



Activity and reproductive capability of *Meloidogyne incognita* and sunflower growth response as influenced by root exudates of some medicinal plants

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ARTICLE INFO

Keywords:

Nematode control
Sunflower
Root exudates
Root-knot nematode

ABSTRACT

Two experiments were separately conducted to test the effect of root exudates of seventeen medicinal plant species on hatching and survival of the root-knot nematode, *Meloidogyne incognita*, *in-vitro* and *in-vivo* on infected sunflower cv. Giza 102. *In vitro* tests, rosemary and marjoram root exudates were the best treatments for inhibiting egg hatch at all concentrations. Nematicidal and nematostatic effects of exudates were concentration-dependent, as the anesthetic effect was obvious especially at low concentrations. *In vivo* experiment, marjoram and rosemary root exudates significantly reduced the numbers of galls, egg-masses on roots, eggs/root and reproduction factor (Pf/Pi) in all times of application. Furthermore, immersing juveniles in marjoram exudates for 90 min before inoculation caused approximately 50% reduction in *Meloidogyne incognita* infection and fecundity. Treatments of rosemary at all times of application were the best in improving plant fresh and dry weights. GC/MS/MS analysis revealed that the most abundant components in the root exudates of marjoram and rosemary were flavonoids, phenolics and terpenoids which were suppressive to nematode survival, development and reproduction regardless to their time of application.

1. Introduction

Use of naturally occurring phytochemicals against plant-parasitic nematodes and the broad spectrum of active compounds including; polythienyls, isothiocyanates, glycosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, lipids, terpenoids, sesquiterpenoids, diterpenoids, quassinoids, steroids, triterpenoids, simple and complex phenolics to improve plant growth and minimize nematode populations has been recommended. Some of these materials have nematostatic and/or nematicidal activity on survival and hatching of some phytonematodes (Chitwood, 2002; Adam, 2006; Haroon et al., 2009; Hooks et al., 2010; Khan et al., 2012; Yang et al., 2016; Danahap and Wonang, 2016). *In-vitro* studies, survival and hatching of *Heterodera schachtii*, *Meloidogyne javanica* and *M. incognita* were reduced when nematodes treated with root exudates of mustard, *Sinapis alba* (Grundler et al., 1991), *Tagetes* sp. (Kalaiselvam and Devaraj, 2011), *Ficus sycomorus* (Jada et al., 2013) and *Crotalaria* spp. (Danahap and Wonang, 2016). When Mahajan et al. (1985) evaluated the nematicidal activity of 55 phenolic compounds, they found that 11 out of them exhibited toxicity against *M. incognita* juveniles. Applying root exudates or cultivation of plants possess nematicidal properties in nematode infested soils could

positively reduce the nematode population to lower levels (Olabiya, 2008; Wani, 2010; Tesleem et al., 2014). Incorporation of *Brassica* species as green manure before transplanting of vegetable crops significantly decrease nematode populations without affecting adversely the growth and yield of the subsequent vegetable crop (Monfort et al., 2007). Also, *Fumaria parviflora* chopped fresh plant material incorporation into the soil can decrease the root-knot index of tomato plants, promote tomato growth, and improve yield productivity (Naz et al., 2015). Also, plants treated with selected botanical exudates were found to be stronger and better for resisting nematode infection (Nwauzoma and Adeleke, 2017; Li et al., 2018).

The objective of this research was to test the effect of some medicinal plant root exudates *in-vitro* on hatching and survival of root-knot nematode, *Meloidogyne incognita*. Also, *in-vivo* studies on *M. incognita* reproduction and sunflower growth responses were carried out.

2. Materials and methods

2.1. Collection of plant materials

Seeds and seedlings of 17 medicinal plant species (Table 1) were

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collected from seed companies of the Egyptian commercial markets. They were sown in nursery beds and left growing for 2 weeks.

2.2. Preparation of root exudates from seedlings of the test plants

Young and actively growing test plants of similar sizes were inserted into 250 ml glass beaker containing water, wrapped with aluminum foil and were properly sealed with masking tape. The set-up containing the plants was held in an upright position on a clean bench. Cotton wool was used to support the seedlings in the beaker in an upright position under constant illumination of daylight for two weeks. A source of aeration in each beaker was set. Foliar spray with nutrient solution (NPK 19-19-19 + microelements) 2 g/L was sprayed twice a week. Due to the uptake of the water by plants, periodically, some water was added. The produced root exudates were collected from each plant after two weeks and used directly or kept frozen to be used when needed.

2.3. *Meloidogyne incognita* source

A pure culture of the root-knot nematode, *M. incognita* was obtained from an isolate maintained and propagated on sunflower cv. Giza 102 at the Nematology Division, Zoology and Agricultural Nematology Department, Faculty of Agriculture, Cairo University.

2.4. In-vitro studies

Three concentrations of each plant root exudates were prepared as follow: 1-full concentration (100% root exudate), 2- half-dilution (1 vol of root exudates: 1 vol of distilled water) and 3- one-third dilution (1 vol of root exudates: 2 vol of distilled water). Four Petri dishes were arranged for each concentration. Each replicate received ten full egg masses, then incubated under room temperature $25 \pm 5 \text{ }^\circ\text{C}$ for 5 days. The number of hatched juveniles were recorded. Another ten egg masses/Petri dish were teased off in distilled water and replicated 4 times to serve as a check.

