Archives Of Phytopathology And Plant Protection

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gapp20

Repellency of Palizin® (Coconut Soap) with three laboratory techniques against five stored-product insect pests

Reza Sadeghi[a], Mahmoud Jokar[b], Arsalan Jamshidnia[a] & Asgar Ebadollahi[c]

[a] Department of Entomology and Plant Pathology, College of Aboureihan, University of Tehran, Tehran, Iran

[b] Department of Plant Protection, Urmia University, Urmia, Iran

[c] Young Researchers Club, Ardabil branch, Islamic Azad University, Ardabil, Iran

Published online: 08 Nov 2013.

To cite this article: Reza Sadeghi, Mahmoud Jokar, Arsalan Jamshidnia & Asgar Ebadollahi (2014) Repellency of Palizin® (Coconut Soap) with three laboratory techniques against five stored-product insect pests, Archives Of Phytopathology And Plant Protection, 47:14, 1686-1695, DOI: 10.1080/03235408.2013.855004

To link to this article: http://dx.doi.org/10.1080/03235408.2013.855004

Archives of Phytopathology and Plant Protection, 2014
Vol. 47, No. 14, 1686–1695, http://dx.doi.org/10.1080/03235408.2013.855004

Repellency of Palizin® (Coconut Soap) with three laboratory techniques against five stored-product insect pests

Reza Sadeghi[a], Mahmoud Jokar[b], Arsalan Jamshidnia[a] and Asgar Ebadollahi[c]*

[a]*Department of Entomology and Plant Pathology, College of Aboureihan, University of Tehran, Tehran, Iran;* [b]*Department of Plant Protection, Urmia University, Urmia, Iran;* [c]*Young Researchers Club, Ardabil branch, Islamic Azad University, Ardabil, Iran*

(*Received 8 October 2013; accepted 10 October 2013*)

The heavy economic damages are the major concern in storage that was caused by coleopteran pests. The conventional control that is accomplished by synthetic pesticides created toxicity to non-target organisms, development of resistance and residue's problems. Nowadays, with changes in legislation and consumer demand, alternatives to synthetic pesticide to manage the store product pest are increasingly needed. The repellent compounds could be considered as one of the mentioned methods in storage. In this study, five widespread species of stored product pest including *Sitophilus oryzae* Linnaeus, *Tribolium castaneum* Herbst, *Rhizopertha dominica* Fabricius, *Oryzaephilus surinamensis* Linnaeus and *Lasioderma serricorne* Fabricius were conducted to assay the percentage repellency (PR) of Palizin® (Coconut Soap 65%). Percentage repellency was achieved with beetles which were exposed to 0, 0.5, 1, 5 and 10% concentration using three techniques: filter papers, *Y*-shape tube and cup bioassays. In each of these methods, the means of PR reacted to increasing concentration except *T. castaneum* and *O. surinamensis* after 72 and 48 h. The maximum PR of Palizin® belonged to a concentration of 10% (except *R. dominica* at 5% and 48 h). Among the methods, except filter paper, degradation process resulted to a decreasing trend of PR. In the present experiment, Palizin® can be recommended as a limiting factor of all beetles and the data which were exported by Cub bioassay were closely adapted to reality condition of storage.

Keywords: Cub bioassay; filter paper method; olfactometer tube; pesticide; repellent

1. Introduction

The damage of food stored that was caused by insects and other bio-agents such as rodents and fungal diseases was 50%, approximately, in developing countries, annually (Brader et al. 2002). Among the insect class, the most serious damage is caused by Coleoptera order because of its feeding during both larval and adult stages (Borror et al. 1984). The rice weevil (*Sitophilus oryzae* Linnaeus) (Coleoptera: Curculionidae), the red flour beetle (*Tribolium castaneum* Herbst) (Coleoptera: Tenebrionidae), the lesser grain borer (*Rhizopertha dominica* Fabricius) (Coleoptera: Bostrichidae), the saw-toothed grain beetle (*Oryzaephilus surinamensis* Linnaeus) (Coleoptera: Silvanidae) and the cigarette beetle (*Lasioderma serricorne* Fabricius) are frequently found in extremely high numbers of store products (Garcìa et al. 2005; Halstead 1963). Synthetic insecticides and fumigant compounds are widely used to control and prevent post-harvest pest

*Corresponding author. Email: Asgar.ebadollahi@gmail.com

infestation. However, the use of insecticides has several disadvantages; residues on the oilseed, developing resistance populations, increasing costs of application, worker safety, ozone depletion, toxicity to non-target organisms and consumer concern (Jemberem et al. 1995; Okonkwo & Okoye 1996; Phillips & Throne 2010). The progressive public distress over safety of pesticide and their environmental pollution has originated in growing interest in finding alternative strategies for chemical control for stored-product pest (Rajendran & Sriranjini 2008). Currently, the researchers worldwide have focused on the use of natural components such as plant oils due to their low mammalian toxicity, environmental safety and biodegradable properties (Raja et al. 2001; Papachristos & Stamopoulos 2002).

