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Management of Tomato Parasitic Nematode through Organic Nematicides in Peshawar, Pakistan

S. Ahmad[1*], B. Safia[2], I. Naz[3], I. Khan[4], A. R. Saljoqi[4], G. Jan[1], J. Uddin[4], M. Abdal[5], and S. Wali Khan[1]

ABSTRACT

The root-knot nematode, *Meloidogyne incognita*, is parasitic to the plants and greatly damages the root of many vegetables. The current *in-Planta* study was designed to explore the nematicidal properties of several botanicals (neem oil, garlic oil, castor oil, extracts of *Tagetes patula* and *Datura inoxia* at a standard dose of 2.0%) at District Peshawar, Pakistan. Tomato (cv. Riogrande) was grown in earthen pots for the growing seasons of 2014 to 2016. Pure culture of the root-knot nematodes were grown in laboratory and were applied at the rate of 50 infective juvenile and 200 eggs per root system through soil drench method. Neem oil and *Datura inoxia* gave the best results by having lower number (2.8 and 5.8, respectively) of galls. Adult females and egg masses were also reduced to 0.9 and 4.8, respectively, per root system. All the treatments differences were statistically significant (P≤ 0.05). Plant growth parameters were also upraised with application of medicinal herbs. This study highlights the nematicidal properties of botanicals for the safe and cheap management of the prevalent root knot nematode. Hence, it is recommended to the farmers for the use of these naturally occurring organic nematicides instead of commercial petro-chemicals that have ill effect on our environment.

Keywords: Botanicals, *Meloidogyne Incognita*, Root knot nematode, Tomato.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is sown worldwide for its numerous uses. Its stem is very debile and it easily scampers over other plants or surfaces. . Its fruit red color is due to the pigment called *Lycopene* (Shi and Maguer, 2000). In the east, tomato is a key component of food and every meal is mooted incomplete without it. Khyber Pakhtunkhwa (KPK), Pakistan, is one of the tomato-producing provinces. There are many varieties sown in the KPK but the commercially available varieties are Money Maker, Roma, and Riogrande (Ali *et al*., 2012). Farmers are confronting with many tomato production obstructions, the main culprits are Root-Knot Nematodes (RKNs), early and late blights diseases, along with some serious insect's problems such as fruit borer and mites.

Root-knot nematodes (Genus; *Meloidogyne*) are obligate sedentary endo-parasites with the host range surpassing 5,500 plant species (Trudgill and Blok, 2001). Farmers are using synthetic insecticides for a long time but because of their inauspicious effects on the environment, growers are now looking for the substitute of handling and finagling these pests. Recently, more accent is being given to the naturally occurring organic

[1] Department of Botany, Abdul Wali Khan University Mardan, Pakistan.
[*]Corresponding author; e-mail: sarirplants@gmail.com
[2] Department of Zoology, Islamia College, University Peshawar, Pakistan.
[3] Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan.
[4] Department of Plant Protection, The University of Agriculture, Peshawar, Pakistan.
[5] Department of Rural Development, The University of Agriculture, Peshawar, Pakistan.

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nematicides that have been derived from plant extracts/essential oils as these are considered ecofriendly and inexpensive (Naz *et al*., 2013). Neem (*Azadirachta indica*) is extracted from the leaves and seeds of neem tree. Wani and Bhat (2012) described neem oil can dramatically reduce the root galling index and improve the plant growth status. A research has shown that Garlic (*Allium sativum*) affect the RKN's by curbing their mobility as well as the nematode power of assimilating their food, and interrupts their ability to procreate (Fadzirayi, 2010). Likewise, *Datura inoxia* (*Solanaceae*) is used in old cultures as a toxicant due to the presence of compounds like alkaloids (Adams and Garcia, 2005). In addition, many other plants, for example, castor plant (*Ricinus communis*) has been used to curb J2s (juvenile stage 2) of *M. incognita* (Wani and Bhat, 2012), similarly *Tagetes patula* (*Asteraceae*) has shown pernicious effects against eggs and J2s of *M. incognita* (Hasabo and Noweer, 2005). Keeping in view the importance of the nematicidal effect of plant extracts, this study was undertaken to explore safe and effective plant extracts/oils. In the present study, plant extracts and oils from different plant sources were evaluated to check the *In-planta* nematicidal effect of plant extracts/oils on *Meloidogyne incognita* in tomato and its effect on plant growth parameters for two growing years 2014 to 2016.

