https://doi.org/10.26651/allelo.j/2020-51-1-1294 0971-4693/94 Euro 20.00
Allelopathy Journal 51 (1): 113-124 (September, 2020) *International Allelopathy Foundation* 2020
Table: 2, Figs : 5

Herbicidal, fumigant and insecticidal potential of essential oil from flowers of *Buddleja alternifolia* Maxim

S.X. Yang†, J.Z. Sun1†, J. Yang2†, Y. Yuan2, Y. Yang, J.C. Qin1,
Y. Kuang and D.A. Sampietro3*
Zhejiang Provincial Collaborative Innovation Center for Bamboo Resources and
High-Efficiency Utilization, Zhejiang A & F University, Lin'an, Zhejiang 311300, China
E-Mail: qinjc@jlu.edu.cn; diegoasampietro@gmail.com; kuang_yan_yan@163.com

(Received in Revised Form: July 8, 2020)

ABSTRACT

We investigated the chemical composition, herbicidal and fumigant insecticidal activities of essential oil from the flowers of *Buddleja alternifolia* Maxim. The main constituents of the oil were: ketoisophorone (21.89 %), (Z, E)-Geranyl linalool (3.61 %), dihydrooxoisophorone (7.76 %), *p*-vinyl guaiacol (5.15 %), phenethyl alcohol (3.40 %), safranal (3.25 %) and pentacosane (3.23 %). The fumigant insecticidal activity was investigated by exposing the 7-day-old adults of *Callosobruchus maculatus* and *Sitophilus oryzae* for 24 h at 20 to 320 μL/L air oil concentrations. The concentrations < 20 μL/L air were also assayed to interpolate the lethal concentration of 50 % (LC_{50}). The herbicidal effects of essential oil were evaluated on seed germination and seedling growth of 4-weeds [*Bidens pilosa*, *Lolium perenne* L., *Echinochloa crusgalli* (L.) Beauv. and *Plantago asiatica* L.]. The lowest concentrations of oil causing 100 % mortality of *C. maculatus* were 20 μL/L and *S. oryzae* 40 μL/L, after exposure times of 21 h and 18 h, respectively. The susceptibility of these insects was same at concentrations < 20 μL/L with LC_{50} values of 9.97-12.03 μL/L. The oil at 0.08 % concentration, completely inhibited (100 % Inhibition) the seed germination of 4- test weed species. Its phytotoxic effects on the seedling growth were species specific and *B. pilosa* proved most sensitive weed. Hence, the flower oil may be developed as herbicide or insecticide after more research.

Key words: *Bidens pilosa*, biological activity, *Buddleja alternifolia*, *Callosobruchus maculatus*, chemical composition, *Echinochloa crusgalli*, essential oil, flowers, fumigant, herbicidal, insecticidal, *Lolium perenne*, *Plantago asiatica*, seeds germination, seedlings growth, *Sitophilus oryzae*

INTRODUCTION

Weeds and insect pests are major problems in Chinese agriculture. The test weeds: *Bidens pilosa*, *Lolium perenne* L., *Echinochloa crusgalli* (L.) Beauv. and *Plantago asiatica* L. are major in crop fields and they cause substantial losses in yields (17). Besides the stored grain insects viz., *Callosobruchus maculatus* (Figure 1A) and *Sitophilus oryzae* (Figure 1B) also causes great loss in stored food grains (35). Synthetic chemicals are widely used to control these weeds and insects pests, which has led to the appearance of

---

*Correspondence author, †These authors are joint first authors [1]College of Plant Science, Jilin University, Xi'an Road No. 5333, Changchun, Jilin 130062, China, [2]The State Key Laboratory Breeding Base of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China, [3]LABIFITO. Universidad Nacional de Tucumán. Ayacucho 471 (4000). San Miguel de Tucumán, Argentina.

resistances in insects and weeds and the environmental pollution. Hence, new alternatives are required to control these noxious organisms. There main advantage to use essential oils as insecticides and herbicides because they degrade quickly, hence, are environmentally friendly and their plant sources are readily available (24,36).

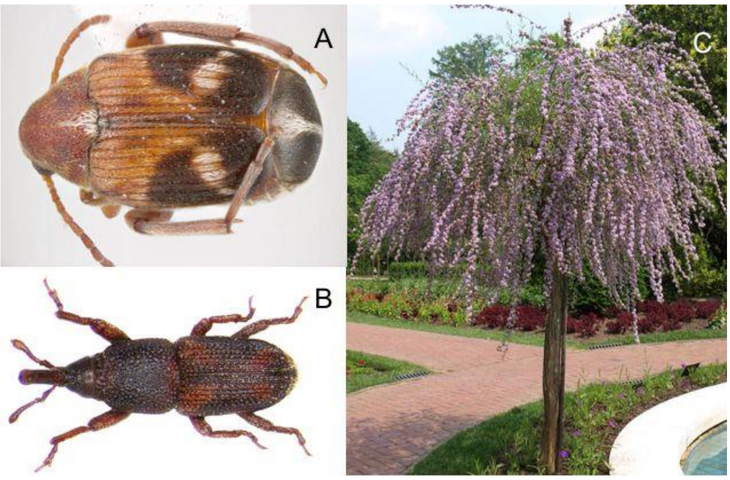

Figure 1. (A) *Callosobruchus maculatus*, (B) *Sitophilus oryzae*, and (C) *Buddleja alternifolia.*

