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Influence of Seeds Mill Extracts as Biopesticides on *Meloidogyne incognita* and Banana Growth

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The effects of methanol extracts of some seeds mill, black seed (*Nigella sativa*), Jojoba (*Simmondsia chinensis*), Olive (*Olea europaea*) alone or in combination with alga, *Chlorella vulgaris* were tested as bio-nematicidal against root-knot nematode (RKN), *Meloidogyne incognita* on banana under greenhouse conditions. The total number of galls, females and egg-masses per root, also nematode soil was significantly reduced compared to untreated nematode control. Results indicated that treatment consisted of *C. vulgaris* + *O. europaea* was the most incorrodible curative for depressing the J2s numbers in soil (51.82%), than the chemical control, oxamyl (65.58%R). The phytochemical analysis of seeds mill (SMEs) and alga approved that, *O. europaea* had the greatest content of phenolic compound (332.14 µg gallic acid/g dry wt). This may elucidate the highly potent of the treatment, *O. europaea* alone or with *C. vulgaris* to control RKNs infestations. The maximum percentage of antioxidant activity (96.41%) was present in *N. sativa*. Furthermore, the greatest enhancement of growth rate was generated using alga, *C. vulgaris* compared with other treatments as well as untreated inoculated plants.

Keywords: Seeds mill, *M. incognita*, Banana, Phytochemical analysis, Phenolic compounds, Antioxidant.

Plant-pathogenic nematodes, (PPNs) are responsible for yield loss in economically crops, especially in tropical and subtropical regions where environmental factors favor their survival and dispersal (Sikora and Fernandez, 2005). Crops protection technology which contains natural products, help to safely control numerous PPNs that affect banana plantain production (Gowen *et al.*, 2005 and Queneherve, 2008). Root-knot nematodes, (RKNs) *Meloidogyne* spp., are the most important, damaging crop production (Koenning *et al.*, 1999) and limiting agricultural productivity and quality (Sasser and Carter, 1985). However, chemical control, especially artificial nematicides have been related with harmful effects on human health, animal, air, ground, and water. In addition, the use of a synthetic chemical nematicides can result in high cost as well as easy to implement (Ferris *et al.*, 1992; Araya and Caswell-Chen, 1994; Abawi and Widmer, 2000 and Chabrier *et al.*, 2004). Hence, there are reasons to develop alternatives strategies to conventional pesticides, alternatives that are of low cost and environmentally friendly; a characteristic that will improve durability of agriculture (Martin, 2003; Javed *et al.*, 2008 and El-Abbassi *et al.*, 2017). Newly, researches has been an increasing attention in phyto-pathogens strategies for pest control with natural substances exerted by many plants, (namely allelopathic compounds), and when they were incorporated into soils, it resulted in exceptional pest inhibition (Ali, 2004; Khan *et al.*, 2016 and Saraf *et al.*, 2014). For instance, many studies aimed at depressing banana-parasitic nematodes using natural plant products have been carried out in many countries (Ioannina *et al.*, 2004; Gahukar, 2012 and Zhao *et al.*, 2017). In addition, some species of algae *i.e.*, *Ulva lactuca*, *Jania rubens*, *Laurencia obtuse* and *Sargassum vulgare* were investigated to control PPN, *Meloidogyne* spp. in banana plantains (El-Ansary and Hamouda, 2014). Algae have been studied as a forceful bio-pesticidal agents (Craigie, 2011), and as a bio-fertilizer for enhancing growth in the crops (Khan *et al.*, 2005 and El-Ansary *et al.*, 2017), because of high content in algae of some micro and macro-nutrient content (Faheed and El-Fattah, 2008). For example, the alga, *Chlorella vulgaris* as a powerful protector have potent activating affect the plant girth (Bilevia, 2013 and Hamouda and El-Ansary, 2017).

The present study was carried out to test the efficacy of three seed mills extracts (SMEs): black seed (*Nigella sativa*), jojoba (*Simmondsia chinensis*) and olive (*Olea europaea*) alone or in combination with a marine alga, *C. vulgaris* on *M. incognita* and banana growth.

Materials and Methods

Pure culture of root-knot nematode:

Eggs of *Meloidogyne incognita* were extracted from tomato (*Lycopersicon esculentum* cv. Castle Rock) roots using sodium hypochlorite solution (Hussey and Barker, 1973). Second stage juveniles (J2s) were collected daily from hatched eggs and were stored at 15°C. The J2s used in the experiments were less than 5 days old.

