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RESEARCH ARTICLE

Chemical characterization, phytotoxic, and cytotoxic activities of essential oil of *Mentha longifolia*

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Abstract

The present study assessed the phytotoxic and cytotoxic potential of the essential oil (EO) extracted from aboveground parts of *Mentha longifolia* (L.) Huds. Gas chromatography–mass spectrometry revealed 39 compounds constituting 99.67% of the EO. The EO was rich in monoterpenoids (mostly oxygenated monoterpenes), which accounted for 89.28% of the oil. The major components in EO were monoterpene ketones such as piperitone oxide (53.83%) and piperitenone oxide (11.52%), followed by thymol (5.80%), and (*E*)-caryophyllene (4.88%). The phytotoxic activities of EO were estimated against *Cyperus rotundus*, *Echinochloa crus-galli*, and *Oryza sativa* (rice) through pre- and post-emergence assays at concentrations ranging from 10 to 250 μg/ml and 0.5–5%, respectively. In pre-emergence assay, the phytotoxic effect of EO was most pronounced on *C. rotundus*, thereby significantly affecting percent germination, plantlet growth, and chlorophyll content. On the contrary, the impact was comparatively lesser on rice, with ~ 40% germination in response to 250 μg/ml of EO treatment. In the post-emergence assay, the spray treatment of EO caused a loss of chlorophyll and wilting in test plants, and subsequently affected the growth of plants, even leading to death in some cases. The cytotoxic activity of EO (at 2.5–50 μg/ml) was studied in meristem cells in onion (*Allium cepa* L.) root tips. EO exposure to the onion roots induced various chromosomal aberrations such as chromosomal bridges, c-mitosis, stickiness, vagrant chromosomes, etc., and negatively affected the mitotic index. At 50 μg/ml, EO treatment triggered the complete death of roots. The study concludes that *M. longifolia* EO has phytotoxic activities due to the mito-depressive effect, along with other physiological effects on target plants. Therefore, EO of *M. longifolia* could be developed into a novel bioherbicide for sustainable management of weeds in agricultural systems.

Keywords Essential oil · Natural herbicides · Weeds · Cytotoxicity · Chromosomal abnormalities

Introduction

Weeds are a hindrance to the growth and yield of associated crops by competing for space and nutrition and causing huge economic losses (Kansas State University 2016). Even in the age of industrialization and technical advancements, weeds remain a key problem. Of late, a number of synthetic chemicals have been tested and applied to counteract the negative impacts of weeds on crops (Gaba et al. 2014). However, the repeated and uncontrolled use of herbicides has ecotoxicological and health concerns and resulted in environmental damage (Zhang et al. 2016) and development of herbicide resistance among weeds (Heap 2019), thus warranting a novel and sustainable weed management approach.

Essential oils (EOs) are pure volatile components of natural plant origin, having a wide array of bioactivities with minimal or zero toxicity to the mammals (Bakkali et al. 2008). Thus, these can provide a useful resource for the management of

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pests, including weeds (Batish et al. 2008; Ibáñez and Blázquez 2018). The current boom in the development of these compounds owes much to their volatility and biodegradability, along with no contamination to the harvest. Also, due to insect repellent/insecticidal nature of many EOs, the oil-based herbicides can simultaneously protect crops from insect pests, without adding any extra input (Isman and Miresmailli 2011; Pavela et al. 2018). Of late, studies have demonstrated the weed-suppressing potential of various EOs in laboratory and pot experiments (Fagodia et al. 2017; Laosinwattana et al. 2018; Sharma et al. 2019). Plant EOs can be appropriately considered as green pesticides for the sustainable management of pests and diseases (Isman 2004). Thus, it is pertinent to investigate EOs from widely used plant species for their weed suppressing and herbicidal activity.

The genus *Mentha* L. (commonly mint; Lamiaceae) has 61 species that are widely spread across the globe, except in South America and Antarctica (Brahmi et al. 2017). Traditionally, many mint species have been used by different tribes and communities for the treatment of diseases and ailments like cold, flatulence, diarrhea, constipation, jaundice, stomachache, and throat infection (Brahmi et al. 2017). *Mentha longifolia* (L.) Huds (wild mint or horsemint) is a native of Europe, Western and Central Asia, and northern and South Africa, and is cultivated mainly for its EOs. It is a perennial herb that grows up to 120 cm in height and spreads mainly through rhizomes. The plant is used in traditional medicine practices for the treatment of various ailments such as bronchitis, headache, cough, nausea, asthma, cold, fever, liver diseases, digestive disorders, and stomach and abdominal disorders (Mikaili et al. 2013; Brahmi et al. 2017). EO of *M. longifolia* possesses a plethora of biological activities such as antispasmodic, anticancerous, antimicrobial, antioxidative, insect repellent, and neuroprotective that can be explored for drug development (Hussain et al. 2010; Mikaili et al. 2013; Zouari-Bouassida et al. 2018). However, there are no reports on the phytotoxic or herbicidal potential of its EO. Thus, the current study aimed to characterize the chemical constituents present in EO of *M. longifolia* and to further explore its phytotoxic and cytotoxic potential.

Materials and methods

Collection of material and EO extraction

The aboveground parts of *M. longifolia* were collected from the bank of Giri River near Yashwant Nagar, Himachal Pradesh, India. The plant material was collected in the morning, packed in airtight bags, and brought to the laboratory. A voucher specimen (PAN 21951) has been deposited in the herbarium (PAN) of Botany Department, Panjab University, India. The plant material was hydrodistilled (2 l distilled water per 2 kg plant material) in Clevenger's apparatus fitted with a condenser (Fagodia et al. 2017). After boiling the material for 4 h, the extracted oil was collected in a glass vial and dehydrated over anhydrous sodium sulfate. Pure oil was then stored in an airtight glass vial at 4 °C until further use for chemical analyses and bioassays.

Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS was carried out on Shimadzu QP2010 system (Shimadzu, Tokyo, Japan) fitted with *ZB*-5MS fused-silica capillary column (nonpolar; 30 m in length and 0.25 mm of internal diameter; thickness of the film was 0.25 μm). Initially, the column temperature was set at 70 °C for 4 min, which was gradually increased to 220 °C at a rate of 4 °C per min. Finally, the temperature was held at 220 °C for 5 min. Helium was used as the carrier gas at a flow rate of 1.1 ml min^{-1}. Two microliters of EO was injected using a split ratio of 1:50 (diluted with *n*-hexane). The ionization energy used for scanning was 70 eV. The mass range for spectra was *m/z* 40–600 amu. The source temperature was 200 °C, whereas the interface temperature was maintained at 220 °C. The constituents were identified based on the matching of mass spectra with those listed in libraries (Wiley 7, NIST 02), and comparison of retention index (RI) calculated relative to a series of *n*-alkane ($C_7 - C_{40}$) with those reported in Adams (2007), NIST Chemistry Webbook, and literature.

Assay of phytotoxic activity

Phytotoxicity of EO was determined in terms of both pre-emergence and post-emergence effects on the test plants: *Cyperus rotundus* L. and *Echinochloa crus-galli* (L.) P. Beauv. and *Oryza sativa* L. (rice). Prior to bioassay, seeds of test species were surface sterilized with 0.5% sodium hypochlorite for 2 min and washed with tap water followed by rinsing with distilled water.

Pre-emergence treatment

Effect of EO on the germination of seeds was studied under laboratory conditions according to the protocol given by Batish et al. (2012). The experiment was carried out using 15 cm Petri dishes lined with a thin cotton wad layer and overlined with Whatman #1 filter paper. Different working concentrations (ranging from 10 to 250 μg/ml) of EO were prepared by dissolving EO in distilled water using Tween 20 (~0.1%, *v/v*). Per Petri dish,10 ml of respective EO treatment or distilled water with Tween 20 (as control) was added. Fifteen seeds of rice or 25 seed each of *E. crus-galli* and *C. rotundus* were equidistantly placed in each Petri dish. To prevent the escape of volatile EO, the Petri dishes were sealed

with double layers of adhesive tape and kept in a growth chamber set at 25 ± 2 °C with a 16-h light/8-h dark photoperiod. For each treatment, including control, five independent replications (Petri dish) were maintained. After 7 days, percent germination, seedling length (tip of root to tip of coleoptile), and photosynthetic pigment content in coleoptiles were determined.

