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NEMATICIDAL ACTIVITY OF GARDEN CRESS BIO ACTIVE INGREDIENTS AGAINST ROOT KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) INFECTED TOMATO TRANSPLANTS

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Abstract

The aim of present study was to evaluate the different extracts of garden cress (*Lepidium sativum)* whole plants and seeds and the combination of top extracts as a biocontrol agents against root knot nematode (*Meloidogyne incognita*) infected tomato transplants in pots experiment. Extracts obtained using different organic solvents from the whole plant and seed of *L. sativum* on J_2 and eggs of *M. incognita* at concentrations of 500 and 1000 ppm. Based on mortality and hatching inhibition, the acetone extract from whole plant (AW) among all extracts gave the strongest activity, which showed 91% and 73% at 1000 ppm, respectively. The next best extracts were the ethyl acetate extract from whole plant (EAW), ethanol extract from seed (ES) and methanol extract from seed (MS). Mixtures of the best four extracts were also assessed for their nematicidal effects against J_2 and egg of *M. incognita*. The mixtures (ES+MS+AW), (ES+MS+AW+EAW) and (ES+MS+EAW) were the most effective treatments at J_2 mortality and egg hatch inhibition, causing over 92% and 81%, respectively. No significant differences among the three mixtures were observed. The strongest nematicidal effect was achieved by the ES + MS + AW mixture, followed by the ES + MS + AW + EAW mixture; with over 78%, 74%, 90% and 67% reduction in the number of galls, egg masses, eggs per root and eggs per egg mass, respectively.*L. sativum* extracts and their mixtures did not adversely affect the ability of tomato plant to flowering, nor did we find any significant differences between the number of fruits per plant or weight of fruits per plant for all extracts and their mixtures compared to control and this is because the experiment was for the period of the seedling and the beginning of production of flowers and fruits only.

Key words: Garden cress - bio active ingredients - Root knot nematode - Tomato transplants.

Introduction

Tomato (*Solanum lycopersicum)* is an important economical vegetable crop in Egypt. However, the crop is being challenged with several constrains that reduce its annual national production, including environmental and biotic stress factors. Root knot nematode (*Meloidogyne incognita*) is one of the most serious plant pests that causes yield reduction in tomato yield by 30-50% (Saravanpriya and Sivakumar, 2005).

Management of nematode problems by chemical control has traditionally been practiced through the direct application of nematicides to agricultural environment. For example, the deleterious effects of soil fumigation treatments that caused severe groundwater contamination have revealed the need for alternative chemical control methods. In the rhizosphere, soil desinfestation can generate additional negative effects on the populations of soil beneficial microbiota leading to the stunting and growth inhibition of many crops, furthermore, human health is negatively affected by residual toxicity (MacDonald and Reichmuth, 1996).

Chemical nematicides are promising solutions with nematode control system, however, their high costs and the hazards they posses made serious limitation on its utilization (Elbadri *et al.,* 2008). Sustainable and environmentally friendly ways to control soil borne plant pathogens is rated as a prospective alternative to commercial soil pesticides and that will be a big prosperity to reduce pollution. This has encouraged research to manage nematode by using some medicinal plant extracts. Different plant parts have been tested to identify the sources of nematicidal natural substances supporting sustainable agricultural practices. Several plants have more environmentally and toxicologically safe selective

that showing efficacious nematicidal potential (Dawar *et al.,* 2008). The use of essential oils has the potential to become an alternative control strategy against plant parasitic nematodes. The root-knot nematodes are the most destructive group of plant parasitic nematodes allover the world and their control is enormously challenging (Ozdemir and Gozel, 2018).

Garden cress (*Lepidium sativum*) is a secondary vegetable crop belongs to brassicaceae family, it is characterized with its medicinal importance which used as stomach worms killer. Garden cress has the ability to grow all over soil types under different climates and does not need special condition for cultivation. It is cultivated for its seeds and fresh leaves are mainly used in salad and have antibacterial, diuretic and stimulant properties. Definition and separation of active ingredients in those plants are needful for using natural methods.

The nematicidalefficiency of top green manure of *L. sativum*on the survival of nematode was tested on potato plants and found that Nematicidal isothiocyanates released after incorporating glucosinolate-containing brassica plants are fully biodegradable and less toxic than their synthetic equivalents, and their use is considered a safer alternative to soil fumigants such as methyl bromide (Fatemy, 2018).

The aim of this work was the evaluation of the extracts of garden cress from whole plants and grounded seeds as a bio-nematicidal controller against *Meloidogyne* root-knot nematode in infected tomato transplants.

Materials and Methods

Garden cress seeds were purchased from a local market in Egypt, seeds were identified at the Department of Vegetable Crops, Faculty of Agriculture, Cairo University.

Seeds of garden cress were planted in plots in September for two successive seasons 2015/2016 and 2016/2017 in the farm of the Department of Vegetable Crops, Faculty of Agriculture, Cairo University.

