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Effect of some environmental parameters on the development of the wheat weevil, *Sitophilus granarius* (Coleoptera: Curculionidae) under laboratory conditions

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Abstract
Adults of laboratory population of the wheat weevil, *Sitophilus granarius* was exposed at the temperature of 20 º, 25 º and 30 ºC and relative humidity of 60%, 65% and 70% RH to determine egg lying, larvae, pupae and adult emergence time and progeny production/reduction in laboratory culture at mentioned environmental conditions. Three replications were used for each application. Twenty pairs of adults were released to get eggs. Data regarding oviposition, fertility and adult emergence was recorded on daily basis. *Sitophilus granarius* has great influence of temperature on its life period. It exhibited high appearance at high temperature in comparison with low temperature. Relative humidity has also excessive influence on the development of *S. granarius*.

Keywords: *Sitophilus granarius*, the wheat weevil, development, temperature, relative humidity

Introduction

Although it came from the Middle East, it is not known exactly where the wheat weevil, *Sitophilus granarius* Linnaeus, 1758 came from. The insect spread around the world through the import and export of grain. It is found mainly in temperate and other cold climates. In hot climates, the rice weevil, *Sitophilus oryzae* and the maize weevil *Sitophilus zeamais* take its place (Davis and Bry, 1985; Jacobs and Calvin, 2001; Karakas, 2020) [1, 2, 3].

The wheat weevil is a snout beetle and belongs to the Coleoptera order. Within this order it belongs to the Curculionidae weevil family. Adults are 3-4mm, dark brown, oval, with long legs, and the front of the head has a long snout, elytra are unmarked, wings (elytra below) are missing and the chest has oval punctures. Larvae are small, white, wrinkled, grub-like larvae (Fava, 1996) [4].

The life cycle of the wheat weevil is highly dependent on temperature and relative humidity. The average lifetime is about one year. The development from egg to adult grain weevil depends on temperature and relative humidity, and is possible between 11 and 30 ºC. The optimum temperature is 30 º C and relative humidity between 70% and 86%. Under these circumstances, the development takes place in 25 days. On average, it takes about two months to develop (Bank and Fields, 1995; Mason and Strait, 1998) [5, 6].

Larvae are immobile, and develop hidden in the grain. There is generally no external evidence that the larvae have been eating and growing inside the seed until after about one month when the adult weevil chews through the seed coat and emerges. Adults leave a hole in the grain, feed and cannot fly. Immediately after the weevil emerges from the kernel it is mature. However, it takes about 14 days before the female begins to lay eggs. In total, the female lays around 150 to 300 eggs (Baker, 1983; Niewiada *et al*., 2005; Cabi.org., 2019) [7, 8, 9].

The grains commonly attacked by grain weevils are rye, wheat, barley, corn, oats, and rice. To a lesser extent, buckwheat, pasta, spaghetti and flour are also affected. The damage caused by the insect consists mainly of quality and weight loss. Also, insects cause reduced feeding, flavour rot and germination. This is especially true for parties in developing countries. Particularly vulnerable to damage are those that have been stored for years in medium to poor storage (Karakas, 2016) [10].

Insect pests associated with stored grain can be divided into three main groups according to their importance, biology and feeding behaviour. These groups are often referred to as primary insect pests, secondary insect pests, and various insect pests. While it is not necessary to identify a specific insect precisely, it is critical to determine whether the insect belongs to one of these three groups in order to determine effective treatment options (Jacobs and Calvin, 2001) [2].

Primary insect pests include those insects considered to be the most damaging pests of stored grain. These pests cause the most damage because they develop within the whole grain kernel. Examples of primary insect pests include the rice weevil, granary weevil, and maize weevil. All of these weevils are similar in appearance and have similar life cycles (Longstaff, 1981) [11].

