J. Agric. Sci. Mansoura Univ., 31 (10): 6761 - 6770, 2006

IMPACT OF CERTAIN ORGANIC AMENDMENTS, *Bacillus thuringiensis* AND OXAMYL ON *Tylenchulus semipenetrans* INFECTING LEMON PLANT

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ABSTRACT

Greenhouse experiment on the influence of dried leaf powder of marigold or horse manure or *Bacillus thuringiensis* in comparison with oxamyl against citrus nematode, *Tylenchulus semipenetrans* infecting lemon plant, *Citrus Limon L* under greenhouse conditions revealed that oxamyl surpassed all treatments tested in increasing plant growth parameters with values of 166.02% and 131.03% for total plant fresh weight and shoot dry weight, respectively. Meanwhile, *B. thurigiensis* ranked second to oxamyl and accomplished the best performance in improving fresh weight of whole plant and shoot dry weight, followed by horse manure application and then marigold powder comparing to nematode alone. On the other hand, oxamyl treatment also surpassed other treatments tested in suppressing nematode population (73.02%) followed descendingly by *B. thuringiensis* (72.37%) and then horse manure (69.54%) and marigold powder (68.98%).

Moreover, all tested components showed significant increase in N, P, and K concentrations in shoot of lemon plants exceeding those of nematode alone. Moreover, the previous treatments clearly increased the total chlorophyll content of lemon plant with range from 80.72% for nematode alone to 2.18% for marigold powder. Moreover, nematode infection without any component added showed the highest record of total chlorophyll content (1996.5 mg/g) as compared to all materials tested .

Keywords:*Bacillus thuringiensis*, Chlorophyll, Citrus nematode, Horse manure, Lemon plant, Marigold powder, Organic soil amendments, Oxamyl.

INTRODUCTION

Citrus trees are considered to be the most produced fruit trees in Egypt and all over the world. About 339533 feddans are grown in Egypt producing 2350247 tons per year (Saad Allah and Meleegy, 2003).

Several plant parasitic nematodes are recorded to attack the root system of citrus plants. The citrus nematode, *Tylenchulus semipenetrans* (Cobb) is the most important one. Infestation by this can result in the disease named " slow decline ". The occurrence of high populations of this nematode caused the encrusted appearance of the root due to soil particles that adhere to egg matrix (dirty roots). However above ground symptoms are wilting, poor fruit production and die back (Jenkins and Taylor, 1967).

Losses caused by citrus nematode can be decreased effectively by adding certain chemical pesticides to the soil. However, environmental, health problems and disturbance in the biological balance of nature that caused as a result of extensive use of nematicides have enhanced scientists to search for another alternative nematode management.

Biological control of plant parasitic nematodes with natural plant extracts and products, animal wastes and microbial agents is an alternative methods. Recently, this trend is receiving greater interest among nematologists, providing effective control against the target nematode and avoiding environmental pollution (Babatola and Oyedunmade, 1992; Stephan, 1995; Nahar *et al.*,1996; Prasad *et al.*,1972; Abd El-Gawad, 1995; Allam, 2000; El-Saedy *et al.*, 2001; Ismail and Fadel, 2004; Hammad, 2005).

