APPLICATION OF SOME ORGANIC ADDITIVES FOR THE MANAGEMENT OF ROOT-KNOT NEMATODE INFECTING CUCUMBER UNDER ORGANIC FARM CONDITIONS

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The management of the root-knot nematode, *Meloidogyne incognita* infecting cucumber (*Cucumis sativus*) roots, using the nematicidal activity of chopped fresh leaves as soil amendments such as; *Metricaria chamomilla, Olea europaea*, *Citrus sinensis, Punica granatum*, *Musa paradisiaca, Datura metal, Eucalyptus globulus, Ricinus communis* and *Ficus benjamina* under organic farm conditions was studied. The chopped fresh leaves of the tested plants were incorporated into the soil at the rate of 100 g/plant. *Eucalyptus globulus* gave the highest reduction of *Meloidogyne* spp. juveniles in soil as well as galls, females and egg-masses in roots since their values averaged 94.54%, 95.04%, 96.38% and 98.00%, respectively, followed by *Metricaria chamomilla* after 45 days post treatment. *Eucalyptus globulus* still gave the most reduction and diminishing nematode numbers after 90 days. *Olea europaea* and *Punica granatum* ranked statistically in the second and third categories, respectively in number of soil juveniles. All treatments significantly improved infected cucumber growth criteria.

Keywords: *Meloidogyne* spp., *Cucumis sativus*, chopped fresh leaves, *Eucalyptus globulus*, soil amendments

Nematodes are among major groups of plant pathogens, and their effective control is essential for profitable production of certain crops (Halbrendt and La Mondia, 2004). Nematicides provide efficient control measure, but their use is very restricted and has been declined drastically during last few years, especially due to high toxicity to mammalians, long

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persistence in the ecosystem, and high costs (Akhtar and Malik, 2000 and Ferraz and Freitas, 2004). Because several major nematicides have been withdrawn from the market, there is an intensive search for alternative and sustainable control measures, especially those based on the use of soil amendments with organic matter (Akhtar and Malik, 2000; Nico et al., 2004 and Cesarano et al., 2017).

The practice of adding organic matter to soil to increase yield is as old as the agriculture itself (Akhtar and Alam, 1993) and has been successfully explored to control some plant parasitic nematodes (Ferraz and Freitas, 2004). The mechanisms of nematode population reduction by soil amendments with organic matter involve stimulation of antagonistic microorganisms, liberation of secondary volatile or not-volatile phytochemicals with nematicidal properties. Besides, the amendments improve the growth of the plants and hence increase the tolerance and plant resistance to nematodes (Chavarría-Carvajal and Rodríguez-Kábana, 1998).

Cucumber (*Cucumis sativus* L.) is a good source of vitamins, minerals, fiber and roughage (Mukhtar et al., 2013), which is one of the reasons why it is grown all over the world. However, this popular vegetable is threatened by tremendous yield losses from *Meloidogyne incognita*, a nematode that attacks cucumber roots, inducing giant cell formation (Wehner et al., 1991; Walters et al., 1993 and Mukhtar et al., 2013). A clearer understanding of how *M. incognita* affects cucumber roots, the effects of infection and the associated changes in host genes expression would be of considerable value in developing strategies to prevent such attacks; however, to date such research has been limited.

Several attempts have been made by various workers for the control of plant parasitic nematodes by organic amendments (Ahmad et al., 2010; Hussain et al., 2011; Abolusoro and Abolusoro, 2012; Ahmad et al., 2013; Kimenju et al., 2014 and Zhang et al., 2019)

Therefore, the present work aims to study the nematicidal potential of chopped fresh leaves of nine plant species in reducing population density of *Meloidogyne incognita* under organic farm conditions.

MATERIALS AND METHODS

Greenhouse experiment was conducted to study the influence of chopped fresh leaves or peels used as soil amendments including, *Metricaria chamomilla* L., *Olea europaea* L., *Citrus sinensis* (L.) Osbec, *Punica granatum* L., *Musa paradisiaca* L., *Datura metal, Eucalyptus globulus* Labill., *Ricinus communis* L. and *Ficus benjamina* L. on galling and reproduction of *Meloidogyne incognita* infected cucumber (*Cucumis sativus*

L.) cv. Shabah roots. A naturally infested soil with root knot nematode greenhouse (60 m x 12 m) was selected in organic farm (SEKEM) for this study and cultivated with one-month old cucumber seedlings in silty clay soil with rows of 8 m long and the distance was 50 cm between the seedlings. Soil amendments were applied at rate of 100 g around each plant as mixed application with the upper soil. There were three replicates for each treatment and an equal number of non-treated replicates to serve as control.

