

Herbicidal effects of essential oils from 11-plant species on barnyard grass (*Echinochloa crus-galli*)

J.C. Han, H.D. Bai¹, X.Z. Ni¹, L.Y. Bai¹ and Z.R. Li^{1*}

Long Ping Branch, Graduate School of Hunan University,

Changsha, Hunan, China, 410125;

E-mail: lizuren88214@sina.com

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ABSTRACT

Echinochloa crus-galli (Barnyard grass) seriously damages crops in China. It has become resistant to many herbicides, hence, new and effective control measures need to be found. We did bioassay to find the herbicidal potential of 11-plant species essential oils on barnyard grass. The essential oils of *Artemisia argyi* Lévl. et Van. (Green Wormwood oil), *Curcuma zedoaria* (Christm.) Rosc. (Turmeric oil) and *Pogostemon cablin* (Blanco) Benth. (Patchouli oil) effectively inhibited the growth of barnyard grass. The green wormwood oil had the most potent herbicidal effects ($EC_{50}=0.0107$ g/mL). After treatment with green wormwood oil, the activities of defence enzymes [catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD)], dramatically increased 8 h after-treatment, and then decreased to initial levels within 72 h. Gas chromatography-mass spectrometry (GC-MS) results revealed that borneol and eudesmol are two major components of green wormwood oil. The herbicidal activity assays revealed that eudesmol was more potent than borneol (Borneol, $EC_{50}=0.0865$ g/mL; Eudesmol, $EC_{50}=0.0134$ g/mL). The experiments indicate that green wormwood oil is a valuable raw material for developing new eco-friendly herbicides for agriculture.

Key words: Allelopathy, *Angelica sinensis*, *Artemisia argyi*, barnyard grass, *Curcuma zedoaria*, defence *Dolomiaea edulis*, enzyme activity, essential oils, *Forsythia suspense*, herbicidal potential, *Lavandula angustifolia*, *Ligusticum chuanxiong*, *Nepeta cataria*, *Ocimum gratissimum*, *Platycladus orientalis*, *Pogostemon cablin*,

INTRODUCTION

The greatest challenge to Chinese agriculture in the 21st century is to improve crop production with environmentally safe solutions (10). Weeds, such as barnyard grass (Fig. 1), are the main factors decreasing crops yield and quality (2). In fact, barnyard grass is major weed competing with rice for natural resources (sunlight, water and nutrients) (13). Eight barnyard grass plants in 1 sqm of paddy field causes 38.22 % reduction in rice yield (7). With the extensive use of herbicides, many glyphosate-resistant biotypes of barnyard grass [*Echinochloa colona*, *E. crus-galli* (L.) P. Beauv] have developed (16,21). Now researchers are finding viable alternative solutions for its control.

Recently environment friendly weed control substances, like essential oils, have been the focus of much research (2,18,22). Many essential oils contain allelochemicals (plant secondary metabolites) with repellent, insecticide and herbicide activities (14,23). For example, essential oil from *Corymbia citriodora* (lemon eucalyptus) reduces the chlorophyll content of weeds and decreased the integrity of leaf membranes and effectively control the weeds (4). In another study, turmeric essential oil significantly inhibited the germination of

*Correspondence author, ¹Collaborative Innovation Center for Field Weeds Control, Hunan University of Humanities and Science, Loudi, Hunan, China, 417000.

weed seeds without affecting the germination and growth of crop seeds (8). Essential oils are extracted from a variety of plants and are environmental friendly. Furthermore, with the emergence of large numbers of herbicide-resistant weeds (14), the demand for targeted herbicides such as biological and botanical herbicides in agricultural production is increasing.



Figure 1. The photograph of single Barnyard grass (Left) and its Population (Right).

This study aimed to (i). to determine the herbicidal activity of essential oils extracted from 11-plants, (ii). to identify their major chemical components and (iii). to find natural products that could be further developed as bioherbicide against *E. crus-galli*.