Buddleja (Loganiaceae) genus has about 100 species, mostly shrubs, widely distributed in tropical and subtropical regions of the world. In China, there are its 29 species, as ornamental plants including *B. alternifolia* (Figure 1C) (9). In China, the *Buddleja* species are widely used in folk medicine to treat the cold, cough, bronchitis, stomach ache and hemostasis (19). Its species have insecticidal, fungicidal, antioxidant, anti-inflammatory, sedative, analgesic, hepatoprotective and diuretic activities (7,13,21,26,28). In *Buddleja* species, flavonoids, benzene compounds, iridoid glycosides, terpenes and saponins are as major active principles (7,11,18). This research aimed to (i). describe for the first time the chemical composition of the essential oil from flowers of *B. alternifolia* Maxim., (ii). determine their insecticidal activities on two storage pests (*Callosobruchus maculatus* and *Sitophilus oryzae*) and (iii). also herbicidal effects on 4-test Weeds (*Bidens pilosa*, *Lolium perenne* L., *Echinochloa crusgalli* (L.) Beauv. and *Plantago asiatica* L.).

MATERIALS AND METHODS

Plant material and essential Oil extraction

Flowers of *B. alternifolia* Maxim (8 kg) were collected in August 2015 from Jinan, Shandong, China. The plant samples were authenticated by Professor Ying Wu and a voucher specimen was deposited at Jilin University, China [N 36°39’, E 117°07’]. Jinan is 54 m above sea level and has an average annual temperature of 14.5 °C and an annual rainfall of 613 mm. The average annual máximum and mínimum temperatures are 19.5°C and 8.5°C, respectively. The flowers of *B. alternifolia* were dried in dark for a week and then powdered in a grinder and hydro-distilled for 6 h using a Clevenger apparatus. The oil obtained after hydro-distillation was dried over anhydrous sodium sulphate, weighed and preserved in a sealed brown flask at 4 °C until further analysis. Oil density was calculated as 850 mg/mL.

GC-MS Analysis

The oil of *B. alternifolia* Maxim was analyzed by GC-MS on a Thermo TRACE GC Agilent 5975, equipped with a HP-5 MS capillary column (30 m×0.25 mm, film thickness of 0.25 μm). The temperature was increased from 60 °C to 280 °C at 8 °C /min; held at 60 °C for 3 min and finally kept at 280 °C for 20 min. Helium was used as carrier gas at a flow rate of 1 mL/min. The essential oil was diluted 1: 100 in acetone and 1 μL of this dilution was injected in GCMS. Other conditions of the GC-MS were: injector temperature kept at 280 °C; split ratio of 30: 1; ionization energy of 70 Ev; temperature of the MS source at 230 °C; MS quadrupole temperature at 150 °C; interface temperature at 280 °C; and mass scan of 35-450 amu. The constituents were identified by comparison of their arithmetic indexes and mass spectra with those stored in the National Institute of Standards and Technology (NIST 2008) and the Wiley 275 databases (27, 32) and reported by Adams (3). The relative amounts of individual oil components were expressed as peak area (%) relative to total peak area.

FUMIGANT TOXICITY ASSAYS

(i) Insects Mortality: The toxicity of the essential oil vapours was tested on 7-day old adults of *C. maculatus* and *S. oryzae* at several oil concentrations and exposure times (23). The fumigant chambers were glass vials (24 ml volume) provided with screw caps. Insects were obtained from cultures on bean seeds and whole rice, respectively. Filter papers (Whatman No. 1) were cut into 1 cm dia pieces and impregnated with oil doses of 0.5, 1, 1.6, 2.1, 4.2, and 8.3 μL to provide fumigant concentrations of 20, 40, 60, 80, 160 and 320 μL/L air, respectively. The impregnated filter papers were then attached to the screw caps of the glass vials. Caps were screwed tightly on the vials, each vial separately contained 20 adults of each inset specie. The cultures were kept in dark at 27±1 °C and 65±5 % relative humidity in BOD incubator. The mortality of insects was recorded every 3 h until 24 h. Insect mortality (%) was calculated using the Abbott correction formula for natural mortality in untreated controls (1). The experiments were twice repeated and set up as completely randomized design where each concentration and control was replicated three times.

