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ORIGINAL RESEARCH ARTICLE

Towards development of a parasitoid cottage industry of the parasitoid wasp *Habrobracon hebetor* (say): optimum rearing and releases conditions for successful biological control of the millet head miner *Heliocheilus albipunctella* (De Joannis) in the Sahel

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Abstract

Augmentative biological control by the parasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) is the most promising strategy to control millet head miner, *Heliocheilus albipunctella* (De Joannis) a major insect pest of pearl millet in the Sahel. As *H. hebetor* survival is somehow challenging during the nine month long off-season when the host, *H. albipunctella* is in diapause, there needs to be a sufficient supply of parasitoids for fresh release each year. Therefore, the aim of this study was to establish a small-scale parasitoid rearing process adjusted to the Sahel conditions that can be scaled-up as necessary. We conducted experiments to fine-tune and standardize the rearing technique of *H. hebetor* for cottage industrial use. The results showed that parasitoids fed with 30% honey solution and supplied daily with one late-larval-stage *Corcyra cephalonica* Stainton (Lepidotera, Pyralidae) produced highest number of progeny. The optimal times for mating and egg fertilization, was achieved when a male and female pair was confined for 24 h in a 30-cc vial. Our findings indicated that, compared with the conventional rearing method -2 females supplied once with 25 *C. cephalonica* larvae-, this new method resulted in 14-times greater parasitoid production. Furthermore parasitoid female can be stored for up to three weeks at fluctuating 23–32°C temperature and 25%–80% relative humidity for numbers accumulations prior to on-farm augmentative releases without altering its fitness.

Keywords Mass rearing · *Corcyra cephalonica* · Feeding medium · Lifespan · Fecundity · Burkina Faso

Introduction

Pearl millet is the world's hardiest warm season cereal crop, surviving on the poorest soils in the hottest, driest regions. Despite this extreme climatic adaptation, pearl millet crops are affected by many biotic factors, including insect pests (Nwanze and Harris 1992). Among these, millet head miner (MHM) *Heliocheilus albipunctella* (De Joannis) (Lepidoptera: Noctuidae) is a major chronic insect pest of millet in the Sahel. Infestations of MHM are more severe in the drier zones of the Sahel (Nwanze and Harris 1992). The MHM larvae feed on the panicle, causing sufficient damage to prevent grain formation (Ndoye 1991; Nwanze and Harris 1992). Outbreaks of MHM occur nearly every year in the Sahel, especially among early planted millet or early maturing material (Eisa et al. 2007). The yield losses resulting from MHM are substantial, ranging from 40% to 85% (Gahukar et al. 1986; Nwanze and Sivakumar 1990; Krall et al. 1995; Youm and Owusu 1998).

Recently, the parasitoid wasp, *Habrobracon* (=*Bracon*) *hebetor* (Say) (Hymenoptera: Braconidae) has been studied for the biological control of MHM in the Sahel (Payne et al. 2011; Ba et al. 2013; Baoua et al. 2014). Augmentative releases of *H. hebetor* led to a maximum of 80% parasitism of MHM larvae (Payne et al. 2011; Ba et al. 2013), resulting in yield increases of at least 30% (Baoua et al. 2014). Although releasing *H. hebetor* controls MHM, further MHM outbreaks

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can occur in subsequent years because there are few alternate hosts for the parasitoid to survive on during the off-season (Kabore et al. 2017). Consequently, new populations of parasitoids must be released each growing season. Therefore, to make a biological control program sustainable, parasitoids must be released every year. There is a growing demand for *H. hebetor* for release in West Africa because of the strong interest and acceptance of new technology by farmers and by local non-governmental organizations (Ba et al. 2013). However, the question remains as to how to produce sufficient numbers of parasitoids to meet the demand. Small-scale cottage industry has been the basis for parasitoid production for augmentative biocontrol in other settings (van Lenteren 2012). Therefore, one option is to establish a community-based cottage industry that can be scaled up to produce parasitoids as necessary. Parasitoid production must be optimized to make such a strategy commercially and technically viable.

