Pak. J. Bot., 55(3): 1051-1063, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-3(6)

CHARACTERISTICS OF EIGHTEEN ESSENTIAL OILS ON *ECHINOCHLOA CRUS-GALLI* AND *GALIUM APARINE* SEEDLING GROWTH

JINGJING XU[1], HAODONG BAI[2], ZHENGDONG BAI[1] AND ZUREN LI[1,2*]

[1]*Hunan Provincial Key Laboratory for Biology and Control of Weeds, Hunan Academy of Agricultural Sciences, Changsha, Hunan 410125, China*
[2]*Collaborative Innovation Center for Field Weeds Control, Hunan University of Humanities, Science and Technology, Loudi, 417000, China*
[*]*Corresponding author's email: lizuren88214@sina.com*

Abstract

The phytotoxicity characteristics of plants can be successfully used for weed control. To enrich the variety of plant-derived herbicides, we extracted the essential oils from 18 plant species by steam distillation and assayed their herbicidal effects on barnyard grass (*Echinochloa crus-galli*) and cleavers (*Galium aparine*). When the target weeds were treated with the 18 essential oils at 0.3 g mL^{-1}, the herbicidal effect on the target weeds was above 80%. The lavender, lemon, and chamomile essential oils showed the best herbicidal effects. The composition of the essential oils was determined by gas chromatography-mass spectrometry (GC-MS). Menthol acetate, linalool, cinene, glycerol triacetate, and 1-menthol, all of which are terpenoids, were found in relatively high levels in the essential oils. We tested each of these five compounds individually for their inhibiting effects and found that the order of effectiveness was: menthyl acetate> glycerol triacetate> linalool> l-menthol > cinene. The five compounds were tested in combination, in which case, their herbicidal effects were reduced. When barnyard grass was treated with lemon essential oil, the defense enzyme activities were found to increase first and then to decrease with the time up to 48h. Clorophyll a and b levels decreased with treatment time, whereby total chlorophyll content also decreased. These investigations present the herbicidal activity of lavender, lemon, and chamomile essential oils and the active compounds. These identified active substances in the essential oils and their inhibitory effects provide references for the development of bio-herbicides from essential oils for the control of weeds in agricultural system.

Key words: Steam distillation, Essential oils, Barnyard grass, *Galium aparine*, Defense enzyme activity, Chlorophyll content

Introduction

Weeds such as barnyard grass (*Echinochloa crus-galli*) and cleavers (*Galium aparine*) greatly reduce crop yields as they compete with crop plants for nutrients, moisture, and light (Oerke, 2006). Indeed, barnyard grass is one of the most harmful paddy weeds in the world, as it shows high seed setting and germination rates. According to previous reports, when eight plants of barnyard grass emerged on one square meter of rice paddy, rice earing rate was reduced by 38.22% (Islam *et al*., 2018). Further, to date barnyard grass has reportedly evolved resistance to nine different herbicides (Heap, 2021). Cleavers is another well-known malignant weed widely distributed in China and other countries, growing mostly in deserts, beaches, ridges, and ditches. It harms the growth of closely planted crops as to they are usually intertwined and grow together with *Sonoma alopecurus* and other weeds (Peng *et al*., 2008). The use of chemical herbicides remains the effective strategy to control these two harmful weeds. However, the abuse of chemical herbicides can result in resistant weeds; furthermore, most chemical herbicides shows strong residual effects on soils and agricultural products, thereby posing a high risk and ultimately a serious threat to human and animal health (Dhungana *et al*., 2019). These side effects of chemical herbicides have led researchers to search for more effective and environment-friendly ways to manage weeds.

The use of allelopathic plants has consequently become an important part of integrated weed management approaches. An allelopathic plant produces biochemical compounds that may directly or indirectly influence another organism in a beneficial or an unfavorable manner (Rice, 1984). Plant allelopathy has been widely investigated; particularly, aromatic plants reportedly produce large amounts of allelopathic substances (Li *et al*., 2019a). Many essential oils are volatiles of low molecular weight produced by aromatic plants, which have been used for weeding. Thus, for example, lemongrass oil was first reported in the 1920s to have potential applications in weeding, as it produces allelochemical d-limonene, which can rapidly dehydrate and kill plant tissues (Tajidin *et al*., 2002; Chaimovitsh *et al*., 2010). Similarly, terpene-based essential oils can effectively inhibit or delay seed germination and impurities (Islam *et al*., 2018). In turn, Fagodia *et al*., (2017) studied the phytotoxicity and cytotoxicity of citrull shell oil, they found that its main component, citral was highly toxic and could be used to control weed growth in agricultural production. Hence, aromatic plants are a crucial source of allelopathic substances.

Aromatic plants are both woody and herbaceous plants of several families that are widely used in medical care, the food industry, eco-tourism, and fruit (Ren *et al*., 2018; Shinwari *et al*., 2018; Anjum *et al*., 2019; Khan *et al*., 2019; Ovais *et al*., 2019; Najeebullah *et al*., 2020). Here, from the resource, we selected representatives' families rich in aromatic plant species: Labiatae, Asteraceae, Chenopodiaceae, Rosaceae, Poaceae, Rutaceae, Annonaceae, Santalaceae, Cupressaceae. Specifically, we selected peppermint (*Menthahaplocalyx*), clary sage (*Salvia japonica* Thunb.), lavender (*Lavandula angustifolia* Mill.), wormwood (*Artemisia absinthium* Linn.), tansy (*Tanacetum vulgare* L.), chamomile (*chamomilla*), wattle (*Mosla soochowensis*), vetivert (*Vetiveria zizanioides*), sweet almonds (*Amygdalus Communis* Vas), bitter almond (*Armeniacae Amarum* Semen) seeds, sweet orange (*Citrus* *sinensis* (L.) peel, neroli (*Citrus bigarradia*), lemon, rue (*Ruta graveolens* L.), grapefruit (*Citrus paradisi* Macf.) peel, ylang-ylang flower (*Cananga odorata*), sandal wood (*Santalum album* L.), and north american cedar (*Thuja occidentalis* Linn.). The essential oils of these 18 plant species were utilized to treat barnyard grass and cleavers seedlings to assay their herbicidal effects. Then, the three most effective ones were selected for gas chromatography-mass spectrometry (GC-MS) analysis. Defense enzymes (catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) and chlorophyll content of barnyard grass seedlings were studied after application of essential oils. This study uncovered that essential oils are the bio-herbicide lead for weeds management in agricultural system.

Materials and Methods

Plant material collection and preparation: Purchased oranges, grapefruits, and lemons were peeled for extraction of the essential oils. The 18 essential oils were all extracted from fresh plant parts (Table 1).

Seed of barnyard grass and cleavers were collected form farm yard at Gaoqiao base in Changsha, China. Fifteen seeds of each species were placed into a plastic cup with 10 g soil. These plants were grown in an artificial greenhouse under a 12 h photoperiod (light / dark cycle), 100-150 μmol m^{-2} s^{-1} and 25/20°C (day/night temperature regime). The 3-stages of barnyard grass and cleavers that grew well and uniformly were selected for use in the subsequent herbicide-activity tests.

Essential oil extraction from the 18 raw plant materials: Nine-hundred grams of dried Labiatae plant material were pulverized using a powder machine (Portable Multifunctional Crusher, Shanghai Industry and Trade Co. Ltd). The 300g of each powdered material were immersed in water (4:1 V/V) and placed in a 3000 mL round bottom flask. The mixture of material and water was conducted using a steam distillation apparatus (Dongguan Kaishengwu Technology Co. Ltd.). Hot water was preheated and added directly to a 2000 mL water vapor generator. In a round-bottomed flask, heating continued to generate steam, the condensed water was then passed, and the distillate were collected using 1000 mL separatory funnels. When it was observed that no more oil droplets dripped from the condenser, the process was deemed to be complete, which took approximately 3 hours. The distillates were left to stand and release the excess water below. 500 mL diethyl ether was added to the remaining distillates, which were then shaken and left to stand. The aqueous layers were removed, and the ether layers were poured into collection bottles, and evaporated. The extraction process was repeated three times. The resultant 18 essential oils were stored in brown glass bottles in a refrigerator at 4°C.

Toxicity tests: The herbicidal activity of the 18 extracted essential oils was evaluated uniformly for barnyard grass and cleavers seedlings (0.5, 1, 2, and 3 g of the mixture added with 9.0 mL of Tween detergent and 0.5 mL of DMF). The essential oil-treated barnyard grass and cleavers seedlings were kept in the artificial greenhouse under the conditions described above. Three pots used as controls were left untreated and the seedling weight was recorded after seven days. The treatments were replicated three times. Based on fresh weight data, the weeding effect was calculated as the previously reported (Li *et al*., 2019b).