The same treatments were tested on the mortality of 500 ± 50 newly hatched juveniles of the nematode. The experiment with the above-mentioned treatments was laid out in a completely randomized design with four replicates. The numbers of dead and living juveniles in the test solutions were observed for 48 h. The cumulative number of the replicates was recorded to determine the live/dead nematodes for each plant root exudates at each time interval. After 2 days, juveniles from each treatment were carefully washed using distilled water for testing their recovery after an additional 2 days. Stick-like nematode larvae which were not recovered after washing by water and had no movement were considered dead.

Table 1
Common and scientific names of medicinal tested plants.

No.	Common name	Scientific name
1	Anise	<i>Pimpinella anisum</i>
2	Basil	<i>Ocimum basilicum</i>
3	Calendula	<i>Calendula officinalis</i>
4	Caraway	<i>Carum carvi</i>
5	Chamomile	<i>Matricaria chamomilla</i>
6	Coriander	<i>Coriandrum sativum</i>
7	Dill	<i>Anethum graveolens</i>
8	Fennel	<i>Foeniculum vulgare</i>
9	Geranium	<i>Pelargonium graveolens</i>
10	Goldenrod	<i>Solidago</i> sp.
11	Horse mint	<i>Mentha longifolia</i>
12	Marjoram	<i>Origanum majorana</i>
13	Peppermint	<i>Mentha piperita</i>
14	Periwinkle	<i>Catharanthus roseus</i>
15	Rosemary	<i>Rosmarinus officinalis</i>
16	Spearmint	<i>Mentha viridis</i>
17	Thyme	<i>Thymus vulgaris</i>

2.5. In-vivo studies

Seeds of sunflower (cv. Giza 102) were planted in 15 cm diameter clay pots filled with steam-sterilized sandy loam soil (1:1, v/v) at the rate of two seeds per pot. After germination, the plants were thinned to one seedling per pot. The pots were arranged in a randomized complete block design (RCBD) on a clean bench in a glasshouse at $32 \pm 5 \text{ }^\circ\text{C}$. All plants were watered when needed and received the same horticultural treatments.

Two-weeks-old seedlings of the sunflower were divided into three groups. Seedlings of the first group were first treated with root exudates of marjoram or rosemary (150 ml/pot) and then inoculated one day after treating with 3000 J₂/pot. Seedlings of the second group were inoculated with 3000 J₂ previously immersed in the same root exudates (the lowest concentration) for 90 min, then inspected before inoculation to confirm that they still have active movement. Seedlings of the third group were inoculated with 3000 J₂/pot and were then drenched, one day after, with the same root exudates. Inoculation was done by slowly dispensing of the previously prepared nematode suspension into 4 holes made in the soil surface around the plant base and were immediately closed as soon as possible. Each treatment was replicated five times, including untreated inoculated pots that served as a check.

Thirty-five days after inoculation, the plants were removed from pots and data on plant growth (total length, total fresh and dry weights) were recorded. A sub-sample of roots from each plant was stained with acid fuchsin/lactophenol (Hooper et al., 2005) and the numbers of galls and egg masses per root were counted. The extraction of nematodes from the roots was carried out using the sodium hypochlorite (NaOCl) technique (Hussey and Barker, 1973) for eggs and sieving for nematodes within the roots (Coyne et al., 2007). The number of eggs/root, reproduction factor (Pf/Pi), the percentages of egg production and nematode reduction were then calculated.

2.6. Chemical analysis

2.6.1. Preparing root exudates for chromatographic analysis

A similar procedure of collecting root exudates which was mentioned before was followed, with some exceptions. The root system of each plant was washed three times using deionized water and transferred to a 100 ml flask containing 50 ml deionized water. The exudates were collected after 24 h, filtered using filter paper and centrifuged at 9000 rpm for 5 min to exclude root border cells. Due to the low concentrations of exudate compounds, subsequent concentration through freeze-drying using lyophilizer (Christ-alpha 2-4 LD plus) was conducted (Dundek et al., 2011). The produced powder was served for the chromatographic analysis.

2.6.2. GC/MS/MS analysis of the active ingredients in root exudates

Root exudates of marjoram and were analyzed by gas chromatography/mass spectrometry and gas chromatography/tandem mass spectrometry (GC/MS/MS). The analysis was carried out using a GC (Agilent Technologies 7890 A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 μm film thickness). The carrier gas was helium with a linear velocity of 1 ml/min. The injector and detector temperatures were 200 °C and 250 °C, respectively. The volume injected 1 μl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250 °C, and acquisition mass range 50–800.

The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Dong et al., 2014).

2.6.3. Statistical analysis

Data were compared by Duncan's Multiple Range Test (DMRT) at the 5% level of probability using computer software, *MSTAT Version 4, 1987*.

