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Effect of extreme low and high temperatures on the almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae)

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Abstract

The different immature stages of the almond moth, *Ephestia cautella* (Walker) were exposed to low temperature of -5°C for different exposure times. Exposure of eggs to 240 to 360 minutes is sufficient to achieve 100% mortality for this stage. Exposed early larval instar to -5°C for 180 minutes is sufficient to achieve 100% mortality. Exposure of the late larval instars to 300 and/or 360 minutes is effective to achieve a complete mortality for the late larval instars of the pest. The calculated LT_{50} and LT_{95} were 113.73 and 208.64 minutes. Exposure of pupae to 300 minutes or more is effective to get a complete mortality for the pupal stage. High temperatures of 45º, 50º, 55º and 60ºC were tested against egg, late larval instars and pupal stages of *E. cautella*. Mortality tended to be increased with the increasing of temperature and exposure time. Exposure time for more than one hour at 45ºC, 15 minutes at 50ºC and 10 minutes at 55ºC were more effective and led to more than 95% mortality for the egg stage of *E. cautella*. Exposure of the late larval instars for more than 97.22, 72.17, 17.65 minutes at 45, 50 and 55ºC is sufficient to achieve more than 95% mortality for the late larval instars as indicated by LT_{95} values. Exposing the pupae to 25 minutes at high temperatures of 50ºC and to 15 minutes at 55ºC is an effective to get complete mortality for the pupal stage. Thus exposure times for more than 90 minutes at high temperature of 45ºC; 33 minutes at 50ºC and/or 11.67 minutes at 55ºC were more effective to achieve more than 95% mortality of the pupal stage of *E. cautella*.

Key words: almond moth, date palm, physical control, IPM program.

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Introduction

Among stored product pests, the almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) is one of the major date palm pests in Egypt. The infestations begin in date palm plantations and continue in storehouse through infested dates and can go through multiple generations (Howard et al., 2001).Besides date palm fruits, dried fig, raisin, rice and maize grains, cereal products, cocoa, chocolate, spices, nuts, dried fruit, processed foods and peanut are reported as hosts of almond moth (Singh & Moore, 1985; Shahhosseini & Kamali, 1989; Hodges & Farrell, 2004; Rees, 2007). Larvae cause a considerable damage by feeding and/or by contaminating stored food with dead bodies and their own products, e.g. excreta, wibbing and silk.

Generally, control of stored product pests is applied using insecticides such as malathion, chlorpyrifos-methyl, phosphine, and methyl bromide (Arthur, 1996). Chemical insecticides have showed numerous environmental problems such as depletion of atmospheric ozone (Fields & White, 2002; Hansen & Jensen, 2002), development of resistance in insects (Sinha & Watters, 1985), mammalian toxicity, disruption of the food chain, proliferation of more harmful insects and sensitive species (Regnault-Roger, 1997). Recently many researchers have been devoted for seeking alternatives against insect pests in warehouses.

Among the alternative control tactics, physical control is one of the most promising methods (Brower & Tilton, 1985; Sharma & Dwivedi, 1997; Faruki et al., 2005; Ayvaz & Tuncbilek, 2006; Ayvaz et al., 2007, 2008; Azizoglu et al., 2010).

The advantages of physical control (low and high temperature) as a pest control procedure includes the absence of undesirable residues in the treated foods, no resistance development by pest insects and a few significant changes in the physicochemical properties or the nutritive value of the treated products (Lapidot et al., 1991; Ahmed, 2001; Zhao et al., 2007).

The present study aimed to study the effect of extremely low and high temperatures on the mortality of the pest. However, results of this study will be useful for understanding the mechanism of population build-up of *Ephestia cautella* on date palm fruits. Moreover, these information will be necessary for the development of an IPM program for date palm pests in Egypt.

Materials and methods

Rearing technique: The experimental insects were collected from infested date fruits of traditional stores and date palm plantations in the New Valley governorate and transferred to laboratory at Plant Protection Department, Faculty of Agriculture, Assiut University during June 2007. Larvae were collected and kept in glass jars 2 Kg and provided with clean semi-dry date fruits (Saidy) as a source of food until pupation. Pupae were individually introduced into glass vials (10x4 cm) and covered with muslin by means of rubber bands until the adult

emergence. The newly emerged adults were transferred into ovi-position cages measuring 30 x 30 x 35 cm at a rate of about 10 pairs males and females / cage. The cages consisted of a wooden bottom and side, the other sides were made of a 2 mm wire mesh and the whole unit was covered with a wood plate. A cloth sleeve was fitted to the wooden side to enable handling the moth. The pest was reared under laboratory conditions for several generations before the study was undertaken to ensure complete adaptation. The present investigations were carried out to study the effect of low temperature and high temperature of 45º, 50º 55º and 60ºC on mortality of egg, larval and pupal stages of the almond moth, *E. cautella*.

