SAARC J. Agric., 24(1): 87-98 (2026) *DOI: https://doi.org/10.3329/sja.v24i1.87918*

SAARC Journal of Agriculture

Journal Homage: https://www.banglajol.info/index.php/SJA

Effect of Temperature on the Parasitism Efficiency of *Habrobracon hebetor* Say against Brinjal Shoot and Fruit Borer

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Abstract

In response to the ecological damage caused by conventional pesticides, bio-control agents offer a sustainable alternative for pest management. The brinjal shoot and fruit borer (BSFB), a devastating lepidopteran pest whose larvae bore into shoots and fruits, making it a critical target for effective bio-control strategies. As *Habrobracon hebetor* Say demonstrates high parasitism and host suppression efficiency against larvae of lepidopteran insect pests, this study investigated the temperature-dependent performance of *H. hebetor* against BSFB at 15°C, 20°C, 25°C, 30°C, and 35°C to determine the optimal temperature range for parasitism efficiency. The experiment was conducted following a single factor completely randomized design (CRD) with three replications. Temperature significantly influenced all assessed parameters: developmental time (DT), emergence success rate (ES), parasitism rate (PR), adult longevity (LR) and sex ratio (SR). DT differed significantly across all regimens and was inversely proportional to the temperatures, ranging 9.8 days at 35℃ to 48.5 days at 15_1 . Emergence success was highest at 30℃ (70%), while the parasitism rate peaked at 25℃ (96.9%). Sex ratios (male: female) varied with temperature, reaching 0.35 at 30℃ and adult longevity was shortest at 35℃ (6.3 days). The highest parasitism at 25℃ and peak emergence at 30℃ suggests a trade-off between parasitism ability and successful adult emergence. These findings indicate that temperature significantly affects the biology and activity of *H. hebetor* against BSFB, with 25–30°C the optimal range for laboratory rearing and field application of *H. hebetor*. This optimum thermal range makes the parasitoid a viable and sustainable bio-control agent against BSFB.

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Received: 17.02.2026 *Accepted: 30.06.2026*

Keywords: Bio-control, *Habrobracon hebetor, Leucinodes orbonalis*, Temperature regime

Introduction

Brinjal (*Solanum melongena* L.), widely popular as eggplant in South and Southeast Asia, is the most important and highly valued vegetables economically and nutritionally. It has become one of the principal and nutritional constituent vegetable crops for a vast number of people in the country, Bangladesh. The brinjal is a year-around crop for around 150,000 resource-poor farmers over an area of nearly 50,000 hectares and occupies an important position in agricultural economy in Bangladesh (Haque & Saha, 2020). Brinjal is a solanaceous crop, and is rich in many vitamins, minerals and fibers which are essential for the regional food and nutritional security (Shirale et al., 2017). The yield of this crop is hampered to a great extent by the brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* Guen. (Lepidoptera: Crambidae), a monophagous pest responsible for a high rate of shoot and fruit infestation that sometimes reach as high as 90% and can cause about 86% loss in marketable yield when not properly controlled (Prodhan et al., 2018; Shelton et al., 2018; Rai et al., 2020).

The destructive potential of *L. orbonalis* is primarily due to its cryptic feeding habit (Iesa, 2021). During vegetative stage the feeding of larvae on tender shoots cause “dead hearts” and stunting of growth (Panwar et al., 2021). In reproductive stage, they attack and bore fruits and spoil the fruits through feeding and excreta and exuviae, which made the fruits unmarketable (Cork et al., 2003; Chakraborti and Sarkar, 2011). As this insect live inside the plants and protected from the contact pesticides, difficult to control (Islam, 2015). Farmers in Bangladesh often depend on broad-spectrum pesticides (Alam and Kiron, 2025) to control this pest which may be used 80-100 sprays per season (Aktar et al., 2009), causing insecticide resistance, damage of natural enemies and thus increased risk for the consumer regarding toxic pesticide residues in the food chains (Reddy et al., 2025).

