

Influence of five plant dried leaf powders on eggplant plants infected with *Meloidogyne incognita* under greenhouse conditions

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Abstract

A greenhouse trail was conducted to study the influence of five plant dried leaf powders i.e. cabbage, sorrel, spinach, rocket and tomato in comparing with oxamyl on root-knot nematode *M. incognita* infecting eggplant under greenhouse conditions. All treatments showed remarkable increase in plant growth parameters as well as reduced nematode criteria. Cabbage dried leaf powder showed higher effectiveness of the tested botanical dried leaf powders and enhanced plant growth parameters with significant increase values. On the other hand, spinach dried leaf powder as soil amendments overwhelmed other treatments in suppressing nematode population in soil (78.3%), root galling number (87.0%), egg-masses (91.5%) and eggs / one gram root (91.5%) respectively. However, the least values of the same nematode criteria was achieved by sorrel treatment with values of 34.0,77.3,90.5 and 89.3 respectively, comparing to nematode alone. The recorded results showed a potential properties of using such plant dried leaf powders as eco-friendly control method instead of chemical pesticides.

Keywords: Eggplant, *Meloidogyne incognita*, control, oxamyl, induced resistance, plant, powder.

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are one of the most wide spread and damaging agricultural pests in the world causing an estimated US \$100 billion loss/ year worldwide (Oka et al., 2000). Root-knot nematodes are obligate parasites and very injurious plant pests for limiting the agricultural productivity. Most cultivated plant species are susceptible to root-knot nematode infection. In Egypt, root-knot nematodes, *Meloidogyne* spp. are becoming a real threat to almost all vegetable crops, especially in the newly reclaimed areas and they have been considered as limiting factors in crop production (Ibrahim et al., 1992). Because of the lack of resistance in plants to most species of root-knot nematode as well as the environmental restrictions on nematicidal use for controlling plant parasitic nematodes; biological control and other eco-friendly control trial have gained recently increasing interest. On the other hand, it has been suggested that the use

of induced resistance in plants could offer a considerable potential for biological control (**Deverall, 1995**). A new strategy for adjusting plant parasitic nematodes is based on the activation of the plant's own defense system via various biotic and abiotic agents. It was very important to find naturally occurring nematicides which may be safe to human health and environmental conditions. Using plant leaves to reduce phytonematodes as soil amendments has been studied by many researchers (**Adegbite and Adesiyun, 2005; Abbas et al., 2009 and Wani, 2006**). There is no literature review demonstrating the efficacy of certain plant dried leaf powders i.e. cabbage, sorrel, spinach, rocket and tomato plants against plant parasitic nematodes. Therefore, the objective of the present investigation was carried out to study the adequacy of the previous plant dried leaf powders in comparison with oxamyl as a nematicide on plant growth response of tomato plants infected with *M. incognita* under greenhouse conditions.

Materials and Methods

1. Source of nematodes:

Second stage juveniles (J2) of *Meloidogyne incognita* (**Kofoed & White**) **Chitwood**, were obtained from a pure culture of *M. incognita* that was initiated by a single egg mass propagated on coleus plants, *Coleus blumei* in the greenhouse of Nematology Research Unit, (NERU) Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt, where this work was carried out. Second stage juveniles (J2) were extracted from soil of coleus plants by sieving and modified Baermann technique (**Goodey, 1957**) counted in a Hawksley counting slide under x 10 magnification then calculated for each 1 ml of the nematode suspension and recorded for preparing nematode inoculation of this work.

2. Nematicide:

Oxamyl (Vydate) 24% L. Methyl-N,N'-dimethyl-N [(methyl) carbamoyl-oxy]-1-thioxamidate, which was used at the rate of 0.3 ml / plant.

