Ecotoxicology and Environmental Safety 171 (2019) 863–870

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Chemical profiling, cytotoxicity and phytotoxicity of foliar volatiles of *Hyptis suaveolens*

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ARTICLE INFO

Keywords:
Essential oil
Chromosomal aberrations
Mitotic index
Natural herbicide
Post-emergent activity

ABSTRACT

In the present study, the essential oil (EO) of *Hyptis suaveolens* has been explored for the first time for its phytotoxic and cytotoxic activities. The phytotoxic activity was assessed against rice (*Oryza sativa*) and its major troublesome weed, *Echinochloa crus-galli*, under laboratory and screenhouse conditions. GC-MS analysis revealed EO to be monoterpenoid (~ 79% monoterpenes) in nature with *α*-phellandrene (22.8%), *α*-pinene (10.1%) and limonene (8.5%) as the major chemical constituents. The laboratory bioassay showed a complete growth inhibitory effect of EO ($\geq$ 2 mg mL^{-1}) towards the germination and seedling growth of *E. crus-galli*. However, the inhibitory effect on rice was much less (~40% inhibition). EO caused visible injury, reduction in chlorophyll content, cell viability and ultimately led to complete wilting of *E. crus-galli* plants. In addition, EO altered the cell division in the meristematic cells of *Allium cepa* as depicted by ~63% decrease in mitotic index. EO exposure induced several aberrations at chromosomal (*c*-mitosis, anaphase bridges, chromosomal breakage, vagrant chromosomes, and sticky chromosomes) and cytological level (cytoplasm destruction, peripheral nuclei, and binucleate cells). The present study concludes that *H. suaveolens* EO possesses phytotoxic activity due to its mitodepressive activity, and could serve as a natural herbicide under sustainable agricultural practices.

1. Introduction

Plants, being a rich source of natural compounds with known phytotoxicity, have been explored for the development of eco-friendly and safer herbicides (Dayan and Duke, 2014). Among various natural compounds, essential oils (hereafter EO) have been considered to be a promising source of natural pesticides (Isman, 2016). These are preferred over synthetic herbicides due to their properties like non-persistence in the soil, non-leachation in groundwater (thus no groundwater contamination), and no or low mammalian toxicity (Isman, 2000). Therefore, weed suppressing property of EOs is regarded as one of the most innovative weed management strategies for sustainable agro-ecosystems (Batish et al., 2008; Dayan et al., 2012). Several EOs have been reported to exhibit phytotoxicity towards the weeds, thus, exhibiting a potential to be used as herbicides. These include: EOs of *Eucalyptus* species (Batish et al., 2004; Batish et al., 2008), *Nepeta meyeri* (Kordali et al., 2015), *Leptospermum scoparium* (Dayan et al., 2011), *Hyssopus officinalis*, *Lavandula angustifolia*, *Majorana hortensis*, *Melissa officinalis*, *Ocimum basilicum*, *Origanum vulgare*, *Salvia officinalis* *Thymus vulgaris*, *Verbena officinalis*, *Pimpinella anisum*, *Foeniculum vulgare* and *Carum carvi* (de Almeida et al., 2010), *Artemisia scoparia* (Kaur et al., 2010), *Pinus nigra* (Amri et al., 2017) and *Citrus aurantiifolia* (Fagodia et al., 2017). In fact, some EO-based commercial herbicides are available in the market for weed control, especially in organic farming (Dayan et al., 2009).

World-over, the genus *Hyptis* (Lamiaceae) consisting of over 400 species of herbs and shrubs, is well-known for a wide spectrum of biological activities, due to the presence of volatile/essential oils (Kuhnt et al., 1995). *Hyptis suaveolens* (L.) Poit. (bushmint, pignut or American mint) is an annual, aromatic herb native to Tropical America. It generally grows wild in wastelands and along the roadsides, water channels and forest margins (Sharma et al., 2017). In India, it has emerged as a problematic weed due to its invasive nature and has been known to displace the native flora and affect the floristic diversity in the invaded areas (Sharma et al., 2017). The aerial parts of *H. suaveolens* are rich in EO that exhibits antioxidant, antimicrobial (Nantitanon et al., 2007), antifungal (Moreira et al., 2010), anticancerous and insecticidal (Conti et al., 2012) activities. However, studies investigating the phytotoxic and cytotoxic properties of EO of *H. suaveolens* are still lacking. We, therefore, conducted a series of experiments to investigate the

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https://doi.org/10.1016/j.ecoenv.2018.12.091
Received 14 March 2018; Received in revised form 5 December 2018; Accepted 27 December 2018

