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Study the effect of different temperature and relative humidity combination on growth and development of *Callosobruchus chinensis* on Bengal gram

[1]Niranjan Mandi, [2]Shanowly Mondal Ghosh, [3]Gautam Chakraborty, [1]Lalatendu Nayak and [4]Uttam Kumar Behera

[1]Assistant Professor, College of Horticulture, Chiplima, Odisha University of Agriculture and Technology, Sambalpur, Odisha, India

[2]Assistant Professor, Department of Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

[3]Professor, Department of Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

[4]Assistant Professor, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

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Corresponding Author: Niranjan Mandi

Abstract

In the laboratory at Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal, the growth and development of the pulse beetle, *Callosobruchus chinensis*, was evaluated under storage conditions on different combinations of temperature (20 °, 30 °, and 40 ° C) and relative humidity (RH) levels (50, 60, 70, 80, and 90%) in Bengal gram during 2022 and 2023. The findings indicated that temperature and relative humidity were significant factors in the development of different stages of the pulse beetle. The most favorable conditions for egg laying were 30 °C and 70% relative humidity, where females were capable of laying up to 84.50 eggs per female. Fecundity and the ovipositional period were not significantly impacted by the low temperature of 20 °C. However, fecundity and the ovipositional period decreased at a high temperature of 40 °C. The most suitable temperature and relative humidity combination for completing the shortest incubation period (5.07 days), larval period (20.10 days), pupal period (4.10 days), and total developmental period (28.49 days) was found to be 30 °C and 70% relative humidity. This combination also produced the highest rates of egg hatching (99.44%) and adult emergence (95.89%). A high temperature of 40 °C in different humidity levels prevented the eggs from hatching. Based to several parameters, a suitable temperature and relative humidity for the growth and development of the pulse beetle, *C. chinensis*, were 30 °C and 70%, respectively.

Keywords: Temperature, relative humidity, *Callosobruchus chinensis*, growth and development

Introduction

Pulses have played a significant role in enhancing the agricultural economy of different countries (Sarwar *et al.*, 2003; Deeba *et al*., 2006) [17, 7]. As one of the richest plant-based sources of protein, pulses are a vital component of the daily diet, particularly in developing countries such as India. Often referred to as the "poor man's meat," they contain 20-30% protein, making them an affordable and nutritious alternative to animal-based proteins (Sharma, 1984; Rahman *et al.*, 2010) [18, 15].

In India, a variety of pulses are cultivated, including chickpea (gram or bengal gram), pigeon pea (tur or arhar), green gram (mung bean), black gram (urd bean), lentil (masur), and pea, among others. Among these, Bengal gram is regarded as one of the most important legumes due to its high protein content and widespread use in human diets. It is often referred to as the "king of pulses" for its nutritional value and significant contribution to pulse production in the country.

Bengal gram (*Cicer arietinum* L.) is a major legume crop grown during the Rabi season in India. Globally, it ranks third among pulse crops in terms of production, after soybean (*Glycine max* L.) and pea (*Pisum sativum*), with an average annual output exceeding 11.5 million tons (Merga & Haji, 2019) [12].

Post-harvest losses of chickpea during storage are influenced by a range of biotic and abiotic factors. In India, approximately 200 insect pest species are known to damage stored grains and grain products. Among these, key insect pests affecting stored pulses include the pulse beetle (*Callosobruchus chinensis* Linn.), the Khapra beetle (*Trogoderma granarium* Everts), and the lesser grain borer (*Rhyzopertha dominica* Fab.). Of these, the pulse beetle is considered the most destructive, as it infests chickpea both in the field and during storage (Rathore & Sharma, 2002) [16].

In the current context, food quality and efficient post-harvest management are critical considerations in agricultural planning and decision-making. It is increasingly important for all stakeholders involved in the storage and preservation of agricultural products to prioritize efforts aimed at minimizing significant post-harvest losses.

