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Impacts of Some Garlic Extracts on Tomato Growth and Physio-Bio Chemistry Under Infection of Root-Knot Nematode, *Meloidogyne incognita*

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ABSTRACT

Root-knot nematode (*Meloidogyne incognita*) is one of the most damaging species of plant nematodes to vegetable crops. The efficacy of ethanolic, methanolic and aqueous extracts of garlic for suppression of *M. incognita* infecting tomato (*Solanum lycopersicum* L.) plants were evaluated under greenhouse conditions. Results showed that all tested extracts caused significant reductions ($P \leq 0.05$) in the nematode criteria, final population, rate of reproduction and percentages of nematode reduction compared to nematicide and non-treated plants. The methanolic extract at 6 ml/plant caused the best effect on the previous criteria (5536, 1.85 and 92.43%) respectively, compared with the non-treated control. On the other hand, all tested extracts decreased the negative effects of root-knot nematode and an enhancement in the growth parameters of tomato plants. Where the methanolic extract achieved the best results at the highest concentration in both measures of vegetative growth and the chemical content of defensive compounds for plants.

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetables in the world. Out of the 170 million tonnes of tomatoes produced globally (FAO 2017). If the issues brought on by root-knot nematodes (*Meloidogyne* spp.) could be avoided, this production would be greater (Jones *et al.*, 2013). These parasites not only damage the tomato plant directly but also increase the tomatoes' susceptibility to other diseases (Fuentes *et al.*, 2017 and van Bruggen *et al*., 2016). The most damaging root-knot nematode species for tomato plants is *Meloidogyne incognita* Chitwood (Kofoid and White). Which also affects a number of other crops important to world trade (Sikora and Fernandez 2005).

Currently, the chemical nematicides are commonly used to control parasitic nematodes on plants, although nematicides show good efficacy (Faria *et al*., 2021), concerns about their residues in the food chain, risks to human health and negative environmental effects have led to the ban of some compounds(Desaeger *et al*., 2020; Mansour, 2004). Bioproducts of plant origin are promising sources to overcome the root-knot nematode problem (Rodrigues, 2022), as some of its products can be used directly as pesticides or

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indirectly to synthesize improved compounds with good efficacy, environmentally safe and economically inexpensive (Khan *et al*., 2022; Ntalli and Caboni 2012). botanical extracts reported to be active against nematodes (Jardim *et al*., 2020 and Andrés *et al*. 2012).

The efficacy of different plant extracts for controlling the plant parasitic nematodes has been studied. (Abo-Elyousr *et al*., 2010; El-Nagdi *et al*., 2014 and El-Nagdi *et al*., 2013). The nematicidal properties of garlic (*Allium sativum* L.) have been reported (Ladurner *et al*., 2014; Bekhiet *et al*. 2010 and Cetintas and Yarba, 2010). In addition to the suppressive effects on nematodes, these extracts have direct and indirect effects on plant growth (Khan *et al*. 2011; Klimpel *et al*. 2011). Hence, the objective of this is to evaluate the effectiveness of ethanolic, methanolic and aqueous garlic extracts to control root-knot nematode, *M. incognita* on tomato plants and its effect on plant growth and the chemical content of defensive compounds under greenhouse conditions.

MATERIALS AND METHODS

Preparation of Garlic Extracts:

Ethanolic, methanolic and aqueous extracts of garlic were prepared by adding 1 kg of garlic cloves to 1L of ethanol, methanol and distilled water, which were then left for 96 h in a dark place, before filtration through Whatman filter paper No. 1. Each extract was arbitrarily termed as a standard solution. The solvents were removed by evaporation. Then the tested rates were prepared by diluting the previous extracts by adding distilled water.

Root-Knot Nematode:

Females and egg masses of M. incognita were isolated from naturally infested tomato roots collected from Ismailia Governorate Egypt. The culture of this nematode was established from single egg masses of adult females previously identified by the morphological characteristics of the perineal patterns (Taylor and Sasser 1978) and reared on tomato plants in a greenhouse.