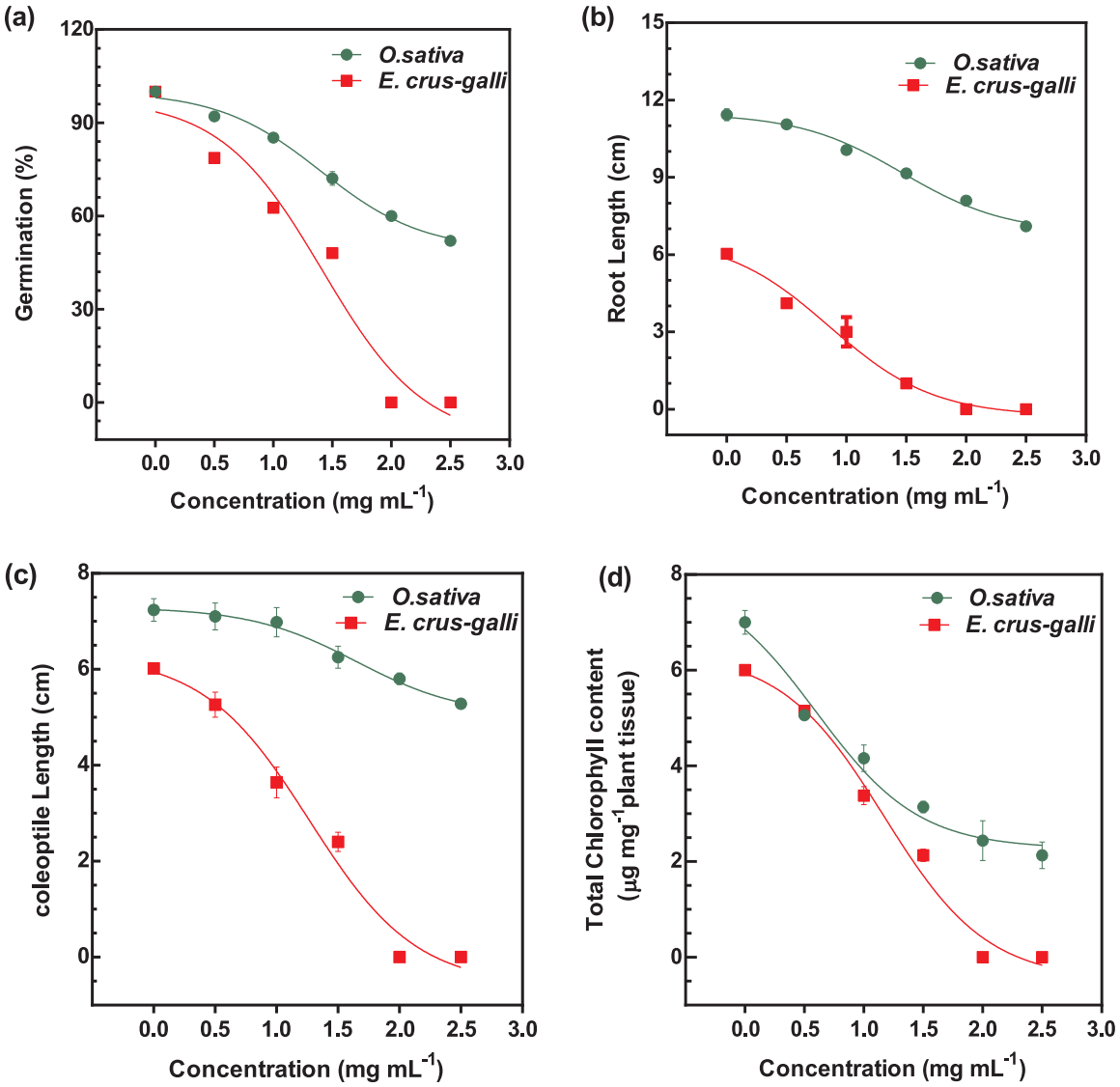

Fig. 1. Dose-response curves showing the phytotoxic effect of *H. suaveolens* EO on: a) germination, b) root length, c) coleoptile length and d) total chlorophyll content in *O. sativa* and *E. crus-galli* measured after 7 days. *Dose-response curves were derived by plotting the concentration (on the x-axis) and percent response (on the y-axis), and analyzed by non-linear fit model: log inhibitor vs. response-variable slope using Graph Pad Prism version 6.*

phytotoxic and cytotoxic properties of *H. suaveolens* EO. The phytotoxicity of *H. suaveolens* EO was evaluated against rice (*O. sativa* L.) and its major weed, *E. crus-galli* (L.) Beauv. (barnyard grass), in a dose-response laboratory bioassay. Cytotoxicity of EO was investigated in terms of alterations in cell division and induction of chromosomal aberrations in root meristems of *Allium cepa* (onion).

2. Material and methods

2.1. Collection of plant material

Fresh leaves of *H. suaveolens* were collected randomly from the plants (at the vegetative stage) growing in wild on the outskirts of Chandigarh (30°45́ 32.62́′ N; 76°44́ 51.01′ E), India. A voucher specimen (PAN# 21101) was deposited in the herbarium of Botany Department, Panjab University, Chandigarh, India. Seeds of rice (*O. sativa* L. var. PAR 115) were purchased from the local seed store, whereas, those of *E. crus-galli* were collected from the infested rice fields around Chandigarh, India.

2.2. Extraction of the EO

EO was extracted from the leaves of *H. suaveolens* by hydro-distillation using Clevenger's apparatus. The leaves (~ 3 kg) were cleaned and placed in round bottom flask fitted with the condenser and boiled in distilled water for 3 h. EO obtained after hydrodistilaltion was dried over anhydrous sodium sulphate and stored at 4 °C until used for chemical analyses and bioassays.

2.3. GC-MS analysis

The chemical composition of the EO was determined using Thermo Scientific Trace 1300 Gas Chromatograph (GC) coupled with Thermo TSQ 8000 Mass Spectrometer (MS), and fitted with Thermo TG 5MS non-polar fused silica capillary column (30 m length × 0.25 mm diameter × 0.25 µm thickness). The initial oven temperature was maintained at 60 °C for 2 min and then increased to 250 °C at the rate of 3 °C min^{-1} and held for 5 min. Helium (He) was used as the carrier gas at a flow rate of 1 mL min^{-1}. Injection volume was 1 µL of the oil. Injector and transfer line temperature was set at 250 °C and 280 °C, respectively. The mass spectra were recorded with ionization energy of