Stephan (1995) determined the efficacy of nematicides and horse manure in controlling *Meloidogyne javanica* on tomato and eggplant. He found that cadusafos (6 g/m²), liquid fenamiphos (5 g/m²) and dichlofenthion (20 g/m²) were the most effective in increasing the yield and reducing nematode infection followed by horse manure. Nahar *et al.* (1996) reported that amendment of soil with all the organic amendments i.e *Tagetes* sp., poultry manure, pigeon manure, poultry + pigeon manure and mustard oil cake reduced root-knot nematode severity and improved growth of tomato plants. Prasad *et al.* (1972) reported the production of a nematicidal toxin by *B. thuringiensis* var. *thuringiensis*. They found that toxin was capable of causing mortality of *M. incognita* juveniles. The toxin produced in the culture medium was described as thermostable and very potent being active even at a 10 fold dilution. These toxins also inhibited root-knot nematode egg hatching. In an evaluation of *B. thuringiensis* for control of phytonematodes, *Helicotylenchus* spp. and *T. semipenetrans*, Abd El-Gawad (1995) found that the nematode suspension treated with *B. thuringiensis* delta- endotoxin had significantly greater numbers of the dead nematodes than water control. Allam (2000) showed that the bioproduct (Agerin) was effective against *T. semipenetrans* on navel orange trees and *M. incognita* and *M. arenaria* on tomato and eggplant. The bioproduct, Agerin and the tested nematicides (Mocap 10% G,Temik 10 %G and Vydate 24% L) were effective in reducing the damage caused by *T. semipenetrans* and *M. javanica* and improving the growth of Washington navel orange and Williams banana plants and their productivity (El-Saedy *et al.*, 2001). Ismail and Fadel (2004) studied the efficacy of three isolates of *B. thuringiensis* at 6.0, 9.0 and 12.0 g/tree (equivalent 1.0, 1.5 an 2.0 kg/feddan) against *T. semipenetrans* on navel orange under field conditions. All doses of the B.t. isolates significantly ($P \leq 0.01$) reduced the nematode population in soil as compared to untreated control. Hammad (2005) evaluated the bio-control activity of supernatant and pellet of B.t. isolate 3 against *M. incognita* on eggplant and indicated that treatment with B.t isolate 3 supernatant significantly reduced number of nematode galls by about 67% and egg-masses by 74%, in comparison with the control.

Therefore, the objective of the persent work was to evaluate and compare the effectiveness of leaf dried powder (marigold), animal manure (horse) Protecto product (*Bacillus thuringiensis*) as a biological agent in comparison with oxamyl as well as N, P, and K ; and total chlorophyll contents status of lemon plant *Citrus limon*.L infected with *T. semipenetrans*, its development and plant growth response under greenhouse condition (17±5 °C) .

MATERIALS AND METHODS

Second stage juveniles of *T. semipenetrans* were obtained from a pure culture propagated on sour-orange, *Citrus aurantium* in the greenhouse of Nematology Research Unit, Agricultural Zoology Department, Faculty of Agriculture, Mansoura University. Nematodes were extracted from soil by sieving and modified Baermann technique (Goodey, 1957).

In this experiment, dried leaf powder of marigold *Tagetes erecta*, and horse manure as soil amendments in comparison with *B. thuringiensis* (Protecto)as biological agent and oxamyl were tested for controlling *T. semipenetrans* on lemon seedlings, *C. limon* under greenhouse conditions.

One dose of 10g of either dried leaf powder of marigold or sun-dried horse manure was separately mixed with 890g steam-sterilized sandy loam soil (1:1) per plastic pots 10cm-diameter watered and kept moist to facilitate proper decomposition of soil components and left on a greenhouse bench for one week. A total of eighteen lemon seedlings (one year-old) was then transplanted to pots (one each) that were filled with sterilized sandy loam soil (1:1).

Fifteen plastic pots were then inoculated with 2000 second stage juveniles of *T. semipenetrans* (N), three of them received a 0.3 ml of oxamyl (Vydate24% E.C) each and *B. thuringiensis* at the rate of 0.01g/pot for another three pots. The inoculated seedlings and those free of any treatment were served as control while the rest seedlings with neither nematode nor any treatment were used for comparison (Ck).

♦Treatments were as follows:

1- N + *B. thuringiensis*,
2- N + Horse manure,
3- N + Marigold,
4- N + Oxamyl,
5- N alone (control) and
6- Plants free of N or any treatment.