Post-emergence treatment

The herbicidal activity of EO spray treatment was studied on mature plants grown in pots as per the protocol of Sharma et al. (2019). Briefly, 15 seeds of each test plant were grown in polypropylene pots filled with 1 kg of garden soil (soil/manure/sand 3:1:1, *w/w*). Each pot was thinned manually to sustain 5 healthy growing plants 2 weeks after emergence. After 2 weeks of thinning, the plants were sprayed with varying concentrations of EO (ranging from 0.5% to 5%) or distilled water (as parallel control) at the rate of 5 ml/pot (~ 1 ml per plant) with a handheld garden sprayer. For each treatment, five pots were maintained as replicates. The effect of EO spray treatment on the growth and physiological parameters was studied after 1, 7, 14, and 21 days of EO treatment.

Chlorophyll content

The chlorophyll content of treated plants was determined according to the method of Hiscox and Israelstam (1979). For this, 25 mg of plant material (coleoptiles) was incubated in 4 ml of dimethyl sulfoxide (DMSO) for 1 h at 60 °C. After 1 h, the optical density was measured at 663 and 645 nm using a double-beam spectrophotometer (Shimadzu UV-1800) against DMSO as blank. The chlorophyll content was calculated using Arnon's equation (Arnon 1949) and expressed on dry weight basis as suggested by Rani and Kohli (1991).

Cytotoxicity assay

The cytotoxicity of EO of *M. longifolia* was evaluated through the *Allium cepa* assay. For this, healthy and uniform bulbs of onion (*Allium cepa* L.; $2n = 16$) were purchased from the market, and dry scales were peeled off without disturbing root primordia. The bulbs were placed above the glass vessels with their lower end dipped in distilled water and kept for 2–3 days at room temperature (25 ± 1 °C) to facilitate for the emergence of adventitious roots. After attainment of 2–3 cm length, these roots were subjected to EO treatment (2.5, 5, 7.5, 10, 25, 50 μg/ml) prepared in distilled water using Tween 20. Parallel setup with methyl methanesulfonate (MMS; 10 μg/ml) and distilled water having the same concentration of Tween 20 were used as a positive and negative control, respectively. After 24 h of treatment, root tips were fixed for overnight in a mixture of ethanol and glacial acetic acid (3:1; *v/v*). Next day, after thorough rinsing, samples were stored in 70% ethanol at 4 °C. For chromosomal studies, the roots were analyzed through squash technique by staining with acetocarmine (Radić et al. 2010). The slides were then observed under a light microscope (Olympus CX21*i* attached with Magnus-Microscope Digital Camera and LCD) to determine different mitotic stages. Cytotoxicity of EO was evaluated in terms of percent chromosomal aberrations (CAs), mitotic index (MI), and phase index as per the method given by Fagodia et al. (2017). In brief, ~ 3000 cells were scored per treatment (~ 1000 cells per slide) to determine MI and PI, whereas ~ 300 dividing cells (~ 100 per slide) were observed to assess CAs.

Statistical analyses

Data collected from all the experiments were analyzed using the statistical program SPSS (ver. 16.0; SPSS Inc., Chicago). The treatment mean values were analyzed by one-way analysis of variance (ANOVA) followed by comparison at $P \leq 0.05$, using post hoc Tukey's test. Dose-response and correlation analyses were determined using GraphPad Prism 6 software, whereas the regression analysis was done using Sigma Plot 8.0.

Results

EO characterization

The hydrodistillation of aerial parts of *M. longifolia* yielded pale yellow colored EO rich in monoterpenoids (mostly oxygenated monoterpenes). Upon GC-MS analyses, 39 compounds constituting 99.67% of the oil were identified (Table 1). The oil was, in general, monoterpenoid in nature with monoterpenes accounting for 89.28% of the total oil, whereas sesquiterpenes constituted only 8.19% of the oil (Table 1). The major components in EO were monoterpene ketones such as piperitone oxide (53.83%) and piperitenone oxide (11.52%), followed by thymol (5.80%) and (*E*)-caryophyllene (4.88%). The density of EO was found to be 0.928 g cm^{-3} at room temperature (25 °C).

Phytotoxicity of *M. longifolia* EO

Effect on seed germination and early growth

EOs caused a significant inhibition of the germination and seedling growth of test plants in a dose-dependent manner (Fig. 1). In pre-emergence assay, *C. rotundus* exhibited the highest sensitivity to the EO, whereas rice plants were least affected. Complete inhibition of germination in *C. rotundus* was observed at > 75 μg/ml EO treatment, whereas no

Table 1 Chemical composition of essential oil obtained from aerial parts of *Mentha longifolia*

Component[a]	RI^{b}_{cal}	RI_{lit}	Percentage[c]
α-Pinene	935	936	0.20
Sabinene	975	975	0.11
β-Pinene	978	978	0.24
3-Octanol	999	996	0.15
Limonene	1031	1029	0.36
Eucalyptol	1033	1032	0.10
β-Ocimene	1039	1037	0.16
γ-Terpinene	1060	1060	0.10
Linalool	1100	1100	0.45
3-Octanol, acetate	1122	1123	0.53
Terpinen-4-ol	1182	1182	0.61
α-Terpineol	1196	1198	0.33
Carvenone	1231	1238[*]	1.44
Carvone	1247	1249	0.10
cis-Piperitone epoxide	1255	1254	2.87
Piperitone oxide	1264	1259	53.83
2-Hydroxy-3-isopropyl-6-methylcyclohex-2-enone	1269	1274	0.90
Isopiperitenone	1273	1272	0.23
Isopulegyl acetate	1286	1272[†]	0.56
Dihydroedulan II	1289	1284[††]	0.58
Thymol	1296	1292	5.80
2-Hydroxypiperitone	1299	1302[#]	4.26
6-Hydroxy carvotanacetone	1305	1310	2.61
Piperitenone	1341	1340	0.17
cis-Pinocarvyl acetate	1345	1339	1.21
Eugenol	1357	1357	0.26
Piperitenone oxide	1366	1366	11.52
(−)-β-Bourbonene	1385	1384	0.25
(+)-3-Carene, 2-(acetyl methyl)-	1387	1380	0.16
β-Elemene	1390	1390	0.17
Nepetalactone, 4aβ,7α,7aβ	1397	1400	1.06
(*E*)-Caryophyllene	1422	1419	4.88
Geranyl acetone	1451	1451	0.58
cis-β-Farnesene	1454	1457	0.36
a-Humulene	1458	1459	0.22
Germacrene D	1483	1482	1.46
δ-Cadinene	1519	1519	0.23
Caryophyllene oxide	1585	1585	0.48
α-Cadinol	1659	1654	0.14
Total identified components (%)			99.67
Monoterpenes hydrocarbons (%)			1.33
Oxygenated monoterpenes (%)			87.95
Sesquiterpenes hydrocarbons (%)			7.57
Oxygenated sesquiterpenes (%)			0.62
Other hydrocarbons (%)			2.20

[a] Compounds are listed on the basis of elution on *ZB-5MS* capillary nonpolar column. Compound identification based on matching of mass spectra with those listed in libraries (Wiley 7, NIST 02) and comparison of calculated RI (retention index) with those reported in literature (Adams 2007; NIST Chemistry Webbook)

