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EFFECT OF *BACILLUS THURINGIENSIS* BERLINER VAR. *KURSTAKI* ON THE LIFE HISTORY OF *HABROBRACON HEBETOR* SAY (HYMENOPTERA: BRACONIDAE)

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ABSTRACT: We examined the potential direct and indirect host-mediated effect of *Bacillus thuringiensis* (Bt) on the developmental aspects of parasitoid *Habrobracon hebetor*, parasitizing *Corcyra cephalonica,* in a Lepidoptera-parasitoid system. Bt variously affected parasitoid developmental stages based on the treatments but none stopped development. Fecundity and progeny sex ratio was significantly lowered by host-mediated Bt exposure but direct Bt ingestion affected only the latter. Bt did not affect incubation and pupal period but the larval period and total lifecycle was prolonged by host-mediated acute Bt exposure and direct Bt ingestion. Host reared in Bt diet shortened larval period and total lifecycle. Female longevity was significantly enhanced by Bt, on host reared in Bt diet, conversely, male longevity was reduced by host-mediated Bt exposure and slightly enhanced by direct Bt ingestion. Except for larval period the development rates were unaffected. Although these aspects highlight the interaction between Bt toxins and Lepidoptera-parasitoid system, it certainly does not undermine the strategy of their combined use, especially as revealed by host reared in low sublethal Bt dose. The study will help to evaluate and formulate appropriate strategies for combined control and offer new avenues for the effective management of stored product pests, like *C. cephalonica*.

KEYWORDS: *Bacillus thuringiensis*; *Habrobracon hebetor*; pest management; *Corcyra cephalonica;* Lepidoptera-parasitoid system; combined biocontrol

