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Research Article

Host location and acceptance by parasitoid, *Habrobracon hebetor* and effect of varying *Bacillus thuringiensis* treatment against rice moth, *Corcyra cephalonica*

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ABSTRACT: The combined effect of microbial pesticide, *Bacillus thuringiensis* Berliner (*Bt*), and insect parasitoid, *Habrobracon hebetor* Say (Hymenoptera: Braconidae) was evaluated for the management of rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae). This parasitoid is very useful in testing host-parasitoid interaction due to its high fecundity and short generation time. The host location and acceptance by parasitoid female, investigated by choice and no choice tests, showed marked preference towards later host instar larvae of *C. cephalonica*. Parasitoid induced mortality of Bt-intoxicated and Bt- reared host larvae was also investigated. *C. cephalonica* mortality was highest (72.00 ± 3.26%) and synergistic when host larvae were exposed to acute Bt treatment in conjunction with the parasitoid. A combined treatment of Bt with *H. hebetor* is an effective strategy in integrated pest control programmes of *C. cephalonica* and other stored grain pests.

KEY WORDS: *Bacillus thuringiensis, Corcyra cephalonica*, Combined treatment, *Habrobracon hebetor*, Pest management

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INTRODUCTION

Pests cause about a 40 per cent reduction in the world's crop yield (Mathew *et al*., 2014). In tropical countries insect pests cause heavy food grain losses in storage, particularly at the farm level, ranging from approximate 10% of the production in India to about 25–40% in Sub-Saharan Africa (Kangade, 2012). *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), the rice moth, is a common notorious pest in tropical and sub-tropical regions of the world infesting stored cereals and cereal commodities. The larvae feed on a variety of stored cereals such as wheat, rice, sorghum, maize, millet, etc., causing serious damage (Verma and Pathak, 2018). It also feeds on a broad range of commodities, including dried vegetable materials and dried fruits like almonds, date palm, nuts, chocolates, biscuits, oilcakes, etc. (Kangade, 2012). High levels of food grain loss can be attributed to inadequate post-harvest management practices and poorly designed storage structures leading to conditions suitable for pest infestation (Anon, 1989). The goal of increasing food availability cannot be achieved without proper management of pest.

The harmful effect of chemical pesticides on non-target organisms is a reality and in South Asia, the use of chemical pesticides in agriculture has seen a sharp increase in recent years particularly in India (Anon, 2005). In the past decades, the indiscriminate use of chemical pesticides has steadily increased (David, 1995; Ranga *et al*., 2007) resulting in unintended effects such as, natural hazards (Pimentel, 1996), development of insect pest resistance (Georghiou, 2012), pest resurgence (Yu *et al*., 2008), outbreak of the secondary pest (Dutcher, 2007) reduction in species diversity (Wilby and Thomas, 2002) alteration of decomposition of organic material and nutrient cycling (Edward, 1980) and objectionable pesticide residue (Metcalf, 1994). Although chemical control is still recognized as an important strategy in an IPM program, the success of any such program along with biological control depends on their judicious integration (Wright and Verkert, 1995; Zhao, 2000; Mahdavi, 2013). Integrating biocontrol agents in this scenario requires sufficient deep knowledge and impact assessment of the pesticide on natural enemies (Croft, 1990). Chemical control, if at all integrated, should be least disruptive to biocontrol agents and should be used only when it is necessary (Mahdavi, 2013).

Habrobracon hebetor Say, 1836, (Hymenoptera: Braconidae), is a gregarious, cosmopolitan ectoparasitoid of the larval stage of stored-grain pyralid moths (Lepidoptera: Pyralidae) such as *C. cephalonica* (Singh *et al*., 2009). This ectoparasitoid has high reproductive rate, short generation time and a large number of host species; hence it is preferred by most researchers for host-parasitoid interactions studies (Gündüz and Gülel, 2005; Singh *et al*., 2014; Singh *et al*., 2016). Moreover being a potential biocontrol agent, it has been effectively used in the control of stored product moths (Yu *et al*., 2003). The females of *H. hebetor* prefer to attack and lay variable numbers of eggs on or near the surface of paralyzed last (fifth) instar host larvae (Antolin *et al*., 1995; Ghimire and Phillips, 2014). It exhibits high fecundity and natural rate of increase, which makes it a promising agent for use against *C. cephalonica*, a very common stored grain pest (Singh *et al*., 2016).