The major challenge in current production is the off-season high temperatures –up to 45 °C– that are unfavorable for parasitoid multiplication. It is not feasible to operate a controlled temperature room because of the lack of electricity in many small communities, and the high cost of energy. At present, parasitoid production is maintained at a slow pace in the off-season on larvae of the rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), and numbers are only increased in mid-June, eight weeks before releases, when the rainy season begins and the temperatures begin to decrease. The temperature becomes more favorable for parasitoid production in mid-July, when the rainy season is well established. This gives a very narrow window for parasitoid multiplication, which limits the number of parasitoids that can be produced in the season.

Another option is to store the parasitoid for few weeks prior to field releases. Cold storage of parasitoids is used in other setting as a valuable tool for increasing the shelf life of parasitoid wasps (Boivin and Colinet 2011). However, community-based cottage industry in remote areas of the Sahel region cannot easily run a cooled environment system for storage of parasitoid. Given the narrow time for parasitoid multiplication, the only option is to store parasitoids at room temperature for few weeks and accumulate numbers needed for field releases. It is then necessary to identify to which extent females could be stored and kept their ability for effective parasitism.

Consequently, it is desirable to improve the mass production process for use in the Sahelian context and for application in the community-based cottage industry that is developing in this region. The objectives of this study were to improve rearing techniques for *H. hebetor* to produce large numbers of parasitoids in a short time. Specifically, we aimed to:

i) Determine the optimal *H. hebetor* male and female confinement conditions for mating and egg fertilization;
ii) Identify the best media for adult feeding and highest parasitoid production
iii) Identify the best media for the longest parasitoid lifespan for storage of adults, accumulation of numbers and use in a timely manner.

Materials and methods

Insect cultures

A colony of *Habrobracon hebetor* was established and maintained in the laboratory on an alternate host, the rice moth *Corcyra cephalonica*. *H. hebetor* individuals were collected from the field from MHM larvae and *C. cephalonica* individuals were collected from stored pearl millet. Both insect colonies were established from wild *H. hebetor* and *C. cephalonica* collected in 2011. Once a year, wild insects were added to the colonies to maintain genetic variability in the population as suggested by Henry et al. (2010). Both insects were reared in the laboratory under ambient conditions. The *C. cephalonica* rearing technique was adapted from that developed by Bal et al. (2002). Wooden cages (20 × 20 × 13 cm) with muslin cloth on three lateral sides and wood at the bottom were used for mass rearing of *C. cephalonica*. A mixture of 1.2 kg millet flour and 1.8 kg millet grain was introduced into the cages and inoculated with approximately 3000 *C. cephalonica* eggs. Subsequent generations were regularly obtained after 30 d at room temperature (average, 26 ± 2 °C). The 3rd and 4th instars of *C. cephalonica* larvae were used for the mass rearing of *H. hebetor*. For this purpose, 25 *C. cephalonica* larvae were confined in a Petri dish for 48 h with two mated *H. hebetor* females. The new generation of *H. hebetor* emerged 8–14 d after confinement.

Study environment

The study was conducted under uncontrolled climatic conditions in the laboratory of Institut de l'Environement et de Recherche Agricole in Kamboinse, Burkina Faso, under a photoperiod of 14 L: 10 D at a fluctuating temperature of 23–32 °C with 25%–80% relative humidity.

Identification of optimal duration of H. Hebetor male and female confinement and container size for mating and egg fertilization

Three sizes of plastic vials (30, 50, and 74 ml) were tested to determine the optimal confinement conditions for mating. One newly emerged unmated female and male pair were kept in each of the containers for 15 min, 1 h, 12 h, 24 h, and 48 h. We used 10 *H. hebetor* pairs corresponding to 10 replicates per

combined type of container and confinement period. After confinement, each female was kept in a Petri dish with one 5th instar *C. cephalonica* larva for 24 h. The female was moved each subsequent day to another Petri dish with another 5th instar *C. cephalonica* larva to parasitize, for a total of seven consecutive days. The female mating or fertility status was indirectly assessed by checking the sex of the progeny produced by each female because unmated/unfertilized females produce only males (Antolin and Strand 1992; Ode et al. 1997).