3. Results

3.1. Egg hatching

Data in (Table 2) reveal that treatments of the aqueous root exudates of medicinal plants affected egg hatching of *M. incognita*. In 100% full concentration, periwinkle, thyme, and horsemint treatments significantly exhibited the lowest % inhibition values (58, 60 and 67%, respectively). On the other hand, the highest inhibition percentages of egg hatching were observed by aqueous exudates of basil, caraway, fennel, marjoram and rosemary attaining 100% as compared with those of check and all other treatments. Moreover, in (1:1) dilution 99, 93 and 90% inhibition of eggs were observed in rosemary, marjoram and basil root exudates, respectively. Meanwhile, treatments of anise, periwinkle and thyme root exudates achieved the lowest egg hatching percentages with values of 41, 45 and 52%, respectively. In (1:2) dilution the maximum inhibition of egg hatching (89%) was obtained by root exudates of rosemary followed by marjoram and basil with 87 and 76% inhibition, respectively. The lowest effective root exudates inhibition was noticed in geranium (6%) followed by goldenrod and thyme with 12 and 21%, respectively.

The results indicate that rosemary root exudates were the best one for suppressing the egg hatching after 5 days of exposure with all concentrations, followed by marjoram and basil with the same dilutions and exposure time. Expectedly, the highest number of hatched eggs was obtained by distilled water (check).

3.2. Juveniles' mortality

Data presented in (Table 3) show the efficacy of root exudates at three concentration levels on *M. incognita* mobility and mortality percentages. Similarly, both parameters were positively proportional to concentration levels. Root exudates full concentration of rosemary, marjoram or basil recorded the highest immobile percent of immobility (100%) after 48 h. However, mortality percent after removing the effect of root exudates by water washing (real dead nematodes) were 44, 87,

and 26% respectively. By (1:1) root exudates dilution of rosemary, marjoram and basil the immobile percentage values of J2 averaged 48, 40 and 37%, respectively; while, calendula, spearmint and check gave the lowest values (9% for all). In case of low dilution of root exudates (1:2), the same treatments of rosemary, marjoram and basil achieved 25, 23 and 22% juvenile immobility, respectively. However, mortality percent dropped down to 6, 2 and 4%, respectively. Horsemint recorded the lowest value (4%) of J2 immobility.

Data illustrated in Fig. (1) show the recovery percentage of *M. incognita* juveniles after 96 h. High recovery percentage was recorded by the full concentration of basil root exudates (74%) followed by rosemary (56%) and thymes (23%); while the least value was obtained by root exudates of peppermint (-31%). The highest percentage of nematode recovery by (1:1) dilution was observed in the root exudates of coriander (25%), and the least one was observed in the root exudates of both rosemary and caraway (-15%). Interestingly, the low dilution of the tested root exudates in most treatments did increase recovery of juveniles except those of periwinkle and rosemary which recorded (-6 and -4%), respectively. This was, undoubtedly, due to the anesthetic effect of the treatments more than the killing effect.

3.3. Effect of the root exudates on *M. incognita* reproduction

The influence of root exudates of marjoram and rosemary on population and reproduction of *M. incognita* infecting sunflower varied according to botanical type and application procedure (Table 4). Application of all root exudates of the tested plants significantly reduced all nematode-related parameters (galls, egg-masses, eggs/root and eggs/egg-mass) in all treatments. However, treatment of the immersed juveniles in rosemary exudates showed pronounced reductions in all nematode parameters. Sunflower plants treated with marjoram exudates at all application times showed the maximum effect on gall formation but versus effect on the number of egg-masses. The lowest treatments which reduced the number of egg-mass were marjoram both post and pre inoculation.

The number of eggs per root system was significantly decreased as the soil drenched with exudates, even immersed juveniles in root exudates of rosemary treatment showed a significant reduction in eggs/root as compared to inoculated un-treated plants. The lowest number of eggs/root was recorded in case of the plants inoculated with immersed juveniles in root exudates of rosemary followed by rosemary pre

Table 2
Influence of medicinal plants root exudates on egg hatching of *M. incognita* after 48 h.

Treatment	100% Full concentration		Half conc. (1 root exudates: 1 distilled water)		One-third conc. (1 root exudates: 2 distilled water)	
	Total	(%) Inhibition	Total	(%) Inhibition	Total	(%) Inhibition
Anise	7 f	99	1123 b	41	1238 b	41
Basil	0 f	100	182 gh	90	498 cd	76
Calendula	63 e	88	447 e	77	1146 b	46
Caraway	0 f	100	300 e-h	84	329 cd	38
Chamomile	62 e	92	798 c	58	1020 b	52
Coriander	6 f	99	325 e-g	83	1139 b	46
Dill	20 f	98	215 f-h	89	661 c	69
Fennel	0 f	100	860 c	55	1298 b	38
Geranium	115 d	86	348 e-g	82	1990 a	6
Goldenrod	33 ef	96	330 e-g	83	1853 a	12
Horse mint	269 b	67	762 c	60	1291 b	39
Marjoram	0 f	100	140 hi	93	281 d	87
Peppermint	17 f	98	596 d	69	1250 b	41
Periwinkle	223 c	58	292 e-h	45	311 cd	42
Rosemary	0 f	100	14 i	99	227 d	89
Spearmint	20 f	98	361 ef	81	1098 b	48
Thyme	215 c	60	257 f-h	52	420 cd	21
Check (Distilled water)	816 a	-	1908 a	-	2110 a	-
LSD P < 0.05	14.63		67.99		148.23	
LSD P < 0.01	19.51		90.66		197.64	

Means in the same column followed by different letter(s) are significantly different at $p < 0.05$ according to Duncan's Multiple range tests.
Inhibition (%) = ((Total in control - Total in treatment)/Total in control) × 100.