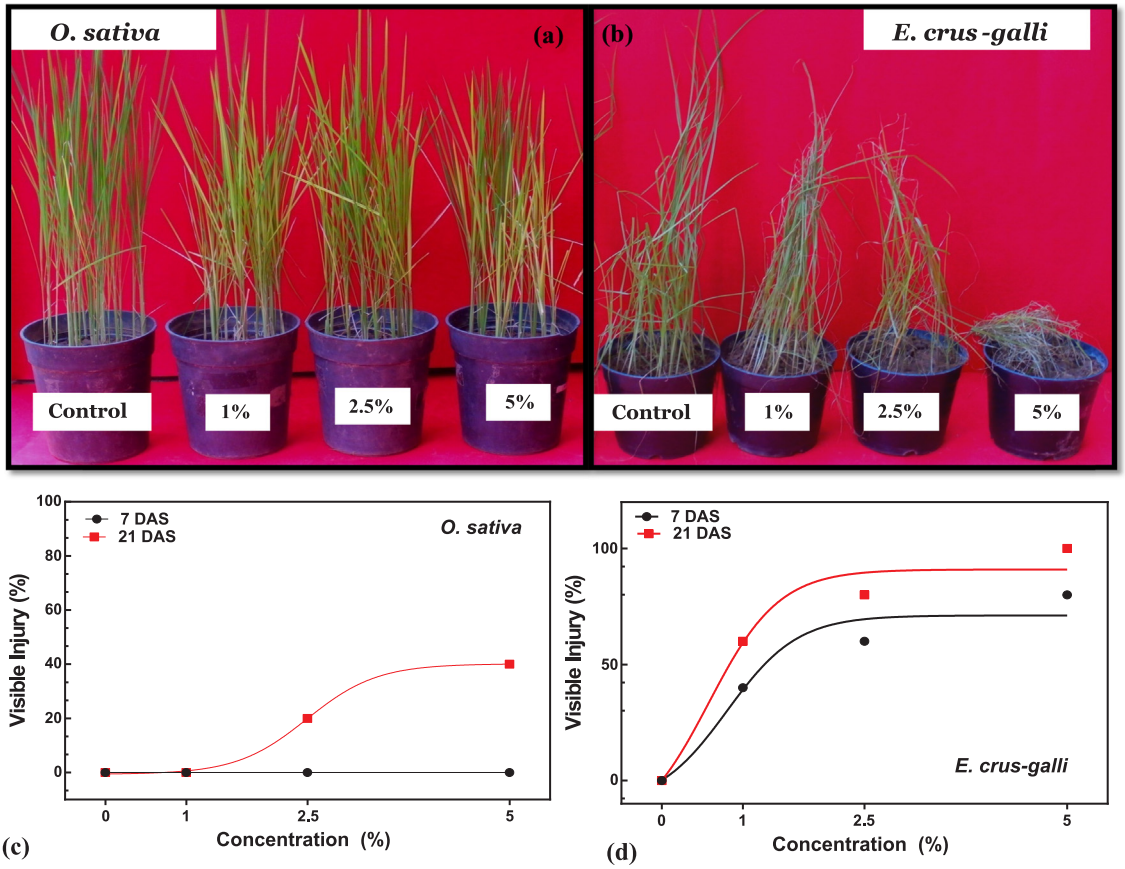

Fig. 2. Post-emergent effect of *H. suaveolens* EO on visible injury in *O. sativa* (a, c) and *E. crus-galli* (b, d), determined at 21 DAS.

70 eV over a scan mass range of *m/z* 30–400 amu and MS source temperature of 240 °C with split injection ratio of 1:150. Peak areas (%) were calculated automatically through the data analysis software XCalibur 2.2SP1 and Foundation 2.0SP1.

2.4. Identification of chemical constituents of EO

EO constituents were identified by comparing their retention indices (RI) and mass fragmentation pattern with those of MS Library search (NIST, 2017; Wiley, 275) or present in the literature (Adams, 2007). The RI values of different chemical constituents were calculated by using the retention times of a homologous series of *n*-alkanes (C_8-C_{32}, Sigma-Aldrich) running in parallel with EO under similar conditions and calculated as per Hérent et al. (2007).

2.5. Phytotoxic studies

2.5.1. Seed germination and seedling growth bioassay

The phytotoxic effect of *H. suaveolens* EO was evaluated against rice and its major weed (i.e. *E. crus-galli*) in a dose-response laboratory bioassay. A stock solution (2.5 mg mL^{-1}) of EO was prepared by dissolving the requisite amount in Tween-20 (used as a surfactant) and final volume was made with distilled water. The stock solution was further diluted to get working concentrations, *i.e.*, 0.5, 1, 1.5 and 2 mg mL^{-1}. A similar set-up without *H. suaveolens* EO but with the treatment of Tween-20 in distilled water served as control. Pre-imbibed seeds of both the test plants (*O. sativa* and *E. crus-galli*) were placed equidistantly in 15-cm-diameter Petri dishes lined with a double layer of Whatman #1 filter paper moistened with 10 mL of EO (0.5–2.5 mg mL^{-1}). Immediately after the treatment, the Petri dishes were sealed with Parafilm®. For each treatment, there were five independent replicates. All the Petri dishes were placed in a completely randomized manner inside a growth chamber set at 25 ± 2 °C temperature, 75% RH and a 16 h/8 h light/dark photoperiod of ~150 μmole m^{-2} s^{-1} photon flux density. After 7 days, the number of germinated seeds was counted and seedling length (from tip of roots to tip of coleoptile) was measured. The coleoptiles were collected from all the treatments for the determination of total chlorophyll content.

2.5.2. Post-emergent activity of H. suaveolens EO

Ten seeds of each test plant were sown in polypropylene pots (Φ = 15 cm) filled with 500 g of garden soil (soil: sand: manure 3: 1: 1, *w/w*). Two-week after seedling emergence, pots were thinned to 5 equal-sized healthy plants per pot, and allowed to grow for another four weeks. After six weeks, these were sprayed with 1%, 2.5%, and 5% (*v/v*) of *H. suaveolens* EO or distilled water (as parallel control) using a common garden sprayer at the rate of 10 mL/plant, and a total of 100 mL pot^{-1}. For each treatment, five independent (pot) replicates were maintained in a completely randomized design and a total of 20 pots were maintained for each plant species comprising of 4 treatments. After 7 and 21 days of spray (DAS), the treated plants were examined for visible injury in terms of percent chlorotic and necrotic areas. In addition, the leaves were sampled from all the treatments for the estimation of total chlorophyll content and measurement of cell viability.

2.6. Estimation of total chlorophyll content

Chlorophyll was extracted from the leaves/coleoptiles (25 mg) of *O. sativa* and *E. crus-galli* in dimethyl sulphoxide (4 mL) as per Hiscox and Israelstam (1979). It was quantified as per Arnon (1949) and expressed on the dry weight basis as suggested by Rani and Kohli (1991).

