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Organic Management of *Meloidogyne incognita* on Grapes in Relation to Host Biochemistry

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Abstract: The organic amendments, pigeon droppings, smashed garlic, meals of soybean, cotton seed and peanut as well as the nematicide, vydate 10% granules significantly suppressed the reproductivity of *Meloidogyne incognita* reproductivity on grape under greenhouse conditions. Peanut meal at 50 and 100 g/pot performed the best results in reducing nematode counts and successfully smashup its build up, followed by pigeon droppings treatments. Nematode infection resulted in increasing root proteins over those in healthy seedlings. All treatments, including vydate significantly increased root protein when compared to infected plants. Pigeon dropping, smashed garlic and the nematicide significantly increased root content of lipids over the infected check and in many cases over the healthy plants. Nematode infection also increased root contents of total soluble sugars and total carbohydrates. Seed meals of soybean and peanut as well as the nematicide significantly increased total soluble sugars over the infected and healthy plants. All treatments significantly improved dry matter content which was always higher than those in infected untreated plants. In field trial, there was noticeable decrease in soil population one month after treatment especially with cotton seed meal which was almost similar to vydate 10% G. Amendments at the end, outmatched the nematicide in diminishing the accumulative average of juveniles, cotton seed meal gave resultes twice as much as vydate.

Key words: Organic amendments, root-knot nematode, grape, root analysis

INTRODUCTION

From time memorial, it has been the habit of man to add any available wastes of crops or animal origin to increase crop yield. Recently, the potential of amendaments, animalcular manures, plant (dry or green) and their extracts, composts, oil cakes, crop wastes and agro-industrial waste to control phytopathogenic nematodes has become recognizable. The abreast effects of such material interpreted to outpouring research work all over the world (Mankau, 1962, 1963; Mankau and Minteer, 1962; Oteifa *et al*., 1964; Singh and Sitaramaiah, 1967; Varagas, 1972; Desai *et al*., 1973; Khan *et al*., 1974; Habich, 1975; Castillo *et al*., 1976; Egunjobi and Afolami, 1976; Sitaramaiah and Singh, 1978; Badra *et al*., 1979; Taha and Saleh, 1979; Singh, 1980; Singh *et al*., 1980; Main and Rodriguez-Kabana, 1982; Bhattacharge and Goswami, 1989; Saeed, 2005). Poultry droppings, litters and dungs significantly affected nematode reproduction (Striling, 1990; Bobatola and Oycdummade, 1992; Sarker and Main, 1995; Akhter and Mahmood, 1997; Farahat *et al*., 1999; Maareg *et al*., 2000; Al-Rehiayani, 2001; Nour El-Deen, 2002; El-Ghonaimy, 2006). Cotton seed, linseed oil cakes, castor pomace, eucalyptus leaves, neem cake and extraction of garlic have been found successful in reducing root galling

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(Johnson, 1974; Nath *et al*., 1982; Nandal and Bhatti, 1983; Gupta and Sharma, 1985, 1990; Lanjeswar and Shukla, 1986; Singh and Singh, 1988; Akhtar and Alam, 1989; Akhter *et al*., 1990; Darekar and Mhase, 1990; Farahat *et al*., 1994, 1999; Verma and Anwar, 1995; Anjum *et al*., 1996; Amin and Youssif, 1998; El-Ghonaimy, 2006).

Present research was designed not only to confirm the previous results but also to study the organic management in grape field infested with the root knot nematode and the effect of such materials on grape roots biochemistry.

MATERIALS AND METHODS

Greenhouse Experiment

Three months old grape seedlings (Thompaon seedless) with uniform size transplanted into 20 cm diameter clay pots filled with steam sterilized sandy loam soil in experimental area of Nematology Diviaion, Faculty of Agriculture, Cairo University were ioculated with 5000 J_2 of the root-knot nematode, *M. incognita*/pot. Organic materials, pigeon droppings, smashed garlic, meals of soybean, cotton seed and peanut were applied at the doses of 25, 50 and 100 g/pot. However, vydate 10% granules was applied at the rate 0.5, 1 and 2 g/pot. All treatments were applied twice, the first at the beginning of experiment and the second one-month after.

