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Comparison of the developmental time of *Bracon hebetor* (Hymenoptera: Braconidae) reared on five different lepidopteran host species and its relationship with digestive enzymes

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Abstract. *Bracon* (*Habrobracon*) *hebetor* Say is a polyphagous parasitoid of lepidopteran larvae, including those of the family Pyralidae. There are many reports that this parasitoid attacks the larvae of stored product and field crop pests. However, there is little information on the biological parameters of this parasitoid attacking different lepidopteran hosts or the effect of the hosts on the digestive enzymes of the parasitoid. Hence, in the current study the effect of different lepidopteran hosts, *Ectomyelois ceratoniae*, *Plodia interpunctella*, *Ephestia kuehniella*, *Helicoverpa armigera* and *Malacosoma disstria*, on the biological parameters and digestive enzymes (α-amylase and proteases) of this parasitoid were investigated. The parasitoid performed better on stored product pests, such as *E. kuehniella* and *P. interpunctella*, than field crop pests, such as *H. armigera* and *M. disstria*. For example, in terms of percentage egg hatch, rate of development, off-spring sex ratio and adult dry mass *Bracon hebetor* did much better when it parasitized stored product insects than field crop insects ($P < 0.05$). Interestingly, the quality and quantity of the proteases and α-amylase of the parasitoid larvae parasitizing stored product and field crop pests differed. The greatest activity of these enzymes was recorded in the gut of those parasitoids that were reared on stored product insects (*P. interpunctella* and *E. kuehniella*). It is concluded that stored product insects, which feed on a diet rich in sugar and glycogen, provide physiological conditions that are more suitable for the parasitoid than field crop insects, which feed on diet rich in terpenes and tannins.

INTRODUCTION

Habrobracon (*Bracon*) *hebetor* Say (Hym.: Braconidae) is an idiobiont, gregarious, polyphagous ectoparasitoid of many lepidopteran larvae. It attacks the larval stages of several species of Lepidoptera including *Ephestia kuehniella*, *Plodia interpunctella*, *Ephestia cautella*, *Corcyra cephalonica*, *Galleria mellonella*, *Helicoverpa armigera*, *Ectomyelois ceratoniae*, *Amyelois transitella*, *Tineola bisselliella*, *Sitotroga cerealella* and *Malacosoma* sp. (Lep.: Lasiocampidae) (Brower et al., 1996; Grieshop et al., 2006; Ghimire & Philips, 2010; Kishani-Farahani et al., 2012). Thus, it is a good potential biological control agent of storage (Press et al., 1982; Keever et al., 1986) and field pests (Uwais et al., 2006; Imam et al., 2007). This parasitoid prefers to parasitize the final stages of its hosts' larvae (Akinkurolere et al., 2009) and paralyzes them by stinging and injecting them with venom, after which the female lays eggs on the surface of the paralyzed host. Embryonic development under laboratory conditions (27°C) is reported to be 0.9 days on *Corcyra cephalonica*, 1.68 on *Spodoptera litura*, 1.43 on *H. armigera*, 1.12 on *G. mellonella*, 1.28 on *M. testutalis* and 1.63 on *E. vittella* (Dabhi et al., 2011). Total development time (egg to adult) of *H. hebetor* on different hosts varied from 5.06 days for the male parasitoid on *S. litura* and 31.76 days for the female parasitoid on *C. cephalonica* (Dabhi et al., 2011). The rapid growth rate (RGR) and short development time of *H. hebetor* contributes to its success, which is dependent on many morphological and physiological adaptations. One morphological adaptation is that its larvae have a blind midgut, which has no direct connection to the hindgut, which prevents larval parasitoids from contaminating the body of their hosts and enables them to rapidly consume and store food. In addition, they store nitrogenous waste as urate granules in their haemocoel, which is a mode of storage excretion (Baker & Fabrick, 2000). Baker & Fabrick (2000) studied the changes that occur in the plasma proteins of the host (*P. interpunctella*) and protein digestion during parasitization by *H. hebetor*. There are no significant changes in the haemolymph proteins of the host 72 h post-parasitization. In addition, the main endoproteases of the parasitoids are trypsin- and chymotrypsin-like enzymes (serine proteases). Kryukova et al. (2011) report that the parasitoid's venom interacts with the host's immune system. Investigations of the biology of *H. hebetor* parasitizing seven lepidopteran hosts, *C. cephalonica*, *S. cerealella* Oliver, *Galleria mellonella* L., *Maruca testulalis* Geyer, *H. armigera* (Hübner) Hardwick, *S. litura* Fab. and *Earias vittella*, reveal that *C. cephalonica* and *S. cerealella* are the best hosts for this parasitoid in terms of low male production, fecundity, percentage egg hatch and growth index (Dabhi et al., 2011). In this study the biology of the parasitoid was investigated using five different (except for *H. armigera*) species of stored product and field crop lepidopteran pests. In addition, the effect of the host on the activities of the digestive enzymes of the parasitoid larvae was studied.

