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Effect of temperature on stored product pests *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchidae)

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Abstract

Two stored product insects, *Tribolium confusum* and *Callosobruchus maculatus* were exposed to different temperature degree (50, 55, 60, 65, 70 and 75 °C) and different exposure periods (10, 15, 20, 25, 30, 35 and 40 minutes). Increase in temperature level or exposure time resulted in an increase in average mortality. Complete mortality of *T. confusum* was achieved in 50, 55, 60, 65, 70 °C at 25, 20, 15, 15 and 10 minutes of exposure times respectively. 100% Mortalities of *C. maculatus* was achieved in 50, 55, 60, 65, 70 and 75 °C at 40, 25, 25, 20, 15 and 10 minutes of exposure times respectively. Loss percentage of wheat flour and emergence percentage of adult insects were significantly decreased ($P < 0.05$) with increasing of temperature levels and exposure times. There was no significant effect of temperature and exposure time on cowpea seeds germination which concluded that Thermal disinfection is one of the means of physical insect control.

Keywords: Temperature, mortality, emergence, stored-products insects, germination

Introduction

Agricultural products have to undergo a series of operations such as harvesting, threshing, cleaning, bagging, transportation, storage, and processing, from the field to the consumer, and there are appreciable losses in crop yield at all these periods caused either by biological agents, namely birds, rodents, insects, mites and fungi or by environmental factors such as moisture, temperature and type of storage structure [1]. It is estimated that more than 20,000 species of pests destroy approximately one-third of world's food production in the field and storage, valued annually at more than 100 billion dollar [2]. A major problem in production, storage and marketing of the cereals and legumes is the infestation by the insects. Postharvest losses caused by insects are estimated from up to 9% in developed countries and to 20% or more in developing countries [3]. They reduce the quality of stored product directly by feeding damage and indirectly by producing webbing and frass [4]. The quantitative and qualitative damage to stored grain and grain product from insect pests may amount 20-30% and 5-10% in the tropical and temperate zones respectively [5]. Among all of the storage-insects, two of the most destructive beetle species in stored grain are *Tribolium confusum* and *Callosobruchus maculatus* [6].

Flour beetles (*Tribolium*. spp) are the most extensive pest in the world. The beetles feed as adult and larva and cause damage resulting in economic losses [7]. *T. confusum* is associated with human stored products. The association between human and *Tribolium* may be as old as 4000 years. These beetles are incapable to infest whole grain and therefore it is secondary pests of grains such as wheat, barley, maize, rice, oats, and rye. Their typical habitat is in stored flour of these grains, therefore they are commonly called flour beetle. These insects may also be found in other types of stored foods materials such as pulses, chocolate, nuts and even spices like red pepper and ginger [8].

The brunched beetles are the only significant agricultural pest insects of stored pulses in Africa and Asia that presently ranged in tropical and sub-tropical area of the world. *C. maculatus* is one of the most distractive storage pests of *Vigna* spp. such as cowpea, green gram, mung bean, black gram and adzuki in the tropics. Pods of cowpea attacked by *C. maculatus* in storage for 8 months could have as much as 50% of seeds damages [9].

Therefore, control of Stored-product pests to prevent economic losses caused by insects is important [1].

In order to preserve the stored product material several measures have been employed from time to time. To manage these stored-products pests, different strategies are tried by the scientists. But still it is continued to be a threatening pest in post-harvest stored products. The use of chemical pesticides has been employed in the storage, which had positive effect on the pest, but associated with major problem that they continued to remain hazardous to man and the environment. Consumer and environmental concerns over the use of chemical control method have generated interest in non-chemical alternatives such as desiccation, impact, exclusion and elevated temperatures [10], because they do not leave chemical residues on food and negative effect on environment. High temperature has been used extensively to manage insects using a different type of methods such as hot air, radio frequencies, microwave and fluidized beds [11, 12, 13]. Insects die due to exposure of high temperature levels, as they limited physiological capacity to thermoregulate [11]. There has been a lot of research on Conventional Heat Treatment for disinfestation of number of stored commodities [14, 15, 16, 17, 18, 19, 20, 21]. There are a few studies that have reported the effect of extreme temperature for the management of *Tribolium* spp and bruchids. Temperature (>82°C) is required in the host media to control the immature stages of flour beetles and granary weevils [22]. Egg, larva and pupa of bruchids are trapped within the seed and therefore, they are excellent target for management using elevated temperatures [23]. The guidelines of using high temperature for controlling *C. maculatus* have been developed by [24].

