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Research Article

Effect of augmentative releases of the parasitoid, *Habrobracon hebetor* Say (Hymenoptera: Braconidae) using plastic cups on *Heliocheilus albipunctella* De Joannis (Lepidoptera: Noctuidae) in the Sahelian region of Burkina Faso

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ABSTRACT: The most important insect pest of the millet crop in Burkina Faso is the Millet Head Miner (MHM), *Heliocheilus albipunctella* De Joannis (Lepidoptera: Noctuidae). To reduce its damage, the use of the parasitoid, *Habrobracon hebetor* Say (Hymenoptera: Braconidae) constitutes the most promising control strategy. The present study aims to know the effect of augmentative releases of *H. hebetor* on this pest using a new parasitoid release technique. This new technique release consists of a recycled plastic cups containing *Corcyra cephalonica* Stainton larvae parasitized individually at different times (8, 12 and 24 hours) by *H. hebetor* females. These cups were installed in the millet fields of different villages (release villages) to control this pest *H. albipunctella* by *H. hebetor*, and maitained the control villages that didn't receive any release. Our findings showed that plastic cups containing parasitized larvae at different times can be used to produce parasitoids. Thus, *C. cephalonica* larvae parasitized in 24 hours produced 280 *H. hebetor*, unlike larvae parasitized in 8 and 12 hours. Emerged *H. hebetor* had a male-biased sex ratio when the time of parasitism of *C. cephalonica* larvae by *H. hebetor* females was 24h. Releasing parasitoids into millet fields reduced the length of mines by 3.80 cm and two times higher parasitism of the pest by *H. hebetor* in the villages where parasitoids were released as compared to control villages. Finally, a millet yield gain of 31% was obtained in the parasitoid released fields. This new release technique of *H. hebetor* could be used in biological control programmes against MHM in the Sahel.

KEYWORDS: Augmentative release, biological control, *Habrobracon hebetor*, *Heliocheilus albipunctella*, plastic cups

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INTRODUCTION

In Burkina Faso, millet, *Pennisetum glaucum* (L.) R. Br., is the third most important cereal after maize and sorghum. Its production is estimated at 957253 tonnes for an area of 1065735 hectares in 2020 (DGESS, 2021). In the Sahelian region of Burkina Faso, millet remains the main cereal produced and consumed by rural households. However, millet cultivation is hampered by biotic factors, notably insect pests (Kadri *et al.,* 2019). Among insect pests, the Millet Head Miner (MHM), *Heliocheilus albipunctella* De Joannis (Lepidoptera: Noctuidae) is the most devastating species (Boly *et al.,* 2022). The larvae cause considerable damage. They feed on the grains of the millet spike, preventing their formation (Gahukar and Ba, 2019). In the Sahelian region of Burkina Faso, MHM damage is observed almost every year (Kabore *et al.,* 2017), affecting 96% of millet fields and 30 to 75% of millet spikes (Kabore, 2018). Depending on the year, sowing location and cultivated variety, the damage is more severe on early-maturing millet (Eisa *et al.,* 2007; Gahukar and Ba, 2019). Yield losses vary between 10 and 85% (Thiaw *et al.,* 2017; Gahukar and Ba, 2019; Oumarou *et al.,* 2019). Given the extent of damage caused by this pest, control strategies involving the use of insecticides, host plant resistance and cultural management practices (Gahukar and Ba, 2019) have been tested with limited success and applicability (Nwanze and Harris, 1992). Thus, biological control with