Developing cost-effective and efficient management strategies for *Callosobruchus chinensis* requires detailed and accurate knowledge of its bio-ecology under varying macro- and micro-ecological conditions. Such understanding is essential for predicting population dynamics and identifying the key mortality factors that regulate pest abundance. These insights are crucial for designing successful pest management approaches. Further investigation is needed in this area, particularly considering the significant influence of abiotic factors such as temperature and relative humidity on pest infestation levels.

The present study was undertaken to investigate the effect of different combinations of temperature and relative humidity on the growth and development of *Callosobruchus chinensis* in Bengal gram.

Materials and Methods

Under laboratory conditions during 2022 and 2023, the effects of different temperature and relative humidity combinations on *C. chinensis* in Bengal gram were examined to determine which combinations affected fecundity, ovipositional period, incubation period, the total developmental period, adult emergence, grain damage and grain weight loss. Temperature levels of 20 °C, 30 °C, and 40 °C, along with relative humidity levels of 50%, 60%, 70%, 80%, and 90%, were maintained using a BOD incubator. The experiment was laid out in a Completely Randomized Design (CRD) with three replications for each treatment combination.

Maintenance of the insect culture

To maintain the stock culture of the pulse beetle, *C. chinensis*, the healthy and sound Bengal gram grains were cleaned and sieved to get removal of any leftover grains or insects. To get removal of any hidden or visible insect infestations, the grains were sterilized for eight hours at 60±5ºC. To increase their moisture content, these grains were conditioned in an incubator for at least a week at 27±2ºC and 65±5% relative humidity. For mass rearing, the adult *C. chinensis* was taken off from the AICRP on the chickpea seed godown at Kalyani. The adults were raised on preconditioned Bengal gram grains in plain glass jars with a volume of 2.5 liters. These jars' relative humidity and temperature were kept at 65±5% and 27±2ºC, respectively. Freshly emerged adults from this stock culture were collected and used as the parental population for subsequent experimental studies.

Methodology

Three pairs of recently emerged adults (male and female) were released separately in three plastic containers containing Bengal gram grains in order to study the fecundity and oviposition period. Every day until a female died, the total number of eggs laid by a female was counted and the oviposition period was also noted. First, newly emerging adults of the pulse beetle, *C. chinensis*, were taken out from the stock culture in order to determine the developmental period (from egg to adult) and the percentage of adult emergence. With 250 grams of Bengal gram grains in a glass jar, thirty pairs of adult pulse beetles were released. A piece of muslin cloth was tied over the jar's mouth and closed with a rubber band. For twenty-four hours, they were allowed to lay eggs. The experiment start the next day with taking out of the grains with a single egg. Thirty such grains, each with one egg, were placed individually into test tubes. The test tubes were covered with pieces of muslin cloth and maintained under different combinations of temperature and relative humidity levels, as described previously. The findings were recorded the oviposition period, fecundity, developmental period (from egg to adult stage) and adult emergence (%).

Method of observation

Under a stereo zoom binocular microscope, the grains were examined every day. As per Singh and Pandey's (2001) [22] procedure, observations were made on the duration of larval period and pupal stages, adult emergence and egg hatching. The duration of the egg stage was determined by observing the transparency of the eggshell, with the appearance of a black spot indicating imminent hatching. Upon hatching, the larvae bored into the grain just below the egg's attachment site. The larvae remained inside the grain and the appearance of a capped exit hole ('window' or dark spot) marked the beginning of the pupal stage. Adults emerged by cutting open these windows in the seed coat. Based on adult emergence, the total number of days taken for the combined larval and pupal development was recorded for each insect."

Statistical analysis

The experiment was conducted using a completely randomized design (CRD) with three replications under storage conditions. The experiment's data was subjected to statistical analysis after the required transformation.

Results and Discussion

The present investigation was carried out to study the effect of selected physical factors, such as temperature and humidity, on the development of the pulse beetle, *Callosobruchus chinensis*. The results of this study are presented herein.

Effect on ovipositional period

The influence of three temperature levels (20 °C, 30 °C, and 40 °C) combined with five relative humidity (RH) levels (50%, 60%, 70%, 80%, and 90%) on the oviposition period of the pulse beetle was studied. The results are presented in Table 1.

