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Full Length Research Paper

Repellent effect of sirinol (garlic emulsion) against *Lasioderma serricorne* (Coleoptera: Anobiidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) by three laboratory methods

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To alleviate insect pest problems in storage, synthetic pesticides are recommended; but their use may create toxicity to non-target organisms, development of resistance and residues in treated products. Under such circumstances, the need for research to find a safe, convenient, durable and economically logical method is necessary. The application of repellents could be considered as a new control method in storage. In this study, percentage repellency (PR) of Sirinol (garlic emulsion) was assayed on adults of cigarette beetles, *Lasioderma serricorne* (F.) and red flour beetles, *Tribolium castaneum* (Herbst) using three techniques, Petri-dish, Y- shape olfactometer tube and leaky glass. The insects were exposed to 0, 0.5, 1.5 and 10% concentration of Sirinol that is a botanical compound and PR of the adults was determined. In each of the three methods, the maximum PR of Sirinol was about 10% concentration, and the quantities were equal to 58.56, 42.58 and 26.29% for *L. serricorne* and 70.99, 55.47 and 38.72 for *T. castaneum* in Petri-dish, Y- shape olfactometer tube and leaky glass techniques, respectively. The interaction between concentration and time (concentration × time) was not significant for the adults of the two species. It was shown that *T. castaneum* was more susceptible to Sirinol than *L. serricorne.* Data analysis revealed that PRs of Sirinol were not significantly different between the time of 12 and 72 h.

Key word: Sirinol, repellent, percentage repellency, garlic.

INTRODUCTION

Conservation of reserved food grain stocks is necessary to ensure a continuous supply at stable prices (Talukder, 2005). Losses are the most serious problem from insect infestation in grain storage, particularly in the developing countries, where poor sanitation and use of inappropriate storage facilities all encourage insect attack (Talukder et al., 2004, 2005). It was estimated that over 20,000 species of field and storage pests destroy approximately one-third of the world's food production, valued annually at over $100 billion, among which the highest losses (43% of potential production) occur in developing Asian and African countries (Jacobson, 1982; Ahmed and Grainge, 1986). Control of these pests relies on the widespread use of various synthetic chemical insecticides and fumigants.

However, the application of various synthetic insect-cides and fumigants to grain storage over the years has led to a number of problems such as environmental pollution, pesticide residue in food grains, development of insecticide resistance and toxicity to non-target organisms (Yusof and Ho, 1992; Lorini and Galley, 1999; Cosimi et al., 2009; Sousa et al., 2009). The increasing public concern over pesticide safety and possible damage to the environment has resulted in increasing attention being given to natural products for the control stored pests

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Abbreviation: **PR**, Percentage repellency.

(Rajendran and Sriranjini, 2008). In this context, many plant products have been evaluated for their toxic properties against different stored grain pests, especially in the form of essential oils (Regnault-Roger, 1997; Rajendran and Sriranjini, 2008). In recent years, many researchers have focused on the search for natural products derived from terrestrial plants as natural insecticides. Terrestrial plants are known to contain a rich source of bioactive metabolites which show antifeedant, repellent and toxic effects in a wide range of insects (Rajendran and Sriranjini, 2008; Ukeh et al., 2009; Mondal and Khalequzzaman, 2010).

On the other hand, production of repellents derived from plants may be easier and less expensive than synthesis of some more complex attractive semio-chemicals (Shadia, 2011). The repellents are desirable chemicals as they offer protection with minimal impact on ecosystem, and repel the insect-pests from the treated materials by stimulating olfactory or other receptors of insects. According to Dethier et al. (1960), an insect repellent is a chemical stimulus, which causes the insects to make oriented movements away from the source of stimulus. Repellents from plant origins are considered safe in pest control operations as they minimize pesticide residues; ensure safety of the people, food, environment and wildlife (Khan, 1982; Talukder and Howse, 1995; Talukder et al., 2004). The plant extracts, powders and essential oils from different bioactive plants were reported as repellents against different economically important stored product insects (Xie et al., 1995; Tripathi et al., 2000; Owusu, 2001; Khan and Gumbs, 2003; Boeke et al., 2004; Talukder et al., 2004; Stancic et al., 2011). Garlic (*Allium sativum* L.) has an effective range of insecticidal repellent (Fields et al., 2001), antifeedant, bactericidal, fungicidal and nematicidal (Loth et al., 2007).