Secondary insect pests do not cause as much damage to stored grain as the primary pests. However, their presence and feeding damage can be a major concern. Unlike the primary pests, the secondary insect pests feed and develop outside of the whole grain kernel. In fact, most of these secondary pests depend on the presence of cracked and broken kernels for their development. Examples of secondary pests include the red flour beetle, confused flour beetle, flat grain beetle, saw-toothed grain beetle, and Indian meal moth (Holscher, 2000) [12].

A number of other pests can, at times, be associated with stored grain. These various pests include the foreign grain beetle, fungus beetles, psocids, and mites. These pests are fungus feeders that do not feed directly on clean, high quality grain. As a result, their presence indicates a serious moisture-related problem associated with the stored grain (Mookerjee and Chawla, 1964) [13].

An effective integrated pest management program for stored grain has many important components. Among these are

sanitation, empty grain bin treatments, grain cleaning, the use of grain protectants, temperature and moisture management, and insect monitoring (Thomas *et al*., 2002; Huang and Subramanyam, 2005) [14, 15].
The importance of temperature and moisture management in preserving grain quality and minimizing insect-related problems cannot be overstated. It is crucial that the grain mass be reduced to 10 ºC or less and 12% to 13% or less moisture as soon as possible after storage. Reducing the grain mass to these levels will slow insect development and will limit both mold growth and the possible formation of hot spots. As stated earlier, reducing the grain mass to these temperature and moisture levels will also prolong the effectiveness of any grain protectant that may have been applied. Effective control of stored product insect pests can be obtained when 50-60 °C is maintained for 24-26 hours. Age of the insect also impact the level of tolerance to temperature with constant humidity and time of exposure. At the same time, relative humidity play a vital role in survival of any pest as well as increases the toxic effect of insecticide so it is helpful in sinking the resistance developed in insects (Imura and Nakakita, 1984; Maier *et al*., 1996) [16, 17].
Insects in stored grain can be controlled by manipulating the physical environment or applying physical treatments to the grain and insects. The variables defining the physical environment that are usually controlled are: temperature, relative humidity or grain moisture content and relative composition of atmospheric gases in the intergranular air. Physical treatments include mechanical impact, physical removal and barriers to prevent to entrance of insects, abrasive and inert dusts, ionizing irradiation, light and sound (Bank and Fields, 1995; Adler, 2010) [18,19].
In this study, the effects of different temperature and humidity combinations on different developmental stages of the wheat weevil were investigated. This research is aimed to provide support to physical control, which is one of the methods of controlling stored seed pests.

Materials and Methods

Applications were carried out in the animal physiology laboratory of the Ankara University, Science Faculty, Department of Biology, between the years 2020-2021.

Insect supply

The wheat weevil adults were supplied from the stock culture of the laboratory of the Plant Protection Department, Faculty of Agriculture, Ankara University, Ankara.

Insect culture technique

The wheat weevil was feed on soft wheat seeds in the glass jars (1 L) having high moisture content so that *S. granarius* easily laid eggs and show better development. Wheat seeds were sterilized at 70 ºC for 15-20 minutes to eliminate the previous infestation of different species of insects, fungi and other microorganisms. The wheat weevil was reared at controlled temperature 26 ± 2 ºC and relative humidity in the range of 65 ± 5% RH which is the best condition for its development. The dark (D) and light (L) periods (12h: D – 12h: L) were maintained according to best development of weevil because *S. granarius* is very sensitive to the light periods for meeting and proper development. After 3 days, the adult weevils were removed to obtain a homogeneous stock of adult weevil and this process was repeated for the next three generations. The homogeneous population is preserved for further experiments.

Temperature and relative humidity applications

Twenty pairs of insects obtained from laboratory culture were released to 100 grams of sterilized wheat seeds in a glass jar (500 ml) and then placed inside an incubator was maintained at one of three different temperature (20, 25 and 30 ºC) levels. The relative humidity applications (60, 65 and 70% RH) within the incubators were maintained by using saturated salt solutions. All incubators were held at a photoperiod of 12h: D – 12h: L hours for the ideal life cycle of the wheat weevil, *S. granarius*. Oviposition was allowed for 72h. This time period of egg laying of mature females of wheat weevils ensures that sufficient amount of eggs are laid. After 72h, released insects were removed and their total number of eggs laid on randomly selected sixty grains of wheat were counted with help of a binocular. These eggs were maintained for life stages of the wheat weevil, *S. granarius*.

