The Efficiency of Using Some Natural Compounds for Management of Citrus Nematode *Tylenchulus semipenetrans*

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Abstract

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The efficacy of chitosan and propolis as well as alcohol and aqueous extracts of cabbage, garlic and ziziphus as bio agents was conducted against *Tylenchulus semipenetrans* infecting sour orange (*Citrus aurantium* L.) under greenhouse conditions. All the tested bio-agents significantly reduced in the numbers of nematodes in soil, galls formation and egg masses than those in the untreated (control) and improved plant growth. Results indicated a highly significant increase in total plant shoot fresh and dry weight, plant length was performed for cabbage alcohol extracts (82.1, 104.73 and 119.7%), followed by garlic alcohol extracts (79.5, 103.38 and 100.0%), respectively. Alcohol extracts achieved the highest reduction rate in nematode population with values of 95.92% for cabbage alcohol extract, followed by 94.92% for garlic alcohol extract and 88.73% for garlic aqueous extract, whereas Oxamyl as a systemic nematicide gave the least reduction rate of 60.49%. Results obtained support using bio-nematicides as biological control agents of *T. semipenetrans* nematode.

Keywords: Biological control, extracts, *Tylenchulus semipenetrans*, citrus.

Introduction

Citrus aurantium L., commonly known as sour-orange, is usually utilized as a flavoring and acidifying agent for food (Karabıyıklı *et al.*, 2014). Besides the essential oil and its components (Barceloux *et al.*, 2008; Moraes *et al.*, 2009), the fruits of *C. aurantium* are sources of flavonoid-type compounds with diverse biological effects (Kang *et al.*, 2011). Additionally, it was reported that flavonoid glycosides were isolated from the plant and the biogenic amine and flavanone contents have been determined (Zhang *et al.*, 2017).

Citrus nematode *Tylenchulus semipenetrans* Cobb (1913) is one of the most important root nematodes of plant trees, and it has been found in every citrus growing region of the world (Duncan, 2005; Milne, 1977). In Egypt, citrus nematodes are widely spread in citrus orchards, causing dieback, poor root and vegetative growth, yellow leaves and reduced yield (Oteifa & Shaarawi, 1964; Oteifa & Tarjan, 1965), and further studies on this nematode were conducted by several workers (Ahmed, 1974; Abd-El-Gawad *et al.*, 1994; Abou-El-Naga *et al.*, 1984; Amen & Hassabo, 1995; Bakr *et al.*, 2011; El-Nagdi *et al.*, 2010; Montasser *et al.*, 2012). Because of the worldwide distribution and importance of citrus nematode, it is essential to develop effective management system for its control.

The use of chemicals for nematode control on large scale is an expensive approach and not environment friendly. Thus, it is important to search for a more appropriate control measure which can be made available to small growers. Several workers demonstrated suggested some plant extracts possess nematicidal properties (Awan *et al.*,1992; Nandal & Bhatti, 1983; Sharma & Trivedi, 1992). Application of such plant extracts to nematode infested soil affects nematode directly and stimulates soil microbes that reduce nematode populations (Ahmad *et al.*, 2004; Nandal & Bhatti, 1986; Reddy *et al.*, 1996).

The present study was conducted to evaluate the efficacy of some bio-nematicides to manage *T. semipenetrans* infecting *Citrus aurantium* plants under greenhouse conditions.

Material and Methods

Source of citrus nematode inoculum

Second stage juveniles of *T. semipenetrans* were obtained from a pure culture propagated on sour orange, *Citrus aurantium* L. in the greenhouse of Nematology Research Unit, Agricultural Zoology Department, Faculty of Agriculture, Mansoura Univ., Egypt. Nematodes were extracted from soil by sieving and modified Baermann technique (Goodey, 1957), and inoculum level was determined. One month old sour orange seedlings were grown singly in 20 cm diameter plastic pots filled with steam-sterilized sandy loam soil. Two weeks later, seedlings were inoculated separately with 2000 infective stages/plant of *T. semipenetrans* by pouring the nematode suspension into four holes in the soil around the root system of each seedling. After inoculation, the holes were closed by pressing the soil and watered. Simultaneously, the bio-nematicides i.e. chitosan and propolis (5g/pot) were incorporated into soil and watered. Whereas alcohol and aqueous plant extracts of

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cabbage garlic and ziziphus at a concentration of 5ml/pot were applied as soil drench. Treatments used were as follows: (a) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans*, (b) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans* + chitosan (5g/pot), (c) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans* + propolis (5g/pot), (d) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans* + cabbage aqueous extract (5ml/pot), (e) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans* + cabbage alcohol extract (5ml/pot), (f) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans* + garlic aqueous extract (5ml/pot), (g) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans* + garlic alcohol extract (5ml/pot), (h) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans* + ziziphus aqueous extract (5ml/pot), (i) Three sour-orange seedlings inoculated with 2000 infective juveniles of *T. semipenetrans* + ziziphus alcohol extract (5ml/pot) and Three sour-orange seedlings free of nematode and any treatment.