The present study was conducted to develop an Integrated Pest Management protocol (IPM) which might be an alternative to the practices being adopted presently for the control of insect pests of stored grains at farm level but are safe, economical, easy to apply and nature friendly. For this purpose, efficacy of garlic emulsion (Sirinol) as a repellent was appraised by applying different concentrations against cigarette beetles, *Lasioderma serricorne* (F.) that is important on dried herbs and attacks tobacco products frequently (Riudavets et al., 2002), and red flour beetles, *Tribolium castaneum* (Herbst), one of the most widespread and destructive stored-product pest throughout the world (Zapata and Smagghe, 2010), using three method bioassays in laboratory conditions.

MATERIALS AND METHODS

Test insects

Tests were carried out on 7 ± 2 day old *L. serricorne* and *T. castaneum* adults. The insects were kept in the stored products in an insect rearing room of the Entomology Department of Urmia University at 25 ± 2°C and 65 ± 5% relative humidity (RH). As a rearing medium, diet of wheat at 14% moisture content mixed with yeast was used for the two species of insects.

Garlic emulsion (Sirinol)

In this study, 85% Sirinol EC (5% of alicin extracted from garlic bean, 75% of fuel oils, 20% of surfactant and retentive; Kimia Sabzavar Co., Iran) was used to control the adults of two stored product insects. Sirinol is considered nontoxic to mammals (rat oral acute LD_{50} is >5000 mg kg^{-1}).

Bioassays

Four concentrations of 0.5, 1, 5 and 10% Sirinol were prepared. Distilled water was used as a solvent. In each bioassay, percentage repellency (PR) was recorded after exposure. It should be mentioned that to select appropriate concentrations and times for original tests, preliminary tests were carried out.

Repellent activity

The repellent action of the garlic emulsion against the insects was evaluated by the three methods as follows:

Petri-dish bioassay technique

The Petri dish chamber test was used to determine the repellency of garlic emulsion to insects (Ajayi and Olonisakin, 2011). A choice bioassay system was used to evaluate repellency of the four concentrations of Sirinol. Different test concentrations were applied to a half filter paper disc (Whatman no. 1, 12 cm diameter; Whatman Co., Germany) as uniformly as possible with a pipette (1 ml of each prepared concentrations). Half of the bottom of a Petri dish was covered with the treated filter paper, while the other half was covered with a filter paper disk impregnated with 1 ml distilled water. Ten unsexed adults were put into each Petri dish (at the center) and the lid was sealed in place with parafilm. Ten replicates were run for each tested concentration so that 100 adults were assayed per concentration. The test was carried out under the same environmental conditions described for the rearing. The numbers of insects on the two half paper disks were recorded after 12, 24, 48 and 72 h from the beginning of the test.

Y-shape olfactometer tube bioassay technique

A "Y" shaped olfactometer with 3 connected glass tubes (10 cm long, 1 cm diameter) with an opening at the intersection of 3 arms for a vacuum pump was used as the olfactometer. The opening on the intersection of the arms facilitated the air circulation in the olfactometer (Paranagama et al., 2004). The ends of two tubes of the olfactometer were connected with perforated, plastic, transparent and wide mouthed bottles (250 ml) through the lids and other end of the tube was used to introduce insects. Using Whatman no.1 filter papers (2.5 cm × 2.5 cm), one treated with a known amount of 1 ml Sirinol and the other treated with equal amount of distilled water were hung separately. The middle of the bottles was connected to the two tubes using metal wires after air-drying for 10 min. The olfactometer was placed horizontally on a white background in daylight. After switching on the vacuum pump, ten test insects were introduced into the olfactometer. The number of insects that moved into the Sirinol treated and distilled water

Table 1. Variance analysis of different treatments of the two experimented insects' repellency in the Petri-dish technique.