All treatments were distributed in a randomized complete block design. Soil and root samples at a depth of 15-30 cm were taken after 45 and 90 days from adding soil amendments

Soil subsamples collected from each plant were carefully mixed and an aliquot of 200 g were processed for nematode extraction by sieving and Baermann–pan technique (Southey, 1970). Counts of second stage juveniles (J2) in soil were determined by means of Hawksley counting slide and stereoscopic microscope according to Siddiqui (1986). Root samples of one g were washed with gentle flow of tap and dried by pressing lightly between plotting papers. Then roots were cut into small pieces, mixed quiet and a given weight was processed for staining as described by Byrd et al. (1981) Also, average numbers of eggs/egg-mass were determined by hand picking four randomized selected egg-masses per each replicate. Egg-masses were shaken in 0.5% sodium hypochlorite for 2 min to release eggs from gelatinous matrix (Hussey and Barker, 1973). The released eggs were suspended in water and counted under stereoscopic microscope. The reduction percentage in gall formation, egg-mass production and juveniles number in soil were calculated in relative to the corresponding values of the check treatments, according to the following formula:

R% = (Control-Infected)/ Control) ×100

The final population and nematode build-up were calculated for all treatments.

Final population (FP) including number of juveniles in soil + egg-masses + females.

The rate of nematode increase (FP/IP) was detected by dividing the nematode final population by the nematode initial population.

Plant growth response based on values of plant growth response as indicated by shoot length and fruit number were determined and calculated for all treatments.

Statistical analysis was carried out according to the procedure "ANOVA" as recorded by Snedecor and Cochran (1980). Means of treatments were compared by Duncan's Multiple Range Test at 5% level of probability. These steps were accomplished using SPSS Program version 16.

RESULTS

Through a greenhouse experiment, eight chopped fresh leaves of *Eucalyptus globulus, Metricaria chamomilla*, *Olea europaea*, *Ricinus communis, Datura metal, Punica granatum*, *Ficus benjamina and Musa paradisiaca* besides peels of *Citrus sinensis* at dose of 100 g/plant were used as soil amendments. These soil amendments were mixed with soil to determine their suppressing effect on *Meloidogyne* spp. infecting cucumber, *Cucumis sativus*. Data in table (1) show that all treatments significantly affected *Meloidogyne* spp. and could arrest its reproduction rate compared with check treatment after 45 days, based on reduction percentage of juveniles number, galls, females, egg-masses and eggs per egg-mass. The final population and nematode buildup were diminished that occurred with all treatments. So, a successfully gradual scale for the effectiveness of used plants can be done as follows, *Eucalyptus globulus* was the most effective plant followed by *Metricaria chamomilla* > *Olea europaea* > *Citrus sinensis* > *Ricinus communis* > *Datura metal* > *Punica granatum* > *Ficus benjamina* > *Musa paradisiaca.* This scale was related with the final population reduction and nematode build-up when compared with untreated check. The highest percentage of final population reduction was achieved by *Eucalyptus globulus,* followed by *Olea europaea* since they were recorded 94.75 and 94.55%. While the lowest reduction in the final population was obtained by Ficus *benjamina* (71.01%). Also, the calculated rates of buildup revealed the same trend with *Eucalyptus globulus*. Treatment the nematode folded 0.83 vs. 24.21 folds in check treatments (Table 1). Data on response of *Meloidogyne incognita* infected cucumber plants to fresh chopped plant leaves as soil amendments 90 days after treatments are tabulated in table (2). *Eucalyptus globulus, Olea europaea* and *Punica granatum* were the most effective treatments, which caused significant decrement in juveniles number present in the rhizosphere (94.54%, 94.54% and 92.71%, respectively). The least reduction percentage in juvenile's number was presented in *Musa paradisiacal* (70.90%) as well as *Ficus benjamina* (70.90%) and *Ricinus communis* (74.55%). A significant reduction in the galls number per gram plant was observed in *Eucalyptus globulus* (95.04%), followed by *Punica granatum* (94.05%), *Olea europaea* (92.57%) and *Musa paradisiacal* (91.08). While *Ricinus communis* (68.81%), *Ficus benjamina* (71.78%) and *Metricaria chamomilla* as well as *Datura metal* recorded 75.24%. Thus, counts of egg-masses /g of most treatments were significantly reduced than that of the check. Higher percentage reductions in egg-masses production were recorded when using *Eucalyptus globules* (98.00%), *Punica granatum* (97%), followed by *Olea europaea* (95.00%). The lower % reductions in egg

Table (1). Effect of certain fresh chopped leaves and curl peel as soil amendments on *Meloidogyne incognita* infected cucumber (*Cucumis sativus*) roots in SEKEM organic farm after 45 days.