Table 3
Survival of *M. incognita* juveniles as influenced by medicinal plant root exudates after 48 h.

Treatment	100% Full concentration			Half conc. (1 root exudates: 1 distilled water)			One-third conc. (1 root exudates: 2 distilled water)		
	Total (Live + Dead)	(%) Immobile	(%) Mortality	Total (Live + Dead)	(%) Immobile	(%) Mortality	Total (Live + Dead)	(%) Immobile	(%) Mortality
Anise	425 c-e	52	94	414 e-g	18	29	515 a-d	6	3
Basil	337 g	100	26	391 g	37	12	347 e-g	23	4
Calendula	400 ef	19	65	438 d-g	9	28	456 b-f	8	2
Caraway	441 cd	23	86	516 a-d	23	39	455 b-f	3	2
Chamomile	403 ef	57	84	529 a-c	17	19	416 d-g	2	6
Coriander	374 f	91	92	553 a-c	18	15	471 a-e	1	2
Dill	341 g	84	76	536 a-c	28	42	467 a-e	2	3
Fennel	418 de	93	90	411 e-g	17	44	468 a-e	0	5
Geranium	415 de	75	2	483 b-e	15	19	556 a-c	2	9
Goldenrod	502 a	89	56	477 c-f	13	15	433 c-g	0	3
Horse mint	433 c-e	7	75	421 d-g	20	39	580 ab	4	3
Marjoram	335 g	100	87	400 fg	40	20	328 fg	22	2
Peppermint	403 ef	11	53	548 ab	17	21	401 d-g	5	3
Periwinkle	491 ab	87	11	486 b-e	10	21	347 e-g	6	15
Rosemary	303 h	100	44	384 g	48	32	303 g	25	6
Spearmint	459 bc	81	14	499 a-d	9	17	359 e-g	2	4
Thyme	474 bc	9	77	500 a-d	16	16	337 e-g	9	1
Check (Distilled water)	514 a	9	7	570 a	9	8	588 a	5	2
LSD P < 0.05	16.13			34.98			51.67		
LSD P < 0.01	21.51			46.64			68.89		

Means in the same column followed by different letter(s) are significantly different at p < 0.05 according to Duncan's Multiple range tests.

Mortality (%) = (Dead juveniles in treatment/total juveniles in treatment) × 100.

Immobile (%) = (Immobile juveniles in treatment/Total in treatment) × 100.

