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Effect of *Bacillus thuringiensis* on oviposition and parasitization by parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) on *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae)

Mathew Isaac L[1], Singh Deepak*[2] and C P M Tripathi[3]

[1,2] Department of Zoology, St Andrews College, Gorakhpur, Uttar Pradesh, India
[3]Department of Zoology, DDU Gorakhpur University, Gorakhpur, Uttar Pradesh, India

ABSTRACT

We examined the sublethal effect of *Bacillus thuringiensis* (*Bt*) on the biocontrol efficiency of *Habrobracon hebetor* during combined biocontrol of stored grain pest, *Corcyra cephalonica*. Parasitization and mortality of the host by parasitoid was investigated under various *Bt* diets and parasitoid combinations. *Bt* treatments reduced parasitization but increased the mortality. Acute *Bt* treatment at LC_{50} showed the highest mortality (73.0 ± 3.00) with least relative parasitization (34.6 ±3.36). *H. hebetor* tended to maximize profitability and showed tradeoff between oviposition and paralyzation. The parasitoid's enhanced attack response to vigorous less contaminated hosts, the ability to recognize and withhold oviposition on contaminated hosts, and the opportunity offered due to host's weakened defense mechanisms seem to play an important role. *Bt* is known to affect various aspects of Lepidoptera-parasitoid and assessing the risks associated with the use of *Bt* products and their potential sublethal effect on non-target organisms, including biological control agents, is a priority. The study will help to evaluate and assess the use of combined biocontrol, agents to formulate effective strategies for the efficient management of stored product pests like *C. cephalonica*.

KEYWORDS :Bacillus thuringiensis, Habrobracon hebetor, Corcyra cephalonica, combined biocontrol

[*]Corresponding Author

Dr. Deepak Singh

Assistant Professor

Dept of Zoology, St Andrew's College

Gorakhpur, UP, India 273001

Email: ceaesac@gmail.com

Mob: +91 9793816620

INTRODUCTION

Pests and pathogens damage over 40% of the world's crop yield and insects alone contribute to nearly half of that damage[1,2]. In the scenario of global warming, climate change and regular pest outbreaks [3] the emerging challenge is not only to manage pests but also to minimize the damage 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[4,5]. Stored-grain pyralid moths, such as *Corcyra cephalonica* Stainton, 1866, (Lepidoptera: Pyralidae)[6], are notorious pests as its larval stage is responsible for severe damage to stored grains and a wide range of other food commodities in tropical and subtropical regions of the world. [7,8,9,10].

The challenges of pesticide resistance, secondary pest outbreaks, risk to natural enemies and non-target species, environment pollution due to pesticide residues and public health concerns have forced us to look for other alternatives to conventional insecticides[11,12,13]. The urgent need to develop safe alternatives for the protection of grain and grain products has led to the methods of integrated pest management (IPM). It promotes the deliberate use of natural regulatory mechanisms, like natural enemies to suppress and regulate a pest population[14,15] and minimizes the use of synthetic pesticides[12]. 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 are very effective biocontrol agent of many pests. *Habrobracon hebetor* Say (1836) (Hymenoptera: Braconidae)[16], is one such cosmopolitan ectoparasitoid which parasitizes the larval stage of several stored-grain pyralid moths[17,18]. Gravid female *H. hebetor* stings and paralyzes host larvae and lay varying numbers of eggs on the surface or near it [19,20]. 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 [21,22,23,24]. These qualities make it a very potent natural biocontrol agent against *C. cephalonica* [20,24].

Greathead (1995)[25] has suggested integrating biological and chemical control in various ways, one of which is using selective insecticides and natural enemies[26]. 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[27,28]. As a successful eco-friendly biopesticide against major lepidopteran pests, it has great potential in IPM programmes[29,30]. 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[31,32,33]. Involving the combination of *Bt* and a parasitoid in an integrated biocontrol strategy has mostly been successful[29,34,35,36]. Normally *H. hebetor* paralyzes far more number of host larvae than needed for oviposition/ parasitization. It returns afterward and oviposits on only a few paralyzed larvae[37]. Unparasitized paralyzed larvae may continue to live for nearly a month before death.