The present study was designed to identify, different temperature-exposure time combinations needed to kill the *C. maculatus* and *T. confusum* in related stored products. The objectives of the investigation reported here were to collect baseline data in the laboratory on the effect of exposure time and treatment temperature on mortality, egg laying, emergence and storage loss percentages of two mentioned species of storage insects. Also to determine swelling and germination potential of cowpea seeds, held at an oven.

Methods and materials

This study was conducted from Nov 10, 2015 to Mar 20, 2016

Samples used

The flour of wheat variety (*Triticum vulgare*) and cowpea seeds variety (*Vigna sinensis*) obtained from the market of Jeddah City, Saudi Arabia were selected for the study. The whole flour of wheat and cowpea seeds were sieved and cleaned from husks, dust or any inert materials. The conditioned samples were then stored at room temperature in sealed bags in the laboratory of plant protection department of Arid Land Agriculture, King Abdulaziz University until used for the experiments.

Stored-grain insects and culture preparation

Two major stored-grain insects *T. confusum* and *C. maculatus* were selected for the study. For culture preparation cleaned and sterilized (heating at 70 °C for 1hr) wheat flour and cowpea samples were placed in glass jars separately to reabsorb moisture. Then, transfer amounts 300gm cowpea beans and 400gm wheat flour to depth of 5 cm to separately sterilized culture jars. Adults of flour beetle and cowpea beetle (200-300 and 200-250 insects respectively) from previous culture were added in to each jar and the jar were

sealed with muslin and placed at 30±2 °C and 75±5% RH. Respectively after two weeks and one week the insects were sieved out, discarded or transferred to another jar. Adult insects of flour beetle 10-15 days after emergence and cowpea beetle 2-3 days after emergence were used for experiment work, according to [25].

Oven and high temperature treatment of samples

For the high temperature treatment, samples were treated with conveyor of high temperature energy using a JSR Oven (Model: Json-250, Desc: Natural convection oven, Volts: 220 VAC' 50/60HZ, Watts: 2.5kV' 11.4A' IP) (fig 1). Samples were exposed to different temperature degrees (50, 55, 60, 65, 70 and 75 °C). Each degree of temperature was carried out at all different exposure periods (10, 15, 20, 25, 30, 35 and 40 min) for all treatments, of conveyor, for determination of adult mortality, emergence of *C. maculatus* from infested cowpea seeds, reduction percentage of emerged progeny adults and oven treated cowpea seeds swelling and germination.



Fig 1: JSR oven with exposed sample of insects.

Determination of mortality

The experiments were conducted with wheat flour and cowpea seed samples. Batches of 10 pairs (10-15 days old of *T. confusum* adults) and 5 pairs (2-4 days old of *C. maculatus* adults) were placed in Petri dishes (9 cm in diameter containing 20gm wheat flour and 10gm cowpea seed respectively). The samples were subjected to high temperature conveyor energy, at different temperature degrees (50, 55, 60, 65, 70 and 75 °C) and different exposure periods (5, 10, 15, 20, 25, 30, 35 and 40 minutes) on adults. After one hour from treatment, the percentages of live and dead insects were counted. The sample was held at room temperature for three hours and the insects were checked for mortality again.