Developmental tests

For fertility test, thirty females were released in different glass jars (425 ml) to observe the fertility rate in different (4, 6, 8, 10 and 12) days.
For larval emergence test, thirty eggs were released in petri dishes (90 mm diam.) at each temperature and relative humidity combination. The data was taken about egg hatching after 3, 4, 6, 8 and 11 days.
For pupae emergence test, the hatched eggs were released in different petri dishes to observe the larval time periods. Larvae took prolong time period to complete its four instars so data about pupae formation were taken after 18, 21 24, 28, 30 and 34 days.
For adult emergence test, the thirty pupae were transferred into new petri dish with great care. The data regarding adult emergence was observed after 4, 6, 8, 10 and 12 days.

Statistical analysis

Data on the developmental stages of the insect was analysed by a two way analysis of variance (ANOVA) and the Tukey test was used as a post-hoc analysis (the multiple comparison techniques to determine the difference between groups in researches) for the interaction and effect of temperature and relative humidity. Mean values of the entire developmental time within one replicate of each treatment were calculated.

Results

The results showed that temperature and relative humidity greatly affect the life cycle of *S. granarius* ($F= 3.68$; $P= 0.041$). The egg lying of wheat weevil was found maximum (24.00 ± 0.57) at 30 ºC and 70% RH after 12 days but when the decrease in temperature from 30 ºC to 25 ºC and 20 ºC, relative humidity from 70% to 65% and 60%, the egg lying by wheat weevils also reduced to 13.00 ± 0.58, 9.66 ± 0.88 and 9.33 ± 0.33 respectively as mentioned in Table 1. The combined effect of temperature and relative humidity influenced the larval emergence from eggs of *S. granarius* ($F= 4.36$; $P= 0.063$) as Table 2 showed that larval emergence was also increased from 10.33 to 26.33 as both factors increased from lowest point (20 ºC and 60% RH) to highest point (30 ºC and 70% RH) after 11 days of observation. The results regarding pupae emergence was also greatly significant different due to temperature and relative humidity variation (F= 4.05; P= 0.053). The pupae emergence increased from 10.33 ± 0.33 and 26.33 ± 0.33 as both combined treatments (30 ºC and 70% RH) but decreased to 15.33 ± 0.39 and 15.00 ± 0.45 as both factors fall down to 25 and 20 ºC, 65 and 60% RH respectively after 31 days of observation as shown in Table 3. The increase in adult emergence from pupae of wheat weevil was observed in Table 4 from 10.33 ± 0.33 to 24.67 ± 0.67 due to the effect of temperature and relative humidity (F= 3.88; P= 0.05). So F and P values showed that both temperature and relative humidity affect the whole life period of wheat weevil.

Table 1: Mean values of Egg Lying Period ± Standard Error (ELP ± SE) of *Sitophilus granarius* after different days under different temperatures (ºC) and relative humidity (RH).

