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STUDIES ON SURVEYING NEMATODES, HOST SUITAPILITY AND CONTROL OF *Meloidogyne incognita* ON GRAPEVINE BY CERTAIN COMPOUNDS WITH REFERENCE TO NEMATODE INVESTATION AND YIELD.

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ABSTRACT

Percentage of frequency occurrence, population density and prominence value were determined for the plant parasitic nematodes associated with some grape varieties in Behira governorate. Data revealed that stunt nematodes *Tylenchorhynchus* spp. and the root Knot nematode *Meloidogyne* spp. were found with highest prominence value on Flame, King-Ruby, Perlette, Superior and Thompson varieties as compared to other nematode genera. Host suitability of varieties Early Superior, Flame, King Ruby, Perlette, Superior and Thompson to infection of root- knot nematode *M. incognita* was tested under greenhouse conditions. The varieties Early Superior, Flame and Superior were rated as susceptible hosts. The relationship of grapevine yield to *M. incognita* densities was studied. Five grapevine varieties were tested to carry out the experiment in South Tahrir. Association between *M. incognita* densities and yield was variable, even when the vines were separated according to vigor of vines, varieties and yield. The highest density of *M. incognita* was associated with the medium yield while the low densities of *M. incognita* associated with the low production of all varieties except Thompson variety. The result showed that the high production trees were more infected than low production trees in the same variety as shown with King Ruby and Flame. Biological treatments i.e. Diple 2x, EM1 and mycorrhyzal fungi (*Glomus* spp.) as well as certain organic soil amendments namely chopped garlic cloves, (*Allium sativum*) and dried powdered leaves and stems of liquor ice (*Glycyrrhiza globra L.*) and chamomile (*Matricari chamomile*) were examined for controlling *M. incognita* infecting Superior variety. Results indicted that all treatments showed significant effect in reduction of galling and reproduction of *M. incognita* on grapevine as compared to untreated trees. However the application of chopped garlic cloves at the rate of 80 gm/ tree gave the highest reduction in galling and reproduction of *M. incognita* was followed by mycorrhyzl fungi (*Glomus* spp.) at the rate of 1ml/ 100ml water as compared to control treatment while the least effect was recorded with dried powder of chamomile plant at the rate of 40 gm / tree . The most tested treatments significantly reduced nematode reproduction of *M. incognita* in soil and roots of grapevine cv. Superior compared to the untreated check.

Keywords: Grapevine, *Meloidogyne incognita*, Survey , host- suitability, infestation and yield and control.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is considered one of the most important economic fruit crops in Egypt. Plant parasitic nematodes especially root- knot nematodes are known as pests of grapevine mainly in tropical and subtropical areas of the world. The root – knot nematode *Meloidogyne* spp. have been recognized as a potential serious problem to the grapevine productivity. The effect of *Meloidogyne* spp on the growth and yield of vine yards was studied in Egypt (EL-Gindi *et al.*, 1976). (Annonymous, 1986) indicated that nematodes are a particular problem in grapes.

Many attempts has been conducted in most grape production areas around the world to assess the amount of damage caused by nematodes. The root-knot nematodes *M.ncognita, M. javanica, Tylenchorhynchus* spp., *Tylenchulus semipentrans, Xiphinema americanum* and *Helicotylenchus* spp. were recorded associated with grapevine at high frequencies occurrence and population densities (Riad, 1982a; Loubser and Meyer, 1988a). Suitability of grape varities to root - knot nematode *M. incognita* infection was studied by many investigators. The varieties King Ruby, Flame Superior and Romy Ahmer were susceptible, while Cleoptra and Thompson varieties were rated as resistant and moderately resistant respectively . (Riad *et al.*, 1982b; Melakeberhan and Ferris, 1987and Mahmoud, 2003). These studies also were oriented to determine the relation ship of grapevine yield to root knot nematode, *M. incognita* densities (Ferris and Mckenry, 1975; Quader *et al.*, 2002; and Korayem, 2006).