In this study, we investigated the effects of the combination of Bt with *H. Hebetor* on the mortality and extent of parasitization of *C. Cephalonica* under various Bt diets. Although various studies have been done on combining *Bt* with the parasitoid, the effect of *Bt* in a Lepidoptera-parasitoid system is yet to be fully understood[38,39,40]. Moreover, emphasis should be paid to the sublethal effects of *Bt* on the behavior and the biology of parasitoids and predators[41]. Since host quality strongly influences the preferences of the parasitoid, the ability to successfully identify and determine host quality and profitability is of prime importance for the fitness of the gregarious idiobiont ectoparasitoid[20,42,43]. This 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*

MATERIALS AND METHODS

All insect cultures, assays, and experiments were conducted at 27 ± 2^{o}C, 70 ± 10% relative humidity and 12:12 L:D photoperiod. Culture methods followed the procedure as described by Singh (2004)[44].

Rearing of the pest

Eggs of *C. cephalonica* were obtained from the Central Integrated Pest Management Centre (CIPMC), Gorakhpur and allowed to develop in coarsely ground mixed grain diet in large plastic containers of size 45cm × 25cm × 15cm [24,30]. Emerging males and females were paired in a beaker (250ml) covered with a black muslin cloth. The collected eggs were again used for culture. After 3-4 generations, full-grown larvae were used for culture of the parasitoid *H. hebetor*. Larvae were also reared in mixed grain diets fortified with *Bt* at LC_{10} and LC_{25}. 4th instar larvae were used in mortality experiments[45,46].

Rearing of the parasitoid

Adults of *H. Hebetor* were collected from the CIPMC, Gorakhpur. Males and females were paired in a beaker (250ml) having 10 full grown 5th instar larvae of *C. cephalonica*, covered with a fine muslin cloth[24,30]. 30% honey solution was provided as food[47,48,49,50]. After parasitization, hosts were kept separately for further development of the parasitoid. The new generation of parasitoids was paired again similarly, and the third generation the adults were used for the parasitization and mortality experiments[24,30,45,46].

Estimation of lethal concentrations and preparation of diet

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 used for the lethal concentration assays and mortality experiments. Estimation of lethal concentrations parameters of *Bt* on *Corcyra cephalonica* followed the method used by Oluwafemi et al (2009)[40]. Larval mortality was recorded after 24, 48 and 72 hours of initial inoculation. Lethal concentration for 48 hours was used in further experiments[40]. The LC_{50}, LC_{25} and LC_{10} values (with 95% confidence limits) of *Bt* on *C. cephalonica* 4th instar larvae were 36.31 (29.953 – 45.704), 12.52 (10.46 – 14.81) and 4.80 (3.64-5.99) mg/ mL respectively. LC_{50}, $LC_{25,}$ and LC_{10} *Bt* diets were prepared using these concentrations.

Effect of combining Bt and Parasitoid on Corcyra Cephalonica Larval Mortality

Five treatments with varying combination of *Bt* and parasitoid were carried out using ten 4^{th} instar *C. cephalonica* larvae in 500mL beakers with 10g diet following the method by Mathew et al. (2018)[45,46]. 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:

***Bt* treatment.** Larvae were placed with *Bt* treated diet at LC_{50}.

Parasitoid treatment. Larvae were placed with untreated diet then after 4 hours exposed to gravid female parasitoid for 24 hours.

***Bt*-parasitoid combined treatment.** Larvae were placed with *Bt* LC_{50} -treated diet then after 4 hours exposed to gravid female parasitoid for 24 hours.

***Bt* LC_{10} reared larvae-parasitoid combined treatment.** Larvae reared on *Bt* LC_{10} -treated diet were placed with *Bt* LC_{10} –diet for 4 hours and then exposed to gravid female parasitoid for 24 hours.

***Bt* LC_{25} reared larvae-parasitoid combined treatment.** Larvae reared on *Bt* LC_{25} -treated diet were placed with *Bt* LC_{25} –diet for 4 hours and then after 4 hours exposed to gravid female parasitoid for 24 hours.