MATERIALS AND METHODS

I. Plant material and essential oils

Barnyard grass seeds were collected in August 2019 from Gaoqiao Town, Changsha County, Changsha City, Hunan Province. These were stored in Hunan Provincial Key Laboratory for Biology and Control of Weeds, Hunan Academy of Agricultural Sciences, Changsha, Hunan Province, China (28° 12' 20" North latitude, East longitude 113° 4' 49" ; altitude : 44.9 m and average annual rainfall : 1361.6 mm.). Twenty uniform barnyard grass seeds were sown from March- June 2020 in plastic pots (dai: 9 cm; ht: 9 cm) with 20 g soil. The pots were kept in greenhouse (photoperiod : 12 h, light intensity: 100-150 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and day/night temperature: 25/23 °C). At 2-leaf stage, thinning was done keeping only 16-healthy barnyard grass seedlings/pot for follow-up experiments.

Eleven essential oils (Table 1), including Green *Artemisia argyi* Lévl. (wormwood oil), *Lavandula angustifolia* Mill. (Lavender oil), *Platyclus orientalis* (L.) (Cypress oil), *Ocimum gratissimum* (Basil oil), *Angelica sinensis* (Oliv.) (Angelica oil), *Ligusticum chuanxiong* Hort. (Chuanxiong oil), *Curcuma zedoaria* Christm) (Turmeric oil), *Dolomiaea*

edulis (Franch.) (Woodbalm oil), *Forsythia suspensa* (Thunb.) (Forsythia oil), *Pogostemon cablin* (Blanco) (Patchouli oil) and *Nepeta cataria* L. (Nepeta oil) were purchased from Yisen Yuan Plant Fragrance Co., Ltd.

Table 1. The sources of plant essential oils and their inhibitory effects on barnyard grass growth

#	English name	Botanical name	Inhibition rate (%) at 0.1 g/ml
1	Green wormwood oil	<i>Artemisia argyi</i> Lévl. et Van. oil	90
2	Lavender oil	<i>Lavandula angustifolia</i> Mill. L.oil	36
3	Cypress oil	<i>Platycladus orientalis</i> (L.) Franco cv. <i>Pendula</i> oil	13
4	Clove basil oil	<i>Ocimum gratissimum</i> L. oil	34
5	Angelica oil	<i>Angelica sinensis</i> (Oliv.) <i>Diels</i> oil	14
6	Chuanxiong oil	<i>Ligusticum chuanxiong</i> Hort. oil	44
7	Turmeric oil	<i>Curcuma zedoaria</i> (Christm.) Rose. oil	27
8	Wood balm oil	<i>Dolomiaea edulis</i> (Franch.) Shih oil	8
9	Forsythia oil	<i>Forsythia suspensa</i> (Thunb.) Vahl oil	2
10	Patchouli oil	<i>Pogostemon cablin</i> (Blanco) Benth. oil	48
11	Nepeta oil	<i>Nepeta cataria</i> L. oil	11

II. Allelopathy assay

In this study 3-Bioassays for Allelopathic potential were done as Under:

(i). Bioassay with 11- Essential Oils: The experimental treatments consisted of two factors (i). 11-plants essential oils (Green Wormwood oil, Lavender oil, Cypress oil, Clove basil oil, Angelica oil, Chuanxiong oil, Turmeric oil, Wood balm oil, Forsythia oil, Patchouli oil, Nepeta oil) herbicidal activity on barnyardgrass and (ii). Oil concentrations: 4-[(0, 0.01, 0.05, 0.1 g/mL) The treatments were replicated 3-times in experimental design.

Essential oil solutions were mixed with 0.5 mL N, N-Dimethylformamide(DMF), then brought to 10 ml volume with 0.6 % Tween-80 water solution. Barnyard grass seedlings kept in green house were sprayed at 0.01 g/mL, 0.05 g/mL and 0.1 g/mL concentrations with each of the 11 essential oils as per treatments using a 3WP-2000 sprayer. Seven days later, a photograph of each plant was taken and the fresh weight was measured. In line with previously reported studies (12), weed-control effects were evaluated based on the fresh weight data.

(ii). Bioassay with *Artemisia argyi* (worm wood) oil: Among the 11-test Essential oils, the *Artemisia argyi* (worm wood) oil showed maximum herbicidal activity, hence, it was studied further. Its bioactivity on barnyardgrass was studied at 6-concentrations (0, 0.01, 0.03, 0.05, 0.08, 0.1 g/mL) in pot culture. The pots were kept in greenhouse (photoperiod : 12 h, light intensity: 100-150 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and day/night temperature: 25/23 °C). Thereafter to identify the most herbicidal components, worm wood oil was analysed with GCMS and 93 compounds peaks were found. Among these two compounds Eudesmol and Borneol were most abundant.