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MATERIAL AND METHODS

Insect host rearing

Carob moths, *Ectomyelois ceratoniae* Zeller (Lep.: Pyralidae) were originally obtained from infested pomegranate orchards, Indian meal moths, *Plodia interpunctella* Hübner (Lep.: Pyralidae), from an infested raisin store and Mediterranean flour moths, *E. kuehniella* Zeller (Lep.: Pyralidae) from infested flour in a bakery, cotton bollworm, *H. armigera* Hübner (Lep.: Noctuidae) from infested tomato field and forest tent caterpillars, *Malacosoma disstria* Hübner (Lep.: Lasiocampidae) from an infested galbanum field (*Ferula gummosa* Boiss). These species were reared on their respective hosts at 25 ± 1°C (except for carob moth and cotton bollworm, which were reared at 29 ± 1°C and 27 ± 1°C, respectively), 65 ± 5% RH and 16L: 8D h in a growth chamber.

Parasitoid rearing

The parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) was collected from a raisin store infested with *P. interpunctella*. After rearing a population of the parasitoid on *P. interpunctella*, the parasitoid was transferred and reared for four generations on each of the hosts mentioned above at 27 ± 1° C, 55 ± 5% RH and 16L : 8D h in a growth chamber.

Developmental time

In order to determine the developmental time of the parasitoid, fifty mated female parasitoids (48–72 h old parasitoid) were placed with three hundred larvae of each moth species and allowed to parasitize them for 24 h. Then, 40 eggs were selected under stereoscope (all but one of the eggs on a host larva were removed using a needle) on larvae of each of the host species, which were then individually transferred to a Petri dish (6 × 0.5 cm), which was kept in a growth chamber at the 27 ± 1° C, 55 ± 5% RH and 16L : 8D h and the embryonic, larval and pupal periods, and development time recorded. In addition, the numbers of males and females were recorded and the off-spring sex ratio (female/total) calculated. To obtain the percentage egg hatch, the number of eggs that hatched on each host was recorded. Adult dry mass was measured using the method of Nicol & Mackauer (1999).

Enzyme extraction

Enzymes were extracted using Bandani et al.'s (2009) method. Briefly, the midguts of fourth instar larvae in a 10 mMNaCl solution were carefully dissected under a stereomicroscope (Stemi SV6 ZEISS, Germany). Midguts (200 midguts/ml) were separated and homogenized in a pre-cooled homogenizer (Teflon pestle). The homogenates from preparations were transferred to 1.5 ml centrifuge tubes and centrifuged at 15,000 g for 15 min at 4°C. The supernatants were pooled and stored at –20°C and used as an enzyme source in subsequent analysis.

α-Amylase activity

α-Amylase (protein concentration of 2.0 mg/ml) activity was assayed using the dinitrosalicylic acid (DNS) procedure (Bernfeld, 1955) and 1% soluble starch solution as the substrate as described by Bandani et al. (2009). Briefly, 10 µl of the enzyme solution, 20 µl of the substrate solution and 70 µl of phosphate buffer pH 7.0 were mixed and incubated at 35°C for 30 min after which DNS was added to stop the reaction. The absorbance was read at 540 nm. A blank without substrate but with α-amylase extract and a control containing no α-amylase extract with substrate were run simultaneously with the reaction mixture. All assays were performed in triplicates and with three replications.

