J. Adv. Zool. 2021: 42(1) : 148-153 **ISSN-0253-7214**

TEMPERATURE DEPENDENT DEVELOPMENT RATES OF PARASITOID, *HABROBRACON HEBETOR*, DIRECTLY FED ON *BACILLUS THURINGIENSIS* VAR. *KURSTAKI*

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ABSTRACT : The interaction of temperature and directly ingested *Bacillus thuringiensis* (*Bt*), on the developmental rates of the parasitoid *Habrobracon hebetor* progeny was investigated using *Corcyra cephalonica* 4th instar larvae as host. Development rates increased with temperature rise. Regression analysis revealed highly significant (p <.01) good fit of the equation for incubation, larval and total lifecycle period, while significant (p <.05) for larval period in untreated diet fed *H. hebetor*, However, under the influence of *Bt*- honey diet, larval period (p >.05) was not a good fit of the equation. Although r- values were overall lower and regression showed no significant fit of the equation for larval periods, significantly higher development rate was observed towards higher temperatures. However, rates during pupal period and total lifecycle showed highly significant (p <.01) good fit of the equation. Significant interactions between *Bt* and temperature were observed during larval ($F_{3,72}$= 45.443, p <.001) and pupal periods ($F_{3,72}$= 3.932, p <.05), however, no such significant interaction occurred during incubation period ($F_{3,72}$= 1.643, p= .187). Mean developmental rates during total life cycle also showed significant interaction between the factors, $F_{3,72}$= 4.684, p <.01. Temperature was way more influential in all stages, as a factor (p < .001), than *Bt*. This study emphasizes the importance of temperature as an ecofactor during combined biological control. Interaction between higher temperatures and *Bt*, within the tolerance limit, may actually be beneficial through faster development during larval stage; although the same cannot be said of the other parameters of its life history.

KEYWORDS : *Bacillus thuringiensis*, *Habrobracon hebetor*, Parasitoids, temperature, biocontrol.

INTRODUCTION

Temperature sets the limits of biological activity in arthropods and regulates population dynamics and seasonal occurrence of insect pests and their parasitoids[7]. Both parasitoids and predators are helpful in regulation of pest population because they are lethal and tend to be density-dependent mortality factor[3]. They are more frequently used than predators in pest suppression programmes[3]. *Habrobracon hebetor* Say[20], (Hymenoptera: Braconidae) attacks many Lepidopteran larvae destructive to stored grain products, mainly moths in the family Pyralidae. It works as a potential biocontrol agent against stored grain Lepidopteran pests, like *Corcyra cephalonica* Stainton[23], (Lepidoptera: Pyralidae) and many others[14,17]. *C. cephalonica* is a primary pest of all cereals, particularly rice, but is also capable of damaging all kind of food grains causing tremendous economic impact[16].

Integration of two or more biocontrol agents are being intensely considered and promoted to complement the effects of each other because of their environmental safety and pest selectivity. The suitability of combining microbial biopesticide, *Bacillus thuringiensis* (*Bt*), and other biological control agents, such as an insect parasitoid, for pest management of stored cereals have been evaluated to a large extent[12,15]. Interactions between parasitoids and their hosts are strongly influenced by changing in the temperature. It affects the parasitoid in several ways directly or indirectly by reducing survival, retarding development or suppressing its reproduction[4,5,13] and likely to influence the successful use of parasitoids. Although the effect of temperature[6,10,21] and several other factors on parasitoids have been studied, the influence of direct *Bt* ingestion, and the interaction involving temperature on the developmental rates of the parasitoid *H. hebetor* is being investigated.

MATERIALS AND METHODS

Insects cultures were maintained (Singh, 2004) from insects obtained from Central Integrated Pest Management Centre (CIPMC), Gorakhpur, Uttar Pradesh, India. Rearing of the pest and parasitoid was done according to Singh[22] *et al.*, 2014, and Mathew[11] *et al.*, 2018. The *Bt* used, was the commercial formulation Dipel DF (*B. thuringiensis* var. *kurstaki*, strain ABTS-351, 32 MIU g^{-1} [millions of International Units per gram]) from Valent Biosciences Corporation, USA.

Mixed untreated grain diet of equal proportions of Wheat, Maize, Rice, and Sorghum was used in rearing as well as control experiments. Whereas, for study of direct effect of *Bt*, similar number of larvae were exposed to a gravid parasitoid female fed on 10% honey solution containing *Bt* at the rate of 500 ìg/ml for 24 hours[9,12]. They were covered with a muslin cloth, kept at constant temperatures of 20, 25, 30 and 35°C, 70 ± 10% relative humidity and 12:12 L:D photoperiod in 10 replicates each. Each replicate was exposed to a gravid female parasitoid for 24 hrs. The beakers were observed after the period for any larval mortality/ parasitization; afterward, the parasitized host larvae were incubated and carefully monitored daily for various life stages[15].

Statistical Analysis

Data from *Bt* treatments on different development parameters were subjected to analysis of variance (One Way and Two Way ANOVA) and mean separation tests were conducted with Tukey's B test using SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA) Statistical Analysis Software. Pearson correlations were also carried out between developmental parameters of *H. hebetor* and temperature.