Increasing awareness towards environmental and public health hazards have resulted in emphasis on biological control based IPM strategies. The solitary ectoparasitoid *H. hebetor* Say is being considered as an important natural enemy for biological control of lepidopteran pests in brinjal crop (Ghimire & Phillips, 2010; Niranjana, 2017). *H. hebetor* is a gregarious idiobiont parasitoid where its females paralyze host larvae and subsequently oviposit on the host bodies, whereas its larvae feed ecto-symbiotically, prevent the larval feeding, and kill the host larvae of crop at the end (Kryukova et al., 2024). Field application efficacy of these agents is being evaluated, and it varies with conditions in crops. Abiotic factors significantly affect parasitoid's performance; temperature being the important one. Since parasitoids are poikilotherms, their development, survival, fecundity and longevity are regulated by temperature within certain ranges (Chown et al., 2002; Roy et al., 2002). Their activity and growth rate increases from low up to optimum and decreases sharply beyond their upper limit (Ismail et al., 2023; Huey & Kingsolver, 1989). Discrepancy of thermal requirements between hosts and their natural enemies may limit their effectiveness (Hance et al., 2007; Thomas & Blanford, 2003). Extreme temperatures influence the development rate, lifespan, oogenesis, searching behavior and sex-ration of *H. hebetor* (Eliopoulos & Stathas, 2008; Huang et al., 2024). High temperatures accelerate the rate of development but decrease their longevity, whereas low temperatures slow down development and population building. (Islam et al., 2019).

Though the effects of *H. hebetor* have been the subject of prior research, little data exists on its performance on the specific climatic conditions prevailing in South Asia against *L. orbonalis*. With rising variation of ambient temperatures in the region with climate change, an understanding of the range of favorable thermals for *H. hebetor*, would help to optimize mass-rearing and effectiveness in field application of *H. hebetor*, as well as assessing its reliability for integration as an IPM component in the sustainable production of brinjal. The objective of the present study was to determine the impact of distinct constant temperatures on the biological performance of *H. hebetor* utilizing *L. orbonalis* as the host.

Materials and Methods

Experimental Layout

The study was conducted using a Completely Randomized Design (CRD) with five temperature conditions (15°C, 20°C, 25°C, 30°C, and 35°C). Each of these treatments was replicated three times, resulting in a total of 15 experimental units, including replications. All experimental units were maintained under consistent climatic conditions, with a constant relative humidity (RH) of 65-70%, an essential ecological parameter that ensured the integrity and accuracy of the study.

Rearing Techniques for BSFB and Parasitoids

The eggs of *L. orbonalis* (Brinjal Shoot and Fruit Borer, BSFB) were obtained from naturally infested brinjal fields to confirm the viability and aggressiveness of the pest. The infected brinjal plants were collected and brought to the laboratory, after selection. Fresh brinjal plants (flowering or fruiting stage) were employed to serve as a comfortable milieu for the BSFB larvae. Plants were checked weekly to avoid incomplete infestation of larvae. *H. hebetor* rearing the parasitoid species were collected from the Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur for *H. hebetor* rearing. Reared artificially in the bio-control laboratory on *Galleria mellonella* as host, the parasitoid was then mass reared. Corcyra larvae were placed in the parasitoid rearing chambers and *H. hebetor* wasps attacked them for egg laying. Colonies of stock *Leucinodes orbonalis* were maintained on fresh brinjal shoots and fruits by the collected larvae from the naturally infested field on the brinjal and reared in the laboratory. Parasitoid *H.*

hebetor were obtained from the Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur and maintained on larvae of *Galleria mellonella* as a factitious host with regular supply of fresh host larvae to continue parasitoid production and regular cleaning of rearing container and replacement for minimizing contamination and nutritional limitation. Newly emerged adult females of *H. hebetor* and the healthy third instar larvae of *L. orbonalis* were taken for the temperature bioassay.