MATERIALS AND METHODS

Maintenance of Nematode Inoculums

For the collection of eggs of *M. incognita,* the areas of Malakand division, Pakistan, were visited. The infected galled roots of tomatoes were collected and samples were brought to the laboratory of plant pathology and stored in a refrigerator at 4˚C until further use (Naz *et al.,* 2013). Firstly, the identification of nematode species was done based on perennial pattern of the adult female. After 24 hours the roots were washed gently to remove any grit and then were cut into small pieces. Then, roots were placed in the distilled water for 24 hours in order to soften the root tissues. Roots were immingled along with 100 mL of water in the electric blender in order to tease the galls for collection of eggs, after 40 seconds, 1% sodium hypochlorite was added for the extraction of eggs (Abid *et al*., 1997). The water containing roots pieces were then passed through a sieve size of 38 µm, eggs were collected and kept in saline solution in order to avoid hatching for further use in experiment.

Preparation of Plant Extracts

The leaves of *Datura inoxia* and *Tagetes patula* were collected from the gardens of University of Peshawar in the month of March. Sun dried (25-27˚C) leaves were powdered into an electric blender to a particle size of approximately 1 mm (Naz *et al*., 2013). Ten gram of dried *Datura inoxia* and *Tagetes patula* leaves powder were separately added to 100 mL of water. The dried powder (leaves) and water were then blended in electric blender for 3-4 minutes. The mixture was then left as such for 72 hours and percolated through muslin cloth. The filtrate obtained was considered as a 'standard concentration (100%). Other treatments including carbofuran, neem oil, castor oil and garlic oil were purchased from Peshawar local pesticide market and were applied at standard dose.

In-planta Greenhouse Experiment on Tomato Inoculated with *Meloidogyne incognita.*

In-planta experiments were conducted in the greenhouse of the Plant Pathology Section at the Agricultural Research Institute (ARI), Tarnab-Peshawar, Pakistan. The experiment was repeated twice for two consecutive years (2014/2015 and

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2015/2016) prior to precision. A total of seven treatments including two essential oils, two plant extracts, and one commercial pesticide (positive check) and simple distal water served as negative check as shown in Table 1. Each treatment consisted of single tomato plant/pot and replicated five times in a Completely Randomized (CR) design. The seeds of tomato cultivar Rio-grande were sown in sterile soil beds in the month of March, 2014 and 2015. After 21 days, the seedlings were transplanted into 15 cm diameter earthen pot containing soil (Clay: sand at 2:1 combination) sterilized at 100˚C for about 6 hours (Naz *et al*., 2013). Seven days after transplantation, each pot was inoculated with approximately 200±15 eggs and 50±15 J2s (per plant) of *M. incognita*. Nematode suspension containing eggs and J2s of *M. incognita* was applied through sterilized micro-pipette by making 4 holes around each tomato seedling and nematode suspension was dispensed into holes at the rate of 10 mL pot^{-1}. Five days after inoculation of J2s, plant extracts/oil suspension was applied to potted tomato at a standard rate as shown in Table 1. Extracts/oil suspension (100 mL) was applied to the potted tomato as a root drench method.

Data Collection

The data were recorded on the RKN and plant parameters. The RKN's parameters included number of adult females per 10 g of roots, number of egg masses per 10 g of roots, and number of galls per root system. Plant length (cm), shoot weight (g), root fresh weight (g), and number of flowers were recorded for three weeks as experiment needed to be terminated (after 60 days of inoculation) due to the expected completion of RKN's two generations lifecycles.

