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Full Length Research Paper

Evaluation of the mortality of two stored-product insects by garlic emulsion (Sirinol) in combination with low air pressure

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Although, there are a large number of suggested potential alternatives to methyl bromide for disinfestations of durable commodities, development alternatives are likely to be costly. The combined factors of methyl bromide phase-out, the gradual development of insect resistance to fumigants and undesirable effects of some fumigants residues in foodstuff, have led to the idea of using controlled atmosphere. In this research, adults of *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (Fabricius) were exposed to various air pressures (first phase) and air pressures together with 150 µL/L Sirinol (second phase) at 27 ± 2°C and 65 ± 5% relative humidity in various durations and mortality rates were determined. Complete mortality of *T. castaneum* adults was obtained within 1, 3 and 6 h when were subjected to 10, 30 and 50 mmHg, respectively. Adults of *R. dominica* showed 96.25 and 60% mortality during 3 and 10 h when exposed to 10 and 30 mmHg, respectively. It was revealed that by using 150 µL/L Sirinol in pressure of 50 mmHg for 4 h, mortality rates exceed 86.25 and 42.5% for adults of *T. castaneum* and *R. dominica*, respectively. Mortality percentage of the insects which were treated with Sirinol was more than the cohorts that were under vacuum conditions without any Sirinol treatment. Ultimately, data analysis showed that *T. castaneum* adults are noticeably susceptible to low air pressure and the combination of low air pressure and Sirinol.

Key words: Fumigant, Sirinol, low pressure, stored-products insects.

INTRODUCTION

Control of insect-pest infestation in storage may cause special problems on stored products. In many storage systems, methyl bromide and phosphine are the most economical fumigants for management of stored-grain insect pests. Methyl bromide however, depletes the ozone layer (Cassanova, 2002). Additionally, some stored-product insects are found to have developed resistance to methyl bromide and phosphine (Subramanyam and Hagstrum, 1995; Champ and Dyte, 1977). Since the announcement of methyl bromide's removal, researchers have been concentrating on finding an effective and appropriate alternative insect control agent. Although, there are some potential substances for methyl bromide, but due to some limitations and drawbacks it is difficult to identify a suitable compound for replacement (Bell et al., 1996). Application of modified atmosphere (vacuum) is one of the considered methods.

The limitation of applying vacuum as a replacement for methyl bromide in ambient temperature is the long duration of fumigation time. In fact, this interval is similar to the time needed for fumigation with phosphine (Navarro and Donahaye, 1990). Mbata and Phillips (2001) evaluated the effect of temperature and exposure periods to vacuum on the mortality of three species of stored-products insects, which were exposed to low pressure. The results indicated an inverse relationship between temperature and exposure time. These problems have highlighted the need for the development of natural products derived from plants as an alternative to conventional insecticides. Many types of spices and herbs are known to possess insecticidal activities

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(Tripathi et al., 1999), especially in the form of essential oils (Shaaya et al., 1991). They do not leave residues toxic to the environment and have medicinal properties for humans with lower toxicity to mammals (Duke, 1985). Essential oils are potential sources of alternative compounds to currently used fumigants.

Various studies have demonstrated fumigant activity of various essential oils against various stored product insects (Shaaya et al., 1991, 1997; Tunç et al., 2000; Lee et al., 2003). Toxicity of various essential oils and their volatile constitutes against all life stages of the flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) indicated that two essential oils, garlic and onion had potent fumigant activities (Isikber, 2010). The insecticidal and repellent activity of garlic has been widely reported (Graigne et al., 1985; Rahman and Motoyama, 2000; Amiri Besheli, 2009). The bulb of garlic has been reported to possess insect controlling properties with repellant, antifeedant, bactericidal, nematicidal and fumigant mode of action that kill aphids and other soft bodied pest (Grainge et al.,1985). Osipitan and Mohammed (2008) reported the ability of garlic to repel borers, fleas, ticks and thrips. Rahman and Motoyama (2000) reported 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.

The use of both, vacuum technology and botanical extract, does not release ozone-depleting chemicals such as methyl bromide into the air. There is little or no information on the use of low pressure to control of stored product insects in combination with botanical extract. Present studies were conducted to develop an Integrated Pest Management protocol (IPM) which might be an alternate to the practices being adopted presently for the control of insect pests of stored grains but are safe, economical, easy to apply and nature friendly. For this purpose, mortality rate of low air pressure alone and in combination with garlic emulsion (Sirinol) against adults of *Tribolium castaneum* (Herbst) one of the most widespread and destructive stored-product pest throughout the world (Zapata and Smagghe, 2010) and *Rhyzopertha dominica* (Fabricius) that attacks grain seeds and causes high loss (Bousquet, 1990) was investigated in this research.