(iii). Bioassay with major chemical compounds (Eudesmol and Borneol) : Bioassay was done in greenhouse (photoperiod : 12 h, light intensity: 100-150 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and day/night temperature: 25/23 $^{\circ}\text{C}$). Two main chemical components of green wormwood oil, eudesmol and borneol, were sprayed on barnyard grass plants to determine their allelopathic and herbicidal effects. Eudesmol and borneol were purchased from Biofount Biotechnology Co., Ltd. (Beijing). Barnyard grass was sprayed with the eudesmol and borneol at 0.01, 0.03, 0.05, 0.08, and 0.1 g/mL, made in Tween solution. The fresh weight of barnyard grass was recorded after 7 days. The inhibition rate of each essential oil component on barnyard grass was calculated based on the differences in fresh weight as per Li *et al.* (12). Three experiments were conducted to confirm the accuracy of the results.

III. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical components of green wormwood oil were determined by GC-MS using a GCMS-QP2010 (Shimadzu Corporation, Kyoto, Japan) using a Rtx-5MS column (30 m \times 0.25 mm ID \times 0.25 μm df, Restek, Bellefonte, PA, USA). The carrier gas, high-purity helium, had a flow rate of 1.0 mL/min, with a split ratio of 2.0. The gasification temperature was 300 $^{\circ}\text{C}$. The temperature program began with an initial temperature of 60 $^{\circ}\text{C}$, held for 1.0 min; followed by a temperature increase to 240 $^{\circ}\text{C}$ at a rate of 10.0 $^{\circ}\text{C}\cdot\text{min}^{-1}$, for 1.0 min; followed by a temperature increase to 280 $^{\circ}\text{C}$ at a rate of 20.0 $^{\circ}\text{C}\cdot\text{min}^{-1}$, for 6.0 min. The injection volume was 1.0 μL , and solvent delay time was 2.45 min. The Electron Ionization (EI) source was operated at an electron energy of 70 eV, ion source temperature of 250 $^{\circ}\text{C}$, and an interface temperature of 250 $^{\circ}\text{C}$. The scanning mass range was m/z 33-600, and the mass spectra of detected compounds were searched against three standard libraries: NIST05, NIST05s and WILY7. The area of the ion chromatographic peak represented the relative amount of each component.

IV. Defence enzyme activities

Ten mL green wormwood oil (0.05 g/mL) was sprayed on 2-leaf stage barnyard grass with a 3WP-2000 sprayer. Green wormwood oil 0.5 g was mixed with 0.5 mL DMF, then brought to 10 ml volume with 0.6 % Tween-80 water solution. Leaf samples were taken at 1, 4, 8, 24, 48 and 72 h after treatment and stored at -70°C until subsequent determination of defence enzymes (CAT, POD and SOD) activities. The activities of defence enzymes were determined using Kits from Nanjing Jian Cheng Bioengineering Institutes, according to their instructions (Table 2). The absorbance of enzyme activity reaction system was measured at 405 nm (CAT), 420 nm (POD) and 550 nm (SOD), respectively. Enzyme activity was calculated according to the formula given in the kit.

Table 2. The defence enzyme kits used in this study

Items	Manufacturer	Cat. No.
CAT	Nanjing Jian Cheng Bioengineering Institute	A007-1-1
POD		A084-3
SOD		A001-1

V. Statistical analysis

Analysis of variance of the data was calculated by LSD method based on the calculated inhibition rates, using the fresh weight data obtained in the biometric experiment. The standard deviation and p-value were used to analyze the error in Microsoft Excel 2019. A toxicity regression equation and correlation coefficient were analyzed by the quantitative data mechanism in Data Processing Software (DPS, version 9.01), and the single-factor statistical analysis Student-Newman-Keuls (SNK) method was used for variance analysis in DPS (20). All bar and line graphs in this study were obtained using Origin 9.0.

RESULTS AND DISCUSSION

Allelopathic effects of essential oils on barnyard grass

Eleven essential oils were sprayed at four concentrations (0, 0.01, 0.05 and 0.1 g/mL) on barnyard grass. After 7-days, all essential oils inhibited the growth of barnyard grass to varying degrees (Fig. 2). Wormwood oil had the greatest inhibition effect (90 %), while turmeric and patchouli oils had intermediate inhibition effects (~ 40 %). Wormwood oil at 0.05 g/mL inhibited the growth of barnyard grass by > 70 % (Fig. 3). At 0.03 g/mL, inhibition was about 50 %. Thus, among the 11 plant-based essential oils tested, wormwood oil was most allelopathic to barnyard grass, and the effect was dose dependent.