Gas chromatography-mass spectrometry (GC-MS): The chemical composition of the three essential oils (lavender essential oil (No.16)), lemon essential oil (No.17) and chamomile essential oil (No.18) that demonstrated best weed control effects were identified using GC-MS ITS-40 (GC-ITMS; Finnigan MAT, San Jose, CA).

The chromatographic conditions for the three essential oil were as follows: chromatographic column: Rxi-50 column (30 m×0.25 mmID × 0.25 μm df). Carrier gas: High-purity helium, carrier gas flow rate: 1.0mL/min, split ratio: 25, gasification temperature of 300°C. Temperature program: initial temperature: 60°C, hold for 2.0 min; temperature increase to 240°C at a rate of 10.0°C min^{-1}, hold for 2.0min; temperature increase to 280°C at a rate of 20.0°C min^{-1}, hold for 6.0min. Injection volume: 0.4 μL solvent delay time 2.55min. Ion source: EI source, electron energy: 70eV, ion source temperature: 250°C, interface temperature 250°C, Scanning mass range: m/z 33-500, mass spectrometry search standard library: NIST05, NIST05s and WILY7 three libraries.

Qualitative analysis of each oil sample was carried out using MS spectrometry, and the resultant spectra were matched with those of the NIST library, the Medicinal and Aromatic Plant and Drug Research Centre (TBAM) Library, and the Wiley GC-MS Library (Juma *et al*., 2019). The relative amount of each identified compound in each extract depended on the peak acreage of the total ion chromatograms (TIC) (Zhang *et al*., 2014).

Herbicidal activity of the major chemical compounds: Indoor toxicity tests were conducted by spraying the 5 major compounds (linalool, menthyl acetate, cinene, glycerol triacetate, and L-Menthol) present in lavender, lemon, and chamomile essential oils. Linalool, menthyl acetate, cinene, glycerol triacetate, and L-menthol were purchased from Macklin Co Ltd (Shanghai, China). Barnyard grass and cleavers seedlings were sprayed with Tween 9.0 mL and 0.5 mL of DMF added with each of the five compounds at concentrations of 0.001, 0.005, 0.01, 0.05, 0.1, and 0.2 g mL^{-1}. After seven days, the fresh weight of the barnyard grass and cleavers seedlings were determined, and the calculations were performed as previously described (Li *et al*., 2019b).

Linalool and menthyl acetate contents in lavender essential oil were 34.84% and 28.1%, respectively, of total components of the essential oil; in turn, glycerol triacetate and cinene contents in lemon essential oil were 41.67% and 28.53%, respectively; and finally, glycerol triacetate and l-menthol contents in chamomile essential oil were 63.07% and 3.14%, respectively. We simulated the proportions of the three essential oils and prepared as the treatments a1, a2, b1, b2, c1, and c2, as summarized in (Table 2).

Table 1. Information of the 18 plant species used for extraction of essential oils.

No.	Scientific name	Plant part used	Cultivars	Acquisitiondate
1.	Sweet almonds	Peeled sweet almonds	*Amygdalus Communis* Vas	2019.3.20
2.	Mint	Fresh mint leaves	*Mentha Canadensis* Linnaeus	2019.3.21
3.	Sweet orange	Fresh sweet orange peel	*Citrus sinensis* (L.)	2019.3.21
4.	Ylang	Ylang	*Cananga odorata*	2019.3.22
5.	Sandalwood	Sandalwood	*Santalum album* L.	2019.3.22
6.	Rock orchid	Rock orchid root	*Dendrobium speciosum*	2019.3.22
7.	Rue	Rue	*Ruta graveolens* L.	2019.3.22
8.	Wormwood	Wormwood	*Artemisia absinthium* Linn.	2019.3.22
9.	North American cedar	Cedar	*Thuja occidentalis* Linn.	2019.3.22
10.	Grapefruit	Grapefruit peel	*Citrus paradisi* Macf.	2019.3.21
11.	Bitter almonds	Peeled bitter almonds	*Armeniacae Amarum Semen*	2019.3.21
12.	Tansy	Tansy	*Tanacetum vulgare* L.	2019.3.22
13.	Happy sage	Happy sage stems and leaves	*Salvia japonica* Thunb.	2019.3.22
14.	Orange blossom	Lime flower	*Citrus junos* Sieb. ex Tanaka	2019.3.23
15.	Earth wattle	Earth wattle	*Mosla soochowensis*	2019.3.20
16.	Lavender	Lavender	*Lavandula angustifolia* Mill.	2019.3.22
17.	Lemon	lemon peel	*Ruta graveolens* L.	2019.3.21
18.	Chamomile	Chamomile	*chamomilla*	2019.3.22

Table 2. Treatment combinations prepared using the five main compounds from three most effective essential oils.

Compounds	Linalool (mL)	Menthyl acetate (mL)	Glycerol triacetate (mL)	Cinene (mL)	L-Menthol (mL)	Tween 80 (mL)	N, N-dimethylformamide (mL)
a1	0.35	0.28	-	-	-	9.5	0.5
a2	3.5	2.8	-	-	-	9.5	0.5
b1	-	-	0.42	0.29	-	9.5	0.5
b2	-	-	4.2	2.9	-	9.5	0.5
c1	-	-	0.63	-	0.3	9.5	0.5
c2	-	-	6.3	-	3	9.5	0.5

Defense enzyme activities and chlorophyll content: Lemon essential oil (0.5 g) was mixed with 9.5 mL of Tween and 0.5 mL of DMF and sprayed onto barnyard grass seedlings. Samples were collected 1, 2.5, 6, 8, 24, and 48 h after treatment, and stored at -70°C until the activities of defense enzymes (CAT, POD, and SOD) and chlorophyll content were measured. The crude extracts of CAT, POD, and SOD were obtained for determination of defense enzyme activity using the corresponding enzyme analysis kit (Nanjing Jiancheng Biotechnology Research Institute) after instructions by the manufacturer. CAT, POD, and SOD activities were determined by measuring absorbance at 405, 420 and 550 nm, respectively (Wang *et al.*, 2017). The results were defined as relative activity (%) and the last value was compared with the initial value.

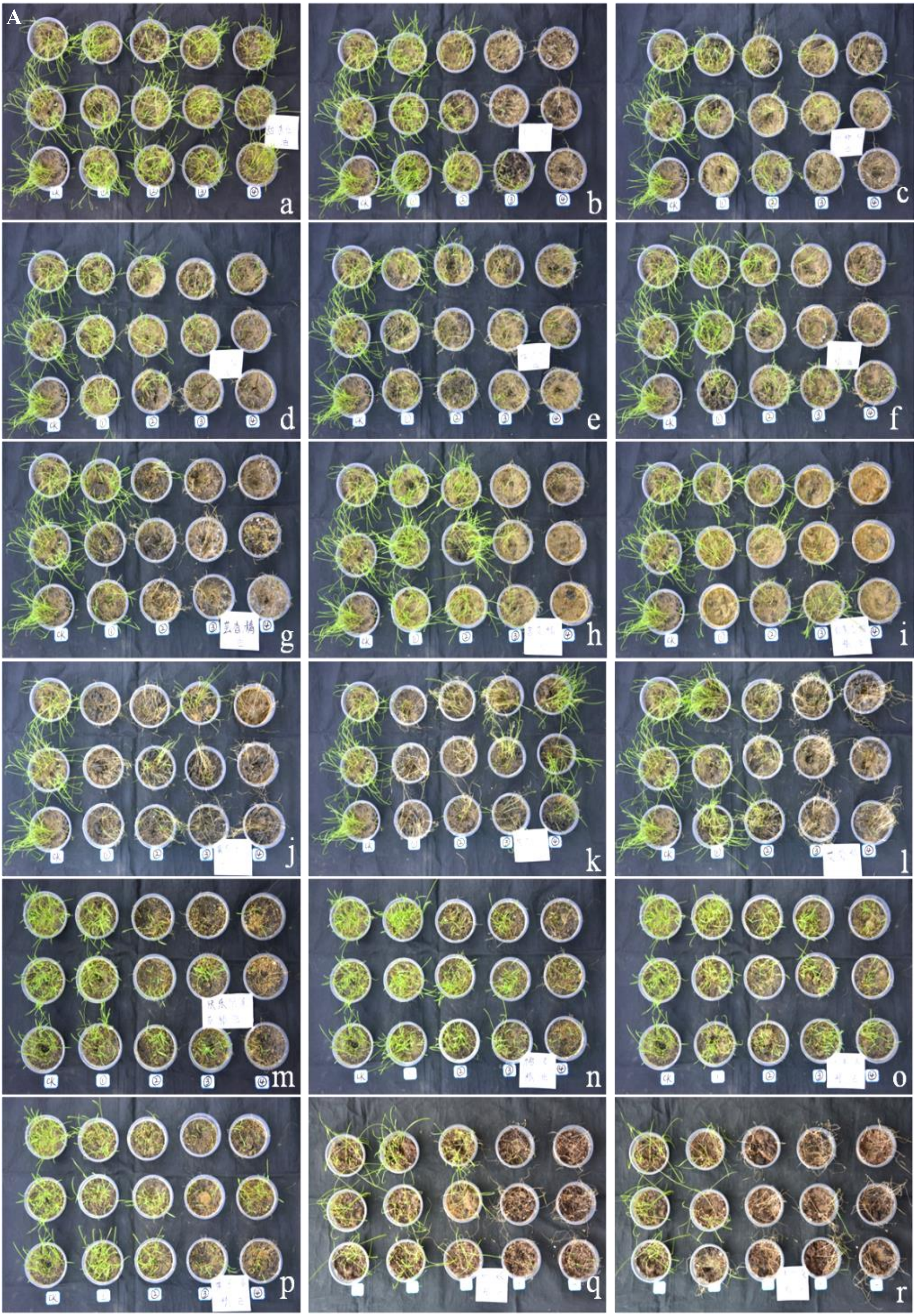
The extraction and quantitative determination of chlorophyll a and b were performed by placing 0.2 g of freshly chopped barnyard grass seedlings treated with lemon essential oil in a capped test tube, and adding 25 mL of 80% acetone and 25 mL of 95% ethanol. Samples were then soaked in a dark room for 24 h until leaf color faded completely. Absorbance at 663 and 645 nm was then determined using a UV spectrophotometer (Shanghai Liangguang Technology Co., Ltd.).

