Destruction Kinetics of Adults of The Rust-Red Flour Beetle by Direct Exposure to Salt-Heat Stress

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ABSTRACT

The present study aims in general to investigate the effect of heat stress as a way to control some types of insects that infesting grains or other stored-products and as a gateway to the knowledge of insect resistance to heat. The effect of heat stress in the mobility of killing the adults of the rust-red flour (*Tribolium castaneum*) through the contact with different concentrations of hot salt solution of sodium chloride (0.0, 0.01, 0.5 and 1.0% at three different temperatures (45, 50 and 55°C) was determined. The results show that the level of interaction that kill the tested insect can be expressed as zero order kinetic model and the killing rate constants (*k*) at the concentration of 0.0% NaCl were 0.005, 0.115 and 0.946 s^{-1} at temperatures 45, 50 and 55°C, respectively. The activation energy for the tested insect under sodium chloride and hot water stress was calculated by Arrhenius equation and it was ranging between 432 and 489 KJ $mole^{-1}$. The increase of sodium chloride concentration was found to have a clear impact on killing the adults of *T. castaneum*.

INTRODUCTION

Contact insecticides and fumigants have been used for a long time to protect stored products from insect pests (White and Leesch, 1996; Daglish, 2006). In response to a growing market demand for foodstuffs that are free of pesticide residues, and because stored-product insects are developing resistance to insecticides, such chemicals have been under increasingly restrictive policies over the past years (Donahaye, 2000; Zettler and Arthur, 2000; Kljajic and Peric, 2005; Collins, 2006).

The Rust-red flour beetle (*Tribolium castaneum*) (Coleoptera: Tenebrionidae) is a pest species that attacks stored food products in cereal mills, food processing plants, grocery stores, households, and ships. The Rust-red flour beetle is reddish brown with antennae that ends as a prominent three-segmented club (Bousquet, 1990). It has a length of 3mm to 4 mm and can fly when conditions are hot and humid. It is considered to be a serious pest species that attacks a very wide range of foodstuffs, especially oilseed cake, groundnuts, cereals, milled cereal products, beans and other dried food products. The female lays tiny white eggs that hatch after about 9 days. The larvae are creamy to light brown with two dark projections on the last body segment. Larvae remain feeding outside of the grain. The larval stage duration ranges from about 22 to over 100 days depending on temperature. Adults emerge from the pupa after about 8 days and they live for three years feeding on the host (Walter, 1990).

Interest of using heat treatment to provide quarantine security against pests in fresh and stored agricultural commodities has increased in the wake of regulatory actions over the use of pesticides. Considering the pesticide effects on humans and the environment, implementation of the US Food Protection Act of 1996 will further limit the use and availability of some widely used chemical fumigants, especially methyl bromide (UNEP, 1995). Therefore, it is necessary to search for a suitable replacement for the process of fumigation by methyl bromide. Treatment and thermal stress both raise the temperature around cereal or by using the techniques of electric shock connect or microwave heating or hot air and others may be one of the options likely to be successful. The effect of heat stress or heat/cooling can be suitable methods for controlling stored products insects. The knowledge of the fundamental kinetics for thermal death of insects allows the prediction of lethal time (LT) over a range of temperature.

The determination of the killing constant rate (*k*) would be useful for choosing the optimal effective temperature that can be used as thermal stress on the insect as a method of controlling stored products insects instead of using the hazardous chemical compounds applied to stored products either sprayed or fumigated. The determination of the killing constant rate would be useful for the possible development of relatively high-temperature/short-time thermal treatment processes that may kill insects while having minimal impact on product quality. In developing a kinetic model to describe the thermal kill of those adults of the Rust-red flour beetle *T. castaneum*, a classical kinetic model can be used to determine the order of reaction and then the activation energy based on the dependence of reaction rate on temperature can be determined. Arrhenius equation can also be used for the calculation of the activation energy (Ea) in a fixed killing constant rate. The activation energy for the thermal activation of the tested insect can be estimated from the relationship between *k* and *T* on an Arrhenius plot (Tang *et al.*, 2000).

