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Effect of temperature and relative humidity on development of *Sitophilus oryzae* L. (coleoptera: curculionidae)

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Abstract
Study was conducted to determine the different life stages of *Sitophillus oryzea* and the effect of various combinations of temperature (25, 30 and 35 °C) and R.H. (55, 60 and 65%) under lab conditions in university of agriculture Faisalabad in 2015-16. Three replications were used for each treatment. Ten pairs of adults were released to get eggs. Data regarding oviposition, fertility and adult emergence was recorded on daily basis. For this effort, first temperature (25 °C) was in practice on decontaminated rice which exposed slow development of *S. oryzae* while highest temperature (35 °C) presented very high developmental rate of all life stages of *S. oryzae.* So, result presented *S. oryzae* has great influence of temperature on its life span. It exhibited high appearance at high temperature in comparison with low temperature. Relative humidity has also excessive influence on the development of *S. oryzae.* At 65% R.H. the maximum mean value of adult appearance was significantly higher as compared to than that of 55%. It is stated that different relative humidity level such as 55, 60 and 65%, 65% level is most favorable for its development and 55% level is less favorable for its growth as compared to other levels.

Keywords: climatic regimes, controlled atmosphere, lab conditions, management, rice weevil, stored pests

1. Introduction

Rice is infested by many insect pests during storage but *Sitophilus* are most destructive and wide spread species in the world particularly in warm and tropical areas [1] but *Sitophilus oryzae* (L.) cause most critical infestation in many cereal crops as primary pest like in rice, wheat, maize and other split pea [2]. Rice weevil can damage the whole grain but can also survive on other commodities such as pasta and split pea [3,4]. The infestation caused by *S. oryzae* (L.) is mostly due to feeding of adults and grub on whole cereal grains [5]. *S. oryzae* showed more growth on polished rice grains than other varieties [6]. It causes quality and weight losses of cereal grains and produce grain dust [7] which attract the secondary pest attack including *Tribollium castenium* (H.), *Oryzaephilus surinamensis* (L.*), Cryptolestus ferugenius* (S.) and mites which are secondary insect pests which feed on broken grains and grain dust [8]. The activity of rice weevil allows to attack of pathogens by manipulating the internal temperature and relative humidity of infested grains [7,8]. *S. oryzae* (L.) have four life stages egg, larvae, pupae and adult. First three stages remain within the cereal grain while adult remain outside of grain and complete its further life cycle [9]. The adult of rice weevil is able to survive up to two years under unfavorable environmental conditions and may transfer to field crops due its flying capability [10]. Females can lay up to 300 eggs during its whole life cycle [11]. Maximum development takes place at 28℃ and 75% relative humidity and greater growth, time period as both factor increases [12]. Rice weevils can be controlled utilization of several grain protectants like malathion, methopereetc to save grains from infestation [13] but it develops resistant against many protectant chemical [5,13]. Now days, fumigation is used prominently to control rice weevil but resistant has been developed due to their residues and toxicity [5]. However, methyl bromide which is used as prominent fumigant since 1930s but now it is banned due to its residual its environmental toxicity under the Montreal Protocol in 2005 [14]. Several control measures are adopted to control insect pest on grain such as use of different botanicals like Pea, Neem, Eucalyptus, Datora and clove oil extracts [15,16].

Many time period as both factor increases [12]. Rice weevils can be controlled utilization of several grain protectants like malathion, methopereetc to save grains from infestation [13] but it develops resistant against many protectant chemical [5,13]. Now days, fumigation is used prominently to control rice weevil but resistant has been developed due to their residues and toxicity [5]. However, methyl bromide which is used as prominent fumigant since 1930s but now it is banned due to its residual its environmental toxicity under the Montreal Protocol in 2005 [14]. Several control measures are adopted to control insect pest on grain such as use of different botanicals like Pea, Neem, Eucalyptus, Datora and clove oil extracts [15,16]. Many Dusts like DE are used to control stored grain pests since centuries which commercially formation takes place in many countries since 1960s to control stored cereals pests which kills the insects through rupturing the cuticle [17,18,19,20] and still insects are not resistant to diatomaceous Earth [21,22] but it did not show good results in low quantity and it reduces the grain quality as well [4]. Several biological controlling agents like Pathogens and Parasitoids are helpful to lower the pest population below ETL [23, 24] but less effective for export purpose [25]. The other methods are used including heating or lowering the temperature [26], Physical anxiety [27] and lowering the oxygen level [28] to control the population of insect pests of stored grains but these physical methods are very expensive for conventional use [29]. Aeration is an effective method to control insects like ambient aeration and chilled aeration in autumn and summer storage [30] while in the case non-aeration increase in pest population than aerated stored grains [31]. Introduction of low pressure in air tight bans is another effective method to avoid to extensive use of insecticide at appropriate temperature [29]. The use of manipulated temperature to regulate the stored grain insects is old and effective methodology [32]. Although controlled atmosphere technology has been identified since 1900 [33]. Effective control of stored product insect pests can be obtained when 50-60 °C is maintained for 24-26 hours [34,35]. Age of the insect also impact the level of tolerance to temperature with constant humidity and time of exposure [36]. The horizontal and vertical implementation of temperature may cause non-uniform spreading of heat so a portion of the treatment may be over or under treated [35] and these treatments showed a huge difference in results which causes the heating block [37]. 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 [3, 38].