S.V	*L. serricorne*				*T. castaneum*			
	df	Mean square	F	Significance	df	Mean square	F	Significance
Factor A (concentration)	3	2018.393	13.146	0.000**	3	7048.027	35.329	0.000**
Factor B (time)	3	1074.949	7.001	0.000**	3	1272.593	6.379	0.000**
AB (concentration × time)	9	21.493	0.140	0.998^{ns}	9	374.054	1.875	0.060^{ns}

$^{n.s}$*P* is not significant; **p* is significant at 0.05 level; ***p* is significant at 0.01 level. *Insect

treated bottles were recorded within 1, 2, 4, 8, 12 and 24 h. Four concentrations of Sirinol were tested separately and each concentration was replicated 10 times. Placement of the Sirinol treated and distilled water treated filter papers were interchanged randomly in subsequent replicates.

Glass bioassay technique

The glass bioassay technique (Mohan and Fields, 2002) determines the response of insects to potential repellents by specifying the number insects that were kept away from treated source or grain (Pretheep et al., 2004). In this technique, leaky plastic glasses with equal pores were used that allow only insects and not grain to pass through. Different concentrations (0.5, 1, 5 and 10%) of garlic emulsion were used into wheat. After air-drying for 10 minutes, wheat was put on the glasses and 10 insects of each species were released at the top of glasses. Glasses were covered by a muslin cloth and then placed in other glasses. Controls (concentration of 0%) with wheat treatment by distilled water were maintained to record natural movement. All experiments were conducted at a room conditions. The number of trapped insects was determined at 4 different intervals (12, 24, 48 and 72 h) after the introduction of the insects. There were ten replicates per treatment.

PR was calculated as follows in each three methods:

$$PR = 100 \times (C-T)/(C + T)$$

Where, C is the numbers of insects on the untreated area and T is the numbers of insects on the treated area (Nerio et al., 2009).

Data analysis

PR data was analyzed using analysis of variance after transforming with arcsine. All negative PR values were treated as zero (Udo, 2005, 2011). Data were subjected to univariate analysis using SPSS (SPSS Inc., 1993). Data of experiment were analyzed by a completely randomized design using factorial arrangements of treatments (ten replicates for each treatment). The analysis of data was performed on each dependent variable and the treatments were compared for significance with ANOVA. Mean separation was determined using the Tukey's test.

RESULTS

Table 1 shows that the F values of concentration and time were significant for *L. serricorne* and *T. castaneum* in the Petri-dish technique. The interaction between concentration and time (concentration × time) were not significant for *L. serricorne* and *T. castaneum*. Moreover, the highest repellency of Sirinol for *L. serricorne* and *T. castaneum* in the Petri-dish technique was observed in concentration of 10% and time of 72 h (Figure 1). In addition, Table 2 shows that the F values of concentration and time were significant for *L. serricorne* and *T. castaneum* in the olfactometer technique. The interaction between concentration and time (concentration × time) were not significant for *L. serricorne* and *T. castaneum*, while the highest repellency of Sirinol for *L. serricorne* and *T. castaneum* in the olfactometer technique was observed in concentration of 10% and time of 2 h (Figure 2).

Furthermore, Table 3 shows that the F values of the concentration were significant for *L. serricorne* and *T. castaneum* but the F values of the time and the interaction between concentration and time (concentration × time) were not significant for *L. serricorne* and *T. castaneum* in the glass technique. The highest repellency of Sirinol for *L. serricorne* in the glass technique was observed in concentration of 10% and time of 72 h (Figure 3). Tables 4 and 5 show that adults of *T. castaneum* were more susceptible to Sirinol than adults of *L. serricorne* because the amounts of PR for *T. castaneum* in different concentrations and times were higher than the amounts of *R. dominica* in the same condition.