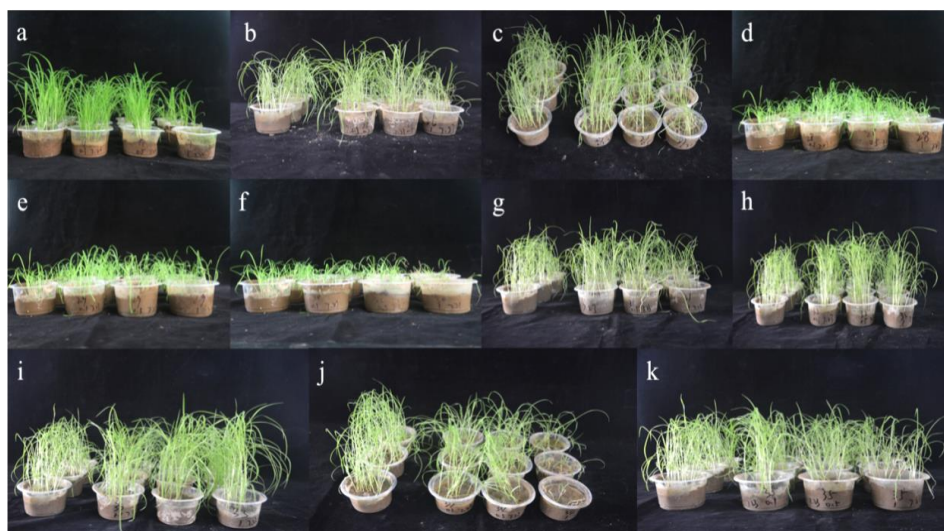


Figure 2. Herbicidal effects of 11-plant essential oils at 0, 0.01, 0.05 and 0.1g/ mL on barnyard grass. a: Green *Artemisia argyi* Lévl. et Van. oil; b: *Lavandula angustifolia* Mill.oil; c: *Platycladus orientalis* (L.) Franco Pendula oil; d: *Ocimum gratissimum* L. oil; e: *Angelica sinensis* (Oliv.) Diels oil; f: *Ligusticum chuanxiong* Hort. oil; g: *Curcuma zedoaria* (Christm.) Rosc. oil; h: *Dolomiaea edulis* (Franch) Shih oil; i: *Forsythia suspensa* (Thunb.) Vahl oil; j: *Pogostemon cablin* (Blanco) Benth oil ; k: *Nepeta cataria* L. oil.

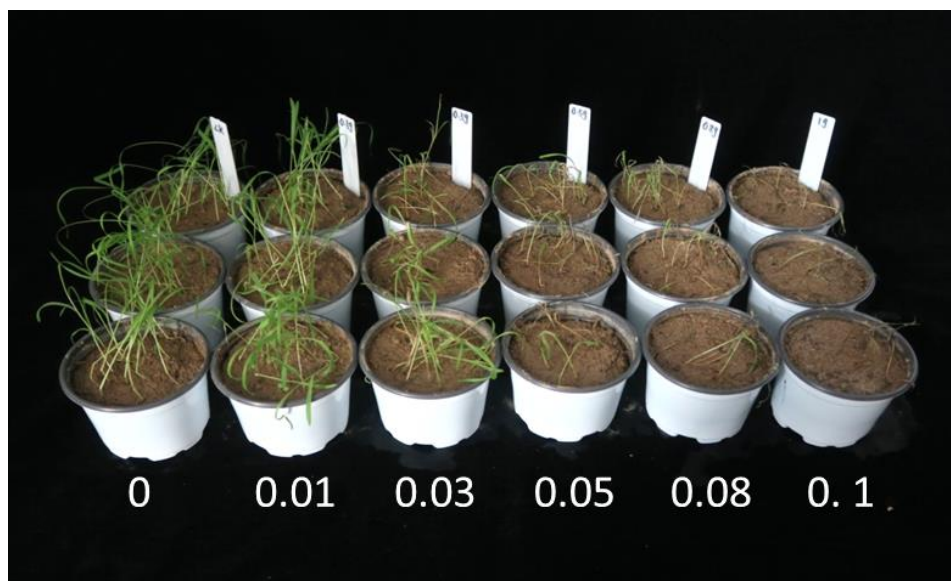


Figure 3. The inhibitory effects of wormwood oil on barnyard grass seedlings at 0, 0.01, 0.03, 0.05, 0.08 and 0.1 g/mL.

