https://doi.org/10.26651/allelo.j/2024-62-1-1487 0971-4693/94 Euro 20.00
Allelopathy Journal 62 (1): 71-84 (May, 2024) *International Allelopathy Foundation* 2024
Table: 2, Figs: 5

Herbicidal potential of essential oils of *Mentha piperita*, *Chrysopogon zizanioides*, *Mentha arvensis*, and *Pelargonium graveolens* to control wild oat

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(Received in revised form : March 4, 2024)

ABSTRACT

Aromatic and medicinal plants release volatile allelochemicals which influence the organisms in their environment. A pot culture experiment was conducted to determine the phytotoxic activity of essential oil emulsions (EOEs) of *Mentha piperita* L., *Chrysopogon zizanioides* L., *Mentha arvensis* L. and *Pelargonium graveolens* L. with two herbicides (clodinafof-propargyl 15 % WP and sulfosulfuran 75 % WG) on growth and physiological parameters of *Avena fatua* L. (wild oat) and on soil enzyme activities. Post-emergence application of EOEs and chemical herbicide were applied on 5-week old *A. fatua* plants. We found that several EOEs with varying concentrations and chemical herbicides reduced ($p \leq 0.05$) the *A. fatua* plant growth (shoot length, root length, fresh and dry weight of plant), physiological parameters and also affected the post-harvest soil enzyme activities and soil microbial biomass carbon (SMBC). Chlorophyll content was considerably decreased, but increased proline accumulation and electrolyte leakage in *A. fatua* plants indicating membrane rupture and loss of integrity. Since essential oils interfere with plant growth and physiological processes and have exceptional inhibitory effects on the *A. fatua* plant, the allelochemicals present in these donor plants may be used as active ingredients to develop natural herbicides.

Keywords: Allelochemicals, *Avena fatua*, chemical herbicides, chlorophyll content, *Chrysopogon zizanioides*, Essential oil emulsions (EOEs), *Mentha arvensis*, *Mentha piperita*, *Pelargonium graveolens*, post-emergence, soil enzyme, weed control.

INTRODUCTION

Synthetic herbicides increased environmental contamination and human toxicological risk (20). The development of alternative weed management techniques required significant effort due to the rise in herbicide-resistant weeds and environmental concerns over the continuous use of synthetic herbicides with various modes of action (12). Hence, the popularity of organic farming and the demand for eco-friendly herbicides in traditional agriculture have recently speeded up the development of natural product-based herbicides (21). One alternative is the allelopathic method (32). The interference process known as allelopathy occurs when living or dead plants release allelochemicals that have a direct or indirect, positive or negative effect on the recipient plants (usually unfavourably). It could be very important in both natural and controlled ecosystems (13).

Aromatic plants are abundant in phenolic and essential oil (EO) components and are major source of allelochemicals with herbicide properties and also interact with other plant

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(11,30,39). Essential oils (EOs) are complex mixture of volatile constituents derived from plants and are frequently used for their herbicidal properties (39,49). These oils may be effective replacement of traditional synthetic pesticides because of their minimal impact on the environment and human health (49). For natural herbicides, p-hydroxyphenyl pyruvate deoxygenase in plant enzymes is their primary target site (HPPD). The essential oil contains monoterpene, sesquiterpene, oxygenated chemicals, and sesquiterpene alcohols, have allopathic effects. Volatile oils and their components are inhibitors of seed germination and reduce plant growth (6,9,19,30,39,44). The volatile oil from *A. scoparia* decreased the growth of *Cyperus rotundus* and *Phalaris minor* (43). Likewise essential oils of aromatic plants of *Thymbra capitate*, *Santolina chamaecyparissus*, *Eucalyptus camaldulensis* and *Mentha piperita* have inhibitory effects on *Erigeron bonariensis* weed growth (47).

China and India are the largest producers and exporters of MAPs. Natural products from MAPs have lesser harmful effects on human systems rather than their synthetic analogues (4,38).

The common wild oat, *Avena fatua* L., is most pervasive, economically damaging and herbicide-resistant weed species that threaten cereal crops. *A. fatua* L. competition reduced crop yields up to 70 %. Natural products-based herbicides are required for the sustainable control of *A. fatua* L. to prevent further herbicide resistance (10,29).

Figure 1. Photographs of aromatic plants used in this study. *Mentha piperita* - in population (1a), single plant (1b), *Chrysopogon zizanioides*- population (2a), single plant (2b), *Mentha arvensis* - in population (3a), single plant (3b), *Pelargonium graveolens*- in population (4a), single plant (4b)

The essential oils are phytotoxic to weed germination, however, there are few studies to evaluate essential oil emulsions on phytotoxicity and soil microbial activity. For a safe and effective weed management, it is essential to screen these essential oil emulsions for both their herbicidal activity and their impact on the soil microorganism. The essential oil emulsion, not only serves as an herbicide but also has no negative impact on the soil microbiological activities. This study aimed to investigate the herbicidal efficacy of *Mentha piperita*, *Chrysopogon zizanioides*, *Mentha arvensis* and *Pelargonium graveolens* (Figure 1) essential oil emulsions (EOEs) and two herbicides against *Avena fatua* L. plant in terms of effect on plant growth, physiological processes (photosynthesis, membrane integrity, severity of damage) and soil microbial enzymatic activities.

