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USE OF RESISTANT CULTIVARS , NPK FERTILIZATION, AND BIOPRODUCTS FOR CONTROLLING NEMATODES AFFECTING CUCUMBER AND SQUASH IN THE NEWLY RECLAIMED LANDS.

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ABSTRACT

Several genera of nematodes were found to be occurring on wilted cucumber and squash in the newley reclaimed lands in El-Behera governorate. The root-knot nematodes *Meloidogyne spp.* were the most frequent and occurred on mean frequency of 30.75 % of the total surveyed cucumber and squash soil samples. These were followed by the nematode genera *Pratylenchus, Tylenchus, Ditylenchus, Helicotylenchus, and Trichodorus* with mean frequencies of 17.19%, 16.61%, 12.82%, 11.24%, and 11.14%, respectively. The *Aphelenchus, Aphelenchoides, Rotylenchus, Xiphinema*, and *Tylenchorhynchus* were also recovered but at frequencies less than 10%. Meantime, Fusarium spp. were also frequent on wilted plants and recovered from 60% and 45% of the tested cucumber and squash, respectively, and followed by *Rhizoctonia solani* but at much lower frequency of 16% and 13%, respectively. The present study confirmed the association of the Fusarium wilt and the root-knot nematodes *(Meloidogyne spp.)* infection on cucumber and squash. The cucumber cv. Americana Beta-alpha and squash cv. Nubaseed Alexandria were found to be the most tolerant varieties tested, against the M. javanica and *M. incognita* and the Fusarium wilt and suppressed root galling/ plant by more than 60% on both cultivars compared to the most susceptible ones. Meantime, the balanced NPK fertilization of 4.6, 5.7 and 1.1 g/pot on cucumber, and the 2.3, 3.4 and 2.3 g/pot on squash significantly decreased mean

nematode (*M. incognita*) infection, assessed in terms of numbers of galls, egg masses and second stage of juveniles, by 73.3 % on cucumber, and by 68.7% on squash compared to the unfertilized infested control. Also, the use of bioproduct agents Nemaless and Agerin effectively decreased mean nematode (*M. incognita*) infection. Nemaless at 2.0 ml/ pot, however, was the most suppressive with the mean of 78.2 %, on cucumber and 71.3 %, on squash. Meantime, the bioproduct Agerin at 6.0 g/pot significantly suppressed the nematode infection on cucumber and squash by 71.7 % and 58.83%, respectively. Moreover, the suppressive effect of the two bioproducts tested was not significantly different from the Furazed chemical nematicide and significantly enhanced growth characteristics of cucumber and squash.

Keywords: Cucumber, Squash, Cultivars, *Meloidogyne javanica*, *M. incognita*, NPK, Agerin, Nemaless, Fungi, *Fusarium oxysporum*, Furazed.

INTRODUCTION

There is a great need for intensifying the agricultural production in Egypt. Cucurbits, in this respect, were considered as a major vegetable food as constituted about 29.88% of the total cultivated area of the vegetable crops in Egypt (Ministry of Agriculture and land reclamation, Economic Affairs Sector, 2004). Cucumber (*Cucumis sativus*) and squash (*Cucurbita pepo*) are among the most important cucurbitaceous crops and two of the leading export vegetables. The newly reclaimed land in El-Behera governorate was considered a major area for cucurbits cultivation where a lot of investments were conducted in this respect. Unfortunately, cucumber and squash in such newly reclaimed land, are suffering from infection with several plant parasitic nematodes which could restrict their cultivation in such area. The use of the chemical nematicides was a quick solution. The impact, however, on public health and the environment was horrifying. Consequently, search for non-chemical nematicides, eco-friendly means, more safer methods, low toxicity products to human and animals, were considered. Various methods were suggested worldwide, where exhibited effectiveness to control nematodes on cucurbits and various crops. This included the use of resistant varieties, the nutritional management, and the use of certain bio-products (Darekar and Bele, 1990; Lvezic *et al.*,1997; Silva *et al.*, 1998; Devappa *et al* ., 1998; Heikal, 2001; Lashein, 2002, Leontopulos *et al.*, 2004, Sikora and Fernandez, 2005). The present study therefore, was conducted to determine nematode genera affecting cucumber and squash in the newly reclaimed land in El-Behera governorate, to identify the possible role of the fungal contribution-particularly the Fusaria- to the incited nematode infection, to investigate the use of resistant cultivars, the NPK fertilization, and certain bio-products to control the root-knot nematode (*Meloidogyne*) infection on cucumber and squash.

MATERIALS AND METHODS

Nematode Survey:

A nematode survey was conducted during the 2004 – 2005 growing seasons. A total of 530 soil and root samples were collected from different fields of cucumber and squash in EL-Nubaria region. Samples were collected at the depth of 20-30 cm from the rhizosphere. A volume of 500 ml from each collected soil sample was successively wet-seived through 100, 325, 400 mesh sieves. The obtained nematode samples were further purified using the Baermann pan technique for 48 hrs according to Goody, (1957). The extracted samples were placed in 1.0 ml counting slide and the nematodes genera were counted and identified. Identification of the root-knot nematode species was based on the morphological characteristics of the perineal patterns of adult females according to the key given by Taylor et al., (1955) and the description of Taylor and Sasser, (1978). Frequency of the recovered nematode genera was calculated as percentage of the total collected samples from each of cucumber and squash plants.

Isolation and identification of fungi associated with wilting of cucumber and squash:

Diseased cucumber and squash roots were collected during the 2002 - 2004 growing seasons from different fields of El-Behera governorate. Root samples were washed thoroughly with tap water,

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cut into small pieces, surface sterilized by 3% hypochlorite sodium (NOCl) solution for three minutes, rinsed several times in sterilized distilled water, dried between two sterilized filter papers, and placed on PDA medium in Petri dishes and incubated at 22 + 1C for 3 – 5 days. Hyphal tip or single spore of the developing fungi were transferred to PDA plates. Inoculum of each purified culture was transferred into PDA slants and incubated at 25 C .The developed fungi were identified according to Gilman, (1957) and Booth (1971). Stock cultures were maintained on PDA slants and kept in refrigerator at 5°C for further studies.

Cultivar reaction of cucumber and squash to nematode infection.

- Preparation of nematode inoculum.

