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Management of the Root-Knot Nematode, *Meloidogyne incognita* on Tomato Plants by Pre-Planting Soil Biofumigation with Harvesting Residues of Some Winter Crops and Waste Residues of Oyster Mushroom Cultivation under Field Conditions

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Abstract

A field trial was carried out to study the effect of pre planting soil biofumigation with air-dried powders of harvesting residues (leaves) of artichoke, cauliflower, sugar beet, table beet, taro and turnip) and waste residues of oyster mushroom cultivation (applied at 300g/m^2), comparing to the chemical nematicide fosthiazate 10%G (Nemathorin®) applied at 12.5 kg/feddan for the management of the root-knot nematode, *Meloidogyne incognita* on tomato plants cv. Alisa.

All treatments greatly managed *M. incognita* infection on tomato plants. Numbers of root galls, nematode egg masses, final population (Pf) and reproduction factor (Rf) in all treatments were significantly reduced as compared to control plants (infected with nematode only). Treatment with fosthiazate 10% provided the maximum reduction (91.47%) of nematode infection on tomato plants, followed by treatments with leaf powders of taro (90.49%), turnip (84.95%), artichoke (82.72%), table beet (81.37%), cauliflower (79.23%), and sugar beet (76.98%). Treatment with waste residues of oyster mushroom cultivation recorded the minimum reduction (67.93%). On the other hand, all treatments significantly increased growth criteria of tomato plants comparing to the control ones. The increase of total fresh weight was ranged between 69.4-223.7% and fruit yield per plant was considerably increased by 134.3-709.9%. It seems that soil biofumigation with plant residues studied herein may consider a promising and economic strategy in managing root-knot nematode infection on tomato plants, particularly in programs of non chemical control practiced in organic farming.

Key words: Root-knot nematode, soil Biofumigation, nematode management, Tomato.

Introduction

The root-knot nematode (RKN), *Meloidogyne* spp. has been referred as one of the most widespread nematodes severely injuring vegetables in Egypt. It causes high losses and seriously damaged their crop production particularly in infested fields of sandy soils (**Ibrahim *et al.*, 2010**).

Regarding deleterious side effects of the synthetic nematicides on the humans and environment, research of alternative methods of nematode management is a major challenge for many nematologists throughout the world. Among the alternatives, soil biofumigation has shown promising results as a sustainable strategy to manage plant-parasitic nematodes, soil-borne pathogens, insects and weeds (**Bailey and Lazarovits, 2003, Matthiessen and Kirkegaard, 2006 and Ploeg, 2008**). The concept of soil biofumigation was initially defined as the biocidal action of volatile compounds resulted during the decomposition of plant tissues of *Brassica* plant species incorporated into the infested soil (**Angus *et al.*, 1994 and Kirkegaard and Sarwar, 1998**), but it was later expanded to include non *Brassica* plant species, several plant and agro-industrial residues, and wastes of farm animals (**Ploeg, 2008**).

A lot of investigations have been made about utilizing many plant residues and agro-industrial wastes as organic soil amendments or pre planting soil biofumigants in the management of plant-parasitic nematodes and other plant pathogens were carried out by many authors (**Akhtar and Alam, 1993; Ploeg and Stapleton. 2001; Mashela, 2002; Bailey and Lazarovits, 2003; López- Pérez *et al.*, 2005; Piedra Buena *et al.*, 2006 & 2007; Monfort *et al.*, 2007; Hassan *et al.*, 2010; López-Pérez *et al.*, 2010; D'Addabbo *et al.*, 2011; El-Nagdi and Abd Elfattah, 2011; Khattak and Khattak, 2011; Anita, 2012; Chindo *et* al., 2012; Karavina and Mandumbu, 2012; Masheva *et al.*, 2012; Aslam and Saifullah, 2013; Kruger *et al.*, 2013 and Youssef and Lashein, 2013**).

Following harvesting times of many different winter crops and vegetables, million tonnes of plant residues were produced in the Egyptian fields (**Tantawi, 2004**). Therefore, the aim of the current study is the utilization of some of these plant residues and wastes of oyster mushroom cultivation as pre-planting soil biofumigants in the management of *Meloidogyne incognita* on susceptible tomato plants under field conditions.