(ii) LC_{50} (Fumigant concentration required to kill 50% of the insects in 24 h): The oil concentrations of 3, 6, 9, 12, 15 and 18 μL/L air as fumigant were also tested to be interpolates as LC_{50} values for both *C. maculatus* and *S. oryzae*. Assay conditions were the same as in the mortality test, except that glass bottles of 50 ml with screw lids were used as fumigant chambers. The experiments were twice repeated and set up as completely randomized design where each concentration and control was replicated five times. Insect mortality (%) was calculated as previously depicted. The number of surviving insects contained in each bottle were recorded 24 h after exposure. The LC_{50} values were calculated by Probit regression analysis procedure provided by SPSS 19.0 statistics, with 95 % intervals, based on the Finney (15) method.

HERBICIDAL ASSAYS

The seeds germination and seedling growth bioassay of seeds of 4-test weeds: *B. pilosa*, *L. perenne*, *E. crusgalli* (L.) Beauv and *P. asiatica* were done in Petri dishes assays (22). The undeveloped and immature seeds were discarded by floating in distilled water. Then selected seeds were sterlised with 1% potassium permanganate for and repeatedly rinsed 4-times with distilled water. The seeds were sterlised 15 min before culture. The sterile petri dishes (9 cm dia) were lined with two layers of filter papers Then, 30 seeds of test weeds (*B. pilosa*, *L. perenne*, *E. crusgalli* or *P. asiatica*) were placed into separate dishes. The oil volumes of 1.20, 2.35 and 4.70 µL were first dissolved in 0.25 mL of DMSO and then diluted with 5 ml sterile distilled water to get oil concentrations of 0.24, 0.47 0.94 µL/mL, respectively. The final DMSO concentration in each emulsion was 5 %. These oil concentrations were expressed in percentages based on the oil density as 0.02, 0.04 and 0.08 %, respectively. Five ml dilution was added to each Petri dish. Control consisted of 5 mL of sterile distilled water containing 5 % DMSO. Lids of the Petri dishes were closed with an adhesive tape to prevent the loss of moisture and contaminations. The dishes were kept in growth chamber [25 ± 2 °C, 12 h fluorescent light and relative humidity: 80 % (12)]. The rate of seed germination (%) of the total seeds geminated, and root and shoot elongation were recorded after one week. The experiments were repeated thrice. Percentage inhibition of seeds germination and shoot and root elongation (I) were calculated as under:

$$I = \frac{T - C}{C} 100$$

Where, T: Mean of root length, shoot length or seed germination (%) in ‘a’ treatment, C: Mean of root length, shoot length or seed germination in negative control.

Data Analysis

Data of the insecticidal and herbicidal assays were subjected to oneway analysis of the variance, and the significance among means was evaluated by the Duncan’s new multiple range test ($p < 0.05$). The statistical analyses were performed in SPSS 19.0 statistics.

RESULTS AND DISCUSSION

Chemical Composition of essential oil

The hydro-distillation of flowers of *B. alternifolia*, yielded 0.4 % (v/w) pale yellow oil. The GC-MS analysis of this oil identified 41 constituents, which accounted for 88 % of its composition (Table 1). The major components were ketoisophorone (21.8 %), dihydrooxoisophorone (7.76 %), *p*-vinyl guaiacol (5.15 %), phenethyl alcohol (3.40%), (Z, E)-Geranyl linalool (3.61 %), safranal (3.25 %) and pentacosane (3.23 %). The contents of remaining compounds were < 3 %. Ketoisophorone dominates the composition of floral scents of butterfly-pollinated *Buddleja* species such as *B. davidii* (6). This compound is less important in bee-pollinated species [*B. crispa*, *B. asiatica*, *B. macrostachya*, *B. myriantha* and *B. forrestii* (16)]. The flower scents of *B. davidii* also contains the dihydrooxoisophorone and benzenoids (20), these irregular terpenes and phenethyl alcohol are main attractants for butterfly pollinators to the flowers of *B. alternifolia* (16). The major constituents (Z, E)-Geranyl linalool, *p*-vinyl guaiacol, safranal and pentacosane are reported for the first time in flower scent of a *Buddleja* species.

Table 1. Constituents of the essential oil from *Buddleja alternifolia*.

Compound name	Relative content (% area)	AIcalc[a]	AI[b]
3-Methyl butanoic acid	1.15	642	645
3-methyl-1-butanol	1.29	758	762
3-Methyl butanenitrile	0.47	920	926
Benzaldehyde	0.56	956	952
1-Octen-3-ol	0.37	978	974
Limonene	0.21	1028	1024
Benzyl alcohol	0.65	1031	1026
β-Isophorone	1.67	1049	1044
Phenethyl alcohol	3.40	1050	1059
Linalool	1.64	1105	1095
Nonanal	0.89	1111	1100
3-Cyclohexene-1-methanol	1.86	1115	1108
Isophorone	1.46	1115	1118
Ketoisophorone	21.89	1135	1140
Eucarvone	1.87	1140	1146
(-)-Terpinen-4-ol	1.31	1170	1174
Dihydrooxoisophorone	7.76	1180	1183
α-Terpineol	2.02	1189	1186
Safranal	3.25	1190	1196
Benzofuran, 2,3-dihydro-	0.88	1200	1208
β-Cyclocitral	0.43	1218	1217
GrandlureIII	2.73	1220	1226
β-fenchyl acetate, exo-	1.07	1235	1229
Phellandral	0.45	1270	1275
p-vinyl guaiacol	5.15	1350	1355
β-Damascenone	0.31	1380	1386
Geranyl acetone	0.75	1458	1453
α-curcumene	0.81	1480	1482
3-oxo-ionone	0.32	1510	1500
Caryophyllene oxide	0.30	1580	1582
Dihydroactinidiolide	0.48	1585	1588
Widdrol	0.27	1595	1599
1-Hydroxy-p-menth-3-one	0.46	1720	1726