Determination of emerged progeny of adults, population density in infested samples and the loss percentages after storage

Batches of 800 adults of red flour beetles (10-14 days old), and 300-400 adults of cowpea beetles (2-4 days old) were placed on sterilized related sample (800gm of wheat flour and 400gm cowpea seeds respectively) in separately glass jar. After one week, all adult insects were removed. The infested culture of flour and cowpea seeds were divided into samples (20gm and 10gm of each respectively), and placed in Petri dishes. The samples were treated with conveyor exposed to different temperature degrees (60, 70 and 80°C) at three deferent exposure times (10, 15 and 20 minutes). Samples were cooled to room temperature and were transferred to glass jars (0.4L), covered with muslin and placed under laboratory conditions. For the reed flour beetle after 6 weeks,

number of F₁ emerged adults were counted. After four months, the means of emerged adults were counted and the loss of the wheat flour weight was calculated from the following equation:

$$\text{Loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where:

W₁: weight of the sample before the treatment and the storage.

W₂: weight of the sample after the treatment and the storage.

In cowpea beetle before that to transfer the sample to class jars, the laid eggs for each treatment were counted. After three weeks, the number of emerged adults, emergence (%) and reduction (%) were calculated from the following equation:

$$\text{Emergence (\%)} = \frac{E}{G} \times 100$$

Where:

E = No. of emerged adults.

G = No. of laid eggs.

The reduction percentage in the number was calculated by the following equation:

$$\text{Reduction (\%)} = \frac{E_C - E_T}{E_C} \times 100$$

Where:

E_C: mean of number emerged adult in control.

E_T: mean of number emerged adult in treatment.

A minimum three replicates were done for all the mortality experiments. Control mortality was determined by allowing the sample and the insects to pass on the conveyor at zero power.

Determination of germination and swelling tests

A. Germination

The germination test was accomplished to seed of each treatment for cowpea seeds with slight modification. Sixty seeds from each treatment with high temperature were divided in to three replicates, placed on Petri dishes containing cotton layer (instead of filter paper) soaked with tap water and covered with tissue paper. Germinated seeds were recorded after 4 days. The obtained results of germination test were recorded for all treatments and control.

B. Swelling test

Three grams of treated cowpea seeds, by conveyor at high temperature degree, were weighted and submerged by tap water in Petri dish. After 1 and 5 hours, the seeds were dried with tissue paper and reweighted for the calculation the absorbed water. Three replicates were done for each treatment and control.

Statistical analysis

All obtained data were statistically analyzed using Finney (1971) [26] software. Comparisons among the means of the various treatments were performed, using the revised least significant different (L. S. D) at < 0.05 level. Angular transformation was done for the percentage values.

Results

Mortality of *T. confusum* and *C. maculatus* were increased with increase of temperature level and exposure time (table 1 and 2). Mortality of adult stages of *T. confusum* and *C. maculatus* was different ($P < 0.05$). *T. confusum* was susceptible than *C. maculatus* due to higher temperatures and exposure times. While adult stages of both insects were susceptible to higher levels of oven energy and shorter exposure times. Mortality of *T. confusum* measured after conveyor heating at 50°C for 10, 15, 20 and 25 minutes of

exposure periods were 1.6, 10.2, 66.0 and 100.0 percent, complete mortalities were achieved in 55, 60, 65, 70 °C at 20, 15, 15 and 10 minutes of exposure times respectively (table 1). Increase in temperature level or exposure time resulted in an increase in average mortality. The mortality of *C. maculatus* adult was, similarly increased as mortality of *T. confusum* with increase of temperature rates or exposure times. Mortalities were started in 50, 55, 60, 65 and 70 °C at 25, 20, 15, 10 and 10 minutes of exposure periods respectively. And complete mortalities were achieved at 40, 25, 25, 20, 15 and 10 minutes of exposure times respectively. There was complete mortality in 75 °C at all exposure times (table 2). In the control mortalities were zero at all exposure times.