Plant material production

Three lines of 5m long and 50cm wide from the land were cultivated with the seeds of garden cress. All cultural practices were done whenever required. The plants were harvested at flowering time within a period of 70-80 days from cultivation. The whole flowered plants were dried at room temperature for 10 days and then were grainded after that with an electric mill to powder, also some of the purchased seeds were grainded to powder.

Extract preparation

Methanol (M), Ethanol (E), Acetone (A), Butanol (B) and Ethyl acetate (EA) were used for extraction and Fractionation of bioactive compounds from the whole plants powder (W) and seed powder (S) of garden cress.

Twenty grams of both whole plants powder or seeds powder were soaked separately in 200 ml of the different solvents at room temperature for 24 h and the samples were filtered after this period using filter papers. The filtrates obtained were dried in rotary evaporator to evaporate the solvents. The deposit of each solvent was used in our experiment by dissolving the respective dried extracts in distilled water contained 0.3% Tween 20 as an emulsifier material as a stock solution for each solvent (mostsolutions had anoily nature) which used to irrigate our tomato seedlings in the pots.

Nematode suspensions preparation

Eggs of *Meloidogyne incognita* were extracted from heavily galled tomato roots using 1.5% sodium hypochlorite (NaOCl) as described by Hussey and Barker (1973). Suspension of eggs was used directly or placed on a modified Baermann dish and incubated at 25 ± 2°C for 7-10 days to separate hatched J_2s (Hooper *et al.,* 2005). The hatched J_2s were collected daily and stored at 6^0C. The juveniles up to 4 days old were used in experiments. Next, the number of nematode J_2 was adjusted to 500 nematodes ml^{-1}.Nematode suspensions were prepared by appropriate dilution with distilled water and the numbers of nematodes were counted under a binocular microscope 40×.

In-vitro test

Well-plate bioassay was used to evaluate the nematicidal activity of all the fractions from the powder of whole plants and seeds of *L. sativum* against J_2s and eggs of *M. incognita*. One ml of nematode suspension containing approximately 400 J_2 or 1000 ± 50 eggs were placed in each well of a 24-microwell plate then one ml of the stock solution was added at concentrations of 1000 and 2000 μg ml^{-1}. Therefore, the final concentrations of the plant extract in J_2 or egg suspension were 500 and 1000 μg ml^{-1}. Control samples contained distilled water with 0.3% Tween 20. All treated and control plates were covered with a lid and stored at 25 ± 1°C. J_2 vitality was determined under a binocular microscope 40×. Nematodes were considered to be dead if they did not move when probed with a fine needle. At the end of the exposure time, J_2 were transferred into fresh distilled to discriminate between nonmotile and dead J_2. Percentages of J_2 mortality were calculated for each well after 48 h. The hatch inhibition effect of the extracts was based upon the number of unhatched eggs at 7 days post-treatment,

as observed under a microscope. Percentages of unhatched eggs were calculated for each well.

The best four extracts that resulted in mortality or hatch inhibition more than 80% of the nematode population were selected for a mixture treatment test and the analysis of GC-MS. This test was performed to determine whether mixing bioactive compounds of different extracts might result in a synergistic effect that is more extensive nematicidal potential than the effect caused by each extract individually. Each mixture treatment was made up either two or three or four extracts. These extracts were equally mixed (1:1, v:v) in the mixture and the final concentration after adding nematode suspension (J_2 or egg) was 1000 ppm. The four best extracts were used individually as positive controls while distilled water with Tween 20 was used as a negative control. Percentages of J_2 mortality and egg hatching inhibition were recorded after 2 and 7 days from incubation, respectively.

Pot test

Tomato (*S. lycopersicum*) seeds *var.* Peto 86 were purchased from local seeds market. For producing seedlings the seeds of tomato were grown in seedling trays. Four weeks old tomato seedlings were transplanted into 10 cm plastic pots containing 400g sterilized mixture of field-soil and sand (1:1, v:v). One week later, 1, 000 J_2 in 3 ml water were inoculated into four holes at 2 cm distance from the stem of each plant. After one day from nematode inoculation, the best four extracts or their mixtures (11 mixtures) were added as soil drenches around the plant base, with 30 ml solution at concentration 1000 ppm for each pot. Untreated pots served as control. Each treatment was arranged in a randomized block design in the greenhouse. Pots were kept in the greenhouse at 25 ± 2°C and 16-h photoperiod. Fifteen days after nematode inoculation the fresh weight and length of root, the number of leaves, galls, egg masses and eggs were determined for each pot. Plant length, vegetative height, number of flowers/plant number of fruits/plant and fruits weight/plant were recorded after inoculation with nematode.

GC-MS analysis

The best four extracts were analyzed to Identify the biochemical compounds Glucosinolate by using GC-MS in the department of chemistry of medicinal plants, national research centre with GC system Mass Hunter GC/MS Acquisition B.07.03.2129 18-May-2015 Copyright © 1989-2014 Agilent Technologies, Inc. according to Hussein (2016) and Hussein *et al.,* (2017).

Statistical analysis

Statistical analyses were performed by IBM SPSS Statistics Version 16. Data were subjected to an analysis of variance (One-way AVOVA). Differences between means were reported as significant if $P < 0.05$ using Tukey's test.

