Behavioral and Developmental Responses of Habrobracon hebetor (Hymenoptera: Braconidae) to Larvae of Helicoverpa armigera (Lepidoptera: Noctuidae) Inoculated With Various Concentrations of Bacillus thuringiensis var. kurstaki (Bacillales: Bacillaceae)

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Abstract

Bacillus thuringiensis Berliner subsp. kurstaki (Btk) and Habrobracon hebetor Say are both biological control agents of Helicoverpa armigera Hubner. The present study evaluated their compatibility for combined application against this pest by examining the acceptability of Btk-inoculated hosts for H. hebetor females and testing for negative life-history impacts on developing progeny. Second-instar H. armigera larvae fed for 72 h on potted chickpea plants treated with three concentrations of Btk (LC15, LC35, and LC70) and were then used in bioassays of parasitoid development and parasitism behavior. Survival of parasitoids was significantly reduced, and immature development prolonged, on hosts fed chickpea plants treated with LC35 and LC70 Btk, but not on plants treated with LC15 Btk. Parasitoids failed to discriminate against hosts treated with LC35 or LC70 Btk in choice tests, but attacked fewer hosts treated with LC15 Btk, paralyzing and parasitizing more healthy hosts, and laying more eggs on them. In contrast, a no-choice test revealed that more hosts treated with LC35 and LC70 Btk were paralyzed compared with control or LC15-treated hosts, but the numbers of hosts parasitized and eggs laid did not vary among Btk treatments. Thus, females required an experience with healthy hosts, as they had in the choice test, to discriminate against diseased ones. We conclude that H. hebetor and Btk are compatible for joint application against H. armigera, which could potentially improve biological control of this pest.

Key words: biological control, entomopathogen, host selection, parasitism, survival

The ectoparasitoid Habrobracon hebetor Say is an important biological control agent of Helicoverpa armigera Hubner and other lepidopteran pests (Ba et al. 2014, Mbate and Warsi 2019). This gregarious idiobiont ectoparasitoid has a fast growth rate, short generation time, high fecundity, and a broad host range, all of which contribute to its effectiveness as a biocontrol agent in integrated pest management (IPM) programs (Ghimire and Phillips 2014, Kabore et al. 2017). Typically, a female of H. hebetor first paralyzes several host larvae by stinging them and injecting them with venom. Once the venom has taken effect and paralyzed the larva, the wasp returns to lay a variable number of eggs (ca. 8–30) on some of them, such that many more hosts are often paralyzed than are used for oviposition ( Hagstrum and Smittle 1977, Antolin et al. 1995, Ghimire and Phillips 2014). Although this parasitoid is often released alone in augmentation biological control programs against lepidopterous pests of field crops in Iran, it can also be used in combination with entomopathogens such as Bacillus thuringiensis subsp. kurstaki Berliner (Btk) (Allahyari et al. 2020a).

The entomopathogen Btk has become an important biocontrol agent for H. armigera in many countries (Gujar 2005; Tabashnik et al. 2015, Nascimento et al. 2018). Because H. hebetor preferentially parasitizes late-instar caterpillars, whereas Btk is more effective against early instars (Sedaratian et al. 2014), the combined use of both agents is an attractive alternative to chemical insecticides for controlling H. armigera. However, the simultaneous application of a parasitoid and an entomopathogen raises questions about potential interactions between them, such as the ability of parasitoids to discriminate between healthy hosts and infected ones, and the
consequences for progeny development when they do not. There are reports of Btk having negative impacts on the biology and life history of parasitoids (De Bortoli et al. 2017), including Campocletis chloridiae Uchida (Hymenoptera: Ichneumonidae) (Mohan et al. 2008), Palmistichus elaeis Delvarre and LaSalle (Hymenoptera: Eulophidae) (Rolim et al. 2020), and also H. hebetor (Sedaratian et al. 2014).

