THE EFFECT OF HEAT TREATMENT ON LOSSES OF DIFFERENT LIFE STAGES FOR *TRIBOLIUM CONFUSUM* DUVAL (COLEOPTERA: TENEBRIONIDAE)

Saeideh loni*, Maryam Moarefi and Reyhaneh Habibie Karahrodi*****

* Department of Entomology, Islamic Azad University, Arak Branch, Young Researchersclub of Arak, IRAN. E-mail: Loni_s2001@yahoo.com

** Department of Agronomy, Faculty of Agriculture, Islamic Azad University, Karaj Branch, Eram Blvd, Azadi St., Mehrshahr, P.O. Box 31876-44511, Karaj, IRAN.

*** Department of Entomology, Islamic Azad University, Arak Branch, Young Researchersclub of Arak, IRAN.

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ABSTRACT: *Tribolium confusum* Duval is an important pest of food processing technology in the world. The use of elevated temperatures or heat shock treatments is a very effective method for managing storage pests. In this study in order to evaluate the mortality of this insect by the use of elevated temperatures, effects of five constant temperatures including 35, 45, 50, 55 and 60°C for 5, 10, 15, 20 and 30 minutes have been studied on different developmental stages of this pest (5-day larvae, 15-day larvae, pupae and adult). After heating, the samples were kept under temperature of 28±1°C and relative humidity of 65±5%. Results indicated the most susceptive and the most heat-tolerant stage of the insect's growth were respectively related to the 15-day larvae and pupal stage. Furthermore, it was revealed that the minimum controlling temperature for the 5-day and 15-day larvae is 50°C and for pupae and adult insects is respectively 55°C and 50°C. These results provides, considering the possibility of pest's existence in different developmental stages in an infected mass, 55 °C for 15 minutes would be effective for managing and controlling life stages of *Tribolium confusum*.

KEY WORDS: *Tribolium confusum*, heat treatment, developmental stages.

Tribolium confusum Duval (Col., Tenebrionidae) is one of the pests of storage crops and attacks about 100 types of these crops. This pest mostly attacks the tropical and semi-tropical regions. Larval old cuticle and excreta of adult insects seriously decrease the quality of flour by entering poison into flour (Songa & Rono, 1998; Hollingworth et al., 2002).

Today, physical methods such as nuclear radiation and physical factors (sound, light, etc.) and chemical methods are being used for controlling *Tribolium confusum* (Bank & Fields, 1995). One of the prevalent methods of controlling storage pest is to use fumigants. Methyl bromide is one these pesticides used for controlling storage pests in food processing technology (Makhijani & Gurney, 1995).

Methyl bromide is one of the most significant chemical compounds with unfavorable effects on ozone layer (Anonymous, 1992; 1993). According to the Montreal Protocol, usage of methyl bromide is permitted in countries like Iran until 2015 (Makhijani & Gurney, 1995). Several methods can be replaced for methyl bromide (Dean, 1911). One of these methods is to use thermal treatments which are of the most important techniques used in food processing technology during the past 80 years (Fields, 1992; Dowdy, 1999; Wright et al., 2002; Mahroof et al., 2003; Roesli et al., 2003).

In some cases, heating treatment is done by exposing foods to 50-60°C temperature for 24 to 36 hours in order to kill all insects existing in the food (Dowdy, 1999; Mahroof et al., 2003). In heating, short period of time is considered for high temperature in order to avoid affecting the quality of crops (Evans, 1986; Tang et al., 2000; Wang et al., 2002). Besides, overheating in the environment causes damage to the processing equipment and machinery used in food processing technology. Moreover, less heating in this part would affect the pests' survival as well (Dowdy, 1999; Mahroof et al., 2003).

At present, economic importance of using these methods for controlling storage pests has been paid special attention (Oosthuizen, 1935; Wright et al., 2002), and many researchers have proved that if heating does not cause complete mortality of the pests, if may leave undesirable effect on reproduction, fertilization and development of the insects progeny (Arbogast, 1981; Gonen, 1977; Kawamoto et al., 1989; Lale & Vidal, 2003; Okasha et al., 1970; Proverbs & Newton, 1962; Saxena et al., 1992; Tikku & Saxena, 1985; Tikhu & Saxena, 1990). Being exposed to a sub lethal temperature (44°C) may cause complete or incomplete mortality of different species of *Tribolium castaneum* in the larvae or pupal stage.

