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Chemical composition, fumigant toxicity and repellency of *Zhumeria majdae* essential oil against *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae)

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Abstract
Chemical composition of the essential oil from *Zhumeria majdae* and its fumigant and repellent activity were investigated against *Rhyzopertha dominica*. Dry aerial parts of the plant were subjected to hydrodistillation using a modified Clevenger-type apparatus and the chemical composition of the volatile oil was studied by GC-MS. Fourteen components (99.36% of the total composition) were identified. Linalool (47.29%), camphor (34.33%), geraniol (3.95%), limonene (3.33%) and camphene (2.98%) were found to be the major constituents of the oil. The mortality of 1-3 days old adults of the insects increased with increase in concentration and exposure time from 3 to 24 h. In all of concentrations, total mortality was achieved after 12 and about 21 h of exposure, respectively. The oil had strong toxicity against all of stages of *Rhyzopertha dominica*. On the basis of LC_{50} values, larval stages were the most tolerant and adult the most susceptible stage in this species. In general, susceptibility of eggs was significantly decreased with increasing their age, while older larvae were more tolerant than young ones. Also, the essential oil had significantly repellency to *R. dominica.* The many diverse bio-effects of *Z. majdae* essential oil confirmed that it is a good candidate for management of *R. dominica*.

Keywords: *Zhumeria majdae*, Essential oil, fumigant toxicity, repellency, stored product pests

Introduction
In stored grain, insect damage may account for 10-40% of loss worldwide [1]. *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) beetle is a serious pest as it attacks various products such as grains, dried fruits, nuts, dough, sugar, candies, tobacco, dried meat, and a number of plant products meant for human consumption [2] and is considered one of the most destructive pests owing to its high incidence and the great difficulty in avoiding the losses that it causes to grains [3].This insect is mainly controlled using synthetic insecticides (organophosphates or pyrethroids) and fumigation, phosphine being remarkable as the main fumigant used [3,4]. These insecticides carry about such serious problems as contamination of the environment, lethal effects in non targeted organisms, toxic residues on stored grain, increasing costs of application and insect resistance [5, 6]. Therefore, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly.
An alternative to synthetic pesticides is the use of natural compounds, such as essential oils that result from secondary metabolism in plants. They should cause less damage to human and environmental health than conventional insecticides. Many of them degrade rapidly and do not accumulate in the body and environment while some are very pest specific and do little or no damage to other organisms. The insecticidal activity of a large number of essential oils and other plant extracts has been assessed against several major agricultural pests [7, 1, 8, 9, 10, 11]. In recent years, there has been an increasing amount of literature on the effect of essential oils of plants such as *Mentha spicata* L. [12]; *Artemisia absinthium* (Asteraceae) [13], three Iranian Eucalyptus species [14] *Ocimum basilicum* L., *Citrus aurantium* L., *Mentha spicata* L. and *Croton pulegiodorus* Baill [15] against *R. dominica*. *Zhumeria majdae* Rech. F. & Wendelbo (Lamiaceae) is one of the important, endemic and unique medicinal plants of Hormozgan province in Iran. This plant with a strong and pleasant odor belongs to the *Lamiaceae* family. Our previous study indicated that its essential oils had sever effect on mortality and repellency of *Callosobruchus maculatus* and *Tribolium confusum* [16].

The aim of this study was to evaluate the chemical composition of the essential oil from *Z. majdae* and its efficacy as a fumigant and repellent in the management of *Rhyzopertha dominica*. This approach will allow discovering natural and safer agents for the development of bio-rational insecticides.

Materials and Methods

Plant materials

Aerial parts of *Z. majdae* were collected at flowering stage from Tange-zagh Mountains, North of Bandar Abbas, Hormozgan province, Iran at an altitude 1200 m, in May 2016. The plant material was dried naturally on laboratory benches at room temperature (24-25˚C) for 5 days until crisp. The dried material was stored at -24˚C until needed and then hydrodistilled to extract its essential oil.

Extraction and analysis of essential oil

Aerial parts of the plant were subjected to hydrodistillation using a modified Clevenger-type apparatus. Conditions of extraction were: 50 g of air-dried sample; 600 ml distilled water and 2 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (6% w/w) was calculated on a dry weight basis. Extracted oil was stored in a refrigerator at 4˚C.

Gas chromatography (GC) analysis was carried out on a HP-6890 gas chromatograph equipped with a flame ionization detector (FID) and a HP-5MS (non-polar) capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The oven temperature was held at 60˚C for 3 min then programmed at 5˚C/min to 260˚C. Other operating conditions were as follows: carrier gas Helium, at a low rate of ml/min; injector temperature 250˚C; detector temperature 280°. GC mass spectrometry (GC-MS) analysis was performed with a HP-6890 GC coupled with HP-5973N with the same characteristics as the one used in GC. The ionization energy was 70 eV with a scan time of 1 s and mass range of 40-300 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. Identification of the constituents of the oil was made by comparison of their mass spectra and retention indices (RI) with known compounds or published spectra.