Effects of feeding medium on longevity and fecundity of *H. hebetor* and population growth potential

Adult *H. hebetor* were fed three different substrates: diluted sugar, diluted honey, and distilled water. The treatments were as follows: i) 10% honey solution; ii) 30% honey solution; iii) 10% sugar solution; iv) 30% sugar solution; v) distilled water; and vi) no feeding substrate (control). Newly emerged females and males were individually kept in Petri dishes and fed with one of the substrates. For each treatment 15 males and 15 females corresponding to 15 replicates were used. Cotton wool soaked with the substrate was the feed supply for the females and males, and was replaced daily until the individuals died. The number of live and dead insects was counted daily and lifespan of the adults was recorded.

The same test was duplicated with eight supplemental females per treatment (i.e. eight replicates). *H. hebetor* female and male pair was kept in a 30 ml vial for 24 h for mating. This time, in addition to the above-mentioned feeding substrate, the females were provided with a 5th instar *C. cephalonica* larva each day to parasitize. The females were kept with the larva in a Petri dish for 24 h and moved each day to a new Petri dish with another larva until the female died. The Petri dishes were incubated until *H. hebetor* progeny emerged. This time, in addition to female lifespan, the number of eggs laid per female, number of emerging adults, and the sexes of the emerging adults were recorded. The following parameters of *H. hebetor* populations were calculated: intrinsic rate of natural increase (*Rm*), net reproductive rate (*Ro*), mean generation time (*T*), and population-double time (*DT*) using Birch (1948) formula.

Effect of maternal ages of H. Hebetor on parasitism and progeny

Newly emerged *H. hebetor* females were kept individually in Petri dishes for 2, 7, 14 or 21 days, during which time they were fed daily with fresh cotton wool soaked with 30% honey solution. *H. hebetor* females were then confined with one male in a 30-ml vial for 24 h for mating. Each female was then provided with a 5th instar *C. cephalonica* to parasitize. The females were kept with the larva in a Petri dish for 24 h and moved each day to a new Petri dish with another 5th instar *C. cephalonica* larva until the female died. The Petri dishes were incubated until *H. hebetor* progeny emerged. For each age, 20 females (i.e. 20 replicates) were used. The number of paralyzed and parasitized host-larvae, number of eggs laid per female, number of emerging progeny, sexes of the emerging adults, and female lifespan were recorded.

We calculated the following parameters of *H. hebetor* populations: intrinsic rate of natural increase (*Rm*); net reproductive rate (*Ro*); mean generation time (*T*); population-double time (*DT*); and finite rate of increase (λ).

H. hebetor first generation progeny emerging from the previous experiment were used to identify the effect of maternal parent age on subsequent –daughter- parasitoid generation performance. The emerging *H. hebetor* females were kept by pair with a male for 24 h for mating and then transferred to a Petri dishes with a 5th instar *C. cephalonica* to parasitize. A total of 16 females (i.e. 16 replicates) from each age group were used for this test. The parasitoid females were kept with the host-larva in a Petri dish for 24 h and moved each day to a new Petri dish with another larva until the female died. The Petri dishes were incubated until *H. hebetor* progeny emerged. The number of eggs laid per female, number of emerging progeny, and the sexes of the emerging adults were recorderd.

Data analysis

Data were subjected to an analysis of variance (ANOVA) (PROC GLM) using SAS software version 9.1 (SAS 2003). When ANOVAs revealed significant differences, means were separated by the Student–Newman–Keuls test at the 5% level. Percentage values of parasitized and paralyzed larvae were arcsine transformed prior to statistical tests. Life table parameters were analyzed and based on specific age females. The jackknife procedure was used to estimate pseudo values life table parameters (Maia et al. 2000).

Results

Optimal H. Hebetor male and female confinement conditions for egg fertilization

For all containers, the proportion of mated females increased significantly with longer duration of confinement (30-ml: df = 4; F = 12.29; $P < 0.0001$; 50-ml: df = 4; F = 3.75; $P = 0.010$; 74-ml: df = 4; F = 3.98; $P = 0.007$) (Fig. 1). Regardless of the duration of male and female confinement, the rate of successful egg fertilization was significantly higher in the 30-ml vial (F = 3.72; $P = 0.027$) (Fig. 1) than in the 50-ml and 74-ml vials. All of the females successfully mated when confined with males for 24-h in the 30-ml vial (Fig. 1).