Effect of low temperature (-5ºC): The time at which 50% (LT_{50}) and 95% (LT_{95}) of the treated insects died, was determined by cooling groups of 80 eggs, early (1-2 days old), late larval instars(>25 days old) and pupae. Four replicates of 20 individuals of each stage for each treatment were exposed to temperature of -5ºC for 30, 60, 120, 180, 240, 300 and 360 minutes. After exposure, individuals were transferred to laboratory conditions of temperature ranged from 25 to 30 °C and 60 to 65% relative humidity. Eggs and pupae were left undisturbed at room temperature and laboratory conditions for 7 days and considered to be dead if no hatch or emergence was noted by that time. Early and late larval instars were assumed to be dead if they did not respond to a gentle prodding after 24 hours recovery at laboratory temperature. Our observations clearly indicated that we can distinguish between them after about one day.

Effect of high temperatures: The effect of high temperature on the mortality of egg, larval and pupal stages of the almond moth was determined. Temperatures were 45º, 50º, 55º and 60ºC and the exposure times to each temperature were 5, 10, 15, 20 and 25 minutes. For each temperature, separate sets of individuals were used for the different exposure intervals. About 80 eggs (<24 hrs old), 100 late larval instar and 30 pupae were exposed to each temperature. After exposure, individuals were removed from the oven and placed under laboratory conditions for subsequent assessment. Control mortality under laboratory conditions was negligible through the test and no corrections were necessary for treatment comparisons. If all individuals were dead after initial exposures as recorded at 60 °C, no further tests were conducted. In case of eggs, mortality was evaluated using a binocular microscope (Paralux 4-10× magnification power). Estimation of mortality on the egg, larval and pupal stages were made as in the aforementioned treatment (low temperature treatment). The values of LT_{50}, LT_{95} and slopes were calculated by a probit analysis using SPSS V. 10 system (SPSS Inc., 1999).

Results

Effect of low temperature (-5ºC): Low temperature of -5ºC was tested against egg, early and late larval instars and pupal stage22 of *E. cautella*. Data in Tables (1 – 4) showed the percentages of mortality in relation to exposure time at -5ºC. The calculated values of LT_{50} and LT_{95} are given in Table 5.

Effect of low temperature (-5ºC) on egg stage: Table (1) revealed that the exposure for 60, 90, 120, 180 and 240 minutes was able to achieve 40.00, 60.00, 86.25, 92.50, and 100.00% mortality, respectively. Exposure time to 4 hrs was sufficient to achieve complete mortality for the egg stage. The LT_{50} and LT_{95} values were calculated as 65.26 and 156.99 minutes (slope = 4.09). It is clear that about 160 minutes at -5ºC were sufficient to kill 95% of the egg stage (Table 5).

Effect of low temperature (-5ºC) on early larval instars: Early larval instars were exposed to -5ºC for different times from 30 to 360 minutes. Table (2) revealed that larval mortalities increased with the increase of exposure time up to 180 minutes. Exposure early larval instars to-5ºC for 180 minutes was sufficient to achieve a complete mortality of this stage. The LT_{50} and LT_{95} values were calculated as 96.94 and 157.34 minutes, respectively (slope = 7.09) (Table 5).

Effect of low temperature (-5ºC) on late larval instars: Late instar larvae of *E. cautella* were exposed for different times to -5ºC. Mortality rates among treated individual larvae of the almond moth showed a time-mortality trend as presented in Table (3). Data showed that the different exposure times resulted in considerable mortalities of the tested larvae. Recorded mortality values among the used exposure times were 6.25, 17.50, 75.00, 95.00 and 100.00% for the exposure times of 90, 120, 180, 240 and 300 minutes, respectively. Complete mortality was achieved for larvae exposed to -5ºC at 300 minutes or more. It is clear that the exposure time at -5ºC for one hour or less did not lead to any mortality and the larvae completed their development after this time of exposure. The LT_{50} and LT_{95} values were calculated as 143.11 and 226.53 minutes (slope = 8.19). Thus exposure time for more than 226.53 minutes was sufficient to kill 95% of late larval instars as indicated by the value of LT_{95} (Table 5).