Experimental design and treatments:

Two months old banana plants cv. Grande-Naine were obtained from the Tissue Culture Lab of the Genetic Engineering and Biotechnology Research Institute (GEBRI). Plants were cultivated in 30 cm diameter pots containing about 4.5 kg of sterilized soil (clay: sand, 1:3) with pH 7. The temperature of the soil was about 28 ± 2 °C for most of the experiment time. Thirty-six pots were inoculated with 3,000 J2s per pot at the planting time. Seven days later, 12 pots were treated with 0.5 g drying methanol extracts of each seeds mill, Black seed (*Nigella sativa*), Jojoba (*Simmondsia chinensis*) and Olive (*Olea europaea*). Also, 12 pots were treated with previous treatments and sprayed on leaves with 50 ml of the green alga, *Chlorella vulgaris* (each ml contains 104 cells). Each treatment was replicated 4 times. Four inoculated pots were sprayed only with 50 ml of *C. vulgaris*. Another 4 inoculated pots were treated with 1 ml of oxamyl (Vydate 24% L). The remaining four inoculated pots served as inoculated and untreated control (nematode check). Four more pots served as untreated and non-inoculated check. The tested materials were added to the soil in 3 holes around the plant and followed by the addition of 50 ml of water. The pots were arranged in a completely randomized design in a greenhouse of the (GEBRI). Plants were evaluated after 45 days of inoculation. The roots were washed carefully by tap water to remove the soil and stained with Phloxine B "3.5 g in 750 ml distilled water + 250 ml acetic acid 5%" solution for 5 min to facilitate counting of all nematode stages in the root under light binuclear. Number of galls, females and egg-masses were counted. Also, juveniles per 250 g soil were extracted according to Cobb's sieving and decanting method by using sieves (60 meshes and 325 meshes). Plant growth parameters, shoot length (cm), shoot weight (g), root length (cm), root weight (g), and corm weight (g) were also recorded.

Determination of total phenolic content (TPC) and antioxidant activity (AOA) of different seeds mill extracts (SMEs) and alga:

The total phenolic content (TPC) of all the tested materials was determined by the Folin Ciocalteu method (Singleton and Rossi, 1965) using spectrophotometer (UV-200-RSLW scientific). The absorbance was measured at 750 nm and compared to gallic acid calibration curve. The TPC was calculated by comparing the absorbance with the gallic acid calibration curve according to the formula:

TPC (µg /g) = C x V / g,

Where:

C = concentration of the gallic acids equivalent from standard curve (µg /ml)

V = volume of the extract used (ml)

g = weight of extract (g)

The contents were expressed as gallic acids (µg GAE/g dry wt).

The free radical scavenging activity was estimated by 1,12-picryl-diphenyl-hydrazyl (DPPH) assay (Ghasemzadeh *et al*., 2010) to calculate percentage of AOA.

Statistical analysis:

All data were subjected to analysis of variance (ANOVA) (Sokal and Rohlf, 1995). Significance of the variable mean differences was determined using Duncan's multiple range tests ($p \leq 0.05$). All analyses were carried out using SPSS 16 software.

Results

Effects of seeds mill extracts (SMEs) on root-knot nematode, RKN (Meloidogyne incognita) reproductivity:

The nematicidal actions of the (SMEs) like, Black seed (*Nigella sativa*), Jojoba (*Simmondsia chinensis*), Olive (*Olea europaea*) alone or in combination with alga, *Chlorella vulgaris* showed significant reduction in nematode criteria when compared to nematode check but no significant differences were achieved within treatments (Table, 1). Number of galls, females, egg-masses in root and Juveniles-2 (J2s) number in soil were significantly ($P \leq 0.05$) inhibited than untreated nematode check. Results stipulated that *C. vulgaris* + *O. europaea* treatment was the most incorrodible curative for decreasing the J2s numbers in soil (51.82% reduction, R), compared to the nematicides check, oxamyl (65.58%R). On the contrary, the treatment *C. vulgaris* was the lowest forceful remedy for inhibiting of J2s, being 39.45%R (Table 1).

Estimation of total phenolic content (TPC) and antioxidant activity (AOA) of the different seeds mill extracts (SMEs):

TPC and AOA were estimated in the different seeds extracts of *N. sativa*, *S. chinensis*, *O. europaea* and alga, *C. vulgaris* (Figs.1&2). The phytochemical analysis of all the treatments indicated that *O. europaea* had the greatest compound of TPC (332.14 µg gallic acid/g dry wt). Such properties may explain the greater potential of *O. europaea* for reducing the population of RKN, *M. incognita*.

The maximum percentage of AOA (96.41%) was present in *N. sativa*. Unlike, the treatment *C. vulgaris* showed the lowest percentage of AOA, being 63.29% (Fig. 2).

Table 1. Effect of some seeds mill extracts and alga on root knot-nematode, *M. incognita* reproductivity.