The present investigation is designed to use the interaction effect of heat shocks and sodium chloride solutions to determine the killing constant rate of one of the more serious insect that attacking wheat grain and other stored products. Also, the time and the temperature of heat treatment necessary for the destruction of these insect can be predicted.

MATERIALS AND METHODS

A. Insect species tested

A susceptible strain of the rust-red flour beetle, *Tribolium castaneum* (Herbst) was collected in 2008 from a wheat flour stock and colonies have been cultured using incubators at constant temperature of 27°C and a relative humidity ranged between 60-70% for several generations. The age of the adults that have been used for the test was about 7 to 14 days.

B. Experiments

Ten insects (adult stage of the Rust-red flour beetle *T. castaneum*) were counted and replaced in a glass tube (1.5 cm diameter X 10 cm length). Salt solutions of different sodium chloride concentrations of 0.01, 0.50 and 1.00 % were prepared. A free sodium chloride solution (water only) was used as 0.00% sodium chloride. The glass tube was dipped in the chosen concentration of sodium chloride solution heated as water bath to give a desired temperature. Three levels of temperature were used (45, 50 and 55°C) and these levels have been selected after running preliminary experiments to find out the effective temperature range that can kill the insect. The bottom of the two opened ends glass is closed by a fine wire net that can hold the insects in the tube (Fig. 1). The insects were dipped in those solutions for different intervals (2-240 s). At each constant temperature, the insects were immersed for different and varying periods (2, 4,6,8,10s at 55°C; 15, 30, 45,60,75,90 s at 50°C and 90,180,210,240 s at 45°C). After dipping the insects in a specific concentration at the chosen temperature for a defined time, the tube that holding the insects were immersed in cold water of 20°C for 20 seconds. The insects were allowed to dry and the number of living insects was recorded 24 hours post-treatment. Each treatment was done in 4 replicates.

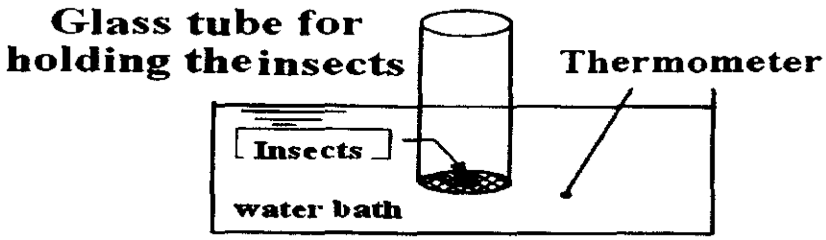

Figure1: Exposing the adults of *T. castaneum* to heat by dipping in hot water bath containing a specific sodium chloride solution.

C. Kinetic study

To calculate the killing rate constant, the following equation was used:

$$-dN/dt = kN^n \quad (1)$$

where, (dN)= change in number of insects, (dt) = change in time, (k) = the killing rate constant of the insect, (N) = the number of insects, and (n): the kinetic order of reaction. For a zero-order reaction the integration of Eq. (1) yields,

$$N_0 - N = kt \ , \quad (2)$$

where, (N_0) = the number of insects at zero time, (N) = the number of insects at any time, (k) = the constant killing rate of the insect, and (t): the time.

The effect of temperature on k was expressed by the Arrhenius equation,

$$k = A \exp(-Ea/Rt), \quad (3)$$

where, (A) = a coefficient of frequency [$1 sec^{-1}$], (Ea) = the activation energy to change the total number of insects [$kJmole^{-1}$] which was estimated from the slope of the line resulted from the regression of Ln(k) values against the reciprocal of the absolute temperature of the transaction, (R) = the universal gas constant [8.314 J $mole^{-1}K^{-1}$], and (T) = absolute temperature [°K].

