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RESEARCH ARTICLE

BIOLOGICAL EFFECTS OF DIFFERENT TEMPERATURES ON VARIOUS STAGES COWPEA BEETLE *CALLOSOBRUCHUS MACULATES* FABRICIUS (COLEOPTERA: BRUCHIDAE)

Zamani[1] .M, Ansari pour[2*].A and Aghasi[3] .K

[1,2]Agriculture faculty, Islamic Azad University, Khorramabad branch, Khorramabad, Iran
[3]Agriculture faculty, Islamic Azad University, Arak branch, Arak, Iran

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ABSTRACT

Cowpea beetle is the most important causing damage factors to stored products including cereal. It is one of the factors that decline the stored products. One factor that has a direct impact on pest life stage is temperature. It is research the effects of 35°c to 60°c on the various life stages of the pest temperatures from egg to adult stage was studied. Each of the temperatures within 5 to 30 minutes was studied. The tests done in a completely randomized design and Duncan's test were used for statistical comparison as significant. Study showed a temperature of 55°c to 60°c control 100% in all stages of biological pest and all times and loss biological different stage pest at 50°c is significant. Pupation stage is the most resistant of the life stages of pests resistant to temperature and also with increasing temperature and time, the mortality rate of pest increased and these two variables are correlated with each other.

INTRODUCTION

Legumes are as one of the main plant sources of protein-rich, is (Shewry and Halford, 2002). Population growth in two recent decades has led to increased consumption of protein, especially red meat. Due to the high consumption of animal protein that create problems for humans, because of this increase production of protein, especially vegetable protein that are healthier than animal protein and they are valuable resources in the diet is inevitable (Hardinge and Stare, 1954; Keys and Anderson, 1957; Olson et al., 1958). Many factors are causing damage to crops such as grains. The factors causing damage include: pests, weeds and plant pathogens. Legumes are such as agricultural products stored in warehouses (Bagheri Zenouz, 2007). Stored pest always are the cause of eliminating of grains in warehouses and insects have a special place among this factors. Among the orders of insects that harm and damage the stored products a lot and especially to the grain can mention the Coleoptera (Bagheri Zenouz, 2007). Some Coleoptera damage that feed of stored grains for 3 to 5 months storage in warehouses is reacting to 100 percent (Keita et al., 2001). Storage pests also reduce the quality and quantity of product (Micu and Peteranec, 2009), reduce the lower marketable yields and power of germination (Lale and Abdulrahman, 1999). Among the coleoptera the beetles of the Bruchidae family are important in terms of damage to stored products, the larvae feed dried beans grains and several generations per year (Bagheri Zenouz, 2007). Among this family cowpea beetle *C. maculates* F. is one of the most dangerous stored products pest and cause damage to the wide range of stored cereal including lentil, pea, chickpea, cowpea and broad bean. This pest has a wide range in tropical and semitropical areas (Bagheri Zenouz, 2007; Mahfuz and Khalequzzaman, 2007). Cowpea beetle of crop entered warehouse from the farm and the amount of farm population is a major factor that influences on the increasing population of the pest in stock (Olubayo and Port, 1997). Because of get out of each cowpea beetle from the seeds of beans about 25% of the seed weight decreased, damaged seeds often don`t germinate and high pollution of products to this beetles reducing the quality and mold growth (Rees, 2004), this pest destroys about 24% of warehouse legumes in Nigeria, so that annual loss on cowpea over 2900 tones has been reported (Ogunwolu and odulami, 1996). Cowpea seeds after 3 to 5 months of storage 100% are destroyed by cowpea beetle and the product weight 60% declined (Keita et al., 2001). The health hazards caused by *Callosobruchus* among the legume grains should not be overlooked, 180 species of bacteria from the species *C. analis* and *C. maculatus* has been isolated and purified, among them 5 species are human pathogenic and 8 species are from common pollution bacteria, one of them is rotten food and 7 species are insect pathogens through dust, water and other resources were allocated randomly into the gut of the insect (Kumari and Neelgund, 1983). Scientists concluded that different species of the genus *Callosobruchus* beside the high storage losses, are able to damage the health of the consumers' of this kind of food products. In the twentieth century due to the increased awareness of the dangers of chemical pesticides used pest control on human health and sustainability of pest in the environmental, the preferences use of pest in the consumers differs (Mohandass et al., 2006). Nowadays to control storage pests use the chemical pesticides especially fumigant, this compounds have effects such as pesticide residues in food, the negative impact on the environment, pest resistance against pesticides and poisoning for consumers, so it is necessary to attempt to access low-risk control methods (Alavanja et al., 1990; Fields, 1998). The problems that chemical pesticides create, the need to use alternative methods for pest control existence, several methods such as using the physical methods for example using high and low temperatures, nuclear radiation (radiation) and use of physical agents (noise, light, etc.)