The pooled mean of the two years' ovipositional periods at 20 ° C in 2022 and 2023 varied from 5.62 to 6.33 days in different relative humidity. The data obtained on the effect of relative humidity (RH) showed clear that the ovipositional period (6.33 days) was longer at 70% RH followed by 60% (6.33 days), 80% (6.01 days), and 5.82 days at 50% RH, while the minimum (5.62 days) was at 90% RH.

The pooled mean oviposition period of females at 30 °C varied from 3.89-4.93 days in different relative humidity. The shortest mean ovipositional period at this temperature was 3.89 days observed at 50% relative humidity, while the longest oviposition period was 4.93 days at 70% RH followed by 4.80 days at 60%, 4.41 days at 80% and 3.96 days at 90% RH.

Considerable variation was observed in the pooled mean ovipositional period of different relative humidity levels at 40 °C over the two years of study. The mean ovipositional duration ranged from 2.18 to 3.30 days. The maximum oviposition period was recorded at 70% relative humidity (3.30 days) followed by 80% (3.03 days), 60% (3.01 days) and 50% RH (2.56 days). The shortest ovipositional period, 2.18 days, was recorded at 90% relative humidity.

With respect to the combined effects of temperature and relative humidity on the ovipositional period, the longest duration (6.33 days) was observed at 20 °C and 70% relative humidity. At 40ºC and 90% relative humidity, the shortest period (2.18 days) was recorded.

Effect on fecundity

The results showing the effect of different combinations of temperature and relative humidity on the ovipositional potential of *C. chinensis* (L.) are presented below (Table 1).

At 20 °C, the pooled mean ovipositional potential over both years ranged from 48.67 to 64.67 eggs per female. The highest fecundity was recorded at 70% RH (64.67 eggs) followed by 60% RH (60.17 eggs), 80% RH (55.83 eggs) and 50% RH (55.33 eggs). The lowest fecundity was observed at 90% relative humidity with 48.67 eggs per female.

The average fecundity of females kept at 30 °C ranged from 59.17 to 84.50 eggs per female, with the highest and lowest being at 70% RH (84.50 eggs) and 50% RH (59.17 eggs), respectively, based on the pooled mean of the two years. The female exposed to 60%, 80%, and 90% relative humidity had an average fecundity of 70.67, 77.67, and 67.33 eggs, respectively.

In different humidities, fecundity at 40 °C ranged from 7.0 to 14.0 eggs per female. At this temperature, the lowest average fecundity was 7.00 eggs in 50% relative humidity followed by 7.67 eggs in 90% RH and the highest was 14.00 eggs in 70% RH followed by 11.33 eggs in 60% and 80% RH.

Combining temperature and relative humidity levels showed that 30 °C and 70% RH was the best combination for egg laying and females laid the most eggs (84.50/female) on this condition. At 40 °C and 50% relative humidity, the lowest fecundity was observed (7.00 eggs/female). The results are in accordance with the findings of Priyadarshini (2018) [14], Sharma (2017) [19], Choudhary (2016) [6], and Bajiya (2009) [1], who observed that 30 °C and 70% relative humidity were optimum conditions for egg laying. These results also support the work of Chakraborty and Mondal (2016) [4] and Mainali (2015) [9].

Effect on incubation period

Average incubation periods at 20 °C ranged from 7.19 to 8.87 days. The incubation period occurred the longest at 50% relative humidity (8.87 days) followed by 60% (7.82 days), 70% (7.29 days), and 80% (7.25 days). The shortest was 7.19 days at 90% relative humidity. At this temperature, it is evident that the incubation period shortened as the relative humidity level increased.

The average mean of the incubation periods of both years at 30 °C varied from 5.07 to 5.34 days under different humidity conditions. 70% RH had the shortest pooled mean incubation period (5.07 days), which was at par with 80% RH (5.08 days) and 90% RH (5.10 days). Incubation period at 50% RH were the longest (5.34 days) followed by 60% RH (5.22 days).

