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Evaluation of Essential Oil and its Main Active Ingredients of Chinese *Litsea cubeba* Against Two Stored-Grain Insects

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Abstract: Essential oils obtained from different plants showed several types of pesticide properties; some of them may be considered alternatives to insecticides for pest control. The main purpose of this research was to detect the chemical composition of the essential oil derived from the fruits of *Litsea cubeba* (Lauraceae). In addition, to evaluate the contact and fumigant toxicity and repellent activities of the essential oil and two main active ingredients against the adults of two stored grain insect pests; riceweevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) in the laboratory. Twenty-one components were identified in the essential oil with gas chromatography-mass spectrometry (GC-MS). The main constituents included E-citral (geranial) (28.49%), Z-citral (neral) (21.57%) and D-limonene (18.82%) followed by Citronella (3.20%), β-pinene (2.85%), Terpinol-4-ol (2.46%), β-Thujene (2.34%) and Geraniol (2.25%). Citral (Z/E-citral) and D-limonene separated as main components from the essential oil. Results from the three compounds; the essential oil, citral and D-limonene showed strong contact toxicity against *S. oryzae* with LD_{50} 7.51, 7.75 and 29.57 µg/l, while fumigation had higher toxicity on the same insects 4.44, 4.89 and 16.68 µg/l, respectively. In the case of the adults of *T. castaneum*, those were more resistant to the essential oils and its main active compounds. The contact toxicity of essential oils, citral and D-limonene against *T. castaneum* presented LD_{50} values 253.66, 349.47 and 3803.20 µg/l, while the fumigation toxicity recorded 204.34, 296.64 and 592.08 µg/l, respectively. Otherwise, the essential oils, citral and D-limonene were strong repellents against *S. oryzae* at 60 nL cm^2, being 81.83 and 53.30%, respectively after two and four hours post-treatment. Whereas, the repellency percentages for *T. castaneum* were 85.76 and 82.33%, respectively, at the same conditions mentioned before. At the same concentrations, the essential oil had more repellent effect on *T. castaneum* than *S. oryzae*. Thus, the essential oils of *L. cubeba* might be potential to be developed as a natural contact and fumigant insecticides or repellents for control of the two insect species under study.

Key words: Essential oil · *Litsea cubeba* · Contact · Fumigation · Toxicity · Repellency and Stored product insects

INTRODUCTION

Stored grains, cereals and their products are important sources of global food. For this reason, the effective storage of these prime products is necessary for human's life [1]. Wheat, rice and maize are the most consumed grains as human and animal food [2]. All of these crops are traditionally included in cropping patterns in Egypt. After harvest, these commodities are stored for up to one year or more to obtain good marketing rates. This prolonged storage makes these products vulnerable to various losses, mainly, by insect pest infestations.

Globally, insect pests which infest the stored-grain products cause the highest quantitative and qualitative loss from 10 to 40% [3, 4]. In Egypt, rice is one of the most important crops. After harvesting and storage, it is susceptible to insect pests infestation. The most important insect that infests rice in the store and the field

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is the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). It is a problematic primary insect pest of the stored rice, wheat and maize grains [5]. These insects which the whole grain had been originated in India and spread afterward all over the world by commerce. Now, it has an international distribution. It is, also, a serious pest in Egypt. Both of larvae and adults feed on the whole grains. They also feed on many kinds of grains, including wheat, corn, oats, rye, barley, sorghum and grain products, specifically macaroni. The rice weevil adult is able to fly and is attracted to the artificial lights. The rice weevil complete the egg and larval stages inside a seed kernel or an alternative human-made products (like macaroni) products [6]. Also, among the insects that attack the stored grain products, the red flour beetle, *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae) causing up to 40% reduction in grain weight [7]. The damage of red flour beetle not only causes significant losses in grain weight because of the consumption of grains, but also resulting in increased temperatures and moisture conditions that lead to rapid growth of fungi, including toxic species [8].

Currently, recommended pest control measures in stored products rely mainly on the use of chemical pesticides or fumigants that pose potential health risks to warm-blooded animals, development of insects' resistance, the threat of environmental pollution and the pests spread [9]. The solution of these problems has necessitated searching for alternative environmentally safe methods to combat these insect pests [10]. The use of essential oils or their components with low toxicity for mammals can effectively prevent insect pests, especially in storage [11]. Investigations in many countries confirmed that some plant essential oils not only expel insects but also have contact and fumigant toxicity against stored grain pests, as well as showing inhibition of feeding or harmful effects on the reproductive system of insects [12].

Essential oils and their components in many plants, including medicinal herbs, spices and fruits, have been successfully evaluated for insecticidal or repellent activity against stored product insects; they proved to be more effective than traditional pesticides [13]. May Chang, *Litsea cubeba* (Lour.) is a perennial plant that can be found in different regions of China and can be extracted from leaves, bark or fruits [14]. Conceptually, the essential oil of this plant is toxic to many insects and acts as a repellant to mosquitoes; *Aedes aegypti* L. [15]. Additionally, it also has a fascinating property as biochemical control on the other stored-product insects. The previous research showed that the essential oil of *L. cubeba* could control the maize weevil *S. zeamais* Motschulsky for its apparent contact toxicity, fumigant toxicity and repellent activity on this pest [16]. All plant parts of *L. cubeba* are used medically as anti-bacterial, anti-allergic and anti-hysterical [17]. The same authors, also, reported that *L. cubeba* oil has anti-fungal properties in vitro against many pathogens such as *Alternaria alternate* (Fr.) Keissl., *Aspergillus niger* van Tieghem, *Candida albicans* (CP Robin) and *Fusarium spp*. and *Helminthosporium spp.*

The present study was carried out to estimate the contact, fumigant toxicity and repellency of *L. cubeba* essential oil and two main active ingredients derived from it against adults of the two stored grain insect species, the rice weevil, *S. oryzae* (L.) and the red flour beetle, *T. castaneum* (Herbst.).

