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Controlling the Root-Knot Nematode, *Meloidogyne incognita* on Chamomile, *Matricaria chamomilla* L. by Using Some Biocides and Latex-Bearing Plants

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ABSTRACT

The tested biocides and latex-bearing plants significantly ($p < 0.05$ and/or 0.01 levels) decreased the number of *Meloidogyne incognita* juveniles in soil, females and egg-masses on roots, total final nematode population and consequently the rate of nematode build-up and improving the growth and yield of chamomile plants throughout two successive seasons (2016 and 2017) as compared to check plants. The suppression in the previous stages and build-up of the nematode or increase in all plant growth and yield parameters evidently varied according to the type of the tested substances. Thereupon, using of *Bacillus thuringiensis karastaki* (the major component for Dipel 2x product) seemed to be the most effective biocides in controlling *M. incognita* followed by using of *Bacillus subtilis* (the major component for Rhizo-N product) while, using of the active ingredient of Plant-Guard product, *Trichoderma harzianum* was least effective. Also, chopped shoots of *Pedilanthus rithymaloides* gave highest reductions in numbers of juveniles, females, egg-masses and consequently the rate of build-up (94%, 62%, 64%, 93% and 93% respectively) followed by *Cryptostegia grandiflora*, *Calotropis procera* and *Euphorbia pulcherrima*. These compounds also

showed significant ($p < 0.05$ and/or 0.01 levels) improvement in growth and yield of chamomile plants as compared to untreated control.

Keywords: Root-knot nematode, *Meloidogyne incognita*, Chamomile, Biocides, Latex-bearing plants.

INTRODUCTION

Matricaria chamomilla L. (Asteraceae) is widely cultivated in Egypt for its medicinal and flavouring properties. More than 14 genera of plant-parasitic nematodes were infested chamomile plants in Egypt [1] where *Meloidogyne incognita* and *Rotylenchulus reniformis* were widely distributed in chamomile fields. Ismail and El-Nagdi [2] found that an increase in the initial inoculum level of *R. reniformis* reduced the fresh and dry root and shoot weights and flower yield with corresponding decrease in nematode build-up. Application of biocontrol agents involving no risk to human health or non-target soil organisms has been suggested as an alternative to the hazards and costs associated with chemical control of nematodes. Several bacteria and fungi in soil have been demonstrated to be effective control agents for specific nematode pests [3,4]. As well as, studies on the role of some biocontrol agents such as *Nematophagous* fungus and *Bacillus* species for the management of plant-parasitic nematodes have been undertaken by some workers [5-11]. Moreover, a variety of organic additives of plant origin such as latex – bearing plants have been evaluated for their suitability as nematode controlling agents [12-18]. So, the present study reports the efficacy of three biocides and fourteen of latex bearing plants throughout two successive seasons against *Meloidogyne incognita* on chamomile, *Matricaria chamomilla* L and effect on growth and yield of the plant in Egypt.

MATERIALS AND METHODS

Tested of biocides against Meloidogyne incognita on chamomile

Three products of biocides i.e., Plant-Guard containing an Egyptian a strain of the fungus *Trichoderma harzianum* (30×10^6 live cell/cm³), Rhizo-N containing Egyptian a strain of the bacteria *Bacillus subtilis* (30×10^6 live cell/g) and Dipel-2x containing an American strain of the bacteria *Bacillus thuringiensis* karastaki (32×10^3 IU/mg) were used as soil treatment throughout two successive seasons, 2016 and 2017. Plant-Guard and Rhizo-N products were produced by El-Nasr company for fertilizers and biocides, Egypt but, Dipel-2X product was produced by Abbott company, USA and these compounds were applied at three doses

(1.25, 2.5 and 5 g/ pot; on base lower dose, recommended dose and higher dose). Seeds of chamomile, *Matricaria chamomilla* were sown in 20 cm diam., clay pots filled with 2 kg autoclaved soil mixture (sand : clay, 1 : 1 – v : v) and after germination only one healthy plant was kept in each pot. Three weeks later of germination, five replicates were prepared from each treatment and each plant was inoculated with 1000 freshly hatched second stage juveniles of *Meloidogyne incognita*. Nematode inoculated untreated pots served as control. The treatments were applied three days after nematode inoculation

Tested of latex - bearing plants against M. incognita infected chamomile

Thirteen different shoots and one stem only of latex-bearing plants i.e., *Euphorbia lactea*, *E. mauritanica*, *E. royleana* (leaves), *E. royleana* (stems), *E. pseudocactus*, *E. nubica*, *E. pulcherrima*, *Cryptostegia grandiflora*, *Tabernaemontana divaricara*, *Carissa carandas*, *Synadenium grantii*, *Pedilanthus rithymaloides*, *Plumeria rubra* and *Calotropis procera* were collected from the Experimental Farm of the Botanical Orman Garden, Giza, Egypt and these plants were chopped and about 40 g (2% - w : w) of each plant were incorporated into clay pots (20 cm) filled with 2 kg autoclaved soil mixture (sand : clay, 1:1 – v : v) throughout two successive seasons, 2016 and 2017.