Soil amendments	Nematodes number before treatment			Nematodes number after treatment												
	In soil /200 g	Galls/ g	Females	In soil/ 200 g	% R	Galls/ g	% R	Females	% R	Egg masses/g	% R	Eggs/ egg mass	% R	F.P	% R	RB
Musa paradisiaca	-	178	178	1000b	64.28	35b	80.76	39bc	84.70	24b	61.29	292bc	36.65	1063	67.77	5.97
Metricaria chamomilla	-	184	202	400d	85.71	11e	93.99	12e	95.29	7.33de	88.17	248bc	46.20	419	87.92	2.07
Datura metal	-	144	144	800b	71.42	37b	79.67	44b	82.74	14c	77.41	297bc	35.57	858	73.99	5.95
Punica granatum	-	236	236	900b	67.85	12de	93.40	14e	94.50	11cd	82.25	258bc	44.03	925	71.96	3.91
Ficus benjamina	-	158	163	867bc	69.03	30bc	83.51	14e	94.50	12cd	80.64	234c	49.24	921	72.08	5.82
Ricinus communis	-	204	224	733bc	73.82	28bc	84.61	30cd	88.23	10cd	83.87	287bc	37.74	773	76.56	3.45
Citrus sinensis	-	158	158	733bc	73.82	22cd	87.91	24d	90.58	8cde	87.09	322b	30.15	765	76.81	4.78
Olea europaea	-	214	235	600cd	78.57	22cd	87.91	24d	90.58	7.67de	87.62	283bc	38.61	632	80.84	2.68
Eucalyptus globulus	-	253	253	333d	88.10	7e	96.15	7e	97.25	3e	95.16	279bc	39.47	343	89.60	1.38
Check	-------	152	167	2800a	-------	182a	-------	255a	-------	62a	-------	461a	---	3299	-------	18.66

*In each colum, means followed by the same letter are not differ significantly at $p \leq 0.05$ according to Duncan's multiple range test
*R= Reduction = (Control-Infected)/ Control) ×100
* FP = Final population included number of juveniles in soil + egg- masses + females.
*RB =Rate of buildup (PF/PI) = Final population PF/ Initial population PI (Norton, 1978).

Table (3). Plant growth parameters (length and fruits number) of cucumber (*Cucumis sativus*) affected by *Meliodogyne* spp. on treated by fresh chopped leaves and orange curl peel as soil amendments in SEKEM organic farm.

Parameters	Plants / Days	*Musa paradisiaca*	*Metricaria chamomilla*	*Datura metal*	*Punica granatum*	*Ficus benjamina*	*Ricinus communis*	*Citrus sinensis*	*Olea europaea*	*Eucalyptus globulus*	Control
Length	7	41.66	45.00	50.00	45.00	50.00	51.67	46.67	41.67	48.33	46.66
Fruit		18.00	24.00	24.00	1800	24.00	18.00	24.00	18.00	18.00	6.00
Length	15	63.33	65.00	63.33	68.30	63.33	75.00	69.33	55.00	68.33	60.00
Fruit		18.00	18.00	18.00	24.00	30.00	24.00	18.00	20.00	18.00	12.00
Length	30	86.33	88.33	96.66	80.00	96.66	102.66	80.00	71.67	93.33	86.66
Fruit		24.00	24.00	24.00	18.00	24.00	24.00	18.00	18.00	24.00	18.00
Length	45	131.67	128.33	126.67	131.67	138.33	135.00	118.33	93.33	130.00	110.00
Fruit		24.00	24.00	24.00	18.00	36.00	24.00	20.00	18.00	36.00	18.00
Length	60	148.33	148.33	140.00	145.00	155.00	150.00	141.66	112.67	145.00	123.33
Fruit		24.00	24.00	24.00	2400	24.00	24.00	24.00	24.00	20.00	12.00
Length	75	175.00	185.00	176.00	183.33	179.33	175.00	175.00	151.67	181.66	141.66
Fruit		24.00	36.00	24.00	24.00	24.00	24.00	18.00	24.00	20.00	12.00
Length	90	211.00	210.00	203.33	206.66	196.66	191.66	188.33	175.00	196.66	173.00
Fruit		24.00	30.00	24.00	2400	30.00	30.00	18.00	24.00	20.00	6.00
Mean Plant length		122.62 ±12.85a	124.28 ±12.80a	122.29 ±11.83a	122.86 ±12.72a	125.86 ±11.86a	125.71 ±10.87a	116.43 ±11.22a	99.76 ±10.33a	122.62 ±11.91a	105.95 ±9.47a
Mean Fruit number		22.86 ±0.18a	26.28 ±0.21b	23.40 ±0.12a	23.40 ±0.15a	26.28 ±0.20b	24.84 ±0.19b	25.44 ±0.29b	20.58 ±0.19a	26.58 ±0.27b	12 ±0.15a