Combining more than one method of biological control for pest management can be a more efficient strategy. *Bacillus thuringiensis* Berliner *(Bt)*, a Gram-positive, spore-forming soil bacteria, has not only emerged as the major ecofriendly biopesticide against major pests but also a key source of genes for developing transgenic crops expressing δendotoxins to provide pest resistance (Singh and Mathew, 2015). *Bt* is a member of the *Bc* (*Bacillus cereus)* group and has emerged as the most successful microbial pesticide having great potential in IPM programmes (Blumberg *et al*., 1997; Singh and Mathew, 2015). Strategies involving the combination of *Bt* and a natural enemy has shown varying effects of *Bt* on pest and its natural enemies. Integrating *B. thuringiensis* with a parasitoid has shown satisfactory control of lepidopterous pests (Blumberg *et al*., 1997). *Bt* has been shown to have no adverse effects on parasitoid development or its emergence when used against pest populations (Weseloh and Andreadis, 1982; Ulpah and Kok, 1996; Oluwafemi *et al*., 2009). Surgeoner and Farkas (1990) have recommended the use of *Bt* insecticide, such as Dipel, for use in integrated pest management programs and they have also shwed that it is harmless to most of the beneficials tested. Oluwafemi *et al*. (2009) have reported that a combination of *Bt* with *H. hebetor* as a biological control agent, resulted in successful control of *Plodia interpunctella* population and has recommended the use of *Bt* in combination with a parasitoid for the control of other lepidopteran pests. However, Erb *et al*., (2001) reported that *Bt* can adversely affect certain parasitoids by causing enhanced or decreased parasitoid larval development times and altered parasitoid sex ratios (Erb *et al*. 2001). Due to premature host death it could reduce the parasitism (Vail *et al*., 1972), decreased parasitoid survival (Nealis and van Frankenhuyzen, 1990) and lower parasitoid emergence rates (Atwood *et al*., 1997).

Several experimental data are available on the capacity of parasitoids to search, attack and successfully develop on different stages of the same host (Canale and Loni, 2006, Akinkurolere *et al*., 2009). While foraging parasitoids may encounter different host developmental stages which are vulnerable to attack, these hosts may differ in their profitability in terms of fitness, so parasitoid becomes selective for particular stages of their hosts (Godfray and Hunter, 1994). In this study, we aim to assess the outcome and predictive accuracy by choice and no choice test in laboratory condition to improve the method and interpretation of host specificity of *H. hebetor* and the suitability of using *Bt* - parasitoid combination for the control of *C. cephalonica.*

MATERIAL AND METHODS

All the insect cultures were maintained (Singh, 2004) and the assays and experiments were conducted at 27 ± 2°C, 70 ± 10% RH and 12:12 L:D photoperiod.

Rearing of the pest

To maintain the culture of *Corcyra cephalonica*, the eggs of rice moth were obtained from the Central Integrated Pest Management Centre, Gorakhpur (CIPMC, GKP) and kept with coarsely ground mixed grain diet in specially designed large plastic containers of size 45 cm × 25 cm × 15 cm. The containers were observed daily for the hatching of the eggs and the diet was replenished regularly after consumption and damage by the larvae. After 3–4 generations, full-grown larvae of rice moth from this culture were taken to feed and rear the parasitoid *Habrobracon hebetor* (Singh *et al*., 2015; Singh *et al*., 2016). Larvae were also reared in mixed grain diets with Bt at LC_{10} and LC_{25}. These larvae were used in mortality experiments.

Rearing of the parasitoid

For the culture of *Habrobracon hebetor*, adults were collected from the CIPMC, GKP. Male and female insect were paired in a beaker (250ml) having 10 full grown 5th instar larvae of *Corcyra cephalonica*, covered with a fine muslin cloth (Singh *et al*., 2015; Singh *et al*., 2016). After the third generation, adults were utilized in experiments.

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] from Valent Biosciences Corporation, USA.