The data related to effect of temperature and exposure time on emergence and loss of wheat flour after 4 months of red flour beetle and cowpea beetle from infested related samples are given in Table 3 and 4. Emergence of both insects from infested sample was decreased with increase of temperature degree and exposure times. After treatment of the infested samples with three high temperature degrees (60, 70 and 80°C) and three different exposure periods (10, 15 and 20 minutes), after 6 weeks and 4 months the mean number of emergence of *T. confusum* (98.0, 81.8 and 52.5) and Loss of wheat flour % after 4 months (27.5, 25.8 and 17.2) were significantly reduced ($P < 0.05$) with increasing of temperature and exposure times (table. 3). The mean numbers of laid eggs of *C. maculatus* for each treatment were 110.2, 90.1, 107.0, 101.3, 98.1, 106.2, 103.0, 102.0 and 107.8 respectively. After three weeks, the numbers of emerged adults in 60°C at three mentioned exposure time were 54.8, 38.4 and 26.7 and in 70°C were 45.3, 1.1 and 0.0. There was no emergence of adult in the treatments treated with 80°C and exposure times. Mean of the mean number of emergence and emergence % for three exposure times were significantly decreased at $P < 0.05$ with increasing of temperature degrees and reduction % of emergence were increased with increase of temperature degree and exposure times.

Result of germination and swelling of treated cowpea seeds with high temperature degrees (50, 55, 60, 65, 70 and 75) at different exposure times (40, 25, 25, 20, 15 and 10 minutes) that gave 100% of mortality of *C. maculatus* are presented in table 5. Mean of the swelling percentage followed by the same letters are not significantly different at $P < 0.05$. There were no significant different at $P < 0.05$ between the mean of cowpea seeds germination treated with high temperature degrees and exposure times.

Table 1: Effect of temperature and time of exposure on mortality of *T. confusum*.

Temperature (C)	Adult mortality (%) after certain exposure time (min) ± SD			
	10	15	20	25
50	1.6 ± 0.2	10.2 ± 3.0	66.0 ± 6.6	100.0
55	1.8 ± 0.3	17.8 ± 4.7	100 ± 00	-----
60	37.0 ± 4.0	100.0 ± 00	-----	-----
65	85.7 ± 8.2	100.0 ± 00	-----	-----
70	100 ± 00	-----	-----	-----
LSD <0.05	A:	2.58		
	B:	3.40		

A: LSD <0.05 for the effect of exposure time comparisons.

B: LSD <0.05 for the effect of different temperature degree comparisons.

#Angular transformation was done for the percentage values.

#All the obtained data were statistically analyzed using costat (1986) software.

#Comparisons among the means of various treatments was performed using the revised least significant difference (L.S.D) at <0,05 level.
Angular transformation was done for the percentage values.

Table 2: Effect of temperature and time of exposure on mortality of *C. maculatus* adult

Temperature (C)	Mortality (%) after exposure time (min) ± SD						
	10	15	20	25	30	35	40
50	0.0	0.0	0.0	14.3±4.1	42.2±3.2	72.5± 1.1	100±00
55	0.0	0.0	8.4±4.3	100±00	100±00	100±00	-----
60	0.0	10.1±2.3	35.4±3.5	100±00	-----	-----	-----
65	9.3± 4.6	95.1±2.2	100± 00	-----	-----	-----	-----
70	55.2±5.0	100±00	-----	-----	-----	-----	-----
75	100± 00	-----	-----	-----	-----	-----	-----
LSD <0.05	A: B:	1.987 2.701					

A: LSD <0.05 for the effect of different temperature degrees comparisons.

B: LSD <0.05 for the effect of different exposure time comparisons.

Angular transformation was done for the percentage values.

Table 3: Effect of temperature and exposure time on *T. confusum* population density from infested wheat flour

Temperature	Exposure time (min)	Mean of emerged adults from infested grains after			Loss of wheat flour% after 4 months	
		6 weeks ± SD	4 months ± SD	mean	% ± SD	Mean
Control		46.4 ± 7.1	189.7±6.0	118.2d	32.8±3.0	32.8d
60	10	43.0 ± 3.0	178.0±10.1	98.0c	29.5±1.1	27.5c
	15	37.7 ± 5.1	162.2±8.2		29.0±1.1	
	20	35.2 ± 5.3	133.0±6.3		24.0±2.2	
70	10	39.2 ± 3.8	160.0±3.7	81.8b	27.8±1.0	25.8b
	15	29.4 ± 2.0	138.0±13.4		27.1±1.3	
	20	19.0 ± 2.0	104.0±5.0		22.0±1.1	
80	10	34.0 ± 2.6	136.0±2.6	52.5a	26.0±1.3	17.2a
	15	20.3 ± 2.2	124.0±6.2		25.0±1.4	
	20	00.0 ± 0.0	00.0±0.0		0.5±0.1	
LSD <0.05	A			4.23		1.51
	B			3.12		4.23
	C			11.53		