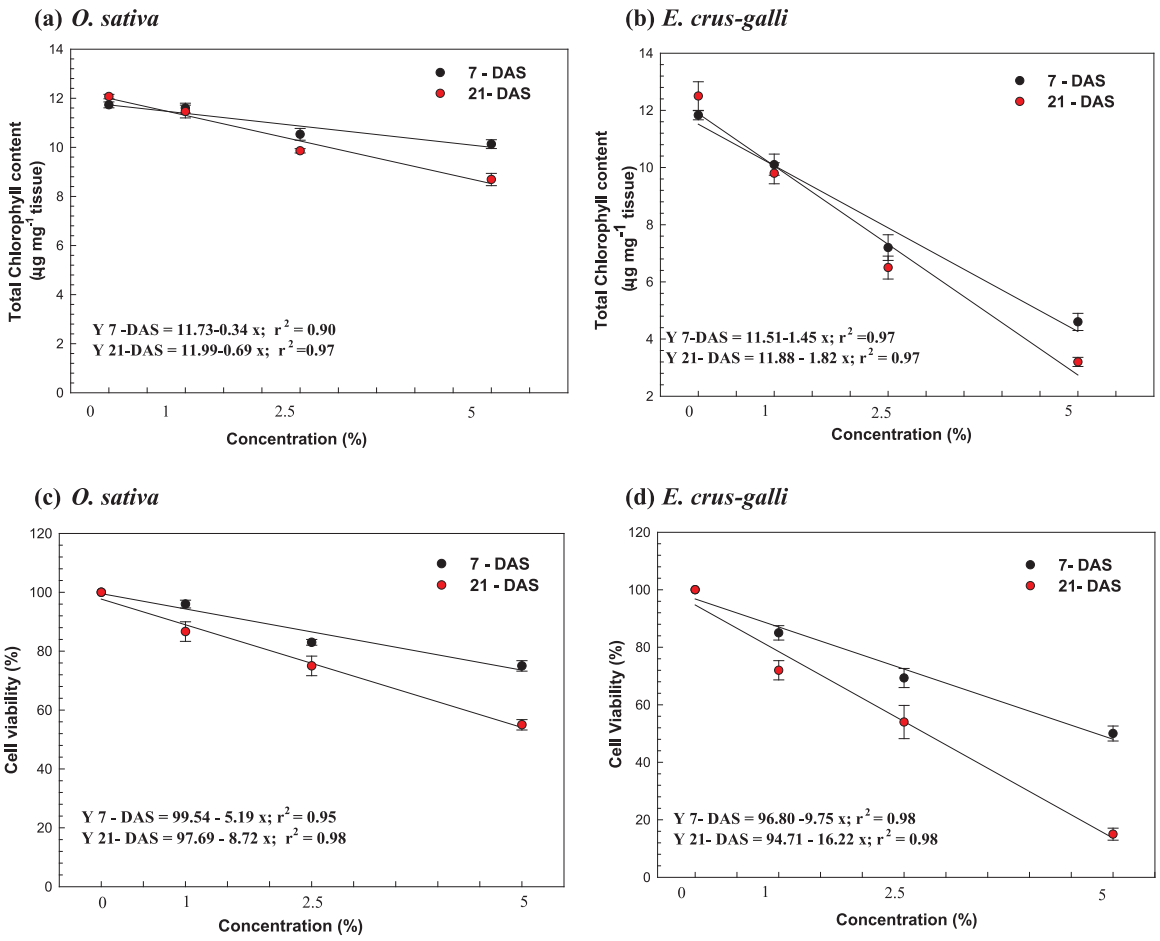

Fig. 3. Effect of post-emergent spray treatment of *H. suaveolens* EO on chlorophyll content and cell viability in *O. sativa* (a, c) and *E. crus-galli* (b, d) measured at 7 DAS and 21 DAS. *Vertical bars along each data point represent the standard error of the mean. Data were analyzed by linear regression. Black lines represent regression lines, and R^2 represents the coefficient of determination. All regressions were significant at $p \leq 0.05$.*

2.7. Determination of cell viability

Cell survivability was determined indirectly from the fresh tissue using 2,3,5-triphenyl tetrazolium chloride (TTC) as per Singh et al. (2006a). TTC captures the electrons from the mitochondrial electron transport chain and forms a red-colored formazan, which is indicative of tissue viability/survival. The absorbance of formazan was read at 530 nm on Shimadzu UV-1800 double beam spectrophotometer using ethanol as blank and the values were expressed with respect to the control.

2.8. Cytogenetic analysis

Effect of *H. suaveolens* EO on the process of cell division was studied using squash technique method. Onion (*A. cepa*), a well-known model plant, was chosen for the study due to its relatively large-sized cells with the fewer number of large-sized monocentric chromosomes ($2n = 16$) that stain well (Fiskesjö, 1997). Healthy and uniform-sized onion bulbs were scrapped and their dry scales were peeled off. They were set for rooting in distilled water for about 2–3 days, till fresh adventitious roots attained a length of 2–3 cm. Onion roots were then subjected to various treatments of *H. suaveolens* EO (*i.e.,* 0.5, 1.5 and 2.5 mg mL^{-1}) along with distilled water (*i.e.,* 0; negative control) and 100 µM methyl methane sulfonate (MMS) as a positive control, for 24 h. After treatment, the roots were fixed in ethyl alcohol and glacial acetic acid (3: 1; *v/v*) for 24 h. The fixed roots were then rinsed with distilled water and finally stored in 70% (*v/v*) ethyl alcohol at 4 °C until further use. Thereafter, the roots were hydrolyzed in 1 N hydrochloric acid for 1 min at 25 °C and stained with acetocarmine (chromosomal stain) for 30 min. After this, the roots were placed on slide and their tips were removed using forceps, macerated in a drop of 40% acetic acid, squashed and observed under the light microscope (Getner, India; model 66475). The different mitotic stages in the onion root-tip cells were observed. To determine MI and phase index, approximately 6000 cells (~2000 cells in each of the three slides) were observed per treatment and control. The frequency of aberrations was expressed in terms of percentage in relation to the number of cells in mitosis. The most frequent abnormalities are shown in photomicrographs (Fig. 4). Mitotic index, phase index and chromosomal aberrations were calculated as per Fagodia et al. (2017).

