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EFFECT OF DIFFERENT TEMPERATURES ON DEVELOPMENTAL PERIODS AND REPRODUCTION OF FRUIT FLY (*Bactrocera cucurbitae*)

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ABSTRACT

Considering the economic value of cucurbits and the crop losses due to fruit fly infestation, the current experiment was carried out in controlled laboratory conditions to investigate the effects of temperature on the growing durations and reproduction of fruit fly as a response to climate change. At first, the culture of cucurbit fruit fly (infested bitter gourd fruits) was collected from the experimental field of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University and then the emerged larvae were artificially reared on bitter gourd slices under laboratory conditions at 25°C, 30°C and 35°C. Differences among the three tested temperatures for different developmental periods were significant, but the differences in male-female ratio at different temperatures were not statistically significant. The duration of life stages was significantly shorter when reared at high temperature (35°C) than when reared at low temperature (25°C). The incubation period and the durations of larval and pupal development of the insect decreased from 17.07±0.09 hours to 15.87±0.03 hours, 5.31±0.03 days to 3.40±0.08 days and 8.83±0.01 days to 8.08±0.04 days, respectively due to the increase of temperature from 25°C to 35°C. The mating duration, fecundity, hatching percentages were maximum at 25°C (3.54±0.01 days, 74.93±0.07 and 86.67±0.03%, respectively). Although some reproductive parameters were favored at 25°C, the results clearly indicate that an increase in temperature in the context of climate change would benefit the insect with more females and more generations per year due to having a short life cycle.

Keywords: *Bactrocera cucurbitae*, fruit fly, life stages, temperature impact

ABSTRAK

Mengambil kira nilai ekonomi cucurbit dan kerosakan tanaman yang disebabkan oleh infestasi lalat buah, satu eksperimen telah dijalankan di dalam makmal kawalan untuk mengkaji kesan

suhu ke atas tempoh pertumbuhan dan pembiakan lalat sebagai respon ke atas perubahan cuaca. Sebagai permulaan, kultur lalat buah cucurbit (diinfestasi oleh buah peria) telah berjaya dikumpulkan dari kawasan ekperimen di Jabatan Entomologi, Bangabandhu Sheikh Mujibur Rahman Agricultural University dan kemunculan larva telah dibiakkan secara tiruan pada hirisan peria di dalam suhu makmal 25°C, 30°C dan 35°C. Perbezaan antara tiga suhu kajian bagi tempoh perkembangan adalah signifikan, manakala perbezaan bagi nisbah jantan-betina bagi suhu berbeza adalah tidak signifikan. Tempoh peringkat hidup adalah lebih pendek secara signifikan apabila dibiakkan pada suhu (35°C) daripada suhu rendah (25°C). Tempoh inkubasi dan tempoh bagi perkembangan larva dan pupa serangga berkurang dari 17.07±0.09 jam ke 15.87±0.03 jam, 5.31±0.03 hari ke 3.40±0.08 hari dan 8.83±0.01 hari ke 8.08±0.04 hari, masing-masing disebabkan penurunan suhu dari 25°C ke 35°C. Tempoh pengawanan, pembiakan, peratusan penetasan adalah maksimum pada 25°C (3.54±0.01 hari, 74.93±0.07 dan 86.67±0.03%, masing-masing). Meskipun, parameter pembiakaan adalah sesuai pada 25°C, hasil menunjukkan peningkatan suhu dalam konteks perubahan cuaca memberikan kebaikan kepada serangga di mana lebih banyak betina dan lebih banyak generasi per tahun disebabkan kitar hidup yang pendek.

Katakunci: *Bactrocera cucurbitae*, lalat buah, kitar hidup, impak suhu

INTRODUCTION

Cucurbits are the most important vegetable group in Bangladesh, accounting for 66.0% of vegetable growing land and 11.0% of total vegetable production (IPM CRSP 2004). Several insect pests have been identified as causing varied degrees of harm to cucurbit crops (Sani et al. 2020). Among them the melon fly or cucurbit fruit fly has been identified as the key pest. The two major species, *Bactrocera cucurbitae* and *B. tau* (Walker), create extensive infestations in cucurbits, with the former being a significant danger (Wee & Oii 2022). *Bactrocera cucurbitae* is the most damaging insect pest of cucurbits in tropical and subtropical areas including Bangladesh. It has more than 81 host species (Dhillon et al. 2005). Based on the cucurbit species and season, the extent of economic loss or damage caused to different cucurbitaceous vegetables by this insect varied from 30 to 100% (Dhillon et al. 2005; Nath & Bhusan 2006). The *B. cucurbitae* infestation caused a significant yield reduction in bitter gourd crops (Pilania et al. 2021).