Each treatment was replicated three times and randomly arranged on a greenhouse bench at 17±5 °C. Plants were received water as needed. During the period of the experiment, plants were protected against mite and insect attack by conventional pesticides. Sixty days after nematode inoculation, plants were harvested. Data dealing with length and fresh weights of shoots and roots and shoot dry weight were also measured and recorded.

Infected lemon roots were stained in 0.01 hot acid fuchsin in lactic acid (Byrd *et al.* 1983), examined for the number of developmental stages and females. Nematodes were then extracted from soil by sieving and modified Baermann technique (Goodey, 1957).

Regarding to N, P, and K determination, 0.2g of dry lemon seedling shoot was subjected to chemical analysis. Total nitrogen content was determined by Kjeldahl method (A.O.A.C, 1980) modified by distilling the ammonia into saturated boric acid solution and titration with (0.1 NaCL) standard. Total phosphorus was colorimetrically determined using the

chlorostannous reduced Molybdophosphoric Blue colour method, while total potassium was Flams photometry estimated as described by Jakson (1967).

Chlorophyll content was spectrophotometrically measured in leaves of the harvested plants using Fadeel's method (1962) Chlorophyll concentration were calculated according to Wellburn and Lichtenthaler (1984). The content of chlorophyll was then expressed in ug/g. F. wt. of the leaves.

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) followed by Duncan's multiple-range test to compare means at $P< 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Data in Table (1) documented plant growth of lemon seedlings, *C. limon* infected with *T. semipenetrans* as influenced by the addition of dried powdered leaves of marigold, *T. erecta* or horse manure or *B. thuringiensis* (with the trade name, Protecto) in comparison with oxamyl under greenhouse conditions at 17 ± 5°C. It is clear that all tested components obviously improved the fresh weight of whole plant as well as shoot dry weight percentages.

Table 1: Plant growth response of lemon plants infected with *Tylenchulus semipenetrans* treated with certain organic amendments; *Bacillus thuringiensis* (Protecto) and oxamyl under greenhouse conditions (17 ± 5°C).

Treatments	* Plant growth response							
	Length (cm)		Fresh weight (g)		F.wt.of whole plant (g)	% of increase	Shoot dry wt. (g)	% of increase
	Shoot	Root	Shoot	Root				
B.t. + N	14.03 bc	20.92 a	1.89 a	0.74b	2.63a	155.33	0.65 a	124.13
Horse manure+ N	14.23 bc	17.96 ab	1.71ab	0.78 b	2.49ab	141.74	0.55 ab	89.65
Marigold+ N	12.97 bc	18.40 a	1.35 ab	0.71 bc	2.06ab	100.00	0.42 b	44.82
Oxamyl+ N	15.97 a	19.33 a	1.92 a	0.82 a	2.74ab	166.02	0.67 a	131.03
N. alone	11.23 c	14.17 c	0.63 c	0.40 c	1.03 c	--------	0.29 c	-------
Plants free of N and untreated	12.00 c	16.83 ab	0.78 bc	0.39 c	1.17 c	---------	0.36 b	-------

N= *T. semipenetrans* at 2000 second stage juveniles (J2)/Pot.
B.t.= *Bacillus thuringiensis*
*** Each number presented the mean of three replicates.**
Means in each column followed by the same letter did not differ significantly at P< 0.05 according to Duncan's multiple-range test.

Apparently, oxamyl surpassed all other treatments in increasing plant growth parameters with values of 166.02% and 131.03%, respectively as compared to nematode alone (Table 1 & Fig. 1, 2). Meanwhile *B. thuringiensis* (Protecto) ranked second to oxamyl, whereas it accomplished the best performance in improving fresh weight of whole plant and shoot dry weight, followed by horse manure application with values of 155.33% and 124.13%; and 141.74% and 89.65%, respectively, and then marigold powder

with values for the same plant growth parameters amounted to 100% and 44.82%, respectively as compared to nematode alone.

