

EFFECT OF FRESH AND DRY NEEM LEAVES APPLICATIONS ON ROOT-KNOT NEMATODE MELOIDOGYNE JAVANICA ON TOMATO PLANTS

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ABSTRACT

This experiment was carried out to evaluate neem fresh and dry leaves at 1 and 2% of soil weight against root-knot nematode, Meloidogyne javanica on tomato plants under greenhouse condition. Results found that all nematode parameters i.e. number of galls, egg masses, females, developmental stages/root system, eggs/egg mass, juveniles/250 g soil, nematode final population (Pf) and reproduction factor (Rf) significantly reduced by neem leaf applications either as a fresh or dry at both doses compared to plants treated with nematode alone. Results indicated that neem powder was most effective treatment in reducing all nematode parameters compared to the fresh application. Neem powder at 2% recorded the highest reduction percentage of all nematode parameters compared to fresh leaf treatments at both levels. The reduction percentages of number of galls, egg masses, females/root system, eggs/egg mass, juveniles/250 g soil, final population (Pf) and reproduction factor (Rf) were 80, 80, 78, 47, 78, 86 and 86 reduction %, respectively.Plant growth parameters i.e. shoot and root lengths, shoot and root fresh weights and shoot dry weight were also affected by neem leaf applications. The highest increase percentage was recorded with neem powder treatment at 2% compared to fresh leaves treatment except shoot dry weight of 2% fresh leaves treatment, which recorded the highest percentage of efficacy. Antioxidant enzymes activity i.e. peroxidase phenoloxidase, catalase as well as carbohydrates and phenols were also significantly enhanced with both neem applications at 2%.

Key words: Organic amendments, *Lycopersicon esculentum*, Antioxidant enzymes, *Azadirachta indica*.

INTRODUCTION

Tomato plant, *Lycopersicon esculentum* Mill. considered one of the most important and widely cultivated vegetables in Egypt. It is highly susceptible to infection by rootknot nematodes, *Meloidogynes* pp., such as *M. incognita* and *M. Javanica* (Khan *et al.*, 1984).

Root-knot nematodes are cosmopolitan in distribution, occur in soil. Many noxious chemicals have been tried over the last few decades but only a few have stood the test of time. Most of these compounds are expensive and out of the reach of many farmers. Among various control strategies, the use of organic amendments has gained the interest of scientists because they pose few to no environmental hazards (Hussain *et al.*, 2011). The incorporation of organic material into the soil reduces root-knot nematodes densities, resulting in an increase in yield (Muller & Gooch, 1982). Nonchemical methods i.e. cultural and biological methods are considered good substitutes for chemicals and provide satisfactory control of root-knots in vegetables and other crops (Khan, 2007). The use of organic soil amendments is the cheapest and most effective way to control plant diseases caused by nematodes (Hussain *et al.*, 2011).

Some antagonistic plant species and parts to Meloidogyne spp. are leaves and flowers of marigold (Tagetes sp.); leaves, roots and seeds of neem (Azadirachta indica), and leaves and seeds of chinaberry (Melia azadirach) (Rather et al., 2007). Neem (A. indica A. Juss.) is a the mahogany member of family. Meliaceae. It is native from the Indo-Pakistan subcontinent and is known to many people as a "wonder tree" due to its many uses in medicine, agriculture,

industry, etc. It has been found, mainly in the last decade or so, that neem materials can affect more than 200 insect species as well as mites, nematodes, fungi, bacteria, and even a few viruses (Ahmad *et al.*, 1996; Walia and Gupta, 1995). Neem leaves is one of the most used plant parts in nematode management as the leaves have various compounds such as nimbin, nimbidin, azadirachtin, salannin, thionemon and meliantriol (Hussain *et al.*, 2011).

The aim of this work was to evaluate fresh and dry neem leaves applications as a soil amendment at 1 and 2% of soil weight against root-knot nematode, *Meloidogyne javanica* on tomato plants under greenhouse condition.

Materials and Methods

Neem leaves powder was applied to soil pots to manage root-knot nematode, *Meloidogyne javanica*. Air dried neem leaves was grinded in a grinder and sieved through a 25 um sieve to obtain a fine powder. Powder and fresh chopped leaves were applied at two different levels 1 and 2% of soil weight (w/w), 10 and 20 g/kg soil.