2.9. Statistical analysis

All the experiments were conducted in a randomized design manner with five independent replicates per treatment including the control. The data of all the experiments were analyzed by one-way ANOVA (analysis of variance) followed by a comparison of mean values using *post-hoc* Tukey's test at $Pp \leq 0.05$ using SPSS software version 16.0 (SPSS Inc., Chicago, IL). For laboratory dose-response bioassay, the data for germination and seedling growth were analyzed by non-linear fit model: log inhibitor *vs*. response-variable slope by plotting the concentration (on the *x*-axis) and percent germination, root length, coleoptile length and total chlorophyll content (on the *y*-axis) using the software GraphPad Prism version 6. For post-emergent spray treatment bioassay, the data were analyzed by linear regression models.

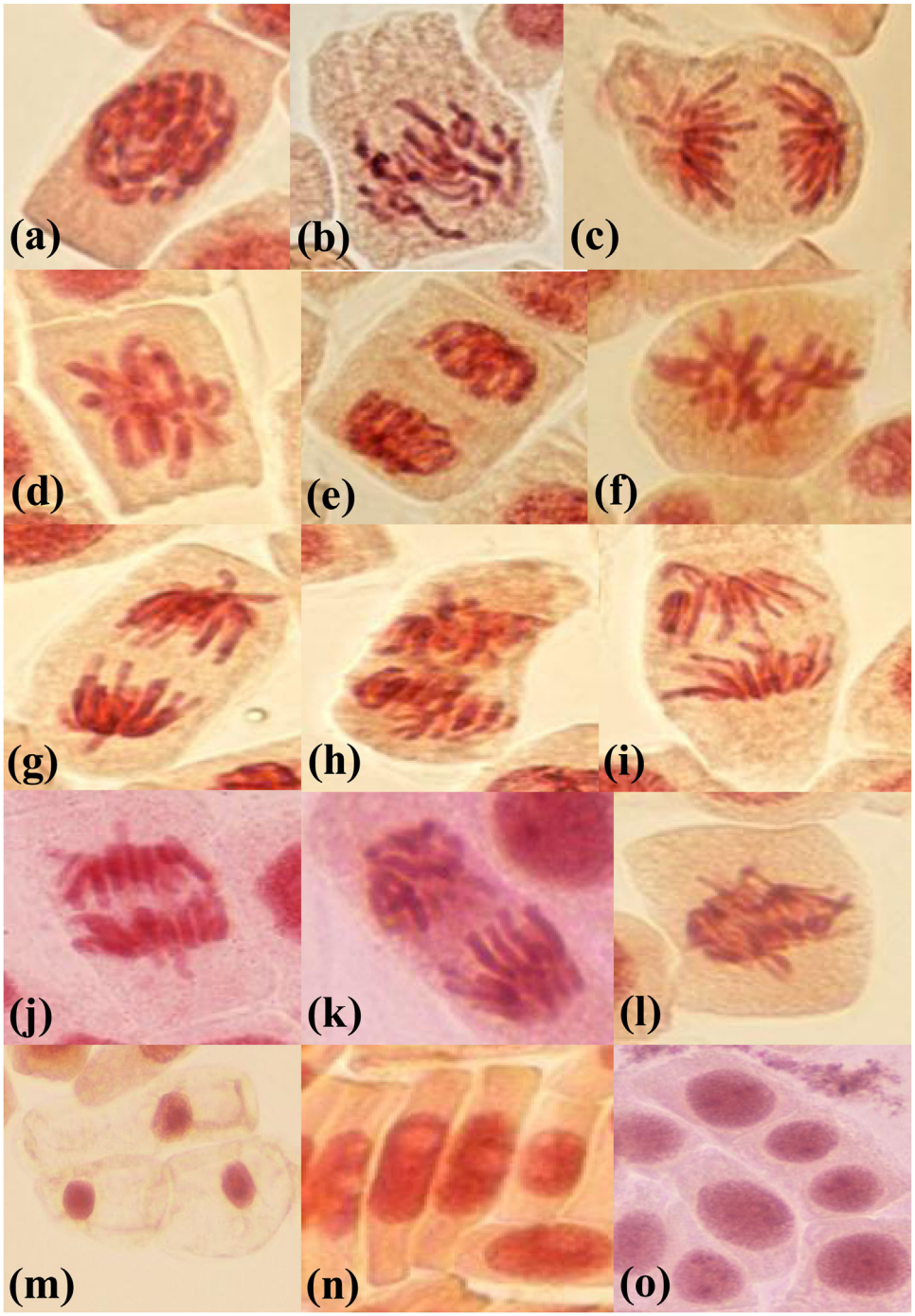

Fig. 4. Different types of chromosomal and cytological aberrations induced by *H. suaveolens* EO in *A. cepa* root tips after 24 h of treatment. *Chromosomal aberrations*: chromosomal breakage at prophase, metaphase and anaphase (a, b and c); sticky chromosomes at metaphase and anaphase (d and e); *c*-mitosis (f); abnormal and distorted anaphase (g, h and i); laggard at anaphase (i); multipolar anaphase (j); vagrant chromosome (k); anaphase with multiple bridges (l); *Cytological aberrations:* destructed cytoplasm with peripheral nuclei (m); elongated cells with extended interphase (n); and binucleate cell (o).

3. Results

3.1. Chemical characterization of H. suaveolens EO

The GC-MS analysis of EO extracted from the leaves of *H. suaveolens* led to the identification of 28 different chemical constituents, representing 99.67% of the compounds in the oil (Table 1; Fig. 5). In general, the oil was of monoterpenoid nature with ~79.33% of monoterpenes (predominantly monoterpene hydrocarbons) (Table 1). The major monoterpenes identified in the oil were: *α*-phellandrene, *α*-pinene, *allo*-ocimene, limonene, *β*-thujene, γ-terpinene, and *o*-cymene.