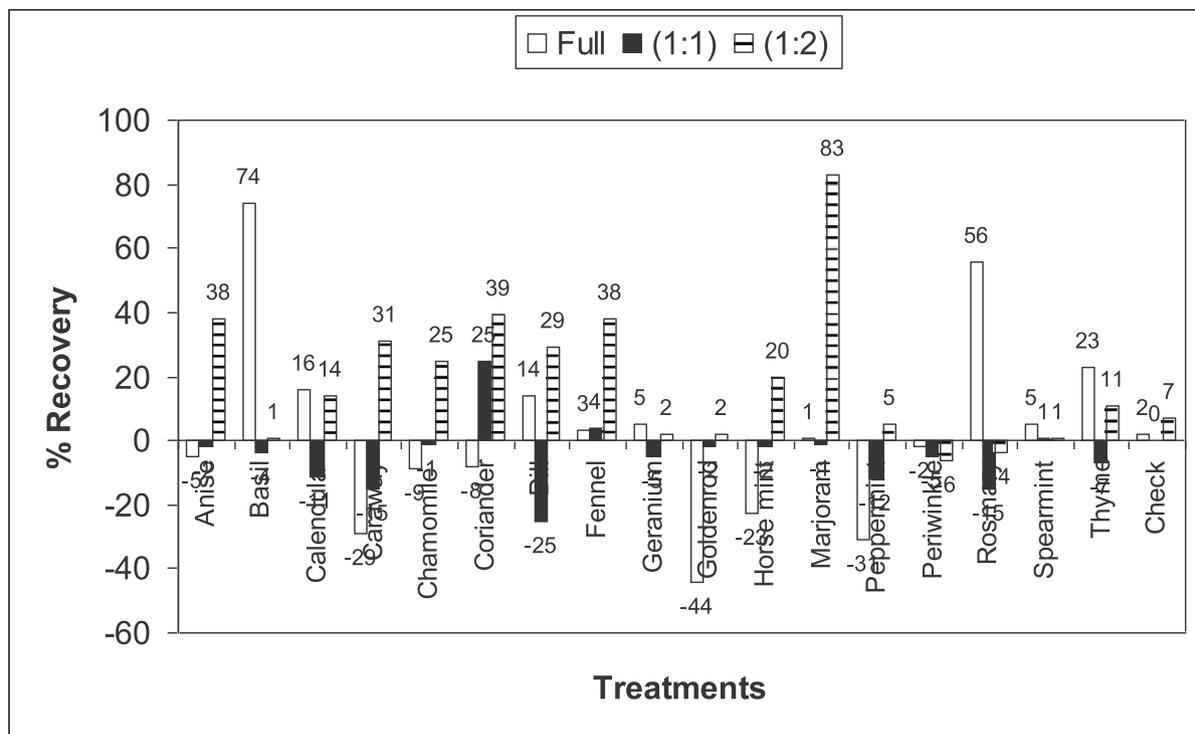


Fig. 1. Percentage of nematode recovery after 96 h.

inoculation, immersed juveniles in marjoram and then rosemary post-inoculation as compared to nematode check. It was obvious that the highest number of eggs/root was recorded in the nematode check.

The nematode reproduction factor per plant root system was significantly decreased as the soil drenched with such root exudates. Also, the immersed juvenile treatments significantly reduced the nematode population with all root exudate treatments as compared to both pre and

post-inoculation ones. The minimum reproduction factor (3.0) per root system was recorded in the case of plants inoculated with immersed juveniles in rosemary root exudates followed by those immersed in marjoram and rosemary post-inoculation (3.6 and 3.7, respectively) as compared to the other treatments. Whereas, the highest nematode reproduction factor (9.1) was recorded from the nematode check.

The maximum % reduction in total eggs/root system over the check

Table 4
Reproductivity of *M. incognita* as influenced by root exudates and time of application.

Treatment	Time of application	Galls/Root	Egg-masses/Root	Eggs/Egg-mass	Eggs/Root	Pf/Pi
Marjoram	Pre inocula	*35 d ±1.333	49 ab ±2.186	276 bc ±10.536	13524 bc ±1137.524	4.5 bc ±0.380
	Immersed juveniles	30 d ±4.256	41 bc ±2.887	267 bc ±11.566	10947 c ±951.215	3.6 c ±0.318
	Post inocula	38 d ±1.202	53 a ±1.528	300 b ±8.743	15900 b ±909.974	5.3 b ±0.302
Rosemary	Pre inocula	102 bc ±4.631	43 bc ±2.028	241 cd ±10.970	10363 c ±966.188	3.5 c ±0.321
	Immersed juveniles	94 c ±2.186	40 c ±1.000	224 d ±5.364	8960 c ±432.315	3.0 c ±0.143
	Post inocula	106 b ±4.041	47 abc ±2.963	238 cd ±13.421	11186 c ±949.823	3.7 c ±0.315
Check (Nematode only)		118 a ±1.528	49 ab ±3.756	555 a ±20.036	27195 a ±2818.002	9.1 a ±0.939
LSD P < 0.05		9.66	5.86	28.69	2963.87	0.99
LSD P < 0.01		13.17	7.99	39.12	4041.65	1.35

Means in the same column followed by different letter(s) are significantly different at $p < 0.05$ according to Duncan's Multiple range tests.

* Mean \pm = Standard error.

(Fig. 2) was recorded in case of rosemary immersed juveniles (67.1%) followed by rosemary pre inoculation, marjoram immersed juveniles and rosemary post-inoculation (61.9, 59.7 and 58.9%) respectively, as compared to that of the check treatment. Also, the minimum % reduction in nematode population/root over the check was recorded when drenched with marjoram exudates post-inoculation (41.5%). The highest % egg production was achieved with marjoram exudates post-inoculation treatment (58.5%). Treatment with immersed juveniles in rosemary root exudates showed 32.9% egg production followed by rosemary exudates pre-inoculation (38.1%) compared to the check treatment (Fig. 3).

3.4. Effect of root exudates on sunflower growth criteria

Total plant height was increased with all root exudates treatments at all application time (Table 5). Poor performance was recorded in nematode check treatment. Rosemary root exudates recorded the maximum plant length (71.6 cm) in the treatment of drenched pre-inoculation, followed by those of post inoculation (69.7 cm) and then those of immersed juveniles (63.3 cm) as compared to check. As for marjoram root exudates, the maximum plant length (66.9 cm) was recorded in the case of post-inoculation treatment followed by pre inoculation one (60.3 cm), while the minimum length value was recorded in those plants inoculated with immersed juveniles (57.3 cm).

The maximum % increase in plant length (48%) was recorded when the soil was drenched with root exudates of rosemary pre inoculation followed by that of post-inoculation 44% (Fig. 4A). The minimum % increase in plant height was recorded in plants inoculated with immersed juveniles in marjoram (18%) followed by plants treated with marjoram root exudates pre inoculation (25%).