Insect rearing

Rhyzopertha dominica were reared on wheat (*Triticum monococcum* L.). To remove any likelihood of contamination, the incorporated wheat was refrigerated at –10°C for 72 h. Adult insect, 1-3 days old, was used for all experiments. The cultures were maintained in the dark in growth chamber sets at 27±1˚C and 65±5% RH. All experiments were carried out under the same environmental conditions.

Bioassay with adults

To assess 50% lethal concentrations of adult insects, different dilutions were prepared to evaluate mortality of insects after a preliminary dose-setting experiment. Twenty adult insects of *R. dominica* were put into 40 ml glass bottles with screw lids. The insects had no contact with filter paper and stayed at the bottom of the vial throughout the experiment. Concentrations of the essential oil tested on *R. dominica* were 6, 9, 13, 19, 26, 36 µl/l air. Control insects were kept under the same conditions without any essential oil. Each concentration was replicated five times. The number of dead and live insects in each bottle was counted 24 h after initial exposure to the essential oil. The treatment bottles were monitored at least 48 h after recording the data and no affected insects recovered. Probit analysis (Finney, 1971) was used to estimate LC_{50} values using SAS software version 8. Any two LC_{50} values whose 95% confidence limits did not overlap have been regarded as significantlt different [17].

Another experiment was designed to determine the action of the oil on adults. Filter papers (2 cm diameter) were impregnated with oil at doses calculated to give the fumigant concentrations (62.9 to 440.3 µl/l air). Then the filter paper was attached to the under surface of the screw cap of a glass vial volume 27 ml. The cap was screwed tightly on the vial containing twenty adults (1 days old) of each species of insect separately. Each concentration and control was replicated five times. Mortality was recorded after 3, 6, 9, 12, 15, 18, 21 and 24 h from commencement of exposure. In this experiment, new batches of insects were prepared for each time, so that mortality for each exposure time was investigated independently. When no leg or antennal movements were observed, insects were considered dead. The Abbott formula was used to correct for natural mortality in control [18]. The data analysis was performed using the statistical software SPSS 16.0.

Bioassay with immature stages

To secure eggs and larvae of the same age, the following procedure was followed. The number of 200 Adult insects, male and female were collected from the stock culture after emergence and put together in plastic containers for 1 day in order to ensure mating and laying eggs. After one day, insects were picked up from containers to obtain different developmental stages of known age.

In order to determine the ovicidal and larvicidal activity of *Z. majdae* oil, glass jars of 40 ml volume with screwed metallic caps were used as exposure chambers. The caps were screwed tightly on the vial containing 20 eggs and larvae. Ovicidal activity and larvicidal activity of the plant oil were investigated against 1 and 4 days old eggs of *R. dominica*, A filter paper (2 cm diameter), treated with different concentrations of essential oil was pasted on the inner surface of the cover of each vial. All the closed vials were kept in the dark and five replicates were set for each concentration. After 24 h of fumigation, the dead eggs and larvae were recorded and unhatched eggs were counted as dead. Probit analysis [19] was used to estimate LC_{50} values using SAS software version 8 (SAS Institute 1997).

Repellency bioassay

The repellent effect of essential oil against adults of both species was evaluated using a Y-tube olfactometer model RZR. The olfactometer consists of a main brunch with 2 arms. Different test solutions were prepared by diluting 50, 85, 120 and 160 µl of essential oil in 0.5 ml of acetone.

Each solution was applied on 2 g of wheat as treated arm. In the other arm, food was treated with acetone only and used as control. Treated and control arms were air-dried for 15 min to evaporate the solvent completely. For each concentration, 30 starved adult insect of *R. dominica* for 24 h were released at the main brunch of olfactometer, individually. In fact, to avoid effects of pseudo-replication, each adult insect was individually placed in olfactometer. Each insect was allowed to choose one of the arms. After 15 min, pitch situation of each insect was recorded. Percentage repellency was calculated using the formula of [9] as follows:

$\%R = \frac{C-E}{T} \times 100$, where C and E are the number of insects in control tube and treatment tube, respectively. T is the number of total insects released in the main brunch of olfactometer. All data was analyzed as nonparametric data via χ^2 test using SPSS 16.0. Before that, all data has been weight cases.

Results

Chemical constituents of *Zhumeria majdae*

Results of the GC-MS analysis for *Z. majdae* essential oil are presented in table 1. A total of 14 chemical compounds constituting 99.36% of the chemical constituents were identified. The five major constituents were linalool (47.29%), camphor (34.33%), geraniol (3.95%), limonene (3.33%) and camphene (2.98%).