MATERIALS AND METHODS

Insect Cultures: Fresh wheat was purchased from one of the grain stores. The grains were washed and kept in a freezer at -20°C under closed conditions for a week or until they were used in the experiments to prevent any insects' infestation. About five hundred adults of the rice weevil, *S. oryzae* were collected from infested wheat grains from Wuhan city, Hubei province, China. Rice weevils were kept in glass jars (13 cm diameter × 17 cm height) covered with a fine mesh. These insects were grown on sterilized whole wheat in a rearing room at 26 ± 1°C and 65 ± 5% RH in complete darkness. Also, the red flour beetle *T. castaneum* was obtained from infested wheat flour. The culture condition was 28±1? and 70-80% relative humidity. The insects were reared in glass containers (0.5 L) containing wheat flour mixed with yeast 10:1 (w/w).

Plant Material and Essential Oil Extraction: One kg *L. cubeba* fruits were purchased from Wuhan city, Hubei Province, China. The fruits were dried at room temperature for one week and ground to a powder using a grinding mill. The ground powder was subjected to hydrodistillation by a Clevenger type apparatus for four h and extracted with n-hexane. Anhydrous sodium sulfate was used to remove water after extraction [18]. The distilled essential oils were stored in a refrigerator at 4°C until being used in the experiments.

Gas Chromatography-Mass Spectrometry: Components of the essential oil were separated and identified by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) on an Agilent 6890N gas chromatograph hooked to an Agilent 5973Nmass selective detector. The same column and analysis conditions were used for both GC-FID and GC-MS. Those were equipped with a flame ionization detector and a capillary column with HP-5MS (30 m × 0.25 mm × 0.25 µm). The GC settings were as follows: The initial oven temperature was held at 60°C for one minute and ramped at 10°C/min to 180°C/ min and then ramped at 20°C/min to 280°C for 15 min. The injector temperature was maintained at 270°C. The samples (1 µl, diluted to 1% with hexane) were injected neat, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0 ml /min. Spectra were scanned from 20 to 550 m/z at two scans per second. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in the laboratories. The retention indices were determined to a homologous series of n-alkanes (C8-C24) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 (Standard Reference Data, Gaithersburg, MD, USA) and Wiley 275 libraries (Wiley, New York, NY, USA) or with mass spectra from the literature [19, 20]. Component relative percentages were calculated based on the normalization method without using correction factors.

Contact Toxicity: The contact toxicity of *L. cubeba* essential oil and two main active ingredients against the adults of two stored-grain insects were tested as described by Liu and Ho [21]. Range-finding studies were run to determine the appropriate testing concentrations of the essential oil of *L. cubeba*. A serial dilutions of the essential oil and two main active ingredients (5 concentrations) were prepared in n-hexane. The adults of *S. oryzae* were exposed to essential oil vapors (5; 10; 15; 20 and 25 µl/l air) for 24 h. However, because of high tolerance of *T. castaneum*, adults were treated with higher doses (50; 100; 150; 200 and 250 µl/l air) and longer exposure periods (24, 72 and 144 h) than *S. oryzae*. Aliquots of 0.5 µl per insect were topically applied dorsally to the thorax of both insect species, using a Burkard Arnold micro-applicator. The control was set up using 0.5 µl n-hexane per insect. Ten insects (one week old) were used for each concentration and control and the experiment was replicated four times. Both the treated and control insects were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators at 26±2°C and 70±5 % R.H.. Results from all replicates were subjected to probit analysis to determine LD_{50} values according to Sakuma [22].

Fumigant Toxicity Bioassay: The fumigant activities of *L. cubeba* oil and the two main active ingredients against *S. oryzae* and *T. castaneum* adults were tested as described by Liu and Ho [21]. The same concentrations in contact bioassay were used again in fumigant toxicity bioassay. A Whatman filter paper (2.0 cm diameter) was placed on the underside of the screw cap of a glass vial (2.5 cm diameter, 5.5 cm height and 24 ml. volume). Ten microliters of the essential oil were added to the filter paper. The solvent was allowed to evaporate for 15 seconds before the cap was placed tightly on the glass vial (with ten mixed sex insects, about one week old) to form a sealed chamber. Those were incubated for 24 hours at 26±2°C and 70±5% R.H. for *S. oryzae* and 24, 72 and 144 hrs for *T. castaneum*. Mortality of insects was recorded and results from all replicates were subjected to probit analysis to determine LC_{50} values [22].

Repellency Tests: The repellent activities of *L. cubeba* essential oil and the two main active ingredients against *S. oryzea* and *T. castaneum* were investigated with the method reported by Chaubey [23]. The experiments set was with five concentrations (75, 60, 45, 30 and 15 nL/cm^2) and two observation times (two and four h). The essential oil and two selected compounds were diluted with n-hexane. Filter paper (9 cm in diameter) was symmetrically cut into two pieces and 500 ??l dilutions of each concentration were dropped evenly on half of the filter paper, while the other half was treated with 500 ??l of n-hexane as a negative control. After being air-dried for 30 s, both two pieces were stuck to the bottom of the Petri dish (9 cm in diameter) with solid glue abreast. Twenty insects were placed at the center of the disk and covered quickly with lids. All the above procedures were repeated four times for each concentration. Counts of insects on each half of the paper were recorded separately. The following formula calculated the percent repellency (PR) of each test:

$$PR\ (\%) = [(Nx-Nt) / (Nc+Nt)] \times 100$$

where Nc is the number of insects in the negative control half and Nt is the number of insects in the tested half. The percent repellency transformed to arcsine square root values for analysis of variance (ANOVA) and the effect on the transformed arcsine of the percent repellency of different treatments (the essential oil, the two compounds and the control). Obtained data at each concentration and exposure time were analyzed separately. Significant differences in repellence rates among treatments given by the analysis of Tukey's test (SPSS).

Statistical Analysis: The data were corrected using data from treatments and the control according to Abbott's formula [24] and the data were subjected to probit analyses using LDP line software according to Finney [25] to estimate LC_{50} and LC_{99} values of the essential oils against each stored product insect species. Mortality percentages for different exposure times were subjected to analysis of variance (one-way ANOVA) using the same statistical program (SPSS 2001) for probit analysis [26]. Means were separated at the 5% significance level by the least significant difference (LSD) test.

