Scientific Research Publishing

Advances in Entomology, 2025, 13(1), 126-138
https://www.scirp.org/journal/ae
ISSN Online: 2331-2017
ISSN Print: 2331-1991

Evaluation of Two Methods of Production and Release of the Parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) for the Control of Pearl Millet Head Miner *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae) in Maradi (Niger)

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How to cite this paper: Amadou, L., Oumarou, M.N., Abdoulaye, O., Laminou, S., Yacouba, I., Baoua, I., Ba, M.N. and Muniappan, R. (2025) Evaluation of Two Methods of Production and Release of the Parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) for the Control of Pearl Millet Head Miner *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae) in Maradi (Niger). *Advances in Entomology*, **13**, 126-138.
https://doi.org/10.4236/ae.2025.131009

Received: November 14, 2024
Accepted: January 11, 2025
Published: January 14, 2025

Abstract

Pearl millet, *Pennisetum glaucum* (Leeke) R. Br, is the main cereal crop in Niger. This crop is seriously attacked by the millet Head miner (MHM), *Heliocheilus albipunctella* (de Joannis) (Lepidoptera, Noctuidae) causing significant yield losses. This study to optimize biological control of this pest was carried out in Niger in the laboratory and in a farming environment. In the laboratory, the larval paralysis and emergence of the parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) were compared between release jute bags, plastic boxes and cardboard boxes. In a farming environment, direct releases were carried out with plastic boxes and releases with jute bags in 12 villages of the Maradi region during the cropping seasons of 2021 and 2022. The results indicated that 25 larvae of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) in the boxes were all paralyzed by 2 females of *H. hebetor* within 7 hours 30 minutes. The emergence of adults began on the 7th day after the beginning of the experiment for a period of two weeks and three weeks respectively in the boxes and jute bags. The production varied from 107.08 to 110.17 parasitoids and was comparable between the Jute bags, plastic boxes and cardboard boxes. In Farmers' fields, the parasitoid release with the two methods caused the parasitism rates that varied from 64.32 to 66.52% depending on the

year but in 2022 the rate of parasitism was higher in the fields with direct releases using plastic boxes (72.66%) compared to those released with jute bags (56.35%). Plastic boxes and cardboard boxes can be used for the production of the *H. hebetor* parasitoids. These results can be recommended to the cottage industries in the Sahel in order to improve the production and release methods of *H. hebetor* and make them more adapted to farmers' fields.

Keywords

Biological Control, Parasitoid Release, *Hebrobracon hebetor*, *Heliocheilus albipunctella*, Niger

1. Introduction

Pearl millet, *Pennisetum glaucum* Leek R. Br, is the staple food for the population in the Sahelian agroecological belt in west and central Africa and it is cultivated by millions of poor farmers. With a production of 2,146,706 tonnes in 2021, Niger occupies the third rank among the producing countries [1]. Pearl millet is a drought-resistant cereal that is well adapted to poor soils, and it is one of the crops that has adapted to Sahelian conditions and traditional eating habits [2]. However, this crop is faced with abiotic and biotic constraints which lead to a low yield ranging from 0.5 to 0.6 t/ha. Among these constraints, the millet head miner (MHM), *Heliocheilus albipunctella* de Joannis (Lepidoptera, Noctuidae) constitutes one of the main constraints for millet production in the Sahel and in Niger [3]. This lepidopteran develops on millet panicles [4] and can cause yield loss of 100% (416.66 - 500 USD per farmer) during periods of high infestations [5] [6].

Thus, to reduce the damage of this insect pest and increase the productivity of pearl millet, several control methods have been recommended, including mechanical, varietal, chemical and biological control [6]. In recent years, biological control using the parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is the method used against MHM by millet producers in the Sahel. Parasitoid releases have been carried out using jute bags containing mainly millet grains as a diet of support for several years. The bag placed in the field, gradually releases the *H. hebetor* parasitoid [7]. A set of 15 release bags cause more than 80% mortality of the MHM larvae and a millet yield gain of 34% [8]. After the insects release, the jute bag and the pear millet grains used are not generally recovered or recycled.