The data were analyzed using the software computer program "Statistxs".

RESULTS AND DISCUSSION

The results revealed that zero order kinetic model can be used to describe the thermal destruction successfully (Fig. 2) and these findings are consistent with the level of interaction that had been registered for the fruit fly *Ceratitis capitata* (Jang, 1986 and 1991) as the slope of the line reflects the killing constant rates.

The killing constant rate(*k*)

The constant rate for the process of killing the adult insects at different temperatures and different levels of concentrations of sodium chloride

solution are presented in Table 1. As seen in Table 1, the constant rate values of insect killing vary from 0.003 s^{-1} as a minimum, at the temperature of 45° C and a maximum value of 0.946 s^{-1} at the temperature of 55° C. It is noticed that the average values of the correlation coefficient (r^2) are high and generally accepted except in certain transactions, especially when the adults were immersed in distilled water at a temperature of 45°C and this class of temperature seems to be less effective in the killing process.

The activation energy (Ea)

Fig. 3 illustrates the relationship between ln*k* values and the reciprocal of absolute temperature (1000/*T*) at four concentrations of sodium chloride solutions. Table (2) shows the values of Ea of the full stage (adult) of the Rust-red flour beetle *T. castaneum*. It is noted that the values of Ea for the different NaCl concentrations are very close and varied from 432 to 489 $KJmol^{-1}$. However, it seems that the insect is more sensitive to temperature change when high concentrations of NaCl were used and that perhaps due to the influence on the osmosis of salt which make the exposed tissues are easily to be burn.