The PR of the Sirinol was 58.56, 42.58 and 26.29% for *L. serricorne*, using Petri-dish, Y- shape olfactometer and leaky glass techniques, respectively (Table 4). According to Tukey's grouping, the differences were not significant for the both species between the times in glass technique (Table 5). Results also showed that the highest PR for each of the two species occurred in concentration of 10% in each of three techniques, which meant that PR increased as concentration increases, although, this was not accurate about the times; for example in olfactometer technique, PR for the time of 12 h was less than the PR for the times of 1, 2, 4 and 8 h (Tables 4 and 5).

DISCUUSION

These results are consistent with the study of Kain and Kovash (1999) who reported the ability of garlic to protect crops against variety of insect pests. Likewise, Grainge et

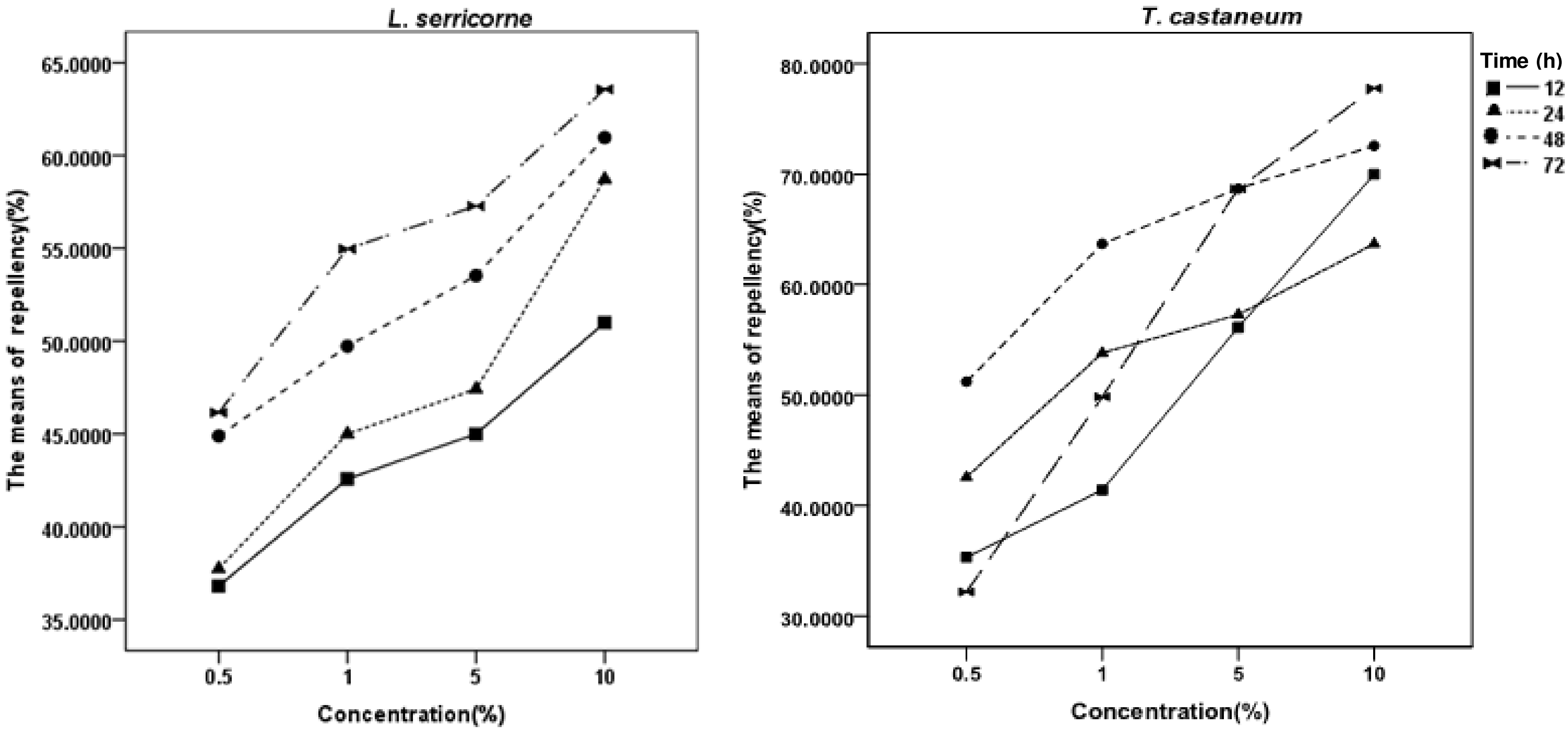

Figure 1. The comparison of repellency of Sirinol in the different concentrations and times for *L. serricorne* and *T. castaneum* in the Petri-dish technique.

Table 2. Variance analysis of different treatments of the two experimented insects' repellency in the olfactometer technique.

S.V	*L. serricorne				*T. castaneum			
	df	Mean square	F	Significance	df	Mean square	F	Significance
Factor A (concentration)	3	13131.420	165.821	0.000**	3	16665.351	121.146	0.000**
Factor B (time)	5	2373.109	29.967	0.000**	5	2733.745	19.872	0.000**
AB (concentration × time)	15	114.142	1.441	0.130^{ns}	15	62.603	0.455	0.960^{ns}

$^{n.s}$*P* is not significant; **p* is significant at 0.05 level; ***p* is significant at 0.01 level. *Insect

