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Eucalyptus kruseana Muel essential oil: chemical composition and insecticidal effects against the lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae)

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Abstract. This research studied the fumigant toxicity, repellent property, durability and anti-nutritional efficiency of essential oil of *E. kruseana* on the adults of lesser grain borer, *Rhyzopertha dominica* F. The essential oil was extracted using Clevenger apparatus and its chemical composition analyzed by GC-MS. 1,8-cineole (69.0%), α-thujene (10.0%), E-caryophyllene (5.87%) and Trans-pinocarveol (4.4%) were identified as main components. The results indicated that LC_{50} of *E. kruseana* essential oil was 27.98 µl/l air and increasing the concentration of essential oil caused the increases in the mortality. LT_{50} values were calculated as 4.16, 3.63 and 2.87 h at 100, 200 and 500 µl/l air, respectively. The calculated LT_{50} so as to study the durability of *E. kruseana* essential oil on *R. dominica* adults was 6.47 days. It was clear from the repellency index that *E. kruseana* essential oil has strong repellency at 70, 140 and 280 µl/l air concentration. The results showed that *E. kruseana* essential oil has significantly affected the RGR (Relative Growth Rate), RCR (Relative Consumption Rate) and FDI (Feeding Deterrence Index) of *R. dominica* adults and it was concentration-dependent. The Efficiency of Conversion of Ingested food (ECI) has decreased while the concentrations are not statistically different at 5% level compared to the control group. The above findings suggest that the essential oil of *Eucalyptus kruseana* can play an important role in pest control and reduce the need for synthetic insecticides and also the risks associated with their use.

Key words: essential oil, *Eucalyptus kruseana*, fumigant toxicity, repellency, durability, anti-nutritional, *Rhyzopertha dominica*.

Introduction

Insects are considered as the basis of problems in agricultural products storage since they affect the quality and quantity of the products. The damages and the destroying of the products after cultivating derived by the damage of insects and other living factors in the world are estimated as 10% to 40% annually (Mohan & Fields 2002). *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) is the main pest of wheat and other storage products in dry condition which are found in many storage in the World. The adult ones migrate through the ducts, cracks and chasms to bigger storages, entering the store pit including huge containers of grains and descend huge masses of wheat followed by their penetration into them (Nansen et al. 2004, Jia et al. 2008).

It should be considered in controlling the pests that those methods to be used which are safe for human and tractable animals and which do not cause damages to the environment. Currently, extreme use of phosphine and other artificial pesticides in controlling storage pests bring about serious problems such as resistance of insects, environment pollution, residual in food products and lethal effects in non-targeted organisms (Bughio & Wilkins 2004, Jovanovic et al. 2007, Aboua et al. 2010).

In this regard, natural products are generally preferred because of their innate biodegradability and less harmful compounds affecting non-target organisms (Regnault-Roger et al. 2012). Plant essential oils are usually extracted from various parts of the plant. The main characteristics of the essential oils are that they are easily extractable, eco-friendly, biodegradable, possess low or no toxicity against mammals, and are very effective against a wide spectrum of insect pests (Lucia et al. 2012). In some countries, essential oils are traditionally used through fumigant or contact action to protect grains against storage pests, a suitable method to preserve products stored in warehouses and on small farms (Isman et al. 2011).

Eucalyptus has been prized as a rich source of essential oil. The oil has been used commercially in food, flavoring, and perfumery, and in the pharmaceutical industries (Singh et al. 2009). In addition, the essential oils of the *Eucalyptus* species possess important biological activities including analgesic, antibacterial, anti-inflammatory, anti-malarial and antioxidant properties (Cimanga et al. 2002, Elaissi et al. 2012). Although previous reports focused on the potential effectiveness of selected essential oils and their components as insecticides (Isman 2006, Tripathi et al. 2009, Regnault-Roger et al. 2012), there are no study on the bio-efficiency of *Eucalyptus kruseana* Muel essential oil against insect pests. In this study, the fumigant toxicity, repellency, durability and anti-nutritional effects of *Eucalyptus kruseana* essential oil as natural bio-pesticides on adults of *R. dominica* along with the chemical composition of this oil were assessed in laboratory condition.