Statistical analysis

Data was by one-way ANOVA and significant difference among were determined using the LSD test at 5 % significance level using the DPS V17.10 software (Huang *et al.*, 2015).

Results

The inhibiting effect of essential oils on barnyard grass seedlings: The inhibitory effects of 18 essential oils on barnyard grass was studied by bioassay experiments. The 18 essential oils sprayed evenly on barnyard grass seedling in a range of concentrations. After one week, it was found that, except sweet almond oil, all essential oils showed herbicidal effects on the growth of barnyard grass seedlings (Fig. 1A). When 0.05 g mL^{-1} of essential oil treatments was used, sweet orange, ylang ylang, sandalwood, vetivert, rue, clary sage, lavender, lemon, and chamomile essential oils could inhibit barnyard grass seedling by over 50% (Fig. 1B). When 0.2 g mL^{-1} essential oils treatments were performed, the same essential oils could inhibition growth by above 90% in the target weed. At 0.05 g mL^{-1}, bitter almond essential oil had a strong herbicidal effect on barnyard grass, reaching 75.20%; however, when the concentration was 0.3 g mL^{-1}, the inhibition rate on growth was only 37.33%. Peppermint, wormwood, tansy, neroli, and soil wattle essential oils all showed relatively weak inhibition rates: only when they were applied at a high concentration (e.g., 0.1 mg L^{-1}), did inhibition of barnyard grass growth reach approximately 50%. In summary, the essential oils extracted from rue, lavender, lemon, and chamomile essential oils showed the strongest herbicidal effects on barnyard grass seedlings and may be useful as raw material for bio-herbicide development.



Cont'd. next page

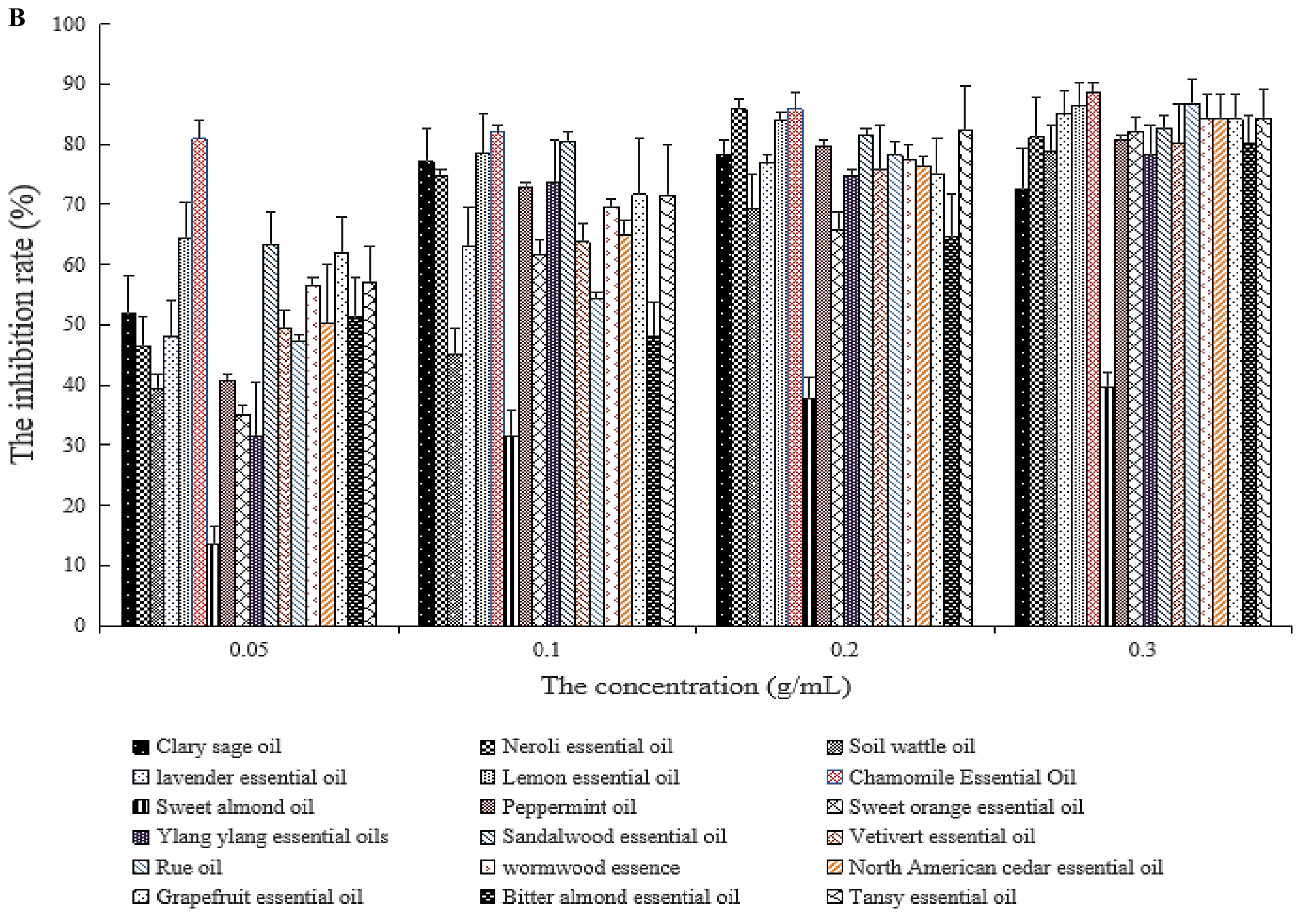
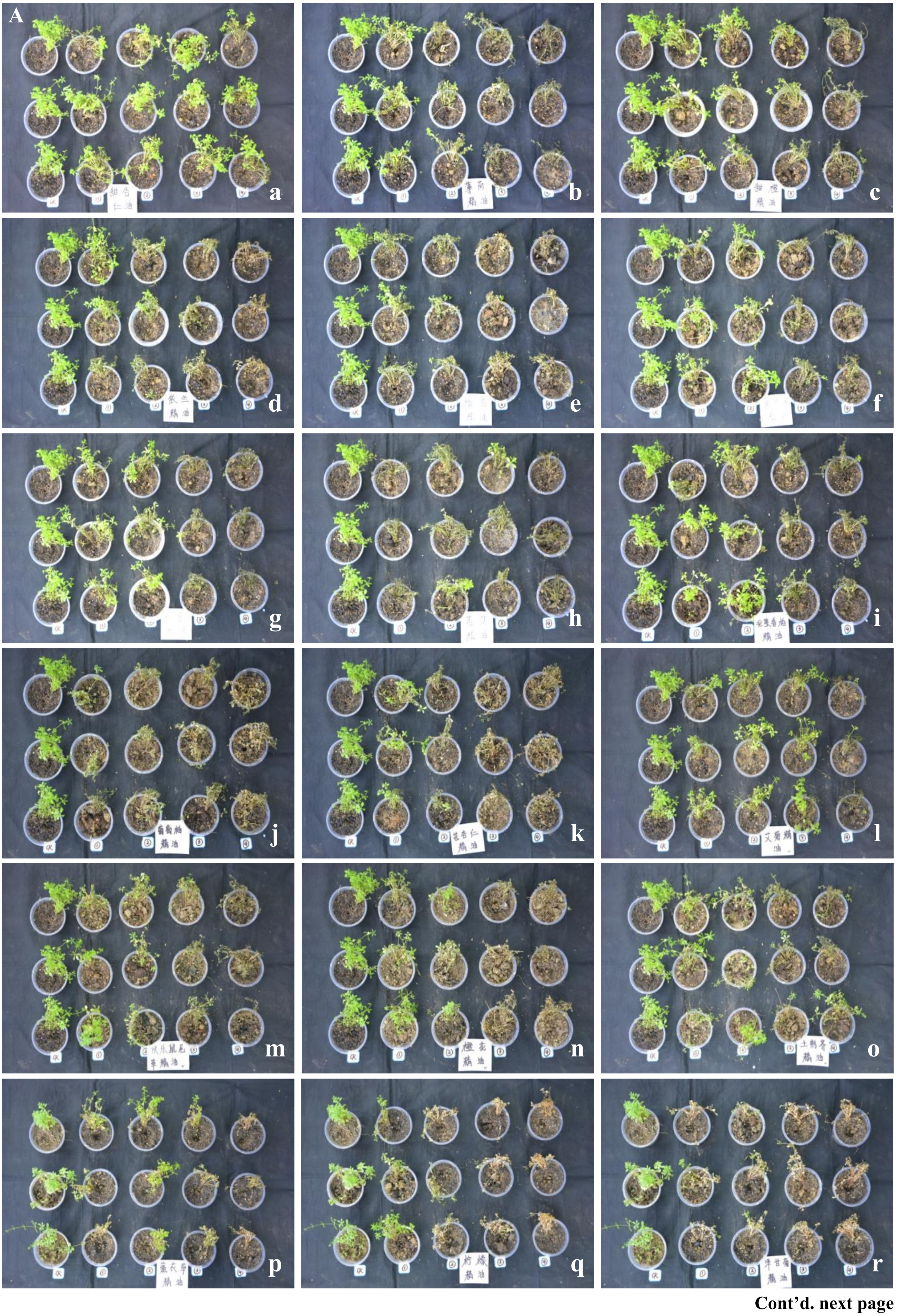