All treatments were replicated four times and the experiment was repeated twice.

Results and Discussion

Nematicidal activity of garden cress extracts towards J_2 and eggs of *M. incognita*

The effects of extracts obtained using different organic solvents from the whole plant or seeds of *L. sativum*on J_2 and eggs of *M. incognita* atconcentrations of 500 and 1000 ppm were determined table 1. All extracts at the both concentrations had significant effects ($P \leq 0.05$) on J_2 mortality and hatch inhibition in comparison with the control. Percentages of mortality and hatch inhibition were dependent on the plant part (whole plant, seed) or solvent used; and were increased as the concentration of the extracts increased. However, significant differences between some concentrations were not detected using Tukey's test. Based on mortality and hatching inhibition, the acetone extract from whole plant

Table 1: Effect of *L. sativum* extracts on juveniles mortality and egg hatching of *Meloidogyne incognita*.

Plant part	Extract	Conce. (ppm)	% J2 Mortality*	% hatchInhibition*
Whole (W)	Acetone (AW)	500	76.5 ± 5.5^{bcd}	60.0 ± 4.8^{abc}
		1000	91.2 ± 4.7^{a}	73.0 ± 3.6^{a}
	Butanol (BW)	500	49.7 ± 6.4^{ijk}	22.2 ± 3.8^{i}
		1000	$63.0 \pm 4.9^{e-h}$	30.5 ± 4.0^{hi}
	Ethanol (EW)	500	$59.0 \pm 6.3^{f-i}$	41.5 ± 3.4^{fgh}
		1000	$69.7 \pm 4.6^{c-f}$	56.5 ± 5.1^{cde}
	Methanol (MW)	500	$51.0 \pm 4.3^{g-j}$	32.2 ± 4.5^{ghi}
		1000	$59.2 \pm 4.5^{f-i}$	$46.5 \pm 3.4^{d-f}$
	Ethyl acetate (EAW)	500	$69.7 \pm 5.5^{c-f}$	44.7 ± 2.7^{efg}
		1000	81.2 ± 4.7^{abc}	61.2 ± 7.9^{abc}
Seed (S)	Acetone (AS)	500	$61.2 \pm 4.7^{e-i}$	41.2 ± 4.7^{fgh}
		1000	$74.2 \pm 5.2^{b-e}$	57.7 ± 7.8^{bcd}
	Butanol (BS)	500	37.2 ± 3.3^{k}	19.5 ± 3.1^{i}
		1000	44.0 ± 5.3^{jk}	24.0 ± 5.3^{i}
	Ethanol (ES)	500	$66.0 \pm 3.9^{c-f}$	$51.0 \pm 2.9^{c-f}$
		1000	85.5 ± 6.5^{ab}	70.5 ± 6.5^{ab}
	Methanol (MS)	500	$72.0 \pm 4.3^{c-f}$	39.5 ± 5.5^{fgh}
		1000	80.7 ± 3.8^{abc}	62.2 ± 4.6^{abc}
	Ethyl acetate (EAS)	500	$51.5 \pm 5.2^{g-j}$	31.0 ± 4.3^{hi}
		1000	$64.2 \pm 5.2^{d-g}$	41.7 ± 7.3^{fgh}
	Control		1.0 ± 0.9^{l}	5.0 ± 2.5^{j}

*Values are means ± SD of four replicates, % mortality after 48h, % hatch inhibition after 7days. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test.

(AW) among all extracts gave the strongest activity, which showed 91% and 73% at 1000 ppm, respectively. The next best extracts were the ethyl acetate extract from whole plant (EAW), ethanol extract from seed (ES) and methanol extract from seed (MS), all of them caused over 80% mortality and 62% hatch inhibition, at concentration of 1000 ppm. No significant differences were found among these four extracts. Therefore, the four extracts were selected for testing their mixtures also well.

Influence of mixtures of the best four extracts on J_2 and egg hatching

Mixtures of the best four extracts AW, EAW, ES, MS were also assessed for their nematicidal effects against J_2 and egg of *M. incognita*. Results showed that all tested mixtures had significant toxic effects on J_2 mortality and egg hatching compared to the control Fig. 1 and 2. Most of the mixtures resulted in significantly higher ($P \leq 0.05$) percentages of mortality than those that caused by each extract individually. The same trend occurred in hatching inhibition test but only with few mixtures. This suggests that mixing their components led to synergistic toxicity. However, this positive toxic effect varied greatly amongst the different mixtures. In comparison with other mixtures and / or individuals, the mixtures (ES+MS+AW), (ES+MS+AW+EAW) and (ES+MS+EAW) were the most effective treatments at J_2 mortality and egg hatch inhibition, causing over 92% and 81%, respectively. No significant differences among the three mixtures were observed. In contrary, the mixture of MS+AW+EAW showed toxic effect less than each extract alone and was the least amongst the tested mixtures, causing J_2 mortality and hatch inhibition not exceed 71% and 52%, respectively; indicating that there is a negative interaction of its components.