Culture of root-knot nematode *Meloidogyne javanica* was established from single egg mass of adult females and identified by the morphological characteristics of the female perineal patterns as described by Taylor and Sasser (1978). Pure culture was reared on susceptible tomato plants in a greenhouse at $30\pm 5^{\circ}$ C.

Treatments were applied by mixing the either the plant powder or fresh chopped leaves with 2 kg sandy clay soil mixture (2:1, v/v) into the plastic pots (15 cm in diam.). Powders and fresh leaves were applied to soil pots one week before tomato seedlings transplanting to allow decomposing the organic amendments. Three weeks-old tomato seedlings cv. GS were transplanted into pots (one seedling/pot). Two thousand nematode eggs and juveniles (J_2) were inoculated per plant by pippetting into three holes made around the plant roots at the same time of tomato transplanting. Each treatment was replicated three times. Untreated pots served as a control. Pots were watered daily and fertilized once a week with 5 ml of 2 g/l N:P:K (20:20:20), obtained from the International Egypt Company for Agricultural and Industrial Developing.

Treatments were carried out as follows:

(1) Nematode alone.

(2) Neem (powder or fresh leaves) + Nematode.

(3) Control (untreated plants).

Plants were uprooted 8 weeks after nematode inoculation, roots washed with tap water to avoid the soil particles. Number of galls, egg masses, females, developmental stages / root system and eggs / egg mass, J₂/250 g soil, final nematode population (P_f), as well as reproduction factor (R_f) were determined. Nematode infected roots were dipped into phloxine-B staining solution of 0.15 g/l for 20 min. to staining and count egg-masses according to Daykin and Hussey (1985). To obtain nematode females, root system of each plant was cut into 2 cm pieces and dipping in a beaker full of tap water for 4 days under laboratory conditions until the root pieces became soft. Roots were then washed with tap water through 250 and 500 mesh sieves to separate the females from therootdebris (Mahdy,2002).

Final nematode population (P_f) was counted according to the equation:

P_f = (No. of egg masses/plant X No. of eggs/egg mass)+No.of females/plant + No. of developmental stages/plant + No. of juveniles/250 g soil.

Rate of nematode reproduction factor (R_f) was recorded according to the equation: $R_f = P_f/P_i$ (P_i = initial population) (Norton, 1978).

Reduction percentages were recorded according to the following equation: Reduction% = [Nematode alone – Treatment ÷ Nematode alone] × 100.

Plant growth characters i.e. shoot and root length (cm), shoot and root fresh weights (g) and shoot dry weight were also recorded. Increase percentage (Efficacy) was recorded according to the following equation: Efficacy % = [Treatment – Nematode alone \div Nematode alone] × 100.

Chemical constituents i.e. total carbohydrates, total sugars and phenol compounds were also determined in dry leaves according to Dubois *et al.* (1956) and Snell and Snell (1959),respectively. Antioxidant enzymes i.e.phenoloxidase, peroxidase and catalase enzymes activity were measured in fresh leaves according to the method described by Broesh (1954), Fehrman and Dimond (1967), Bach and Oparin (1968), respectively.

Data were statistically analyzed according to analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics. Crop, Rockville, MD, 1995), Variance homogeneity for all treatments was confirmed by the Bartlett test. The comparison between means was carried out by Duncan's Multiple Range Test (Duncan, 1955).

Results

Data in Table (1) showed that incorporation both tested dry and fresh neem leaves at 1 and 2% of soil weight were effective in reducing all nematode parameters compared to nematode alone. Both doses significantly reduced the most nematode parameters i.e. number of galls, egg masses, eggs / egg mass, and number of J_2 / 250 g soil compared to treated plants with nematode alone.

Data in Table (1) showed that the most effective treatment in reducing nematode parameters was the application of neem as a dry powder at 2% compared to fresh one. The highest reduction percentage of galls (80%) was obtained with 2% dry powder, whereas the least one (59%) was obtained at 1% of fresh neem leaves.