When temperature and relative humidity were combined, the longest incubation period (8.87 days) was recorded at 20 °C and 50% relative humidity, while the shortest (5.07 days) was recorded at 30 °C and 70% relative humidity. These results correspond with the findings of Mainali *et al*.(2015) [9], Sharma (2017) [20], Choudhary (2016) [6], Priyadarshini (2018) [14] and Bajiya (2009) [1], who found that when the temperature increased the incubation period shortened. In a similar vein, Mandi and Ghosh (2008) [10] observed that shorter incubation period correlated with higher temperatures and relative humidity.

Effect on hatching percentage

The impact of a constant temperature of 20 °C in combination with varying relative humidity levels on egg hatching is presented in Table 1. At this temperature, the pooled mean hatching percentage ranged from 50.56% to 72.22% across different humidity levels. The highest hatching rate (72.22%) was observed at 70% relative humidity, while the lowest (50.56%) occurred at 50% RH. At other humidity levels, hatching percentages ranged from 59.44% to 65.00%.

At 30 °C, the pooled mean percentage of egg hatching ranged from 85.00% to 99.44% across different relative humidity levels. The highest hatching percentage (99.44%) was recorded at 70% RH, which was statistically at par with 60% RH (97.22%) and 80% RH (96.11%). The lowest hatching rate was observed at 50% RH (85.00%), followed by 90% RH (89.44%).

The combined impact of temperature and relative humidity showed that the highest egg hatching percentage (99.44%) occurred at 30 °C and 70% RH, whereas the lowest hatching rate (50.56%) was recorded at 20 °C and 50% RH.

The impact of 40 °C temperature in combination with 50%, 60%, 70%, 80%, and 90% relative humidity on *Callosobruchus chinensis* (L.) was also investigated. No egg hatching was observed at any of the humidity levels at this temperature. This finding corroborates the observations of Singh (2004) [21], who reported a complete failure of egg hatching in *C. chinensis* at 40 °C, regardless of humidity. Similarly, Lale and Vidal (2003) [8] reported no development at 40 °C in two related species, *C. maculatus* (F.) and *C. subinnotatus* (Pic).

Table 1: Effect of different combination of temperature and relative humidity levels on ovipositional period, fecundity, incubation period and hatching percentage of pulse beetle, *C. chinensis* in 2022 and 2023 (pooled mean)

	Ovipositional period (days)*			**Fecundity***			**Incubation period (days)***		**Hatching (%)****	
Relative humidity (%)	**20 °C**	**30 °C**	**40 °C**	**20 °C**	**30 °C**	**40 °C**	**20 °C**	**30 °C**	**20 °C**	**30 °C**
50	5.82 (2.61)	3.89 (2.26)	2.56 (1.89)	55.33 (7.51)	59.17 (7.50)	7.00 (2.76)	8.87 (3.14)	5.34 (2.52)	50.56 (45.30)	85.00 (67.22)
60	6.13 (2.67)	4.80 (2.41)	3.01 (2.00)	60.17 (7.82)	70.67 (8.47)	11.33 (3.48)	7.82 (2.97)	5.22 (2.50)	60.56 (51.09)	97.22 (81.21)
70	6.33 (2.71)	4.93 (2.44)	3.30 (2.08)	64.67 (8.10)	84.50 (9.24)	14.00 (3.85)	7.29 (2.88)	5.07 (2.47)	72.22 (58.19)	99.44 (88.25)
80	6.01 (2.65)	4.41 (2.33)	3.03 (2.01)	55.83 (7.54)	77.67 (8.87)	11.33 (3.51)	7.25 (2.87)	5.08 (2.47)	65.00 (53.73)	96.11 (78.71)
90	5.62 (2.57)	3.96 (2.23)	2.18 (1.79)	48.67 (7.04)	67.33 (8.26)	7.67 (2.92)	7.19 (2.87)	5.10 (2.47)	59.44 (50.44)	89.44 (71.21)
S.Em±	0.03	0.04	0.04	0.14	0.15	0.32	0.01	0.01	1.32	1.92
CD at 5%	0.08	0.11	0.12	0.44	0.48	NS	0.04	0.03	4.19	6.11

* The figures given in parentheses are square root transformed values.
** The figures given in parentheses are angular transformed values.