Benzyl benzoate	2.10	1752	1759
2,6-Dimethyl-3,5,7-octatriene-2-ol,	1.47	1828	1824
Z,Z-Benzyl salicylate	1.01	1860	1864
Farnesyl acetone	0.32	1915	1919
Methyl hexadecanoate	0.35	1928	1921
Hexadecanoic acid, methyl ester	0.35	1930	1924
(Z,E)-Geranyl linalool	3.61	1998	1998
(E,E)-Geranyllinalool	0.93	2031	2026
Heneicosane	0.61	2083	2100
Methyl linoleate	1.29	2185	2200
Tetracosane	0.49	2350	2400
Pentacosane	3.23	2510	2500
Hexacosane	0.63	2585	2600
Heptacosane	2.42	2689	2700
Octacosane	0.48	2795	2800
Nonacosane	0.64	2900	2900
Fatty acid derivatives	16.13		
Benzenoids	15.61		
Hydrocarbonated monoterpenes	0.21		
Oxygenated monoterpenes	48.48		
Hydrocarbonated sesquiterpenes	0.81		
Oxygenated sesquiterpenes	2.25		
Oxygenated diterpenes	4.54		
	88.06		

[a] Arithmetic indexes calculated (AIcalc) from retention times in relation to series of *n*-alkanes on a HP-5 MS capillary column were compared with arithmetic indexes (AI) provided by Adams (3).

Fumigant Activity

The exposure time required to kill 100 % adults of *C. maculatus* and *S. oryzae* was decreased, with the increase in concentration of flower oil (Figure 2). However at all concentrations of oil, the mortality was higher in *C. maculatus* than *S. oryzae*. For example, *C. maculatus* and *S. oryzae* exposed to 160 µL oil/L air reached 100 % mortality after 3 and 6 h, respectively. At 320 µL oil/L air, all adults of *C. maculatus* died in few minutes, while those of *S. oryzae* took 3 h. The lowest concentration of oil producing 100 % mortality was 20 µL/L for *C. maculatus* and 40 µL/L for *S. oryzae*. The exposure time associated with these concentrations were 21 h and 18 h, respectively. The insecticidal activity of essential oil often is attributed to its major chemical constituents (10). However, there is no information about the fumigant toxicity of major substances of *B. alternifolia* oil on *S. oryzae* and *C. maculatus*, although some of them have semiochemical or insecticidal activities. In addition to the semiochemical (ketoisophorone, dihydroxyoxophorone and phenethyl alcohol), the *n*-pentacosane at very low conc. act as repellent against *S. zea* and as an attractant to *C. maculatus* (4,29). The *p*-vinylguaiacol showed moderate insecticidal activity (LD_{50} = 63.75 µg/adult) in topical applications on adults of *S. zea* (8), while, safranal and geranyllinalool are biocidal to insect pests (25).

Minor constituents of flower oil (linalool and α-terpineol) have strong insecticidal activity (14,26). Hence, the synergic effects between the minor and major constituents could not be ignored.

