Egyptian Journal of Biological Pest Control, 21(2), 2011, 159-163

Lethal Effects of Low Atmosphere Pressures on Various Developmental Stages of *Tribolium castaneum* (Herbst) and *Ephestia kuehniella* (Zeller) under Laboratory Conditions

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(Received: August 23, 2011and Accepted: September 19, 2011)

ABSTRACT

Decreased atmosphere pressures were applied to create low oxygen atmosphere to be used as an efficient alternative method of methyl bromide and phosphine fumigations for controlling the stored product pests. In the current study, efficacy of some low pressures of atmosphere (20, 40, 60, 80 and 100 mm Hg on adult and 20 and 40 mm Hg on developmental stages) were investigated against two common stored-product insects; the red flour beetle [*Tribolium castaneum* (Herbst)) (Coleoptera: Tenebrionidae)] and the Mediterranean flour moth [*Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae)] to determine their mortality rate in relation to different low pressures at 30°C. Adults of *T. castaneum* required 0.54 and 32.69 hr. as exposure period at 20 and 100 mm Hg, respectively to achieve 50% mortality (LT_{50}). While, these LT_{50} values were 1.42 and 36.42 hr for *E. kuehniella*, respectively. LT_{50} values for eggs, larvae and pupae of *T. castaneum* in 20 mm Hg were 4.92, 0.86 and 5.35 mm Hg, respectively, while those of *E. kuehniella* were10.13, 3.60 and 0.95 hr, respectively. The values at 40 mm Hg for eggs, larvae and pupae of *T. castaneum* were 15.58, 6.06 and 11.78 hr, respectively. While those for *E. kuehniella* were 33.55, 9.58 and 3.41 hr, respectively. These results indicated that the susceptibility of these insects to reduced atmospheric pressure increased by decreasing the atmosphere pressure. Application of decreased atmospheric pressure as one of the safe methods in pest management has a potential efficacy for controlling stored-product insects.

Key words: Atmosphere pressure, *Tribolium cstaneum, Ephestia kuehniella*, non-chemical control.

INTRODUCTION

Agricultural and animal stored products are threatened by more than 1200 species of pests (Rajendran, 2002). Two major stored product pests namely, *Tribolium castneum* (Herbst) and *Ephestia kuehniella* (Zeller) were chosen for current study. Both species are worldwide pests of stored products which hardly damages wheat and flour. In many storage systems, fumigants, especially methyl bromide and phosphine are the most economical and common tools for managing stored product pests not only due to their ability to kill a broad range of pests but also because of their easy penetration into the commodity and leaving minimal residue (Mueller, 1990 and Lee *et al.*, 2004). However, due to the potential ozone-depleting property and high toxicity to warm-blooded animals, including human beings, the use of methyl bromide, the most effective fumigant, was restricted for controlling the quarantine pests (Dansi *et al.*, 1984 and Anonymous, 1991).

The procedure of fumigation by phosphine, which is also widely employed, may become restricted as well, because it can make the stored-product insects resistant to fumigants. The potential genotoxicity of phosphine is also one of the reasons why some researchers support its restriction (Bell and Wilson, 1995 and Meaklim, 1998). Based on the above-mentioned reasons, it seems wise to find alternative ways of controlling stored-product pests. Controlled atmosphere, including low oxygen, high carbon dioxide concentrations and reduced pressure are also efficient ways of controlling stored-product insects, especially in the adult stage. Modified atmosphere treatments are healthy, environment-friendly ways of controlling the pests which threaten a large number of stored-products (Philips and Throne, 2010).

Since the phase-out of methyl bromide in 2005, the controlled atmosphere procedures have been adopted as feasible alternative treatments in the most developed countries. These methods have been also tested in the laboratories and under industrial conditions and were used for many years to control various insect and mite species (Fleurat-Lessard, 1990; Adler *et al.*, 2000 and Navarro, 2006). It has been demonstrated by previous studies that low pressure, produced by low O_2 concentrations and high concentration of CO_2, can control stored-product insects in storages (Philips and Throne, 2010).