augmentative releases of the parasitoid, *Habrobracon* (= *Bracon*) *hebetor* Say (Hymenoptera: Braconidae) appeared to be the most effective control method. Parasitism rates of over 80% have been recorded in millet fields (Ba *et al.*, 2014; Amadou *et al.*, 2017; Kabore *et al.*, 2017; Baoua *et al.*, 2018). The parasitoids are reared on *Corcyra cephalonica* Stainton larvae (Lepidoptera: Pyralidae). They are then released using small jute bags (7 × 10 cm) containing a mixture of millet grains and flour, together with 25 larvae of *C. cephalonica* and two mated *H. hebetor* females (Ba *et al.,* 2014; Kabore *et al.,* 2017). The jute bags are tied to trees in millet fields or to the ceiling of straw granaries. Emerging parasitoids escape through the mesh of the jute bags and disperse to parasitize MHM larvae in millet fields (Kabore *et al.*, 2017). According to Ba *et al.* (2014), jute bags produce up to 70 parasitoids in 14 days, with a male-biased sex ratio. Fifteen bags cover an area of 3 km^2 (Baoua *et al.,* 2018). Although effective, the technique of producing parasitoids using jute bags can have some inconveniences. Indeed, for unknown reasons, some *H. hebetor* females initially introduced into jute bags containing the *C. cephalonica* larvae which are supposed to parasitize them and produce offspring can leave the bags without parasitizing them (Kabore, 2018). Thus, unparasitized larvae will be able to develop and spread in the environment (Kabore, 2018). In addition, studies have shown that the parasitism rate of *C. cephalonica* larvae in jute bags (7 × 10 cm) is between 78 and 88% (Ba *et al.,* 2014). Given that some millet granaries are not far from millet fields and that *C. cephalonica* is a stored product pest, it is necessary to avoid spreading it while trying to control MHM. It is thus necessary to refine the *H. hebetor* releases technique to limit the ecological risks while maintaining the effectiveness of the biological control programme. This could be achieved by direct releases of *H. hebetor* adults as suggested by Amadou *et al.* (2019), but this would be difficult when parasitoids must be transported from place to place (several tens of kilometres). For better control of MHM by *H. hebetor*, it is preferable to improve the current method of *H. hebetor* release. We wanted to know the effect of augmentative releases of *H. hebetor* on MHM using a new parasitoid release technique. This parasitoids release technique uses recycled plastic yoghurt cups containing only *C. cephalonica* larvae parasitized by *H. hebetor*. Specifically, we sought to: (i) know the number of parasitoids, the sex ratio of adults emerging from recycled plastic cups according to the time of confinement of *H. hebetor* and its surrogate host, and (ii) evaluate the efficiency of *H. hebetor* emerging from recycled plastic cups on the MHM in the field.

MATERIALS AND METHODS

Study environment

The study was conducted in two phases. The first phase was carried out in the laboratory of the "Institut de l'Environnement et de Recherches Agricoles "in Dori, in northern Burkina Faso, at a temperature between 26-32°C and a relative humidity of 60-80%. The second phase of the experiment consisted of field releases conducted in 2015 and 2016 in farmers' fields in villages located in the Seno province,