Preliminary assay of LC_{50}

Bioassay of *Bt* on *Corcyra cephalonica* was carried out as per Oluwafemi *et al*. (2009). Dipel DF was serially diluted using 1, 2, 4, 8, 16, 32 and 64 mg per mL of distilled water. 1mL distilled water served as control. The dilutions were incorporated into artificial diet @ 0.2 mL/g and allowed to dry. The treated diets were transferred into 250 mL Borosil glass beakers @ 10 g/beaker. Twenty *C. cephalonica* 4th instar larvae were introduced into each beaker and maintained in the laboratory. Larval mortality was recorded after 24, 48 and 72 h of initial inoculation. The experiment was replicated five times. LC_{50} for 48 h was used in further experiments (Oluwafemi *et al*., 2009). Also, LC_{25} and LC_{10} for 48 h were used to prepare *Bt* treated diets to rear host larvae for mortality experiments.

Host location and acceptance by parasitoid female

Choice and no-choice tests were utilized to determine the preferences of the parasitoid in host location and acceptance (Oluwafemi *et al*., 2009).

Choice test

Ten *C. cephalonica* larvae, two of each instar (I to V), reared on a mixed diet, were placed in a 500mL beaker covered with a muslin cloth. A control, without parasitoid, was also set up. All experiments including control were replicated 20 times. Larval mortality was observed after 8 h of introducing gravid female *H. hebetor*.

No choice test

Ten first instar larvae of *C. cephalonica* which were reared on a mixed diet, were placed in a 500 mL beaker covered with muslin cloth. The same protocol was followed for 2nd, 3rd, 4th and 5th host-instars. Controls were also set up. All treatments were replicated 20 times. Larval mortality was observed after 8 h of introducing gravid female *H. hebetor*.

Effect of Combining *Bt* and Parasitoid on *Corcyra Cephalonica*

In this experiment, there were six treatments with each bioassay being carried out using ten *C. cephalonica* larvae of 4th instar in 500 mL beakers with 10 g diet in 5 replicates each. It was covered with a muslin cloth. Varying treatments of Bt and parasitoid were:

Untreated (control): *Corcyra cephalonica* larvae were placed with untreated mixed grain diet.

Bt treatment: *Corcyra cephalonica* larvae were placed with Bt treated mixed grain diet at LC_{50}.

Parasitoid treatment: *Corcyra. cephalonica* larvae were placed with untreated mixed grain diet then after 4 hours exposed to gravid female parasitoid for 24 h.

***Bt*-parasitoid combined treatment:** *Corcyra cephalonica* larvae were placed with Bt treated mixed grain diet at LC_{50} then after 4 h exposed to gravid female parasitoid for 24 h.

***Bt* LC_{10} reared larvae-parasitoid combined treatment:** *Corcyra cephalonica* larvae reared on Bt LC_{10} -treated mixed diet were placed in the same diet and then after 4 hours exposed to gravid female parasitoid for 24 h.

***Bt* LC_{25} reared larvae-parasitoid combined treatment:** *Corcyra cephalonica* larvae reared on Bt LC_{25} -treated mixed diet were placed in the same diet and then after 4 hours exposed to gravid female parasitoid for 24 h.

The experiments were observed after 24 h for the number of larval mortality/parasitization (Oluwafemi *et al*., 2009).

Statistical analysis

Data from choice and no choice tests were corrected for that of control using Abbott's Formula: Corrected mortality (%) = $(P - P_0) / (100 - P_0) \times 100$, where P is the percent mortality of treated insects, P_0 is the percent mortality of insects in the untreated control (Abbott 1925).

The LC_{50} and other LC values for 24, 48 and 72 h (with 95% confidence limits) were calculated by using POLO - Plus 2.0 program (Leora Software, 2005) and Probit Analysis Statistical Method and mortality data of preliminary screening tests and different treatments 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.

RESULTS AND DISCUSSION

The LC_{50} value (with 95% confidence limits) of Bt on *C. cephalonica* 4th instar larvae for 24, 48 and 72 h were 65.813 (52.946 – 85.689), 36.311 (29.953 – 45.704) and 17.745 (15.350 – 20.742) mg/mL respectively.

Choice test

In Choice test, the mean percent mortality (±Standard Error) of 1st, 2nd, 3rd, 4th and 5th instar larvae of *C. cephalonica* due to parasitoid *H. hebetor* were 5.00 ± 3.44, 10.00 ± 4.59, 45.00 ± 6.18, 65.00 ± 5.26 and 85.00 ± 5.25 respectively (Fig. 1).