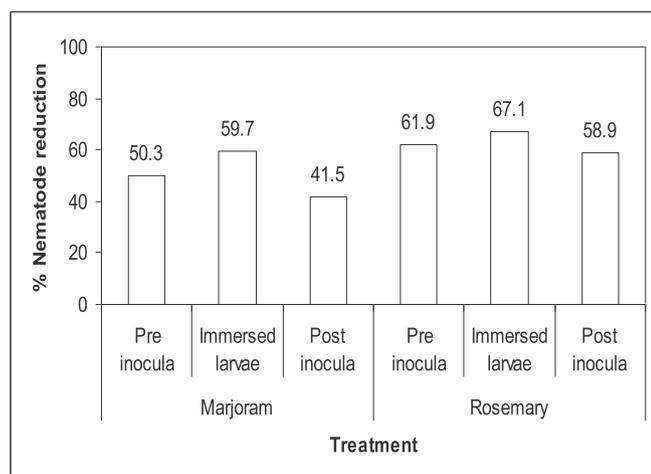


Fig. 2. % Nematode reduction as influenced by root exudates of marjoram and rosemary.

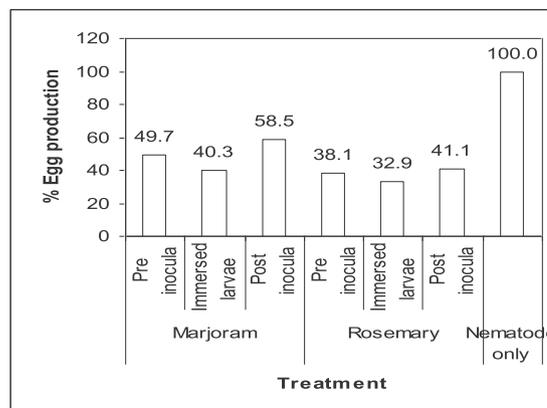


Fig. 3. % Egg production of *M. incognita* as influenced by root exudates of marjoram and rosemary.

Pronounced plant fresh weight was recorded in treatments of most drenched root exudates, pre and post-inoculation of rosemary achieving (9.8 and 9.7 g), respectively. While plants of marjoram immersed juveniles and post-inoculation treatments gained (9.4 and 9.3 g), respectively, followed by those of pre inocula of marjoram and rosemary immersed juveniles (8.4 and 8.8 g, respectively). Comparatively the minimum plant fresh weight was recorded in plants of inoculated untreated treatment (6.5 g).

The maximum % increase in plant fresh weight was recorded when the soil was drenched with root exudates of rosemary (51%). While the minimum % increase (29%) was recorded in plants treated with root exudates of marjoram pre inoculation (Fig. 4B).

Accordingly, all treatments significantly did improve plant dry weight. The maximum increase in dry weight was observed in plants treated with root exudates of rosemary in both pre and post-inoculation treatments (2.4 g) followed by root exudates of marjoram in both immersed juveniles and post-inoculation treatments (2.3 g). Meanwhile, minimum dry weight (2 g) was observed in those plants treated pre-inoculation with root exudates of marjoram.

The % increase in plant dry weight was significantly different from the check at $P < 0.05$ (Fig. 4C). Considerable effect of root exudates of rosemary was observed with pre and post-inoculation treatments (50%), which were the best treatments, against *M. incognita*. On the other hand, the minimum % increase was observed in marjoram pre inoculation treatment (25%).

3.5. Chemical contents of rosemary and marjoram root exudates

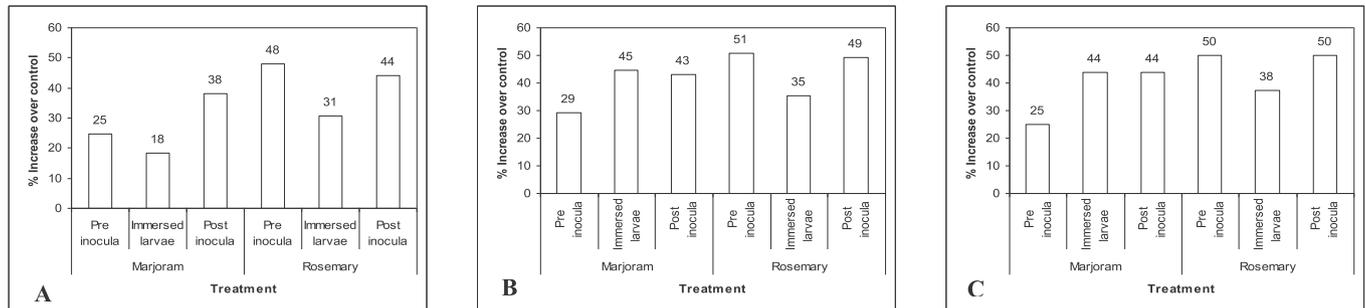
Components of rosemary and marjoram root exudates were analyzed by GC/MS/MS (Tables 6 and 7). The extracts comprised 6 main chemical classes of components. Flavonoids, terpenoids, phenolic, glycosides and organic acids were highly present with different concentrations.

Table 5Effect of medicinal plants root exudates on growth parameters of sunflower infected with *M. incognita* as influenced by time of application.

