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Modulations in the developmental rate of parasitoid, *Habrobracon hebetor* exposed to *Bacillus thuringiensis* during biocontrol of rice moth, *Corcyra cephalonica*

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Abstract

The sublethal effect of *Bacillus thuringiensis* (*Bt*) on the developmental parameters of *Habrobracon hebetor* was assessed during combined biocontrol of stored grain pest, *Corcyra cephalonica*. All stages, except the incubation period, were significantly affected. Correlations were studied between the modulations in developmental rates due to different *Bt* treatments. Correlation for development on host larvae reared on *Bt* LC_{10} diet was highest (r=.998, p<.01) with that of the untreated control. *H. hebetor* was more affected by acute LC_{50} and direct ingestion of *Bt*, than long-term low concentration exposure. The correlation between the effects of acute exposure and direct ingestion was in fact very high (r=.999, p<.01). The experiment illustrates the desirability of using low concentration of the biopesticide in long-term strategy. The study will help to evaluate the use of biocontrol agents in combination with *Bt* to formulate effective strategies for the efficient management of stored product pests like *C. cephalonica*.

Keywords: *Bacillus thuringiensis*; *Habrobracon hebetor*; *Corcyra cephalonica*; Combined biocontrol; Developmental rates

1. Introduction

Pests and pathogens damage more than 40% of the world's crop yield and insects alone contribute to nearly half of that damage. In the scenario of global warming, climate change and regular pest outbreaks, the challenge has always been not only to manage pests, but also to minimize the adverse effects of the various methods to the environment. In India and other developing countries, most of which lie in the warmer latitudes, the problem is aggravated by the highly favorable environment for the insect pests and an ever increasing and expanding human population [1,2]. Stored-grain are severely affected by pyralid moths, such as *Corcyra cephalonica* Stainton, 1866, (Lepidoptera: Pyralidae), a notorious pest as its larval stage responsible for severe damage to stored grains and a wide range of other food commodities in tropical and subtropical regions of the world [3,4]. The urgent need to develop safe alternatives in view of global awareness for the environment, and for the protection of grain and grain products have led to the methods of integrated pest management (IPM). It promotes the deliberate and strategic use of natural regulatory mechanisms, like natural enemies to suppress and regulate a pest population and minimizes the use of synthetic pesticides [5]. Biological control for pest management utilizes natural enemies of pest and therefore a multifaceted approach using a combination of mutually compatible biocontrol agents is a desirable and efficient strategy.

Parasitoids have been found to be very effective biocontrol agent of many pests. *Habrobracon hebetor* Say (1836) (Hymenoptera: Braconidae) [6], is one such cosmopolitan ectoparasitoid which parasitizes the larval stage of several stored-grain pyralid moths [7]. Gravid female *H. hebetor* stings and paralyzes host larvae and lay varying numbers of eggs on the surface or near it. Its considerable range of host species, high reproductive rates and short generation time not only makes it a good biocontrol agent but also an important subject of various bio-control researches on the Lepidoptera-parasitoid system [8]. These qualities make it a very effective natural biocontrol agent against *C.*

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cephalonica. Involving the combination of biopesticides like *Bt*, and a parasitoid in an integrated biocontrol strategy has mostly been successful [9]. The microbial insecticide, *Bacillus thuringiensis* Berliner (*Bt*), is a Gram-positive spore-forming bacteria found in soil, occupying more than 90% of the biopesticide market [10]. As a successful eco-friendly biopesticide against major lepidopteran pests, it has great potential in IPM programmes. Insects belonging to the orders Coleoptera, Diptera and Lepidoptera have been found to be susceptible to the parasporal crystalline inclusions, δ-endotoxins, produced during the sporulation process [11].