Every single treatment was replicated 4 times and four pots inoculated with nematode as well as four untreated healthy checks. All treatments were arranged in a randomized design on a bench in the greenhouse at 32±5°C receiving similar horticulture treatments. After 120 days plants were lifted out, data of nematode counts were recorded.

Root Chemical Analysis

Protein, lipids, total soluble sugars (TSS), total carbohydrates (TCH) and dry matters (ASH) were determined in 1 g dry roots of each treatment following the standard methods of the Association of Official Analytical Chemists (AOAC, 1990).

Field Trial

A viticulture sandy soil field located at El-Nobaria (Cairo-Alex. Desert Road), naturally infested with the root-knot nematode, *M. incognita* was treated separately with cotton seed and peanut meals (0.5 kg/tree). The nematicide, vydate 10% G (40 g/tree) was applied as a comparable chemical treatment. Treatments were marked and labeled as permanent sampling sites. The experimental area was divided into a randomized block design. Each treatment was replicated four times (four trees for each treatment). Soil samples were periodically taken every month after application for 7 months starting from May to November 2001 to asses the nematode soil population. At the end of experiment, the accumulative averages and total nematode counts recovered from the soil were calculated. Efficacies of above mentioned materials were estimated by the using the formula of Henderson and Tilton (Anonymous, 1981):

$$= \left(1 - (\frac{J_2 \text{ sample pop. of treated plots after application}}{J_2 \text{ sample pop. of treated plots before application}} \times \frac{J_2 \text{ sample pop. of check plots before application}}{J_2 \text{ sample pop. of check plots after application}}\right) \times 100$$

Statistics

Data were statistically analyzed according to Duncan's multiple range test at the p = 0.05 significance level by MSTAT version 4 (1987).

RESULTS

Effect of Organic Amendments on *M. incognita* Reproductivity under Greenhouse Conditions

Data presented in Table 1 reveal that all of organic amendments applied at three rates (25, 50 and 100 g/pot) significantly suppressed gall formation, number of eggmasses, final population as well as the calculated nematode build up (Pf/Pi) and egg production as measured by eggs/eggmass and eggs/g root when compared with the nematode untreated check. However, differences in nematode suppression were noticeable among treatments and/or doses. For instance, peanut meal treatments at 50 and 100 g/pot performed smashing reductions in all nematode counts and successfully smashed nematode build up and egg production followed by pigeon droppings treatments. Although, no clear proportional effects were observed among materials/doses and gall formation and eggamsses of smashed garlic, cotton seed, soybean pomace, the effect on egg production increased steadily by increasing material dose. Concerning, vydate 10%, the nematode galls, eggmasses and fecundity were sharply declined. Values of build up were eliminated with no significant differences between peanut and soybean meals with preponderance of the highest doses applied.

Effect of Organic Amendments on Grape Roots Chemical Components under Greenhouse Conditions

Data in (Table 2) revealed that root protein increased significantly in most treatments including vydate 10%, irrespictive of their concentrations, when compared with the nematode inoculated check

Table 1: Reproductivity of *M. incognita* as influenced by addition of some organic amendments