Experimentation

To estimate biological performance and rate of parasitism of *Habrobracon hebetor,* 60 third instar larva of *Leucinodes orbonalis* were transferred into a ventilated container (Height 20 cm, Diameter 15 cm). 10 newly emerged, one day old adult female *H. hebetor* wasp were released in each of experimental trial in container for the parasitism and allowed to expose in the container for the period of 24 hours. Fine mesh sieve was covered on the top of the container to allow ventilation and to restrict the escape of parasitoids in the environment. These experiments were conducted at the controlled laboratory condition i.e. relative humidity 65-70% and light regime 14:10 h (L:D). The experiment conditions facilitate the controlled host-searching and sting behavior of parasitoid in enclosed area. After 24 hours of parasitism, adult parasitoids were removed from the container and the larval of the host was examined for daily record of emergences of parasitoids.

Parasitism was confirmed by observing host paralysis and deposition of *H. hebetor* eggs or larvae on the external body of the host. The numbers of parasitized larvae, parasitoid larvae/pupae and emerged parasitoid adults for each replicate were recorded. Emerging adult parasitoids were separately recorded as males and females. Sex of adults was discriminated by the help of stereoscope according to external structural features of antenna and terminal abdominal parts. Emergence success was estimated by percent of parasitized larvae to emerged adults.

Parameters studied

The biological performance of *Habrobracon hebetor* was evaluated in terms of DT, ES, PR, SR and AL at different temperature levels. All parameters were estimated at five temperatures (15°C, 20°C, 25°C, 30°C and 35°C) to simulate the diversity of environmental conditions. The number of days required for each specific parasitoid species from oviposition on BSFB larvae to emergence was recorded (DT = developmental time). ES (Emergence Success Rate) The number of emerged adults was visually counted and ES as a % of the total parasitized larvae that produced emerged adults. The rate of parasitism (PR) was determined by dividing the number of parasitized larvae by the total exposed, with parasitism being confirmed when presence of larvae in BSFB were observed and calculated using the formula:

$$\text{Parasitism Rate (\%)} = \left(\frac{\text{Number of parasitized larvae}}{\text{Total larvae exposed}}\right) \times 100$$

The sex ratio (SR) was calculated as the number of males emerging from parasitized larvae to that of females, i.e. male : female. The Adult Longevity (AL) was assessed through the life span of parasitoids, as both sexes at each temperature took out average longevity from adult emergence to death.

Statistical analysis

Analysis of Variance (ANOVA) was used to test the hypothesis that the temperature treatments were different for the five parameters (DT, ES, PR, SR and AL). We estimated the statistical significance of temperature effects at $p < 0.01$. Tukey's HSD test was used to perform post-hoc comparisons to find out which temperatures have significant impact on parameters.

Results

Effect of temperature on DT, ES, PR, SR and AL of *H. hebetor* against BSFB

Analysis of variances (Table 1) revealed that the effect of temperature, exerted a statistically significant impact on developmental time, emergence success, parasitism rate, sex-ratio and adult longevity of *H. hebetor*.

Table 1. One-way ANOVA investigating the effect of temperature on on DT, ES, PR, SR and AL of *H. hebetor* against BSFB

Variable	Source	DF	SS	MS	F-value
DT	Treatment	4	3072.69	768.172	4448.87**
	Error	10	1.73	0.173	
	Total	14	3074.41		
PR	Treatment	4	6210.76	1552.69	522.09**
	Error	10	29.74	2.97	
	Total	14	6240.50		
ES	Treatment	4	8048.22	2012.06	1401.15**
	Error	10	14.36	1.44	
	Total	14	8062.58		
AL	Treatment	4	4187.67	1046.92	1460.81**
	Error	10	7.17	0.72	
	Total	14	4194.83		
SR	Treatment	4	1.6067	0.4017	2619.63**
	Error	10	0.0015	0.000153	
	Total	14	1.6082		