General protease activity

A general proteolysis assay was done using a slightly modified version of the methods of Saadati & Bandani (2011) and Gatehouse et al. (1999). Briefly, 10 µl of enzyme extract and 50 µl of substrate solution (Azocasein 2%) were mixed with 40 µl of 20 m M phosphate buffer at pH 7.0. After 60 min incubation 100 µl of 30% trichloroacetic acid (30% TCA) was added to the reaction mixture, which was then kept at 4°C for 30 min and then centrifuged at 15000 g for 15 min to precipitate the substrate that was not hydrolyzed. Finally, 100 µl 1 M NaOH was added to 100 µl of the assay mixture and absorbance measured at 405 nm.

Electrophoresis

Electrophoretic detection of proteolytic enzymes was done using the procedures described by Laemmli (1970) and Dastranj et al. (2013). PAGE for proteolytic activity was first separated on a 10% (W/V) gel co-polymerized with 0.1% gelatin and stacked on a 4% gel with 0.05% SDS. Electrophoresis was conducted at 4°C until the leading dye reached the bottom of the gel. Then, the gel was rinsed with distilled water and washed with 2.5% (V/V) Triton X-100 for 60 min followed by incubation in phosphate buffer (pH 7.0) for about 6 h. Finally, the gel was stained as described by Dastranj et al. (2013).

Amylolytic activity in the gel was detected using the procedures described by Mehrabadi et al. (2012). Briefly, PAGE was performed by separating in 8% (W/V) gel and stacked in 4% gel with 0.05% SDS. Electrophoresis was run until the blue dye reached the bottom of the gel, after which the gel was removed and rinsed with distilled water and left in a solution of 1% (V/V) Triton X-100 for 15 min. The gel was then taken and put in a solution of a phosphate buffer (pH 7) containing 1% starch solution, 2 mM CaCl2 and 10 mMNaCl for 1.5 h. Finally, the gel was treated with a solution of 1.3% I2 and 3% KI to stop the reaction and stain the un-reacted starch background. Zones of α-amylase activities appeared as light bands against a dark background.

Protein determination

Protein concentration was measured using Bradford (1976) method with bovine serum albumin as a standard.

Statistical analysis

Data were compared using the one-way analysis of variance (ANOVA) in SAS 9.1 (SAS Institute, Cary, NC, U.S.A.) followed by a Duncan multiple range test, with significant differences at $P < 0.05$.

Table 1. Developmental times (D.T.) (days) of the different stages and total developmental time of *H. hebetor* reared on five different species of lepidopteran hosts.

HOST	Embryonic D.T.	Larva D.T.	Pupa D.T.	Total D.T. (adult emergence)
Plodia interpunctella	1.89 ± 0.007^{c}	5.25 ± 0.079^{b}	5.60 ± 0.040^{e}	12.76 ± 0.112^{c}
Ephestia kuehniella	2.35 ± 0.031^{b}	5.55 ± 0.069^{b}	6.02 ± 0.015^{d}	13.92 ± 0.09^{d}
Ectomyelois ceratoniae	2.24 ± 0.047^{b}	5.63 ± 0.059^{b}	6.59 ± 0.015^{c}	14.46 ± 0.039^{e}
Helicoverpa armigera	2.71 ± 0.031^{a}	5.68 ± 0.041^{b}	7.36 ± 0.063^{b}	15.73 ± 0.041^{b}
Malacosoma disstria	2.73 ± 0.047^{a}	6.28 ± 0.039^{a}	7.68 ± 0.015^{a}	16.70 ± 0.07^{a}

Means indicated by different letters are significantly different (Duncan, $P < 0.05$).

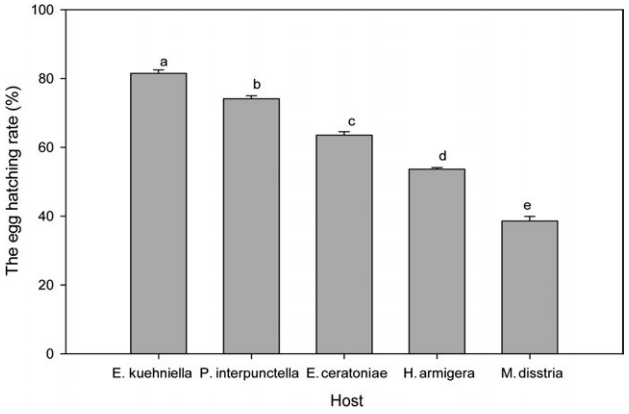

Fig. 1. Percentage egg hatch of *H. hebetor* reared on five different species of lepidopteran hosts. Means indicated by different letters are significantly different (Duncan, $P < 0.05$).