Many researchers have previously studied the mortality of stored-product insects, such as *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Tribolium castaneum* (Herbst), *Lasioderma serricorne* (F.), *Oryzaephilus surinamensis* (L.) and *Callosobruchus maculatus* (F.) (Finkelman *et al.*, 2006; Mbata and Philips, 2001, 2004, 2005 and 2009). But pressures used in this study, were not investigated in previous researches as well life stages of experimental insects.

The goal of this research was to determine effective required exposure period to several low pressures at 30°C to achieve 50% mortality (LT_{50}) for adults and developmental stages of *T. castaneum* and *E. kuehniella*.

MATERIALS AND METHODS

Insects

The red flour beetle, *T. castaneum* and the Mediterranean flour moth, *E. kuehniella* were selected for this study. All experimental insects were obtained from insect cultures in stored-products lab and were reared at 29±2°C and 65±5% relative humidity, in darkness, on the diets of ground wheat and yeast for *T. castaneum* and flour for *E. kuehniella* using standard cultural techniques (Donahaye, 1990). 1-2 days old eggs were obtained by sieving 500 ml ovipositional jars. Thirty eggs of *T. castaneum* were arranged on a strip of double-coated transparent tape, which had one surface attached to one side of a dark filter paper. To obtain first larval instar, eggs were placed in 100 ml jars. After 1-2 days, the larvae were separated daily and were used for experiments. 2-3 days old pupae and 1-4 days old adults were obtained by daily separating of the jars containing last larval instars and pupae, respectively.

For obtaining eggs of *E. kuehniella,* adults were placed in a funnel and caged by gauze. A dark filter paper was placed below the funnel and 1-2 days old eggs were daily used. Thirty eggs of *E. kuehniella* laid within 24 h, were arranged on a strip of double-coated transparent tape, which had one surface attached to one side of a dark filter paper. The strips of filter paper were placed singly in glass vials, which were subsequently covered with plastic caps that had the center section removed and replaced with muslin screen to allow ventilation. For obtaining first larval instars, eggs were placed in 100 ml jars. After 1-2 days, the larvae were separated daily and were used for experiments. 2-3 days old pupae and 1-4 days old adults were obtained as mentioned for *T. castaneum.*

Bioassays tests

For reducing the pressure, a vacuum pump Model DSE42 was utilized along with nine-liter-capacity containers equipped with two gates for air input and output and a manometer. Also, 25 ml vials were used for caging experimental insects. Preliminary tests were carried out in the containers using the vacuum pump to obtain appropriate bioassay pressures. First of all, normal pressure of experiments in Urmia (Iran) was calculated using the procedure suggested by Evett and Liu (1988) which indicated a pressure of 653 mm Hg. Based on the results of the preliminary tests, five different pressure conditions (20, 40, 60, 80, and 100 mm Hg) were selected for adult insects' treatment. Other stages of insects were treated with 20 and 40 mm Hg of atmosphere pressure.

Temperature and relative humidity are very effective factors in low pressure experiments and thus all experiments in this study were carried out in an incubator with 30±2°C and 40±5% RH. Each experiment was replicated three times on three different days for each stage of the two experimental insects.

For recording the data of adults, the adults that did not move when lightly probed or shacked in light and mild heat were considered dead. Other developmental stages were considered dead when they did not develop furthermore to adults.

All experiments were carried out in 3 replicates and 30 cases were presented in each of them.

Data analysis

To analyze the quantitative data on mortality, the SPSS software version 16 was used. The Probit analysis was used to estimate LT_{50} values. The data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significance of differences (HSD) test to assess the statistical differences between the means at an alpha level of $\alpha = 0.01$ and 0.05.

