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Impact of Four Organic Acids on *Meloidogyne Incognita* Infecting Tomato Plants under Greenhouse Conditions

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ABSTARCT

A pot trail was conducted in order to study the influence of four organic acids i.e. salicylic , citric , oxalyic and humic acids at two concs.0.1& 0.05% in comparison with oxamyl against *Meloidogyne incognita* infecting tomato cv.9065FI under greenhouse(24±2C°).Results indicated that all tested treatments clearly improved tomato plant characters and reduced nematode development. Among the organic acid tested, salicylic acid at the two concs. surpassed other application in values of percentage increase of plant length (23.37&40.54%), number of leaves (28.03&55.3%), number of branches (16.7&54.2), total plant fresh weight (32.3&57.23%) and shoot dry weight (25.2&33.84%), and achieved the highest percentage reduction values of final nematode population(78.08%), number of egg- masses (89.47%), respectively. In the mean time, oxalic acid applications gave the least values in this respect comparing to the check. Salicylic acid and humic acid at 0.05% since their eggmasses indices were in par (2) vs (4) for nematode alone. Among tested concentrations , 0.1 g/L of such organic acids was higher in values of N, P , K , C , O.M and total chlorophyll as compared to nematode alone , whereas the concentration of 0.05g/L of the four organic acids ranked first in percentage increase values of total phenol which were amounted to 8.39 , 6.36 , 4.43 and 2.68% for salicylic , citric , oxalic and humic acids comparing to nematode alone , respectively.

Keywords: Salicylic , citric , oxalyic, humic, acid, *Meloidogyne incognita*, nematode , oxamyl.

INTRODUCTION

The root knot nematodes, *Meloidogyne* spp. are one of the most harmful nematode pests in both tropical and subtropical crop production regions and cause extensive economic damage worldwide **(Rehman *et. al.*, 2012).** Tomato is preferable host to several species of root-knot nematodes. Modern way of nematode management is totally based on the nematicides as higher population growth demands increase in crop production. On the other hand these nematicides are not only toxic to the root-knot nematodes but also accumulate in plant. These nematicides often lead to environmental pollution and even the depletion of stratospheric zone **(Wheeler and Starr 1987).** Hence, there is the most importance need for an eco-friendly substitute for nematode management. Many occurring compounds are known to possess nematicidal activity such as organic acids. Organic acids released during the decomposition of raw organic materials are one of many factors contributing to reduce nematode damage **(McBride *et al.*, 2000),** but little is known about the direct effects of low-molecular-weight organic acids for nematode control. A new strategy for adjusting plant parasitic nematodes is based on the activation of the plant's own defense system through various biotic and abiotic agents such as organic acids, vitamins and inorganic minerals. The possibility of using four organic acids in the control of *M. incognita* infecting tomato plant was evaluated under greenhouse conditions.

MATERIALS AND METHODS

1. Nematode stock culture, propagations and preparing nematode inoculum:

To collect and determine the inocula of *Meloidogyne incognita* eggs; *M. incognita* was previously identified according to **Taylor *et al.* (1955).** Infected root systems with heavy eggmasses of *M. incognita* of various growing coleus, *Coleus blumei* plants, grown in 25 cm-diam plastic pots filled with sterilized clayey soil, at the Nematology Research unit (NERU). Unit, Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt, were well washed and cleaned by running tap water, then placed in a plastic container with enough solution of 1.0% NaOCl for 60 seconds, shaked vigorously (manually) then quickly passed through nested sieves and thoroughly washed the collected eggs with tap water to remove the bleach (**Hussey and Barker, 1973**) . Eventually, the number of eggs per unit volume of water was counted and then plants were inoculated directly with eggs according to the design of each experiment of this work which was carried at the greenhouse of Nematological Research Unit (NERU).

2-Pesticide

Oxamyl (Vydate 10 G) Methyle-N-N-dimethyl-(N-(methyl) carbomycocyl)-1-Thioxamidate.

3-Preparation of Tested Organic Acids:

Four organic acids i.e. salicylic, oxalic, citric and humic were separately prepared by using 0.05&0.1g for each that added to 1 Liter distilled water to be 0.05% and 0.1% concentrations respectively.