[b] Retention index (RI) calculated relative to a series of *n*-alkane ($C_7 - C_{40}$) on *ZB-5MS* column. RI_{cal}: calculated values; RI_{lit}: literature values (Adams 2007; NIST Chemistry Webbook; *DB-5MS; HP-5MS*); [†] Karioti et al. 2007; [††] Skaltsa et al. 2003; Brada et al. 2006; [‡] Nurzyńska-Wierdak et al. 2018; [*] Joshi 2014

[c] Percentage of constituents was calculated from GC peak areas

germination was observed in *E. crus-galli* at 250 μg/ml EO treatment. Rice was the most resistant test plant; at 250 μg/ml EO treatment, ~60% inhibition in seed germination in rice was observed (Fig. 1a). Similarly, EO interfered with coleoptile and root length in test plants. Treatment with 250 μg/ml of EO resulted in ~58% and 68% reduction in coleoptile and root length in rice (Fig. 1b, c). In contrast, in *E. crus-galli*, significant inhibition was observed in coleoptile

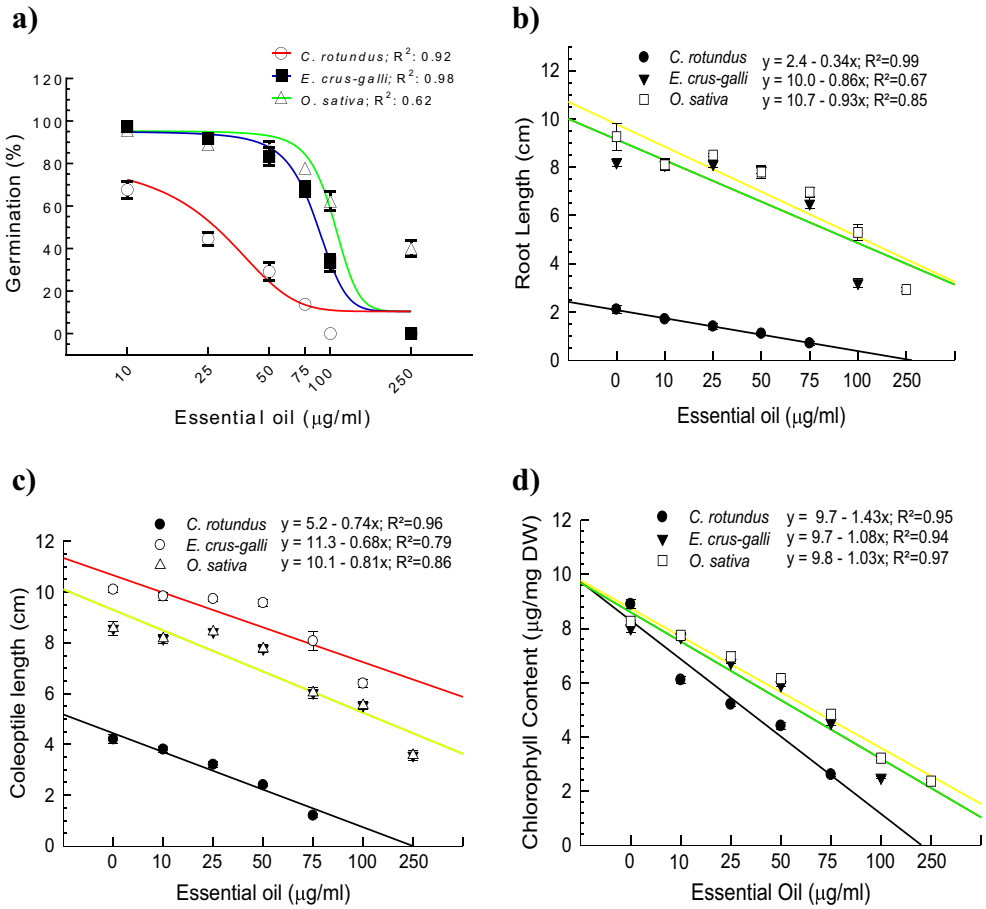

Fig. 1 Effect of the *Mentha longifolia* essential oil on (**a**) germination, (**b**) root length, (**c**) coleoptile length, and (**d**) total chlorophyll content in *Cyperus rotundus, Echinochloa crus-galli,* and *Oryza sativa* in pre-emergence assay, observed after 7 days

and root length upon 75 µg/ml EO treatment, whereas root and coleoptile growth in *C. rotundus* was inhibited at 10 and 25 µg/ml, respectively (Fig. 1b, c). EO treatment caused ~ 70% and ~ 69% reduction in the chlorophyll content in *C. rotundus* (at 75 µg/ml) and *E. crus-galli* (at 100 µg/ml), respectively, whereas the reduction was much less in rice (~ 56% at 100 µg/ml) (Fig. 1d).

Effect of the foliar spray

Foliar spray of EO caused a reduction in root and shoot length and chlorophyll content in test plants (Figs. 2, 3, 4). In general, the phytotoxic effect was less on rice than on weeds, *E. crus-galli*, and *C. rotundus.* EO spray treatment resulted in wilting within 24 h of the spray and even led to the death of plants. In *C. rotundus*, treatment of 5% EO induced the death of above-ground parts within a week of treatment. However, *E. crus-galli* showed some resistance to wilting but eventually dried up after 14 days of the spray treatment. In contrast, rice plants showed some injury but did not dry up completely; rather, these showed signs of recovery after 14 days (data not presented).

Root length declined significantly in *C. rotundus* and *E. crus-galli* upon ≥ 2.5% EO treatment at ≥ 7 days after spray. It was decreased by 15–29% during 14 to 21 days after spraying with 2.5% EO in *C. rotundus* (Fig. 2a), whereas ~ 20–21% and 14% reduction was noticed in *E. crus-galli* (Fig. 2b) and rice (Fig. 2c). Likewise, EO spray treatment significantly reduced shoot length in *C. rotundus* at ≥ 1% and in *E. crus-galli* at ≥ 2.5%, when measured 7 days after treatment. Shoot length declined by 20–25% in *C. rotundus* (Fig. 3a) and by 25–32% in *E. crus-galli* (Fig. 3b) during 7–21 days after spray treatment with 2.5% EO. In contrast, reduction in shoot length in rice was 12–17% during the same duration (Fig. 3c) Chlorophyll content also declined significantly by 51–72% in *C. rotundus* (Fig. 4a) and 46–60% in *E. crus-galli* (Fig. 4b) during 7 to 21 days after spray with 2.5% EO treatment. In contrast, the chlorophyll content in rice declined by only 34–35% only during 7–21 days after treatment with 2.5% EO (Fig. 4c).

Effect on cell division and mitotic index

The cytotoxicity of EO was evaluated in terms of changes in mitotic index (MI; Table 2) and chromosomal aberrations (CAs; Table 3, Fig. 5) in onion root tip cells. EO inhibited mitosis at ≥ 50 µg/ml treatment, whereas at lower concentrations, EO interfered with mitotic division and caused

