doi: 10.11594/jtls.07.03.01
THE JOURNAL OF TROPICAL LIFE SCIENCE
OPEN ACCESS Freely available online
VOL. 7, NO. 3, pp. 189 – 196, September, 2017
Submitted May 2017; Revised June 2017; Accepted June 2017

Bioactivity of *Citrus hystrix* D.C. Leaf Extract Against Cigarette Beetle *Lasioderma serricorne* (F.)

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ABSTRACT

The major control of pest stored *Lasioderma serricorne* for using synthetic pesticides, look like phosphine. Although quite effective, synthetic pesticides have a negative effect on the environment, such as pest resistance, deadly natural enemies, residues that are harmful to the environment and human health. The use of botanical pesticides as an alternative control be the focus this study. Botanical pesticides are selective to the target, safe for non-target insects and the environment. This research studies the repellent and fumigant activity of the leaf extract of *Citrus hystrix* against all *L. serricorne* life stage. The results of GC-MS analysis of leaf crude extracts of *C. hystrix* with N-hexane solvent showed the highest percentage in the citronellal compound (86.43%). *C. hystrix* leaf extract showed stronger fumigant against pupae and eggs, compared adults, and larvae. The toxicity of the leaf extract of *C. hystrix* increased with increasing concentration. At a concentration of 60 ppm, fumigant activity showed the toxicity of 98.75% (pupae), 93.75% (eggs), 86.25% (adults) and 76.25% (larvae). Sequentially the LC50 value of fumigant activity from the highest to the lowest as follows; larvae 47.56 ppm, adults 43.42 ppm, eggs 31.61 and pupae 29.63 ppm. Extract of leaves of *C. hystrix*, have character repellent against *L. serricorne*. At a concentration of 60 ppm the IR value of 66% including repellent class IV, which means strong repellent level. Based on the results of the research, extracts of leaves of *C. hystrix* has a fumigant activity and repellent for controlling *L. serricorne*.

Keywords: *Lasioderma serricorne, botanical insecticide, Citrus hystrix, fumigant, repellent, life stage*

INTRODUCTION

One of the processes of post-harvest management is the storage and drying tobacco in the stored. Storage area affects the quality of the material stored. The stored tobacco leaf are can become the growing place of pest. *Lasioderma serricorne* known to breed on the kinds of seeds, spices, and mainly tobacco, as well as attacking commodities during storage and processing [1]. After eggs hatching, the larvae burrowing down into tobacco will eat small galleries through the tobacco material [2]. Various control measures against storage pests can be applied individually or in the integrated control program, which includes chemical, physical and biological control, environmental manipulation and the use of resistance varieties [3]. In the case of mass multiplication of stored product pests, which are frequently used are inorganic insecticide and contact insecticide [4].

Pest control stored which is often carried out is by the application of active pesticide ingredient with a phosphine fumigation method [5]. Although the use of fumigant and contact insecticide active ingredient phosphine is very effective, it has become the world's attention due to its negative effects, such as ozone depletion, environmental pollution, kill insect non-target, pest resistance, and pesticide residues are left behind [6]. The development of resistance to phosphine in *L. serricorne* has occurred [7]. Control alternative indicated by the plant products, namely, essential oils and their components [8]. The majority of the active ingredients secondary metabolites issued on plants as chemical defenses against pest organisms [9]. The natural compounds from plant sources are believed to have the advantage over conventional pesticides regarding toxicity to mammals is low, the rapid degradation and local availability [8]. In the context of agricultural pest management, botanical insecticides are most suitable for use in the pro-

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How to cite:
Ikawati S, Dhuha MS, Himawan T (2017) Bioactivity *of Citrus hystrix* D.C. Leaf Extract Against Cigarette Beetle *Lasioderma serricorne* (F.). J. Trop. Life. Science 7 (3): 189 – 196.

duction of organic foods in industrialized countries, but it can play a greater role in the production and post-harvest food protection in developing countries [10].