Many studies have shown that essential oils can inhibit weed growth. For example, the essential oil of *Thymbra capitata* (L.) Cav. effectively inhibited the seedling growth of *Avena fatua* by 96.56 % at 0.25 $\mu\text{L}/\text{mL}$, of *Amaranthus retroflexus* by 90.56 % at 0.5 $\mu\text{L}/\text{mL}$ and of *Echinochloa crus-galli* by 68.70 % at 1 $\mu\text{L}/\text{mL}$ (22). Ammar *et al.* found that ginger and turmeric essential oils at the highest test concentration (1 g/mL), completely inhibited the seed germination of *Multiflorum* and significantly affected that *P. oleracea* and *E. crus-galli* (1). In another study, 3-essential oils (eucalyptus, lavender and pine), were inhibitory to weeds *Portulaca oleracea* L. and *Pleuropterus multiflorus* (Thunb.) Nakai, but were less toxic to tomato and cucumbers. In their study, lavender essential oil decreased the hypocotyl and radicle growth of *Multiflorum* by 87.8 % and 76.7 % at 1 $\mu\text{L}/\text{mL}$ dose (9). Li. *et al.* (17) proved that the essential oils of basil, thyme and lemon balm had more bio-herbicidal effects than sage and goldenrod essential oils, on the germination and early seedling growth of velvetleaf weed (17). It is evident that essential oils are getting an increasingly important role in weed control. Our study revealed 50 % inhibitory effects on barnyard grass growth at 0.03 g/mL concentration of wormwood oil, suggesting that this oil may be promising for development of new herbicides.

GC-MS analysis of wormwood oil and the herbicidal activity of its major components

Wormwood oil was analyzed by GC-MS (Fig. 4) and 93 chemical components were identified (Table 3). Two major chemical components in wormwood oil were eudesmol (21.94 %) and borneol (6.3 %). The allelopathic activity of these two compounds were tested (Fig. 5). At 0.03g/mL concentration, the inhibitory effects on fresh weight of eudesmol on

Table 3. Total ion chromatograms of wormwood oil compounds detected by GC-MS analysis

Peak Number	Retention Time	Integration Start Time	End Time of Integration	Peak Area (A)	Peak Area (%)
1	4.430	4.370	4.477	123887	0.06
2	4.730	4.690	4.803	199020	0.09
3	5.026	4.977	5.057	454462	0.20
4	5.085	5.060	5.160	476702	0.21
5	5.223	5.173	5.323	3413494	1.53
6	5.371	5.337	5.417	75323	0.03
7	5.476	5.430	5.520	1550115	0.70
8	5.546	5.520	5.617	561113	0.25
9	5.828	5.777	5.883	3165784	1.42
10	5.914	5.883	6.003	1500058	0.67
11	6.074	6.013	6.093	767287	0.34
12	6.132	6.093	6.217	3222760	1.45
13	6.318	6.267	6.393	1283980	0.58
14	6.507	6.467	6.570	1998498	0.90
15	6.636	6.580	6.680	4415936	1.98
16	6.720	6.677	6.743	1846400	0.83
17	6.797	6.747	6.873	48921667	21.94
18	6.977	6.940	7.047	947512	0.43
19	7.177	7.123	7.227	4994769	2.24
20	7.348	7.290	7.410	2804399	1.26
21	7.540	7.490	7.603	7298298	3.28
22	7.681	7.630	7.753	1134910	0.51
23	7.864	7.817	7.920	1909568	0.86
24	8.008	7.970	8.053	9141823	4.10
25	8.174	8.120	8.210	1678249	0.75
26	8.241	8.210	8.297	1281956	0.58
27	8.470	8.427	8.503	997973	0.45
28	8.537	8.507	8.563	1656608	0.74
29	8.582	8.563	8.613	574851	0.26
30	8.641	8.613	8.660	847232	0.38
31	8.695	8.660	8.747	7801125	3.50
32	8.886	8.807	8.937	5246487	2.36
33	9.022	8.943	9.080	14033088	6.30
34	9.165	9.113	9.207	8462731	3.80
35	9.244	9.217	9.267	232023	0.10
36	9.301	9.267	9.320	551240	0.25
37	9.356	9.323	9.413	5896408	2.65
38	9.435	9.413	9.450	138270	0.06
39	9.469	9.450	9.490	141158	0.06
40	9.512	9.490	9.557	363570	0.16
41	9.604	9.570	9.633	856895	0.38
42	9.716	9.680	9.737	441612	0.20
43	9.774	9.740	9.817	3758831	1.69
44	9.915	9.880	9.933	305377	0.14
45	9.954	9.937	10.000	396321	0.18
46	10.049	10.020	10.100	223251	0.10
47	10.148	10.117	10.163	121010	0.05
48	10.197	10.163	10.257	507025	0.23
49	10.394	10.343	10.453	796797	0.36