			Nematode counts			Nematode fecundity			
Treatments	Dose g/pot	Galls	Eggm-asses	Final population	Pf/Pi	Eggs/ Eggmass	Egg production (%)	Eggs/ g root	Nematode reduction (%)
Pigeon dropping	25	50^{efgh}	34^{fghi}	8860	1.8^{ijkl}	78^{hij}	8	222^{hi}	84
	50	51^{efg}	31^{ghi}	9074	1.8^{ijkl}	84^{hi}	8	215^{hi}	83
	100	65^{d}	41^{def}	9836	2.0^{hijk}	78^{hij}	10	209^{hi}	82
Smashed garlic	25	76^{c}	47^{d}	16947	3.4^{de}	191^{b}	27	746^{c}	68
	50	131^{b}	76^{b}	32973	6.6^{b}	210^{a}	48	1065^{b}	39
	100	134^{b}	70^{b}	20036	4.0^{c}	166^{c}	35	646^{de}	63
Cottonseed meal	25	83^{c}	56^{b}	17152	3.4^{de}	145^{d}	25	679^{cd}	38
	50	80^{c}	45^{de}	15448	3.1^{ef}	148^{d}	20	511^{f}	71
	100	60^{def}	46^{d}	15055	3.0^{ef}	110^{ef}	15	361^{g}	72
Soybean meal	25	61^{de}	46^{d}	13535	2.7^{fg}	114^{ef}	16	582^{ef}	75
	50	48^{gh}	36^{fghi}	10448	2.1^{hi}	90^{gh}	10	271^{gh}	81
	100	53^{efg}	40^{def}	10220	2.0^{hijk}	73^{ij}	9	292^{gh}	81
Peanutmeal	25	49^{fgh}	37^{efgh}	9559	1.9^{ijk}	91^{gh}	10	280^{gh}	82
	50	37^{i}	28^{i}	7000	1.4^{l}	68^{j}	6	127^{i}	87
	100	40^{hi}	30^{hi}	8182	1.6^{jkl}	73^{ij}	7	168^{i}	85
Vydate10% granules	0.5	65^{d}	45^{de}	11724	2.3^{gh}	120^{e}	16	337^{g}	78
	1.0	54^{efg}	39^{defg}	9440	1.9^{ijk}	100^{fg}	12	301^{gh}	82
	2.0	50^{efgh}	35^{fghi}	7934	1.6^{jkl}	90^{gh}	10	165^{i}	85
Inoculated only		197^{a}	157^{a}	53718	10.7^{a}	210^{a}	100	1940^{a}	-

Means followed by the same letter(s) whithin a column in each block are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test

$$\%\ \text{Egg production} = \frac{\text{Eggs/eggmass} \times \text{No. of eggmasses of treatment}}{\text{Eggs/eggmass} \times \text{No. of eggmasses of highest treatment}} \times 100$$

$$\%\ \text{Nematode reduction} = \frac{\text{Final pop. of check} - \text{final pop. of treatment}}{\text{Final pop. of check}} \times 100$$

Table 2: Chemical analysis of grape roots infected with *M. incognita* and treated with some organic amendments

		Root chemical analysis				
Treatments	Dose g/pot	Protein	Lipids	TSS	TCH	ASH
Pigeon dropping	25	7.5^{e}	1.2^{hi}	1.1^{hi}	14.2^{k}	77.1^{e}
	50	12.7^{a}	1.8^{d}	0.7^{jk}	18.6^{h}	66.9^{o}
	100	9.1^{b}	3.2^{a}	0.7^{jk}	18.6^{h}	69.0^{m}
Smashed garlic	25	8.1^{d}	1.3^{h}	1.0^{ij}	18.2^{i}	72.4^{k}
	50	7.1^{f}	1.2^{hi}	1.1^{hi}	25.8^{c}	66.0^{p}
	100	6.3^{g}	2.7^{b}	1.6^{g}	14.2^{k}	76.8^{f}
Cotton seed meal	25	7.9^{d}	2.7^{b}	1.3^{ghi}	28.6^{b}	60.7^{r}
	50	8.6^{c}	0.6^{k}	0.6^{k}	20.0^{g}	70.8^{l}
	100	8.8^{c}	1.0^{j}	1.4^{gh}	22.2^{d}	68.0^{n}
Soybean meal	25	5.2^{j}	1.6^{f}	5.9^{c}	14.2^{k}	79.1^{d}
	50	3.9^{kl}	0.5^{l}	6.8^{b}	13.7^{l}	81.8^{c}
	100	5.5^{hi}	1.1^{ij}	4.7^{e}	18.3^{i}	75.1^{g}
Peanut meal	25	2.5^{o}	0.2^{m}	7.8^{a}	20.6^{f}	76.7^{f}
	50	4.0^{k}	0.2^{m}	6.6^{b}	21.3^{e}	74.6^{h}
	100	5.3^{ij}	0.1^{m}	4.5^{e}	22.0^{d}	72.5^{k}
Vydate 10% granules	0.5	5.2^{j}	1.2^{hi}	5.9^{c}	20.0^{g}	73.6^{j}
	1.0	5.7^{h}	2.3^{c}	4.5^{e}	17.9^{j}	74.1^{i}
	2.0	3.7^{lm}	1.5^{g}	5.0^{d}	8.6^{n}	86.2^{a}
Healthy		3.2^{n}	1.0^{j}	0.5^{k}	12.0^{m}	84.3^{b}
Inoculated only		3.5^{m}	1.7^{e}	1.9^{f}	29.2^{a}	65.6^{q}