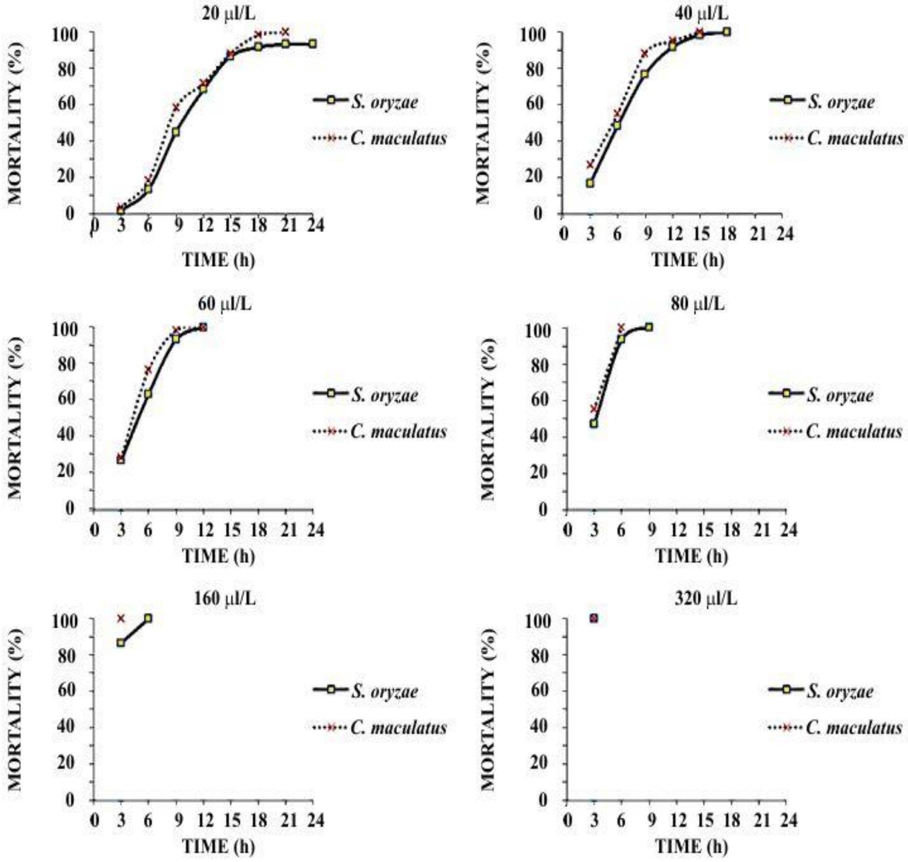

Figure 2. Fumigant toxicity of flower oil from *Buddleja alternifolia* against (*Callosobruchus maculatus* and *S. oryzae*) at various concentrations and exposure times. The assays were done in growth chamber (at 27 °C with a relative humidity of 65 %).

The LC_{50} and LC_{95} values (Table 1) were calculated from adult insects exposed for 24 h to flower oil concentrations < 20 µL/L air. Here, the 95 % confidence intervals indicated that *C. maculatus* (LC_{50} = 9.97 µL/L; LC_{95} = 19.15 µL/L) had the same sensitivity to the oil as *S. oryzae* (LC_{50} = 12.03 µL/L; LC_{95} = 26.95 µL/L) (Table 2). Our LC_{50} values were several times higher than commercial fumigants (methyl bromide and phosphine) and effectively controlled the storage insect pests (31). However, these fumigants deplete the ozone and are harmful to the environment, which did not occur with essential oils. The flower oil showed similar or higher biocidal effects than other plant essential oils reported as strong insecticidals to *C. maculatus* and *S oryzae*. For example, orange peel oil had lower fumigant effects than *B. alternifolia* flower oil with LC_{50} values of 21.87 µL/L air on *C. maculatus* and 60 µL/L air on *S. oryzae* (31). Lee *et al.* (23) recorded LC_{50} values against *S. oryzae* for essential oils of Myrthaceae species ranged from 19 to 100 µl/L air. The LC_{50} of essential oil from flowering parts of *Perovskia abrotanoides* (Lamiaceae) was 18.75 µl/L air (5). These data suggest that the development of insecticidal agents based on *Buddleja alternifolia* flower oil deserves further research.

Table 2. Calculated lethal concentration of 50 % (LC_{50}) and 95 % (LC_{95}) flower oil of *B. alternifolia* based on bioassay on *C. maculata* and *S. oryzae*

Insect species	LC_{50} (µL/L)[a]	LC_{95} (µL/L)[a]	Regression equation	Standard error	Chi-square (χ^2)
S. oryzae	12.03 (11.35~12.75)	26.95 (23.64~32.30)	y=-9.07+8.40x	0.74	4.55
C. maculatus	9.97 (8.24~11.61)	19.05 (15.29~32.28)	y=-10.46+10.47x	0.85	16.63

[a] 95 % lower and upper fiducial limits are shown in parenthesis.

Allelopathic Herbicidal Activity

The flower oil of *B. alternifolia* also inhibited the seeds germination and the seedlings root and shoot elongation of all test weeds (*B. pilosa*, *L. perenne*, *E. crusgalli* and *P. asiatica*)

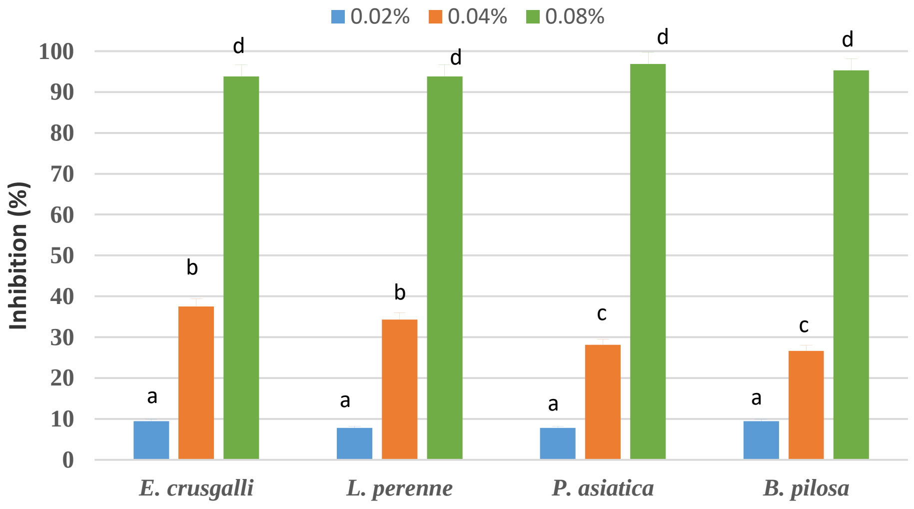

Figure 3. Effects of flower oil from *Buddleja alternifolia* on seed germination of test weeds (*E. crusgalli*, *L. perenne*, *P. asiatica* and *B. pilosa*). Different letters on the bars indicate significant differences (Duncan's new multiple range test, $p < 0.05$).