50	10.592	10.547	10.613	120387	0.05
51	10.641	10.617	10.663	295518	0.13
52	10.687	10.667	10.757	419935	0.19
53	10.814	10.757	10.913	1737213	0.78
54	10.956	10.920	10.977	234823	0.11
55	11.003	10.980	11.033	158808	0.07
56	11.437	11.360	11.463	2373122	1.07
57	11.491	11.463	11.527	1953485	0.88
58	11.547	11.527	11.570	144860	0.07
59	11.590	11.570	11.633	272384	0.12
60	11.694	11.650	11.730	372015	0.17
61	11.869	11.803	11.907	16364401	7.35
62	12.028	11.990	12.073	541359	0.24
63	12.196	12.160	12.233	432806	0.19
64	12.337	12.277	12.413	546431	0.25
65	12.495	12.453	12.547	1620012	0.73
66	12.674	12.640	12.687	134680	0.06
67	12.704	12.687	12.760	243642	0.11
68	12.889	12.797	12.933	16286802	7.31
69	12.979	12.940	13.013	412441	0.19
70	13.117	13.067	13.173	835802	0.38
71	13.345	13.283	13.393	1586186	0.71
72	13.584	13.543	13.657	740448	0.33
73	13.702	13.667	13.747	862417	0.39
74	13.796	13.743	13.840	1326939	0.60
75	13.894	13.847	13.930	278662	0.13
76	13.962	13.937	14.020	203228	0.09
77	14.072	14.033	14.127	284334	0.13
78	14.180	14.130	14.223	465640	0.21
79	14.553	14.523	14.583	85144	0.04
80	14.679	14.640	14.727	400976	0.18
81	15.004	14.937	15.057	1517619	0.68
82	15.112	15.057	15.167	3006463	1.35
83	15.743	15.713	15.793	631329	0.28
84	15.979	15.930	16.043	2826190	1.27
85	16.117	16.067	16.177	335639	0.15
86	17.817	17.787	17.863	147097	0.07
87	18.197	18.157	18.247	182363	0.08
88	19.024	18.957	19.067	506128	0.23
89	19.285	19.243	19.333	791274	0.36
90	19.409	19.357	19.440	164304	0.07
91	19.739	19.700	19.823	898799	0.40
92	20.995	20.963	21.027	298165	0.13
93	21.666	21.617	21.703	184840	0.08

barnyard grass was more than 80 %, while the inhibitory effects of borneol on barnyard grass were only 30 %-40 %. The EC₅₀ value of eucalyptol and borneol was 0.0134 g/mL and 0.0865 g/mL, respectively, indicating that eudesmol was more inhibitory than borneol. The EC₅₀ value of worm wood oil was 0.0107 g/mL. The regression equations and EC₅₀ values of these compounds on barnyard grass are shown in Table 4. Hence, the eudesmol may be used to develop new bioherbicides for barnyard grass control.