Means followed by the same letter(s) whithin a column in each block are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test. TSS = Total soluble sugars, TCH = Total carbohydrates, ASH = Dry matters

which had relatively higher protein content than helthy plants. Earnest significant incrase was achieved with pigeon droppings, smashed garlic and cotton seed meal. The next were soybean and peanut meals. Significant elevation of root protein content was detected in smashed garlic (25 and 50 g/pot) and cotton seed meal treatments. The most eminent significant increase was in pigeon dropping treatment having 50 g/pot. Yet, the least and extrinsic protein increase was in the highest dosage of vydate 10%.

Root lipids were lower in un-inoculated than inoculated controls and much more lower in all amended treatments. Reductions in root lipids quantities were greater in meals of cotton seed at 25 and 50 g/pot and soybean treatments and greatest in peanut meal treatments. However, few treatments enhanced significantly lipids magnitude, for instance, the highest dosage of pigeon dropping, smashed garlic, lowest dose of cotton seed meal and intermediate one of vydate 10%.

Inoculated non-treated plants had more total soluble sugars and total carbohydrates than healthy ones. But negative correlation was found between total soluble sugars and pigeon dropping, smashed garlic and cotton seed meal treatments at all dosages used. Contrary, affluent significant promotion was achieved with soybean, peanut meals and vydate 10% treatments at all dosages. However, that promotion attenuated by increasing dosage of soybean and peanut meals.

A reverse action was found in the dry matter (ASH) content, which was much lower in inoculated than healthy plants. All treatments/dosages significantly improved ASH content (but cotton seed meal at 50 g/pot) and was always more than inoculated ones. The maximum quantity of dry matter was given by vydate 10% at 2 g/pot, whereas the minimum was in smashed garlic at 50 g/pot treatment.

Effect of Selected Treatments on *M. incognita* Population under Field Conditions

Data as presented in Table 3 and Fig. 1 circumstantiate that there was noticeable decrease in soil nematode population one month after treatment with amendments especially with that of cotton seed meal which was almost similar to vydate 10% treatment. The accumulative averages of juveniles recovered from soils of the treated plants reflected to considerable extent the arresting prohibitive effects of organic amendments which outlasted the nematicide and performed better suppressive action. Cotton seed meal (0.5 kg/tree) achieved 50% reduction of the total nematode soil population as compared with untreated check and was twice as much as vydate 10%.