Supplemental Table 1. Effect of treatment on DT, ES, PR, SR and AL of *H. hebetor*

Treatment	DT	ES	PR	SR	AL
15°C	48.50 ± 0.3a	5.50 ± 0.3e	40.60 ± 1.3c	1.2767 ± 0.09a	51.50 ± 0.9a
20°C	21.67 ± 0.3b	17.27 ± 0.6d	92.50 ± 1.2a	0.9933 ± 0.061b	29.67 ± 0.4b
25°C	13.30 ± 0.9c	35.67 ± 0.9c	96.90 ± 1.7a	0.5400 ± 0.021d	13.67 ± 0.3c
30°C	11.90 ± 2.0d	70.00 ± 0.6a	87.00 ± 0.6b	0.3500 ± 0.031e	9.67 ± 0.2d
35°C	9.80 ± 0.6e	51.67 ± 0.9b	85.30 ± 0.5b	0.8300 ± 0.031c	6.33 ± 0.3e

Values are mean ± standard error (SE), within each variable column, means followed by different letters are significantly different according to Tukey's HSD.

Developmental Time (DT)

Developmental time significantly decreased with increasing temperature. The shortest developmental time of *H. hebetor* was recorded at 35°C, with a mean duration of 9.8 days, whereas the longest developmental time was recorded at 15°C, requiring 48.5 days for development. This indicates that temperature directly affected the developmental rate of *H. hebetor*, with development becoming faster as temperature increased (Figure 1, Supplemental Table 1).

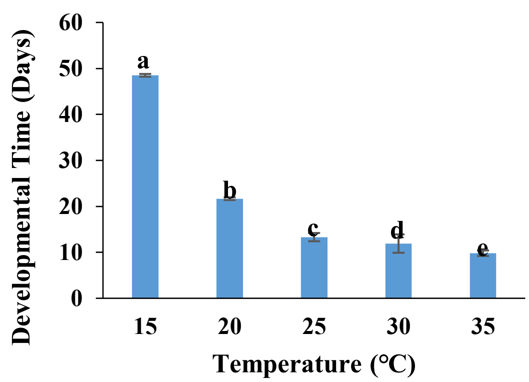

Fig. 1. Variation in developmental time of *H. hebetor* under the influence of different temperature conditions. The different letters above the bar are significantly different at the $p < 0.01$ level as per the Tukey's HSD test.

Emergence Success Rate (ES)

Emergence success of *H. hebetor* was significantly different in all temperature treatments. The highest and lowest percentage of emergence rates 70% was recorded at 30°C and 5.5% at 15 ºC, respectively. The results that emerged adults concentrated in the optimum temperature (30ºC) while low temperatures (15 ºC) drastically reduced their amount. Bars with different letters differ significantly according to Tukey’s HSD test ($P < 0.01$) (Figure 2, Supplemental Table 1).

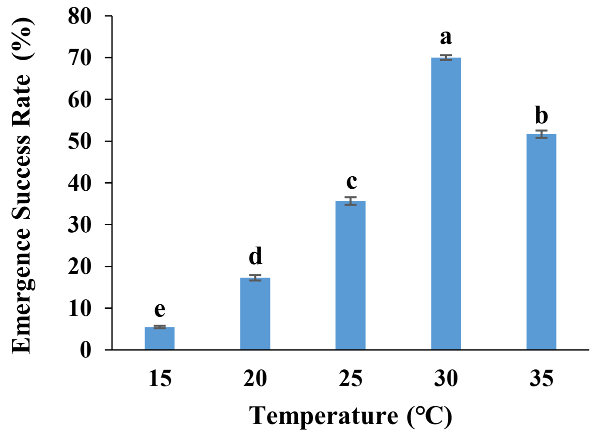

Fig. 2. Effect of temperature on emergence success rate. The different letters above the bar are significantly different at the $p < 0.01$ level as per the Tukey's HSD test.