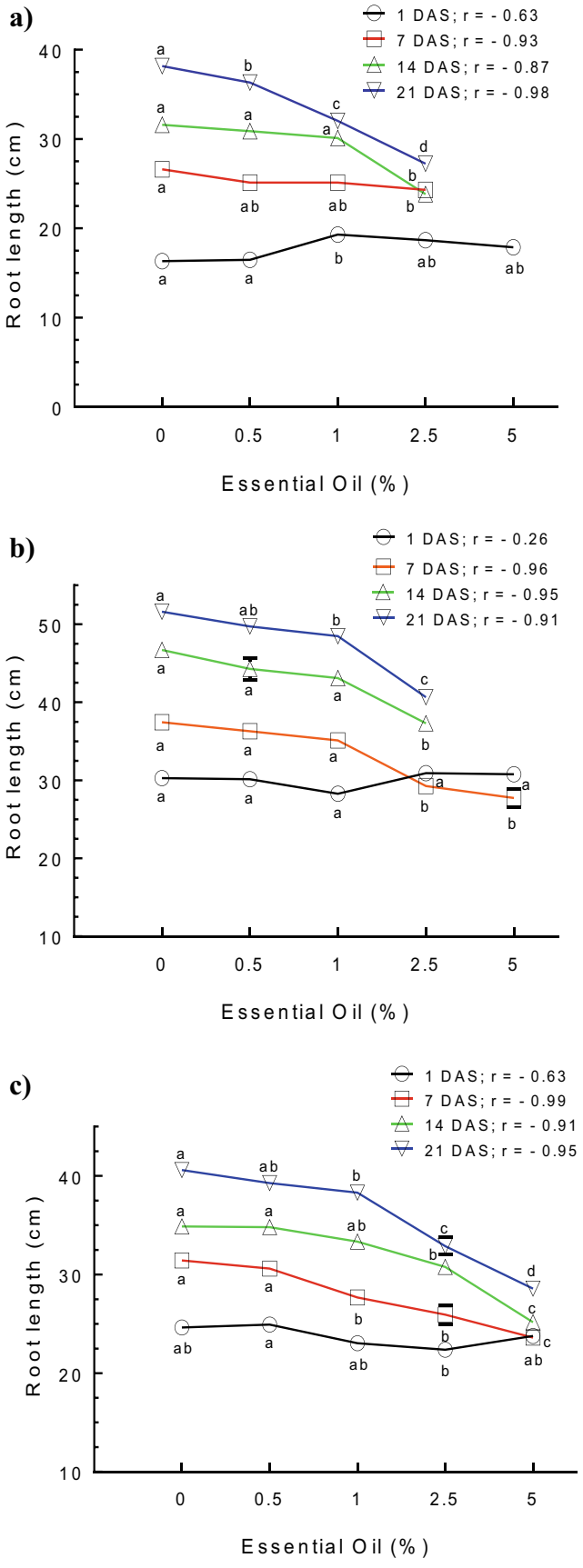

Fig. 2 Effect of spray treatment of *Mentha longifolia* essential oil on the root length in (**a**) *Cyperus rotundus*, (**b**) *Echinochloa crus-galli*, and (**c**) *Oryza sativa*, observed at 1, 7, 14, and 21 day(s) after foliar spray (DAS). Different alphabets along a curve represent significant difference at $P \leq 0.05$ applying post hoc Tukey's test

alteration in MI, besides inducing CAs. After 24 h of treatment, EO caused a significant reduction in MI in a treatment-dependent mode. Interestingly, at 25 μg/ml of EO treatment, the decline (~66%) in mitotic activity was greater than that observed with the positive control, MMS (Table 2). As regards phase index, the prophase and telophase indices declined in response to EO treatment, whereas metaphase and anaphase indices increased with EO concentration (Table 2). In the negative control (distilled water), very few abnormalities (4.4 ± 0.42%), mostly in the form of *c*-mitosis and stickiness, were seen (Table 3). However, in response to EO treatment, different physiological (action on the spindle) and clastogenic (action on chromosome) aberrations were observed in a concentration-dependent manner. Among physiological aberrations, *c*-mitosis, and vagrant and sticky chromosomes appeared in higher frequency, whereas among the clastogenic aberrations, ring chromosomes had the highest percentage. Treatment with 25 μg/ml of EO induced a tenfold increase in CAs over that in the negative control (Table 3).

Discussion

GC-MS analysis of the EO of *M. longifolia* revealed that the oil was rich in monoterpenes. Piperitone oxide and piperitenone oxide were the major constituents of oil, followed by thymol, (*E*)-caryophyllene, and 2-hydroxy piperitone. These observations are in parallel with the earlier studies on *M. longifolia* EO, which also reported piperitone oxide (Hussain et al. 2010) and piperitenone oxide as the major components (Segev et al. 2012; Saeidi et al. 2016). Literature reports from India showed the presence of carvone in EO from Jammu (Shahi et al. 2002); piperitenone oxide, *trans*-piperitone oxide, and *cis*-piperitone oxide in EO from Sirmaur (Himachal Pradesh) and Garhwal Himalayas (Singh et al. 2008; Verma et al. 2015) as the major components in the EO of *M. longifolia*. The variation in chemical composition may be attributed to the vast morphological diversity of the species (Anwar et al. 2017), and various intrinsic (age, plant part, and genetic characteristics) and extrinsic (climatic conditions, soil type, harvesting time, and drying, extraction, and analytical methods) factors (Barra 2009; Saeidi et al. 2016). Due to these variations, the EO of *M. longifolia* has been reported to show a large number of different chemotypes depending on whether the dominant component of the EO is 1,8-cineole (Asghari et al. 2018), carvone (Anwar et al. 2017), menthol (Hajlaoui et al. 2010), menthone (Soilhi et al.

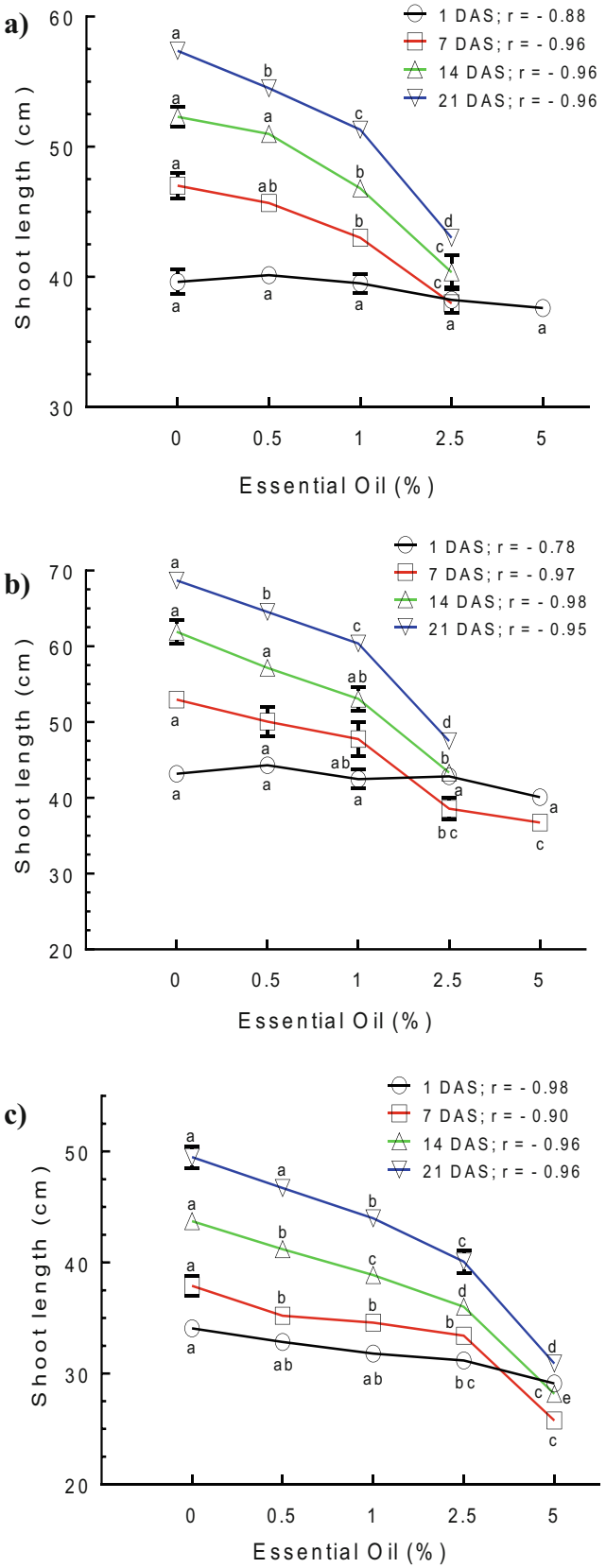

Fig. 3 Effect of spray treatment of *Mentha longifolia* essential oil on the shoot length in (**a**) *Cyperus rotundus*, (**b**) *Echinochloa crus-galli*, and (**c**) *Oryza sativa*, observed at 1, 7, 14, and 21 day(s) after foliar spray (DAS). Different alphabets along a curve represent significant difference at $P \leq 0.05$ applying post hoc Tukey's test