The nematodes were multiplied on tomato plants cv. Rutegers, grown in plastic pots (25cm diameter) filled with sterilized soil-sand mix (1:2, v/v) at 25 C under greenhouse conditions. Tomato roots, infested with root-knot nematodes, were removed from the pots and gently washed with tap water. Roots were cut into small pieces and macerated for two periods of 10 seconds each at high speed by using a Waring blender. This method released the highest number of nematodes from roots. The macerated root solution was then placed in a Duran bottle containing sodium hypochlorite (Clorox), water was added to adjust the final concentration of sodium hypochlorite to 1.05% as described by Hussey and Barker, (1973). The solution in the Duran bottle was vigorously shaken for 3 minutes to release the eggs from the egg matrix. The solution was then poured through different size sieves to remove the root tissue. Eggs were collected on the 25 micrometer (µm) sieve and washed several times with tap water to remove residual sodium hypochlorite. Extracted eggs were then counted under the microscope according to Hussey and Barker, (1973). Egg-masses of root-knot nematodes were stained by dipping the roots in 0.015 % Phloxine B solution for 20 minutes and then stained roots were washed with tap water to remove the residual Phloxine B. Numbers of galls and egg masses present in the roots were recorded (Taylor and Sasser, 1978).

Pathogenicity and cultivar reaction of cucumber and squash to nematode.

These were conducted under greenhouse conditions. The tested cultivars of cucumber were the Americana Beta-Alpha, Americana IBB, Beta-Alpha (Hybrid Japanese) and Denamarky Beta-Alpha. Also, the tested squash cultivars were, Egypt for development, Heitic Alexandria, Nubaseed Alexandria and El-Maamora. Additionally, the tested cucumber and squash cultivars were obtained from seed Dept. Ministry of Agriculture, El-Giza. Four replicate pots were used for each treatment. Each pot was sown with 3 surface sterilized cucumber or squash seeds. After one week from sowing pots were infested with 5000 freshly hatched 2^{nd} stage juveniles (J2) and eggs/pot. Final number of nematodes, root galls, eggmasses, and were recorded 45 days after inoculation.

Pathogenicity and cultivar reaction of cucumber and squash to *Fusarium spp.*

The most common *Fusarium* sp. (*F.oxysporum*) was chosen for the pathogenicity tests. Incoula of the fungus was prepared by growing in conical flasks containing 150 g autoclaved medium consisting of sorghum grains, sand and water at the rate of 1:20:4 (Abd El-Rehim,1984) and incubated at 25°C for 15 days . Sterilized pots, 25 cm diameter, were filled with autoclaved sandy-loam soil and then inoculated with the inoculum of the tested fungus at the rate of 5 g/kg soil . Pots were divided into two groups 1^{st} group sown after one week of fungal infestation with three surface-sterilized cucumber seeds and the 2^{nd} group sown with sterilized squash seeds. Cultivars used as aforementioned in the preceeding paragraph. Check treatments were carried out in pots filled with sterilized sandy loam soil (non-inoculated). Four replicates were used for each treatment. Data of decayed seeds and pre- or post- emergence damping-off seedlings were recorded at 10 days intervals for a period of two months. Data obtained were statistically analyzed using Complete Randomized Block Design (Snedecor and Cochran, 1973).

Effect of the interactions between fungi and nematodes on nematode infection:

Culture of *M. incognita* was prepared as previously stated. Also, one of the most common *Fusarium* species, i.e. *F.oxysporum* recovered in the present study was chosen for implementing the

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interaction study with nematode. Discs of the fungal growth on PDA were transferred to conical flasks containing sterilized sand sorghum medium (Abdel-Rehim, 1984). Then, conical flasks were incubated at 22 ± 1°C for 15 days. Sterilized pots (35 cm diameter) were filled with mixture of autoclaved sandy clay (1:1) soil and mixed thoroughly with the inoculum of the tested fungus at the rate of 5gm / kg soil. After the establishment of the tested fungus, or otherwise stated, each pot sown with three surface sterilized seeds of cucumber or squash. The tested cultivar of cucumber was Denmarky Beta-Alpha while squash cultivar was EL-Maamora as these cultivars were extremely susceptible in the previous pathogenicity test. Check treatments were carried out in pots without inoculation containing sterilized sandy clay soil. After one week from seedlings emergence, pots were infested with 5000 freshly hatched juveniles and eggs / pot. Four replicates were used for each treatment.

Treatments were as follows: 1- Non treated control . 2- Inocula of *F . oxysporum* infested soil one week before sowing. 3- *M . incognita* infested soil, one week after seedling emergence. 4- A mixture of *M . incognita* and *F. oxysporum* infested soil, one week after seedling emergence. 5- *F. oxysporum* infested soil, one week before sowing, followed by *M. incognita* infestation, one week after seedling emergence. 6- Inocula of *M. incognita* infested soil, one week after seedling emergence and followed by *F. oxysporum,* two weeks after seedling emergence. 7- After 45 days of sowing date, the plants were gently removed from soil. Number of galls/plant, numbers of egg masses/plant, J2 and eggs/gm and root fresh weight, were recorded. The root and shoot length and also, fresh and dry weight were recorded. Data obtained were statistically analyzed according to Snedecor and Cochran (1973).

Effect of different rates of NPK fertilization on nematode infection.

Under the greenhouse conditions, 25-cm diam. plastic pots were prepared and sown with seeds of cucumber and squash cultivars which were mostly susceptible to root-knot infection in the previous tests. Then, ammonium sulphate (20.6 % N) was added at three different rates 2.3, 4.6 and 7.5 g / pot for cucumber and 1.1, 2.3 and 3.4 g / pot for squash, however , calcium super phosphate 15 % was added at (

2.9, 5.7 and 8.6) and (1.7, 3.4 and 5.1) gm / pot with two tested cucumber and squash crops, respectively. Potassium sulphate (48 %) was also, added at (0.6, 1.1 and 1.7) and (1.1, 2.3 and 3.4) g / pot with two tested cucumber and squash crops respectively. Super phosphate was added, before sowing while, ammonium sulphate was added twice after15 and 30 days of sowing date. Potassium sulphate was added during sowing and 20 days after sowing. Inocula of *M .incognita* were prepared as previously described under pathogenicity tests. One week after seedling emergence, pots were infested with a mixture of 5000 eggs and freshly hatched juveniles of *M. incognita* per pot. Three replicates were used in each treatment and experiments were terminated 8 weeks after sowing. Number of root galls and egg masses (surface egg masses) were recorded. Also, number of egg masses root system (egg masses), and final numbers of J2 in roots were recorded as previously described.