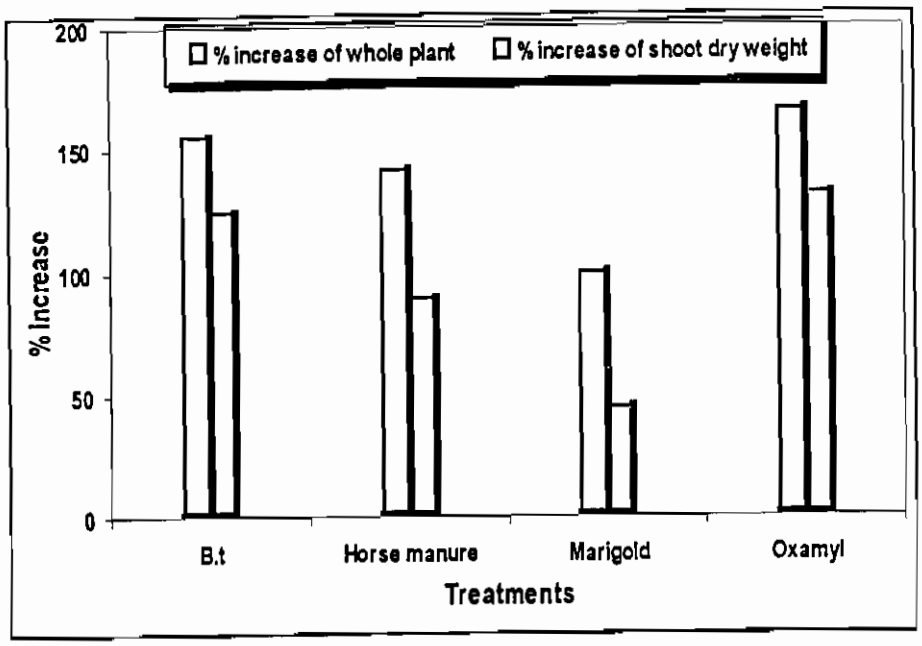

Fig. (1): Plant growth response of lemon infected with *Tylenchulus semipenetrans* treated with certain organic amenders; B.t. (Protecto) and oxamyl under greenhouse conditions (17±5°C).

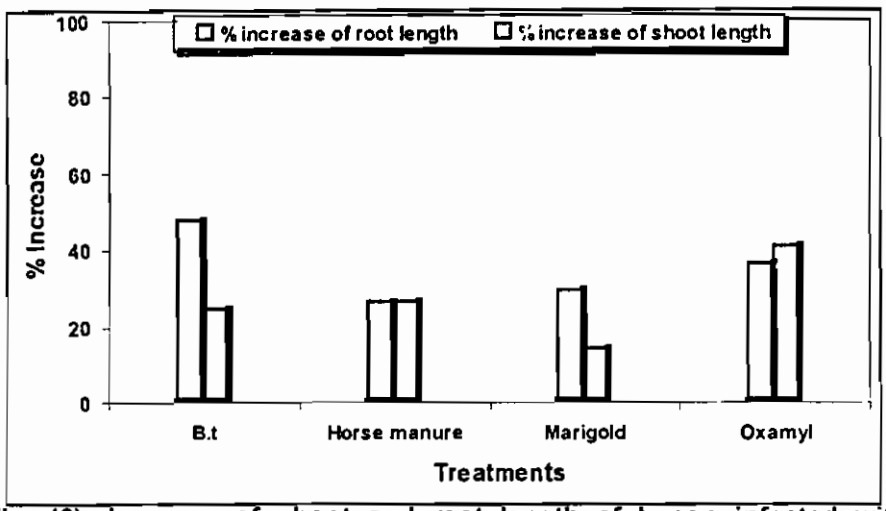

Fig. (2): Increase of shoot and root length of lemon infected with *Tylenchulus semipenetrans* treated with certain organic amenders; B.t. (Protecto) and oxamyl under greenhouse conditions (17±5°C).