MATERIALS AND METHODS

Test insects

Tests were carried out on of *T. castaneum and R. dominica*. The insects were kept in the stored products insects' rearing room of the Entomology Department of Urmia University at 25 ± 2°C and 65 ± 5% relative humidity (R.H.). As rearing medium, diet of wheat flour mixed with yeast (17:1 w/w) was used for *T. castaneum* (Donahaye, 1990) and wheat for *R.* dominica.

Experimental setup

The vacuum chamber was made of steel and its capacity was 6 l. Two faucets were set on the lid to control the flow of air or other gases in and out of the chamber, along with a barometer and an opening on the lid with a septum in it for injection. One of the faucets is connected to a vacuum pump (Varian DSE 42, Germany) to fix the operating pressure in the chamber.

Relative air pressure in Urmia

Normal pressure of experiments in Urmia city (Iran) (653.5 mmHg) was calculated by the method of Evett and Liu (1988). Due to the calculated air pressure and the scales of the barometer, other low air pressures were created by vacuum pump. The following data were used for the calculation; air pressure in seaside (height 0): 760 mmHg = 101.33 KN/m^2; height 2 km: 596 mmHg = 79.5 KN/m^2; height 1.3 km (Urmia). Using 2 km as 101.3 - 79.5 and 1.3 km as x = P =14.1895; total air pressure was calculated as;

Air pressure in Urmia (P) = 101.33 – 14.1895 = 87.14 KN/m^2 = 653.5 mmHg

Garlic emulsion (Sirinol)

In this study, 85% Sirinol EC (5% of alicin which was 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 was considered nontoxic to mammals (rat oral acute LD_{50} is >5000 mg kg^{-1}).

Experimental protocol

First phase (low pressure exposition)

Twenty-four hours prior to each test, the insects, Sirinol and other needed equipment were transferred to a room with adjustable temperature and humidity. The test was performed in the temperature of 27 ± 2°C and the relative humidity of 65 ± 5%. Adults of *T. castaneum* and *R. dominica* were randomly collected from the rearing containers and each were exposed to air pressures of 10, 30, 50, 70 and 90 mmHg during the exposure period of 1, 2, 3, 4, 5, 6, 8, 10 and 12 h (Table 1). Each treatment had four replications and the number of insects used in each treatment and replication was 20. After each time interval for a definite air pressure expired, the container lid was removed and the mortality was recorded. As for control treatments, in each case after achieving the required air pressure, the container lid was removed immediately and mortality was recorded. No mortality was observed in any control treatment. It should be mentioned that to select appropriate pressures and times for original tests, preliminary tests carried out.

Second phase (combination of low pressure and Sirinol exposition)

Adults of *T.* castaneum and *R.* dominica were exposed to both 150 µL/L of Sirinol and air pressures of 50 and 90 mmHg for 1, 2, 3, 4, 6 and 7 h. For each treatment there were four replications and the number of insects used in each replication for any treatment was 20. The test was carried out at 27 ± 2°C and relative humidity of 65 ± 5%. At the initiation of experiment, the adult insects of each species were transferred to vacuum chamber and the definite air

Table 1. Low pressures and exposure times using for two insect species at the first phase.

Time (h) \ P(mmHg)	10	30	50	70	90
1	C; D	C; D	C; D	C; D	C; D
2	D	C; D	C; D	C; D	C; D
3	D	C; D	C; D	C; D	C; D
4		D	C; D	C; D	C; D
5		D	C; D	C; D	C; D
6		D	C; D	C; D	C; D
8		D			C
10		D			C
12					C

C: *Tribolium castaneum*; D: *Rhyzopertha dominica*.

pressure was applied using the vacuum pump. Then, 150 µL/L of Sirinol was injected through the opening on the lid to the container using an Oxford pipette which was attached to a needle-head gauge 18. When the exposure time for any individual test expired, the chamber lid was removed and mortality was recorded.