Each treatment was replicated three times and randomly arranged on a greenhouse bench at 27±4ºC. Plants were watered regularly and treated horticulturally as recommended. Plants were harvested 45 days after inoculation with nematode juveniles. Infected plants were uprooted and washed with tap water and the number of galls, nematode developmental stages, egg masses in roots were recorded after staining with lactic acid fuchsin (Byrd *et al.*, 1983). Nematode reproduction rate was then calculated. Data on shoot and roots length as well as fresh weight were recorded and regarded as plant growth criteria. Data were analyzed by means of Duncan's multiple-range test (1955).

Results and Discussion

Data presented in Table 1 reveal that all tested components i.e. chitosan and propolis as well as alcohol or aqueous plant extract of cabbage, garlic and ziziphus affected growth of sour-orange plants infected with *T. semipenetrans* at different levels. It was evident that among the treatments, alcohol extract achieved the highest increase in total plant fresh weight, plant length and shoot dry weight with values of 82.1, 104.73 and 119.7% for cabbage alcohol extracts, followed by 79.5, 103.38 and 100.0% for garlic alcohol extracts and then 78.3, 100.34 and 105.63%, for ziziphus alcohol extracts, respectively. Moreover, treatments with aqueous extract tested in this study showed slower improvement in plants growth than alcohol extract. Cabbage extract led to a total plant fresh weight, plant length and shoot dry weight shoot increase by 81.6, 104.73 and 119.7%, respectively, followed by 75.9, 103.38 and 100.0% increase, respectively, when garlic extract was used, and 80.1, 101.35 and 105.63% increase, respectively, when ziziphus extract was used, compared with the untreated control. Next to plant extracts, use of chitosan led to 97.30, 64.7 and 84.51% increase and the use of propolis led to 83.78, 80.28and 60.4% increase in the same parameters, respectively. Moreover, Oxamyl nematicide alone had the least effect on percentage increase of plant growth parameters of 29.6, 58.11 and 40.85 % for the same parameters, respectively, compared to the untreated control.

Table 1. Efficacy of chitosan and propolis as well as alcohol and or aqueous plant extracts on growth parameters of sour-orange plants infected with *Tylenchulus semipenetrans* under greenhouse conditions (27±4ºC).

	Plant Growth Parameters*									
	Length (cm)		Total length (cm)	Increase %	Fresh weight (g)		Total plant fresh Wt. (g)	Increase %	Shoot dry weight (g)	Increase %
Treatments	Shoot	Root			Shoot	Root				
Chitosan	34.1 f	39.7 g	73.8 h	64.7	2.62 f	3.0 e	5.62 e	97.30	1.28 hi	84.51
Propolis	33.9 f	38.6 h	72.5 i	60.4	2.60 f	2.82 f	5.43 f	83.78	1.27 i	80.28
Cabbage alcohol extract	38.6 a	43.4 a	82.1 a	81.6	2.87 a	3.18 ab	6.05 a	104.73	1.55 a	119.7
Garlic alcohol extract	37.5 c	42.0 b	79.5 b	75.9	2.80 bc	3.21 a	6.01 a	103.38	1.41 c	100.0
Ziziphus alcohol extract	38.0 b	40.3 ef	78.3 d	73.2	2.82 ab	3.1 cd	5.92 b	100.34	1.42 c	100.0
Cabbage aqueous extract	37.2 c	41.0 cd	78.2 d	73.1	2.76 cd	3.15 abc	5.91 b	100.33	1.35 d	90.14
Garlic aqueous extract	36.1 d	40.3 ef	76.4e f	68.8	2.72 d	3.08 cd	5.80 d	62.84	1.32 ef	87.32
Ziziphus aqueous extract	38.3 ab	41.1 cd	79.4 p	75.9	2.85 ab	3.11 bcd	5.79 d	101.35	1.45 b	105.63
Oxamyl	26.3 i	32.3 l	58.6 m	29.6	2.28 i	2.39 j	4.67 i	58.11	1.0 l	40.85
N. alone	20.7 k	24.5 n	45.2 o	-	1.12 k	1.83 l	2.95 k	-	0.71 n	-
Control Plants	23.1 j	26.2 m	49.2 n	-	2.05 j	2.23 k	4.28 j	-	0.83 m	-

N=2000 J_2 of *T. semipenetrans*

* Each value represents the mean of three replicates.