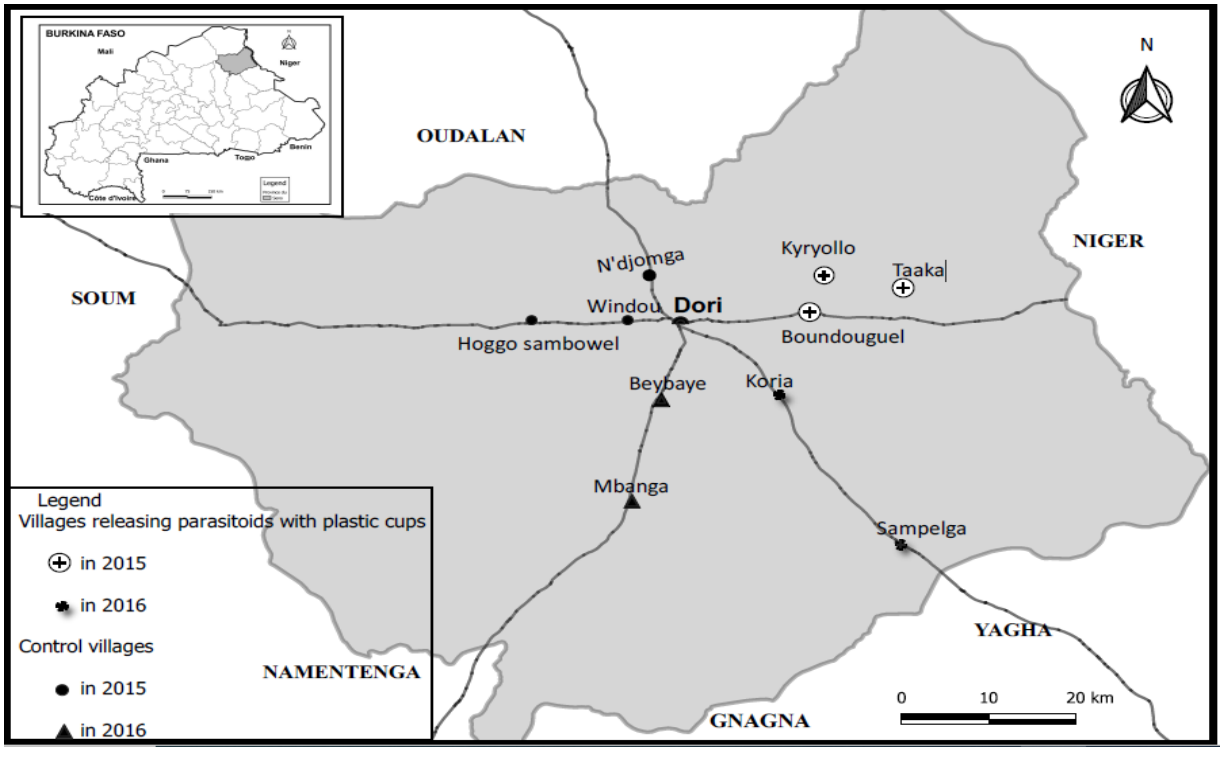

Figure 1. Map showing villages selected for parasitoids release using plastic cups and control villages.

northern Burkina Faso (Figure 1). The Seno province is located in the Sahel region of Burkina Faso. This province is known to be endemically infested with MHM (Kabore *et al.*, 2017). It has an unimodal rainfall pattern with a rainy season extending from June to October. Total rainfall of 513 mm and 497 mm were recorded in 2015 and 2016, respectively. Pearl millet, especially the local variety, is the main cereal crop. It covers more than 70% of the cultivated area and is generally associated with cowpea and sorghum (Boly *et al.*, 2022). The selection of villages for the experiment was based on similarities between villages. Villages were similar in that the same local variety of millet was cultivated on sandy soils using a pick, and farmers did not use inputs, such as fertilizers or pesticides for its production because of its low market value.

Mass rearing of *H. hebetor* and *C. cephalonica*

A population of *H. hebetor* was established from parasitized MHM larvae. These larvae were collected from millet fields. *H. hebetor* population is maintained in the laboratory on *C. cephalonica* at the "Institut de l'Environement et de Recherches Agricoles" at Dori, Burkina Faso. The mass rearing of these insects was carried out regularly in the laboratory at a temperature between 24 and 32°C and a relative humidity of 65-85%. The photoperiod during mass rearing was 12:12 (L: D). *C. cephalonica* is reared on a mixture of millet grains and flour in wooden cages (20 × 20 × 13 cm). The parasitoid, *H. hebetor*, is reared on all larval stages of *C. cephalonica* except the second and first instars. The two insects were reared using the technique described by Ba *et al.* (2013). Each year, a population of wild parasitoids captured in the millet fields were added to the laboratory colony to maintain genetic variability in the population as suggested by Henry *et al.* (2010).