Bt toxins have the potential to affect natural enemy development by diminishing the growth and vigor of their hosts, but can also make the host more vulnerable to attack by other natural enemies (Mohan et al. 2008). However, there exists variance among parasitoid species in susceptibility to Bt toxins; immature survival of Meteorus pulchrinicornis Wesmael (Hymenoptera: Braconidae) reared on larvae of Spodoptera litura E. (Lepidoptera: Noctuidae) fed diet amended with the field rate of Btk was not significantly different from controls (Walker et al. 2007). Similarly, Chilcutt and Tabashnik (1999) reported that Btk treatment of Plutella xylostella (Lepidoptera: Plutellidae) larvae had no effect on oviposition preference of the endoparasitoid Cotesia plutellae Kurjumov (Hymenoptera: Braconidae), and direct feeding of the pathogen to wasps in honey had no detrimental effects. However, prolonged feeding of host larvae on Btk-contaminated diet was detrimental to parasitoid survival because of premature host mortality. Therefore, the ability of parasitoids to discriminate against infected hosts can be an important factor affecting parasitoid fitness, biocontrol efficiency, and persistence in the field when these agents are used in combination (Wan et al. 2019).

Parasitoid fitness is intrinsically linked to host quality because the latter affects juvenile survivorship, fecundity, and the body size of emerging adults (Vinson and Ivantsch 1980, Ghimire and Phillips 2014). Natural selection should favor parasitoids that maximize their fitness by distinguishing and avoiding unsuitable hosts (Jiang et al. 2014). The present study was conducted to quantify the negative effects of Btk on development and survival of H. hebetor when they parasitize larvae of H. armigera inoculated with various concentrations of Btk and to determine whether H. hebetor females are able to discriminate inoculated hosts from healthy ones. The results were expected to clarify the compatibility of these two biocontrol agents for joint application in integrated pest management programs for H. armigera.

Materials and Methods

Plant Culture

Three to five seeds of chickpea, Cicer arietinum L. (cv. Bivanij), were sown in each plastic pot (10 cm ht x 7 cm diam) filled with a mixture of soil (30%), coco peat (30%), peat moss (30%), and perlite (10%). The pots were held at a daytime temperature of 23 ± 3°C, lowered to 20 ± 3°C at night in a greenhouse at the Department of Horticulture, Ilam University. Plants were watered every 2–3 d, as required, and used in rearing and bioassays when they reached the six to eight leaf stages (8–12 d).

Insect Colonies

Insect rearing and all experiments were carried out in climate-controlled chambers set to 28 ± 1°C, 60 ± 5% RH, and a 16:8 (L:D) photoperiod. An H. armigera colony was established by collecting larvae (ca. 250) from chickpea fields in Ilam Province, Iran, in the spring of 2018. Each larva was transferred to a ventilated plastic container (4 x 6 x 8 cm) and fed an artificial diet based on that of Twine (1971) with some modification: bean flour (205 g), yeast (35 g), wheat germ (30 g), sunflower oil (5 ml), formaldehyde 37% (2.5 ml), agar (14 g), ascorbic acid (3.5 g), methyl-parahydroxy-benzoate (2.2 g), sorbic acid (1.1 g), and distilled water (700 ml). Larvae were provided with a piece of diet (ca. 1 cm³) that was refreshed daily until they pupated. Emerged moths were transferred to a transparent Plexiglas oviposition chamber (20 x 30 x 30 cm), 30–40 per container, and provisioned daily with 10% honey solution provided on cotton balls in open Petri dishes (6 cm diameter) and allowed 4–5 d to mate. The top and walls of the chamber were covered with a fine mesh that served as an oviposition substrate. The mesh nets bearing eggs were collected daily and transferred to plastic bags held under the same physical conditions. After hatching, neonate larvae were transferred to potted chickpea plants (three or four per plant) and allowed to feed 4 d until they reached the second instar, whereupon they were either used in experiments or transferred to larval rearing containers (as above) to maintain the colony.