The following study of different temperature and humidity combinations will be explained the egg laying, time period, hatching time, larval instar development and pupal development time period. There are following main objects of the experiment.

- To identify the total developmental stage period of the *Sitophilus oryzae.*
- To identifying the best combination of temperature and relative humidity to reduce the insect population
- To identify the rate of oviposition at different temperature and relative humidity.
- To identify the survival of rice weevil at different temperature and relative humidity combinations.

2. Materials and Methods

Experiments were performed in the Stored Grain Research, Training and Storage Management Cell of Department of Entomology, University of Agriculture, Faisalabad, during the year 2015-16.

2.1 Insect Collection

The wild population of *Sitophilus oryzae* L. was collected by random selection of different silos from grain market Faisalabad.

2.2 Insect Rearing

Collected rice weevil was feed on rice in the plastic jars having high moisture content so that *S.oryzae* easily laid eggs and show better development. Rice was sterilized at 70 °C for 15-20 minutes to eliminate the previous infestation of insects, fungi and other microorganisms. Rice weevil was reared at controlled temperature 32±2 °C and relative humidity in the range of 70±5% which is best condition for its development. Dark and light hours (12: L and 12: D) was maintained according to best development of weevil because *S.oryzae* is very sensitive to the light hours for meeting and proper growth. After 72 hours, the adult rice weevil was remove to get homogenous stock of adult weevil and this process was repeated for next three generations. Homogeneous population was maintained for further experiment.

2.3 Temperature and relative humidity effect on fecundity, larval, pupae development and adult emergence.

Ten adult pairs of *S. oryzae* from the laboratory colony was released in small jarson rice grains which was dried to a consistent moisture level and then placed inside incubator chambers was maintained at one of three different temperature (25, 30 and 35 °C) levels. The relative humidity levels within the incubator chambers were maintained by using saturated salt solutions as described in Winston and Bates (1960). All incubators were held at a photoperiod of 12:12 (L: D) hours for proper flight and meeting of the rice weevil. Oviposition was allowed for 72 h. A 72-h period for egg laying ensures that sufficient amount of eggs are laid. After 72 h, released insects were removed and their total number of eggs laid on randomly selected fifty grains of rice were counted with help of compound microscope. These eggs were maintained for egg fertility, larva and pupae life and adult emergence.

2.3.1 Fecundity rate of females of *Sitophils oryzae*

Thirty females were released in different jars to observe the fecundity rate in different days (3, 5, 7, 9 and 11 days).

2.3.2 Larvae emergence or egg hatching of *Sitophilus oryzae*

The thirty eggs were released in patri dishes at each temperature and relative humidity combination. The data was taken about egg hatching after 3, 5, 7, 9 and 11 days.

2.3.3 Pupae emergence of *Sitophilus oryzae*

The hatched eggs were released in different patri 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 17, 20, 23, 27, 30 and 33 days.

2.3.4 Adult emergence of *Sitophilus oryzae*

The thirty pupae were transferred into new patri dish with great care. The data regarding adult emergence was observed after 3, 5, 7, 9 and 11 days.

2.4 Statistical data evaluation

Data on the number of eggs laying, larval and pupae developmental times and rates of adult emergence was analyzed by a two-way analysis of variance (ANOVA), and the Tukey test was used as a post hoc analysis for the interaction and effect of temperature and RH (PROC GLM; SAS Institute, 2000). Mean values of all developmental time within a replication of each treatment was calculated.