Fruit flies of cucurbits remain active throughout the year in most of the tropical countries. But weather conditions and host species are important drivers to determine their activities; hence prevailing environmental conditions have a remarkable influence on their population dynamics. Because insects are typical ectothermic animals, variations in ambient temperature have a significant impact on them (Chen & Ma 2010; Ma et al. 2014). In recent years, the increase in climate temperature associated with the increase of greenhouse effects has become the most important environmental factor affecting insect growth and reproduction. Higher temperatures can affect oviposition (Kurz et al. 2008), mating (Bale et al. 2002), and thermostatic behavior (Björkman et al. 2011) of insects in either a favorable or negative way. Extreme heat can also have an impact on the reproductive system of insect. The temperature above 31°C is harmful for the growth and reproduction of *B. cucurbitae* (Dhillon et al. 2005). Having a thorough knowledge of the population ecology of the target pest is essential for devising effective pest management strategies. Since life tables are the only source of accurate and in-depth information on insect survival, population growth, stage specific changes, and reproduction, the life table research should be given utmost importance in ecologically sound pest management strategies. Therefore, the current study was designed to investigate the

impacts of temperature on the durations of *B. cucurbitae* life stages as well as potential changes in its fecundity and adult sex ratio.

MATERIALS AND METHODS

Laboratory Conditions and Insect Culture

The experiment was carried out in incubators (Model: IN110, Memmert, Germany, w×h×d: 745×864×584 mm) in the laboratory of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. The initial culture of cucurbit fruit fly was collected from the experimental field of the Department of Entomology, BSMRAU. The infested bitter gourd fruits were kept above a sand layer to raise larvae and pupae of *B. cucurbitae* for laboratory research. The pupae were then collected from the sand and transported to petri dishes for the emergence of adult flies. The standard laboratory conditions (25°C temperature, 50% RH and 12L:12D photoperiod) were maintained throughout this process (Kandakoor et al. 2019). To study the effect of temperature on the insect at egg, larval and pupal stages, different individuals were taken from the initial reared insect.

Rearing of Adult Fruit Fly

Adult *B. cucurbitae* were kept in a wooden framed cage (60 x 40 x 40 cm) with metal screens on either side for adult treatment. Three replicates were used with 25 adults at each replicates for each treatment (25°, 30° and 35°C temperature). The rearing was done in incubators at the treatment temperatures where 50% RH and 12L:12D photoperiod were maintained for every case. For adult feeding, a 10% W/V glucose solution kept in a 50 mL beaker was supplied inside the cage (Mir et al. 2014). To keep the solution within the reach of adult fruit flies, a cotton swab was placed in the beaker with half submerged in the glucose solution and the other half remaining over the rim. Each cage contained slices of bitter gourd for oviposition. To prevent decay, these slices were changed every day with new ones. The mating period, oviposition period, fecundity, and sex ratio were all recorded on a daily basis. To record the mating duration of the insect, observation was done for five days. Reproductively mature females were maintained in separate cages (one female per cage) from 08:00 to 20:00 hours a day at the treatment temperatures. A male was released in each cage daily at 08:30, and observations were made at 30-minute intervals till 20:00 hours. When mating began between the insects, copulation duration was recorded. The entire procedure for recording mating duration was repeated three times with 10 pairs of insects for each of treatment temperature.

Treatment of Eggs

Egg treatment included 75 eggs, which were divided into three replicates (3 treatments, 3 replicates, 25 units) and each with 25 eggs was set in clean petri dishes. Five slices of dietary bitter gourd were placed on petri dishes for natural environmental conditions. A tissue paper layer was placed on the surface of the diet slices. Under a binocular microscope (Model: MSC-B201, 40×-100×, USA), 25 eggs were counted. These were meticulously transferred onto tissue paper. The petri dishes were then covered and shifted to an incubator which was set to three different temperatures (25°, 30° and 35°C). Each Petri dish was examined under a stereo microscope (Model: Ladybird Micro Zoom 1240 Trino, Micros, Austria) at 2-hour intervals to determine egg hatching. The number of hatched eggs, incubation period, and rate of development were all recorded.