Effect of bioproducts on nematode infection:

The granular nematicide Furazed 10 G (carbofuran), the bio-products Agerin (*Bacillus thuringiensis* compound obtained from Nematology Lab. Dept, Plant Pathology, Faculty of Agriculture. Alexandria University) and Nemaless biocide (obtained from Soil, and Environment Research Institute. ARC, Giza) were used in the present study. Pots and root-knot inoculum were prepared as previously described. Pots were distributed in two sets, the 1stset sown with cucumber cultivar Denmarky Beta-Alpha while, the 2nd set sown by squash cultivar El-Maamora. These two cultivars were the mostly susceptible to root-knot infection in the previous tests. Each pot sown with three seeds of the tested cucumber or squash cultivars. After one week from seedling emergence, pots were infested with a mixture of 5000 eggs and freshly hatched juveniles of *M. incognita*/pot which was the most aggressive in the previous tests. Pots in each set were divided into seven groups (treatments) of 4 replicates. The 1st and 2nd group received the granular nematicide , Furazed 10G (carbofuran), was applied at the two rates 3.0 and 1.5 g / pot, the 3 rd and 4th group received the bio-product Agerin that applied in two doses of 6.0 g and 3.0 g / pot, respectively. The 5th and 6th group received the bio-product Nemaless which was applied also in two concentrations of 2.0 and 1.0 ml /pot. The 7th group was inoculated with nematode and left untreated to serve as control. Furazed and Agerin were applied once in the soil, after inoculation with nematode in a circle around the plant stem, 5cm depth Nemaless was applied weekly for 1st month (3 times). The experiments were terminated 10 weeks after sowing . Each pot sown with 3 seeds. Pots were arranged in randomized complete block design. Cucumber and squash roots were gently removed from the soil and washed with running tap water. Length, fresh and dry weight of roots and shoots and numbers of galls, egg-masses, J2 and eggs in roots were recorded .

RESULTS

Nematode genera occurring on wilted cucumber and squash:

Eleven genera of nematodes were recovered from the rhizosphere of wilted cucumber and squash plants (Table, 1). The *Meloidogyne* was the most frequent genus as it constituted 34 % and 27.5% of the tested cucumber samples (250) and squash (280), respectively, with a mean of 30.75% on both crops. This was followed by the *Pratylenchus* as it constituted 20.8% and 13.57% on both crops, respectively, with the mean of 17.19%. The *Tylenchus, Ditylenchus* and *Helicotylenchus*. however, occurred with averages of 16.61%. 12.82% and 11.24% respectively, and almost in similar frequencies on both crops. Meantime, the *Aphelenchus*, *Aphelenchoides* and *Rotylenchus* were recovered in frequences 9.20%, 7.04% and 6.59%, respectively (Table. 1).

Fungi associated with wilted cucumber and squash:

Data presented in Table (2) showed that several species of *Fusarium* were isolated from the surveyed region with frequencies ranged between 5% and 22%. *Fusarium oxysporum* was the most frequent fungus as constituted 22% and 19% in cucumber and squash, respectively. *R. solani* was also isolated but with frequencies of 16% and 13% on both crops, respectively. Meantime, *Pythium ultimum* was isolated at the frequency of 15% and 12% from both crops, respectively. Also, *Alternaria alternata, Macrophomina phaseolina, Sclerotinia sclerotiorum, Aspergillus niger, Rhizopus stolonifer and Trichoderma harzianum* were recovered, but at the range of 2% to 9% on both crops, respectively.

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Table 1 : Frequency of genera of plant parasitic nematodes recovered from cucumber and squash rhizosphere collected from the newly reclaimed land in El-Beheira Governorate , during 2004-2005 growing seasons.

Nematode genera	Number of soil samples containing the nematode genera		% Frequency		% Mean frequency
	Cucumber	Squash	A cucumber	B squash	A + B
Aphelenchoides	20	17	8	6.07	7.04
Aphelenchus	21	28	8.4	10	9.2
Ditylenchus	32	36	12.8	12.85	12.82
Helicotylenchus	33	26	13.2	9.28	11.24
Meloidogyne	85	77	34	27.5	30.75
Pratylenchus	52	38	20.8	13.57	17.19
Rotylenchus	16	19	6.4	6.78	6.59
Trichodorus	28	31	11.2	11.07	11.14
Tylenchorhynchus	9	5	3.6	1.78	2.69
Tylenchus	42	46	16.8	16.42	16.61
Xiphinema	14	10	5.6	3.57	4.59
Total samples	250	280	-	-	-

$$\text{\% Frequency} = \frac{\text{Number of samples containing certain}}{\text{Total number of samples tested}} \times 100$$

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Table 2 : Frequency of fungi recovered from cucumber and squash plants showed wilt symptoms collected from different fields in the newly reclaimed lands in El-Beheira Governorate, during 2002-2004 growing seasons.

Isolated fungi	* % frequency of fungi isolated from	
	Cucumber	Squash
Alternaria alternata (Fr.) Keissler.	3	4
Aspergillus niger Van Tighem.	4	2
Fusarium moniliforme Sheld.	12	6
Fusarium semitectium Berk and Rav.	9	5
Fusarium oxysporum Schlecht.ex Fr.,	22	19
Fusarium solani (Mart.) App. And Wr	17	15
Macrophomina phaseolina (Maub.) Ashby	4	7
Pythium ultimum Trow.	15	12
Rhizoctonia solani Kuhn	16	13
Rhizopus stolonifer (Her.ex Fr Lind)	4	6
Sclorotinia sclerotiorium	7	9
Trichoderma harizianum	2	3

$$\% \text{ Frequency} = \frac{\text{No. of plant samples from which the fungus recovered}}{\text{Total plant samples tested}} \times 100$$

Cultivar reaction of cucumber and squash to nematode infection:

All the tested cucumber cvs., Americana Beta Alpha, American IBB, Beta – Alpha (Hybrid Japanese) and Denamarky Beta-Alpha varied in their susceptibility to both *M. javanica* and *M. incognita* (Table 3). A significant number of galls (32.5-71.8/ plant), egg masses (52.5-65.9/ plant) and number of the 2nd stage juveniles (36.3- 48.8/g fresh root) were produced by *M. javanica* on the tested cucumber cultivars. Meantime, for most of the criteria tested, *M. incognita* was more aggressive than *M. javanica*, since most of the disease criteria incited by *M. incognita* were significantly higher. The cv. Americana Beta-Alpha of cucumber exhibited the highest tolerance to both *M. javanica* and *M. incognita* as the lowest numbers of galls (23.5 & 35.2

Table 3 : Reaction of cucumber cultivars to infection with *M . javanica* and *M . incognita* under greenhouse conditions*.