3. Prepare plant dried leaf powders:

Fresh leaves of certain tested plants i.e. Cabbage *Brassica oleracea*, Sorrel *Rumex vesicarius*, Spinach *Spinacia oleracea*, Rocket *Eruca Sativa*, and tomato *Solanum lycopersicum* were collected from farmers, Dakahlia governorate and botanically identified then sun dried and were separately ground to fine powders in a grinder and separately added at the rate of 5g/plant / pot according to the design of the experiment.

4. Greenhouse experiment:

A pot trial was set-up to study the effect of five plants dried leaf powders i.e. (Cabbage) *Brassica Oleracea*, (Sorrel plant) *Rumex vesicarius*, (Spinach) *Spinacia oleracea*, (Rocket) *Eruca Sativa* (tomato) *Solanum lycopersicum* in comparing with oxamyl at the recommended dose on root-knot nematode *M. incognita* infecting

eggplant plant cv. Melana (*Solanum melongena* L.) under greenhouse conditions ($25\pm 2^{\circ}\text{C}$). Sixty four plastic pots (10cm-diam). containing 900 g steam sterilized sand loamy soil (1:1, v:v) with one 30 day-old sterilized eggplant seedlings each were used in this study. One week after eggplant seedlings transplanting, 3000 J_2 of *M. incognita* were inoculated to fifty six seedlings each and eight non inoculated were used as control. One week later, treatments were added to eight seedlings each and mixed with soil, while eight seedlings with nematode received oxamyl at the rate of 0.3 ml / plant. Another eight seedlings (pots) with nematode only were left without any treatment. Treatments were as follows:

1. N+ cabbage powder (5g/pot),
2. N + Sorrel powder (5g /pot),
3. N + Spinach powder (5g /pot),
4. N + Rocket powder (5g / pot),
5. N + tomato powder (5g /pot),
6. N + Oxamyl (0.3ml /plant),
7. Nematode alone and
8. Plant free of nematode and any treatments.

Each treatment was replicated eight times. Plastic pots were arranged in a randomized complete block design and irrigated with tap water as needed. Plants were harvested 45 days after nematode inoculation, and plant growth criteria i.e. shoot and root lengths; and fresh weights, as well as shoot dry weights were determined and recorded. Number of *M. incognita* (J_2) in 250 g. of soil/pot were extracted by sieving and modified Baermann technique (**Goodey, 1957**) counted in a Hawksely counting slide under $\times 10$ magnification then calculated for each pot and recorded. Infected roots of each plant per treatment were washed with tap water, fixed in 4% formalin for 24 hrs and stained in 0.01 lactic acid-fuchsin (**Byrd et al., 1983**) and then examined for the number of galls, and egg-masses. Eggs were extracted from root system using 1.0% sodium hypochlorite (**Hussey and Barker, 1973**) and number of eggs/ one gram root was recorded. The root gall index (RGI) and egg mass index (EI) were estimated according to the scale given by (**Taylor and Sasser, 1978**) as follows: 0= no galls or egg-masses, 1= 1-2 galls or egg-masses, 2= 3-10 galls or egg-masses, 3= 11-30 galls or egg-masses, 4= 31-100 galls or egg-masses and 5= more than 100 galls or egg-masses.

5. Data analysis:

The obtained data were subjected to ANOVA, followed by Duncan's multiple ranges to compare means (**Duncan, 1955**). All calculations were performed with COSTAT software.

Results and Discussion

Data represented in Table (1) documented the response of eggplant plant to tested botanical plant dry powders under the stress of *M. incognita* in comparison with oxamyl as a nematicide under greenhouse conditions. All treatments showed remarkable increase in plant growth parameters in terms of plant length, plant fresh and dry weights with various degrees. It was evident that cabbage powder showed higher effectiveness of the tested botanical powders to enhance plant growth parameters and achieved significant increase values of plant length (81.2%), total plant fresh weight (60.1%) and shoot dry weight (88.6%), followed by spinach leaves treatment with values of 62.3, 36.6 and 77.1% for the same plant growth criteria, respectively, comparing to nematode alone. (Table 1). Meanwhile, plant receiving the rocket and tomato dried leaf powders treatments showed considerable percentage increase values of plant length (50.0 %), total plant fresh weight (33.9%) and shoot dry weight (71.4%), respectively. However, plant treated by tomato or sorrel dried leaf powders gave the least percentage increase values of plant length (29.0 & 38.0%), total plant fresh weight (27.9 & 5.5%), shoot dry weight (37.1 & 37.1%), respectively. Meanwhile, oxamyl as a systemic nematicide recorded moderately values in ameliorating plant growth values that averaged 42.4, 19.7 and 28.6% for plant length, total plant fresh weight and shoot dry weight, respectively, comparing to nematode alone.