Treatment	Number per root						Number per 250g soil	
	Galls	%R	Females	%R	Females with egg-masses	%R	Juveniles 2	%R
N. sativa	203.37b	9.35	214.57b	32.34	214.57b	19.57	2197b	42.58
S.chinensis	194.35b	20.07	218.93b	29.22	218.93b	23.03	2166.75b	43.37
Olea europaea	179.4b	20.05	226.71b	27.5	226.71b	22.11	2028.25b	46.99
Ch. vulgaris	205.89c	8.24	237.22b	24.14	237.22b	29.78	2316.5b	39.45
C. vulgaris + N. sativa	183.81b	18.07	222.11b	28.97	222.11b	28.55	2011.25b	47.41
C. vulgaris + S. chinensis	191.52b	14.64	223.97b	28.43	223.97b	25.17	2000b	47.73
C. vulgaris + O. europaea	173.21b	22.8	205.69b	33.16	205.69b	33.45	1843.5b	1.82
Chemical check, Oxamyl	128.81a	42.59	175.06a	44.01	175.06a	47.19	1316.75a	65.58
Nematode check	224.37d	0	312.69c	0	312.69c	0	3826c	0

Means followed by the same letter (s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.
% R= percent reduction compared with nematode check

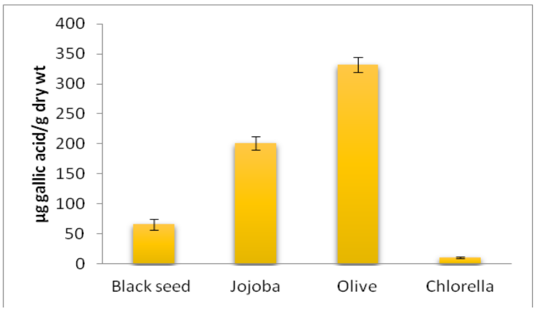

Fig. 1. Total phenol content of different seeds mill extracts and alga.

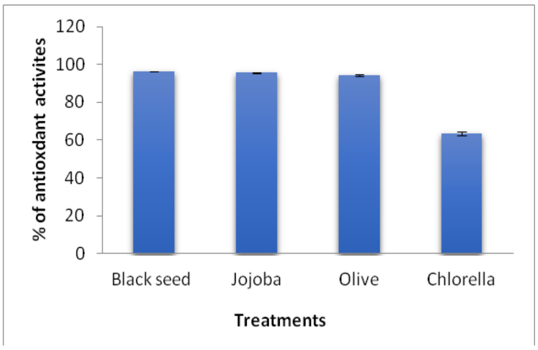

Fig. 2. Antioxidant activity of the tested treatment

Enhancement of plant health:

Most of various (SMEs) and alga improved significantly (P≤0.05) plant health and this improvement was greater on all of the banana plant parameters compared to nematode control (Table 2).

Table 2. Effect of seeds mill extracts alone or in combination with alga, *C. vulgaris* on banana growth parameters.

Treatment	Shoot		Root		Corm
	length (cm)	weight (g)	length(cm)	weight(g)	weight(g)
N. sativa	49.75b	73.23b	67.25c	51.27d	28.75bc
S. chinensis	50.25b	83.29b	55bc	44.15cd	23.65bc
Olea europaea	40a	39.04a	40.75b	29.59b	19.63a
Ch. vulgaris	53.5b	110.48c	46.75b	67.32f	32.24c
C. vulgaris+N. sativa	50b	69.33b	57.25bc	43.66cd	19.85a
C. vulgari +S. chinensis	37.75a	43.04a	49.25b	37.62bc	19.15a
C. vulgaris O. europaea	36.5a	40.91a	43b	30.81bc	17.31a
Chemical check, Oxamyl	36.25a	36.71a	40.25b	26.46b	18.21a
Nematode check	35.5a	46.35a	18.5a	13.65a	19.41a
Check	41.25a	46.35a	52.5bc	31.1bc	22.65bc

Means followed by the same letter (s) within a column are not significantly different (p ≤0.05) according to Duncan's multiple range test.

The greatest enhancement of banana growth rate was generated using alga, *C. vulgaris* in comparison with all the treatments as well as nematode check. Overall, the highest effect of shoot weight of all the plants was extremely higher than nematode control. Yet lesser effects on most banana growth were obtained by incorporating the oxamyl treatment.

