https://doi.org/10.26651/allelo.j/2023-58-2-1427 0971-4693/94 Euro 20.00
Allelopathy Journal 58 (2): 165-182 (March, 2023) *International Allelopathy Foundation* 2023
Table: 6, Figs: 7

Chemical composition and nematicidal activity of essential oil and piperitenone oxide of *Mentha longifolia* L. against *Meloidogyne incognita*

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(Received in revised form: August 29, 2022)

ABSTRACT

The essential oil (EO) from the aerial parts of *Mentha longifolia* was obtained by hydro-[distillation method and its chemical composition was determined using GC-MS. This oil was rich in oxygenated monoterpenes (64.91 %) and sesquiterpenes hydrocarbons (1.62 %). Piperitenone oxide (62.91 %) was identified as major compound along with limonene; germacrene-D; 1, 8-cineole; piperitenone; linalool propanoate and p-mentha-1, 8-diene-2-one. Piperitenone oxide (PO) was purified from the oil using column chromatography and identified by1D and 2D NMR and GC-MS. Its EO and PO were evaluated at different concentrations (31.25 to 1000 ppm) for their nematicidal activity against *M. incognita* in lab conditions. These effectively killed the nematodes with $LC_{50/96h}$ values of 92.74 and 34.15 ppm, respectively. In pot study, they were applied to tomato plants as bare root dipping and soil drenching at 1000 and 2000 ppm under polyhouse condition. PO showed promising nematicidal activity and suppressed nematode infestation and multiplication (9.5 galls/root and 11 egg masses/root) on tomato roots compared to EO (13 galls/root and 15.5 egg masses/root) as a soil dresser at 2000 ppm with increased plant biomass and Fluopyrum 400 SC @ 500 g a.i. ha^{-1} (6.5 galls/root and 5.2 egg masses/root). Piperitenone oxide showed potent nematicidal activity against *M. incognita* for the first time and could be used as an alternate to synthetic nematicide against *M. incognita*.

Key words: Column chromatography, essential oil, hydrodistillation, *Meloidogyne incognita*, *Mentha longifolia*, nematicide, protected cultivation, root knot nematode, *Solanum lycopersicum*, tomato**.**

INTRODUCTION

Root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is one of the major nematode pests under the protected cultivation of tomato and is currently managed using toxic and synthetic nematicides due to a lack of environmentally benign nematicides. Globally it reduces crop yields by 12-14 % i.e., annual losses of > $125 billion (31,38). These also attack the tomato crop (*Solanum lycopersicum* L.), grown worldwide and rich source of lycopene, vitamins, minerals, antioxidants and organic acids (34). Annually in India, the *Meloidogyne* spp., (root knot nematodes) cause 23 % yield losses in tomato. Major nematode problems occur in protected cultivation of tomato crop, perhaps due to congenial environment for nematode multiplication (42). Plant parasitic nematodes cause 21.3 % losses in crops (29). Currently synthetic nematicides are used to control *Meloidogyne* spp., due to lack of safer alternative from plant origin. Most of the nematicides are toxic to non-targeted organisms and environment. Essential oils bearing plant species

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are an excellent source of biologically active constituents (terpenes, flavonoids, alkaloids, saponins and acetogenins) and are nematicidal to plant parasitic nematodes, hence, these can be an excellent source of natural nematicides (3). Plant extracts and essential oils-based pesticides are called "Botanical Pesticides" (32), and can be used to control crop pests (22,23,24,27). These natural pesticides contain alkaloids, flavonoids, terpenoids, essential oils etc (7,8,26). Essential oil and their constituents inhibit the acetylcholinesterase (AChE) activity (4).

Mentha longifolia L., an herbaceous perennial and aromatic plant is native to Europe, Africa and India. It is rich in essential oil and other bioactive compounds with numerous medicinal and pest control properties (39). Piperitenone, a monoterpenoid ketone has therapeutic property, which upon epoxidation changed to piperitenone oxide (20). Its essential oils and compounds have not been evaluated against *M. incognita*. Therefore, the essential oil and piperitenone oxide, isolated from *M. longifolia* were evaluated for egg hatching and nematicidal activities against *M. incognita* under lab and polyhouse conditions. The chemical composition of the oil was also determined.

MATERIAL AND METHODS

Collection of plant material

The fresh shoots of *M. longifolia* were collected from Krishi Vigyan Kendra, Shikohpur, Gurugram, Haryana, India (Altitude: 249 m, 28° 22.29' N latitude and 76° 59.34' E longitude, mean annual rainfall:596 mm and temperature:5.1 to 44 °C).

Isolation of essential oil

The fresh shoots were washed with tap water, shade dried (300 g) and subjected to hydro-distillation for 4 h using Clevenger-type apparatus. The distillate obtained after 4 h was extracted with diethyl ether using separatory funnel and the ethereal layer was dried over anhydrous sodium sulphate. The ether was removed and stored at 4 °C for further analysis.

Gas chromatography Analysis

Analysis of the *M. longifolia* essential oil was done using a Gas Chromatography-Flame Ionization Detector (GC-FID, Shimadzu model 2010 Plus) equipped with a capillary column DB-5 (30 m × 0.25 mm, film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate of 1mL min^{-1} (split mode). The oven temperature was 60 °C, then programmed at 3°C min^{-1} to 230°C with split mode (1:20) and injection volume was 0.3 μL. The injector and the detector temperatures were kept at 250 °C and 260 °C, respectively. The relative percentages of the individual components were calculated based on GC peak area (FID response) without using correction factors.

Gas Chromatography-Mass Spectrometry Analysis

The oil analysis was carried out using GC/MS (Focus-DSQ, Thermo) equipped with TG-5MS capillary column (30 m x 0.25 mm i.d.; film thickness 0.25 μm). Chromatographic conditions were as follows: injector temperature was 250 ºC, Helium as carrier gas at a flow-rate of 1 mL min^{-1} and injection volume was 0.2 μL (1000 ppm in hexane), respectively. The column temperature was 60 ºC and programmed at 3 ºC to 250 ºC min^{-1} and held for 5 min. with split ratio of 1:20. The MS transfer line and source temperatures were 270 ºC and 200 ºC. The GC column was coupled directly to single quadrupole mass spectrometer in EI mode at 70 eV with the mass range of 35-400 a.m.u at 1 scan/s. The identification of individual compounds was carried out on the basis of retention time and retention index using a homologous series of n-alkanes (C_8-C_{20}, Sigma-Aldrich) by comparing their mass spectra with NIST Mass Spectral Library (Version 2.0 d. 2005) studying the fragmentation patterns of individual components and literature (2).

Isolation and identification of main compound from oil

The *M. longifolia* essential oil (2 mL) was adsorbed on silica-gel (100 g, 100-200 mesh) and column chromatographed over silica-gel using hexane and the polarity of the eluant was increased using acetone. Elution of the column with hexane : acetone (98 : 2) gave a colourless viscous liquid (1.0 g).

***In-vitro* Nematicidal Bioassay**

(i). Preparation of test solutions

The test solution (10000 ppm) of essential oil and piperitenone oxide were prepared in distilled water with Tween-80 (3 %) and were mixed for 30 min using magnetic stirrer followed by its serial dilution to obtain 2000, 1000, 500, 250, 125, 62.5 and 31.25 ppm concentrations for nematicidal activity and stored at room temperature (25 ºC).

(ii). Isolation and purification of juveniles (J_2s) and eggs

The population of *M. incognita* was maintained on tomato plants (*Solanum lycopersicum* L. cv. Pusa Ruby) at Division of Nematology, ICAR-IARI, New Delhi, India in sick microplots. Heavily infected tomato plants were uprooted, and their roots were washed with running tap water to remove soil particles. Egg masses were handpicked by using sterile forceps and transferred to vial containing 0.5% (v/v) sodium hypochlorite (NaOCl) and shaken well for 3 min. The egg mass suspension was then passed through a series of filters with pore sizes of 74, 45 and 25 µm. Sterilized eggs that were retained on the 25 µm filter were collected with sterile distilled water (21) and allowed to hatch in modified Baermann funnels at 28 °C to get freshly hatched second stage juveniles (J_2s) (48).