Materials and Methods

Collection of plant materials:

Leaves of artichoke (*Cynara scolymus* L.), cauliflower (*Brassica oleracea* L.), sugar beet, table beet (*Beta vulgaris* L.) and turnip (*Brassica rapa* L.) were collected from the production fields in Alexandria governorate, and leaves of Taro (*Colocacia esculenta* L.) were collected from Shanawan province, Menoufiya governorate during the period from March to April, 2013. Waste residues of the oyster mushroom, (*Pleurotus ostreatus* (Jacq.) P. Kumm) cultivation (a combination of rice straw + spent fungal mat) were obtained from the production unit of oyster mushroom at the Integrated Protection Laboratory (Sabaheya, Alexandria). All collected plant materials were left to complete air drying for 2-3 weeks and exposed to over drying in an electrical oven at below 45ºC for 3 days, then well-ground in a home mill and sieved through a 1 mm pore aperture stainless screen to provide the fine powder.

Field experiment:

Five field microplots (10 m long X 50 cm width, each) of sandy soil (composed of sand 92%, clay 5% and silt 3%, its EC = 1.12 dsm^{-1} and pH = 7.2) naturally infested with the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood in a private orchard at El-Ma'moura region, Alexandria governorate, were selected to carry out this experiment. Each microplot was split to nine partitions (50 X 50 cm each) with thick plastic sheets inserted to a depth 40-50 cm in order to avoid cross contamination through the drainage. Initial nematode population (Pi) was determined in 1 kg soil sampled from each soil partition by extraction using a combination of Cobb's sieving and centrifugal sugar floatation techniques (**Ayoub, 1980**).

Powders of studied plant materials at 300g/m^2 soil (equivalent to 1260 kg/feddan) were thoroughly mixed to the upper 20-25 cm of soil surface and irrigated soon to the field capacity in order to make proper decomposition (**Kagai *et al.*, 2012**). Each treated partition received 75 gm of plant powder and covered with a thin layer of transparent plastic sheet to keep the decomposition products of plant residues under soil surface (**Anita, 2012**). A check treatment infected with nematode only (control) and a comparative treatment with the chemical nematicide fosthiazate 10% (Nemathorin®) were included. Granules of the nematicide at the rate of 3g/m^2 soil (equivalent to the recommended dose 12.5kg/feddan) were thoroughly mixed to the upper 20-25 cm of soil surface and watered soon without covering.

Healthy and uniform tomato seedlings (*Solanum lycopersicum*) cv. Alisa (40 days old with 4-5 true leaves) reared in a transplanting tray (11 X 19 wells) were purchased from a nursery of vegetables at Rashid province, Behera governorate. Following 15 days of decomposition, plastic covers were removed and all soil partitions were planted at the same time with a tomato seedling (**Anita, 2012**).

All treatments were distributed within each microplot in a completely randomized block design and maintained under natural conditions during the period from May to August, 2013. The study field area was hand-weeded and plants received their needs of water and fertilizers at the recommended doses along the time of experiment.

Three months after transplanting, tomato plants were carefully displaced from each microplot partition, and roots were gently washed using tap water to discard adhering soil particles and wiped between several layers of tissue papers and fresh weights of shoot and root systems of all experimental plants were recorded. Also, fruit yield per each experimental plant was weighted. Root galls caused by nematode were counted and roots were stained with an aqueous solution of Phloxine B (0.15 g/l. tap water) for 15 min and rewashed with tap water to remove stain residuals to show nematode egg masses (in red) for counting (**Holbrook *et al.*, 1983**). Final nematode population (Pf) was estimated after extracting nematode eggs from tomato roots by shaking with 1% sodium hypochlorite and counted (**Hussey and Barker, 1973**), and nematode second stage juveniles (J_2) by extraction from soil (**Ayoub, 1980**). Finally, nematode reproduction factor (Rf) was calculated according to the formula Rf = Pf ÷ Pi, where Pi is the initial nematode population (**Oostenbrink, 1966**).