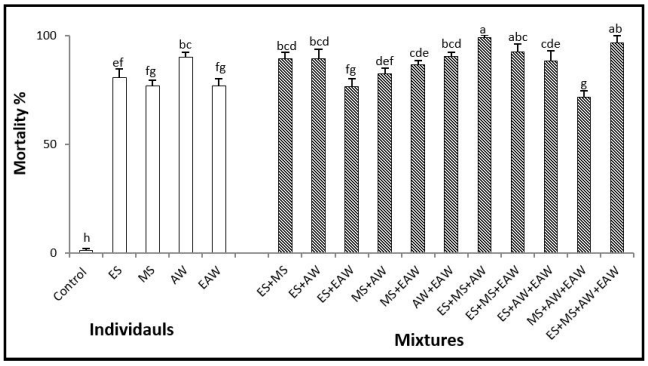


Fig. 1: Mortality of *M. incognita* juveniles after exposure to the best four extracts and their mixtures. Error bars represent standard deviations. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test (n = 4).

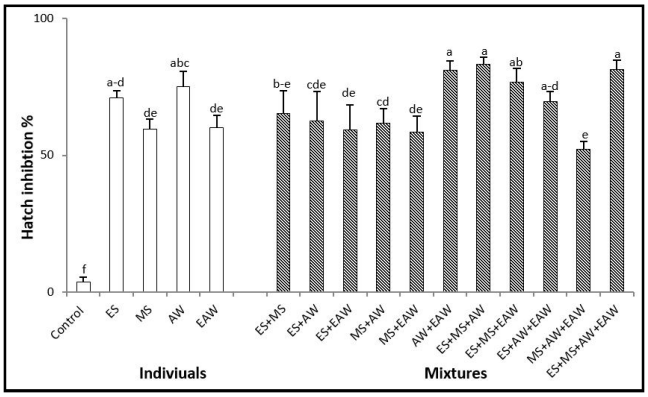


Fig. 2: Hatch inhibition of *M. incognita* eggs after the best four extracts and their mixtures. Error bars represent standard deviations. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test (n = 4).

Influence of the best extracts and their mixtures

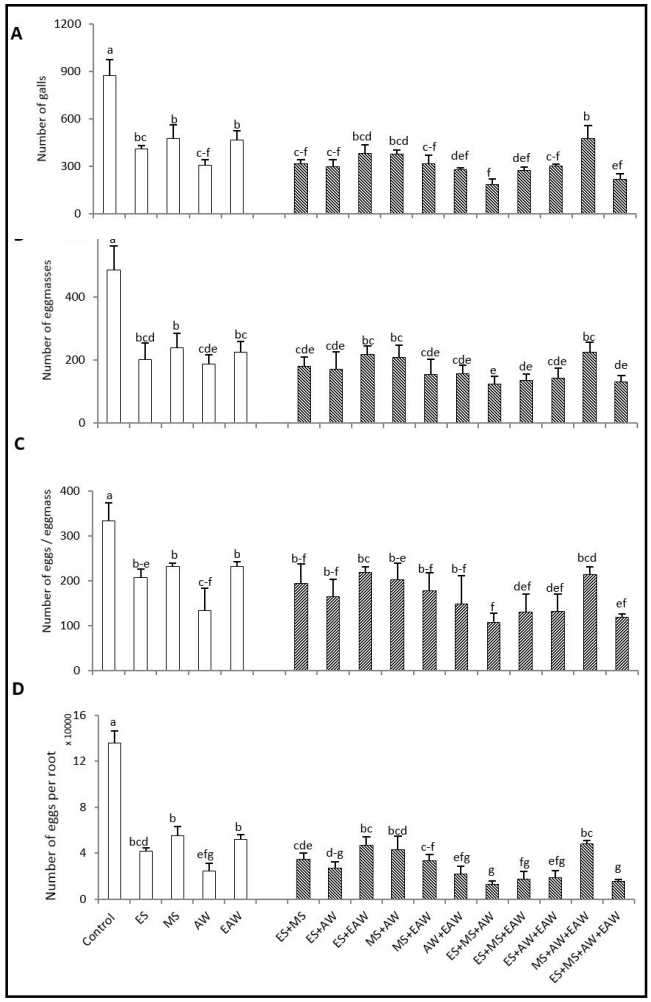


Fig. 3: Effect of plant extracts and their mixtures on *M. incognita* reproduction on tomato roots. Numbers of galls (A), egg masses (B), eggs per egg mass (C) and eggs per root (D). Error bars represent standard deviations. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test (n = 4).

on reproduction of *M. incognita* on tomato

The nematicidal activity of the best four extracts and their mixtures against *M. incognita* on tomato plants were assessed. All plant extracts used either alone or mixtures significantly reduced all nematode parameters measured on tomato roots compared to the untreated control Fig. 3 (A-D). Among the individual treatments, AW had the best nematicidal effect at reducing numbers of galls, egg masses, eggs per egg mass and eggs per root, followed by ES then MS and EWA. It caused over a 94% reduction in the number of eggs per root, compared to the untreated control. It was clear that the tested mixtures gave better results at reducing galls, egg masses and eggs than those that achieved by their constituents individually, in most cases. The strongest nematicidal effect was achieved by the ES + MS + AW mixture, followed by the ES + MS + AW + EAW mixture; with over 78%, 74%, 90% and 67% reduction in the number of galls, egg masses, eggs per root and eggs per egg mass, respectively. No significant differences between both mixtures were detected. Other mixtures gave great results similar to those that was achieved by the best treatment individually (AW), except for mixtures (ES + EAW), (MS + AW) and (MS + AW + EAW), all of them reduced all nematode parameters moderately.