Fig. 1. Herbicidal effects of 18 essential oils on barnyard grass seedlings.
A: (a) swe*et al*mond; (b) peppermint; (c) sweet orange; (d) ylang ylang; (e) sandal wood; (f) vetivert; (g) rue; (h) wormwood; (i) North American cedar; (j) grapefruit; (k) bitter almond; (l) tansy; (m) clary sage; (n) neroli; (o) soil wattle; (p) lavender; (q) lemon; (r) chamomile. ck: untreatment, essential oils concentrations of 0.05, 0.1, 0.2, 0.3 g mL^{-1}. All experiments are conducted at the same time, and they have the same control group.
B: ck: untreat control, essential oils concentrations tested included 0.05, 0.1, 0.2 and 0.3 g mL^{-1}. All experiments are conducted at the simultaneously. The control group was the same in all cases

The inhibiting effect of essential oils on cleavers seedlings: Figure 2A shows the effects of the 18 extracted essential oils on cleavers seedling growth. Among all essential oils tested, that from swe*et al*mond was the only one to show no obvious herbicidal effects on the Cleaver's seedlings. As can be in Fig. 2B, herbicidal effects increased with increasing oil concentration. At 0.05 g mL^{-1}, the inhibitory effect of peppermint, sweet orange, ylang ylang, vetivert, rue, neroli, and lavender essential oils on cleavers seedling was less than 50%, whereas when the concentration was increased to 0.1 g mL^{-1}, cleavers seedling growth inhibition exceeded 60%. Further, at 0.3 g mL^{-1}, the herbicidal inhibition reached 80%. As Figure 2B shows, at 0.05 g mL^{-1}, sandalwood, wormwood, north American cedar, grapefruit, bitter almond, tansy, clary sage, lemon, and chamomile essential oils caused over 50% inhibition in cleavers seedlings, and at 0.1g mL^{-1}, the inhibition increased to more than 70%, indicating that cleavers seedlings were more sensitive to these essential oils. When the treatment concentration used was 0.3 g mL^{-1}, growth inhibition caused by most essential oils was approximately 80%. Figure 2 shows that the sandalwood, grapefruit, lemon, and chamomile essential oils had the strongest herbicidal effects on the cleavers, whereby, their plant parts might be used for developing plant-based bio-herbicides.

GC-MS analysis of lavender, lemon, and chamomile essential oils and the herbicidal activity of the major components: Essential oils obtained from lavender, lemon, and chamomile showed favorable herbicidal effects on both target weeds under study here. These three essential oils were analyzed using GC-MS (Fig. 3), and 31 of the main chemical components were identified. (Table 3) presents the 12 main chemical components in the lavender essential oil, including linalool (34.84%) and menthyl acetate (28.1%). In turn, among the 10 main chemicals detected in the lemon essential oil obtained from lemon, including cinene, glycerol triacetate, and L-linalool accounted for 28.53%, 41.67% and 7.14% of all components, respectively. Lastly, the 10 major chemicals detected from the chamomile essential oil included glycerol triacetate (63.07%), lilial (4.64%), and 6-methyl-gamma.-ionone (4.07%). GC-MS analysis showed that these three essential oils contained five main components, namely, linalool, menthyl acetate, cinene, L-menthol, and glycerol triacetate, all of which are terpenoids.



Cont'd. next page

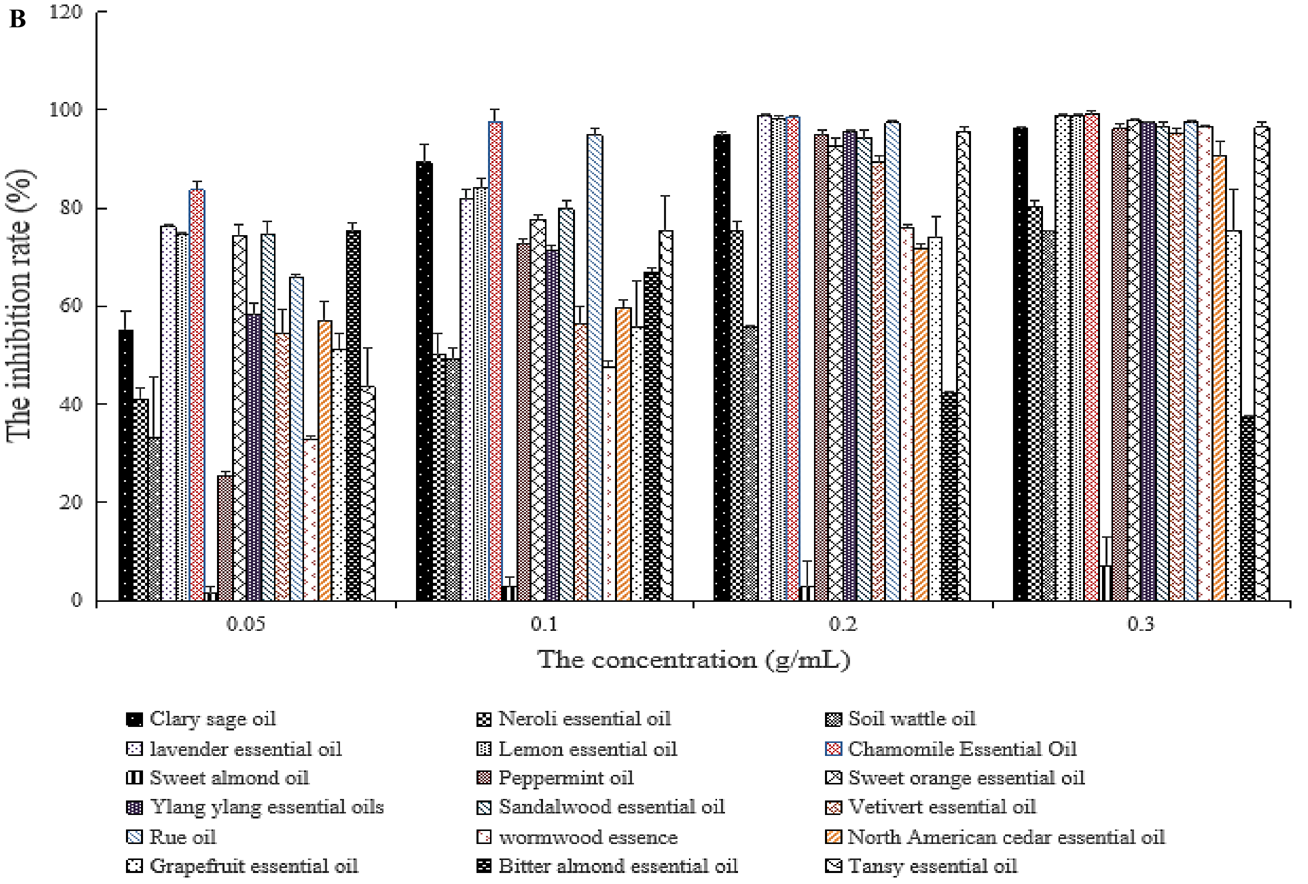


Fig. 2. Toxicity of 18 essential oils to cleavers.
A: (a) swe*et al*mond; (b) peppermint; (c) sweet orange; (d) ylang ylang; (e) sandalwood; (f) vetivert; (g) rue; (h) wormwood; (i)north american cedar; (j) grapefruit; (k) bitter almond; (l) tansy; (m) clary sage; (n) neroli; (o) soil wattle; (p) lavender; (q) lemon; (r) chamomile. ck: untreatment, essential oils concentrations of 0.05, 0.1, 0.2, 0.3 g mL^{-1}. All experiments are conducted at the same time, and they have the same control group.
B: ck: untreat control, essential oils concentrations tested included 0.05, 0.1, 0.2, and 0.3 g mL^{-1}. All experiments are conducted at the simultaneously. The control group was the same in all cases.