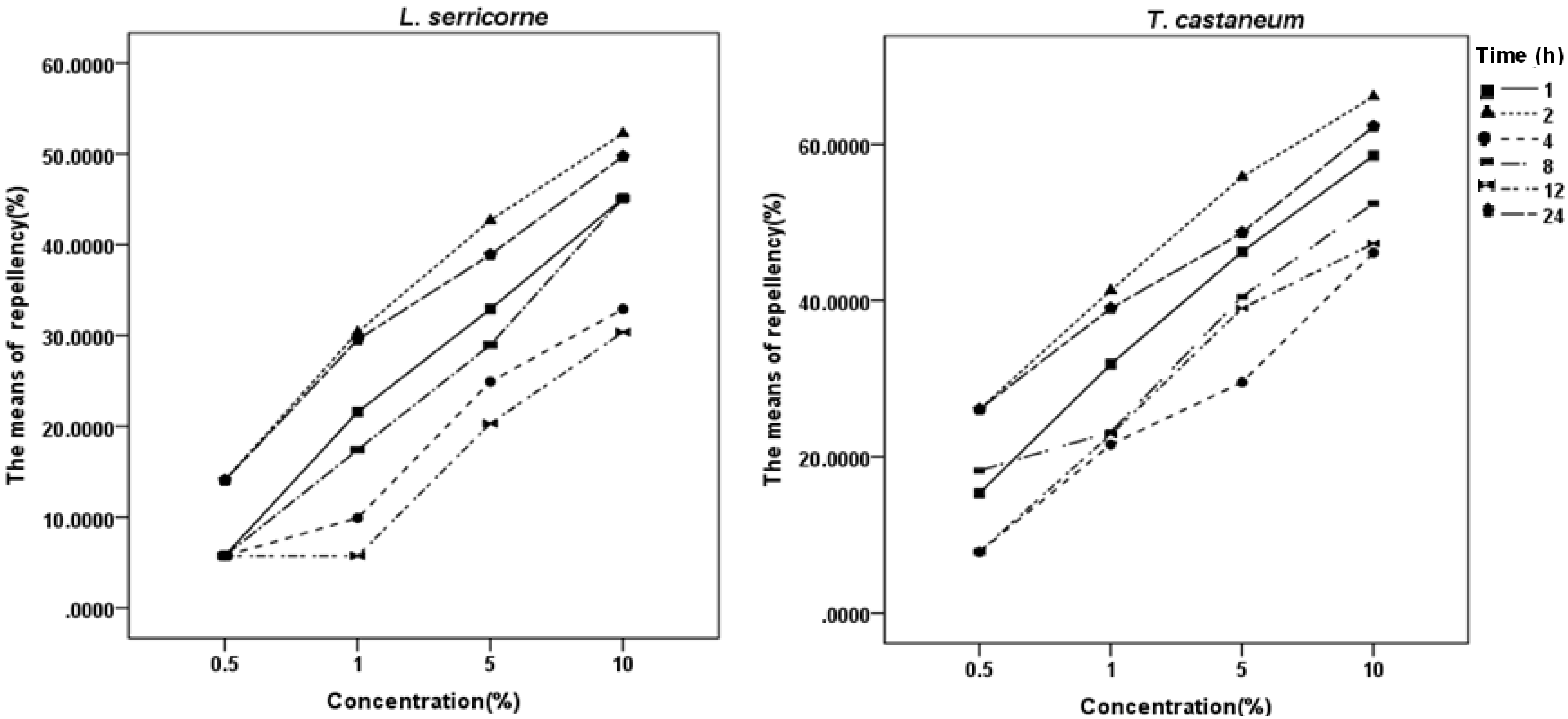

Figure 2. The comparison of repellency of Sirinol in the different concentrations and times for *L. serricorne* and *T. castaneum* in the olfactometer technique.

Table 3. Variance analysis of different treatments of the two experimented insects' repellency in the glass technique.

S.V	**L. serricorne*				**T. castaneum*			
	df	**Mean square**	**F**	**Significance**	**df**	**Mean square**	**F**	**Significance**
Factor A (concentration)	3	2691.373	36.553	0.000**	3	7836.749	110.550	0.000**
Factor B (time)	3	145.935	1.982	0.118^{ns}	3	172.223	2.429	0.067^{ns}
AB (concentration × time)	9	44.477	0.604	0.837^{ns}	9	35.813	0.505	0.910^{ns}

$^{n.s}$*P* is not significant; **p* is significant at 0.05 level; ***p* is significant at 0.01 level. *Insect

