



Timing of releases of the parasitoid *Habrobracon hebetor* and numbers needed in augmentative biological control against the millet head miner *Heliocheilus albipunctella*

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Abstract *Heliocheilus albipunctella* de Joannis (Lepidoptera: Noctuidae) is one of the major insect pests of pearl millet in the Sahel. The native parasitoid, *Habrobracon hebetor* Say (Hymenoptera: Braconidae), is currently being promoted for augmentative biological control of the pest in the Sahel. The current study was carried out to identify the right time for releases of the parasitoid using either pearl millet growing stage, or pest occurrence as reference, and to determine the optimal number of parasitoids needed to cover a given area. Our results indicate that release of parasitoids at the panicle emergence stage or six - weeks after first sight of eggs of *H. albipunctella* lead to highest parasitism of *H. albipunctella* larvae by *H.*

hebetor. The dose of 800 parasitoids for a distance of 3 km radius was enough for controlling *H. albipunctella*. The implications of the results are discussed toward cost effective and practical recommendation adapted to the Sahelian conditions.

Keywords Parasitism · Pearl millet growing stage · *Corcyra cephalonica* · Niger · Sahel

Introduction

Pearl millet *Pennisetum glaucum* (L.) R. Br. (Poaceae) is a cereal crop grown in tropical countries. In Africa, it is mainly grown in the Sahelian countries. Niger is the largest producer with 7.2 million hectares of cultivated area (FAO 2019). Niger has, however, one of the lowest pearl millet grain yields with only 500 kg ha⁻¹ (FAO 2019). This low productivity is due to many abiotic (drought, poor soil) and biotic constraints including insect pests (Nwanze and Harris 1992). The millet head miner (MHM) *Heliocheilus albipunctella* de Joannis (Lepidoptera: Noctuidae) is one of the major chronic insect pests of pearl millet in Niger and the entire Sahel (Gahukar and Ba 2019). Infestations of the MHM are more severe in the drier zones of the Sahel (Nwanze and Harris 1992). Damage is due to *H. albipunctella* larvae that feed on the panicle and prevent grain formation (Nwanze and Harris 1992; Gahukar and Ba 2019). Almost every

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year MHM outbreaks are observed in the Sahel, especially on early-planted or early maturing pearl millet, with yield losses up to 85% (Gahukar et al. 1986; Nwanze and Sivakumar 1990; Krall et al. 1995; Youm and Owusu 1998).

Biological control has emerged as the most attractive solution for controlling the MHM and the gregarious parasitoid *Habrobracon* (= *Bracon*) *hebetor* Say (Hymenoptera: Braconidae) is considered a natural enemy with great biocontrol potential (Gahukar et al. 1986; Bhatnagar 1987). Recently, augmentative releases of *H. hebetor* have been successful against the MHM in Niger (Payne et al. 2011; Ba et al. 2013, 2014; Baoua et al. 2014, 2018). This has led to parasitism of up to 80% of the MHM larvae (Ba et al. 2013, 2014), resulting in 34% increase in grain yield (Baoua et al. 2014).

The parasitoids released from a 15 cm × 25 cm jute bag can produce an average of 70 adult parasitoids over a three-week period (Baoua et al. 2018). Recently it has been demonstrated that when a set of 15 bags are deployed in one place, the parasitoids could disperse to a distance of 3 km from the point of release over a period of five weeks (Baoua et al. 2018). However, the study did not explore the exact quantities of parasitoids needed for a given area of pearl millet.

To rationalize the current biological control program, it is critical to know the exact number of parasitoids needed to cover a given area. Moreover, timely release of *H. hebetor* is essential for successful control of the MHM. Several studies have shown the critical need for timely release of agents for effective augmentative biological control programs (van Lenteren 2012; El-Heneidy et al. 1991; Neuville et al. 2016). This information is needed since the technology is being transferred to farmers in Niger (Amadou et al. 2017; Guerçi et al. 2018). Farmers need to know the exact number of parasitoids to be released, and the correct time of release, either at a specific crop developmental stage or at the first appearance of the pest.

The main objective of this study was to identify the best time for releases of *H. hebetor* as a function of MHM occurrence, pearl millet's growth stage, and to determine the optimum number of parasitoids needed to cover a given area. It will allow to determine the best time to release the parasitoid *H. hebetor* and number of parasitoids needed in a given area for effective control of MHM.