Treatment of Larvae

For larvae treatment, 75 larvae were divided into three replicates for each treatment with each replicate containing 25 larvae. Each petri dish included 25 larvae, which were kept at the tested temperatures (25°C, 30°C, and 35°C). Once the larvae reached the third instar, the petri dishes were placed inside a plastic container with a sand layer at the bottom. The larvae moved out from the petri dishes onto the sand for pupation. The plastic containers were monitored on a daily basis for pupae and when the pupae were formed, they were sieved out from the sand. The larval growing period and developmental rate were all noted.

Treatment of Pupae

Pupae treatment included 75 pupae per temperature, with 25 pupae maintained in three sand-filled petri plates as three replicates. The petri dishes were covered using plastic nets, and kept separately at 25°C, 30°C, and 35°C temperatures till adult emergence. Then the data were all documented. The presence of a pointed ovipositor was considered to identify the females, and the data were presented as sex ratio indicating the ratio of females to males in the tested population.

Data Analysis

One-way Analysis of variance followed by Tukey HSD posthoc test (at 5.0% level of significance) was performed to compare the difference among the treatments in terms of life stage durations, fecundity, egg hatching percentage and sex ratio of the insect. All the statistical analyses to determine the temperature effect on life stages of fruit flies were performed through statistical packages of R program (version 4.1.2).

RESULTS

The differences in egg incubation periods of *B. cucurbitae* at the tested temperatures were statistically significant (Table 1). The shortest mean incubation duration (15.87±0.03 hours) was obtained at 35°C, while the longest (17.07±0.09 hours) one was recorded at 25°C. The mean egg-hatching percentage ranged from a low of 86.1±0.06% at 35°C to a high of 86.67±0.03% at 25°C. The total larval periods of *B. cucurbitae* were 5.31±0.03 days, 4.36±0.02 days and 3.40±0.08 days at 25°C, 30°C and 35°C respectively. As listed in Table 1, the pupal stage followed the same developmental pattern as the egg and larval stages. The pupal development period decreased from 8.83±0.01 days at 25°C to 8.08±0.04 days at 35°C. The mating duration of *B. cucurbitae* decreased from 3.54±0.01 hours at 25°C to 2.15±0.06 hours at 35°C. Pre-oviposition periods were found to be longer (13.98±0.01 days) at 25°C and shorter with increasing temperatures (at 30°C, it was 12.51±0.01 days and at 35°C, it was 10.18±0.02 days). Oviposition durations were longer at 25°C (21.08±0.04 days) and shorter at 30°C (18.15±0.03 days) and 35°C (14.08±0.04 days). The fecundity was the highest (74.93±0.07%) at 25°C and then decreased to 72.47±0.09% at 30°C and 71.03±0.09% at 35°C. The mean egg-hatching percentage ranged from a low of 86.1±0.06% at 35°C to a high of 86.67±0.03% at 25°C. Sex ratio was found 1.13±0.03, 1.08±0.04, and 1.03±0.03 at 35°C, 30°C and 25°C, respectively (Table 1).

Table 1. Durations of different life stages of *Bactrocera cucurbitae*. Each data is the mean of 3 replications

Variables (Different life stages of fruit fly)	**Mean±S.E.**		
	T3 (25°C)	**T2 (30°C)**	**T1 (35°C)**
Egg incubation period (hour)	17.07±0.09a	16.67±0.0b	15.87±0.03c
1st Instar (day)	0.91±0.01a	0.76±0.01b	0.54 ±0.02c
2nd Instar (day)	1.77±0.01a	1.45 ±0.01b	1.05 ±0.05c
3rd Instar (day)	2.64±0.02a	2.15 ±0.01b	1.81±0.04c
Total maggot period (days)	5.31±0.03a	4.35 ±0.02b	3.40 ±0.08c
Prepupal period (days)	0.93±0.01a	0.70±0.02b	0.57±0.01c
Pupal period (days)	8.83±0.01a	8.37±0.01b	8.08 ±0.04c
Mating period (Hours)	3.54±0.01a	3.16±0.01b	2.15±0.06c
Pre-oviposition period (days)	13.98±0.01a	12.51±0.0b	10.18±0.02c
Oviposition period (days)	21.08±0.04a	18.15±0.03b	14.08±0.04c
Fecundity	74.93±0.07a	72.47±0.09b	71.03±0.09 c
Hatching %	86.67±0.03a	86.30±0.06b	86.10±0.06 c
Sex ratio (Female:Male)	1.03±0.03a	1.08±0.04a	1.13±0.03 a

Means within a row followed by same letter are not significantly different according to Tukey's HSD post hoc test at <0.05 significance level.