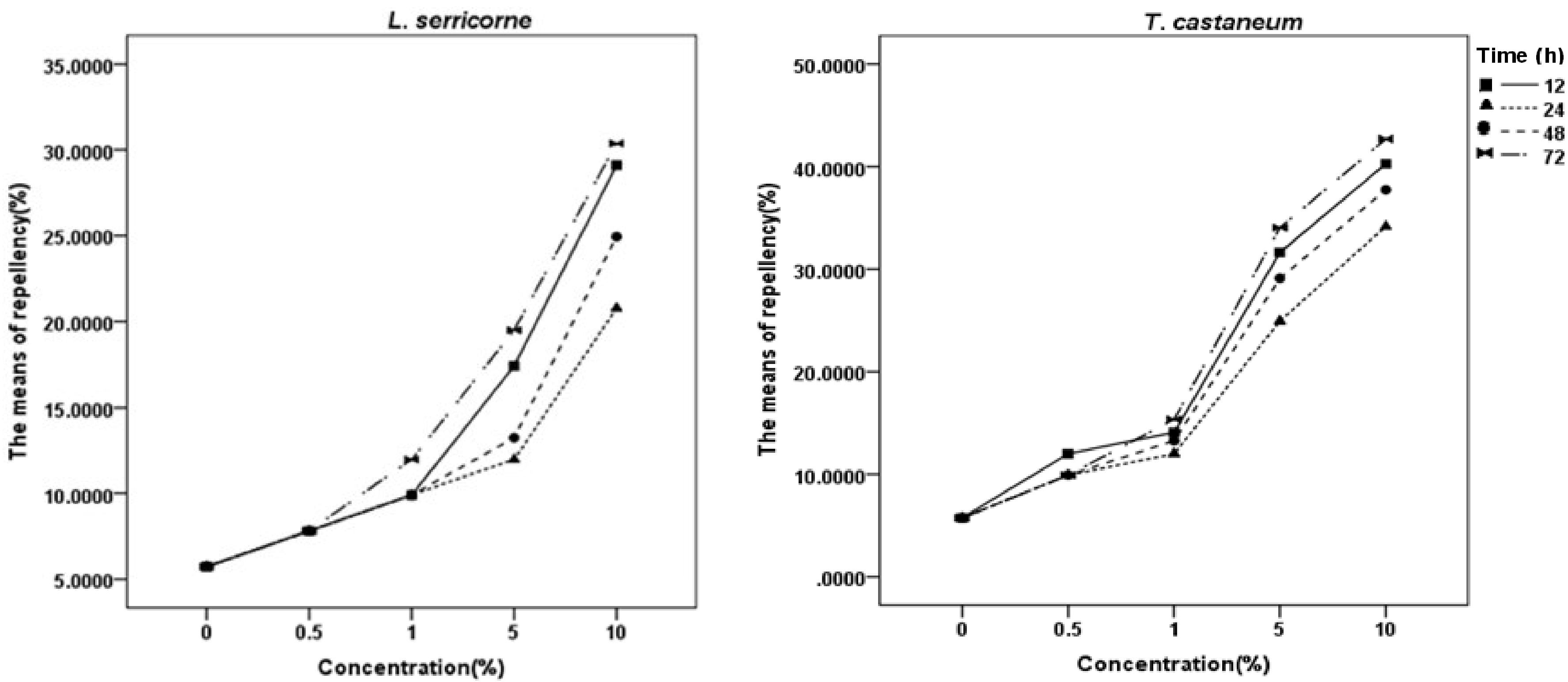

Figure 3. The comparison of repellency of Sirinol in the different concentrations and times for *L. serricorne* and *T. castaneum* in the glass technique.

al. (1985) reported that garlic has insect controlling properties that repel and make the host less favorable and less prone to attack and infestation by insects. Buba et al. (2007) also reported the effectiveness of garlic at controlling *Bemisia tabaci* Gennadius and *Megalurothrips sjostedti* Trybom in cowpea.

From this study, it seems that the effectiveness of the garlic emulsion is directly related to the quantity applied. However, the relatively high insect repellency caused by 1 ml Sirinol treatment indicated the high potency and repellent activity of garlic emulsion. Our result therefore indicated that the high concentrations will cause high PR rates, which was also supported by other researchers (Bouda et al., 2001; Lee et al., 2001; Owusu, 2001; Park et al., 2003; Wang et al., 2006). The repellency of over 70% in the concentration of 10% of Sirinol against *T. castaneum* adults in Petri-dish technique irrespective of application rate is suggestive of the high repellent activity of garlic. This is in agreement with the reports of Graigne et al. (1985) and Osipitan and Mohammed (2008) that indicated the insecticidal, repellence, antifeedant, and fumigative effects of garlic. In addition, Rahman and Motoyama (2000) also reported the repellency effects of garlic clove, grated garlic and its volatile extract applied on brown rice (*Nilaparvata lugens* Stål), maize weevil (*Sitophilus zeamais* Motschulsky) and red flour beetle (*Tribolium* sp.), and suggested that the active volatile compounds are likely to be sulfide compounds produced by rapid degradation of allicin. Rahman and Schmidt (1999) by GC-MS analysis showed that allicin was a principle segment of garlic extract and had repellency effects on pests.

Data analysis revealed that Sirinol's PR had no significant difference between the time of 12 and

Table 4. The results of repellency effect of the Sirinol on *L. serricorne* and *T. castaneum* adults at different concentrations in three techniques.