#Means followed by the same letters are not significantly different at P<0.05.

A: LSD for the effect of temperature degree comparisons.

B: LSD for the effect of exposure times (10, 15 and 20 min) comparisons.

C: LSD for the effect of emergence time (6 weeks and 4 months) comparisons.

Table 4: Effect of temperature and exposure time on emergence of *C. maculatus* from infested cowpea seeds

Temperature (C)	exposure time (min)	Mean of laid eggs ± SD	Mean no. of emerged adult		Emergence%		Reduction%
			±SD	mean	% ± SD	mean	
Control		140.0 ± 2.3	80.2 ± 4.5	80.2 ^d	57.2 ± 3.3	57.1 ^d	00.0
60	10	110.2 ± 5.5	54.8 ± 3.3	39.9 ^c	49.7 ± 3.2	38.9 ^c	31.6
	15	90.1 ± 2.2	38.4 ± 4.1		42.6 ± 1.4		52.1
	20	107.0 ± 6.6	26.7 ± 3.8		24.9 ± 2.5		66.7
70	10	101.3 ± 7.2	45.3 ± 2.5	15.5 ^b	44.7 ± 3.0	15.2 ^b	43.5
	15	98.1 ± 4.0	1.1 ± 1.0		1.1 ± 1.0		98.6
	20	106.2 ± 4.7	0.0 ± 0.0		0.0 ± 0.0		100.0
80	10	103.0 ± 7.5	0.0 ± 0.0	0.0 ^a	0.0 ± 0.0	0.0 ^a	100.0
	14	102.0 ± 8.6	0.0 ± 0.0		0.0 ± 0.0		100.0
	20	107.8 ± 3.3	0.0 ± 0.0		0.0 ± 0.0		100.0
LSD <0.05	A			3.40		1.49	
	B			2.22		0.707	

#Means followed by the same letters are not significantly different at P<0.05.

A: LSD <0.05 for the effect of different temperature degrees comparisons.

B: LSD <0.05 for the effect of exposure time (10, 15 and 20) comparisons.

Angular transformation was done for the percentage values.

Table 5: Effect of temperature and time of exposure on swelling and germination of cowpea seeds

Temperature (C)	exposure time (min)	Swelling time (hr)	Swelling%		Germination after 4 days% ± SD
			% ± SD	mean	
Control		1	45.6 ± 0.2	68.5 ^a	100.0 ± 0.0
		5	91.4 ± 3.1		
50	40	1	52.1 ± 1.8	74.2 ^b	97.2 ± 3.4
		5	96.2 ± 1.3		
55	25	1	50.0 ± 3.5	72.9 ^b	98.1 ± 4.3
		5	95.8 ± 4.1		
60	25	1	55.0 ± 3.7	72.6 ^b	100.0 ± 0.0

		5	90.2 ± 2.5		
65	20	1 5	51.0 ± 1.1 95.2 ± 1.5	73.1 ^b	97.5 ± 4.4
70	15	1 5	49.9 ± 2.3 89.8 ± 1.8	69.8 ^a	100.0 ± 0.0
75	10	1 5	52.6 ± 1.9 94.1 ± 2.3	73.3 ^b	96.4 ± 4.3
LSD <0.05				3.421	N.S

#Means followed by the same letters are not significantly different at $P \leq 0.0$.

Germination and swelling test were done only to temperature and time of exposure that gave 100% mortality of *C. maculatus*.

NS. Not significant.