3. Results

The results revealed that temperature and relative humidity greatly affect the fecundity of *Sitophillus oryzae* (F= 3.39, P= 0.031). The egg laying of rice weevil was found maximum (23.66 ± 0.67) at 35 °C and 65% R.H. after 11 days but when the decrease in temperature from 35 °C to 30 and 25 °C and relative humidity from 65% to 60 and 55%, the egg laying by rice weevil also reduced to 8.66 ± 0.33 and 7.33 ± 0.88 respectively as mentioned in table-1. The combined effect of relative humidity and temperature influenced the larval emergence from eggs of *S.oryzae* (F=3.22 & P= 0.036) as table 2 showed that larval emergence was also increased from 9.67 to 24.00 as both factors increased from lowest point (25 °C and 55%) to highest point (35 °C and 65%) after 10 day of observation. The results regarding pupae formation was also greatly significant different due to temperature and relative humidity variation (F=3.00 & P=0.046). The pupae formation increased from 8.33 to 24.63 as both combined treatments (35 °C and 65%) but decreased to 14.67 ± 0.67 and 13.33 ± 0.33 as both factors fall down to 30 and 25 °C, 60 and 55% respectively after 30 days of observation as shown in table 3. The increase in adult formation from pupae of rice weevil was observed in table 4 from 9.33 to 26.33 due to the effect of temperature and relative humidity (F=3.31 & P= 0.031). So, F and P value showed that both temperature and humidity affect the whole life span (fecundity, larvae emergence, pupae formation and adult emergence) of rice weevil.

Table 1: Mean values of egg laying (± S.E) of *Sitophillus orzae* after different days under different temperature (T=25, 30 and 35 °C) and different relative humidities (H= 55, 60 and 65%). Each column, mean value followed by same latter are not significant to each other; Tukey and HSD test ≤ 0.05; *= Significant, ** = Highly Significant, NS = Non-Significant

Treatment	Egg laying ± S.E Days				
	3 days	5days	7 days	9 days	11 days
T_1H_1	2.33 ± 0.33 C	3.33 ± 0.33 C	3.33 ± 0.33 C	5.33 ± 0.88 C	7.33 ± 0.88 C
T_1H_2	7.33 ± 0.33 B	8.33 ± 0.33 B	8.33 ± 0.33 B	8.66 ± 0.88 B	9.66 ± 0.88 B
T_1H_3	10.33 ± 0.33 F	11.00 ± 0.57F	11.00 ± 0.57F	11.33 ± 0.66 F	12.66 ± 0.33 F
T_2H_1	4.67 ± 0.33EF	5.66 ± 0.33 EF	5.66 ± 0.33 EF	7.66 ± 0.33 EF	8.66 ± 0.33 EF
T_2H_2	9.33 ± 0.33 EF	10.33 ± 0.33 EF	10.33 ± 0.33 EF	12.66 ± 1.20 EF	14.33 ± 1.45 EF
T_2H_3	14.33 ± 0.67 E	15.33 ± 0.67 E	15.33 ± 0.67 E	16.66 ± 0.33 E	18.33 ± 0.66 E
T_3H_1	8.67 ± 0.33 D	9.66 ± 0.33 D	9.66 ± 0.33 D	10.66 ± 0.33 D	12.33 ± 0.66 D
T_3H_2	13.00 ± 0.58 D	14.00 ± 0.58 D	14.00 ± 0.58 D	16.00 ± 0.57D	18.33 ± 1.20D
T_3H_3	18.67 ± 0.33 A	19.33 ± 0.33 A	19.33 ± 0.33 A	21.66 ± 0.88 A	23.66 ± 0.67 A
ANOVA	F= 3.07* P = 0.043	F= 3.00* P= 0.046	$F= 0.84^{ns}$ P= 0.50	F= 2.98* P= 0.046	F= 3.39* P= 0.031

Table 2: Mean values of larvae emergence (± S.E) of *Sitophillus orzae* after different days under different temperature (T=25, 30 and 35 °C) and different relative humidities (H= 55, 60 and 65%). Each column, mean value followed by same latter are not significant to each other; Tukey and HSD test ≤ 0.05; * = Significant, ** = Highly Significant, NS = Non-Significant