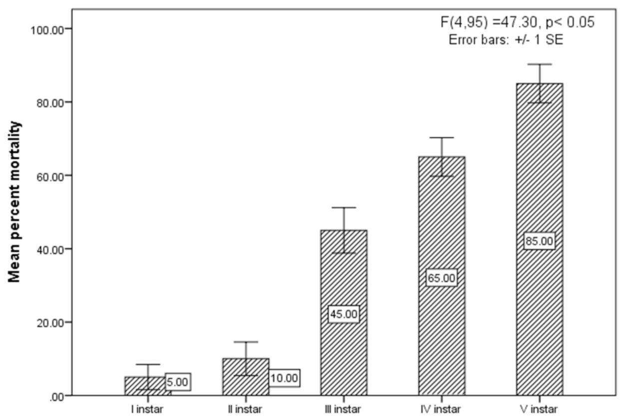

Fig. 1. Mean percent mortality of *Corcyra cephalonica* larvae in Choice test.

One-way Analysis of Variance (ANOVA) yielded statistically significant difference between the mortality of all instar larvae of *C. cephalonica* ($F_{(4,95)} = 47.302$, $p < .05$) (Table 1). Tukey Post Hoc Test for multiple comparisons revealed statistically significant difference between the mortality of 1st instar (5.00 ± 3.44, $p < .05$) when compared to 3rd instar (45.00 ± 6.18, $p < .05$), 4th instar (65.00 ± 5.26, $p < .05$) and 5th instar (85.00 ± 5.25, $p < .05$) larvae. Similarly, statistically significant difference was seen between the mortality of 2nd instar (10.00 ± 4.59, $p < .05$) when compared to that of 3rd, 4th and 5th instar larvae. But there was no statistically significant difference between the mortality of 1st instar (5.00 ± 3.44, $p = .955$) and 2nd instar larvae (10.00 ± 4.59, $p = .955$).

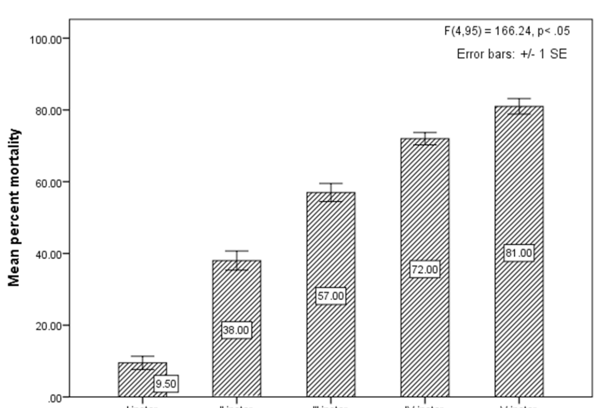

Fig. 2. Mean percent mortality of *Corcyra cephalonica* larvae in No Choice test.

The parasitoid was provided the opportunity to parasitize any out of the five host larvae instars. The lowest mortality was recorded in 1st and 2nd instars. Whereas, the highest mortality was recorded in 5th instar larvae. *H. hebetor* shows preference towards larger sized instars for laying eggs.

No Choice Test

In No Choice test the mean percent mortality (±Standard Error) of 1st, 2nd, 3rd, 4th and 5th instar larvae of *C. cephalonica* due to parasitoid *H. hebetor* were 9.50 ± 1.85, 38.00 ± 2.67, 57.00 ± 2.52, 72.00 ± 1.72 and 81.00 ± 2.16 respectively (Fig. 2).

One-way Analysis of Variance (ANOVA) yielded statistically significant difference between the mortality of all instar larvae of *C. cephalonica* ($F_{(4,95)} = 78.536$, $p < .05$) (Table 1). Tukey Post Hoc Test for multiple comparisons revealed statistically significant difference between the mortality among 1st instar (9.50 ± 1.85, $p < .05$), 2nd instar (38.00 ± 2.67, $p < .05$), 3rd instar (57.00 ± 2.52, $p < .05$),4th instar (72.00 ± 1.72, $p<.05$) and 5th instar (81.00 ± 2.16, $p < .05$) *C. cephalonica* larvae.

H. hebetor showed significantly increasing parasitization of 3rd, 4th and 5th instar host larvae. 5th instar larvae being less active and motile is the easiest target. *H. hebetor* shows preference towards larger sized instars for laying eggs.