MATERIALS AND METHODS

The pot experiment was conducted from December 2020 - March 2021 at Research Farm CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow [26°5' N latitude and 80°5' E longitude, altitude 120 m above MSL (mean sea level)]. The soil used in this experiment was loam soil and filled in 30 cm dia and 30 cm height pot (soil: sand: manure: 3:1:1 w/w). During study period, the experimental site receives rainfall (4.6 mm) only once in February month, recorded maximum temperature (35.21 °C) and minimum (6.16 °C), relative humidity (89.85 % - 25.28 %), during the experimental period.

Plant material and essential oil emulsion (EOEs)

Seeds of weed plant *A. fatua* L. were collected from a nearby farmer’s field and air dried in shade. For the studies, air-dried seeds with moisture contents greater than 12 % were used (26). Essential oil of *Mentha piperita*, *Chrysopogon ziznioides*, *Mentha arvensis* and *Pelargonium graveolens* were collected from Research Farm CSIR-CIMAP, Lucknow, Uttar Pradesh, and Research Centre CSIR-CIMAP, Pantnagar, Uttarakhand, India. Different concentration of essential oils was obtained by dissolving the required concentration of essential oil in distilled water with the help of emulsifier agent Tween 80 (0.1 % v/v). The mixture was stirred at 3000 rpm for 25 min using homogenizer and obtained emulsion were stored at (25 ± 1°C). For 30 days, the stability of emulsions was monitored. Throughout this time, no phase separation was noticed.

Experimental details

Pot experiment was conducted to assess the comparative phytotoxicity of essential oil emulsion (EOEs) and chemical herbicides against *A. fatua* plant as a post-emergence herbicide. The treatments were : 4-essential oil emulsion of aromatic and medicinal plants *Mentha piperita*, *Chrysopogon zizanioides*, *Mentha arvensis*, *Pelargonium graveolens* with 3-concentrations (2 %, 4 %, 8 %) of each EOEs and two chemical herbicides [clodinafof-propargyl 15 % WP (0.1 %), sulfosulfuran 75 % WG (0.0132 %)]. In each pot 10 seeds of *A. fatua* were sown and kept under natural light conditions and irrigated daily. Foliar application of EOEs and chemical herbicides at 30 ml per pot were applied on 5-weeks old weed plants. The treatments were replicated thrice in complete randomised design (CRD). Weed plants without foliar application of EOEs and chemical herbicide were taken as control.

Weed plants uprooted after seven days of foliar application of treatment their growth parameters (shoot length, root length, fresh weight and dry weight) were measured. For the dry weight, weed plants were kept in a hot air oven for 48 h at 60 °C. Soil microbial enzymes and soil microbial biomass carbon (SMBC) in post-harvest soil were also assayed.

Physiological parameters

Visible injury

Visible injury was observed visually on the 1st and 7th days after the foliar spray of essential oil emulsion and chemical herbicides. Scoring of visual injury was done by following the severity indexing ranging from 0-10 and calculated in percent (%) according to Rao (36).

Chlorophyll and carotenoid

Fresh leaf samples (0.25 g) were homogenized in a mortar with 80 % acetone to measure total chlorophyll, chlorophyll *a*, *b*, and carotenoid. Following Arnon's procedure (5), the extract was centrifuged at 7500 rpm for 10 min and the absorbance was measured at 663 and 645 nm to estimate total chlorophyll, chlorophyll *a*, chlorophyll *b* and at 480 and 510 nm to evaluate carotenoids by spectrophotometer (MODEL: UV-1800 240V, SHIMADZU EUROPA GmbH®). All calculations of photosynthetic pigments are made according to the following equations:

Chlorophyll:

Chl a= [12.7(A_663)-2.69(A_645) × volume]/ [1000 × wt. of sample]
Chl b= [22.9(A_645)-4.689(A_663) × volume]/ ([1000 × wt. of sample])
Carotenoid = [7.6 (A_480)-1.48 (A_510) × Volume]/ [1000 × wt. of sample]

Relative electrolyte leakage

Seven days after the foliar spray on the weed plant, relative electrolyte leakage (REL) was analyzed. Conductivity was measured after distilled water added to 100 mg of plant leaves for 30 minutes (C1). After the conductivity measurement, test tubes containing plant leaves were boiled for 20 minutes and the conductivity was measured again (C2). Using the formula below, REL was determined and expressed in % (26).

