Efficacy of Monoterpenes against the Cowpea Weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), in Stored Cowpea Seeds

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ABSTRACT

In the present study the contact and fumigant toxicities of seven monoterpenes namely, (*R*)-carvone, 1-8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene and (*R*)-linalool, were evaluated against the adults of *Callosobruchus maculatus* F. The potential of the most effective monoterpenes for control the insect in stored cowpea seeds was also examined. The tested monoterpenes showed potent contact toxicity against the adults of *C. maculatus* within 24 h of exposure. Among the tested monoterpenes, cuminaldehyde (LC_{50} = 19.66 μg/cm^2) was the most toxic compound, followed by (*R*)-carvone, (*S*)-fenchone and geraniol, while (*R*)-linalool and 1-8-cineole were the less effective compounds. In the fumigant toxicity experiments, (*R*)-linalool, cuminaldehyde and (*R*)-carvone revealed the strongest toxicity among the tested compounds with LC_{50} values of 0.08, 1.33 and 1.41 μl/l, respectively. The other monoterpnes had remarkable fumigant toxicity except for geraniol which had moderate activity. When mixed with cowpea seeds, three monoterpenes, (*R*)-carvone, cuminaldehyde and geraniol caused high mortality percentages of the adults of *C. maculatus*, after one week of treatment. These monoterpenes also caused significant reduction in laid eggs and emerged adults after 5 weeks of treatment compared with control. Interstingly, complete mortality of adults, and no laid eggs and emerged adults were observed when the seeds treated with cuminaldehyde and (*R*)-carvone at the application rates of 2, 3 and 4 mg/g. The present study suggests that monoterpenes, (*R*)-carvone, cuminaldehyde and geraniol, may be potential seed protectants and could be used in controling *C. maculatus*.

Key words: Contact toxicity; fumigant toxicity; monoterpenes; Callosobruchus maculatus; stored cowpea.

INTRODUCTION

The pulse beetle, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), is one of the most destructive pests attacking leguminous grains, such as cowpeas, lentils, green gram, and black gram (Raja *et al.* 2000; Park *et al.* 2003; Aboua, 2004). The larvae feeding on seeds causes quantitative and qualitative losses consisting in seed perforation and reductions in weight, leading to market value decrease and the seeds become unsuitable for human consumption and replanting (Ajayi and Lale 2001; Tapondjou *et al.* 2002).

Contact insecticides and fumigants are currently the most common methods used to protect stored products from insect pests (Daglish, 2006). However, the intensive use of these chemicals may lead to serious problems, such as development of insect resistance, persistence of residues and widespread environmental hazards. In response to the above mentioned problems, such chemicals have been under increasingly restrictive policies over the past years (Zettler and Arthur, 2000; Collins, 2006). Therefore, there is worldwide interest in developing alternative insect control agents. One of these alternatives is to use plant derivatives against agriculturally important insects. Among the plant derivatives, essential oils and their major constituents, monoterpenes, are considered potential insect control agents since they possess remarkable insecticidal activity against the stored product insects.

Monoterpenes are main constituents of the majority of plant essential oils. They can be classified into two major groups: monoterpene hydrocarbons that include acyclic aliphatic, monocyclic aliphatic, and dicyclic aliphatic and oxygenated monoterpenes that include acyclic monoterpenoids, monocyclic monoterpenoids, and dicyclic monoterpenoids. The latter group includes many alcohols, aldehydes, ketones, ethers, and acids (Templeton, 1969). In addition to their other bioactivities, monoterpenes possess acute contact and fumigant toxicity to insects (Waliwitiya *et al.* 2005; Samarasekera *et al.* 2008), repellent activity (Watanabe *et al.* 1993), antifeedant activity (Hummelbrunner and Isman 2001; Argandoña *et al.* 2002), as well as development and growth inhibitory activity (Karr and Coats, 1992).

Many researchers have described the fumigant and contact toxicities of some monoterpenes against other stored products insects, such as *Sitophilus oryzae* and *Tribolium castaneum* (Rice and Coats 1994; Prates *et al.* 1998; Kim and Ahn, 2001; Park *et al.* 2003; Lee *et al.* 2001, 2003, 2004; Abdelgaleil *et al.* 2009). However, there were no studies on the toxicity of monoterpenes against *C. maculatus* as well as on the effectiveness of monoterpenes for control this insect in stored seeds. Therefore, the present work was carried out to evaluate the fumigant and contact toxicities of seven monoterpenes against *C. maculatus*. In addition, the efficacy of monoterpens for controling the insect in stored cowpea seeds was also examined.