Correlation matrixes were employed to analyze the correlation among the variables (Figure 1). Only sex ratio had a negative correlation with all other variables. It had the highest negative correlation with hatching which was 72.42%, followed by prepupal period (64.79%). On the contrary, hatching had the highest positive correlation with prepupal period which was 98.11% followed by fecundity (97.11%) and least positive correlation with a mating period (85.79%).

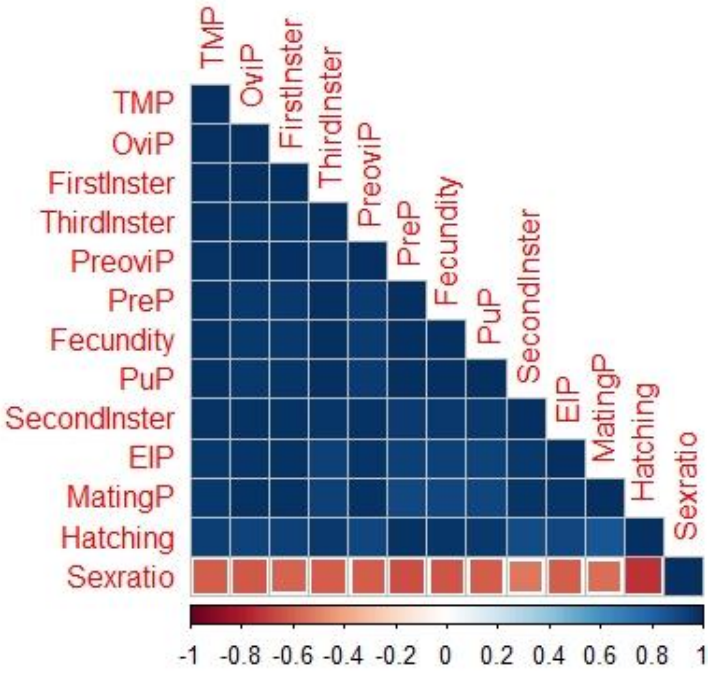


Figure 1. Correlation matrix among the different variables. Correlation plots represent the order wise relationship corresponding to color gradient between different variables (PreP – Prepupal period; PuP – Pupal period; MatingP – Mating period; PreoviP – Preoviposition period; TMP – Total maggot period; OviP – Oviposition period; EIP – Egg incubation period)

The heatmap dendrogram represents the result of a hierarchical clustering calculation of the variables (Figure 2). Based on the distance, the result of clustering is presented as the distance or similarities between the clustered rows or columns. In the heatmap, treatment 1 (35°C) was closely associated with treatment 2 (30°C) as they offered a short distance and treatment 3 (25°C) was in a distant clade. Sex ratio alone was shown in distant clade from all other life stages variables.

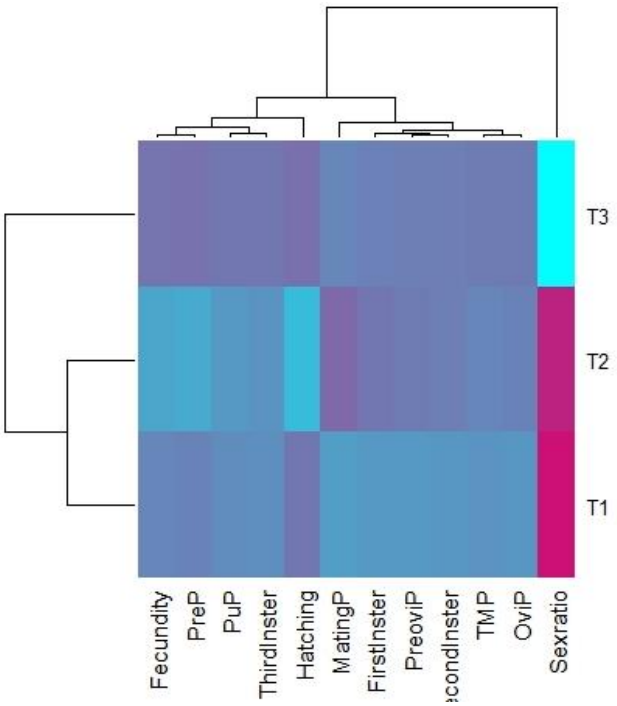


Figure 2. Heatmap dendrogram to visualize the result of a hierarchical clustering of the analyzed parameters). (PreP – Prepupal period; PuP – Pupal period; MatingP – Mating period; PreoviP – Preoviposition period; TMP – Total maggot period; OviP – Oviposition period and T1, T2, T3 represent 3 different treatment/temperature)