(iii). Juvenile's mortality bioassay

It was done in 24 well tissue culture plate. The nematode suspension of 100 J_2s/10µL was poured into each well including control (31.25 to 1000 ppm) and 1mL concentrations of test solutions were added and mixed thoroughly. Tween-80 (3.0%) in distilled water and sterile distilled water were taken as negative control. Velum Prime® (Fluopyram 400 SC) nematicide at 500 ga.iha^{-1} was used as positive control. The experiments were done thrice and each treatment was replicated 4 times. Whole set up was incubated at 28±2 °C. Observations in each treatment were recorded after 24, 48, 72 and 96 h of exposure, all dead and alive J_2s were counted with counting dish under stereoscopic binocular microscope. Mortality of nematodes was determined by keeping immobile nematodes in fresh distilled water for 24 h and observed under stereoscopic binocular microscope. Mortality rates were calculated using Abbott's formula (1).

(iv). Egg hatching bioassay

Nematode egg suspension of 100 eggs/10µL was poured into 24 well tissue culture plate and 1 mL of different concentrations of test solutions were added and mixed. Tween-80 (3.0%) in distilled water and sterile distilled water were taken as negative control. Velum Prime® (Fluopyram 400 SC) at 500 g.a.iha^{-1} was taken as positive control. Whole set up was incubated at 28±2 °C. Observations on egg hatching were recorded at 2, 4, 6 and 8 days of exposure in each treatment. Hatching (%) was calculated by counting the number of hatched and unhatched eggs under stereoscopic binocular microscope.

***In-vivo* pot experiments**

Pot experiments were conducted in polyhouse (Centre for Protected Cultivation Technology, ICAR-IARI, New Delhi, India) in complete randomised design with 4-replications. Tomato seeds (NS 4266) were sown in horticultural nursery media, cocopeat: vermiculite: perlite (3: 1: 1). Irrigation and fertilizer were given as per the plants requirement and maintained for 21 days. Tomato seedlings of 4 to 5 leaf stage were transplanted into 15 cm size earthen pots containing steam-sterilized soil (sand: soil, 1:1, w/w). Two experiments were conducted in polyhouse. First, tomato roots were dipped in two different concentrations (2000 and 1000 ppm) of test solutions of *M. longifolia* essential oil and piperitenone oxide for 15 min. Treated seedlings were transplanted into earthen pots inoculated with *M. incognita* juveniles @ 2 J_2s/cc of soil (2 days post transplanting). Another experiment was done, where 2-concentrations (2000 and 1000 ppm) of test solutions of *M. longifolia* essential oil and piperitenone oxide were applied twice as soil drench (100 $mLpot^{-1}$) : first, 1-day before inoculation (2 J_2s/cc of soil) and another 7-days after the first treatment and later on, 21 days old tomato seedlings were transplanted into earthen pots with treated soil. Irrigation, fertilizer and other agronomic practices were given as per the crop requirement. Chemical (non-fumigant nematicide) Velum Prime® (Fluopyram) is a succinate dehydrogenase inhibitor that blocks cellular respiration, causing nematode death (17) was used as positive control. Each treatment was replicated four times. After twelve weeks of nematode inoculation, the tomato plants were uprooted and washed gently with running water to remove adhering soil. Plant growth [shoot length (cm), shoot weight (g), root length (cm), root weight (g)] and nematode infestation (number of galls/root, number of egg masses/root) parameters were recorded.

Statistical analysis

All the statistical analyses (ANOVA) were performed using STAR v. 2.0.1. Tukey´s honest significant difference (HSD) tests ($P < 0.05$). Data from four replicates were pooled and analysed. LC_{50}(ppm) were calculated using Probit analysis (SPSS statistical package, v 16.0)

RESULTS AND DISCUSSION

Chemical profiling of essential oil

The aerial parts of *M. longifolia* contained 1.17 % essential oil on fresh weight basis by hydrodistillation method. GC-MS analysis of the essential oil showed the presence of 16-compounds, representing 84.53 % of the oil (Table1 and Figure 1). The oil was rich in oxygenated monoterpenes (64.91 %), followed by sesquiterpenes hydrocarbons (1.62 %), monoterpenes hydrocarbons (1.4 %) and other compounds. piperitenone oxide (62.91 %) was the major compound in the oil. While other compounds were: limonene (0.88 %), germacrene-D (0.7 5%), 1,8-cineole (0.75 %), piperitenone (0.37 %), 2-methyl-3-phenylpropanal (0.36 %), linalool propanoate (0.33 %) and p-mentha-6,8-diene-2-one (0.23 %) and 17 % compounds remained unidentified.

Table 1. Chemical constituents of the essential oil of *Mentha longifolia L.*

Compound	KI*	KI	Amount (%)
α-Thujene	924	922	0.06
α-Pinene	932	930	0.10
Sabinene	975	974	0.14
Myrcene	990	991	0.18
Octanol	988	989	0.10
p-Cymene	1024	1026	0.04
Limonene	1029	1028	0.88
1,8-Cineole	1031	1030	0.75
4-Terpineol	1177	1178	0.23
trans-dihydrocarveol	1193	1191	0.10
Linalool propanoate	1377	1379	0.33
p-Mentha-6,8-diene-2-one	1423	1422	0.23
Piperitenone	1340	1341	0.37
Piperitenone oxide	1366	1367	62.9
Unidentified	-	-	17.0
Germacrene-D	1484	1486	0.75
trans-Calamenene	1522	1524	0.22
Monoterpenes hydrocarbons	-	-	1.4
Oxygenated monoterpenes	-	-	64.91
Sesquiterpenes hydrocarbons	-	-	1.62
Alkane hydrocarbon derivatives	-	-	1.0
Unidentified	-	-	17.0
*Adams, 2007, KI= Kovat's Index calculated using C_8-C_{20} hydrocarbons in DB-5 capillary column			

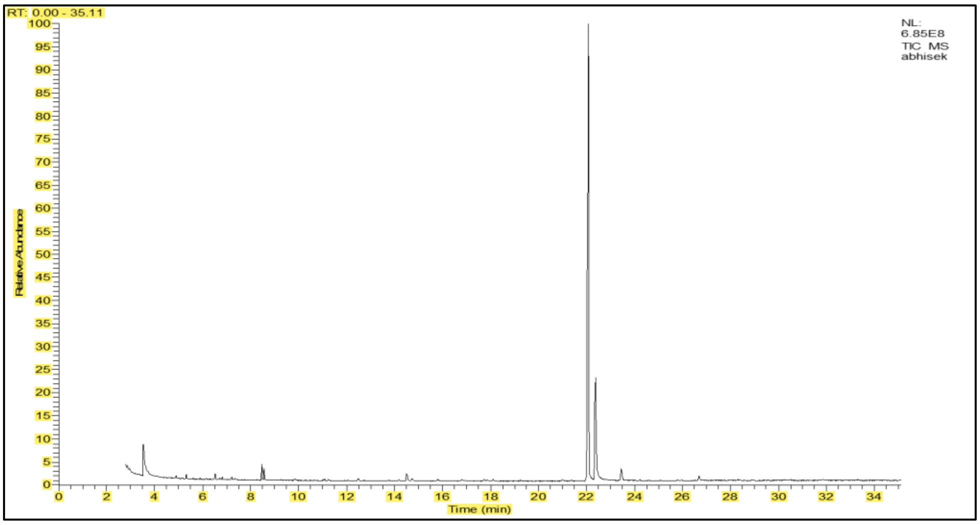

Figure 1. Total ion chromatogram of the essential oil of *Mentha longifolia* L.