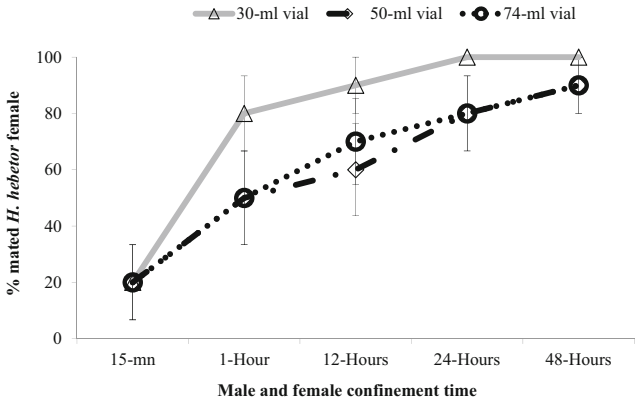

Fig. 1 Percentage of mated females of *H. hebetor* (% means ± SE) when confined with males at different times with different types of containers

Effects of feeding media on H. Hebetor adult longevity, female fecundity, and emerging adult sex ratio and population growth potential

The lifespan of *H. hebetor* males and females increased significantly from roughly 4 d to ~32 d when fed with either sugar or honey solutions in absence of host-larvae (Table 1). When *H. hebetor* females were supplied daily with a new *C. cephalonica* larva, the addition of feeding substrate did not significantly affects female lifespan (Table 1). In that case, the females remained fertile throughout their entire lifespan and laid up to ~860 eggs. However females fed with 30% honey produced higher number of progeny (Table 1).

The effects of feeding media values on life table parameters of *H. hebetor* are presented in Table 2. The net reproductive rate (R_0) was the only parameter that differed significantly between treatments. There was no significant difference for other parameters regardless of feeding substrate as far as *H. hebetor* female was supplied daily with one fresh *C. cephalonica* larvae (Table 2). Projected number of produced progeny reached up to ~290,000 individuals within 1 month (Table 2).

Effect of H. Hebetor maternal parent age on progeny production and first generation fitness

The parasitoid *H. hebetor* was able to paralyze and parasitize *C. cephalonica* larvae regardless of the age of the female. However significantly higher effective parasitism with deposit of eggs was recorded with younger females (Table 3). Likewise, younger female laid eggs during a significant longer time and deposited significantly more eggs (Table 3). In contrast the sex ratio of the progeny became female-biased as the maternal parent got older (Table 3). Consequently, all life table parameters, of *H. hebetor* were significantly affected by the maternal parent age (Table 4). The intrinsic rate of increase and the finite rate of increase got higher with older females. On the contrary, the net reproductive rate, the generation time,

Table 1 *H. hebetor* adults lifespan, females fecundity and progeny when feed on different substrate in absence or presence of host-larvae

Substrate	Without *C. cephalonica* host-larvae		With *C. cephalonica* host-larvae		
	Male lifespan (days±SE)	Female lifespan (days±SE)	Female lifespan (days ±SE)	Total number eggs laid per *H. hebetor* female (Means ±SE)	Total number *H. hebetor* progeny (Means ±SE)
No substrate	3.93 ± 0.15c	5.80 ± 0.44b	30.00 ± 0.75a	750.25 ± 14.04b	359.00 ± 6.53ab
Distilled water	6.93 ± 0.67c	8.80 ± 1.03b	30.00 ± 0.85a	762.87 ± 16.81ab	337.00 ± 26.69ab
10% Sugar	18.66 ± 3.01b	23.86 ± 1.89a	31.62 ± 1.11a	804.00 ± 20.45ab	265.00 ± 9.80b
30% Sugar	19.60 ± 2.10b	26.46 ± 2.82a	30.50 ± 0.98a	816.87 ± 34.85ab	278.12 ± 17.12b
10% Honey	18.26 ± 2.36b	25.86 ± 3.42a	29.00 ± 1.29a	805.20 ± 28.20ab	369.00 ± 31.43ab
30% Honey	29.73 ± 2.41a	32.06 ± 4.24a	32.00 ± 0.79a	857.12 ± 31.52a	386.60 ± 54.99a
	Df = 5; F = 20.84; $P < 0.0001$	Df = 5; F = 15.86; $P < 0.0001$	Df = 5; F = 1.24 $P = 0.29$	Df = 5; F = 2.29 $P = 0.04$	Df = 5; F = 3.47 $P = 0.005$