In the recent studies, susceptibility of *L. serricorne*, *O. surinamensis*, *R. dominica*, *S. oryzae* and *T. castaneum* to plant-derived materials was evaluated. For example, *Agastache foeniculum* (Pursh) Kuntze essential oil was tested for toxicity against adults of *O. surinamensis* and *L. serricorne* by Ebadollahi et al. (2010a). The influence of different concentrations of the essential oil vapours on adult mortality was significant. *O. surinamensis* was more susceptible than *L. serricorne* at the exposure time 24 h. In the other study, fumigant toxicity of essential oils of *Eucalyptus globulus* Labill and *Lavandula stoechas* L., grown in Iran, was demonstrated against *L. serricorne* and *R. dominica*. Mortality increased as the exposure time and the dose of the essential oils increased (Ebadollahi et al. 2010b). Strong insecticidal activity of essential oils from *Foeniculum vulgare* Mill. and *Satureja hortensis* L. was indicated against *Sitophilus granarius* L. and *S. oryzae*. LC_{50} values indicated that *S. granarius* was more susceptible than *S. oryzae* to essential oils at the exposure times 24 and 48 h (Ebadollahi 2011). In the study of Ebadollahi et al. (2013), *Agastache foeniculum* essential oil caused high mortality on *T. castaneum* larvae with fumigation test. Study on the effect of this oil on total carbohydrate, lipid and protein contents demonstrated that all of them were decreased with increasing of concentrations. Furthermore, inhibition of esterase and glutathione-S-transferase activities was also observed.

As part of our ongoing research on natural insecticides from the flora of Iran, the aim of this study was to evaluate the repellent efficacy of Palizin® as a commercial plant origin material by three bioassay methods against five mentioned stored-product insect pests. This approach will allow us to identify natural and safer agents for the development of bio-rational insecticides to manage these coleopteran insect pests.

2. Materials and methods

2.1. Insect rearing

All experimental tests were conducted on 7 ± 2-day-old adults of *S. oryzae*, *T. castaneum*, *R. dominica*, *O. surinamensis* and *L. serricorne* under 25 ± 2 °C and 65 ± 5% relative humidity (r. h.) and 10:14 h light: dark photoperiod. The adults of both sexes were obtained from the stock culture of long-established laboratory colonies kept in the section of stored-products insects' rearing room of the Entomology Department of Tehran University. Test insects were reared in soft non-infested wheat at 14% m.c. (*S. oryzae*), whole meal wheat flour plus 5% brewer's yeast (19:1) (*R. dominica*), ground wheat enriched with glycerin and yeast (*T. castaneum* and *O. surinamensis*) and whole-wheat flour with 5% yeast (*L. serricorne*) (Childs & Overby 1983; Ogendo et al. 2008) in the plastic boxes (20 × 25 × 15 cm) covered by a lid with a plastic mesh to circulate air. Adults were removed and transferred to another box with new seeds or

flour, every seven days. In this way, adults were obtained in the next generations from each box with similar age (±7 days). These adults were used in bioassays (Cosimi et al. 2009).

2.2. *Coconut Soap (Palizin®)*

This coconut soap was provided as commercial product formulised by Kimia Sabzavar Company with active ingredient 65% and soluble liquid. The commercial properties included: common name: coconut soap, trade name: Palizin® and application group: insecticidal and miticidal soap. Distilled water was utilised for diluting gel and preparing 0.5,1, 5 and 10% concentrations.

2.3. *Repellent activity*

Four concentrations of 0.5, 1, 5 and 10% of Palizin® were prepared. The distilled water was used as a solvent. In each bioassay, percentage repellency (PR) was recorded after exposure. The amount of concentrations and times for checking the susceptibility of each insect to Palizin® were determined by pretest. All bioassays were carried out in the dark incubators at 28–30 °C and 70–80% relative humidity (r. h.). The beetles were regarded dead when the legs or antenna were found immobile when prodded with a pencil. The repellent action of the Palizin® against the insects was evaluated by three methods.