Storage pest control with botanical pesticides may provide some bioactivity [11]. Such compounds can act as a fumigant [6], a contact insecticide [6, 12], antifeedant [13, 14], as a repellent [11, 15], and also may result in some biological parameters such as growth rate, lifespan, and reproduction [11, 13, 16]. Fumigation and repellent activity plays a major role in controlling insect pests in stored products. Ability insecticide plant essential oils that are contact and fumigant have been widely demonstrated against stored product pests. Some plant compounds have been tested against insects that attack stored products in order to develop new control techniques [17, 18].

Kaffir lime (*Citrus hystrix* D.C.) is one of the plants is known to have a high level of essential oil that can be used as botanical pesticides. Toxicity test against corn beetle (*Sitophilus zeamais* Motschulsky) using oil extract 30 to 120 uL of *C. hystrix* resulted in 100% beetle mortality in the 5 hour test period [19]. Therefore, this research aimed to study the activity of the fumigant and repellent leaf extract of *C. hystrix* against tobacco beetle *L. serricorne*.

MATERIALS AND METHODS

Insects rearing

Cigarette beetles were collected from infested, stored, tobacco and reared in a 600 mL plastic container containing oatmeal and yeast (95%: 5%) were mixed thoroughly. Rearing conducted in the laboratory rearing with a temperature of 27 ± 2°C and a relative humidity of 75 ± 5%. After oviposition, the insect parents were removed. The new emerging adults were used for rearing back and test insects.

Preparation of the extract

Extraction of *C. hystrix* (Figure 1) carried out by maceration method. The leaves of *C. hystrix* cleaned then dried for 24 hours at room temperature. It aims to reduce the water content, thereby speeding up the process of extraction and more resistant to microbes. Lime leaves cut into small pieces with a knife. Maceration performed with material and solvent ratio of 1: 4. Extraction leaves of *C. hystrix* using non-polar solvents, n-hexane 96% p.a. Then stirring using an orbital shaker at 120 rpm for 24 hours. Maceration results were filtered using filter paper Whatman no. 1. After filtered, the extraction is then separated from the n-hexane using a rotary vacuum evaporator. Evaporator temperature is set around 60°C. The evaporated then filtered and put into jars and sealed. It is intended that the filtrate does not evaporate, and then stored in a refrigerator at 4°C.

Gas chromatography/mass spectrometry (GCMS)

Chemical constituents were analyzed by gas chromatography-mass spectrometry (GC-MS). GCMS analysis: GCMS analysis of N-hexane plants extract was determined by GCMS Shimadzu Model QP-2010 Plus Mass Spectrometer under the following conditions: DB – Polyethylene Glycol coated fused silica capillary column (30 m length × 0.25 mm ID × 0.25 µm film thickness): Helium Carrier Gas (1.34 mL/minute); 250°C injector temperature; 240°C interface temperature; 200°C Ion Source Temperature. Column temperature programmed at 60°C with 10°C / minute rise to 230°C. For GCMS detection an ionization energy of 70 ev was used. As much as 50 mg of N-Hexane leaf extract sample were taken separately and made up to 10ml with N-hexane, from which 1 µL of the sample was injected (split mode) in the column. The components were identified based on a comparison of the retention times with those found in the literature and on computer matching spectral MS data against Wiley 8 Library (comparison quality > 80%).

Fumigant activity

Larvae, pupae, and adults. Testing fumigant activity against *L. serricorne* done by exposing as many as 20 larvae (age 10 – 12 days), 20 pupae (aged 1 – 2 days), and 20 adults, without distinction of male and female (age 5 – 7 days) on each concentration tested. Each concentration of dissolved in 1 mL of acetone. The concentration is 0 (control), 20, 30, 40, 50, and 60 ppm. Tests conducted in a glass bottle of 250 mL.

Each concentration is dripped evenly on a piece of

Figure 1. Kaffir lime (*C. hystrix* D.C.)

filter paper with a size of 7 × 9 cm and dried (10 minutes). Then hung on a bottle cap with a pin. Filter paper mounted such that it is not in contact with the wall. After the test bottles stored indoors at a temperature of 27 ± 2°C and a relative humidity of 65 ± 5%. After exposure for 48 hours, respectively (larvae, pupae, and adults) were transferred into Petri dishes. Mortality of *L. serricorne* larvae and adults as soon as possible were observed, the insect is considered dead when touched does not show move. Pupae observed daily for 10 days, the emerging adults were recorded and pupae not be/reach adults stage considered dead.