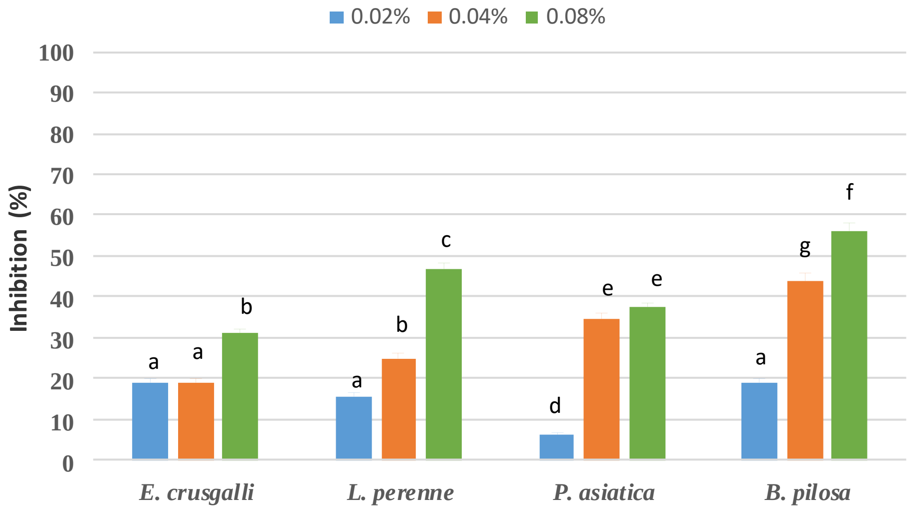

Figure 4. Effects of flower oil from *Buddleja alternifolia* on root growth of test Weeds (*E. crusgalli*, *L. perenne*, *P. asiatica* and *B. pilosa*). Different letters on the bars indicate significant differences (Duncan's new multiple range test, $p < 0.05$).

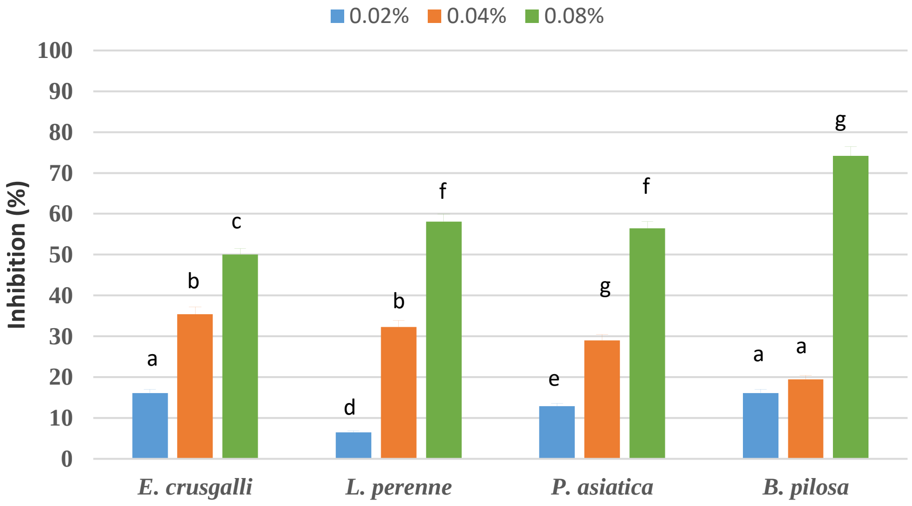

Figure 5. Effects of flower oil from *Buddleja alternifolia* on shoot elongation of test Weeds (*E. crusgalli*, *L. perenne*, *P. asiatica* and *B. pilosa*). Different letters on the bars indicate significant differences (Duncan's new multiple range test, $p < 0.05$).