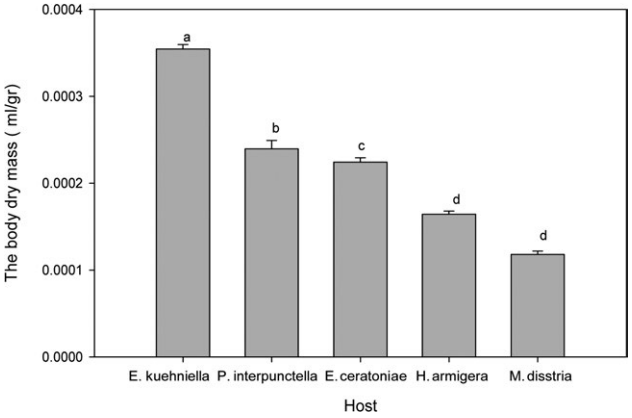

Fig. 2. Adult dry mass of *H. hebetor* reared on five different species of lepidopteran hosts. Means indicated by different letters are significantly different (Duncan, $P < 0.05$).

RESULTS

Developmental time

The duration of the embryonic development of *H. hebetor* depended on the host on which the parent developed (Table 1).

The shortest embryonic period was recorded for the eggs that were laid by parasitoids that developed on *P. interpunctella* and the longest for those that developed on *M. disstria*. The statistical analysis of our findings placed the duration of the embryonic period of *H. hebetor* into one of three groups. The first group included those with the longest embryonic periods, i.e., eggs laid by the parasitoids reared on *H. armigera* and *M. disstria*. The second group those with an intermediate embryonic period, i.e., eggs laid by the parasitoids reared on *E. kuehniella* and *E. ceratoniae*. Finally, the third group included the eggs with the shortest embryonic period, i.e., eggs laid by the parasitoid reared on *P. interpunctella* ($F = 73.57$, $df = 4$, $P < 0.001$) (Table 1).

Larval period of the parasitoid on the five hosts differed significantly, with this longest period recorded for those that developed on *M. disstria* (6.28 days) and shortest for those that developed on *P. interpunctella* (5.25 days). Larval developmental times on *E. kuehniella*, *E. ceratoniae* and *H. armigera*, were 5.55, 5.63 and 5.68 days, respectively ($F = 26.25$, $df = 4$, $p < 0.0001$) (Table 1).

The pupal periods of the parasitoids reared on the different lepidopteran hosts also differed significantly, with the shortest period recorded for those reared on *P. interpunctella* (5.6 days) and the longest for those reared on *M. disstria* (7.68 days). Pupal developmental times on *E. kuehniella*, *E. ceratoniae* and *H. armigera* were 6.02, 6.56 and 7.33 days, respectively ($F = 777.37$, $df = 4$, $p < 0.0001$).

Total developmental times from egg to adult of this hymenopterous parasitoid reared on *P. interpunctella*, *E. kuehniella*, *E. ceratoniea*, *H. armigera* and *M. disteria* were 12.76, 13.92, 14.46, 15.73 and 16.7 days, respectively, and differed significantly on the different hosts ($F = 406.33$, $df = 4$, $p < 0.0001$).

Percentage egg hatch

The percentage egg hatch recorded for the parasitoids reared on the five different hosts differed significantly ($F = 450.815$, $df = 4$, $P < 0.001$). The percentage egg hatch was highest for the parasitoids reared on *E. kuehniella* (81.47%) and lowest for those reared on *M. disstria* (38.57%). The percentage egg hatch was 74.1, 63.52 and 53.63%, respectively for those reared on *P. interpunctella*, *E. ceratonia* and *H. armigera* (Fig. 1).

TABLE 2. The off-spring sex ratio of *H. hebetor* reared on different species of lepidopteran hosts.