Eggs

Testing the activity of the fumigant against eggs is done by exposing the eggs (age 0 – 24 hours) at each concentration tested. Testing the eggs is laid on a round plate cloning. The tool is made of mica acrylic with a size of 5 × 4 cm by the hole. Each round consists of 20 cloning microwell plates (D: 3 mm; inside 2 mm). Each microwell is filled one egg, each test used as many as 20 eggs. The plate that already contains eggs is stored in 750 mL jars. Furthermore, the procedures for treatment of such steps on the previous treatment. After 48 hours, the plate was transferred to the maintenance of a clean environment. Observations of hatching eggs done under a stereo microscope after 10 days and the eggs that did not hatch considered dead.

All the number of dead insects in every stage were recorded and percentage of insect mortality were calculated. If there were dead insects in the controls, the percentage of mortality needs to be corrected using Abbot's formula [20].

Repellent activity

Observation of repellent activity conducted on each concentration used Petri dish olfactometer (Figure 2). Each petri dish olfactometer consists of treatment and control. Each concentration is dripped into pieces of filter paper (4 × 7 cm). The concentration is 20, 30, 40, 50, and 60 ppm. For control treatment only drops of acetone. Then the filter paper is inserted into the bottle, and the bottle sealed with a cap on olfactometer. Each test using 20 adults of *L. serricorne* and placed in the middle of a petri dish. Petri dishes sealed with parafilm coated. Observations made the response after 2 hours by counting the number of insects in response to each treatment and control.

Variable observations observed in repellency test is to count the number of insects in response to the control and treatment. To determine the response of test insects repellent can be calculated using the formula calculating the value Repellent Index (IR) based on the Pascual-Villalobods and Robledo's formula [21]:

$$IR = \{(C - T)/(C + T)\} \times 100\%$$

Note:
IR: Repellency Index (%)
C: Number of insects in response to the control
T: Number of insects that the response to treatment

If the IR value is positive, it indicates repellent properties, while IR negative value indicates of the attractant

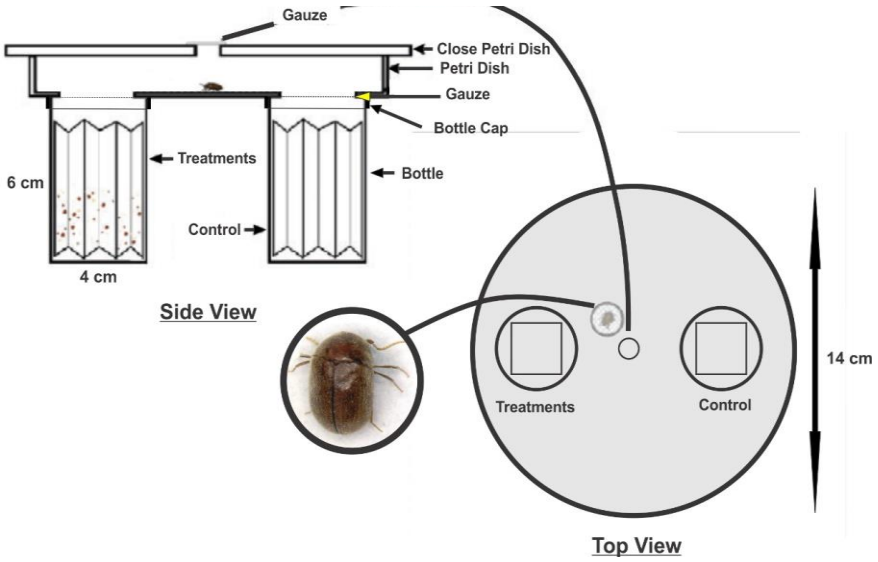

Figure 2. Petri dish olfactometer

properties [21]. To determine the level of repellency used the following criteria [22].