The pots were watered immediately after incorporation, for ensuring proper decomposition of the latex additives. Untreated pots served as control. Two weeks later, seeds of chamomile were sown in the pots and after germination only one healthy plant was kept in each pot and five replicates were prepared from each treatment. Each plant was inoculated with 1000 freshly hatched second stage juveniles of *Meloidogyne incognita*. The pots were arranged in a randomized complete block design under greenhouse conditions at $25 \pm 5^\circ\text{C}$. Seventy days after nematode inoculation, chamomile plants were gently uprooted and the juveniles in the soil were extracted by sieving and centrifugation technique [19]. Also, numbers of females and egg-masses were counted from the whole root system. Total final nematode population and rate of nematode build-up (final population/initial population – Pf/Pi) were determined. Length, fresh and dry weights of both shoot and root systems were recorded. Numbers, fresh and dry weights of flowers were determined. The percentage reduction or increase in the nematode population or plant growth parameters as compared to check plants was determined.

Statistical analysis

Data were analyzed statistically using the Fishers Least Significant Differences (LSD).

RESULTS AND DISCUSSION

Effect of biocides against Meloidogyne incognita and growth of chamomile

The tested biocides with their various doses significantly ($p < 0.05$ and/or 0.01 levels) decreased the juveniles number in soil, females and egg-masses on roots, total final nematode population and consequently the rate of nematode build-up as compared to check plants. With few exceptions, no statistical differences were recorded between the three doses of each biocide in the previous nematode stages and their build-up (Table 1). The obtained reduction greatly varied based on the type of the evaluated treatments. So, the highest suppression in numbers of juveniles in soil, females, egg-masses and consequently the rate of nematode build-up was comparatively more with using of *Bacillus thuringiensis* karastaki (the major component for Dipel 2x product), followed by using of *Bacillus subtilis* (the major component for Rhizo-N product) but, application of the active ingredient of Plant-Guard product, *Trichoderma harzianum* was least effective as shown in Table 1. The obtained data are agreement in with those of [20-25]. Amin and with related to suppressing nematodes by using *Trichoderma harzianum* on other hosts. Two isolates of *T. harzianum* could antagonize *Meloidogyne arenaria* by producing anti-nematodal compounds that directly affect nematodes or make the plant roots less attractive [20]. Some possible mechanisms have been suggested to be involved in *Trichoderma*, s antagonism : a) The production of volatile or nonvolatile antibiotics by the fungus [26] b) space- or nutrient- (carbon, nitrogen, iron, etc.) limiting factors that compete with the host [27]; and c) direct mycoparasitism, whereby the host-fungus cell wall is degraded by the lytic enzymes secreted by *Trichoderma* [28]. In addition [29] Stephan et al., reported that *T. harzianum* has dual effect because it provided significant control of root rot and root-knot disease complex on tomato. With respect to *Bacillus thuringiensis* or *B. subtilis*, our data revealed that they reduced number of the different stages of *Meloidogyne incognita* and consequently restricted the development and build-up of the nematode on chamomile plants. Our findings agree with those obtained [3,6-10,25,30]. Moreover, the bacteria *B. subtilis* is able to produce some polypeptides antimicrobials antibiotics i.e., subtilin, tyrothricin and polymyxin which may be effect on nematode development and its reproduction. Also, the nematocidal activities of several known polypeptide antibiotics of microbial origin including polymyxin were screened by Otoguro et al., [31] who found that these antibiotics possess significantly strong nematocidal activities against the pine wood nematode, *Bursaphelenchus lignicolus*

Table 1: Effect of some biocides on the development and reproduction of *Meloidogyne incognita* infecting chamomile plants.

(Means of two successive seasons, 2016 & 2017).