in a concentration dependent manner. There were no interspecific differences in the weeds germination with complete inhibition at 0.08 % (Figure 3). The inhibitory effects of oil were species specific on seedling growth. The highest inhibition in root length (45 % and 59 %) of *B. pilosa* was with 0.04 and 0.08 %, respectively, followed by *L. perenne* with 48 % inhibition at 0.08 % (Figure 4). In remaining species (*E. crusgalli* and *P. asiatica*) inhibitions were < 40 % at 0.08 %. In shoot length, the interspecific differences occurred at the highest concentration (0.08 %) assayed with reductions of 74 % (*B. pilosa*), 61-58 % (*L. perenne* and *P. asiatica*) and 51 % (*E. crusgalli*) (Figure 5). There is variability in essential oil *in-vitro* phytotoxicity due to several factors including the test receptor plants and the environmental conditions (34). The observed phytotoxicity was likely due to the higher contents of oxygenated monoterpenes and benzenoids in oil. Principal component analyses relating the composition of essential oils with their phytotoxic activity on seed germination and seedling growth showed that, those with highest herbicidal effect on weeds were rich in oxygenated monoterpenes and benzenoids instead of sesquiterpenes or hydrocarbonated terpenes (33). The monoterpenes and the benzenoids act on the cell membranes by changing their permeability which in turn affects the cell division, respiration, photosynthesis and other physiological processes (2).

CONCLUSIONS

The essential oil from flowers of *B. alternifolia* was rich in oxygenated monoterpenes, hence, exhibited strong insecticidal and herbicidal effects. Further research is needed to confirm the reproducibility of oil composition and to develop formulations to control the insect pests in grain storage and same weeds in field conditions.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (21702187, 31870332, 31470414); Zhejiang Public Welfare Research Project (LGN19B020001); Zhejiang Key Research and Development Program (2020C03090, 2018C02050); National Science & Technology Fundamental Resources Investigation Program of China (2018FY100800); The National Key Research and Development Program of China (2018YFD0401403); National Science & Technology Fundamental Resources Investigation Program of China (2018FY100800); National Undergraduate Innovation and Entrepreneurship Training Program (201910341055).

REFERENCES

1. Abbott, W.S. (1925). A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18:** 265-267.
2. Abrahim, D., Braguini, W.L., Kelmer-Bracht, A.M. and Ishii-Iwamoto, E.L. (2000). Effects of four monoterpenes on germination, primary root growth and mitochondrial respiration of maize. *Journal of Chemical Ecology* **26**: 611-624.

3. Adams, R.P. (1989). *Identification of Essential Oils by Ion Trap Mass Spectroscopy*. Academic Press, New York, USA.
4. Adhikary, P., Mukherjee A. and Barik, A. (2014). Role of surface wax alkanes from *Lathyrus sativus* L. seeds to attract the *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Products Research* **59**: 113-119.
5. Arabi, F., Moharramipour, S. and Sefidkon, F. (2008). Chemical composition and insecticidal activity of essential oil from *Perovskia abrotanoides* (Lamiaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *International Journal of Tropical Insect Science* **28**: 144-150.
6. Chen, G., Sun, H., Sun, W. and Norman, E. (2011). *Buddleja davidii* and *Buddleja yunnanensis*: Exploring features associated with commonness and rarity in Buddleja. *Flora* **206**: 892-895.
7. Chong, L. (1993). Iridoids from *Buddleja Alternifolia* Maxim. *Journal of Lanzhou University (Medical Science)* **19:** 209-211.
8. Chu, S.S., Jiang, G.H. and Long-Liu, Z. (2011). Insecticidal Components from the Essential Oil of Chinese Medicinal Herb, *Ligusticum chuanxiong* Hort. *E-Journal of Chemistry* **8**: 300-304.
9. Dai, W. and Castillo, C. (2007). Factors affecting plant regeneration from leaf tissues of *Buddleja* species. *Hortscience* **42:** 1670-1673.
10. Dias C.N. and Moraes, D.F. (2014). Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: review. *Parasitology Research* **113**: 565-592.
11. Ding, N., Yahara, S. and Nohara, T. (1992). Structure of mimengosides A and B, new triterpenoid glycosides from *Buddlejae Flos* produced in China. *Chemical & Pharmaceutical Bulletin* **40:** 780-782.
12. Dudai, N., Poljakoff-Mayber, A., Mayer, A.M., Putievsky, E. and Lerner, H. (1999). Essential oils as allelochemicals and their potential use as bioherbicides. *Journal of Chemical Ecology* **25:** 1079-1089.
13. El-Domiaty, M.M., Wink, M., Aal, M.M.A., Abou-Hashem, M.M. and Abd-Alla, R.H. (2009). Antihepatotoxic activity and chemical constituents of *Buddleja asiatica* lour. *Zeitschrift fur Naturforschung C.* **64:** 11-19.
14. Enan, E. (2001). Insecticidal activity of essential oils: Octopaminergic sites of action. *Comparative Biochemistry and Physiology. C: Pharmacology, Toxicology and Endocrinology* **130:** 325-337.
15. Finney, D.J. (1971). *Probit Analysis*. 3rd edition Cambridge University, London.
16. Gong, W.C., Chen, G., Vereecken, N.J., Dunn, B.L., Ma, Y.P. and Sun, W.B. (2015). Floral scent composition predicts bee pollination system in five butterfly bush (*Buddleja*, Scrophulariaceae) species. *Plant Biology* **17**: 245-255.
17. Gui, B.J., Wei, J.G. and Zi, H.L. (2014). Allelopathic potentials of volátiles form *Ageratum conyzoides* L. on weeds and vegetables. *Journal of Henan Agricultural Sciences* **43**: 92-95. (Chinese).
18. Guo, H., Koike, K. and Li, W. (2004). Saponins from the flower buds of *Buddleja officinalis*. *Journal of Natural Products* **67:** 10-13.
19. Houghton, P. (1984). Ethnopharmacology of some *Buddleja* species. *Journal of Ethnopharmacology* **11:** 293-308.
20. Joshi, S., Mishra, D., Bisht, G. and Khetwal, K.S. (2012). Comparative study of essential oil composition of *Buddleja asiatica* and *Buddleja davidii* aerial parts. *International Journal of Green Pharmacy* **6**:23-25.
21. Juarez, Z.N., Bach, H., Sanchez-Arreola, E., Bach, H. and Hernandez, L.R. (2016). Protective antifungal activity of essential oils extracted from *Buddleja perfoliata* and *Pelargonium graveolens* against fungi isolated from stored grains. *Journal of Applied Microbiology* **120:** 1264-1270.
22. Kong, C.H., Hu, F., Xu, T. and Liu, Y.H. (1999). Allelopathic potential and chemical constituents of volatile oil from *Ageratum conyzoides*. *Journal of Chemical Ecology* **25:** 2347-2356.
23. Lee, B. H., Annis, P. C., Tumaalii, F. and Choi, W. S. (2004). Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. *Journal of Stored Products Research* **40**: 553-564.
24. Lee, D.H., Ha, N. and Bu, Y.M. (2006). Neuroprotective effect of *Buddleja officinalis* extract on transient middle cerebral artery occlusion in rats. *Biological & Pharmaceutical Bulletin* **29:** 1608-1612.
25. Lemaire, M., Nagnan, P., Clement, J.L., Lange, C., Peru, L. and Basselier, J.J. (1990). Geranyllinalool (diterpene alcohol): An insecticidal component of pine wood and termites (Isoptera: Rhinotermitidae) in four european ecosystems. *Journal of Chemical Ecology* **16**: 2067-2079.

