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Comparative suppressive effect of some organic acids against *Meloidogyne incognita* infecting tomato

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Abstract

The comparative suppressive potential of some organic acids known as resistance inducing chemicals viz., salicylic acid (SA) and its two derivatives; acetylsalicylic acid (ASA) and 3,5-dinitrosalicylic acid (DNSA), along with L-ascorbic acid (AA), oxalic acid (OA) and citric acid (CA) for managing *Meloidogyne incognita* infecting tomato plants was investigated under greenhouse conditions. The results showed that all tested organic acids as well as the application methods significantly reduced tomato root galls and 2nd stage juvenile numbers in soil compared with control. Except DNSA and AA, foliar application of tested organic acids was more effective in reducing nematode galls than soil drench application. Foliar application of ASA caused superior effect in the reduction of J_2 in soil (100 %) followed by AA (89.44 %), OA (88.25 %) and SA (70.69 %). CA (96.52 %) was tended to be the most effective chemicals in reducing J_2 in soil when applied as soil drench. All treatments enhanced shoot and root length of tomato as well as shoot weight compared with the untreated check. Only SA and DNSA significantly increased the root weights. DNSA enhanced the growth indices of tomato plant when used as foliar spray more than soil drenching and opposite trend was observed for OA. These chemical activators have potential to suppress *M. incognita* infection through the stimulation of tomato tolerance.

Keywords: Chemical inducers, induced resistance, nematodes, tomato, soil drench, foliar spray.

Plant parasitic nematodes worldwide are responsible for 125 billions of dollars' worth for crop losses every year (Chitwood, 2003). Most losses are mainly due to the root-knot nematodes, *Meloidogyne* species (Wesemael *et al*., 2010). *Meloidogyne incognita* is one of the most distributed and severely affected tomato productions in Egypt (Ibrahim & Mokbel, 2009). Various strategies have been extensively used to manage phytonematodes in infested areas such as organic amendments, biological control and chemical nematicides. Although, chemical nematicides are the most rapid and effective control measure, they have withdrawn from the market due to human health and environmental hazards (Rich *et al*., 2004). There is an urgent need to find alternative methods which are safe and environment friendly.

The application of exogenous SA and other organic acids (chemical inducers) is probably one of the possible alternatives and environmentally safe management practices for protecting plants from various pathogenic infections (Walters *et al*., 2013).

The nematicidal potential of different organic acids which are either released from organic material during the decomposition processes (McBride *et al*., 2000; Abdel-Rahman *et al*., 2008) or produced as secondary metabolites by the nematode antagonistic fungi (Zuckerman *et al*., 1994; Jang *et al*., 2016) has been reported. Many areas of plant production can use organic acids as a plant growth regulator or soil conditioner for enhancing plant resistance against plant pathogen.

This approach might be help in increasing the plant growth and its yield (Scheuerell & Mahaffee, 2004). Little investigations have been done on chemical inducers against root-knot nematodes as compared with that carried out against other plant pathogens. Therefore, with an aim to develop an integrated strategy involving the use of some resistance inducing chemicals for the successful sustainable management of *M. incognita* is necessary.

The objectives of the present investigation were to study the *in vivo* comparative suppressive effect of some chemical inducers on tomato resistance to *M. incognita* infection and to compare the efficacy of the two methods of inducer application (foliar spray and soil drench). Moreover, the growth indices of tomato in the treated and untreated inoculated plants as compared to control were also examined under glasshouse conditions.

Materials and Methods

Nematode inoculum: The root-knot nematode, *M. incognita* eggs were isolated and extracted with 1% NaOCl solution for 2 min from infected roots of eggplant (*Solanum melongena* L.). The eggs were collected on a 37µ sieve and gently washed with distilled water prior to use in the course of study (Hussey & Barker, 1973).

Organic acids and a nematicide: Organic acids *viz*., Salicylic acid (SA) and its two derivatives; acetylsalicylic acid (ASA) and 3, 5-dinitrosalicylic acid (DNSA), along with L-ascorbic acid (AA), oxalic acid (OA) and citric acid (CA) used in the present study were purchased from Sigma Aldrich, St Louis, Missouri, USA. Aqueous solution of such chemical was prepared in order to obtain the concentration required. The nematicide, Oxamyl (10%G) obtained from E. I. du Pont de Nemours & Company Inc. was used for comparison.