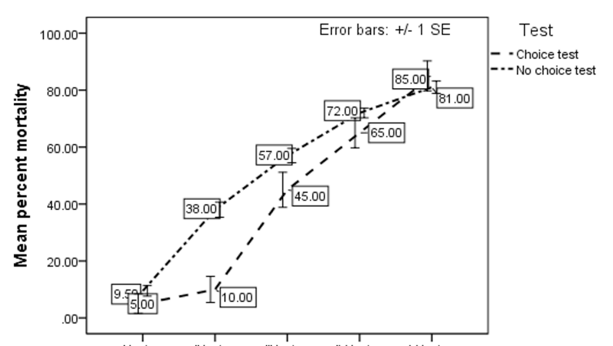

Fig. 3. Compared mean percent mortality of *Corcyra cephalonica* larvae in Choice and No Choice test.

Table 1. Percentage mortality of *Corcyra cephalonica* larval instars by *Habrobracon hebetor* under no-choice and choice conditions. Mortality is expressed as percentage mean ± SE

Instar larvae	*No-choice test*		*Choice test*	
	Control	*Treatment*	*Control*	*Treatment*
1st	2.00 ± 1.26a	9.50 ± 1.85a	2.50 ± 1.45a	5.00 ± 3.44a
2nd	0.00 ± 0.00a	38.00 ± 2.67b	0.00 ± 0.00a	10.00 ± 4.59a
3rd	0.00 ± 0.00a	57.00 ± 2.52c	0.00 ± 0.00a	45.00 ± 6.18b
4th	0.00 ± 0.00a	72.00 ± 1.72d	0.00 ± 0.00a	65.00 ± 5.26c
5th	0.00 ± 0.00a	81.00 ± 2.16e	0.00 ± 0.00a	85.00 ± 5.25d

Means followed by different letters in each column are significantly different ($P < 0.05$) using Tukey's B test

Table 2. Percentage mortality of *Corcyra cephalonica* (4th instar larvae) after exposure to *Bt*, parasitoid and *Bt*–parasitoid combined treatments

Treatments	Mortality ± SE	Confidence Limits
Control	0.00 ± 0.00a	0.00 – 0.00
Bt	23.00 ± 2.13b	18.17 – 27.83
P	35.00 ± 2.69c	28.92 – 41.08
Bt–P (host larvae exposed 4hrs in LC_{50})	72.00 ± 3.26d	64.61 – 79.39
Bt–P (host larvae reared on LC_{10} diet)	67.00 ± 2.13d	62.17 – 71.83
Bt–P (host larvae reared on LC_{25} diet)	65.00 ± 3.42d	57.27 – 50.85

Means followed by different letters in each column are significantly different ($P<0.05$) using Tukey's B test. (Control=clean untreated diet; *Bt* = diet treated with *Bt* at LC_{50} dose; P = parasitoid (*Habrobracon hebetor*); *Bt*–P = *Bt*–parasitoid (combined treatment). *Bt* = *Bacillus thuringiensis*.

Effect of Combining *Bt* and *Habrobracon hebetor* against *Corcyra Cephalonica*

Significantly high mortality rates were observed in all *Bt*, parasitoid and *Bt*-Parasitoid treatments (Table 2). One-way Analysis of Variance (ANOVA) yielded statistically significant difference between the *C. cephalonica* mortality among treatments ($F_{(5,54)} = 130.697$, $p < .05$). Tukey Post Hoc Test for multiple comparisons revealed statistically significant difference between the mortality in control experiment (0.00 ± 0.00, $p < .05$) when compared to that in treatments of Bt (23.00 ± 2.13, $p < .05$), parasitoid (35.00 ± 2.69, $p < .05$), *Bt*–P (larvae exposed 4hrs in LC_{50}) (72.00 ± 3.26, $p < .05$), *Bt*–P (larvae reared on LC_{10} diet) (67.00 ± 2.13, $p < .05$) and *Bt*–P (larvae reared on LC_{25} diet) (65.00 ± 3.42, $p <.05$).