The ethanol extract of *L. sativum* produced the greatest inhibition zone against *E. coli* ATCC 25922 (Hala Abushady *et al.,* 2016).

After 24 h exposure to cress extracts, 97% on average of the J_2 of *Globodera rostochiensis*, the golden cyst nematode, became immobile. As time of exposure extended, this effect became more profound and almost 100% of the J_2 lost their mobility in plant extracts (Zanbouriand Fatemy, 2014).

Effects of *L. sativum* extracts and their mixtures on the growth of tomato transplants

Young tomato seedlings are the best developmental stage for root-knot nematode inoculation and that compatible with the results of Atamian *et al.,* (2012). There were no significant differences founded between *L. sativum* extracts and their mixtures on the plant length comparing with the negative control, but the opposite founded with the positive control which gave the smallestplant length. The same results founded with the vegetative height of tomato transplants. For root length, no significant differences were found between all treatments and both negative and positive control, but we observed that the length of the positive control root was almost double the vegetative height which decreased significantly and this was not done by adding the extracts and their mixtures, but rather near plant growth from the negative control. Roots are the principle organs

Table 2: Tomato plants parameters measurement according to the extracts of garden cress and their mixtures.

Treat-ments	Plant length (cm)	Vegetative height (cm)	Root length (cm)	Plant dry weight (g)	Root fresh weight (g)	Num. of leaves	Num. of flowers	Num. of fruits/plant	Fruits weight/ plant (g)
- control	36.00 ab	16.50 ab	19.50 ab	4.40 def	3.03 abc	11.00 ab	2.50 bcde	0.50 a	0.30 a
+ control	25.00 c	8.50 c	16.50 b	3.28 f	2.24 cd	9.00 bcd	3.75 ab	0.25 a	0.25 a
ES	32.25 b	13.75 b	18.50 ab	3.85 ef	1.96 d	8.25 cd	0.75 ef	0.75 a	0.90 a
MS	36.00 ab	17.75 a	20.50 ab	7.60 ab	2.72 bcd	10.00 abc	0.75 ef	0.00 a	0.00 a
AW	38.25 ab	16.25 ab	19.75 ab	8.58 a	3.46 ab	10.25 abc	1.50 def	0.25 a	0.99 a
EAW	35.25 ab	15.75 ab	19.50 ab	7.00 abc	3.62 ab	9.00 bcd	2.75 abcd	0.75 a	0.70 a
ES+MS	33.50 b	15.75 ab	17.75 b	5.28 cdef	3.23 ab	9.25 abcd	1.75 cdef	0.25 a	0.20 a
ES+AW	34.50 b	15.50 ab	19.00 ab	5.38 cdef	3.07 abc	9.00 bcd	1.00 def	0.00 a	0.00 a
ES+EAW	36.00 ab	14.75 ab	21.25 ab	5.30 cdef	3.05 abc	10.75 abc	1.00 def	0.00 a	0.00 a
MS+AW	31.50 b	15.75 ab	15.75 b	6.48 abcd	3.05 abc	9.75 abc	0.50 f	0.75 a	0.18 a
MS+EAW	37.50 ab	16.00 ab	21.50 ab	7.00 abc	3.80 ab	9.75 abc	0.25 f	0.25 a	0.10 a
AW+EAW	35.25 ab	14.25 ab	21.00 ab	5.68 bcde	3.05 abc	10.25 abc	0.25 f	0.50 a	0.45 a
ES+MS+AW	31.75 b	16.00 ab	15.75 b	5.00 cdef	3.05 abc	9.75 abc	1.50 def	0.25 a	0.28 a
ES+MS+EAW	37.25 ab	15.25 ab	22.00 ab	6.28 bcd	2.70 bcd	9.25 abcd	1.00 def	0.50 a	0.85 a
ES+AW+EAW	35.75 ab	16.75 ab	19.00 ab	5.38 cdef	2.23 cd	11.75 a	3.50 abc	0.25 a	0.16 a
MS+AW+EAW	32.75 b	17.00 ab	15.75 b	5.48 bcdef	2.97 abc	6.75 d	4.50 a	0.25 a	0.18 a
ES+MS+AW+EAW	42.25 a	16.25 ab	26.00 a	5.75 bcde	3.22 ab	9.75 abc	3.50 abc	0.50 a	0.38 a
$LSD_{0.05}$	7.6056	3.5461	7.9222	2.2197	0.9462	2.5756	1.9793	0.8140	1.0316

Methanol(M), Ethanol (E), Acetone (A), Butanol (B) and Ethyl acetate (EA) were used for extraction and Fractionation of bioactive compounds from the whole plants powder (W) and seed powder (S) of garden cress.

responsible for nutrients and water uptake, in additions, root system plays a very essential role in foraging plants in different soil types (Hazman and Brown, 2018).