Pot experiment: Under glasshouse conditions, one month-old of two tomato seedlings (*Lycopersicon esculentum* Mill. cv. Super Strain B) transplanted into 15 cm diameter clay pots filled with steam sterilized sandy clay loam soil were inoculated with 5000 nematode eggs/ pot after two days from transplanting time. Each of the tested organic acid was applied two days before inoculation, either as soil drench or foliar spraying at the rate of 10 mM in a total volume of 30 ml sterilized distilled water. Pots were sprayed and/or drenched with 25 ml sterilized distilled water left as check treatment. All treated pots including controls (non-inoculated and untreated inoculated with *M. incognita*) were arranged in a complete randomized block design and each treatment was replicated three times.

All treatments (Table 1) were compared to Oxamyl (10% G) which was applied sequentially after transplantation at 10 ppm (0.01 g a. i/kg soil). The tomato resistance to *M. incognita* was estimated in terms of 2^{nd} juveniles in the soil and tomato root galling. At the end of experiment (50 days), plants were gently removed from pots. The number of galls per root system and the number of 2^{nd} stage juveniles were counted. Plants growth was also recorded.

Statistical analysis: The statistical analysis was performed according to the SAS program (SAS Institute, 1998) after transformation of the data to values of square root for X + 1. Data were subjected to factorial ANOVA design followed by least significant difference (LSD) test to determine the significant differences among means at the probability level of 0.05.

Results and Discussion

The differences in the effectiveness on galls at all tested organic acids were statistically significant ($p \leq 0.05$) from the control treatment and the differences between the two methods of application for all treatments were also significant (Table 1). Generally, OA (58.07 %) treatment was the most effective compound followed by AA (56.92 %), DNSA (48.80 %) and SA (47.61 %) in decreasing root galling. No significant differences at ($p \leq 0.05$) between ASA (26.05 %) and CA (28.82 %) were noticed in respect to the effectiveness for galls. Interestingly, the effects of AA and OA on gall formation were significantly ($p \leq 0.05$) similar to that of oxamyl (62.10 %), whereas the efficacy of SA, ASA, DNSA and CA were less than that of the nematicide.

With regard to the methods of application used, it appeared that foliar spray of SA, ASA, OA and CA gave higher reduction in galls than soil drenching. The percent reductions of these chemicals were 74.24, 32.49, 77.21 and 38.07, respectively. However, no significant differences were noticed between SA and OA as well as between ASA and CA in this respect. On the other hand, DNSA (69.44 %) and AA (68.17 %) were more effective in reducing tomato root galls when used as soil drench than foliar spray (Table 1).

All of tested chemical elicitors suppressed 2^{nd} stage juveniles number in the soil compared to control treatment but without significant differences ($p \leq 0.05$) among them and Oxamyl. In general, ASA (93.71 %) treatment was the most effective treatment while DNSA (62.30 %) was considered to be the least effective one. CA (78.88 %), OA (75.82 %), AA (74.92 %) and SA (64.55 %) were seen with intermediate effectiveness for J_2 in the soil. Except DNSA, significant differences between the two methods of application for all treatments were observed. Spray application of ASA caused superior effect in the reduction of J_2 in soil (100 %) followed by AA (89.44 %), OA (88.25 %) and SA (70.69 %). On the contrary, CA was tended to be the most effective chemicals in reducing J_2 in soil when applied as soil drench (Table 2).

Regarding the effect of the tested chemical compounds and Oxamyl on tomato growth characteristics which listed in Table (3) all treatments exhibited significant differences ($p \leq 0.05$) in increasing the tomato shoot length comparable to control treatment. Also, all of the tested treatments significantly ($p \leq 0.05$) increased the shoot weight except AA and OA which gave the same effect as control treatment. Except OA, all treatments significantly promoted the root length compared to control treatment. No significant differences among the tested treatments on root weight were observed except the treatments of SA and DNSA which significantly increased the root weights.

As for the method applied, it appeared that there were significant differences between foliar spray and soil drench for all tested treatments on the tomato growth indices except SA on shoot length, AA and CA on root length as well as SA and ASA on root weight. DNSA enhanced the growth indices of tomato plant when used as foliar spray more than that of soil drench and vice versa was noticed with OA (Table 3).

It is the ultimate goal of using chemically-induced resistance as a part of integrated pest management program. In the present study, L-AA (vitamin C) was found to be effective in tomato inducing resistance against *M. incognita* (Arrigoni *et al.*, 1979). They also found that the amount of AA in susceptible tomato cultivars was reported to be lower than in resistance cultivars.