Given the severe infestations of MHM, the high demand for parasitoids and the emergence delay of 14 to 21 days from the jute bags, the cottage industries in Niger design and sell the plastic release boxes containing parasitoids which are released directly in the infested pearl millet fields. The effects of this direct release technique compared to the usual method with jute bags have never been evaluated. However, the release boxes have been used in the studies for determining the right release periods and the required number of parasitoids to be released in Niger [9]. This information is needed as farmers purchase plastic boxes containing the

parasitoids rather than jute-release bags containing the larvae of the parasitoids. The main objective of this study is to determine the possibility of direct releases of *H. hebetor* using recyclable cardboard boxes or recyclable plastic boxes without the use of a millet-based support diet and to assess the parasitism rates of the MHM direct releases compared to gradual releases of the *H. hebetor* by jute bags.

2. Materials and Methods

2.1. Study Area

The field experiments were conducted from July to September during 2021 and 2022 cropping season in farmers' fields of the Maradi region.

The laboratory experiments were carried out at the Entomology laboratory of INRAN (Institut National de la Recherche Agronomique du Niger) in Maradi, located in south-central Niger (13˚30'00" north latitude and 07˚06'06" east longitude). The activity was carried out from August 10th to October 10th, 2021 under an ambient temperature of 28.5 to 35˚C and an average relative humidity of 68 to 74%.

The field release experiments were carried out in 12 early sowing villages in the Madarounfa department of the Maradi region.

These villages were selected based on an MHM infestation level of 25%, which can cause economic damage to crops. They were identified during a survey at the millet heading stage. A group of six villages were surveyed each year. The villages were divided into two groups, and each group of three villages was assigned to one of the following treatment: (1) Parasitoid release with Jute bags and (2) parasitod release with plastic boxes.

2.2. Animal Material

The parasitoid *H. hebetor* and its host *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) used in this experiment were collected from established colonies in the INRAN-Maradi entomology laboratory. The *C. cephalonica* larvae used for the production of *H. hebetor* were reared on a mixture of millet and cowpea.

Parasitoid *H. hebetor* and its host *C. Cephalonica* Stainton used in this experiment were collected, from established and maintained colonies in the INRAN-Maradi entomology laboratory. The *C. cephalonica* larvae used for the production of *H. hebetor* were reared on a mixture of millet and cowpea.

2.3. Preparation of Boxes and Bags for Release to the Laboratory

The plastic boxes used have a conical shape, the base has 3.1 cm radius and a height of 4.2 cm. For aeration, the boxes were covered with lids that have a tight-mesh fabric disc of 1.5 cm in diameter. Twenty-five larvae of *C. cephalonica* and two mated females of *H. hebetor* were introduced into the boxes and kept at laboratory till 90% to 95% emergence of adults.

Cardboard tea boxes measuring 12 cm in length, 5.5 cm in width and 6 cm in height. A window of 5 cm * 2 cm was cut on the upper side of the box and covered with a transparent plastic film to observe the interior of the box. Two windows of 2 cm * 2 cm were cut on the lateral sides of the box and closed with a fine mesh fabric. Then, 25 larvae of *C. cephalonica* and two fertilized females of *H. hebetor* were introduced.

The release bag is an 8 cm * 16 cm jute bag containing 40 g of millet as a support diet into which 25 larvae of *C. Cephalonica* and two mated females of *H. hebetor* were released. The whole set up was placed in transparent canvas bags for easy counting of emerged parasitoids. To release it in a farming environment, the plastic box and the release bag were prepared as described above.

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Cardboard tea boxes measuring 12 cm in length, 5.5 cm in width and 6 cm in height. The upper side of the box has a window of 5 cm × 2 cm covered by a transparent plastic film to observe the interior of the box, two windows of 2 cm × 2 cm were made on the lateral sides of the box and closed with a fine mesh fabric. Then, 25 larvae of *C. cephalonica* and two fertilized females of *H. hebetor* were introduced.

The release bag is an 8 cm × 16 cm jute bag containing 40 g of millet as a support diet into which 25 larvae of *C. Cephalonica* and two mated females of *H. hebetor* were inserted. The whole was placed in transparent canvas bags, thus making it possible to maintain the parasitoid and ease the counting of emerged parasitoids. To release it in a farming environment, the plastic box and the release bag were prepared as described above.