Cucumber cultivars	No of galls/plant				No. of egg masses/plant				No. of eggs and 2nd stage juveniles /gm fresh root			
	Cont.	M.J.	M.I.	Mean	Cont.	M.J.	M.I.	Mean	Cont.	M.J.	M.I.	Mean
Americana Beta-Alpha	0.0	23.5	35.2	29.35	0.0	52.5	53.8	53.15	0.0	36.3	34.8	35.55
American IBB	0.0	35.8	37.75	36.78	0.0	54.2	55.8	55.0	0.0	44.0	48.5	46.25
Beta-Alpha (Hybrid-Japanese)	0.0	49.5	70.0	59.75	0.0	61.4	66.2	63.8	0.0	47.5	50.0	48.75
Denamarky Beta-Alpha	0.0	81.8	71.9	76.85	0.0	65.9	68.0	66.59	0.0	48.8	52.6	50.7
Mean	0.0	45.1	56.2	51.8	0.0	58.5	60.95	59.64	0.0	44.15	46.48	45.32

* This was assessed in terms of number of galls/ plant, number of egg-masses/ plant, and number of eggs and 2nd stage juveniles/g fresh root of the tested nematodes, 45 days after inoculation. Data are means of four replicates. Cont. = uninfested control, M. j. = *Meloidogyne javanica*, M.i. = *Meloidogyne incognita*

L.S.D at 0.05 for			
Cultivars	10.08	9.09	6.3
Nematode	7.07	1.8	2.2
Interaction			
CVS.X nematode	11.0	17.0	4.40

galls / plant), number of eggmasses (52.2 & 53.8 eggmasses / plant), and number of 2nd stage juveniles (36.3 & 34.8/ gm fresh root) for both nematode species were recorded, respectively. The American IBB and the cv. Beta-Alpha (Hybrid Japanese), meantime, showed less tolerance while the cv. Denamarky Beta-Alpha was the most susceptible and exhibited the highest number of galls (71.8 and 81.9 galls /plant), number of eggmasses (65.9 and 68.0/plant), and number of 2nd stage juveniles (48.8 and 52.6/g fresh root) incited by *M. javanica* and *M. incognita*, respectively. For squash, both *M. javanica* and *M. incognita* were pathogenic to the tested squash cvs. Egypt for development; Heitic Alexandria; Nubseed Alexandria and El-Maamora (Table 4). A significant number of galls (23.8 - 61.1/plant), egg masses (51.3 – 72.7/plant) number of 2nd stage juveniles (41.0 - 124.25/g root fresh weight) were incited by *M. javanica* on the tested squash cultivars. In case of *M. incognita*, the number of galls of 25.1 – 64.9/plant, the number of eggmasses of 47.3-73.4/plant, and the number of 2nd stage juveniles of 53.25 – 112.0 /g root fresh weight, were recorded on the squash cultivars (Table 4). Squash cv. Nubaseed Alexandria inoculated with *M. javanica* and *M. incognita* was the most tolerant squash cultivar tested. It showed the lowest number of galls (23.8, 25.1 galls /plant), the lowest number of egg-masses (51.3, 47.3/plant), the lowest number (41.0, 53.25/g root fresh weight) of 2nd stage juveniles and eggs with the two nematode species, respectively (Table 4). The cv. El-Maamora, however, was the most susceptible as exhibited the highest number of galls (61.1, 64.9 galls/plant), the highest number of egg-masses (72.7, 73.4 /plant) and high number of 2nd stage juveniles (124.2, 112.0 /g root fresh weight) when inoculated with *M. javanica* and *M. incognita*, respectively (Table 4).

Table 4 : Reaction of squash cultivars to infection with *M . javanica* and *M. incognita* under greenhouse conditions*.

Squash cultivars	No of galls/plant				No. of egg masses/plant				No. of eggs and 2nd stage juveniles / gm root fresh weight			
	Cont.	M.J.	M.I.	Mean	Cont.	M.J.	M.I.	Mean	Cont.	M.J.	M.I.	Mean
Egypt for development	0.0	50.3	52.57	51.43	0.0	61.7	63.8	62.75	0.0	58.75	67.25	63.0
Heitic Alexandria	0.0	35.4	36.3	35.85	0.0	55.6	57.2	56.4	0.0	73.5	72.0	72.75
Nubaseed Alexandria	0.0	23.8	25.1	24.4	0.0	51.3	47.3	49.3	0.0	41.0	53.25	47.125
El-Maamora	0.0	61.1	64.9	63.0	0.0	72.7	73.4	73.05	0.0	124.25	112.0	118.125
Mean	0.0	42.6	44.72	44.8	0.0	60.33	60.42	60.37	0.0	74.28	76.13	75.25

* This was assessed in terms of number of galls/ plant, number of egg-masses/ plant, and number of eggs and 2nd stage juveniles/g fresh root of the tested nematodes, 45 days after inoculation. Data are means of four replicates. Cont. = uninfested control, M. j. = *Meloidogyne javanica*, M.i. = *Meloidogyne incognita*

Cultivars	9.12	4.08	13.8
Nematode	10.1	2.07	13.3
Interaction			
CVS.X nematode	10.20	5.14	16.54

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Reaction of cucumber and squash to *Fusarium oxysporum* :

Data obtained in Table (5) indicated that all the tested cucumber cultivars were susceptible by varied degrees to *F. oxysporum*. Pre-and post-emergence damping-off developed on the cultivars inoculated with *F. oxysporum* ranged between 8.33% and 41.66% for pre-emergence damping-off and between 16.67% and 50% for post-emergence damping-off. The cv. Americana Beta-Alpha, however, was the most tolerant as it showed the lowest percentage of total damping-off (25.0 %) by *F. oxysporum* infection. In addition, data in Table (6) showed that, all the tested squash cultivars were susceptible to *F. oxysporum* as 8.33-33.33% and 4.33-50% were recorded for the pre- and post-emergence damping-off, respectively. The lowest pre- and post-emergence damping-off 8.33 % and 4.33%, were recorded on cv. Alexandria Nubaseed.