A laboratory culture of H. hebetor was maintained on late instars of Ephesia kuehniella (Zeller) obtained from the Plant Protection Bureau of Ilam Province. The parasitoid colony was established by collecting parasitized H. armigera larvae (ca. 60) or pupae (ca. 30) from chickpea fields in Ilam Province, Iran, in the spring of 2018. These were reared out under the same physical conditions as described above for H. armigera. Emerging wasps were transferred to plastic Petri dishes (9 cm diameter), fed with a 10% honey solution on strips of paper (1 x 3 cm). Generations were started by placing two male and two female wasps in each of a series of Petri dishes (as above) and providing each dish with 10–12 fifth-instar larvae of E. kuehniella daily. Adult wasps were introduced to clean Petri dishes with new hosts daily until both female wasps died. Parasitized host larvae were kept until emergence of adult wasps and the colony was reared for eight generations before use in the experiments. All experiments employed 3-d-old mated females that had each been provided with three late-instar larvae of E. kuehniella on each of two successive days to verify successful mating and oviposition prior to use.

Helicoverpa armigera Bioassay

A wettable powder formulation of Bacillus thuringiensis subsp. kurstaki (Belthirul, 32,000 IU/mg) was obtained from Probelte Pharma, SL (Madrid, Spain). Five concentrations of Btk were prepared (250, 625, 1,250, 2,000, and 2,500 ppm), and in each treatment, the foliage of potted chickpea plants was immersed for 10 s in one of the Btk suspensions (n = 90 plants for each concentration), then air-dried on a laboratory bench for 10 min; control plants were immersed in distilled water. Each treated chickpea plant was then placed in a ventilated plastic container (12 cm diameter x 12 cm height). Second-instar H. armigera larvae were transferred to Btk-treated chickpea plants, one larva per plant. The basal container was covered with a second container (15 cm diameter x 13 cm height) ventilated by means of a fine mesh glued to an aperture cut in the bottom (7 cm diameter). Both containers had sloping sides, so that a larva-proof seal was obtained when the larger container was placed upside down over the smaller one (Allahyari et al. 2020b). The second-instar H. armigera larvae were then left to feed on the Btk-treated chickpea plants for 3 d under the same physical conditions as the insect colony. Larval mortality was recorded on day 3 post-inoculation, and lethal concentrations were determined using Probit analysis.
Parasitoid Bioassay

After determining the lethal concentrations of Btk in the first bioassay, second-instar larvae of H. armigera were starved for ca. 8 h and then transferred to chickpea plants treated with one of three concentrations of Btk (LC_{15}, LC_{35}, and LC_{70}), or distilled water as controls, one larva per plant, and allowed to feed for 72 h. In total, 85, 120, 250, and 60 larvae were fed plants treated with LC_{15}, LC_{35}, LC_{70}, and water, respectively, in consideration of lower expected host survival at higher concentrations. Surviving larvae were isolated in plastic Petri dishes (6 cm diameter) and provisioned with artificial diet, as described above, for the remainder of their development. Experimental wasps were held as couples in Petri dishes for 2 d after emergence and provided E. kuehniella larvae to ensure successful mating. Next, two female wasps, each 3 d old, were transferred to each Petri dish containing a single Btk-treated or control larva of H. armigera for 24 h. After the wasps were removed, a single parasitoid egg was left on each larva and parasitized larvae were held under the standard experimental conditions until emergence of adult wasps. The duration of immature parasitoid development, numbers of pupae formed, and numbers of adults emerging were all recorded. After emergence, adults were transferred to Petri dishes (6 cm diameter) and provisioned with diluted honey (10%) on strips of paper (1 x 3 cm) every other day until death of the last wasp to estimate adult longevity.

