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Terpene Rich Essential Oil of *Dracocephalum kotschyi* Boiss as Efficient Alternative to Synthetic Chemicals in Management of *Callosobruchus maculatus* Fabricius

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ABSTRACT: The application of plant-derived essential oils has been revealed to proficiently insect pest control agents, meaningfully decreasing the side-effects caused by chemical insecticides. In the present study, the toxicity of essential oil isolated from *Dracocephalum kotschyi* has been assessed against cowpea weevil (*Callosobruchus maculatus* Fabricius) as one of the most damaging and cosmopolitan insect pest of stored beans. The other objective of current study was to identify the chemical composition of *D. kotschyi* essential oil as one of the medicinal plants endemic to Iran. Bioassays results revealed sound fumigant toxicity of essential oil, in which a concentration of 16.41 µl/l produced up to 50% insect mortality after 72 h. Increases in concentrations of the essential oil and exposure times augmented the insect susceptibility so that the highest mortality (96.25%) was achieved at a highest tested concentration (44.12 µl/l) after 72 h. A concentration of 44.12 µl/l was enough to achieve the 50% mortality of tested insects within 10.761 h. The 24 h-LC_{50} value of essential oil was 24.947 µl/l which decreased to 17.794 µl/l after 72 h. Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil exposed terpenes including geranyl acetate, citral, z-citral, methyl geranate, limonene, α-pinene and α-campholenal were the main components. Based on the results of present study, the terpene rich essential oil of *D. kotschyi* may be considered as promising alternative to the synthetic chemicals for *C. maculatus* management.

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Cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae), is a primary insect pest of legume grains which causes high quantitative and qualitative losses on legumes including notable decreases in weight, nutritional value, and the potential of seed germination (Badii et al., 2011). The application of chemical insecticides and fumigants such as organophosphates and phosphine is a main method in the management of *C. maculatus*. Due to the increasing resistance of insect pests to chemical pesticides such as phosphine and pesticide residues in food and environment, the use of eco-friendly and safe agents is needed (Kang et al., 2013; Loddé et al., 2015).

In recent decades, the use of essential oils extracted from aromatic plants has been considered as efficient alternatives to chemical insecticides (Isman and Grieneisen, 2014). The insecticidal effects of some plant-derived essential oils against *C. maculatus* have been approved in some recent studies, indicating possible application of plant essential oils in *C. maculatus* management (Viteri Jumbo et al., 2018; Moura et al., 2019; Alves et al., 2019).

Dracocephalum kotschyi Boiss., known as Zarrin–Giah and belonging to the Lamiacae family, is one of the endemic medicinal plants to Iran (Jalaei et al., 2015). This plant has traditionally used as an additive to fit the taste of tea and yogurt and as a folk medicine because of its antispasmodic and analgesic properties (Jahaniani et al., 2005). Several terpenes and aliphatic compounds such as 1,8-cineole, carvacrol, carvone, caryophyllene, citral, limonene, menthone, and *α*-terpineol have been identified in the essential oils isolated from different parts of *D. kotschyi* (Sonboli et al., 2019). Caused by such complex chemical composion, the *D. kotschyi* essential oil exposed diverse biological activities including antibacterial, anticancerous, antioxidant, and immunomodulatory properties (Jalaei et al., 2015; Sadraei et al., 2015).

The current study aimed to investigate the fumigant toxicity of *D. kotschyi* essential oil against *C. maculatus*, hoping that it could be considered as a new botanical-derived insecticide. The other objective was to identify the chemical composition of *D. kotschyi* essential oil and to investigate the probable relationship between its components and insecticidal activity.

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MATERIAL AND METHODS

Insect rearing: The original populations of *C. maculatus* were collected from infested cowpea seeds from Moghan Region (Ardabil province, Iran). Insects reared on disinfested cowpea (*Vigna ungiculata* (L.)) in 700 ml transparent plastic jars under laboratory conditions at 27 ± 2 °C, 75 ± 5% relative humidity and a 12 h photo period (Moura et al., 2019). To prevent flocking of the insects and infesting of rearing jars, old seeds were substituted with new ones monthly. The emerged adults were isolated and used for the production of a new generation. Finally, one to seven day-old adults were selected for bioassays.