Table (5): Reaction of cucumber cultivars *to F. oxysporum* infection under greenhouse conditions.

Cucumber cultivars	Pre-emergence damping-off %	Post-emergence damping-off %	Total
American Beta-Alpha	8.33	16.67	25.0
American IBB	16.67	25.00	41.67
Beta-Alpha (Hybrid Japanese)	25.00	50.00	75.0
Denamarky Beta-Alpha	41.66	33.33	74.99
Control (uninoculated)	0.0	0.0	0.0
Mean	18.33	25	
L.S.D. at 0.05	.7.70	10.55	

*** Data are mean of four Replicates. Data are recorded 15, and 45 days after infestation for the pre- and post-emergence damping-off, respectively..**

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Table (6): Reaction of squash cultivars to *F. oxysporum* infection under greenhouse conditions.

Squash cultivars	Pre-emergence damping-off %	Post-emergence damping-off %	Total
Egypt of Development	16.67	33.33	50.0
Heitic Alexandria	16.67	25.00	41.67
Alexandria Nubaseed	8.33	4.33	12.66
El-Maamora	33.33	50.00	83.33
Control (noninoculated)	0.0	0.0	0.0
Mean	15	23.33	
L.S.D. at 0.05	5.65	10.71	

*** Data are mean of four Replicates. Data are recorded 15, and 45 days after inoculation for the pre- and post-emergence damping-off, respectively.**

Effect of the interaction between fungi and nematodes on nematode infection:

Data presented in Table (7) showed that inoculation with *M. incognita* alone to Denamarky Beta-Alpha resulted in a considerable number of root galls (57.2/plant), eggmasses (51.9/plant), and 2nd stage of juveniles (52.2 /g fresh root). However, intensive root galling (83.1/plant), eggmasses (71.3/plant) and 2nd stage of juveniles (103.8 /g fresh root) occurred when the nematode infestation was accompanied by *F. oxysporum* inoculation while root galling of 80.9/plant, eggmasses of 73.7/plant, and 2nd stage juveniles and eggs of 119.2, were recorded when fungal inoculation preceeded the nematode infestation. On the other hand, when the nematode infestation preceeded the fungal infection, a lower number of galls (64.7/plant), eggmasses (54.9/plant), and 2nd stage juveniles (92.6/plant) were recorded. However, the occurred root galls and number of 2nd stage juveniles and eggs/g fresh root, in this case were

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still significantly higher than in the case of infestation with nematode alone (Table, 7). For squash, data presented in Table (8) revealed a trend typically similar to that obtained on cucumber. However, more intensive egg-masses (217.8 /plant) were developed on the co-infestation of *F. oxysporum* and *M. incognita* (Table 8).

Table (7): Interaction between *F. oxysporum* and *M. incognita* and their effect on cucumber (cv. Denamarky Beta-Alpha) disease syndrome*..

Treatment	No. of galls / plant root	No. of egg-masses/plant	No. of 2nd stage juveniles and eggs/g fresh root
Control (non-inoculated)	0.0	0.0	0.0
F. oxysporum	0.0	0.0	0.0
M. incognita	57.2	51.9	52.2
M. incognita + *F. oxysporum* (comb)	83.1	71.3	103.8
M. incognita followed by F. oxysporum	64.7	54.9	92.6
F. oxysporum followed by M. incognita	80.9	73.7	119.2
L.S.D. at 0.05	7.19	8.84	20.83

*** This was assessed in terms of number of galls/ plant, number of egg-masses/ plant, and number of eggs and 2nd stage juveniles/g fresh root of the tested nematodes. Data are means of four replicates. Data were recorded 45 days after sowing,**

Table (8): Interaction between *F. oxysporum* and *M. incognita* and their effect on Squash (cv. El-Maamora) disease syndrome.

Treatment	No. of galls / plant root	No. of egg-masses/plant	No. of 2nd stage juveniles and eggs/g fresh root
Control (non-inoculated)	*0.0	0.0	0.0
F. oxysporum	0.0	0.0	0.0
M. incognita	51.6	156.2	61.6
M. incognita + *F. oxysporum* (comb)	74.5	217.8	117.4
M. incognita followed by *F. oxysporum*	58.6	163.0	99.0
F. oxysporum followed by *M. incognita*	72.1	164.2	97.2
L.S.D. at 0.05	11.17	10.99	10.79

*** This was assessed in terms of number of galls/ plant, number of egg-masses/ plant, and number of eggs and 2nd stage juveniles/g fresh root of the tested nematodes. Data are means of four replicates. Data were recorded 45 days after sowing,**

Effect of NPK fertilization on nematode infection to cucumber and squash.

Significant reductions in root galling, number of eggmasses, and number of eggs and 2nd stage juveniles were occurred in cucumber and squash plants in most of NPK applications compared to the untreated infested control (Table 9 and 10). In cucumber (Table 9), the highest reduction in root galls (26.9/plant), eggmasses (21.3/plant) and number of 2nd stage juveniles and eggs (13.8/g fresh weight) were recorded in plants treated with NPK at rate of (4.6, 5.7,and 1.1 g/pot). This was followed by rate (6.9, 8.6 and 1.7 g/pot) and the rate (2.3, 2.9 and 0.6 g/pot). The different rates of phosphorus alone did not exhibit a significantly effect on galls and eggmasses formation, but significantly decreased number of 2nd stage juveniles and eggs (66.8 /g root fresh weight) at the low P application (2.9 g/pot). The higher rates of P (5.7- 8.6 g/pot), however, tended to increase root galling and number of eggmasses. Meantime, nitrogen application alone at the different rates tested decreased numbers of galls, eggmasses, and eggs and 2nd stage juveniles to 52.9-66.9/plant, 48.3-57.8/plant and 18.4-

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28.8/g root fresh weight, respectively. The same effect was recorded in case of the potassium application alone (Table 9). Significant reductions in numbers of root galls (21.0 /root), egg-masses (29.9/root system), J2 and eggs (16.1/g fresh weight) were found in plants treated with NPK at rate of (2.3, 3.4, and 2.3 g/pot) Table (10). This was followed by the rates of (3.4, 5.1, and 3.4 g/pot) and the rate of (1.1, 1.7, and 1.1 g/pot) where numbers of galls/plant, egg-masses/plant and 2nd stage juveniles & eggs/g fresh weight were (2.3, 31.1, and 18.2) and (28.3, 33.1, and 17.9), respectively.