Choice Test

Second-instar larvae of H. armigera were fed for 3 d on chickpea plants treated with one of three concentrations of Btk (LC_{15}, LC_{35}, and LC_{70}), or distilled water as a control, as described above. Treated larvae that survived each of three Btk concentrations, and healthy larvae (controls), were isolated in plastic Petri dishes (9 cm diameter), which had been divided into eight equal radial sections by means of a wire mesh to isolate a single larva in each section and prevent cannibalism among them (Fig. 1). The wire mesh was coarse enough to permit wasps to move easily through the partitions. Four treated and four healthy larvae were arranged alternately among the eight sections of each dish, and each was provisioned with a piece of artificial diet (ca. 1 cm³). A single, 3-d-old female wasp was introduced to each Petri dish and allowed forage for 24 h, whereupon the wasp was removed and all paralyzed and parasitized hosts, as well as total eggs laid, were recorded. If a larva was still alive but showed no movement when stimulated by a fine brush, it was considered to be paralyzed and parasitized if it received at least one egg (all parasitized larvae were paralyzed, but not all paralyzed larvae were parasitized). Altogether, 60 female wasps were tested at each concentration, one female per replicate. Data for replicates in which the wasp was killed by the host larvae were discarded.

No-Choice Test

A no-choice test was conducted using the same procedures as the choice test, except that eight Btk-treated larvae from each of three concentrations (LC_{15}, LC_{35}, and LC_{70}) or eight healthy larvae (as control) were placed in each Petri dish (9 cm diameter) and a 3-d-old female wasp was introduced to each (n = 60) as described above. Data for replicates in which the wasp were killed by the host larvae during the experiment were discarded.

Statistical Analyses

Lethal concentrations of Btk were calculated by SPSS software package (SPSS 1998) using mortality data of the bioassay subjected to Probit analysis (Finney 1971). Parasitoid survival was analyzed by Kaplan–Meier (α = 0.05). Developmental times were compared by one-way analysis of variance (ANOVA; SPSS 1998), and Fisher’s least significant difference (LSD) test was used to separate means (α = 0.05). Mean numbers of paralyzed and parasitized larvae and numbers of eggs laid in the choice test were compared by paired t-test (two tailed). In the nonchoice test, the data were analyzed by one-way ANOVA (SPSS 1998) followed by Fisher’s LSD test to separate means (α = 0.05).

Results

Helicoverpa armigera Bioassay

The lethality of Btk against second-instar larvae of H. armigera was measured after 72 h of continuous feeding on treated chickpea plants and increased significantly with the concentration applied (Table 1).

Parasitoid Bioassay

When second-instar H. armigera larvae fed for 72 h on chickpea plants treated with different concentrations of Btk, survival was 100% (60/60) in controls, compared with 89.4% (76/85) in the LC_{15} treatment, 62.5% (75/120) in the LC_{35} treatment, and 19.2% (48/250) in the LC_{70} treatment. When surviving H. armigera larvae were each parasitized with a single egg, parasitoid survival was significantly reduced in the LC_{70} and LC_{35} treatments, whereas the LC_{15} treatment survival was not significantly different from controls (Fig. 2). Although the incubation period of parasitoid eggs hatching on H. armigera larvae was not affected by Btk treatment (F_{1,241} = 0.18, df = 3,255; P = 0.910), larval development was significantly delayed in the LC_{35} treatment (F = 6.54, df = 3,155; P < 0.001) and total immature development was delayed by both the LC_{35} and LC_{70} treatments, but not by the LC_{15} treatment (F = 5.12, df = 3,153; P = 0.002; Table 2). Pupation time was not significantly different among treatments (F = 1.63, df = 3,153; P = 0.190), but the longevity of male (F = 3.03, df = 3,83; P = 0.030) and female (F = 2.78,
Table 1. Lethality of various concentrations of Bacillus thuringiensis var. kurstaki against second-instar larvae of Helicoverpa armigera (n = 90 per concentration) derived from Probit analysis (df = 3, intercept = 6.7 ± 0.62) with 95% confidence intervals (CIs)

| Lethality | Concentration (ppm) | 95% CIs       | χ²  | P     |
|-----------|---------------------|---------------|-----|-------|
| LC15      | 201.9               | 147.8–254.7   | 4.96| 0.174 |
| LC25      | 371.7               | 301.3–438.7   |     |       |
| LC70      | 871.7               | 762.4–1,003.0 |     |       |