Effect of low temperature (-5ºC) on pupal stage: Table (4) revealed that exposure times of 90, 120, 180, 240 and 300 minutes were able to kill 14.00, 62.00, 81.48, 92.59, and 100.00%, respectively. Exposure time for 5hrs or more was sufficient to achieve 100% mortality for the pupal stage. The LT_{50} and LT_{95} values were calculated as 113.73 and 208.64 minutes (slope = 5.85). Thus exposure time for 208.64 minutes was sufficient to achieve 95% mortality of the pupal stage as indicated by the value of LT_{95} (Table 5). Generally, the increase of the exposure time induced regular increase in mortality percentages. Lethal time to reduce survival by 50% (LT_{50}) of eggs, early, late larval instars and pupae of *E. cautella* at -5ºC were 1.09, 1.62, 2.39 and 1.90 hrs and those by LT_{95} were 2.61, 2.62, 3.78 and 3.47 hrs, respectively.

Effect of high temperature: The effect of high temperatures of 45º, 50º, 55º and 60ºC on the mortality of the egg, late larval instars and pupal stage of the almond moth *E. cautella* was estimated. Percentages of mortality in relation to temperature and exposure times are shown in Tables (6-8). The calculated LT_{50} and LT_{95} values are given in Table (9).

Table 1: Mortality (%) of *E. cautella* eggs after exposure for different times to low temperature of -5ºC.

		Mortality	
Exposure time (minutes)	Number of larvae	Numbers	Percentage (%)
30	80	0	0.0
60	80	32	40.00
120	80	69	86.25
180	80	74	92.50
240	80	80	100.00
300	80	80	100.00
360	80	80	100.00

Table 2: Mortality (%) of *E. cautella* early larval instars after exposure for different times to low temperature of -5ºC.

		Mortality	
Exposure time (minutes)	Number of larvae	Numbers	Percentage (%)
30	80	0	0.0
60	80	9	11.25
120	80	54	67.50
180	80	80	100.00
240	80	80	100.00
300	80	80	100.00
360	80	80	100.00

Table 3: Mortality (%) of *E. cautella* late larval instars after exposure for different times to low temperature of -5ºC.

		Mortality	
Exposure time (minutes)	Number of larvae	Numbers	Percentage (%)
30	80	0	0.00
60	80	0	0.00
120	80	14	17.50
180	80	60	75.00
240	80	76	95.00
300	80	80	100.00
360	80	80	100.00

Table 4: Mortality (%) of *E. cautella* pupae after exposure for different times to low temperature of -5ºC.

		Mortality	
Exposure time (minutes)	Number of larvae	Numbers	Percentage (%)
30	27	0	0.00
60	27	0	0.00
120	27	17	62.96
180	27	22	81.48
240	27	25	92.59
300	27	27	100.00
360	27	27	100.00

Table 5: Regression equations, LT_{50} and LT_{95} (minutes) expressed the effect of low temperature of -5ºC on egg, larval and pupal stages of *E. cautella*.

Stages	Slope±SD	Regression equations	LT_{50} (minutes)	LT_{95} (minutes)
Egg	4.09±0.15	Y=-7.61+4.09x	65.26	156.99
Early instars larva	7.09±0.35	Y=-14.24+7.09x	96.94	157.34
Late instars larva	8.19±0.33	Y=-17.82+8.19x	143.11	226.53
Pupa	5.85±0.21	Y=-12.17+5.85x	113.73	208.64

Effect of high temperature on egg stage: Table (6) revealed that mortality tended to be increased with the increase of temperature and exposure time. Mortalities of *E. cautella* 24.00, 28.00, 36.00, 48.00 and 88%; 76.00, 88.00, 92.00, 96.00 and 100.00%; and 88.00, 92.00, 96.00, and 100% were recorded when eggs were exposed to high temperature of 45º; 50º and 55ºC for 5, 10, 15, 20 and 25 minutes, respectively. Exposure time of 25 and 20 minutes is effective to achieve 100.00% mortality for the egg stage at temperatures of 50º and 55ºC, respectively. The LT_{50} and LT_{95} values were calculated as 13.52 and 59.06 minutes (slope = 2.07) for temperature of 45ºC; 1.37 and 12.59 minutes (slope= 1.94) for temperature of 50ºC and 0.33 and 7.88 minutes (slope= 1.65 ±0.21) for the temperature of 55ºC. Thus exposure times 59.06, 12.59, and 7.88 minutes at 45º, 50º and 55ºC , respectively were sufficient to achieve 95% mortality of the egg stage as indicated by LT_{95} value (Table 9).