Data analysis

Mortality data from all the bioassays were analyzed with SPSS software (SPSS Inc, 1993). The data of experiment were analyzed by a completely randomized design using factorial arrangements of treatments (four replicates for each treatment). The analysis of data was performed on each dependent variable using the treatments were compared for significance with ANOVA. Mean separation was determined using the Tukey's test.

RESULTS AND DISCUSSION

Mortality percentages of *T. castaneum* and *R. dominica* are shown in Table 2 (first phase results). Adults of *T. castaneum* showed 98.75% of mortality after being exposed to air pressure of 10 mmHg for 1 h, which was equal to the mortality percentage of this insect after exposure to air pressure of 50 mmHg for 6 h. The highest mortality percentage of *R. dominica* was obtained after 3 h of exposure to air pressure of 10 mmHg. Table 1 displays that adults of *R. dominica* are less susceptible to low air pressure compared to the adults of *T. castaneum*. It could be postulated that to control adults of *R. dominica* by using vacuum, one of the following methods should be applied:

(1) Producing ultra low pressures
(2) Increasing the exposure time of the insect under vacuum conditions

The second method is economic and feasible and the exposure time is equivalent to that of fumigation with phosphine. LT_{50} (the time required to kill 50% of the population at a certain dose or concentration) and LT_{95} (lethal time of toxicant expected to kill 95% of population) values for the adults of tested insects are shown in Table 3. Comparing upper and lower limits of LT_{50} and LT_{95} values for two insect species in the air pressures of 30 and 50 mmHg showed that the difference between susceptibility of these species is significant.

Table 4 displays the mortality percentages of the second phase of the tests. In comparing the data of Tables 2 and 4, it was concluded that the mortality of *T. castaneum* adults (which showed no mortality after being exposed to air pressure of 90 mmHg for 12 h) was increased by rate of 12.5% after exposure of 150 µL/L of Sirinol and air pressure of 90 mmHg for 7 h. Therefore, the effect of vacuum on the insects' mortality was increased in the presence of Sirinol. Injection of 150 µL/L of Sirinol in the air pressure of 50 mmHg for 4 h resulted in 86.25% of mortality, which is comparable to 81.25% mortality obtained by air pressure of 50 mmHg after 5 h without Sirinol injection. In the case of *R. dominica*, mortality percentage of adult insects was recorded as 46.25% after exposure to 150 µL/L of Sirinol and air pressure of 50 mmHg for 5 h which is equal to the mortality rate in the air pressure of 30 mmHg for 8 h without presence of Sirinol. Overall, results show that *T. castaneum* adults are more susceptible to low air pressure and the combination of low air pressure and Sirinol compared to the *R. dominica*. Table 5 shows that F values of pressures, times and the interaction between pressures and time periods (pressure × time) are significant on the insects. The results also showed that there was a significant difference in the mortality between low air pressures for adults of *T. castaneum* and *R. dominica* in the second phase (Tables 6 and 7). Moreover, Figures 1 and 2 show that the influence of reduce pressure and Sirinol at the air pressure of 50 mmHg and 7 h was more than the other pressures and times, because the highest mortality of *T. castaneum* and

Table 2. Effect of vacuum in controlling adults of *T. castaneum* and *R. dominica*.

Number of *R. dominica* adult	Exposure time (h)	Air pressure (mmHg)	Mortality (%)	Number of *T. castaneum* adult	Exposure time (h)	Air pressure (mmHg)	Mortality (%)
80	1	10	31.25	80	1	10	98.75
80	1	30	0	80	1	30	31.25
80	1	50	0	80	1	50	0
80	1	70	0	80	1	70	0
80	1	90	0	80	1	90	0
80	2	10	70	80	2	30	81.25
80	2	30	2.5	80	2	50	23.75
80	2	50	0	80	2	70	0
80	2	70	0	80	2	90	0
80	2	90	0	80	3	30	100
80	3	10	96.25	80	3	50	50
80	3	30	8.75	80	3	70	0
80	3	50	0	80	3	90	0
80	3	70	0	80	4	50	76.25
80	3	90	0	80	4	70	2.5
80	4	30	16.25	80	4	90	0
80	4	50	2.5	80	5	50	81.25
80	4	70	0	80	5	70	5
80	4	90	0	80	5	90	0
80	5	30	26.25	80	6	50	98.75
80	5	50	6.25	80	6	70	8.75
80	5	70	0	80	6	90	0
80	5	90	0	80	8	90	0
80	6	30	37.5	80	10	90	0
80	6	50	11.25	80	12	90	0
80	6	70	0				
80	6	90	0				
80	8	30	46.25				
80	10	30	60				

Table 3. LT_{50} and LT_{95} values measured by hour for the adults of *T. castaneum* and *R. dominica*.