Al-Sayed (1990) showed that spray of AA on tomato plants succeeded in decrement of tomato galls, reproduction of *M. incognita* and promoted plant growth. Aqueous solutions of AA at 1000 µg/ml diminished *M. javanica* populations, egg-masses and females in the root of tomato plant (Osman, 1993).

Moreover, application of AA as soil drench against *M. javanica* in potato plants (Osman *et al*., 2016) and *M. incognita* infecting sugar beet (Maareg *et al*., 2014), showed its potential effect in suppressing population and reproduction of root-knot nematodes and improved plant yields. Similar results are recorded in the present study that tomato plants sprayed or drenching soil with

AA at 10mM caused significant reduction in galls and 2nd juveniles of *M. incognita* infecting tomato and increased plant growth parameters.

There are several possible mechanisms for how organic acids may be promoting plant growth and increasing its resistance against nematode infestation. The AA function for enhancing the resistance of plant against pathogenic stress especially against root-knot nematodes during the development of plant systems has been reported (Osman *et al*., 2013). It is modulated the complex of biochemical pathway reactions such as enzymatic reactions, induction of proteins synthesis, and the production of different defense metabolites that tolerate the plant against stress response (Khan *et al*., 2011). However, another function of AA is to perform as an effective radical scavenger to eliminate the reactive oxygen species (ROS) in plants which consequently led to protect plants against oxidative stress (Pastori *et al*., 2003). All of which are responsible for resistance in plants to pathogenic nematodes.

The obtained results regarding OA and CA are in full synchronization with that reported by Zaki *et al*., (2004) who found that OA was more effective in larval death (>90%), reduction (98%) in hatching eggs was observed and inhibit penetration of *M. javanica* into tomato roots. Also, soil treated with *Aspergillus niger* culture filtrate, citric and oxalic acids, each at 10%, caused significant decrease in root galls and in *M. arenaria* egg-masses on tomato plants. Both acids significantly enhanced plant growth (Mokbel *et al.*, 2009). Moreover, Zuckerman *et al*., (1994) found that nematicidal properties of both oxalic acid and citric acid as a fungal metabolites that secreted in culture filtrate of *A. niger*. Oxalic acid was found to be effective against root-knot nematodes and its nematicidal potential is most related to the strong acidity of the acid (Jang *et al*., 2016). Among the organic acid tested, however, oxalic acid applications at 0.05 or 0.1 % gave the least efficient organic acid in reducing *M. incognita* population (El-Sherif *et al*., 2015). Besides, the nematicidal potential of low-molecular-weight organic acids such as acetic acid and/or lactic acid against root-knot nematodes has been declared (Seo & Kim, 2014). In addition to exogenous organic acids like OA and CA are beneficial in protecting plants from various stresses via induced resistance, OA suppresses the host plant oxidative burst (Apel & Hirt, 2004). This could be explained the high nematicidal activity of OA caused in lowering media pH (Legendre *et al*., 1993). Organic acids are also implicated in adjusting physiological processes of plants, including decreasing lipid peroxidation and boosting antioxidant enzyme activity (Apel & Hirt, 2004).

In the present study, the potential use of SA and its derivatives as chemical inducers in defense responses of tomato to *M. incognita* in terms of reducing tomato galls and nematode population in soil are in well agreement with those obtained by several authors: Osman (1993) reported that SA was found to reduce the number of 2ndjuveniles and other developmental stages of *M. javanica* in tomato plants. Spray of SA induced suppression of *M. incognita* infecting plants (Nandi *et al*., 2000), this effect was likely to be due to the signaling role of SA in inducing plant resistance to pathogen. Also, Pankaj & Sharma (2003) found that plant growth of okra significantly increased at 50 and 100 µg SA/ml whether applied as seed soak, drench and spray and was at par with Carbofuran treatment. However, there was a decrease in plant growth with higher SA concentrations. The authors suggested that SA might have induced some resistance in okra against *M. incognita*. Soil application of SA reduced root-knot severity on chamomile seedlings and gave significant flower yields (Pandey & Kalra, 2005). Moderate reduction (20-25%) of *Meloidogyne* spp. reproduction was observed on tomato plants treated with either root dip or soil-drench of SA (Molinari, 2008). SA as soil drench was the most effective treatments that gave a defense response in tomato for a long time (Molinari & Baser, 2010). Application of SA reduced tomato root galls and eggs of *M. incognita* (Mukherjee

Table 1. Effect of some organic acids on the number of galls of *Meloidogyne incognita* on tomato plants in a pot experiment.