Table 9 : Effect of NPK fertilization on root-knot nematode incidence on cucumber (cv. Denamarky Beta-Alpha) sown in potted soil inoculated with *M. incognita* .

Treatments g/pot	Mean no. of galls / plant	Mean no. of egg masses / plant	Mean no. of 2nd stage juveniles and eggs / g fresh root.
N 2.3 - 0 – 0	66.9	57.8	28.8
N 4.6 - 0 - 0	60.1	52.4	21.9
N 7.5 - 0 – 0	52.9	48.3	18.4
Mean	60	52.8	23.3
P 0 - 2.9 - 0	83.6	88.2	66.8
P 0 - 5.7 - 0	88.6	103.7	82.1
P 0 - 8.6 – 0	93.4	109.8	92.3
Mean	88.5	100.6	80.4
K 0 - 0 - 0.6	66.2	58.1	31.2
K 0 - 0 - 1.1	61.9	52.3	26.2
K 0 - 0 - 1.7	59.6	42.4	23.7
Mean	62.6	50.9	27.03
NP 2.3 - 2.9 - 0	71.8	77.0	58.4
NP 4.6 - 5.7 - 0	75.4	68.6	51.7
NP 7 - 8.6 - 0	81.9	51.8	44.8
Mean	76.4	65.8	51.6
NK 2.3 - 0 - 0.6	76.1	79.8	59.2
NK 4.6 - 0 - 1.1	77.7	70.1	56.2
NK 7 - 0 - 1.7	82.0	65.2	50.1
Mean	78.6	71.1	55.2
PK 0 - 2.9 - 0.6	46.7	63.7	59.6
PK 0 - 5.7 - 1.1	36.9	51.8	27.8
PK 0 - 8.6 - 1.7	33.6	46.7	23.6
Mean	39.1	54.1	37
NPK 2.3 - 2.9 - 0.6	31.2	26.8	14.9
NPK 4.6 - 5.7 - 1.1	26.9	21.3	13.8
NPK 6.9 - 8.6 - 1.7	28.1	23.4	17.2
Mean	28.7	23.8	15.3
Control inoculated non treated 0 - 0 – 0	82.8	103.0	103.9
L.S.D at 0.05	10.45	9.78	11.58

*** Data are means of three replicates.**

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Table 10 : Effect of different levels of NPK fertilizers on root-knot nematode incidence on squash (cv. El-Maamora) sown in potted soil inoculated with *M. incognita* .

Treatments g/pot	Mean no. of galls / plant	Mean no. of egg masses / plant	Mean no. of 2nd stage juveniles and eggs / g fresh root.
N 1.1 - 0 – 0	63.8	55.8	27.1
N 2.3 - 0 - 0	58	50.4	20.7
N 3.4 - 0 – 0	50.1	46.4	17.8
Mean	57.3	50.9	21.9
P 0 – 1.7 – 0	80.6	86.6	58.7
P 0 – 3.4 - 0	85.6	103.3	81.0
P 0 – 5.1 – 0	89.9	110.1	91.3
Mean	85.4	100.0	77.0
K 0 - 0 – 1.1	65.3	56.2	30.3
K 0 - 0 – 2.3	59.9	49.9	25.9
K 0 - 0 – 3.4	55.8	41.3	19.3
Mean	60.3	49.1	25.2
NP 1.1 - 1.7 - 0	69.6	76.4	57.1
NP 2.3 - 3.4 - 0	72.9	68.0	50.9
NP 3.4 - 5.1 - 0	79.8	49.9	43.7
Mean	74.1	64.8	50.6
NK 1.1 - 0 - 1.1	74.1	78.4	58.1
NK 2.3 - 0 - 2.3	75.6	68.4	55.1
NK 3.4 - 0 - 3.4	83.6	63.4	49.1
Mean	77.8	70.1	54.1
PK 0 - 1.7 - 1.1	44.7	60.7	28.6
PK 0 - 3.4 – 2.3	36.8	50.7	26.2
PK 0 - 5.1 – 3.4	32.6	45.7	22.3
Mean	38.03	52.4	25.7
NPK 1.1 - 1.7 - 1.1	28.3	33.1	17.9
NPK 2.3 - 3.4 - 2.3	21.0	29.9	16.1
NPK 3.4 - 5.1 - 3.4	23.0	31.1	18.2
Mean	24.1	31.3	17.4
Control inoculated non treated 0 - 0 - 0	79.6	106.8	93.4
L.S.D at 0.05	5.96	2.9	

*** Data are means of three replicates.**

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Effect of bioproducts on *Meloidogyne incognita* and growth characteristics of cucumber and squash:

Effect on root-knot nematode incidence on cucumber and squash

It was evident that the treatment with Furazed (10 % carbofuran) 10G at 3.0 g/pot, on cucumber (cv. Denamarky Beta-Alpha), significantly decreases the number of galls (15.2 /plant), number of egg-masses (10.4/ plant), and number of 2^{nd} stage juveniles (3.2/g root fresh weight) compared to untreated infested control. However, Nemaless at 2.0 ml/pot and Agrein at 6.0 g/pot exhibited an reduction effect on the nematode propagules which was not significantly different from Furazed effect for the different criteria tested. For squash, a similar trend was recorded where Furazed 10G at 3.0 gm/pot showed the highest reduction effect followed by Nemaless at 2.0 ml/pot which was not significantly different from Furazed effect for the different criteria tested while the treatment with Agrein at 6.0 g/pot was significantly lower than Furazed 10G at 3.0 g/pot for most aspects (Fig.1).

Effect on growth characteristics of cucumber and squash

Growth characteristics of cucumber and squash significantly improved by all tested treatments. Plant length, fresh and dry weight per plant increased with all bio-products treatments compared to untreated infested control. However, treatment with the nematicide Furazed 10G at 3.0 g/pot gave the highest enhancement effect. This was followed by Nemaless (at 2.0ml/pot) and Agrein (at 6.0 g/pot) and they exhibited effect, mostly, not significantly different from Furazed 10G (Fig. 2).