Treatment	Larval emergence ± S.E Days				
	2 days	3 days	5 days	7 days	10 days
T_1H_1	3.66 ± 0.33D	4.67 ± 0.33D	5.66 ± 0.40 D	7.66 ± 0.40 D	9.67 ± 0.40 D
T_1H_2	6.33 ± 0.33 CD	7.33 ± 0.33 CD	7.33 ± 0.37 CD	10.33 ± 0.37 CD	12.33 ± 0.37CD
T_1H_3	9.33 ± 0.33 CF	10.33 ± 0.33 CF	8.33 ± 0.39 CF	13.33 ± 0.39 CF	15.33 ± 0.39 CF
T_2H_1	4.66 ± 0.33 EF	5.66 ± 0.33 EF	11.33 ± 0.33 EF	8.33 ± 0.67 EF	10.33 ± 0.67EF
T_2H_2	8.33 ± 0.33 EF	9.33 ± 0.33 EF	6.33 ± 0.33 EF	11.66 ± 0.67 EF	13.67 ± 0.67 EF
T_2H_3	13.00 ± 1.00 E	14.00 ± 1.00 E	10.33 ± 1.11 E	17.00 ± 1.00 E	19.00 ± 1.00 E
T_3H_1	8.66 ± 0.33B	9.66 ± 0.33 B	15.00 ± 0.45 B	12.66 ± 0.45 B	14.67 ± 0.45B
T_3H_2	12.00 ± 0.57 B	13.00 ± 0.57 B	14.00 ± 0.60 B	16.00 ± 0.57 B	18.00 ± 0.57 B
T_3H_3	17.66 ± 0.33 A	18.66 ± 0.33 A	19.66 ± 0.33 A	22.00 ± 0.57 A	24.00 ± 0.57 A
ANOVA	F= 3.68* P= 0.023	F= 3.68* P= 0.023	F= 3.45* P=0.029	F= 3.22* P= 0.036	F= 3.22* P= 0.036

Table 3: Mean values of pupae formation (± S.E) of *Sitophillusorzae* after different days under different temperature (T=25, 30 and 35 °C)and different relative humidities (H= 55, 60 and 65%).Each column, mean value followed by same latter are not significant to each other; Tukey and HSD test ≤ 0.05; * = Significant, ** = Highly Significant, NS = Non-Significant

Treatment	Pupae formation/transmission ± S.E Days					
	17 days	20 days	23 days	27 days	30 days	33 days
T_1H_1	0.33 ± 0.33 C	5.00 ± 0.33 C	5.33 ± 0.33 C	5.00 ± 0.33 C	8.33 ± 0.33 C	5.00 ± 0.33 C
T_1H_2	5.33 ± 0.33 B	8.33 ± 0.33 B	10.33 ± 0.33 B	8.33333 ± 0.33 B	13.33 ± 0.33 B	8.33333 ± 0.33 B
T_1H_3	8.33 ± 0.33 F	11.67 ± 0.33 F	15.33 ± 0.33 F	11.67 ± 0.33 F	16.33 ± 0.33 F	11.67 ± 0.33 F
T_2H_1	2.67 ± 0.33 EF	6.33 ± 0.33 EF	7.33 ± 0.33 EF	6.33 ± 0.33 EF	10.67 ± 0.33 EF	6.33 ± 0.33 EF
T_2H_2	7.33 ± 0.33EF	10.67 ± 0.33 EF	12.33 ± 0.33 EF	10.67 ± 0.33EF	14.67 ± 0.67 EF	10.67 ± 0.33EF
T_2H_3	12.33 ± 0.67 E	14.67 ± 0.33E	19.33 ± 0.67 E	14.67 ± 0.33E	20.33 ± 0.67 E	14.67 ± 0.33E
T_3H_1	6.67 ± 0.33 D	14.00 ± 0.33 D	10.33 ± 0.33 D	14.00 ± 0.33 D	14.67 ± 0.33 D	14.00 ± 0.33 D
T_3H_2	11.00 ± 0.57 D	12.00 ± 0.57 D	18.00 ± 0.57 D	12.00 ± 0.57 D	19.00 ± 0.57 D	12.00 ± 0.57 D
T_3H_3	16.67 ± 0.33 A	18.67 ± 0.33 A	23.33 ± 0.33 A	18.67 ± 0.33 A	24.67 ± 0.33 A	18.67 ± 0.33 A

ANOVA	F= 3.46* P= 0.043	F= 3.00* P= 0.043	F= 3.07* P= 0.04	F= 3.00* P= 0.046	F= 3.00* P=0.046	F=2.22^{NS} P= 0.10

Table 4: Mean values of adult formation (± S.E) of *Sitophillus orzae* after different days under different temperature (T=25, 30 and 35 °C) and different relative humidities (H= 55, 60 and 65%). Each column, mean valaue followed by same latter are not significant to each other; Tukey and HSD test ≤ 0.05; Df= degree of freedom; * = Significant, ** = Highly Significant, NS = Non-Significant.