MATERIALS AND METHODS

1. Chemicals

The monoterpenes, (*R*)-carvone (98%), 1-8-cineole (99%), cuminaldehyde (98%), (*S*)-fenchone (98%), geraniol (98%), (*S*)-limonene (96%) and (*R*)-linalool (95%) were purchased from Sigma-Aldrich Chemical Co., Steinheim. Chemical structures of these compounds are shown in Fig. 1.

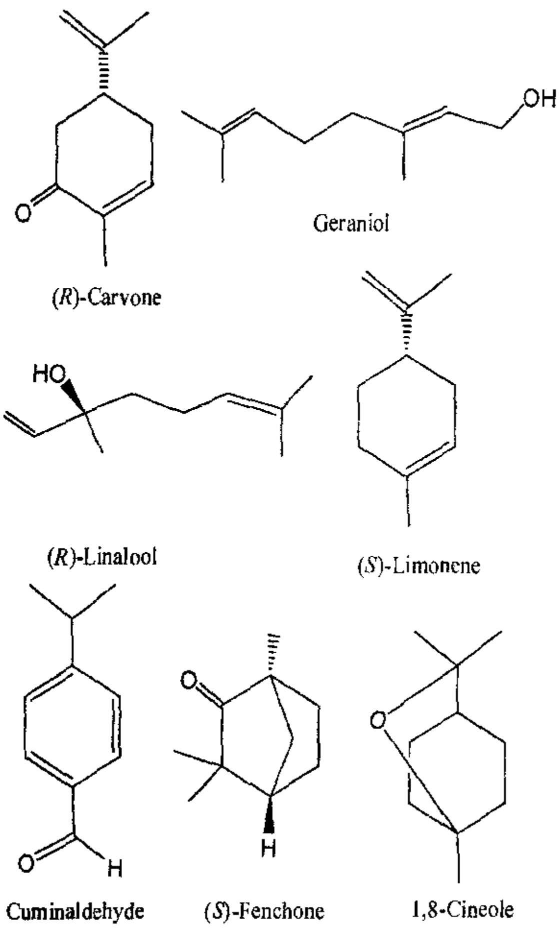

Fig. 1: The chemical structures of monoterpenes.

2. Test insect

Culture of the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), was maintained in our laboratory over 5 years without exposure to insecticides and reared on sterilized cowpea seeds. The seed moisture content was equilibrated at 13%. Insect rearing and all experimental procedures were carried out at 26±1°C and 70±5% R.H. and a 12:12 light:dark photoperiod. Adults used in toxicity tests were 2-3 days post-emergence.

3. Contact toxicity assay

The insecticidal activity of the monoterpenes against the adults of *Callosobruchus maculatus* was determined by direct contact application (Qi and Burkholder, 1981). A series of dilutions of monoterpenes were prepared using acetone as a solvent. Aliquots of 1 ml of the dilutions were applied on the bottom of a glass Petri dish (9 cm diameter) to give a range of concentrations (2.5 -200 μg/cm^2). After solvent evaporation for two minutes, 20 adults were introduced into each Petri dish. Control dishes with and without solvent were conducted. All treatments were replicated three times. The mortality percentages were recorded after 24 hours of treatment and LC_{50} values were calculated according to Finney (1971).

4. Fumigant assay

The toxicity of the monoterpene vapours against the adults of *C. maculatus* was evaluated by using a modified fumigant toxicity assay as described by Huang *et al.* (2000). One litre glass jars were used as fumigation chambers. Monoterpenes at volumes of 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2.5, 5, 10, 20, 40, 60, 80 and 100 μl were applied on filter paper pieces (2 ×3 cm) attached to the undersurface of screw caps of the glass jars. The caps were screwed tightly onto the jars containing 20 insects. Three replicates of each control and treatment were set up. Number of dead insects was recorded after 24 hours of treatment. The mortality percentages were calculated and LC_{50} values were determined as previously described.