Materials and methods

Study environment

The experiments were conducted during three consecutive cropping seasons between June and September from 2015 to 2017 in farmers' pearl millet fields in the regions of Tahoua and Zinder in Niger. This agroecosystem has a unimodal rainfall pattern with the rainy season extending from May to October. In the Tahoua region, a total annual rainfall of 442 mm, 516 mm, and 613 mm was recorded in 2015, 2016, and 2017, respectively. The Zinder region had an annual rainfall of 528 mm, 491 mm, and 434 mm in 2015, 2016, and 2017, respectively. The maximum daily temperature during the pearl millet growing season ranged from 24–39 °C. During this period, the area has contiguous pearl millet fields covering almost 80% of the cultivated area, usually in association with cowpea, *Vigna unguiculata* (L.) Walp. (Fabaceae). Farmers usually grow two to three local pearl millet varieties on 3–10 ha. The MHM infestation (panicle bearing eggs and/or larvae and/or mine) varied from year to year and between locations. In the Tahoua region the infestation (attacked panicles) varied from 31 to 44%, with 2015 being the least infested year and 2017 the highest. In Zinder it varied from 16 to 49% with 2015 being the least infested year and 2017 the highest. No irrigation, chemical fertilizers or pesticide are applied in the pearl millet crop.

Parasitoid rearing

Habrobracon hebetor was collected from a culture established from field-collected MHM larvae. *Habrobracon hebetor* larvae were reared on the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and maintained in the laboratory throughout the study period at fluctuating room temperatures (mean = 26 ± 2 °C) at the entomology Laboratory of INRAN (Institut National de la Recherche Agronomique du Niger) in Maradi. Rice moths were reared on a mixture of pearl millet grain and flour in wooden cages (20 × 20 × 13 cm), and the parasitoids were reared on third and fourth instar *C. cephalonica* larvae using the technique described by Ba et al. (2014).

Assessing the timing for release of parasitoids based on pearl millet growing stage

The experiment was carried out in a total of 36 villages in the Tahoua region with a different set of 12 villages for each season. The villages lie between latitude 13°53' and 14°44'N, and longitudes 05°19' and 06°18'E. The villages were selected randomly within an area with endemic presence of the MHM. In selected farms the millet was planted from May 22 to June 5 in all three years. The selected villages had not been subjected to any *H. hebetor* releases for at least two years prior to the experiment. The selected twelve villages were divided into four groups of three and assigned to one of the following treatments: (1) three villages each were supplied with 15 parasitoid bags at the pearl millet panicle emergence stage, (2) three villages each with 15 parasitoid bags at the pearl millet male flowering stage, (3) three villages each with 15 parasitoid bags at the pearl millet grain filling stage, and (4) three “control villages” did not receive any parasitoids. All three villages, in the same treatment, were separated by at least 5 km and all groups of villages of different treatments were at least 15 km away from each other (Ba et al. 2014). The parasitoids were released using jute bags of 15 cm × 25 cm containing 200 g of pearl millet grains, 100 g of pearl millet flour, 25 *C. cephalonica* larvae (a mixture of third and fourth instars) and two mated *H. hebetor* females. In each release village, the parasitoid bags were evenly distributed within five pearl millet fields (three bags per farmer's field) using the method described by Ba et al. (2014). The jute bags were suspended to the ceiling of traditional straw granaries located in farmers fields and emerging parasitoids were able to escape through the jute mesh and straw granaries and disperse to parasitize MHM larvae in millet fields (Ba et al. 2013, 2014). In each village the bags were evenly distributed to five farmers (three bags per farmer), one in the center of the village and one in each direction (E, W, N and S) with each of E, W, N and S farm 500 m away from the farm in the center of the village (Ba et al. 2014). Each bag produced typically 60–70 parasitoids (Baoua et al. 2018).

Assessing time of release of *H. hebetor* after first sight of eggs of MHM

This experiment was carried out in a total of 27 villages in the Tahoua region in three successive seasons in 2015, 2016, and 2017 with a different set of nine villages for each season. The villages lie between latitude 13°53' and 14°44'N, and longitudes 05°19' and 06°18'E. The villages were selected randomly within an area of endemic presence of the MHM. In selected farms the millet was planted between May 22 and June 5 in all three years. The selected villages had not been subjected to any *H. hebetor* releases for at least two years prior to the current experiment. Each year the selected nine villages were divided into three groups and assigned to one of the following treatments: (1) three villages that were each supplied with 15 parasitoid bags four weeks after first sight of MHM eggs, (2) three villages that were each supplied with 15 parasitoid bags six weeks after first sight of MHM eggs, and (3) three “control villages” that did not receive any parasitoids. Daily observations were conducted in selected villages at pearl millet jointing stage to determine the date of first sight of MHM eggs. The selection of villages and parasitoid releases were the same as described in the previous experiment.

Assessing numbers of *H. hebetor* adults to be released to cover an area of 3 km radius

This experiment was carried out in the Zinder region in a set of 12 villages in three successive seasons in 2015, 2016, and 2017 with a different set of 12 villages for each season. The villages lie between latitude 10°11' and 13°29'N, and longitudes 08°00' and 09°08'E.