Isolation and identification of main compound from oil

A pure compound piperitenone oxide (PO) (1.0 g) was isolated and identified as by GC-MS and NMR from the essential oil of *M. longifolia* by column chromatography over silica-gel using hexane-acetone (98:2) mixture. EI-MS spectrum of this isolated compound showed a molecular ion peak at *m/z* 166.0, corresponding to the molecular formula $C_{10}H_{14}O_2$ in GC-MS analysis. NMR spectra of the compound also confirmed its identity. Its NMR spectral data were found similar to reported earlier (41). Its identity was further confirmed by comparing the mass fragmentation pattern and Kovat's index by GC-MS analysis and literature (2) and based on above study the compound was identified as piperitenone oxide (PO) (Figure 2). In ^{1}HNMR spectrum (Figure 3), a singlet appeared at δ 3.25 ppm (1H, *s*) was assigned to the proton attached to C-2 proton while those appeared at δ 2.65-2.27 ppm (2H, *m*) and δ1.84 2.17 ppm (1H, *m*) were linked to C-5 and C-6 carbon atoms in its HSQC spectrum. Three singlets appeared at δ 1.81, 1.89 and δ2.11 ppm showed the presence of three methyl groups in the compound. ^{13}CNMR spectrum of the compound showed the presence of ten carbon atoms and a peak appeared at δ 198 ppm showed the presence of carbonyl group in the compound. Its DEPT spectra showed the presence of three methyl, two methylene, one methine and four quaternary carbon atoms. The linkage of each proton to its carbon atom was confirmed using HSQC and HMBC correlation studies (Table 2).

Table 2. ^{1}H-NMR (400MHz) and ^{13}C-NMR (100MHz, in $CDCL_3$) values of piperitenone oxide with DEPT and HMBC correlations

H/C	^{1}H-NMR value (δ)	^{13}C NMR value (δ)	DEPT	HBMC correlations
1	-	63.3	C	-
2	3.25 (s)	63.5 (s)	CH	C-1, C-3, C-4, C-7, C-8
3	-	198.5	C	-
4	-	149.3	C	-
5	2..65-2.27 (2H, m)	23.1(m)	CH2	C-1, C-3, C-4, C-6, C-8
6	1.84-1.93(1H_a, m) 2.12-2.17(1H_b, m)	27.8 (m)	CH2	C-1, C-4, C-5
7	1.81(3H, s)	21.8 (m)	CH3	C-1, C-2, C-6,
8	-	127.6	C	-
9	1.89(3H, s)	23.1(s)	CH3	C-1, C-3, C-4, C-7, C-8
10	2.11(3H, s)	23.1 (s)	CH3	C-3, C-4, C-5, C-8

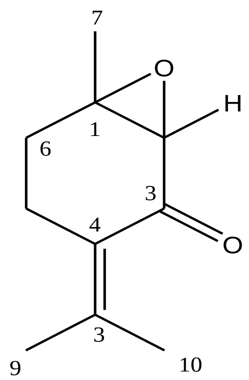

Figure 2. Structure of piperitenone oxide

We did chemical profiling of essential oil to know its major and minor compounds, which had the nematicidal activity against *M. incognita*. As previously reported, PO was the main constituent of mint oil in various species of Lamiaceae family and its contents varied with species e.g. *Mentha x villosa* (55.4 to 98 %) (43), *M. microphylla* (32.9 % to 65 %) (45), *M. spicata* (94.8 %) (37), *M. rotundifolia* (80.8 %) (40), *M. longifolia* (14.7 % to 83.7 %) (25), *M. aquatica* var. *crispa* (91.2 %) from South Vietnam (44), *Satureja parvi folia* (69.8 %) from Argentina (11), endemic *S. kallarica* (71.2%) from Iran (18), *Calamintha incana* (66.6 %) from Turkey (47), *C. nepeta* ssp. *glandulosa* (52 %) from Belgium (28). Apart from Lamiaceae family, PO is also predominant constituent of *Lippia* spp. of Verbenaceae ranging from 22.9 % to 72 % (14). Apart from natural sources, PO can be produced by chemical synthesis by epoxidation of piperitenone, condensation of mesityl oxide in tetrahydrofuran (THF) with methyl vinyl ketone in THF and a solution of Triton-B and by several other chemical routes (9,35).

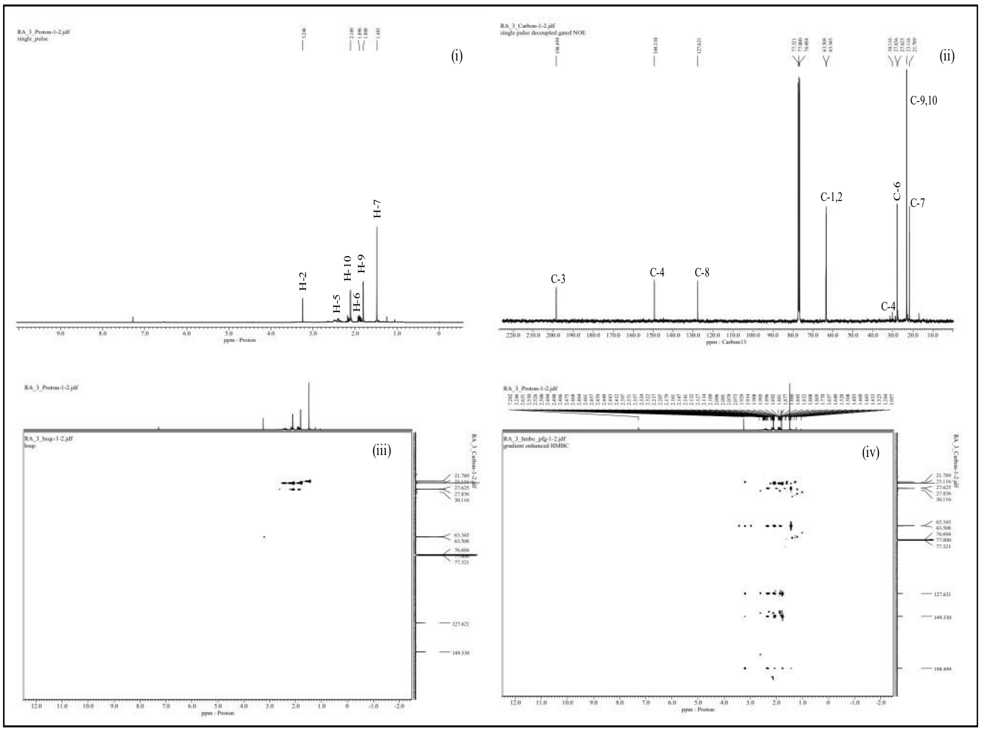

Figure 3. NMR spectra (i. ^{1}H-NMR, ii. ^{13}C-NMR, iii. HSQC and iv. HMBC) of isolated compound (in $CDCl_3$)

PO of *Mentha* species possess different biological activities like cardiovascular, hypotensive, bradycardic, insecticidal, trypanocidal, schistosomicidal, antimicrobial and antinociceptive properties (10). It exhibited central analgesic activity in mice and rats (19) highly toxic to larvae of mosquito spp. including the *Aedes aegypti* (46), trypanocidal activity against epimastigote and trypomastigote forms of *Trypanosoma cruzi*, decreased

Table 3. Effects of Essential Oil (EO) and piperitenone oxide (PO) on J_2s mortality and egg hatching of *M. incognita* at different concentrations and exposure period