2.3.1. *Petri-dish bioassay technique or filter paper method*

The Petri dish chamber test was used to determine the repellency of Palizin® to the insects according to Caballero-Gallardo et al. (2012). This experimental was used a no-choice bioassay system to measure repellency of the four concentrations of Palizin®. Per different treatment of each concentration, a volume of 1 cc was uniformly put on a half filter paper disc (Whatman No. 1, 12 cm diameter) with a pipette. The other half filter paper disc was impregnated with distilled water as a positive control, utilising the same experimental condition. The halves were air-dried for 20 min to remove the solvent, reattached with adhesive tape and held onto glass Petri dishes. Ten unsexed adults were put into each Petri dish at the centre, and the lid was sealed within place with Para film. Ten replicates were used for each tested concentration so that 100 adults were assayed per concentration. The test was carried out under the same conditions described for the rearing. The numbers of adults on the two half paper discs were counted after 12, 24, 48 and 72 h exposure.

2.3.2. *Y-shape olfactometer tube bioassay technique*

The *Y*-shape olfactometer was assembled by three linked glass tubes (10 cm long, 1 cm diameter) which were an opening to facilitate the air circulation in the olfactometer (Paranagama et al. 2004). At the crossing of three arms there is a pump to circulate air through each arm of the Y-tube olfactometer at a rate of 1 L/min. Two perforated plastic, transparent, wide-mouthed bottles (250 ml) were connected with the terminal of two arms which were used to introduce insects and the other arm of the olfactometer was connected with vacuum pump. Two Whatman No. 1 filter papers (2.5 cm × 2.5 cm) were saturated with 1 cc of Palizin® as treatment and the other with an equal amount of

distilled water as a control. After air-drying for 10 min, the filter papers were hung by wires in the middle of the bottles separately. To start the bioassay, the olfactometer was placed horizontally on a white background in daylight by turning on vacuum pump and conducting 10 test insects into the olfactometer. The numbers of beetles which motivated into Palizin® and distilled water treated bottles were recorded within 1, 2, 4, 8, 12 and 24 h. This technique with four doses of Palizin® was replicated 10 times.

2.3.3. Cup bioassay technique

According to Mohan and Fields (2002), movement of insects in the treated grains revealed the interaction between repellent and attractant abilities. It is worth mentioning that this technique was a simple and rapid mimic of storage conditions. The cup bioassay was conducted in two disposable cups in which the perforated opaque cup (7 cm diameter × 10 cm high) containing crop was sprayed with the concentration and transparent cup (7 cm diameter × 9 cm high) was used to count the insects. The opaque cup, with a mesh bottom which just passed the insects through their pores and a lid to prevent the escape of flying insects, was put above the transparent cup, tightly. The insects that left through the sides were trapped in a Petri dish below the cup. The crops (200 g) were sprayed with different concentrations (0.5, 1, 5 and 10%) of Palizin® and by distilled water as control treatment. After air-drying for 10 min, ten unsexed adults were released from the centre of the crop mass in the container through a long-stemmed funnel. In comparative surveying, the number of insects leaving the treated grain with control treatments showed potential repellents. All experiments were run at 25 ± 1 °C, 75 ± 10% relative humidity (r.h.). The repellency was determined in terms of the adults' runaway reaction rate from the treatment container. The number of trapped insects was counted at four different intervals (12, 24, 48 and 72 h) after the introduction of the adults. There were 10 replicates per treatment. Juliana and Su (1983) described a comfortable classification for mean repellency value that calculated the PR of compounds from 0 to *V*; class 0 (PR = 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (PR = 40.1–60%), class IV (PR = 60.1–80%) and class V (PR = 80.1–100%).

2.4. Data analysis

Because PR data were percentage value, we are using arcsine transforming (Arcsin$\sqrt{x}$). PR data were analysed using the variance with ANOVA and checked by Turkey's test in SPSS software at completely randomised design using factorial arrangements of treatments (10 replicates in each treatment).

3. Results

3.1. Petri-dish bioassay technique or filter papers methods

The repellent activity of tested concentrations of Palizin® on each insect species is shown in Tables 1 and 2. In this technique, the highest repellent activity for insects at all intervals assayed belonged to 10% concentration. However, for *R. dominica*, the maximum repellency reaction was recorded at 5% and after 48 h. In the case of *T. castaneum* and *O. surinamensis*, after 72 and 48 (h) respectively, no significant difference was found even at different concentrations (Table 2). Among five beetles, adults of *O. surinamensis* had most potential repellency to Palizin® (65.36–84.07). Adults of

Table 1. Variance analysis of the repellency of Palizin® in the filter paper technique.