Data presented in Table (2) showed the impact of horse manure, marigold powder, *B. thuringiensis* (Protecto) and oxamyl on nematode population of *T. semipenetrans* infecting lemon plants under greenhouse conditions at (17 ± 5°C). Results indicated that all tested treatments significantly reduced the total number of juveniles and males in soil; and females in root as well as nematode population densities (Table 2& Fig. 3) as compared to pots received nematode alone.

Table 2: Influence of horse manure, dried leaf powder of marigold as organic amenders, and *Bacillus thuringiensis* (Protecto) in comparison with oxamyl on percentage of reduction of *Tylenchulus semipenetrans* under greenhouse conditions (17 ± 5 °C).

Treatments	* Nematode population in		Final population (Pf)	% Reduction
	Soil	Root		
	Juveniles + Males	Females		
B.t. + N	800.00 b	20.67 b	821.33 b	72.37
Horse manure + N	879.33 b	26.33 b	905.67 b	69.54
Marigold + N	894.00 b	28.33 b	922.33 b	68.98
Oxamyl + N	784.67 c	17.33 b	802.00 b	73.02
N. alone	2930.00 a	43.00 a	2973.00 a	----

N= *T. semipenetrans* at 2000 second stage juveniles (J2)/Pot.
B.t.= *Bacillus thuringiensis*
* Each number presented the mean of three replicates.
Means in each column followed by the same letter did not differ significantly at P< 0.05 according to Duncan's multiple-range test.
% of nematode reduction = $\frac{\text{N alone} - \text{N of each treatment}}{\text{N alone}} \times 100$

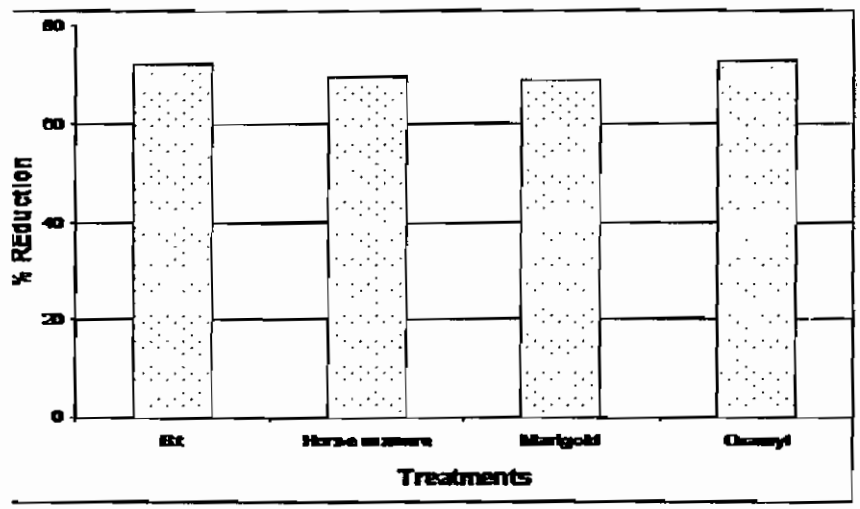

Fig. (3): Influence of horse manure, dried leaf powder of marigold as organic amenders,and B.t. (Protecto) in comparison with oxamyl on percentage of reduction of *Tylenchulus semipenertrans* under greenhouse conditions (17±5°C).