Treatment	Time of application	Plant length (cm)		Plant fresh weight (g)		Plant dry weight (g)	
Marjoram	Pre inocula	*60.3 de	±0.529	8.4 b	±0.058	2.0 c	±0.018
	Immersed juveniles	57.3 e	±0.551	9.4 ab	±0.674	2.3 ab	±0.159
	Post inocula	66.9 bc	±1.848	9.3 ab	±0.153	2.3 ab	±0.064
Rosemary	Pre inocula	71.6 a	±1.617	9.8 a	±0.252	2.4 a	±0.055
	Immersed juveniles	63.3 cd	±1.386	8.8 ab	±0.100	2.2 bc	±0.046
	Post inocula	69.7 ab	±1.550	9.7 a	±0.088	2.4 a	±0.052
Check (Nematode only)		48.4 f	±1.617	6.5 c	±0.321	1.6 d	±0.055
LSD P < 0.05		4.21		0.69		0.18	
LSD P < 0.01		5.74		0.94		0.24	

Means in the same column followed by different letter(s) are significantly different at p < 0.05 according to Duncan's Multiple range tests.

* Mean ± = Standard error.

**Fig. 4.** % Changes in plant length (A), fresh weight (B) and dry weight (C) of sunflower plants infected with *M. incognita* as influenced by root exudates of marjoram and rosemary.

Flavonoids, mainly flavones, were the major compounds in all exudates, except marjoram in which the major component was terpenoids. In marjoram root extract, the maximum class's recorded was terpenoids (29.22%) followed by flavonoids (25.89%) and phenolics (20.49%), while the minimum was carboxylic acid (13.99%) followed by glucosides and aldehyde (6.20 and 4.21%), respectively. Flavonoids were the maximum chemical class observed in root exudates of rosemary (53.26%) followed by coumarins, terpenoids and phenolics (14.58,

12.90 and 12.17%), respectively; meanwhile, the minimum classes observed were esters and glycosides (5.33 and 1.74%), respectively. In general, there were proportional significant suppression effects of the chemical classes and ratios on the RNA activity, penetration and reproduction of the nematode.

Table 6

The main classes and compounds of marjoram root exudates extracted by GC/MS/MS.

Name	RT (Min)	Area sum %	Sub-class	Class	%
Coniferyl aldehyde	21.162	4.21	-	Aldehyde	4.21
Benzoic acid 2,6-dihydroxy	23.211	13.99	Benzoic	Carboxylic acid (Benzoic)	13.99
Luteolin tetramethyl ether	15.1	4.45	Flavones	Flavonoids	25.89
5,7,3',4',5'-Pentahydroxyflavone	13.483	1.54			
7,4'-Dimethoxy-3-hydroxyflavone	13.889	7.09			
3-Hydroxy-2',4',5'-trimethoxyflavone	14.443	1.11			
5,7,2'-Trimethoxyflavone	14.695	0.83			
Nobiletin	17.289	4.27			
3,2',4',5'-Tetramethoxyflavone	18.081	1.1			
3'-4'-Dimethoxy-3-hydroxy-6 methylflavone	4.203	2.36			
2-Hydroxychalcone	12.961	1.83	Chalcones		
2'-hydroxy-2,5,6'-trimethoxychalcone	14.915	1.31			
Apigenin 8-C-glucoside	15.604	4.92	Conjugated glycosides	Glycosides	6.20
Salysilic acid β-D-O-glucuronide	11.295	1.28			
3,4,5-Trimethoxycinnamic acid	5.275	2.19	-	Phenolics	20.49
Propyl gallate	20.099	1.55			
p-cresol,2,2'-methylenebis[6-tert-butyl-	22.089	15.41			
Probuco	20.387	1.34			
Phytol	20.675	1.34	Diterpenes	Terpenoids	29.22
cis-sesquibabinene hydrate	21.716	0.55	Monoterpene		
cis-Thujopsene	16.631	0.98	Sesquiterpenes		
α-Gurjunene	21.274	6.16			
Caryophyllene	18.635	0.86			
Neoclovene	21.373	8.96			
Guaiol	19.09	1.91			
Cubebol	15.334	1.17			
Hexa-hydro-farnesol	20.968	7.29			

Table 7

The main classes and compounds of rosemary root exudates extracted by GC/MS/MS.

Name	RT (Min)	Area sum %	Sub-class	Class	%
apigenin 8-C-glucoside	13.817	1.74	Flavone glycoside	Glycoside	1.74
7-Methoxy-3-(4-methoxyphenyl)-4-methylcoumarin	15.159	4.31	-	Coumarins	14.58
Fraxidin	16.784	3.52			
6,7,8-Trimethoxycoumarin	20.333	2.86			
5,7-Dimethoxy-4-methylcoumarin	4.082	3.89			
Rosmarinic acid	19.937	5.33	Ester of caffeic acid	Esters	5.33
3',4'-Dimethoxy-3-hydroxy-6-methylflavone	13.042	6.09	Flavones	Flavonoids	53.26
3,6,2'-Trimethoxyflavone	13.965	3.6			
3,2',4',5'-Tetramethoxyflavone	22.54	10.1			
5-Hydroxy-3',4',5',6,7,8-Hexamethoxyflavone	21.229	9.4			
7,3',4',5'-Tetramethoxyflavone	4.761	4.68			
3-Hydro-7,8,2'-trimethoxyflavone	7.594	2.32			
6,3',4'-trimethoxyflavone	8.814	10.44			
4'-Hydroxy-2'-methyl-3,4,5-trimethoxychalcone	3.37	4.33	Chalcones		
2,5-Dimethoxy-2'-hydroxy-5'-methylchalcone	11.254	2.3			
3,4,5-Trimethoxycinnamic acid	5.617	2.99	-	Phenolics	12.17
3,4,5-Trimethoxycinnamic acid	6.405	3.06			
Resorcinol sulfoxide	21.972	6.12			
Γ-Tocotrienol	15.825	4.16	Vitamin E family	Terpenoids	12.9
Cis-sesquisabinene hydrate	4.325	3.36	Monoterpene		
longiborneol	23.094	2.25	Sesquiterpene		
epi-γ-Eudesmol	18.478	3.13			