Treatment	Adult formation ± S.EDays				
	3 days	5days	7 days	9 days	11 days
T_1H_1	1.67 ± 0.33 B	3.67 ± 0.33 B	7.67 ± 0.33 D	7.67 ± 0.33 D	9.33 ± 0.33 D
T_1H_2	5.67 ± 0.33 F	9.67 ± 0.33 F	14.67 ± 0.33 F	16.33 ± 0.33F	17.33 ± 0.33 F
T_1H_3	9.67 ± 0.33 F	13.67 ± 0.33 F	17.67 ± 0.33 F	19.67 ± 0.33 F	22.67 ± 0.33 F
T_2H_1	4.33 ± 0.33 E	8.33 ± 0.33 E	9.33 ± 0.33 E	11.33 ± 0.67 E	12.33 ± 0.33 E
T_2H_2	8.67 ± 0.33DE	12.67 ± 0.33DE	14.67 ±0.33DE	18.33 ± 0.33DE	19.33 ± 0.33DE
T_2H_3	11.67 ± 0.33 D	15.33 ± 0.67 D	18.33 ± 0.88 D	21.00000 ± 0.57 D	22.00 ± 0.57 D
T_3H_1	7.67 ± 0.33 C	11.667 ± 0.33 C	14.67 ± 1.00 C	15.33 ± 0.88 C	16.33 ± 0.33 C
T_3H_2	13.00 ± 0.57 C	17.00 ± 0.57 C	18.00 ± 0.57 C	20.00 ± 0.57C	22.00 ± 0.33 C
T_3H_3	18.00 ± 0.57 A	22.00 ± 0.57 A	22.67 ± 0.33 A	24.67 ± 0.67 A	26.33 ± 0.33 A
Anova	F= 4.11** P= 0.01	F= 4.59** P= 0.01	F= 6.49** P= 0.001	F= 3.88** P= 0.01	F= 3.31* P= 0.03

4. Discussion

Sitophilus oryzea L. is the primary and internal feeder of maize, wheat, barley particularly of rice [39]. The current study was conducted to evaluate the effect of temperature (25,30 and 35 °C) and relative humidity (55,60 and 65%) on the fecundity, egg hatching, pupae formation and adult emergence and it showed the significantly difference regarding to the time period for different developmental stages.

The egg laying rate by female of *S. oryzae* increased as temperature and humidity increased and vice versa. The gradual rise in percentage of egg laying of *S. oryzae* as days increased but during 5 to 9 days maximum increased was observed in egg laying percentage was observed in present experiment. The rate of larvae emergence also significantly affected by both factors (relative humidity and temperature. Larval stage of rice weevil is very prolonged than approximately all other stored grain insect pests but its duration and rate of pupa emergence from larval stage varied as environmental conditions was manipulated. The present results were quit accordance of Imura [40] and Mainali *et al.* [41] recorded the egg laying period became shorter at 35 °C and maximum egg laying was observed at 30 and 35 °C while Okelana *et al.* [42] described that at 50-70% R.H. supported greater development of weevil then 90% but in some cultivar, fungi and bacterial development resist the growth of weevil when temperature is favorable. Similarly, Lale and vidal [43] determined the effect of relative humidity (30, 60 and 90%) and temperature (25, 30, 35 and 40 °C) on growth pattern of *Callosobruchus maculatus* and *Callosobruchus subinnotatus* reared on Bambara groundnut and they described that Optimum temperature for *C. maculatus* and *C. subinnotatus* was 30 and 35 °C respectively. Progeny developments were restricted of both species at 40 °C. The adult emergence also required more time at low temperature and humidity while its time period was also decreased as temperature rose up to 30 and 35 °C in combination of high relative humidity (60 and 65%) which are also described by Riaz *et al.* [44] during studying the *Trogoderma granarium.* He mentioned that highest rate of oviposition was obtained at 30 °C or 35 °C but reduced at 20 °C or 40 °C. Development from egg to adult was lowest at 35 °C but extended at 25 °C. No larva was converted into pupa at extreme temperature. So, the results indicate that growth restricted at both extreme temperatures while showed maximum development at 35 °C. Similarly, relative humidity affect the life cycle of rice weevil as described by Dermott and Evans [45] who treated the *R. dominica*, *S. cerealella* and *S. oryzae* for proper control by manipulate the surrounding and decrease the moisture contents by exposing hot air (70,80 and 60 °C) for various range of time periods such as 4 to 12 minutes. The significant results were obtained when temperature ranging from 59 to 65 °C to increase the wheat grain temperature. Due to these treatments, the internal and primary insect pests such as *R. dominica* and *S. oryzae* were also controlled and save the stored gains. So, increase in relative humidity will increase the population growth of these insects including *S. oryzae.*

5. Conclusion

From the present study, it is concluded that the at 30 and 35 °C, the development time period of rice weevil was very short so it show greater population growth so below 30 °C or above 35 °C which was not suitable for development hence temperature and relative humidity can greatly affect the life stage of internal pests like *Sitophilus oryzae* and can be used as combination with any chemical or physical measures because these factors can increase the efficacy and reduce the resistance of the pest against them.

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