Parasitism Rate (PR)

The parasitism rate differed significantly among the different temperatures. The parasitism rate was highest at 25°C (96.9%), followed by at 20°C (92.5%) but it did not differ significantly between these two temperatures, which indicated the similar parasitism efficiency under those conditions. Moreover, there was no significant difference in parasitism rate between 35°C and 30°C. Nevertheless, the parasitism rate decreased at 15°C where only 40.6% of larvae was parasitized (Figure 3, Supplemental Table 1). This suggests that low temperatures do not lead to optimal development and parasitism of *H. hebetor*.

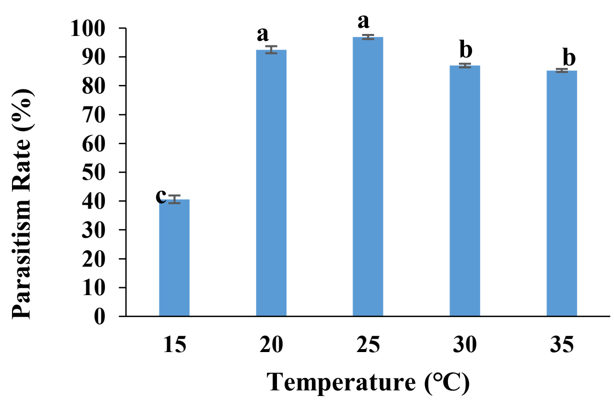

Fig. 3. Influence of temperature on the parasitoid rate. The different letters above the bar are significantly different at the $p < 0.01$ level as per the Tukey's HSD test.

Sex Ratio (SR)

The sex-ratio varied significantly at all temperatures. The greatest male: female ratio (1.27) occurred at 15°C and the least (0.35) at 30°C, demonstrating that temperature is a key factor to sex-biasing; lower temperatures were associated with male-biased progeny, but warm and hot exposures reduce the proportionality number of males in higher degree than females do (Figure 4, **Supplemental Table 1**).

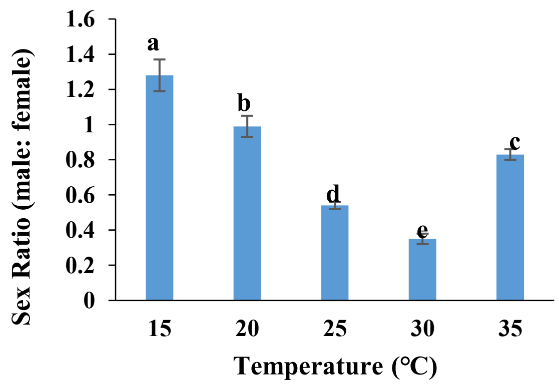

Fig. 4. Influence of temperature on the sex ratio of the parasitoid. The different letters above the bar are significantly different at the $p < 0.01$ level as per the Tukey's HSD test.

Adult Longevity (AL)

There were strong adult longevity differences between the temperature treatments. The longest and shortest lifespans were attained by *H. hebetor* at 15°C (51.5 d), and 35°C (6.3d) respectively. This means that the colder the temperatures, the longer a parasitoid can live, while warmer weather makes it shorter-lived (Figure 5, **Supplemental Table 1**).

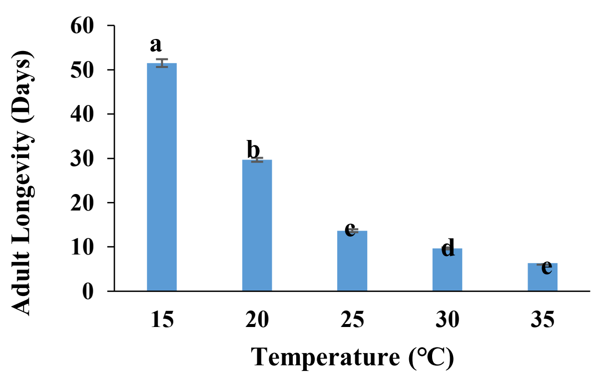

Fig. 5. Effect of temperature on adult longevity of *H. hebetor.* The different letters above the bar are significantly different at the $p < 0.01$ level as per the Tukey's HSD test.