RESULTS AND DISCUSSION

The Chemical Composition of the Essential Oil: The chemical compositions of the essential oil derived from *L. Cubeba* fruits collected from Wuhan, China are shown in (Table 1). The main constituents of *L. cubeba* essential oil are E-citral (geranial) (28.49%), Z-citral (neral) (21.57%) and D-limonene (18.82%) followed by Citronella (3.20%), β-pinene (2.85%), Terpinol-4-ol (2.46%), β-Thujene (2.34%) and Geraniol (2.25%). A Twenty one components were identified in the essential oil of *L. cubeba*, accounting for 98.31% of the total oil (Table 1). The result is popular to previous work. Yang *et al.* [27] Mentioned 33 compounds of *L. cubeba* essential oil and E/Z-citral and D-limonene were the most common ingredients. Ko *et al.* [14] found that 17 compounds from which E-citral (41.3%), Z-citral (30.1%) and methylheptenone (5.6%) from *L. cubeba* mature fruits extraction which were collected from Chiang Mai province, China. While Si *et al.* [28] extracted *L. cubeba* essential oil from 8 locations in China and they found that up to 59 compounds and the major ingredients were E-citral and Z-citral. The chemical constituents of essential oil may vary in the components and amounts due to harvest time and local, climatic and seasonal factors as well as storage duration of medicinal herbs [29]. For example, fruit essential oil collected from Fujian, Jiangxi, Guizhou, Hunan, Yunnan and Sichuan Provinces contained geranial (44.4-50.0%), neral (34.2-37.4%) as its main constituents whereas D-limonene was only a minor constituent (0.7–5.3%) (Si et al., 2012). Wang and Liu (2010) reported that neral (63.75%) and limonene (7.38%) were the two main constituents followed by methyl heptenone (3.54%), camphene

Table 1: Chemical composition of the essential oil from *L. cubeba*.

No.	Compound	Retention index	(%) Composition
1	α-Pinene	931	1.37
2	β-Thujene	967	2.34
3	β-Pinene	981	2.85
4	6-Methyl-5-hepten-2-one	996	0.40
5	D-Limonene	1025	18.82
6	Eucalyptol	1029	1.07
7	Linalool	1123	1.23
8	cis-Verbenol	1143	1.78
9	4-Terpineol	1174	1.99
10	Z-Citral	1240	21.57
11	E-Citral	1560	28.49
12	beta-Myrcene	1680	1.12
13	Geraniol	1710	2.25
14	Camphene	1721	1.75
15	Terpinol-4-ol	1750	2.46
16	ß - Myrcene	1821	1.20
17	Linalool acetate	1843	1.65
18	Methylheptenone	1890	0.50
19	Citronella	1920	1.25
20	Camphor	1953	1.02
21	Citronella	1975	3.20
	Total		98.31

(3.12%), α-pinene (2.87%) and p-cymene (2.14%) in the essential oil of *L. cubeba* from southern areas of China. The essential oil collected from Nanchang, Jiangxi Province, China by Wang *et al.* [30] contained limonene (26.25%), geranial (25.97%), neral (21.90%), β-pinene (6.20%), β- phellandrene (4.51%) and á-pinene (3.80%) as the main components of the fruit oil. However, the main components of the fruit oil from Nantou, Taiwan were found to be geranial (37.16%), neral (28.29%), D-limonene (22.90%) and β-myrcene (2.06%) [31]. The above findings suggest that further studies on plant cultivation and essential oil standardization are needed because chemical composition and content of constituents of the essential oil varies greatly with the plant population.

The Contact Toxicity of the Oil and Isolated Constituents: Contact toxicity of *L. cubeba* essential oils, citral and D-limonene, were investigated with two previously mentioned stored product insect species. Increased exposure period and doses of essential oils and its components caused higher mortality rates of both insects. The mortalities of essential oil, citral and D-limonene against *S. oryzae* adults were 81.1; 73.30 and 39.9 % at 25 µl/l air dose, respectively after 24 h exposure. The red flour beetle *T. castaneum* was more tolerant than rice weevil, so at the same dose 25 µl/l air, it didn't show the activity to *T. castaneum*. Therefore, as the doses and exposure time were increased,

Table 2: LC_{50} and LC_{99} values of the contact toxicity of *L. cubeba* essential oil and its two main active ingredients against the adults of *S. oryzae* and *T. castaneum*

Insects	Samples	Time (h)	LC_{50} ^{a} (µl/l air)	LC_{99} ^{a} (µl/l air)	Slope±SD	Chi Square (χ^2)	p-Value	R.
S. oryzea	Essential oil	24 h	7.51	442.79	1.31±0.24	4.63	0.20	0.927
	Citral		7.75	1665.00	0.99±0.24	1.85	0.60	0.948
	D-limonene		59.57	7447.48	1.10±0.28	3.99	0.26	0.893
T. castaneum	Essential oil	24 h	253.66	5670.34	1.72±0.28	7.98	0.04	0.914
	Citral		349.47	13256.47	1.47±0.28	6.59	0.086	0.899
	D-limonene		288.24	54622.47	1.02±0.25	0.51	0.91	0.984
	Essential oil	72 h	130.72	2297.08	1.86±0.26	10.47	0.01	0.918
	Citral		151.43	9908.57	1.28±0.25	3.54	0.31	0.941
	D-limonene		347.47	14320.03	1.44±0.28	3.01	0.38	0.944
	Essential oil	144 h	81.68	1454.31	1.86±0.25	6.21	0.10	0.946
	Citral		97.63	2375.22	1.67±0.25	11.59	0.008	0.558
	D-limonene		288.24	54622.47	1.02±0.25	0.51	0.91	0.984

[a] Dose (µg/L air); mean mortality of the control with n-hexane ≤ 1%.