		Numbers of / root system					\ 0		
Treatment	Doses % /2 kg soil	Galls	Reduction %	Egg masses	Reduction %	Eggs/ egg mass	Reduction %	J2/ 250 g soil	Reduction %
Dourdor	1	29.3 c	72	29.0 c	69	96.0 d	40	1833.3 d	66
Powder	2	20.7 d	80	19.3 e	80	85.0 e	47	1166.7 e	78
Fresh	1	42.7 b	59	44.0 b	53	122.7 b	24	3000.0 b	44
	2	29.7 c	71	24.7 d	74	116.7 c	28	2000.0 c	62
Nematode alone		103a	-	94.3 a	-	161.3 a	-	5333.3 a	-

 Table (1): Effect of fresh and dry neem leaves on numbers of galls and egg masses/plant, eggs/ egg mass and J2/ 250 g soil of *M. javanica* infecting tomato plants and reduction %.

Means, in each column, followed by different letters are significantly different according to Duncan's Multiple Range Test (p < 0.05).

Table (2): Effect of fresh and dry neem leaves on numbers of females, nematode developmental stages, final population (Pf) and reproduction factor (Rf) of *M. javanica* infecting tomato plants and reduction %.

		Numbers of / root system							
Treatment	Doses % / 2 kg soil	Females	Reduction %	Develop. stages	Reduction %	Pf	Reduction %	Rf	Reduction %
Powder	1	28.3 c	64	17.7 d	76	4663.3 d	78	2.3 c	78
FOWLEI	2	17.0 e	78	16.3 d	78	2840.5 e	86	1.4 c	86
Fresh	1	40.3 b	48	34.7 b	54	8473.8 b	59	4.2 b	59
116511	2	23.0 d	71	26.7 c	64	4932.2 c	76	2.5 bc	76
Nematode	alone	78.0 a	-	74.7 a	-	20696.6 a	-	10.3 a	-

Means, in each column, followed by different letters are significantly different according to Duncan's Multiple Range Test (p < 0.05). Pf = Final population. Rf = Reproduction factor

Egg masses were significantly reduced with all applied treatments compared to nematode alone. The highly reduction% recorded when neem powder was applied at 2% by approximately 80%, followed by fresh leaves at 2% by 74%, whereas the least one recorded 53% at 1% with fresh chopped leaves as shown in Table (1).

The same trend of results was obtained with eggs/egg mass as they significantly reduced the number of eggs/egg mass compared to the plants treated with nematode alone. Application neem leaves powder at 2% recorded also the highest reduction% of eggs/egg mass was 47%, while fresh leaves application recorded approximately 28% at 2%. The lowest reduction percentage of eggs/egg mass obtained with fresh chopped leaves at 1% by 24% (Table, 1). In this manner, number of J2 was inhibited in soil amended with neem powder at 2% as the reduction percentage was 78%, compared to 62% reduction by fresh chopped leaves at 2%. The lowest reduction % (44%) was recorded with 1% fresh leaves as shown in Table (1).

Results in Table (2) cleared that dry decreased the numbers leaves of final nematode females, nematode population (Pf) and consequently the reproduction factor (Rf) compared to fresh leaves application. Results indicated also that dry leaves powder at 2% was the most effective treatments in reducing number of nematode females by 78 and 85% for nematode final population and reproduction factor, respectively. The lowest percentage of reduction was obtained with the treatment of 1 % fresh chopped leaves

Treatment	Doses %	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Efficacy %	Fresh root weight (g)	Efficacy %	Dry shoot weight (g)	Efficacy %
Powder	1	86.0 b	23.3 b	34.3 b	33.5	3.1 b	-	3.7 b	-
	2	90.0 a	30.0 a	39.2 a	52.5	6.6 a	120	6.3 a	70.3
Fresh	1	61.7 d	14.5 d	25.5 d	-	2.9 b	-	3.6 b	-
	2	78.3 c	20.0 c	30.7 c	14.9	6.0 a	100	6.4 a	73
Nematode alone		61.0 d	13.0 d	25.7 d	-	3.0 b	-	3.7 b	-

Table (3): Effect of fresh and dry neem leaves on the plant growth parameters of root-knot nematodes infected tomato plants.