3.2. Effect of H. suaveolens EO on germination and early growth

Dose-response curves showed the growth inhibitory effect of *H. suaveolens* EO on the germination and early seedling growth of both the test plants. At the highest concentration of oil (2.5 mg mL^{-1}) treatment, a complete inhibition (i.e. 100%) of seed germination was observed in *E. crus-galli*, whereas ~ 48% reduction was observed in *O. sativa* (Fig. 1a). Not only the emergence, but the seedling growth (measured in

Table 1
GC-MS profile of EO extracted from the leaves of *H. suaveolens*.

Peaks	RT[a]	Compound[b]	RI[c]	Area (%)[d]	Methods of Identification[e]
1	5.14	*β*-Thujene	884	8.07	RI-MS
2	5.28	*α*-Pinene	894	10.09	RI-MS
3	5.54	Camphene	905	0.39	RI-MS
4	5.91	*α*-Phellandrene	917	22.78	RI-MS
5	6.61	Terpinolene	940	1.97	RI-MS
6	6.76	*o*-Cymene	945	4.2	RI-MS
7	7.29	*α*-Terpinene	962	0.29	RI-MS
8	10.67	Limonene	1056	8.48	RI-MS
9	11.36	γ-Terpinene	1074	4.24	RI-MS
10	19.82	*p*-Cymene	1272	1.57	RI-MS
11	23.72	*allo*-Ocimene	1364	8.67	RI-MS
12	7.5	*cis*-*β*-Terpineol	969	0.21	RI-MS
13	8.66	*cis*-*p*-Menth-2-en-1-ol	1005	0.26	RI-MS
14	9.29	(−)-4-Terpineol	1021	1.96	RI-MS
15	12.02	*cis*-Sabinene hydrate	1091	2.02	RI-MS
16	12.33	Linalool	1099	0.95	RI-MS
17	12.49	α-Fenchol	1103	0.59	RI
18	13.6	(*E*)-Ocimene oxide	1128	1.37	RI
19	17.41	1,8-cineole	1217	1.72	RI-MS
20	12.81	α-Bergamotene	1110	2.67	RI-MS
21	24.02	*β*-Elemene	1371	0.47	RI-MS
22	26.18	*β*-Caryophyllene	1423	9.47	RI-MS
23	28.61	Germacrene D	1483	0.17	RI-MS
24	28.85	*α*-Humulene	1489	1.01	RI-MS
25	14.81	Caryophyllene oxide	1456	2.33	RI-MS
26	15.94	*α*-*trans*-Bergamotol	1183	3.11	RI
27	18.78	(*E*)-*β*-Farnesene	1248	0.46	RI-MS
28	21.27	(*Z*) − 3-Hexenol	1306	0.15	RI-MS
		Total identified		99.67	
		Monoterpene hydrocarbons (%)		70.75	
		Oxygenated monoterpenes (%)		9.08	
		Sesquiterpene hydrocarbons (%)		13.79	
		Oxygenated sesquiterpenes (%)		5.9	
		Aliphatic compound (%)		0.15	

[a] RT: Retention time.
[b] Chemical compound.
[c] RI: Kovats index.
[d] %age: Percentage of chemical compound.
[e] Identification: identification was based on comparison of retention indices on TG 5MS column using homologous series of *n*-alkanes (C_8–C_{32}); "RI-MS" means that identification was based on retention indices and comparison with NIST 2.0 libraries.

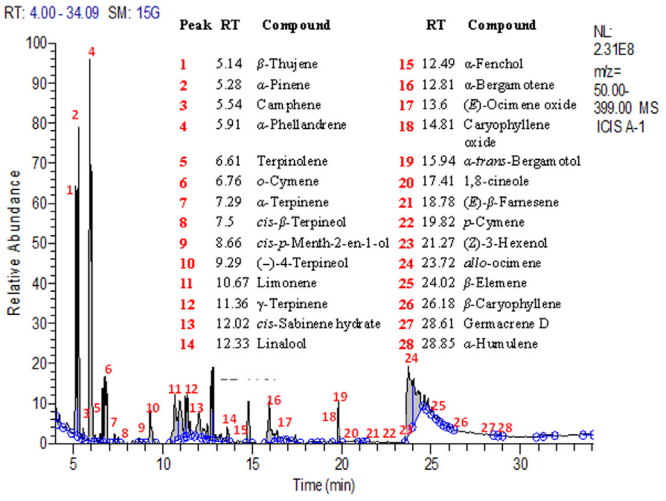

Fig. 5. Total Ion Chromatogram (TIC) of *H. suaveolens* EO upon GC-MS analysis.

terms of root and coleoptile length) was also reduced severely in response to EO treatment (Fig. 1b,c). The root length of *O. sativa* and *E. crus-galli* was reduced by 37% and 100%, respectively, compared to their respective controls upon treatment with 2.5 mg mL^{-1} of *H. suaveolens* EO (Fig. 1b). Similarly, the coleoptile length was also reduced significantly ($p \leq 0.05$) by ~ 27% and 100% in *O. sativa* and *E. crus-galli* as compared to the control (Fig. 1c). Further, a significant ($p \leq 0.05$) reduction of 69% and 100% was observed in the chlorophyll content in *O. sativa* and *E. crus-galli,* respectively, upon comparison with their respective controls, when treated with 2.5 mg mL^{-1} EO (Fig. 1d). In general, a greater phytotoxic effect was observed on weed (*E. crus*-galli) than on the crop plant (*O. sativa*).

3.3. Effect of foliar spray treatment of H. suaveolens EO

a) Visible injury

Post-emergent spray treatment with *H. suaveolens* EO caused visible injuries in a dose-dependent manner (Fig. 2). In *O. sativa*, no visible injury was noticed at 7 DAS upon treatment with *H. suaveolens* EO (Fig. 2c). However, at 21 DAS, yellow colored patches (chlorosis) indicating minor injuries (0–20%) were noticed on the foliar parts of *O. sativa* seedlings in response to 2.5% oil emulsion. On the treatment with the highest concentration (5%, *v/v*), chlorosis and temporary wilting (20–40%) were noticed on *O. sativa;* however, these recovered with time (Fig. 2a).

