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Fumigant toxicity and repellent effect of three Iranian *Eucalyptus* species against the lesser grain beetle, *Rhyzopertha Dominica* (F.) (Col.: Bostrichidae)

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Abstract

In the present study, fumigant toxicity, repellent property and durability of essential oils of *Eucalyptus dundasii* Maiden, *Eucalyptus floribunda* F. Muell and *Eucalyptus kruseana* F. Muell were evaluated against the adults of *Rhyzopertha dominica* (F.). The essential oils were isolated with hydro-distillation method by Clevenger apparatus. Results of fumigant toxicity showed that as the concentration and exposure time increased, the mortality also increased. LC_{50} values for *E. dundasii*, *E. floribunda* and *E. kruseana* were achieved as 41.69, 34.39 and 27.98 µl/l air, respectively. For Repellent Index, it was found that all essential oils have repellent effect at concentrations of 70, 140 and 280 µl/l air and *E. kruseana* oil showed the highest repellency as compared to other essential oils. In the evaluation of durability of fumigant toxicity, it found that *E. kruseana* essential oil has more stable.

Keywords: Bio-effects, essential oil, *Eucalyptus dundasii*, *Eucalyptus floribunda*, *Eucalyptus kruseana*, *Rhyzopertha dominica*

1. Introduction

Although stored grains can be destroyed by insects, fungi, and vertebrate pests, insect pests are often the most important because of the favorable environmental conditions that promote their development. Among the most serious economic insect pests of grains, internal feeders such as lesser grain borer, *Rhyzopertha dominica* F., are primary insect pests. *R. dominica* is a destructive insect pest of stored grains. Both larvae and adults of the pest attack whole, sound grains and cause extensive damage [10]. Due to the high potential and wide host range of products such as wheat, barley, rice and oats it was considered as major stored product insect pest [22].

Although effective synthetic insecticides such as methyl bromide or phosphine for control of stored product pests are available, there is a global concern about their negative effects causing ozone depletion, environmental pollution, and toxicity to non-target organisms, and pesticide residues [5, 7, 18].

Recently, essential oils have received a great deal of attention as pest control substances [19]. They are a complex mixture of volatile organic compounds produced as secondary metabolites whose functions are other than the nutrition. Plant essential oils are considered insect-control agents because they act as bioactive chemicals, are selective in action and have little or no harmful effects on environment and non-target organisms [24].

The genus *Eucalyptus* from *Myrtaceae* family comprises about 800 species [20] and its leaves have been reported to contain aromatic oils with a characteristic odour whose recovery by steam distillation produces essential oils [8, 20]. The *Eucalyptus* essential oil has been used commercially in food, flavoring, and perfumery, and in the pharmaceutical industries and its pesticidal effects has been evaluated by many workers [4]. *Eucalyptus* contains a rich source of bioactive constituents, possessing fungicidal, insecticidal and herbicidal activities [27].

As part of our ongoing research on natural insecticides from the flora of Iran, three *Eucalyptus* species including *E. dundasii* Maiden, *E. floribunda* F. Muell and *E. kruseana* F. Muell were tested against *R. dominica* adults. This approach will allow us to identify natural and safer agents for the development of bio-rational insecticides to manage of stored product insect pests.

2. Material and methods

2.1. Insect rearing

The insect species used in this study i.e. lesser grain beetle borer, *R. dominica* was procured from insect breeding room located in Entomology Section of Evin Research Center, Tehran, Iran. The insects were not affected by any material primarily. 1 kg wheat in cube containers was used for insect rearing. For ventilation, the containers were covered by lace cloth. In order to remove any probable contamination to other insects, the applied wheat was stored in refrigerator for 72h at -10 °C. The incubators with 27 ± 1 °C and relative humidity of 65 ± 5% were used for insect rearing and experiments. Experiments were done within spring and summer of 2012 and in all experiments adult insects aged as 1 to 7 old days were used.