Data presented in Table (2) reveal that all tested components showed protection performance in eggplant plant against nematode infection in terms of reduction percentage of nematode criteria on such host plant, It was clear that nematode reproduction were significantly affected by all tested treatments. Among the tested applications spinach leaves as soil amendments overwhelmed other treatments in suppressing nematode population in soil (78.3%), root galling number (87.0%), egg-masses (91.5%) and eggs /one gram root (91.5%), followed by tomato application with values of 76.8, 87.0, 90.5 and 90.8% for the same nematode criteria, and then cabbage or rocket that gave the intermediate reduction values which were amounted to 72.5 or 71.6; 86.2 or 82.5%; 89.4 or 91.1% and 91.0 or 89.3% for nematode population in soil, number of galls, egg-masses and eggs / one gram root respectively, comparing to nematode alone (Table 2). However, the least values of the same nematode criteria was achieved by sorrel treatment with values of 34.0, 77.3, 90.5 and 89.3 for number of nematode in soil, number of galls, egg-masses and eggs / one gram root respectively, comparing to nematode alone.

In the present study, five botanical dried leaf powders i.e. sorrel, spinach, cabbage, rocket and tomato showed nematicidal properties against the target pest, *M. incognita* infecting eggplant as well as all tested materials significantly caused ameliorating plant growth and suppressed nematode criteria as well.

Table (1): Plant growth response of eggplant plant cv. Melana infected with *M. incognita* under the stress of tested five botanical dried-leaf powders under greenhouse conditions.

Treatments (plant dried-leaf powders)	Length (cm)		Total	Increase %	Fresh weight (g)		Total F.W (g)	Increase %	Shoot D.W (g)	Increase %
	Shoot	Root			Shoot	Root				
Cabbage	18.1±4.1ab	32.5±2.6a	50.6±4.9a	81.2	17.0±5.2 bc	12.3±7.0 a	29.3±11.4a	60.1	6.6±2.0 a	88.6
Sorrel	14.0 ±4.4bc	24.1±6.4abcd	38.1±3.7b	38.0	15.0±5.2bc	4.3±0.7b	19.3±4.9ab	5.5	4.8±1.8a	37.1
Spinach	14.7±4.1bc	30.0±2.7ab	44.8±4.9ab	62.3	20.1±7.7ab	4.9±2.2b	25.0±8.4a	36.6	6.2±1.6a	77.1
Tomato	13.3±4.9 bc	22.4±8.1bcd	35.6±8.2b	29.0	18.0±4.7abc	5.4±2.6 b	23.4±5.4ab	27.9	4.8 ±1.8a	37.1
Rocket	13.3±4.9bc	28.1±5.0abc	41.4±5.0ab	50.0	18.3±2.5abc	6.3±2.2b	24.5±3.6ab	33.9	6.0±2.4a	71.4
Oxamyl	18.6±3.9ab	20.6±7.1cd	39.3±8.8b	42.4	16.6±1.8bc	5.3±1.8b	21.9±2.7ab	19.7	4.5±2.1a	28.6
Nematode alone	10.0±5.1c	17.6±5.0d	27.6±9.9c	----	11.7±5.8c	6.6±4.0 b	18.3±8.6b	----	3.5±1.4a	----
Untreated plants	21.0±4.5 a	24.0 ±6.6 bcd	45.0±7.8ab	63.0	24.4±3.1a	4.1±1.2b	28.5±2.4a	55.7	5.6±2.7a	60.0
L.S.D 0.05	4.49	6.15	6.99	-	4.8	3.32	6.66	-	2.03	-
F	5.09	6.007	7.93	-	4.60	5.13	2.82	-	2.14	-
P	.0002	.0001	.00001	-	.0004	.0001	.0136	-	.0534	-

N= 3000 J2 of *M. incognita*.