Parameter	*R. dominica*			*T. castaneum*		
Air pressure (mmHg)	30	50	70	10	30	50
Total number of insects	80	80	80	80	80	80
Lower limit of LT_{50}	1.1372	2.77321	8.2518	1.19655	7.36819	7.77375
LT_{50}	1.26217	2.97999	11.73957	1.3538	8.1614	10.20243
Upper limit of LT_{50}	1.3805	3.18177	70.39777	1.50025	9.30435	29.43761
Lower limit of LT_{95}	2.24503	5.69476	13.70173	2.80312	20.93004	12.4527
LT_{95}	2.55669	6.34011	27.1715	3.3027	27.39696	21.46557
Upper limit of LT_{95}	3.08271	7.30387	971.0032	4.22921	40.5897	192.4303

R. dominica was observed at the mentioned pressure and time.

Previously, many studies were performed on mortality properties of modified atmosphere and reduced pressure on stored-product insects (Bare, 1948; Calderon et al., 1966; Calderon and Leesch, 1983; Navarro and Donahaye, 1987; Locatelli and Doalio, 1993; Finkelman et al., 2004; Mbata et al., 2004, 2005, 2009). Reduced pressure or vacuum causes low O_2 and high CO_2 concentrations by metabolic arrest and losses water

Table 4. Effect of vacuum and Sirinol in controlling adults of *T. castaneum* and *R. dominica*.

Total number of adult	Sirinol amounts (µL/L)	Air pressure (mmHg)	Exposure time (h)	Mortality (%) *T. castaneum*	Mortality (%) *R. dominica*
80	150	90	6	1.25	0
80	150	90	7	12.5	7.5
80	150	50	1	12.5	5
80	150	50	2	33.75	17.5
80	150	50	3	56.25	30
80	150	50	4	86.25	42.5
80	150	50	5	95	46.25
80	150	50	6	95	47.5
80	150	50	7	100	53.75

Table 5. Variance analysis of combination of 150 µL/L Sirinol and low pressure (50 and 90) for adults of *T. castaneum* and *R. dominica* mortality in 0, 1, 2, 3, 4, 5, 6 and 7 h exposure times.

Insect	*T. castaneum*				*R. dominica*			
S.V	df	Mean square	F	Sig.	df	Mean square	F	Significance
Factor A (pressure)	2	26801.535	3481.968	.0001**	2	8679.159	6694.802	.0001**
Factor B (time)	7	1682.282	218.557	.0001**	7	484.124	373.437	.0001**
AB (pressure × time)	14	1246.756	161.975	.0001**	14	351.063	270.798	.0001**

** Significant at the 1% level.

Table 6. Multiple comparisons of pressure for adults of *T. castaneum*.

Air pressure (mmHg)		Mean Difference (I - J)	Standard error	Significance	95% Confidence interval	
					Lower bound	Upper bound
653.5	50	-51.447556*	.6935974	.000	-53.107420	-49.787691
	90	-2.756286*	.6935974	.000	-4.416150	-1.096421
50	653.5	51.447556*	.6935974	.000	49.787691	53.107420
	90	48.691270*	.6935974	.000	47.031406	50.351135
90	653.5	2.756286*	.6935974	.000	1.096421	4.416150
	50	-48.691270*	.6935974	.000	-50.351135	-47.031406

*The mean difference is significant at the 0.01 level.

through opened spiracle is lethal for insects (Phillips and Throne, 2010; Mitcham et al., 2006). Influence of low pressure and CO_2 was studied by Navarro et al. (2004) and Isikber et al. (2002). Their results showed that the combination of propylene oxide with low pressure or CO_2 can provide a potential alternative to methyl bromide for quarantine treatment of commodities where rapid disinfestations techniques and high level of insect mortality are essential. Toxicity of methyl bromide alone and in combination with carbon dioxide or under reduced pressure was studied by Donhaye and Navarro (1989). Their results showed significant difference between methyl bromide alone and mixed by CO_2 and reduced pressure. Navarro et al. (2004) exposed all stages of *T. castaneum* to propylene oxide fumigant (PPO) alone and in combination with low pressure (100 mmHg) at 4 and 8 h. They indicated that LD_{50} (the amount of poison per unit weight which will kill 50% of the particular population of the animals species employed for the tests) and LD_{99} (lethal dose of toxicant expected to kill 99% of population)

Table 7. Multiple comparisons of pressure for adults of *R. dominica*.