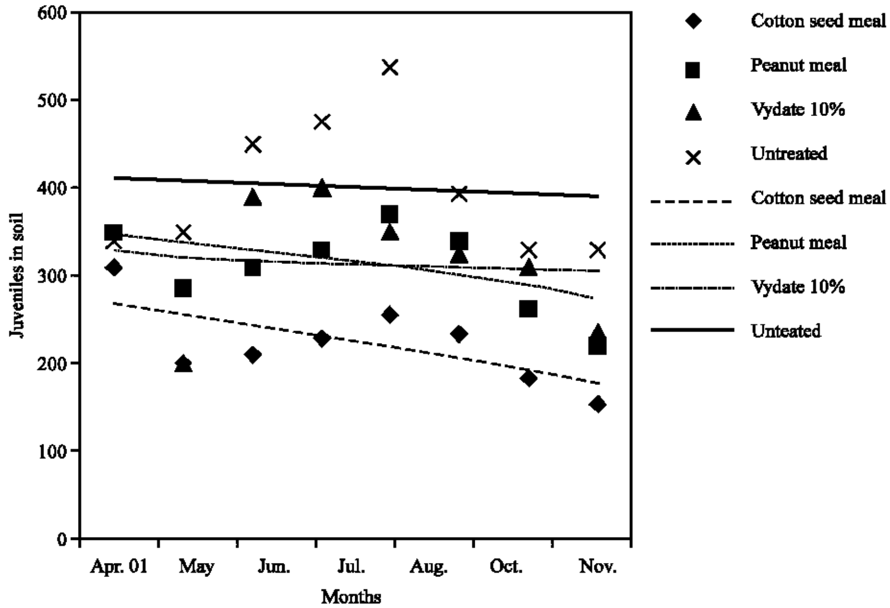

Fig. 1: Influence of some organic amendments on the behavior of *M. incognita* population in sandy soil grape field

Table 3: Effect of some organic amendments on *M. incognita* soil population in sandy soil grape field

	Juveniles/250 g soil		
Treatments	Accumulative average	Total	Total reduction (%)
Cotton seed meal (0.5 kg/tree)	209 (44.9)	1463^{c}	50
Peanut meal (0.5 kg/tree)	303 (29.2)	2118^{b}	27
Vydate 10% granules (40 g/tree)	316 (19.3)	2211^{b}	24
Check (untreated)	416	2914^{a}	-

Means followed by the same letter(s) whithin a column in each block are not significantly different (p = 0.05) according to Duncan's multiple range test. Number between braktes calculated by formula of Henderson and Tilton (Anonymous, 1981)

DISCUSSION

Application of non living organic materials, large or small, simple or complex, of plant or animal origin, chemical or non chemical to the soil is known to have beneficial effects on soil nutrients (as substrate for microorganisms), soil physical conditions (water retention, cation exchange capacity, soil aggregation), soil biological activity and crop performance (Kang *et al*., 1981; Wade and Sanchez, 1983). In addition, the increasing evidence that organic materials as safe, low cost are an alternative method for nematode management. Organic materials were pestilential and possessed significant reduction in *M. incognita* development, total numbers of galls, eggmasses, egg production, size of nematode population and subsequent nematode build up. Potency of these materials on nematode population was more or less up to the comparable nematicide vydate 10% and seemed to be dependent on their nature and concentration. Addition of pigeon dropping or peanut meal to the soil overmatched vydate 10% in reducing the nematode final population. Present results are in coincidence with those of (Verma and Anwar, 1995; Akhter and Mahmood, 1997; Amin and Youssif, 1998; Farahat *et al*., 1994, 1999; Maareg *et al*., 2000; Al-Rehiayani, 2001).

The mechanism involved in nematode control by addition of organic materials are variable and complementary. The nematode control achieved by decomposition of crop residues in soil was due to liberation of fatty acids (Patrick *et al*., 1965; Sayre *et al*., 1965; Sitaramaiah and Singh, 1978; Badra *et al*., 1979; Montasser, 1991b; El-Naggar *et al*., 1993). The high content of fatty acids of botanical meals or oils are the expectant chemicals produced during decomposition of such chemicals and affect nematode bionomics. Accumulated toxins of the decomposing products (Johnson, 1974; Alam *et al*., 1973), tannins in nematotoxic polyphenols (Tayler and Murant, 1966), marked increase in numbers of natural enemies that are parasitic or predacious on nematodes (Mankau, 1962 and 1963), changed physical and chemical properties of soil which inimical to phytonematodes (Alam *et al*., 1973), or increased host resistance to nematode infection (Alam *et al*., 1977; Alam *et al*., 1980). Also, rapid decomposing of organic matter caused rapid kill of nematodes (Hollis and Rodriguez-Kabana, 1966) due to rapid increase of butyric and propionic acids. These organic acids have contact nematicidal action on free stages of parasitic nematodes (Ponchillia, 1972; Sitaramaiah and Pathak, 1979; Al-Sayed *et al*., 1988; Browning *et al*., 2004). Present results prove that these acids may have a direct role in biological defense mechanism or indirect one since they increased proteins and fatty acids in the root tissues.