2.4. Experimental Design

The laboratory trial consists of three treatments: plastic box, cardboard box and release bag and 12 replications. The boxes were placed on bench and the jute bags were hung on a wire fixed horizontally. Each box and bag contained 25 larvae of *C. cephalonica* and two mated females of *H. hebetor*. To assess the paralysis of *C. Cephalonica* by *H. hebetor*, the plastic boxes and cardboard boxes were examined as soon as the parasitoid is in contact with the host.

The field experiment consisted of 12 villages located at least 5 km apart, selected in the department of Djirataoua in Maradi region (**Table 1**, **Figure 1**). For each year, direct releases were carried out with plastic boxes containing parasitoids in 3 villages and releases with jute bags in 3 other villages. At each village, releases were carried out in 4 fields located at the four sides of the village distant at least 500 to 1000m from the houses following the method described by [8] [10] Per field, 300 adults of *H. hebetor* (including 50% females) contained in three plastic

boxes were released directly (equivalent of 3 bags of release). Regarding the release using jute bags, 12 bags were placed per village; 3 bags per field. The release bags were protected with 5 liters cans with bases cut-out and were hung on trees in the pearl millet fields.

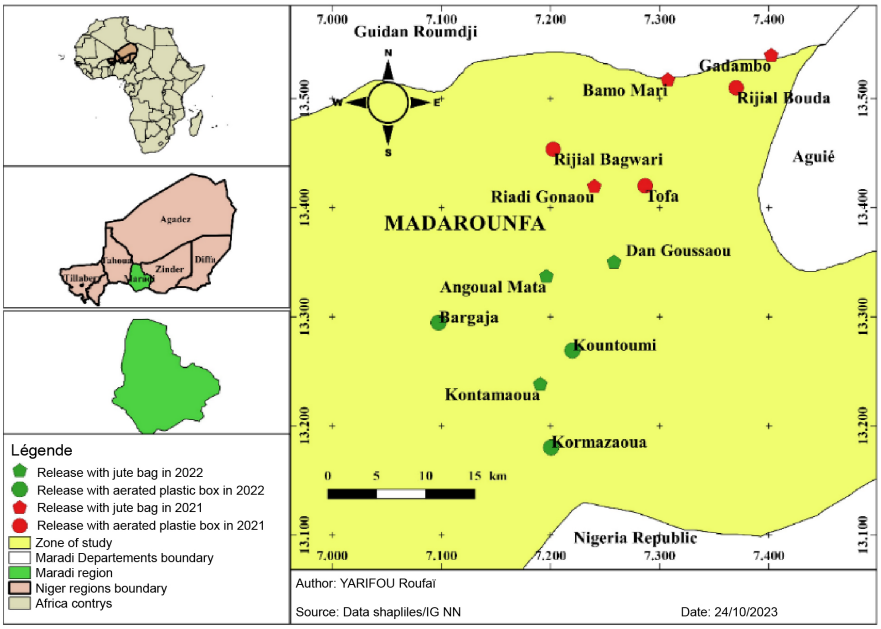

Figure 1. Map of location of study sites.

Table 1. Study villages and treatments.

Villages	Treatments	Year
Riadi Gonao Bamo Mari Gadambo	Parasitoids release with jute bag	2021
Rijial bagwari Tofa Rijial Bouda	Parasitoids release with plastic box	2021
Angoual Mata Dan Goussaou Kontamaoua	Parasitoids release with jute bag	2022
Bargaja Kountoumi Kormazaoua	Parasitoids release with plastic box	2022

2.5. Data Collection

In the laboratory, the evolution of the paralysis of *C. cephalonica* in the boxes and the emergence of *H. hebetor* in the different treatments was recorded. Observations on paralysis began 30 minutes after preparation (introduction of *H. hebetor* females with *C. cephalonica* larvae). The number of *C. cephalonica* larvae that had lost their motility was counted every 30 minutes.

For the emergence of *H. hebetor*, observations began six days after the device was established. These daily observations were carried out every morning from 10 a.m. and the following data were recorded: Number of males and females of *H. hebetor*; and *C. cephalonica* adults emerged.