Technique	Means of PR (%)									
	***L. serricorne**					***T. castaneum**				
Concentration (%)	**0.0**	**0.5**	**1.0**	**5.0**	**10.0**	**0.0**	**0.5**	**1.0**	**5.0**	**10.0**
Petri-dish technique	-	41.4^{c}	48.07^{bc}	50.81^{b}	58.56^{a}	-	40.33^{d}	52.19^{c}	62.70^{b}	70.99^{a}
Olfactometer technique	-	8.51^{d}	19.09^{c}	31.45^{b}	42.58^{a}	-	16.9^{d}	29.97^{c}	43.31^{b}	55.47^{a}
Glass technique	5.74^{c}	7.82^{c}	10.42^{bc}	15.54^{b}	26.29^{a}	5.74^{d}	10.42^{cd}	13.66^{c}	29.93^{b}	38.72^{a}

For each insect species, the means in the same rows followed by the same letters do not differ significantly ($p > 0.05$) as determined by Tukey's test. *Insect.

Table 5. The results of repellency effect of the Sirinol on *L. serricorne* and *T. castaneum* adults at different times in three techniques.

Technique	Means of PR															
	***L. serricorne**								***T. castaneum**							
Time (h)	**1**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**1**	**2**	**4**	**8**	**12**	**24**	**48**	**72**
Petri-dish technique	-	-	-	-	43.85^{c}	47.22^{bc}	52.28^{ab}	55.49^{a}	-	-	-	-	0.71^{b}	54.34^{b}	64.04^{a}	57.12^{ab}
Olfactometer technique	26.33^{b}	34.89^{a}	18.37^{c}	24.31^{b}	15.54^{c}	33.06^{a}	-	-	38.001^{bc}	47.33^{a}	26.28^{d}	33.59^{cd}	29.25^{d}	44.02^{ab}	-	-
Glass technique	-	-	-	-	13.99^{a}	11.24^{a}	12.33^{a}	15.08^{a}	-	-	-	-	0.74^{a}	17.34^{a}	19.15^{a}	21.54^{a}

For each insect species, means in the same column followed by the same letters do not differ significantly ($p > 0.05$) as determined by Tukey's test. *Insect

72 h (Table 5), which demonstrated the garlic emulsion as persistence. Our results therefore showed that Sirinol's repellent effect was not decreased by passing time in contrast to the report of Liu and Ho (1999) that the repellency power of an essential oil of *Evodia rutaecarpa* Juss. decreased after 5 h. As a result, repellency power of essential oils for their high volatility (Owolabi et al., 2009; Ajayi and Olonisakin, 2011) decreases more rapidly than the other botanical formulation such as garlic emulsions for the same period. Many plant products such as essential oils have been screened for their repellent activity against stored grain pests (Regnault-Roger, 1997; Cosimi et al., 2009; Nerio et al., 2009). Other studies have shown that *T. castaneum* can also be repelled by essential oils from *E. rutaecarpa* (Liu and Ho, 1999), *Ocimum gratissimum* L. (Ogendo et al., 2008) and *Artemisia vulgaris* L. (Wang et al., 2006).

Based on our results, adults of *T. castaneum* were repelled by Sirinol even at very low concentration, thus, adults of *T. castaneum* were more susceptible to Sirinol than adults of *L. serricorne.* It seems that the higher movement ability of *T. castaneum* than other stored-product insects such as *L. serricorne* is the dominant reason for more effect of botanic extract on it (Liu and Ho, 1999; Tripathi et al., 2000). Researches carried out worldwide during the last three decades have significantly extended our knowledge on botanical pesticides. Many plant derived natural products active against insects could be produced from locally available raw materials, perhaps in many cases right at the site of usage, so as to be relatively inexpensive (Talukder, 2006). In this study, garlic emulsion (Sirinol) was effective at managing the population of two major stored product insects. It may therefore be one of the alternative control options in our immediate environment.

Conclusion

The results of this study showed that garlic emulsion at all the levels tested had effective repellent and could be considered for integration with other effective control options in the management of *L. serricorne* and *T. castaneum*. Therefore, the use of this botanical compound could reduce the serious health hazards to human life, insect pest resistance, resurgence, environmental pollution, ecological imbalance and residues in market produce.

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