Conc. (ppm)	24 HAT	48 HAT	72 HAT	96 HAT	2 DAT	4 DAT	6 DAT	8 DAT
	J2s mortality (%)				**Egg hatching inhibition (%)**			
Essential Oil								
1000	36.0^{b} ± 1.3	54.7^{b} ± 0.7	70.7^{b} ± 0.9	80.7^{b} ± 0.9	100.0^{a}± 0.0	100.0^{a}± 0.0	100.0^{a}± 0.0	96.0^{a}± 0.4
500	26.5^{c} ± 1.0	45.7^{c} ± 1.1	54.5^{c} ± 0.6	75.2^{c} ± 0.5	96.0^{b}± 0.4	95.2^{b}± 0.5	91.7^{b}± 0.5	87.2^{b}± 0.6
250	22.5^{d} ± 0.6	40.0^{d} ± 0.9	50.5^{d} ± 0.9	67.2^{d} ± 0.6	81.7^{c}± 1.1	80.0^{c}± 0.9	77.5^{c}± 0.6	75.2^{c}± 0.7
125	11.7^{e} ± 0.8	22.7^{e} ± 0.9	42.0^{e} ± 0.9	52.5^{e} ± 0.6	77.2^{d}± 0.6	76.0^{d}± 0.7	75.0^{c}± 0.7	70.5^{d}± 0.6
62.5	8.25^{f} ± 0.5	15.5^{f} ± 0.6	31.7^{f} ± 0.8	45.2^{f} ± 0.5	70.2^{e}± 1.1	69.2^{e}± 1.3	66.2^{d}± 1.0	61.2^{e}± 0.9
31.25	5.5^{f} ± 0.3	9.5^{g} ± 0.6	19.5^{g} ± 0.6	32.7^{g} ± 0.6	65.2^{f}± 0.9	60.5^{f}± 0.6	61.2^{e}± 1.4	58.5^{e}± 0.9
Piperitenone oxide								
1000	81.0^{b} ± 0.8	97.25^{a} ± 0.9	100.0^{a} ± 0.0	100.0^{a} ± 0.0	100.0^{a}± 0.0	100.0^{a}± 0.0	100.0^{a}± 0.0	100.0^{a} ± 0.0
500	72.5^{c} ± 0.6	91.0^{b} ± 0.8	100.0^{a} ± 0.0	100.0^{a} ± 0.0	100.0^{a}± 0.0	100.0^{a}± 0.0	100.0^{a}± 0.0	100.0^{a} ± 0.0
250	43.5^{d} ± 1.0	54.0^{c} ± 1.3	82.0^{b} ± 1.3	97.0^{a} ± 1.1	94.2^{b}± 0.5	92.0^{b}± 0.6	91.5^{b}± 0.6	90.2^{b}± 0.5
125	30.7^{e} ± 0.9	43.0^{d} ± 1.1	51.0^{c} ± 0.8	88.0^{b} ± 2.1	90.7^{c}± 0.9	88.5^{c}± 1.0	86.7^{c}± 0.5	84.7^{c}± 0.5
62.5	26.7^{f} ± 1.3	32.5^{e} ± 0.6	38.7^{d} ± 0.9	61.5^{c} ± 1.0	88.2^{d}± 0.5	86.0^{c}± 0.4	82.7^{d}± 1.0	79.7^{d}± 0.5
31.25	21.0^{g} ± 0.8	29.2^{e} ± 0.9	31.5^{e} ± 1.0	53.7^{d} ± 1.3	86.0^{e}± 0.4	83.2^{d}± 0.9	79.2^{e}± 0.9	78.5^{d}± 0.6
T-80 (3%)	0.0^{h} ± 0.0	0.0^{f} ± 0.0	0.0^{f} ± 0.0	0.0^{e} ± 0.0	34.7^{b}± 0.9	23.2^{b}± 0.9	15.5^{b}± 1.0	14.0^{b} ± 1.1
SDW	0.0^{h} ± 0.0	0.0^{f} ± 0.0	0.0^{f} ± 0.0	0.0^{e} ± 0.0	28.2^{c}± 0.7	17.2^{c}± 0.6	15.2^{b}± 0.5	9.7^{c}± 0.5
Velum Prime®	91.0^{a} ± 0.8	95.7^{a} ± 0.5	100.0^{a} ± 0.0	100^{a} ± 0.0	100.0^{a}± 0.0	100.0^{a}± 0.0	100.0^{a}± 0.0	100.0^{a} ± 0.0

Data is mean of 4- replicates ± SE. According to Tukey's honest significant difference (HSD) test, the same letter indicates data in each column is not significantly different ($p < 0.05$). HAT: hours after treatment, DAT: days after treatment, SDW: sterile distilled water, T-80: tween 80.

adult worms motility of *Schistosoma mansoni* (Plathelminths) at 70.96 µgmL^{-1} until 96 h of observation (33), induced hypotensive, bradycardic and acute cardiovascular effects in rats (19), antibacterial activity against *Staphylococcus aureus* and antifungal activity against *Candida albicans* (6).

***In-vitro* nematicidal activity of essential oil and piperitenone oxide on J_2s mortality**

Essential Oil (EO) and piperitenone oxide (PO) nematicidal activity were evaluated in *in-vitro* (Table 3). The highest juvenile mortality rate of 80.75 % and 100 %, respectively was achieved at 1000 ppm after 96 h., remaining concentrations mortality rate ranged from 45.25 % to 75.25 % and 61.50% to 100 %, respectively at 62.5 to 500 ppm after 96 h. with $LC_{50/96h}$ (Table 4) values of 92.74 and 34.15 ppm, respectively. The immobilized juveniles were transferred to distilled water for their revival test but none of them revived in any of the above concentrations after 96 h. The PO pure compound proved more lethal to *M. incognita* than crude essential oil and the rate of mortality was directly proportional to its concentration and exposure period (Figure 4). There was no mortality in Tween 80 (3%) and sterile distilled water control. Chemical (non-fumigant nematicide) Velum Prime®(Fluopyram) is a succinate dehydrogenase inhibitor that blocks cellular respiration, causing nematode death (17) acted as positive control with $LC_{50/96h}$ value of 3.46 ppm (Table 4).

Table 4. Comparative toxicity (LC_{50}) of Essential Oil (EO) and piperitenone oxide (PO) in comparison with Velum prime® as a positive control on *M. incognita* J_2s. LC_{50} (ppm) were calculated using SPSS statistical package (v 16.0)

Test samples	Exposure period (h)	Heterogeneity		*LC_{50}	Fiducial limit (ppm)	
		Chi-Square Tests (χ2)	df	ppm	Min.	Max.
Essential Oil	24	0.82	4	2578.26	1405.69	7404.15
	48	1.86	4	645.3	472.21	993.7
	72	1.97	4	260.7	196.3	361.58
	96	0.7	4	92.74	65.75	122.55
Piperitenone oxide	24	10.16	4	221.65	136.26	390.55
	48	28.61	4	116.59	45.18	237.17
	72	23.75	4	78.63	37.89	130.25
	96	7.04	4	34.15	19.03	47.72
Velum Prime®	24	1.43	4	0.05	0	1.76
	48	0.16	4	0.14	0	2.19
	72	1.42	4	1.99	0.09	6.77
	96	2.76	4	3.46	0.17	9.55

*LC_{50} = Lethal concentration calculated to give 50 % mortality, EO: Essential oil, PO: piperitenone oxide

***In-vitro* bioassay of essential oil and piperitenone oxide on egg hatching**

The effects of EO and PO were evaluated on egg-hatching of *M. incognita* (Table 3). Results revealed that the hatching was merely 4 % and 0 % respectively, at 1000 ppm 8 days after incubation. Remaining concentrations of 500 to 62.5 ppm also showed lower hatching rates:12.75 % to 38.75 % and 0.00 % to 20.25 %, respectively after 8 days of incubation with $LC_{50/8}$ days (25.79 and 8.72 ppm, respectively) (Table 5). The unhatched