Furthermore, the microbial breakdown of nitrogen containing substances in soil via processes of mineralization might be as operative against nematodes as an increase in predacious nematodes, nematode-trapping fungi or their toxins (Walker, 1992). NH_3 or possibly nitrite produced are among principle compounds responsible for the decreased nematode populations. Also, the direct or indirect influence of pH, Ca^+ ions, moisture (Dubey, 1968). Present resultes proved that infected untreated grape roots contained higher values of total protein than healthy roots. Reports of Singh *et al*. (1978), Ganguly and Dasgupta (1980), Arya and Tiagi (1982), Sharma and Raj (1987) and Romabati and Dahanachand (2000) indicated to similar results. They found that total protein increased in galled roots and that increase was proportional to the degree of infection. By adding organic amendments to infected plants, profusion of total protein content was observed. However, that increase was conditional by amendment nature not its concentrations. Peanut meal at its lowest dose was the only treatment that reduced root total protein as compared to the inoculated-untrated check.

Vydate 10% G increased the total protein content at all doses, the highest was in the middle and lowest rate was achieved by its highest dose. Singh *et al*. (2000) reported different results, they noticed large numbers of polypeptides and protein bands in case of inoculated untreated than phenamiphos, triazophos and carbosulfan treatments.

Higher amounts of lipids, total soluble sugars and total carbohydrates were recorded in the infected untreated grape roots than healthy ones. Controvert results were obtained in literature, Epstein (1972) found that the amount of simple sugars in root of marigold infected with *Longidorus africans* was twice as much as healthy roots. Meanwhile, lower amounts of carbohydrates and lipids were reported by Hanounik *et al*. (1975), Jatala and Jehnsen (1976), Massoud (1980) and Kheir and Abadir (1982) in different crops and nematode species. Results of Melakeborhan *et al*. (1990) indicated that *M. incognita* didn't affect the concentration of reducing but non reduced sugers increased in susceptible and decreased in moderately resistant variety. The amount of lipids, total soluble sugars and total carbohydrates increased or decreased as a result of organic materials application not only according to their nature but also concentration. Lipids have been decreased with more ratios than infected untreated roots in cotton seed, soybean and peanut meals at their middle dosages but increased at pigeon droppings and smashed garlic highest dosages.

Redundant increase in total soluble sugars values were obvious in all soybean and peanut meals treatments. Yet, it decreased in pigeon droppings, smashed garlic and cotton seed meal but not as much as healthy plants. Total carbohydrates decreased in the amended treatments as compared to the inoculated check but slight increase appeared if it compared to the healthy plants.

Concerning the effect of vydate 10%, general reductions in lipids and total carbohydrates contents were recorded except in its middle dose. While high amounts of total soluble sugars were observed.

Lower amounts of dry matter content was found in the infected check than healthy plants. Some reports indicated to increase in mineral's content in galled roots (Oteifa and El-Gindi, 1962; Shafiee and Jenkins, 1963). But Singh and Choudhury (1974) reported that dry matter content was dependent on the host or its susceptibility. Changeable promotion in dry matter content was apparent (but not to the levels of healthy plants) in most organic additives.

Analysis of to the above mentioned chemicals indicate to more or less constant patterns in the total protein and dry matter contents in grape roots and oscillated ones in lipids, total soluble sugars and total charbohydrate contents. That was imputable according to hosts, its reststance or susceptibility, nematode species and their nutritional demands, time of harvest, which part of plant was analyzed, foliage or roots (Singh and Choudhury, 1974; Tayal and Agrawal, 1982; Kheir and Abadir, 1982; Singh *et al*., 2000) and we add the nature of the applied materials.

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