Table 1: Chemical constituents of the essential oil from *Zhumeria majdae*

Compounds	Retention Index	Composition (%)
α-pinene	939	0.99
Camphene	954	2.98
3-octanone	984	1.08
Limonene	1029	3.33
Terpinolene	1089	0.66
Linalool	1097	47.29
Camphor	1146	34.33
Borneol	1169	1.02
Terpinen-4-ol	1177	0.68
-terpineol***α***	1189	0.77
Z-citral	1318	0.98
Geraniol	1253	3.95
Geranial	1267	0.99
-caryophyllene***β***	1419	0.85

Fumigant toxicity

In all cases, considerable difference in mortality of insects to essential oil vapour was observed with different concentrations and times. From the table 3, it can be seen that, *Z. majdae* oil was toxic to *R. dominica*. The mortality of the pest was increased with increase in concentration and with exposure time from 3 to 24 h (Table 2). At the lowest concentration (62.9 µl/l air), mortality of *R. dominica* reached 26% with a 3 h exposure. Concentrations from 188.7 to 440.3 µl/l air and exposure time of 6 h were enough to obtain more than 50% kill of *R. dominica*. In all of the concentrations, the oil yielded more than 80% mortality after 9 h exposure. Total mortality of this pest was achieved after 12 h of exposure, in all concentrations.

Table 2: Percentage mortality of adults of *Rhyzopertha dominica* exposed to various concentrations and periods of exposure time to essential oil from *Zhumeria majdae* impregnated on filter paper disks.

		Mortality ± SE						
Con. (µl/l air)	Time (h)							
	3	6	9	12	15	18	21	24
62.9	26±2.1 ^{d}	43±1.3 ^{c}	84±4.2 ^{b}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}
188.7	27±1.3 ^{d}	52±1.3 ^{c}	87±2.1 ^{b}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}
314.5	35±1.6 ^{d}	61±2.5 ^{c}	89±2.7 ^{b}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}
440.3	43±1.6 ^{d}	67±1.3 ^{c}	91±2.7 ^{b}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}
*Means followed by the same letter in a row within each concentration are not significantly different using Tukey's test at $p<0.05$.								

Data probit analysis, on the basis of LC_{50} values, showed that larval stages were the most tolerant and adult was the most susceptible stage. Results showed that 1 day old eggs of *R. dominica* (LC_{50} = 53.72 µl/l air) were more tolerant than 4 days old eggs (LC_{50} = 36.21 µl/l air**)**. Also, 1 day old larvae of *R. dominica* (LC_{50} = 112.2 µl/l air) was more sensitive than 8 days old larvae (LC_{50} = 414.97 µl/l air) (no overlap in 95% confidence limits). In general, susceptibility of eggs was significantly decreased with increasing their age, while older larvae were more tolerant than young ones (Table 3).

Table 3: Fumigant toxicity of *Zhumeria majade* essential oil against adults and immature stages of *Rhyzopertha dominica*

stage	LC_{50} (µl/l air)	b±SE	df	χ^2	P-value
Adult	15.81 (13.9-18.0)	2.24±0.247	4	3.86	0.42
L_1 (1 day old)	112.2 (99.1-126.3)	2.49±0.25	4	0.36	0.99
L_8 (8 days old)	414.97 (395.5-434.1)	6.35±0.66	4	0.39	0.98
E_1 (1 day old)	53.72 (50.32-57.12)	4.62±0.48	4	3.36	0.50
E_4 (4 day old)	36.21 (32.6-40.4)	2.63±0.31	4	1.42	0.84
95% lower and upper fiducial limits are shown in parenthesis.					

Repellency

The essential oil of *Z. majdae* strongly repelled *R. dominica* adult. A χ^2 test indicated that the oil was repellent to *T. confusum* adults at concentrations of 50 µl and was highly significant at 160 µl when the repellency was 90.23% (Table 4). At the lowest concentration (50 µl) the repellency was (33.33%). Commonly, the repellency was increased with concentration in all case. In this experiment, the behavior of this pest was observed. At the onset, *R. dominica* were agitated, moved frequently and showed exceptional excitement. But Thus, *Z. majdae* oil has potential for use with at least some stored product insects as a repellent.

Table 4: Repellent effects of the essential oil from *Zhumeria majdae* to adults of *Rhyzopertha dominica* using Y-tube olfactometer model RZR.

