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EFFICACY OF CHOPPED FRESH LEAVES OF SOME PLANTS ON CONTROLLING *Meloidogyne javanica* INFECTING TOMATO PLANTS

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ABSTRACT

Eight chopped fresh leaf plants namely; cabbage, lantana, castor bean, radish, watercress,, peppermint, camphor and datura were mixed with soil pots at two doses of 1 and 2 % of soil weight for controlling root-knot nematode, *Meloidogyne javanica* infecting tomato plants under greenhouse condition. Results revealed that all tested chopped leaf plants at both doses were effective in reducing nematode parameters compared to plants treated with nematode alone. Moreover, results confirmed that there are no significant differences between different chopped leaf plants with both doses in reducing nematode parameters. Cabbage and camphor chopped leaves at 2% significantly increased root fresh weight by 100%. Shoot fresh and dry weights were significantly increased with all tested chopped leaves at both doses except radish, watercress and peppermint chopped leaves. The activities of antioxidant enzymes i.e. phenoloxidase, peroxidase and catalase also showed high significant enhancement with all treatments at both doses compared with plants treated with nematode alone. The highest significant increase in antioxidant enzymes was obtained with cabbage and camphor chopped fresh leaves at 2%.

Key words: Control, soil amendments, root-knot nematodes, tomato, *Lycopersicon esculentum*, antioxidant enzymes

INTRODUCTION

Root knot nematodes (*Meloidogyne* spp.) cause conspicuous root galls and serious reduction in yield of several host plants. They are of major economic significant throughout the tropics and warmer regions of the world. Infected plants suffer vascular damages, which disturb water and mineral uptake (Luc *et al*., 2005). Although, chemical nematicides hold major promise in nematode control (Adegbite and Adesiyan, 2001 and Oyedunmade *et al.,* 1992). The high cost, harmful effects on environment, flora and fauna in cultivated area (Tanweer and Hisamuddin, 2012) as well as their non availability at the time of need. For these reasons, several researchers have investigated the safety control methods i.e. soil amendments as they have been explored as a method of suppressing plant-parasitic nematodes (Akhtar and Malik, 2000 and Chitwood, 2002).

The aim of this study was to evaluate eight chopped leaves plants at two doses against root-knot nematode *Meloidogyne javanica* infecting tomato plants under greenhouse condition.

MATERIALS AND METHODS

In this study eight different plants namely: cabbage (*Brassica aleracea*); Lantana *(Lantana camara*); castor bean (*Ricinus communis*); radish (*Raphanus sativus*); watercress (*Eurica sativus*); peppermint (*Menthe piperita*); camphor (*Eucalyptus citriodora* L.); datura (*Datura stramonium*) were used as a chopped fresh leaves against *Meloidogyne javanica* infecting tomato plants. The chopped fresh leaves of the eight plants were mixed thoroughly with 2 kg of non-sterilized sandy-clay mixture soil (2:1, v/v) of 15 cm diam plastic pots at 1 and 2% of soil weight (w/w), one week before tomato transplanting. The pots were kept moist under greenhouse condition for one week to allow the chopped leaves to decay. Three weeks-old tomato plants *Lycopersicon esculentum* Mill. cv. GS were singly transplanted into pots (one

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seedling/pot). Two thousand newly hatched 2nd stage juveniles of *M.* javanica were pipetted into three holes around tomato roots.

Culture of root-knot nematode, *M. javanica* was established from single egg mass of adult females previously identified using morphological characteristics of female perineal patterns as described by Taylor and Sasser (1978). Pure culture was reared on susceptible tomato plants in a greenhouse at 30± 5°C. Nematode eggs were extracted from heavily galled tomato roots using sodium hypochlorite (NaOCl) solution technique as described by Hussey and Barker (1973). Root-knot juveniles (J_2) were collected from hatched eggs (Oostenbrink, 1960). Fifty four pots were used in this experiment. Each treatment was replicated three times. Fifty one pots were inoculated with 2000 J_2 of *M. javanica*. Three pots were left untreated with chopped leaves or nematode to serve as a control.

