

Effect of Solar Heater Boxes and Constant Temperatures on Mortality and Developmental Stages of *C. maculatus*

¹Ragaa Mohammed Elbashier Elhadaa, ²Rita Muhamad Awang,
²Dzolkhifli Omar, ³Uma Rani Sinniah and, ⁴Ionel Vaieriu Grozescu

¹Department of Plant Protection, Faculty of Agricultural Studies, Sudan University of Science and Technology

²Department of Plant Protection, Faculty of Agriculture, University Putra Malaysia, 43400 UPM, Serdang

³Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM, Serdang.

⁴Faculty of Engineering, SEG: University College, Kota damansara

Abstract: Cardboard and Plywood were the materials which used for solar heater boxes to control adult and developmental stages of cowpea beetle *Callosobruchus maculatus* (F.) according to their effectiveness in previous study on solar heaters. The effect of solar heat on hatchability, oviposition, 4th instars and mortality was evaluated. Adult mortality, hatchability, oviposition and adult emergence were significantly affected by solar heater boxes treatment. A temperature of 66.4°C was achieved in solar heater boxes within 15 minutes which caused 100% mortality of adults and 4th larval instars of *C. maculatus* compared to 0% mortality after 1, 2 and 3 days for untreated adults. Heat treatment decreased hatchability by 70% and 66.7% in Plywood and Cardboard respectively comparing with untreated eggs. To verify the effect of solar temperatures, another experiment was done using the oven. Adults of *C. maculatus* and eggs were exposed to constant temperatures to evaluate the effect on oviposition, adult emergence and mortality. Times needed for 100% adult's mortality were 10, 20, 40 and 50 minutes with temperatures of 70, 60, 50 and 40°C respectively. The oviposition was also affected significantly by different temperatures.

Keywords: *C. maculatus*, solar heater boxes, constant temperatures

I. Introduction

In many regions, the losses of the quality and production of legume seeds are strict. Therefore it is necessary to develop technologies such as special storage facilities (Ishimoto *et al.*, 1996). With the establishment of stored-product research and development at the beginning of 20th century have come the many strategies for protection and disinfestation that we see today against the overabundance of insect pests that face up to the grain and food industry. The current status of stored product insect management in developed countries is in a period of transition from chemical methods to non-chemical methods, and hence there is great potential for using heat in stored products and associated structures. The use of heat to disinfest grain and structures considered as one feasible alternative to pesticides. It is comparatively rapid and does not leave any insecticide residues (Beckett *et al.*, 2007). Different types of solar heaters using solar heating to disinfest grains, has been found to control stored products insects effectively (Murdock and Shade, 1991; Ntougam and Kich, 1992; Ntougam *et al.*, 1996; Bank, 1998; Nahdy *et al.* 1998; Qaisrani and Bank, 2000; Chauhan and Ghaffar, 2002; Lale and Vidal, 2003; Mekasha *et al.* 2006; Tilley *et al.*, 2007). This experiment aims to determine the influence of heat treatment on developmental stages of cowpea weevil *C. maculatus*.

II. Material And Methods

1- Insect culture development

Seeds of adzuki bean which introduced from supermarket were sterilized through heat treatment at 70°C by putting in an oven for two hours. Stock culture of *C. maculatus* from about 5 pairs (plate 5) was developed on the disinfested adzuki bean seeds (plate 6). The culture was maintained and allowed reproduce in the laboratory at room temperature in closed aquariums to avoid cross infestation. The aquarium was covered with muslin for aeration.

2- Temperature recording

Temperatures were sensed with "Type J" thermocouples fitted to "Intech Micro2100-A16" data logger. The temperature between seeds was detected using a 5mm diameter thermocouple to sense the temperature that can affect the adults and eggs outside the seed. While temperature within seeds was measured using 1mm diameter thermocouple inserted into the seed to sense the temperature which can affect the developmental stages. The 1 mm hole inside the seed was drilled with battery operated Standard PBC drill pit, 14.500 rpm. The

hole around the thermocouples was sealed with instant filler to prevent air from entering seed cavity. 1mm and 5mm thermocouples were placed outside at about 15cm above the ground to sense air temperature.