RESULTS AND DISCUSSION

Tables (1-4) summarize the results of probit regression analysis on mortality for reduced pressure on eggs, first instar larvae, pupae and adults of *T. castaneum* and *E. kuehniella*. By reducing the pressure, LT_{50} values decreased concurrently depending on the species and stage of the experimental pest. Also, resistance ratio (RR_{50}) as indicated by the LT_{50} of resistant species to susceptible species showed amounts of differences in susceptibility by using this pest's control method. As shown in table (1), the LT_{50} values for eggs of *T. castaneum* at 20 and 40 mm Hg were 4.92 and 15.58 hr, respectively. While the values for *E. kuehniella* were 10.13 and 33.55 hr, respectively. *T. castaneum* eggs were more susceptible than *E. kuehniella* eggs in all the modified pressures.

In table (2), the LT_{50} values obtained for *T. castaneum* larvae at 20 and 40 mm. Hg were 0.86 hr and 6.06 hr, respectively, opposed to 3.60 hr and 9.58 hrs for larvae of the flower moth. The table shows also that the susceptibility of the tested insects had increased by decreasing the atmosphere

Table (1): Toxicity of low pressures values of 20 and 40 mm Hg on eggs of *T. castaneum* and *E. kuehniella*

	T. castaneum		*E. kuehniella*	
	20 mm Hg	40 mm Hg	20 mm Hg	40 mm Hg
LT_{50}	4.92	15.58	10.13	33.55
Slope (b)	2.17	2.90	2.59	3.54
F	43.91**	96.89**	119.07**	77.70**
RR_{50}^{b}			2.06	2.15

**Significant in level %1. [b] Resistance Ratio (RR) is LT_{50} of resistant species/ LT_{50} of the susceptible species.

Table (2): Toxicity of low pressures values of 20 and 40 mm Hg on larvae of *T. castaneum* and *E. kuehniella*

	T. castaneum		*E. kuehniella*	
	20 mm Hg	40 mm Hg	20 mm Hg	40 mm Hg
LT_{50}	0.86	6.06	3.60	9.58
Slope (b)	2.71	2.42	1.69	2.64
F	27.01**	65.41**	67.08**	73.09**
RR_{50}^{b}			4.19	1.58

**Significant in level %1. [b] Resistance Ratio (RR) is LT_{50} of resistant species/ LT_{50} of the susceptible species.

Table (3): Toxicity of low pressures values of 20 and 40 mm Hg on pupae of *T. castaneum* and *E. kuehniella*

	T. castaneum		*E. kuehniella*	
	20 mm Hg	40 mm Hg	20 mm Hg	40 mm Hg
LT_{50}	5.35	11.78	0.95	3.41
Slope (b)	2.33	2.61	2.66	0.18
F	64.61**	77.01**	105.61**	62.87**
RR_{50}^{b}	5.63	3.45		

**Significant in level %1. [b] Resistance Ratio (RR) is LT_{50} of resistant species/ LT_{50} of the susceptible species.

pressure from 40 to 20 mm Hg. As previous results larvae of *T. castaneum* were more susceptible than *E. kuehniella* larvae in the used modified pressures.

Table (3) depicts the LT_{50} values of pupal stage of *T. castaneum* and *E. kuehniella*. In this table, the LT_{50} values for *T. castaneum* pupae at 20 and 40 mm Hg were 5.35 hr and 11.78 hr, respectively, while these values were 0.95 and 3.41 hr in case of pupae of *E. kuehniella*, demonstrating that the susceptibility of these insects increased as the atmosphere pressure were reduced from 40 to 20 mm Hg and also showed that, unlike other stages, in pupae of *T. castaneum* were more tolerant than those of *E. kuehniella* at the same pressure.

Concerning the effect of atmosphere pressure on adults of *T. castaneum* and *E. kuehniella* were concerned, table (4) showed that at 20, 40, 60, 80 and 100 mm Hg., the LT_{50} values of these reduced pressures for adults of *T. castaneum* were 0.54, 1.44, 7.29, 17.80 and 32.69 hr, respectively, while that for *E. kuehniella* were 1.42, 5.81, 11.11, 24.58 and 36.42 hr., respectively. These results showed that *T. castaneum* adults were more susceptible than those of *E. kuehniella* in the same pressure.

