Ji, P. and Gardener, B.B.M. (2009). Evaluation of an antibiotic producing strain of *Pseudomonas fluorescens* for suppression of plant-parasitic nematodes. J. Nematol. ,41: 234–240.

Trudgill, D.L. and Block,V.C. (2001). Apomictic polyphagous root–knot nematodes : exceptionally successful and damaging biotrophic root pathogens. Annual Review of Phytopathology, 39 : 53 – 77.

Wei, L.; Shao, Y.;Wan, J.; Feng, H.; Zhu, H.; Huang, H.and Zhou, Y.(2014). Isolation and characterization of a rhizobacterial antagonist of root-knot nematodes. PLoS ONE,9: 1–7.

Whitehead , A.G. (1988). Plant nematode control. CAB Int., Walling ford, UK, 384.

Xiong, J.; Zhou, Q.; Luo, H.; Xia, L.;Li, L.;Sun, M. and Yu, Z.(2015). Systemic nematicidal activity and biocontrol efficacy of *Bacillus firmus* against the root-knot nematode *Meloidogyne incognita*. World J. Microbiol. Biotechnol.,31:661–667.

Zhang, F.; Peng, D.; Ye, X.; Yu, Z.; Hu, Z.;Ruan, L.and Sun, M.(2012). In vitro uptake of 140 kDa *Bacillus thuringiensis* nematicidal crystal proteins by the second stage juvenile of *Meloidogyne hapla*. PLoS ONE,7: 1–8.

تأثير مواد مكافحة مختلفة على نيماتودا تعقد الجذور *Meloidogyne incognita* التى تصيب نباتات الخيار

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أختبر معمليا كل من التركيز القياسى ١٠٪ ، ونصف القياسى ٥٪ لثلاثة زيوت (ديفر ٩٧٪ مركز قابل للأستحلاب ، زيت البردقوش ، زيت البرتقال) بالإضافة إلى نوعين من البكتيريا وهما *Bacillus megaterium* و *B. poylmyxa* والتى أظهرت تأثيرا كمبيدات نيماتودية على كتلة البيض والطور اليرقى الثانى لنيماتودا تعقد الجذور *Meloidogyne incognita*. ولقد تأثرت نسبة فقس البيض والموت فى الطور المعدى بالتركيز المستخدم وفترة التعريض للمواد المختبرة. وأعطت كل من بكتيريا *B. megaterium* ، زيت ديفر ٩٧٪ وزيت البردقوش أعلى تأثير، بينما أعطت بكتيريا *B. poylmyxa* أقل تأثير. فيما يتعلق بنسبة الموت للطور المعدى ، فقد أظهرت النتائج بعد ٧ أيام من المعاملة أن بكتيريا *B.poylmyxa* وزيت كل من ديفر ٩٧٪ والبردقوش سجلت أعلى نسبة موت للطور المعدى (٧٥,١٤ ، ٧٣,٠٠ ، ٦١,٨٥ ٪) وذلك بعد التعريض للتركيز القياسى ١٠ ٪ و نسب موت (٥٣,٠٠ ، ٦٧,٤٢ و ٥٢,٥٧ ٪) عند التعريض لنصف التركيز القياسى ٥٪ على التوالى. تحت ظروف الصوبة ، أظهرت النتائج أن من بين جميع المواد المختبرة ، كان الأوكساميل ، مسحوق اوراق المورينجا الجاف وبكتيريا *B. megaterium* أكثرهم تأثيرا فى خفض العقد المتكونة على جذور نباتات الخيار بواسطة نيماتودا تعقد الجذور. وسجل اقل عدد من العقد والعقد التى يزيد قطرها عن ٤ مم فى النباتات المعاملة بمبيد الأوكساميل والمورنيجا وبكتيريا *B. megaterium*. أوضحت النتائج أن كل من المورينجا وبكتيريا *B. megaterium* يمكن استخدامهما فى مكافحة نيماتودا تعقد الجذور *M. incognita* وزيادة محصول نباتات الخيار.