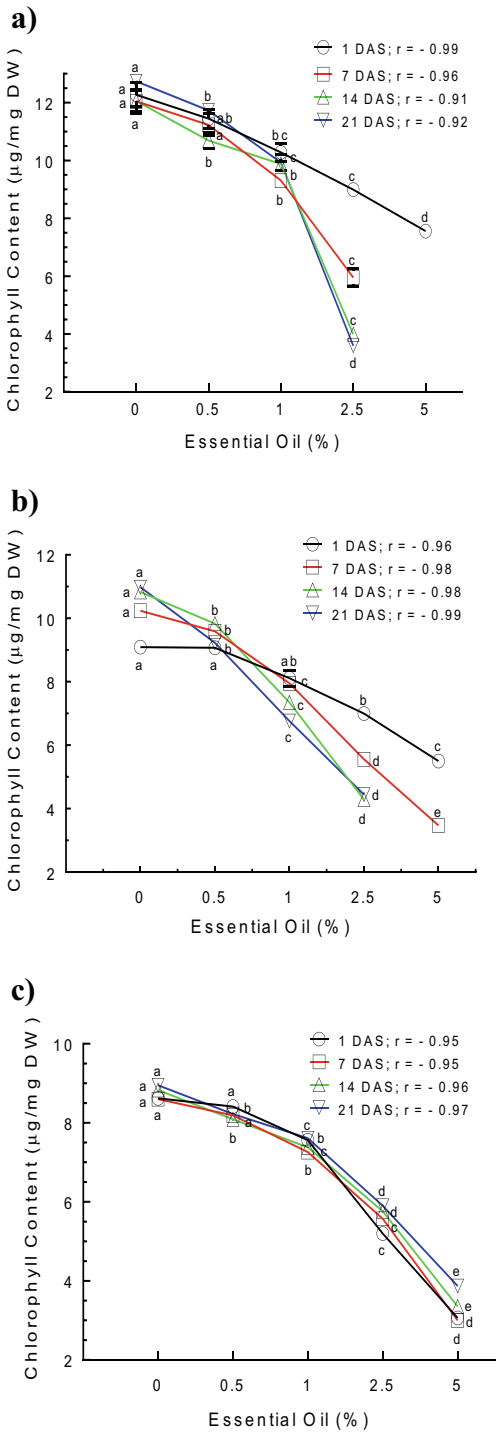

Fig. 4 Effect of spray treatment of *Mentha longifolia* essential oil on the chlorophyll content in (**a**) *Cyperus rotundus*, (**b**) *Echinochloa crus-galli*, and (**c**) *Oryza sativa*, observed at 1, 7, 14, and 21 day(s) after foliar spray (DAS). Different alphabets along a curve represent significant difference at $P \leq 0.05$ applying post hoc Tukey's test

2019), piperitone (Okut et al. 2017), piperitone oxide (Hussain et al. 2010), piperitenone (Segev et al. 2012), piperitenone

Table 2 Effect of the *Mentha longifolia* essential oil on mitotic and phase index in onion root tip cells

Treatments	Mitotic index (%)*	Phase index (%)*			
		Prophase	Metaphase	Anaphase	Telophase
Negative Control (0 μg/ml)	10.5 ± 1.21a	49.1 ± 6.63a	17.4 ± 2.63a	10.9 ± 3.3a	22.7 ± 3.58ab
Essential oil (μg/ml)					
2.5	9.6 ± 1.24ab	28.1 ± 4.67b	23.7 ± 4.74a	19.7 ± 4.17ab	28.9 ± 3.45a
5	8.2 ± 0.73ab	20.9 ± 2.35b	31.9 ± 3.92a	26.4 ± 3.14ab	19.8 ± 4.46ab
7.5	7.5 ± 0.79abc	12.9 ± 1.39b	35.3 ± 4.09a	32.3 ± 1.89b	17.7 ± 3.48ab
10	6.0 ± 0.39bcd	22.4 ± 2.55b	34.7 ± 1.75a	29.0 ± 4.94ab	13.5 ± 4.09ab
25	3.6 ± 0.39d	25.3 ± 1.56b	32.7 ± 4.09a	31.1 ± 6.06b	10.9 ± 0.99b
MMS (Positive control; 10 μg/ml)	4.1 ± 0.15d	27.3 ± 1.88b	35.7 ± 5.98a	21.5 ± 2.24ab	15.5 ± 3.12ab

*Data represented as mean ± standard error. Different alphabets in a column represent significant difference at $P \leq 0.05$ applying post hoc Tukey's test

oxide (Saeidi et al. 2016), or pulegone (Soilhi et al. 2019). In particular, the monoterpene-rich EOs have been reported to exhibit pronounced weed-suppressing activity as compared to the sesquiterpene-rich EOs (Amri et al. 2012). Therefore, in the present investigation, the inhibitory activity of the oil can be a consequence of several monoterpene-rich constituents (89.28%), thus warranting its phytotoxic potential as a bioherbicide.

We investigated the phytotoxic potential of *M. longifolia* EO against *O. sativa*, *C. rotundus,* and *E. crus-galli* in pre- and post-emergence assays). The test plants exhibited a varying degree of decline in seedling growth and chlorophyll content, and rice showed the maximum resistance to EO. Our findings are in conformity with those of Üstüner et al. (2018) who reported that the EO from leaves of *M. longifolia* caused 100% inhibition of *Rumex crispus* and *Convolvulus arvensis* at 5 μg/cm^2 oil concentration under in vitro conditions. However, not much has been done to explore the phytotoxic potential of EOs from *Mentha* species, in general. Nevertheless, studies have demonstrated the phytotoxic potential of EOs obtained from *M. piperita* and *M. spicata* (Azirak and Karaman 2008; Mahdavikia and Saharkhiz 2015). In fact, a number of fragrant plants of the family Lamiaceae have been reported to possess phytotoxic activities (Angelini et al. 2003; Batish et al. 2012; Hazrati et al. 2018; Sharma et al. 2019). In the post-germination assay, EO spray treatment caused visible injuries to the plants and wilting due to the loss of chlorophyll content. The loss in chlorophyll content might have occurred due to the impairment of photosynthetic machinery or enhanced degradation of the chlorophyll caused by EO treatment (Kaur et al. 2010). The phytotoxic activity of EO can be attributed to the major components of the oils, as suggested by previous studies (Amri et al. 2012; Fagodia et al. 2017; Laosinwattana et al. 2018; Sharma et al. 2019). However, whether the major components exert phytotoxic effects alone or in synergism with

Table 3 Chromosomal abnormalities observed in the roots meristematic cells of the onions exposed to the essential oil of *Mentha longifolia*

Treatments	Type of aberrations (%)*										Total aberrant cells (%)
	CM	CB	AB	SC	DS	BN	MA	P	V	CR	
Negative Control (0 μg/ml)	23.1	ND	7.7	38.5	7.7	ND	7.7	ND	7.7	7.7	4.4 ± 0.42b
Essential oil (μg/ml)											
2.5	20.0	8.6	5.7	17.1	8.6	2.9	8.6	ND	20.0	8.6	13.1 ± 0.62c
5	23.5	ND	3.9	27.5	13.7	1.9	7.8	ND	15.7	5.9	22.1 ± 0.76c
7.5	15.4	ND	13.5	28.8	7.7	1.9	3.8	5.8	21.1	5.8	25.7 ± 1.58d
10	27.5	ND	13.0	11.6	10.1	ND	14.5	1.4	15.9	5.8	32.4 ± 2.02e
25	18.0	ND	16.0	24.0	12.0	ND	8.0	4.0	10.0	8.0	44.7 ± 1.26f
MMS (10 μg/ml)	19.7	1.6	9.8	34.4	9.8	3.3	6.6	3.3	4.9	6.6	52.1 ± 0.86a

*Data represented as mean values of 3 observations (for types of aberrations) and mean ± standard error (for total aberrant cells). Different alphabets (in the last column) represent significant difference at $P \leq 0.05$ applying post hoc Tukey's test.