And that may be caused by nematode suffering of tomato plant table 2. This was different for plant dry weight where the highest dry weight was given with the treatments AW, MS, MS+AW, EAW and EAW+MS with significant differences between them with the positive and negative control and that indicating positive effects of these extracts and their mixtures on tomato plant growth table 2.

For the fresh weight of tomato roots the treatment ES gave less significant weight compared to negative control and there were no significant differences between the other treatments compared to both control negative and positive, indicating that these extracts and their mixtures did not affect significantly the weight of roots as happened with root length before table 2.

The treatment EAW+AW+MS gave the lowest number of leaves on the tomato plant compared to negative control with significant differences. The number of flowers were the highest with the treatments EAW+AW+MS+ES, EAW+AW+ES, EAW and the positive control compared to negative control table 2.

The above shows that *L. sativum* extracts and their mixtures did not adversely affect the ability of tomato plant to flowering, nor did we find any significant differences between the number of fruits per plant or weight of fruits per plant for all extracts and their mixtures compared to control and this is because the experiment was for the period of the seedling and the beginning of production of flowers and fruits only. The treatments EAW, EAW + AW + ES, EAW + AW + MS, ES + MS + AW + EAW and the positive control activated flowers production on tomato plants compared to negative control table 2.

The maximum increase in the plant growth parameters was founded in plant treated with the mixture ES + MS + AW + EAW with the best effect as a nematicidal control so that means we had mixtue contain toxic compound to nematode and stimulate comounds of the plant parameters as showed in table 3, 4 and 5 for the GC-MS analysis for the individual extracts of this mixture.

GC-MS analysis

Chromatogram obtained from GC-MS analysis is shown in Tables 3-5 and Figs. 4-7. The list of compounds matched with the NIST library search in the GC-MS analysis is tabulated in tables 3-5. The compounds found in gas chromatogram were subjected for mass spectrometry and the results are matched with known compounds in the NIST library.

It was found that the damage effect of acetone extract from whale plant showed the hieghest mortality and hatching inhibition ratio which showed 91% and 73% at 1000 ppm, respectively table 1 as biological control agents of *M.eincognita*, this extracts contained Formamide, N-methyl which had an effect on nematode by decreasing glutathione content and that feebleness nematode like what happened with animal tumour cell by using this compound as a drug to stop increasing of the cancer cell can induce differentiation in selected malignant cell lines, are known to increase doubling times, inhibit clonigenicity and to effect responses against particular human colon carcinomas. At concentrations which inhibit growth and clonigenicity according to Cordeiro and Savarese (1986) and Cordeiro and Savarese (1984).

Chatoui *et al.,* (2016) found that methanol and ethyl acetate of *L. sativum* seed extracts are most active on the whole of the bacteria tested and revealed a significant antibacterial activity against *Rhodococcus equi*.

This leads to the fact that the use of these natural extracts, which have the ability to resist the nematode *M. incognita* did not have any negative impact on the growth of tomatoes, which encourages the use of such extracts are harmless to the environment.

Chemical nematicides had high effectiveness against the root-knot nematodes, biological control of plant parasitic nematodes by natural extracts has been considered a more natural and environmentally acceptable alternative to such chemicals. Thus, the overall goal of such biocontrol agents is the identification and deployment of highly effective extract to nematode before their development into registered plant protection products (Suarez *et al.,* 2004, Abd Elgawadand Mohamed, 2006).

Non-host Brasicaceae species are used as cover crops for reducing population levels of different parasitic nematode species (Nyczepir and Thomas 2009), also release of allelochemicals after incorporation in the soil (Halbrendt 1996) and further increase in nematode natural enemies in soil (Wang *et al.,* 2002).

Conclusion

Garden cress plants and seed contain compound have a good nematicidal activity against root knot nematode (*Meloidogyne incognita*) infected tomato transplants in pots experiment, by using different extracts and combination of these extracts. The damage effect of acetone extract (AW) from whole plant showed the hieghest mortality and hatching inhibition ratio. The strongest nematicidal effect was achieved by the ES + MS + AW mixture, followed by the ES + MS + AW + EAW mixture; with over 78%, 74%, 90% and 67% reduction in the number of galls, egg masses, eggs per

Table 3: GC-MS analysis of ethanol (ES) and methanol (MS) with seeds extracts.