Reduction (%) of nematode Pf was calculated using Henderson and Tilton' formula (**Henderson and Tilton, 1955**), as follows:

$$\text{Reducation}(\%) = \left[1 - \left(\frac{a}{b} \times \frac{c}{d}\right)\right] \times 100$$

Where:

a = nematode population density in the treatment after application.

b = nematode population density in the treatment before application.

c = nematode population density in the untreated control before application.

d = nematode population density in the untreated control after application.

Samples (50g) of fine powder of all studied plant residues were sent to the Department of Soil and Agricultural Chemistry, Faculty of Agriculture, Saba Pasha, Alexandria University for the determination of its carbon and nitrogen contents and C:N ratios.

Statistical analysis:

Analysis of variance (ANOVA) between studied treatments for all parameters was statistically done using a SAS program, and means were compared by Fisher's protected LSD values at 5% level of probability (**SAS, 1997**).

Results and Discussion

All treatments studied in this experiment greatly managed *M. incognita* infection on tomato plants cv. Alisa. Numbers of root galls, nematode egg masses, final population (Pf) and reproduction factor (Rf) in all treatments were significantly (P = 0.05) reduced as compared to the control plants infected with nematode only (Table 1). Fosthziazate 10% was the superior treatment which reduced nematode infection on tomato plants by 91.47%. This finding is agreed with results of **Kesba, 2011 and Radwan *et al.*, 2012**. They found that fosthziazate 10% was the most effective treatment against the RKN as compared to the other treatments with organic materials or bioproducts of microbial origin.

Also, incorporation of all studied plant residual powders to *M. incognita*-infested soil before planting of tomato plants significantly reduced nematode infection. The maximum reduction (90.49%) was achieved by treatment with taro leaf powder, while the minimum one (67.93%) is obtained by treatment with powder of oyster mushroom residues. Other treatments suppressed nematode infection by 76.98-84.95% (Table 1). Nematicidal potential of brassicaceous and non brassicaceous plant residues applied to soil as soil amendments or pre-planting soil biofumigants towards RKN were reported by other authors (**Ploeg and Stapleton, 2001; Lopez-Perez *et al*., 2005 & 2010; Monfort *et al*., 2007; El-Nagdi and Abd El Fattah, 2011; Anita, 2012, Youssef and Lashein, 2013 and Barros *et al*., 2014)**.

Moreover, utilizing residues of the spent mushroom compost as effective soil amendment in controlling RKN infection on tomato plants was previously studied by **D'Addabbo *et al*., 2011; Khattak and Khattak, 2011 and Aslam and Saifullah, 2013**.

On the other hand, all treatments significantly increased growth parameters and fruit yield performance of tomato plants as compared to the control plants. The maximum increase of total plant fresh weight (223.7%) was provided by treatment with artichoke leaf powder, while the minimum one (69.4%) was obtained by treatment with powder of oyster mushroom residues. Application of Fosthiazate 10% gave a considerable increase reached to 156.1% (Table 2).

Tomato plants grown in soil amended by leaf powder of table beet produced the maximum increase of fruit yield (709.9%), followed by treatments with leaf powders of turnip (625.2%), artichoke (543.1%), taro (532.6%), cauliflower (509.1%) and sugar beet (459.8%), respectively comparing to control plants. Minimum increase of fruit yield (134.3%) was recorded for treatment with oyster mushroom residues, while application of fosthiazate 10% gave an increase reached to 470.5% (Table 2). Many authors greatly supported our findings (**Mashela, 2002; Lopez-Perez *et al*., 2005 & 2010; Monfort *et al*., 2007; Piedra Buena *et al*., 2007; Hassan *et al*., 2010; El-Nagdi and Abd Elfattah, 2011; Anita, 2012 and Youssef and Lashein, 2013**).

A number of mechanisms and modes of action have been proposed and discussed to explain observed beneficial effects of organic soil amendments on plants grown in the presence of nematodes. Among them, release of naturally occurring nematicidal compounds (such as polythienyls, isothiocyanates, glucosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, fatty acids and their derivatives, sesquiterpenoids, diterpenoids, quassinoids, steroids, triterpenoids and phenolic compounds) during biodegradation of organic plant materials (**Chitwood, 2002**), stimulation of natural enemies of nematode and improve plant growth due to the increase of soil fertility which indirectly affect the plant tolerance or resistance to nematode infection (**Oka, 2010 and Chindo *et al.*, 2012**).