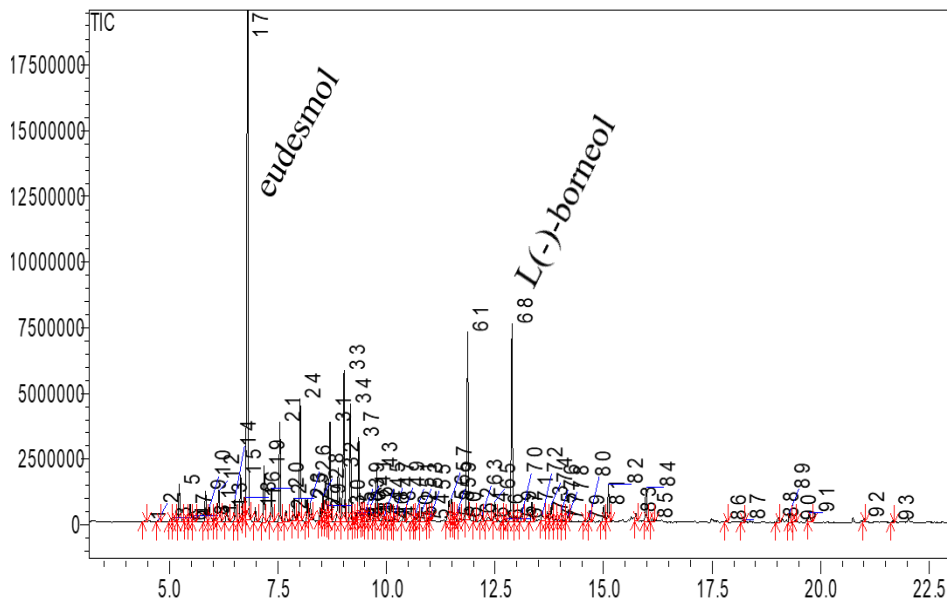


Figure 4. Total ion chromatogram from the GC-MS analysis of wormwood oil

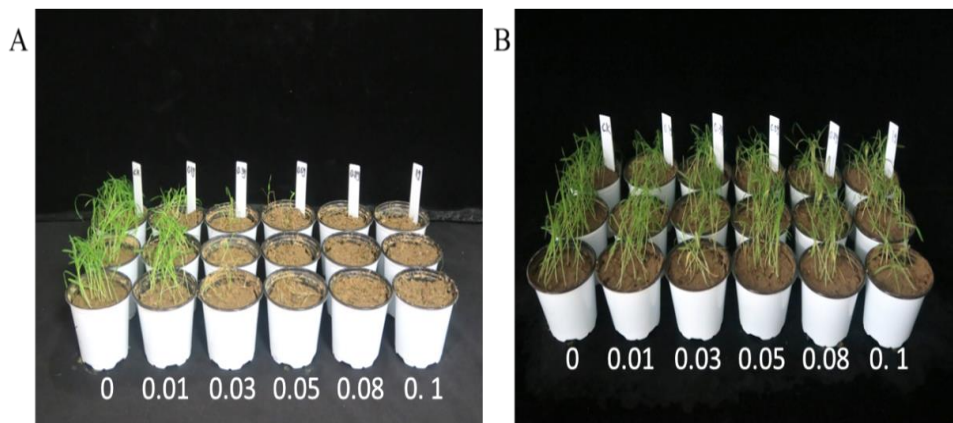


Figure 5. The herbicidal activity of eudesmol (A) and borneol (B) at 0, 0.01, 0.03, 0.05, 0.08 and 0.1 g/mL.

Table 4. The regression equations and EC₅₀ values of wormwood oil, eudesmol and borneol.

Compound	Regression formula	Correlation coefficient	EC ₅₀ (g/mL)	95 % confidence limits	p-value
Green wormwood oil	$y=1.884x+6.825$	0.9609	0.0107	0.0642~0.1791	0.0092
Eudesmol	$y=2.388x+9.470$	0.9796	0.0134	0.0097~0.0185	0.0035
Borneol	$y=1.505x+6.5995$	0.9808	0.0865	0.0674~0.1109	0.0032

Both eudesmol and borneol are monoterpenoids. Monoterpenes are terpenes composed of two isoprene units with the molecular formula C₁₀H₁₆ (5). Monoterpenes are the main constituents of many essential oils showing herbicidal activity. For example, Andriana *et al.* (3) proved that the content of monoterpene compounds in an essential oil is related to its ability to inhibit weed growth. They reported that the *Piper cubeba* essential oil contains 16.54 % more monoterpenes than *Piper nigrum*, and the inhibitory effects of *P. cubeba* essential oil on the elongation of *Bidens pilosa* roots and shoots were 4.16 and 37.74 % stronger than *P. nigrum* (3). In another study, Sharma *et al.* (19) reported that 0.2 mg/mL *Hyptis suaveolens* essential oil, which contains 79 % monoterpenoids, completely inhibited the growth of barnyard grass and was less inhibitory (40 %) to growth of rice (19). In this study, the monoterpene compound (eudesmol) was the major compound (21.93 %) in the wormwood essential oil. Moreover, compared with the original essential oil, the EC₅₀ value of eudesmol (0.0134 g/mL) on barnyard grass was higher than wormwood oil (0.0107 g/mL). That is probably because other minor chemicals might also be involved, but it needs to be verified. Hence, eudesmol, the main compound of wormwood oil, is a promising lead compound for new herbicides.