Linalool, menthyl acetate, cinene, L-menthol, and glycerol triacetate were tested for their herbicidal effect on barnyard grass seedlings (Fig. 4). The regression equations and EC_{50} values of these compounds for barnyard grass are presented in (Table 3). The EC_{50} values for menthyl acetate, linalool, cinene, glycerol triacetate, and l-menthol were 0.0029, 0.0045, 0.0266, 0.0035, and 0.0081 g mL^{-1}, respectively. Therefore, their herbicidal effect ranked in the following order: menthyl acetate> glycerol triacetate> linalool> l-menthol > cinene.

Barnyard grass seedlings were treated with different mixture of these five compounds (Fig. 5). The mixture of glycerol triacetate and l-menthol had a strong herbicidal effect on the seedlings at high concentrations, but no significant herbicidal effects can neither be seen in Figure 5B or 5C. We speculate that this may be because the combination of glycerol triacetate and cinene makes the two herbicidal effects mutually antagonistic, similarly, the mixture of linalool and menthyl acetate seemingly makes the two separate herbicidal effects mutually antagonistic, thereby weakening the herbicidal effect of the combined compounds.

Defense enzyme activities: After treatment with the lemon essential oil, POD, SOD, and CAT activities in the barnyard grass first increased and then decreased (Fig. 6). Chlorophyll a, chlorophyll b, and total chlorophyll contents decreased with increasing treatment times. CAT, POD, and SOD activities were 62.39, 23.86, and 73.47 U/gFW in the control group, respectively. In contrast, 1 and 24 h after treatment, CAT activity was increased to 86.92 U/gFW and 484.69 U/gFW, respectively, but then decreased to 76.81 U/gFW 48 h after treatment. Similarly, POD activity increased with time and peaked at 175.07 U/gFW after 8 h and then decreased to 139.91 U/gFW after 24 h and to 60.79 U/gFW after 48 h. Similarly, SOD activity increased to a peak of 407.32 U/gFW after 24 h, but then decreased to 57.47 U/gFW after 48h.

Chlorophyll a and chlorophyll b in the control group were 1306.6 and 301.1 mg/L, respectively, while total chlorophyll content was 1607.7 mg/L. At 0.5 h after treatment, chlorophyll a decreased to 813.5 mg/L, chlorophyll b increased to 467.2 mg/L, and total chlorophyll content decreased to 1280.7 mg/L. With time, chlorophyll a, chlorophyll b, and total chlorophyll decreased. After 48 h, chlorophyll a was reduced to 484.1 mg/L, chlorophyll b was reduced to 108.6 mg/L, and the total chlorophyll was only 592.7 mg/L. In general, chlorophyll was reduced in barnyard grass after treatment with the lemon essential oil.

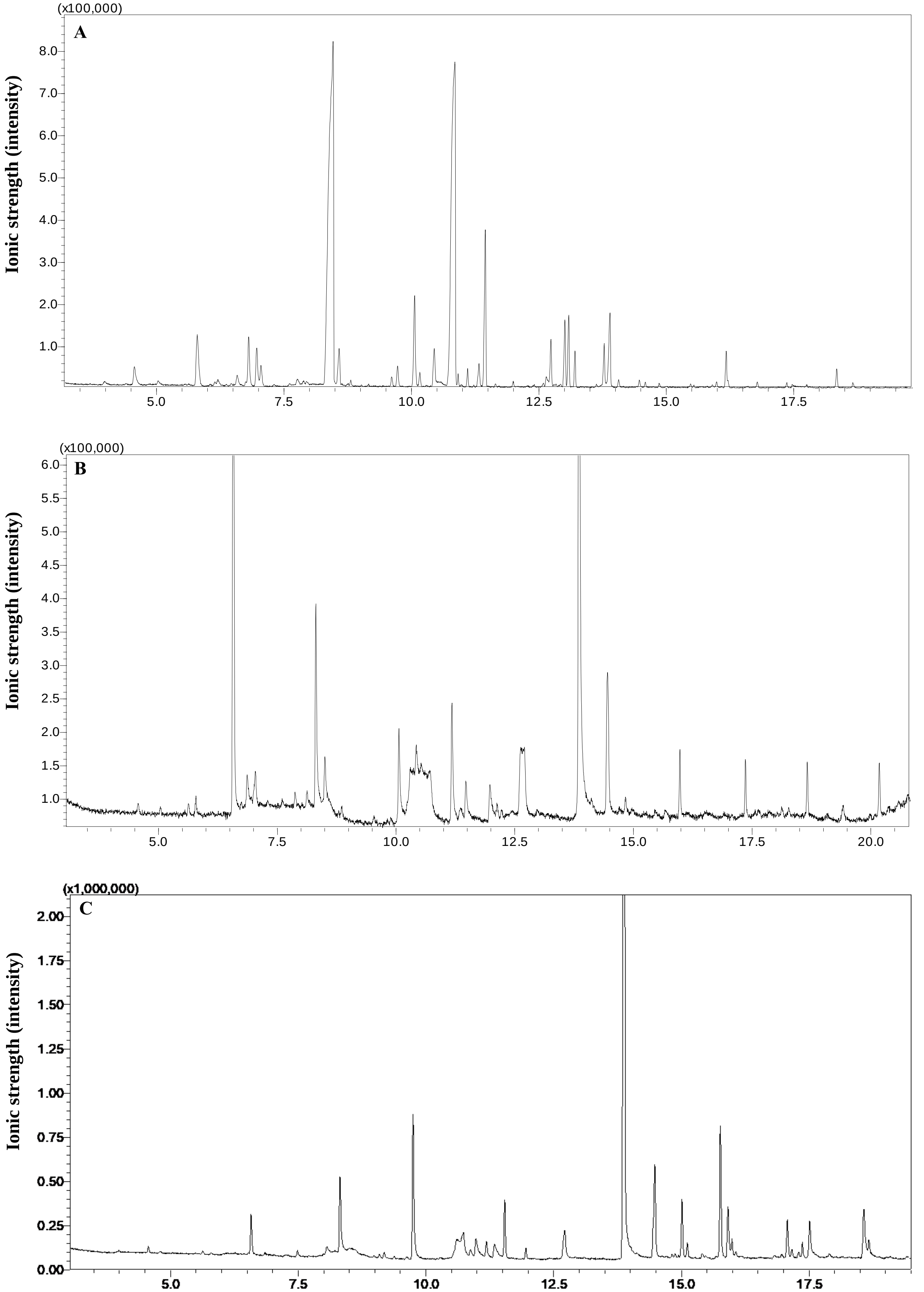


Fig. 3.Total ion chromatography of (a) lavender, (b) lemon, and (c) chamomile essential oils using the GC/MS chromatography.

Table 3. Chemical composition of the three most effective essential oils as determined by GC-MS.