		Mean Difference (I - J)	Standard error	Significance	95% Confidence Interval	
					Lower bound	Upper bound
653.5	50	-29.373546*	.2846492	.000	-30.054746	-28.692345
	90	-1.780677*	.2846492	.000	-2.461878	-1.099476
50	653.5	29.373546*	.2846492	.000	28.692345	30.054746
	90	27.592869*	.2846492	.000	26.911668	28.274070
90	653.5	1.780677*	.2846492	.000	1.099476	2.461878
	50	-27.592869*	.2846492	.000	-28.274070	-26.911668

*The mean difference is significant at the 0.01 level.

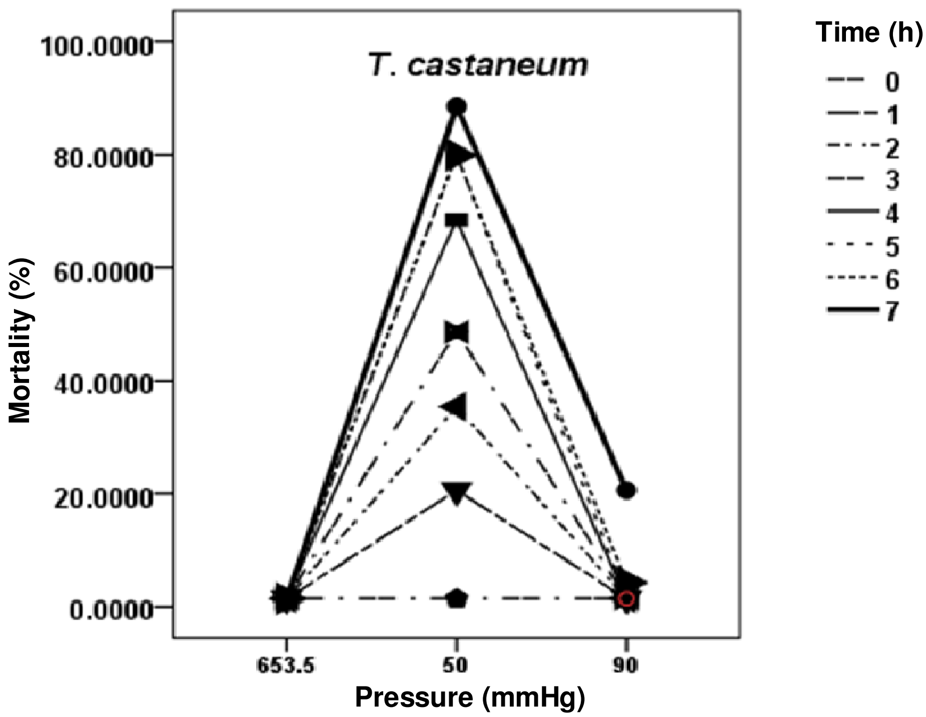

Figure 1. The comparison of mortality of *T .castaneum* in the different times and pressures.

of propylene oxide in combination with the vacuum were lower than the used of it without vacuum. They also showed that the pressure of 100 mmHg had synergistic effect on the toxicity of propylene oxide used against different stages of *T. castaneum.* These results are similar to the results obtained in the second phase of current research.

In addition, our results show a synergistic effect between Sirinol and 50 and 90 mm Hg pressures, so that after using Sirinol under low pressures, the time needed to obtain similar mortality of *T. castaneum* adults at the mentioned pressures decreased. The time needed to obtain LT_{99} for adults of *T. castaneum* at 20 mm Hg pressure and temperature of 25℃ has been reported 2.7 h by Calderon et al. (1966). This period is near to the LT_{95} achieved for adults of *T. castaneum* (2.55 h) at the pressure of 30 mmHg and temperature of 27 ± 2℃ in the current study. The time required for control adults of *T. castaneum* in the temperature of 25℃ and pressure 32.5 mmHg for 3 h has been reported by Phillips et al. (2000), which is similar to the time obtained in the current study. The results of this study indicated that adults of *R. dominica* had 60% mortality after taking 10 h under the pressure of 30 mmHg and temperature of 27 ± 2℃ does not match with the results of Phillips et al. (2000) who reported 100% mortality of *R. dominica* adults after 3 h exposure in the similar conditions (temperature 25℃ and pressure 32.5 mm Hg). This difference may be due to the