Insect	Time	df	*F*	*P* value
L. serricorne	72	3	3.19	0.035
	48	3	14.13	0.018
	24	3	4.26	0.011
	12	3	18.56	0.025
R. dominica	72	3	6.39	0.001
	48	3	13.55	0.002
	24	3	16.97	0.023
	12	3	14.68	0.0001
O. surinamensis	72	3	5.49	0.003
	48	3	0.918	ns
	24	3	3.10	0.038
	12	3	13.65	0.0001
T. castaneum	72	3	0.80	0.003
	48	3	3.02	ns
	24	3	2.66	0.038
	12	3	3.85	0.0001
S. oryzae	72	3	2.25	0.098
	48	3	2.16	0.109
	24	3	1.40	0.258
	12	3	19.76	0.0001

ns = non significant.

Table 2. Results of repellency tests undertaken with Petri dishes technique.

		Mean repellency (%)[a]			
Insect	Concentration (%)	12 h	24 h	48 h	72 h
S. oryzae	0.5	(III) 52.22^{c}	(III) 55.32^{c}	(III) 58.21^{c}	(IV) 60.44^{c}
	1	(III) 54.31^{bc}	(III) 59.54^{c}	(IV) 62.31^{c}	(IV) 70.38^{b}
	5	(III) 60.35^{b}	(IV) 68.23^{b}	(IV) 78.36^{b}	(IV) 75.78^{b}
	10	(IV) 71.28^{a}	(IV) 75.25^{a}	(V) 80.41^{a}	(V) 82.21^{a}
T. castaneum	0.5	(IV) 63.37^{b}	(IV) 62.77^{b}	(IV) 64.03^{b}	(IV) 67.03^{ns}
	1	(IV) 65.88^{b}	(IV) 66.02^{ab}	(IV) 70.25^{ab}	(IV) 71.4^{ns}
	5	(IV) 66.54^{b}	(IV) 71.25^{ab}	(IV) 70.41^{ab}	(IV) 68.62^{ns}
	10	(IV) 78.06^{a}	(IV) 78.06^{a}	(IV) 78.87^{a}	(IV) 75.61^{ns}
O. surinamensis	0.5	(IV) 65.36^{b}	(IV) 68.62^{c}	(IV) 73.22^{ns}	(IV) 70.77^{b}
	1	(IV) 67.65^{b}	(IV) 73.10^{ab}	(IV) 78.87^{ns}	(IV) 68.62^{b}
	5	(IV) 72.24^{b}	(IV) 77.08^{ab}	(IV) 74.63^{ns}	(IV) 71.72^{b}
	10	(V) 85.86^{a}	(V) 82.28^{a}	(V) 80.49^{ns}	(V) 84.07^{a}
R. dominica	0.5	(III) 55.88^{c}	(III) 55.71^{c}	(IV) 62.21^{b}	(IV) 62.61^{b}
	1	(IV) 61.59^{bc}	(IV) 64.55^{b}	(IV) 68.31^{b}	(IV) 66.83^{b}
	5	(IV) 66.02^{b}	(IV) 69.94^{ab}	(IV) 76.92^{a}	(IV) 74.49^{a}
	10	(IV) 77.08^{a}	(IV) 74.30^{a}	(III) 58.52^{c}	(IV) 76.92^{a}
L. serricorne	0.5	(III) 53.89^{c}	(III) 56.31^{b}	(IV) 60.99b	(III) 59.87^{b}
	1	(III) 58.25^{bc}	(III) 59.66^{b}	(IV) 67.76a	(IV) 71.22^{a}
	5	(IV) 60.33^{b}	(IV) 61.86^{ab}	(IV) 66.83a	(IV) 69.08^{ab}
	10	(IV) 72.53^{a}	(IV) 67.00^{a}	(IV) 69.12a	(IV) 72.70^{a}

[a]The original data were transformed into arcsine square-root percentage values before ANOVA tests.
Classification for mean repellence values are shown in the parenthesis. Different letters indicate significant differences according to Tukey test at $p = 0.05$. Columns with the same letter are not significantly different. The means for the water controls are nil or small and these values were not included in the calculation of these mean values.

R. dominica (at 12 and 24 h) and *L. serricorne* (at 12 h) indicated that the highest repellency rate related to increasing of concentrations.

3.2. Y-shape olfactometer tube bioassay technique

Repellency results of adults differed significantly among the four treatment concentrations per exposure period (Table 3). There was a significant relationship between concentration and PR, just like above (Table 4). However, this bioassay method expressed closer significant ($P \leq 0.05$) relationship between dose and repellent effect of all of the beetle species. For example, *S. oryzae* were exposed for 4 h or *L. serricorne* for 8 and 12 h. The maximum and minimum repellencies among the tested species were observed with *L. serricorne* (53.78–85.86) and *S. oryzae* (24.32–59.61).