Statistical analysis

All the percentage of mortality data were subjected to statistical analysis without transformation using Analysis of Variance (ANOVA). If it is significantly different, then the test continued using the Least Significant Difference test (LSD) at level 5% error by using *SPSS v21*. Median Lethal Concentration LC_{50} from fumigation test on the eggs, larvae, pupae, and adults calculated using Probit Analysis program [23]. The data Repellent Index (IR) analysis with Kruskal-Wallis nonparametric analysis using *SPSS v21* application.

RESULTS AND DISCUSSION

Chemical constituents

Based on the test results of GC-MS, the chemical constituents of *C. hystrix* leaf extracts are shown in Table 1. GCMS analysis of *C. hystrix* leaf extracts obtained four constituents were identified, with a total of 100% (Table 1). In detail, the leaf extract of *C. hystrix* showed the highest percentage in the compound citronellal (86.43%), citronellol (11.48%), beta-linalool (1.65%), and sabinen (0.44%) respectively. Different types of plants extracts showed differences in the number and percentage of chemical constituents [24]. Essential oils of plants may contain hundreds of different constituencies, but certain components will be present in larger quantities [9]. Acyclic look like linalool and citronellal also make the components of essential oils [25].

The major constituent in *C. hystrix* was β-citronellal, representing 66.85% of the total oil with more than four constituent results [26]. In this study, the major component also characterized β-citronellal, but with a higher percentage (86.43%). (-)-Citronellal was main constituent (81%) of the *C. hystrix* leaf oil [27]. In another study, β-citronellal was not found in the *C. hystrix* oil [19]. The different in constituent characterization results might be caused the difference in technique for extraction, a variety of *C. hystrix*, and the difference in cultural practices. Furthermore, it has been reported that overall toxic effect of plant extracts may be contributed by many chemical constituents [28]. The mechanism for underlying the possible interaction among constituents is important to be understood by determining the toxic effect of each chemical constituents of plant extract by applying them individually or in combination on the insect test.

Table 1. Chemical constituents analysis of *C. hystrix* leaf extracts by GCMS

Retention time (min)	*Compound name*	*Percentage (%)*
6.565	Sabinen	0.44
8.576	Beta-Linalool	1.65
9.480	Beta-Citronellal	86.43
10.634	Citronellol	11.48

Fumigant toxicity

Fumigant toxicity assay of *C. hystrix* leaf extracts on adults, pupae, larvae, and eggs of *L. serricorne* performed during 48 hours of exposure showed that *C. hystrix* leaf extract has strong fumigant toxicity against all stages of *L. serricorne* (Figure 3). The constituents of plants volatiles could enter test insects either through the cuticle or the spiracle [29]. The probable reason for the death of insects when exposed to volatiles compounds could be either due to interference in gaseous exchange in respiration or asphyxiation [30]. Fumigant activity of the extract compounds cause interference in the respiratory system and can increase the activity of sensory nerves are high, leading to death due to disruption of some insect body systems [31].

The best concentration contained at 60 ppm as the mean percentage mortality of more than 50% in all stages of *L. serricorne*. The percentage of insect mortality from the highest were at pupae, eggs, adults and larvae stage, which the percentage sequentially, 98.75%, 93.75%, 86.25%, and 76.25%. This means pupae and eggs stage are more vulnerable and sensitive than larvae and adults stage. Wherein, the LC50 value from the lowest was at pupae eggs stages (29.63 ppm), (31.61 ppm), adults (43.42 ppm), and larvae (47.56 ppm) (Table 2). The smaller value of LC50 means the more effective. The other study showed that the toxic effect of the *Elsholtzia stauntonii* extract influenced by treatment dose and stage of insect development [32].

The surface of the eggs chorionic *L. serricorne* contained aeropyl and also micropyl located at the poles eggs [33]. Micropyl is a hole, which the eggs of *L. serricorne* contains micropyl between 7 until 11 with the functions as breathing hole [34]. Because of that the fumigant activity of *C. hystrix* leaf extracts could entering through aeropyl, and micropyl holes, which caused the eggs failed to grow and eventually die. *L. serricorne* insect very active during larvae and adults stage, because the stage of larvae lots foraging to grow and develop [35]. Meanwhile, when the adult's stage is sucking fluids and eat a bit less, its makes larvae and adults more resistant than the eggs and pupae stage.