3. Effect of solar heat on developmental stages of *C. maculatus*

3.1 Effect of solar heat treatment on oviposition and adult mortality of *C. maculatus*

Fifteen pairs of newly emerged adults for each treatment were introduced to one kilogram of clean adzuki bean seeds in Perspex tray in the middle of each solar heater box. The adults were exposed to heat treatment instantly after introduction. The exposure times for treatment were, 15, 30, 45, 60, 75, 90, and 120 minutes and untreated adults were included as a control. The experiment was done in completely randomized design with three replications. After each treatment the adults were separated from the seed by sieving through 3mm mesh sieve to avoid eggs that could be oviposited instantly before or at some point in the early time of exposure when the generated heat could not influence the adults. The adults then introduced to about 100 clean seeds and kept in the laboratory under room temperature. Between-seed temperatures were measured as explained in section 2. Dead insects were counted within 1, 2 and 3 days after treatment and percent mortality was found. Eggs oviposited during the life period of the adults were counted to evaluate the influence of solar heat treatment on oviposition.

3.2 Effect of solar heat treatment on egg hatchability of *C. maculatus*

Newly emerged adults were positioned within disinfected seeds in aquarium and allowed to oviposit. The next day the adults were removed by sieving through 3mm mesh sieve. For each treatment solar heater boxes containing one kilogram of adzuki bean seeds in a Perspex tray were interspaced with 30 eggs and subjected to heat instantly after removal of the adults. The eggs were exposed to heat for 15, 30, 45, 60, 75, 90, and 120 minutes and untreated eggs were included as a control. The experiment was done in completely randomized design with three replications. Between-seed temperature was recorded as explained in section 2. The treated eggs then were kept in the laboratory under room temperature for six days. Hatched and unhatched eggs were noticed daily using binocular microscope. Hatching eggs were known by the movement of larva and the different in shell color according to presence of proteins. The percent of egg hatchability was counted by dividing the proportion of hatched eggs to the total number of eggs multiply by 100.

3.3 Effect of solar heat treatment on 4th larval instar of *C. maculatus*

Newly emerged adults were introduced to clean seeds of adzuki bean in aquarium and allowed to oviposit for one day. The next day the adults were sieved through 3mm mesh sieve. Seeds with eggs were separated and kept in the laboratory under room temperature condition. According to Mekasha, 2004 the result of the study on the biology of developmental stages of the insect, time required for eggs to reach fourth instar from the first day of oviposition, was 18 days. Consequently, 30 seeds with eggs for each treatment were distributed within one kilogram of adzuki bean seeds and exposed to heat at this respective period to treat the fourth instar larva. The fourth instar larvae exposed to heat for 15, 30, 45, 60, 75, 90, and 120 minutes. Untreated check was included. The treated larvae from each treatment were kept in the laboratory under room temperature in Petri dishes. Number of emerged adults was noticed and percent of adult emergence was found in order to evaluate the effect of heat in the 4th larval development. The experiment was done in completely randomized design in three replications. Within-seed temperatures were recorded as illustrated in section 2.

4. Effect of constant heat on *C. maculatus*

4.1 Effect of constant heat on oviposition and adult mortality of *C. maculatus*

To verify the effect of solar temperatures on the cowpea weevil *C. maculatus*, another experiment was done using the oven. Fifteen pairs of newly emerged adults for each treatment were introduced to 50 grams of clean adzuki bean seeds in Petri dish. The adults were exposed instantly after introduction to temperatures of 70, 60, 50 and 40°C. The exposure times for treatments were, 10, 20, 30, 40, 50, 60, and 70 minutes and untreated adults were included as a control. After each treatment the adults were separated from the seed by sieving through 3 mesh sieve to avoid eggs that could be oviposited instantly before or at some point in the early time of exposure when the heat could not influence the adults. The adults then introduced to about 100 clean seeds and kept in the laboratory under room temperature. Dead insects were counted within 24, 48 and 72 hours after treatment and percent mortality was found. Eggs oviposited during the life period of the adults were counted to evaluate the influence of solar heat treatment on oviposition.