Principal component analysis (PCA) was performed with the variables of the collected data on the life stages of fruit flies in 3 different treatments and it was found that more than 97% of the variations presented in the scree plot (Figure 3) were explained by the first two components of PC1 and PC2. As a result, in the PCA biplot analysis, two dimensions were considered to explain the variances and their respective contribution the variances resulted from the studied variables referring to dimension 1 (Dim 1) and dimension 2 (Dim 2) (Figure 4).

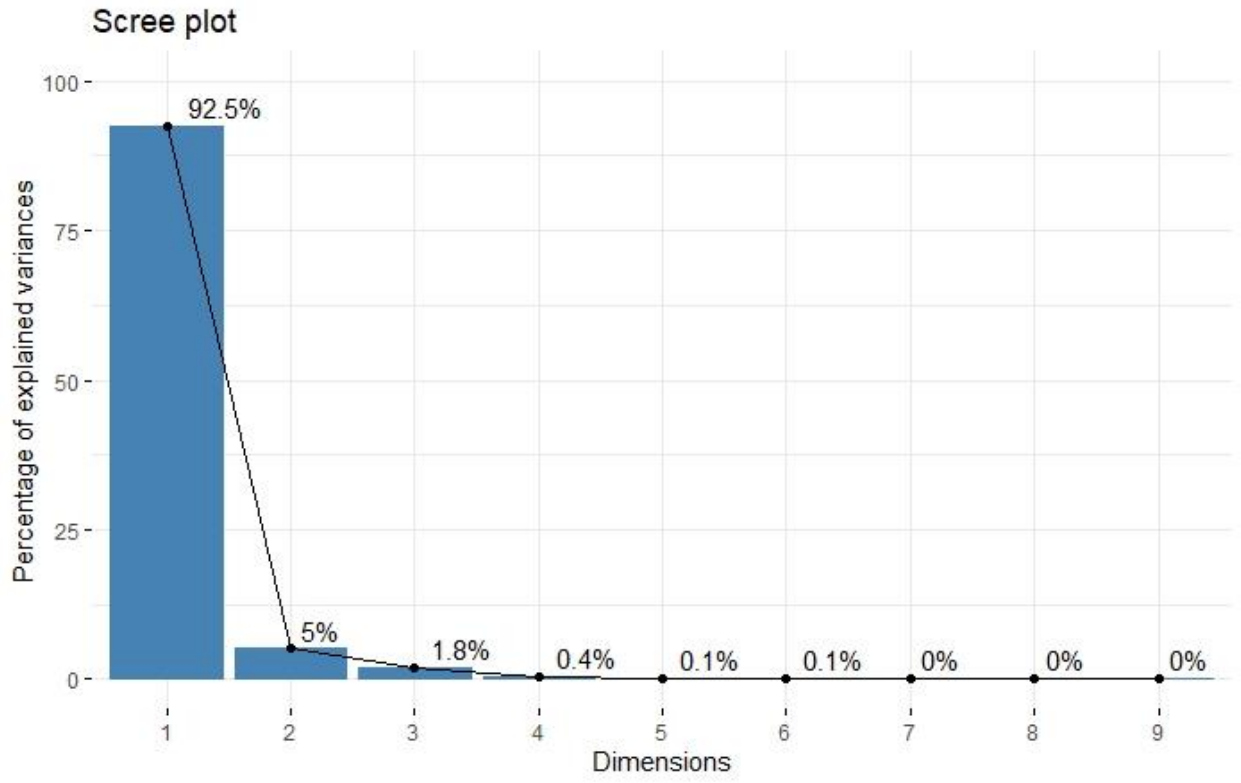
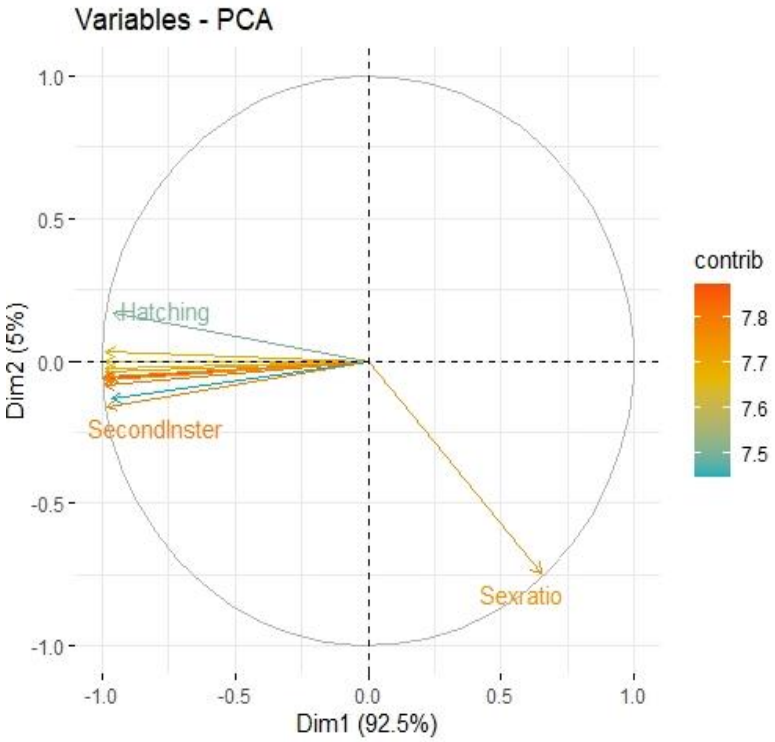
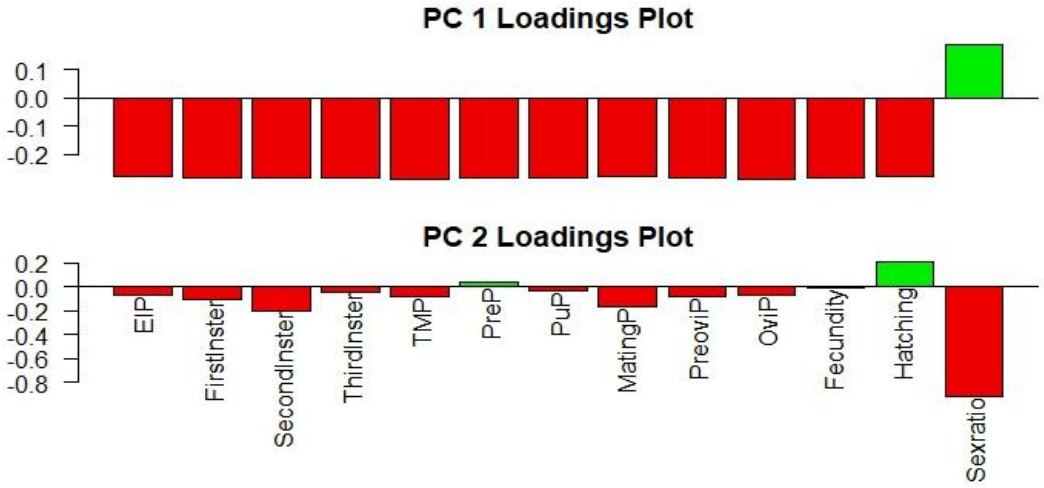