The use of chemicals to control phytoparasitic nematodes has always been an expensive remedy and may also reduce populations of beneficial antagonistic microorganisms in soil. However, great interest has given among nematologists to use alternative nematode management practices because of environmental and health problems associated with nematicide use. The nematicidal activity of certain plant products applied in soil as organic amendment including medicinal plant were suppressed the build up of root – knot nematode *M. incognita* (Gupta and Sharma, 1993; Ali, 1994; Ali *et al.*, 1997; Shady, 2001; Shawky and Moisa 2005). The biological treatments i.e. EM1, Diple 2x and mycorrhyzal fungi were used as microbial pathogen to control root knot nematode *M. incognita* (Osman *et al.*, 1988; Carling *et al.*, 1989; Mena *et al.*, 1996; Zawam, 1999; Radwan *et al.*, 2004; and EL- Hadad, 2005).

The objective of the present study were to provide information on the occurrence and population density of the most common pathogenic nematodes associated with grapevine and to evaluate host suitability of certain varieties to the infection of *M. incognita*. The present investigation aimed also to evaluate the relations ship between the infection of root–knot nematode *M. incognita* and the yied of same grapevine varieties. The effect of certain biological compounds and some organic soil amendments to control *M. incognita* infecting grapevine in Egypt.

MATERIALS AND METHODS

1-Survey study:

Survey was carried out to determine the plant parsitic nemtodes associated with certain grapevine varieties at the newly reclaimed sandy soil of South Tahrir and to study the distribution and density of the root knot nematode *Meloidogyne* spp. Soil and root samples were collected from the rhizosphere of the varieties Flame, King Ruby, Perlette, Superior and Thompson. Aliquot of 250 gm soil sample was taken and soaked in tap water for nematode extraction by sieving through, 60 and 325 mesh sieves.

Nematode present in the suspension were collected from the fine sieve and extracted by the modified Bermann pan technique (Goody, 1963). The final volume of extracted suspension was descend to about 20 ml. Recovered nematode genera from each sample was counted under a microscope using the eelworm counting slide. Genera identification was based on morphology of adult and juvenile forms according to (Mai & Lyon, 1975) and (Siddiqui, 1986) . Percentage of frequency occurrence (%F.O), population density per 250 gm soil (P.D) and prominence value (P.V.) of the identified genera was calculated according to (Norton, 1978) .

2- Host Suitability of some grapevine varieties to *M. incognita* under greenhouse conditions:

Greenhouse test was conducted to determine the susceptibility of selected grapevine varieties to *M. incognita* .The tested varieties were Early Superior, Flame, King Ruby, Perlette, Superior and Thompson. To monthes old seedling of each grapevine variety grown in pot of 20 cm diameter and were filled with steam sterilized sandy loam soil and they were inoculated with 3000 newly hatched second stage juveniles of *M. incognita* . Eight pots were used for each variety, of which four pots were inoculated while others were left free and served as control . All pots were arranged in block design system. Four month after nematode inoculation plants were uprooted and root system were washed from adhering soil. Number of juveniles per pot, galls, egg masses, eggs per egg- mass and developmental stages per root system were counted. The nematode reproduction factor (RF) was calculated for each variety. Root gall index (R G I) were determined according to the scale given by (Taylor and Sasser, 1978) as follows: 0 = no galls, 1 = 1-2 galls, 2 = 3 – 10 galls, 3 = 11 –30 galls, 4 = 31 – 100 galls and 5 = more than 100 galls. Host suitability was measured according to the scale of (Hadisaeganda and Sasser, 1982) on the basis of root gall index as follows: RGI range of 0.0 – 1.0 = highly resistant (HR), 1.1- 3.0 = very resistant (VR), 3.1 – 3.5 = moderately resistant (MR), 3.6 - 4 = slightly resistant (SR) and 4.1 – 5 = susceptible (S). Host suitability was also measured by using the designations based on the relation between root gall index and nematode reproduction (R. Factor) according to (Canto – Saenz, 1983) as follows: (RGI ≤ 2 & R ≤ 1) resistant (R) , RGI ≤ 2 & R> 1) tolerant (T), RGI≥ 2 & R ≤ 1) hyper – susceptible (HS) and (RGI$\geq$ 2 & R$\geq$ 1) susceptible (S). Data were statistically analyzed using (F) test according to (Snedecor, 1966) and least significant differences between treatments were calculated at 5 %.