Values followed by the same letters in the same column are not significantly different at P=0.05 based on Duncan's multiple-range test.

Data as depicted in Table 2 verify that the tested bioagents i.e. chitosan, propolis and aqueous and alcohol extracts of cabbage, garlic and ziziphus were significantly effective in reducing numbers of *T. semipenetrans* juveniles recorded from soil, egg masses and females, final population and the subsequent calculated rates of build-up as compared to those of the inoculated untreated check. Alcohol extract achieved the highest reduction rate in nematode population with values of 95.92% for cabbage alcohol extract, followed by garlic alcohol extract (94.56%) and zizipus aqueous extract (88.73%), whereas oxamyl as a systemic nematicide gave the least value of 60.49% compared to the nematode treatment alone.

Data presented in Table 3 show the influence of chitosan, propolis and aqueous and alcohol extracts of cabbage, garlic and ziziphus in comparison with oxamyl treatment on nitrogen (N), phosphorus (P) and potassium (K) concentrations, total chlorophyll content and total phenol content in sour-orange infected with *T. semipenetrans* under greenhouse conditions. It was evident that N, P, and K concentrations and total phenol content were significantly reduced, whereas that of total chlorophyll content sharply increased by nematode infection alone. All tested components showed significant increase in N, P, and K concentrations and total phenol content as well as reduction in total chlorophyll content compared with the nematode alone treatment (Table 3).

Table 2. Impact of chitosan and propolis as well as alcohol and aqueous plant extracts on development, population density and reduction rate of sour-orange plants infected with *Tylechulus semipenetrans* under greenhouse conditions (27±4ºC).

	Nematode Population in*					
	Soil	Root/plant		Final		Reduction***
Treatments	juveniles	Females	Egg masses	population	Rf**	%
Chitosan	466.0 de	8.66 f	1.33 f	476 de	0.238	82.86
Propolis	752.0 c	24.8 b	10.33 c	786 c	0.393	71.8
Cabbage alcohol extract	113.6 g	0.0 i	0.0 g	113.6 g	0.056	95.92
Garlic alcohol extract	151.6 g	0.0 i	0.0 g	151.6 g	0.075	94.56
Ziziphus alcohol extract	350.6 ef	0.0 i	0.0 g	350.6 ef	0.175	87.41
Cabbage aqueous extract	347.0 ef	0.0 i	0.0 g	347 ef	0.173	87.54
Garlic aqueous extract	314.3 f	0.0 i	0.0 g	314.3 f	0.157	88.73
Ziziphus aqueous extract	381.0 def	0.0 i	0.0 g	381 ef	0.175	86.32
Oxamyl	1073.6 b	21.3 cd	5.66 d	1100.6 b	0.550	60.49
N. alone	2709.6	54.0 a	23.0 a	2786.6 a	1.393	-

N=2000 J_2 of *T. semipenetrans.*

*Values in the table are the mean of three replicates. Each value followed by the same letters in the same column are not significantly different at P=0.05 according to Duncan's multiple-range test.

** Rf (Reproduction factor) = Final population (Pf) / Initial population (Pi)

***% of nematode reduction = [(N alone – N of each treatment) / (N alone)] x 100

Table 3. Nitrogen, phosphorus and potassium concentrations in addition to chlorophyll and total phenol content in fresh shoot of sour-orange plant infected with *Tylenchulus semipenetrans* treated with chitosan and propolis as well as alcohol or aqueous extract under greenhouse conditions (27±4ºC).