Plant materials and extraction of ssential oil: Foliage of *Dracocephalum kotschyi* was collected at flowering stage from Esfahan, Iran. Specimens were shaed-dried on laboratory benches at room temperature within 1 week and the dried materials were ground to obtain a fine powder. Each 100 g powder was subjected to hydro-distillation using a one-liter capacity Clevenger apparatus with 500 ml disttiled water within 3 h. Extra water from essential oil was removed by anhydrous sodium sulfate and obtained essential oil was refrigerated at 4°C.

Fumigant toxicity: The bioassays were performed with 20 one to seven day-old unsexed *C. maculatus* adults in 340 ml glass jars, sealed with a screw cap, as fumgant chamber. After a concentration-setting experiment, *D. kotschyi* concentrations ranged from 11.76 to 44.12 µl/l were conducted for fumigation. The concentrations were applied on 2 × 3 filter papers suspended from the jar caps which were reinforced sealed with silicone glue. Jars without essential oil concentration asigned as the controls. Four replicates were subjected to each concentration and dead insects were counted after 24, 48 and 72-h exposure times.

Chemical characterization of essential oil: Chemical composition of the *Dracocephalum kotschyi* essential oil were assessed using gas chromatography (Agilent 7890B) coupled with mass-spectrometer (Agilent 5977A). The analysis was carried out by a HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The temperatures of the injector was 280 °C and the column temperature adjusted from 60 to 350 °C.

The carrier gas was helium (99.999%) with flow rate of 1 ml/min. Essential oil was diluted in methanol, and 1 µl solution was injected (split 1:10 at 0.75min).

The identification was accomplished by comparing components mass spectra with spectra available in the the equipment database: Wiley 7n.1 mass computer library and National Institute of Standards Technology (NIST).

Statistical analysis: Data were analyzed using one way variance analysis (ANOVA) and the Tukey's HSD test at probability of 0.05 was used to separate the means of insect mortalities. Probit analysis was used to estimate LC_{50} (Lethal Concentrations to kill 50% of insect), LT_{50} (Lethal Time to kill 50% of insect), the data heterogeneity and the regression lines detailes using SPSS software version 24.

RESUTLS AND DISCUSSION

Analysis of variance revealed that the tested concentrations of *D. kotschyi* essential oil including 11.76, 16.41, 22.82, 34.74 and 44.12 µl/l ($F = 225.319$ and $p < 0.0001$) and the 24, 48 and 72 h-exposure time ($F = 104.328$ and $p < 0.0001$) had statistically significant effects on the mortality of *C. maculatus*. *D. kotschyi* essential oil showed considerable toxicity against the adults of *C. maculatus* so that a 16.41 µl/l concentration caused up to 50% mortality after 72h. Compare mean with Tokey HSD test at $\alpha = 0.05$ indicated that the lowest (25.00%) and highest (44.12%) mean mortalities observed at 11.76 and 44.12 µl/l concentrations, respectively (Figure 1). According to table 1, increases in the essential oil concentration significantly decreased the LT_{50} values so that it was reduced from 161.187 to 10.761 h by 11.76 and 44.12 µl/l, respectively. The LC_{50} value of *D. kotschyi* essential oil was also decreased from 24.947 µl/l within 24 h-exposure times to 17.794 µl/l after 72 h (Table 2). In other words, the susceptibility of *C. maculatus* generally increased with increasing essential oil concentration and exposure time.

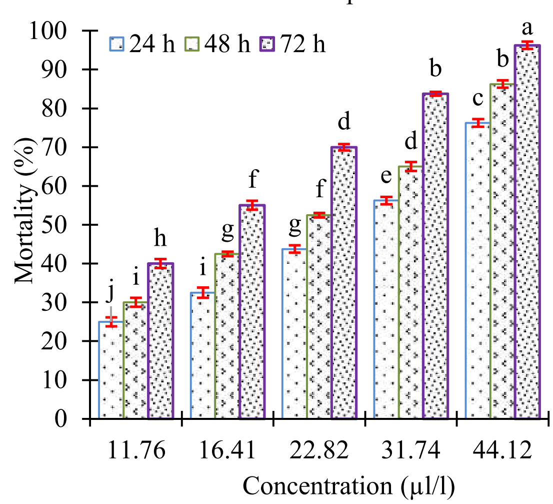

Fig. 1: Fumigant toxicity of the essential oil of *Dracocephalum kotschyi* against the adults of *Callosobruchus maculatus*. Columns are means of mortality percentage and bars SD (standard Deviation). Means recorded by the dissimilar letters in the columns are statistically different according to the Tukey test at α = 0.05.