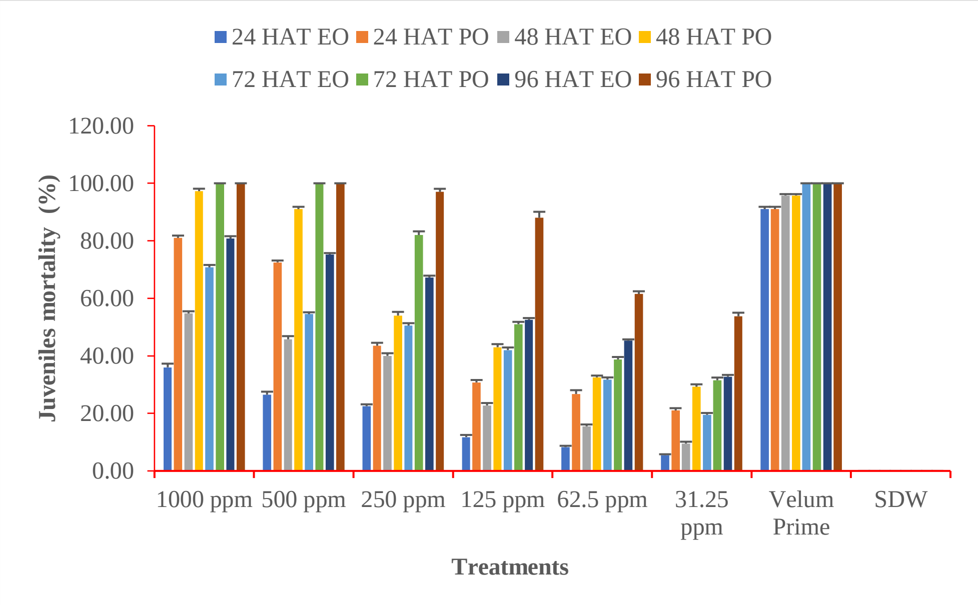

Figure 4. Effects of Essential Oil (EO) and piperitenone oxide (PO) on *M. incognita* J_2s mortality. HAT: hours after treatment, SDW: sterile distilled water

Table 5. Comparative toxicity (LC_{50}) of Essential Oil (EO) and Piperitenone Oxide (PO) in comparison to Velum prime® as a positive control on egg hatching inhibition of *M. incognita*. LC_{50} (ppm) were calculated using SPSS statistical package (v 16.0)

Test samples	Exposure period (days)	Heterogeneity		*LC_{50}	Fiducial limit (ppm)	
		χ2	df	ppm	Min.	Max.
Essential Oil	2	11.44	4	19.05	1.04	44.17
	4	11.18	4	23.27	2.45	49.33
	6	11.9	4	23.04	1.422	52.47
	8	5.88	4	25.79	13.03	39.82
Piperitenone oxide	2	6.37	4	3.35	0.36	9
	4	8.93	4	4.52	0	19.16
	6	8.94	4	7.54	0.03	23.55
	8	11.4	4	8.72	0	27.84
Velum Prime®	2	1.91	4	3.77	0.19	10.07
	4	5.54	4	1.96	0.03	7.17
	6	5.53	4	1.8	0.02	6.92
	8	5.53	4	1.8	0.02	6.92

*LC_{50} = Lethal concentration calculated to give 50 % hatching inhibition, EO: Essential oil, PO: piperitenone oxide

eggs were transferred to distilled water for hatching test but no juveniles emerged at 250-1000 ppm after 8 days, however only few juveniles emerged with low mobility at 32.5 to 125 ppm concentrations after 8 days. Egg hatching of *M. incognita* was dose-dependent on EO and PO (Figure 5). The emergence of J_2s were inversely related to concentration and exposure period. Hatching was high (86.00 % and 90.25 %), in Tween 80 (3 %) and sterile distilled water, respectively as negative controls. Chemical (non-fumigant nematicide) Velum Prime®(Fluopyram) acted as positive control with $LC_{50/8\ days}$ value of 1.80 ppm (Table 5).

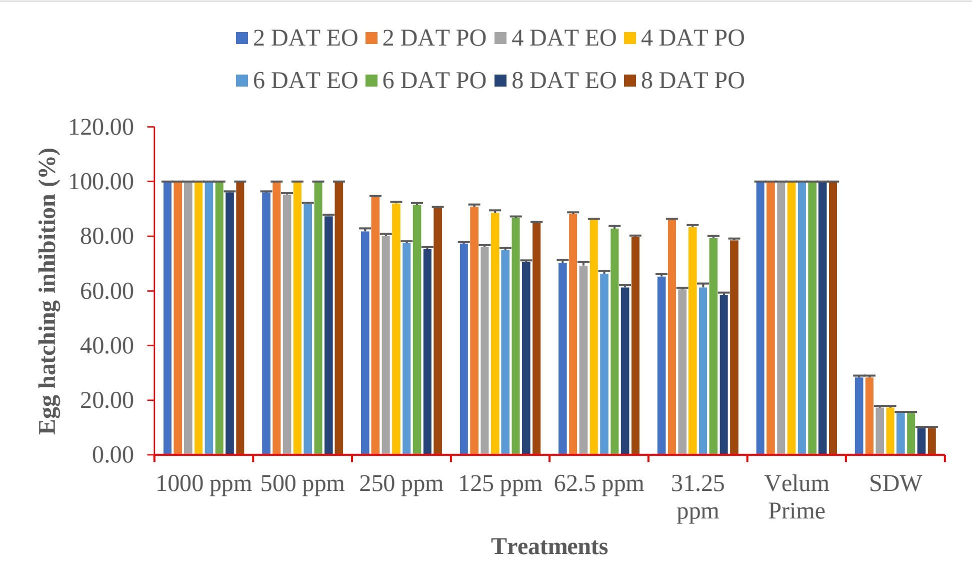

Figure 5. Effects of Essential Oil (EO) and piperitenone oxide (PO) on *M. incognita* egg hatching. DAT: Days after treatment, SDW: Sterile distilled water

***In-vivo* nematicidal activity of essential oil and piperitenone oxide**

Based on the *in-vitro* nematicidal activity, the disease control efficacy of EO and PO (Table 6) were assessed for control of root-knot nematode (*M. incognita*) disease in tomato grown in pots in polyhouse. In bare root dip treatment, the EO and PO test solutions effectively suppressed the nematode infestation on tomato roots with 13 galls/root, 15.5 egg masses/root and 9.5 galls/root, 11 egg masses/root, respectively. Whereas, EO and PO applied as soil drenching at 2000 ppm, suppressed the nematode infestation (10.75 galls/root, 13.7 egg masses/root and 7.00 galls/root, 9.7 egg masses/root, respectively) and stimulated the tomato shoot and root biomass (Table 6) 12-weeks after inoculation. Soil drenched with EO and PO reduced the nematode infestation and enhanced the plant biomass than bare root dip treated plants (Figures 6 and 7). However, maximum nematode infestation (43.2 galls/root, 52.5 egg masses/root) and reduction in plant growth (95 cm) were in untreated tomato plants. Whereas, soil dressed with Velum Prime® (Fluopyram)

Table 6. Effects of bare root dipping and soil drenching with Essential Oil (EO) and piperitenone oxide (PO) on the infestation of the root-knot nematode *M. incognita* and the growth of tomato (cv. NS 4266)