Each day emerged adults of *H. hebetor* from each treatment were captured and transferred into the mating cages using a vacuum aspirator.

In the field, observations were carried out in two phases, before the releases to record the level of MHM infestation and the natural parasitism of *H. hebetor* and at three weeks after the releases. In each field, 100 pearl millet panicles chosen at random (400 panicles per village) were examined; the number of miners per panicle; the number of dead larvae parasitized by *H. hebetor* were recorded.

The infestation rate = panicle infestation rate: it is the proportion of panicles attacked over the total panicles examined.

$$\text{Equation (1): } \mathbf{T} = (n/N) * 100;$$

With **T** = infestation rate, n = Number of attacked panicles; N= Total number of panicles analyzed.

The rate of parasitism = it is the proportion of miners with parasitoid cocoons compared to the total number of miners.

$$\text{Equation (2): } \mathbf{P} = (x/Y) * 100;$$

With **P** = rate of parasitism, **x** = Number of miners with parasitoids cocoons; **Y** = Total number of miners recorded.

2.6. Data Analysis

Data analysis was carried out using SPSS software version 20. The mean and standard deviation of the emergences of *H. hebetor* and *C. cephalonica* were calculated. The t-test and the analysis of variance (ANOVA) were used to compare the means. The chi-square test was used to compare the parasitism in the fields with the two methods of release. Before taking up ANOVA, ASIN transformation was calculated using the formula below:

$$\text{Equation (3): } Tr = \text{ASIN}\sqrt{\text{Proportion}}$$

The proportion was obtained by making the rate divided by 100 (Rate/100).

3. Results

3.1. Evolution of Paralysis Rate of *C. cephalonica* Larva by *H. hebetor* Depending on the Type of Box

The females of *H. hebetor* introduced into the boxes paralyzed the *C. cephalonica* larvae in both types of boxes with a similar dynamic (**Figure 2**). Observations began 30 minutes after the introduction of the two females of *H. hebetor* in the boxes containing 25 larvae of *C. cephalonica* each. One hour after the introduction of the females of *H. hebetor*, it was observed that 25% of the larvae were paralyzed for the two treatments then 50% of the larvae after 3 hours. The two females took 4.5 hours to paralyze 75% of *C. cephalonica* larvae. The total paralysis of the larvae

is reached at 7 hours and 30 minutes for both treatments. Paralysis was not observed in the jute bags as the larvae infiltrated the diet of support and bag material will not allow to record the data.

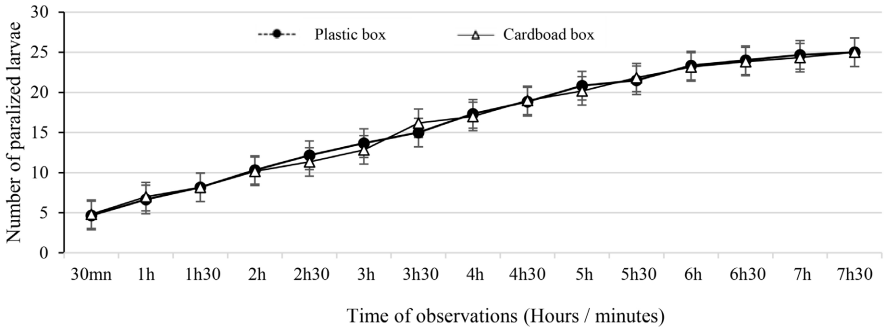

Figure 2. Paralysis growth of *C. cephalonica* larvae by *H. hebetor* depending on the type of box.

3.2. Emergences of *H. hebetor* Depending on Treatments

Observations began 6 days after the preparation of the different treatments. Twenty-three observations were carried out from August 18th to September 9th, 2022. The first emergences were noted from the 7th day for all treatments then continued for 14 days for the plastic box and cardboard box treatment while the emergences continued for up to 21 days for the release bags treatment (**Figure 3**). The peak of emergence was observed on the 16th day after the establishment of the experiment for the plastic boxes with an average daily emergence of 16 *H. hebetor* per plastic box. For the cardboard box treatment, the peak was observed at the same day with an average emergence of 14 individuals per cardboard box. However, the release bags treatment showed its peak on the 11th day with an emergence of 12 parasitoids per bag.