Materials and methods

Insects rearing

Insects used in this study was lesser grain beetle borer, *Rhyzopertha dominica,* which were prepared from insect breeding room located in Entomology Section of Evin Research Center, Tehran, Iran. The insects were not affected by any material primarily. In order to breed the insects, 1 kg wheat in cube containers made from fiberglass were used. In order to run the 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. In all the experiments, adult insects aged as 1 to 7 old days were used.

Plant material and essential oil extraction

The leaves of the *Eucalyptus kruseana* were collected from Koshkak Station (32° 2' 45.60'' N, 48° 51' 25.20'' E, 150 m a. s. l.) which was located in 1km distance of Koshkak village, Shoshtar town, Khozestan province, Iran within summer of 2012. Then, they were dried under shadow and appropriate ventilation conditions. in order to 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 continues coated with aluminum coverage.

Chemical analysis of essential oil

Gas Chromatograph (GC): Gas Chromatograph is Thermo-UFM model equipped with F.I.D. detector and Eurochrom 2000 data processor, Ph-f cylinder (lengthen as 10m, inner diameter of 0.1mm and constant phase layer thickness of 0.4 micron) which is non-polar. Cylinder thermal programming ranged from 60 to 285 °C speeding by 3 °C per minute is made in 5/8 minutes. The transporting gas is helium whose pressure is set as following: 3 kg/cm^3, fusion ratio of 100:1 to dilute the sample, injection section temperature of 280 °C and detection temperature of 280 °C.

Gas Chromatography-Mass Spectrometry (GC/MS): The gas chromatograph apparatus is Varian 3400 model connected to mass spectrometer with Saurn 2 software whose cylinder is the same as that of GC. Overhead cylinder pressure is 35 Psi and ionization energy equals to 70 ev. Thermal programming of cylinder is ranged from 40 to 25 °C and is set as follow: thermal speeding raises up by 4 °C per minute, thermal degree of injection shield is 260 °C and transfer line temperature is 270 °C.

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 20, 22.5, 27.5, 35 and 45 µl/l air from *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 treated 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 *E. kruseana* 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 oils, 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 deaths. 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/lair 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 tightening by strips. This experiment was repeated three other times. Twenty numbers of adult insects were placed in the test glasses after one day of placing essential oil within the glasses, and the numbers of dead insects were counted after 24 h. So, insects aged as three days were added to the containers and the number of dead insects were recounted after 24h. This trend followed for 5, 7, and etc. days of insects living. 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 tightening with strips. The durability of essential oil was evaluated for 15 days and the number of deaths was recorded.

Essential oil repellency

In order to study the essential oil repellent index, Lopez et al. (2008) 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 are 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 are placed whose ratio is 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.

In order to categorize the repellent effect of essential oil s, Tapondjou et al. (2005) method is used. Five groups are 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%.

Nutritional indexes

Five g of wheat were put into the 40 ml test glasses and the seeds were treated with 0.5, 1, 1.5, 2 and 3 µl of eucalyptus which were diluted in 1 ml of acetone. In the control treatment, 1 ml acetone was used only. Twenty weighed adult insects were released in the containers and were kept there to be fed for three days. Following three days of treatment, both the insects and wheat were weighed and nutritional indexes were estimated as follow (Huang et al. 2000);

Relative Growth Rate = RGR = (A-B)/(B×Day) that A was alive insect weights in mg for each one, B was initial insect weight in mg for each one and Day was treatment duration (three days). Relative Consumption Rate (RCR) = RCR = D/(B×Day) that D was the amount of ingested food in mg for each one. Efficiency of Conversion of Ingested food (ECI) = % ECI = RGR/RCR × 100 and Feeding Deterrence Index (FDI) = % FDI = (C-T)/C × 100 that C was the amount of ingested food in the control group (mg for each one) and T was the amount of ingested food in the treatment group (mg for each one).