Effect of the time of confinement of *H. hebetor* and *C. cephalonica* larvae for offspring production

Mated *H. hebetor* females were individually introduced into a plastic cup. Plastic cups were made from recycled 100ml capacity yogurt cups. Each cup contained a larva of the last instar of *C. cephalonica*. The confinement times for the *H. hebetor* female and the *C. cephalonica* larva were set as follows: i) 8h; ii) 12h; and iii) 24 hours. Twenty-five *H. hebetor* females were used for each confinement time. After the confinement time, the females were removed from the plastic cups and introduced into a new plastic cup containing a new larva of the same stage of *C. cephalonica.* Parasitized larvae were introduced into plastic cups (25 larvae/plastic cup). The larvae parasitized are distinguished from larvae paralyzed by the presence of eggs and prepupae of *H. hebetor* on the remains of *C. cephalonica*. Fifteen replicates were performed for each confinement time. Each plastic cup containing the parasitized larvae was covered with a muslin cloth to trap emerging parasitoids. The plastic cups were kept in the laboratory for two weeks. *H. hebetor* offspring were counted and sexed daily. They were then removed from the plastic cups.

Preparation of plastic cups for the release of *H. hebetor* in millet fields

Plastic cups made from recycled yogurt pots were perforated with about 50 small holes of 0.5 cm diameter to allow *H. hebetor* adults to exit (Figure 2). We then introduced into each of the cups, 25 5th instar larvae of *C. cephalonica*, previously parasitized by females of *H. hebetor*. The larvae were individually subjected to parasitism of one mated *H. hebetor* female for 24 hours in a plastic cup. A set of 15 plastic release cups was prepared. The 15 plastic release cups were placed in the millet fields. Emerging parasitoids escaped through small holes and dispersed into millet fields to parasitize MHM larvae.

Effect of *H. hebetor* release on MHM damage, parasitism and millet yield

This experiment was conducted with a group of six villages in 2015 and repeated in 2016 with another group of four villages. The ten villages were randomly chosen in the Seno province, where MHM is endemic. All these villages showed similarities in terms of the nature of the soil, farming practices and the local variety used for sowing. The villages were divided into two groups of 3 villages in 2015 and 2 villages in 2016, and assigned to one of the following treatments: (i) "Release villages" with plastic cups. Each village receives 15 plastic cups of parasitoids. (ii) "Control villages" that did not receive plastic cups of parasitoids. All villages with the same treatment were separated by at least 5 km and all groups of villages with different treatments were

Figure 2. Plastic release cup containing 25 last instar larvae of *C. cephalonica* parasitized by *H. hebetor.*

at least 15 km apart from each other (Kabore *et al.*, 2017). In each village subjected to parasitoid release, the plastic cups were evenly distributed in 5 millet fields (3 plastic cups per field). The five millet fields were selected based on the method described by Ba *et al.* (2014). Plastic cups were hung in trees present in millet fields using nylon string. Data on MHM damage (number and length of mines) and larvae parasitized by *H. hebetor* were collected 35 days after parasitoid release in the millet fields. Hundred millet spikes with the presence of MHM mines were randomly selected from each of the five millet fields in each of the villages (those receiving parasitoids and control villages). Thus, a total of 3000 and 2000 millet spikes were observed, respectively, in 2015 and 2016. Data on millet grain yield were also collected at harvest from spikes taken from an area of 16 m^2 (4 × 4 m) randomly in each of the 5 fields of the different villages and treatments. The panicles were threshed and grain weight and grain yield were measured (in kilogram per hectare) and grain moisture content was adjusted to 12%.

Data analysis

Data collected in the laboratory and the farmers' fields were subjected to an analysis of variance (ANOVA PROC GLM) and an independent *t*-test, respectively, to compare the release villages with the control villages using SAS software version 9.1 (SAS, 2003). When ANOVAs and the t-test were significant, means were compared by the Student–Newman–Keuls test at the 5% level.

RESULTS

Parasitoids emergence according to the time of confinement of *H. hebetor* and *C. cephalonica* larvae

The mean number of *H. hebetor* offspring emerging per plastic cup and the proportion of female parasitoids varied significantly with confinement time (Table 1). The number of emerging adults was higher when the confinement time was 24 hours. In addition, the sex proportion (= sex ratio) of emerging parasitoids was female-biased when *H. hebetor* was given a shorter time to parasitize *C. cephalonica* larvae (Table 1). There was no significant difference in the duration of parasitoid emergence (Table 1). Parasitoids emerged in less than 4 days regardless of the confinement time (Table 1).