3.3. Cup bioassay

The results achieved with cup bioassay were overlapped by the two methods mentioned above. The means of PR monotonically showed a significant relation with concentration (Table 5). According to Table 6, *T. castaneum* was the best for the evaluation of PR because of its maximum movement rate (40.28–56.93 at dose: 10%, 12–72 h). In

Table 3. Results of repellency tests undertaken with Y-shape olfactometer tube bioassay technique.

		Mean repellency (%)[a]				
Insect	Concentration (%)	2	4	8	12	24
S. oryzae	0.5	(II) 28.92^{c}	(II) 28.39^{d}	(II) 29.84^{c}	(II) 26.79^{c}	(II) 24.32^{c}
	1	(III) 41.73^{b}	(II) 36.23^{c}	(II) 32.21^{c}	(II) 31.95^{c}	(II) 28.40^{c}
	5	(III) 47.90^{b}	(III) 44.68^{b}	(III) 41.74^{b}	(III) 40.56^{b}	(II) 34.39^{b}
	10	(III) 56.70^{a}	(III) 59.61^{a}	(III) 54.11^{a}	(III) 47.50^{a}	(III) 43.85^{a}
T. astaneum	0.5	(IV) 60.37^{c}	(IV) 63.00^{b}	(IV) 62.91^{b}	(IV) 60.25^{c}	(III) 58.07^{b}
	1	(IV) 64.84^{bc}	(IV) 68.46^{ab}	(IV) 68.08^{ab}	(IV) 64.12^{b}	(IV) 62.70^{b}
	5	(IV) 70.38^{bc}	(IV) 71.79^{a}	(IV) 69.36^{a}	(IV) 67.66^{ab}	(IV) 71.83^{a}
	10	(IV) 73.89^{a}	(IV) 74.24^{a}	(IV) 71.10^{a}	(IV) 70.20^{a}	(IV) 73.78^{a}
O. surinamensis	0.5	(III) 44.74^{c}	(III) 46.44^{b}	(III) 45.30^{b}	(III) 40.90^{b}	(II) 35.86^{c}
	1	(III) 51.76^{bc}	(III) 47.90^{b}	(III) 45.86^{b}	(III) 42.09^{b}	(II) 37.66^{bc}
	5	(III) 58.37^{ab}	(III) 54.45^{a}	(III) 50.58^{ab}	(III) 48.78^{a}	(III) 42.41^{ab}
	10	(IV) 61.65^{a}	(III) 54.99^{a}	(III) 54.29^{a}	(III) 52.54^{a}	(III) 45.86^{a}
R. dominica	0.5	(III) 59.8^{c}	(IV) 61.20^{c}	(IV) 60.90^{c}	(IV) 62.47^{b}	(IV) 60.58^{b}
	1	(IV) 65.79^{b}	(IV)64.28^{bc}	(IV) 66.00^{b}	(IV) 65.74^{b}	(IV) 62.10^{b}
	5	(IV) 70.05^{a}	(IV) 67.27^{b}	(IV)69.38^{ab}	(IV) 66.19^{b}	(IV) 62.56^{b}
	10	(IV) 72.66^{a}	(IV) 72.49^{a}	(IV) 72.78^{a}	(IV) 71.52^{a}	(IV) 67.33^{a}
L. serricorne	0.5	(III) 55.20^{c}	(III) 53.87^{c}	(III) 56.38^{d}	(III) 55.78^{d}	(III) 55.80^{c}
	1	(IV) 62.92^{b}	(IV) 61.65^{b}	(IV) 63.73^{c}	(IV) 62.92^{c}	(III) 55.71^{c}
	5	(IV) 67.66^{b}	(IV) 68.17^{b}	(IV) 73.35^{b}	(IV) 73.35^{b}	(IV) 71.89^{b}
	10	(V) 84.07^{a}	(V) 85.86^{a}	(V) 85.86^{a}	(V) 84.07^{a}	(V) 82.28^{a}

[a]The original data were transformed into arcsine square root percentage values before ANOVA tests.
Classification for mean repellency values are shown in the parenthesis. Different letters indicate significant differences according to Tukey test at $p = 0.05$. Columns with the same letter are not significantly different. The means for the water controls are nil or small and these values were not included in the calculation of these mean values.

Table 4. Variance analysis of the repellency of Palizin® in the olfactometer technique.