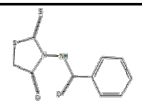
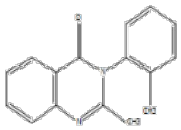
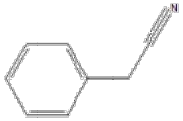
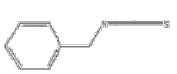
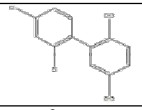
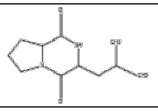
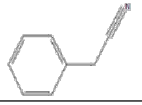
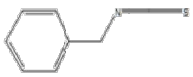
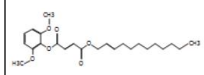
Peaks	Absorbance Name	Phytochemical Compound	M.W. g/mol	Retention Time (m)	Chemical structure	Pharmacological properties
5	Ethanol	N-(4-Oxo-2-thioxo-thiazolidin-3-yl)-benzamide-C10H8N2O2S2	252	4.2		Insecticidal and antifungal activities. https://pubchem.ncbi.nlm.nih.gov/compound/482274#section=Chemical-Co-Occurrences-in-Literature
7		Methaqualone-C16H14N2O	250.301	14		sedative, hypnotic agent that was used for insomnia, but was taken off of the market, in the U.S., in 1983 due to its high risk of abuse. Overdose load to death.National Center for Biotechnology Information. PubChem Compound Database; CID=6292, https://pubchem.ncbi.nlm.nih.gov/ compound/6292 (accessed Sept. 10, 2018).
8		Benzyl nitrile or Benzyl cyanide- C8H7N	117.151	15.5		Poisonous. May be fatal if inhaled, swallowed, or absorbed through skin. Contact may cause burns to skin and eyes.National Center for Biotechnology Information. PubChem Compound Database; CID=8794, https://pubchem.ncbi.nlm.nih.gov/compound/8794 (accessed Sept. 10, 2018
9		Benzyl isothiocyanate or Benzene, (isothiocyanatomethyl)-C8H7NS	149.211	23.5		Food additives - Flavoring AgentsNational Center for Biotechnology Information. PubChem Compound Database; CID=2346, https://pubchem.ncbi.nlm.nih.gov/compound/2346 (accessed Sept. 10, 2018).
11		1,1'-Biphenyl, 2,4-dichloro-2',5'-dimethyl-C14H12Cl2	251.15	29.7		Useful for treating hyper-proliferative disorders.https://pubchem.ncbi.nlm.nih.gov/compound/619502#section=Depositor-Supplied-Patent-Identifiers&fullscreen=true
13		Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-C11H18N2O2	210.277	34.8		Antioxidant activity assessed as hydroxyl radical scavenging activity by electelec spin resonance method with antibacterial activity.https://pubchem.ncbi.nlm.nih.gov/compound/7074739#section=Depositor-Supplied-Patent-Identifiers.
4	Methanol	Benzyl nitrile -C8H7N	117.151	15.6		Potentially toxic compound non- carcinogenic (not listed by IARC) National Center for Biotechnology Information. PubChem Compound Database; CID=8794, https://pubchem.ncbi.nlm.nih.gov/compound/8794 (accessed Sept. 10, 2018).
5		Benzyl isothiocyanateor Benzene,(isothiocyanatomethyl)-C8H7NS	149.211	23.4		Food additives - Flavoring AgentsNational Center for Biotechnology Information. PubChem Compound Database; CID=2346, https://pubchem.ncbi.nlm.nih.gov/compound/2346 (accessed Sept. 10, 2018).
6		Fumaric acid, 2,6-dimethoxyphenyldodec-2-en-1-yl ester- C24H34O6	418.53	34.8		https://www.chemeo.com/cid/77-379-7/Fumaric%20acid%2C%202%2C6-dimethoxyphenyl%20dodec-2-en-1-yl%20ester

Table 4: GC-MSanalysis of acetonewith whole plant extract (AW).