Essential oil	Compounds	Fomula	Peak area (%)
Lavender essential oil	Alpha-Pinene	$C_{10}H_{16}$	1.15
	2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane	$C_{10}H_{16}$	0.18
	Myrcene	$C_{10}H_{16}$	2.75
	Trans-beta-Ocimene	$C_{10}H_{16}$	1.79
	Eucalyptol	$C_{10}H_{18}O$	0.7
	Linalool	C_{10}H18O	34.84
	p-Menth-1-en-4-ol	C10H18O	3.21
	Menthyl acetate	$C_{12}H_{22}O_2$	28.1
	2-Isoproprny-5-methyl-4-hexenyl acetate	$C_{12}H_{20}O_2$	5.08
	Caryophyllene	$C_{15}H_{24}$	2.08
	Bay pine(oydter) oil	$C_{12}H_{20}O_2$	2.06
	Pentadecane	$C_{15}H_{32}$	2.74
Lemon essential oil	Cinene	$C_{10}H_{16}$	28.53
	Antifoam	$C_8H_{16}O$	1.46
	Eukalyptol	$C_{10}H_{18}O$	1.54
	L-linalool	$C_{10}H_{18}O$	7.14
	NOnylaldehyde	$C_9H_{18}O$	1.74
	Capric aldehyde	$C_{10}H_{20}O$	4.11
	Alpha-methylbenzyl acetate	$C_{10}H_{12}O_2$	4.7
	Z-citral	$C_{10}H_{16}O$	1.37
	Cital a	$C_{10}H_{16}O$	2.43
	Glycerol triacetate	$C_9H_{14}O_6$	41.67
Chamomile essential oil	L-linalool	$C_{10}H_{18}O$	3.14
	L-Menthol	$C_{10}H_{20}O$	5.72
	4-tert-butylcyclohexyl acetate	$C_{12}H_{22}O_2$	2.01
	Glycerol triacetate	$C_9H_{14}O_6$	63.07
	6-methyl-.gamma.-ionone	$C_{14}H_{22}O$	4.07
	Beta-ionone	$C_{13}H_{22}O$	1.97
	Lilial	$C_{14}H_{20}O$	4.64
	2-phenoxyethyl isobutyrate	$C_{12}H_{16}O_3$	2.01
	Dihydro methyl jasmonate	$C_{13}H_{22}O_3$	1.54
	Alpha-n-Hexyl-beta-phenylacrolein	$C_{15}H_{20}O$	2.71

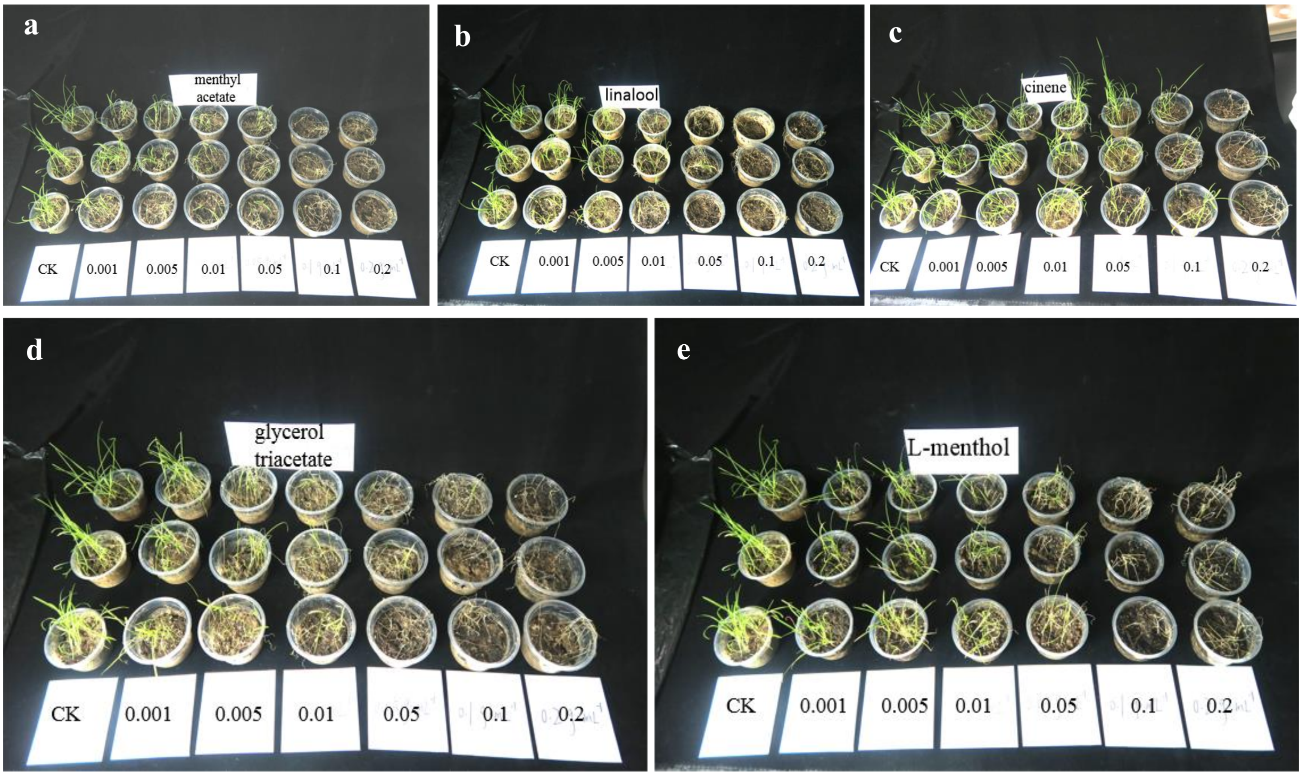


Fig. 4. Determination of the toxicity of five compounds identified from the herbicidal essential oils to barnyard grass (ck:control, 0.001 g mL^{-1}, 0.005 g mL^{-1}, 0.01 g mL^{-1}, 0.05 g mL^{-1}, 0.1 gmL^{-1}, 0.2 g mL^{-1}) (a) menthyl acetate; (b) linalool ; (c) cinene; (d) glycerol triacetate; (e) l-menthol. All experiments are conducted at the same time, and they have the same control group.

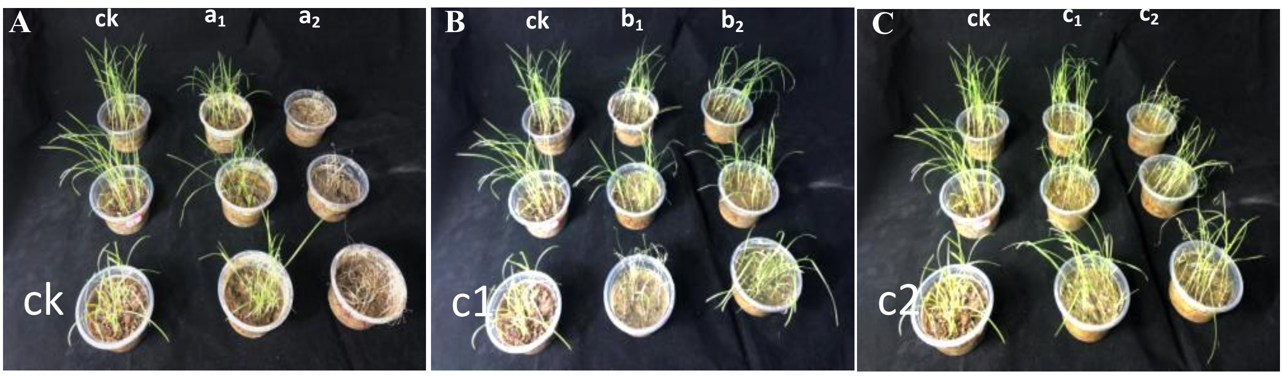


Fig. 5. Determination of toxicity of several compounds present in essential oils to barnyard grass seedlings.
All experiments were conducted simultaneously. The control group was the same in all caese. Seedlings were treated with triacetate and l-menthol (A), glycerol triacetate and cinene (B), linalool and menthyl (C), according to the proportion in which they are present in the natural essential oil. Barnyard grass seedlings were treated with the dosage shown in Table 3. ck:control;a1,a2,b1,b2,c1,c2 represents different mixtures, detailed information is shown in Table 3.

Discussion

Essential oils from 18 plant species were assayed for herbicidal effects on barnyard grass and cleavers seedlings. Among them, the lavender, lemon, and chamomile were the best. The composition of these three essential oils were determined by GC-MS and we found five terpenoid compounds present at relatively high levels in these plant materials. We investigated the five compounds separately and found that their herbicidal effects ranked as follows: menthyl acetate> glycerol triacetate> linalool> l-menthol> cinene. When the five compounds were mixed and sprayed on barnyard grass seedlings, the herbicidal effects were found to be reduced. This indicates antagonistic effects among these compounds when they are mixed. The different essential oils tested were differentially effective for control of the two target weeds studied.

The phytotoxicity of essential oils strongly inhibited the growth of barnyard grass and cleavers seedlings. Furthermore, the sensitivity of cleavers to the essential oils was greater than that of barnyard grass, as the same dose of essential oil was more likely to kill cleavers seedlings. Essential oils from lavender and peppermint reportedly showed a certain inhibitory effect on the germination of *Amaranthus retroflexus* seeds (Campiglia *et al*., 2007). Similarly, orange peel oil had a certain phytotoxicity at high concentration (1000 μg/mL^{-1}) and can inhibit duckweed growth (Erukainure *et al*., 2016). Furthermore, lemongrass oil had strong toxicity to wild oats and barnyard grass, and can be used in agricultural production to control weeds growth (Fagodia *et al*., 2017). Singh *et al*., (2009) showed that the essential oil of *Artemisia salicola* had strong herbicidal activity against *Cyperus rotundus*, *Phalaris minor*, and *Avena fatua*. Thus, different essential oils had different show differential toxic effects on target weeds (Xu *et al*., 2017; Hazrati *et al*., 2018).