Greenhouse Experiment:

The previous dilutions were added as a soil drench as follows: Tomato cv. Super Hybrid F1 A of two-week-old seedlings was transplanted to pots 25 cm diam. containing 2 kg filled with sandy loam soil (1:1v/v). One week later, each pot was inoculated with 3000 freshly hatched second-stage juveniles of the *M. incognita* obtained from the previous culture. The prepared extracts were added three days after infection in the natural growing season (2022). Also, the nematicide, oxamyle (oxamyle 24%) at the rate of 5ml/pot (equivalent to 2L /feddan = 4,200 m^2) was tested as a comparison. There were three replicates for each treatment, and a similar number of pots with nematode only served as the control. All the pots were arranged in a completely randomized block design and watered as needed. After four weeks, the plants were carefully uprooted. The soil was extracted using the sieving and decanting method (Seinhorst, 1956). The nematode criteria; numbers of root galls, developmental stages, nematodes in soil, number of egg mass, number of eggs per egg mass and final population were counted according to Young (1954) and Bybd Jr *et al*., (1983). The total number of juveniles in the soil and the total number of whole roots were recorded according to Hussey and Barker, (1974). The length and weight of the shoots and roots as well as the dry weight of the shoots were recorded.

The nematode final population (P_f) is estimated by the following formula:

$$\mathrm{Pf} = [\mathrm{no.\,Egg/masses} \times \mathrm{no.\,Eggs\,/\,Egg - masses}] + [\mathrm{Developmental\ stage/root}] + [\mathrm{Juveniles\ in\ soil}] + [\mathrm{Adult\ females/root}].$$

The Build-up was calculated by dividing the final population by the initial one. The reduction percentages of root-knot nematode were calculated by:

$$\textbf{Reduction\%} = \left(\frac{\text{final population of control - final population of treatment}}{\text{final population of control}}\right) \text{x}100$$

The increase percentages in growth parameters were calculated by:

$$\textbf{Reduction/increase \%} = \frac{\text{C-B}}{\text{C}} \text{x}100$$

Where, C = value of control, B= value of treatment.

Statistical Analysis:

All data were subjected to Analysis of Variance (ANOVA) using SAS package, by Duncan's Multiple Range Test (Duncan 1955) (P ≤0.05)

RESULTS AND DISCUSSION

Effect of Deferent Garlic Extracts on Root-Knot Nematode, *M. incognita* Infecting Tomato Plants Under Greenhouse Conditions:

The impacts of ethanolic, methanolic and aqueous extracts of garlic against root-knot nematodes, *Meloidogyne incognita* infecting tomato plants were evaluated under greenhouse conditions. Data presented in Table 1 revealed that all the tested treatments caused a significant reduction (P≤0.05) in the nematode criteria; numbers of root galls, developmental stages, nematodes in soil, number of egg mass, number of eggs per egg mass, final population, rate of nematode reproduction and percentages of nematode reduction where significantly reduced (P≤0.05) as compared with control and nematicide.

Table 1: Effect of deferent garlic extracts on root-knot nematode, *M. incognita* infecting tomato plants under greenhouse conditions.

Treatments	Rate (ml)	Root-knot nematode, *M. incognita*								RGI**	Reduction %
		Galls/root	Juveniles in soil (250 g)	In root				Final population	Build-up		
				Developmental stages	Females	No. of egg-masses/root	No. of eggs/egg-mass				
Ethanolic extract	2	109cd	1177c	91cd	114c	110b	119c	14472	4.82	5	80.20
	4	94cd	1169d	85cde	101cd	107b	108d	12911	4.30	4.3	82.34
	6	67f	1121e	83cde	94cd	91c	103d	10671	3.56	4	85.40
Methanolic extract	2	82e	1081f	59d	66d	73d	101d	8579	2.86	4	88.26
	4	54g	1037g	47d	58d	62e	97d	7156	2.39	4	90.21
	6	37h	850h	29d	49e	48f	96d	5536	1.85	4	92.43
Aqueous extract	2	125b	1366b	118b	136b	128b	134b	18772	6.26	5.3	74.32
	4	118c	1328b	95cd	118c	116	119c	15345	5.12	5	79.01
	6	107cd	1309b	100c	94cd	106b	115c	13693	4.56	5	81.27
Oxamyle	5	9i	45i	31d	27f	6g	37e	325	0.11	2	99.56
Control	-	387a	1632a	211a	232a	265a	268a	73095	24.37	5.3	-
LSD		6.39	132.46	41.43	8.69	9.53	7.84	-	-	-	-

Each value represents the mean of three replicates. ** Root Gall index (RGI) was calculated according to Taylor and Sasser, (1978). Values followed by the same letter are not statistically different according to Duncan's multiple-range test. ($P \leq 0.05$).