For each parameter means were compared by a Student–Newman–Keuls test at the 5% level, with different alphabetic letters indicating significant differences

Table 2 Life table parameters of *H. hebetor* feeding on different substrate in presence of *C. cephalonica* host-larvae and projected number of progeny produced

Substrates	Net reproductive rate (Ro ± SE)	Intrinsic rate of increase (Rm ± SE)	Generation time (T ± SE)	Population doubling time (DT ± SE)	Projected number of *H. hebetor* progeny produced in 1 month
No substrate	167.25 ± 15.00a	0.49 ± 0.03a	10.45 ± 0.74a	1.43 ± 0.09a	213,108
Distilled water	123.75 ± 21.69ab	0.40 ± 0.01a	11.83 ± 0.64a	1.74 ± 0.04a	85,118
10% Sugar	104.95 ± 12.92b	0.44 ± 0.08a	11.48 ± 1.97a	1.78 ± 0.36a	84,504
30% Sugar	100.63 ± 12.03b	0.52 ± 0.03a	8.91 ± 0.90a	1.35 ± 0.10a	69,642
10% Honey	126.38 ± 13.88ab	0.37 ± 0.04a	13.20 ± 1.49a	1.90 ± 0.21a	164,645
30% Honey	169.80 ± 7.55a	0.38 ± 0.01a	13.37 ± 0.48a	1.83 ± 0.07a	289,944
	Df = 5; F = 4.28; $P = 0.009$	Df = 5; F = 1.78; $P = 0.16$	Df = 5; F = 2.10; $P = 0.11$	Df = 5; F = 1.46; $P = 0.24$	

For each parameter means were compared by a Student–Newman–Keuls test at the 5% level, with different alphabetic letters indicating significant differences

Table 3 Percentage host larvae parasitized, number eggs laid, progeny produced, progeny sex ratio in relation to different *H. hebetor* female ages

Parental females age (days)	Paralyzed host-larvae (% ± SE)	Parasitized host-larvae (% ± SE)	Number of days of laying (Means ± SE)	Total number eggs laid per *H. hebetor* female (Means±SE)	*H. hebetor* female proportion (% ± SE)
2	99.31 ± 0.37	98.68 ± 0.60a	31.15 ± 2.30a	833.45 ± 65.96a	43.78 ± 2.21b
7	97.89 ± 1.00	92.86 ± 1.91bc	32.37 ± 1.20a	675.00 ± 36.45b	51.10 ± 6.16ab
14	99.20 ± 0.48	96.66 ± 0.91ab	23.33 ± 1.96b	504.33 ± 55.92c	58.77 ± 4.77a
21	99.44 ± 038	89.95 ± 1.79c	19.70 ± 1.95b	426.30 ± 46.98c	60.55 ± 3.25a
	DF = 3; F = 1.35; $P = 0.26$	DF = 3; F = 7.50; $P = 0.0002$	DF = 3; F = 3.02; $P = 0.03$	DF = 3; F = 11.99; $P < 0.0001$	DF = 3; F = 3.10; $P = 0.03$

For each parameter means were compared by a Student–Newman–Keuls test at the 5% level, with different alphabetic letters indicating significant differences

Table 4 Life table parameters of *H. hebetor* in relation to different maternal female ages

Parental females age (days)	Net reproductive rate (Ro)	Intrinsic rate of increase (R_m)	Generation time (T)	Population doubling time (DT)	Finite rate of Increase (λ)
2	151.90 ± 12.06ab	0.334 ± 0.007b	14.55 ± 0.55a	2.02 ± 0.04a	1.41 ± 0.01b
7	167.93 ± 7.61a	0.385 ± 0.03ab	14.64 ± 1.61a	1.98 ± 0.20a	1.47 ± 0.04ab
14	93.52 ± 10.79b	0.41 ± 0.02ab	11.17 ± 0.89ab	1.72 ± 0.10a	1.51 ± 0.03ab
21	114.80 ± 31.46ab	0.44 ± 0.02a	10.21 ± 0.90b	1.592 ± 0.06a	1.56 ± 0.03a
	DF = 3; F = 3.53; $P = 0.02$	DF = 3; F = 3.52; $P = 0.02$	DF = 3; F = 4.68; $P = 0.007$	DF = 3; F = 2.77; $P = 0.05$	DF = 3; F = 3.60; $P = 0.02$

For each parameter means were compared by a Student–Newman–Keuls test at the 5% level, with different alphabetic letters indicating significant differences

and the population doubling time varied between treatments but could not really be correlated to the age of the maternal parent a (Table 4). The subsequent generation of *H. hebetor* was not significantly affected by the age of the maternal parent (Table 5). All the subsequent generation of females laid similar number of eggs, and had similar number of progeny, with no significant difference of sex ratio, regardless of the age of the maternal parent they derived from (Table 5).