3- Relationship between *M. incognita* density and yield of certain grapevine varieties:

The study of the correlation between yield and nematode population was carried out in naturally infested orchard with *M. incognita* in Behira governorate and grown with certain grapevine varieties i.e. Flame, King-Ruby. Perlette, Superior and Thompson. Soil and root samples were collected from these varieties at the begining of the season. Five samples were obtained from each variety (three trees for each). The number of second stage juveniles per 250 gm soil and nematode population in 2 gm root were counted. Data on yield per feddan were estimated during fruit harvest of grapevine for each variety.

4- Effect of certain treatments in controlling *M. incognita* infecting grapevine under field condition:

This experiment was conducted in naturally infested orchard growing with cv Superior in Behira Governorate to determine the effect of some bioagents and organic soil amendments to control *M. incognita* . Seven trees were used for each treatment and other seven trees were served as control. Before application, samples were taken from all treatments to estimate the initial nematode population. The treatments of this experiment were as follow:

1- Diple 2x, Agriculture research center commercial bioproduct as bioagent containing an isolate of *Bacillus thuringiensis* was added to the soil at rate of 2 gm / 1 liter water.
2- EM1 (effective micro – organisms) commercial bioproduct containing group of micro organism include photosynthetic bacteria, lactic acid bacteria, yeasts, actinomyccetes and fungi (Mashhour, *et al.*, 2001) was added at the rate of 0.5 m1 /1L water.
3- Mycorrhyzal fungi (*Glomus* spp.) was added at the rate of 1 ml / 100 ml water.
4- Chopped garlic cloves at rates of 40, 60, 80 gm/ tree .
5- Dried liquorice powder was added at the rate of (40,60.80 gm / tree).
6- Dried chamomile powder (40,60.80 gm / tree).
7- Control treatment (untreated trees).

After four months soil and root samples were collected from the rhizosphere of the treated trees. the second stage juveniles of *m. incognita* were counted in 250 gm soil and galls, egg – masses, developmental stages/ one gm root and eggs / egg mass was recorded for each replicate. nematode reproduction factor (rf) and reduction in nematode population were estimated. data were subjected to statistical analysis according to (snedacor, 1966) and least significant differences between treatments were calculated.

RESULTS AND DISCUSSION

1- Plant parasitic nematodes associated with certain grapevine varieties:

Data in Table (1) show the list of nematode genera recovered from the rhizosphere of certain grapevine varieties in Behira Governorate. Resultes indicated that the genera were detected on the varieties, Flame, King-Ruby, Perlette, Superior and Thompson were *Meloidogyne, Tylenchulus, Tylenchorhynchus, Xiphinema* and *Longidorus.* The root-knot nematodes were determined with the highest frequency occurrence (%F.O.), population density (PD) on Flame and Superior followed by Perlette, and King-Ruby with frequency values of 73.5, 70.6, 64.7 and 58.8 and population densities in 250gmsoil of 62.4, 60.3, 53.5 and 60 respectively . The obtained results are in a good line with those reported by (Khan, 1988; Vadivelu *et al.,* 1992; Robians *et al.*, 1995 and Park – So Deuk, *et al.*, 1999). They indicated that root knot nematodes, *M. javanica* and *M. incognita* were found with high occurrence and high numbers in grapevine. They also reported that the predominant species on grapes was *M. incognita* .