$$REL = \frac{(C1)}{(C2)} \times 100$$

Proline

Using a colorimetric approach and employing proline's reactivity to ninhydrin and L-proline as a reference, proline accumulation in leaves was estimated (7). The fresh tissue sample (0.5 g) was homogenized in 2 ml of 3 % sulfosalicylic acid and centrifuged at 10,000 rpm for 10 min. After that, take 2 ml of supernatant in test tube and reacted with 2 ml of acid ninhydrin reagent and 2 ml of GAA and kept on a water bath at 100 °C for 1 h, cooled on ice. 4 mL of toluene was thoroughly and kept for 30 min. Separate the layers and absorbance was recorded at 520 nm (7). The results were calculated and expressed in µmol g^{-1} on the basis of fresh weight leaf tissue. A proline standard curve was used to determine the proline concentration.

Soil sampling and analysis

From a depth of 0 to 15 cm, post-harvest soil samples from the experimental pots were taken and following biological characteristics were examined:

(i). **Acid phosphatase and alkaline phosphatase activity:** Tabatabai and Bremmer (45) and Eivazi and Tabatabai (17) method was used to evaluate the activity. To test the acid phosphatase and alkaline phosphatase activity, soil samples were incubated with p-nitrophenyl phosphate in modified universal buffer (pH 6.5 and 11.0, respectively).

(ii). **Soil dehydrogenase activity:** Casida (14) method was used to assess its activity. By transporting hydrogen from organic molecules to inorganic acceptors, dehydrogenase plays a significant part in the biological oxidation of soil organic matter.

(iii). **Soil Microbial Biomass Carbon (SMBC):** The Fumigation extraction method of Vance *et al*., (46) was used to quantify SMBC. The amount of soluble C retrieved from soil after and before fumigation correlate with their presence in microbial cells and C released from microbial cells after chloroform fumigation is directly quantified.

Statistical analysis

With the help of the Windows software package SPSS 20, statistical analyses for various recorded observations were carried out and ANOVA was applied. The significance between the values was evaluated using Duncan's test. At 5 % ($p \leq 0.05$) level of significance was used in the calculations to determine the results.

RESULTS

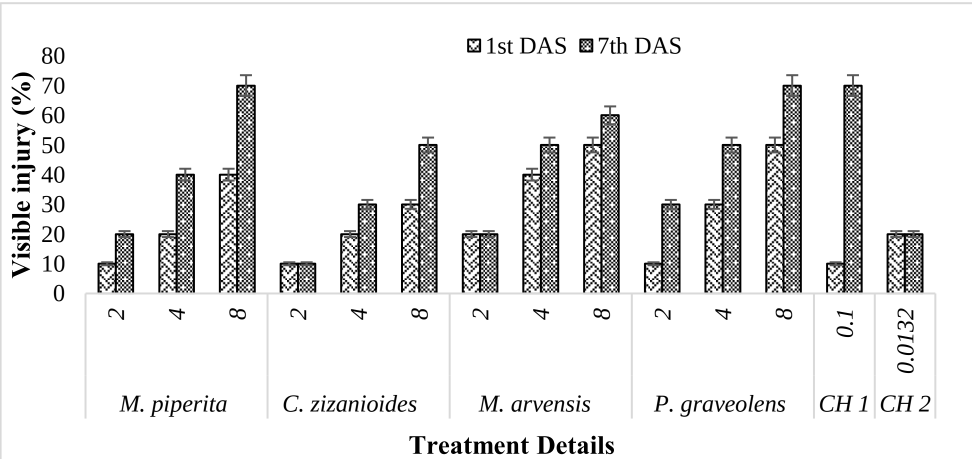

Figure 2. Visible injury after foliar spray of EOEs/chemical herbicides at 1st and 7th days after spray (DAS) ***CH 1 - Clodinafof- propargyl 15 % WP, CH 2- Sulfosulfuran 75 % WG.

*Y-Axis indicates the concentration (%) of essential oil emulsion such as *Mentha piperita*, *Chrysopogon zizanioides*, *Mentha arvensis*, *Pelargonium graveolens* and Clodinafof-propargyl 15 % WP, Sulfosulfuran 75 % WG

Visible injury

Visible injury in *A. fatua* plants was recorded 1st and 7th day after foliar application (Figure 2). Highest injury was recorded by *Mentha piperita*, *Pelargonium graveolens* at 8 %, and clodinafof-propargyl 15 % WP treatment after 7th day of foliar application which was 70 % injury than other treatments. *Chrysopogon zizanioides* at 2 % showed same visible injury 1st and 7th day after application and showed minimal injury symptoms than other treatment. With the time visible injuries were increased in all treatment except *Chrysopogon zizanioides* treatment at 2 % and sulfosulfuran 75 % WG treatment.