5. Cowpea seed treatment and insect exposure

Stock solutions of the test monoterpenes were prepared in acetone. Fifty grams of cowpea seeds were placed in 300 ml glass jars. Cowpea seeds in glass jars were treated with 1 ml of the stock solutions of the test monoterpenes. Each compound was tested at application rates of 1, 1.5, 2, 3 and 4 mg/g. The control jars were treated with acetone. All jars were shaken manually for approximately 2 min to achieve equal distribution of the monoterpenes through the entire seed mass. The jars were left for 30 minutes for complete evaporation of the solvent. Twenty adults of *C. maculatus* were separately introduced into each jar. Three replicates were set up for each treatment and control. The jars were covered with cheesecloth fastened by rubber bands to prevent escape of insects and to ensure proper ventilation. The jars were kept at 26±1°C and 70±5% R.H. The adult mortality and the number of laid eggs were examined after one week of treatment. Then 5 weeks later, the number of emerged adults of *C. maculatus* was counted and the adult emergence percentages were calculated. The

(1982) was used to determine the reduction percentage in the number of progeny: % = (1 - *x/y*) × 100, where *x* = the number of adults emerged in the treatment; *y* = the number of adults emerged in the control.

6. Statistical analysis

The concentration-mortality data were subjected to Probit analysis to obtain the LC_{50} values using the SPSS 12.0 software program (Statistical Package for Social Sciences, USA). The values of LC_{50} were considered to be significantly different, if 95% confidence limits did not overlap. The mortality, laid eggs and emerged adults data were submitted to a one-way analysis of variance (ANOVA). Mean separations were performed by Student-Newman-Keuls (SNK) test and differences at P = 0.05 were considered as significant.

RESULTS AND DISCUSSION

1. Contact toxicity of monoterpenes

The insecticidal activity of the seven tested monoterpenes was evaluated against the adults of *C. maculatus* using the residual film method. The values of LC_{50}, 95% confidence limits, slopes and other parameters generated from regression lines are given in Table 1. All of the tested monoterpenes exhibited remarkable insecticidal activity. Cuminaldehyde showed the strongest insecticidal activity with LC_{50} value of 19.66 μg/cm^2, followed by (*R*)-carvone, (*S*)-fenchone and geraniol as their LC_{50} values were 32.55, 38.62 and 39.0 μg/cm^2, respectively. On the other hand, (*R*)-linalool and 1-8-cineole represented the lowest insecticidal activity in this assay. To the best of our knowledge, there were no reported studies on the contact toxicity of monoterpenes against *C. maculatus*. However, few studies have discribed the contact toxicity of some monoterpenes against the other common stored product insects, *Sitophilus oryzae* and *Tribolium castaneum* (Prates et al. 1998; Kim and Ahn, 2001; Park *et al.* 2003; Garcia *et al.* 2005; Abdelgaleil *et al.* 2009),

2. Fumigant toxicity of monoterpenes

Data of the fumigant toxicity of the test monoterpenes on *C. maculatus* adults are given in Table 2. Except for geraniol which revealed a moderate toxicity, all of the tested compounds exhibited promising insecticidal activity. (*R*)-Linalool (LC_{50} = 0.08 μl/l), cuminaldehyde (LC_{50} = 1.33 μl/l) and (*R*)-carvone (LC_{50} = 1.41 μl/l) showed the strongest toxicity among the tested compounds. In addtion, (*S*)-fenchone and 1-8-cineole had potent insecticidal activity since their LC_{50} values were 3.84 and 4.63 μl/l, respectively. The fumigant toxicity of some essential oils has been demonstrated against *C. maculatus*. For example, The essential oils of *Ageratum conyzoides*, *Citrus aurantifolia*, *Melaleuca quinquenervia*, *Carum copticum*, *Vitex pseudo-negundo*, *Artemisia scoperte Ocimum basilicum* and *O. gratissimum* were found to possess fumigant toxicity against *C. maculatus* (Ke'ita *et al.* 2001; Negahban *et al.* 2006; Sahaf and Moharramipour, 2008; Aboua *et al.* 2010). The fumigant toxicity of these oils was attributed to their active monoterpene constituents. In addition, it has been reported that some of the test monoterpenes (i.e., 1-8-cineole, geraniol, (*S*)-limonene, (*R*)-linalool and (*S*)-fenchone) possessed fumigant toxicity towards *S. oryzae* and *T. castaneum* (Prates *et al.* 1998; Lee *et al.* 2001, 2003, 2004; Abdelgaleil *et al.* 2009).