However, hosts treated with LC15 Btk were paralyzed more often than healthy hosts in both choice and no-choice bioassays, suggesting that their infections rendered them more vulnerable to initial attacks by the parasitoid, without rendering their behavior sufficiently abnormal to cue wasp avoidance, even when they were presented with healthy alternatives. Host defensive responses represent a hazard to wasps, as these large host larvae are sometimes able to injure or kill attacking parasitoids (Mohan et al. 2008). Hosts treated with LC0 were also paralyzed more often than controls in the no-choice test, but the reverse was true in the choice test, suggesting that disease symptoms in this treatment were pronounced enough that wasps were more likely to avoid them when given a chance to compare them to healthy hosts. Allahyari et al. (2020a) showed that H. armigera larvae inoculated with sublethal concentrations of a nucleopolyhedrovirus, HearNPV, were more often paralyzed by H. bebetor females than controls and inferred reduced defensive responses in infected hosts. Sometimes, Bt-infected hosts can be more susceptible to parasitism than untreated hosts, due to their slower development and smaller size (Maskarenhas and Luttrell 1997, Erb et al. 2001). However, H. bebetor females in the no-choice test parasitized similar numbers of LC15-treated and LC70-treated hosts as they did healthy ones, and laid equal numbers of eggs on each. Therefore, whatever infection-related factors increased their susceptibility to attack did not alter their acceptability for oviposition for these females.

Parasitoid females discriminated against hosts treated with the LC70 Btk concentration in the choice test and paralyzed and parasitized fewer of them compared with healthy hosts. We conclude that these hosts inoculated with higher doses of Btk displayed sufficiently abnormal symptoms that their acceptability to parasitoids for oviposition was reduced. However, they were parasitized at similar rates and received similar numbers of eggs in the no-choice test. The different results of these two tests suggest that females require experiences with healthy hosts, as they had in the choice test, to discriminate disease symptoms in infected ones. Similarly, Akinkurolere et al. (2009) reported that female H. bebetor parasitized more less-preferred, early-instar larvae of Plodia interpunctella Hubner (Lepidoptera: Pyralidae) when they were offered in no-choice situations, having had no experience with larger ones, and Wan et al. (2019) found that Microplitis pallidipes Szepligeti (Hymenoptera: Braconidae) required prior experience with healthy larvae of Spodoptera exigua (Lepidoptera: Noctuidae) before they could discriminate against those with nucleopolyhedrosis virus (NPV) infections.

Development and survival of H. bebetor larvae were both negatively affected when they developed on hosts subjected to the LC0 and LC15 Btk treatments. Although immature development was delayed by less than 1 d, the adult longevity of wasps was also reduced significantly. It has been argued that natural selection should favor the avoidance of entomopathogen-infected hosts, which should select for parasitoids that can distinguish them from healthy ones (Flexner et al. 1986, Jiang et al. 2014). For example, both the growth and immature survival of the solitary endoparasitoid C. chlorideae were reduced in a dosage-dependent manner, relative to controls, when their H. armigera larval hosts fed continuously on a diet contaminated with sublethal losses.

Choice Test

Female parasitoids failed to discriminate between healthy hosts and those treated with the LC15 of Btk, paralyzing and parasitizing similar numbers of them, and laying similar numbers of eggs on them (Table 3). When LC15-treated hosts were available, females paralyzed more treated hosts than healthy ones, but parasitized equal numbers of them and laid similar numbers of eggs on them. When LC70-treated hosts were available, parasitoids paralyzed and parasitized more healthy hosts than treated ones and laid more eggs on them.