4-Controlling *Meloidogyne incognita* Infecting Tomato Plant By Certain Organic Acids in Comparison with Oxamyl Under Greenhouse Conditions.

Greenhouse experiment was conducted to examine the influence of four organic acids i.e. citric, humic, oxalic and salicylic acid in comparison with oxamyl on tomato cv.6090FI, plants under the stress of *M. incognita* infection at(24±2C°).Forty four plastic pots (15cm-diam) containing 1600g steam- sterilized clay sand(1:1,v:v)with one tomato seedling 30 days-old each were used in this study .One week after transplanting tomato seedlings ,forty ones were inoculated with 2000 *Meloidogyne incognita* eggs each inoculated where tomato seedlings were sprayed by either 0.05 or 0.1 %concentrations of each organic acids under study at the rate of 4 replicates/level per each organic acid. This process was repeated four times at one week intervals and aluminum foil covered the soil surface of each pot to prevent drops of the liquid. Oxamyl at the rate of 0.3g/pot/seedlings for 4 replicates was added at the first time of spraying materials of the tested components where four seedlings with nematode only and another four seedlings without nematode and any treatment left for control .Each treatment was replicated four times and treatments were as followed :

1-N+citric acid (0.05% 15ml sprayed),
2-N+citric acid (0.1% 15ml sprayed),
3-N+humic acid (0.05% 15ml spraye,d)
4-N+humic acid (0.1% 15ml sprayed),
5-N+oxalic acid (0.05% 15ml sprayed),
6-N+oxalic acid (0.1% 15ml sprayed),
7-N+salicylic acid (0.05% 15ml sprayed),
8-N+salicylic acid (0.1% 15ml sprayed),
9-N+oxamyl (0.3g/plant),
10-N alone, and
11-Plant free of Nematode or any treatment.

Plastic pots were then arranged in randomized complete block design in the greenhouse and treated horticulturally, protected against mites and insects by conventional pesticide and irrigated with water as needed. Plants were harvested after 45 days from nematode inoculation and plant growth criteria i.e. plant length, fresh weight of shoot and root, shoot dry weight were determined and recorded.

Infected tomato roots of each concentration per each organic acid/replicate, oxamyl treatment and nematode alone were washed with tap water separately fixed in 4% formaline for 24 hrs and stained in acid fuchsin **(Byrd, *et al.*,1983)** and examined with stereoscopic microscope for the number of galls, eggmasses , developmental stages and femals , of *M. incognita* and recorded. *Meloidogyne incognita* (js) was extracted from soil/plastic pot in 250g/replicate the through sieving and modified baermann technique **(Goodey , 1957),**counted by Hawksely counting under ×10 magnification microscope , recorded and calculated for each pot. The root gall index (RGI) and egg mass index (EI) were estimated according to the scale given by **Taylor and Sasser (1978)** as follows: 0= no galls or egg-masses, 1= 1-2 galls or egg-masses, 2= 3-10 galls or egg-masses, 3= 11-30 galls or egg-masses, 4= 31-100 galls or egg-masses and 5= more than 100 galls or egg-masses

Statistical analysis:

Statistically, the obtained data were subjected to analysis of variance (ANOVA) **(Gomez and Gomez, 1984),** followed by Duncan's multiple range Tested (DMRT) to compare means **(Duncan, 1955).**

Chemical analysis:

Nitrogen(N), phosphorus(P), potassium(K), organic matter(OM) and carbon(O.C) as well as chlorophyll content were determined according to Kjeldahl methods **(A.O.A.C., 1980).**

Determination of total phenols:

In this experiment, total phenols were determined after harvesting in fresh leaves bases using the Folin- Ciocalteau reagent (Kaur and Kapoor 2002). Total catechol equivalents by the following equation:

$$T=c\times V/m\times 100$$

Where:

T- Total content of phenolic compounds, in mg of catechol/100 g of fresh weight material.
c- The concentration of catechol established from the calibration curve, in mg/ml.
V- The volume of extract in ml.
m-The weight of pure plant ethanolic extracting.