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1.INTRODUCTION

Insect pests have always been a major challenge facing mankind in the provision of food. Particularly in the tropics and sub-tropics, where most developing countries lie, the problem increases manifold due to a favorable warmer environment suitable for a wide range of insects [1] and global climate change [2]. The highly conducive environment of agroecosystem and the remarkable ability of insect pests to adapt and evolve biotypes further aggravate the problem [3]. One-fifth of the world's total crop production is estimated to be damaged by insects annually. The losses in storage range from approximate 10% of the production in developing countries, like India, to about 25-40% in Sub-Saharan Africa particularly at the farm level [4,5,6]. Commercial formulates based on *Bacillus thuringiensis* (Bt) [7] has emerged as the most successful microbial pesticide having great potential in biocontrol programmes modern integrated pest management (IPM) [8,9,10]. It has been used to control several insect pests successfully [11] and has become an alternative to chemical strategies due to its specificity, regulation of pest population and non-persistence in the environment. *Bt* is a Gram-positive, soil bacteria, producing one or more inclusion bodies of δ-endotoxins during sporulation, which have been found to be toxic for invertebrates, primarily insect species in the orders Coleoptera, Diptera and Lepidoptera [12,13,14]. It is also a key source of genes providing pest resistance in genetically modified plants and microorganisms by transgenic expression of δ-endotoxins [15,16,17,18,19]. In accordance with the philosophy and methodology of IPM, the concept of biological control is based on "enemy release hypothesis" [20] which involves the deliberate use of biotic agent to suppress and/or regulate a pest population [21,22]. The ability of self-perpetuation [23], lack of resistance [24] and lack of adverse side effects [25,26,27] gives them a distinct advantage over other pest control methods [18]. A better strategy for a successful biological control may require using more than one natural enemy [28]. Synergistic interactions have often been observed when biopesticides have been used in combination with other natural enemies. Bt treatment may be used to complement the effects of other biological control agents, such as parasitoids, because of their environmental safety and pest selectivity [8,19,29]. *Habrobracon hebetor* [30] (Hymenoptera: Braconidae), is one such gregarious, cosmopolitan ectoparasitoid of the larval stage of several stored-grain pyralid moths [31,32]. Stored-product moths, such as *Corcyra cephalonica* Stainton, 1866, [33] (Lepidoptera: Pyralidae), are among the most destructive insects of stored grain, processed food and a broad range of other food commodities throughout the world [34,35,36,37,38,39,40]. *H. hebetor* is a promising natural enemy against *C. cephalonica* [41,42]. High reproductive rate, short generation time and considerable range of host species make it a potent agent for biocontrol of stored product pests, and a mass-produced parasitoid suitable for study of host-parasitoid system and various bio-control research as well [42,43,44,45]. Gravid *H. hebetor* females prefer to attack last instar host larvae by stinging and lay variable numbers of eggs on or near the surface after it becomes paralyzed [41,46]. The suitability of combining Bt and other biological control agents, such as an insect parasitoid, for pest management of stored cereals have been evaluated by laboratory assays [18,47]. Previous studies on strategies involving the combination of *Bt* and parasitoids have shown varying effects of *Bt* on pest and its natural enemies. Satisfactory control of lepidopterous pests by integrating *B. thuringiensis* with a parasitoid has been reported [8,48] without apparent negative effects on parasitoid life history [47,49,50]. Bt insecticide, such as Dipel, have been recommended for use in integrated control programs [51]. High reduction of *P. interpunctella* population has been reported by applying a combination of *Bt* with *H. hebetor* as a biological control agent and the use of Bt in combination with a parasitoid has been recommended for control of other lepidopteran pests [47]. However, *Bt* has also been shown to affect certain