4.2 Effect of constant heat on hatchability and adult emergence of *C. maculatus*

Newly emerged adults were positioned within disinfected seeds in aquarium and allowed to oviposit. The next day the adults were removed by sieving through 3mm mesh sieve. For each treatment, 9cm Petri dish containing 50grams of adzuki bean seeds were interspaced with 30 eggs and subjected to heat instantly after removal of the adults. The eggs were exposed to 40, 50, 60, and 70°C for 10, 15, 30, 40, 50, 60, and 70 minutes

and untreated eggs were included as control. The experiment was done in completely randomize design with three replications. The treated eggs next were kept in the laboratory under room temperature for six days. Hatched and unhatched eggs were noticed daily using binocular microscope. Hatching eggs were known by the movement of larva and the different in shell color according to present of proteins. The percent of egg hatchability was counted by dividing the proportion of hatched eggs to the total number of eggs multiply by 100. Newly emerged adults were introduced to clean seeds of adzuki bean in aquarium and allowed to oviposit for one day. The next day the adults were sieved through 3mm mesh sieve. Seeds with eggs were separated and exposed instantly to heat treatment at temperatures; 40, 50, 60, and 70°C for 10, 20, 30, 40, 60, and 70 minutes and untreated eggs were included as control check. The experiment was conducted in completely randomize design with three replications. The treated eggs then were kept in the laboratory under room temperature until the adults emerged. The percent of adult emergence were found.

III. Data Analysis

Percents of mortality, hatchability, and adult emergence data were *root square* transformed while normal data was used for oviposition. Statistical Analysis Software (SAS) was used to analyze the data using analysis of variance (ANOVA) procedure and Duncan Multiple Range Test (DMRT) was used for mean comparison.

IV. Results And Discussion

4.3.1 Effect of solar heat treatment on oviposition and adult mortality of *C. maculatus*

Adult mortality was significantly affected by solar heat treatment (Figure 1). Exposure to heat for 15 minutes increased between-seed temperature in solar heater boxes to 66.4°C and 67.4°C for Cardboard and Plywood respectively which caused 100% mortality of adults. When the exposure time was extended to 30, 45, 60, 75, 90, and 120 minutes between-seed temperatures increased to 69.2, 70.2, 70.9, 71.2, 71.9, 72.4°C respectively and certainly caused complete mortality for the adults. However, in untreated check none of the adults that were not exposed to heat treatment were killed. Solar heat treatment on adults was also affected the oviposition. All the adults of *C. maculatus* were died immediately without laying eggs for all exposure times. These results were in agreement with earlier findings. Fields (1992) mentioned that, the thermal death point for the cowpea weevil *C. maculatus*, is 57°C. All the life stages of this insect were killed when exposed for one hour to this point (Murdock and Shade, 1991).

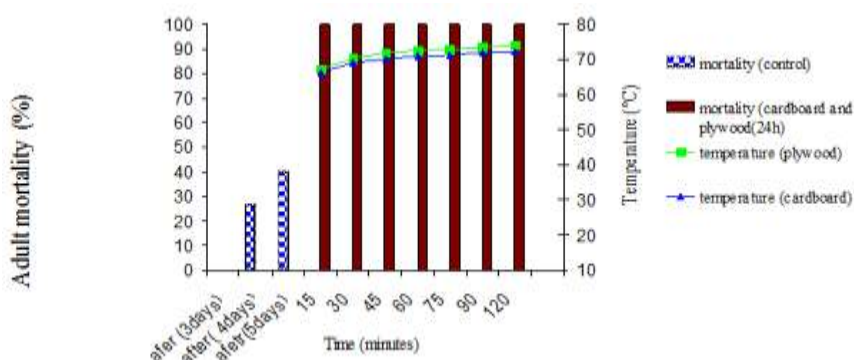


Figure 1. Effect of temperature of solar heater boxes on adult mortality of *C. maculatus*

A study done by Murdock and Shade (1991) on *C. maculatus* showed that, when the adults exposed to solar heat with temperature of 65°C complete mortality of all stages was achieved. Furthermore, according to Mekasha (2004) exposure to solar heat of adults of this insect in one kilogram of adzuki bean seeds for 45 minutes increased between seed-temperatures to 69.6°C and resulted in 100 % mortality of the adult bruchids and restricted the oviposition. Maina and Lale (2004) investigated the effect of solar heat on *C. maculatus*, their results indicated significant effect on oviposition and adult progeny. Furthermore, study done by Lale and Ajayi (2001) resulted in complete adult mortality of *C. maculatus* when exposed to solar heat for 7, 14 and 24h.