Means, in each column, followed by different letters are significantly different according to Duncan's Multiple Range Test (p < 0.05). Efficacy %= Treatment – N alone/ N alone × 100.

Table (3) cleared that all evaluated dry and fresh neem leaves markedly affected the vegetative plant growth parameters i.e. shoot and root lengths, and weights as well as dry shoot weights compared to treated plants with nematode alone. highest Results confirmed that the percentage of shoot and root fresh weights encouragement recorded by applying powder leaves at 2% by 52.5 and 120%, respectively. The exception was recorded with dry shoot weight as the treatment of fresh leaves at 2% recorded the highest percentage of efficacy (73%) and the least one observed with fresh and dry leaves at 1%.

Activity of some antioxidant enzymes, carbohydrates and phenols in leaves of tomato plants infected with root-knot nematode,*Meloidogyne javanica* treated with fresh and dry neem leaves.

It can be noticed from the data in Tables (4 and 5) that antioxidant enzymes activity i.e. peroxidase, phenoloxidase and catalase as well as the chemical carbohvdrates components i.e. and affected phenols were bv neem application either as dry powder or fresh chopped leaves at both different levels 1 and 2% compared to nematode alone.

Data in Tables (4 and 5) revealed that the highly significant activity of peroxidase, phenoloxidase and catalase as well as the highly content of chemical components i.e. carbohydrates and phenols achieved by the dry and fresh leaves application at 2% followed by fresh chopped leaves at 2% compared to nematode alone.

Results found that there is no significant difference between both fresh and dry leaves at 2% in its effect on the enzymes activity and chemical component, except peroxidase and carbohydrates with fresh chopped leaves at 2%.

Table (4): Activity of some antioxidant enzymes in tomato plants infected with root-knot nematode,
Meloidogyne javanica and treated with fresh and dry neem leaves.

Treatment	Freatment (O.I		Phenoloxidase (O.D.g ⁻¹ Drw.) after 45 min.	Catalase mM. H ₂ O ₂ mg ⁻¹ min ⁻¹		
Powder	1	0.88 b	0.66 c	27 ab		
Fowder	2	0.91 a	0.90 a	29 a		
Fresh	1	0.69 d	0.77 b	26 ab		
FIESH	2	0.79 c	0.87 a	29 a		
Nematode alone		0.18 e 0.22 d		19 c		
Untreated (control)		0.19 e	0.39 d	20 c		

Means, in each columns, followed by different letters are significantly different according to Duncan's Multiple Range Test (p < 0.05). Drwt= dry weight.

Table (5): Effect of fresh and dry neem leaves application on total carbohydrates and ph	enols in
tomato plants infected with Meloidogyne javanica.	

Treatment	Levels %	Carbohydrates (mg / 100 g Dr wt)	Total phenols (mg / 100 g Dr wt)		
Dourdor	1	2812.5 b	0.057 b		
Powder	2	5000.0 a	0.076 a		
Freeh	1	2187.5 c	0.053 b		
Fresh	2	2500.0 bc	0.075 a		
Nematode alone		625.0 d	0.0036 d		
Untreated	l (control)	937.5 d	0.0045 c		

Means, in each columns, followed by different letters are significantly different according to Duncan's Multiple Range Test (p < 0.05). Drwt= dry weight.

The interested data showed that there was a negative correlation between shoot and root fresh and dry root weights; number of galls; egg masses; number of eggs and reproduction factor (Table 6).

Moreover, there was negative correlation between peroxidase, phenoloxidase, catalase and number of galls, egg masses, number of eggs and reproduction factor (Table 7).

Table (6): Correlation between plant growth parameters and number of galls, egg masses, egg	gs
and reproduction factor with fresh and dry neem leaves application.	