parasitoids adversely by causing reduced parasitism [52], lower survival rates of parasitoid due to premature host death [53] and lower parasitoid emergence rates [54,55,56]. It may affect larval developmental period [57,58] and alter parasitoid sex ratios [47,59,60]. *H. hebetor* is a potent biocontrol agent against *C. cephalonica* [42,61] preferring later instars and combination of *Bt* with *H. hebetor* as biocontrol agent resulted in a high reduction of *C. cephalonica* population [62]. *Bt* can, therefore, be used along with parasitoid in combined treatment of *C. cephalonica* and other lepidopteran pests [63]. Biopesticides like Bt have moved out of narrow “niche” biological control products into the mainstream of forest and commercial farming. Understanding interactions between parasitoids and pathogens such as Bt are becoming increasingly important as integrated pest management regimes involving combinations of biocontrol agents are being used more frequently [60]. Considerable work has been directed towards assessing effect of Bt products for the risks associated with its use and its potential to affect biological control agents and other non-target organisms [47,64]. Sublethal effects of Bt on other trophic levels, such as parasitoids of the target and nontarget pests, can range from synergistic to competitive depending on various interacting factors [65] but needs to be well understood [66]. Although various Pyralid hosts have been used in the studies done to investigate the effect of Bt on *H. hebetor* in a Lepidopteran host-parasitoid system, *C. cephalonica* has not been much used. Interference of *Bt* with reproduction and development of *H. hebetor* using *C. cephalonica* in the Lepidoptera-parasitoid system needs to be correctly assessed so as to utilize the full potential of such combined treatment for pest control. Parasitoid foraging or oviposition may not only be influenced by attributes of the Bt-infected host but also by the direct or indirect effect of the pathogen itself [60,67]. In this study, we examined the potential direct and indirect effect of Bt on the aspects of life history of the parasitoid, *H. hebetor*, in a Lepidoptera-parasitoid system under laboratory conditions. Bt-intoxicated and Bt-reared *C. cephalonica* larvae were used to evaluate indirect host-mediated effects on parasitoid. Effect of direct treatment of Bt, through Bt-honey feed, on the parasitoid was also evaluated. 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. For this, we used laboratory assays with host Lepidoptera and parasitoid that were reared on artificial diets, to which commercial Bt formulation was added [68]. Such information is necessary to assess the suitability of integrated systems based on biological agents with different modes of action, like Bt-parasitoid combination, and to develop appropriate strategies for development and deployment for the control of stored grain pest like *Corcyra cephalonica* [69]. This should offer new insight into the effective management of stored grain pests in line with the concept of integrated pest management (IPM).

2. MATERIALS AND METHODS

All insect cultures and experiments were conducted at 27 ± 2°C, 70 ± 10% relative humidity and 12:12 L:D photoperiod. Culture methods were based on established methods [70,71].

Rearing of the pest

Eggs of stored grain pest, *Corcyra cephalonica*, rice moth, were obtained from the Central Integrated Pest Management Centre (CIPMC), Gorakhpur. The culture was kept and maintained coarsely ground mixed grain diet in plastic containers (45cm × 25cm × 15cm). The containers were observed daily and the nutrient was replenished regularly after consumption and damage by the larvae. Newly emerged males and females adults were paired in a beaker (250ml) covered with a black muslin cloth. The collected eggs were again kept with fresh nutrient in plastic containers. A mass culture of *C. cephalonica* was, thus, maintained. After 3-4 generations, full-grown larvae of rice moth from this culture were taken to maintain the parasitoid *Habrobracon hebetor* culture [42,70,71]. Larvae were also reared in mixed grain diet with Bt at LC_{10} for use in the experiment. The 4th instar larvae were preferred over the 5th in the Bt treatment experiments as the latter becomes less active and stops feeding thereby compromising the Bt ingestion required.