Conc. (ppm)	Nematode infestation parameters		Plant growth parameters			
	Number of galls/root	Number of egg masses/ root	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
Bare root dip treatment						
EO- 2000	13.0^{c} ± 0.8	15.5^{c} ± 0.6	127.75^{ab} ± 1.10	35.5^{a} ± 0.6	212.2^{a} ± 1.9	21.0^{cd} ± 0.5
PO- 2000	9.5^{b} ± 0.6	11.0^{bc} ± 0.7	137.0^{a} ± 0.8	38.0^{a} ± 0.4	217.7^{a} ± 1.1	23.2^{abc} ± 0.6
EO- 1000	19.2^{b} ± 0.6	20.5^{b} ± 0.6	122.5^{b} ± 0.64	32.5^{b} ± 0.6	199.7^{b} ± 4.2	19.4^{d} ± 0.3
PO- 1000	12.7^{b} ± 0.6	13.2^{b} ± 1.0	129.0^{b} ± 0.9	33.5^{b} ± 1.0	207.2^{b} ± 1.4	21.4^{c} ± 0.3
Soil drenching						
EO- 2000	10.7^{c} ± 0.9	13.7^{d} ± 0.9	134.7^{a} ± 1.7	34.7^{a} ± 0.6	211.7^{a} ± 1.8	22.1^{b} ± 0.4
PO- 2000	7.0^{b} ± 0.8	9.7^{c} ± 0.5	140.5^{a} ± 1.0	37.5^{a} ± 0.9	215.2^{a} ± 1.5	23.5^{ab} ± 0.3
EO- 1000	14.7^{b} ± 0.5	19.7^{c} ± 1.1	124.2^{b} ± 1.4	33.2^{ab} ± 0.5	210.2^{a} ± 3.4	20.3^{c} ± 0.4
PO- 1000	9.7^{b} ± 0.5	11.0^{c} ± 0.4	134.0^{ab} ± 1.3	35^{ab} ± 0.9	212.7^{a} ± 1.5	22.1^{c} ± 0.3
UIC	43.2^{a} ± 1.3	52.5^{a} ± 1.7	95.5^{c} ± 1.5	25.7^{c} ± 0.8	153.5^{c} ± 2.8	23.7^{ab} ± 0.3
UNIC	0.0^{d} ± 0.0	0.0^{d} ± 0.0	111.2^{b} ± 2.3	28.2^{b} ± 0.5	168.0^{b} ± 2.1	23.1^{bc} ± 0.4
Tween 80 (3%)	39.7^{b} ± 1.3	45.0^{b} ± 1.0	93.7^{c} ± 1.1	23.7^{c} ± 0.9	159.5^{c} ± 2.3	22.8^{c} ± 0.1
Velum Prime®	6.5^{c} ± 0.3	5.2^{b} ± 0.6	129.5^{a} ± 1.8	31.7^{a} ± 0.9	214.5^{a} ± 1.7	24.5^{a} ± 0.3

Data shown correspond to the mean of four replicates ± SE. According to Tukey's honest significant difference (HSD) test, the same letter indicates data in each column is not significantly different ($p < 0.05$). T-80: tween 80, EO: Essential oil, PO: piperitenone oxide, UIC: Untreated inoculated control, UNIC: Untreated non inoculated control.

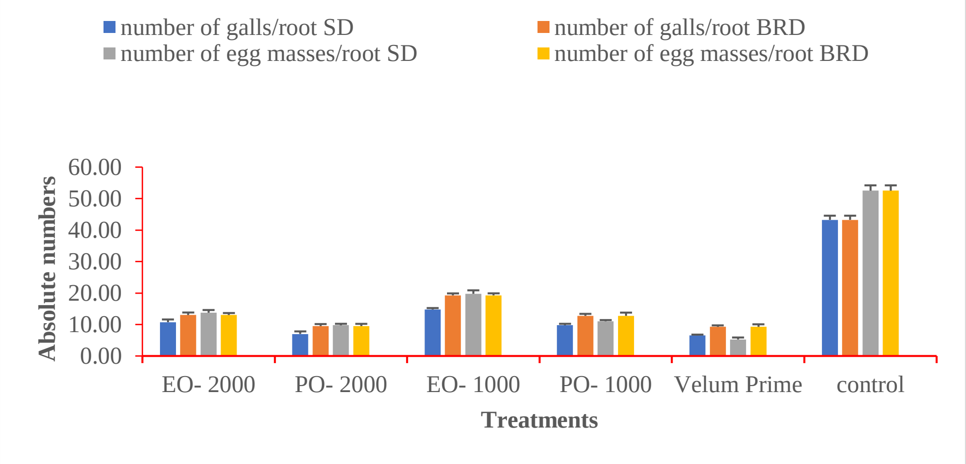

Figure 6. Effects of bare root dipping and soil drenching with Essential Oil (EO) and piperitenone oxide (PO) on the infestation of the root-knot nematode *M. incognita*. SD: soil drenching, BRD: bare root dipping

as positive control decreased the infestation (6.5 galls/root, 5.2 egg masses/root) of *M. Incognita* and increased plant growth and biomass. The disease control efficacy of PO was significantly higher than Velum Prime® (Fluopyram).

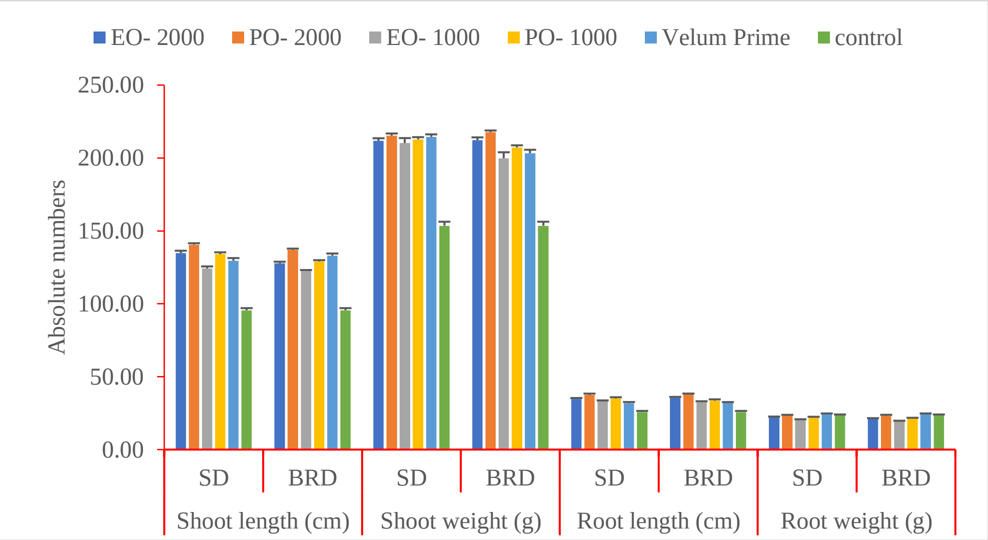

Figure 7. Effects of bare root dipping and soil drenching with Essential Oil (EO) and piperitenone oxide (PO) on the growth of tomato (cv. NS 4266). SD: soil drenching, BRD: bare root dipping