RESULTS AND DISCUSSION

Data presented in Table1 reveal the influence of four organic acids i.e. salicylic , citric , oxalyic and humic acid at two concs.0.1& 0.05% in comparison with oxamyl at the recommended dose against *Meloidogyne incognita* infecting tomato cv.9065FI under greenhouse(24±2C°).Results indicated that all tested treatments obviously increased the percentage values of tomato plant characters. Among the organic acid tested, salicylic acid at the two concs .surpassed other treatments in values of percentage increase of plant length (23.37&40.54%), number of leaves (28.03&55.3%), number of branches (16.7&54.2), total plant fresh weight (32.3&57.23%) and shoot dry weight (25.2&33.84%), followed by that humic acid treatments in this respect comparing with nematode alone, respectively (Table1), Moreover, plant receiving citric acid treatments showed the intermediate position in this respect, whereas, oxalic acid treatments gave the least values of percentage increase of plant length (15.3&19.0%),number of leaves (10.6&18.2%),number of branches(1.7&8.3%)total plant fresh weight (5.2&9.7%) and shoot dry weight (13.07 &17.7%) comparing to nematode alone, respective,(Table 1).In the meantime, plants receiving none of the tested organic acids and free of nematode showed reasonable percentage increase values of 11.7,11.43 and15.38% for numbers of branches/ plant, total plant fresh weight and shoot dry weight compared to nematode alone. It is interesting to note that oxamyl as a systematic nematicide ranked first in percentage increase values of number of leaves (65.15%)0nly,whereas salicylic acid(0.05%),ranked first in percentage increase values of plant length(40.54%),total plant fresh weight (57.23)shoot dry weight(33.84%).compared to nematode alone, respectively (Table1).

Table (1) : Impact of four organic acids at two concentrations applied as foliar spraying application on tomato plant cv. 9065 FI under the stress of *Meloidogyne incognita* infection in the green house conditions (24±2C^0).

Treatments	Conc.%	*Plant growth response													
		Length (cm)		Total plant length (cm)	** Inc.%	No. of leaves	** Inc.%	No. of branches	** Inc. %	Fresh weight (g)		Total plant F. Wt (g)	** Inc.%	Shoot dry weight (g)	** Inc.%
		shoot	root							Shoot	root				
salicylic acid +N	0.1	56.7 d	28.8c	85.5d	23.37	42.25 d	28.03	7.0	16.7	14.11 cd	4.54 a	18.65 d	32.3	1.63 a	25.2
Oxalic acid +N	0.1	51.0e	29.9b	80.9f	15.3	36.50 f	10.60	6.1	1.7	10.54 g	4.29 b	14.83 g	5.2	1.47 b	13.07
Citric acid +N	0.1	56.2d	27.0d	83.2e	20.1	41.50 d	25.8	6.75	12.5	12.48 e	4.53 a	17.01 e	20.6	1.48 b	13.84
Humic acid +N	0.1	56.6d	26.7d	83.3e	20.2	41.80 d	26.7	7.0	16.7	13.63 d	3.83 d	17.46 e	23.8	1.60 ab	23.1
Salicylic acid +N	0.05	59.6b	37.8a	97.4a	40.54	51.25 b	55.3	9.25	54.2	17.67 a	4.50 a	22.17 a	57.23	1.47 b	33.84
Oxalic acid +N	0.05	58.4c	24.1f	82.5e	19.0	39.00 e	18.2	6.50	8.3	12.33 e	3.38 e	15.71 f	9.7	1.53 ab	17.7
Citric acid +N	0.05	58.8c	25.0ef	83.8e	20.9	45.50 c	37.9	7.75	29.2	14.40 c	4.10 c	18.50 d	31.20	1.56 ab	20.0
Humic acid +N	0.05	59.8b	28.6c	88.4c	26.7	46.30 c	40.2	8.25	39.6	16.27 b	4.03 c	20.30 c	43.9	1.58 ab	29.23
Oxamyl +N		67.2a	25.5e	92.7b	39.0	54.50 a	65.15	9.0	50.0	17.32 a	3.86 d	21.18 b	50.2	1.57 ab	20.76
N alone		47.6f	21.8g	69.4g	_	33.00 h	_	6.0	_	11.00 fg	3.10 f	14.10 h	_	1.30 c	_
Plant free		48.0f	22.0g	70.0g	1.01	34.00 g	3.03	6.7	11.7	11.50 f	4.20 bc	15.70 f	11.43	1.50 ab	15.38
L.S.D		0.746	1.313	2.579		0.911				0.609	0.153	0.645		0.089	

N = 2000 eggs of *M. incognita* * Each figure is the mean of four replicates.