Pots were arranged using randomized block design under greenhouse condition. Plants watered daily and fertilizered weekly with a 5 ml of 2 g/l N:P:K (20:20:20), obtained from the International Egypt Company for Agricultural and Industrial Developing. Pots were maintained 60 days after nematode inoculation. At the end of experiment, plants were gently removed, roots were carefully washed and the following measurements were determined: number of galls, egg masses, females and developmental stages/root system, number of eggs/egg mass, number of J_2/250 g soil, final nematode population (P_F) as well as reproduction factor (RF). Egg masses were counted by dipping the roots into phloxine-B staining solution (0.15 g/l tap water) for 20 min. according to Daykin and Hussey (1985). Females were collected by cutting the root system of each plant into 2 cm pieces and submerged in a beaker full of tap water for 4 days, at root temperature, until the root pieces became decayed. Then, the decayed roots were washed with tap water through 250 and 500 mesh sieves to separate females from the root debris (Mahdy, 2002).

Final nematode population (P_f) was assessed according to the following equation: P_f= (No. of egg-masses/plant × No. of eggs / egg mass) + No. of females/plant + No. of developmental stages/plant + No. of J_2/250 g soil. Reproduction factor (R_f) = P_f / initial population (P_i) was also recorded (Norton, 1978).

Plant growth parameters i.e. shoot and root fresh weights and shoot dry weight were recorded.

Activity of Antioxidant Enzymes:

The activity of antioxidant enzymes i.e. phenoloxidase, peroxidase and catalase activities were estimated in tomato fresh leaves of different treatments according to Broesh (1954); Fehrman and Dimond (1967) and Bach and Oparin (1968).

Statistical analysis:

Data were analyzed according to standard 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 at $p<0.05$. Correlations between all parameters were done according to Waller and Duncan (1969).

RESULTS

Data presented in Table (1) reported that all evaluated chopped leaf plants either at 1 or 2 % of soil weight were effective in reducing all nematode parameters compared to nematode alone. Results confirmed that there are no significant differences between the most evaluated chopped fresh leaf plants and it's both doses in reducing the nematode parameters (Table 1).

Results shown in Table (1) cleared that the reduction percentage of galls was ranged between 39 and 79%. The highest reduction percentage of number of nematode galls between the evaluated chopped fresh leaf plants was obtained with cabbage followed by lantana and camphor at 2% with 79, 77 and 74

reduction %, respectively. The lowest reduction percentage less than 50% obtained with radish, watercress and peppermint at 1% by 39, 41 and 46%, respectively compared to nematode alone.

Egg masses were also significantly affected by all incorporated fresh chopped leaves compared to plants treated with nematode alone. Reduction percentage was convincing with cabbage fresh chopped leaves followed by lantana, camphor and castor by 52, 44, 42 and 41% at 2%, respectively (Table 1).

Results revealed that cabbage, lantana, castor, camphor and datura had the same effect at both used doses in reducing all nematode parameters. The exception noticed with number of eggs/egg mass as the fresh chopped leaves of cabbage of cabbage at 2% was the most effective treatment as the reduction percentage was 71%, followed by lantana at 2% by 68% as shown in Table (1). Results also revealed that all evaluated plants significantly reduced the mean number of 2nd stage juveniles in soil pots compared to plants treated with nematode alone (Table 1). The highest reduction percentage (72%) was recorded with chopped fresh cabbage leaves at 2%, followed by that of lantana at 2% by 70%. The lowest effect recorded with radish at 1% by 43%, followed by watercress and peppermint by 46 and 48% at 1%, respectively (Table 1).

Nematode final population (P_f) and reproduction factor (R_f) were also significantly affected by all evaluated plants at both doses compared to plants treated with nematode alone (Table 2). Results confirmed that the highest reduction percentage in both nematode parameters was recorded with fresh chopped leaves at 2% by 84%, followed by lantana by 80% at 2%, cabbage at 1% by 79%, camphor at 2% by 78% and castor at 2% by 76% for both nematode parameters. The lowest reduction percentage of P_f and R_f were recorded with watercress, peppermint and radish chopped fresh leaves at 1% with 16, 19 and 23 reduction %, respectively.

Table (1). Effect of eight chopped fresh leaf plants on numbers of galls and egg masses/plant, eggs/ egg mass and J_2/ 250 g soil of *M. javanica* infecting tomato plants and reduction %.