Data presented in Table (3) showed the impact of certain organic amendments i.e. horse manure or marigold dried leaf powder or the bio-control agent, *B. thuringiensis* (Protecto) in comparison with oxamyl on N, Pand K concentrations as well as total chlorophyll content in lemon plants infected with *T. semipenetrans* under greenhouse conditions at 17 ± 5°C. It was evident that nitrogen and potassium concentrations were remarkably reduced by nematode infection. Moreover, all tested treatments showed significant increase in N, P, and K concentrations exceeding those of nematode alone. The highest increment in nitrogen concentrations was achieved by the applications of oxamyl (124.13%), followed by horse manure (78.81%) and the bio-control agent, *B. thuringiensis* (68.48%), whereas, marigold powder gave the least value (25.94%) . However, the bio-control agent with value of 90.08% ranked second to oxamyl with value of 135.5% in increasing phosphorus concentration, followed by horse manure (40.49%) then marigold (19.63%). Similar trend was noticed with potassium concentrations with values of 175.92%, 158.31%, 70.9% and 58.51%, respectively. With respect to the effect of nematode infection to lemon plant in pots received oxamyl or *B. thuringiensis* or horse manure or marigold powder as well as nematode alone on the total chlorophyll content, results indicated that all of the previous treatments obviously increased the total chlorophyll content of lemon plants with range from 1996.5 mg/g (for nematode alone) to 1128.9 mg/g (for marigold powder). Moreover, nematode infection without any component added showed the highest record of total chlorophyll content (1996.5 mg/g) as compared to all materials tested (Table 3).

Table 3: Influence of *T. semipenetrans* infection to lemon plant treated with horse manure, dried leaf powder of marigold, and *Bacillus thuringiensis* (Protecto) and oxamyl on the content of N, P, K and chlorophyll A, B under greenhouse conditions (17 ± 5°C).

Treatments	* N mg/gm	* P ppm	* K ppm	* Chlorophyll content		Total. mg/g
				A mg/g	B mg/g	
B.t. + N	32.60 a	0.46 ab	39.60 a	769.50 b	497.50 b	1267.00
Horse manure + N	34.60 a	0.34 b	26.20 ab	756.00 b	394.00 c	1150.00
Marigold + N	24.37 ab	0.29 bc	24.30 bc	707.30 b	421.60 b	1128.90
Oxamyl + N	43.37 a	0.57 a	42.30 a	831.30 b	429.33 b	1260.63
N. alone	19.35 b	0.24 c	15.33 c	1233.00 a	763.50 a	1996.50
Plant free of N and untreated	40.53 a	0.26 c	36.33 a	641.70 c	463.00 b	1104.70

N= *T. semipenetrans* at 2000 second stage juveniles (J2)/Pot.
B.t.= *Bacillus thuringiensis*
*** Each number presented the mean of three replicates.**
Means in each column followed by the same letter did not differ significantly at P< 0.05 according to Duncan's multiple-range test.

Regarding the impact of *B. thuringiensis* (Protecto) as biological agent in comparison with dried leaf powder leaves of marigold or horse manure or oxamyl against *T. semipenetrans* infecting lemon plant, *C. limon*

L. *B. thuringiensis* (Protecto) ranked second to oxamyl and accomplished the best performance in improving plant growth parameters, followed by horse manure and then marigold powder. Similar trend was obtained for suppressing citrus nematode population. These results were in accordance with those reported by El-Saedy *et al.*, (2001) in respect to *B. thuringiensis* (Agerin) on *M. javanica* and *T. semipenetrans* infesting Washington navel orange and Williams banana plants; and Ismail and Fadel, (2004) in respect to B.t. isolates on citrus nematode; and Hammad, (2005) in respect to B.t. isolates 3 (supernatant and pellet) on root-knot nematodes infecting eggplant roots.

Moreover, results of the present investigation could be attributed to the presence of nitrogen in the organic amenders i.e. marigold powder or horse manure or the metabolites produced by *B. thuringiensis* (Aronson *et al.*, 1986) as well as the production of some growth regulators that involved in root absorption area. Also, the production of a nematicidal toxin by *B. thuringiensis* var. *thuringiensis* which inhibited to root-knot nematode eggs may be involved (Prasad *et al.*, 1972).