Statistical Analysis

Experimental data of pot experiment was analyzed by one-way analysis of variance (ANOVA) (Ozdemir and Gozel, 2017) using STATISTIX (8.0 Version; Analytical Software). Least Significant Difference (LSD) test was used for the statistical difference among the plant extracts/oils at $P \leq 0.05$.

RESULTS

Data for the growing season of 2014/2015 shows that maximum galls were present in negative control (20.80), while least number of galls were observed in carbofuran (2.0) treated plants followed by neem oil (8.80) per root system at $P \leq 0.05$, as shown in Table 2. Maximum root weight was gained by plants in the negative control (21.60 g) due to the presence of large number of galls. Statistically significant ($P \leq 0.05$) difference was observed among treatments (Table 2).

Data for the growing season of 2015/16 shows significant ($P \leq 0.05$) difference among the treatments as shown in Table 6. Results shows that maximum galls were present in the negative control (14.4 per root system), while the least number of galls were observed in carbofuran (1.75) treated plants followed by neem oil (2.8) per root

Table 1. List of treatments used in *in-planta* experiments for the growing seasons 2014/2015 and 2015/2016.

S.No	Treatments	Chemical Group	Application Rate (%)
T1	Control +	Standard (Carbofuran)	1 g 1000 g^{-1} of soil
T2	Control -	Simple Distilled Water (SWD)	-
T3	Neem oil	Botanical (Oil)	2%
T4	Garlic oil	Botanical (Oil)	2%
T5	*Tagetes patula*	Botanical (Extract)	2%
T6	*Datura inoxia*	Botanical (Extract)	2%
T7	Castor oil	Botanical (Oil)	2%

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Table 2. Effect of plant extracts/oils on number of galls, Galling Index (GI) and root fresh weight per root system of tomato inoculated with *Meloidogyne incognita* under greenhouse conditions for two growing years.

S. No	Extracts/oil	2014/2015			2015/2016		
		Number of galls per root system	Galling Index (GI) [a]	Root weight fresh (g)	Number of galls per root system	Galling Index (GI)	Root weight fresh (g)
T1	Carbofuran (+) [b]	2.00 e	1.0	14.60 c	1.75 d	1.0	11.7 d
T2	Water (-) [c]	20.80 a	3.0	21.60 a	14.4 a	3.0	19.2 a
T3	Neem oil @ 2.0%	8.80 d	2.0	14.60 c	2.8 d	2.0	12.9 cd
T4	Garlic oil @ 2.0%	15.20 bc	3.0	15.60 c	9.3 b	2.0	13.4 cd
T5	*Tagetes patula* extracts @ 2.0%	14.20 bc	3.0	17.60 b	9.8 b	2.0	14.6 bc
T6	*Datura inoxia* extracts @ 2.0%	11.00 cd	3.0	18.60 b	5.8 c	2.0	13.4 cd
T7	Castor oil @ 2.0%	17.20 ab	3.0	21.60 a	10.4 b	3.0	15.6 b
LSD at 0.05%		4.8598		1.9648	1.4		2.0

[a] GI = Galling Index (0 to 5 scale (Where 0= No gall, 1= 1-2 Galls, 2= 3-10 Galls, 3= 11-30 Galls, 4= 31-100 Galls and 5= Galls above 100 per root system. [b] Carbofuran (Positive standard; applied @ 1.0 g pot^{-1}).[c] Water (Control negative, simple distal water). [a-d] Means followed by the same letters do not differ significantly by LSD test ($P \leq 0.05$).

system. Statistically significant root weight gain by plants in the control (19.2 g) was achieved due to the presence of large number of galls. The then followed treatments were castor oil (15.6 g) followed by *Tagetes patula* and garlic oil (14.6 g respectively) at $P \leq 0.05$ as shown in Table 2.

The minimum number of egg masses were found in carbofuran (8.82) and neem oil (9.80) treated plants, while the maximum number of egg masses was found in the negative control (31.48) for the growing season of 2014/2015. The least number of adult females was found in plants treated with neem oil (1.92), carbofuran (2.80), *T. patula* (3.76) and *D. inoxia* (4.26) per 10 g of roots as shown in Table 3.