Defence enzyme activities

Changes in the activity of defense enzymes were measured to determine the herbicidal mechanism of wormwood oil. After it was sprayed, the activities of POD, SOD and CAT in barnyard grass were measured periodically, up to 72 h (Fig. 6). The activities of CAT, POD and SOD in the control group were 1.08, 38.15 and 1.67 U/mg protein,

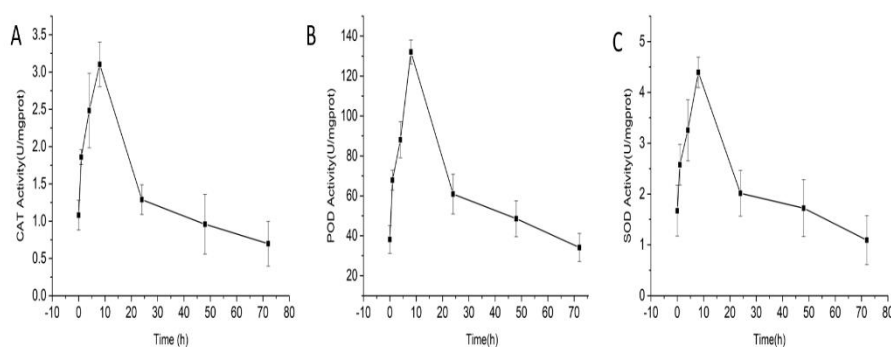


Figure 6. Effects of wormwood oil at 0.05 g/mL on changes in activities of antioxidant defence enzymes A: CAT, B: POD and C: SOD in barnyard grass seedlings.

respectively. CAT activity increased to 3.10 U/mg protein 8 h after wormwood oil treatment, then decreased to 0.70 U/mg protein after 72 h. Similarly, POD reached its highest activity of 132.04 U/mg protein 8 h after treatment, and then decreased to 34.13 U/mg protein by 72 h. Similarly, SOD activity reached a maximum of 4.40 U/mg protein at 8 h, and then decreased to 1.09 U/mg protein at 72 h.

It appeared that wormwood oil decreased the activity of defence enzymes and inhibited the growth of barnyard grass. Some studies have shown that essential oils changes the enzyme activities of the organism. For instance, Korona-Glowniak *et al.* (11) demonstrated that thyme, lemongrass, cedar and lemon balsamic oils can inhibit urease activity of *Helicobacter pylori* (11). The essential oil of *Convolvulus arvensis* L. lowered the activities of CAT, POD, and SOD, eventually leading to electrolyte leakage (15). In addition, spraying the essential oil of cloves enhanced the activity of citrus defence-related enzymes [β -1, 3-glucanase (β -Glu), chitinase (CHI), phenylalanine aminolyase (PAL), POD and polyphenol oxidase (PPO)] (6). Essential oils could therefore be potential candidates for the development of new green herbicides.

CONCLUSIONS

The 11-essential oils of plant origin (Green Wormwood oil, Lavender oil, Cypress oil, Clove basil oil, Angelica oil, Chuanxiong oil, Turmeric oil, Wood balm oil, Forsythia oil, Patchouli oil, Nepeta oil) were screened for their herbicidal effects on barnyard grass. The wormwood oil over 0.05 g/mL concentration killed the seedlings of test Barnyard grass. Wormwood oil was analyzed by GC-MS and 93 compounds were detected. Its 2- major components: eudesmol and borneol were herbicidal, however, the Eudesmol had higher herbicidal activity than borneol. The wormwood oil had strong allelopathic inhibitory effects on test weed, hence, it may be used to develop bioherbicides to control *E. crus-galli*.

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DECLARATION

Z.R. Li and L.Y. Bai designed and planned experiment. H.D. Bai and X.Z. Ni did enzyme activity experiments. J.C. Han conducted indoor bioassay experiments and wrote article. The author shave declared that no competing interests exist.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

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

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