After 24 hours observations were done for the number of paralyzed larvae[40]. Paralyzed host larvae were observed until the emergence of parasitoid larvae to ascertain the number of oviposited or parasitized larvae and the rest were counted for mortality.

Statistical Analysis

Any mortality in untreated setup was used to ascertain corrected mortality (%) = (P- P_0) / (100–P_0) × 100, where P is the percent mortality of treated insects; P_0 is the percent mortality of insects in the untreated control[51].

The LC values were calculated using POLO - Plus 2.0 program (Leora Software, 2005) and Probit Analysis Statistical Method. Parasitism and mortality data of different treatments were analyzed by analysis of variance (One Way ANOVA) and the means were tested for separation with Tukey's HSD using SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software.

RESULTS

All combinations of *Bt* and *H. hebetor* resulted in high mortality. All paralyzed host larvae died. Significantly high mortality was observed when parasitoid was allowed to attack host larvae treated with *Bt* LC_{50} fortified diet (37.0 ±2.13, $p< .001$). A synergistic effect was seen as the mortality was significantly higher than either parasitoid or *Bt* when used alone[46]. The extent of ovipositing was affected by *Bt* and showed significant variation among various *Bt*-parasitoid combinations ($F_{(3,36)}$= 10.93, $p< .001$). Ovipositing was highest on host larvae reared on *Bt* LC_{10} diet (40.9 ±2.35, $p< .05$) followed by LC_{25} and those exposed to 4 hrs in *Bt* LC_{50}. The proportion of oviposited larvae among paralyzed or dead ones was significantly reduced by *Bt* treatments ($F_{(3,36)}$= 39.91, $p< .001$), especially when host larvae was acutely intoxicated in LC_{50} (34.6 ±3.36, $p< .05$), whereas, larvae reared on low doses of *Bt* showed significantly higher ratio.

Table No. 1. Percentage mortality and oviposition of *Corcyra Cephalonica* 4th instar larvae after exposure to parasitoid and *Bt*–parasitoid combined treatments.

Treatments	Mortality± SE	Oviposited ± SE	Oviposited among paralyzed± SE
P (control)	37.0 ± 2.13a	28.0 ±2.50a	74.8 ±3.17d
Bt–P (host larvae exposed 4hrs in LC_{50})	73.0 ± 3.00b	25.0 ±1.67a	34.6 ±3.36a
Bt–P (host larvae reared on	66.0 ± 1.63b	40.9 ±2.35b	62.0 ±3.25c

LC_{10} diet)			
Bt–P (host larvae reared on LC_{25} diet)	64.0 ± 3.06b	31.0 ±1.39a	48.6 ±2.05b

Means ± SE followed by different letters in each column are significantly different (P<0.05) using Tukey's B test.; *Bt* = *Bacillus thuringiensis;* P = parasitoid (control) (*Habrobracon hebetor*); *Bt*–P = *Bt*–parasitoid (combined treatment).