Susceptibility of *C. maculatus* to the fumigation of plant essential oils has been reported in some recent studies. For example, fumigant toxicity of candela (*Vanillosmopsis arborea* baker), peppermint (*Mentha piperita* L.), parsley (*Petroselinum sativum* L.), river red gum (*Eucalyptus camaldulensis* Dehnh.), and Persian hogweed (*Heracleum persicum* Desf.) with 24 h-LC50 values of 5.23, 25.70, 489.50, 26.10, 136.40 µl/l was approved against *C. maculatus*, respectively, and the toxicity was decreased according to prolonged exposure times (Izakmehri et al., 2013; Massango et al., 2017; Saeidi and Mirfakhraie, 2017; Moura et al., 2019).

Table 1: LT_{50} values and related linear regression data of *Dracocephalum kotschyi* essential oil against *Callosobruchus maculatus* adults

Concentration (µl/l)	LT_{50} with 95% confidence limits (h)	χ^2 (df = 1)	Slope ± SE	Intercept	Significant
11.76	161.187 NC	0.506	0.852 ± 0.389	-1.881	0.477*
16.41	61.066 (46.086 – 121.893)	0.415	1.183 ± 0.376	-2.112	0.519*
22.82	34.251 (21.929 – 43.881)	1.784	1.340 ± 0.375	-2.057	0.182*
31.74	20.846 (10.003 – 28.000)	3.146	1.558 ± 0.391	-2.055	0.076*
44.12	10.761 (3.084 – 16.887)	1.828	1.927 ± 0.484	-1.988	0.176*

* Since the significance level is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits. NC: Noun Calculated; and χ^2: Chi-square.

Table 2: LC_{50} values and related linear regression data of *Dracocephalum kotschyi* essential oil against *Callosobruchus maculatus* adults after 24, 48 and 72 h

Time (h)	LC_{50} with 95% Confidence Limits (µl/l)	χ^2 (df = 3)	Slope ± SE	Intercept	Significant
24	24.947 (22.297 – 28.201)	2.313	2.355 ± 0.297	-3.291	0.510*
48	20.702 (18.051 – 23.428)	0.106	2.124 ± 0.293	-2.795	0.991*
72	14.794 (13.033 – 16.331)	2.131	3.221 ± 0.343	-3.769	0.546*

* Since the significance level is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits. χ^2: Chi-square.

Table 3: Chemical profile of the essential oil of *Dracocephalum kotschyi* Boiss from Iran

Compound	Classification	Retention Time (minute)	Percentage
α-Pinene	Monoterpene hydrocarbon	5.236	9.25
β-Myrcene	Monoterpene hydrocarbon	6.283	0.56
Cymene	Monoterpene hydrocarbon	6.947	0.71
Limonene	Monoterpene hydrocarbon	7.033	9.57
Ocimene	Monoterpene hydrocarbon	7.176	0.36
1,8-Cineole	Monoterpenoid	7.090	0.78
Linalool Oxide	Monoterpenoid	7.914	0.37
γ-Campholenal	Monoterpenoid	8.274	1.42
Linalool	Monoterpenoid	8.475	1.83
α-Campholenal	Monoterpenoid	9.133	4.98
Terpinen-4-ol	Monoterpenoid	10.495	0.59
α-Terpineol	Monoterpenoid	10.889	0.84
Myrtenal	Monoterpenoid	11.073	1.00
Cyclohexylallene	Heterocyclic hydrocarbon	11.324	4.61
Verbenone	Monoterpenoid	11.490	0.88
Z-Citral	Monoterpenoid	12.509	11.58
Carvol	Monoterpenoid	12.606	1.67
Citral	Monoterpenoid	13.625	15.43
Geraniol	Monoterpenoid	13.756	1.01
Methyl geranate	Monoterpenoid	15.770	9.59
Nerol	Monoterpenoid	17.281	2.04
Geranyl acetate	Monoterpenoid	18.019	16.10
Methyl palmitate	Fatty acid methyl ester	30.710	0.37
Methyl-7,10-octadecadienoate	Fatty acid methyl ester	31.872	0.24
Methyl stearate	Fatty acid methyl ester	32.026	0.22
Monoterpene hydrocarbon			20.45
Monoterpenoid (Oxygenated monoterpene)			70.11
All terpenes			90.56
Others			5.44
Total			96.00