2- Susceptibility of certain grapevine varieties to *M. incognita:*

Five grapevine varieties i.e., Early Superior, Flame, King – Ruby, Perlette, Superior and Thompson were tested for their susceptibility to root – knot nematode, *M. incognita* under green house conditions. Data in Table (2) demonstrated that the above mentioned varieties were considered as susceptible hosts according to (Canto- Saenz, 1983) . The varieties Perlette, Flame, Superior, and Early Superior were rated as susceptible while the varieties Thompson and King – Ruby were slightly resistant according to (Hadisoeganda and Sasser, 1982). The present results are in harmony with the findings reported by (Hassan, 1985; Melakeberhan *et al* ., 1988 and Mahmoud, 2003) who showed that King Ruby, Flame and Superior were susceptible to *M. incognita* while Thompson was moderately resistant.

3-Relationship between grapevine yield and nematode densities:

Relationship between the population densities of *M. incognita* and the yield of certain grapevine varieties was tested under filed conditions. Data in Table (3) and Fig. (1) revealed the relation between *M. incognita* density and the yield of the varieties Flame, King –Ruby, Perlette, Superior and Thompson. The results indicated that the yield was affected by the density of inoculum of *M. incognita*, but this effect deferred according to the varieties. These results can be explained by (Ferris and Mckenry, 1975 and Quader *et al.*, 2002) who illustrated that correlations between *M. incognita* densities and vine performance were variable and the damage threshold of the noot –knot nematode for grapevine will vary between varieties. The results showed that the highest density of *M. incognita* was correlated with the medium production while the low yield trees had less number of nematode density in all varieties except Tomson variety.

It means that the high production trees were more infected with *M. incognita* than low production trees in the same variety as shown with King-Ruby and Flame. This correlation is not clear in the cases of Superior and Perlette. This results can be explained on the base that the low production trees had very weak root system and less root feeders than the root system of the high production trees which can able to carry more nematode reproduction.

Table (1): Percentage of frequency occurrence, population density and prominence value of plant parasitic nematodes associated with certain grape varieties in Behira governorate

Nematode genera	Flame			King-Ruby			Superior			Perlette			Thompson		
	%F.O	P.D	P.V	%F.O	P.D	P.V	%F.O	P.D	P.V	%F.O	P.D	P.V	%F.O	P.D	P.V
Meloidogyne	73.5	624	534.9	58.8	60	460	70.6	60.3	506.7	64.7	53.5	430.3	26	25.6	130.5
Tylenchulus	35.3	31.7	188.3	25.9	22.2	112.9	32.4	45.5	259	2.7	33.3	173	17.7	11.4	48
Tylenchorhynchus	47.0	58.8	403.1	38.2	33.8	209	41.2	48.6	311.9	40.8	32.9	210.1	15	9	34.9
Xiphinema	26.5	31.1	160.0	20.6	25.7	116.6	20.4	32	173.5	14.7	20	76.7	14.7	8.2	31.4
Longidrus	17.7	16.7	70.2	14.6	16	61.1	23.5	18.8	91.1	11.8	15	51.5	8.8	6.5	19.3

$$\text{Population density (P.D)} = \frac{\text{Total number of individuals of a genus}}{\text{Number of samples containing this genus}}$$

$$\text{Frequency occurrence (\%F.O).} = \frac{\text{Number of samples containing a genus}}{\text{Number of collected samples}} \times 100$$

$$\text{Prominence value (P.V.)} = \text{population density} \times \sqrt{\text{frequency occurrence}}$$

Table (2): Host suitability of certain grape varieties to *M. incognita* infection under greenhouse conditions