Rearing of the parasitoid

Adults of *Habrobracon hebetor* Say. (Hymenoptera: Braconidae), were collected from the CIPMC, Gorakhpur. Male and female insects were paired in a beaker (250 ml) having 10 full grown Vth instar larvae of *Corcyra cephalonica*, covered with a fine muslin cloth. The adults were provided 30% honey solution as food [72,73,74,75] through a thin glass tube having honey mixed with distilled water and plugged with cotton. After parasitization, the parasitoids were withdrawn from the beaker and hosts were kept separately for further development. After completing its development, the new generation of adult wasps was paired again in a similar manner for fresh egg laying. After the third generation, the adults were utilized in the experiments [42,71].

Bacillus thuringiensis

Commercial formulation based on *B. thuringiensis* selected for the assays was Dipel DF (*B. thuringiensis* var. *kurstaki*, strain ABTS-351, 32 MIU g^{-1} [millions of International Units per gram]).

Bt treated diets

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 [68]. 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 [47]. Bt-treated parasitoid diet was prepared using 10% honey solution containing *B. thuringiensis* var. *kurstaki*, at the rate of 500 µg/ml [76].

Bt treatment of *H. hebetor*

In this experiment, there were four treatments including control, with each bioassay being carried out using ten *C. cephalonica* larvae of 4th instar in 500mL beakers with 10g diet. It was covered with muslin cloth, kept at 27 ± 2ºC, 70 ± 10% relative humidity and 12:12 L:D in 10 replicates each [71].

1. *Untreated (control)* - *C. cephalonica* larvae reared on fresh untreated mixed grain diet were exposed to a gravid female parasitoid for 24 hours.

   The effect of Bt on the parasitoid was assessed by two different methods. In the first method, the host-mediated indirect effect of Bt on *H.hebetor* was assessed by allowing it to parasitize two variations of Bt-treated *C. cephalonica* larvae.
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.
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.

   In the second method, the direct effect of Bt on *H.hebetor* was assessed using treated parasitoid diet.
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 *B. thuringiensis Kurstaki*, at the rate of 500 µg/ml for 24 hours [76].

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 [47,71].

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 HSD using SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software.

3. RESULTS AND DISCUSSION

Results

Fecundity of parasitoid *H.hebetor* was found to be significantly reduced by Bt and its mode of intake ($F_{(3,36)} = 24.95$, $p < .001$) (Table 1). It was significantly low with a mean ± SE of 23.20 ±0.77, $p < .001$, when host larvae exposed 4hrs in LC_{50} diet was used, followed by Bt LC_{10} reared host larvae (57.40 ±4.22, p< .05), whereas the mean fecundity in direct Bt-honey diet fed parasitoids (68.20 ±5.91, p=.53) was not significantly lower than in untreated control (Fig 1).

Table 1. Effect of varying Bt treatment on reproduction and development of *H. hebetor*

Variables	**Untreated Parasitoids** (Control)	**Bt treated Parasitoids**		
		Host larvae exposed 4hrs in LC_{50} diet	Host larvae reared on LC_{10} diet	Fed directly on 500µg/mL Bt-Honey diet
Fecundity	74.20 ±5.47a	23.20 ±0.77b	57.40 ±4.22c	68.20 ±5.91ac
Progeny sex ratio	0.53 ±0.01a	0.22 ±0.09b	0.43 ±0.05ac	0.24 ±0.03bc
Male (%)	46.61	78.18	56.81	76.25
Female (%)	53.39	21.82	43.19	23.75
Incubation period*	1.49 ±0.02a	1.42 ±0.03a	1.45 ±0.02a	1.43 ±0.02a
Larval period*	1.95 ±0.05a	2.80 ±0.23b	1.80 ±0.08a	2.50 ±0.11b
Pupal period*	6.05 ±0.14a	6.30 ±0.15a	5.85 ±0.15a	5.80 ±0.08a
Total lifecycle (egg to adult)*	9.50 ±0.18a	10.40 ±0.31b	9.10 ±0.16a	9.70 ±0.13ab
Male adult longevity*	5.60 ± 0.45a	2.80 ±0.25b	3.20 ±0.25b	5.90 ±0.43a
Female adult longevity*	23.00 ±2.53a	28.80 ±2.72ab	34.40 ±1.69b	30.00 ±2.53ab