* Corresponding author: **Ansari pour. A**
Agriculture faculty, Islamic Azad University, Khorramabad branch, Khorramabad, Iran

to control of storage pests has been used (Banks and fields, 1995). One of the alternative methods to pesticides is using the high temperatures or heating, heating is the most important method using in food industrial during the last 80 years (Fields, 1992; Mahroof et al., 2003). Using the high temperature for the first time in France to control of Angoumois Grain Moth (*Sitotroga cerealella* Olivier) was performed (Fields, 1992). In Australia, for pest control of storage products are placed one minute at a temperature of 56°c to 76°c (Fields, 1992). Considering that cowpea beetle one of the most important pest stored legumes, in this research it has been tried to examine the effect of different temperatures on various stages of its life and to know what is the best temperature for physically combat to this pest and also the use amount of chemical pesticides that used in warehouses reduced and in this way provide a method for further research in this items for other researchers.

MATERIALS AND METHODS

Breeding of cowpea beetle (C. maculatus)

Male and female insect were collected from infected storage seed, these insects was breeding in temperature conditions 30±2°C and relative humidity 60±5 percent in incubator in the darkness on legumes such as cowpea.

Effects of high temperatures at different times on pest`s eggs

Experiment in glass containers with a net cap with 100 ml was done. In each glass there were 10 cowpea seeds containing a single day, for these experiment temperatures 35, 40, 45, 50, 55 and 60°C with using incubator was applied. Containers at the time 5, 10, 15, 20 and 25 minutes at thermal conditions were placed and then got out of incubator to 30°C situation. After 5 days (first instar larvae inside the seed) the number of dead eggs were counted using binocular (Criterion mortality not to enter the first instar larvae into the seed). This experimental designed completely randomized factorial experiment, each treatment was replicated 5 times. Data from the test after normality testing with SAS 9.1 software for analysis and Duncan test was used for comparison as significant.

The effect of high temperatures on the first instar larvae

One day-old eggs were prepared as previous experiments, after 5 days that first instar larvae imported the seeds, ten beans contains first instar larvae were placed in a glass containing a volume of 100 ml. for this experiment temperatures 35, 40, 45, 50, 55 and 60°C was applied. In each temperature containers at 5, 10, 15, 20 and 25 minutes at thermal conditions were placed and after that got out of the incubator and transferred at 30°C situation, with spliting Seeds mortality of first instar larvae was calculated. The experiment designed completely randomized and factorial experiment. Factors included temperature in 6 levels and 5 levels of time data from the test after normality testing with SAS 9.1 software for analysis and Duncan test was used for comparison as significant.

The effect of high temperature at different times over the 11-day larval stage and Pupa

Seeds contain one day eggs in temperature condition 30°C and 60% humidity were placed, after 16 days containing 11 days larvae (five days is the time for egg hatched) and after 18 days insects were into the pupation. The effects of different temperatures at different times such as previous tests were performed. Criterion was to mortality of 11-day Larvae and pupa and not to get out the adults. This experiment designed completely contingency randomized and factorial experiment each treatment replicated 5 times. Experiment`s data after mortality test with SAS9.1 software statistically analyzed Duncan test were used for statistical comparison as significant.