Treatments and (type of active ingredient)	Dose ml/pot* g/pot &	Mean number of nematodes			Total final nematode population	Rate of build-up (Pf / Pi)
		In soil	On roots			
		Juveniles	Females	Egg-masses		
Plant-Guard (<i>Trichoderma harzianum</i>)	1.25*	525 (87)**	45 (30)	13 (86)	383 (91)	0.38 (91)
	2.50*	340 (91)	39 (39)	4 (96)	383 (91)	0.38 (91)
	5.0*	275 (93)	36 (44)	4 (96)	315 (92)	0.32 (92)
Rhizo-N (<i>Bacillus subtilis</i>)	1.25	207 (95)	35 (45)	4 (96)	246 (94)	0.25 (94)
	2.50	152 (96)	31 (52)	2 (98)	185 (95)	0.19 (95)
	5.0	110 (97)	29 (55)	2 (98)	141 (97)	0.14 (95)
Dipel 2X (<i>B. thuringiensis karastaki</i>)	1.25	195 (95)	32 (50)	4 (96)	231 (94)	0.23 (94)
	2.50	142 (96)	30 (53)	2 (98)	174 (96)	0.17 (96)
	5.0	97 (98)	29 (55)	2 (98)	128 (97)	0.13 (97)
Control	-	3910	64	90	4064	4.1
LSD 5%		640	9	14	420	0.5
LSD 1%		810	12	19	670	0.7
Note: # Figures are means of five replicates.						
** Figures in parenthesis indicate percentage reduction from control.						

With the different rates of all the tested treatments significantly ($p < 0.05$ and/or 0.01 levels) increased chamomile growth and flower yield parameters – with few exceptions – as compared to untreated plants (Table 2). Significant variations at 0.05 and/or 0.01 levels in all shoot and root systems growth and flower yield criteria were recorded in some treatments.

Also, statistical differences, with some exceptions, were observed between the tested three rates of each biocide in all plant growth and flower yield parameters (Table 2). The increase in chamomile growth and flower yield parameters greatly varied according to the type of the studied biocides. So, the highest increase in shoot length was attained by using Plant-Guard product (ranged from 30-74%) followed by Rhizo-N were 9-57% and Dipel 2X product (ranged from 9-35%) while the highest increase in some parameters viz. fresh and dry weights of shoot, root dry weight were obtained by using Rhizo-N product followed by

Dipel 2X and Plant-Guard. Also, the highest increase in root fresh weight and numbers, fresh and dry weights of flowers were achieved by using Rhizo-N product followed by Plant-Guard and Dipel 2x products. The improvement in chamomile growth as compared to the untreated control could be attributed to minimizing the nematode populations. Moreover, [20] Windham et al., 1986 stated that *T. harzianum* has been used to enhance plant growth.

Table 2: Effect of some biocides on the growth of chamomile infected with *Meloidogyne incognita*. (Means of two successive seasons, 2016 & 2017).

Treatments	Dose ml/pot* and g/pot	Shoot growth			Root growth			Flower yield		
		Length (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)	No.	Fresh wt. (g)	Dry wt. (g)
Plant-Guard (<i>Trichoderma harzianum</i>)	1.25*	30 (30)**	4 (33)	3 (50)	10 (11)	4 (33)	2 (33)	50 (67)	5 (67)	2 (122)
	2.50*	32 (39)	5 (67)	4 (100)	13 (44)	5 (67)	3 (100)	70 (133)	7 (133)	3 (233)
	5.0*	40 (74)	7 (133)	4 (100)	14 (56)	7 (133)	3 (100)	90 (200)	9 (200)	5 (456)
	1.25	25 (9)	5 (67)	3 (50)	10 (11)	4 (33)	2 (33)	60 (100)	6 (100)	2 (122)
Rhizo-N (<i>Bacillus subtilis</i>)	2.5	33 (44)	6 (100)	4 (100)	13 (44)	5 (67)	2 (33)	100 (233)	10 (233)	5 (456)
	5	36 (57)	15 (400)	5 (150)	16 (78)	7 (133)	3 (100)	140 (367)	13 (333)	5 (456)
	1.25	25 (9)	7 (133)	3 (33)	10 (11)	3 (0)	1.5 (0)	30 (0)	3 (0)	2 (122)
Dipel 2X (<i>B. thuringiensis karastaki</i>)	2.5	26 (13)	9 (200)	4 (100)	11 (22)	4 (33)	2 (33)	40 (33)	4 (33)	2 (122)
	5	31 (35)	11 (267)	5 (150)	15 (67)	7 (133)	3 (100)	80 (167)	8 (167)	4 (344)
Control	-	23	3	2	9	3	1.5	30	3	0.9
LSD 5%		3	2	0.7	3	2	0.7	35	4	2
LSD 1%		4	3	0.9	4	3	0.9	48	5	3

Note: # Figures are means of five replicates.
** Figures in parenthesis indicate percentage increase from control.