Means in each column followed the same letter(s) did not differ at P<0.05 according to Duncan's multiple-range test.

Data in Table(2) illustrate the impact of four organic acids, i.e. Salicylic, citri , oxalic ,and humic at two concentration 0.1&0.05% comparing to oxamyl at the recommended dose on *Meloidogyne incognita* infecting tomato plant cv.9065FI under greenhouse condition(24±2C^{o}).Results revealed that nematode parameters were adversely affected by the tested organic acids at the two concentrations. Plant receiving salicylic acid either at 0.1 or 0.05% surpassed other tested organic acids in reducing number of total nematode population (pf),galls and eggmasses on roots, since its concentration at 0.05% achieved the highest percentage reduction values of final nematode population(78.08%), number of egg- masses (89.47%) followed by that of humic acid with values of78.08,83.63,and 87.30 values of final nematode population, number of galls and eggmasses comparing to nematode alone, respectively (Table 2).Plant receiving oxalic acid treatments either at 0.1% or 0.05% gave the least reduction percentage values of final nematode population (70,97&70.17%), number of galls (67.01&81.03%),egg- masses (66.6&84.21%) compared to nematode alone, respectively, whereas citric acid treatments (0.1&0.05) achieved the intermediate reduction percentages values in this respect (Table2). It is worthy to observe that oxamyl as a systemic nematicide ranked first in diminishing nematode parameters i.e. final population (90.7%),number of galls (90.32%) and eggmasses (98.14%),respectively. Moreover, *Meloidogyne incognita* reproduction factor was adversely affected by all organic acids treatments tested at two concentrations. Such rates of nematode reproduction were ranged between 0.35 to 0.49 vs. 1.67for nematode alone. Meanwhile, salicylic acid treatment either at (0.1or0.05%) gave the least value of reproduction factor (RF) which were amounted to 0.43 or 0.35 vs 1.67for nematode alone ,whereas oxalic acid at the same two concs. showed the highest values of RF that appointed to 0.48 or 0.49, vs 1.67 for nematode alone ,respectively. It is worthy to note that oxamyl ranked first in diminishing nematode build-up with value of 0.15 VS 1.67 for nematode alone.(Table2).

Likewise, significant results were noticed between eggmasses indices of all tested treatments and nematode alone, since they ranged from 2 and 3 for humic acid and salicylic acid respectively at 0.1% vs 4 for nematode alone .Similar trend was observed for salicylic acid and humic acid at 0.05% since their eggmasses indices were in par (2) vs (4) for nematode alone. It is worthy to note that all tested applications gave considerable reduction in root galls and eggmasses numbers with root galls indices appointed 3 for the two two concs ,(0.1 & 0.05%)tested except of oxalic acid 0.1% (4)vs 4 for nematode alone (Table 2).

Table (2): Nematode parameters of *Meloidogyne incognita* infecting tomato plant cv. 9065 FI as influenced by four organic acids at two concentrations applied as foliar spraying treatments under green house conditions (24±2C^0).