Host	Off-spring sex ratio[1]
Plodia interpunctella	0.42 ± 0.66^{a}
Ephestia kuehniella	0.62 ± 0.89^{b}
Ectomyelois ceratoniae	0.51 ± 0.59^{c}
Helicoverpa armigera	$0.39 \pm 0{\cdot}70^{d}$
Malacosoma disstria	0.26 ± 0.55^{e}

[1]The sex ratio [female/(male + female)] of *H. hebetor* off-spring. Means indicated by different letters are significantly different (Duncan, $P < 0.05$).

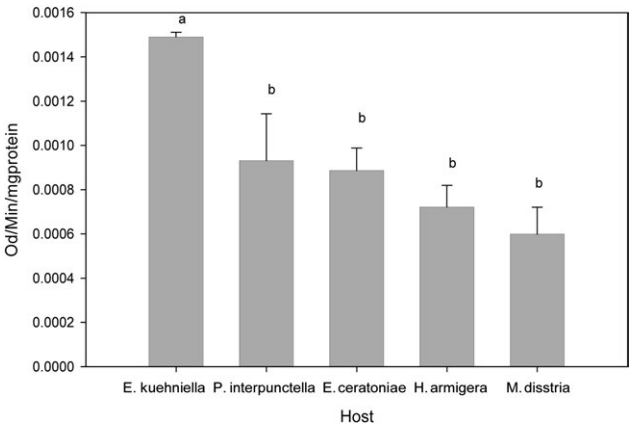

Fig. 3. Protease activity recorded in midgut extracts of larvae of *H. hebetor* reared on five different species of lepidopteran hosts. Each column represents the average of three independent measurements. Means indicated by different letters are significantly different (Duncan, $P < 0.05$).

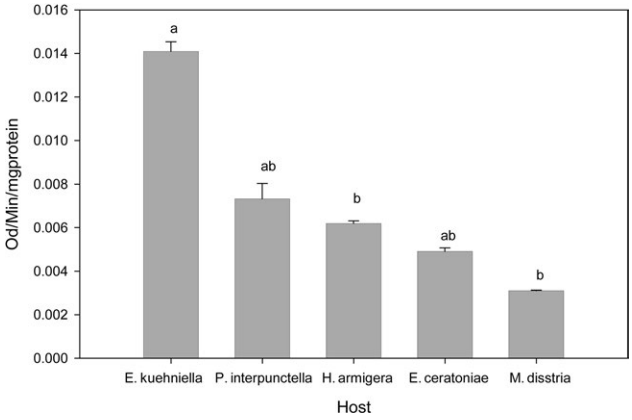

Fig. 4. Amylase activity recorded in midgut extracts of larvae of *H. hebetor* reared on five different species of lepidopteran hosts. Each column represents the average of three independent measurements. Means indicated by different letters are significantly different (Duncan, $P < 0.05$).

Sex ratio

The off-spring sex ratio (female/total) of *H. hebetor* reared on the different hosts differed significantly. The sex ratio was greatest for those reared on *E. kuehniella* (0.62) and lowest for those reared on *M. disstria* (26.1) (Table 2). The sex ratio was 0.42, 0.51 and 0.39 for those reared on *P. interpunctella*, *E. ceratoniae* and *H. armigera*, respectively, and differed significantly on the different hosts ($F = 278.706$, $df = 4$, $P < 0.001$) (Table 2).

Adult dry mass

There were significant differences in the adult dry mass of the parasitoid reared on the different insect hosts ($F = 217.47$, $df = 4$, $P < 0.0001$) (Fig. 2). The greatest dry mass was recorded when they were reared on *E. kuehniella* and the lowest when reared on *M. disstria*. There were no significant differences in parasitoid dry mass when they were reared on *H. armigera* and *E. ceratoniea* (Fig. 2).