Insect	Time	df	*F*	*P* value
L. serricorne	24	3	27.02	0.001
	12	3	38.87	0.001
	8	3	45.20	0.001
	4	3	33.00	0.001
	2	3	29.10	0.001
R. dominica	24	3	6.34	0.001
	12	3	5.42	0.003
	8	3	11.61	0.001
	4	3	18.31	0.001
	2	3	26.98	0.001
O. surinamensis	24	3	7.34	0.001
	12	3	8.19	0.001
	8	3	5.71	0.002
	4	3	3.58	0.017
	2	3	5.56	0.003
T. castaneum	24	3	8.19	0.001
	12	3	2.69	0.006
	8	3	3.87	0.018
	4	3	6.05	0.001
	2	3	7.66	0.001
S. oryzae	24	3	35.26	0.001
	12	3	15.14	0.001
	8	3	27.87	0.001
	4	3	36.84	0.001
	2	3	27.21	0.001

ns = non significant.

Table 5. Variance analysis of the repellency of Palizin® in the cup bioassays technique.

Insect	Time	df	*F*	*P* value
L. serricorne	72	4	10.46	0.0001
	48	4	18.33	0.0001
	24	4	23.70	0.0001
	12	4	31.84	0.0001
R. dominica	72	4	1.87	0.0001
	48	4	0.911	0.0001
	24	4	5.26	0.0001
	12	4	6.44	0.0001
O. surinamensis	72	4	11.42	0.0001
	48	4	12.95	0.0001
	24	4	5.94	0.0001
	12	4	9.871	0.0001
T. castaneum	72	4	9.87	0.0001
	48	4	10.91	0.0001
	24	4	24.18	0.0001
	12	4	24.18	0.0001
S. oryzae	72	4	23.97	0.0001
	48	4	23.31	0.0001
	24	4	18.37	0.0001
	12	4	16.67	0.0001

ns = non significant.

Table 6. Results of repellency tests undertaken with Cub bioassay technique.

Insect	Concentration (%)	Mean repellency (%)[a]			
		12	24	48	72
S. oryzae	0 (Control)	(I) 13.08^{c}	(I) 9.50^{cd}	(I) 16.65^{bc}	(I) 9.50^{c}
	0.5	(I) 2.35^{d}	(I) 4.14^{d}	(I) 5.93^{d}	(I) 7.71^{c}
	1	(I) 16.18^{bc}	(I) 17.3^{bc}	(I) 10.32^{cd}	(I) 9.50^{c}
	5	(II) 22.04^{b}	(I) 19.90^{b}	(I) 19.09^{b}	(II) 23.17^{b}
	10	(II) 32.36^{a}	(II) 35.21^{a}	(II) 41.49^{a}	(II) 36.71^{a}
T. castaneum	0	(I) 14.70^{c}	(I) 15.68^{d}	(I) 10.32^{d}	(I) 12.92^{b}
	0.5	(I) 13.37^{c}	(II) 31.00^{c}	(II) 29.17^{c}	(I) 12.33^{b}
	1	(II) 23.60^{c}	(II) 37.20^{bc}	(II) 28.63^{c}	(I) 16.99^{b}
	5	(II) 37.8^{b}	(III) 44.49^{b}	(III) 42.98^{b}	(II) 37.69^{a}
	10	(III) 56.03^{a}	(III) 56.93^{a}	(III) 51.33^{a}	(III) 40.28^{a}
O. surinamensis	0	(I) 13.08^{c}	(I) 11.29^{bc}	(I) 13.08^{b}	(I) 14.87^{a}
	0.5	(I) 11.13^{c}	(I) 9.50^{c}	(I) 5.93^{b}	(I) 12.57^{a}
	1	(I) 15.52^{bc}	(I) 19.44^{ab}	(I) 8.53^{b}	(I) 4.14^{b}
	5	(II) 22.19^{b}	(II) 20.57^{ab}	(II) 22.85^{a}	(I) 15.50^{a}
	10	(II) 33.69^{a}	(II) 29.01^{a}	(II) 29.67^{a}	(II) 20.57^{a}
R. dominica	0	(I) 11.29^{c}	(I) 5.93^{c}	(I) 9.50^{c}	(I) 9.50^{d}
	0.5	(I) 12.92^{c}	(I) 12.92^{b}	(I) 16.49^{a}	(I) 12.92^{c}
	1	(I) 19.09^{bc}	(I) 19.09^{ab}	(I) 14.7^{b}	(I) 16.99^{b}
	5	(II) 28.41^{a}	(I) 17.30^{ab}	(I) 13.56^{b}	(I) 16.49^{b}
	10	(II) 25.97^{ab}	(II) 24.27^{a}	(I) 17.30^{a}	(II) 22.33^{a}
L. serricorne	0	(I) 11.29^{d}	(I) 13.08^{c}	(I) 7.71^{d}	(I) 7.71^{b}
	0.5	(I) 19.90^{c}	(I) 14.87^{c}	(I) 19.09^{c}	(I) 15.52^{b}
	1	(II) 27.60^{b}	(II) 29.07^{b}	(II) 25.14^{bc}	(II) 24.32^{a}
	5	(II) 31.61^{b}	(II) 27.60^{b}	(II) 27.60^{b}	(II) 28.86^{a}
	10	(III) 45.60^{a}	(II) 37.89^{a}	(II) 36.65^{a}	(II) 30.62^{a}