Treatment	Method of application	Mean galls (x)/ root system	Transformed data ($\sqrt{x+1}$)	(%) over control
Untreated check		201.83	14.05	-
SA	Foliar spray	52.00	7.27	74.24
	Soil drench	159.5	12.67	20.97
	Mean	105.75	9.97	47.61
ASA	Foliar spray	136.25	11.71	32.49
	Soil drench	162.25	12.77	19.61
	Mean	149.25	12.24	26.05
DNSA	Foliar spray	145.00	12.06	28.16
	Soil drench	61.67	7.83	69.44
	Mean	103.34	9.95	48.80
AA	Foliar spray	109.67	10.47	45.66
	Soil drench	64.25	7.94	68.17
	Mean	86.96	9.21	56.92
OA	Foliar spray	46.00	6.86	77.21
	Soil drench	123.25	11.13	38.93
	Mean	84.63	8.99	58.07
CA	Foliar spray	125.00	11.21	38.07
	Soil drench	162.33	12.73	19.57
	Mean	143.67	11.97	28.82
Oxamyl	0.01 g a.i/kg soil	76.50	8.74	62.10
$LSD_{0.05}$ (A)	-	-	1.00	-
between				
treatments	-	-	0.58	-
$LSD_{0.05}$ (B)				
between methods	-	-	1.79	-
$LSD_{0.05}$ (A x B)				

Each organic acid; salicylic acid (SA), acetylsalicylic acid (ASA), dinitrosalicylic acid (DNSA), ascorbic acid (AA), oxalic acid (OA) or citric acid (CA) was applied once either as foliar spray or soil drench at the rate of 10 mM.
Each figure is the average of three replicates.

Table 2. Influence of some organic acids on the number of *Meloidogyne incognita* J_2 infected tomato plants in a pot experiment.

Treatment	Method of application	Mean 2nd juveniles /250 g soil	Transformed data (√x+1)	(%) over control
Untreated check		126.23	11.22	-
SA	Foliar spray	37.00	4.19	70.69
	Soil drench	52.50	7.24	58.41
	Mean	44.50	5.72	64.55
ASA	Foliar spray	0.00	1.00	100
	Soil drench	15.89	3.59	87.41
	Mean	7.95	2.30	93.71
DNSA	Foliar spray	41.17	5.48	67.38
	Soil drench	54.00	6.31	57.22
	Mean	47.59	5.90	62.30
AA	Foliar spray	13.33	2.80	89.44
	Soil drench	50.00	6.03	60.39
	Mean	31.67	4.42	74.92
OA	Foliar spray	14.83	2.92	88.25
	Soil drench	46.22	5.50	63.38
	Mean	30.53	4.21	75.82
CA	Foliar spray	48.93	6.08	61.24
	Soil drench	4.39	1.92	96.52
	Mean	26.66	4.00	78.88
Oxamyl	0.01 g a.i/kg soil	5.77	2.09	95.43
$LSD_{0.05}$ (A) between treatments	-	-	4.02	-
$LSD_{0.05}$ (B) between methods	-	-	2.32	-
$LSD_{0.05}$ (A x B)	-	-	5.50	-

Each organic acid; salicylic acid (SA), acetylsalicylic acid (ASA), dinitrosalicylic acid (DNSA), ascorbic acid (AA), oxalic acid (OA) or citric acid (CA) was applied once either as foliar spray or soil drench at the rate of 10 mM. Each figure is the average of three replicates.

Table 3. Effect of some organic acids on the growth parameters of tomato plants infected with *Meloidogyne incognita* in a pot experiment.