I		No. of			Fresh	_	
Treatment	No. of galls	egg masses	No. of eggs	Fresh shoot weight	root	Dry shoot weight	Reproduction factor
Reproduction factor	0.996***	0.969*	0.871	-0.966*	-0.840	-0.648	-
No. of galls	-	0.970*	0.862	-0.964*	-0.871	-0.692	0.996**
No. of egg masses		-	0.732	-0.882	-0.933	-0.692	0.969*
No. of eggs			-	-0.965*	-0.531	-0.797	0.871
Fresh shoot weight				-	0.728	0.504	-0.966*
Fresh root weight					-	0.957*	-0.840
Dry shoot weight						-	-0.648

Table (7): Correlation between antioxidant enzymes activity and number of galls, egg masses, eggs and reproduction factor with fresh and dry neem leaves application.

Treatment	Peroxidase	Phenoloxidase	Catalase	No. of galls	No. of egg masses	No. of eggs	Reproduction factor
Reproduction factor	- 0.874	- 0.349	- 0.798	- 0.692	0.969*	0.871	-
Peroxidase	-	0.612	0.556	- 0.836	- 0.762	- 0.889	- 0.874
Phenoloxidase		-	0.756	- 0.417	- 0.497	- 0.078	- 0.349
Catalase			-	- 0.825	- 0.915	- 0.436	- 0.798
No. of galls				-	0.970*	0.862	0.996**
No. of egg masses					-	0.732	0.969*
No. of eggs						-	0.871

DISCUSSION

Our results revealed that incorporation the fresh and dry neem leaves at both used levels proved to be efficient in decreasing nematode population with positive effects on growth parameters of tomato plants compared to the treated plants with nematode alone.

Usha Mehta and Sundararaj (1995) revealed that application of all neem products as a organic amendments enhanced the growth of plants as measured by shoot and root length and weight. The reduction in nematode population can be attributed to the nematicidal activity of the neem component in neem leaves (Egunjobi and Larrinde, 1975) as well as the neem amendment proving the efficacy of the antagonistic microorganism.

Pandy (2000) evaluated the influence of three organic materials i.e. neem cake; nees leaf powder (*Adhatoda vasica*) and leaf powder of *Murraya koenigii* (Spreng) and two nematicides on *M. incognita* reproduction on *Mentha arvensis*. Results revealed that the maximum reduction in *M. incognita* populations and improving plant growth occurred at high doses of neem cake followed by nees leaf powder (*A.* *vasica*). Reduction in nematode population density by application of plant leaf powder of *A. vesica* and *M. Koenigii* might have resulted from a toxic effect on the nematodes or they might have interfered with chemicals affecting the susceptibility of menthol mint towards root-knot nematode. Inhibition of *M. incognita* reproduction resulted in the significant improvement in *M. arvensis* growth.

Neem contains various compounds that are toxic to many groups of insects, arthropods as well as nematodes, among which azadirachtin, a triterpenoid of the limonoid class, is the most active compound for the inhibition of nematode development (Mordue (Luntz) *et al.*, 2005).

Hussain *et al.*, (2011) found that neem *A. indica* caused maximum reductions in the number of galls, egg masses and reproduction factor (Rf) of the nematode and increases all growth parameters of okra.

Amendments not only change physical and chemical soil properties, but also support a wide variety of antagonistic fungi, bacteria, microorganisms like etc...(Jaffee et al., 1998; Timm et al., 2001) that through completion, antibiosis or parasitism can retard the populations of plant disease-inciting agents like nematodes, bacteria and fungi. Addition of soil amendments results in a considerable increase in the liberation of CO₂ through saprophytic activities the of soil saprophytes which can suppress the activities of disease causing agents (Papavizas and Davey, 1992).

Sayre (1980)postulated two hypotheses, which explain the effectiveness of soil amendments in two ways. The decomposition products from soil amendments are directly toxic to plant nematodes and manipulation of soil microbial populations by addition of amendments initiates a cascade of events of favoring the build-up bacteria. microbivorers, nematode-trapping funai and other soil antagonists that destroy parasitic nematodes. The breakdown of organic matter releases compounds into soil that may be toxic to nematodes. Hussain et al., (2011) confirmed the nematicidal effect of neem is attributed to naturally occurring chemicals i.e. azadirachtin, nimbin, salannin, nimbidin, kaempferol, thionemone, quercetin and others.

Devakumar *et al.,* (1985) identified limonoids in neem that were highly active against nematode. The limonoids are compounds belonging to the betafuranotriterpenoid group.

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