Many of brassicaceous plant residues contain high quantities of sulfur compounds called glucosinolates (GLSs) which can be converted (into soil during bio decomposition) to isothiocyanates and other related compounds by enzymatic hydrolysis occurred by the endogenous myrosinase. Isothiocyanates are highly toxic to plant-parasitic nematodes, many plant pathogens and insects. Other phytochemical constituents found in brassicaceous plants such as phenols and ascorbic acids, may compliment the activity of GLSs (**Zasada and Ferris, 2004; Antonious *et al.*, 2009 and Avato *et al.*, 2013**). Therefore, the nematicidal activity of cauliflower and turnip leaf powder in the current study is attributed to presence of GLSs and their derivatives.

It was reported that the soil pH (7.3) is optimal for enzymatic hydrolysis of GLSs to isothiocyanates (**Zasada and Ferris, 2004**). However, pH of the soil used in the present study (=7.2) is too close to the optimal one. This observation supported the nematicidal potential of cauliflower and turnip leaf powders studied herein.

On the other hand, artichoke leaves are rich in the phenolic compounds such as cynaroside and caffeic acid (**Orlovskaya *et al.*, 2007**). Similarly, leaves of beetroot (*Beta vulgaris*) had a considerable content of phenolic compounds and fatty acids (**Biondo *et al.*, 2014**). Also, **Aslam and Saifullah (2013)** reported that phenolic compounds present in spent mushroom compost have an antimicrobial activity, which could be an effective biocontrol of RKN on tomato plants.

Aqueous extract of taro leaves had a potential anthelmintic activity against the earth worm, *Pheritima posthuma* and traditionally used in curing intestinal worm infection of humans (**Kubde *et al.*, 2010**), antimicrobial, and anticancer properties (**Wei *et al.*, 2011**). Phytochemical constituents of taro leaves are including alkaloids, glycosides, flavonoids, phenolics, saponins, sterols, essential oils and tannins (**Subhash *et al.*, 2012**). Nematicidal activity of these constituents naturally occurred in many plant species was previously reported by **Chitwood, 2002**.

It's worthy to note that this study probably the first report on the nematicidal activity of taro leaves and its possible use in the management of RKN on a tomato plants.

It was proposed that potential of nematode suppression by organic soil amendments generally depends on the amount of the amendment used, C:N ratio, and time of biodegradation (decomposition) in soil. Also, grinding of the dried plant residues may be important to make decomposition process easily done because it increases the surface area of the organic amendment. Moreover, organic materials with C:N ratio less than 20:1 have higher biodegradation rates in soil and often

Table (1): Effect of pre-planting soil biofumigation with harvesting residues of some winter vegetables and crops and waste residues of oyster mushroom cultivation (applied at 300g/m^2), comparing to the chemical nematicide fosthiazate 10%G (at 12.5kg/feddan) on management of the root-knot nematode, *Meloidogyne incognita* (*Mi*) infecting tomato plants cv. Alisa under field conditions.