Figure 3. Principal component analysis (PCA) among the life stage variables of fruit fly in 3 different treatments

The contribution of different variances in PCA is presented in Here dimension 1 alone contributed 92.5% and dimension 2 contributed 5%. Sex ratio had positive loading on PC1 and all other variables negatively influenced this. On the other hand, for PC2, sex ratio had very strong negative loading while both hatching percentage and prepupal duration showed positive loadings (Figure 4a & b).



(a)



(b)

Figure 4 a-b. Principal component analysis representing different variables for 3 different treatments. (EIP – Egg incubation period; PreP – Prepupal period; PuP – Pupal period; MatingP – Mating period; PreoviP – Preoviposition period; TMP – Total maggot period; OviP – Oviposition period)

The results on fruit fly developmental stages were significantly varied due to the temperature differences. However, mere visual inspection cannot properly detect the most significant variable that needs to be focused on for selecting the best temperature in response to the fruit fly population development. Therefore, all the studied dependent variables were analyzed using PCA to explore the relative variability among the different fruit fly developmental processes for effective temperature selection. Results from the biplot of the

temperatures (Figure 5) revealed that T3 (35 °C) is located in a distinct position in relation to the Dim1 where sex ratio was found as a strong positive contributor in the variations while T1 (25 °C) has a distinct position with the most remarkable contributor of the hatching that has strong positive contribution in the life stage of fruit fly in response to the Dim2 (Figure 5). Meanwhile, T2 (30 °C) was found to interact with both dimensions, and most of the variables were in a negative position except sex ratio. From these findings, it can be stated that sex ratio and hatching were the most significant characteristics to evaluate the life stage of the fruit fly which were remarkably influenced by the temperature of T3 (35 °C) and T1 (25 °C).