The herbicidal effects of plant essential oils can be elucidated by determining the allelochemical constituents of the essential oils. The main components of the essential oils in *Artemisia* spp. are monoterpenoids, accounting for 71.6% of all components of their essential oils, among which, myrcene is the most abundant (29.27%), followed by limonene (13.3%) (Singh *et al*., 2009). The chemical components of lavender, lemon, and chamomile essential oils were present in Tables 2 and include alcohols and esters. These compounds present in plant essential oils are known to inhibit the growth processes of target species. Many plants can release phytotoxic monoterpenes, such as pinene and limonene, to inhibit weed growth (Angelini *et al*., 2003). Some essential oil ingredients, such as linalool and limonene reportedly inhibit seed germination (Martino *et al*., 2010). We mixed the five compounds identified from the three essential oils according to the information summarized in Table 2, and treated barnyard grass seedlings with a spray of these mixtures. We found that the compounds were not synergistic, but antagonistic. Thus, clearly the herbicidal effects of any given essential oil results from the combined actions of the various compounds present in it, but when these compounds are mixed with other active ingredients, the results might be very different and herbicidal effects may be significantly reduced due to antagonistic effects between components from oils.

Plant wilt damage largely depends on the effectiveness of plant antioxidant system, which includes enzymatic and non-enzymatic reactions. In the experiments reported herein, lemon essential oil was sprayed on barnyard grass seedlings, and POD, SOD, and CAT activities were found to increase and then decrease with time. Studies have found that CAT activity in plants is closely related to the amount of salt, and consequently, to plant drought resistance (Ariasmoreno *et al*., 2017). Additionally, heat shock can induce POD, which plays an important role in minimizing oxidative damage (Iba, 2002). After the lemon essential oil treatment, the chlorophyll a and b, and total chlorophyll content decreased, suggesting that the essential oil might have caused oxidative damage to the chloroplast. Indeed, studies have shown that lipid peroxidation can cause reduced chlorophyll synthesis and impaired chloroplast function (Singh *et al*., 2009). Consistently with our results, Bagavathy and Xavier found that chlorophyll a/b content and total chlorophyll content of sorghum plants treated with eucalyptus leaf extracts were reduced, as a result of chloroplast dysfunction (Bagavathy & Xavier, 2007). Lemon essential oils inhibited barnyard grass seedling, which may be explained by its negative effect on defense enzymatic activities and chlorophyll content.

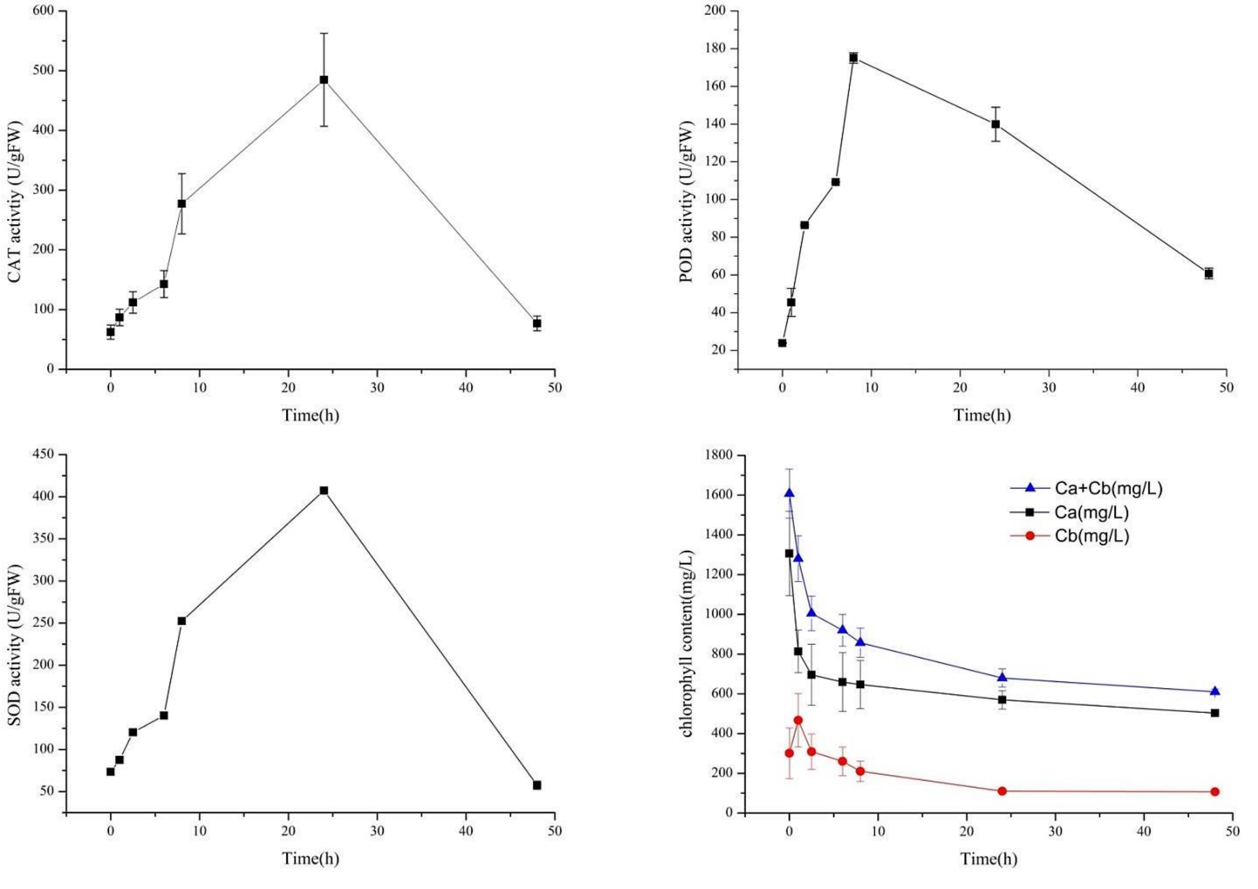


Fig. 6. Catalase (CAT, A), peroxidase (POD, B), superoxide dismutase (SOD, C) activities, and chlorophyll content (D) at the 3-leaf stage in barnyard grass seedlings treated with 5 mL of lemon essential oil (0.2 g mL^{-1}). Ca: chlorophyll-a; Cb: chlorophyll-b.

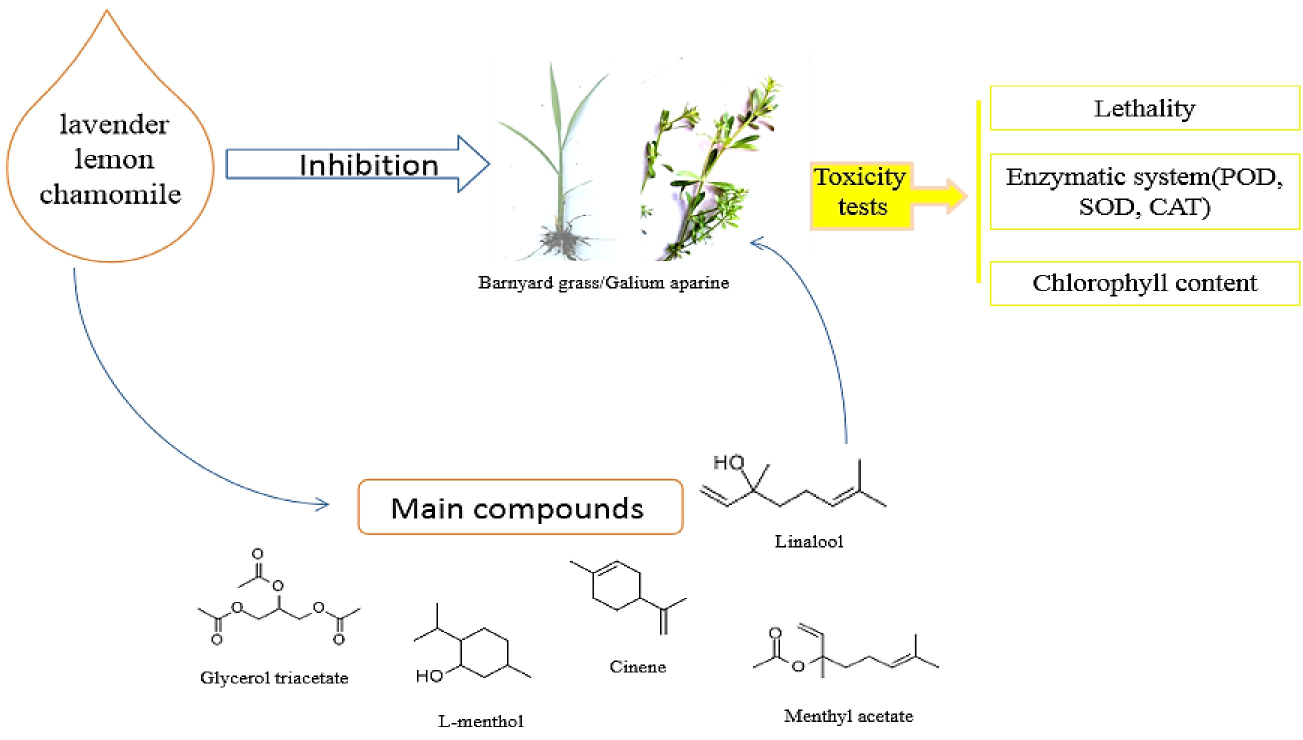


Fig. 7. Schematic representation of the herbicidal effect of three essential oils on barnyard grass and clovers seedlings.