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

Parasitoid progeny sex ratio also showed significant reduction in all Bt treatments ($F_{(3,36)} = 8.21$, p < .001), it was lowest in Bt exposed (0.22 ±0.09, p< .05) followed by direct Bt-fed (0.24 ±0.03, p< .05) parasitoids as compared to the untreated control (0.53 ±0.01), with the Bt reared showing no statistically significant difference (0.43 ±0.05, p=.54) (Fig 2). Bt treatments similarly decreased incubation period and was shortest in Bt exposed parasitoids (1.42 ±0.03, p=.17) but there was no

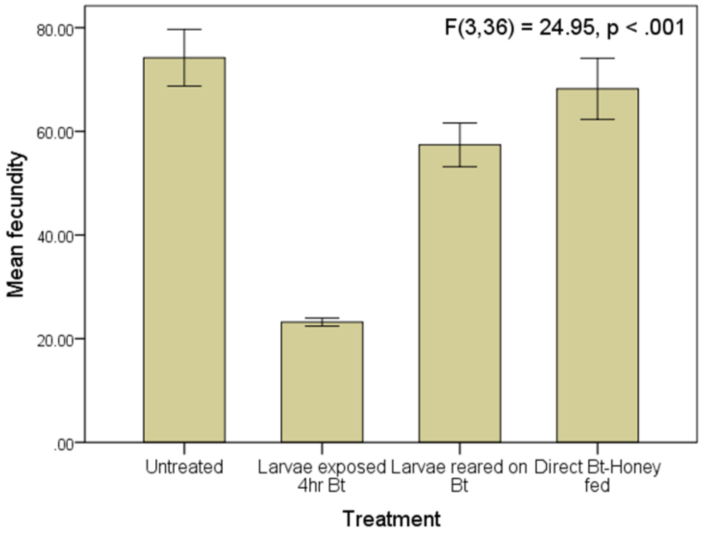

Fig 1. Mean fecundity of *H. hebetor* under varying Bt treatment

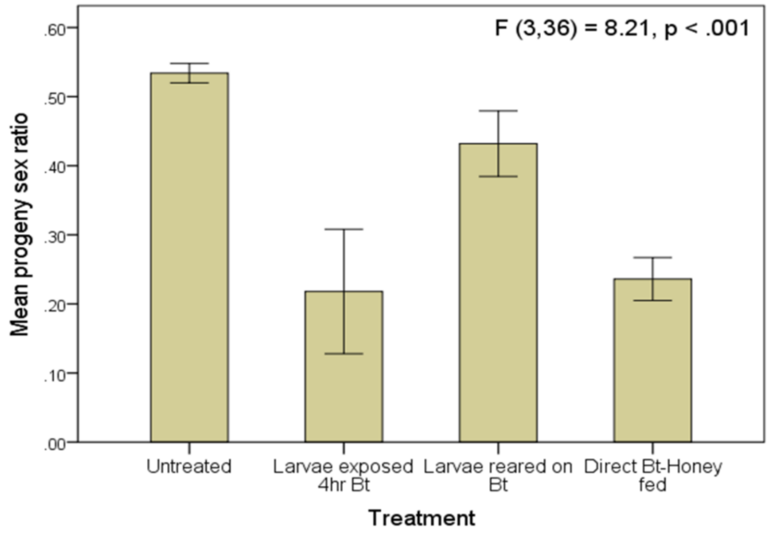

Fig 2. Progeny sex ratio of *H. hebetor* under varying Bt treatments

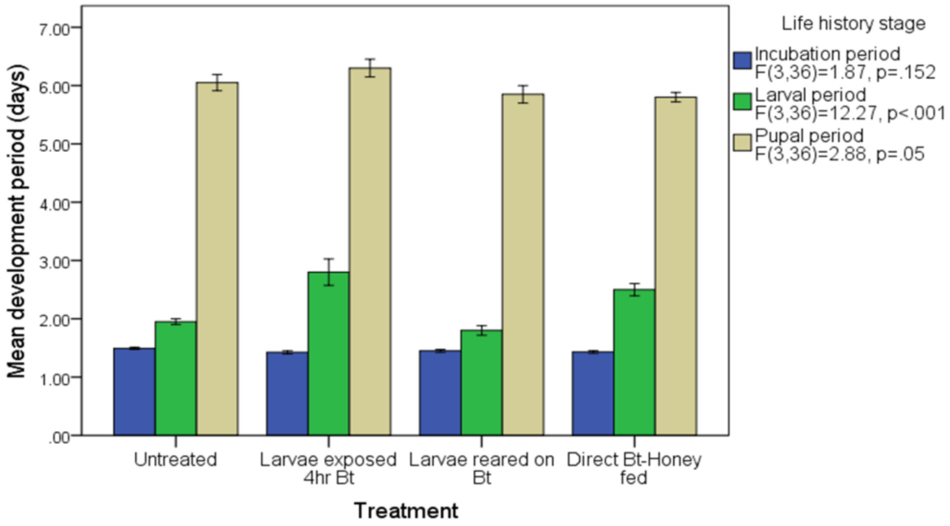

Fig 3. Mean development period of life history stages of *H. hebetor* under varying Bt treatments