In addition, the effect of the tested extracts of the same extract differed significantly. Where data indicated that treatment with methanol extract at 6 ml/plant caused the best effect on the previous nematode criteria (37, 850, 29, 49, 48, 96, 5536, 1.85 and 92.43%) respectively, compared with non-treated control (387, 1632, 211, 232, 265, 268, 73095 and 24.37) respectively. With significant effects as compared to chemical and nematicide. (oxamyle). Followed by treatment with methanol extract at 4 ml/plant which showed (54, 1037, 47, 58, 62, 97, 7156, 2.39 and 90.21%) and methanol extract at 2 ml/plant (82, 1081, 59, 66, 73, 101, 8579, 2.86 and 88.26%) respectively, for the previous nematode criteria. In addition, the middle rank was occupied by the ethanol extract at 4 ml/plant (94, 1169, 85, 101, 107, 108, 12911, 4.30, 4.3 and 82.34%) and 6 ml/plant (67, 1121, 83, 94, 91, 103, 10671, 3.56 and 85.40%) and the highest rate of the aqueous extract 6ml/plant (107, 1309, 100, 94, 106, 115, 13693, 4.56 and 81.27%), respectively. And in the last place in terms of effect, the ethanolic at 2 ml/plant (109, 1177, 91, 114, 110, 119, 14472, 4.82 and 80.20%) and aqueous extract at 2/plant (125, 1366, 118, 136, 128, 134, 18772, 6.26 and 74.32%) and 4/plant (118, 1328, 95, 118, 116, 119, 15345, 5.12 and 79.01%) achieved the least effect compared to the control, respectively.

Considering the effect of the tested extracts on the ability of nematode to galls formation on the roots (RGI), all the tested extracts caused a significant reduction ($P \leq 0.05$). Where the methanolic extract achieved the best effect in all the tested rates (4,4 and 4), followed by the higher concentration of the ethanolic extract (4) compared to the control (5) and oxamyle (5), respectively. The same results were found by Cetintas and Yarba (2010) and Jones *et al*., (2013) who noted that the garlic and extract were more effective in reducing the nematode infestation. Consequently, reducing the number of egg-mass and root galling formations caused by *M. incognita* than the other plant essential oils tested and garlic deserves serious consideration for inclusion into nematode management tactics. This result is also in line with the previously described assay with garlic (Al-Shalaby, 2009 and El-Saedy *et al.,* 2014)

Plant Growth Response to Deferent Garlic Extracts Against Root-Knot Nematode *M. incognita* Infecting Tomato Plants Under Greenhouse Conditions:

On the other hand, all tested treatments decreased the negative effects of root-knot nematode and an enhancement in the growth parameters of tomato plants, but the effect varied through all tested garlic extracts. Data presented in Table 2 reported that all the tested treatments caused significant effects ($P \leq 0.05$) on root and shoot length and shoot dry weight. The highest root and shoot length and shoot dry weight were recorded in treatment with ethanolic extract at 6 ml/plant (53.70, 74.26 and 3.55g) and 4 ml/plant (50.84, 70.47 and 3.00g), followed by methanolic extract at 6 ml/plant (51.00, 73.27 and 2.87g), compared to the control (28.80, 47.13 and1.83g) respectively. While the aqueous extract achieved the least significant effect at all tested rates compared to the control and nematicide.

Generally, by evaluation of the percentage of plant growth parameters, the results indicated that the ethanolic extract at higher concentration (6ml/plant) achieved the best significant effect (86.50, 57.57 and 93.62%) and methanolic extract (77.11, 55.48 and 56.74%) followed by aqueous extract (45.73, 37.35 and 70.92), for the previous parameters respectively.

Table 2: Effect of deferent garlic extracts on tomato growth parameters infected by *M. incognita* under greenhouse conditions.