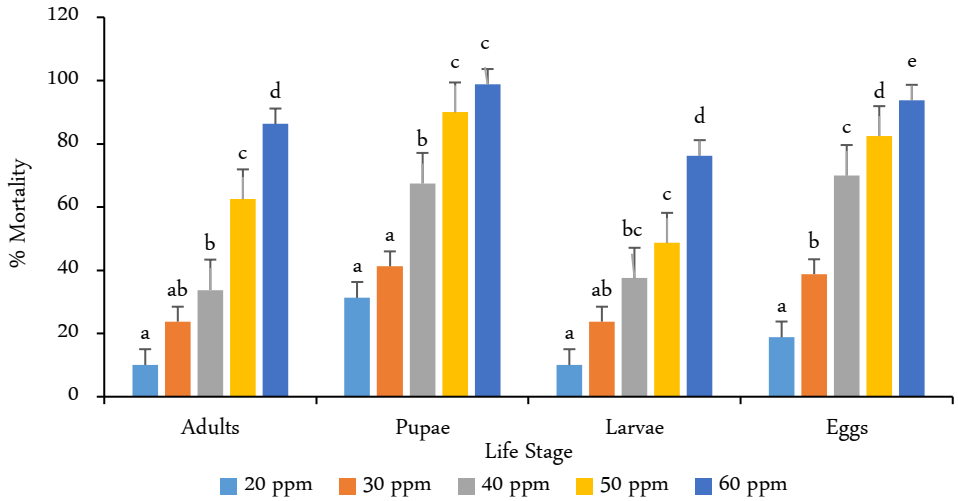

Figure 3. Fumigant toxicity of five level concentration of *C. hystrix* leaf extracts against *L. serricorne* after 48 hours. Note: the levels in the five concentrations block no connected by the same letter are significantly different ($P \leq 0.05$) between concentrations based on LSD test (Error bars indicate the Standard deviation of means)

Table 2. Lethal concentration of *C. hystrix* leaf extracts on four stages of *C. ferrugineus* after a 48-h exposure period

Phase	*Regression equations*	*SE*	*LC_{50} (ppm)**	*LC_{90} (ppm)*	*Limits LC_{50} (ppm)**	
					Under	*Upper*
Adults	y = -2.9038 + 4.8264 x	0.9897	43.42	80.02	33.06	64.26
Pupae	y = -1.6892 + 4.5449 x	0.8992	29.63	56.72	18.95	37.35
Larvae	y = -1.5641 + 3.9136 x	0.9320	47.56	101.10	38.92	68.28
Eggs	y = -2.3121 + 4.8751 x	0.9193	31.61	57.91	27.13	35.68

* LC_{50} and LC_{90} = the concentration of which 50% and 90% of the insects died

Nevertheless, these study results show the potential of *C. hystrix* leaf extracts in controlling the cigarette beetle, with the best time for application is at pupae stage. Formulation of plants extracts and application trials in extensive tobacco storage are needed to establish the practicality and effectiveness of plants extracts as cigarette beetle controlling technique. The advantage of using volatile oils are not particularly dangerous to consumers because they are removed during washing and easily evaporate during cooking [36].

Repellent activity

The response of *L. serricorne* adults on repellent activity test based on Pascual-Villalobods and Robledo's formula obtained positive IR value (Table 3). This indicates that the leaf extract of *C. hystrix* tested on *L. serricorne* is repellent at all concentrations. The ethanolic extract from *C. hystrix* has the highest repellent activity to Sitophilus oryzae compared with mintweed and kitchen mint [37]. Statistical analysis Kruskal-Wallis nonparametric generate value P-value less than 0.05 α. It shows that the extract of leaves of *C. hystrix* application significantly affects the response of *L. serricorne* who refused (repellent). Differences in IR caused by different levels of concentration, which higher the concentration the higher of its IR value (Figure 4). Based on the coefficient of determination R_2 0.9766 which is close to 1, can be interpreted that the concentration and IR have a strong relationship. These values indicate that the IR is affected by the level of concentration of extract of leaves of *C. hystrix* and both affect each other by 97.66%.