Effect of high temperature on late larval instars: Late larval instars of *E. cautella* were exposed to 45º, 50º, 55º and 60ºC for 5, 10, 15, 20 and 25 minutes. Mortality rates among treated larvae showed a time-mortality trend as presented in Table (7). R ecorded mortality values among the used exposure times were 4.00, 8.00 and 20% when the larvae exposed to 45ºC for 15, 20 and 25 mints, respectively. Exposure time of 25 minutes at high temperature of 55ºC is sufficient to get 100% mortality for the late larval instars of the pest (Table 7). The calculated LT_{50} and LT_{95} values were 40.15 and 97.22 minutes at 45ºC; 10.95 and 72.17 minutes at 50ºC and 2.87 and 17.65 minutes at 55ºC, respectively. Thus exposure time for more than 97, 72, 17 minutes at 45º, 50º and 55ºC is sufficient to achieve more than 95% mortality for the late larval instars as indicated by LT_{95} values (Table 9).

Effect of high temperature on pupal stage: Data in Table (8) indicate that exposure times of 5, 10, 15, 20 and 25 minutes were able to kill 15.00, 22 .00, 33.00, 44.00 and 58.00%, respectively at temperature of 45ºC. At 50 and 55ºC, mortality increased with the increase of exposure time. Exposure time to 25 minutes at 50ºC and 15 minutes at 55ºC is sufficient to achieve a complete mortality for the pupal stage. The LT_{50} and LT_{95} values were calculated as 23.18 and 90.29; 6.13 and 33.58; 1.82 and 11.67 minutes when the pupal stages exposed to 45º, 50º and 55ºC, respectively. Thus exposure times for more than 90 minutes at high temperature of 45ºC; 33 minutes at 50ºC

and / or 11.67 minutes at 55ºC were sufficient to achieve more than 95% mortality of the pupal stage (Table 9). Our results, on the effect of high temperatures of 45º, 50º, 55º and 60ºC on the different stages of the pest concluded that the exposure period more than 97.22 minutes at 55ºC is sufficient to obtain a sufficient mortality of different life stages of *E. cautella*.

Table 6: Mortality (%) of *E. cautella* eggs after exposure for different times to high temperatures of 45º, 50º, 55º and 60ºC.

Temp. (ºC)	Mortality (%) Exposure time (minutes)				
	5	10	15	20	25
45	24.00	28.00	36.00	48.00	88.00
50	76.00	88.00	92.00	96.00	100.00
55	88.00	92.00	96.00	100.00	100.00
60	100.00	100.00	100.00	100.00	100.00

Table 7: Mortality (%) of *E. cautella* late larval instars after exposure for different times to high temperatures of 45º, 50º, 55º and 60ºC.

Temp. (ºC)	Mortality (%) Exposure time (minutes)				
	5	10	15	20	25
45	0.00	0.00	4.00	8.00	20.00
50	36.00	44.00	56.00	64.00	72.00
55	72.00	76.00	88.00	92.00	100.00
60	100.00	100.00	100.00	100.00	100.00

Table 8: Mortality (%) of *E. cautella* pupae after exposure for different times to high temperatures of 45º, 50º, 55º and 60ºC**.**

Temp. (ºC)	Mortality (%) Exposure time (minutes)				
	5	10	15	20	25
45	15.00	22.00	33.00	44.00	58.00
50	44.00	55.00	66.00	88.00	100.00
55	77.00	88.00	100.00	100.00	100.00
60	100.00	100.00	100.00	100.00	100.00

Discussion

Results of the present study clearly indicate that the susceptibility to sub-zero temperature differ according to the developmental stages. Generally, egg stage and early larval instars were the most susceptible to -5ºC (lowest LT_{50} and LT_{95}); while late larval instars and pupal stage were more cold-tolerant stages (highest LT_{50} and LT_{95}). There is a wide variation in cold tolerance among stages of stored-product insects, as demonstrated by many authors (David et al, 1977; Fields, 1992; Donahaye et al., 1995; Abdelghany et al. 2010; Loganathan et al. 2011) and others. In

the present study, egg stage was the most cold-susceptible stage. This conclusion is in agreement with the general theory that the egg is the most cold-susceptible stage of most stored-product insects (Fields, 1992). The pupal stage of *E. cautella* was found to be the least sensitive stage to -5ºC for which LT_{99} of 144 hrs were required (Donahaye et al., 1995). Numerous studies have been undertaken on the influence of temperature close to 0ºC and sub-zero temperatures on stored product insects. Imai & Harada (2006) mentioned that low temperature storage may present feasible alternative to chemical fumigation of stored tobacco. Beckett (2011) demonstrated that low temperatures will have a considerable impact on the population parameters of insects and hence can be available method of control. However, disinfestations of stored-product insects with sub-zero temperatures are well documented (Fields, 1992; Bank &Fields, 1995; Donahaye et al., 1995; Collins et al., 2006). The present data clearly indicate that exposure to sub-zero temperatures could result in a high mortality of this major pest of stored products, thus providing a useful tool for its control in respect to Integrated Pest Management Programs (IPM).