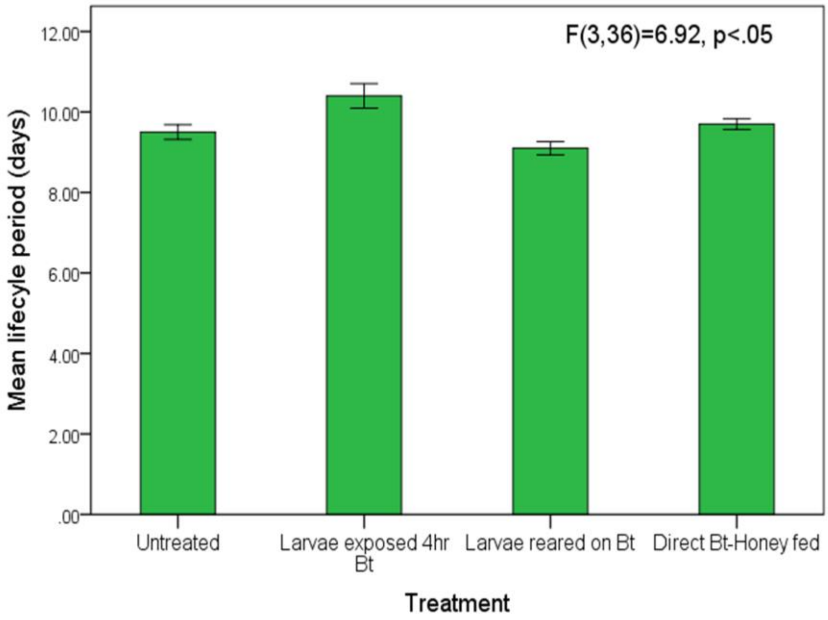

Fig 4. Mean lifecycle period of *H. hebetor* under varying Bt treatments

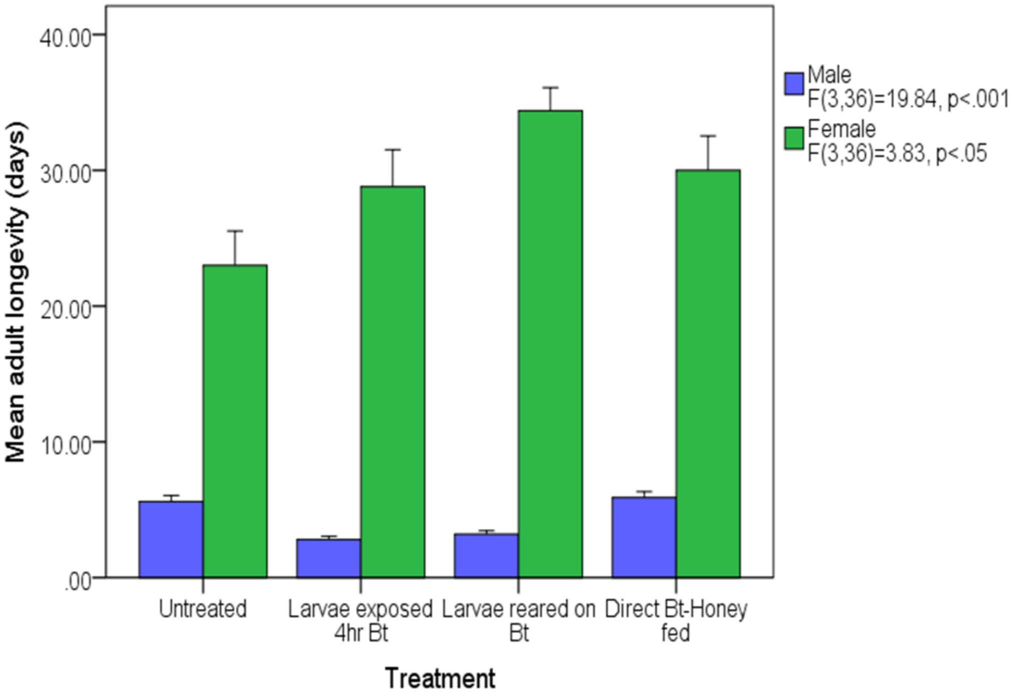

Fig 5. Mean adult longevity of *H. hebetor* under varying Bt treatments

statistically significant difference ($F_{(3,36)} = 1.87$, p=.15) among them (Fig 3). Significant lengthening of larval period ($F_{(3,36)} = 12.27$, $p< .001$) was observed in Bt exposed (2.80 ±0.23, $p< .001$) followed by direct Bt fed parasitoids (2.50 ±0.11, $p< .05$) (Fig 3). Pupal periods did not vary significantly ($F_{(3,36)} = 2.88$, p=.05) and was shortest in direct Bt-fed *H.hebetor*. Interestingly pupal period of Bt exposed parasitoids was longer than even the control (Fig 3). Bt treatment significantly affected the life cycle duration (egg to adult) of parasitoid ($F_{(3,36)} = 6.92$, $p< .05$). It was longer in Bt exposed parasitoids significantly (10.40 ±0.31, $p< .05$), followed by direct Bt fed ones. *H. hebetor* parasitizing Bt reared host, whereas, showed slight shortening of lifecycle period (Fig 4). The life history stage most affected by Bt was the larval period in all Bt treatments except where host larvae were reared on Bt LC_{10} diet. .Adult longevity was significantly affected by Bt treatments in both males ($F_{(3,36)} = 19.84$, $p< .001$) and females ($F_{(3,36)} = 3.83$, $p< .05$) (Fig 5). Host-mediated indirect Bt treatment significantly shortened the lifespan of males, with the lowest in Bt exposed (2.80 ±0.25, $p< .001$) followed by Bt reared (3.20 ±0.25, $p< .001$), except in direct Bt fed (5.90 ±0.43, p= .93) parasitoids where the longevity was slightly more than that in control. Female longevity increased in all Bt treatments showing a significant effect of Bt with the Bt reared parasitoids showing significantly enhanced longevity (34.40 ±1.69, $p< .05$).

Table 2. Effect of varying *Bt* treatment on development rate of *H. hebetor*

Development rates	Untreated	Bt treated		
		Host larvae exposed 4hrs in LC_{50} diet	Host larvae reared on LC_{10} diet	Parasitoid directly fed on 500µg/mL Bt-Honey diet
Incubation Period	0.67 ±0.01a	0.70 ±0.01a	0.69 ±0.04a	0.70 ±0.70a
Larval period	0.52 ±0.02a	0.38 ±0.03b	0.57 ±0.03a	0.41 ±0.02b
Pupal period	0.166 ±0.004a	0.159 ±0.004a	0.172 ±0.004a	0.173 ±0.002a
Total Life Cycle (Male)	0.108 ±0.002a	0.095 ±0.002b	0.108 ±0.002b	0.103 ±0.001b
Total Life Cycle (Female)	0.106 ±0.002a	0.097 ±0.003b	0.110 ±0.002a	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.