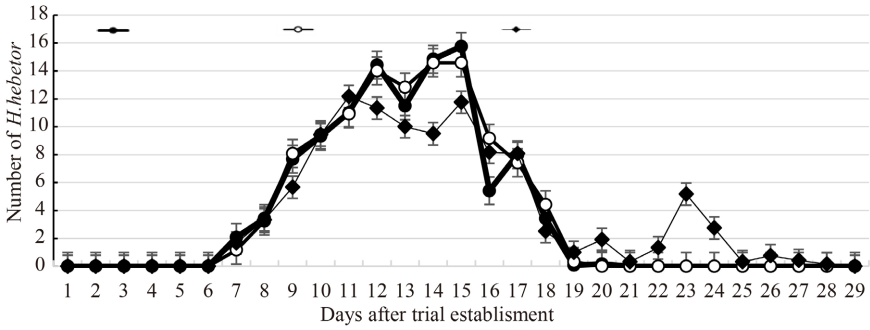

Figure 3. Evolution of *H. hebetor* emergence depending on the treatments.

3.3. Average Number of Emerged Individuals of *H. hebetor* and *C. cephalonica* Per Treatments

The average emergences of *C. cephalonica* were different between the treatments, the larvae in the plastic and cardboard boxes were 100% parasitized (**Table 2**) while few larvae of *C. cephalonica* introduced into the release bags escaped the

parasitism of *H. hebetor*.

Table 2. Average number of *H. hebetor* and *C. cephalonica* per boxes and jute bag.

Treatments	Total *H. hebetor* (Male + Femelle)	*H. hebetor* Female	*C. cephalonica* Adult
Plastic Box	107.08 ± 3.276	66.83 ± 2.98	0
Cardboard Box	110.17 ± 4.81	73 ± 3.78	0
Jute Bag	107.75 ± 3.77	69.25 ± 3.22	11.67 ± 1.23
Average	108.33 ± 3.95	69.69 ± 28.06	--
ANOVA	F = 0.16 P = 0.85	F = 0.86; P = 0.43	F = 90.44; P < 0.001

3.4. Infestation of Pearl Millet Panicle by MHM in the Field

In 2021, the percentage of infested panicle was 63% in villages with the direct release and 67% for villages with the jute bag release with a significant difference between the treatments (P = 0.02). An average number of 2.68 miners per infested panicle was obtained with a slight difference (Table 3).

In 2022, the rate of panicles infestation by MHM was not statistically different (P > 0.5) between the two treatments. An average rate of 63% of panicle attacked was obtained. The number of miners recorded was 2.16 miners per infested panicle without significant difference between the two release methods (P = 0.26).

Table 3. Percentage of infested panicles and number of miners per panicle.

Treatments	2021		2022	
	Infested Panicle (%)	Number of Miners per Panicle	Infested Panicle (%)	Number of Miners per Panicle
Direct Release with Plastic Boxes	63.66 ± 1.42 b	2.92 ± 0.05 a	62.91 ± 0.38	2.13 ± 0.10
Release with Jute Bags	70.4 ± 4.94 a	2.47 ± 0.04 b	62.16 ± 3.00	2.19 ± 0.08
Average	67.03 ± 2.70	2.68 ± 0.03	62.54 ± 1.36	2.16 ± 0.06
T-test	t = −3.16; ddl = 5195; P = 0.02	t = 6.37; ddl = 3394; P < 0.001	t = −0.26; ddl = 1194; P = 0.59	t = 0.41; ddl = 746; P = 0.26

a b: Means followed by the same letter are not significantly different (LSD, 5%).

3.5. Parasitism Rate of MHM Larvae by *H. hebetor*

Before the parasitoids release, the natural parasitism rate ranged between 7 to 11% %. After three weeks of release, observation in the fields revealed the presence of dead larvae with *H. hebetor* cocoons in the panicle. In 2021, the rate of larval parasitism per infested panicle was an average of 66.52% with non-significant differences (X^2 = 14.03; p = 0.23) between the direct release method and that of the jute bag (Table 4).