CM c-Mitosis, *CB* chromosomal breakage, *AB* anaphase bridge, *SC* sticky chromosome, *DS* distortion in the spindle, *BN* binucleate cells, *MA* multipolar anaphase, *P* polyploidy, *V* vagrant, *CR* chromosome rings, *ND* not detected

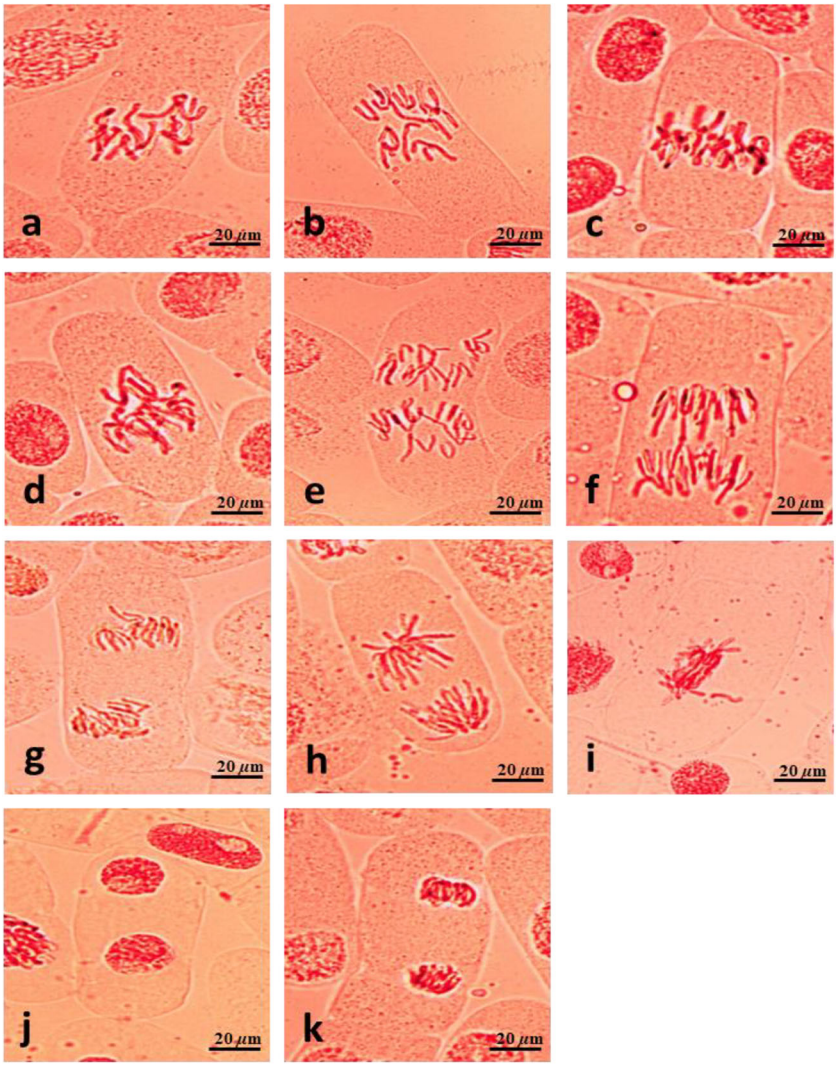

Fig. 5 Different types of abnormalities observed in the root meristematic cells of the onion in response to the *Mentha longifolia* oil. Chromosomal aberrations: **a** c-mitosis, **b** chromosome ring, **c** stickiness in metaphase, **d** disoriented metaphase, **e** multipolar anaphase, **f** anaphase bridge, **g** disoriented spindle, **h** vagrant, **i** chromosome break, **j** binucleate cell, **k** stickiness in telophase

other components could not be ascertained. Nevertheless, EO treatment significantly affected plant growth and development. Phytotoxic effect of EO was more pronounced on root growth than on shoot growth, which is in corroboration with earlier studies (Singh et al. 2006; Fagodia et al. 2017).

Further, the EO was assessed for its cytotoxic activities as the plant growth depends on cell division, expansion, and differentiation of the cells. Any alteration in the cell cycle leads to abnormal growth or inhibition of growth in living organisms (Rojas et al. 1993). To evaluate the cytotoxic potential of any compound in vivo, *A. cepa* bioassay is a very reliable model due to its high sensitivity and reproducibility of results (Pathiratne et al. 2015). In the present study, cytotoxicity was measured in terms of the effect on MI and CAs induced upon EO treatment. MI is a common parameter used to assess the cytotoxicity of any substance (Xu et al. 2016). In our study, MI declined with increasing dose of EO treatment. This might be attributed to the disturbances in the cell cycle, leading to a decline in the number of cells entering mitosis (Sudhakar et al. 2001). The effect on MI is also related to the reduction of root length in target plants (Çelik and Aslantürk 2010) and has consequences on a plant's DNA synthesis (Qin et al. 2015). Previously, the role of monoterpenes in preventing the entry of cells into the mitotic phase has been well described (de Assis Alves et al. 2018).

A decline in MI observed in the study may be due to the loss of DNA or inhibition of nucleoprotein synthesis (El-Ghamery et al. 2003). Of late, studies have reported a decline in MI in the growing root tip cells, in response to EO or its major components (Aragão et al. 2015; Gogoi et al. 2018; Sharma et al. 2019). Besides inducing a decline in MI, EO treatment also induced CAs (both physiological and clastogenic) in the treated cells. *c*-Mitosis and sticky chromosomes appeared to be the most common indicators of physiological action. The stickiness in chromosomes may arise owing to the toxicity of EO on the chromosomal organization (Pinheiro et al. 2015). Stickiness is irreversible and can lead to chromosomal bridge formation (Carvalho et al. 2018) and

cell death, consequently declining the MI (Zhang et al. 2014). *c*-Mitosis occurs when chromosomes' centromere fails to attach to the mitotic spindle, thus causing impairment in the cell cycle (Freitas et al. 2016). In the present study, EO treatment resulted in lower MI than the positive control but induced less CAs in the cell cycle; thus suggesting that both processes are independent of each other. Therefore, the effect of EOs on cell division and chromosomal behavior seems to be responsible for its phytotoxicity towards target plants, along with some other physiological effects.

Conclusions

The present study demonstrated the phytotoxic potential of the EO of *M. longifolia*, having piperitone oxide and piperitenone oxide as the major components. The oil displayed a greater effect on weeds than the rice crop and, therefore, can be exploited for the management of weeds in the agricultural fields. Since EOs are generally considered as safe compounds to use and biodegrade relatively easy, these could reduce the chemical burden on agricultural systems. However, the use of natural products as lead compounds in bioherbicides is quite challenging, as several roadblocks to achieving this goal such as yield, volatility, solubility, and impact on nontarget organisms need to be resolved first. As such, these EOs routinely remain underutilized, and advances in field trials could greatly alter this landscape, leading to their large-scale commercialization.

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Author contributions DRB and HPS conceived the idea for this study. DRB, RKK, SSY, and HPS designed the study. NS conducted the experiments. NS, HPS, and DRB carried out the analysis. NS wrote first version of manuscript. All authors interpreted results and contributed to the following versions of the manuscript to produce the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing or conflict of interest.