4.3.2 Effect of solar heat treatment on egg hatchability of *C. maculatus*

Results of the effect of solar heat treatment on the hatchability of *C. maculatus* were represented in figure 2. Exposure of eggs to heat for 15 minutes increased the temperature between seeds to 66.4°C for Cardboard and 67.4°C for Plywood and decreased hatchability of eggs to 26.7 % and 30 % respectively which is significantly ($p \leq 0.05$) lower from untreated eggs (83.3%). Extended exposure time to 30 minutes and more, of heat treatment caused 100 % mortality of egg.

These results were nearly similar to some researcher findings. An experiment done by Mekasha (2004) indicated that, solar heat treatment of *C. maculatus* eggs reduced hatchability to 33.4% with temperature mean of 45.1°C for 15 minutes. However, extension of exposure times to heat for more than 30 minutes restricted egg hatchability. Moreover, Lale and Ajayi (2001) found that solarization for 7, 14 and 24 hours caused 100% mortality of eggs for *C. maculatus*.

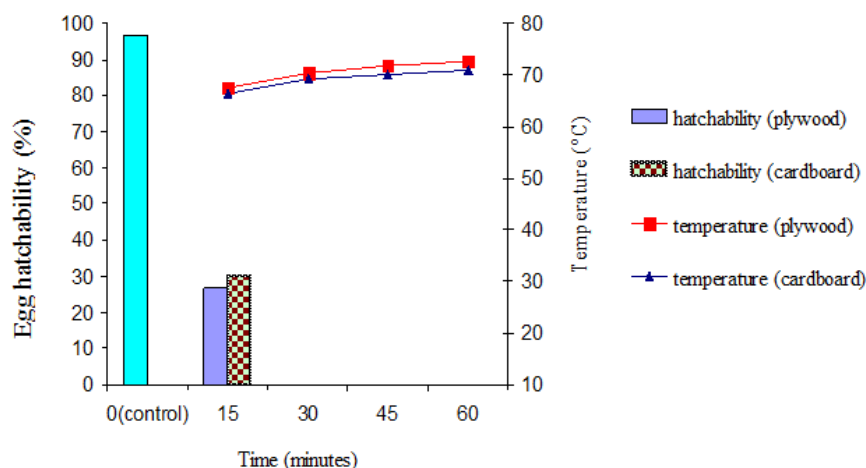


Figure 2. Effect of temperature of solar heater boxes on egg hatchability of *C. maculatus*

4.3.3 Effect of solar heat treatment on 4th larval instar of *C. maculatus*

Solar heat treatment was observed to influence the fourth larval instar of the insect and adult emergence was significantly affected (Figure 3). Exposure of 4th instars to heat for 15 minutes increased the temperature within seeds to 63.0°C for Plywood and 62.5°C for Cardboard solar heater boxes and decreased adult emergence to 23.3% and 26.6% respectively which is significantly ($p \leq 0.05$) different when compared to adult emergence from untreated larvae (82.2%). When the exposure times of heat treatment extended to 30 minutes and more the temperatures also increased and none of the treated larvae were able to reach adult stages.