Discussion

Thermal treatment has been extensively investigated by several researchers as an alternative method of controlling insects. In the current study High temperature degrees and different exposure times for each degree of temperature were evaluated in lab condition for the management of two stored product pests (*T. confusum* and *C. maculatus*). At higher temperature levels, shorter exposure time at low temperature level, high exposure times were required to kill insects. For example, at 70 and 75 °C, 10 minutes exposure period was needed to achieve 100% mortalities of *T. confusum*, and *C. maculatus* adults respectively, whereas 25 and 40 minutes exposure times, 50 °C temperature was enough to achieve 100% mortalities of *T. confusum* and *C. maculatus* adults respectively. Increased temperature levels had significant effect on mortality; exposure times estimated for 100% mortality were decreased among the insects tested. These results are agreement with [27-37, 24], all these studies used different species and strain of insects, different sources of heat, different rates of heating and the insects were reared and tested on different stored products. Further research is required to determine which of these factors is responsible for the differences in insects' specific tolerance to heat.

There is a wide variation between species in their ability to survive at high temperature [11, 38]. At high temperature, 54 and 55 °C, the time required to kill 90% of young larvae of *S. paniceum* was 0,06 h, whereas it was took 1.3, 0.63 and 0,25 h, to kill 99% of *T. castaneum*, *L. serricorne* and *T. confusum*, respectively [39]. In the present study *T. confusum* was susceptible than *C. maculatus* due to high temperature and shorter exposure time. Different species of insects have different susceptibilities to the high temperature treatment that the survival at 49 °C of adult *O. surinamensis* = *T. confusum* < *O. Mercator* = *Cathartus quadricollis* < *Gibbium psylloides* = *S. granarium* < *Trogoderma variable* = *T. castaneum* = *S. oryzae* < *R. dominica* = *C. pusillus* < *Lasioderma serricorne* [40].

Insects die by exposing to high temperature degrees, because of their limited physiological capacity to thermoregulate [11]. Higher temperature presumably increased the respiratory and metabolic rates of exposed insects and thus caused more rapid mortality from increased stress due to low oxygen [41, 42].

In the present investigation infested samples exposed to different temperature degrees for different exposure periods resulted in 100% mortality at higher temperature degree and high exposure time. The mean numbers of emerged adults and emergence percentage were decreased with increasing of temperature levels and exposure times. This in conformity with the findings of [43] which reported that exposure of *C. maculatus* adults to solar heat at a temperature of 50 °C for 2, 4 and 6 h, decreased the oviposition, retarded egg development and 100% adult mortality in *Vigna subterranean*. 100% adult mortality was observed in black gram seeds when exposed to different exposure periods to sun light [14]. Emergence of red

flour beetle decrease with increase in temperature and exposure time [34]. The effect of sun light on the population build-up of *Bruchidius atrolineatus* and *C. maculatus* was registered by [44]. It is evident from the present study that high temperature provided adequate protection of stored products against infestation by *T. confusum* and *C. maculatus*. It offers a great prospect for successful protection of stored material against attack by mentioned insects for small and medium storage and does not require any extra financial investment.

In the present research Germination and swelling test were done only to temperature and time of exposure that gave 100% mortality of *C. maculatus*. There was no significant effect of temperature and exposure time on cowpea seeds germination. This in conformity with finding of [29]; found that the temperatures and exposure times necessary to disinfest the cowpeas had no significant on the cooking time and germination of cowpea seeds. But different with finding of [45]; studied that germination of treated mung bean seeds was reduced significantly due to expose to microwave and increasing temperature and exposure times. Possible reasons for these differences are all these studies use different sources of heat and different rates of heating. The effect of high temperature treatment of cowpea seeds, using different source of heat and heating rates on germination, swelling, cooking time and protein content should be investigated.

Conclusion

With regard to the results of this study it is evident that high temperature have effective role in the management of stored product pests. Therefore, the results presented here would lead to a reduction in the economic losses associated with infestation of *T. confusum* and *C. maculatus*, and minimize injury to stored products in the storage.

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