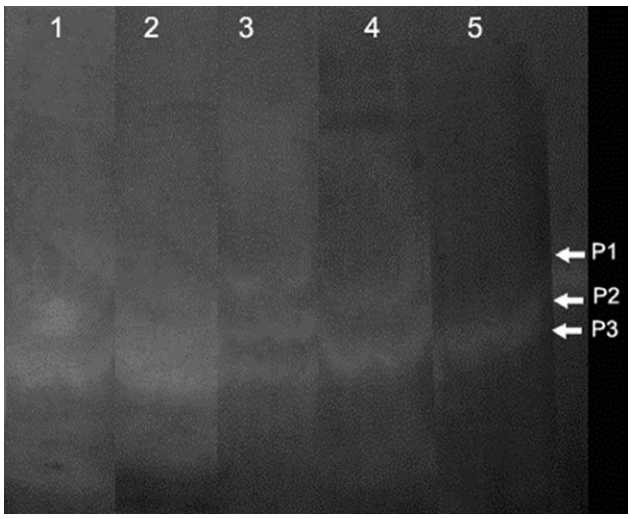

Fig. 5. Proteolytic activity recorded electrophoretically in midgut extracts of larvae of *H. hebetor* using 1% gelatin as the substrate. Numbers are as follow: 1 – *Ectomyelois ceratoniae*, 2 – *Ephestia kuehniella*, 3 – *Malacosoma disstria*, 4 – *Plodia interpunctella*, 5 – *Helicoverpa armigera.*

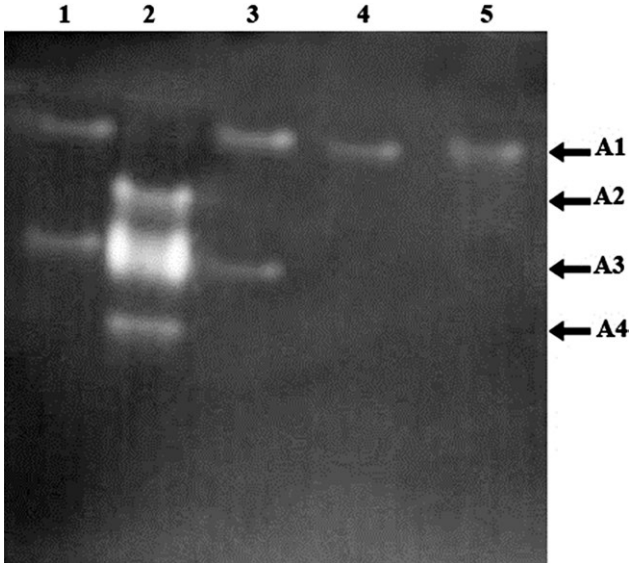

Fig. 6. Amylolytic zymogram of midgut extracts of larvae of *H. hebetor* reared on five different species of lepidopteran hosts using 1% starch as the substrate. Numbers are as follow:1 – *Ectomyelois ceratoniae*, 2 – *Ephestia kuehniella*, 3 – *Malacosoma disstria*, 4 – *Plodia interpunctella*, 5 – *Helicoverpa armigera.*

Protease activity

The level of general protease activity recorded in the midgut of the parasitoid larvae was highest when they were reared on *E. kuehniella* (Fig. 3), being almost two times that recorded for larvae reared on the other hosts. The lowest protease activity was recorded in the midguts of larvae reared on *M. disstria.* The order of the protease activity recorded in the midguts of the larvae reared on the different hosts was *E. kuehniella* > *H. armigera* > *E. ceratoniae* > *P. interpunctella* > *M. disstria* (Fig. 3).

In the gel assay (Fig. 5) three protease bands (P1, P2, and P3) were recorded when the parasitoid was reared on *E. kuehniella* and *M. disstria*, only one (P3) band when it was reared on *H. armigera* and two bands (P2 and P3) when reared on *E. ceratoniae* and *P. interpunctella*.

Amylase avtivity

The level of amylase activity recorded in the midguts of the parasitoid larvae was highest when they were reared on *E. kuehniella*, followed by *P. interpunctella*, *H. armigera*, *E. ceratoniae* and *M. disstria* (Fig. 4). The lowest α-amylase activity was recorded in the midguts of larvae reared on *M. disstria*. Interestingly, the gel assay revealed three clear amylase bands (A2, A3 and A4) for the parasitoid larvae reared on *E. kuehniella* and fewer bands for those reared on the other hosts (Fig. 6). Only two faint amylase bands (A1 and A3) were recorded for the parasitoid larvae reared on *E. ceratoniae* and *M. disstria,* and one faint band (A1) for those reared on *P. interpunctella* and *H. armigera* (Fig. 6).