Table 9: Regression equations, LT_{50} (minutes) and LT_{95} (minutes) expressed the effect of high temperature (45º, 50º, 55º and 60ºC) on egg, larval and pupal stages of *E. cautella*.

Stages	Temp. (ºC)	Slope±SD	Regression equations	LT_{50} (minutes)	LT_{95} (minutes)
Egg	45	2.07±0.06	Y=-2.46+2.07	13.52	59.06
	50	1.94±0.10	Y=-0.71+1.94x	1.37	12.59
	55	1.65±0.21	Y=-0.06+1.65x	0.33	7.88
	60	-	-	-	-
Larva	45	4.28±0.23	Y=-6.86+4.28x	40.15	97.22
	50	1.33±0.05	Y=-1.37+1.33x	10.95	72.17
	55	1.7±0.008	Y=-0.81+1.77x	2.87	17.65
	60	-	-	-	-
Pupa	45	1.77±0.07	Y=-2.42+1.77x	23.18	90.29
	50	2.45±0.06	Y=-2.09+2.45x	6.13	33.58
	55	2.65±0.29	Y=-1.18+2.65x	1.82	11.67
	60	-	-	-	-

Eggs stage was the most sensitive stage to high temperatures followed by pupae, and late larval instars. Our results show also that more than 59.06 minutes at 45ºC of exposure were sufficient to reach a 95% mortality of the egg stage at 45ºC. Increase in temperature decreased the exposure time for all stages of the pest. On the other hand, the more extreme temperature, the more quickly all stages of *E. cautella* die, with death occurring in a few minutes at 60ºC. Results concerning the effect of heat treatment on the different developmental stages of *E. cautella* have been matched early by Fields (1992), Marouf et al. (2003), Adler (2006), Zhao et al. (2007), Ben-Ialli et al. (2009) and El-Naggar & Mikhaiel (2011). Fields (1992) reported that the more extreme temperature the more quickly insects die, with death occurring in a few minutes at 55 ºC. He

added also that most stored-product pests are killed within hours after they are exposed to temperatures of 50ºC or more. Neven (2000) demonstrated that increasing the treatment temperature markedly increased the egg mortality rate, with 100% mortality achieved after 180, 120, 60, 40 and 30s at temperature of 54º, 60º, 65º, 70º and 75ºC, respectively. She explained that, this mortality is generally due to physiological and biochemical modifications (agglutination of protein associated with different egg constituents) that occur within the egg during heat treatment-with the mortality rate rising as the extent of modifications increases. Marouf et al. (2003) and Zhao et al. (2007) elucidate that as temperature rises the treatment time required to obtain the same disinfestations level with *Ephestia kuehniella Zeller* eggs decreases. Insect eggs are very susceptible to temperature above 50ºC. Adler (2006) tested the efficacy of heat on the Mediterranean flour moth *E. kuehniella* in laboratory experiments at 45°C, 50°C and 55°C. Eggs, pupal and young larval stages were found to be the most tolerant surviving up to 60 minutes at 45°C, up to 7 min. at 50°C and up to 5 min at 55°C. Fitting a log trend through the data gave lethal exposure times of 660 minutes at 45°C, 27 min at 50°C and 7.2 min at 55°C. Survival insects were detected in most samples where temperature did not exceed 50°C. Ben-Ialli et al. (2009) reported that below 50ºC, very long treatment times (> 60 min) were required to achieve 100% mortality. However, treatment time was short (< 3 min) at temperatures over 54ºC. Generally, in the laboratory experiments, the lethal time to reduce survival by 95% of eggs, early and late larval instars and pupae of *E. cautella* at -5°C were 2.61, 2.62, 3.78 and 3.47 hours, respectively. However, concerning the effect of high temperature of 45°, 55°, and 60°C, it could be concluded that exposure time of more than 97.22 minutes at 55°C is sufficient to achieve a complete mortality of different life stages of *E. cautella*.It is clear that the manipulation by extreme temperatures is providing a useful tool for the control of the almond moth, *E. cautella* in respect to Integrated Pest Management Program.

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