Discussion

The female determines the sex ratio of offspring in hymenopteran haplodiploid parasitoid wasps through mating (Godfray 1994; Harvey and Gols 1998; Jarosik et al. 2003). Usually, males develop from unfertilized eggs and females from fertilized eggs (Harvey and Gols 1998; Damiens et al. 2003; Jarosik et al. 2003). This feature is also observed in *H. hebetor* (Benson 1973; Holloway et al. 1999). Thus, females must mate to produce fertilized eggs and to produce females among the progeny. The production of many fertilized eggs and female progeny are essential for the mass production of *H. hebetor* in an augmentative biological control program. Our results showed that the best conditions for successful egg fertilization were confinement of female and male pairs for 24 h in small vials (30 ml), rather than larger vials (50–74 ml). In general, *H. hebetor* females fight and reject the male at first during courting, and so the male needs time to continue the courtship until the female is ready to mate. Larger vials offer females more space to escape from the male during courting. The small vials offered less space for the female to escape, increasing the chances of mating within 24 h. This corroborates the findings of Ode et al. (1995), who reported that successful mating was achieved in 15 min when male and female pairs were confined in Petri dishes. Ghimire (2008) also concluded that larger containers are less suitable for fecundity, because parasitoid *Bracon hebetor* females spend more time searching for the host and less time parasitizing the host in larger containers than in smaller containers.

The parasitoid wasp progeny develop on individual hosts, which are their entire food source. However, the adults also sometimes feed on the same host larva. This is known as host feeding, and it supplies the adult parasitoids with nutrients needed for egg maturation and to increase fecundity (Jervis and Kidd 1986; Heimpel and Collier 1996; Dai et al. 2014). In our experiment, feeding parasitoid adults with either sugar or honey solution in the absence of a host larva increased their life expectancy from 3 to 5 d to 23–32 d. This is consistent with the results of previous studies (Temerak 1983; Ode et al. 1996; Radhika and Chitra 1998; Burger et al. 2004). However, the addition of the host larvae led to 3.41–5.17 fold increase in life expectancy of female that were not given any substrate or only water. In the presence of the host-larvae, the addition of sugar or honey did not increase *H. hebetor* female lifespan. However the addition of the 30% honey solution substantially increased fecundity and progeny production as observed for other parasitoids (Hossain and Haque 2015). Surprisingly. the sugar solution did not enhance *H. hebetor* fecundity and offspring contrarily to previous findings in other settings (Tena et al. 2015). This could be related to the quality of the sugar as suggested by Tian et al. (2016).

In our study, the oviposition rates and offspring production rates were similar to those obtained in previous studies. Our results showed that the fecundity of the parent *H. hebetor* was ~860 eggs/female and the offspring production was ~ 390/female, compared with 200–400/female in other studies (Nikam and Pawar 1993; Yu et al. 1999; Chen et al. 2011). Consequently, the *Ro* and *Rm* values were higher and the *T* and *DT* values were lower than those obtained in previous studies (Amir-maafi and Chi 2006; Eliopoulos and Stathas 2008; Saadat et al. 2016), indicating rapid reproductive potential of the parasitoid population. We propose that these differences are due to the improved mass rearing procedures in our study. In our new rearing method, the female was supplied with one new larva per day, whereas 15–30 larvae were supplied simultaneously in some previous studies (Nikam and Pawar 1993; Eliopoulos and Stathas 2008; Yu et al. 1999; Chen et al. 2011). With conventional rearing methods, when

Table 5 Percentage host larvae parasitized, number of eggs laid, progeny produced, progeny sex ratio of subsequent generation of *H. hebetor* developing from maternal parent of different ages