2.2. Plant material and essential oil extraction

The leaves of the three *Eucalyptus* species including *E. dundasii*, *E. floribunda* and *E. kruseana* were collected from Koshkak Stattion located in 1km distance of Koshkak village, Shoshtar town, Khozestan province, Iran within summer of 2012 and shade dried. In order to prepare extract of essential oil, first dried leaves were crushed and powdered using electric mills, 70 g of the crushed specimen accompanied by 1000 ml distilled water and essential oil was extracted using glass Clevenger apparatus at 80 °C categorized by distillation method. The time of essential oil extraction was 4 h for each of the samples. Collected oil was stored in refrigerator at 4 °C in glass container coated with aluminum coverage.

2.3. Fumigant toxicity

In order to determine the fumigant toxicity of *E. kruseana* essential oil on adult insects, firstly effective concentrations of essential oil were obtained for the mortality of 20 to 80% of treated insects during the initial tests. The tests were run in glass cylinder containers having shield with volume of 40 ml as fumigant chambers. Twenty adult insects were posited in the glass. Concentrations of 30, 37.5, 45, 57.5 and 75 µl/l air of *E. dundasii* essential oil, 25, 27.5, 35, 40 and 50 from µl/l air of *E. floribunda* essential oil and 20, 22.5, 27.5, 35 and 45 µl/l air of *E. kruseana* essential oil were chosen. Then, they were put on filter paper using sampler. Immediately, the glasses were recapped, the sides of the cap were covered with strips so as to prevent the outlet of essential oil. The number of died insects were counted after 24 h. In these experiments, those insects incapable of moving their heads, antennae and body were considered as dead. This test was repeated four times with control groups. All procedure was done for controls without essential oil concentrations.

For studying of lethal time values, three concentrations of 100, 200 and 500 µl/l air from each essential oil higher than LC_{50} was chosen to be used for analyzing the rate of death. 20 adults' insects were put into cylinder glasses volume as 40 ml. Mentioned concentrations were used on the filter paper which attached inside of glasses caps. In order to prevent the outlet of essential oil s, caps of the glasses were covered with strips. This experiment was treated four times with control groups. This means that separate tests were used for each time interval. After the passage of time, the containers were opened and the number of dead insects was counted. Those insects incapable of moving their heads, legs, antennae and body were considered as dead ones.

In order to determine the durability of essential oil in fumigant toxicity, four concentrations were used so as to cause higher rates of mortality. The obtained results were only acceptable at the highest concentration from statistic aspect. Cylinder-like capped glasses with 40 ml volume were used. Twenty µl/l air concentration of the essential oil was treated on the filter paper inside the glasses caps. The glasses were capped and in order to ascertain the impenetrability of air, they were tightened by strips. This experiment was repeated three times. Twenty adult insects were placed in the test glasses after one day of placing essential oil within the glasses, and the mortality was counted after 24 h. So, insects aged as three days were added to the containers and mortality was recounted after 24 h and followed for 5, 7, and etc. days. The glasses were capped before the transmission of insects and when insects are completely placed in the containers, they were recapped again to be kept until the last moment of treatment. In order to make sure of impenetrability of air into the containers, they were tightened with strips. The durability of essential oil was evaluated for 7, 13 and 15 days for *E. dundasii*, *E. floribunda* and *E. kruseana*, respectively.

2.4. Repellent activity

In order to study the essential oil repellent index, Lopez *et al.* [16] method was applied. In doing so, two holes were perforated in two sides of the plastic container and each hole would be connected to the other using 5cm pipe. Hence, three plastic containers would be connected and those containers to be put in the sides of the middle one will be considered as control and treated containers. 40 wheat seeds were placed into the control container on which 1ml of acetone has been spilled. In the treatment containers, concentration of 2.8, 5.6 and 11.2 µl from the essential oil was placed whose ratio was 4:1 to be diluted with acetone. 50 adult insects aged as 1 to 7 days to be kept hungry for 24h were placed in the middle container. The containers were capped during the experiment and the number of insects were counted in each container after 24 h followed by the estimation of repellent index. This test was repeated three times.

Essential oil repellent Index (RI) was calculated as follow: RI= 2G/ (G+P). That G= the number of adult insects in treatment area and P= the number of adult insects in control area. For each calculated RI, the mean and standard deviation were determined. If the mean is lower than 1-SD, essential oil concentration has repellent property. If the man is higher than 1+SD, essential oil concentration has attractant property. If the man falls between 1-SD and 1+SD essential oil concentration is neutral.