Data for the growing year 2015/2016 revealed that the least number of egg masses was found in carbofuran (4.7) and neem oil (4.8) treated plants, while the maximum number of egg masses was found in the negative control (19.8). A statistical difference was observed among the treatments. Least number of adult females was found in plants treated with neem oil (0.9) and carbofuran followed by *T. patula* (1.3) and *D. inoxia* (1.6) per 10 g of roots as shown in Table 3.

Data in Table 4 reveal that statistically significant ($P \leq 0.05$) difference was found in shoot length for the growing year of 2014/2015. Maximum shoot length was observed in plants treated with neem oil (55.0 cm) followed by carbofuran (54.0 cm), while the negative control plants achieved only 16.8 cm shoot length. High root length was observed in carbofuran (34.0 cm) treated plants followed by neem oil (30.4 cm). Statistically significant ($P \leq 0.05$) difference was observed among all the treatments over the negative control.

Results for the growing year 2015/16 shows in Table 4 reveal that maximum increase in shoot length was observed in plants treated with neem oil (58.4 cm) followed by carbofuran (54.0 cm) and *D.*

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Table 3. Effect of different plant extracts/oils on mean number of egg masses and number of females per 10 g of roots of tomato inoculated with *M. incognita* under greenhouse conditions for two growing years.

S. No	Extracts/Oil	2014/2015		2015/2016	
		Number of egg masses per 10 g of roots	Number of adult females per 10 g of roots	Number of egg masses per 10 g of roots	Number of adul females per 10 g of roots
T1	Carbofuran (+)[A]	8.82 f	2.08 c	4.7 e	1.0 d
T2	Water (-)[B]	31.48 a	14.50 a	19.8 a	7.8 a
T3	Neem oil @ 2.0%	9.80 f	1.92 c	4.8 e	0.9 d
T4	Garlic oil @ 2.0%	21.90 c	7.28 b	9.1 c	2.4 c
T5	*Tagetes patula* extracts @ 2.0%	17.68 d	3.76 c	11.7 b	1.3 cd
T6	*Datura inoxia* extracts @ 2.0%	15.06 e	4.26 c	6.2 d	1.6 cd
T7	Castor oil @ 2.0%	27.72 b	14.66 a	12.0 b	5.9 b
LSD at 0.05%		1.6384	2.5486	0.9	1.1

[a] Carbofuran (Positive standard; applied @ 1.0 g pot^{-1}). [b] Water (Control negative, simple distal water). [a-f] Means followed by same letters do not differ significantly by LSD test (P≤ 0.05).

Table 4. Effect of different plant extracts/oils on mean shoot and root length per root system of tomato inoculated with *M. incognita* under greenhouse conditions for two growing years.

S. No	Extracts/Oil	2014/2015		2015/2016	
		Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
T1	Carbofuran (+)[A]	54.0 a	34.0 a	51.6 b	40.8 a
T2	Water (-)[B]	16.8 d	9.20 d	22.6 e	19.6 c
T3	Neem oil @ 2.0%	55.0 a	30.4 ab	58.4 a	38.8 a
T4	Garlic oil @ 2.0%	30.6 c	20.2 c	39.0 c	30.4 b
T5	*Tagetes patula* extracts @ 2.0%	40.0 b	25.0 bc	41.8 c	38.0 a
T6	*Datura inoxia* extracts @ 2.0%	45.0 b	28.0 ab	54.0 b	40.2 a
T7	Castor oil @ 2.0%	20.2 d	12.8 d	32.8 d	31.0 b
LSD at 0.05%		7.78	6.91	3.1	4.0

[a] Carbofuran (Positive standard; applied @ 1.0 g pot^{-1}). [b] Water (Control negative, simple distal water). [a-e] Means followed by same letters do not differ significantly by LSD test (P≤ 0.05).