Response *L. serricorne* who repellent due to their smell disliked by insects. The smell is a compound leaf extract of *C. hystrix* vaporized. The content of *C. hystrix* leaf extract contains compounds citronellal and citronellol which has repellent properties. So when contained insects that approached the smell of the insects will stay away because they do not like the smell. The odor can be responded insects through the respiratory system of insects. The properties of citronellol and geraniol that make repellency effect against mosquitoes [38]. In the present study, the lowest IR value was obtained at a concentration of 20 ppm, so in the lowest class. While the

Tabel 3. The repellent index of *C. hystrix* leaf extract against *L. serricorne* adults

Concentration (ppm)	The mean value IR (%)	Class IR*
20	8	I
30	30	II
40	40	II
50	58	III
60	66	IV

* Repellency class: 0 = < 0.1 % IR, I = 0.1 to 20 % IR, II= 20.1 to 40 % IR, III= 40.1 to 60 IR, IV=60.1 to 80% IR, and V = 80.1 to 100% repellency index [22].

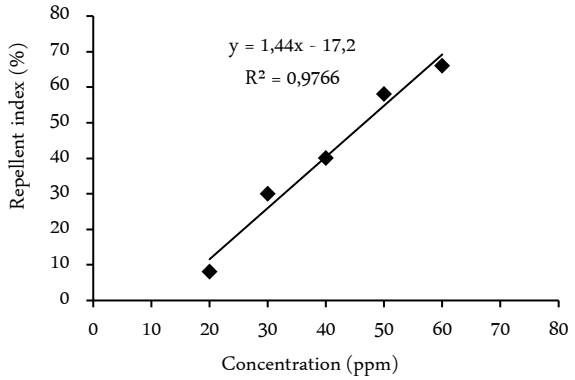

Figure 4. The relation between the concentrations with IR

value of IR and the highest class present in concentrations of 60 ppm which are included in the level strong repellent (Class IV). In another study, essential oil of Kaffir lime based on IR also categorized ad class IV [19].

However, this study shows that at appropriate rates of application, the *C. hystrix* leaf extract could exhibit both fumigant and repellent toxic actions against the cigarette beetle. The studies about the effectiveness period (LT_{50}) and formulation development for weevil control by *C. hystrix* leaf extract must be determined to reduce cost. However, further studies under bulk storage conditions and safety of the extract in other organisms also needed before recommending the large-scale use of these plants extracts.

CONCLUSION

Based on the results of the study the bioactivity of *C. hystrix* leaf extract has a fumigant activity against adults, pupae, larvae and eggs *L. serricorne*. Sequentially LC_{50} value fumigant activity highest to lowest as follows; larvae 47.56 ppm, adults 43.42 ppm, eggs 31.61 and pupae 29.63 ppm. Leaves extract of *C. hystrix* also are repellent against *L. serricorne* with the best repellent index value contained at a concentration of 60 ppm with an index value of 66% repellent and belongs to a class IV repellent which means it has a strong repellent level. It shows that *C. hystrix* leaf extract has great potential as one of the stored pest control components.

ACKNOWLEDGMENT

This research was financially support by University of Brawijaya Malang through BOPTN research grant 2015.