The effect of high temperature at different times on adults of cowpea beetles

10 adult insects, 1 to 3 days cowpea beetles were placed into a glass containing a volume of 100 ml. For these experiment temperatures 35, 40, 45, 50, 55 and 60°C were applied with incubator. In each temperature containers were applied at 5, 10, 15, 20 and 25 minutes in thermal condition and after that containers were out of incubator and were applied in 30°C thermal condition and mortality rates of adults insects in vitro counted. This experiment designed completely randomized and factorial experiment, each treatment replicated 5 times. The experiment test after mortality test statistically analyzed with SAS9.1 software. Duncan test were used for statistical comparison as significance.

RESULTS

Effect of different temperature at different times on cowpea beetles egg were analyzed, among the different temperatures there was a significance (Table 1), more losses are at 55, 60°C and in both temperature in 5 minutes the mortality rate of cowpea beetle is 100 percent and the lowest mortality is in 35, 40 temperatures respectively 0 and 2% that is not a significant difference also at temperature, also at temperatures 45 and 50°C respectively 10, 52 percent of cowpea beetles wasted (table 2). Based on variance analysis table, among the different temperature at different times on the pest eggs there are significant differences (Table 1). The most losses were at the highest times mean 25 minutes since in 35°C with increasing the time of eggs heating, the losses increases and respectively at 5, 10, 15, 20 and 25 minutes, mortality was 0, 0, 0, 12 and 16% or at 50°C at mentioned times respectively the mortality was 52, 64, 78, 100 and 100% that means with increasing time the mortality increase and have a significant differences. Also based on variance analysis data showed a significant interaction between temperature and time (Table 2). Since increasing the time from 15 to 20 minutes in 40°C only caused increasing the mortality for 4% but at 50% this increase causing 22% mortality of cowpea beetles (table 2). The effects of different temperatures at different times on first instar larvae of cowpea beetles were analyzed among the different temperature, there are significant differences that showed the effect of temperature on an insect larvae pest (Table 3). The most losses are in the temperatures 55, 60°C and in both temperatures in five minutes the mortality rate of an one-day cowpea beetles is 100% in temperatures 35 and 40°C. The first instar larvae and no losses and don`t have a significant difference, also in temperatures 45 and 50°C respectively4 and 24% of the first instar larvae of a cowpea beetle were lost (Table 4). Based on results the most losses at the highest toll means 25 minutes had been showed so that even in temperature 40°C with increasing time of exposure of first instar larvae the mortality elevated and respectively in times 5, 10, 15, 20 and 25 minute, the mortality were 0, 2, 4, 10 and 14% at 50°C at the mentioned time the mortality respectively were 42, 50, 64, 82 and 100%, means with increasing the time, the mortality increases and have significant differences with each other (table 4), also based on analysis data the interaction between temperature and time has been significant (Table 3).

Table 1 Statistical analysis of the effect of high temperatures at different times on eggs of four-point bean beetle

Variable	df	Mean square	Sum of squares	F
Temperature	5	10.6462	53.2311	2415.36**
Time	4	0.6101	2.4407	140.5**
Temperature * Time	20	0.1216	2.4328	28.01**
Error	120	0.0043	0.5211	
Total	149		58.6259	

** Significant at the one percent level

Table 2 Comparison of mean ± standard error caused by the high temperatures at different times on the egg of four-point bean beetle

Temperature °C	Time				
	5	10	15	20	25
35	0±0.00	0±0.00	0±0.00	12±2.45	16±2.44
40	2±2.45	4±2.45	8±2.45	12±2.45	26±2.45
45	10±0.00	14±2.45	20±3.16	24±2.45	28±2.45
50	52±3.47	64±4.20	78±2.44	100±0.00	100±0.00
55	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
60	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Duncan comparison test has been conducted

Table 3 Statistical analysis of the effect of high temperatures on the larvae of four-point bean beetle