Generation 1 developing from maternal parent of different ages	Number *H. hebetor* eggs laid per parasitized host-larvae (Means±SE)	Total number eggs laid per *H. hebetor* female (Means ±SE)	Total Number *H. hebetor* progeny (Means±SE)	*H. hebetor* female proportion (% ± SE)
2	26.74 ± 0.66	835.00 ± 82.18	368.5 ± 31.48	40.73 ± 2.42
7	25.89 ± 1.24	808.17 ± 86.09	416.37 ± 41.19	38.33 ± 2.05
14	27.48 ± 1.00	818.62 ± 70.69	356.12 ± 29.00	43.61 ± 2.60
21	26.00 ± 1.02	802.37 ± 56.98	401.75 ± 38.84	42.16 ± 2.00
	DF = 3; F = 1.21; $P = 0.31$	DF = 3; F = 0.40; $P = 0.99$	DF = 3; F = 0.62; $P = 0.60$	DF = 3; F = 0.97; $P = 0.41$

two females are supplied once with 25 stage 3 and 4 *C. cephalonica* larvae, it led to a progeny of 35 individuals per female (Ba et al. 2013, 2014). Total projected production corresponds to 20,000 *H. hebetor* individuals within 1 month, requiring 13,700 *C. cephalonica* larva. The new method requires almost half the number of host larvae per *H. hebetor* parent female to produce more than 14 times more parasitoid offspring within 1 month. This is a highly significant increase in number of parasitoids that can be produced in a 1 month.

Our findings indicated that *H. hebetor* females fed with 30% honey solution and kept at fluctuating 23–32 °C temperature and 25%–80% relative humidity for up to 21 days, can still effectively paralyze and parasitize the host larvae. They first paralyze the host by stinging and then lay a variable number of eggs on the ventral surface of the larva, as described previously (Hagstrum and Smittle 1977; Antolin et al. 1995). 14–21 day-old females laid less eggs and had lower progeny than newly emerged and 7 day-old females. Similar findings have been reported after storage of *H. hebetor* at cold temperatures for more than 20 days (Chen et al. 2011). However, with cold storage of *H. hebetor*, the age of the maternal parents had no effect on parasitism and reproduction of F1 generation (Chen et al. 2011). Even though 21 day-old females produced half the number of eggs of newly emerged female, they parasitized ~90% of host-larvae,which is a very good rate of parasitism. As females get older, their progeny had significantly higher number of females. This is known as sex ratio manipulation and has been extensively described by King (1987). This is very interesting as our mass production sometimes lack enough number of females. Our data suggests that *H. hebetor* can be stored at room temperature for up to 21 days before mating, for maintaining, accumulating large numbers, and increase of female numbers in our mass rearing program for augmentative field releases.

These results have several implications: i) The current rearing technique can be significantly improved for cost-effective mass parasitoid production; ii) parasitoid multiplication can start 4 weeks prior to on-farm release in mid-July when the rainy season is well established and temperatures have cooled; iii) parasitoid adults can be stored for 3 weeks for timely release as long as they are fed with sugar or honey solution, thereby allowing a constant supply to control MHM; and iv) parasitoids can be released more than 2 weeks before MHM larvae are present in the field and survive on other food sources, such as nectar, fruit, pollen, and honeydew (Heimpel and Collier 1996; Jervis et al. 1996; Tooker and Hanks 2000; Burger et al. 2004; Rahat et al. 2005).

Even though *H. hebetor* mass cultured on *C. cephalonica* has been successfully used for augmentative releases against the MHM in the Sahel (Kabore et al. 2017; Baoua et al. 2018), the field fitness of the parasitoid derived from the new proposed rearing method need to be further investigated. As indicated in other settings, rearing conditions can affect field performance of released parasitoids (Collier and Steenwyk 2004; Gandolfi et al. 2003; Bloem et al. 2004; Joyce et al. 2010; Sepúlveda et al. 2017).

Conclusion

In summary, we have developed a method to make better use of the host larvae resource to obtain higher production of *H. hebetor* parasitoids within a short time. This is an important step towards establishing a viable cottage industry for the augmentative biological control of MHM in the Sahel. Further investigations on the economic implications of the new method will advance the business model for *H. hebetor* commercialization.

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