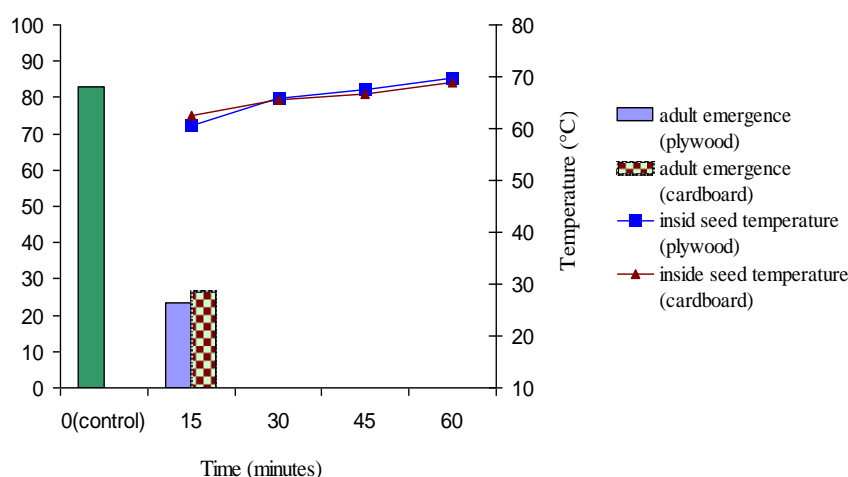


Figure 3. Effect of temperature of solar heater boxes on adult emergence of *C. maculatus*

Similar findings were reported by Mekasha (2004). When the larvae exposed to heat for 15 minutes, the temperatures within seed increased to 61.1°C and significantly reduced number of adult emergence and no adult emergence was noticed when exposure time extended to 30 minutes and more. Moreover, Murdock *et al* (1991) found that, exposed to solar heat at temperature of 57°C for one hour is sufficient to kill all developmental stages of *C. maculatus*. It seems that larvae stages need more time of exposure to cause complete mortality than that needs for adult mortality. In fact, larvae stages of this insect spend the whole life time inside the seeds. Lale, (1998) reported that, the seed cotyledons most likely protect developing larvae of *C. maculatus* against the solarization lethal effects. This may be why significantly higher percentages of adult emerged from larvae which exposed to the sun 15 minutes than from larvae exposed 30 minutes and more.

4.3.4 Effect of constant heat on developmental stages of *C. maculatus*

Exposed to heat at 70°C strongly affected adult mortality and egg hatchability. Complete mortality was achieved instantly when adults exposed for just 10 minutes or less. Hatchability was 0.0 % compared to 96.7% for untreated eggs. Exposed to heat at 60°C caused complete mortality instantly in 20 minutes of exposure while 100% mortality was achieved after one day in 10 minutes of exposure. No oviposition or hatchability was observed in these particular times (table 1).

Table 1. Effect of constant heat on mortality and developmental stages of *C. maculatus*

Exposure time (minutes)	Temperatures		
	60°C		
	Mortality (%)	Hatchability (%)	Oviposition (%)
CK ^a	0.0	96.7	82.3
10	100 (24h)	0.0	0.0
20	100	0.0	-

CK^a Untreated control

Table 2 and 3 showed the effect of the constant temperature on oviposition, hatchability, adult mortality and adult emergence of *C. maculatus* when exposed to 50°C and 40°C for different exposure times. The results indicated that, oviposition and egg hatchability were significantly affected by these points of temperature when compared to untreated check. Oviposition and hatchability percent decreased when exposure time increased.

Table 2. Effect of constant heat on developmental stages and mortality of *C. maculatus*

Exposure time (minutes)	Temperatures (50°C)					Adult emergence (%)
	*Oviposition (Eggs)	Hatchability (%)	Adult mortality (%)			
			24h	48h	72h	
CK ^a	82.3 ^a	96.7 ^a	0.0 ^b	0.0 ^c	0.0 ^c	82.2 ^a
10	33.0 ^b	90.0 ^{ab}	3.3 ^{ab}	10.0 ^{bc}	20.0 ^b	50.0 ^b
20	21.3 ^c	73.3 ^{bc}	6.7 ^{ab}	10.0 ^{bc}	30.0 ^b	46.7 ^b
30	9.5 ^d	60.0 ^c	6.7 ^{ab}	20.0 ^{ab}	53.3 ^a	30.0 ^{cb}
40	0.0 ^e	36.7 ^d	10.0 ^a	30.0 ^a	60.0 ^a	13.3 ^c
C.V%	16.5	13.5	50.6	47.7	16.3	28.9

CK^a Untreated control

*¹ No of eggs/female, during life.