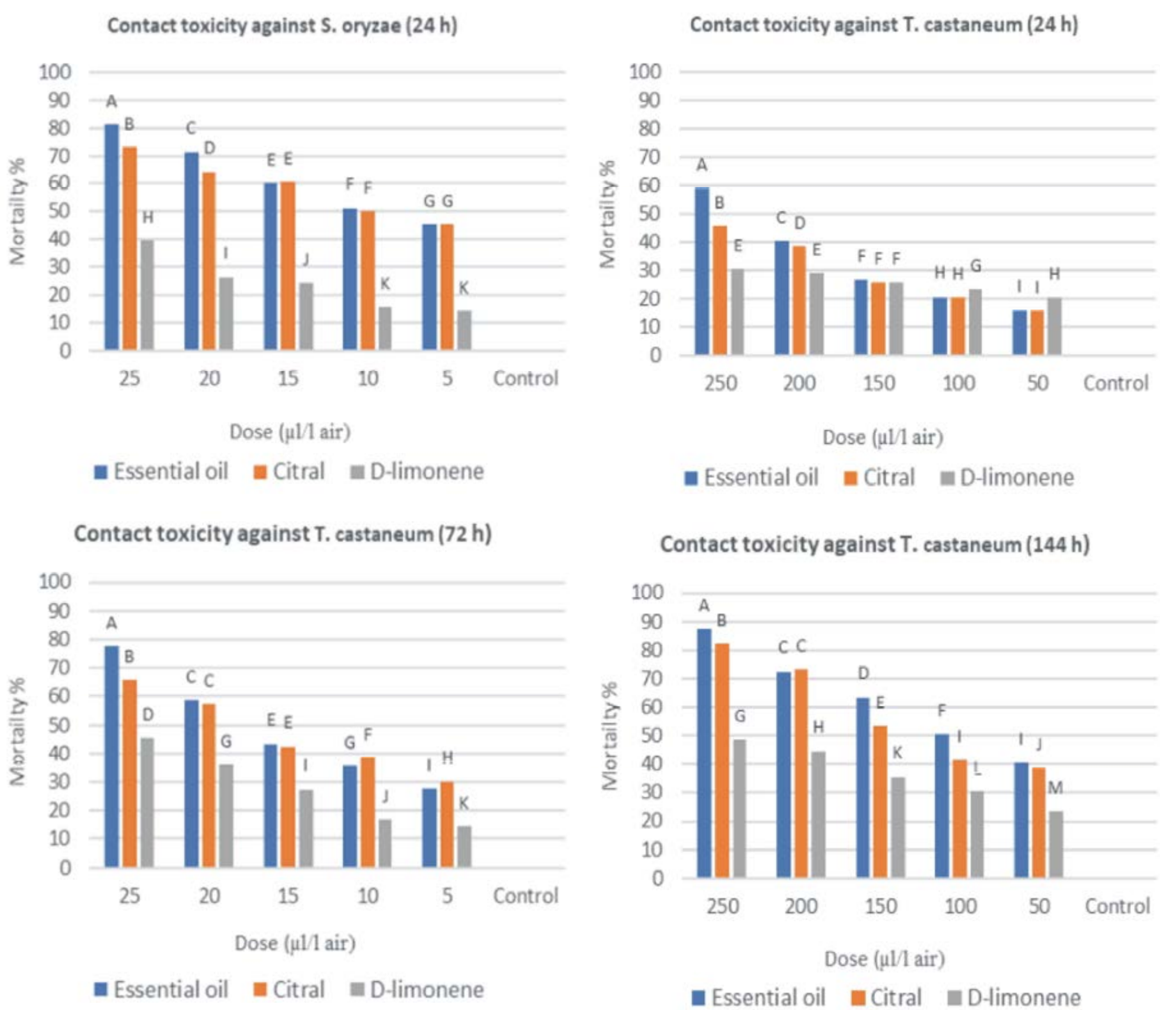

Fig. 1: Mortality Percentages of contact toxicity of *L. cubeba* essential oil and its two main active ingredients against the adults of *S. oryzae* and *T. castaneum* after exposure periods. Letters above columns indicate significance between doses. Columns with the same letter are not significantly different.

the percentage mortalities of essential oils, citral and D-limonene against *T. castaneum* adults were 87.70; 82.20 and 48.80 %, respectively at 250 µl/l air dose after 144 h exposure. On the other hand, probit analysis showed that LC_{50} values of *L. cubeba* essential oil, citral and D-limonene against *S. oryzae* were 7.51, 7.75 and 59.57 µl/l air, respectively. LC_{99} values revealed that *S. oryzae* was more susceptible to the essential oil 442.79 µl/l air than citral and D-limonene 1665 and 7447.48 µl/l air, respectively (Table 2). In case of *T. castaneum*, the LC_{50} and LC_{99} values of the essential oil were 81.68 and 1454.31 µl/l air, respectively, at the highest exposure time (144 h). The doses required for 99% mortality (LC_{99}) for using citral and D-limonene against *T. castaneum* were 2375.22 and 54622.47 µl/l at 144 h exposure, respectively (Table 2).

Similar studies were conducted with other essential oils. Cassia (*Cinnamomum cassia* (L.) and cinnamon oil (*Cinnamomum zeylanicum* Blume) were tested with *Sitophilus oryzae* (L.) and the results showed that the LC_{50} values on *S. oryzae* were 100 mg/cm^2 and 20 mg/cm^2 after 48 h [32]. Tapondjou *et al.* [33] found that the essential oils from *Eucalyptus saligna* Sm. and *Cupressus sempervirens* L. were toxic to *S. zeamais* and *T. castaneum* but *E. saligna* oil was more toxic to both insects than *C. sempervirens*. The LC_{50} values of *S. zeamais* and *T. castaneum* were 0.36 and 0.48 µL/cm^2 at 3 days after exposure, being more toxic than *L. cubeba* essential oils in this study. Furthermore, microapplicator was also used to apply on insect thorax for contact toxicity. *Litsea cubeba* essential oil was reported against *S. zeamais* adults [14]. *Alpinia conchigera* Griff., *Zingiber zerumber* Smitt, *Curcuma zedoaria* (Berg.) Roscoe are all from the Zingiberaceae. The essential oils were extracted from rhizomes and the essential oils were tested with *S. zeamais* and *T. castaneum* adults. The three essential oils and their major compounds (terpinen-4-ol and isoborneol) were toxic to both insects [34]. It can be seen that essential oils could be used to control the insects, but the effectiveness depends on the insect species. Therefore, botanical insecticides should replace the chemical insecticides, but the risk to user and environment need to more research studies.