Effect of some latex - bearing plants on development of Meloidogyne incognita and growth of chamomile

In Table 3 the data showed that the root-knot nematode, *M. incognita* reproduced freely in untreated pots. Using of all tested fresh chopped shoots or stems of latex-bearing plants significantly reduced at $p < 0.05$ and/or 0.01 levels the population density of *M. incognita* as expressed by the number of juveniles in soil, females and egg-masses on roots, total final nematode population and consequently the rate of nematode build-up as shown in Table 3.

Significantly differences at 0.05 and/or 0.01 levels in the previous nematode stages and their build-up were noted within some of the tested treatments. The reduction values in development and build-up of the nematode clearly varied based on the type of the evaluated materials. Therefore, *Pedilanthus rithymaloides* chopped shoots gave highest reductions in values of juveniles, females, egg-masses and consequently the rate of nematode reproduction (94%, 62%, 64%, 93% and 93%; respectively) followed by *Cryptostegia grandiflora*, *Calotropis procera* and *Euphorbia pulcherrima*, but using of chopped shoots of *Plumeria rubra* was least effective in reducing all the previous values (73.3%, 81.1%, 66%, 73.3% and 73.9%; respectively) followed by *E. mauritanica*, *E. pseudocactus* and *Tabernaemontana divaricaria* as shown in Table 3.

Table 3: Effect of some latex-bearing plants on the development and reproduction of *Meloidogyne incognita* infecting chamomile plants. (Means of two successive seasons, 2016 & 2017).

Treatments	Mean number of nematodes				
	In soil Juveniles	On root		Final population	R=P _f /P _i
		Females	Egg-masses		
<i>Euphorbia lactea</i>	420 (89)*	45 (48)	26(63)	491 (88)	0.5 (88)
<i>E. mauritanica</i>	560 (86)	40 (54)	32 (54)	632 (85)	0.6 (85)
<i>E. royleana</i> (leaves)	420 (89)	40 (54)	35 (50)	495 (88)	0.5 (88)
<i>E. royleana</i> (stems)	370 (91)	42 (51)	30 (57)	443 (89)	0.4 (90)
<i>E. pseudocactus</i>	550 (86)	37 (57)	41 (41)	628 (85)	0.6 (85)
<i>E. nubica</i>	390 (90)	32 (63)	21 (70)	443 (89)	0.4 (90)
<i>E. pulcherrima</i>	360 (91)	41 (52)	31 (56)	432 (90)	0.4 (90)

<i>Cryptostegia grandiflora</i>	320 (92)	50 (42)	50 (29)	420 (90)	0.4 (90)
<i>Tabernaemontana divaricara</i>	540 (86)	41 (52)	30 (57)	611 (85)	0.6 (85)
<i>Carissa carandas</i>	370 (91)	41 (52)	35 (50)	446 (89)	0.5 (88)
<i>Synadenium grantii</i>	530 (87)	40 (54)	31 (56)	601 (85)	0.6 (85)
<i>Pedilanthus rithymaloides</i>	240 (94)	33 (62)	25 (64)	298 (93)	0.3 (93)
<i>Plumeria rubra</i>	670 (83)	40 (54)	34 (51)	744 (82)	0.7 (83)
<i>Calotropis procera</i>	330 (92)	36 (58)	23 (67)	389 (91)	0.4 (90)
Control	3960	86	70	4116	4.1
LSD 5%	196	41	15	170	0.2
LSD 1%	295	52	20	240	0.3
Note: # Figures are means of five replicates.					
** Figures in parenthesis indicate percentage increase from control.					

Regarding of chamomile growth, all the plants grown in amended soil with chopped latex-bearing plants showed significant improvement at 0.05 and/or 0.01 levels in all shoot, root and flower yield parameters – with few exceptions- as compared with untreated control (Table 4).

Table 4: Effect of some latex-bearing plants on the growth of chamomile infected with *Meloidogyne incognita*. (Means of two successive seasons, 2016 and 2017).