Effect on larval period

The average larval period of *C. chinensis* (L.) at 20 °C ranged from 31.03 to 36.35 days at different humidities, according to the pooled mean (Table 2). At 70% relative humidity, the minimum average period a larval stage needed to finish developing at this temperature was 31.03 days, whereas at 50% RH, the maximum was 36.35 days. The average larval period was 32.53 days at 80%, 33.55 days at 90% and 34.22 days at 60% RH, respectively.

At 30 °C, the pooled mean larval period of *Callosobruchus chinensis* (L.) of both years in different RH, ranging from 20.10 to 22.83 days. The shortest average larval period (20.10 days) was observed at 70% RH, while the longest (22.83 days) occurred at 50% RH. At other humidity levels, the average larval periods were 21.52 days at 60% RH, 20.66 days at 80% RH and 20.99 days at 90% RH.

The combination of temperature and relative humidity showed that 30 °C and 70% relative humidity was most suitable for completing the shortest larval development period (20.10 days), while 20 °C and 50% relative humidity was observed longest larval development period (36.35 days). The current findings are consistent with the results reported by Mandi and Ghosh (2008) [10], who found that the maximum larval period was 47.00 days at 20 °C and 30% relative humidity, and the minimum was 18.48 days at 30 °C and 60% relative humidity. Similar results were found by Bhargava *et al.* (2014) [2], Mainali *et al.* (2015) [9], Choudhary (2016) [6], Sharma *et al.* (2017) [19], and Priyadarshini (2018) [14], all of which completely corroborate our present findings.

Effect on pupal period

The average time required for pupal development at 20 °C ranged from 5.51 to 6.74 days in 70% and 50% relative humidity, according to the combined mean of the two years. For 60%, 80% and 90% relative humidity, the corresponding values were 6.52, 5.85 and 6.22 days, respectively.

The average duration for the pupal stage to fully mature at 30 °C varied from 4.10 to 5.13 days depending on the humidity. 70% RH (4.10 days) had the shortest average pupal period followed by 80% RH (4.33 days), 90% RH (4.92 days) and 60% RH (5.01 days). When reared at 50% relative humidity, the longest average pupal period was recorded 5.13 days.

When temperature and relative humidity were combined, the minimum average pupal period was 4.10 days at 30 °C and 70% relative humidity, while the longest average duration of pupal period was 6.74 days at 20 °C and 50% relative humidity. These results are consistent with the work of Pimentel *et al.* (2003) [13], Chandrakantha *et al.* (1987) [5], Aldana and Alfonso (1985), and Mansour *et al.* (1976) [11], who studied *C. maculatus* (F.) and observed that the pupal stage was decreased when the temperature increased from 20 °C to 30 °C and higher. Similarly, Mandi and Ghosh (2008) [10] and Boshra (1993) [3] noted an increase in the larval and pupal periods at lower temperatures of 20 °C.

Effect on total developmental period

At 20 °C temperature, the pooled mean of the average overall development periods in both years varied between 42.98 and 50.60 days at different humidity levels. At 70% relative humidity, the minimum average time needed to complete the entire development period was 42.98 days followed by 44.68 days at 80%, 45.75 days at 90% and 47.87 days at 60%. At 50% relative humidity, the maximum average time needed to complete the entire development period was 50.60 days.

A temperature of 30 °C and different relative humidity had an impact on the pooled mean average time needed for the entire development period, which varied from 28.49 to 31.58 days. The shortest total developmental period was 28.49 days when the beetle was raised at 70% RH. This was followed by 29.45 days at 80%, 30.00 days at 90% and 30.14 days at 60% RH. However, the longest total development period (31.58 days) was noted for beetles reared at 50% RH.