REFERENCES

1. Dimetry NZ, Barakat AA, El-Metwaly HE et al. (2004) Assessment of damage and losses in some medicinal plants by the cigarette beetle (*Lasioderma serricorne* F.). Bulletin of the national Research Centre Egypt 29: 325 – 333.
2. Ashworth JR (1993) The biology of *Lasioderma serricorne*. Journal of Stored Products Research 29 (4): 291 – 303. doi: 0.1016/0022-474X(93)90044-5.
3. Lo PK (1986) Recognition and control of insect pests in stored grains. Post-harvest prevention of paddy/rice loss 171-186. Taipe, Taiwan Council of Agriculture - Asian Productivity Organization.
4. Gasch T (2014) The use of semiochemicals for stored product protection. In Conference of Siebentes Nachwuchswissenschaftlerforum: 26 – 28 November 2014; Quedlinburg, Germany.
5. Imai T, Harada H (2006) Low-temperature as an alternative to fumigation to disinfect stored tobacco of the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). J. Applied Entomology and Zoology 41: 87 – 91. doi: 10.1303/aez.2006.87.
6. Shaaya E, Kostjukovski M, Eilberg J, Sukprakarn C (1997) Plant oils as fumigants and contact insecticides for the control of stored-product insects. Journal of Stored Products Research 33 (1): 7 – 15. doi: 10.1016/S0022-474X(96)00032-X.
7. Rajendran S, Narasimhan KS (1994) Phosphine resistance in the cigarette beetle *Lasioderma serricorne* (Coleoptera: Anobiidae) and overcoming control failures during fumigation of stored tobacco. International Journal of Pest Management 40 (2): 207 – 210. doi: 10.1080/09670879409371883.
8. Ebadollahi A, Safaralizadeh MH, Pourmirza AA (2010) Fumigant toxicity of *Lavandula stoechas* L. oil against three insect pests attacking stored products. Journal of Plant Protection Research 50 (1): 56 – 60. doi: 10.2478/v10045-010-0010-8.
9. Rajendran S, Sriranjini V (2008) Plant products as fumigants for stored-product insect control. Journal of Stored Products Research 44 (2): 126 – 135. doi: 10.1016/j.jspr.2007.08.003.
10. Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated

world. Annual Review of Entomology 51: 45 – 66. doi: 10.1146/annurev.ento.51.110104.151146.