References

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry, 4th edn. Allured Publishing Corporation, Carol Stream

Amri I, Gargouri S, Hamrouni L, Hanana M, Fezzani T, Jamoussi B (2012) Chemical composition, phytotoxic and antifungal activities of *Pinus pinea* essential oil. J Pest Sci 85:199–207. https://doi.org/10.1007/s10340-012-0419-0

Angelini LG, Carpanese G, Cioni PL, Morelli I, Macchia M, Flamini G (2003) Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. J Agric Food Chem 51:6158–6164. https://doi.org/10.1021/jf0210728

Anwar F, Alkharfy KM, Najeeb-ur-Rehman AEHK, Gilani AUH (2017) Chemo-geographical variations in the composition of volatiles and the biological attributes of *Mentha longifolia* (L.) essential oils from Saudi Arabia. Int J Pharmacol 13:408–424. https://doi.org/10.3923/ijp.2017.408.424

Aragão FB, Palmieri MJ, Ferreira A, Costa AV, Queiroz VT, Pinheiro PF, Andrade-Vieira LF (2015) Phytotoxic and cytotoxic effects of *Eucalyptus* essential oil on lettuce (*Lactuca sativa* L.). Allelopathy J 35:259–272

Arnon DI (1949) Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1–15. https://doi.org/10.1104/pp.24.1.1

Asghari B, Zengin G, Bahadori MB, Abbas-Mohammadi M, Dinparast L (2018) Amylase, glucosidase, tyrosinase, and cholinesterases inhibitory, antioxidant effects, and GC-MS analysis of wild mint (*Mentha longifolia* var. *calliantha*) essential oil: a natural remedy. Eur J Integr Med 22:44–49. https://doi.org/10.1016/j.eujim.2018.08.004

Azirak S, Karaman S (2008) Allelopathic effect of some essential oils and components on germination of weed species. Acta Agr Scand B-S P 58:88–92. https://doi.org/10.1080/09064710701228353

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils–a review. Food Chem Toxicol 46:446–475. https://doi.org/10.1016/j.fct.2007.09.106

Barra A (2009) Factors affecting chemical variability of essential oils: a review of recent developments. Nat Prod Commun 4:1147–1154. https://doi.org/10.1177/1934578x0900400827

Batish DR, Singh HP, Kohli RK, Kaur S (2008) *Eucalyptus* essential oil as a natural pesticide. For Ecol Manag 256:2166–2174. https://doi.org/10.1016/j.foreco.2008.08.008

Batish DR, Singh HP, Kaur M, Kohli RK, Singh S (2012) Chemical characterization and phytotoxicity of volatile essential oil from leaves of *Anisomeles indica* (Lamiaceae). Biochem Syst Ecol 41:104–109. https://doi.org/10.1016/j.bse.2011.12.017

Brada M, Bezzina M, Marlier M, Lognay GC (2006) Chemical composition of the leaf oil of *Mentha rotundifolia* (L.) from Algeria. J Essent Oil Res 18:663–665. https://doi.org/10.1080/10412905.2006.9699198

Brahmi F, Khodir M, Mohamed C, Pierre D (2017) Chemical composition and biological activities of *Mentha* species, in Aromatic and Medicinal Plants - Back to Nature, ed Hany A. El-Shemy, IntechOpen, https://doi.org/10.5772/67291. Available from: https://www.intechopen.com/books/aromatic-and-medicinal-plants-back-to-nature/chemical-composition-and-biological-activities-of-mentha-species

Carvalho A, Leal F, Matos M, Lima-Brito J (2018) Effects of heat stress in the leaf mitotic cell cycle and chromosomes of four wine-producing grapevine varieties. Protoplasma 255:1725–1740. https://doi.org/10.1007/s00709-018-1267-4

Çelik TA, Aslantürk ÖS (2010) Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with *Allium* test. Biomed Res Int 2010(189252):1–8. https://doi.org/10.1155/2010/189252

de Assis Alves T, Pinheiro PF, Praça-Fontes MM, Andrade-Vieira LF, Corrêa KB, de Assis Alves T, da Cruz FA, Júnior VL, Ferreira A, Soares TCB (2018) Toxicity of thymol, carvacrol and their respective phenoxyacetic acids in *Lactuca sativa* and *Sorghum bicolor*. Ind Crop Prod 114:59–67. https://doi.org/10.1016/j.indcrop.2018.01.071

El-Ghamery AA, El-Kholy MA, El-Yousser MA (2003) Evaluation of cytological effects of Zn^{2+} in relation to germination and root growth

of *Nigella sativa* L. and *Triticum aestivum* L. Mutat Res 537:29–41. https://doi.org/10.1016/S1383-5718(03)00052-4

Fagodia SK, Singh HP, Batish DR, Kohli RK (2017) Phytotoxicity and cytotoxicity of *Citrus aurantiifolia* essential oil and its major constituents: limonene and citral. Ind Crop Prod 108:708–715. https://doi.org/10.1016/j.indcrop.2017.07.005

Freitas AS, Cunha IMF, Andrade-Vieira LF, Techio VH (2016) Effect of SPL (spent pot liner) and its main components on root growth, mitotic activity and phosphorylation of histone H3 in *Lactuca sativa* L. Ecotoxicol Environ Saf 124:426–434. https://doi.org/10.1016/j.ecoenv.2015.11.017

Gaba S, Fried G, Kazakou E, Chauvel B, Navas ML (2014) Agroecological weed control using a functional approach: a review of cropping systems diversity. Agron Sust Develop 34:103–119. https://doi.org/10.1007/s13593-013-0166-5

Gogoi R, Loying R, Sarma N, Munda S, Pandey SK, Lal M (2018) A comparative study on antioxidant, anti-inflammatory, genotoxicity, anti-microbial activities and chemical composition of fruit and leaf essential oils of *Litsea cubeba* Pers. from north-East India. Ind Crop Prod 125:131–139. https://doi.org/10.1016/j.indcrop.2018.08.052

Hajlaoui H, Ben AF, Snoussi M, Noumi E, Bakhrouf A (2010) Effect of *Mentha longifolia* L. ssp. *longifolia* essential oil on the morphology of four pathogenic bacteria visualized by atomic force microscopy. Afr J Microbiol Res 4:1122–1127

Hazrati H, Saharkhiz MJ, Moein M, Khoshghalb H (2018) Phytotoxic effects of several essential oils on two weed species and tomato. Biocatal Agric Biotechnol 13:204–212. https://doi.org/10.1016/j.bcab.2017.12.014

Heap I (2019) The International Survey of Herbicide Resistant Weeds. Available at www.weedscience.org. Accessed 21 October 2019

Hiscox JD, Israelstam GF (1979) A method for extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57:1332–1334. https://doi.org/10.1139/b79-163

Hussain AI, Anwar F, Nigam PS, Ashraf M, Gilani AH (2010) Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. J Sci Food Agric 90:1827–1836. https://doi.org/10.1002/jsfa.4021

Ibáñez MD, Blázquez MA (2018) Phytotoxicity of essential oils on selected weeds: potential hazard on food crops. Plants (Basel) 7(4):79. https://doi.org/10.3390/plants704007

Isman MB (2004) Plant essential oils as green pesticides for pest and disease management. ACS Symp Ser 887:41–51. https://doi.org/10.1021/bk-2004-0887.ch004

Isman MB, Miresmailli S (2011) Plant essential oils as repellents and deterrents to agricultural pests. ACS Symp Ser 1090:67–77. https://doi.org/10.1021/bk-2011-1090.ch005

Joshi RK (2014) Aroma profile of *Mentha arvensis* Linn. Growing in Belgaum region of Karnataka, India. J Essent Oil Bear Pl 17:1397–1399. https://doi.org/10.1080/0972060X.2014.884776

Kansas State University (2016) Left uncontrolled, weeds would cost billions in economic losses every year. ScienceDaily. Retrieved August 16, 2019 from www.sciencedaily.com/releases/2016/05/160516130720.htm