Essential oils containing PO, Lactone group and other constituents possesses nematicidal potential. The essential oils of *Lippia turbinate* and *L. juneliana* (Lamiaceae) at 666 µL L^{-1} concentration with piperitenone oxide (24.8 % and 36.5 %, respectively) as main constituents caused juvenile mortality (99.66 % and 96.79 %, respectively) of *Meloidogyne* spp. (15). Aqueous extracts and essential oils *Mentha* × *piperita*, *Mentha spicata* ($EC_{50/72h}$ of 358 mgL^{-1}), and *Mentha pulegium* containing abundant terpenes [menthofuran ($EC_{50/48h}$ of 127 mgL^{-1}) isomenthone, menthone, menthol, pulegone, and carvone ($EC_{50/48h}$ of 730 mgL^{-1})] exhibited significant nematicidal activity against *Meloidogyne incognita* (12). The essential oils of *Alpinia galanga* (100 %), Caraway (22.3%), *Eugenia caryophyllata* (9.4 %), *Cinnamonum zeylanicum* (7.2 %), *Mentha pulegium* (2.4 %) and *Foeniculum vulgare* (2.1%) are nematicidal to eggs of *Meloidogyne hapla* (25). Whereas, essential oils of *Mentha arvensis*, *M. spicata*, *S. montana*, *Thymus vulgaris* and *T. zygis* strongly suppressed egg hatching (86-99 %) in *M. javanica* 5 days after incubation (5). Essential oils of *Eucalyptus camaldulensis*, *E. saligma* and *E. urophylla* caused mortality and inhibited the hatching of *Meloidogyne exigua* infesting coffee (30). Essential oils of *Lippia alba*, *L. sidoides* and *Cymbopogon citratus* showed *in-vitro* nematicidal effects against *M. incognita* race 1, by reducing the hatching and increasing the mortality of hatched juveniles and it was due to the presence of substances viz., thymol, carvacrol, citronellal, citral, geraniol, linalool and limonene constituents (16). The essential oil and their constituents are nematicidal due to the presence of phenols, aldehydes and alcohols, which may interact with the cytoplasmic membrane and damages the structure of polysaccharides, lipids, phospholipids and promoting membrane depolarization, such as those of the mitochondria (5). Monoterpenoids (borneol, carveol, citral, geraniol and α-terpineol) exhibited anti-hatching and juvenile mortality of *M. incognita* mainly due to terpenoids in their chemical structure and reactive groups (16). Citronellol and geraniol significantly reduces root galling induced by *M. incognita* and *M. javanica* (16). Essential oil from *Artemisia absinthium*, *Citrus bergamia*, *Eucalyptus citriodora*, *Hypericum perforatum*, *Lavandula officinalis*, *Mentha arvensis*, *Ocimum basilicum*, *Piper nigrum*, *Thymus serpylluma* and *Zingiber officinale* were tested against *Meloidogyne incognita* on tomato plants in greenhouse conditions, these significantly enhanced the plant growth and reduced the root galling and egg mass formation at 5 % (36). Essential oils of *Cinnamomum camphora* (L.) J. Presl. (camphorwood), *C. verum* J. Presl. (cinnamom), *Eucalyptus citriodora* Hook (lemon eucalyptus), *E. globulus* Labill. (Blue gum), *Mentha piperita* L. (peppermint), *Pelargonium asperum* Ehrh. ex Willd. (*Bourbon geranium*), *Ruta graveolens* L. (common rue), *Schinus molle* L. (false pepper) and *Syzygium aromaticum* (L.) (clove) are highly larvicidal, ovicidal and suppressive to *M. incognita* infection in tomato plants and also improved the plant growth (13).

CONCLUSIONS

The essential oil from the aerial parts of *Mentha longifolia* are rich in piperitenone oxide. *In-vitro* purified piperitenone oxide from its crude oil showed promising nematicidal activity against *M. incognita*. Nematicidal activity of piperitenone oxide was reported for the first time in this study and was found more active than crude oil. Further, nematicidal activity of piperitenone oxide is required at field level so that it can be used for the development of nematicide of plant origin as an alternative to synthetic and toxic nematicides for nematode management.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, Joint Director and Dean (Education), ICAR-Indian Agricultural Research Institute, New Delhi, Delhi 110012, India - for providing facilities.

DECLARATION

We declare that all authors of this Ms have made substantial contributions. We have not excluded any author that substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

REFERENCES

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18:** 265-267.
2. Adams, R.P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry* (Vol. 456). Allured publishing corporation, Carol Stream, IL.
3. Aissani, N., Balti, R. and Sebai, H. (2018). Potent nematicidal activity of phenolic derivatives on *Meloidogyne incognita*. *Journal of Helminthology* **92:** 668-673.
4. Almeida, R.N., Hiruma, C.A. and Barbosa-Filho, J.M. (1996). Analgesic effect of rotundifolone in rodents. *Fitoterapia* (Milano) **67:** 334-338.
5. Andrés, M.F., González-Coloma, A., Sanz, J., Burillo, J. and Sainz, P. (2012). Nematicidal activity of essential oils: a review. *Phytochemistry Reviews* **11:** 371-390.
6. Arruda, T., Antunes, R.M., Catão, R.M., Lima, E. O., Sousa, D.P., Nunes, X.P. and da Cunha, E.V. (2006). Preliminary study of the antimicrobial activity of *Mentha* x *villosa* Hudson essential oil, rotundifolone and its analogues. *Revista Brasileira de Farmacognosia* **16:** 307-311.
7. Banaras, S., Javaid, A. and Khan, I.H. (2020). Potential antifungal constituents of *Sonchus oleraceous* against *Macrophomina phaseolina. International Journal of Agriculture and Biology* **24:** 1376-1382.
8. Baratta, M.T., Dorman, H.D., Deans, S.G., Figueiredo, A.C., Barroso, J.G. and Ruberto, G. (1998). Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal* **13:** 235-244.
9. Bergmann, E. and Bracha, P. (1959). A simple synthesis of piperitenone. *The Journal of Organic Chemistry* **24:** 994-995.
10. Božović, M., Pirolli, A. and Ragno, R. (2015). *Mentha suaveolens* Ehrh. (Lamiaceae) essential oil and its main constituent piperitenone oxide: Biological activities and chemistry. *Molecules* **20:** 8605-8633.
11. Cabana, R., Silva, L.R., Valentão, P., Viturro, C.I. and Andrade, P.B. (2013). Effects of different extraction methodologies on the recovery of bioactive metabolites from *Satureja parvifolia* (Phil.) (Lamiaceae). *Industrial Crops and Products* **48:** 49-56.
12. Caboni, P., Saba, M., Tocco, G., Casu, L., Murgia, A., Maxia, A. and Ntalli, N. (2013). Nematicidal activity of mint aqueous extracts against the root-knot nematode *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* **61:** 9784-9788.
13. D'Addabbo, T., Argentieri, M.P., Laquale, S., Candido, V. and Avato, P. (2020). Relationship between chemical composition and nematicidal activity of different essential oils. *Plants* **9:** 1546.
14. de Abreu Mates, F.J., Machado, M.I.L., de Vasconcelos Silva, M.G., Craveiro, A.A. and Alencar, J.W. (2000). Essential oils of *Lippia alnifolia* Schau. (Verbenaceae) and *Lippia*a *gracillis* HBK, two aromatic medicinal shrubs from northeast Brazil. *Journal of Essential Oil Research* **12:** 295-297.
15. Duschatzky, C.B., Martinez, A.N., Almeida, N.V. and Bonivardo, S.L. (2004). Nematicidal activity of the essential oils of several Argentina plants against the root-knot nematode. *Journal of Essential Oil Research* **16:** 626-628.
16. Echeverrigaray, S., Zacaria, J. and Beltrão, R. (2010). Nematicidal activity of monoterpenoids against the root-knot nematode *Meloidogyne incognita*. *Phytopathology* **100:** 199-203.
17. Feist, E., Kearn, J., Gaihre, Y., O'Connor, V. and Holden-Dye, L. (2020). The distinct profiles of the inhibitory effects of fluensulfone, abamectin, aldicarb and fluopyram on *Globodera pallida* hatching. *Pesticide Biochemistry and Physiology* **165:** 104541.
18. Ghasemi Pirbalouti, A., Vosoghi, N., Craker, L. and Shirmardi, H.A. (2014). Chemical composition of the essential oil of *Satureja kallarica* Jamzad. *Journal of Essential Oil Research* **26:** 228-231.
19. Guedes, D.N., Silva, D.F., Barbosa-Filho, J.M. and Medeiros, I.A. (2002). Muscarinic agonist properties involved in the hypotensive and vasorelaxant responses of rotundifolone in rats. *Planta Medica* **68:** 700-704.
20. Hendriks, H. and Van Os, F.H. (1976). Essential oil of two chemotypes of *Mentha suaveolens* during ontogenesis. *Phytochemistry* **15:** 1127-1130.
21. Hussey, R.S. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* **57:** 1025-1028.
22. Iqbal, T., Hussain, A.I., Chatha, S.A.S., Naqvi, S.A.R. and Bokhari, T.H. (2013). Antioxidant activity and volatile and phenolic profiles of essential oil and different extracts of wild mint (*Mentha longifolia*) from the Pakistani Flora. *Journal of Analytical Methods in Chemistry*, 2013.
23. Javaid, A. and Khan, I.H. (2016). Management of collar rot disease of chickpea by extracts and soil amendment with dry leaf biomass of *Melia azedarach* L. *Philippine Agricultural Scientist* **99:** 150-155.