*² from treated 4th instars

For each set of comparison means followed by the same letters are not significantly different ($p \geq 0.05$)

Adult mortality significantly affected by heat treatments at 48 hours for both points of temperatures. From tables 2 and 3 it was clear that, heat treatment was also affected

adult emergence from treated fourth instars when they exposed to heat at 40°C and 50°C for different exposure times.

Haines (1991) mentioned that the suitable temperature for most favorable oviposition and development of *C. maculatus* is 30-35°C. Study done by Lale and Vidal (2003b) indicated that difference in temperature could have huge effect on oviposition and

development of *C. maculatus*. Oviposition was decreased significantly at temperature of 40°C and the eggs failed to develop into adults.

Table 3. Effect of constant heat on developmental stages and mortality of *C. maculatus*

Exposure time (minutes)	Temperatures (40°C)					* ² Adult emergence (%)
	* ¹ Oviposition (Eggs)	Hatchability (%)	Adult mortality (%)			
			24h	48h	72h	
CK ^a	82.3 ^a	96.7 ^a	0.0 ^b	0.0 ^d	0.0 ^d	82.2 ^a
10	48.2 ^b	96.7 ^a	0.0 ^b	3.3 ^d	10.0 ^{dc}	70.0 ^{ab}
20	36.1 ^c	90.0 ^{ab}	0.0 ^b	6.7 ^{dc}	13.3 ^{dc}	66.7 ^{ab}
30	27.4 ^d	90.0 ^{ab}	0.0 ^b	16.7 ^{bc}	23.3 ^{bc}	66.7 ^{ab}
40	14.1 ^e	70.0 ^{bc}	0.0 ^b	20.0 ^{ab}	30.0 ^{abc}	53.3 ^{ab}
50	9.4 ^{ef}	60.0 ^c	3.3 ^{ab}	30.0 ^a	36.7 ^{ab}	50.0 ^{ab}
60	4.7 ^f	53.3 ^c	10 ^a	30.0 ^a	46.7 ^a	33.3 ^b
C.V%	11.8	14.3	87.0	30.5	29.1	34.2

CK^a Untreated control

*¹ No of eggs/female, during life.

*² from treated 4th instars

For each set of comparison means followed by the same letters are not significantly different ($\rho \geq 0.05$)

Lale (1998) mentioned that, a previous study was done by Iloba and Osuji (1986). They found that adults of *C. maculatus* when exposed to temperatures higher than 40°C survived up to 4 hours at 47.5°C but death occurred at 51.3°C in 15 minutes. Furthermore, an experiment in an oven conducted by Murdock *et al* (1991) resulted in complete mortality of larvae, pupae and adults of *C. maculatus* when exposed to heat of up to 57.3°C for one hour. The mortality of an insect stage at high temperatures is a function of temperature and exposure time. At any given temperature, mortality increases with an increase in exposure time. In temperature above 55°C, there is rapid mortality where the insect is dead in minutes or seconds (Beckett *et al.*, 2007). High temperatures are known to lower egg production and to stop the ability of movement and finally caused death in adult insects (Lale, 1998). Death of bruchids at high temperatures may result from various factors. Their limited physiological ability to thermoregulate, denatured of proteins, nucleic acids, lipids and carbohydrates or the interruption of the balance of metabolic process which build up toxic production (Murdock *et al.*, 1997; Lale, 1998; Beckett *et al.*, 2007). In this study, the decline of oviposition as well as the mortality of eggs, adults, and 4th instars' larvae might have been due to a combination of the adverse effect of high temperatures.

V. Conclusion

Adult mortality, hatchability, oviposition and adult emergence were significantly affected by solar heater boxes treatment. Complete mortality was achieved when adults exposed to heat for 15 minutes at 66.4°C compared to 0% mortality after 24, 48 and 72 hours for untreated adults. Times needed for 100% adult's mortality were 10, 20, 50 and 70 minutes with temperatures of 70, 60, 50 and 40°C respectively.

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