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inoxia (54.0 cm), while the negative control plants reached 22.6 cm of shoot length. Statistically significant difference was found among all the treatments over the negative control. Maximum root length was observed in carbofuran (40.8 cm) and neem oil (30.4 cm) treated plants, while the least root length achieved by the control negative was 19.6 cm.

Maximum shoot fresh and dry weights were achieved with carbofuran (48.40 g fresh and 27.40 g dry weight) followed by neem oil (45.60 g fresh and 27.20 g dry weight), while the least weights were observed in the negative control (17.20 g fresh and 10.20 g dry) as shown in Table 5, for the growing year 2014/2015.

Data in Table 5 for the growing year 2015/2016 reveal that maximum shoot fresh and dry weights were achieved with carbofuran (53.2 g fresh and 28.0 g dry weight) followed by neem oil (48.6 g fresh and 29.0 g dry weight), while the least weights were observed in the negative control (22.4 g fresh and 14.8 g dry weight).

Statistically significant ($P \leq 0.05$) difference was observed in number of flowers for all the treatments over the negative control as shown in Figures 1 and 2.

DISCUSSION

In the present study, essential oils (neem, garlic and castor) and plant extracts (*Tagetes patula* and *Datura inoxia*) were evaluated against *Meloidogyne incognita*, keeping in view the prevailing qualitative and quantitative losses of the main vegetables growing in Pakistan. Outstanding outcomes were achieved through these studies: neem oil and *Datura inoxia* proved to be the best plant-based products that could be used as an alternative to toxic petrochemicals. Our studies indicate that using these plant-based nematicides in higher concentrations provide efficient, economical and eco-friendly management of *Meloidogyne incognita*. This study conformed the nematicidal properties of these plant-based nematicides for their application in IPM (integrated pest management) programs for sustainable agriculture cropping including organic farming, as these plant-based nematicides have minimal residual effects.

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Table 5. Effect of different plant extracts/oils on mean shoot weight (fresh and dry) per root system of tomato inoculated with *M. incognita* under greenhouse conditions for two growing years.

S.No	Extracts/Oil	2014/2015		2015/2016	
		Shoot weight (Fresh) (g)	Shoot Weight (Dry) (g)	Shoot weight (Fresh) (g)	Shoot Weight (Dry) (g)
T1	Carbofuran (+) [a]	48.40 a	27.40 a	53.2 a	28.0 ab
T2	Water (-) [b]	17.20 e	10.20 e	22.4 e	14.8 e
T3	Neem oil @ 2.0%	45.60 a	27.20 a	48.6 b	29.0 ab
T4	Garlic oil @ 2.0%	39.40 bc	22.60 bc	39.8 d	26.6 b
T5	*Tagetes patula* extracts @ 2.0%	36.40 c	20.20 c	36.0 d	23.6 c
T6	*Datura inoxia* extracts @ 2.0%	39.40 bc	24.0 b	43.2 c	30.8 a
T7	Castor oil @ 2.0%	23.0 d	15.20 d	24.0 e	19.2 d
LSD at 0.05%		3.00	2.65	3.1	2.7

[a] Carbofuran (Positive standard; applied @ 1.0 g pot^{-1}). [b] Water (Control negative, simple distal water).
[a-e] Means followed by same letters do not differ significantly by LSD test ($P \leq 0.05$).