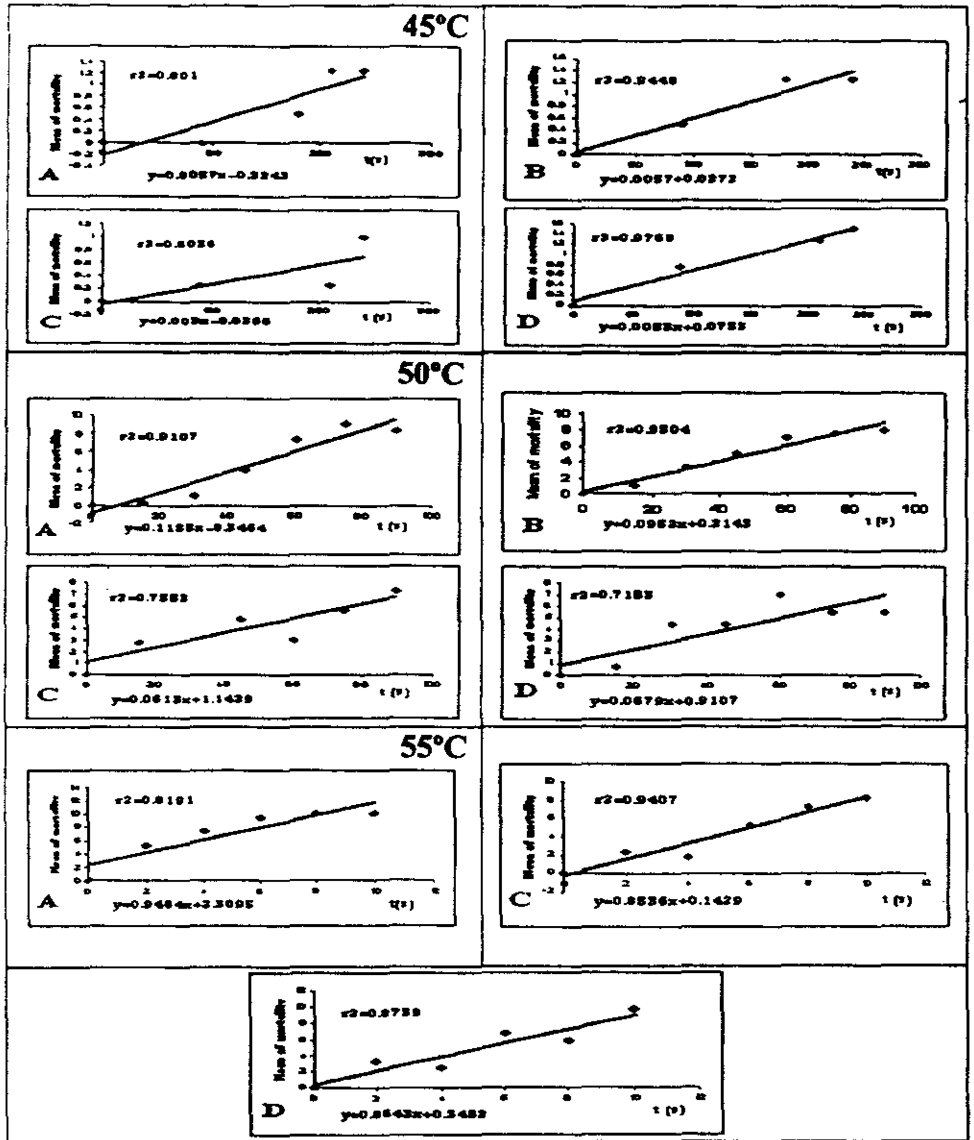

Fig. (2): Mean mortality percentages of *T. castaneum* adults against time at 45, 50 and 55° C in the presence of NaCl concentrations: A (0.0%), B (0.01 %), C (0.5 %) and D (1.0 %).

Table 1: A constant average rate of thermal killing (k s^{-1}) and standard deviation, for the full stage (adult) of *T. castaneum* at different temperatures and different concentrations of sodium chloride solution

Temperature °C	NaCl Conc. (%)	k s^{-1}	S.E	r^2	adjusted r^2	S.D
	0.00	0.0057	0.2774	0.801	0.735	0.32290
	0.01	0.0057	0.1517	0.945	0.917	0.17617
	0.50	0.0030	0.2857	0.609	0.414	0.33129
45	1.00	0.0059	0.1064	0.977	0.965	0.12340
	0.00	0.1155	0.8747	0.911	0.893	1.28380
	0.01	0.0950	0.5262	0.950	0.940	0.77230
	0.50	0.0619	0.8522	0.755	0.706	1.25070
50	1.00	0.0680	1.0353	0.715	0.658	1.51920
	0.00	0.9460	1.3467	0.819	0.774	1.86080
	0.50	0.8530	0.6491	0.941	0.926	0.89690
55	1.00	0.8640	0.9939	0.874	0.842	1.37330

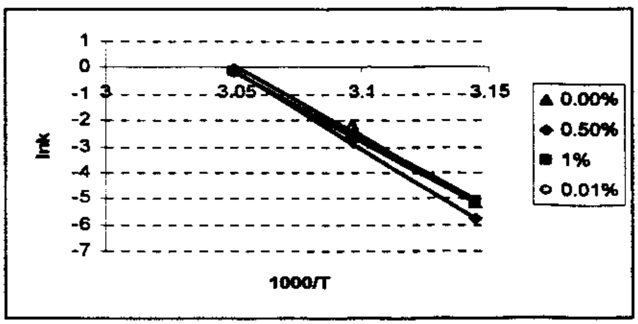

Fig. 3: Arrhenius plot for temperature effects on thermal death (killing) rates of the adult of the Rust-red flour beetle *T. castaneum* at 0.00, 0.01, 0.50 and 1.00% concentrations of NaCl

Table 2: Activation energy for *T. castaneum* at different concentrations of sodium chloride solution

NaCl%	Ea (kJ $mole^{-1}$)	r^2
0.00	443	0.99
0.01	477	1.00
0.50	489	0.99
1.00	432	0.99

The Ea values for *Tribolium castaneum* obtained in this study (432-489 kJ mol^{-1}) are comparable with those values that previously calculated for the Caribbean fruit fly (445 kJmol^{-1}) (Moss and Chan, 1993) and for the Codling moth (5^{th} instar larvae) (472 kJmol^{-1}) (Wang *et al.*, 2002b) (Table 3). The present results are in agreement with those obtained by Tang *et al.* (2000) which mentioned also that this sort of data provides an opportunity for the possible development of relatively high-temperature/short-time thermal treatment processes that may kill insects while having minimal impact on product quality. The increase of sodium chloride concentration was found to have a clear impact on killing the adults of *T. castaneum*.