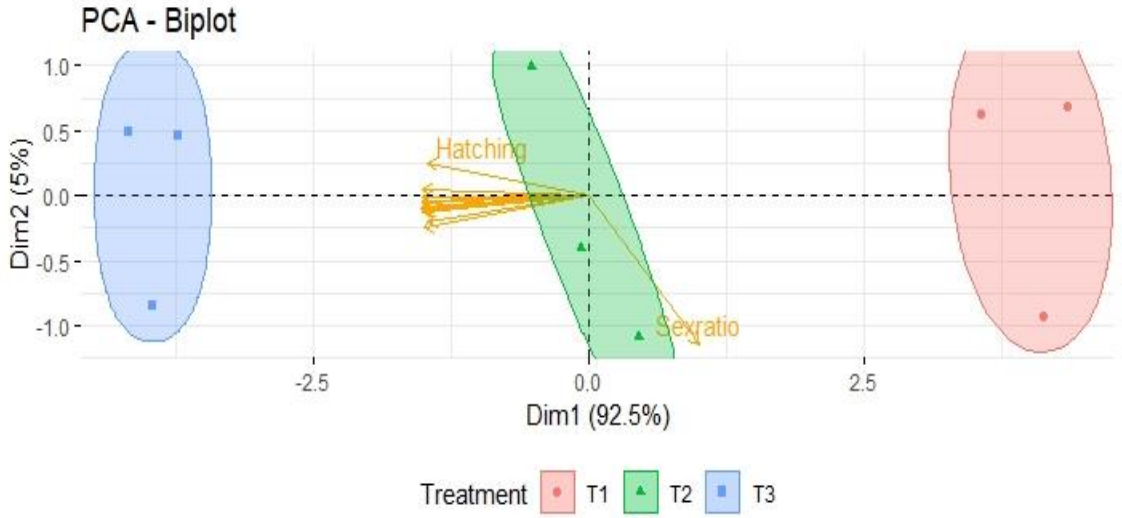


Figure 5. Biplot generated through principal component analysis corresponding with different treatments. (Dim1 = Dimension 1; Dim2 = Dimension 2; T1= 35°C; T2 = 30°C; T3 = 25°C)

DISCUSSION

Since insects are ectothermic organisms, the change in prevailing temperature can affect them either negatively or positively. Consequently, the life events of insects vary at different temperatures. The current findings showed that an increase in temperature from 25°C to 35°C was favorable for a faster life cycle of *B. cucurbitae* by reducing the developmental period. Individuals raised at 35°C completed their life cycles within a significantly shorter time. On the other hand, the mating time, oviposition period, fecundity, and hatching percentage were all maximum at 25°C and as the temperature increased, these reproductive parameters decreased.

The current results indicated that the duration of all immature stages (egg incubation period, larval period and pupal period) decreased with the increase of temperature. According to some other research findings, an increase in temperature gradually shortens the length of the immature stages in the optimum temperature range (Li et al. 2015). Kandakoor et al. (2019) reported that the egg, larval, pupal and preoviposition periods of *B. cucurbitae* showed negative correlation (–0.83571, –0.93257, –0.76333 and –0.22307, respectively) with temperature when increased from 16°C to 36°C. Fetoh et al. (2012) also noted similar results that the developmental duration of the eggs, larvae and pupae of *B. zonata* showed a significant decrease with a rise of temperature from 20°C to 35°C. Kandakoor et al. (2019) observed that

when the temperature was raised from 24°C to 28°C, the time for completing the development from egg to oviposition was reduced from 28.92 to 25.39 days. Duyck et al. (2004) reported that the time required for the development of eggs and pupae significantly decreased over the temperature range of 15–30°C in *B. zonata*. Vayssières et al. (2008) reared *B. cucurbitae* on cucumber and observed that the insect took 17.2 days at 25°C and 13.2 days at 30°C for the preadult development. Huang and Chi (2011) found that the overall preadult growth of *B. cucurbitae* occurred in 15.1 days at 25°C.