*In each column, means followed by the same letter are not differ significantly at $p \leq 0.05$ according to Duncan's multiple range test

-masses production was recorded when used *Ficus benjamina* (65%), followed by *Ricinus communis* (77%) and *Citrus sinensis* (83%). Inhibition of egg production was more pronounced in the treatment with *Ricinus communis* (67.22%), followed by *Eucalyptus globulus* (66.74%). Considerable values of the nematode final population and rates of buildup were detected by all treatments. The higher final population reduction percentage was achieved by *Eucalyptus globulus,* followed by *Olea europaea* and recording 94.75% and 94.55%, respectively. The lowest reduction final population was obtained by *Ficus benjamina* (71.01%). Also, the calculated rates of buildup revealed the same trend in *Eucalyptus globules*. Treatment of the nematode folded 0.83 vs. 24.21 folds in check (Table 2).

Improvement of plant growth parameters in terms of shoot length and fruit number was variable and proportional with the tested chopped leaves of *Eucalyptus globulus, Metricaria chamomilla*, *Olea europaea*, *Ricinus communis, Datura metal, Punica granatum*, *Ficus benjamina and Musa paradisiaca* as well as peels of *Citrus sinensis* applied at dose of 100 g/plant as soil amendment (Table 3). It is interesting to notice that, plant growth parameters were highly responded to materials application, in spite of nematode infection. Although, all materials increased shoot length of treated plants, differences among values of such parameter were mostly insignificant. *Musa paradisiaca* and *Metricaria chamomilla* gave the best result in length of shoots of 211 and 210 cm, respectively compared to check. On other hand, *Olea europaea* and *Citrus sinensis* curl peels recorded the least results of 175 and 188.33 cm in length of shoots. While, *Metricaria chamomilla* and *Eucalyptus globulus* gave the best results in fruits number.

DISCUSSION

The applications of organic amendments into soil led to build-up of micro-organisms, including natural enemies of nematodes. Such organic matters are known to have compounds of nematicidal effect released during its decomposition in the soil and is directly responsible for suppression of nematode population. The presence of high concentration of low molecular weight of acetic, propionic, butyric, isovaleric and valeric fatty acids as well as responsible fraction of phenols and hydrogen sulfide in the organic manure, i.e. poultry and pigeon droppings clearly proved to be highly nematotoxic (Badra et al., 1979), however ammonia, nitrites, phenols, hydrogen sulfide and wide range of volatile fatty acids and organic acids are among the chemical produced during the decomposition of plant residues and affected nematode reproduction. The high oil content of *Eucalyptus*

Table (3). Plant growth parameters (length and fruits number) of cucumber (*Cucumis sativus*) affected by *Meliodogyne* spp. on treated by fresh chopped leaves and orange curl peel as soil amendments in SEKEM organic farm.

Parameters	Plants / Days	*Musa paradisiaca*	*Metricaria chamomilla*	*Datura metal*	*Punica granatum*	*Ficus benjamina*	*Ricinus communis*	*Citrus sinensis*	*Olea europaea*	*Eucalyptus globulus*	Control
Length	7	41.66	45.00	50.00	45.00	50.00	51.67	46.67	41.67	48.33	46.66
Fruit		18.00	24.00	24.00	1800	24.00	18.00	24.00	18.00	18.00	6.00
Length	15	63.33	65.00	63.33	68.30	63.33	75.00	69.33	55.00	68.33	60.00
Fruit		18.00	18.00	18.00	24.00	30.00	24.00	18.00	20.00	18.00	12.00
Length	30	86.33	88.33	96.66	80.00	96.66	102.66	80.00	71.67	93.33	86.66
Fruit		24.00	24.00	24.00	18.00	24.00	24.00	18.00	18.00	24.00	18.00
Length	45	131.67	128.33	126.67	131.67	138.33	135.00	118.33	93.33	130.00	110.00
Fruit		24.00	24.00	24.00	18.00	36.00	24.00	20.00	18.00	36.00	18.00
Length	60	148.33	148.33	140.00	145.00	155.00	150.00	141.66	112.67	145.00	123.33
Fruit		24.00	24.00	24.00	2400	24.00	24.00	24.00	24.00	20.00	12.00
Length	75	175.00	185.00	176.00	183.33	179.33	175.00	175.00	151.67	181.66	141.66
Fruit		24.00	36.00	24.00	24.00	24.00	24.00	18.00	24.00	20.00	12.00
Length	90	211.00	210.00	203.33	206.66	196.66	191.66	188.33	175.00	196.66	173.00
Fruit		24.00	30.00	24.00	2400	30.00	30.00	18.00	24.00	20.00	6.00
Mean Plant length		122.62 ±12.85[a]	124.28 ±12.80[a]	122.29 ±11.83[a]	122.86 ±12.72[a]	125.86 ±11.86[a]	125.71 ±10.87[a]	116.43 ±11.22[a]	99.76 ±10.33[a]	122.62 ±11.91[a]	105.95 ±9.47[a]
Mean Fruit number		22.86 ±0.18[a]	26.28 ±0.21[b]	23.40 ±0.12[a]	23.40 ±0.15[a]	26.28 ±0.20[b]	24.84 ±0.19[b]	25.44 ±0.29[b]	20.58 ±0.19[a]	26.58 ±0.27[b]	12 ±0.15[a]