Treatments	Nematode Parameters*											
	Nematode population in			Final population (Pf)	Red%	RF	NO. of galls	Red %	RGI	No. of Egg-masses	Red %	EI
	Soil (j_2) (1kg)	Roots										
		Females	Dev. stages									
Salcylic acid (0.1%)	840.5 e	19.25 d	11.0 fg	870.75 f	74.0	0.43	14.0 f	85.45	3	9.0 de	88.8	3
Oxalic acid (0.1%)	925.0 c	36.0 b	11.5 f	972.5 c	70.97	0.48	31.75 b	67.01	4	27.0 b	66.6	3
Cetric acid (0.1%)	870.5 d	21.5 c	15.5 d	907.5 d	72.91	0.45	17.75 c	81.55	3	14.0 c	82.7	3
Heumic acid (0.1%)	816.75 f	35.5 b	39.25 b	891.5 e	73.38	0.44	16.75 d	82.59	3	13.0 c	83.0	2
Salcylic acid(0.05%)	686.5 i	15.5 f	8.0 h	710.0 i	78.08	0.35	13.75 f	85.27	3	8.5 e	89.47	2
Oxalic acid (0.05%)	972.0 b	14.0 g	13.25 e	999.25 b	70.17	0.49	18.25 c	81.03	3	12.75 c	84.21	3
Cetric acid (0.05%)	812.0 g	18.0 e	10.5 g	840.5 g	74.91	0.42	16.25 de	83.11	3	12.5 c	84.52	3
Heumic acid (0.05)	696.5 h	17.75 e	19.75 c	734.0 h	78.08	0.36	15.75 e	83.63	3	10.25 d	87.30	2
Oxamyl	304.75 j	2.25 h	4.5 i	311.5 j	90.70	0.15	4.5 g	95.32	2	1.5 f	98.14	1
N alone	3150.75 a	109.75 a	89.5 a	3350.0 a	_	1.67	96.25 a	_	4	80.75 a	_	4
L.S.D	0.939	0.937	0.771	1.864			0.541			1.320		

N=2000 eggs of *M. incognita* * Each figure in the mean of four replicates.
Means in each column followed the same letter(s) did not differ at P<0.05 according to Duncan's multiple-range test.

Data as depicted in Table(3) summarize the chemical compositions of tomato leaves, i.e. N, P and K;C, O.M ,chlorophyll contents and total phenol as influenced by *Meloidogyne incognita* infection under the stress of applying four organic acids i.e. salicylic, humic, citric and oxalic at two concentrations(0.05 & 0.1 %) four times at 7 days intervals in comparison with oxamyl in the greenhouse conditions(24±2C^{o}). Obviously , results revealed that all tested treatments significantly achieved high percentage increase values of all items under study. Among tested concentrations , 0.1 g/L of such organic acids was superior in values of N, P , K , C , O.M and total chlorophyll as compared to nematode alone , whereas the concentration of 0.05g/L of the four organic acids ranked first in percentage increase values of total phenol which were amounted to 8.39 , 6.36 , 4.43 and 2.68% for salicylic , citric , oxalic and humic acids comparing to nematode alone , respectively (Table3).Oxamyl as a systemic nematicide showed the highest percentage increase values of N(80.53%) , P(26.04%) ,K(34.13%) ,C(7.14%) O.M(7.09%) , total chlorophyll(11.7%) and total phenol(10.52%) as compared to nematode alone . It is interesting to note that plant free of any treatment and nematode showed the less value of total phenol(0.79%), comparing to nematode alone (Table3).

Table (3): Nitrogen (N) Phosphorus (P), Potassium(K), Total phenol and Total chlorophyll contents in leaves of tomato CV.9065 FI infected with *Meloidogyne incognita* under stress of four organic acids at two concentration applied separately as foliar spraying in comparison with oxamyl in the green house conditions(25± 3c^{o}).