24. Javaid, N., Shah, M.H., Khan, I.H., Javaid, A. and Waleed, S.M. (2020). Herbicidal activity of *Ageratum conyzoides* against parthenium. *Pakistan Journal of Weed Science Research* **26:** 137-146.
25. Jeon, J.H., Ko, H.R., Kim, S.J. and Lee, J.K. (2016). Chemical compositions and nematicidal activities of essential oils on *Meloidogyne hapla* (Nematoda: Tylenchida) under laboratory conditions. *The Korean Journal of Pesticide Science* **20:** 30-34.
26. Khan, I.H. and Javaid, A. (2019). Antifungal, antibacterial and antioxidant components of ethylacetate extract of quinoa stem. *Plant Protection* **3:** 125-130.
27. Khan, I.H., Javaid, A., Al-Taie, A.H. and Ahmed, D. (2020). Use of neem leaves as soil amendment for the control of collar rot disease of chickpea. *Egyptian Journal of Biological Pest Control* **30:** 98.
28. Kirimer, N., Baser, K.H.C., Özek, T. and Kürkçüoglu, M. (1992). Composition of the essential oil of *Calamintha nepeta* subsp. *glandulosa*. *Journal of Essential Oil Research* **4:** 189-190.
29. Kumar, V., Khan, M.R. and Walia, R.K. (2020). Crop loss estimations due to plant-parasitic nematodes in major crops in India. *National Academy Science Letters* **43:** 409-412.
30. Lima salgado, S.M., Campos, V.P., Das gracascardoso, M. and Soares salgado, A.P. (2003). Eclosão e mortalidade de juvenis de segundoestádio de *Meloidogyne exigua*emóleosessenciais. *Nematologia Brasileira* **27:** 17-22.
31. Lu, H., Xu, S., Zhang, W., Xu, C., Li, B., Zhang, D. and Liu, F. (2017). Nematicidal activity of trans-2-hexenal against southern root-knot nematode (*Meloidogyne incognita*) on tomato plants. *Journal of Agricultural and Food Chemistry* **65:** 544-550.
32. Mahmoud, S.S. and Croteau, R.B. (2002). Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends in Plant Science* **7:** 366-373.
33. Matos-Rocha, T.J., dos Santos Cavalcanti, M.G., Barbosa-Filho, J.M., Lúcio, A.S.S.C., Veras, D.L., Feitosa, A.P.S. and Brayner, F.A. (2013). *In-vitro* evaluation of schistosomicidal activity of essential oil of *Mentha* x *villosa* and some of its chemical constituents in adult worms of *Schistosoma mansoni*. *Planta Medica* **79:** 1307-1312.
34. Mourvaki, E., Gizzi, S., Rossi, R. and Rufini, S. (2005). Passionflower Fruit - A" New" Source of Lycopene? *Journal of Medicinal Food* **8:** 104-106.
35. Nakanishi, O., Fuiitani, M., Ichimoto, I. and Ueda, H. (1980). An improved process for the synthesis of piperitenone from mesityloxide and methyl vinyl ketone. *Agricultural and Biological Chemistry* **44:** 1667-1668.
36. Ozdemir, E. and Gozel, U. (2018). Nematicidal activities of essential oils against *Meloidogyne incognita* on tomato plant. *Fresenius Environmental Bulletin* **27:** 4511-4517.
37. Padalia, R.C., Verma, R.S., Amit, C., Velusamy, S. and Chanotiya, C.S. (2013). Essential oil composition of sixteen elite cultivars of Mentha from western Himalayan region, India. *Maejo International Journal of Science and Technology* **7:** 83-93.
38. Postnikova, O.A., Hult, M., Shao, J., Skantar, A. and Nemchinov, L.G. (2015). Transcriptome analysis of resistant and susceptible alfalfa cultivars infected with root-knot nematode *Meloidogyne incognita*. *PLoS One* **10:** e0118269.
39. Prakash Singh, Ravendra Kumar, Om Prakash, Mahesh Kumar, Anil Kumar Pant, Valary A. Isidorov and Lech Szczepaniak. (2017). Re-investigation of chemical composition, pharmacological, antibacterial and fungicidal activity of essential oil from *Mentha longifolia* (L.) Huds. *Research Journal of Phytochemistry* **11:** 129-141.
40. Riahi, L., Elferchichi, M., Ghazghazi, H., Jebali, J., Ziadi, S., Aouadhi, C. and Mliki, A. (2013). Phytochemistry, antioxidant and antimicrobial activities of the essential oils of *Mentha rotundifolia* L. in Tunisia. *Industrial Crops and Products* **49**: 883-889.
41. Shanmugam, P.V., Saroj, A., Maurya, R., Yadav, A., Gupta, N., Samad, A. and Chanotiya, C.S. (2017). Enantioselective GC analysis of C3-oxygenated p-menthane type Indian *Mentha spicata* var. viridis 'Ganga' essential oil. *Natural Product Communications* **12:** 1934578X1701200330.
42. Sharma, H.K., Pankaj and Lal, J. (2008). Effects of chemicals on root-knot nematode, *Meloidogyne incognita* infecting tomato under polyhouse cultivation. *Indian Journal of Nematology* **38:** 124-125.
43. Teles, S., Pereira, J.A., Santos, C.H., Menezes, R.V., Malheiro, R., Lucchese, A.M. and Silva, F. (2013). Effects of geographical origin on the essential oil content and composition of fresh and dried *Mentha* × *villosa* Hudson leaves. *Industrial Crops and Products* **46:** 1-7.
44. Thach, L.N., Nhung, T.H., My, V.T. and Tran, H.A. (2013). The new rich source of rotundifolone: *Mentha aquatica* L. var. *crispa* oil from microwave-assisted hydrodistillation. *Journal of Essential Oil Research* **25:** 39-43.

45. Tomei, P.E., Uncini Manganelli, R.E., Flamini, G., Cioni, P.L. and Morelli, I. (2003). Composition of the essential oil of *Mentha microphylla* from the Gennargentu Mountains (Sardinia, Italy). *Journal of Agricultural and Food Chemistry* **51:** 3614-3617.
46. Tripathi, A.K., Prajapati, V., Ahmad, A., Aggarwal, K.K. and Khanuja, S.P. (2004). Piperitenone oxide as toxic, repellent, and reproduction retardant toward malarial vector *Anopheles stephensi* (Diptera: Anophelinae). *Journal of Medical Entomology* **41:** 691-698.
47. Tümen, G., Baser, K.H.C., Kürkcüoglu, M. and Demirçakmak, B. (1995). Composition of the essential oil of *Calamintha incana* (Sm.) Boiss. from Turkey. *Journal of Essential Oil Research* **7:** 679-680.
48. Viglierchio, D.R. and Schmitt, R.V. (1983). On the methodology of nematode extraction from field samples: Baermann funnel modifications. *Journal of Nematology* **15:** 438.

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