2.5. Statistical analysis

Lethal concentration (LC_{50}) and lethal time (LT_{50}) values were estimated by probit analysis using SPSS software. In order to categorize the repellent effect of essential oils, Tapondjou *et al.* [26] method was used. Five groups were considered based on the mean of repellent percent: Class 0: PR= 0–0.1%, Class I: PR= 0.1–20%, Class II: PR= 20.1–40%, Class III: PR= 40.1–60%, Class IV: PR= 60.1–80% and Class V: PR= 80.1–100%.

3. Results

The adults of *R. dominica* was very susceptible to the essential oils of *E. dundasii*, *E. floribunda* and *E. kruseana* in the evaluation of fumigant toxicity and the LC_{50} values were calculated as 41.69, 34.39 and 27.98 µl/l air, respectively. On the other hand, *E. kruseana* essential oil was more effective than essential oils of *E. floribunda* and *E. dundasii* (Table 1).

Table 1: LC_{50} values of fumigant toxicity of three essential oils against adults of *R. Dominica*.

Essential oil	**LC_{50} (µl/l air) (95% Confidence Limits)**	**LC_{90} (µl/l air) (95% Confidence Limits)**	**Slope ± SE**	**χ^2 (df= 1)**
E. kruseana	27.98 (23.29-31.69)	65.32 (55.04-87.83)	7.99 ± 1.27	39.28
E. floribunda	34.39 (30.07-38.01)	77.07 (64.90-104.12)	8.40 ± 1.29	41.89
E. dundasii	41.69 (32.84-49.86)	142.61 (108.01-232.55)	5.51 ± 0.88	38.76

LT_{50} values of *E. dundasii*, *E. floribunda* and *E. kruseana* essential oils on *R. Dominica* adults are shown in table 2. The LT_{50} of 100, 200 and 500 µl/l air from *E. kruseana* essential oil on *R. Dominica* adults was calculated as 4.16, 3.63 and 2.87 h, respectively while these were 4.28, 3.92 and 3.13 h for *E. floribunda* and 5.54, 3.76 and 2.05 h for *E. dundasii*. It was obvious that the essential oil of *E. kruseana* can effective on *R. Dominica* in minimum time (Table 2).

Table 2: LT_{50} values of fumigant toxicity of three *Eucalyptus* essential oils against adults of *R. Dominica*.

Essential oil	**Concentration (µl/l air)**	**LT_{50} (h) (95% Confidence Limits)**	**χ^2 (df= 1)**	**Slope ± SE**
E. kruseana	100	4.16 (3.70-4.50)A	50.12	10.81 ± 1.59
	200	3.63 (3.10-3.99)A	35.96	11.82 ± 1.97
	500	2.87 (2.19-3.23)B	19.39	13.21 ± 3.00
E. floribunda	100	4.28 (3.91-4.59)A	96.04	9.12 ± 0.93
	200	3.92 (3.54-4.24)A	79.21	10.32 ± 1.15
	500	3.13 (2.46-3.55)B	28.27	11.17 ± 2.10
E. dundasii	100	5.54 (5.07-5.92)A	94.33	10.01 ± 1.03
	200	3.76 (3.31-4.18)B	84.22	9.05 ± 0.98
	500	2.05 (1.58-2.40)C	44.44	8.64 ± 1.29

* Comparison was done according to Preisler method [23].

The obtained results reveal that the effect of essential oils has declined by the passage of time. The calculated LT_{50} so as to study the durability of *E. dundasii*, *E. floribunda* and *E. kruseana* essential oil on *R. dominica* adults was 3.71, 5.44 and 6.47 days respectively and *E. kruseana* essential oil was durable than others but there was no significant difference between this essential oil with *E. floribunda* (Table 3).

Table 3: LT_{50} values for stability of three *Eucalyptus* species against adults of *R. Dominica* at concentration of 500 µl/l air**.**

Essential oil	**LT_{50} (day) (95% Confidence Limits)**	**χ^2(df= 1)**	**Slope ± SE**
E. kruseana	6.47 (5.73-7.27)A	133.90	-5.27 ± 0.45
E. floribunda	5.44 (4.57-6.39)A	102.74	-3.78 ± 0.37
E. dundasii	3.71 (3.28-4.14)B	71.74	-7.56 ± 0.89

* Comparison was done according to Preisler method [23].