Con. (µl)	Number of insect			χ^2	df	P-value	Repellency (%)
	Control	Treated	No response				
50	14	7	9	2.33	1	0.13	33.36
85	17	6	7	5.26	1	0.02	70.35
120	22	4	4	12.46	1	0.00	76.8
160	25	2	3	19.59	1	0.00	90.23

Discussion

Results of our study showed that the most components of the essential oil were linalool (47.29%) and camphor (34.33%). Linalool (a terpene alcohol chemical) and camphor (a terpenoid) are naturally occurring compounds, which can be found in various flowers and over 200 species plants. According to a study by Rustaiyan, *Z. majdae* essential oil mainly comprises of monoterpenes (about 97%). The ratio of linalool to camphor in this species was almost 1:1 in 1988, whereas in 1990, this ratio was reported to be approximately 2:1 [20]. In a study conducted by Ebadollahi *et al*. indicated that linalool (58.3%) and camphor (25.9%) are the main components of *Z. majdae* essential oil [21]. In a similar study by Majrouhi *et al*., 22 components were identified in the essential oil of *Z. majdae* including 11 monoterpene hydrocarbons (13.8%), eight oxygenated monoterpenes (83.7%), and two sesquiterpenes (0.6%), representing nearly 99% of the total composition [22]. These results concur with Soltanipour *et al*. in which *Z. majdae* had linalool (60.4%) and camphor (26.5%) as the major constituents [23]. The differences observed between the amounts of components can possibly due to season, time and stage of harvest. *Zhumeria majdae* oil showed strong fumigant activity and strong repellency against *R. dominica* and it can be an effective repellent substance against this pest. From a practical point of view, a possible use of this oil as insecticide should take into consideration that longer exposure periods are needed to reduce the tolerance of *R. dominica*

In addition, *Z. majdae* was characterized by a rapid knockdown effect, hyperactivity, convulsion and paralysis and death against insects. Rapid mortality is one of the main spots here, because insects die quickly and no progeny are produced. Jacobson pointed out that the most promising botanical insect control agents are in the families of Annonaceae, Asteraceae, Canellaceae, Lamiaceae, Meliaceae and Rutaceae [24]. Studies have not been reported previously concerning the activity of *Z. majdae* as fumigant on insect pests. However, the insecticidal activity of some essential oils from Lamiaceae and other plants has been evaluated against a number of stored product insects. For example, Rozman *et al*. obtained positive results by treating *Tribolium castaneum* (Herbst), *Sitophilus oryzae* and *Rhyzopertha dominica* with the essential oils of *Lavandula angustifolia, Rozmarinus officinalis, Thymus vulgaris* [25]. Papachristos and Stamopoulos showed that *Lavandula hybrid* Rev. had toxic activity against *Acanthoscelides obtectus* (Say) eggs [1]. In related studies, oils from three *Mentha* spp. and two lamiaceae oils, ZP51 and SEM76 had LC_{50} values of 0.5-2.8 µl/l air against major coleopteran pests of stored food commodities [26, 1]. Several reports indicated that monoterpenoids was lethal to the insects through inhibition of the activity of the acetyl choline esterase (AChE) enzyme [27]. Our results also compare favorably with phosphine LC_{50} values of 8-12 µg/l after 20 h treatment against some stored product pests [28].

The results revealed that the most tolerant stage was the larva and the most sensitive the adult. Eggs of *R. dominica* were more tolerant than the adults. Papachristos and Stamopoulos suggested two explanations of the egg's greater tolerance [1]. The first was that since monoterpenoids are known neurotoxins, their ovicidal activity will only become apparent when the embryo's nervous system begins to develop. Hence, the recorded very low vulnerability to vapours of the 1-day old eggs is due to the fact that the embryo's nervous system has not yet developed. The second explanation is that the permeability of the egg's external surface (chorion and/or vitelline membrane), which is lower at the start of embryogenesis, obstructs the diffusion of vapours into the young eggs. A third explanation put forward by Emekci *et al*. was that since respiration rates are much lower at the egg stage than at active stages such as adult, there is a lower rate of air exchange and consequently monoterpenoid diffusion into the egg. Concerning the larval stage, we observed that susceptibility to vapours varied with age. In fact, as the larva develops it becomes less susceptible [29].

The genus *Zhumeria* is a member of the large and evolutionary advanced plant family Lamiaceae. This plant grows only in Iran. Iran is located in arid and semi-arid areas and has many endemic aromatic plants from different families. It therefore seems very worthwhile to mount a comprehensive screening program to determine the insecticidal efficacy of such plants. *Zhuueria majdae* is used as medicine and is considered to be less harmful than most conventional insecticides. Apart from a natural origin, *Z. majdae*, like most of other plant essential oils, can pose fewer or lesser risks to human health and in the environment [30]. There is a need to conduct further studies on essential oils against other insects particularly in the presence of commodity load to establish its efficacy as a fumigant.

In conclusion, the results of this and of earlier studies indicate that essential oils including, *Z. majdae*, are a bio-source of biologically active vapors which may potentially prove to be efficient insecticides. For the practical application of the essential oils as insecticides, further studies which deal with the development of formulations are necessary to improve efficacy and durability as well as to reduce costs.

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