In 2022, the rate of parasitism was higher in fields with direct releases (72.66%) compared to fields with jute bag releases (56.35%).

There were slight variations in 2022 between 2 to 3 villages in both treatments which may be due to the differences of sowing date, the rain fall distribution and probably the millet varieties used by farmers (Table 4).

Table 4. Rate of *H. hebetor* parasitism on MEM larvae.

Treatments	Parasitism Rate in 2021	Parasitism Rate in 2022
Direct Release with Plastic Boxes	64.97 ± 10.096	72.66 ± 31.91 a
Release with Jute Bags	57.4 ± 6.994	56.35 ± 30.74 b
Average	66.52 ± 2.01	64.32 ± 32.1
Khi-Deux	$X^2 = 14.03$; P = 0.23	$X^2 = 1.79$; P = 0.002

a b: Means followed by the same letter are not significantly different (LSD, 5%).

4. Discussion

The paralysis progressed uniformly in the cardboard and plastic boxes despite the difference in the raw materials used to manufacture them. All the *C. cephalonica* larvae introduced into the boxes were paralyzed by the two *H. hebetor* females on the same day within 7 hours, 30 minutes after assembled.12.5 larvae were paralyzed per female. This result is similar to those of [11] who reported an average paralysis rate of 8.19 larvae per female of *H. hebetor* each day. But lower than 30 larvae were paralyzed by a single female of this parasitoid as demonstrated in Burkina Faso [12]. These results show that during incubation the production and breeding boxes should not be moved before complete paralysis and parasitism.

The results of current study indicate that the production of the parasitoid *H. hebetor* is possible in recycled black tea cardboard boxes and plastic boxes due to the almost similar number of the parasitoids obtained with the usual jute bags. For the three treatments, the beginning of emergences at one week after incubation and the peaks observed in the second week are consistent with the results obtained by [10] from jute bags. The long emergence time of parasitoids from jute bags than in cardboard and plastic boxes could be due to the diet of support contained in the jute bag and would have given temporary shelter to *C. cephalonica* larvae before being paralyzed while the larvae contained in the boxes are physically exposed to *H. hebetor* parasitism. Therefore, the emergences recorded in the bags during the third week seem to be obtained from late parasitized *C. cephalonica* larvae.

The emergence time of *H. hebetor* of 21 days from jute bags has been reported by several studies in the Sahel. Therefore, the boxes reduced the duration of parasitoid emergence and increased the rate of parasitism of *C. cephalonica*. Experiments carried out in Burkina Faso and Niger recorded the parasitism rates varying from 78.14% to 87.19% using jute bags for release of *H. hebetor* [10] even though all the larvae were not parasitized. The adults of *C. cephalonica* observed in the

release bags come from larvae which escaped the parasitism of *H. hebetor*.

Given the *H. hebetor* production, the total average number of parasitoids emerged from the jute release bags, the similar parasitoids production from the two types of boxes. The mini cardboard boxes of back tea could be recycled and used in the cottage industries. The average production for all the treatments (108.33 parasitoids) is higher than the average of 70 parasitoids per jute bag obtained by [10] On the other hand, the number is lower than that reported by [12] who reported an average of 151.9 individuals per female. This difference could be attributed to the number of *C. cephalonica* larvae subjected to *H. hebetor* females in the boxes. In this case study, production was obtained through the parasitism of 25 larvae of *C. cephalonica* by two females of *H. hebetor*.

The results obtained indicate that it is possible to use the boxes for the production and marketing of *H. hebetor* parasitoid and also, the boxes can serve to release the parasitoids in pearl millet fields for the management of the millet head miner.

The MHM infestation levels above 60% in the study villages during the two years show that this pest still constitutes one of the main constraints of the crop; these rates are close to those of previous studies [6].

Releases carried out in infested fields with a natural parasitism rate of 7 to 11% are consistent with the rate reported by Ba et al. 2013. The MHM parasitism rate for releases with plastic boxes was higher or comparable to the parasitism rates of 35% to 70% and lower to90% recorded by previous studies of releases with jute bags [7] [8] [10]. The parasitism rates of of *H. hebetor* on *H. albipunctella* larvae in this study were higher than the rate of 34.56% obtained by [13].