Predicted values of the lethal times, LT_{50}, LT_{95}, LT_{99} and $LT_{99.98}$ are calculated from Eq. (2) and presented in Table 4. Both the temperatures of 45 and 50 ° C will not be suitable or appropriate for the commercial applications since they need long time to cause death and such lengthy exposure would not be desired. On the other hand, the treatment of 55 ° C would be more practical as it produced 95% mortality after an exposure time of about 11 s. At 50 ° C, the time that required to cause 95% kill was found to be of a range of 82-153 s and these results are in agreement with those of Johnson *et al.* (2003) who found that the time required to produce 95% mortality of the 5^{th} larval instar of the Indian meal moth (*Plodia interpunctella*) was 114 s (about 1.9 min.) at a temperature of 50 ° C. The suggested effective temperature of 55 ° C for *T. castaneum* can be easily produced by the aid of radio frequency treatments which give a range of temperature of 50 - 55 ° C.

Figure 4A & B show the Thermal Death Time (TDT) curves at different temperatures with 0.5 and 1.0% NaCl, respectively. Those curves were developed by plotting N/N_0 against the time (s) for each suggested temperature to obtain the minimum exposure time required to achieve

100% mortality of *Tribolium castaneum* adults. It is noticed that as the temperature increases, the heating time to cause a specific kill decreases.

Therefore, the higher heating rate provides greater mortality since it requires smaller lethal time and since heat treatment is being considered as an alternative quarantine treatment.

Table 3: Comparisons of activation energies for thermal kill of insects and microorganisms with the Rust-red flour beetle (*T. castaneum*) due to heat treatment.

Insects/ organism	Temperature range (°C)	Activation energy Ea (kJmol^{-1})	Source
The Rust-red flour beetle (*T. castaneum)*	45-55	289-432	**The Present Study**
Mediterranean fruit fly			
Eggs	45-47	784	Jang (1986)
First instar	45-48	656	Jang (1986)
Melon fly			
Eggs	43-46	518	Jang (1986)
First instar	45-48	650	Jang (1986)
Oriental fruit fly			
Eggs	43-46	958	Jang (1986)
First, early and late third instar	43-48	209-401	Jang (1986,1991)
Caribbean fruit fly (eggs)	37-42	440	Moss and Chan (1993)
	43-50	445	Moss and Chan (1993)
Queensland fruit fly (eggs)	42-48	538	Waddell *et al.* (2000)
Navel orangeworm (fifth-instar)	46-54	510-520	Wang *et al.*(2002a)
Codling moth (fifth-instar)	46-52	472	Wang *et al.*(2002b)
Microorganisms (spores)	100-130	222-502	Lund (1977)

Table 4: Lethal time (LT) (s) calculated by the kinetic model (zero order) to achieve different mortalities of the rust-red flour beetle at three temperatures with different NaCl concentrations.