Growth parameters

A. fatua plants were uprooted on 7-days after foliar application of treatment and growth parameters (shoot and root length, fresh and dry weight) were recorded (Table 1). Shoot length reduction was highest in treatment of *Pelargonium graveolens* at 8 % (43.22 %) and root length reduction by *Mentha arvensis* treatment at 8 % (54.08 %) over control. Fresh weight of *A. fatua* seedlings was severely reduced by *Mentha piperita* and *Mentha arvensis* followed by *Pelargoium graveolens* treatment at 8 % over control respectively 73.93 %, 71.27 %, 70.43 % while chemical herbicide clodinafof- propargyl 15 % WP and sulfosulfuran 75 % WG reduced fresh weight of seedling 65.56 % and 41.78 % respectively. Dry weight of seedling was maximum decreased by *Mentha piperita* (77.18 %) and *Mentha arvensis* (76.63 %) followed by *Pelargonium graveolens* (71.07 %) treatment at 8 % than control. Within the treatment, *Chrysopogon zizanioides* showed non-significant results. *Chrysopogon zizanioides* treatment at 2 % reduced the shoot length 7.46 % while increased the fresh weight of seedling (11.52 %) with respect to control (Table 1).

Table 1. Effects of EOEs/chemical herbicides on growth parameters of *Avena fatua* L.

Treatments	EOEs Conc. (%)	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)
Control	-	44.43f	10.89f	52.57h	19.02e
M. piperita	2 (%)	35.00e	9.89ef	43.78g	13.94d
	4 (%)	33.22de	7.17abcd	37.12ef	12.75cd
	8 (%)	27.67bc	5.61ab	13.70a	4.34a
C. zizanioides	2 (%)	41.11f	8.33cde	58.63i	13.45cd
	4 (%)	34.78e	7.44bcd	33.11de	13.08cd
	8 (%)	33.67de	5.83ab	40.56fg	9.33b
M. arvensis	2 (%)	33.33de	6.36abc	42.95g	11.13bc
	4 (%)	32.11de	5.44ab	18.30abc	5.87a
	8 (%)	29.89cd	5.00a	15.10ab	4.45a
P. graveolens	2 (%)	29.72cd	9.05def	21.85c	6.85a
	4 (%)	26.39bc	6.83abcd	19.62bc	6.15a
	8 (%)	25.22b	5.94abc	15.54ab	5.50a
Clodinafof- propargyl 15 % WP	0.1 (%)	29.79cd	7.49bcd	18.10abc	5.60a
Sulfosulfuran 75 % WG	0.0132 (%)	20.32a	7.01abcd	30.60d	6.55a

Mean followed by same letter within one column (Shoot length, root length, fresh weight, dry weight) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test).

Effects on chlorophyll and carotenoid

Total chlorophyll, Chl *a*, Chl *b* and carotenoid content were recorded after 7-days of treatment application and maximum reduction in total chlorophyll and carotenoid found in treatment *Mentha arvensis* at 8 % which were 0.488 mg g^{-1} FW and 2.618 mg g^{-1} FW respectively followed by *Mentha piperita* (total chlorophyll- 0.505 mg g^{-1} FW, carotenoid- 3.096 mg g^{-1} FW) and *Pelargonium graveolens* (total chlorophyll- 0.552 mg g^{-1} FW, carotenoid- 2.618 mg g^{-1} FW) treatment (Table 2). Chl *a* reduction was maximum recorded by *Chrysopogon zizanioides* at 4 % (0.305 mg g^{-1} FW) followed by *Mentha piperita* at 8 % (0.363 mg g^{-1} FW) and *Mentha arvensis* at 8 % also (0.363mg g^{-1} FW). Chl *b* was lowest in *Mentha arvensis* at 8 % (0.126 mg g^{-1} FW) followed by *Chrysopogon zizanioides* at 4 % (0.139 mg g^{-1} FW) and *Mentha arvensis* at 8 % (0.143mg g^{-1} FW). Total chlorophyll and carotenoid in chemical herbicides clodinafof-propargyl 15 %WP were recorded 0.648 mg g^{-1} FW and 3.456 mg g^{-1} FW respectively where as in sulfosulfuran 75 % WG treatment 1.253 mg g^{-1} FW total chlorophyll and 5.067 mg g^{-1} FW carotenoid recorded. Maximum chlorophyll was found in control (1.708 µ mole g^{-1} FW) while carotenoid recorded after treatment by *Mentha piperita* at 2 % treatment (5.787 µ mole g^{-1} FW) (Table 2).