For instance, only 1.3% of the eggs laid by female *Tribolium castaneum* are transformed into pupa when exposed to 44°C temperature and relative humidity of 75% for 8 hours (Oosthuizen, 1935).Mortality of *T. castaneum* in 50°C temperature and 15 to 30 minutes exposure time when the insects is not fed in post-treatment recovery period (for one day) is less than 29%. Prolongation of recovery period to 7 days results in 51-65% mortality. But in conditions when the amount of food is suddenly decreased or food is considered for insect at the time for recovery, survival of adult insects increases (Dowdy, 1999). High temperatures leave undesirable effects on reproduction of storage pests. When *Trogoderma granarium* Everts. , are exposed to 45°C temperature for 48 to 72 hours in pupal stage, adults made out of these pupae are not capable of regeneration (Saxena et al., 1992).When the 1-day, 2-day, 3-day and old pupae of *T. castaneum* are in 45°C temperature for 48 to 72 hours, development of the next generation would be completely stopped since survival of pupal stage is entirely disturbed. When the 2 or 3-day pupae of *T. castaneum* are kept in 45°C temperature for 48 hours, the produced female insects do not produce and larvae. But then the 1-day pupae are kept in 45°C temperature for 48 to 72 hours, they do not survive(Saxena *et al.*, 1992). In another research, when kept in 35°C temperature for 7 days and then in 26.5 °C temperature, the two-week female *Sitophilus granarius* (L.) resulted fewer adults in comparison with the females kept in 26.5 °C temperature (Gonen, 1977). According to the importance of the usage of non-chemical methods in controlling storage pests and role of temperature as a safe factor for environment, this research studies the effects of 35, 45, 50, 55 and 60°C temperature on mortality of *T. confusum* at different developmental stages.

MATERIALS AND METHODS

Insects Rearing

T. confusum was reared under laboratory conditions at 28±1°C and 65±5% relative humidity in darkness. For this purpose, pastry flour has been used in all tests as the food ration for the pest. 500g flour has poured in a plastic disposable dish with 13cm height and 15cm diameter and adult types of this pest were put in the dishes. After a while when the surface of the food area was being covered larvae's old cuticle, a new culturing environment was prepared and the adult insects and other life stages were transferred to the new environment from the previous one by a bolter.

Temperature treatment

Test was done on different developmental stages of the insect including 5-day larvae, 15-day old larvae, 3-4 day old pupae, and 7-day old adult insects. Thermal treatments included temperatures of 35, 45, 50, 55 and 60°C for 5, 10, 15, 20 and 30 minutes together with the experimental group. This test was done based upon factorial in the form of completely randomized design in 4 replications, and each unit of test included 25 insects. To conduct the studies, the first thing required was a population of the same age. To collect young larvae of the same age, the insect rearing dishes each contain 500gr flour were prepared and about 1000 male and female adult insects were released in them. After 24 hours and adults were collected by bolter and the dishes were put in an incubator until hatching of the eggs. After hatching in the sixth day and larvae's birth, the larvae were given five days for activity and then in the fifth day the 5-day old larvae were collected from the environment and put in the petri for being tested. 20gr flour was also given to the larvae for their nutrition. After performing thermal treatments, the samples were exposed to rearing condition and the mortality rate was recorded after collapse of this period. The case was done in regard to old larvae.

To study the effect of different temperatures on pupal stage of this pest, first we needed pupae of the same age. To collect pupae of the same age, all the pupae available in the rearing environment were collected first, so that even a single pupae was not left in the environment. Therefore, all the pupae appeared in the next day were almost of the same age.

Pupae of the same age were selected randomly from the environment and undergone thermal treatments. To achieve 3-4 day old insects, all the adult insects available in the rearing environment were collected by a bolter; therefore, the adult insects appeared in the next day were all of the same age. Then the 3-4 day old insects were collected randomly from the environment, and the samples' mortality was recorded after performing thermal treatments. Statistical data analysis was done using SAS and SPSS software.

RESULTS

The results achieved from the statistical analysis in all stages indicated that there was a significant difference of about 1% between different treatments in comparison with the experiment. Based on the obtained information, 60°C temperature has caused 100% mortality in all life stages of the pest for all durations. In 5-day larvae stage, 55°C temperature for 10 to 30 minutes and 50°C for 30 minutes caused 100% mortality and there was no significant difference between them. In 50°C for 20, 15 and 10 minutes the observed mortality was respectively 89, 80 and 72%.

At this stage, increase of time was effective on increase of mortality rate, so that by increasing the time from 5 minutes to 10 minutes in 50°C temperature, the mortality rate was increased from 29% to 72% (table 1-3). Therefore, the best controlling temperature for this stage is 50°C for 30 minutes.