MHM damage following *H. hebetor* releases

The length of mines due to MHM, which represents the extent of MHM damage, was significantly lower in villages in which *H. hebetor* was released in 2015 and 2016 than in control villages (2015: $t_{1,2998}$ = -17.01, P < 0.0001; 2016: $t_{1,1998}$ = -11.62, P < 0.0001) (Figure 3).

Parasitism of MHM following *H. hebetor* releases

In both successive years, the number of MHM larvae parasitized by *H. hebetor* per millet spike was significantly higher in villages where the parasitoid plastic cups were placed than in the control villages (2015: $t_{1,2998}$ = 18.47, P < 0.0001; 2016: $t_{1,1998}$ = 9.97, P < 0.0001) (Figure 4a).

Likewise, the percentage of millet spikes with at least one parasitized larva by *H. hebetor* was significantly higher in the sites subject to plastic cup releases than in control villages in both years (2015: $t_{1,2998}$ = 19.51, P < 0.0001; 2016: $t_{1,1998}$ = 7.79, P < 0.0001) (Figure 4b).

Lastly, the proportion of mines with the presence of *H. hebetor* cocoons was significantly higher in beneficiary villages from releases than in the control (2015: $t_{1,2998}$ = 21.13, P < 0.0001; 2016: $t_{1,1998}$ = 9.51, P < 0.0001) (Figure 4c).

Yield of millet following *H. hebetor* releases

The millet grain weight was significantly greater in villages where parasitoids were released than in control villages in both years (Table 2). Depending on the treatment, a yield gain of 31% depending on the year was obtained compared to control villages (Table 2).

DISCUSSION

The present study clearly shows that recycled plastic cups of 100 ml capacity can be used for the release of *H.*

Table 1. Number of emerging offspring of *H. hebetor*, their sex ratio and duration of *H. hebetor* emergence according to confinement time

Confinement time	Number of emerging offspring of *H. hebetor* (Means ± SE)	Proportion of female (= Sex ratio) *H. hebetor* (% ± SE)	Duration of the emergence of *H. hebetor* in the day (Means ± SE)
8 hours	176.93 ± 10.67 b	51.14 ± 1.67 b	3.33 ± 0.16 a
12 hours	200.20 ± 19.96 b	61.72 ± 1.07 a	3.60 ± 0.19 a
24 hours	280.20 ± 11.51 a	48.10 ± 2.94 b	3.86 ± 0.16 a
	$F_{2, 42}$ = 13.64 ; P<0.0001	$F_{2, 42}$ = 12.10 ; P<0.0001	$F_{2, 42}$ = 2.40 ; P= 0.10

¥ SE = Standard Error

¥ The different alphabetical letters in the same column indicate significant differences.