4. Discussion

Root exudates of 17 medicinal plants caused various mortality percentages of J2 of *M. incognita* in *in-vitro* tests. The highest significant effect on the nematode was obtained by marjoram, rosemary at its full concentration and 1:1 dilution. Contact nematicidal action of plant root exudates on plant-parasitic nematodes has been reported by Nandal and Bhatti (1983) and Salako (2002). Such action is greatly affected by biologically active ingredients that can kill or inhibit the growth of nematodes (Huang et al., 1981; Sharma and Scolari, 1984). The mortality of juveniles may be attributed to several compounds exude from plant roots which are different in both quality and quantity causing variable contact nematicidal effects. These nematicidal properties may have no or limited effects on egg hatching and nematode body penetration. Also, they could inhibit metabolic reactions such as those of respiratory enzymes, acetylcholinesterase enzyme, and hydrolysis of acetylcholine by acetylcholinesterase and esterases that function in various metabolic systems (Aguillera et al., 1984; Atkinson and Fowler, 1990; Nile et al., 2017). Moreover, root exudates of *Ficus sycomorus* (Jada et al., 2013), *Crotalaria* spp. (Danahap and Wonang, 2016) and *Allium fistulosum* (Li et al., 2018) had a direct lethal effect on root-knot nematode (RKN) larvae. Toxic substances of root exudates of certain plants may include; spectine, autofine, phenols, saponins, alkaloids, tylophonine and glucosinolates (Peterson and Clinch, 1994; Monfort et al., 2007; Jada et al., 2013). Also, the effect of root exudates of many plants was noticed on egg hatching which promoted a good nematicidal and ovicidal activity.

The present results provide insight into the role of marjoram and rosemary root exudates in suppressing *Meloidogyne incognita* infection, fecundity and increasing resistance of sunflower against it under greenhouse conditions. Generally, flavonoids, phenolics, terpenoids, coumarins and benzoic acid represent more or less 90% of the total phytochemicals found in marjoram, rosemary root exudates. Consequently, treatment of root diffusates containing such compounds had a prolonged nematicidal effect against nematode penetration, development and reproduction. A large number of terpenoids and their derivatives are inhibitory to juveniles of RKN (Ohri and Pannu, 2009). Also, the phenolic compounds were found to be involved in plant defense and have provided resistance against nematode attack (Ohri and Pannu, 2010). Phenolic compounds disrupt the energy generation mechanism suppressing the oxidative phosphorylation. Also, they interfere with parasites' cell surface glycoprotein causing death (Bauri

et al., 2015). Identification of flavonoids as a natural nematicide from root exudates that contribute to chemotactic attractions or repulsion of nematodes towards or away from plant roots may lay a foundation for the development of natural nematicide products (Chin et al., 2018). Disorientation of nematode infective stages in soil may be another acceptable explanation to inhibit nematode infectivity into plant roots. Dutta et al. (2012) reported similar results concerning nematicidal and nematostatic compounds from tomato exudates. Also, Yang et al., 2016 stated similar results concerning the effect of resistant tomato root exudates on *Meloidogyne incognita* infection.

The tested root exudates improved plant growth criteria. Similar results were obtained by Tiyagi et al. (1986), Adam (2006) and Danahap and Wonang (2016) on tomato; Ameen and Hasabo (1995) on sour orange and Li et al. (2018) on cucumber. Also, the study provides a valuable contribution to the potential use of such plants as a companion plant to protect sunflower plants against RKN infection. Previous studies also reported that using companion plants, such as marigold (*Tagetes patula* L.), basil (*Ocimum basilicum* L.), lettuce (*Lactuca sativa* L.), white mustard (*Sinapis alba* L.) and *Brassica* species in greenhouse cultivation of vegetable crops suppressed the development of *Meloidogyne* spp. (Monfort et al., 2007; Tringovska et al., 2015). In a molecular study, Dong et al. (2014) concluded that lauric acid released from crown daisy can regulate *M. incognita* chemotaxis and blocks infection, thereby reducing nematode damage. Besides, *Mi-flp-18* expression is down-regulated during the parasitism of J2s that encounter high concentrations of lauric acid in the tomato-crown daisy intercropping system. We identified flavonoids, phenolics and terpenoids as major chemical classes of the tested plant root exudates that could inhibit the activity of J2 and decreased hatchability of RKN eggs.

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