ºC / %RH	ELP ± SE / Days				
	4	**6**	**8**	**10**	**12**
20 / 60	3.33 ± 0.33 c	5.33 ± 0.88 c	5.33 ± 0.88 c	7.33 ± 0.88 c	9.33 ± 0.33 c
20 / 65	8.33 ± 0.33 b	9.33 ± 0.33 b	9.33 ± 0.33 b	9.66 ± 0.88 b	10.33 ± 0.33 b
20 / 70	11.00 ± 0.57 f	12.33 ± 0.66 f	12.33 ± 0.66 f	12.66 ± 1.20 f	13.00 ± 0.58 f
25 / 60	5.00 ± 0.33 ef	6.33 ± 0.33 ef	6.33 ± 0.33 ef	8.66 ± 0.88 ef	9.66 ± 0.88 ef
25 / 65	10.33 ± 0.33 ef	11.00 ± 0.57 ef	11.00 ± 0.57 ef	13.00 ± 0.58 ef	15.33 ± 0.67 ef
25 / 70	15.33 ± 0.67 e	16.00 ± 0.57 e	16.66 ± 0.33 e	17.00 ± 1.00 e	19.33 ± 0.33 e
30 / 60	9.33 ± 0.33 d	10.33 ± 0.33 d	10.33 ± 0.33 d	11.33 ± 0.66 d	13.00 ± 0.58 d
30 / 65	14.00 ± 0.58 d	14.33 ± 1.45 d	14.33 ± 1.45 d	16.00 ± 0.57 d	18.33 ± 0.66 d
30 / 70	19.33 ± 0.33 a	20.33 ± 0.67 a	20.33 ± 0.67 a	22.00 ± 0.57 a	24.00 ± 0.57 a
Statistical analysis	F= 4.07 ** P= 0.063	F= 4.00 ** P= 0.066	F= 0.90 * P= 0.50	F= 3.39 ** P= 0.046	F= 3.68 ** P= 0.041

Each column, mean value followed by same latter are not significant to each other.
The Tukey HSD test ≤ 0.05. (* non-significant, ** significant, *** extremely significant)

Table 2: Mean values of Larvae Emergence Period ± Standard Error (LEP ± SE) of *Sitophilus granarius* after different days under different temperatures (ºC) and relative humidity (RH).

ºC / %RH	LEP ± SE / Days				
	3	**4**	**6**	**8**	**11**
20 / 60	4.67 ± 0.33 d	5.33 ± 0.88 d	6.33 ± 0.33 d	8.33 ± 0.33 d	10.33 ± 0.33 d
20 / 65	7.33 ± 0.33 cd	8.33 ± 0.33 cd	8.66 ± 0.88 cd	11.33 ± 0.66 cd	13.00 ±0.58 cd
20 / 70	10.33 ± 1.11 cf	11.66 ± 0.67 cf	9.67 ± 0.40 cf	14.67 ± 0.45 cf	16.00 ± 0.57 cf
25 / 60	5.66 ± 0.40 ef	6.67 ± 0.33 ef	12.66 ± 0.45 ef	9.67 ± 0.40 ef	11.33 ± 0.33 ef
25 / 65	9.66 ± 0.88 ef	10.33 ± 1.11 ef	7.33 ± 0.33 ef	12.33 ± 0.37 ef	14.67 ± 0.45 ef
25 / 70	14.00 ± 1.00 e	15.00 ± 0.45 e	11.33 ± 0.33 e	18.00 ± 0.57 e	21.66 ± 0.88 e
30 / 60	9.66 ± 0.33 b	10.33 ± 1.11 b	16.00 ± 0.57 b	13.33 ± 0.39 b	15.33 ± 0.39 b
30 / 65	13.00 ± 0.58 b	14.00 ± 0.58 b	15.00 ± 0.45 b	17.00 ± 1.00 b	19.33 ± 0.33 b
30 / 70	18.66 ± 0.33 a	19.33 ± 0.33 a	21.66 ± 0.88 a	24.00 ± 0.57 a	26.33 ± 0.33 a
Statistical analysis	F= 4.67 ** P= 0.043	F= 4.68 ** P= 0.043	F=4.48 ** P= 0.053	F= 4.38 ** P= 0.063	F= 4.36 ** P= 0.063

Each column, mean value followed by same latter are not significant to each other.
The Tukey HSD test ≤ 0.05. (* non-significant, ** significant, *** extremely significant)

Table 3: Mean values of Pupae Emergence Period ± Standard Error (PEP ± SE) of *Sitophilus granarius* after different days under different temperatures (ºC) and relative humidity (RH).