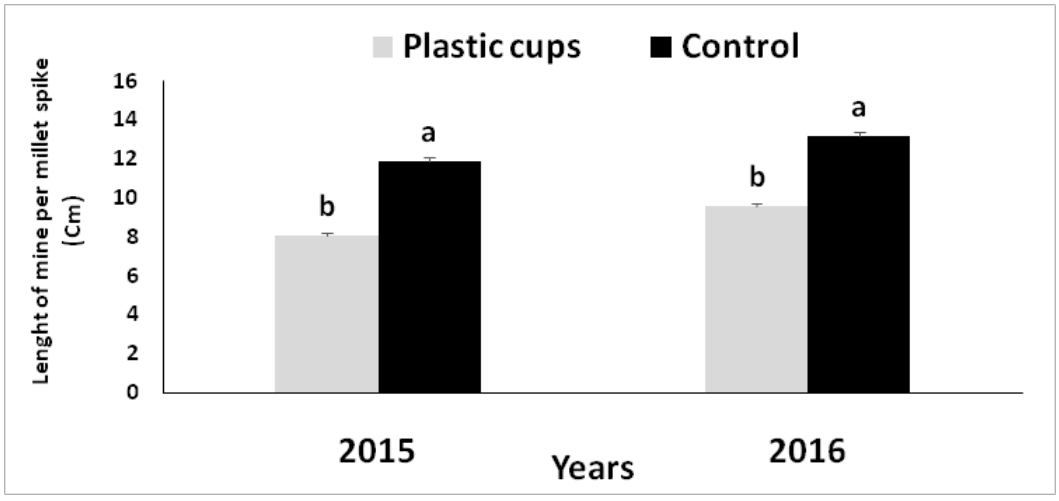

Figure 3. The mean length of mines per millet spike in control villages and parasitoid release villages.