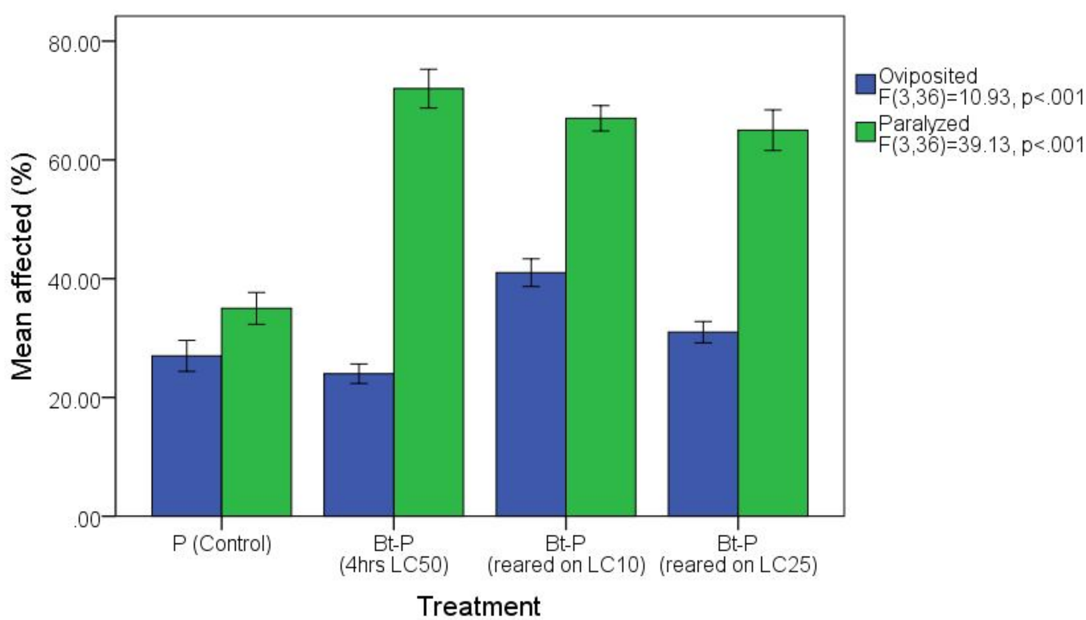

Fig No. 1. Effect of *Bt* on the relative proportion of mortality and oviposition of *C. cephalonica* larvae by *H. hebetor*

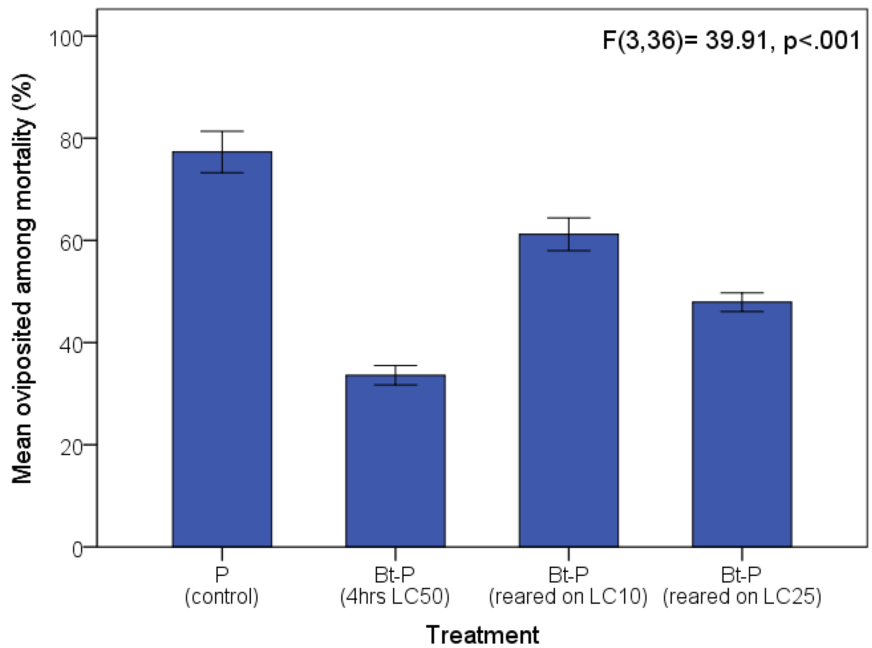

Fig No. 2. Effect of *Bt* on the extent of oviposition by *H. hebetor* on parasitized *C. cephalonica* larvae.

DISCUSSION

H. hebetor preferably parasitizes later instars of lepidopteran hosts as they are more profitable in terms of ease of locating and as better host for developing larvae. It has excellent flying and searching abilities and relatively long lifespan allowing for a wider dispersal in the release area and the potential for establishing a functional population on severely pest infested sites, thus making only an initial single inoculation enough[52]. *H. hebetor* is known to withhold or reduce ovipositing when the larval stages are not preferred or the quality of host is low[53]. The parasitoid tends to paralyze significantly more larvae than it can parasitize and oviposit. Host larvae fed on *Bt*-treated diets may not be similarly intoxicated, therefore, profitability may vary leading to host selection by the parasitoid. The lower ratio of oviposition in *Bt*-treated hosts points to this, implying that the most profitable ones were parasitized.