ºC / %RH	PEP ± SE / Days					
	18	**21**	**24**	**28**	**31**	**34**
20 / 60	1.67 ± 0.33 c	7.33 ± 0.33 c	7.66 ± 0.40 c	7.33 ± 0.38 c	10.33 ± 0.33 c	7.66 ± 0.40 c
20 / 65	6.33 ± 0.33 b	10.67 ± 0.33 b	12.33 ± 0.33 b	10.67 ± 0.33 b	15.00 ± 0.45 b	10.67 ± 0.33 b
20 / 70	9.67 ± 0.40 f	12.00 ± 0.57 f	16.67 ± 0.33 f	12.00 ± 0.57 f	18.67 ± 0.33 f	13.33 ± 0.33 f
25 / 60	3.66 ± 0.33 ef	7.33 ± 0.33 ef	8.33 ± 0.33 ef	7.33 ± 0.33 ef	11.00 ± 0.57 ef	7.33 ± 0.33 ef
25 / 65	8.33 ± 0.33 ef	11.00 ± 0.57 ef	13.33 ± 0.33 ef	11.00 ± 0.57 ef	15.33 ± 0.33 ef	11.00 ± 0.57 ef
25 / 70	13.33 ± 0.33 e	15.33 ± 0.33 e	20.66 ± 0.88 e	15.33 ± 0.33 e	21.66 ± 0.88 e	15.33 ± 0.33 e
30 / 60	7.33 ± 0.33 d	15.33 ± 0.33 d	11.67 ± 0.33 d	15.33 ± 0.33 d	15.33 ± 0.39 d	15.33 ± 0.33 d
30 / 65	12.33 ± 0.33 d	13.33 ± 0.33 d	19.00 ± 0.57 d	13.33 ± 0.33 d	21.66 ± 0.88 d	13.33 ± 0.33 d
30 / 70	18.67 ± 0.33 a	19.33 ± 0.67 a	24.67 ± 0.33 a	19.33 ± 0.67 a	26.33 ± 0.33 a	19.66 ± 0.33 a
Statistical analysis	F= 4.57 ** P= 0.063	F= 4.05 ** P= 0.063	F= 4.11 ** P= 0.063	F= 4.05 ** P= 0.053	F= 4.05 ** P= 0.053	F= 3.07 * P= 0.100

Each column, mean value followed by same latter are not significant to each other.
The Tukey HSD test ≤ 0.05. (* non-significant, ** significant, *** extremely significant)

Table 4: Mean values of Adult Emergence Period ± Standard Error (AEP ± SE) of *Sitophilus granarius* after different days under different temperatures (ºC) and relative humidity (RH).

ºC / %RH	AEP ± SE / Days				
	4	**6**	**8**	**10**	**12**
20 / 60	2.33 ± 0.33 b	4.67 ± 0.33 b	8.66 ± 0.88 d	8.67 ± 0.33 d	10.33 ± 0.33 d
20 / 65	6.33 ± 0.33 f	10.67 ± 0.33 f	15.33 ± 0.67 f	17.67 ± 0.33 f	18.33 ± 0.33 f
20 / 70	10.33 ± 0.33 f	14.67 ± 0.33 f	18.33 ± 0.33 f	20.00 ± 0.57 f	23.33 ± 0.33 f