Treatment	Nematode Pi/ kg soil	No. / plant root				Nematode Pf	Reduction (%)*	Rf	Reduction (%)*	Mean of reductions
		Root galls	Reduction (%)	Egg masses	Reduction (%)					
Mi-infected plants only (Control)	5541 ± 654	1084.2 a	-	945.6 a	-	193496 a	-	34.95 a	-	-
Fosthiazate 10% + *Mi*	4954 ± 663	93.6 e	91.37	73.2 f	92.26	15494 f	91.04	3.07 f	91.22	91.47
Artichoke leaf powder + *Mi*	5220 ± 753	171.2 cd	84.21	160.6 cd	83.02	33045 cd	81.87	6.37 de	81.77	82.72
Cauliflower leaf powder + *Mi*	4893 ± 714	198.8 cd	81.66	185.6 cd	80.37	38536 cd	77.45	7.88 c	77.45	79.23
Oyster mushroom residues + *Mi*	5535 ± 616	328.4 b	69.71	312.0 b	67.01	62998 b	67.41	11.33 b	67.58	67.93
Sugar beet leaf powder + *Mi*	4770 ± 700	220.4 c	79.67	207.4 c	78.07	41596 c	75.03	8.69 c	75.14	76.98
Table beet leaf powder + *Mi*	4945 ± 643	185.2 cd	82.92	170.4 cd	81.98	34013 cd	80.30	6.90 d	80.26	81.37
Taro leaf powder + *Mi*	5688 ± 629	105.0 e	90.32	89.2 ef	90.57	18944 ef	90.46	3.29 f	90.59	90.49
Turnip leaf powder + *Mi*	5156 ± 758	149.6 de	86.20	138.8 de	85.32	28571 de	84.13	5.54 e	84.15	84.95
LSD at $P = 0.05$		61.847		58.255		12028		0.9338		

- Original values are averages of five replicates (one plant each).
- Means followed by different letter(s) within a column are significantly different according to Fisher's LSD value at $P = 0.05$.
- Pf (final population of nematode) = final eggs extracted from roots + final second stage juveniles extracted from soil.
- Rf (Reproduction factor of nematode) = Pf ÷ Pi, where Pi = initial population of nematode.
- Reduction (%) of root galls, nematode egg masses and Rf value = (Control – Treatment) ÷ Control X 100.

* Reduction (%) of nematode Pf was calculated using Henderson and Tilton' formula.

Table (2): Effect of pre-planting soil biofumigation with harvesting residues of some winter vegetables and crops and waste residues of oyster mushroom cultivation (applied at 300g/m^2), comparing to the chemical nematicide fosthiazate 10%G (at 12.5kg/feddan) on growth performance and yield of tomato plants cv. Alisa infected by the root-knot nematode, *Meloidogyne incognita* (*Mi*) under field conditions.

Treatment	Shoot fresh weight (g)	Increase (%)	Root fresh weight (g)	Increase (%)	Total fresh weight (g)	Increase (%)	Fruit yield per plant (g)	Increase (%)
Mi-infected plants only (Control)	89.2 f	-	22.4 e	-	111.6 f	-	70.6 e	-
Fosthiazate 10% + *Mi*	221.6 bcd	148.4	64.2 bc	186.6	285.8 bcd	156.1	402.8 c	470.5
Artichoke leaf powder + *Mi*	283.5 a	217.8	77.8 a	247.3	361.3 a	223.7	454.0 bc	543.1
Cauliflower leaf powder + *Mi*	198.9 de	123.0	61.5 c	174.6	260.4 d	133.3	430.0 c	509.1
Oyster mushroom residues + *Mi*	151.6 e	70.0	37.5 d	67.4	189.1 e	69.4	165.4 d	134.3
Sugar beet leaf powder + *Mi*	209.8 cd	135.2	59.5 c	165.6	269.3 cd	141.3	395.2 c	459.8
Table beet leaf powder + *Mi*	264.7 ab	196.7	75.4 a	236.6	340.1 ab	204.7	571.8 a	709.9
Taro leaf powder + *Mi*	286.5 a	221.2	69.7 abc	211.2	356.2 a	219.2	446.6 bc	532.6
Turnip leaf powder + *Mi*	251.4 abc	181.8	72.2 ab	222.3	323.6 abc	190.0	512.0 ab	625.2
LSD at $P = 0.05$	49.083		10.269		57.309		78.224	

- Values are averages of five replicates (one plant each).
- Means followed by different letter(s) within a column are significantly different according to Fisher's LSD value at $P = 0.05$.
- Increase (%) was calculated according to the following formula:

$$\text{Increase}(\%) = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

nematicidal activities (**McSorely and Gallaher, 1995a&b, Ritzinger and McSorley, 1998 and Mashela, 2002**). Fortunately, C:N ratios of all plant residues used in the present study (except waste residues of oyster mushroom cultivation) are within the recommended range to achieve these effects (Table 3).

Table (3): Carbon and nitrogen contents and C:N ratio of studied plant residues and organic waste materials.