Discussion

The biological efficacy and demographic stability of *Habrobracon hebetor* Say in combating the Brinjal Shoot and Fruit Borer (BSFB), *Leucinodes orbonalis*, are primarily regulated by thermal constraints, with our findings identifying a physiological optimum range of 25°C to 30°C. An incisive examination of Developmental Time (DT) demonstrates a quintessential non-linear ectothermic reaction, wherein the reduction from 48.50 days at 15°C to 9.80 days at 35°C aligns with the metabolic theory of ecology. This acceleration is notably significant in relation to the host's biology; Dhaliwal and Aggarwal (2021) demonstrated that *L. orbonalis* development likewise reaches its zenith at 30°C, indicating a substantial degree of phenological synchronicity between the parasitoid and the pest at this temperature threshold. Nonetheless, this accelerated advancement incurs a significant fitness cost manifested as diminished Adult Longevity (AL). The significant reduction in lifespan to merely 6.33 days at 35°C supports the "rate of living" hypothesis, indicating that heat-induced metabolic surges deplete the parasitoid's finite lipid reserves, a trade-off recently measured by Huang et al. (2024), who observed nearly identical lifespan changes across the same temperature range.

Our data reveals a functional decoupling between maternal behavior and offspring development. The percentage parasitism was highest at 25°C (96.9%) indicating the best conditions of parasitism of BSFB larvae by female adults, while the Emergence Success (ES) reached its maximum at the higher threshold of 30°C (70.0%). This indicates that whereas mothers like cooler environments for foraging and stinging, the biochemical processes necessary for larval eclosion and pupal metamorphosis require elevated thermal energy, a detail corroborated by Sahragard et al. (2008). The elevated efficiency at 25–30°C confirms *H. hebetor's* role as a principal natural adversary of BSFB, as emphasized by Mannarakkoth and Hegde (2018) and Srinivasan (2008).

The most significant demographic discovery is the dynamics of the Sex Ratio (SR); the population transitioned from an imbalanced male bias at 15°C (1.27) to a predominantly female progeny at 30°C (0.35). The sex ratio varied significantly between the two temperature treatments, with a male biased sex ratio at 15°C and a significantly female biased sex ratio at 30°C. This phenomenon could be linked to a temperature dependent effect on the fertilization mechanism though was not directly investigate in the study. Moreover, a significant discrepancy arises when juxtaposing our findings with those of Farag et al. (2015), who documented greater resilience at 35°C utilizing *Galleria mellonella*. However, we saw a significant decline in efficiency in *H. hebetor* in high temperature treatment at 35℃ suggests that higher temperatures might have an undesirable influence on the biological efficiency of *H. hebetor* on *L. orbonalis*. Although quality of host and mechanisms that are against the stresses are not determined in this study, it’s necessary for us to consider host as the one affecting the performance of the parasitoids under the condition of high temperatures in further study. The substantial statistical significance ($p<0.01$)

indicates that for effective integrated pest management (IPM) in brinjal, the mass-rearing and field releases should be adjusted at 25-30°C to achieve female-biased populations and maximal host suppression.

Conclusion

Temperature has a decisive impact on *Habrobracon hebetor's* performance. While development is fastest at higher temperatures, parasitism peaks at 25–30℃, and other traits like emergence, sex ratio, and longevity show clear temperature-dependent patterns. These results suggest that temperatures ranging between 25-30℃ may be optimum conditions for production and release of *H. hebetor* targeting BSFB, due to the highest parasitism and lowest emergence success in 25℃, and greatest emergence success in 30℃.

Acknowledgement

This research was carried out with the support of the Higher Education Acceleration and Transformation (HEAT) sub-project (PIN-13473). We sincerely appreciate the funding, guidance, and research support provided by this program, which greatly contributed to the successful completion of our work.

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