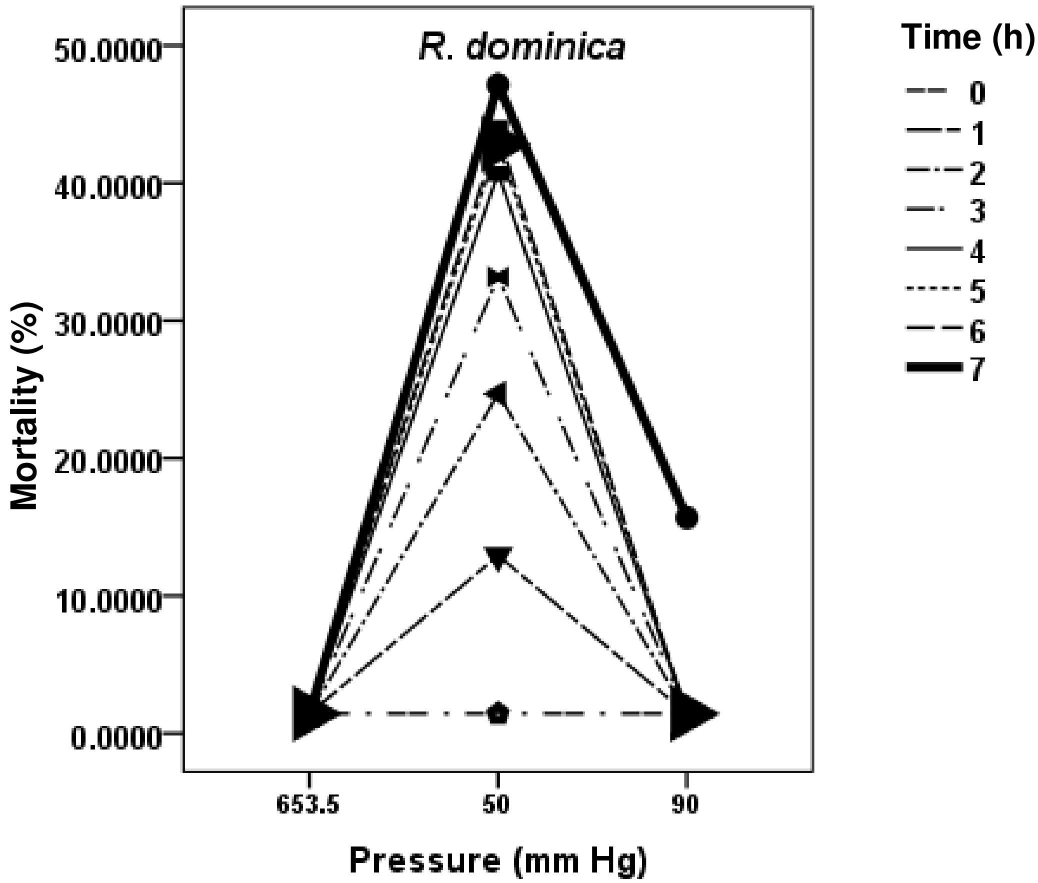

Figure 2. The comparison of mortality of *R. dominica* in the different times and pressures.

differences in insects' species, the insects' ages and circumstances of the tests.

So far, Sirinol whose toxicity has already been reported by Amiri (2009) was used and it was revealed that application of Sirinol in the air pressure of 50 mmHg provide the mortality rate similar to the state without Sirinol injection and decreases the fumigation duration considerably. From this study, it can be seen that the use of Sirinol with vacuum clearly resulted in significant reductions of LT_{50} and LT_{90} values for adults of *T. castaneum* and *R. dominica*. However, data without garlic emulsion indicated that there was less mortality of the adults to vacuum alone. In conclusion, the results suggest that Sirinol has a synergistic effect on the test insects when exposed together with low air pressures, and combination of garlic emulsion (Sirinol) with vacuum can be a potential as an alternative application to the most commonly used commercial fumigants, methyl bromide and phosphine.

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