No-Choice Test

In a no-choice situation, similar numbers of hosts were paralyzed in the LC15 treatment as in controls, but more hosts were paralyzed in the LC15 and LC25 treatments (Table 4). However, paralyzed hosts in LC15 were not significantly different from those in LC0. There were no differences among treatments in the numbers of hosts parasitized or the numbers of eggs laid.

Discussion

Female parasitoids did not discriminate against second-instar H. armigera treated with the LC15 concentration of Btk in either choice or no-choice tests, and similar numbers were paralyzed and parasitized as healthy ones. This would suggest that parasitoids did not detect any symptoms of Btk infection in these hosts, which is ostensibly characterized by sluggish movement and weight loss (Blumberg et al. 1997).
concentrations of Btk (Mohan et al. 2008). Similarly, Rolim et al. (2020) reported negative impacts on *P. elaeisis* when this endoparasitoid developed in host larvae of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), many of which extended to the subsequent generation. These impacts included impaired foraging behavior and reduced reproduction and longevity that the authors inferred would reduce compatibility between these biocontrol agents.

These various studies suggest that infection with Bt will reduce the suitability of lepidopteran hosts for parasitism, but provided the hosts survive long enough for completion of wasp development, the parasitoid can survive. Sedaratian et al. (2014) examined *H. hebetor* larvae developing on *H. armigera* larvae treated with sublethal concentrations of Btk and found negative effects on their development, survival, and reproductive rate, which was reflected in diminished life table parameters such as finite and intrinsic rates of increase. However, they did not assess wasp behavioral responses to inoculated hosts. Bt-infected hosts may be smaller and provide poorer nutrition for parasitoid growth and development (Romeis et al. 2006), so a failure to discriminate against infected hosts under field conditions could potentially impair parasitoid reproductive success and reduce fitness. Our behavioral observations indicate that *H. hebetor* has some ability to discriminate against infected hosts, but it depends on both the degree of infection and whether the wasp has an opportunity to compare them to healthy hosts. Given that foliar applications of Btk have relatively short persistence in the field, many pest larvae will probably consume sublethal doses and remain as suitable hosts for parasitism by *H. hebetor*. Any ability of wasps to discriminate against heavily infected hosts could aid biological control outcomes by shifting more parasitism toward uninfected and sublethally infected host larvae.

### Table 2. Mean (± SE) duration of immature stages and adult longevities (in days) of *Habrobracon hebetor* when reared on *Helicoverpa armigera* larvae which fed on Btk-treated chickpea plants for 72 h

| Life stage          | n   | Control       | LC$_{15}$      | LC$_{35}$      | LC$_{70}$      |
|---------------------|-----|---------------|----------------|----------------|----------------|
| Egg                 | 60  | 1.3 ± 0.06 a  | 1.3 ± 0.05 a   | 1.3 ± 0.05 a   | 1.3 ± 0.07 a   |
| Larva               | 47  | 2.3 ± 0.09 c  | 2.5 ± 0.10 bc  | 2.7 ± 0.14 b   | 3.1 ± 0.15 a   |
| Pupa                | 47  | 5.7 ± 0.10 a  | 5.9 ± 0.13 a   | 6.1 ± 0.14 a   | 6.1 ± 0.21 a   |
| Total immature      | 47  | 9.3 ± 0.13 c  | 9.7 ± 0.20 bc  | 10.1 ± 0.24 ab | 10.5 ± 0.28 a  |
| Male longevity      | 26  | 4.4 ± 0.37 a  | 3.6 ± 0.33 ab  | 3.1 ± 0.42 b   | 2.7 ± 0.52 b   |
| Female longevity    | 21  | 10.7 ± 0.72 a | 9.0 ± 0.94 ab  | 7.3 ± 1.04 b   | 6.9 ± 1.54 b   |

Means within rows followed by the same letter were not significantly different among treatments (ANOVA followed by Fisher’s LSD, α = 0.05).