Depending upon the quality of conditions and timing of interaction, the interactions between parasitoids and *Bt* can range from synergistic to competitive[54,55] several studies have shown slower development in *Bt* infected larvae which makes them sluggish and more susceptible to attack by parasitoids[56,57]. Furthermore, ingestion of a sublethal dose of *Bt* may change host attributes that may result in host-mediated effects that can influence foraging and ovipositing priorities of the parasitoids[55]. Several studies have shown the ability of female parasitoids to discriminate between *Bt*-treated and untreated larvae, by parasitizing mostly the latter. Experiments on *Bt* plants have shown that parasitoids may avoid sublethally affected *Bt*-fed host larvae in which their offspring is unlikely to develop[58,59,60]. Choice and no choice tests done by Erb et al (2001)[55] seemed to suggest an effect of decreased host vigor. Weseloh (1980)[61] found that parasitoid attacked vigorous host rather than less active ones, as host movement and contact with the host integument were the attack eliciting factors. *Bt*-treated host larvae may not be similarly intoxicated, therefore, vigor may vary leading to host selection by the parasitoid. However, this alone does not explain the lower rate of parasitism among paralyzed *Bt* infected host larvae in this experiment.

Parasitoids are sensitive to changes in host quality, therefore, the altered host defense behavior of a weakened *Bt* infected larvae may result in a higher rate of attack by parasitoid[60,62]. Since *H. hebetor* can discriminate between varying larval profitability, it may prefer hosts where less time and energy is expended while searching. This may lead to higher rate of attack and paralyzation of host larvae. Moreover, the parasitoid may withhold or reduce the number of eggs[43,53]. Therefore a combination of inherent nature of attacking less infected vigorous larvae and a new opportunity to maximize paralyzation of weakened Bt-infected larvae may lead to higher mortality. Larvae paralyzed by *H. hebetor* do not survive long and a prior *Bt* infection is more likely to accelerate death. *H. hebetor* showed marked preference towards later instars (4th and 5th) than earlier stages in choice tests[46,63]. These early instars being more active, move deep into the infested food in contrast to the later instars[64]. *Bt* is more lethal to early host instars than the later ones and thus can not only complement but also synergize the lethal effect. The host mortality was greater when a combination of *Bt* and *H. hebetor* was used than either used alone. This synergistic effect seen in the mortality was in agreement with Chilcutt and Tabashnik (1997b)[65], Oluwafemi et al. (2009)[40] and Ebrahimi et al (2012)[66]. Navon (1993)[67] reported on several studies on the effect of *Bt* on parasitoids of susceptible insects species, but the effects have been negligible. Studies have also shown the effect of *Bt* on life history parameters of parasitoid[29,55]. *H. hebetor* had lower fecundity when reared on *Bt* infected host. This may be due to insufficient resources for parasitoid larval growth in a dying *Bt* contaminated host larvae[40,68,69].

Sublethal effects of *Bt* on the behavior and the biology of natural enemies of pest needs to be emphasized as host quality strongly influences the preferences of the parasitoid. A combined treatment integrating *Bt* with parasitoid for pest control can yield better results[70] partly due to the synergic effect involved in mortality. Stored grain pests, like *C. cephalonica*, the rice moth, can be controlled by integrating parasitoid *H. hebetor* [24,71], which attack the late host instars, and *Bt*[72], which is more toxic to early instars. Therefore, *Bt* can be safely used with parasitoid in combined biological control strategies against lepidopteran pests including *C. Cephalonica*[73]. *Bt* is known to affect various aspects of the Lepidoptera-parasitoid system and therefore, assessing the risks associated with the use of *Bt* products and their potential to affect non-target organisms, including biological control agents system, should be emphasized. The aspects of parasitoid choices and resulting larval mortality in *Bt*-contaminated hosts should be considered in an IPM program, to utilize and conserve parasitoid populations effectively.

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