According to the current findings, the mean egg-hatching percentage was comparatively lower (86.1±0.06%) at 35°C than at 25°C (86.67±0.03%). This result was closely related to Prasad and Hasan (2018) who found 87.50±2.5% mean egg-hatching when reared at 34.36 to 25.46°C. Mir et al. (2014) also found an average fruit fly egg-hatching percentage of 86.10±0.5 in between 23.97±0.66°C and 16.17±0.81°C. Temperature has a significant impact on the reproductive characteristics of insects. Extreme temperature regimes reduce reproductive rates because of decreased viable egg formation as a result of poor mating. Although excessive temperature is detrimental to insects, increasing temperature within a given threshold can offer organisms better survivability in complex environmental conditions through certain metabolic pathways, such as saccharide metabolism and fat metabolism (Qian et al. 2017; Van Dooremalen and Ellers 2010). The best reproductive rates in *B. carambolae* were obtained at 25-27°C (Danjuma et al. 2018). On the other hand, temperature below 20°C and above 32°C caused higher mortality of *B. cucurbitae;* hence, found to be less favourable for the insect (Kandakoor et al. 2019).

Mir et al. (2014) reported 75.8±12.49% fecundity of *B. cucurbitae* and Lanjar et al. (2013) found 50-91 eggs per female *Bactrocera* fly during her entire life cycle in the laboratory. The present findings also found similar fecundity of *B. cucurbitae* ranging from 71.03±0.09% at 35°C to 74.93±0.07% at 25°C. The increase of temperature showed a significant reduction in the fecundity of the insect. The results are in line with those of Huang et al. (2020) who found that the fecundity of *B. tau* showed a downward trend when the temperature was increased from 24°C to 40°C.

In the current study, sex ratio was found higher (1.13±0.03) at 35°C but decreased to 1.03±0.03 at 25°C. The findings are consistent with Mir et al. (2014) who found sex ratio 1.10 ± 0.14 within temperature range of 13-35.5°C. According to Huang et al. (2020), treatment temperatures ranging from 24°C to 38°C favored the females of *B. tau* in female/male ratio by producing an excess of females which would maintain or increase the population size. However, the differences in sex ratio at treatment temperatures in the present study were not statistically significant.

The correlation matrix among the developmental durations and reproductive parameters of *B. cucurbitae* indicated that a longer prepupal period and higher fecundity resulted in high hatching percentage of egg. Interestingly, high egg hatching rate was associated to produce more males than females. As a result, high egg hatching at 25°C did not stimulate the population growth due to having fewer females than males. PCA analysis showed that the sex ratio, hatching and prepupal period were the most promising variables affecting the life stage study of fruit fly. PCA biplot showed that 25 °C and 35 °C had very strong influence on both sex ratio and egg hatching percentage of the insect while 30 °C had an intermediate influence.

The average daily temperature ranges from 24 to 32 °C across Bangladesh based on different seasons (WorldData.info 2023). The current findings clearly indicated that the increase of temperatures to 35°C would have significant positive impact on the life cycle of *B. cucurbitae* compared to 25°C, which is a very serious matter of concern not only for the country but also for the world in the context of global climate change.

CONCLUSION

Considering the overall findings, it can be concluded that the increase of temperature to 35°C would favor the population growth of *B. cucurbitae* in terms of producing more females and a short life cycle leading to more generations per year. Since the cucurbit crops are more likely to face higher infestations of *B. cucurbitae* at high temperature, the rise of global temperature which is also a global concern has high probability to affect the production of cucurbit crops.

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AUTHORS DECLARATIONS

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Conflict of Interest

There is no conflict of interest among the authors.

Ethics Declarations

Ethics declarations are not applicable for this research.

Data Availability Statement

This manuscript has no accessible data.

Authors' Contributions

T.I. Tora: Data collection, conducting research and writing the manuscript; M.M. Rahman: Conceptualization, correspondent, data analysis, research supervision, review and editing the manuscript; M. Afroz: Reviewing and editing the manuscript, M.R.U. Miah: Review and editing the manuscript; M.R. Amin: Review and editing the manuscript; M.H. Kabir: Review and editing the manuscript; M.M. Rahman: Review and editing the manuscript; J. Hassan: Statistical analysis of the data and J.C. Biswas: Review and editing the manuscript.

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