Fumigation Toxicity: Data concerning the fumigation toxicity of *L. cubeba* essential oil and two main active ingredients against *S. oryzae* and *T. castaneum* are shown in (Table 3). It is quite clear that the mortality percentages by the essential oil were dependent on the applied dosage and exposure times. Increasing doses caused significant increase in mortality. The mortalities by essential oil, citral and D-limonene against *S. oryzae* adults were 100; 95.50 and 62.20 %, respectively at 25 µl/l air dose after 24 h exposure (Fig. 2). Probit analysis showed that LC_{50} values of *L. cubeba* essential oil, citral and D-limonene against *S. oryzae* were 4.44, 4.89 and 16.68 µl/l air, respectively. LC_{99} values revealed that *S. oryzae* was more susceptible to the essential oil (27.87) µl/l air than citral and D-limonene (115.04 and 634.05 µl/l air, respectively) (Table 3). In that context, the mortality values, significantly, increased depending on the increase in essential oil doses and exposure times. When *T. castaneum* adults were exposed to *L. cubeba* oil, citral and D-limonene. The mortality values reached 100, 94.4 and 61.1%, respectively at 250 µl/l air dose after 144 h exposure. *T. castaneum* adults were more resistant to *L. cubeba* essential oil and its main compounds than *S. oryzae*. LC_{50} and LC_{99} values of the essential oil against *T. castaneum* were 5.00 and 2220.28 µl/l air, respectively at the longest exposure period (144 h). The doses required for 99% mortality LC_{99} for using citral and D-limonene against *T. castaneum* were 5.02and 169.14 µl/l, respectively at 144 h exposure (Table 3).

The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility, they have fumigant activity that might be of importance for controlling stored-product insects [35]. In the current study, the essential oil obtained from *Litsea cubeba* and its constituents showed insecticidal activity against the adults of *S. oryzae* and *T. castaneum*. The red flour beetle was the highest tolerant species against this oil as the doses required to kill the adults of this species were much higher than those needed to kill *S. oryzae* for all treatments. Also, adults of *S. oryzae* were more susceptible to *L. cubeba* oil than the other constituents. The toxic effects of *L. cubeba* oil could be attributed to significant components such as E-citral (geranial) (28.49%), Z-citral (neral) (21.57%) and D-limonene (18.82%). The high toxicity of citral and D-limonene was reported against several stored product insects such as lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrychidae), maize weevil *S. zeamais* (Coleoptera: Curculionidae), cigarette beetle Lasioderma serricorne (Coleoptera: Anobiidae) and flat grain beetle *Cryptolestes pusillus* (Coleoptera: Laemophloeidae) [34; 27]. The high volatility of this toxic mono and sesquiterpene compounds likely delivered fumigant toxicity by vapor action via the respiratory system, but further work is

Table 3: LC_{50} and LC_{99} values of the fumigation toxicity of *L. cubeba* essential oil and its two main active ingredients against the adults of *S. oryzae* and *T. castaneum*

Insects	Treatments	Time (h)	LC_{50} (µl/l air)	LC_{99} (µl/l air)	Slop±SD	Chi Square (÷^{2})	p-Value	R.
S. oryzea	Essential oil	24 h	7.51	442.79	1.31±0.24	4.63	0.20	0.927
	Citral		7.75	1665.00	0.99±0.24	1.85	0.60	0.948
	D-limonene		59.57	7447.48	1.10±0.28	3.99	0.26	0.893
T. castaneum	Essential oil	24 h	253.66	5670.34	1.72±0.28	7.98	0.04	0.914
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	D-limonene		288.24	54622.47	1.02±0.25	0.51	0.91	0.984
	Essential oil	72 h	130.72	2297.08	1.86±0.26	10.47	0.01	0.918
	Citral		151.43	9908.57	1.28±0.25	3.54	0.31	0.941
	D-limonene		347.47	14320.03	1.44±0.28	3.01	0.38	0.944
	Essential oil	144 h	81.68	1454.31	1.86±0.25	6.21	0.10	0.946
	Citral		97.63	2375.22	1.67±0.25	11.59	0.008	0.558
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[a] Dose (µg/L air); mean mortality of the control with n-hexane ≤1%.