Table 2. Effects of EOEs/chemical herbicides on Total chlorophyll, Chlorophyll *a*, *b*, carotenoid, REL and proline content in *Avena fatua* L. leaves

Treatments	EOEs Conc (%)	T. Chl. (mg g^{-1} FW)	Chl. *a* (mg g^{-1} FW)	Chl. *b* (mg g^{-1} FW)	Carot. (mg g^{-1} FW)	REL (%)	Proline (µ mole g^{-1} FW)
Control	Control	1.708i	1.264i	0.444g	5.398j	7.255a	0.127e
M. piperita	2 (%)	1.580i	1.149h	0.431g	5.787i	10.687cd	0.081a
	4 (%)	0.765g	0.568f	0.198bc	3.102cd	11.244cd	0.152f
	8 (%)	0.505ab	0.363b	0.143a	3.096cd	12.017de	0.172g
C. zizanioides	2 (%)	1.545i	1.168h	0.377f	4.770g	11.939de	0.092bc
	4 (%)	0.444a	0.305a	0.139a	2.601a	13.151ef	0.125e
	8 (%)	0.597cde	0.415cd	0.183b	3.424e	14.711gh	0.175g
M. arvensis	2 (%)	0.725fg	0.521e	0.204bc	2.929bc	14.046fg	0.087ab
	4 (%)	0.666ef	0.446d	0.220cd	3.338de	15.413h	0.185h
	8 (%)	0.488ab	0.363b	0.126a	2.618a	18.253i	0.231i
P. graveolens	2 (%)	0.752g	0.505e	0.247d	4.323f	8.736b	0.095c
	4 (%)	0.580cd	0.401bc	0.179b	2.671ab	10.564c	0.118d
	8 (%)	0.552bc	0.370b	0.182b	2.968bc	13.440fg	0.306j
Clodinafof-propargyl 15 % WP	0.1 (%)	0.648de	0.449d	0.199bc	3.456e	13.092ef	0.487k
Sulfosulfuran 75% WG	0.0132 (%)	1.253h	0.925g	0.329e	5.067h	10.711cd	0.087ab

Mean followed by same letter within one column (T. Chl., Chl. *a*, Chl. *b*, Carot., REL, proline) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test); *T. Chl.- Total Chlorophyll, Chl. *a*- Chlorophyll *a*, Chl. *b*- Chlorophyll *b*, Carot.- Carotenoid, REL- Relative electrolyte leakage).

Effects on relative electrolyte leakage

Relative electrolyte leakage was recorded on 7-days after foliar application of treatment, and found that electrolyte leakage increased in all the treatments over control. Relative electrolyte leakage was highest recorded at 8 % and 4 % by *Mentha arvensis* treatment, which was 60.25 % and 52.92 % respectively than control followed by *Pelargonium graveolens* 46.01 % at 8 % treatment. In chemical herbicides clodinafof-propargyl 15 %WP and sulfosulfuran 75 %WG were 44.58 % and 32.25 % respectively compared to control. The lowest relative electrolyte leakage was recorded after treatment of *Pelargonium graveolens* at 2 % (16.95 %) with respect to control (Table 2). With increasing the concentration of essential oil, increased electrolyte leakage was observed.

Effects on proline accumulation

Proline accumulation in seedlings was recorded on 7-days after foliar application of treatment. Proline is an amino acid which indicates that a plant is in stress when more proline accumulates. With the concentration of essential oil, a trend of increased content of proline was found. Proline accumulation in seedlings varied with the type of essential oil. The highest proline accumulation was recorded in chemical herbicides clodinafof-propargyl 15 % WP (0.487 µ mole g^{-1} FW) and after treatment of *Pelargonium graveolens* at 8 % (0.306 µ mole g^{-1} FW) followed by 0.231µ mole g^{-1} FW by *Mentha arvensis* at 8 % (Table 2). The lowest accumulation of proline was found after treatment of *Mentha piperita* at 2 % (0.081 µ mole g^{-1} FW) followed by sulfosulfuran 75 % WG treatment (0.087 µ mole g^{-1} FW) and *Mentha arvensis* at 2 % (0.087µ mole g^{-1} FW).

Soil microbial enzymes

Acid phosphatase, alkaline phosphatase, and dehydrogenase soil enzyme were assayed in post-harvest soil (Figure 3 and Figure 4). Maximum acid and alkaline phosphatase activity were recorded in chemical herbicides clodinafof-propargyl 15 % WP were 314.57 µg PNP g^{-1} h^{-1} and 531.04 µg PNP g^{-1} h^{-1} respectively. After treatment of *Pelargonium graveolens* at 4 % acid and alkaline phosphatase activity were found at 251.30 µg PNP g^{-1} h^{-1} and 508.55 µg PNP g^{-1} h^{-1} respectively while at 8 % recorded 209.21 µg PNP g^{-1} h^{-1} and 452.97 µg PNP g^{-1} h^{-1}. Alkaline phosphatase activity was found at 530.40 µg PNP g^{-1} h^{-1} after treatment of *Mentha arvensis* at 8 % (Figure 3). Dehydrogenase activity was also maximum recorded in chemical herbicides clodinafof-propargyl 15 % WP (489.64 µg TPF g^{-1} h^{-1}) followed by *Pelargonium graveolens* at 2 % (418.41 µg TPF h^{-1} g^{-1} soil) and *Pelargonium graveolens* at 8 % (381.31 µg TPF g^{-1} h^{-1}). The lowest dehydrogenase was recorded after treatment of *Mentha arvensis* at 4 % (116.54 µg g^{-1}) (Figure 4). Results indicated that soil microbial enzymes changed with a concentration of essential oil and type of treatment (i.e. essential oil). No trends were found with the concentration of essential oil and gives a mixed effect.