Discussion

Many compounds present in seeds mill wastes could celebrate a favorable selection for the control of many pests (El-Abbassi *et al*., 2017). In addition, algae have been studied as potential pesticidal agents for pest control (Craigie, 2011). In vivo study of the different seeds mill extracts (SMEs), *N. sativa*, *S. chinensis* and *O. europaea* alone or in combination with alga, *C. vulgaris* confirmed its useful effectiveness for RKNs control. Overall, counts of galls, females and egg-masses in the roots and juveniles-2 (J2s) in the soil were greatly reduced compared with the nematode control. For instance, the best effective treatment in depressing J2s in soil was *C. vulgaris* + *O. europaea* treatment (51.82% R) than oxamyl (65.58% R). All treatments of SMEs became more effective to RKNs control in banana plants because of their highly protection after addition and press by their combination with the algal suspension. Many studies aimed at inhibiting PPNs using compounds from plant extracts have been investigated in many countries (Gahukar 2012 and Zhao *et al*., 2017). Sustainable mechanisms involved in plant-nematode inhibition are production of some nematicidal compounds, like ammonia and fatty acids, during biodegradation. In addition to the current mechanisms for nematode reduction, that some plant extracts have the potential of toxic compounds like, thienyls, alkaloids, phenols, sesquiterpens, diterpens and polyacetylene for the control of nematodes (Pavaraj *et al*., 2012 and Pretali *et al*., 2016), or increase in PPN antagonists (McSorley, 2011 and Briar *et al*., 2016). These lead to enhance of antagonistic microorganisms and increase in plant resistance (Oka, 2010). Interestingly, the phytochemical analysis of all the SMEs and alga showed that *O. europaea* had the greatest content of phenolic compound (332.14 µg gallic acid/g dry wt). The maximum percentage of antioxidant activates (96.41%) was present in *N. sativa*. Memorable, phenolics and lignifications compounds were related with resistance of host plant toward a kind of many pests (Chitwood, 2002). Also, algae possess a wide range of mainly components like, alginic acids, alkaloids, and phenolics, these components have a biopesticidal activity against pests (Ara *et al*., 2002a, 2002b; Kosma *et al*., 2011; El-Ansary and Hamouda, 2014 and Hamouda and El-Ansary, 2017). Generally, all the tested treatments of SMEs alone or in combination with alga enhanced banana growth, this enhancement was noticed on shoots and corms than on roots. The highly effect on most growth parameters was given by alga, *C. vulgaris* than nematode check. Hamouda and El-Ansary (2017) reported that, *C. vulgaris* as a forceful protector against RKNs, have strong stimulating effect on the growth of banana plants, because of high concentration in algae of many minor and major-mineral elements (Faheed and El-Fattah, 2008). In contrast, the greenhouse study using two banana plantain varieties: "Essong" and "Big Ebanga" showed that all neem seeds extractions enhanced plant health significantly than control (Kosma *et* *al*., 2011), because of the neem leaves have a high content of nitrogen (as a good bio-fertilizer) to increase plant growth (Gajalakshmi and Abbasi, 2004).

Our study provided evidence that all treatments of SMEs each alone or in combination with alga may have favourable and eco-friendly nematicides for depressing RKNs, M. incognita in banana plants as an alternative chemical pesticide.

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تـــأثيـــر مستخلصات مطحـــون البذور واستخدامهـــا كمبيدات طبيعية على *Meloidogyne incognita* ونمو الموز

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في هذا البحث تم استخدام مستخلصات الميثانول لمطحون بذور، الحبة السوداء والجوجوبا والزيتون، منفردة أو مخلوطة مع الطحلب *Chlorella vulgaris* مقارنة بالمبيد النيماتودي الأكساميل "الفايديت" ضد نيماتودا تعقد الجذور من نوع *M. incognita* على نبات الموز تحت ظروف الصوبة. و قد أظهرت النتائج أن العدد الكلى لكل من العقد والاناث وأكياس البيض على الجذر و كذلك أعداد اليرقات فى ٢٥٠ جم تربة تم خفضها معنويا مقارنة بالكنترول (النباتات المصابة غير المعاملة). أثبتت التحليلات الكيميائية لكل نوع من أنواع البذور الثلاثة والطحلب، أن محتوى مستخلص مطحون بذور الزيتون هو الأعلى فى كمية الفينولات الكلية (٣٣٢،١٤ ميكروجرام حامض الجاليك لكل جرام جاف وزن). وهو ما يوضح التاثير القوى والفعال للمعاملة السابقة منفردة أو مع الطحلب *Chlorella vulgaris* فى تقليل الإصابة بنيماتودا تعقد الجذور. كما سجل مستخلص مطحون بذور الحبة السوداء أعلى نسبة مئوية من مضادات الاكسدة النشطة (٩٦،٤١%). و من ناحية أخرى، فإن الزيادة الكبيرة فى معدل النمو يظهر باستخدام الطحلب *Chlorella vulgaris* مقارنة بالمعاملات الأخرى والكنترول النيماتودى. كما أدت المعاملات إلى تحسين القياسات الخضرية ونمو النباتات مقارنة بالمعاملة الكنترول (نباتات بدون عدوى و بدون معاملة).