In regard to 15-day larvae, also 55°C temperature for 5 to 30 minutes and 50°C temperature for 15 to 30 minutes caused 100% mortality. 50°C temperature in 5 minutes and 10 minutes respectively causes 86% and 97% mortality. The best controlling temperature for this stage is 50°C temperature for 10 minutes (table 1-3). The results related to pupal stage of the pest indicated that 55°C temperature causes 100% mortality for all durations. At this stage, increase of time from 20 to 30 minutes in 50°C temperature caused the increase of mortality from 11% to 87%. The best controlling temperature for this stage is 55°C temperature for 5 minutes (Table 1-3).

Results related to developmental stage of adult insects indicated that the thermal treatment of 55°C temperature for 5 to 30 minutes and the thermal treatment of 50°C temperature for 30 minutes caused 100% mortality. Increases of time from 15 to 20 minutes in 5°C temperature at this stage increased the mortality rate from 45% to 69%.

CONCLUSION

Based on the results, it has been indicated that increase of temperature decreases the time of reaching to the maximum mortality. It means that in a given period, the higher temperature causes the higher mortality. Therefore, in superheating or decontamination with high temperatures, not only the temperature but also the time has a special importance. According to the conducted tests and achieved results, it can be concluded that the developmental stage of 15-day larvae is the most susceptible and sensitive stage and the pupal stage is the most resistant life stage of *Tribolium confusum* against high temperatures.

According to the fact that in an environment contaminated with pest, all life stages of the pest are available and therefore, what is economically important is the control of harmful stages of the pest using controlling temperature appropriate for the pest's different developmental stage, so that it prevents regeneration. Larval stage of this insect has more significant in view of economy and has the capacity to cause intensive damage. Based on the achieved results, 50°C temperature for 30 minutes suffices for controlling this stage of the pest's life. 55°C temperature in 5 minutes also controls well the pupal stage which is the most resistant stage of pest's life.

15 minutes exposure time in 60°C temperature, control all life stages of *Tribolium castaneum* and *Oryzophilus surinamensis* Linnaeus and this fact accords with the results of the present study (Wilkin & Nelson, 1987). Besides, Boina and Subramanyam (2004) proved that young larvae of *Tribolium castaneum* are in the stage sensitive to increase of temperature. But results of the present research indicated that old larvae are the most sensitive stage. Exposure of one to a few days old pupae and 14-day or older larvae of *Tribolium castaneum* to 50°C for 39 and 60 minutes has harmful effects on reproduction and survival of egg stage to adult stage. Besides, young larvae of this pest tolerate temperatures over 50°C (Mahroof *et al.*, 2003) and this fact completely accords with the present research. Larvae and adult stages of *Tribolium confusum* Duval had 100% mortality when exposed to 47.5 °C temperature for 48 hours or 50°C temperature for 24 hours (Taheri, 1994). Considering the fact that the heating period in this study has been between 24 to 48 hours. Observation of 100% mortality is completely logical and confirms the results of this study.

In general results of this research indicate that heating has the capacity to be used for controlling storage pests , and designing and manufacturing the equipment for defusing equal heat in the mass or the product is of great importance. Furthermore , based upon the achieved results , 55 °C temperature for 15 minutes is recommendable for controlling all developmental stages of the pest.

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Table1-3. Ranking of the interacting between temperature and time in different developmental stages of *T. confusum*.

Average of mortality				Heat duration (min)	Temp. °C
Adult	**Pupae**	**15- days larvae**	**5-days larvae**		
A 100	A 100	A 100	A 100	30	60
A 100	A 100	A 100	A 100	20	60
A 100	A 100	A 100	A 100	15	60
A 100	A 100	A 100	A 100	10	60
A 100	A 100	A 100	A 100	5	60
A 100	A 100	A 100	A 100	30	55
A 100	A 100	A 100	A 100	20	55
A 100	A 100	A 100	A 100	15	55
A 100	A 100	A 100	A 100	10	55
A 100	A 100	A 100	A 95	5	55
A 100	B 87	A 100	A 100	30	50
C 69	C 11	A 100	B 89	20	50
D 54	D 0	A 100	C 80	15	50
E 0	D 0	B 97	D 72	10	50
E 0	D 0	C 86	E 29	5	50
E 10	D 0	D 12	F 2	30	45

Note: in the thermal treatments and in experiment when the mortality rate has been zero, they have not been mentioned in the table.