In this study, we investigated the effects of *Bt* on the developmental parameters of *H. hebetor* during biocontrol of *C. cephalonica*. Although various studies have been done, the effect of *Bt* in a Lepidoptera-parasitoid system needs more investigation [12]. An ideal pest control strategy should ensure the parasitoid quality and its host profitability when using any compatible biopesticide. It is critically important to ensure the fitness of the gregarious idiobiont ectoparasitoid [13]. This study will help to assess and evaluate the suitability of integrating biological agents with biopesticides, like *Bt*, and to develop appropriate strategies for the control of stored grain pest like *C. cephalonica.*

2. Material and methods

2.1. Insect culture and Lethal concentration assays

All insect cultures, assays, and experiments were conducted at 27 ± 2°C, 70 ± 10% relative humidity and 12:12 L:D photoperiod. Culture methods followed the procedure as described by Singh (2004) and Mathew (2019) [13,14]. Lethal concentration values for Dipel DF (*B. thuringiensis* var. *kurstaki*, strain ABTS-351, 32 MIU g-1 [millions of International Units per gram], a commercial formulation based on *B. thuringiensis*, was calculated following the method used by Oluwafemi et al (2009) [12]. The methods were based on the use of concentrations of *Bt* formulation that would allow survival of sufficient hosts and parasitoids to compare both their survival and development [15]. LC values for 48 hours were obtained for *Bt* on *C. cephalonica* 4th instar larvae. The LC_{50} and LC_{10} values (with 95% confidence limits) were 36.31 (29.95 – 45.70) and 4.80 (3.27 – 5.99) mg/mL respectively and were used in the further experiment [12,14,16].

2.2. *Bt* Treatments

Three treatments with varying *Bt* intake regimes were carried out using ten 4th instar *C. cephalonica* larvae in 500mL beakers with 10g diet following the method by Mathew et al. [17]. It was covered with a muslin cloth and done in 10 replicates each. An untreated setup was also set up to correct mortality. Varying treatments were:

2.2.1. Untreated (control)

C. cephalonica larvae reared on fresh untreated mixed grain diet were exposed to a gravid female parasitoid for 24 hours.

The host-mediated effect of *Bt* on the parasitoid was assessed by two different methods.

2.2.2. Host larvae exposed 4hrs in LC_{50} diet

C. cephalonica larvae reared on fresh untreated mixed grain diet were placed with *Bt* treated mixed grain diet at LC_{50} then after 4 hours exposed to a gravid female parasitoid for 24 hours.

2.2.3. Bt-LC_{10} reared larvae

C. cephalonica larvae reared on *Bt* LC_{10}-treated mixed diet were exposed to a gravid female parasitoid for 24 hours.

Direct effect of *Bt* on *H. hebetor* was assessed using treated parasitoid diet.

2.2.4. Bt-treated parasitoid diet

C. cephalonica larvae reared on fresh untreated mixed grain diet were exposed to a gravid parasitoid female fed on 10% honey solution containing *Bt* at the rate of 500 μg/ml for 24 hours [18].

The experiments were observed after 24 hours for any larval mortality/ parasitization afterward the parasitized host larvae were incubated and carefully monitored daily for larvae, pupae and adult emergence [12].

2.3. Statistical Analysis

Data from *Bt* treatments on different development parameters of *H. hebetor* were subjected to analysis of variance (One 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.

3. Results and discussion

Bt treatments significantly affected the developmental rates in all stages except the incubation period. Development rates was generally relatively higher in parasitoid treated with host larvae reared on *Bt* LC_{10} diet as compared to other treatments (Table.1). Incubation rate was insignificantly lengthened by *Bt* treatments ($F_{(3,36)}$ = 1.77, p= .169). However, larval development rate significantly varied among treatments ($F_{(3,36)}$ = 12.93, p < .001), significantly lowered on 4hr *Bt* LC_{50} exposed and directed *Bt*-fed treatments but did not vary significantly from *Bt* LC_{10} treatment. Pupal developmental rates were more or less same among treatments ($F_{(3,36)}$ = 2.86, p = .05) but was slightly high for parasitoids parasitizing host larvae reared in *Bt* LC_{10} and directly *Bt*-fed ones. The overall average development rates also showed the significant effects of *Bt*.

Table 1 Effect of varying *Bt* treatment on development rate of *Habrobracon hebetor*

Development rates	**Untreated**	***Bt* treated**		
		Host larvae exposed 4hrs in LC_{50} diet	**Host larvae reared on LC_{10} diet**	**Parasitoid directly fed *Bt*-Honey diet**
Incubation rate	0.67 ±0.01a	0.70 ±0.01a	0.69 ±0.04a	0.70 ±0.70a
Larval development rate	0.52 ±0.02b	0.38 ±0.03a	0.57 ±0.03b	0.41 ±0.02a
Pupal development rate	0.166 ±0.004a	0.159 ±0.004a	0.172 ±0.004a	0.173 ±0.002a
Total development rate	0.106 ±0.002b	0.097 ±0.003a	0.110 ±0.002b	0.103 ±0.001ab

Means and Standard error followed by different letters in each row are significantly different (*P*<0.05) using Tukey's B test.