Highest larval host mortality was observed in *Bt*–P (larvae exposed 4hrs in LC_{50}) combination and there was no statistically significant difference between it (72.00 ± 3.26, p =.169) and the *Bt*–P (larvae reared on LC_{10} diet) (67.00 ± 2.13, p =.169) and *Bt*–P (larvae reared on LC_{25} diet) (65.00 ± 3.42, p =.056) treatments. Similarly there was no statistically significant difference between larval mortality in *Bt*–P (larvae reared on LC_{10} diet) (67.00 ± 2.13, p = .580) and *Bt*–P (larvae reared on LC_{25} diet) treatments.

The present experiments show that *Habrobracon hebetor* has the capacity to locate and parasitize all stages of *Corcyra cephalonica* but it showed specific preference for 4th and 5th instar larvae when compared with all other stages. Early instars are generally difficult to locate as the food media serves as a refuge. The results are in accordance with the studies done by Sait *et al*. (1997); they showed that it is strenuous for the parasitoid *Venturia canescens* to search and parasitize *Plodia interpunctella* 2nd instar larvae which are concealed in refuge than 5th instar within the same environment (Akinkurolere *et al*., 2009). Our studies reveal that the choice test, host location, and parasitism by *H. hebetor* is negligible and non-significant in early stages (instars). Ode *et al*. (1997) proved that *H. hebetor* can withhold or reduce the number of eggs laid if there are poor quality hosts or non preferred stages. In another study conducted by Akinkurolere *et al*. (2009) it was shown that early instar larvae are active and can push itself deep into the treated diet during feeding in contrast to high instar larvae that tend to move away from the infested diet to the surface for pupation sites (Akinkurolere *et al*., 2009).

In no-choice test the study indicated higher interaction with the later stages (3rd to 5th), again the Ist and 2nd stages are not preferred. 2nd stage, however, showed some acceptance with the no choice test but the best suitable was again the 5th instar. The inability of *H. hebetor* to locate an attack early instar in comparison to later instars could result from the weaker vibration produced by early instars (Akinkurolere *et al*., 2009). The low preference in this study of early instars is due to age-dependent mechanisms which parasitoids used to discriminate between different instars of the same host (Mattiacci and Dicke, 1995a) and also less time and energy is expended while searching the later instars instead of early instars. Therefore, later instars could be easily located and attacked and is more profitable to the parasitoid in a population of mixed age group of host instars (Mattiacci and Dicke, 1995b; Akinkurolere *et al*., 2009). In no-choice test, only the physiological host range can be estimated (Withers and Browne, 2004) so the ability to predict the ecological host ranges of parasitoid by laboratory-based host specificity tests alone has its limitations. A more accurate prediction of ecological host range can be done by choice test (Murray, 2010). Babendreier *et al*. (2005) reported that there is generally an agreement between no choice and choice test result in most insect control studies that have included both (Table 1). The results obtained in this study conformed to the same view (Fig. 3).

Habrobracon hebetor is an effective biocontrol agent especially against host population that wanders and feed on the surface (Akinkurolere *et al*., 2009). Although it parasitizes all instars, it is more effective on later instars, which are more profitable to the parasitoid as less time and energy it wasted to locate and parasitize. *H. hebetor* is a good biocontrol agent against *Corcyra cephalonica* (Deepak *et al*., 2006; Singh *et al*., 2016). In this study, the result demonstrated that a combined treatment of *Bt* and *H. hebetor* against *C. cephalonica* was more effective and lethal than both *Bt* and *H. hebetor* when used alone, thus suggesting a synergistic effect. *Bt* being more lethal in early instar (1st and 2nd), the 3rd to 5th instars are more profitable for *H. hebetor* with 5th instar recording the highest mortality. These results were same as observed by Zhang *et al*. (1995). The combined treatment was significantly more effective and *Bt* did not prevent parasitoid development and reproducibility, which suggests that their lethal effect enhanced when combined together.

The study suggests that *Habrobracon hebetor* prefer later host instars as far as locating the host and parasitization is concerned, and combining *Bt* with *H. hebetor* as biocontrol agent results in a high reduction of *Corcyra cephalonica* population. *Bt* can, therefore, be used along with *H. hebetor* in combined treatment against *C. cephalonica* and other lepidopteran pests. Further studies on the interference of *Bt* with reproduction and development of *H. hebetor* is needed to correctly assess and utilize the full potential of such combined treatment for pest control.

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