Treatments	Chemical components in Leaves															T. phenol mg/100 g	Inc. %
	N %	%Inc.	P %.	%Inc	K	%Inc	C %	%Inc	C / N	O. M	%Inc	Chlorophyll content mg/g F.WT.					
												Chlo. A	Chlo. B	A+ B	Inc. %		
Salicylic acid (0.1%)	1.30	15.04	0.328	5.46	1.75	4.79	33.9	0.89	26.02	58.4	1.55	de 0.513	0.334 gh	0.847 fg	2.17	591.9 c	7.55
Humic acid (0.1%)	1.92	69.91	0.381	22.50	2.15	28.74	35.8	6.54	18.46	61.5	6.40	a 0.550	0.368 c	0.918 b	10.73	560.3 i	1.81
Citric acid (0.1%)	1.54	36.28	0.347	11.57	1.86	11.37	34.4	2.38	22.33	59.2	2.42	c 0.525	0.342 f	0.867 de	4.83	579.6 e	5.32
Oxalic acid (0.1%)	1.73	53.09	0.369	18.64	1.99	1.91	35.1	4.46	20.28	60.3	4.32	b 0.536	0.355 d	0.891 c	7.47	570.8 g	3.72
Salicylic acid (0.05%)	1.22	7.96	0.320	2.89	1.73	3.59	33.7	0.29	27.62	58.1	0.51	0.508 e	0.329 hi	0.837 gh	0.96	596.5b	8.39
Humic acid (0.05%)	1.80	59.29	0.373	19.93	2.08	24.55	35.2	4.76	19.55	60.6	4.84	0.630 f	0.360 d	0.909 i	8.80	565.1 h	2.68
Citric acid (0.05%)	1.43	26.54	0.339	9.00	1.80	7.78	34.2	1.78	23.91	58.9	1.90	0.520 cd	0.337 fg	0.857 ef	3.37	585.3 d	6.36
Oxalic acid (0.05%)	1.62	43.36	0.355	14.14	1.94	16.16	34.7	3.27	21.41	59.7	3.28	0.529 bc	0.348 e	0.877 d	5.79	574.4 f	4.43
Oxamyl	2.04	80.53	0.392	26.04	2.24	34.13	36.0	7.14	17.64	61.9	7.09	0.552 a	0.374 b	0.926 ab	11.70	608.2 a	10.52
N alone	1.13	-	0.311	-	1.67	-	33.6	-	29.73	57.8	-	0.503 e	0.326 i	0.829 h	-	550.3 k	-
Plant free of any treatment	1.16	2.65	0.319	2.57	1.90	13.77	34.0	1.19	24.3:1	60.1	3.97	0.556 a	0.381 a	0.937 a	13.02	554.7 j	0.79
L.S.D												0.008	0.005	0.013		0.169	

N=2000 eggs of *M. incognita* * Each figure in the mean of four replicates.
Means in each column followed the same letter(s) did not differ at P<0.05 according to Duncan's multiple-range test.

Apparently, using organic acids i.e. salicylic, humic, citric and oxalic at two concentrations (0.05 & 0.1 %) four times at 7 days intervals in the present work as foliar spraying process succeeded to improve tomato plant growth criteria and diminish nematode parameters as well as achieved the high percentage increase values of N, P , K , C , O.M and total phenol comparing to nematode alone. Plant receiving salicylic acid either at 0.1 or 0.05% surpassed other tested organic acids in reducing number of nematode population (pf), galls and eggmasses on roots. These findings are in agreement with those of **Zinov'eva *et al.*, 2011** who reported that the pre-planting salicylic acid (S.A) treatment of tomato seeds resulted in an increased resistance of susceptible tomato cultivars to *M. incognita.* This protective effect is higher in the case of SA combined with chitosan, a biogenic elicitor of plant resistance. Their studied preparations stimulate the growth and development of the plants. The increase in the resistance of tomato plants is related to the increased activity of phenylalanine ammonia-lyase and an increased SA content in plant tissues infected with nematodes; both these factors significantly influence nematode development (**Zinov'eva *et al.*, 2011**).Organic acids released during the decomposition of organic materials are one of many factors contributing to reductions in nematode damage (**McBride *et al.*, 2000)** The present research focused on finding compounds that are safe to human and environment. An alternative to pesticide application is that, it may be possible to utilize a scheme of inducible plant defenses**.** The role of organic acid in plant diseases may be due to the correlation between these acids and plant health. The role of humic acid in overcoming the harmful effects of chocolate spot and rust diseases in faba bean plant may be due to the increase in chitinase activity, **(Abd El-Kareem, 2007)** and stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water **(Chen *et al.*, 2004).** Organic acids can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests which

consequently increase yield of plant **(Scheuerell *et al*., 2004)**. Amino acids help to increase chlorophyll concentration in plant leading to higher degree of photosynthesis. Any factor causes increase in photosynthetic pigments will lead to increase carbohydrate content. Also associated with phenolic compounds which play a major role in plant defense **(Hahlbrock and Scheel, 1989).** The present results were in agreement with the findings that reported by **Mahgoob and Zaghlool 2002 (2002)** in respect to the fact of phenolic compounds which play appositive role against plant pathogens.
The present results also give an approach to control *M. incognita* in tomato plant using organic acids that are safer than nematicides. These results should be considered when designing an integrated pest management program for root-knot nematodes or other nematode pathogens in tomato and other crops.

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