Treatments	Rate (ml)	Plant growth parameters					
		Root Length (cm)	*Increase %	Shoot Length (cm)	*Increase %	Dry Weight (g)	*Increase %
Ethanolic extract	2	48.20d	67.40	69.36bc	47.17	2.74ab	49.65
	4	50.84c	76.57	70.47bc	49.54	3.00a	63.83
	6	53.70b	86.50	74.26b	57.57	3.55a	93.62
Methanolic extract	2	44.41de	54.22	63.79bc	35.37	2.90ab	58.16
	4	49.65d	72.42	69.43bc	47.34	2.74ab	49.65
	6	51.00c	77.11	73.27b	55.48	2.87ab	56.74
Aqueous extract	2	36.93f	28.26	60.06bc	27.45	2.42b	31.91
	4	40.78de	41.63	62.82bc	33.30	2.56b	39.72
	6	41.96de	45.73	64.73bc	37.35	3.13a	70.92
Oxamyle	5	57.08a	98.24	90.17a	91.34	3.55a	93.62
Control	-	28.80g	-	47.13c	-	1.83	-
LSD		8.74	-	9.61	-	5.93	-

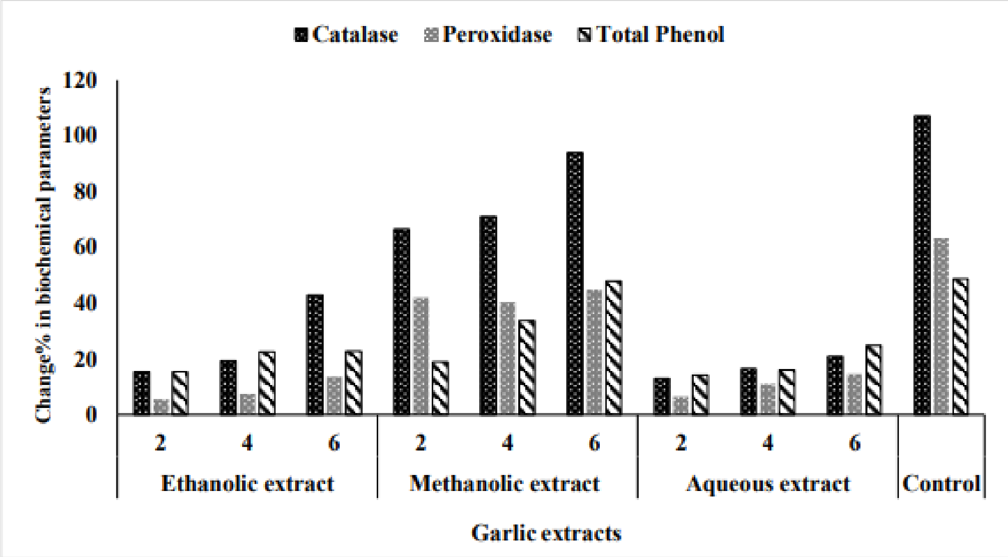
Each value represents the mean of three replicates.. Values followed by the same letter (s) in a column do not significantly differ according to Duncan's multiple range tests, LSD ($P \leq 0.05$).

Efficacy of Deferent Garlic Extracts on Biochemical Parameters of Tomato Plants Infected with *M. incognita* under Greenhouse Conditions:

Previous extracts were evaluated for their effect on the plant content of defensive biochemical compounds against *M. incognita* penetration. Data in Table 3 and Fig1.The results showed that there was a significant effect on the concentration of plant defines chemical compounds such as Catalase (CAT), Peroxidase (PO), Total Phenol (TPH), Polyphenol oxidase (POS), Proline (P) and Salicylic acid (SA). Where, the higher concentration (6ml/plant) of methanolic, ethanolic and aqueous extracts achieved the best significant effect a (0.016, 0.131, 0.534, 0.667, 0.792 and 0.698 µg/g) followed by ethanolic (0.150, 0.205, 0.793, 0.787, 0.892 and 0.789 µg/g), for the previous parameters compared to the control (0.261, 0.238, 1.027, 0.893, 1.197 and 0.841 µg/g), respectively. The same pattern was noted in the increased percentages of CAT, PO, TPH, POS, P and SA. This is partly because of the antioxidant, antibacterial, and antifungal properties of the metabolites this plant produces (Dias *et al*., 2021). Moreover, it has been shown that several of these metabolites are effective against nematodes. (Park *et al*., 2005). The garlic extracts that contain the essential oils (Al-Shalaby, 2009; ElSaedy *et al*., 2014) are also reported to be active against M. incognita. The essential oil of garlic is rich in organosulfur compounds such as diallyl sulphide, allyl methyl trisulphide, ajoene, diallyl disulphide and diallyl trisulphide (Eder *et al*., 2021; Corzo-Martinez and Villamiel 2007). Apparently, sulphur compounds are responsible for the nematicidal activity of garlic essential oil (Park *et al*., 2005).