Treatment	Method of application	Growth indices			
		Fresh shoot		Fresh root	
		Length (cm)	Weight (g)	Length (cm)	Weight (g)
Control (without nematode)		53.08	12.52	22.75	5.65
Control (with nematode)		25.67	5.50	10.25	2.43
SA	Foliar spray	37.63	8.09	19.00	4.53
	Soil drench	38.88	10.67	15.13	3.93
	Mean	38.26	9.38	17.07	4.23
ASA	Foliar spray	37.63	8.14	13.63	2.73
	Soil drench	49.00	13.25	23.88	3.62
	Mean	43.32	10.70	18.76	3.18
DNSA	Foliar spray	45.00	12.44	17.17	5.18
	Soil drench	36.33	8.55	12.42	3.52
	Mean	40.67	10.50	14.80	4.35
AA	Foliar spray	36.25	9.64	14.42	3.84
	Soil drench	27.25	5.88	15.88	2.75
	Mean	31.75	7.76	15.15	3.30
OA	Foliar spray	27.25	4.98	10.50	1.47
	Soil drench	38.88	11.06	17.75	4.85
	Mean	33.07	8.02	14.13	3.16
CA	Foliar spray	50.25	12.86	15.75	3.98
	Soil drench	40.00	9.20	16.67	2.89
	Mean	45.13	11.03	16.21	3.44
Oxamyl	0.01 g a.i/kg soil	42.83	9.01	16.00	3.09
$LSD_{0.05}$ (A) between treatments	-	7.68	2.91	4.52	1.68
$LSD_{0.05}$ (B) between methods	-	4.44	1.68	2.61	0.97
$LSD_{0.05}$ (A x B)	-	12.16	4.36	5.89	2.30

Each organic acid; salicylic acid (SA), acetylsalicylic acid (ASA), dinitrosalicylic acid (DNSA), ascorbic acid (AA), oxalic acid (OA) or citric acid (CA) was applied once either as foliar spray or soil drench at the rate of 10 mM. Each figure is the average of three replicates.

et al., 2012). Seed treatment or soil drench of 50 μM SA caused greater reduction in *M. javanica* egg-masses on the roots of tomatoes (Mostafa nezhad *et al*., 2014). Foliar application of 0.05 and 0.1 % SA surpassed other tested organic acids in improved plant growth indices and in reducing *M. incognita* populations on the roots of tomato (El-Sherif *et al.*, 2015). Soil drench of SA and ASA reduced *M. incognita* reproduction on tomato roots (Anter *et al.*, 2014). Contrary, application of SA as foliar spray or as soil drench did not show any enhancement of tomato resistance against *M. javanica* (Oka *et al*., 1999).

SA is a naturally occurring plant hormone, plays a critical signaling function in plant resistance to pathogens (Klessig *et al*., 2000). SA mediates the oxidative stress precedes the systemic acquired resistance (SAR) development which closely related to the formation of pathogenesis-related proteins and enhancement the activities of antioxidant enzymes (Shirasu *et al*., 1997). Also, SA is contributed in the *Mi-1*resistance gene of tomato that confers resistance to root-knot nematodes (Branch *et al*., 2004).

In our study, a significant reduction in tomato galls and 2nd juveniles in soil in relation to SA application or other tested organic acids, two days before inoculation with *M. incognita* was recorded. These results were in accordance with Arrigoni *et al*., (1979) and Al-Sayed (1990). Pandey & Kalra (2005) showed that several activators including SA and ASA applied as pre-infection suppressed nematode reproduction. Molinari & Baser (2010) mentioned that the effect of the pre-inoculation of SA elucidate the persistence of defense response for such a long time.

Furthermore, three days pre-inoculation treatment of chemical inducers as soil drench including SA, ASA and AA was most effective in reducing nematode population than at or post-inoculation time (Anter *et al*., 2014).

In the present study, no phyto-toxicity was observed on plant treated once by any of the tested organic acids. However, in other publications, phyto-toxicity caused either by high doses or repeated application of chemical inducers has been increasingly documented (Oka *et al*., 1999; Molinari & Baser, 2010; Anter *et al*., 2014). Also in our study, it was stated that the tested chemical inducers may act directly or indirectly on the stimulus to hatching, on juveniles ability to find, penetrate and settlement of the feeding site as well as on the development of nematodes (Cook, 1991).

From the obtained results, it was noticed that one application of all of the tested organic acids either as foliar spraying or as soil drenching induced tolerance to *M. incognita* in terms of the reduction in the tomato gall numbers and 2nd juveniles in the soil. These chemicals might be used as a potential tactic in integrated approach of root-knot nematode management through enhancing tomato plant tolerance to root-knot nematode infection. Such treatments which provide promising levels of control require further evaluation under micro-plot and field conditions. Also, effect of different rates and application frequency of resistance inducing chemicals against *M. incognita* infecting tomato must be needed in the future work.

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