Variable	df	Mean square	Sum of squares	F
Temperature	5	12.3574	61.7877	2095.75**
Time	4	0.3462	1.4151	5873**
Temperature * Time	20	0.0933	1.8678	15.84**
Error	119	0.0059	0.0701	
Total	148		65.8606	

** Significant at the one percent level

Table 4 Comparison of the mean ± standard error caused by the high temperatures at different times on the larvae of four-point bean beetle

Temperature °C	Time				
	5	10	15	20	25
35	0±0.00	0±0.00	0±0.00	0±0.00	2±2.45
40	0±0.00	2±2.45	4±2.45	10±2.45	14±2.45
45	4±2.45	6±2.45	10±0.00	14±2.45	18±2.45
50	42±2.45	50±4.47	64±2.40	82±2.45	100±0.00
55	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
60	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Duncan comparison test has been conducted

Table 5 Analysis of variance of the effects of high temperatures on the larval stage 11 days of four-point bean beetle

Variable	df	Mean square	Sum of squares	F
Temperature	5	13.1731	46.3210	1387.57**
Time	4	0.2070	1.592	21.15**
Temperature * Time	20	0.3260	1.4210	3.44**
Error	119	0.0094	0.7540	
Total	148		50.0890	

** Significant at the one percent level

Table 6 Comparison of the mean ± standard error caused by the high temperatures at various times over 11 days old larvae of on the four-point bean beetle

Temperature °C	Time				
	5	10	15	20	25
35	0±0.00	0±0.00	0±0.00	2±2.45	4±2.45
40	0±0.00	0±0.00	2±2.45	6±4.20	8±3.74
45	0±0.00	2±2.45	4±2.45	8±3.74	12±2.45
50	28±2.45	36±2.45	48±3.74	62±2.45	76±2.45
55	76±2.45	92±3.74	100±0.00	100±0.00	100±0.00
60	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Duncan comparison test has been conducted.

Table 7 Analysis of variance of the effects of high temperatures on the four-point bean beetle pupation

Variable	df	Mean square	Sum of squares	F
Temperature	5	11.6659	58.3295	1639.23**
Time	4	0.4242	1.6962	59.61**
Temperature * Time	20	0.0713	1.4262	10.02**
Error	119	0.0071	0.8540	
Total	148		62.3066	

** Significant at the one percent level

Table 8 Comparison of the mean ± standard error caused by the high temperatures at different times on the four-point bean beetle pupation

Temperature °C	Time				
	5	10	15	20	25
35	0±0.00	0±0.00	0±0.00	2±2.45	4±2.45
40	0±0.00	0±0.00	2±2.45	4±2.45	8±2.45
45	0±0.00	2±0.00	4±2.45	6±3.74	10±2.45
50	18±2.45	32±2.45	40±0.00	50±0.00	60±0.00
55	62±2.45	80±3.60	100±0.00	100±0.00	100±0.00
60	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Duncan comparison test has been conducted.

Table 9 Analysis of variance of the effects of high temperatures on the four-point bean beetle adult

Variable	df	Mean square	Sum of squares	F
Temperature	5	11.8592	59.2860	1422.65**
Time	4	0.3238	1.3950	38.85**
Temperature * Time	20	0.0541	1.0835	6.49**
Error	119	0.0083	1.0083	
Total	148		62.6744	

** Significant at the one percent level

Table 10 Comparison of the mean ± standard error caused by the high temperatures at different times on the four-point bean beetle adult

Temperature °C	Time				
	5	10	15	20	25
35	0±0.00	0±0.00	0±0.00	4±2.45	20±2.45
40	0±0.00	2±2.45	2±2.45	4±2.45	8±2.45
45	4±0.00	6±0.00	8±2.45	10±3.20	12±2.45
50	36±2.45	48±2.45	62±2.45	72±2.45	82±2.45
55	74±2.45	100±0.00	100±0.00	100±0.00	100±0.00
60	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Duncan comparison test has been conducted.