Results

Results of analysis of the *E. kruseana* essential oil summarized and presented in Table 1. Results showed that nineteen compounds were identified in the essential oil. The major components were found to be 1,8-cineole (69.0%), α-thujene (10.0%), E-caryophyllene (5.87%), Trans-pinocarveol (4.4%), α-guaiene (1.97%) and longipinanol (1.0%).

The adults of *R. dominica* was very susceptible to the *E. kruseana* essential oil in the evaluation of fumigant toxicity. Comparison of the means of mortalities using Tukey's test showed that the essential oil had significantly different effects at different concentrations and that higher rates of mortality was observed in higher levels of concentration (Fig. 1). The results showed that the means of mortalities with concentrations of 20 and 22.5 µl/l air performed the same while the means in 27.5, 35 and 45 µl/l air concentrations were different. Concentration of 45 µl/l air has caused the highest mortality against *R. dominica* adults (Fig. 1). The LC_{50} of *E. kruseana* essential oil on the adults of *R. dominica* was 27.98 µl/l air. Since the slope of regression line is positive, the increase of concentration logarithm leads to the increase of the number of mortality probit (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. Comparison of the three concentrations and 95% upper and lower confidence limits using Preisler method showed that 100 and 200 µl/l air concentrations acted the same; however, 500 µl/l air concentration was significantly different from the two others. Comparison of the confidence interval of three concentrations using Preisler method indicated that LT_{50} of 100 and 200 µl/l air concentrations was not significantly different while LT_{50} at 500 µl/l air was significantly different with the two other concentrations (Table 3).

The obtained results reveal that the effect of essential oil has declined by the passage of time. The calculated LT_{50} so as to study the durability of *E. kruseana* essential oil on *R. dominica* adults was 6.47 days. LT_{50} of *E. kruseana* essential oil at 500 µl/l air concentration was calculated as 6.47 days and in day 15, the percent of mortality in treatment and control groups have been the same (Table 4).

Table 1. The results obtained for chemical analysis of essential oil isolated from the leave of *E. kruseana*.

Components	Retention time	Percentage (%)
α-thujene	928	10
α-pinene	935	0.37
β-pinene	976	0.23
Myrcene	987	0.13
α-phellandrene	1002	0.18
α-terpinene	1014	0.84
Limonene	1028	0.2
1,8-cineole	1030	69
Terpinolene	1086	0.2
Trans-pinocarveol	1136	4.4
Pinocarvone	1160	0.44
Terpinen-4-ol	1174	0.52
E-caryophyllene	1420	5.87
α-guaiene	1435	1.97
Trans cadina-1(6),4-2	1474	0.34
Trans muurola-4(14),5-	1491	0.13
bicyclogermacrene	1496	0.13
Zonarene	1527	0.30
Longipinanol	1566	1
Total		96.25

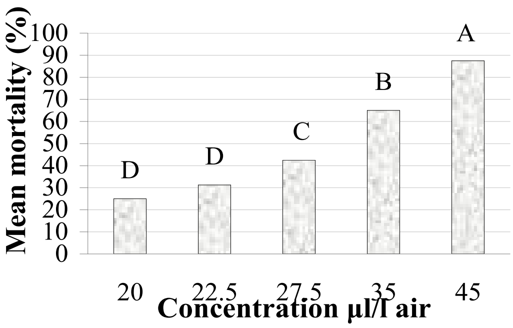

Figure 1. The means mortality of *R. dominica* adults at different concentration of *E. kruseana* essential oil. Different letters over columns indicate significant differences according to Tukey's test at p = 0.05. Columns with the same letter are not significantly different.

Table 2. The Probit analysis of *R. dominica* adults affected by the fumigation of *E. kruseana* essential oil.

LC_{50} (µl/l air) [a]	LC_{90} (µl/l air) [a]	Slope ± SE	Intercept	χ^2 (df= 1)
27.98 (23.29-31.69)	65.32 (55.04-87.83)	7.99 ± 1.27	-11.57	3928.76

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

Table 3. LT_{50} values of fumigant toxicity of *E. kruseana* essential oil against adults of *R. dominica*.