[a]The original data were transformed into arcsine square root percentage values before ANOVA tests. Classification for mean repellency values are shown in the parenthesis. Different letters indicate significant differences according to Tukey test at $p = 0.05$. Columns with the same letter are not significantly different.

contrast, *R. dominica* was slightly reacted to volatile components of Palizin® for low repellency rate (17.30–25.97 at dose 10%, 12–72 h). In this method, a moderate level of repellency was computed for all insect pests.

4. Discussion

By filtering paper method, Nerio et al. (2009) estimated PR of *Sitophilus zeamais* 36 ± 16, 58 ± 12, 86 ± 5 and 91 ± 3% for doses 0.063, 0.126, 0.252 and 0.503 µl/cm^2 and Tapondjou et al. (2005) calculated the PR of the volatile oil from *Eucalyptus saligna* on *S. zeamais* (73 ± 15%) and *T. confusum* (98 ± 4%) as similar as our finding in Table 2. Dose-response was stronger than recent data because it depends on several factors among which are the chemical composition, insect susceptibility, difference on pest specie, dose or condition (Casida 1990). Mentioned authors utilised Acetone for diluting and treatment control (whereas present research diluted with distilled water) and can argue the main reason of differences. Olivero-Verbel et al. (2010) described that this activity had a small tendency to decrease, gradually degrade with an increase in exposure time (2–4 h), whereas our finding demonstrated increasing rates, except Cup bioassay. The most notable reason of phenomena is evaporation pick which happened early exposure duration (1–2). Ryan and Byrne (1988) suggested that the toxic effect

may be attributed to reversible competitive inhibition of acetyl cholinesterase by occupation of the hydrophobic site of the enzyme's active centre. One of the volatile components was established treatment and the control in common filter paper tests was acetone, which was acting as a carrier agent of the active ingredient. But in the present experiment, distilled water played the role of stabiliser: absorbed active ingredients onto the filter paper, and switched the severe and immediate effects to gradual. Many repellency tests were conducted with filter paper, which does not mimic the grain storage conditions because the interaction of the active ingredients of paper and crop surface is contrary. Beetles orient to odours only after initiation of flight, and an important disadvantage of *Y*-shape olfactometer tube is the inadequate space to fly(Louise et al. 1983). In the cup bioassay tests, where repellency is due mainly to the volatile component of Palizin®, is simulating real condition complexes of repellent activity, crop and pest behaviour.

In conclusion, the application of Palizin® is not particularly dangerous to storekeepers since they are commonly used. Washing and cooking eliminate residual in grocery. The results from the study suggested that Palizin® has noticeable repellent properties and could be considered for integration with other effective control options in the stored-product management.

Acknowledgements

The authors thank the Abureihan Campus, University of Tehran for financial supporting this research.

References

Borror DJ, Triplehron CA, Johnson NF. 1984. An introduction to the study of insects. 6th ed. New York, NY: Saunders College Publishing. p. 875.

Brader B, Lee RC, Plarre R, Burkholder W, Kitto GB, Kao C, Polston L, Dorneana E, Szabo I, Mead B, Sullin D, Denning R. 2002. A comparison of screening methods for insect contamination in wheat. J Stored Prod Res. 38:95–115.

Caballero-Gallardo K, Olivero-Verbe J, Stashenko EE. 2012. Repellency and toxicity of essential oils from *Cymbopogon martinii*, *Cymbopogon flexuosus* and *Lippia origanoides* cultivated in Colombia against *Tribolium castaneum*. J Stored Prod Res. 50:62–65.

Casida JH. 1990. Pesticide mode of action, evidence for implications of a finite number of biochemical targets. In: Casida JE, editor. Pesticides and alternatives. Innovative Chemical and Biological Approaches to Pest Control. Amsterdam: Elsevier; p. 11–22.