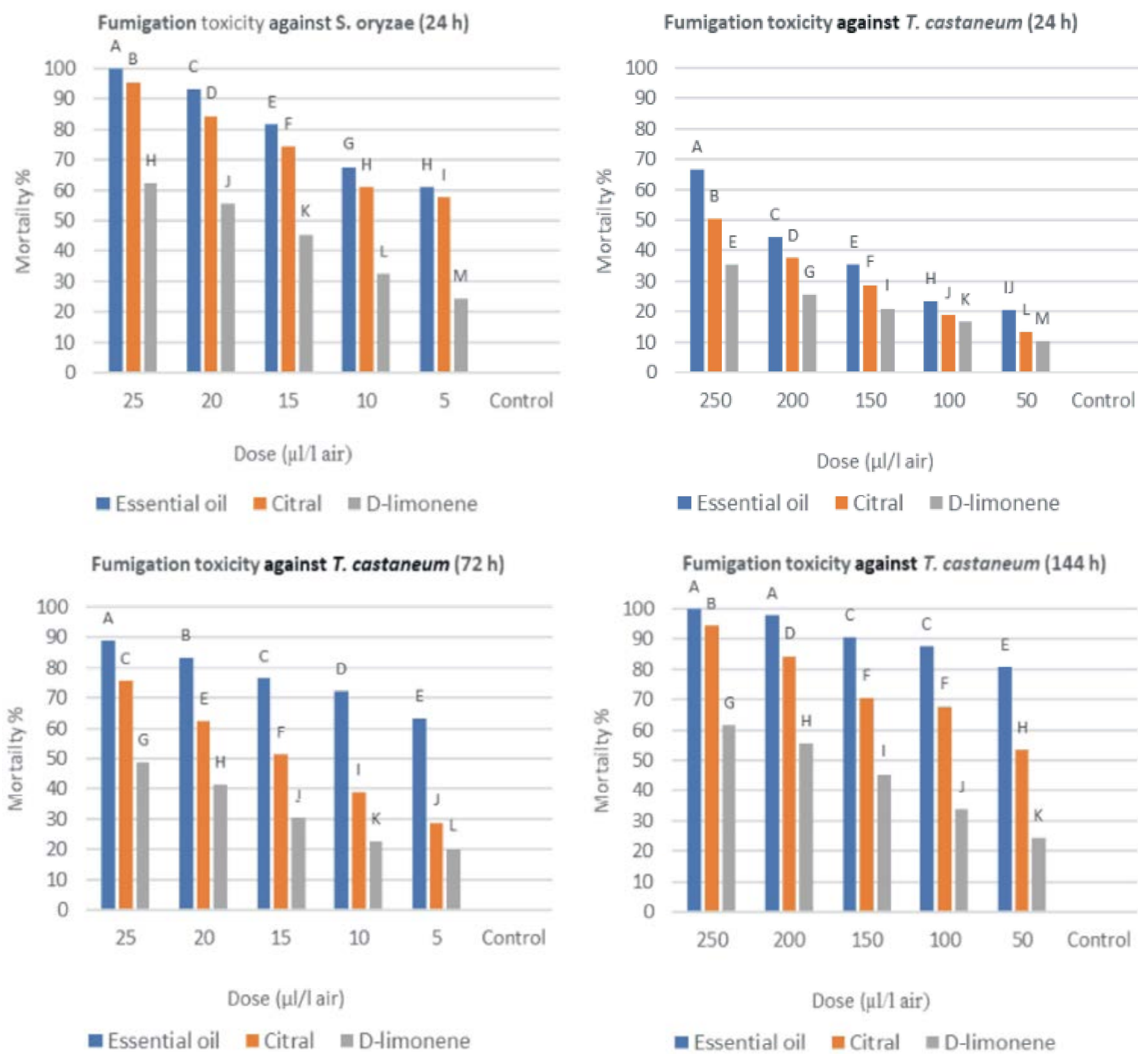

Fig. 2: Mortality percentages of the fumigation toxicity of *L. cubeba* essential oil and its two main active ingredients against the adults of *S. oryzae* and *T. castaneum* after exposure period. Letters above columns indicate significant differences between doses. Columns with the same litters are not significantly different

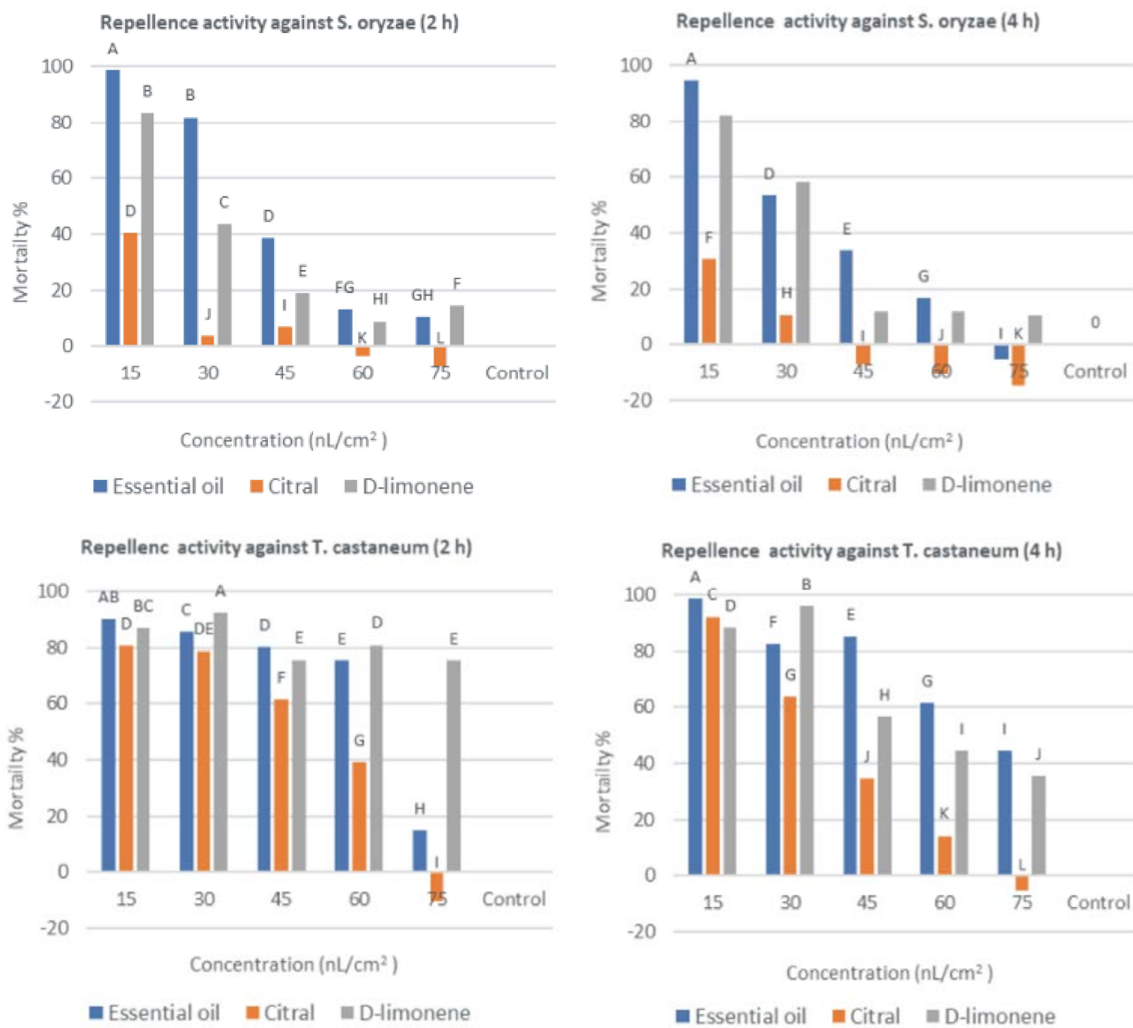

Fig. 3: Repellency percentages (RP) of *L. cubeba* essential oil and its two main active components against *S. oryzae* and *T. castaneum* at 2 and 4 h after exposure. Letters above columns indicate significant differences between doses. Columns with the same letter are not significantly different.

needed to confirm their extract mode of action. Among the five compounds, linalool and D-limonene have been demonstrated to be potent inhibitors of acetylcholinesterase (AChE) activity from several stored-product insects [36]. Considering that the currently used fumigants are synthetic insecticides, fumigant activities of the crude essential oil and citral are quite promising and they showed potential for development as possible natural fumigants for the control of stored product insects. However, for the practical application of the essential oil/compounds as novel fumigants, further studies on the safety of the essential oil/compounds to humans and development of formulation are necessary to improve the efficacy and stability and to reduce cost. Considering that the currently used fumigants are synthetic insecticides, fumigant activities of the crude essential oil and citral are quite promising and they show potentiality for development as possible natural fumigants for the control of stored product insects. However, for the practical use of the essential oil/compounds as novel fumigants, further studies on the safety of these compounds to humans and the development of formulation are needed.