Increasing the time from 15 to 20 minutes at a temperature of 40°C causing 4% first instar larvae mortality but at 50°C this increasing of time causing 18% increasing of a cowpea beetle larvae mortality (table 4). Based on the results different temperature effects in different times on 11 days cowpea beetle larvae stage among the different temperatures there is a significant difference (Table 5). The most the most mortality of 11 day larvae in the temperature 55 and 60°C and in both temperature at 4 minutes the mortality rate is 100% (Table 6). Temperatures 35 and 40 degrees Celsius don`t have a significant effect on this stage of pest and don`t have significant difference. Also these larvae at temperatures 45 and 50°C respectively 0 and 28% died (Table 6). Based on the results the most mortality at highest toll means 25 minutes were showed, so that the temperature of 45°C with increasing the time the death rate went up respectively in 5, 10, 15, 20 and 25 minutes, losses were 0, 2, 4, 8, 12 and 50°C at mentioned times the mortality respectively were 28, 36, 48, 62 and 76%. It means with increasing the time the losses went up and have significant difference (Table 6). Based on the variance analysis data the temperature ant time interaction has been significant so that with increasing time from 15 to 20 minutes at a temperature 35°C only causing 2% death increasing but at 50°C , this time increasing causing 14% death increasing of at 11 day larvae (Table 6). The effects of different temperature at different times on the pupation stage of cowpea beetle were examined the difference temperature there was a significant difference (Table 7). The most mortality at 60° C was observed and for five minutes the mortality rate was 100%. At Temperatures 35, 40 and 45°C there is casualties and no significant difference among the temperatures. Also pupa pest at temperatures 50 and 55°C, 18and 62% were lost (Table 8).

Based on the results the most mortality was in the highest tool means at 25 minutes was observed, at 45°C with increasing the exposure time of a pupa the mortality went up and respectively at 5, 10, 15, 20 and 25 minutes the losses were 0, 0, 2, 4 and 8%, at 55°C and in mentioned times the mortality respectively were 62, 80, 100, 100 and 100% that means with increasing the time the mortality increased and among the variable there are significant difference (Table 7), also based on variance analysis data the interaction effect of temperature and time is significant and increasing time from10 to 15 minutes at 45°C only causing 2% mortality increasing, but at 45°C this time increasing causing 20% pupa pest mortality (Table 8). The effects of different temperature and different times on adult insect of a cowpea beetle were examined, based on the results there was a significant difference (Table 9). The most mortality is at 60°C and in temperature in five minutes the mortality rate of adult insects of a cowpea beetle is 100% and the lowest mortality is observed at temperatures 35, 40 and 45°C that respectively are 0, 0 and 2% and don`t have a significant difference with each other and also at 50 and 55°C respectively 34 and 74% adults insect of a cowpea beetles were lost (Table 10). Based on the results the most losses were at the highest temperature means 25 minutes were observed and at 35°C with increasing exposure time an adult insect the mortality increased and respectively at 5, 10, 15, 20 and 25 minutes the mortality of adult insects was 0, 0, 0, 4 and 20% and at 50°C and in the mentioned times respectively the mortality was 34, 48, 62, 72 and 82% and with increasing the time the mortality went up and have significant difference with each other and also based on variance analysis data the interaction effect of temperature and time has been significant so that increasing time from 10 to 15 minutes at 45°C just causing 2% mortality increasing but at 50°C this increasing time causing 14% mortality increasing of an adult insect cowpea beetle (table 10).

DISCUSSION

The proven researching showed that temperature 55 to 60° C in 5, 10, 15, 20 and 25 minutes control all stages of bio-pest and losses of different biological pest stages at 50°C is remarkable. These results are quite similar to the field believes (Field, 1992). Field believes that most insects over 24 hours in 40°C, 12 hours in 45°C, 5 minutes in 50°C, one minute in 55°C and 5 seconds in 60°C are not left a live and usually eggs are the most sensitive and pupae are the most tolerant stage (Field, 1992), also based on Talekar studies penetration cowpea beetles to the seeds that have a relative humidity less than 9.5% and the influence of temperature from 55 to 60°C remarkably have reduced.

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