Concentration (µl/l air)	LT_{50} (days) [a]	χ^2 (df= 1)	Slope ± SE	Intercept	Comparison using Preisler method
100	4.16 (3.70-4.50)	50.12	10.81 ± 1.52	-6.69	A
200	3.63 (3.10-3.99)	35.96	11.82 ± 1.97	-6.62	A
500	2.87 (2.19-3.23)	19.39	13.21 ± 3.00	-6.06	B

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

Table 4. Calculated LT_{50} values for the durability of fumigant toxicity in *E. kruseana* essential oil at concentration of 500 µl/l air on *R. dominica*.

Concentration (µl/l air)	LT_{50} (days) [a]	χ^2 (df= 1)	Slope ± SE	Intercept
500	6.47 (5.73-7.27)	133.90	-5.27 ± 0.45	4.27

One would say based on the obtained results of repellency experiment, the essential oil of *E. kruseana* was repellent at 70, 140 and 280 µl/l air concentration and the repellency index increases by the increase of concentration percent (Table 5). Based on the means of repellency percent, *E. kruseana* essential oil has the same effects at 140 and 280 µl/l air concentrations and falls into the same group. However, it has lower repellency effect at 70 µl/l air which falls into different group. According to the comparison 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 (Table 5).

Relative Growth Rate (RGR), Relative Consumption Rate (RCR), Efficiency of Conversion of Ingested food (ECI) and Feeding Deterrence Index (FDI) were showed in Table 6. The results of variance analysis showed that *E. kruseana* essential

Table 5. The results of repellency effect of *E. kruseana* essential oil against *R. dominica* adults.

Concentration (µl/l air)	Mean of repellent Indexes ± Standard Deviation (SD)	SD-1	SD+1	Effect	Mean repellent (%) ± SD
70	0.23 ± 0.06	0.93	1.06	repellent	76.79 ± 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 ± 2.06 V

Mean with same letter for each column aren't significant deference.

Table 6. The anti-nutritional effects of essential oil from *E. kruseana* on adults of *R. dominica*.

Concentration (µl/l air)	RGR (F= 9.73)	RCR (F= 9.59)	ECI (F= 0.79)	FDI (F= 4.10)
0	0.053 ^{a} ± 0.01	0.56 ^{a} ± 0.07	21.74 ^{a} ± 7.98	-
12.5	0.027 ^{ab} ± 0.020	0.32 ^{ab} ± 0.14	9.71 ^{b} ± 9.40	43.28 ^{a} ± 21.10
25	0.009 ^{b} ± 0.001	0.27 ^{bc} ± 0.09	9.55 ^{b} ± 3.03	47.05 ^{a} ± 18.72
37.5	0.009 ^{b} ± 0.001	0.25 ^{bc} ± 0.14	6.41 ^{c} ± 6.60	55.39 ^{ab} ± 21.28
50	0.007 ^{b} ± 0.001	0.18 ^{bc} ± 0.13	4.01 ^{cd} ± 1.59	65.71 ^{ab} ± 23.43
75	0.001 ^{b} ± 0.001	0.04 ^{c} ± 0.01	0.74 ^{d} ± 1.07	91.47 ^{b} ± 4.18

Non-similar letters indicate significant difference at 5% level of probability in Tukey's test. RGR = Relative Growth Rate, RCR = Relative Consumption Rate, ECI = Efficiency of Conversion of Ingested food and FDI = Feeding Deterrence Index.

oil has affected the RGR, RCR and FDI of *R. dominica* adults and it was concentration-dependent. The concentration difference with control group was considerable (Table 6). The results of variance analysis showed that the effects of *E. kruseana* essential oil are not significantly different at different concentrations on the index of ECI. In general, one would contribute the main element of caused effects in RGR and RCR to the effects of food deterrent index (Table 6).