Table 3: Efficacy of deferent garlic extracts on biochemical parameters of tomato plants infected with *M. incognita* under greenhouse conditions.

Treatments	Rate (ml)	Biochemical parameters					
		Catalase	Peroxidase	Total Phenol	Polyphenol oxidase	Proline	Salicylic acid
Ethanolic extract	2	0.221b	0.225b	0.868b	0.859b	0.966d	0.810b
	4	0.211b	0.220b	0.796c	0.849b	0.952e	0.794cd
	6	0.150c	0.205bc	0.793c	0.787d	0.892g	0.789cd
Methanolic extract	2	0.087d	0.138d	0.832bc	0.763d	0.819h	0.779cd
	4	0.075d	0.142c	0.680e	0.729e	0.802i	0.762d
	6	0.016e	0.131d	0.534f	0.667f	0.792j	0.698e
Aqueous extract	2	0.228b	0.222b	0.881b	0.827c	1.106b	0.820b
	4	0.218b	0.212bc	0.862b	0.867b	1.095c	0.810b
	6	0.207b	0.203bc	0.772d	0.763d	0.928f	0.807b
Control (Nematode only)		0.261e	0.238	1.027	0.893a	1.197a	0.841a



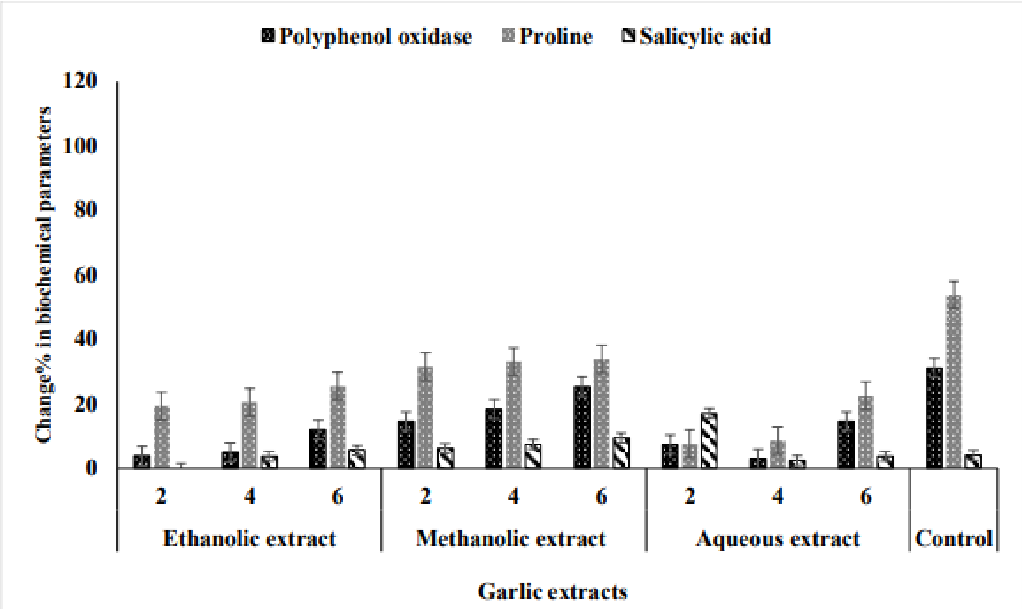


Fig. 1: Efficacy of deferent garlic extracts on change percentage in biochemical parameters of tomato plants infected with *M. incognita* under greenhouse conditions

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