RESULTS AND DISCUSSION

Temperature rise, in general, caused increase in the development rates of all stages. Regression between increasing temperature and the developmental rates of various life stages of the life history of untreated honey fed *H. hebetor*, highly significant ($p < .01$) good fit of the equation for incubation, larval and total lifecycle period, while significant ($p < .05$) for larval period (Table 1). The extent of correlation was very high (r= .999) between temperature and average developmental rate during total life cycle, showing a very linear relationship. However, under the influence of *Bt*- honey diet, larval period (p >.05) was not a good fit of the equation. R values were overall lower and regression showed no significant fit of the equation for larval periods, however, pupal period and total lifecycle showed highly significant (p <.01) good fit of the equation. Although, correlation of larval development rates to temperature was not significant, as compared to the control; significantly higher development rate was observed towards higher temperatures. Two-way ANOVA to examine the effect of *Bt* treatment and temperature on developmental rates showed statistically significant interaction during larval ($F_{3,72}$= 45.443, p <.001) and pupal periods ($F_{3,72}$= 3.932, p <.05), and no significant interaction during incubation period ($F_{3,72}$= 1.643, p= .187). Similarly, total life cycle also showed significant interaction between factors, $F_{3,72}$= 4.684, p <.01. At all stages, temperature was way more influential, as a factor (p < .001), than the *Bt* treatments.

Table 1. Mean developmental rates of various particulars of life history of *Habrobracon hebetor* at various constant temperatures, and the effect of direct Bt ingestion

Parasitoids directly fed untreated 10% Honey diet						
Variables	**Temperature**				**Regression**	**r-value**
	20°C	**25°C**	**30°C**	**35°C**	**equation**	
Incubation Period	0.25 ±0.00Aa	0.45 ±0.00Ab	0.70 ±0.00Ac	0.80 ±0.02Ad	Y = -0.50 +0.04 X	.987**
Larval period	0.32 ±0.01Aa	0.40 ±0.00Ab	0.55 ±0.01Ac	0.57 ±0.01Ac	Y = -0.04+0.02 X	.969*
Pupal period	0.08 ±0.01Aa	0.12 ±0.02Ab	0.17 ±0.01Ac	0.22 ±0.00Ad	Y = -0.11+0.01 X	.997**
Total Life Cycle	0.05 ±0.00Aa	0.08 ±0.01Ab	0.11 ±0.00Ac	0.13 ±0.01Ad	Y = -0.06+0.01 X	.999**
Parasitoids directly fed 500ug/mL Bt-10% Honey diet						
Incubation Period	0.25 ±0.01Aa	0.44 ±0.01Ab	0.72 ±0.02Ac	0.77 ±0.01Ad	Y = -0.46+0.04 X	.974*
Larval period	0.25 ±0.01Ba	0.32 ±0.01Bab	0.43 ±0.01Bb	1.03 ±0.07Bc	Y = -0.84+0.05 X	.884
Pupal period	0.09 ±0.00Ba	0.11 ±0.00Bb	0.18 ±0.01Bc	0.21 ±0.01Ad	Y = -0.08+0.01 X	.983**
Total Life Cycle	0.05 ±0.00Aa	0.07 ±0.00Bb	0.10 ±0.00Ac	0.14 ±0.00Ad	Y = -0.08+0.01 X	.992**

Means and Standard error followed by different lower case letters in the same row, and uppercase letters in the same column between same variable, are significantly different (P<0.05) using Tukey's B test.

* Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

Females *H. hebetor*, being synovigenic, have very limited number of mature eggs when they emerge, and for production of further eggs depend on adult nutrition and host-feeding[8]. Therefore, any toxin present in the food may get absorbed by the gut and can easily get incorporated into the developing egg. This can, in fact, affect the developmental and life history parameters of the parasitoid progeny[12]. Studies have shown that, *Bt* infection of host larvae increases the content of some amino acids, proteins[12,19] and cause double fold increase in lipid concentration. This may lead to a haemolymph more nutritious than otherwise. The observation that, larval development rates are significantly accelerated when female parent is directly fed *Bt*, leads to the fact that some portion of *Bt* toxins may act as rich protein source for eggs developing in the parasitoid. Higher development rates indicate a higher quality of host larvae[12,18] the variations are indicative of nutritive quality of the host larvae compounded by *Bt* and the influences of temperature. Higher temperatures only mobilize more nutrients, so the observations show a good fit of the linear regression equations, as expected, in most respects.

All physiological functions have optimal thermal ranges within which functions are optimal, and negative effects are seen when temperatures depart from this optimal range[2]. In interacting communities, such as parasitoids and their hosts in a grain storage facility, their closely life histories may be closely linked, but may not share the same optimal temperature ranges, development curves, and upper thermal tolerances[1]. As seen in this study, higher temperatures, within the tolerance limit, may actually be beneficial to the development of the parasitoid; although the same cannot be said of the other parameters of its life history. This warrants further future studies in this direction for a holistic approach-based decision. The constant ambient temperatures, at such sites, has to be taken into account when considering an integrated strategy for using Bt in conjunction with parasitoids.

ACKNOWLEDGEMENTS

The authors thank Prof. J.K. Lal, Secretary and Principal, St. Andrew's College, Gorakhpur; and DDU Gorakhpur University, Gorakhpur, for providing laboratory and library facilities during the PhD work, on which this article is based.

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