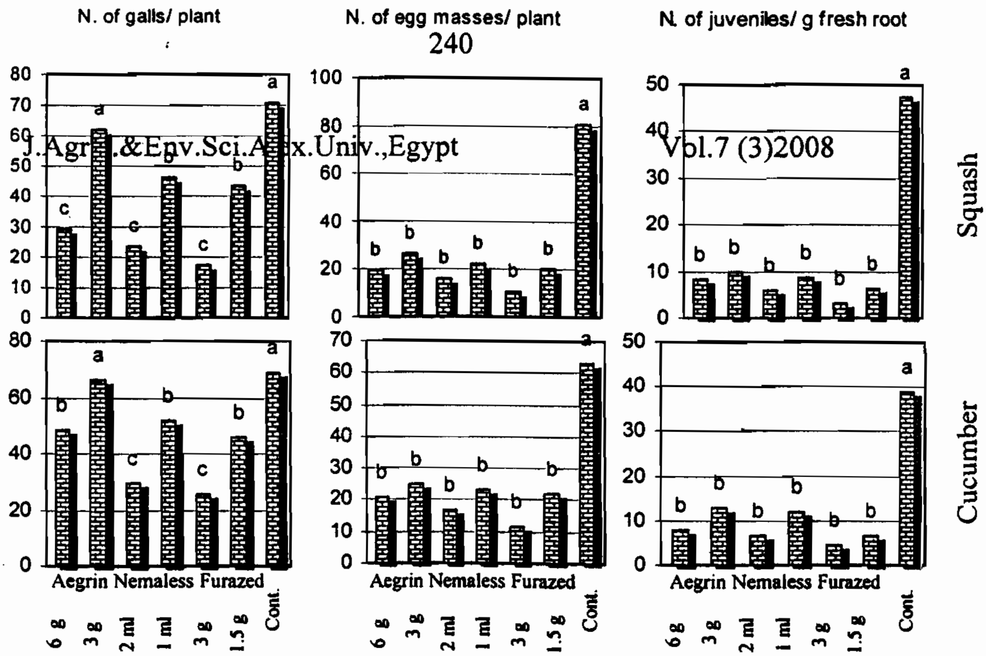

Fig (1): Effect of the bio-products, Agerin and Nemaless, and the Furazed nematicide on disease parameters of *Meloidogyne incognita* on artificially infested cucumber cv. Beta Alfa Denmarky and squash cv. El-Maamora .

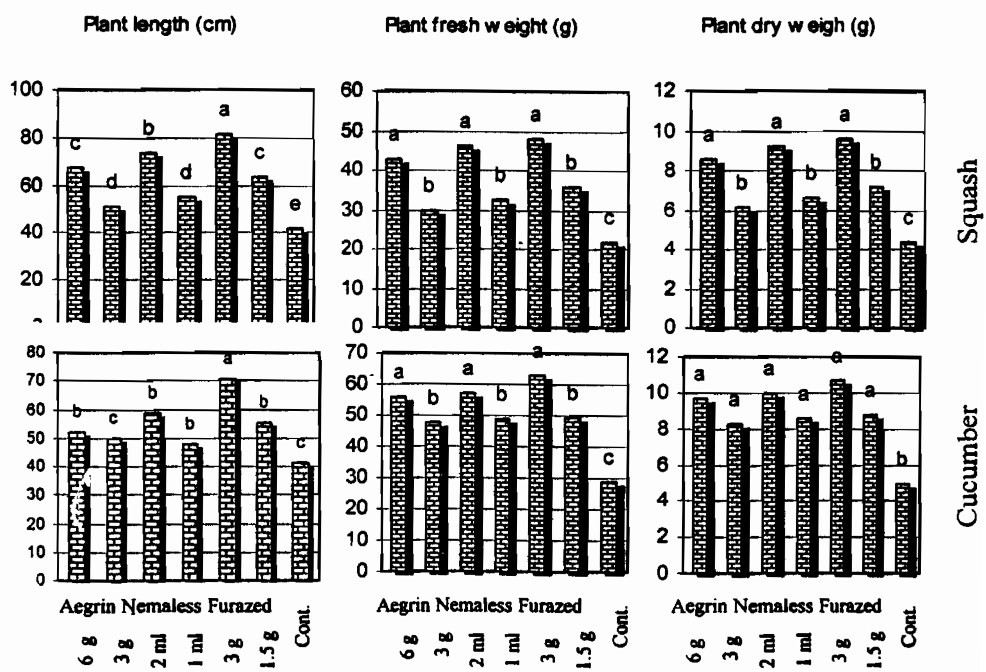

Fig (2): Effect of the bio-products, Agerin and Nemaless, and the Furazed nematicide on disease parameters of cucumber cv. Beta Alfa Denmarky and squash cv. El-Maamora artificially infested with *Meloidogyne incognita*

* Columns sharing the same letter (s) are not significantly different at $p < 0.05$ level of probability