Temperature °C	NaCl Conc. (%)	LT_{50}	LT_{95}	LT_{99}	$LT_{99.98}$
45	0.00	877	1667	1737	1754
	0.01	877	1667	1737	1754
	0.50	1667	3167	3300	3333
	1.00	847	1610	1678	1695
50	0.00	43	82	86	87
	0.01	53	100	104	105
	0.50	81	153	160	162
	1.00	74	140	146	147
55	0.00	5	10	10	11
	0.50	6	11	12	12
	1.00	6	11	11	12

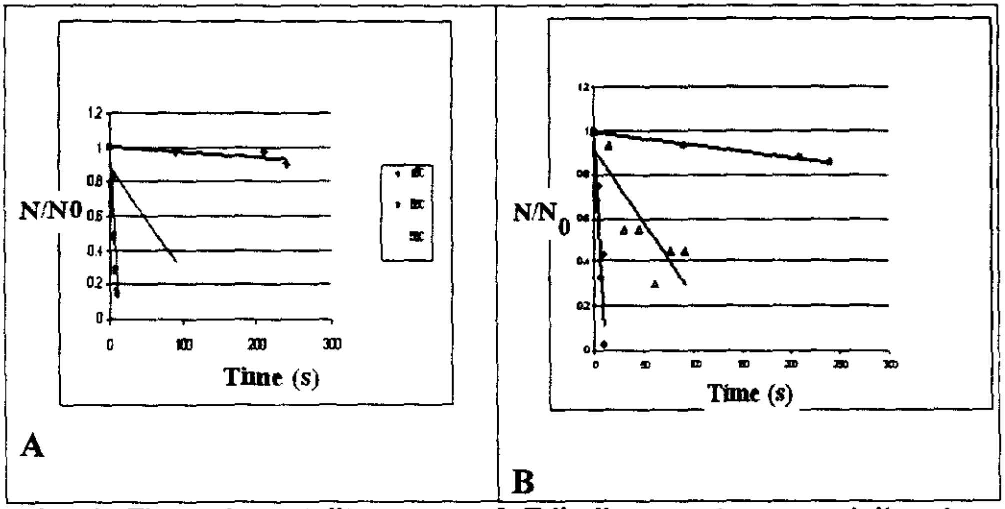

Fig. 4: Thermal mortality curve of *Tribolium castaneum* adults at different temperatures. Lines were obtained by regression using zero order reaction model where N_0 = number of treated insects (40), N = number of surviving insects at: (A) 0.5 and (B) 1.0% Na Cl.

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الملخص العربي

حركية موت الطور الكامل لخنفساء الدقيق الكستنائية تحت تأثير الإجهاد الحراري- الملحي المباشر

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استهدف البحث دراسة تأثير الإجهاد الحراري- الملحي كطريقة لمكافحة بعض أنواع الحشرات التي تصيب الحبوب أ و المنتجات المخزونة الأخرى ، وكمدخل إلى معرفة مقاومة الحشرات للحرارة حيث تمت دراسة تأثير الإجهاد الحراري-الملحي فى حركية قتل الطور الكامل لحشرة خنفساء الدقيق الكستنائية ونلك بتلامسها المباشر مع نسب مختلفة من تركيز محلول ملح كلوريد الصوديوم (0,0،٠٫٠١، ٠٫٥ ، ١٫٠٪) الساخن عند ثلاثة مستويات من درجات الحرارة (٤٥ ،٥٠، ٥٥° م) . وأظهرت النتائج أن رتبة تفاعل قتل الحشرة يمكن التعبير عنه بتفاعل من الدرجة الصفرية وكانت قيم ثابت معدل القتل عند تركيز ٠٫٠% من ملح كلوريد الصوديوم هي ٠٫٠٠٥ و٠٫١١٥ و٠٫٩٤٦/ ثانية عند درجات حرارة ٤٥و٥٠ و٥٥°م على التوالي ، وأمكن حساب طاقة التنشيط للحشرات المختبرة تحت تأثير كلوريد الصوديوم والإجهاد الحراري عن طريق علاقة (معادلة) أرهينياس حيث وصلت هذه الطاقة إلي ما بين ٤٣٢ و ٤٨٩ كيلو جول مول $^{-1}$ خلال درجات الحرارة المقترحة . وقد بينت النتائج أن زيادة تركيز ملح كلوريد الصوديوم ذا أثر واضح على قتل الحشرة الكاملة لخنفساء الدقيق الكستنائية.