Soil microbial biomass carbon (SMBC)

Soil microbial biomass carbon (SMBC) is an important quality indicator of soil. SMBC was decreased in all the treatments of essential oil, while enhancement of SMBC was observed in chemical herbicide treatments over control. SMBC was highest recorded in chemical herbicides clodinafof-propargyl 15 % WP (701.34 µg g^{-1}) and sulfosulfuran 75 %

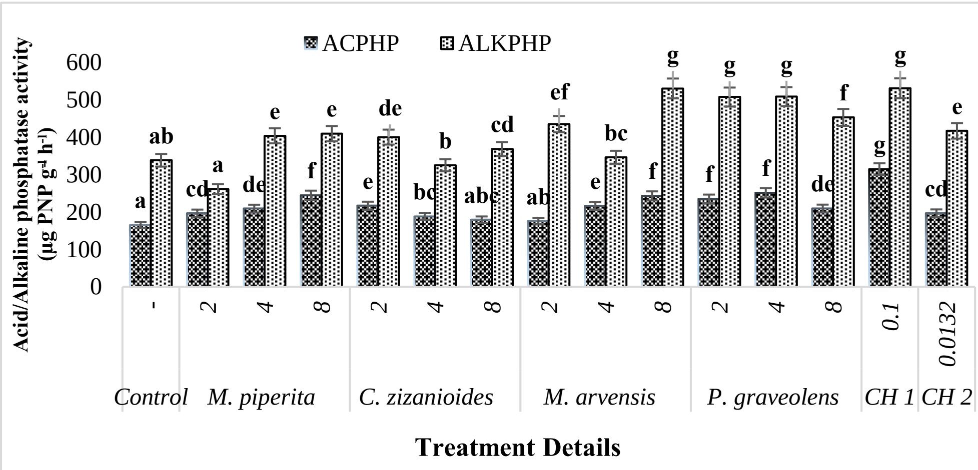

Figure 3. Effects of EOEs/chemical herbicides on soil phosphatase activity in post-harvest soil

Mean followed by same letter within one column (ACPHP, ALKPPHP) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test). *ACPHP- Acid phosphatase, ALKPHP-Alkaline phosphatase, CH 1 - Clodinafof- propargyl 15 % WP, CH 2- Sulfosulfuran 75 %

*Y-Axis indicates the concentration (%) of essential oil emulsion such as *Mentha piperita*, *Chrysopogon zizanioides*, *Mentha arvensis*, *Pelargonium graveolens* and Clodinafof-propargyl 15 % WP, Sulfosulfuran 75 % WG

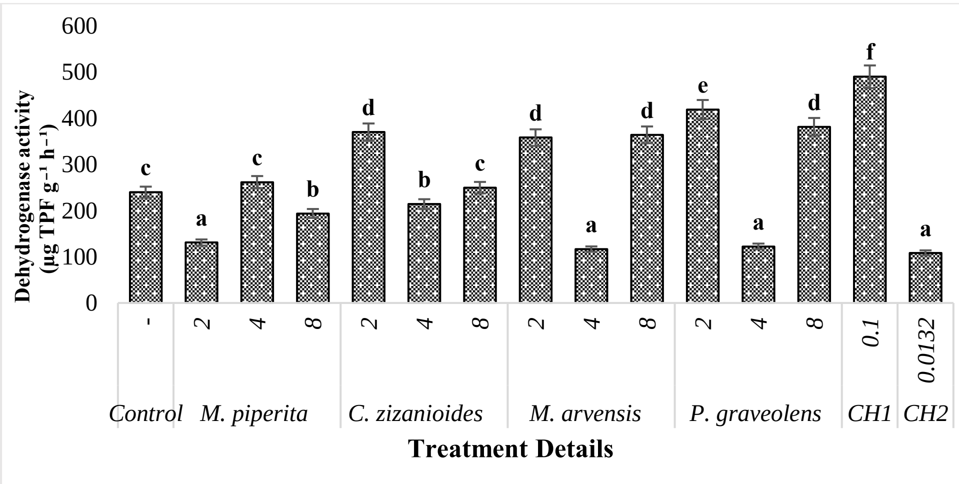

Figure 4. Effects of EOEs/chemical herbicides on soil dehydrogenase activity in post-harvest soil