Based on the obtained results of repellency experiment, the essential oils of *E. dundasii*, *E. floribunda* and *E. kruseana* were repellent at 70, 140 and 280 µl/l air concentrations and for all essential oil the mean of repellent indexes decreased by the increasing of essential oil concentrations (Table 4). According to the comparative analysis of each concentration of *E. kruseana* oil using categorizing method of repellency effect, it can be concluded that the essential oil has significantly different at 70 µl/l air concentration compared to 140 and 280 µl/l air. Also, the two concentrations of 140 and 280 µl/l air are more repellent and act better while all concentrations of essential oils of *E. floribunda* and *E. dundasii* have obtained same categories (Table 4).

Table 4: Repellent index of different concentration of three *Eucalyptus* species against adults of *R. Dominica***.**

Essential oil	**Concentration (µl/l air)**	**Mean of repellent indexes**	**Standard Deviation (SD)**	**SD-1**	**SD+1**	**Effect**	**Mean repellency (%)± SD**
E. kruseana	70	0.23	0.06	0.93	1.06	Repellent	74.79 ± 6.72 **IV**
	140	0.17	0.07	0.92	1.07	Repellent	82.75 ± 7.18 **V**
	280	0.07	0.02	0.97	1.02	Repellent	92.91 ± 20.06 **V**
E. floribunda	70	0.39	0.12	0.87	1.12	Repellent	60.57 ± 12.52 **IV**
	140	0.25	0.04	0.95	1.04	Repellent	74.61 ± 4.98 **IV**
	280	0.21	0.08	0.91	1.08	Repellent	78.75 ± 8.99 **IV**
E. dundasii	70	0.39	0.12	0.87	1.12	Repellent	60.24 ± 12.57 **IV**
	140	0.33	0.03	0.96	1.03	Repellent	67.00 ± 3.82 **IV**
	280	0.25	0.03	0.96	1.03	Repellent	74.56 ± 3.10 **IV**

4. Discussion

Recently, many studies have been done for evaluation of susceptibility of *R. Dominica* to plant essential oils for testing both fumigant toxicity and repellency effect. Ebadollahi *et al.* [11] reported strong insecticidal activity of *Lavandula stoechas* L. essential oil against *Tribolium castaneum* Herbst, *Lasioderma serricorne* F. and *R. Dominica*. The results indicated that all species at different concentrations and exposure times were affected and the increase of concentration led to the increase of mortality rates. *Laurus nobilis* essential oils from Tunisia, Algeria and Morocco were assessed for their repellent and toxic activities against two major stored product pests: *R. Dominica* and *T. castaneum*. Results showed that *L. nobilis* essential oils were repellant and toxic to adults of *R. Dominica* and *T. castaneum*. Repellent and fumigant toxicities were highly dependent upon insect species and oil origin [6]. In another study, the toxicity of *Salvia leriifolia* (Benth) essential oil against granary weevil, *Sitophilus granarius* (L.), and *R.*

Dominica was evaluated by fumigation at 24, 48, and 72 h exposure times. *R. Dominica* was more susceptible than *S. granarius* for all exposure times. LC_{50} values at 24 h were estimated at 79.17 µl/l for *S. granarius*, and 25.87 µl/l for *R. Dominica*. Furthermore, with increasing the exposure time and essential oil concentration, LC_{50} values decreased [14]. The pesticide potentiality of the essential oils from the *Artemisia absinthium* L. against *R. Dominica* and *Spodoptera littoralis*, one of the most dangerous pests of protected crops, was investigated by Dhen *et al.* [9]. The essential oil of *A. absinthium* exhibited strong fumigant toxicity against *R. Dominica* adults with a LC_{50} value of 18.23 µl/l air. Furthermore, this essential oil showed high fumigant activity against *S. littoralis* with a LC_{50} value of 10.59 µl/l air. The results of mentioned studies have supported present study for susceptibility of *R. Dominica* to plant essential oils.