In contrast, in *E. crus-galli*, at 7 DAS severe injuries (40–60%) with appearance of small yellow and dark brown necrotic spots were observed in response to 2.5% *H. suaveolens* EO treatment. However, at 5% EO treatment, severe irreversible injuries (60–80%) followed by the appearance of necrotic spots and complete wilting of plants was noticed (Fig. 2b). At 21 DAS, even at the lowest treatment, *i.e.* 1%, the level of injuries was severe (40–60%) but reversible, while at 2.5% EO treatment, severe injuries (60–80%) were noticed with no recovery (Fig. 2). However, permanent wilting (~100% injury) leading to death of *E. crus-galli* treated plants was observed at 21 DAS upon treatment with 5% *H. suaveolens* EO (Fig. 2b,d). Based on the symptomology of chlorosis and necrosis, it was clear that *H. suaveolens* EO application caused much severe damage to the weed (*E. crus-galli*) as compared to the crop (*O. sativa*).

b) Chlorophyll content and cell viability

In *O. sativa* plants, chlorophyll content declined by 14% and 28% after 7 and 21 DAS, respectively, upon treatment with 5% *H. suaveolens* EO (Fig. 3a). In contrast, in *E. crus-galli* decline was more pronounced and 61% and 74% reduction was observed at 7 and 21 DAS, respectively, (Fig. 3b). Parallel to chlorophyll content, a significant decline in cell viability was also observed in *O. sativa* and *E. crus-galli* in response to *H. suaveolens* EO (Fig. 3c, d). In response to 2.5% oil treatment, cell viability was reduced by ~17% and ~ 31% at 7 DAS and by ~25 and ~45% at 21 DAS in *O. sativa* and *E. crus-galli*, respectively (Fig. 3a, b). At 5% EO treatment, a further decline (25% and 50% at 7 DAS and 45% and 85% at 21 DAS, in *O. sativa* and *E. crus-galli*) in cell viability of the test plants was noticed (Fig. 3c, d).

3.4. Effect of H. suaveolens EO on cell division

Treatment with 2.5 mg mL^{-1} of EO of *H. suaveolens* significantly declined (by 63%) mitotic index and altered the phase indexes of different mitotic stages in root meristems of *A. cepa* (Table 2). EO treatment induced various chromosomal (like chromosomal breakage, sticky chromosomes, *c*-mitosis, abnormal and distorted anaphase, multipolar anaphase, and anaphase bridge) (Fig. 4a-l) and cytological aberrations (like damaged cytoplasm with peripheral nuclei, elongated cells with extended interphase, and bi-nucleated cell) (Fig. 4m–o). The frequency of aberrations in the root meristem cells of *A. cepa* increased upon treatment with *H. suaveloens* EO, and ~ 49% aberrations were observed with 2.5 mg mL^{-1} of EO (Table 3).

Table 2
Effect of different concentrations of *H. suaveolens* EO on the mitotic index and mitotic activity in *A. cepa* root tips exposed for 24 h.

Concentration	Mitotic phases (%)				Mitotic index (% ± S.E)
(mg mL^{-1})	Prophase	Metaphase	Anaphase	Telophase	
Essential oil					
0.05	19.91	29.40	33.54	17.15	9.11 ± 0.25ab
0.5	30.71	20.88	26.30	22.11	7.06 ± 0.31b
1.5	24.00	23.07	30.80	22.11	5.70 ± 0.27cbd
2.5	26.30	31.45	30.52	11.73	3.70 ± 0.10d
Positive control	26.10	24.34	24.34	25.22	3.23 ± 0.40d
Negative control	32.11	23.94	21.45	22.50	10.15 ± 1.25a

Data represented as mean values (for mitotic phases) and mean ± standard error (for mitotic index). Different alphabets (within the last column) represent significant difference at $p \leq 0.05$ applying *post hoc* Tukey's test.

Table 3
Chromosomal aberrations in the root tip cells of *A. cepa* exposed to different concentrations of *H. suaveolens* EO for 24 h.

Treatment	Types of Aberrations (%)							Total aberrant cells (%)
	CM	CB	AB	SC	DA	BN	MA	
Essential oil (mg mL^{-1})								
0.05	18.6	14.00	17.00	26.00	24.00	ND	0.33	20.96 ± 1.10b
0.5	20.0	17.66	22.66	17.66	20.66	1.00	0.33	21.33 ± 5.77b
1.5	15.00	8.33	8.66	12.66	15.33	7.33	2.00	24.57 ± 3.31 b
2.5	16.00	16.66	13.33	18.33	21.00	14.33	3.66	48.51 ± 3.21a
Negative control	3.00	ND	ND	4.00	ND	ND	ND	1.02 ± 0.30c
Positive control	18.33	7.66	12.66	15.00	14.33	3.66	2.00	64.05 ± 6.68a

Data represented as mean values (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 chromosomes; DA: distortion at anaphase; BN: binucleate cells; MA: multipolar anaphase; ND: not detected.

4. Discussion

In the present study, the GC-MS analysis revealed that the EO of *H. suaveolens* was monoterpenoid in nature (~79% of the oil was constituted by monoterpenes). The major chemical constituents reported in EO were: *α*-phellandrene, *α*-pinene, limonene, *β*-thujene, γ-terpinene, *allo*-ocimene, and *o*-cymene. The results of present study are in agreement with some previous studies that have reported the presence of monoterpene hydrocarbons such as *α*-phellandrene, *α*-pinene, limonene, *β*-thujene, γ-terpinene, *allo*-ocimene, and *o*-cymene in the EO extracted from the leaves of *H. suaveolens* (Eshilokun et al., 2005; Conti et al., 2012). The phytotoxic potential of *H. suaveolens* EO was evaluated against *O. sativa* and its major weed *E. crus-galli*. The results indicated that *H. suaveolens* EO exposure inhibited the seedling emergence, seedling growth and the chlorophyll content of both the test plants in a dose-dependent manner. However, the effect was more pronounced on the weed (*E. crus-galli*) as compared to the crop (*O. sativa*).