Table 2 Multiple correlation between development rate of *Habrobracon hebetor* under different *Bt* treatments

Variables	**Untreated Parasitoids (Control)**	**Host larvae exposed 4hrs LC_{50} diet**	**Host larvae reared on LC_{10} diet**	**Parasitoid directly fed *Bt*-Honey diet**
Untreated Parasitoids (Control)	1	0.965*	0.998**	0.974*
Host larvae exposed 4hrs LC_{50} diet	0.965*	1	0.948	0.999**
Host larvae reared on LC_{10} diet	0.998**	0.948	1	0.960*
Parasitoid directly fed *Bt*-Honey diet	0.974*	0.999**	0.960*	1

*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

There was a positive correlation between total developmental rate and larval development rate across different *Bt* treatments, although it was not statistically significant (r = .870, p > .05) (Table 2). Multiple correlations between different *Bt* treatments on development rates of *H. hebetor* showed that development rates on all *Bt* treatments were highly positively correlated with each other. Parasitoid development on host larvae reared on *Bt* LC_{10} diet was the most correlated with the control, showing statistically significant positive correlation (r = .998, p <.01). Highly significant positive correlation between parasitoid development on host larvae exposed for 4hr in LC_{50} diet and those directly fed *Bt*-honey diet (r = .999, p <.01) shows the similarity in the effect of host-mediated and direct *Bt* treatment.

In this study *H. hebetor*, the natural enemy of *C. cephalonica*, showed some significant effect of *Bt* on its developmental rates in life history. Integrated pest management involving the combination of *Bt* and a natural enemy has been successful in reducing pest population without apparent negative effect on the life history of parasitoid [19]. However, this microbial pathogen can act only against the feeding stages of target pests. Therefore, the complexity of insect ecology and their behavior can severely impair the efficiency of *Bt* when used alone [20]. Environmental factors also include the quality and quantity of the food sources provided by the host, and they have an impact on both immature stages during development and some physiological aspects in adults [21]. Generally, any host species can be considered nutritionally suitable if it allows a parasitoid species to develop until maturity [22]. Insect growth, development, and reproduction are positively correlated with the amount and quality of ingested food [14,23].

In the experiments, it was observed that certain developmental stages of the parasitoid were significantly affected by the *Bt*-contaminated host and direct feeding of *Bt*. Host larvae reared on LC_{10} were not severely affected, whereas host larvae in LC_{50} (4hr exposure) became highly intoxicated and thereby reducing the percentage of host larvae exposed to the parasitoid to lay eggs upon. Total lifecycle also showed the similar trend [16] and the same was observed in the development stages as larval period and pupal period where prolonged on severely *Bt* intoxicated host larvae, as reported by Salama et al. (1991) [24] in their study with Bracon brevicornis, suggesting that *Bt* toxins retard growth severely, as the parasitoid lacks sufficient resource upon which to develop [14,25,26]. No matter what the mechanism of interaction between *Bt* and *C. Cephalonica* is, if a parasitoid initially survived, *Bt* had a minor effect on parasitoid development [27], but in the severely intoxicated host and parasitoid fed directly on *Bt* diet, the prolonged development period and effect on longevity showed interaction with *Bt* toxins.

4. Conclusion

The potential direct effect of *Bt* on larval parasitoid within their hosts and the indirect host-mediated effect on parasitoid in a Lepidoptera-parasitoid system does not undermine the strategy of their combined use. Especially, as revealed by the results when host larvae are reared in a low sublethal dose of *Bt*, parasitoids are not much affected and attacks any host larvae that may have survived after its initial instar stages which are very *Bt*-susceptible. The results will be useful for IPM strategies in the field and warehouses application of *Bt* formulations. The study will also help to evaluate and formulate strategy for pest management in stored grains by employing the synergistic effect of combined treatment. Since *Bt* does not prevent parasitoid development and moreover their lethal effects are additive to each other, a combined treatment with *Bt* and parasitoid release could produce better protection against insect pest than either treatment when used singly.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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