Varieties	No. of 2Js/pot	No. of galls /root system	*R. G.I.	No. of egg-masses /root system	No. of eggs/ eggmass	No. of developmental stages / root	R. factor	Host category	
Early Superior	2 5553	495.0	5.0	174	330	737	20.9	S*	S**
Flame	5707.0	363.0	5.0	130	302	879	15.3	S	S
King Ruby	2533.0	56.0	4.0	92	268	201	9.2	SR	S
Perlette	4586.0	181.0	5.0	106	283	534	11.7	S	S
Superior	4946	214.0	5.0	111	311	566	13.3	S	S
Thompson	1654.0	52.0	4.0	38	185	205	3.0	SR	S
L.S.D at 0.05%	1965.9	42.7	0.0	78.03	2107.9	2.9	3.04		

$$\text{R Factor (indicator of nematode reproduction)} = \frac{\text{Nematode final population}}{\text{Nematode initial population}}$$

Host suitability based on

* Root gall index (R.G.I.) according to Hadisoegande &Sasser (1982)

** Root gall index and R factor according to Cant- Saenz (1983)

S = Susceptible, SR = slightly resistant*

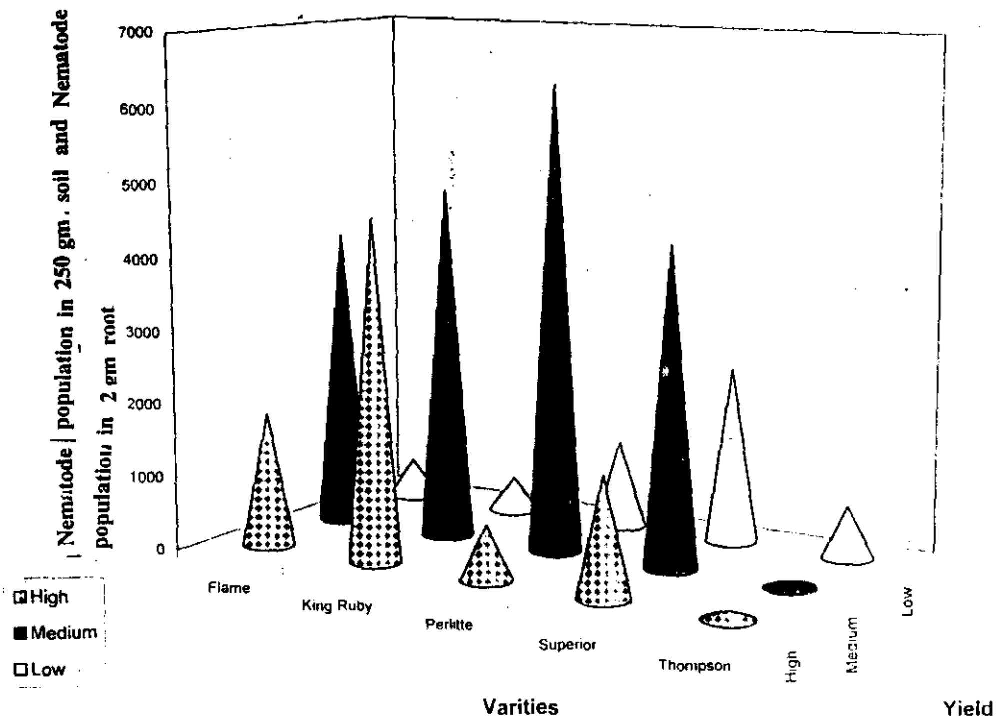

Fig.(1): Relation between yield / fedden of certain grapevine varieties and population nematode *Meloidogyne incognita*

Table (3): The effect of the infection of root knot nematode *Meloidogyne incognita* on the yield of certain grapevine varieties.