Although established fumigant toxicity of essential oils against *C. maculatus* in the above-mentioned studies is confirmed our findings, there are obvious differences between their 24 h-LC_{50} values, which can be due to the origin of plant essential oils. The insecticidal properties of *D. kotschyi* essential oil have been described in one of the previous studies on peach aphid (*Myzus persicae* Sulz.) with LC_{50} values of 4.40 and 0.27 µl/l after 24 and 72 h, respectively (Jalaei et al., 2015). The corresponding LC_{50} values in the present study (24.947 and 14.794 µl/l, respectively) are much higher. This difference may be due to the species of insect pests and disparate chemical components of tested essential oils. So, *D. kotschyi* with considerable insecticidal potential can be offered for further studies in the integrated pest management strategies. Geranyl acetate (16.10%), citral (15.43%), z-citral (11.58%), methyl geranate (9.59%), limonene (9.57%), α-pinene (9.25%), and α-campholenal (4.98%) were identified as main components in the essential oil of *D. kotschyi*. From 96% recognized components in the essential oil, 90.56% were terpene compounds from two hydrocarbon monoterpene (20.45%) and monoterpenoids (70.11%) groups (Table 3).

The chemical components of the essential oil extracted from *D. kotschyi* have been investigated in some previous studies. Jalai et al (2015) showed that Limonene-10-al (73.75%), limonene (19.96%), Menth-1-en-9-ol (1.14%) and Para-limonen-9-ol (1.08%) were the major constituents in the essential oil of the *D. kotschyi* from the Khansar area (Esfahan province, Iran). Of these compounds, only limonene (57.5%) was found in the essential oil of the present study but no other compounds were identified. In another study, from Chadegan area (Esfahan province, Iran) citral (12.5%), z-citral (10.7%), geraniol (10.0%), geraniol acetate (10.0%), α-pinene (9.96%), limonene (8.97%), cyclononadiene (3.94%) and terpinene-4-ol (1.68%) were the major constituents (Sadraei et al., 2015). Geraniol acetate, citral, z-citral, limonene and α-pinene were also identified with high percentages in the present study while geraniol and terpinene-4-ol had lower percentages and cyclononadiene was not detected. These differences can be due to a number of internal and external factors such as growth stage and plant organs used in essential oil' extraction, different geographical conditions, climate changes, and genetic make-up (Fejér et al., 2018; Ale Omrani Nejad et al., 2019). The insecticidal effects of identified terpenes in *D. kotschyi* essential oil include geranyl acetate, citral, limonene, α-pinene, and linalool against *C. maculatus* were also documented (Ajayi et al., 2014; Alves et al., 2019). Therefore, the toxicity of *D. kotschyi* essential oil may be related to these insecticidal agents. However, other slightly detected compounds in *D. kotschyi* essential oil such as geraniol, α-terpineol, 1,8-cineole, and cymene can influence on insecticidal potential (Yeom et al., 2015; Gaire et al., 2019).

The *D. kotschyi* essential oil with high percentage of monoterpene hydrocarbones and monoterpenoids such as geranyl acetate, citral, limonene, α-pinene and linalool, which were recognized as efficient insecticidal agents, indicated considerable toxicity against *C. maculatus* adults. The presence a variety of such terpenes in the essential oils is due to the interaction of plants-herbivores. Indeed, coevolution before herbivores resulted in the production of effective insecticidal agents as secoundary metabolites such as essential oils in aromatic plants. Further, plant essential oils have multiple modes of action, indicating the pest resistance chance before them will be very low. Furthermore, *D. kotschyi* essential oil and many other oils are generally considered as safe and/or harmless bio-agents. According to results of the present study, *D. kotschyi* essential oil can be suggested as an efficient and available biorational insecticide to manage *C. maculatus*. However, evaluation of new formulations to increase essential oil stability, and its side-effetcs on the benefecial organisms such as parasitoid wasps and stored grains must be considered.

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