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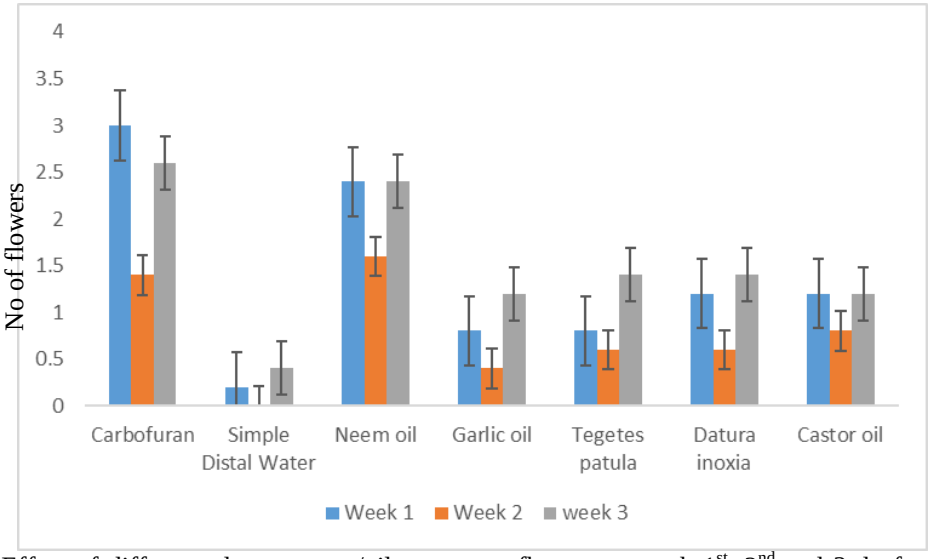

Figure 1. Effect of different plant extracts/oils on mean flowers at week 1st, 2nd and 3rd of tomato plants inoculated with *M. incognita* under greenhouse experiment for the growing season 2014/2015.

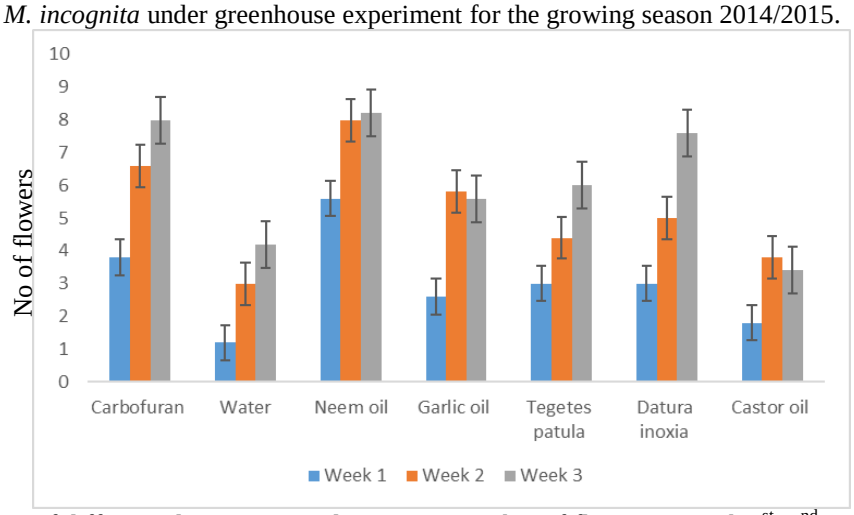

Figure 2. Effect of different plant extracts/oils on mean number of flowers at week 1st, 2nd, and 3rd of tomato plants inoculated with *M. incognita* under greenhouse experiment for the growing season 2015/2016.

organic nematicides has gained a predominant role especially in the olericulture (Ganai *et al*., 2014). Scientists have reported that these plant extracts/oils induce resistance or activate the defense system of the plants against plant pathogenic nematodes (Picard *et al*., 2004). The galled roots are actually the non-infected tissues that swell as a result of abnormal plant defense hormones, as these are defense hormones that directly affect the biology of intruders. The plant extracts/oils served as catalytic compounds that further boost the performance of host plant against RKN (Resha and Savita. 2015)

Application of neem oil gave promising results throughout the experiment. The GI (Galling Index) and the number of adult females of the plants treated with neem oil were dramatically reduced to 1.92 and 9.80, respectively. The present results of neem oil are confirming the previous work of many other researchers (Kiran and Sumayya, 2017; Chedekal and Al-Kayoumi, 2013; Wani and Bhat, 2012). In *in-planta* experiment the number of egg masses and the number of females per 10 g of the roots were less (15.06 egg masses and 4.26 females), which clearly indicate that *Datura inoxia* extracts created the roots environment