Varieties	No. of nematode in 250gm soil	No. of nematode in 2gm root	Yield (ton / faddan)
Flame	1158	656	11.3
	2617	1488	9.3
	339	193	4.8
King-Ruby	2970	1689	12.5
	3090	1757	9.5
	300	170	5.2
Perlitte	465	264	9.7
	4050	2302	6.0
	750	426	4.0
Superior	1027	584	14.2
	2766	1573	10.6
	1534	872	6.3
Thompson	74	45	10.7
	48	27	8.5
	450	256	6.1

4- Effect of some biological compounds, mycorrhyzal fungi and certain organic soil amendments in controlling root–knot nematode, *M. incognita* investing grapevine under field conditions;

Data in Table (4) revealed the effect of all treatments either biological or organic soil amendments on the reproduction of *M. incognita* infesting grapevine cv Superior. All the treatments reduced nematode population. The application of chopped garlic cloves (80 gm / tree) gave the highest reduction percentage in nematode population of *M. incognita* in soil and roots as compared to the other treatments. The reduction percentage was 85%. Compared to control. This result is in harmony with those reported by (Sukul *et al.*, 1974; Rhode, 1978; Ali, 1994 ; Shady, 2001 and Shawky, 2005). They indicated that addition of chopped garlic cloves reduced root galling, number of eggs / root system and nematode population in soil. Nematicidal garlic properties were attributed to allicin diallyl disulfide, ammonia and pyruviic acid (Stoll and Sebeck, 1950). Mycorrhyzal fungi ranked second to garlic in its effect on root-knot nematode, *M. incognita* where mycorrhyzal fungi, *Glomus* spp. was effective in reducing the reproduction of *M. incognita* and the reduction percentage was 78%. The obtained results agree with those reported by (Carling *et al.*, 1989; Zawam, 1999 and El- Haddad *et al.*, 2005) who determined that the galling and eggs number of *M. incognita* were suppressed by inoculation with *Glomus* spp. On the other hand, the dried powder of liquaric (*Glycyrrhiza glabra L.*) at rate of 80 gm / tree had lethal effect against *M. incognita*, the percentage reduction in nematode population was 71compared to control. This inhibition action due to the accumulation of glycyrrhizin material (Abou Zaid, Sh.N. (2000). The commercial bioproduct Diple 2x 2gm/ 1 liter water had also adverse effect on *M. incognita* as it reduced nematode population. Reduction percentage was 66. These results confirm those obtained by (Osman *et al.*, 1988; Mena *et al.*, 1996; Radwan *et al.*, 2004; El- Nagdi and Youssef, 2004). The mode of action of *B. thuringiensis* toxins is mainly inhibition of protein and nucleic acid synthesis (Sebesta *et al.*, 1969). Data also show that the dried powder of chamomile (*Matricaria chamomile*) was the least effective treatment in controlling *M. incognita* as compared to other treatments. The effect of chamomile du to Tri-hydroxy flavon (Hikal and Omar, 1993) . In conclusion, organic soil amendments and biological treatments under investigation exhibited nematicidal activities, since they caused reduction in the nematode population. Such materials can play an important role in the classical and natural biological control of root knot nematode, *M. incognita* infesting grapevine.

Table (4): Efficacy of certain biological compounds and soil amendments in controlling root- knot nematode *Meloidogyne incognita* infesting grapevine cv Superior under field condition.

Treatment	Initial J2s/ 250 gm soil	Final J2s/ 250 gm soil	No. galls /1gm root	No. of egg masses/ 1gm root	No. of eggs / eggmass	No. of developmental stages/ 1gm root	Final population in 250gm/soil and 1gm/root	*R. Factor	**Red%
Diple 2x	1180	1306	27	24	219	75	6640	5.6	66
EM1	1486	1740	34	32	276	100	9516	6.4	61
Chopped Garlic clove (40 gm)	1506	1346	26	25	279	63	8318	5.5	67
Chopped Garlic clove (60gm)	1753	1273	25	23	225	50	6508	3.7	78
Chopped Garlic clove (80gm)	2020	1027	20	14	204	36	4964	2.4	85
Dried powder of shamomile (40 gm)	1173	2586	51	45	297	149	16100	13.7	17
Dried powder of shamomile (60 gm)	1446	2440	48	41	288	120	14368	10.4	37
Dried powder of shamomile (80 gm)	1503	2105	41	34	259	97	12272	8.1	51
Dried powder of liquorices (40 gm)	1413	1387	27	26	262	64	8213	5.7	66
Dried powder of liquorices (60 gm)	1400	1280	22	24	280	45	7372	5.2	69
Dried powder of liquorices (80 gm)	1927	1613	31	30	257	54	9368	4.8	71
Mycorrhyzal fungi	2106	1354	24	21	290	56	7509	3.6	78
Control	1352	3213	102	57	353	156	22364	16.6	0.0
L.S.D at 0.05%	149.12	1624.5	9.31	7.05	39.17	33.26	2270.73	1.14	