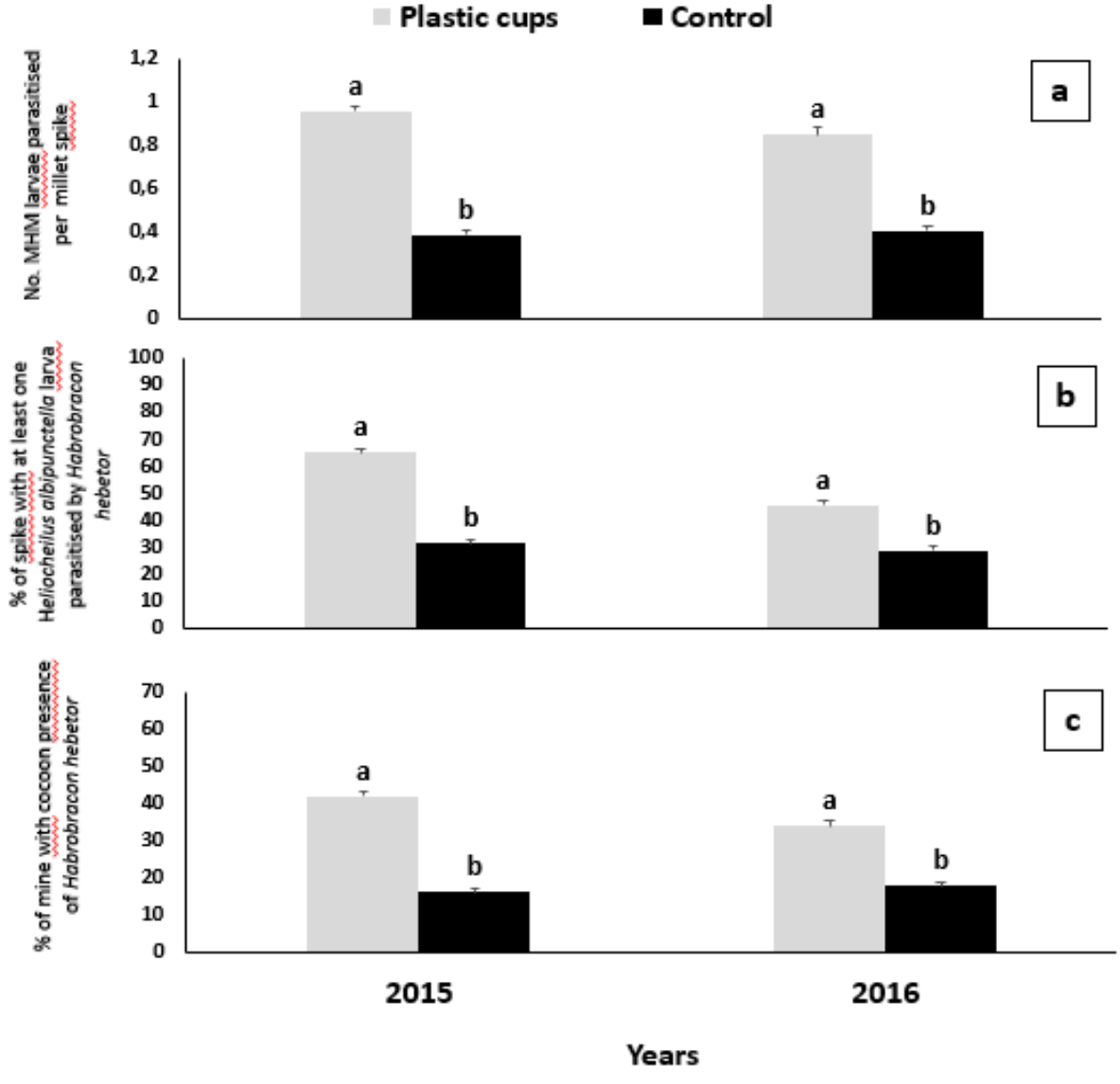

Figure 4 (a). Number of *H. albipunctella* larvae parasitized by *H. hebetor* per millet spike, **(b).** percentage of millet spike bearing at least one *H. albipunctella* larva parasitized by *H. hebetor*, **(c).** proportion of mines with the presence of *H. hebetor* cocoon.

Table 2. Grain yield and gain in yield are a function of the treatments

	2015		2016	
Treatments	**Grain yield (kg/ha) (Means ± SE)**	**Gain in yield**	**Grain yield (kg/ha) (Means ± SE)**	**Gain in yield**
Plastic cups	918.38 ± 44.28 [a]	31.17%	985.85 ± 53.03 [a]	31.19%
Control	632.16 ± 28.70 [b]		678.40 ± 25.61 [b]	
	$t_{1;\ 118} = 5.42$ $P < 0.0001$		$t_{1;\ 78} = 5.22$ $P < 0.0001$	

The different alphabetical letters in the same column indicate significant differences.

hebetor in millet fields to effectively control the millet head miner. Parasitoid emergence in plastic cups shows that all *C. cephalonica* larvae parasitized individually by *H. hebetor* females in 24 h produced more offspring than those parasitized in 8h and 12h. This significant number of parasitoids produced in plastic cups is four times higher than that found in other similar studies of parasitoid production (Ba *et al.,* 2014; Baoua *et al.,* 2018). This result could be explained by the fact that these authors had introduced two mated females into jute bags (7x10 cm) containing a mixture of *C. cephalonica* larvae, millet grains and flour. Thus, all *C. cephalonica* larvae present in the jute bags were not parasitized by the two females (Ba *et al.,* 2014), unlike the plastic cups. Schöller (2000) showed that when the host-parasitoid ratio is 1:1, the rate of parasitism is very high, in contrast to the 5:1 ratio. In other words, the rate of parasitism decreases as the number of host larvae provided to a female *H. hebetor* increases. It would therefore be interesting to parasitize all the host larvae to obtain an important population of *H. hebetor*. For this purpose, several studies have shown that *H. hebetor* female can produce more than 400 individuals during her lifetime (Ghimire and Phillips, 2014; Kabore *et al.,* 2019).

Regarding the duration of *H. hebetor* emergence, it was very shorter than the 14 and 21 days obtained respectively by Ba *et al.* (2014) and Baoua *et al.* (2018).

According to these authors, *C. cephalonica* larvae supplied to the two females were not parasitized at the same time, in comparison with our study. Thus, the offspring of the two *H. hebetor* females emerged over several days. Our findings also showed that the male-biased sex ratio for 24 h of confinement was not different from those obtained in other studies (Eliopoulos and Stathas, 2008; Ba *et al.,* 2014; Baoua *et al.,* 2018). On the other hand, *H. hebetor* offspring emerging after a confinement time of less than 24 hours had a female-biased sex ratio, which is very interesting in the *H. hebetor* production process as our parasitoid rearing systems sometimes lack females. Several studies have shown different results about the sex ratio of the offspring of *H. hebetor*. Thus, some studies have reported a female-biased sex ratio (Farag *et al.,* 2012; Eslampour and Aramideh, 2016; Kabore *et al.,* 2019) and others a male-biased sex ratio (Eliopoulos and Stathas, 2008; Landge *et al.,* 2009; Ba *et al.*, 2014). These conflicting reports on the sex ratio of *H. hebetor* offspring are likely to be due to host larval stages, the age of *H. hebetor* females used, differences in the host larvae used, the host-parasitoid ratio, environmental conditions, the food used to feed the host and the duration of parasitism (Benson, 1973; Ode *et al.,* 1996; Gündüz and Gülel, 2005; Dabhi *et al.,* 2011; Faal-Mohammad-Ali and Shishehbor, 2013; Kabore, 2018). Likewise, the unfavourable rearing conditions described by Ba *et al.* (2014) could be responsible for the male-biased sex ratio.