In the present study, fumigant toxicity and its durability and repellent effects of essential oils from three *Eucalyptus* species were demonstrated against *R. Dominica*. There are many reports that provide sound evidences for insecticidal activities of *Eucalyptus* species that parallel with present results. For example, the essential oils of *E. camaldulensis, E. intertexta* and *E. sargentii* were tested against three major stored-product beetles, *Callosobruchus maculatus* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum*. The mortality of the insects increased with concentration from 37 to 926 µl/l air and with exposure time from 3 to 24 h. The LC_{50} values of *Eucalyptus* essential oils tested in this study ranged from 2.55 to 33.50 µl/l air [17]. In the evaluation of fumigant toxicity of *Artemisisa herba*-alba Asso essential oil on *T. castaneum*, *C. maculatus* and *R. dominica* after 24 h, it was found that *R. dominica* had the highest sensitivity to essential oil with LC_{50}= 76.48 µl/l air and *T. castaneum* with LC_{50}= 564/40 µl/l air was known to have stronger resistance to essential oil. Also, the increase of concentration and passage of time led to the increased rate of mortality [25]. *Eucalyptus* essential oils can have repellent effect on pests. *E. citriodora* essential oil had strong repellent activity on *T. castaneum* insects [21]. Haririmoghadam *et al.* [13] conducted a research in which they applied four concentrations of *E. salmonophloia* and *E. kingsmillii* essential oils on the female adults of *Tetranychus urticae* Koch which repellent index was neutral at 9% and 17% concentrations and was repellent at 23% and 29% concentrations.

The chemical composition of the essential oils of the *Eucalyptus* species has been evaluated in many studies; 1,8-cineole (63.3%) and α-pinene (15.9%) in the study of Abravesh *et al.* [1] and bicyclogermacrene (28.8%), α-pinene (17.7%) and 1,8-cineole (12.1%) in the study of Assareh *et al.* [3] were reported as main components of *E. kruseana* essential oil from Iran. In the study of Elaissi *et al.* [12], the chemical composition of essential oils of fifteen *Eucalyptus* species harvested from the Jbel Abderrahman and Korbous arboreta (North East Tunisia) were analyzed by GC/FID and GC/MS. The main one was 1,8-cineole, followed by spathulenol, *trans*-pinocarveol, *α*-pinene, *p*-cymene, globulol, cryptone, *β*-phellandrene, viridiflorol, borneol and limonene. 1, 8-cineole, major of the components of the *Eucalyptus* essential oil, was evaluated for repellency and toxicity against three stored product coleopterans *Callosobruchus maculatus* F., *R. dominica*, and *Sitophilus oryzae* L. It was found to be moderately repellent to all three species, with a mean repellency in the range of 65–74 % at the highest dose tested (4.0 ml/ml) within 1 h. A toxicity assay revealed that direct topical application was more effective than using impregnated filter paper. The compound was more effective as a fumigant and gave 93–100 % mortality against the entire three pest species at the dose of 1.0 ml/l air under empty jar conditions as compared to treatment of jars filled with grain (11–26 % mortality). The lethal dose and lethal concentration required to kill 50 % of the beetles (LD_{50} and LC_{50}, respectively) varied with the toxicity assay method. LD_{50} values of 0.03, 0.04, and 0.04 ml/insect against *C. maculatus*, *R. dominica* and *S. oryzae*, respectively, were found in the topical application assay, while the LC_{50} values in the fumigant assay were 0.28, 0.33, and 0.46 ml/l against *C. maculatus*, *R. dominica*, and *S. oryzae*, respectively [2]. In the study of Lee *et al.* [15], Six out of 42 essential oils extracted from species of the family Myrtaceae found in Australia were shown to have potent fumigant toxicity against three major stored-grain insects: *S. oryzae*, *T. castaneum* and *R. Dominica*. They indicated; fumigant effects of the essential oils rich in 1,8-cineole were considered to warrant further research into their potential for commercial use. It can be concluded that the insecticidal activity of tested *Eucalyptus* essential oils could be attributed either to the major compound of the oils such as 1, 8-cineole, or to the synergic and/or antagonistic effects of all the components of the oils.

Results of the present and earlier studies demonstrated that the essential oil from *Eucalyptus* species have had toxic properties on the insect pests. The essential oils investigated in the present study are used as pharmaceuticals and in flavoring and are therefore considered less harmful to humans than most conventional insecticides and they can use as safe fumigants for controlling *R. Dominica*. Also, we need some additional studies for formulating and improving methods of application.

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