11. Papachristos DP, Stamopoulos DC (2002) Repellent, toxic and reproduction inhibitory effect of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Products Research 38 (2): 117 – 128. doi: 10.1016/S0022-474X(01)00007-8.
12. Weaver KD, Dunkel VF, Potter CR, Ntezurubanza L (1994) Contact and fumigant efficacy of powdered and intact *Ocimum canum* Sims (Lamiales: Lamiaceae) against *Zabrotes subfasciatus* (Boheman) adults (Coleoptera: Bruchidae). Journal of Stored Products Research 30 (3): 243 – 252. doi: 10.1016/0022-474X(94)90052-J.
13. Saxena RC, Dixit OP, Harshan V (1992) Insecticidal action of *Lantana camara* against Callosobruchus chinensis (Coleoptera: Bruchidae). Journal of Stored Products Research 28 (4): 279 – 281. doi: 10.1016/0022-474X(92)90009-F.
14. Huang Y, Tan JMWL, Kini RM, Ho SH (1997) Toxic and antifeedant action of nutmeg oil against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Journal of Stored Products Research 33 (4): 289 – 298. doi: 10.1016/S0022-474X(97)00009-X.
15. Ndungu M, Lwande W, Hassanali A et al. (1995) *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellents. Entomologia Experimentalis et Applicata 76 (3): 217 – 222. doi: 10.1111/j.1570-7458.1995.tb01965.x
16. Regnault-Roger C (1997) The potential of botanical essential oils for insect pest control. Integrated Pest Management Reviews 2 (1): 25 – 34. doi: 10.1023/A:1018472227889.
17. Regnault-Roger C, Hamraoui A (1994) Inhibition of reproduction of Acanthoscelides obtectus Say (Coleoptera), a kidneybean (*Phaseolus vulgaris*) bruchid, by aromatic essential oils. Crop Protection 13 (8): 624 – 628. doi: 10.1016/0261-2194(94)90009-4.
18. Paranagama P, Abeysekera T, Nugaliyadde L, Abeywickrama K (2003) Effect of the essential oils of Cymbopogon citratus, Cymbopogon nardus and Cinnomomum zeylanicum on pest incidence and grain quality of rough rice (paddy) stored in an enclosed seed box. Journal: Food, Agriculture and Environment 1 (2): 134 – 136.
19. Kerdchoechuen O, Laohakunjit N, Singkornard S, Matta FB (2010) Essential oils from six herbal plants for biocontrol of the maize weevil. HortScience 45 (4): 592 – 598.
20. Abbott WS (1925) A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18 (2): 265 – 267. doi: 10.1093/jee/18.2.265a.
21. Pascual-Villalobods MJ, Robledo A (1998) Screening for anti-insect activity in Mediterranean plants. Industrial Crops and Products 8 (3): 183 – 194. doi: 10.1016/S0926-6690(98)00002-8.
22. Juliana G, Su HCF (1983) Laboratory studies on several plant materials as insect repellents for protection of cereal grains. Journal of Economic Entomology 76 (1): 165 – 175. doi: 10.1093/jee/76.1.154.
23. Chi H (1997) Probit analysis. Taiwan, National Chung Hsing University.
24. Stefanazzi N, Taedoro S, Andriana F (2011) Composition and toxic, repellent and feeding deterrent activity of essential oils against the stored-grain pests *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). Pest Management Science 67 (6): 639 – 646. doi: 10.1002/ps.2102.
25. Koul O, Walia S, Dhaliwal GS (2008) Essential oils as green pesticides: potential and constraints. Biopesticides International 4 (1): 63 – 84.
26. Loh FS, Awang RM, Omar D, Rahmani M (2011) Insecticidal properties of *Citrus hystrix* DC leaves essential oil against *Spodoptera litura* Fabricius. Journal of Medicinal Plants Research 5: 3739 – 3744.
27. Sato A, Asano K, Sato T (1990) The chemical composition of *Citrus hystrix* DC (Swangi). Journal of Essential Oil Research 2 (4): 179 – 183. doi: 10.1080/10412905.1990.9697857.
28. Pratesa HT, Santosa JP, Waquila JM et al. (1998) Insecticidal activity of monoterpenes against *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst). Journal of Stored Products Research 34 (4): 243 – 249. doi: 10.1016/S0022-474X(98)00005-8.
29. Dhar R, Dawar H, Garg S et al. (1996) Effect of volatiles from neem and other natural products on genotrophic cycle and oviposition of *Anopheles stephensi* and *An. culicifacies* (Diptera: Culicidae). Journal of Medical Entomology 33 (2): 195 – 201. doi: 10.1093/jmedent/33.2.195.
30. Michelraj S, Sharma RK (2006) Fumigant toxicity of neem formulations against *Sitophilus oryzae* and *Rhyzopertha dominica*. Journal of Agricultural Technology 2 (1): 1 – 16.
31. Henn T, Weinzieri R (1989) Botanical insecticides and insecticidal soaps. Illionis, University of Illinois at Urbana-Champaign, College of Agriculture, Cooperative Extension Service.
32. Lu JH, Su XH, Zhong JJ (2012) Fumigant acivity of *Elsholtzia stauntonii* extract againts *Lasioderma serricorne*. South African Journal of Science 108 (7/8): 103 – 106. doi: 10.4102/sajs.v108i7/8.556.
33. Gautam SG, Opit GP, Tebbets JS et al. (2014) Egg morphology of key stored-product insect pests of the United States. Annals of the Entomological Society of America 107 (1): 1 - 10. doi: 10.1603/AN13103.

34. Kucerova Z, Stejskal V (2010) External egg morphology of two stored-product anobiids, *Stegobium paniceum* and *Lasioderma serricorne* (Coleoptera: Anobiidae). Journal of Stored Products Research 46 (3): 202 – 205. doi: 10.1016/j.jspr.2010.05.002.
35. Cabrera BJ (2001) Cigarette Beetle, *Lasioderma serricorne* (F.) (Insecta: Coleoptera: Anobiidae). http://edis.ifas.ufl.edu/in384. Accessed: July 2017.
36. Tapondjou AL, Adler C, Fontem DA et al. (2005) Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. Journal of Stored Products Research 41 (1): 91 – 102. doi: 10.1016/j.jspr.2004.01.004.
37. Buatone S, Indrapichate K (2011) Protective effects of mintweed, kitchen mint and kaffir lime leaf extracts against rice weevils, *Sitophilus oryzae*, in stored, milled rice. International Journal of Agriculture Sciences 3 (3): 133 – 139.
38. Maia MF, Moore S J (2011) Plant-based insect repellents: A review of their efficacy, development and testing. Malaria Journal 10 (Suppl 1): S11. doi: 10.1186/1475-2875-10-S1-S11.