### Table 3. Mean (± SE) numbers of *Helicoverpa armigera* larvae (out of n = 8 per female) paralyzed and parasitized by *Habrobracon hebetor* (n = 60 per treatment) in a 24-h choice test, and total number of eggs laid

| Host type | Variable | Healthy | Treated | t    | P     |
|-----------|----------|---------|---------|------|-------|
| LC$_{15}$ (df = 53) | No. of paralyzed | 2.0 ± 0.14 | 2.0 ± 0.10 | 0.32 | 0.751 |
|          | No. of parasitized | 1.4 ± 0.10 | 1.3 ± 0.09 | 0.73 | 0.471 |
|          | No. of eggs laid   | 10.4 ± 0.77 | 9.2 ± 0.67 | 1.29 | 0.204 |
| LC$_{35}$ (df = 56) | No. of paralyzed | 1.9 ± 0.11 | 2.3 ± 0.09 | 2.42 | 0.019 |
|          | No. of parasitized | 1.1 ± 0.07 | 1.3 ± 0.07 | 1.22 | 0.226 |
|          | No. of eggs laid   | 11.0 ± 0.70 | 11.2 ± 0.66 | 0.23 | 0.819 |
| LC$_{70}$ (df = 57) | No. of paralyzed | 2.2 ± 0.11 | 1.9 ± 0.11 | 2.00 | 0.050 |
|          | No. of parasitized | 1.6 ± 0.68 | 1.0 ± 0.94 | 4.33 | <0.001 |
|          | No. of eggs laid   | 12.7 ± 0.88 | 9.1 ± 0.75 | 3.34 | 0.001 |

Treated host larvae were second instars that fed for 72 h on chickpea plants treated with Btk; healthy larvae were fed on plants treated with distilled water (paired t-test, two tailed).

### Table 4. Mean (± SE) numbers of *Helicoverpa armigera* larvae (out of n = 8 per female) paralyzed and parasitized by *Habrobracon hebetor* females (n = 60 per treatment) and numbers of total eggs laid on the healthy (control) or treated hosts in a no-choice test

| Host treatment | No. of hosts paralyzed | No. of hosts parasitized | No. of eggs laid |
|----------------|------------------------|--------------------------|------------------|
| Control        | 3.8 ± 0.21 a           | 2.8 ± 0.16 a             | 19.9 ± 1.4 a     |
| LC$_{15}$      | 3.7 ± 0.17 b           | 2.6 ± 0.14 a             | 21.1 ± 1.4 a     |
| LC$_{35}$      | 4.4 ± 0.15 a           | 2.9 ± 0.14 a             | 20.8 ± 1.2 a     |
| LC$_{70}$      | 4.3 ± 0.13 a           | 2.6 ± 0.13 a             | 20.3 ± 1.2 a     |
| F              | 7.02                   | 1.07                     | 0.16             |
| df             | 3,230                  | 3,230                    | 3,230            |
| P              | 0.005                  | 0.363                    | 0.925            |

Means within columns followed by the same letter were significantly different (one-way ANOVA followed by Fisher’s LSD, α = 0.05).

Host larvae were fed on Btk-treated chickpea plants for 72 h, beginning as second instars, with control plants treated with distilled water. Means bearing different letters are significantly different within columns (one-way ANOVA followed by Fisher’s LSD, α = 0.05).
Furthermore, laboratory studies have shown that *H. armigera* has the ability to evolve resistance to Btk (Lu et al., 2004), and resistant populations have been detected in the field (Liu et al., 2010). This eventuality might be delayed or precluded if releases of *H. hebetor* reduce the survival of resistant genotypes. Allahyari et al. (2020b) found evidence of additivity between Btk and *H. hebetor* in controlling *H. armigera* on chickpea in an earlier field study, which would support their effectiveness in joint application. Thus, even though Btk had some negative effects on *H. hebetor* under laboratory conditions, and the parasitoid failed to discriminate against hosts treated with low concentrations of Btk, we conclude that these two biocontrol agents are potentially compatible for combined application against *H. armigera* under field conditions.

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**Conflict of Interest**

The authors declare no conflicts of interest exist.

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