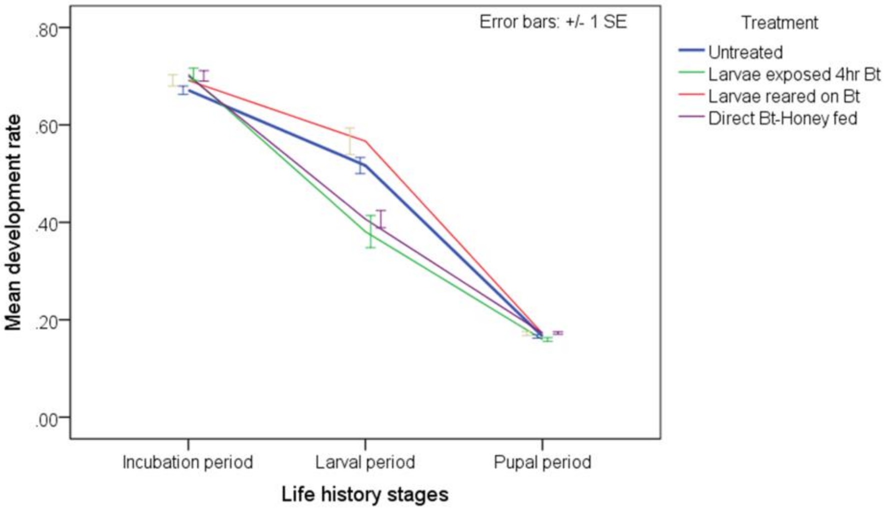

Fig 6. Effect of varying *Bt* treatments on development rate of *H. hebetor*

Development rates of the parasitoid also showed the similar trend (Table 2). It was generally relatively higher in parasitoid treated with host larvae reared on Bt LC_{10} diet as compared to other treatments (Fig 6). Larval period significantly varied among treatments.

DISCUSSION

In this study *H. hebetor*, the natural enemy of *C. cephalonica,* showed some significant effect of Bt on its life history. It has added to the understanding of the interaction of biopesticide Bt with other trophic levels when used against insect pests. 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 [50]. 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 [77]. In addition to environmental factors such as temperature, light, and humidity, the quality and quantity of the food sources provided by the host have an impact on both immature stages during development and some physiological aspects in adults [78]. Generally, any host species can be considered nutritionally suitable if it allows a parasitoid species to develop until maturity [79,80]. Insect growth, development, and reproduction are positively correlated with the amount and quality of ingested food [81,82]. 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 honey diet fortified with Bt. Highest fecundity (egg laid per female) was observed on host reared on LC_{10} in comparison to host larvae exposed for 4 hr in LC_{50} diet, whereas there was no significant difference in the performance of the parasitoid on the untreated host and parasitoid fed directly on Bt mixed with honey diet (Table 1). 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. Parasitoids, like *H. hebetor,* are known to withhold or reduce the number of eggs laid in the absence of a host [77,83] or quality of host [79]. Progeny sex ratio was significantly affected in host larvae exposed to Bt for 4hr and parasitoid fed directly on Bt diet. It was female-biased in untreated diet and highly male-biased in parasitoid exposed to Bt LC_{50} and those directly fed on Bt diet. Observations on the proportion of males in progeny suggest a possible effect of host larval nutrition on sex ratio as in life expectancy and adult emergence in the same species [84]. Though this study is not in full accordance with the observations made by a few workers [77], where the progeny sex ratio was female-biased in both treatments, the study is suggestive that parasitoid directly fed on Bt diet has no effect on fecundity but the progeny was highly male-biased thus showing that Bt substantially affects the female to bias their offsprings towards male. This study showed that the female longevity was significantly affected by Bt when compared with untreated diet [85] but there was no significant difference in the longevity of female adult parasitoid when comparing varying Bt treatments (Fig 5). There was no indication that Bt directly affected adult parasitoid longevity when directly fed on honey solution fortified with Bt in both male and female when compared to the control. Total lifecycle also showed the similar trend and the same was observed in the development stages as larval period and pupal period where prolonged on severely Bt intoxicated host larvae as in the study with *Bracon brevicornis* [85], suggesting that Bt toxins retard growth severely as the parasitoid does not have sufficient resource upon which to develop [67,86]. Regardless of the mechanism of interaction between Bt and *C. Cephalonica,* if a parasitoid initially survived, Bt had a minor effect on parasitoid development [60], 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 using the 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 very Bt-susceptible initial instar stages. The results reveal several aspects that may affect the outcome of IPM strategy in the field and warehouses application of Bt formulations. The study can be utilized to evaluate and formulate strategy for pest management of stored cereals 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 because.

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CONFLICT OF INTEREST

None

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