Comparing the results of contact and fumigant toxicities revealed that the assay method impacted the toxicity of monoterpenes. For example, (*R*)-linalool was the most effective fumigant among the tested compounds but was the less effective contact toxicant. In contrast, geraniol had pronounced contact toxicity but was the weakest fumigant among the tested compounds. These findings suggested that the assay method had a great effect on the efficacy of monoterpenes. Similarly, Prates *et al.* (1998) and Park *et al.* (2003) noticed that the activity of monoterpenes against stored product insects varied with assay methods.

Table 1: Contact toxicity of monoterpenes against the adults of *Callosobruchus maculatus* (F.).

Monoterpene	LC_{50}[a] (μg/cm^2)	95% Confidence limits (mg/cm^2)		Slope ± S.E.[b]	Intercept ± S.E.[c]	(χ^2)[d]
		Lower	Upper			
(*R*)-Carvone	32.55	31.45	33.50	15.83 ± 2.19	-5.02 ± 0.74	0.00
1-8-Cineole	68.83	65.42	72.20	7.94 ± 1.20	-13.01 ± 1.95	0.96
Cuminaldehyde	19.66	17.93	21.23	4.89 ± 0.43	-0.47 ± 0.10	0.54
(*S*)-Fenchone	38.62	13.62	44.99	21.17 ± 1.98	-29.43 ± 2.77	11.73
Geraniol	39.0	7.54	64.64	3.98 ± 0.42	-1.57 ± 0.20	7.66
(*S*)-Limonene	50.67	48.88	53.01	10.01 ± 1.38	-15.10 ± 2.04	0.99
(*R*)-Linalool	78.79	64.64	102.70	1.91 ± 0.25	-1.34 ± 0.15	1.93

[a] The lethal concentration causing 50% mortality after 24 h.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Intercept of the regression line ± standard error.
[d] Chi square value.

Table 2: Fumigant toxicity of monoterpenes against the adults of *Callosobruchus maculatus* (F.).

Monoterpene	LC_{50} [a] (µl/L)	95% Confidence limits (µl /L)		Slope ± S.E. [b]	Intercept ± S.E. [c]	(χ^2) [d]
		Lower	Upper			
(*R*)-Carvone	1.41	0.78	1.89	6.49 ± 0.65	- 0.97 ± 0.12	9.08
1-8-Cineole	4.63	2.22	5.57	2.93 ± 0.88	- 1.95 ± 0.78	1.81
Cuminaldehyde	1.33	1.25	1.41	6.48 ± 0.56	- 0.81 ± 0.12	0.76
(*S*)-Fenchone	3.84	3.06	4.40	3.24 ± 0.48	- 1.89 ± 0.38	0.25
Geraniol	53.47	39.24	89.65	1.14 ± 0.23	- 1.98 ± 0.36	0.03
(*S*)-Limonene	13.34	2.23	21.79	2.06 ± 0.24	- 2.32 ± 0.34	4.15
(*R*)-Linalool	0.80	0.49	1.00	2.28 ± 0.47	- 0.21 ± 0.11	2.61

[a] The lethal concentration causing 50% mortality after 24 h.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Intercept of the regression line ± standard error.
[d] Chi square value.

3. Effect of monoterpenes on mortality of *C. maculatus* in treated cowpea seeds

The results of contact and fumigant toxicities demonstrated that (*R*)-carvone, cuminaldehyde, geraniol and (*R*)-linalool were the most effective against *C. maculatus*. Therefore, these four compounds were tested for their potential to control the insect in stored cowpea seeds. The mortality of *C. maculatus* adults in treated cowpea seeds with different application rates after one week is presented in Table 3. The results showed that there were significant differences between monoterpenes and among the application rates of each monoterpene on the percentages of adult mortality. Cuminaldehyde caused the highest mortality at the application rates of 1 and 1.5 mg/g followed by (*R*)-carvone. Both compounds caused complete mortality of adults at the application rates of 2, 3 and 4 mg/g. At the same time geraniol produced 100 % mortality at the application rates of 3 and 4 mg/g. (*R*)-Linalool was the less effective among the tested compounds; this compound caused 61.2 % mortality at the highest tested application rate (4 mg/g). These results are in good agreement with our previous study on the activity of the tested compounds against *S. oryzae* and *T. castaneum* in which cuminaldehyde, (*R*)-carvone and geraniol were more toxic than (*R*)-linalool against both insects (El-arami *et al.* 2009).