	N*	P	K	Chlorophyll content		Total chlorophyll	Total phenol
Treatments	mg/g	mg/g	mg/g	A mg/g	B mg/g	mg/g	mg/g
Chitosan	43.81 b	0.64 f	45.48 de	821.17 f	511.78 i	1332.98 i	229. 43 i
Propolis	43.58 b	0.45 bi	43.80 f	812.23 fg	528.94 g	1341.17 I	215.30 i
Cabbage alcohol extract	55.73 a	0.76 a	46.93 a	808.72 gh	541.15 e	1349.87 i	272.60 a
Garlic alcohol extract	50.30 de	0.69 cde	45.03 c	800.09 bd	535.66 ef	1335.75 k	236.43 b
Ziziphus alcohol extract	49.57 e	0.73 abc	46.20 bc	786.18 i	452.03 m	1259. 29 a	253.37 b
Cabbage aqueous extract	54.11 b	0.75 a	46.30 abc	793.48 i	461.07 i	1254.55 o	257.33 b
Garlic aqueous extract	52.80 c	0.74 a	46.77 ab	786.18 i	452.03 m	1238.21 q	271.07 a
Ziziphus aqueous extract	50.67 d	0.71 bcd	45.80 cd	771.01 k	470.02 k	1241.03 o	266.77 e
Oxamyl + N	40.94 i	0.51 i	39.77 i	831.73 e	429.27 ri	1261.00 m	261.83 c
N. alone	18.85 m	0.27 i	16.05 k	1213.40 a	761.63 a	1975.03 a	189.23 i
Control Plants	37.43 i	0.25 i	33.63 i	632.52 i	429.27 ri	1061.79 r	194.43 e

N=2000 J_2 of *T. semipenetrans*

*Values in the above table are the mean of three replicates. Each value followed by the same letters in the same column are not significantly different at P=0.05 according to Duncan's multiple-range test.

Among the tested treatments, Cabbage alcohol extract achieved the highest increase (compared to the nematode alone treatment) in nitrogen (N), phosphorus (P) and potassium (K) concentrations and total phenol contents, as their values averaged 55.73, 0.76, 46.93 and 272.60 Mg/g, respectively, followed by cabbage aqueous extract treatment with average values of 54.11, 0.75, 46.30 and 257.33 Mg/g, respectively. Whereas, oxamyl treatment gave the least concentrations of N, P, and K and total phenol content (compared with nematode alone treatment), and reached 40.94, 0.51, 39.77 and 261.83 mg/g, respectively. Results also showed that total chlorophyll contents in sour-orange plants infected with *T. semipenetrans* increased in plants treated with nematode without any of tested applications and reached 1975.03 mg/g. Moreover, there was a negative correlation between different treatments and reduction of chlorophyll content compared with nematode alone treatment, as their values reached 1261.00, 1238.21, 1349.87 mg/g for oxamyl, garlic aqueous extract, and cabbage alcohol extract, respectively (Table 3).

Such results agree with those reported earlier by Ahmad *et al.* (2004), Ayazpour *et al.* (2010), Mousa *et al.* (2011) and Tibugari *et al.* (2012). The inhibition of *T. semipenetrans* population in this investigation may be due to the accumulation of toxic by-products of decomposition and/or increase in phenolic contents that enhance host resistance and kill nematodes that enter the root (Alam, 1991; Sivapalan, 1972). The ability of plant extracts to inhibit and control plant disease is due to some natural compounds such as-sterols, saponins, tannins, alkaloids and flavonoids (Mousa *et al*., 2011).

الملخص

سعدون، س.م.، م.ي. سرجاني، هلال م. منى، علي م.م. رهام وس.ب. جاد. 2022. تأثير استخدام بعض المركبات الحيوية في مكافحة نيماتودا الموالح/الحمضيات وتأثيرها على نمو المجموع الخضري والجذري لأشجار النارنج تحت ظروف البيوت المحمية. مجلة وقاية النبات العربية، 40(4): 346−350. https://doi.org/10.22268/AJPP-40.4.346350

هدفت هذه الدراسة لاختبار كفاءة استخدام خمسة أنواع من المركبات الحيوية، وهي الكيتوزان والبروبليس والمستخلص المائي والكحولي لنباتات الثوم والكرنب والجوجوبا، فى مكافحة نيماتودا الموالح/الحمضيات التي تصيب النارنج تحت ظروف البيوت المحمية. أشارت النتائج إلى أن معاملة التربة بتلك المعاملات أدّت إلى خفض أعداد النيماتودا في التربة وكذلك أعداد العقد الجذرية وعدد البيض داخل كيس البيض على جذور النباتات المصابة مقارنة بالنباتات المصابة غير المعاملة. وأعطى استخدام المستخلص الكحولي للكرنب أفضل النتائج بنسبة خفض لأعداد النيماتودا وصلت إلى 95.92%، يليه المستخلص الكحولي للثوم بنسبة 94.56%. وقد انعكس ذلك على معدّل نمو النباتات، حيث سجلت معدلات نمو النباتات المعاملة بالمستخلص الكحولي للكرنب والثوم أفضل النتائج، ويليها المعاملة بالمستخلص الكحولي للجوجوبا.

كلمات مفتاحية: مكافحة حيوية، مستخلصات، *Tylenchulus semipenetrans*، موالح/الحمضيات.

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