However, the direct releases generated a higher rate of parasitism of MHM larvae in the field than that of releases with jute bag. In 2021 and 2022, the parasitism rates obtained by direct releases were respectively 8% and 16% higher than those of release with jute bags (X^2 = 1.79; P = 0.002). The non-significant difference between the two release methods (X^2 = 14.03; P = 0.23) in 2021 indicate that direct releases were effective and moreover the boxes provide the assurance of a quickly release of female and male parasitoids while the release bag does not allow the sexes verification. Jute bags carry the parasitoid larvae to be transferred to the farmers' fields and the parasitoids emergence will start 4 to 5 days after placement. Regarding these advantages for direct releases, the cottage industries may focus effort to make them available at the right time as the pest attacks occurs each year and the action of the parasitoid can begin on the same day of release. The production and release may be successful as parasitoids are easily transferable to the field using the boxes as found they can live for 29 to 32 days in boxes containing diluted honey solution at 30% [12]. In West Africa there is an increasing demand of *H. hebetor* to control MHM by producers and local non-governmental organizations [14]. Hence the existence of cottage industries in Niger, Burkina Faso and Mali for the production and sale of *H. hebetor* in rural areas [13] [15] contributing to reduce the yield losses of pearl millet.

This release method offers to producers the chance to view the parasitoids

through the transparent walls of the box, and to be sure purchasing live parasitoids at the production and sales units. However, the high temperatures when the boxes are exposed to sunlight can constitute a risk of parasitoid mortality, especially during transportation to the fields. As for the jute bag, a delay of at least 7 days is observed before the emergence of the first adults of *H. hebetor*, and this emergence spreads over a period of 15 days [10]. Therefore, the bags can be placed before the beginning of the infestation as a preventive measure. As reported earlier by [9] the releases carried out during the pearl millet panicle emergence stage are similar in terms of parasitism rate to releases carried out at the flowering and maturity stages. Although the evolution of MHM parasitism by *H. hebetor* with the two methods of release as a function of time was not evaluated in this study. Therefore, further studies should be done to assess the speed of MHM parasitism by *H. hebetor* as recent data for parasitoid sales at the production units and selling points in Niger show the preference of release boxes over jute release bags by the farmers. According to the producers, the attack of the parasitoids on the larvae of the MHM could be more instantaneous if plastic boxes were used rather than jute-release bags. In addition, the cost of production of plastic boxes is 9.09% lower than jute bags, which are mostly sold at the same price to farmers. Though the plastic boxes are not biodegradable but they could be recycled in households or serve as unit for selling chili peppers and fried peanuts in rural areas. Further research is essential to determine and compare the evolution of parasitism in release fields as a function of time and the coverage area as previous studies demonstrated that parasitoids released with release bags can cover a radius of 3 to 5 km [8].

5. Conclusion

The pearl millet head miner is still the main constraint for pearl millet production in the Sahel. Farmers and NGOs prefer purchasing *H. hebetor* parasitoid in plastic containers over jute bags, which is increasing. Further studies are however needed to evaluate the gain concerning the time for the preparation of boxes and compare the cost of using the two methods of release as our finding revealed their efficiency for parasitoid production and the parasitism on MHM at three weeks after parasitoid release.

Acknowledgements

Funding for this research was provided by Norway Embassy in Bamako, Mali through CSAT project (Grnat Number NER-17/005) implemented by IITA (International Institute for Tropical Agriculture) and partner institutions (Institut National de la Recherche Agronomic du Niger and l'Institut d'Economie Rurale) and also additional support from Mc Knight Foundation trough SAHEL IPM project implemented by UDDM-Niger, INRAN-Niger, IER-Mali and ICRISAT. The authors thank farmers who allowed the team to conduct the studies in their fields, and our collaborators. Amina Lona, Mayaki Gaya, Souley Galadima Rabé for their contribution in data collection. The opinions expressed herein are those of the

authors and do not necessarily reflect the views of Norway Embassy or the M Knight.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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