Repellent Activity: The repellent activities of the *L. cubeba* and two main active ingredients against *S. oryzae* and *T. castaneum* adults were evaluated using the area preference method after two and four h post-treatment (Fig. 3). The essential oil, citral and D-limonene showed 98.60; 40.53 and 83.33% repellency against *S. oryzae* adults after two h exposure at 75 nL/cm^2. While after four h post-treatment, the repellence activity decreased at the same concentrations by 94.46, 30.70 and 82.26% for the essential oil, citral and D-limonene, respectively. Also, the repellence activity decreased gradually by the decreasing of the sample concentration, At 45 nL/cm^2 the repellence activity of *L. cubeba* essential oil, citral and D-limonene were 38.76; 6.86 and 18.90 %, respectively after two h post-treatment. Citral produced lowest repellency level after two and four hours with all the concentrations, while the essential oil and D-limonene showed strongest repellence activity against *S. oryzae*. (Fig. 3). On the other hand, the repellence activities of the essential oil of *L. cubeba* and isolated constituents to *T. castaneum* adults were tested using the same way after two and four hours post-treatment (Fig. 3). At the highest concentration 75 nL/cm^2, the *L. cubeba* essential oil showed strong repellency level (90.20% and 98.60%) after two and four hours post-treatment, respectively against *T. castaneum* adults. Its activity decreased gradually by decreasing the sample concentration. As well, at lowest concentration 15 nL/cm^2, the repellence response of *T. castaneum* adults to *L. cubeba* essential oil decreased significantly compared to the higher concentrations (14.76% and 44.53%) at two and four hours, respectively. At all the assayed concentrations, citral exhibited strong repellency level against *T. castaneum*. At 75 nL/cm^2 citral produced strong repellency at two and four hours post-treatment (80.86% and 92.13%, respectively). By decreasing the concentration, the activities of citral were reduced gradually, at 15 nL/cm^2 citral produced lower repellency level at two and four hours post-treatment (10.26% and -5.16%, respectively). More adults were found surviving on areas treated by citral at the lowest concentrations (15 nL/cm^2), indicating that citral may attract *T. castaneum* adults at the low level. While D-limonene exhibited high repellency against *T. castaneum*. At the highest concentration (75 nL/cm^2), D-limonene only showed 87.03% and 88.50% repellency at two and four hours after exposure. While with the decreasing of the concentration, the activity of D-limonene was gradually reduced.

Many essential oils and their constituents have been evaluated for repellency against insects [37]. Based on the previous reports, the essential oil of *L. cubeba* fruits have also been found to be repellent against some insects, e.g., mosquitoes *A. aegypti*, red flour beetles *T. castaneum*, maize weevils *S. zeamais* [15]. In the present study, that the repellency activities of the essential oil and the two copmponents of *L. cubeba* collected from Wuhan, China against *S. oryzea* and *T. castaneum* are reported. These findings, when considered together, suggest that the essential oil and the five compounds showed potentialities for development as natural repellent materials against stored product insect pests.

CONCLUSION

The chemical composition of the essential oil from *L. cubeba* fruits was analyzed. The contact, fumigant and repellent activities of the essential oil and two main active ingredients against two stored-product insects were estimated. This work investigated the insecticidal bioactivity of the essential oil of *L. cubeba* fruits and two main active ingredients against rice weevil, *Sitophilus oryzae* and the red flour beetle, *Tribolium castaneum*. However, further studies are needed to focus on the safety of the studied essential oil on vertebrates and to improve the potency and stability of these crop protection products for practical use.

REFERENCES

1. Stejskal, V., J. Hubert, R. Aulicky and Z. Kucerova, 2015. Overview of present and past and pest-associated risks in stored food and feed products: European perspective. J. Stored Prod. Res., 64: 122-132.
2. Wondatir, Z., A. Adie and A.J. Duncan, 2015. Assessment of livestock production and feed resources at Robit Bata, Bahir Dar, Ethiopia. http://hdl.handle.net/10568/56988 .
3. Fields, P.G., 2006. Effect of *Pisum sativum* fractions on the mortality and progeny production of nine stored-grain beetles. J. Stored Prod. Res., 42: 86-96.
4. Lorini, I. and A.F. Filho, 2004. Integrated pest management strategies used in stored grain in Brazil to manage phosphine resistance. In: Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast Australia, pp: 293-300.
5. Lucas, E. and J. Riudavets, 2002. Biological and mechanical control of *Sitophilus oryzae* L. (Coleoptera: Curculionidae) in rice. J. Stored Prod. Res., 38(3): 293-309.
6. Baker, J.E., F.E. Dowell and J.E. Throne, 1999. Detection of parasitized rice weevils in wheat kernels with near-infrared spectroscopy. Biol. Cont., 16: 80-90.