Regarding the release of parasitoids in millet fields, our findings showed that the release of parasitoids using plastic cups significantly reduced the damage caused by the MHM, resulting in a reduction in the length of mines by 3.80 cm per spike. During the two consecutive years, the number of MHM parasitized larvae by *H. hebetor* was twice higher in the villages receiving parasitoids using plastic cups than in the control villages. This suggests that almost half of the parasitism recorded in the release villages was due to the released wasps, which was similar to previous findings (Ba *et al.,* 2014; Kabore *et al.,* 2017; Amadou *et al.*, 2017; Baoua *et al.,* 2018). In addition, the percentage of spikes with at least one *H. hebetor* parasitized larva and the percentage of mines with *H. hebetor* cocoons were higher in the villages that benefitted from the release of parasitoids than in the control villages during the two consecutive years. Studies conducted by Amadou *et al.* (2017) also showed that the percentage of spikes with at least one larva parasitized by *H. hebetor* was higher in the release villages than in the control villages. This percentage was between 26 and 49% depending on the areas where the parasitoids were released from the jute bags (7 x 10 cm). However, in our study, this percentage was 65%. This difference could be explained by the number of parasitoids released in the millet fields. Indeed, the 15 plastic cups released 4203 parasitoids per village in four days against 900

to 1000 parasitoids released by 15 jute bags in 21 days (Baoua *et al.,* 2018). Given the short emergence and development time of *H. hebetor* of 9-12 days (Ghimire and Phillips, 2014; Kabore, 2018), coupled with a high intrinsic rate of increase (Kabore *et al.,* 2019), we estimate that a population of several thousand parasitoids could be established in one month from a set of 15 cups of parasitoids per village. In addition, the parasitism of MHM larvae by *H. hebetor* obtained in our study from plastic cup releases was higher than that obtained by Bhatnagar (1989). This difference is due to the technique used by this author to release the parasitoids. This author used pieces of bamboo containing 50 to 100 *Ephestia* sp. larvae, which were exposed to the natural parasitism of *H hebetor* before being deposited in the millet fields. After using this technique to obtain a significant number of parasitoids for release, it was found that the parasitoids released were not important because the naturally available parasitoids had paralyzed several *Ephestia* sp. larvae instead of parasitizing them. This shows that the parasitoid release technique using plastic cups is effective in controlling MHM.

According to Baoua *et al.* (2018), a device of 15 jute bags placed in a village can cover an area with a radius of 3 km. This increased yields by more than 30% (Baoua *et al.,* 2014). However, in our study, the large number of parasitoids released in a village using plastic cups increased grain yield by 31%. Therefore, this technique could be used in the biological control of MHM because it has many advantages: (1) the release of parasitoids takes place over a short period, (2) the parasitoid release cups do not release *C. cephalonica* adults and do not require protective equipment, (3) they can be reused several times and are locally available, (4) and the cost of selling 15 plastic cups containing the parasitized larvae is $US 30. In Niger, the cost of a jute bag is $US 3.34 (Guerci *et al.,* 2018). Therefore, reducing the cost of this technology could be much more available to farmers. However, it would be interesting to find a trick to recover the plastic cups after the millet harvest, so that they do not become a phenomenon of environmental pollution over the years. In addition to the recovery of plastic cups, the technique of parasitism of *C. cephalonica* larvae with *H. hebetor* needs to be improved. This could be done by introducing live larvae and females of *H. hebetor* in the plastic cups.

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