Statistically, none of the interactions between the two species, developmental stages of each species and also between the two developmental pressures showed a significant difference (Table 5).

Sensitivity of different species of pests to low pressures varied according to the life stages and their physiological properties. Active stages, adult and larvae, which require higher oxygen demand were more susceptible to low pressures than the eggs and pupae (Philips and Throne, 2010). Mortality of insects exposed to low atmosphere pressures was presumed to be as a result of physiological effects from exposure to low oxygen and high level of carbon dioxide of atmosphere created under vacuum. Reduced pressure or vacuum causes low O_2 and high CO_2 concentrations which ultimately lead to lethal metabolic arrest and loss of water through opened spiracles of the insects (Philips and Throne, 2010 and Mitcham *et al.*, 2006). The sessile stages, (eggs and pupae) of slower rates of respiration and metabolism were more tolerant to reduced pressure values than the active ones (larvae and adults) which are of faster rates of respiration and metabolism. Also, results of current study indicated more tolerance of eggs and pupae than larvae and adults to reduced pressures. As RR_{50} shown, in all stages (except in pupae) and all experimental pressures *T. castaneum* was more susceptible than *E. kuehniella*.

As reported in earlier studies, in agreement with the present results, the egg and pupal stages due to their slower rate of metabolism are more tolerant to low pressure (Mbata *et al.*, 2004 and 2005). In this study, eggs of *T. castaneum* and *E. kuehniella* were found the most tolerant to reduced pressure. Generally, different stages of both insect species under study could be arranged descendingly according to their rates of tolerance to decreased

Table (4): Toxicity of low pressures values on adults of *T. castaneum* and *E. kuehniella*

	T. castaneum					*E. kuehniella*				
	100 mm Hg	80 mm Hg	60 mm Hg	40 mm Hg	20 mm Hg	100 mm Hg	80 mm Hg	60 mm Hg	40 mm Hg	20 mm Hg
LT_{50}	32.69	17.80	7.29	1.44	0.54	36.42	24.58	11.11	5.81	1.42
Slope (b)	9.6	7.96	4.55	8.66	3.13	3.60	4.78	2.30	1.81	6.85
F	182.30**	174.20**	159.01**	569.98**	166.27**	116.73**	89.89**	45.17**	78.59**	82.77**
RR_{50} [b]						1.11	1.38	1.52	4.03	2.63

**Significant in level %1. [b] Resistance Ration (RR) is LT_{50} of resistant species/ LT_{50} of the susceptible

Table (5): Factorial ANOVA of effects of two low pressures two experimental species and four developmental stages of *T. castaneum* and *E. kuehniella*

Source	df	Mean square	F	P
Species	1	341.60	27.02*	0.014
Stage of insect	3	326.89	25.86*	0.012
Pressure	1	628.88	49.74**	0.006
Species×Pressure	1	19.21	1.52ns	0.31
Species×Stage of insect	3	65.08	5.15ns	0.11
Pressure× Stage of insect	3	42.64	3.37ns	0.17

atmosphere pressure as; eggs> pupae> larvae>adults for *T. castaneum,* while for *E. kuehniella* they were eggs> larvae> adults > pupae (Tables, 1-4).

In conclusion, obtained results demonstrated a good efficacy of decreasing the atmosphere pressure on the tested pest species. Also, the low pressure didn't cause disturbance or toxicity on the ecosystem. These points characterize low pressures as potential and important alternative tools for pests' control to substitute the chemical fumigants such as methyl bromide and phosphine. However, more studies are still needed to investigate the mortality effect of these pressures into the bulk of storage commodities.

ACKNOWLEDGMENT

The authors would like to acknowledge Mr. Mehdi Shirmaleki who accepted the responsibility of data analysis.

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