* Each value is the mean of eight replicates.

* Means in each column followed by the same letters(s) did not differ at $p < 0.05$ according to Duncan's multiple range tests.

Table (2): Response of *Meloidogyne incognita* infecting eggplant plant cv. Melana as affected by tested five plants dried leaf powders under greenhouse conditions.

Treatments (dried-leaf powders)	N. Soil	Reduction %	No. of galls	Reduction %	RGI	No. of eggmasses	Reduction %	E. I	Eggs/ 1 gram root	Reduction %
Cabbage	1971.9±614.2c	72.5	25.6±15.16 b	86.2	3.2±0.7bc	6.7 ±3.0 b	89.4	1.9±0.4b	328.0±136.6 b	91.0
Sorrel	4732.75±463.5 b	34.0	42.2±18.8 b	77.3	3.7±0.4 b	6.0±2.61b	90.5	1.9±0.4b	335.5±130.7b	90.8
Spinach	1559.3±283.5 c	78.3	24.1±16.9b	87.0	2.9±0.4bc	5.6±2.7b	91.1	1.8±0.3 b	309.3±82.6b	91.5
Tomato	1663.75±1040.81c	76.8	24.1±16.86b	87.0	2.9±0.35c	6.0±2.6b	90.5	2.0±0b	335.5±130.7b	90.8
Rocket	2040.4±490.6c	71.6	31.6±19.2b	82.5	3.4±0.5 bc	6.2±2.3 b	90.2	2.0±0 b	309.0±82.4 b	91.5
Oxamyl	1288.8±77.9c	82.0	15.4±4.4b	91.7	2.9±0.4c	5.9±2.4b	90.6	2.0±0 b	391.3±85.9b	89.3
N alone	7173.8±1078.0a	0.0	186.0±74.3a	0.0	4.8±0.5a	63.0±19.5a	0.0	4.0±0a	3642.4±137.4a	0.0
L.S.D 0.05	637.25	--	31.61	--	0.47	7.79	--	0.35	109.05	--
F	93.03	--	30.05	--	15.37	62.44	--	40.9	1064.74	--
P	.00001	--	.00001	--	.00001	.00001	--	.00001	.00001	--

* N= 3000 J2 of *M. incognita*.

ox= oxamyl.

* Each value is the mean of eight replicates.

Means in each column followed by the same letters(s) did not differ at p< 0.05 according to Duncan's multiple range tests.