Conclusion

Eighteen different essential oils of plant origin were investigated that have certain herbicidal effects on barnyard grass and cleavers seedlings (Fig. 7). The five main chemical compounds, namely menthol acetate, linalool, cinene, glycerol triacetate, and 1-menthol, present in the three essential oils (lemon, lavender, and chamomile) as to their strong herbicidal effect on the target weeds under study, were terpenoids. Terpenoid herbicides derived from plant essential oils or used as lead compounds, can effectively reduce the problems associated with herbicide residues, weed resistance, and environmental pollution during the application of organic synthetic herbicides. The discovery of new active substances that can be used as herbicide precursors, there is no doubt that plant essential oils are considered to be a significant source.

Acknowledgements

This work was supported by grants from the China Agriculture Research System (CARS-16-E19), Natural Science Foundation of Hunan Province (2021JJ20034), Hunan Key Research and Development Program (2020NK2046) and Scientific-Innovative of Hunan Agricultural Sciences and Technology (2019LS05, 2019LS06, 2019TD03, 2020CX06, 2020CX58 and 2020CX60), China.

References

Angelini, L.G., G. Carpanese, P.L. Cioni, I. Morelli, M. Macchia and G. Flamini. 2003. Essentialoils from Mediterranean Lamiaceae as weed germination inhibitors. *J. Agri. Food. Chem*., 51(21): 6158-6164.

Anjum, S., R. Khadim, S.Z.A. Shah and A. Ghafoor. 2019. Biochemical characterization of geographically diverse Mentha species from Azad Jammu and Kashmir. *Fresen. Environ. Bull.*, 28(2A): 1336-1344.

Ariasmoreno, D.M., J.F. JiménezBremont, I. Maruri-López and P. Delgado-Sánchez. 2017. Effects of catalase on chloroplast arrangement in Opuntia streptacantha chlorenchyma cells under salt stress. *Sci. Rep*., 7(1): 1-14.

Bagavathy, S. and G.S. Xavier. 2007. Effects of aqueous extract of Eucalyptus globulus on germination and seedling growth of sorghum. *Allelopath. J*., 20(2): 395-402.

Campiglia, E., R. Mancinelli, A. Cavalieri and F. Caporali. 2007. Use of essential oils of cinnamon, lavender and peppermint for weed control. *Italian. J. Agron*., 2(2): 171-178.

Chaimovitsh, D., M. Abu-Abied, E. Belausov, B. Rubin, N. Dudai and E. Sadot. 2010. Microtubules are an intracellular target of the plant terpene citral. *Plant. J*., 61(3): 399-408.

Dhungana, S.K., I.D. Kim, B. Adhikari, J.H. Kim and D.H. Shin. 2019. Reduced germination and seedling vigor of weeds with root extracts of maize and soybean, and the mechanism defined as allelopathic. *J. Crop. Sci. Biotech*., 22(1): 11-16.

Erukainure, O.L., O.A. Ebuehi, M.I. Choudhary, M.A. Mesaik, A. Shukralla, A. Muhammad and G.N. Elemo. 2016. Orange peel extracts: Chemical characterization, antioxidant, antioxidative burst, and phytotoxic activities. *J. Dietary. Suppl*., 13(5): 585-594.

Fagodia, S.K., H.P. Singh, D.R. Batish and R.K. Kohli. 2017. Phytotoxicity and cytotoxicity of Citrus aurantiifolia essential oil and its major constituents: Limonene and citral. *Indus. Crops. Prod*., 108(2017): 708-715.

Hazrati, H., M.J. Saharkhiz, M. Moein and H. Khoshghalb. 2018. Phytotoxic effects of several essential oils on two weed species and tomato. *Biocat. Agri. Biotech*., 13(2018): 204-212.

Heap, I. 2021. The international survey of herbicide resistant weeds. Online Internet Saturday.

Huang, S., L. Wang, L. Liu, Y. Hou and L. Li. 2015. Nanotechnology in agriculture, vestock and aquaculture in china. a review. *Agron. Sustain. Develop*., 35: 369-400.

Iba, K. 2002. Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. *Ann. Rev. Plant Biol*., 53(1): 225-245.

Islam, A.M., M. Hasan, M.M. Musha, M.K. Uddin, A.S. Juraimi and M.P. Anwar. 2018. Exploring 55 tropical medicinal plant species available in Bangladesh for their possible allelopathic potentiality. *Ann. Agri. Sci*., 63: 99-107.

Juma, G., Y. Zhang, Q. Liu, Y. Yang, X.P. Wang, S. Yang and Nasiruddin. 2019. Allelopathic effects of native plant species *Dicranopteris dichotoma* on invasive species *Bidens pilosa* and *Eupatorium catarium*. *Allelopath. J*., 48(1): 45-58.

Khan, I., Z.K. Shinwari, N.B. Zahra, S.A. Jan, S. Shinwari and S. Najeebullah. 2019. DNA barcoding and molecular systematics of selected species of family Acanthaceae. *Pak. J. Bot*., 52(1): 205-212.

Li, Z.R., N. Amist and L.Y. Bai. 2019a. Allelopahty in sustainable weeds management. *Allelopath. J*., 48(2): 109-138.

Li, Z.R., Y.B. Liu, X.M. Zhou, X.G. Li and L.Y. Bai. 2019b. Allelopathic herbicidal effects of crude ethanolic extracts of *Veronica persica* (Lour.) Merr. on weeds. *Allelopath. J*., 46(1): 117-128.

Martino, L.D., E. Mancini, L.F. Almeida and V.D. Feo. 2010. The antigerminative activity of twenty-seven monoterpenes. *Mol.*, 15(9): 6630-6637.

Najeebullah, S., Z.K. Shinwari, S.A. Jan, I. Khan and M. Ali. (Year Missing) Ethno-medicinal and phytochemical properties of genus allium: a review of recent advances. *Pak. J. Bot.*, 53(1): 135-144.

Oerke, E.C. 2006. Crop losses to pests. *J. Agri. Sci.*, 144(1): 31-43.

Ovais, M., A.T. Khalil, S.A. Jan, M. Ayaz, I. Ullah, W. Shinwari and Z.K. Shinwari. 2019. Traditional Chinese medicine going global: opportunities for belt and road countries. *Proc. Pak. Acad. Sci.,* 56(3 SI): 17-26.

Peng, X.G., J.X. Wang, M. Duan and J.H. Yang. 2008. The resistance to tribenuron-methyl in *Galium aparine* in winter wheat fields in northern China. *Acta Phyto. Sin*., 35(5): 459-462.

Ren, P., N. Fan, M. Tian and Y.H. Qin. 2018. Research progress on medical effects of essential oils. *China J. Trad. Chinese Med. Pharm*., 33(6): 2507-2511. (In Chinese)

Rice, E.L. 1984. Allelopathy; Academic Press Inc: Norman, Oklahoma.

Shinwari, Z.K., S.A. Jan, A.T. Khalil, A. Khan, M. Ali, M. Qaiser and N.B. Zahra. 2018. Identification and phylogenetic analysis of selected medicinal plant species from Pakistan: DNA barcoding approach. *Pak. J. Bot*., 50(2): 553-560.

Singh, A., D. Singh and N. Singh. 2009. Allelochemical stress produced by aqueous leachate of *Nicotiana plumbaginifolia* Viv. *Plant Growth Regu*., 58: 163-171.

Tajidin, N.E., S.H. Ahmad and A.B. Rosenani. 2002. Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages. *Afri. J. Biotech*., 11(11): 2685-2693.

Wang, J., X.H. Yang, A.S. Mujumdar, D. Wang, J.H. Zhao and X.M. Fang. 2017. Effects of various blanching methods on weight loss, enzymes inactivation, phytochemical contents, antioxidant capacity, ultrastructure and drying kinetics of red bell pepper (*Capsicum annuum* L.). *LWT-Food Sci. Technol.,* 77(11): 337-347.

Xu, S.C., S.J. Zhu and J. Wang. 2017. Design, synthesis and evaluation of novel cis-p-menthane type Schiff base compounds as effective herbicides. *Chinese Chem. Lett.*, 28(7): 1509-1513.

Zhang, X., Q. Chen, S. Li and Q. Xue. 2019. Extraction process of basil essential oil by steam distillation. *Med. Plant*, 10(02): 14-16.

(Received for publication 2 September 2021)