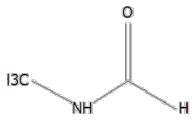
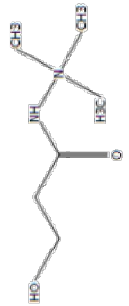
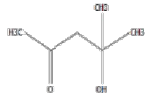
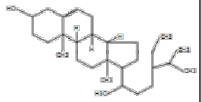
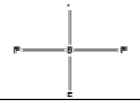
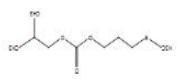
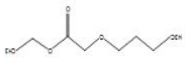
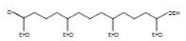
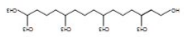
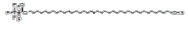
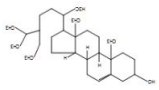
Peaks	Absorbance Name	Phytochemical Compound	M.W. g/mol	Retention Time (m)	Chemical structure	Pharmacological properties
1	Acetone	Formamide, N-methyl -C2H5NO	59.068	1.7		depletes cellular glutathione, a key molecule involved in the antioxidation of reactive oxygen species (ROS) and other free radicals, thereby enhancing ionizing radiation-induced DNA cross-linking in and terminal differentiation of tumor cells. (NCI04). https://pubchem.ncbi.nlm.nih.gov/compound/n-methylformamide#section=Pharmacology-and-Biochemistry.
2		2-(3-hydroxy-1-oxopropyl) -1,1,1-trimethyl-hydrazinium hydroxide inner salt- C6H14N2O2	146.19	1.7		Substances that augment, stimulate, activate, potentiate, or modulate the immune response at either the cellular or humoral level. The classical agents (Freund's adjuvant, BCG, Corynebacterium parvum, et al.) contain bacterial antigens. Some are endogenous (e.g., histamine, interferon, transfer factor, tuftsin, interleukin-1). Their mode of action is either non-specific, resulting in increased immune responsiveness to a wide variety of antigens, or antigen-specific, i.e., affecting a restricted type of immune response to a narrow group of antigens. The therapeutic efficacy of many biological response modifiers is related to their antigen-specific immunoadjuvanticity. https://pubchem.ncbi.nlm.nih.gov/compound/123868#section=EU-Clinical-Trials-Register.
3		Propanal- C3H6O	58.08	2.4		Detoxification of aldehydes can proceed by oxidation to readily metabolized acids, by reduction to alcohols, and by reaction with sulfhydryl groups, particularly glutathione. Under conditions that deplete glutathione levels or result in an inhibition of aldehyde dehydrogenase (eg, Antabuse treatment), the acute and chronic effects of aldehyde toxicity might be more fully expressed. https://pubchem.ncbi.nlm.nih.gov/compound/527#section =Pharmacology-and-Biochemistry.
4		2-Pentanone, 4-hydroxy -4-methyl or Diacetone alcohol-C6H12O2	116.16	4.3		Flavoring agents, absorption ... occurs readily from the lung. https://pubchem.ncbi.nlm.nih.gov/compound/31256#section= Absorption-Distribution-and-Excretion
5		Octatriacontyl pentaflu oropropionate- C41H77F5O2	697.057	25.2		Antioxidant and cytotoxic activities. https://pubchem.ncbi.nlm.nih.gov/compound/91693082
6		.gamma.-Sitosterol -C29H50O	414.71	30.6		plant extracts containing gamma-sitosterol have demonstrated toxicity on in-vitro human cell assays; which may discourage use as a natural supplement. https://drugs.ncats.io/drug/5LI01C78DD

Table 5: GC-MSanalysis of ethyl acetatewith whole plant extract (EAW).

Peaks	Absorbance Name	Phytochemical Compound	M.W. g/mol	Retention Time (m)	Chemical structure	Pharmacological properties
3	Ethyl Acetate	Sulfur tetrafluoride- F4S	108.054	2.6		A highly toxic and corrosive gas. On contact with water, steam, or mineral acids it decomposes and produces toxic and highly irritating fumes. https://cameochemicals.noaa.gov/chemical/4574.
4		Isobutyl (3-(methylthio) propyl) carbonate -C9H18O3S	206.302	2.8		Unknown. https://pubchem.ncbi.nlm.nih.gov/compound/Isobutyl-_3-_methylthio_ propyl_-carbonate#section=Names-and-Identifiers.
5		Ethyl 2-butoxyacetate -C8H16O3	160.213	2.9		Anti-allergic and anti-asthmatic activities. https://pubchem.ncbi.nlm.nih.gov/compound/Ethyl-butoxyacetate #section=Depositor-Supplied-Patent-Identifiers
6		2-Pentadecanone, 6,10,14-trimethyl -C18H36O	268.485	17.9		Flavoring Agents. https://pubchem.ncbi.nlm.nih.gov/compound/6_10_14-Trimethylpentadecan-2-one#section=Uses
7		Phytol- C20H40O	296.539	20.5		Flavoring Agents. https://pubchem.ncbi.nlm.nih.gov/compound/5280435#section=Uses
8		Octatriacontyl pentafluoro-propionate- C41H77F5O2	697.057	25.2		Antioxidant and cytotoxic activities. https://pubchem.ncbi.nlm.nih.gov/compound/91693082
9		Octasiloxane, 1,1,3,3,5,5,7, 7,9,9,11,11,13,13,15,15-hex-adecamethyl- C16H50O7Si8	579.25	26.7		Unknown. https://pubchem.ncbi.nlm.nih.gov/compound/123906482.
11		.gamma.-Sitosterol -C29H50O	414.718	30.6		plant extracts containing gamma-sitosterol have demonstrated toxicity on in-vitro human cell assays; which may discourage use as a natural supplement. https://drugs.ncats.io/drug/5LI01C78DD

root and eggs per egg mass, respectively. No significant differences between both mixtures were detected. Other mixtures gave great results similar to those that was achieved by the best treatment individually (AW). The maximum increase in the plant growth parameters was founded in plant treated with the mixture ES + MS + AW + EAW with the best effect as a nematicidal control so that means we had mixtue contain toxic compound to nematode and stimulate comounds of the plant parameters.

Results of this study conclude that the mixture of the extracts of *L. sativum* contain significant activity as a nematicidal bio-controller by applying these compounds to the soil mixture after planting the seedlings of tomato and these product had no bad significant effects on the growth of the plants, but stimulated the growth of infected plants, despite the presence of nematodes to grow closer to the growth of negative control.

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