25 / 60	5.67 ± 0.33 e	9.33 ± 0.33 e	11.67 ± 0.33 e	13.00 ± 0.57 e	13.33 ± 0.33 e
25 / 65	9.33 ± 0.33 de	13.00 ± 0.57 de	15.33 ± 0.67 de	19.67 ± 0.33 de	20.00 ± 0.57 de
25 / 70	12.33 ± 0.33 d	16.33 ± 0.33 d	19.67 ± 0.33 d	22.00 ± 0.57 d	23.66 ± 0.67 d
30 / 60	8.67 ± 0.33 c	12.67 ± 0.33 c	15.33 ± 0.67 c	16.33 ± 0.33 c	17.33 ± 0.33 c
30 / 65	14.47 ± 0.33 c	18.33 ± 0.33 c	19.67 ± 0.33 c	21.00 ± 0.57 c	23.33 ± 0.33 c
30 / 70	19.33 ± 0.33 a	23.33 ± 0.33 a	23.66 ± 0.67 a	24.00 ± 0.57 a	24.67 ± 0.67 a
Statistical analysis	F= 5.12 *** P= 0.01	F= 5.49 *** P= 0.01	F= 6.29 *** P= 0.01	F= 4.01 *** P= 0.01	F= 3.88 ** P= 0.05

Each column, mean value followed by same latter are not significant to each other.
The Tukey HSD test ≤ 0.05. (* non-significant, ** significant, *** extremely significant)

Discussion

The wheat weevil, *Sitophilus granarius* L. is the primary and internal feeder of maize, barley, rice particularly of wheat. The current study was conducted to evaluate the effect of some environmental conditions such as temperature and relative humidity on the life cycle of *S. granarius* and showed that the significantly difference regarding to the time period for different developmental stages.

Developmental stages of insect depends on many environmental (temperature, humidity, grain water content) and biotic factors (age of females, grain availability, hardness of testa, presence of insects of the same or other species and natural enemies).

In general, the results obtained in this experiment are in agreement with results of recent studies on the efficacy of temperature and humidity in storage insects' control (Hagstrum and Flinn, 2014) [20].

The rate of larvae emergence also significantly affected by temperature and relative humidity. Larval period of *Sitophilus* species is very prolonged than other stored grain insect pests duration and rate of pupae emergence from larval period varied as environmental conditions was manipulated. Mainali *et al*., (2015) [21] showed that the egg laying activity became shorter at 35 ºC and maximum egg lying was observed at 30 and 35 ºC while Okelana *et al*., (1985) [22] described that at 50-70% RH supported greater development of weevil then 90% but in some cultivar and microorganisms development resist the growth of weevil when temperature is suitable.

While adult emergence requires more time at low temperature and humidity, Riaz *et al*., (2014) [23] noted that during the *Trogoderma granarium* study, the highest ovulation rate was achieved at 30 °C or 35 °C, but decreased at 20 °C or 40 °C. Growth from egg to adult was lowest at 35 °C but extended at 25 °C. No larvae turned into pupae in extreme heat.

Similarly, relative humidity affects the life cycle of the rice weevil as described by Dermott and Evans (1978) [24], who treat *Rhyzopertha dominica*, *Sitotroga cerealella* and *Sitophylus oryzae* for proper control by manipulating the environment and reducing moisture content by exposure to hot air. (70, 80 and 60 °C) for various time intervals such as 4 to 12 minutes. Significant results were obtained when the temperature varied between 59 and 65 °C to increase the wheat grain temperature.

The effect of temperature and relative humidity on the development of stored seed pests can also be considered as a physical control method for this type of pest. Stored product pests can only survive and reproduce in a narrow temperature range. At temperatures outside this range, the pest population either remains stationary or dies at fast or low rates. The main source of water of stored product pests is food. The moisture content of foods affects the number of offspring, rate of growth, life expectancy and the rate of adulthood. Stored product pests cannot survive and reproduce at a relative humidity below 50% (Golić *et al*., 2011; Hasan *et al*., 2017) [25, 26].

The data of this study can be evaluated in the future regarding physical management against stored product pests.

Conclusion

From this study, it was concluded that at 25 and 30 °C, the growth time of wheat weevil is very short, therefore it shows more population growth below 25 °C or above 30 °C which was not unsuitable for growth. Temperature and relative humidity can greatly affect the life stage of stored grain pests such as *Sitophilus* species. It can be used in combination with any chemical or physical measure because these factors can increase the pest's effectiveness against them and reduce their resistance.

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