Treatment	Dose % / 2 kg soil	Numbers of / plant				Eggs/egg mass	*Reduction %*	J_2/250 g soil	*Reduction %*
		Galls	*Reduction %*	Egg masses	*Reduction %*				
Cabbage	1	29.0 hij	*71*	18.7 g	45	175.0 m	65	983 efg	66
	2	21.7 j	*79*	16.3 h	52	143.3 q	71	800 g	72
Radish	1	61.3 b	*39*	31.7 b	7	430.0 f	14	1650 b	43
	2	51.0 cd	*50*	25.7 c	24	475.0 d	5	1280 cde	56
Watercress	1	59.7 b	*41*	30.7 b	10	495.0 b	1	1580 bc	46
	2	47.0 de	*54*	25.0 c	27	425.0 g	15	1267def	56
Lantana	1	32.3 ghi	*68*	21.3 ef	37	243.3 l	51	1033 efg	64
	2	23.7 jk	*77*	18.9 g	44	158.3 p	68	883 fg	70
Castor	1	39.4 ef	*61*	24.3 cd	29	266.7 i	47	1167 def	60
	2	31.7 ghi	*69*	20.0 fgh	41	188.3 m	62	1000 efg	66
Peppermint	1	55.0 bc	*46*	30.0 efg	12	490.0 c	2	1500 bcd	48
	2	51.0 cd	*50*	25.0 c	27	450.0 e	10	1440 bcd	50
Camphor	1	37.0 fg	*63*	22.7 de	33	263.7 j	47	1146 defg	60
	2	26.7 ijk	*74*	19.7 fg	42	171.7 o	66	983 efg	66
Datura	1	46.0 de	*55*	24.7 cd	27	320.0 h	36	1067 def	63
	2	34.7 fgh	*66*	22.7 de	33	250.0 k	50	1027 efg	65
Nematode alone		101.3 a	-	34.0 a	-	500.0 a	-	2900 a	-

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$). *P_f = Final population. **R_f = Reproduction factor. Pot contains 2 kg soil.

Table (2). Effect of eight chopped fresh leaf plants on numbers of females, nematode developmental stages, final population (Pf) and reproduction factor (Rf) of M. javanica infecting tomato plants and reduction %.

Treatment	Dose % / 2 kg soil	Numbers of / plant				Pf	Reduction %	Rf	Reduction %
		Females	Reduction %	Nematode developmental stages	Reduction %				
Cabbage	1	22.3 jk	75	28.7 h	64	4306.5 b	79	2.2 lm	78
	2	16.7 k	71	22.3 i	72	3174.8 b	84	1.6 n	84
Radish	1	71.3 b	21	65.7 b	18	15418.0 b	23	7.7 d	23
	2	46.3 cde	19	50.3 cd	37	13584.1 b	32	6.8 e	32
Watercress	1	50.7 c	14	52.0 c	35	16879.2 b	16	8.4 b	16
	2	41.0 efg	54	44.3 ef	49	11977.0 b	40	6.0 g	40
Lantana	1	29.3 hi	67	29.3 h	64	6273.9 ab	69	3.1 j	69
	2	21.0 jk	77	23.7 i	70	3919.6 b	80	2.0 m	80
Castor	1	35.0 gh	61	41.3 f	49	7723.8 b	62	3.9 i	61
	2	27.3 ij	70	29.05 h	64	4822.3 b	76	2.4 l	76
Peppermint	1	48.0 cd	47	49.3 cd	39	16297.3 b	19	8.1 c	19
	2	43.3 def	52	46.0 de	43	12779.3 b	36	6.4 f	36
Camphor	1	31.0 ij	66	40.0 f	50	7203.0 b	64	3.6 j	64
	2	21.7 jk	76	26.7 hi	68	4413.9 b	78	2.2 lm	78
Datura	1	37.7 fg	58	44.0 ef	45	9052.4 b	55	4.5 h	55
	2	30.0 hi	67	33.7 g	58	6765.4 b	66	3.4 k	66
Nematode alone		90.0 a	-	80.3 a	-	20070.3 a	-	10.0 a	-

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$). * Pf = Final population. **R_f = Reproduction factor. Pot contains 2 kg soil.

Fresh shoot and root weights, as well as dry shoot weight of nematode infected plants were markedly affected by amending the fresh chopped leaves of the eight tested plants singly with soil pots compared to plants treated with nematode alone as presented in Table (3). Data showed that all treatments had no significant effect on plant growth parameters when compared with treated plants with nematode alone. Fresh root weight showed significant increase with cabbage and camphor at 2% (Fig. 1A). Results found that chopped fresh leaves of radish, watercress and peppermint had also no significant effect at both used doses on fresh and dry shoot weights of tomato compared to plants treated with nematode alone (Fig. 1B &C).