DISCUSSION

The current study revealed that although *H. hebetor* can parasitize several different lepidopteran hosts, its development time, off-spring sex ratio, percentage egg hatch and

adult dry mass significantly depends on the host ($P < 0.05$). For example, *P. interpunctella* and *E. kuehniella* were the best hosts in terms of all the parameters evaluated. However, in terms of developmental time and off-spring sex ratio *P. interpunctella* was a better host than *E. kuehniella*. For example, total developmental time of the parasitoid reared on *P. interpunctella* was 12.76 days and on *E. kuehniella* 13.92 days. The off-spring sex ratio recorded for the parasitoids reared on the *P. interpunctella* and *E. kuehniella* were 0.42 and 0.62, respectively. However, in terms of percentage egg hatch and adult dry mass, *E. kuehniella* was a much better host than *P. interpunctella*. Both of these hosts (*P. interpunctella* and *E. kuehniella*) are important stored product pests (Heimpel et al., 1997; Darwish et al., 2003). The performance of this parasitoid on field crop pests such as *M. disstria*, a serious pest of deciduous hardwood trees (Batzer & Morris, 1978) and *H. armigera*, a polyphagous and cosmopolitan insect pest of various plant species, including cotton, maize and tomato (Sharma et al., 2004; Srinivas et al., 2004) was poor i.e. it took longer to complete its development on these two hosts. According to Dabhi et al. (2011) the performance of *Habrobracon hebetor* on stored product insects, like *Corcyra cephalonica*, a pest of stored products such as cereals, cereal products, oilseed, pulses, dried fruit, nuts and spices, and *S. cerealella*, a serious pest of cereal kernels, was much better than on *H. armigera*, *S. litura* and *E. vittella.*

Interestingly, the level of activity of the digestive enzymes α-amylase and general proteases in the larvae of the parasitoid was highest in those reared on the two stored product pests (*P. interpunctella* and *E. kuehniella*). For example, in terms of α-amylase activity, three clear amylase bands were recorded for the larvae of the parasitoid reared on *E. kuehniella*, indicating that this host provides better nutrition than the other hosts, which induced a higher digestive enzyme activity.

The activity of enzymes in the guts of larvae reared on other hosts, such as *M. disstria* and *H. armigera* was lower (qualitative measure) and there were fewer isoenzymes (quantitative measure). One explanation of this is that those parasitoid hosts that feed on a diet rich in secondary metabolites, such as tannins and terpenes, which adversely affect the growth of the host are likely to also affect the growth and development of the parasitoid and the activity of its digestive enzymes. It is likely that some compounds in the host inhibited the activity of the digestive enzymes of the parasitoid (Boigegrain et al., 1992) because when reared on these two hosts the activities of both of their digestive enzymes (amylase and proteases) were less and there were fewer isoenzymes in the extracts. The lowest amylase and protease activity were recorded in the guts of the parasitoid larvae reared on *M. disstria* and the highest in the gut of those reared on *E. kuehniella*. The same pattern was recorded for percentage egg hatch, off-spring sex ratio and adult dry mass i.e. in terms of these parameters the performance was much better when the parasitoid was reared on stored product insects, such as *E. kuehniella* and *P. interpunctella*, which accords with findings of Jhansi & Babu (2003) and Dabhi et al. (2011). *B. hebetor* reared on fifth instar larvae of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (natural diet) and in vitro (artificial diet) did better on the natural diet in terms of the time it took them to complete their larval and pupal development, which indicates that the artificial diet was less suitable than the natural diet. A similar situation was recorded in this study as the field crop insects proved to be less suitable hosts than the larvae of stored product pests.

Based on these findings it is concluded that stored product insect pests, such as *P. interpunctella* and *E. kuehniella,* are more suitable hosts than field crop pests, such as *M. disstria* and *H. armigera*, for the parasitoid *H. hebetor* since it performs better on stored product insects. In addition, the parasitoid's digestive enzymes function better qualitatively and quantitatively indicating that stored product insects provide more suitable nutrition for the parasitoid. Thus, it is concluded that of those tested store product insects are the most suitable for the viability of this parasitoid.

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