Mean followed by same letter within one column (DHA) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test). *DHA- Dehydrogenase, CH 1 - Clodinafof-propargyl 15 % WP, CH 2- Sulfosulfuran 75 % WG

*Y-Axis indicates the concentration (%) of essential oil emulsion such as *Mentha piperita*, *Chrysopogon zizanioides*, *Mentha arvensis*, *Pelargonium graveolens* and Clodinafof-propargyl 15 % WP, Sulfosulfuran 75 % WG

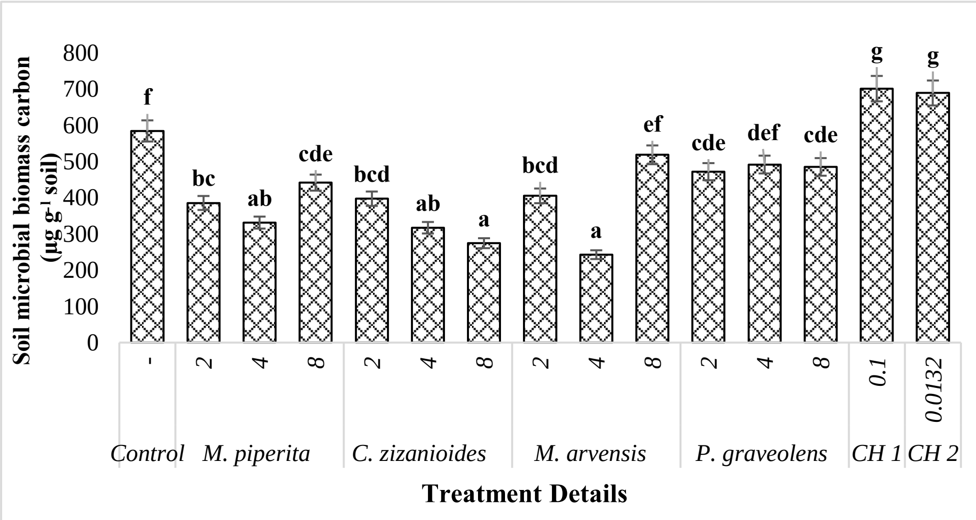

Figure 5. Effects of EOEs/chemical herbicides on soil microbial biomass carbon in post-harvest soil Mean followed by same letter within one column (SMBC) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test). *SMBC- Soil microbial biomass carbon), CH 1 - Clodinafof- propargyl 15 % WP, CH 2- Sulfosulfuran 75 % WG
*Y-Axis indicates the concentration (%) of essential oil emulsion such as *Mentha piperita*, *Chrysopogon zizanioides*, *Mentha arvensis*, *Pelargonium graveolens* and Clodinafof-propargyl 15 % WP, Sulfosulfuran 75 % WG

WG (689.3644 µg g^{-1}). After treatment of *Mentha arvensis* at 8 % have been found 518.55 µg g^{-1} and 491.78 µg g^{-1} by *Pelargonium graveolens* at 4 %. The lowest soil microbial biomass carbon was recorded after treatment of *Mentha arvensis* at 8 % (242.78 µg g^{-1}) (Figure 5). *Mentha piperita* and *Mentha arvensis* gives mixed effect with the concentration of essential oil while in the *Chrysopogon zizanioides* treatment increasing the concentration of essential oil reduction in SMBC was observed. After the application of *Pelargonium graveolens* treatment, within the concentration, SMBC was not significantly affected.

DISCUSSION

According to their allelopathic characteristics, *Mentha piperita* and *Mentha arvensis* are members of the Lamiaceae family, and EOs from this family are frequently documented for their herbicidal activity. EOs from members of the Lamiaceae family significantly decreased the germination and seedling growth of weeds (4,37,44). It is reported that nanoemulsion of peppermint (PNs) showed herbicidal effects on maize and barnyard grass and effective dose of treatment causing 10 % of maize damage (leaf necrosis) while in barnyard grass causing 50-90 % damage. Spraying with the PNs caused significant changes in the length of barnyard grass aerial parts but not its roots. PN4 (5 %), PN5 (10 %), and herbicide, treatment found shortest aerial parts length, 36-40 % shorter than the Control plants (37). By using the cotton swab method, Mirmostafaee *et al*. (31) investigated the effects of 112 essential oils from medicinal and aromatic plants on lettuce seed and seedlings. They found that Lamiaceae (*M. officinalis*, *T. daenensis*, *T. transcaspicus*, *O. vulgare*, *Z. tenuior*, *M. suaveolens*), Geraniaceae (*P. graveolens*) EOs had the greatest degree of germination reduction. Lettuce was found to have decreased in vigor index, radicle length and hypocotyl length (31). The essential oil from mint species (*M. spicata, M. spicata crispa, M. longifolia*) showed strong inhibition of germination. The monoterpenes menthone/isomenthone and linalool were highly active as well, while limonene produced only a weak inhibition (40,41).