Table (3). Effect of different chopped fresh leaves on growth parameters of tomato plants infected with *M. javanica*.

Treatment	Dose %	Root fresh weight (g)	*Increase %*	Shoot fresh weight (g)	*Increase %*	Shoot dry weight (g)	*Increase %*
Cabbage	1	3.2 bc	-	25.5 abcd	*63.5*	6.1 ab	*41.9*
	2	4.6 a	*100*	28.8 a	*84.6*	6.8 a	*58.1*
Radish	1	2.6 c	-	18.5 fgh	-	4.3 gh	-
	2	2.9 bc	-	18.7 fgh	-	4.6f gh	-
Watercress	1	2.8 bc	-	17.3 gh	-	4.5f gh	-
	2	3.2 bc	-	19.2 efgh	-	4.9 defg	-
Lantana	1	3.0 bc	-	23.4 bcde	*50.0*	5.6 bcd	*30.2*
	2	3.6 b	*56.5*	26.2 abc	*67.9*	5.9 bc	*37.2*
Castor	1	2.9 bc	-	24.2 abcd	*55.1*	5.3 bcde	*23.3*
	2	3.3 bc	-	25.3 abcd	*62.2*	5.8 bc	*34.9*
Peppermint	1	2.6 c	-	18.5 fgh	-	4.6 efgh	-
	2	2.8 bc	-	21.5 defg	*37.8*	4.7 efg	-
Camphor	1	3.2 bc	-	24.8 abcd	*59.0*	5.6 bcd	*30.2*
	2	4.6 a	*100*	27.4 ab	*75.6*	6.8 a	*58.1*
Datura	1	2.9 bc	-	22.1 def	*41.7*	5.1 cdef	*18.6*
	2	3.3 bc	-	24.4 abcd	*56.4*	5.6 bcd	*30.2*
Nematode alone		2.3 c	-	15.6 h	-	4.3 gh	-
Untreated		2.6 c	-	18.5 fgh	-	3.9 h	-

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$). Increase % = treatment – N alone / N alone × 100.

Antioxidant enzymes in leaves as affected by applying different fresh chopped leaves in nematode infected tomato plants:

Data presented in Table (4) showed that the most evaluated chopped leaves plants significantly increased the activity of all antioxidant enzymes at both used levels compared to treated plants with nematode alone. The highest value of phenoloxidase recorded with cabbage chopped leaves at 2%, followed by camphor at 2%. Peroxidase and catalase recorded high significant value with cabbage and camphor at 2% compared to plants treated with nematode alone (Table, 4).

Data presented in Table (5) indicated that there was a highly significant negative correlation between antioxidant enzymes activity of catalase and number of galls, eggs and reproduction factor and only significant correlation with number of egg-masses. There was highly negative correlation between peroxidase enzyme activity and number of galls and eggs and only significant correlation with number of egg-masses and reproduction factor. Also, there was only significant correlation between phenoloxiodase and number of galls and reproduction factor and non significant correlation with number of egg-masses when used green manure in controlling nematode infection. Moreover, there was a significant negative correlation between nematode parameters and root fresh weight and shoot dry weight, but

highly significant negative correlation with shoot fresh weight (Table 6).

Table (4). Effect of chopped fresh leaves on the enzymes activity of phenoloxidase, peroxidase and catalase in the leaves of tomato plants infected with *M. javanica*.

Treatment	Dose %	Phenoloxidase O.D.	Peroxidase O.D.	Catalase mM H_2O_2 mg^{-1} min^{-1}
Cabbage	1	0.45 f	0.88 d	2.04b
	2	0.99 a	1.40 a	2.40a
Radish	1	0.35 k	0.22 i	1.30g
	2	0.44 f	0.58 e	1.60de
Watercress	1	0.32 l	0.14 j	0.94h
	2	0.41 g	0.58 e	1.50fg
Lantana	1	0.38 hi	0.29 h	1.70cd
	2	0.88 c	1.30 b	2.10b
Castor	1	0.37 ij	0.28 hi	1.40fg
	2	0.66 d	0.98 c	2.00b
Peppermint	1	0.35 k	0.23 hi	0.89h
	2	0.38 hi	0.61 e	1.40fg
Camphor	1	0.38 hi	0.40 g	1.80c
	2	0.95 b	1.40 a	2.40a
Datura	1	0.36 jk	0.28hi	1.30g
	2	0.58 e	0.90d	2.00b
Nematode alone		0.30 m	0.13j	0.87h
Untreated		0.39 h	0.50f	1.50ef

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Table (5). Correlation between enzymes activity and number of galls, egg masses, eggs and nematode reproduction factor with chopped fresh leaves application.