Childs DP, Overby JE. 1983. Mortality of the cigarette beetles in high-carbon dioxide atmospheres. J Econ Entomol. 76:456–544.

Cosimi S, Rossi E, Cioni PL, Canale A. 2009. Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: Evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). J Stored Prod Res. 45:125–132.

Ebadollahi A. 2011. Susceptibility of two *Sitophilus* species (Coleoptera: Curculionidae) to essential oils from *Foeniculum vulgare* and *Satureja hortensis*. Ecologia Balkanica. 3:1–8.

Ebadollahi A, Safaralizadeh MH, Pourmirza AA, Gheibi SA. 2010a. Toxicity of essential oil of *Agastache foeniculum* (Pursh) Kuntze to *Oryzaephilus surinamensis* L. and *Lasioderma serricorne* F. J Plant Protect Res. 50:215–219.

Ebadollahi A, Safaralizadeh MH, Pourmirza AA. 2010b. Fumigant toxicity of essential oils of *Eucalyptus globulus* Labill and *Lavandula stoechas* L. grown in Iran, against the two coleopteran insect pests; *Lasioderma serricorne* F. and *Rhyzopertha dominica* F. Egypt J Biol Pestic Control. 20:1–5.

Ebadollahi A, Khosravi R, Jalali Sendi J, Honarmand P, Moayed Amini R. 2013. Toxicity and physiological effects of essential oil from *Agastache foeniculum* (Pursh) Kuntze against

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) larvae. Annu Rev Res Biol. 3:649–658.

Garcìa M, Donael OJ, Ardanaz CE, Tonn CE, Sosa ME. 2005. Toxic and repellent effects of *Baccharis salicifolia* essential oil on *Tribolium castaneum*. Pestic Manage Sci. 61:612–618.

Halstead DGH. 1963. The separation of *Sitophilus oryzae* (L.) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) with a summary of their distribution. Entomol Mon Mag. 99:72–74.

Jemberem B, Obeng-Ofori D, Hassanali A, Nyamasyo GNN. 1995. Products derived from the leaves of *Ocimum kilimandscharicum* (Labiatae) as post-harvest grain protectants against the infestation of three major stored product insect pests. B Entomol Res. 85:361–367.

Juliana G, Su HCF. 1983. Laboratory studies on several plant materials as insect repellents for protection of cereal grains. J Econ Entomol. 76:154–157.

Louise EM, van Lenteren JC, Heymans M, Melis M. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiol Entomol. 8:97–106.

Mohan S, Fields PG. 2002. A simple technique to assess compounds that are repellent or attractive to stored product insects. J Stored Prod Res. 38:23–31.

Nerio LS, Olivero-Verbel J, Stashenko EE. 2009. The repellent activity of essential oils from seven aromatic plants grown in Colombia against *Sitophilus zeamais* Motschulsky (Coleoptera). J Stored Prod Res. 45:212–214.

Ogendo JO, Kostyukovsky M, Ravid U, Matasyoh JC, Deng AL, Omolo EO, Kariuki ST, Shaaya E. 2008. Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. J Stored Prod Res. 44:328–334.

Okonkwo EU, Okoye WI. 1996. The efficacy of four seed powders and the essential oils as protectants of cow-pea and maize grain against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Nigeria. Int. J Pestic Manage. 42:143–146.

Olivero-Verbel J, Nerio LS, Stashenko EE. 2010. Bioactivity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils grown in Colombia. Pestic manage Sci. 66:664–668.

Papachristos DP, Stamopoulos DC. 2002. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J Stored Prod Res. 38:117–128.

Paranagama PA, Abeysekera KHT, Nugaliyadde L, Abeywickrama KP. 2004. Repellency and toxicity of four essential oils to *Sitophilus oryzae* L. (Coleoptera: Curculionidae). J Nat Sci Found Sri Lanka. 32:127–138.

Phillips TW, Throne JE. 2010. Bio rational approaches to managing stored product pests. Annu Rev Entomol. 55:375–397.

Raja N, Albert S, Ignacimuthu S, Dorn S. 2001. The effect of plant volatile oils in protecting stored cowpea *Vigna unguiculata* (L.) Walpers against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) infestation. J Stored Prod Res. 37:127–132.

Rajendran S, Sriranjini V. 2008. Plant products as fumigants for stored-product insect control. J Stored Prod Res. 44:126–135.

Ryan MF, Byrne O. 1988. Plant-insect co evolution and inhibition of acetyl cholinesterase. J Chem Ecol. 14:1965–1975.

Tapondjou A, Adle C, Fontem D, Bouda H, Reichmuth C. 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.