26. Mahlke, J.D., Boligon, A.A., Machado, M.M. and Athayde, M.L. (2013). Composition of the essential oil of *Buddleja thyrsoides* Lam. leaves from Brazil. *Natural Products Research* **27:** 1319-1322.
27. McLafferty, F.W. (1989). *Registry of Mass Spectral Data*. Wiley, New York, USA.
28. Mensah, A.Y., Sampson, J. and Houghton, P.J. (2001). Effects of *Buddleja globosa* leaf and its constituents relevant to wound healing. *Journal of Ethnopharmacology* **77:** 219-226.
29. Ndungu, M., Lwande, W., Hassanali, A., Moreka, L. and Chhabra, S.C. (1995). *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellents. *Entomologia Experimentalis et Applicata* **76**: 217-222.
30. Negahban, M., Moharramipour, S. and Sefidkon, F. (2007). Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-productinsects. *Journal of Stored Products Research* **43:** 123-128.
31. Oboh, G., Ademosun, A.O., Olumuyiwa, T.A., Olasehinde, T.A., Ademiluyi, A.O. and Adeyemo, A.C. (2017). Insecticidal activity of essential oil from orange peels (*Citrus sinensis*) against *Tribolium confusum*, *Callosobruchus maculatus* and *Sitophilus oryzae* and its inhibitory effects on acetylcholinesterase and Na+/K+-ATPase activities. *Phytoparasitica* **45**: 501-508
32. Stein, S.E. (1990). *Mass Spectral Database and Software*, Version 3.02, National institute of standards and technology. USA.
33. Verdeguer, M., Amparo Blazquez, M. and Boira, H. (2009). Phytotoxic effects of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils on weeds of Mediterranean summer crops. *Biochemical Systematics and Ecology* **37**: 362-369.
34. Wang, S.Q., He, S.L., Zhang, M.Z., Zhang, Y.X., Wang, Q.Y., Zhang, C.Y., Liu, T.Y., Liu, B., Han, J.Y., Qin, J.C. and Sampietro, D.A. (2019). Chemical composition and allelopathic potential of essential oils from *Eupatorium maculatum* on *Lolium perenne* L. and *Echinochloa crusgalli* L. *Allelopathy Journal* **49**:51-62.
35. Weaver, D.K., Dunkel, F.V. and Ntezurubanza, L. (1991). The efficacy of linalool, a major component of freshly-milled *Ocimum canum* Sims (Lamiaceae), for protection against postharvest damage by certain stored product Coleoptera. *Journal of Stored Products Research* **27:** 213-220.
36. Zhang, X., Li, Z.H. and Feng, J. (2010). Insecticidal activities of extracts from *Buddleja albiflora* Hemsl. against four kinds of pests. *Zhejiang Daxue Xuebao, Nongye Yu Shengming Kexueban* **36:** 192-198.