*R Factor =indicator of nematode reproduction $= \frac{\text{final of nematode population}}{\text{initial of nematode population}}$

**Red% indicate percentages nematode reduction in soil and root (% efficiency accorgding to Handerson & Tilton formula (Anonymous (1981)

$$= 1 - \left(\frac{\text{Population in the treated trees after application}}{\text{Population in the treated trees before application}} \times \frac{\text{Population in the check trees before application}}{\text{Population in the check trees after application}} \right) \times 100$$

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دراسات على حصر النيماتودا وقابلية العائل للإصابة ومكافحة" ميلودجينى إنكوجنيتا "على العنب بمركبات معينة والعلاقة بين الإصابة والمحصول

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تصاب شجيرات العنب بالنيماتودا المتطفلة على النباتات وتعتبر نيماتودا تعقد الجذور من أخطر أنواع النيماتودا التى تصيب العنب. ويهدف هذا البحث إلى عمل حصر لبعض أصناف العنب الهامة اقتصاديًا لتحديد حساسية بعض الأصناف وهى الطومسون وكنج روبى والفليم والبرليت وسبريور، وتأكيد حساسية هذه الأصناف باختبار حساسية تحت ظروف الصوبة.

وأوضحت الاختبارات أن أكثر الأصناف حساسية هي أسبريور وكنج روبى والفليم، وعلى ضوء هذه النتائج تم اختيار الصنف الحساس أسبريور لدراسة تأثير بعض المعاملات الحيوية وهى EMI وفطر الميكروهيزا وبعض المنتجات الطبيعية لنباتات طبية وهى مهروس فصوص الثوم والمسحوق الجاف لنباتات العرقسوس والشيح على نيماتودا تعقد الجذور ميلودجينى إنكوجنيتا التي تصيب صنف أسبريور تحت ظروف الحقل. وأوضحت النتائج أن مهروس فصوص الثوم

بجرعة ٨٠ جم/شجرة وفطر الميكروهيزا معدل ١ مل/١٠٠ مل ماء أكثر المعاملات فاعلية في مكافحة هذا النوع من نيماتودا تعقد الجذور، حيث حدث انخفاض في تعداد النمياتودا بنسبة ٨٥%، ٧٨% على التوالي مقارنة بالكنترول، كما لوحظ تأثير واضح لنيماتودا تعقد الجذور لنبات العرقسوس، حيث كان الانخفاض في تكاثر النيماتودا بنسبة ٧١% عند إضافة المسحوق الجاف لهذا النبات بجرعة ٨٠ جم/شجرة.

وأجريت دراسة للعلاقة بين إنتاجية أشجار العنب والإصابة بنيماتودا تعقد الجذور لبعض أصناف العنب وهى الفليم وكنج روبى والبرليت وسبريور والطومسون وكانت النتائج كالتالي:

١- أعلى تعداد لهذا النوع من النيماتودا في التربة والجذور كان مرتبطا بالمحصول المتوسط الإنتاجية. بينما التعداد المنخفض لهذا النوع من نيماتودا تعقد الجذور كان مرتبطا بالإنتاج المنخفض ماعدا الصنف طومسون.

أوضحت النتائج أن الأشجار ذات الإنتاجية العالية مصابة بتعداد أعلى من نيماتودا تعقد الجذور بالمقارنة بالأشجار المنخفضة الإنتاج في نفس الصنف، كما هو واضح في صنفي كنج روبي والفليم.