Discussion

Recently, many study have been done for evaluation of susceptibility of stored product insect pests specially *Rhyzopertha dominica* to plant essential oils. For example, the insecticides effects of *Lavandula stoechas* L. essential oil against three species of insect pests namely, *Tribolium castaneum* Herbst, *Lasioderma serricorne* (F.) and *R. dominica* were studied by Ebadollahi et al. (2010). The results indicated that all species at different concentrations and in 24, 48 and 72h exposure times were suspected and the increase of concentration led to the increase of mortality rates. In the study of Habashi et al. (2011), the essential oil of *Carum copticum* L. against *R. dominica* was evaluated. LC_{50} values were equal to 19.01 and 15.12 µl/l air in 24 and 48 h exposure times, respectively. In the other 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 of the exposure time and essential oil concentration, LC_{50} values decreased (Hosseini et al. 2013). The pesticide potentiality of the essential oils from the absinthe wormwood, *Artemisia absinthium* L. against *R. dominica* and *Spodoptera littoralis*, one of the most dangerous pests of protected crops, was investigated by Dhen et al. (2014). 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 and LC_{90} value of 41.74 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 and a LC_{90} value of 17.12 µl/l air, too. The results of mentioned studies have supported present study for susceptibility of *R. dominica* to plant essential oils.

The essential oils eucalyptus species comprise of metabolic compositions such as terpenoeids and phenol compositions and include toxic effects against stored product pests (Lee et al. 2004, Tapondjou et al. 2005). In the present study, fumigant toxicity and its durability, repellent and anti-nutritional bio-effects of essential oils from *E. kruseana* were assessed against *R. dominica*. There are many researches that provide sound evidences for insecticidal activities of Eucalyptus species that parallel with present results; the repellency of *Eucalyptus saligna* essential oil against *Sitophilus zeamais* Mostchulsky and *Tribolium confusum* du Val was showed. The results showed that the main components such as cymol, 1,8-cineole, terpineol and α-pinene cause toxic effects and repellency (Tapondjou et al. 2005). The essential oils from *Eucalyptus camaldulensis, Eucalyptus intertexta* and *Eucalyptus sargentii* were tested against three major stored-product beetles, *Callosobruchus maculatus* (F.), *Sitophilus oryzae* (L.) and *T. castaneum*. The mortality of 1 to 7 day old adults 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 were ranged from 2.55 to 33.50 µl/l air (Negahban & Moharramipour 2007). Anti-nutritional effects of *Eucalyptus globulus* and *Lavandula steochas* essential oils against *Tribolium castaneum* adults were illustrated in the study of Ebadollahi (2011). Concentrations of LC_{15} to $LC_{35,}$ those concentrations causing 15% to 35% of mortality, were used for each essence and the results were analyzed after 48 h. Both essential oils were significantly led to the decline of nutrition in the insect and anti-nutritional activity of essential oils increased by the increasing of concentration. In the same study, the anti-nutritional effects and insecticides of *Eucalyptus globulus* and *Gaultheria procumbens* L. demonstrated against *Agrotis ipsilon* (Hufnagel) (Jeyasankar 2012).

In the current study, the main included composition of *E. kruseana* essential oil i.e. 1, 8- cineole was detected using GC-MS and it is supported by many research that 1, 8- cineole has been main components of many eucalyptus species (Sefidkon et al. 2007, Fathi & Sefidkon 2012, Zhang et al. 2012). The researches have indicated that herbal monoterpene such as 1, 8- cineole have shown to be successful in initial analysis of fumigation toxicity (Lee et al. 2003). The insecticidal propriety of many essential oils is mainly attributed to monoterpenes which are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions. Due to their high volatility, they have fumigant and gaseous action which are very important in controlling the stored-product insects. Jointly or independently, these compounds are involved in the bioefficacy of the essential oils used with a range of effects i.e. insecticidal, repellent, antifeeding, and ovicidal activities (Rajendran & Sriranjini 2008). It can be concluded that the toxicity of *E. kruseana* essential oil against mentioned pests is related to their major components such as 1,8-cineole. some researches have demonstrated that essential oils have neurotoxic, citotoxic, phototoxic and mutagenic action among others in different organism (Bakkali et al. 2008, Isman et al. 2011), and the essential oils act at multiple levels in the insects, so the possibility of generating resistance is little probable (Gutiérrez et al. 2009). For all these reasons, we can infer that the plant essential oils especially *E. kruseana* essential oil could be considered as a natural alternative in the control of stored grains insects such as *R. dominica.*

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