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less favorable for the root-knot nematodes penetration. Review of literature revealed that many plants such as *Datura inoxia* bear toxic properties against not only insects but also the root knot nematodes. Toxic nematicidal properties of *Datura* species could be attributed to the presence of alkaloids such as Tigloidine, Tropine, Apoatropine, Hyoscyamine, and Scopolamine (Adams and Garcia, 2005). The current study confirms the results of Nandakumar *et al*., (2017). It is not clear how these essential oils disrupt the activities of RKN. Previously, Oka *et al.* (2000) reported that octopamine is the compound that acts as neurotransmitter is insects. *D. inoxia* also contain the phyto-compound octopamine, which could be the possible compound to inhibit the growth of RKN and ultimately lead to the mortality. Throughout the experiment, the positive control (carbofuran) gave promising results among all other treatments. The maximum numbers of eggs were inhibited from hatching and maximum number of J2s was killed. The number of galls and number of adult females were less in the roots of tomato plants treated with carbofuran, which could be due to the reason that carbofuran application did not allow eggs to hatch. In addition, application of carbofuran created an unsuitable environment around the root zone and thus prevented J2s from the incursion into the roots. As a result, the plant growth dynamism increased staggeringly. Research has disclosed that the carbofuran (Carbamate) acted by subduing acetylcholine-esterase of the nematode nervous system; this is a credible explanation of the effects of carbofuran on root-knot nematodes (Safdar *et al*., 2012).

We presume that these deviations in the nematicidal potency of botanicals against *M. incognita* could be due to differences in the applied doses, their formulation, and application procedures and many other environmental factors. We can conclude from the current response of tomato to RKN and plant oils/extracts that changes that occur after the attack of RKN can be regarded as defense response of the plant. Results indicate that the plant extracts used are potent for their nematicidal properties. It is evident that these plant-based nematicides have certain phytochemicals that have a lethal effect on RKN (Khan *et al*., 2019).

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مدیریت نماتد انگلی گوجه فرنگی با نماتدکش آلی در پیشاور پاکستان

س. احمد، ب. صافیا، ی. ناز، ی. خان، ا. ر. سلجوقی، گ. جان، ج. اودین، م. عبدال، و س. ولی خان

چکیده

نماتد ریشه گرهی، *Meloidogyne incognita*، انگلی است برای گیاهان که صدمه زیادی به ریشه آنها میزند. پژوهش گیاهی حاضر به این منظور طراحی و اجرا شد که خواص نماتدکشی چند عصاره یا اسانس گیاهی (شامل گیاهان neem، روغن سیر، روغن کرچک، عصاره Tagetes patula و Datura inoxia در دُز های استاندارد ۲٪) در ناحیه پیشاور پاکستان بررسی شود. به این منظور، در طی فصل رشدهای ۲۰۱۴ تا ۲۰۱۶، کولتیوار Riogrand گوجه فرنگی در گلدان های سفالی پرورش داده شد.همچنین، کشت خالص نماتد ریشه گرهی در آزمایشگاه اجرا شد و از آنها به مقدار ۵۰ عدد نابالغ (juvenile) و ۲۰۰ تخم به روش خندق خاک (soil drench method) به هر

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سامانه ریشه افزوده شد. بهترین نتایج از نظر تعداد گال از روغن neem و Datura inoxia به دست آمد (به ترتیب ۲/۸ و ۵/۸) . نیز، تعداد ماده های بالغ و توده های تخم در هر سامانه ریشه به ترتیب به ۰/۹ و۴/۸ کاهش یافت. همه تفاوت های آماری تیمارها معنادار($P \leq 0.05$) بود. پارامترها رشد گیاهی نیز در اثرمصرف داروهای گیاهی ارتقا یافت. این پژوهش، خواص نماتدکشی عصاره ها و اسانس های گیاهی را برای مبارزه ومدیریت ایمن و ارزان نماتد ریشه گرهی رایج برجسته کرد. از این رو، به کشاورزان توصیه می شود که این نماتدکش های طبیعی وآلی را به جای مواد پتروشیمیایی تجاری که اثرات مخرب بر محیط زیست دارند استفاده نمایند.

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