The observed growth inhibitory/phytotoxic effect of *H. suaveolens* EO could be attributed to the presence of various chemical constituents reported in the EO, especially the monoterpenes. EOs from aromatic plants of Lamiaceae has been found to exhibit phytotoxicity, inhibiting germination and seedling growth (de Almeida et al., 2010). Several studies have demonstrated that the EOs and their constituents, especially monoterpenes, are phytotoxic in nature (Ahuja et al., 2015; Batish et al., 2007; Singh et al., 2005, 2006a; Tworkoski, 2002). This is further validated from earlier reports demonstrating that EOs rich in monoterpenes are comparatively more phytotoxic than those with a higher percentage of sesquiterpenes (Amri et al., 2012; Kordali et al., 2007). However, whether the observed phytotoxic effect of *Hyptis* oil is due to the individual monoterpenes or combined synergistic effect of various monoterpenes cannot be ascertained from the present study.

Post-emergent application of *H. suaveolens* EO caused various visible injury symptoms, like discolouration of plants, chlorosis and necrotic spots at 21 DAS. Injury symptoms were more prominent in *E. crus-galli* than in *O. sativa*. In addition to growth parameters, a significant reduction in the chlorophyll content was also observed in the leaves of both the test plants. The decline in chlorophyll content in the leaves of test plants may be attributed to either decreased synthesis of chlorophyll or its enhanced degradation or both (Yang et al., 2004). Similar to our study, several other studies have also reported that EOs or their constituents (mainly monoterpenes) reduced the amount of chlorophyll in the leaves of treated plants (Kaur et al., 2010; Kordali et al., 2007).

In general, the growth inhibitory/phytotoxic effect of any chemical compound might have been manifested through its cytotoxic effect. Therefore, the present study was further extended to determine the effect of *H. suaveolens* EO on the cell division of root meristem cells of *A. cepa*. EO of *H. suaveolens* reduced the mitotic activity (by ~63%) in the root meristem cells of *A. cepa*. The observed reduction in mitotic activity might be due to the reduction in the number of dividing cells that further led to a disturbance in cell cycle, thereby an impaired growth (Fiskesjö, 1997). According to Yuet-Ping et al. (2012), reduction in the mitotic index may either be due to an arrest of one or more mitotic phases or due to slowing down of the rate of cell progression. In addition to this, other plausible reason for the interference of *H. suaveolens* EO with mitotic activity could be attributed either to the loss of DNA/protein synthesis or due to the prevention of nucleoprotein synthesis in the cell cycle (El-Ghamery et al., 2003). As per Nishida et al. (2005), EO/monoterpenes have the ability to inhibit DNA synthesis, which prevents the cell from entering into mitosis. Likewise, some other studies have also reported the inhibition of cell division by EOs and their constituents (Singh et al., 2006b; Pawlowski et al., 2012; Fagodia et al., 2017).

Besides, *H. suaveolens* EO exposure also induced various chromosomal aberrations like sticky chromosomes, chromosome bridges, c-mitosis and bi-nucleated cells in the root tip cells of *A. cepa*. The sticky chromosomes may be formed due to the toxic effect of volatile oil on the organization of chromatin (Pinheiro et al., 2015). Sticky chromosomes involve a proteinaceous matrix of chromatin rather than DNA and due to its irreversible nature, stickiness leads to apoptosis and

thereby, decline in the mitotic index (Fernandes et al., 2009; Pawlowski et al., 2012). Since, in sticky chromosomes, chromatin organization is affected, therefore, it may further lead to the formation of chromosome bridges, as observed in the present study. Therefore, chromosome bridges may have arisen as a result of stickiness of chromosomes, which hinder their separation or free movement. According to Jabee et al. (2008), chromosomal bridges arise due to the formation of dicentric chromosomes by breakage. EO may inhibit the spindle formation similar to the effect of colchicine (Badr, 1983). The bi-nucleated cells observed in *H. suaveolens* EO treated root tip cells of *A. cepa* are formed due to the absence of cytokinesis after telophase and are visible in the interphase of next cell cycle (Hayashi and Karlseder, 2013). Therefore, these alterations caused by the EO of *H. suaveolens* in the root meristem cells of *A. cepa* might be due to the presence of monoterpenes or the combined synergistic effect of various monoterpenes reported in the EO (Fagodia et al., 2017; Pawlowski et al., 2012; Pinheiro et al., 2015).

In our study, EO of *H. suaveolens* exhibited greater toxicity against the weed *E. crus-galli* than the crop *O. sativa.* This property of the oil makes it worth exploiting for weed management since the EOs are biodegradable and fall under the category of GRAS (Generally Regarded as Safe) compounds (Isman, 2000; Tworkowski, 2002; Batish et al., 2008; Dayan et al., 2009). However, there are many limitations such as their volatile nature, insolubility in water, and inconsistent efficacy under different environmental conditions, and their impact on non-target organisms (Batish et al., 2008), which need to be ascertained before recommendation of the oil as a viable option for sustainable weed management (Batish et al., 2008). Nevertheless, the solubility of EOs can be enhanced by using adjuvants or making their emulsion with surfactants and making formulations (Ahuja et al., 2015). Further, the solubility, stability and efficacy of EOs can be enhanced by micro- and nano-encapsulation (Bakry et al., 2015).

5. Conclusions

Based on the results of the present study, it is concluded that the EO of *H. suaveolens* possess herbicidal activity against the weed due to its cytotoxic potential. EO, being a natural product, could be suggested as an alternative source for the development of natural herbicides in weed management programmes.

Acknowledgement

Anita Sharma is thankful to the University Grants Commission (New Delhi, India) for the financial support in the form of fellowship.

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