Plant materials	Carbon content (%)	Nitrogen content (%)	C:N ratio
Artichoke leaf powder	12.65	1.32	9.40 : 1
Cauliflower leaf powder	11.90	1.61	7.35 : 1
Oyster mushroom residues	11.53	0.47	24.7 : 1
Sugar beet leaf powder	12.65	1.67	8.16 : 1
Table beet leaf powder	12.28	1.83	6.90 : 1
Taro leaf powder	11.53	1.26	8.29 : 1
Turnip leaf powder	12.65	2.09	6.62 : 1

Bello *et al*., (2004) documented that soil biofumigation provided levels of *M. incognita* control in bell pepper that were similar to levels achieved by using methyl bromide (a chemical fumigant). Our results confirmed this observation, where the most treatments effectively controlled *M. incognita* on tomato plants similar to the chemical nematicide, fosthiazate 10%.

Eventually, we suggested that utilizing harvesting residues of some winter vegetables and crops or waste residues of oyster mushroom cultivation as soil biofumigants (before planting) could become a promising tool in the RKN management on susceptible tomato plants, particularly in organic farming systems.

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الملخص العربي

مكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* على نباتات الطماطم بتدخين التربة حيويًا قبل الزراعة باستخدام متبقيات حصاد بعض المحاصيل الشتوية ومتبقيات زراعة عيش الغراب المحاري تحت ظروف الحقل

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أجريت تجربة حقلية لدراسة تأثير التدخين الحيوي للتربة قبل الزراعة باستخدام مساحيق متبقيات حصاد (أوراق) بعض الخضروات والمحاصيل الشتوية وهي الخرشوف، القرنبيط، بنجر السكر، بنجر المائدة، القلقاس واللفت، وكذا متبقيات زراعة عيش الغراب المحاري (خليط بيئة قش الأرز + متبقيات النمو الفطري) بمعدل ٣٠٠جم/م٢ تربة، مقارنة مع المبيد الكيميائي نيماثورين ١٠٪ (بمعدل ١٢,٥كجم/الفدان) على مكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* على نباتات الطماطم صنف *Alisa* تحت ظروف الحقل. أدت جميع المعاملات إلى خفض معنوي في الإصابة بالنيماتودا على نباتات الطماطم، وقد انخفضت أعداد العقد الجذرية، كتل بيض النيماتودا، الكثافة العددية النهائية للنيماتودا، وكذا قيمة عامل تكاثر النيماتودا، معنويًا بالمقارنة مع النباتات المصابة بالنيماتودا فقط (الشاهد). حققت المعاملة بالمبيد أعلى نسبة خفض في الإصابة بلغت ٩١,٤٧٪، تبعها مباشرة المعاملة بمساحيق أوراق كل من القلقاس (٩٠,٤٩٪)، اللفت (٨٤,٩٥٪)، الخرشوف (٨٢,٧٢٪)، بنجر المائدة (٨١,٣٧٪)، القرنبيط (٧٩,٢٣٪)، وبنجر السكر (٧٦,٩٨٪). سجلت المعاملة بمسحوق متبقيات زراعة عيش الغراب المحاري أقل نسبة خفض في الإصابة (٦٧,٩٣٪). ومن ناحية أخرى، أدت جميع المعاملات إلى زيادة معنوية في مقاييس نمو نباتات الطماطم مقارنة بالنباتات المصابة بالنيماتودا فقط. وقد تراوحت نسبة الزيادة في الأوزان الطازجة الكلية للنباتات في جميع المعاملات بين ٦٩,٤–٢٢٣,٧٪، وقد زاد أيضًا محصول الثمار معنويًّا بنسبة تراوحت بين ١٣٤,٣–٧٠٩,٩٪. إن إستراتيجية التدخين الحيوي للتربة قبل الزراعة باستخدام المتبقيات النباتية في تلك الدراسة تعتبر طريقة اقتصادية واعدة لمكافحة نيماتودا تعقد الجذور على نباتات الطماطم، وخصوصاً في برامج المكافحة غير الكيميائية المتبعة في حالة الزراعة العضوية.