A remarkable increase in N.P.K. concentrations in shoot of lemon plants infected with citrus nematode achieved by marigold powder or horse manure or *B. thuringiensis* or oxamyl as compared to nematode alone. These results agree with the findings reported by Nour-El-Deen, (2002) in respect to *M. incognita* on peach plant. Moreover, pots received 0.01g *B. thuringiensis* showed excess of NPK contents in shoot of lemon plants infected by *T. semipenetrans*, a situation which revealed the highest values of percentage increase for plant fresh weight and shoot dry weight (155.33% and 124.13%) respectively with percentage of nematode reduction reached 72.02%. This result was also in harmony with those of Midan *et al.*, (1985) who stated that excess of potassium in plants diminished the average severity of tomato roots infected with nematode to the lowest limit.

An increment in total chlorophyll content was accomplished by *B. thuringiensis* followed by oxamyl, horse manure and then marigold application, whereas, citrus nematode infection alone surpassed all treatments tested in possessing the highest value of total chlorophyll for shoot of lemon plants. These results were supported by Audebert *et al.*, (2000) who reported that leaf chlorophyll content was greater in higher nematode density plots during their study on the influence of cyst nematode, *Heterodera sacchari* on the water status and growth of rice in Côte d' Ivoire. Moreover these results were reported by Nour-El-Deen, (2002) in respect to *M. incognita* on peach plant.

Obviously, the present investigation showed that the nematicidal activity of the tested soil amendments and the bacterium *B. thuringiensis* as biological agent against citrus nematode on *C.limon* can be varied from component to another. These variations may be attributed to the differences in the chemical nature, composition, concentration of toxic compounds present in these tested materials and methods of application used. The safety of such materials and its low cost is one of its advantage. However, additional researches are needed using plant or animal wastes and the biological agent

bacterium, *B. thuringiensis* in microplot and field experiments before using effectively in integrated pest management (IPM).

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تأثير بعض الاضافات (المحسنات) العضوية و بكتريا "باسيلس ثرونجينسيس" و الاوكساميل على نيماتودا الموالح التى تصيب نباتات الليمون.

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تم دراسة تأثير بعض المواد العضوية مثل مسحوق اوراق القطيفة الجافة و مخلف الخيل و مسحوق بكتيريا"باسيلس ثرونجينسيس" بالمقارنة مع مبيد الأوكساميل على نيماتودا الموالح التى تصيب نباتات الليمون تحت ظروف الصوبة عند درجة حرارة ١٧±٥م حيث أظهرت النتائج ان كل المواد المختبرة ادت الى تحسن ملحوظ و معنوي في مقاييس نمو نباتات الليمون حيث كان مبيدالأوكساميل متفوقا في ذلك واحتلت معاملة البكتريا المرتبة الثانية للمبيد فى الزيادة لهذه المقاييس النباتية ويليها في ذلك معاملة مخلف الخيل ثم معاملة مسحوق القطيفة مقارنة بالنباتات المصابة الغير معاملة كما أدت جميع المعاملات إلى خفض واضح في تعداد النيماتودا في التربة والجذر تتراوح ما بين ٦٨,٩% إلى ٧٣,٠٢% و كان أعلى معدل نقص في تعداد النيماتودا من معاملة مبيد الأوكساميل يليه في ذلك معاملة البكتريا ثم المخلف الحيواني (الخيل) والنباتي (القطيفة) مع عدم وجود أي اختلاف معنوي بينهم على التوالي . كما أدت كل المعاملات المختبرة إلى زيادة معنوية فى نسبة النيتروجين و الفسفور و البوتاسيوم عن تلك الخاصة بالنيماتودا بمفردها. بجانب زيادة المحتوى الكلى للكلوروفيل في أوراق نبات الليمون بمعدلات تتراوح بين ٨٠,٧٢% للنيماتودا بمفردها إلى ٢,١٨% لمسحوق القطيفة كما كان المحتوى الكلى للكلوروفيل في أعلى قيمة وهى ١٩٩٦,٥ مليجرام/ جرام في حالة الإصابة بالنيماتودا لنباتات الليمون بدون إضافة أى من المعاملات المختبرة .