Previously reported data agree with our results, *Mentha piperita*, *Mentha arvensis* and *Pelargonium graveolens* reduced the shoot length, root length, fresh weight and weight of *A. fatua* plant. According to reports, different EO compositions have different effects on the same receptor species in terms of allelopathy (4,14). Our findings demonstrated that the same receptor plant exhibits a variety of responses to the application of various EOs due to their EO compositions. EO with a high concentration of oxygenate monoterpenes had a larger inhibitory effect on seed germination than EO with a high concentration of hydrocarbon components (23). α-Pinene and 1,8 cineole were reported to be extremely phytotoxic against the germination and seedling growth of diverse species in previous studies (1,14,18). Additionally, it is understood that 1,8 cineole prevents the growth and extension of BY-2 cultivated tobacco cells. When compared to the control treatment, higher EO solution concentrations significantly ($p \leq 0.05$) reduced shoot and root growth (in terms of length, fresh and dried weight) (15).

The reduction in photosynthetic pigments (Total chlorophyll, Chl *a*, Chl *b*, and carotenoid content) was increasing with the concentration of EO which agrees with earlier reports (2,5). *S. hortensis* EO application on weed spp. also reported for significant reduction in chlorophyll content (23). Our study also revealed that with the reduction of photosynthetic pigment, an increase in the relative electrolyte leakage (REL) and proline content was found. Previously, various researchers reported that application of EO increased electrolyte leakage, MDA, and proline which agrees with our results (15,22). The highest numbers of dead plants were recorded in the treatment which was showed highest electrolyte leakage, it might be due to the increased membrane permeability carried on by deteriorating the integrity of the membrane, and excessive ROS production (15,22). A pogostemon oil treatment is said to have caused test plants to produce an excessive amount of ROS, which in turn caused oxidative stress. It is previously reported that, a higher proline accumulation and REL indicates that ROS further damaged the integrity of the membrane (15,22,26).

Results showed that the oils had an inhibitory effect on the carbon content of soil microbial biomass and had a mixed impact on the activity of soil dehydrogenase (DHA). Our findings revealed a consistent pattern for both soil phosphatase activity and soil microbial biomass carbon (26,33). In line with their antibacterial properties, EOEs have an inhibitory effect on soil microbial biomass and reported that the soil microbial biomass decreased, and extractable C increased at day 7 as a result of the EO's rapid antibacterial activity (25). The MBC content of the soil was decreased because of these oils' ability to alter the permeability of the microbes' cell walls. Spraying EOEs, on the other hand, increases the activity of the alkaline phosphatase and the acidic soil. Previous results showed a tenfold rise in alkaline phosphatase activity following the spraying of essential oils, but uncertain trend was reported for urease activity (33) and as soil organic content increased, alkaline phosphatase activities also increased (34). The additional carbon source for the microorganisms in the current investigation may be the cause of the increased phosphatase activities (3,26). Various researchers reported that phytotoxicity of EOs showed selectivity towards monocot and dicot species and monocots weed species were more resistant to essential oils compared to dicot weed sp. (22,42) *A. fatua* a monocot weed species and *Mentha piperita*, *Mentha arvensis* and *Pelargonium graveolens* EOs showed inhibitory effect for *A. fatua* weed when foliar application of EOEs were applied. Our findings revealed that these essential oil can be a potential herbicides and further it could be helpful for sustainable weed management.

CONCLUSIONS

For the first time, the phytotoxicity of essential oils emulsions from plants such as *Mentha piperita*, *Chrysopogon zizanioides*, *Mentha arvensis* and *Pelargonium graveolens* against *A. fatua* weed and soil microorganism have been studied with a more practical approach, i.e., *in-vivo* conditions, monitoring their effects in order to know their true potential as a substitute to synthetic chemicals and comparative study with other two chemical herbicides- clodinafof-propargyl 15 % WP, sulfosulfuran 75 % WG. The findings clearly showed that, to varying degrees, the tested EOEs were extremely effective against weeds, greatly limiting their growth characteristics. Although EOEs showed significant promise as natural herbicides, the best application dose must be determined in order to effectively control weeds without having any negative impact on soil microorganisms.

ACKNOWLEDGEMENTS

We acknowledge Director, CSIR-CIMAP for providing necessary facilities and Integral University, Lucknow for providing Ph.D. registration (IU/R&D/2022-MCN 0001699) of the first author.

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 interest.

ETHICAL APPROVAL

Animal models were not used in the experiments conducted for this manuscript.

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