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DISCUSSION

In the last few years, nematode infection and wilt diseases constituted a constant threat and caused big losses in the newly reclaimed lands in El-Behera Governorate. It became key factors for the success and failure of the cucurbits cultivation in this area. Big efforts were focused to control this danger and to save the huge investments conducted in this area. The use of nematicides was of amazing success, however, the impact on the environment and the human health was terrible which made the research for alternative methods rather than the use of the chemical nematicides is very important. The present study revealed the association of the *Meloidogyne incognita* and *M. javanica* , with cucumber and squash wilt in the surveyed areas. The *Meloidogyne* spp. were recovered from almost one third of the surveyed soil samples and proved pathogenicity on a variety of cucumber and squash cultivars. On the other hand, several *Fusarium* species, *Fusarium oxysporum* in particular, were found to be associated with the cucurbits wilt. Meantime, the contribution of the fungus *F. oxysporum* to the nematode infection was also confirmed in the present study which makes the situation even worse. The co-infestation of both *M.incognita* and *F. oxysporum* increased the root-knot infection on cucumber and squash in terms of number of galls/plant, number of egg-masses/ plant, and number of 2nd stage of juveniles/g root fresh weight compared to infection with *M. incognita* only, on both crops. These findings were supported by researches on cucurbits in different parts in Egypt and other parts in the world on different crops. (Choo *et al.*, 1990, Kassab and Ali, 1997, Abd-El-Alim *et al.*, 1999, Maosheng *et al.*,2001). The use of the resistant cultivars was considered the hallmark of any program in the plant diseases control (Agrios, 2005). In the present study, the c.v Americana Beta-Alpha of cucumber and the c.v. Nubaseed Alexandria of squash exhibited good tolerance to both the root-knot and fusarial infection. A considerable low mean number of galling (29.35/ plant; 24.4/ plant), and low percentage of damping-off (25%; 12.66%) were recorded on cucumber and quash cvs., respectively, compared to other varieties where the developed disease parameters were considerably high. These findings were consistent reports on worldwide (Carneiro *et al.*, 2000, Choo *et al.*, 1990 and Khan *et al.*, 1993, 1994 and 2000). The nutritional management was reported to be an important factor in the plant diseases management (Zinov' eva 1986, Ivezic *et al.*,1997, and Silver *et al.*,1998). This should be for the advantages of the host and disadvantages of the pathogen. In the present study, the balanced NPK fertilization of 4.6 - 5.7 - and 1.1 g/pot showed the lowest numbers of galling, egg masses, and Juveniles in soil infested with *M. incognita* which was almost 73.3 % disease suppression on cucumber. The NPK fertilization rate of 2.3, 3.4, and 2.3 g/pot on squash was the most suppressive and exhibited a suppression of 68.7% for the nematode infection. These results were in agreement with several investigators (Zinov'eva 1986, Ivezic *et al.*, 1997 and Silva *et al.*,1998). The use of bioproducts rather than the chemical nematicides was recommended by several investigators (El-Adwy *et al.*, 2001, Keren *et al.*,2000, Hammad, 1999, Heikal,2001 and Lashein, 2002) as an efficient and safer way for the nematode diseases control. In the present study, Nemaless, at 2.0 ml/pot (25cm diameter), was suppressive 71.3-78,2 % to *M. incognita* nematode infection on squash and cucumber. Meantime, the bioproduct Aegrin at 6.0 g/pot significantly suppressed the *M. incognita* nematode infection on both cucumber and squash by 71.7% and 58.83%, respectively. Moreover, the suppressive effect of Aegrin and Nemaless was not significantly different from that of the chemical nematicides Furazed tested which gave the advantage for the use of the bioproducts for the nematode diseases management. It was also notable that the use of such bioproducts was of an enhancement effect for the growth characteristics of both cucumber and squash assessed in terms of the plant height, the plant fresh weight and the plant dry weight, which was in agreement with records of several investigators (Youssef, 1993, Walteres and Wehner, 1997, Radwan *et al.*, 2004, Heikal, 2008). The presented study emphasized the use of the resistant cultivars, the balanced fertilization, and the natural bioproducts as effective components for the nematode diseases management.

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الملخص العربي

استخدام الأصناف المقاومة و التسميد بالعناصر الكبري و المركبات الحيوية لمقاومة النيماتودا التي تصيب الخيار و الكوسة في الأراضي المستصلحة حديثا

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تم عزل وتعريف أجناس عديدة من النيماتود الممرضة للنبات من نباتات خيار وكوسة مصابة بالذبول في الاراضى المستصلحة حديثا في محافظة البحيرة. وكان جنس الميلويدوجين *Meloidogyne* أكثر الأجناس النيماتوديه تواجدا حيث تم عزله بنسبة 30,75% من اجمالي عينات الخيار والكوسه. يلي ذلك جنس براتيلنكس *Pratylenchus* ، تايلنكس *Tylenchus*، داي تيلنكس *Ditylenchus*، هيليكوتيلنكس *Helicotylenchus* ترايكودورس *Trichodorus* بنسب 17,19% ، 16,61% ، 12,82% ، 11,24% ، 11,14% علي التوالي علاوة علي ذلك فقد تواجدت اجناس افيلنكس *Aphelenchus*، افيلنكويدز *Aphelenchoides* ، روتيلنكس *Rotylenchus* ، زيفينما *Xiphinema* ، تلينكو رينكس *Tylenchorhynchus* بنسب اقل من ١٠%. وفي نفس الوقت تم عزل فطر الفيوزاريم *Fusarium* من النباتات المصابة بالذبول بنسب60% و 45% من نباتات الخيار و الكوسة علي التوالي. يلي ذلك فطر ريزوكتونيا سولاني *Rhizoctonia solani* بنسب 16%و13% علي التوالي. وقد أثبتت النتائج وجود ارتباط بين الاصابه بفطر الفيوزاريم و شدة الاصابه بنيماتودا تعقد الجذور علي نباتات الخيار و الكوسه. كما اثبتت الدراسة ان صنف الخيار امريكانا بيتا الفا و صنف الكوسة الاسكندريه نوباسيد كانا أكثر الأصناف المختبرة تحملا للاصابه بكل من نوعي نيماتودا تعقد الجذور *M .incognita , M javanica*. وكذلك الأكثر تحملا للاصابه بالذبول الفيوزاريومي، مما أدي إلي تثبيط تكون العقد الجذريه النيماتوديه بنسبه أكثر من 60% مقارنة بالأصناف الحساسة. في نفس الوقت إدي التسميد بالعناصر الكبري (الآزوت،الفسفور،البوتاسيوم) بمعدلات 4,6 ، 5,7 ، 1,1 جم / أصيص لزراعات الخيار، معدلات 2,3 ، 3,4 ، 2,3 جم/ اصيص في زراعات الكوسه الي تقليل الاصابه بنيماتودا تعقد الجذور *"M. incognita"* كمتوسط لعدد العقد النيماتوديه ، و عدد كتل البيض بنسبة 73,3% ، 68,7% علي كل من زراعات الخيار و الكوسة علي التوالي. كما أكدت الدراسات ان استعمال المركبات الحيوية نيمالس واجرن قد أدي إلي خفض نسبة الاصابه بالنيماتودا ميلويدوجين انكوجنيتا وان المركب الحيوي نيمالس عند استعماله بمعدل2مل/أصيص كان أكثر فاعليه حيث أدي إلي تثبيط الاصابه بنسبة78,2% ، 71,35% علي الخيار و الكوسة علي التوالي . كما ان استخدام الأجرين بمعدل6 جم/أصيص أدي ألي تثبيط الإصابه بنسبة 71,7% ، 85,83% علي الخيار و الكوسة علي التوالي. علاوة علي ما تقدم فان النتائج المتحصل عليها قد أثبتت انه لا توجد فروق معنوية بين استخدام المركبات الحيوية المختبرة و المبيد النيماتودي الكيماوي فيورازد. وقد أثبتت الدراسة أن استعمال المركبات الحيوية قد أدي إلي تحسين الصفات الزراعية والمحصوليه لكل من الخيار و الكوسة.