These materials seemed to have higher toxic action but not as much as oxamyl (nematicide) which overwhelmed in some cases all other tested treatments. Many reports in literature emphasized the role of plant powders in controlling plant parasitic nematode. The nematocidal activity of tested botanical components such as cabbage product is due mainly to their contents of glucosinolates and myrosinase located in different parts of the cell come into contact (**Poulton and Moller, 1993**). The enzymatic hydrolysis of glucosinolates may lead to the formation of bioactive volatile isothiocyanates (**Cole, 1976 and Fenwick et al., 1963**). Obviously, isothiocyanates are thought to be the main components of the pest-pathogen-and weed-suppressive effects that observed after soil incorporation of *brassica* tissue (**Kirkegaard and Sarwar, 1999**) and their inhibitory effect on fungi, bacteria, insects and nematodes and weeds has been amply demonstrated in in-vitro experiments (**Brown and Morra 1997; Harvey, 2002 and Bello et al., 2004**). These various tested materials may play a role in defense mechanisms of the tested host plants against involving the target nematodes. Tomato leaves could be useful as a biological control agent due to a strong antifungal activity against pathogenic fungi and indicating a defense response against plant pathogenic fungi (**Kobayashi, 2012**). The major chemical components of tomato leaves were sugars, mineral composition, organic acids, lycopene, total phenols and hydroxycinnamic acids (**Suárez, et al., 2008**). The chemical composition of *Rumex vesicarius* indicates that it is a good source of minerals, a moderate source of protein and ascorbic acid and high in oxalic acid as well as low in lipids and tocopherol (**Alfawaz, 2004**). *R. vesicarius* is a good source of bioactive compounds due to its content of various phytochemicals such as phenolic and antioxidant compounds (**Beddou, et al., 2015**). These various tested materials may play a role in defense mechanisms of tested host plants against the target nematodes. Antioxidants such as ascorbic acid (vitamin C) and oxalic acid are essential for plant defense to avoid damage from abiotic and biotic stress (**Racchi, 2013**). An increasing demand to environmentally acceptable alternative for traditional pesticides provides an important to search new bio-based strategies in crop protection. Employing induced resistance is one of such strategy, consisting of boosting the natural plant immunity against plant parasitic nematodes. Botanical plant parts have been a valuable source of biologically active compounds especially of used to protect plants from pests and diseases. Botanical inducer resistance can be combined with biopesticides, biological control agents, biostimulants and even chemical pesticides, which could result in reduced pesticide consumption (**Burketova et al, 2015**). The chemical elicitors such as salicylic acid affect production of phenolic compounds and activation of various defense-related enzymes in plants. Their introduction into agricultural practice could minimize the scope of chemical control (**Thakur and Sohal, 2013**).

Conclusion

It could be concluded that tested botanical dried leaf powders showed nematicidal properties against *M. incognita*, which may be considered as an environmentally acceptable alternative for traditional pesticides in crop protection. These various tested materials may also play a role in defense mechanisms of tested host plants against the target nematodes. The topic needs more applicable studies to prove these findings.

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تأثير مسحوق الأوراق الجافة لخمسة نباتات على نبات الباذنجان المصاب بنيماتودا تعقد الجذور "ميلدوجيني انكوجنيتا" تحت ظروف الصوبة الزراعية

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تم إجراء تجربة تحت ظروف الصوبة الزراعية لتقييم خمسة من المساحيق الجافة لأوراق الكرب، والحميض والسبانخ، والجرجير والطماطم بالمقارنة بالمبيد النيماتودي الأوكساميل وتأثير ذلك على نباتات الباذنجان المصابة بنيماتودا تعقد الجذور بمستوي عدوى ٣٠٠٠ يرقة/ نبات وقد أظهرت النتائج ما يلي:

١- أظهرت جميع المعاملات ارتفاعاً ملحوظاً في المقاييس النباتية المختبرة بدرجات متفاوتة وخفض المقاييس النيماتودية.

٢- سجل مسحوق السبانخ المركز الأول في خفض أعداد النيماتودا في التربة (٧٨,٣٪)، وعدد العقد النيماتودية (٨٧,٠٪)، وكتل البيض (٩١,٥٪) وأعداد البيض لكل جرام جذر (٩١,٥٪) على التوالي، مما يوضح وجود خصائص إمكانية استخدام مسحوق أوراق السبانخ الجاف كطريقة للسيطرة على النيماتودا نباتية النطفل المختبرة كوسيلة صديقة للبيئة وبديلة للمبيدات.

٣- في حين حققت المعاملة بمسحوق أوراق الحميض الجافة أقل القيم في هذا السياق بقيم ٣٤,٠، ٧٧,٣، ٩٠,٥، ٨٩,٥ على التوالي لكلا من أعداد النيماتودا في التربة وعدد العقد النيماتودية وكتل البيض وأعداد البيض في واحد جم جذر على التوالي.