Karioti A, Skaltsa H, Gbolade AA (2007) Analysis of the leaf oil of *Syzygium malaccense* Merr. et Perry from Nigeria. J Essent Oil Res 19:313–315. https://doi.org/10.1080/10412905.2007.9699290

Kaur S, Singh HP, Mittal S, Batish DR, Kohli RK (2010) Phytotoxic effects of volatile oil from *Artemisia scoparia* against weeds and its possible use as a bioherbicide. Ind Crop Prod 32:54–61. https://doi.org/10.1016/j.indcrop.2010.03.007

Laosinwattana C, Wichittrakarn P, Teerarak M (2018) Chemical composition and herbicidal action of essential oil from *Tagetes erecta* L. leaves. Ind Crop Prod 126:129–134. https://doi.org/10.1016/j.indcrop.2018.10.013

Mahdavikia F, Saharkhiz MJ (2015) Phytotoxic activity of essential oil and water extract of peppermint (*Mentha*× *piperita* L. CV. Mitcham). J Appl Res Med Aromat Plants 2:146–153. https://doi.org/10.1016/j.jarmap.2015.09.003

Mikaili P, Molaverrostami S, Moloudizargari M, Aghaianshakeri S (2013) Pharmacological and therapeutic effects of *Mentha longifolia* L. and its constituent menthol. Ancient Sci Life 33:129–136. https://doi.org/10.4103/0257-7941.139059

Nurzyńska-Wierdak R, Gruszecki R, Kosior M (2018) Does preservation modify the essential oil content and chemical composition of leaf celery (*Apium graveolens* L. var. *secalinum* Alef.)? Acta Sci Pol-Hortoru 17:27–36. https://doi.org/10.24326/asphc.2018.6.3

Okut N, Yagmur M, Selcuk N, Yildirim B (2017) Chemical composition of essential oil of *Mentha longifolia* L. subsp. *longifolia* growing wild. Pak J Bot 49:525–529

Pathiratne A, Hemachandra CK, De Silva N (2015) Efficacy of *Allium cepa* test system for screening cytotoxicity and genotoxicity of industrial effluents originated from different industrial activities. Environ Monit Assess 187:730. https://doi.org/10.1007/s10661-015-4954-z

Pavela R, Maggi F, Lupidi G, Mbuntcha H, Woguem V, Womeni HM, Barboni L, Tapondjou LA, Benelli G (2018) *Clausena anisata* and *Dysphania ambrosioides* essential oils: from ethno-medicine to modern uses as effective insecticides. Environ Sci Pollut Res 25:10493–10503. https://doi.org/10.1007/s11356-017-0267-9

Pinheiro PF, Costa AV, de Assis Alves T, Galter IN, Pinheiro CA, Pereira AF, Ramos Oliveira CM, Praca Fontes MM (2015) Phytotoxicity and cytotoxicity of essential oil from leaves of *Plectranthus amboinicus*, carvacrol, and thymol in plant bioassays. J Agric Food Chem 63:8981–8990. https://doi.org/10.1021/acs.jafc.5b03049

Qin R, Wang C, Chen D, Björn LO, Li S (2015) Copper-induced root growth inhibition of *Allium cepa* var. *agrogarum* L. involves disturbances in cell division and DNA damage. Environ Toxicol Chem 34:1045–1055. https://doi.org/10.1002/etc.2884

Radić S, Stipaničev D, Vujčić V, Rajčić MM, Širac S, Pevalek-Kozlina B (2010) The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. Sci Total Environ 408:1228–1233. https://doi.org/10.1016/j.scitotenv.2009.11.055

Rani D, Kohli RK (1991) Fresh matter is not an appropriate relation unit for chlorophyll content: experience from experiments on effects of herbicides and allelopathic substances. Photosynthetica 25:655–658

Rojas E, Herrera LA, Sordo M, Gonsebatt ME, Montero R, Rodriguez R, Ostrosky-Wegman P (1993) Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. Anti-Cancer Drugs 4:637–640. https://doi.org/10.1097/00001813-199312000-00005

Saeidi K, Ghafari Z, Rostami S (2016) Effect of drying methods on essential oil content and composition of *Mentha longifolia* (L.) Hudson. J Essent Oil Bear Pl 19:391–396. https://doi.org/10.1080/0972060X.2015.1108246

Segev D, Nitzan N, Chaimovitsh D, Eshel A, Dudai N (2012) Chemical and morphological diversity in wild populations of *Mentha longifolia* in Israel. Chem Biodivers 9:577–588. https://doi.org/10.1002/cbdv.201100108

Shahi AK, Pal S, Dutt P (2002) *Mentha longifolia* (Linn.) Huds: a carvone rich chemotype in Jammu region. Indian Perfumer 46:63–65

Sharma A, Singh HP, Batish DR, Kohli RK (2019) Chemical profiling, cytotoxicity and phytotoxicity of foliar volatiles of *Hyptis suaveolens*. Ecotoxicol Environ Saf 171:863–870. https://doi.org/10.1016/j.ecoenv.2018.12.091

Singh HP, Batish DR, Kaur S, Kohli RK, Arora K (2006) Phytotoxicity of the volatile monoterpene citronellal against some weeds. Z Naturforsch 61:334–340. https://doi.org/10.1515/znc-2006-5-606

Singh HP, Batish DR, Mittal S, Dogra KS, Yadav S, Kohli RK (2008) Constituents of leaf essential oil of *Mentha longifolia* from India. Chem Nat Comp 44:528–529. https://doi.org/10.1007/s10600-008-9124-x

Skaltsa HD, Demetzos C, Lazari D, Sokovic M (2003) Essential oil analysis and antimicrobial activity of eight *Stachys* species from Greece. Phytochemistry 64:743–752. https://doi.org/10.1016/S0031-9422(03)00386-8

Soilhi Z, Rhimi A, Heuskin S, Fauconnier ML, Mekki M (2019) Essential oil chemical diversity of Tunisian *Mentha* spp. collection. Ind Crop Prod 131:330–340. https://doi.org/10.1016/j.indcrop.2019.01.041

Sudhakar R, Ninge Gowda KN, Venu G (2001) Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytologia 66:235–239. https://doi.org/10.1508/cytologia.66.235

Üstüner T, Kordali S, Bozhüyük AU (2018) Herbicidal and fungicidal effects of *Cuminum cyminum*, *Mentha longifolia* and *Allium sativum* essential oils on some weeds and fungi. Rec Nat Prod 12:619–629. https://doi.org/10.25135/rnp.80.18.05.106

Verma RS, Pandey V, Chauhan A, Tiwari R (2015) Essential oil composition of *Mentha longifolia* (L.) L. collected from Garhwal region of Western-Himalaya. J Essent Oil Bear Pl 18:957–966. https://doi.org/10.1080/0972060X.2014.897594

Xu QM, Wang YZ, Liu H, Cheng JS (2016) Physiological responses and chromosomal aberration in root tip cells of *Allium sativum* L. to cerium treatments. Plant Soil 409:447–458. https://doi.org/10.1007/s11104-016-2978-y

Zhang H, Jiang Z, Qin R, Zhang H, Zou J, Jiang W, Liu D (2014) Accumulation and cellular toxicity of aluminum in seedling of *Pinus massoniana*. BMC Plant Biol 14:264. https://doi.org/10.1186/s12870-014-0264-9

Zhang C, Hu R, Huang J, Huang X, Shi G, Li Y, Yin Y, Chen Z (2016) Health effect of agricultural pesticide use in China: implications for the development of GM crops. Sci Rep 6:34918. https://doi.org/10.1038/srep34918

Zouari-Bouassida K, Trigui M, Makni S, Jlaiel L, Tounsi S (2018) Seasonal variation in essential oils composition and the biological and pharmaceutical protective effects of *Mentha longifolia* leaves grown in Tunisia. Bio Med Res Int 2018:7856517. https://doi.org/10.1155/2018/7856517