7. Ajayi, F.A. and S.A. Rahman, 2006. Susceptibility of some staple processed meals to red flour beetle, *Tribolium castaneum* (Herbst) (*Coleoptera: Tenebrionidae*). Pak. J. Biol. Sci., 9: 1744-1748.
8. Magan, N., R. Hope, V. Cairns and D. Aldred, 2003. Post-harvest fungal ecology; impact of fungal growth and mycotoxin accumulation in stored grain. European J. Plant Pathology, 109: 723-730.
9. Zettler, J.L. and F.H. Arthur, 2000. Chemical control of stored product insects with fumigants and residual treatments Crop Protection, 19: 577-582.
10. Phillips, T.W. and J.E. Throne, 2010. Biorational approaches to managing stored-product pests. Ann Rev Entomol., 55: 375-97.
11. Isman, M.B., 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51: 45-66.
12. Rajendran, S. and V. Srianjini, 2008. Plant products as fumigants for stored product insects control. J. Stored Prod. Res., 44: 126-135.
13. Zhang, J.S., N.N. Zhao, Q.Z. Liu, Z.L. Liu, S.S. Du, L. Zhou and Z.W. Deng, 2011. Repellent constituents of essential oil of *Cymbopogon distans* aerial parts against two stored-product insects. J. Agric. Food Chem., 59: 9910-9915.
14. Ko, K., W. Juntarajumnong and A. Chandrapatya, 2009. Repellency, fumigant and contact toxicities of *Litsea cubeba* (Lour.) Persoon against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). Kasetsart Journal, 43: 56-63.
15. Noosidum, A., A. Prabaripai, T. Chareonviriyaphap and A. Chandrapatya, 2008. Excitorepellency properties of essential oils from Melaleuca leucadendron L., (Lour.) Persoon and *Litsea salicifolia* (Nees) on *Aedes aegypti* (L.) mosquitoes. J. Vector Ecol., 33: 305-312.
16. Zhang, W., Z. Zhang, Z. Chen, J. Liang, Z. Geng, S. Guo and Z. Deng, 2017. Chemical composition of essential oils from six *Zanthoxylum* species and their Repellent activities against two stored-product insects. J. Chemistry, Article ID 1287362, pp: 7.
17. Nor Azah, M.A. and S. Susiarti, 1999. *Melaleuca cajuputi* Powell, pp: 126-131 In P.C.M. Janson, E. Westphal and N. Wulijarni-Soetipto (eds.). Plant Resources of South East Asia. Vol. 19. Procea Foundation, Bogor, Indonesia.
18. Papachristos, D.P. and D.C. Stamopoulos, 2004. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae), J. Stored Prod. Res., 38: 117-128.
19. Laribi, B., K. Kouki, A. Mougou and B. Marzouk, 2010. Fatty acid and essential oil composition of 3 Tunisian caraway (*Carum carvi* L.) seed ecotypes. J. Sci. Food Agric., 90: 391-396.
20. Samojlik, I., N. Lakic, K. Mimica-Dakovic-Svajcer and B. Bozin, 2010. Antioxidant and hepatoprotective potential of essential oils of coriander (*Coriandrum sativum* L.) and caraway (*Carum carvi* L.) (Apiaceae). J. Agric. Food Chem., 58: 8848-8853.
21. Liu, Z.L. and S.H. Ho, 1999. Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook against the grain storage insects, *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst). J. Stored Prod. Res., 35: 317-328.
22. Sakuma, M., 1998. Probit analysis of preference data. Appl. Entomol. Zool., 33: 339-347.
23. Chaubey, M.K., 2007. Insecticidal activity of *Trachyspermum ammi* (Umbelliferae), Anethum graveolens (Umbelliferae) and Nigella sativa (Ranunculaceae) essential oils against stored-product beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Afr. J. Agric. Res., 2: 596-600.
24. Abott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
25. Finney, D.J., 1971. "Probit Analysis". Cambridge University Press, Cambridge, London, pp: 333.
26. Steel, R.G., J.H. Torrie and D. Dickey, 1997. Principles and Procedure of Statistics. A Biometrical Approach 3rd Ed. McGraw Hill BookCo. Inc., New York, pp: 352-358.
27. Yang, K., C.F. Wang, C.X. You, Z.F. Geng, R.Q. Sun, S.S. Guo, S.S. Du, Z.L. Liu and Z.W. Deng, 2014. Bioactivity of essential oil of *Litsea cubeba* from China and its main compounds against two stored product insects. J. Asia-Pacific Entomology, 17: 459-466.
28. Si, L.L., Y.C. Chen, X.J. Han, Z.Y. Zhan, S.P. Tian, Q.Q. Cui and Y.D. Wang, 2012. Chemical composition of essential oils of *Litsea cubeba* harvested from its distribution areas in China. Molecules, 17: 7057-7066.

29. Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils - A Review. Food and Chemical Toxicology, 46: 446-475.
30. Wang, Y., Z.T. Jiang and R. Li, 2009. Complexation and molecular microcapsules of *Litsea cubeba* essential oil with β-cyclodextrin and its derivatives. Eur. Food Res. Technol., 228: 865-873.
31. Chen, C.J., Y.H. Tseng, F.H. Chu, T.Y. Wen, W.W. Cheng, Y.T. Chen, N.W. Tsao and S.Y. Wang, 2012. Neuropharmacological activities of fruit essential oil from *Litsea cubeba* Persoon. J. Wood Sci., 58: 538-543.
32. Lee, E., J. Kim, J.R. Choi and D.R. Ahn, 2008. Toxicity of cassia and cinnamon oil compounds and cinnamaldehyde-related compounds to *Sitophilus oryzae* (Coleoptera: Curculionidae). J. Econ. Entom. 101: 1960-1966.
33. Tapondjou, A.L., C. Adler, D.A. Fontem, H. Bouda and C. Reichmuth, 2005. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. J. Stored Prod. Res., 41: 91-102.
34. Suthisut, D., P.G. Fields and A. Chandrapatya, 2011. Contact toxicity, feeding reduction and repellency of essential oils from three plants from the ginger family (Zingiberaceae) and their major components against *Sitophilus zeamais* and *Tribolium castaneum*. J. Econ. Entom., 104: 1445-1454.
35. Regnault-Roger, C. and A. Hamraoui, 1995. Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). J. Stored Prod. Res., 31: 291-299.
36. Lopez, M.D. and M.J. Pascual-Villalobos, 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Ind. Crop Prod., 31: 284-288.
37. Caballero-Gallardo, K., J. Olivero-Verbel and E.E. Stashenko, 2011. Repellent activity of essential oils and some of their individual constituents against *Tribolium castaneum* Herbst. J. Agric. Food Chem., 59: 1690-1696.