The combined effect of temperature and relative humidity indicated that *C. chinensis* (L.) completed its total development in the shortest time (28.49 days) when reared at 30 °C and 70% relative humidity. In contrast, the longest development period (50.60 days) was recorded at 20 °C and 50% RH. These results are in strong agreement with the findings of Mainali *et al.* (2015) [9], Sharma (2017) [20], and Priyadarshini (2018), [14] who also reported that 30 °C and 70% RH were optimal for the development of *C. chinensis* on pulses. Furthermore, previous studies by Mandi and Ghosh (2008) [10], Lale and Vidal (2003) [8], Pimentel *et al.*

(2003) [13], Boshra (1993) [3], Chandrakantha *et al.* (1987) [5] and Mansour *et al.* (1976) [12] similarly observed that the developmental duration of *C. chinensis* and related species was significantly reduced as temperature increased from 20 °C to 30 °C or higher.

Effect on adult emergence percentage

In different humidities, the pooled mean adult emergence percentage at 20 °C varied from 31.78 to 65.03 percent. At this temperature, the highest percentage was 65.03 percent at 70% RH followed by 60% (50.39%), 80% (43.37%) and 50% relative humidity (36.63%), while the lowest percentage of adult emergence was observed at 90% RH (31.78%).

The pooled mean of all successful adult emergence at 30 °C varied from 79.78 to 95.89 percent in various humidities. At this temperature, the highest average adult emergence was 95.89 percent in 70% RH, while the lowest average adult emergence was 79.78 percent in 50% RH followed by 85.28 percent in 90% RH, 90.83 percent in 80% RH and 92.06 percent in 60% RH.

The most effective combination of temperature and relative humidity was determined to be 30 °C and 70% RH, which led to 95.89% adult emergence. In contrast, only 31.78% of adults emerged when 20 °C and 90% RH were coupled. The results are consistent with the findings of Chakraborty and Mondal (2016) [4], who discovered that the ideal temperature and humidity combination for maximum adult emergence was 30 °C and 75% relative humidity. In a similar vein, the current findings are supported by the highest adult emergence percentage of *C. chinensis* (L.) observed by Mandi and Ghosh (2008) [10], Mainali *at el*. (2015) [9], Choudhary (2016) [6], Sharma (2017) [19], and Priyadarshini (2018) [14] at 30 °C and 70% relative humidity.

Table 15: Effect of different combination of temperature and relative humidity levels on larval period, pupal period, total developmental period and adult emergence percentage of pulse beetle, *C. chinensis* in 2022 and 2023 (Pooled mean)

Relative humidity (%)	Larval period (days)*		Pupal period (days)*		Total developmental period (days)*		Adult emergence (%)**	
	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
50	36.35 (6.12)	22.83 (4.89)	6.74 (2.78)	5.13 (2.48)	50.60 (7.18)	31.58 (5.71)	36.63 (37.23)	79.78 (63.26)
60	34.22 (5.94)	21.52 (4.75)	6.52 (2.74)	5.01 (2.45)	47.87 (6.99)	30.14 (5.58)	50.39 (45.21)	92.06 (73.61)
70	31.03 (5.66)	20.10 (4.60)	5.51 (2.56)	4.10 (2.26)	42.98 (6.63)	28.49 (5.44)	65.03 (53.73)	95.89 (78.28)
80	32.53 (5.80)	20.66 (4.66)	5.85 (2.62)	4.33 (2.31)	44.68 (6.76)	29.45 (5.52)	43.37 (41.17)	90.83 (72.36)
90	33.55 (5.88)	20.99 (4.69)	6.22 (2.69)	4.92 (2.44)	45.75 (6.84)	30.00 (5.57)	31.78 (34.30)	85.28 (67.42)
S.Em±	0.02	0.01	0.02	0.02	0.03	0.01	0.45	0.28
CD at 5%	0.06	0.04	0.05	0.06	0.08	0.04	1.44	0.89

* The figures given in parentheses are square root transformed values.
** The figures given in parentheses are angular transformed values.

Conclusion

The present investigation examined how different temperature and relative humidity combinations affected the development of the pulse beetle, *C. chinensis* and found that the most favourable conditions for development were 30ºC and 70% relative humidity. The normal development was prevented by the low temperature of 20 °C at all relative humidity levels. At 40 ° C in different humidity levels, no hatching of eggs was observed. The low temperature of 20 °C has little effect on fecundity and the oviposition period. At a high temperature of 40 °C, however, fecundity and the oviposition period reduced.

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