*In each column, means followed by the same letter are not differ significantly at $p \leq 0.05$ according to Duncan's multiple range test

globulus green leaves and *Olea europaea* may interpret its efficacy in reducing nematode reproduction (Khan et al., 1974; Mian and Rodreguez-Kabana, 1982 and Stirling, 1991). In addition, the use of the proper organic materials to improve soil chemical, physical, and biological parameters which consequently organic matter the activities of major and minor plant-parasitic nematodes in the production system. The functions and interactions of soil organic matter are complex. Some of the benefits of organic matter include the improvement of soil structure, erosion control, water relations, availability of plant nutrients, ion exchange, chelation, buffering capacity, energy for soil organisms, and suppression of plant pathogens (Hodges, 1991). Soil density affects not only root growth but also the penetration of water as well as air permeability. The addition of organic matter significantly changes soil density (Mays et al., 1973; Turner et al., 1994 and Widmer et al., 1997) by the formation and stabilization of soil aggregates (Biswas and Khosla, 1971; Pagliai et al., 1981 and Rose, 1991), which increases soil pore size (Gallardo-Lara and Nogales, 1987), that improving soil structure. Soil structure is of paramount importance in soil productivity and can become the limiting factor of crop yield (Epstein et al., 1976; Mays et al., 1973 and Rose, 1991), where the addition of organic matter can increase available water and so decrease irrigation requirements. So, moisture-holding capacity increases. Different sources of organic matter affect the chemical properties of soils through different ways. One important change resulting from the addition of organic matter is a potential increase in available nutrients, including an increase in organic carbon (Guidi et al., 1983) potassium, calcium, and magnesium (Bengston and Cornette, 1973; Mays et al., 1973 and Duggan and Wiles, 1976). Organic matter also can change the level of available nitrogen in the soil. Nitrogen availability depends on the C/N ratio of the organic source.

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إستخدام بعض الإضافات العضوية كوسيلة لمكافحة نيماتودا تعقد الجذور التي تصيب نبات الخيار تحت ظروف الزراعة العضوية

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في هذه الدراسة تم مكافحة نيماتودا تعقد الجذور على نبات الخيار بإستخدام التأثير الإبادي لمجموعة من الأوراق الطازجة المقطعة والنباتات المستخدمة هي الكاموميل، الزيتون، الرمان، الموز، الداتورة، الخروع، فيكس بينجامينا بالإضافة إلى قشر البرتقال البلدي تحت ظروف الزراعة العضوية في مزرعة سيكم. وتم إستخدام هذه الأوراق بمعدل ١٠٠ جرام/ نبات. وأعطي أوراق نبات الكافور أعلى نسبة في تقليل أعداد اليرقات في التربة، العقد الجذرية لنبات الخيار، عدد الإناث، كتل البيض والتعداد النهائي للنيماتودا على التوالي. يليها أوراق نبات الكاموميل وذلك بعد مرور ٤٥ يوم. واستمرت أوراق نبات الكافورفي إعطاء أعلى نسبة خفض لتعداد النيماتودا بعد مرور ٩٠ يوم، يليها أوراق الزيتون ثم أوراق الرمان في المرتبة الثالثة إحصائيًا بالنسبة للتعداد النهائي للنيماتودا. كما أدت كل المعاملات إلى زيادة معنوية في معدلات نمو نباتات الخيار المصابة بالمقارنة بتلك المصابة وغير المعاملة.