Factor	Peroxidase	Phenoloxidase	Catalase	No. of galls	No. of egg masses	No. of eggs	R_f
R_f	-0.559*	-0.532*	-0.623**	0.942***	0.954***	0.883***	-
Peroxidase	-	0.935***	0.796***	-0.660**	-0.545*	-0.672**	-0.559*
Phenoloxidase	-	-	0.775***	-0569*	-0.441	-0.598**	-0.532*
Catalase	-	-	-	-0.703**	-0.525*	-0.724***	-0.703**
No. of galls	-	-	-	-	0.966***	0.966***	0.942***
No. of egg masses	-	-	-	-	-	0.881***	0.954***
No. of eggs	-	-	-	-	-	-	0.883***

Rf = Reproduction factor.

Table (6). Correlation between plant growth parameters and number of galls, egg-masses, eggs and nematode reproduction factor with fresh chopped leaves application.

Treatment	No. of galls	No. of egg-masses	No. of eggs	Fresh root weight	Fresh shoot weight	Dry shoot weight	R_f
R_f	0.942***	0.954***	0.883***	-0.529*	-0.685**	-0.511*	-
No. of galls		0.966***	0.966***	-0.528*	-0.641**	-0.469*	0.942***
No. of egg-masses		-	0.881***	-0.406	-0.506*	-0.312	0.954***
No. of eggs			-	-0.561*	-0.732***	-0.568*	0.883***
Fresh root weight					0.827***	0.897***	-0.529*
Fresh shoot weight						0.940***	-0.685**
Dry shoot weight							-0.511*

DISCUSSION

Applying the leaves of the eight different plants as a fresh chopped leaves at 1 and 2% of soil weight were significantly reduced the nematode population on tomato roots compared to treated plants with nematode alone. The chopped leaf of cabbage was the best one as it increased the nematode reduction, whereas the radish chopped leaves showed the lowest effective one. The use of such plants as fresh green amendments has been shown to maintain or improve soil organic matter (Moody *et al.,* 1999 and Dunn, 2002), and in some cases reduce plant parasitic nematode population (McSorley, 1999 and Pankhurst *et al.,* 1999). Soil organic matter improves the soil environment in which the root grow well, as well as reducing the sensitivity of plants to nematode injury (Dunn, 2002). The present results generally complement those of other researchers (Bridge, 1987; Minton and Baujard, 1990; McSorley, 1999; Ploeg, 1999 and VanBiljon and Meyer, 2000). Although where contradictions occur they may be due to the differences in susceptibility between cultivars (McSorley and Dickson, 1995). Ekaterini and Prosser (2003) reported that applying the green manure amendments were enhanced the control of nematode, fungi and suppress weeds, improve soil physical conditions (increase water infiltration, decrease erosion). Decomposition of incorporated crop biomass releases nematicidal compounds and enhanced the growth of nematode bioantagonists. The crops yield increases were attributed to nutritional effect of the green manure and the reduction of nematode populations or the modification of factor linked to the nematode populations induced by their cropping (Jean *et al.,* 1992). Some compounds released form decaying plant materials have shown nematode suppressive properties such as ammoniacal nitrogen and isothiocyanates (Crow *et al*, 1996). Isothiocyanates are released and could explain both reduced galling and phytotoxic effect when adding the rapeseed green manure. It has been demonstrated that cabbage contains glucosinolates, which are a unique family of compounds that have many exciting properties which could be used to improve plant protection against nematodes, herbivores and other pathogens. Although their potential has sometimes been in doubt and attempts to use them have met with mixed success which increase antioxidant system by protecting plants

from free radicals produced due to nematode infection. There is evidence that they may find an important role in an integrated pest management system when used wisely (Lazzeri and Manici, 2001).

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