4. Effect of monoterpenes on laid eggs and adult emergence of *C. maculatus* in treated cowpea seeds

The results of the reduction of laid eggs, progeny production and the reduction percentage of *C. maculatus* adults after 5 weeks of seed treatment are presented in Table 3. In general, all of the tested compounds at the tested application rates showed significant reduction in number of laid eggs and emerged adults compared with control. The potency of compounds was in order of cuminaldehyde, (*R*)-carvone, geraniol and (*R*)-linalool. The reduction precentages of adults were very high compring with control, inculding (*R*)-linalool which caused low mortality precentages but caused high reduction percentages of 92.5 at the application rate of 4 mg/g.

Progeny reduction in the treated seeds is perhaps more important than parental mortality, because a seed protectant should protect the seed for a long storage period (Athanassiou *et al.* 2005). In our work, progeny reduction of *C. maculatus* was significantly high on cowpea seeds treated with the tested monoterpenes, which suggest that long-term protection for the treated cowpea seeds. Nevertheless there were no published studies on the efficacy of monoterpenes for control *C. maculatus* in stored seeds, it has been reported that some essential oils could be used for control *C. maculatus* in cowpea seeds (Kèita *et al.* 2001; Ketoh *et al.* 2005; Raja and William, 2008).

Seed germination results of the most effective monoterpenes, cuminaldehyde, (*R*)-carvone and geraniol, at the highest application rate (4 mg/g) revealed that the three compounds decreased the germination percentage of the treated seeds compared with control. (*R*)-Carvone caused the highest reduction in seed germination followed by cuminaldehyde with germination percentages of 45 and 65, respectively, while geraniol had the lowest effect on seed germination.

The presence of naturally occurring insecticidal components has been known for centuries.However, few of these compounds are commercially used in managing stored-product insects. Problems associated with the use of synthetic insecticides, such as the development of resistance, persistence of residues in stored products, and damage to the environment and human health (Lorini and Galley, 1999; Zettler and Arthur, 2000) have generated interest in naturally occurring compounds. Some of active natural products with interesting insecticidal potential such as the tested monoterpenes are isolated from edible plants, therefore they considered being safer to human and environment than other chemicals. The results presented in this study suggest that some monoterpenes such as (*R*)-carvone, cuminaldehyde, geraniol could be efficient protectants against *C. maculatus* in stored cowpea seeds and also could be used in integrated pest management program of this insect.

Table 3: Effect of monoterpenes applied to cowpea seeds on mortality, laid eggs and adult emergence of *Callosobruchus maculatus* (F.).