Treatments	Shoot growth			Root growth			Flower yield		
	L. (cm)	Fresh wt. (g)	Dry wt. (g)	L. (cm)	Fresh wt. (g)	Dry wt. (g)	No.	Fresh wt. (g)	Dry wt. (g)
<i>Euphorbia lactea</i>	47 (57)*	16 (78)	7 (75)	22 (38)	10 (67)	4 (100)	53 (61)	3.3 (57)	2.1 (24)
<i>E. mauritanica</i>	38 (27)	14 (56)	8 (100)	22 (38)	12 (100)	4 (100)	54 (64)	3.2 (52)	1.9 (12)
<i>E. royleana</i> (leaves)	39 (30)	11 (22)	7 (75)	25 (56)	8 (33)	8 (300)	53 (61)	3.2 (52)	2 (18)
<i>E. royleana</i> (stems)	38 (27)	12 (33)	7 (75)	22 (38)	8 (33)	3 (50)	74 (124)	3.6 (71)	2 (18)
<i>E. pseudocactus</i>	42 (40)	16 (78)	8 (100)	22 (38)	10 (67)	4 (100)	70 (112)	3.3 (57)	2 (18)

<i>E. nubica</i>	32 (7)	12 (33)	5 (25)	18 (13)	6 0	2 0	51 (55)	3.1 (48)	1.9 (12)
<i>E. pulcherrima</i>	31 (3)	10 (11)	5 (25)	18 (13)	8 (33)	3 (50)	51 (55)	3.1 (48)	2 (18)
<i>Cryptostegia grandiflora</i>	39 (30)	14 (56)	7 (75)	25 (56)	13 (117)	4 (100)	72 (118)	3.4 (62)	2.1 (24)
<i>Tabernaemontana divaricaria</i>	39 (30)	12 (33)	7 (75)	18 (13)	7 (17)	3 (50)	62 (88)	3.3 (57)	2 (18)
<i>Carissa carandas</i>	36 (20)	10 (11)	6 (50)	22 (38)	8 (33)	3 (50)	43 (30)	2.9 (38)	2 (18)
<i>Synadenium grantii</i>	32 (7)	10 (11)	6 (50)	22 (38)	11 (83)	4 (100)	38 (15)	2.6 (24)	1.9 (12)
<i>Pedilanthus rithymaloides</i>	31 (3)	9 (10)	6 (50)	18 (13)	7 (17)	3 (50)	48 (46)	2.8 (33)	1.9 (12)
<i>Calotropis procera</i>	34 (13)	10 (11)	6 (50)	19 (19)	7 (17)	3 -50	41 (24)	2.8 (33)	1.9 (12)
<i>Plumeria rubra</i>	36 (20)	11 (22)	6 (50)	27 (69)	8 (33)	3 -50	63 (91)	3.2 (52)	2 (18)
Control	30	9	4	16	6	1.7	33	2.5	1.5
LSD 5%	5	4	1	4	3	0.6	20	0.6	0.1
LSD 1%	8	6	2	6	4	0.8	27	0.8	0.2

Statistical variations at 0.05 and/or 0.01 levels in some shoot and root systems as well as flower yield parameters. The increase in plant growth and flower yield parameters greatly varied according to the type of the tested materials. Thus, using of *Euphorbia lactea* attained highest % increase in shoot length and flower dry weight (57% and 24%; respectively), *E. pseudocactus* gave 78% and 100%; respectively increase in shoot fresh and dry weights, using of *E. mauritanica* gave 100% increase in shoot dry weight, *Cryptostegia grandiflora* gave 56%, 117%, 100% and 24% increases in length, fresh and dry weights of roots and flower dry weight; respectively and finally, the data in Table 4 showed that using of fresh chopped of stems of *E. royleana* attained highest % increase in number and fresh weight of chamomile flowers 24% and 71%; respectively. The obtained data agree with those obtained [12-17,32]. They recorded that the effective role of these additives may be attributed to the differences in chemical nature, composition of toxic compounds present in these plant lattices.

CONCLUSION

The reduction in the nematode development and reproduction may be due to suppression in the nematode penetration which was affected by toxic substances of plant latex and in turn it caused in retardation various activities of juveniles such as movement,

feeding, development and/or reproduction. As well as, the improvement in plant growth and yield of the treated inoculated chamomile is undoubtedly due to the nematode diminution and partly due to the fact that these additives also served as organic manure's [14] Siddiqui and Alam, 1988. In addition, [32] Abdel-Rahman et al. found that changes in physical and chemical properties of soil may be inimical to nematodes or may be increasing host resistance according to Ismail and Amin [16]. Over all, it could be concluded that certain plant latex could be used as a source of cheap and effective nematicides against the plant-parasitic nematodes.

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