Application rate (mg/g)	Mortality (%±SE) after 1 week	Mean No. of eggs (± SE) after 1 week	Emerged adults after 5 weeks		
			(Mean No. of adults (± SE)	Emergence (%)	Reduction (%)
control	10.0 ± 0.0g	785 ± 60.77a	471.7 ± 22.36a	60.1	0.0
			(*R*)-Carvone		
1.0	32.0 ± 1.38e	330 ± 21.10e	40 ± 2.64fg	12.1	91.5
1.5	56.0 ± 1.11c	213 ± 16.18fgh	11.0 ± 1.15g	5.1	97.6
2.0	100.0 ± 0.0a	0.0 ± 0.0i	0.0 ± 0.0g	0.0	100.0
3.0	100.0 ± 0.0a	0.0 ± 0.0i	0.0 ± 0.0g	0.0	100.0
4.0	100.0 ± 0.0a	0.0 ± 0.0i	0.0 ± 0.0g	0.0	100.0
			Cuminaldehyde		
1.0	58.0 ± 2.59c	209 ± 17.79fgh	20 ± 1.0g	9.5	95.7
1.5	71.8 ± 1.04b	131 ± 11.02h	13± 2.51g	9.9	97.2
2.0	100.0 ± 0.0a	0.0 ± 0.0i	0.0 ± 0.0g	0.0	100
3.0	100.0 ± 0.0a	0.0 ± 0.0i	0.0 ± 0.0g	0.0	100
4.0	100.0 ± 0.0a	0.0 ± 0.0i	0.0 ± 0.0g	0.0	100
			Geraniol		
1.0	18.4 ± 2.60f	480 ± 21.30d	230 ± 24.86c	47.9	51.0
1.5	53.3 ± 1.78c	262 ± 16.10ef	43.3 ± 1.66fg	16.5	90.8
2.0	75.0 ± 3.00b	146 ± 7.09gh	22 ± 2.51g	15.1	95.3
3.0	100.0 ± 0.0a	0.0 ± 0.0i	0.0 ± 0.0g	0.0	100
4.0	100.0 ± 0.0a	0.0 ± 0.0i	0.0 ± 0.0g	0.0	100
			(*R*)-Linalool		
1.0	13.3 ± 1.66fg	655 ± 14.06b	386 ± 12.23b	58.9	18.1
1.5	19.4 ± 3.27f	580 ± 17.53c	143 ± 11.38d	23.1	69.6
2.0	34.0 ± 3.92e	421 ± 22.83d	99 ± 5.50c	23.5	79.0
3.0	41.0 ± 1.09d	308.7 ± 11.56e	65 ± 4.16f	21.0	86.2
4.0	61.2 ± 1.41c	223 ± 6.43fg	35 ± 2.64fg	15.7	92.5

Data are expressed as means ± S.E. from experiments with three replicates.
Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

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المخلص العربى

كفاءة المونوتربينات فى مكافحة حشرة خنفساء اللوبيا فى بذور اللوبيا المخزونة

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فى هذه الدراسة تم تقييم سمية سبع من المونوتربينات وهى (*R*)-carvone و1-8-cineole وcuminaldehyde و (*S*)-fenchone وgeraniol و(*S*)-limonene وlinalool و(*R*)- على خنفساء اللوبيا بطريقتى المعاملة بالملامسة والتدخين. بالإضافة إلى إن كفاءة بعض هذه المركبات التى أظهرت سمية عالية تم إختبارها لمكافحة هذه الأفة فى بذور اللوبيا المخزونة . المركبات المختبرة أظهرت سمية مميزة بالملامسة خلال ٢٤ ساعة من التعرض. مركب cuminaldehyde (LC_{50} = 19.66 µg/cm^2) كان أعلى المركبات فاعلية يليه كل من (*R*)-carvone و (*S*)-fenchone و geraniol فى حين كانا مركبى (*R*)-linalool و 1-8-cineole أقل المركبات فاعلية فى هذا الإختبار. فى تجارب التدخين مركبات (*R*)-linalool وcuminaldehyde و (*R*)-carvone كانت هى أفضل المركبات سمية حيث كانت قيم الـ LC_{50} لها 0.08 و 1.41 و1.33 ميكروليتر/لتر على الترتيب. المركبات الاخرى كان لها تأثير سام جيد عدا مركب geraniol الذى أظهر سمية متوسطة. عند خلط هذه المركبات مع بذور اللوبيا لمكافحة خنفساء اللوبيا ثلاث مركبات وهى (*R*)-carvone وcuminaldehyde وgeraniol سببت نسب موت مرتفعة للحشرات الكاملة بعد أسبوع من المعاملة. هذه المركبات أيضا سببت خفض معنوى فى نسب و ضع البيض والحشرات الكاملة الناتجة بعد ٥ أسابيع من المعاملة. من الملفت للنظر أنه حدث موت كامل للحشرات ولم يتم وضع بيض ولم تخرج حشرات كاملة بعد ٥ أسابيع من المعاملة بمركبى (*R*)-carvone وcuminaldehyde على معدلات ٢ و ٣ و ٤ مجم/جم. من هذه الدراسة يمكن الإقتراح بإن مركبات (*R*)-carvone وcuminaldehyde وgeraniol ربما يكون لها دور فعال كواقيات للبذور كما أنه يمكن استخدامها فى برامج مكافحة خنفساء اللوبيا.