The villages were selected randomly within an area of endemic presence of the MHM. In selected farms the millet was planted between the third and fourth week of May. The selected villages had not been subjected to any *H. hebetor* releases for at least two years prior to the current experiment. Each year, the selected twelve villages were divided into four groups of three villages and assigned to one of the following treatments: (1) three villages, each supplied with 400 *H. hebetor* adults, (2) three villages, each supplied with 800 *H. hebetor* adults, (3) three villages, each supplied with 1600 *H. hebetor* adults, and (4) three “control villages” that did not receive any parasitoids. The selection of villages was as described

in previous experiments. The parasitoids were released from boxes with a sex ratio of 1:1 at the beginning of the grain filling stage. For the accuracy of number of parasitoids released in this experiment, we placed the exact number of adult parasitoids of 24 h old in boxes for release. The parasitoids released in a given village were evenly distributed in four different pearl millet fields, 300 m apart from the center of the village, and one farm in each direction (E, W, N and S).

Data collection and analysis

Every year we collected at random 200 panicles per field at harvest time in four different fields in each village of the different treatments of the three experiments. This corresponds to 800 panicles per village and a total of 2400 panicles per treatment. The numbers of live larvae (unparasitized), dead larvae without cocoon (unparasitized), mines without larvae (unparasitized) and dead larvae with cocoon (parasitized) were recorded. Larvae parasitized by *H. hebetor* were easily distinguished by the presence of cocoons (Garba and Gaoh 2008). The percentage parasitism was computed by calculating the ratio of total number of parasitized larvae over the total of larvae. Data were all subjected to arcsine transformation prior to analysis of variance using PROC GLM with the SAS software version 9.1 (SAS 2003). When ANOVAs were significant, means were compared by the Student–Newman–Keuls tests at the 5% level.

Results

Parasitism of MHM by *H. hebetor* following releases of parasitoids at different pearl millet developmental stages

In 2015, the parasitism of MHM by *H. hebetor* was significantly higher in fields that received parasitoid bags at the pearl millet panicle emergence stage ($F_{3,1931} = 173.91$; $P < 0.001$) (Fig. 1). In 2016, the highest parasitism was recorded in fields that received parasitoid bags at panicle emergence and flowering stages ($F_{3,3222} = 119.16$; $P < 0.001$) (Fig. 1). However, in 2017, all fields that received parasitoid bags regardless of millet development stage had similar levels of parasitism of MHM by *H. hebetor*, but they

were significantly higher than in control villages ($F_{3,4116} = 66.04$; $P < 0.001$). For all three years the control fields that did not receive any parasitoid bags had 2.5 to 7.4 times less parasitism than fields that received parasitoids (Fig. 1). The three years averages indicate a 5.19–5.43 fold increase in parasitism when parasitoids were released either at panicle emergence or flowering stages as compared to control ($F_{3,9156} = 301.09$; $P < 0.001$).

Parasitism following releases of parasitoids at different dates after first sight of eggs of the MHM

For all three years, the control fields that did not receive any parasitoid bags had 2.02 to 15.65 times lower parasitism than fields that received parasitoids (Fig. 2). In 2015, the highest parasitism of MHM by *H. hebetor* was recorded on fields receiving parasitoid bags six weeks after first sight of eggs of MHM ($F_{2,1486} = 125.80$; $P < 0.001$). However, in both 2016 ($F_{2,2112} = 74.55$; $P < 0.001$) and 2017 ($F_{2,2860} = 361.84$; $P < 0.001$), the highest percentage of parasitism was recorded on fields receiving parasitoid bags four weeks after first sight of MHM eggs (Fig. 2). The three years averages indicate significantly higher parasitism ($F_{3,6462} = 439.19$; $P < 0.001$) on fields receiving parasitoid bags four weeks after first sight of MHM egg (1.53 fold higher than six weeks and 3.76 fold higher than control).

Parasitism following releases of different numbers of *H. hebetor* adults

The releases of 400, 800, and 1600 adults of *H. hebetor* led to significantly higher percentages of parasitism of MHM larvae as compared to the control in all three years (2015: $F_{3,757} = 16.29$; $P < 0.001$; 2016: $F_{3,3345} = 51.24$; $P < 0.001$; 2017: $F_{3,4670} = 74.31$; $P < 0.001$). In 2016, the release of 1600 parasitoids gave a higher percentage of parasitism than the release of 400 parasitoids but the percentage of parasitism between the releases of 800 and 1600 parasitoids did not differ significantly (Fig. 3). The three years averages indicate a 3.11–3.75 fold increase in parasitism when 400–1600 parasitoids were released as compare to the control ($F_{3,8781} = 139.40$; $P < 0.001$).

Fig. 1 Parasitized larvae of millet head miner (MHM), *H. albipunctella* (% mean \pm SE) due to *H. hebetor* following releases of the parasitoid at different pearl millet growing stages in 2015, 2016 and 2017. For each year, bars bearing different letters were significantly different (Student–Newman–Keuls test, $\alpha = 0.05$)

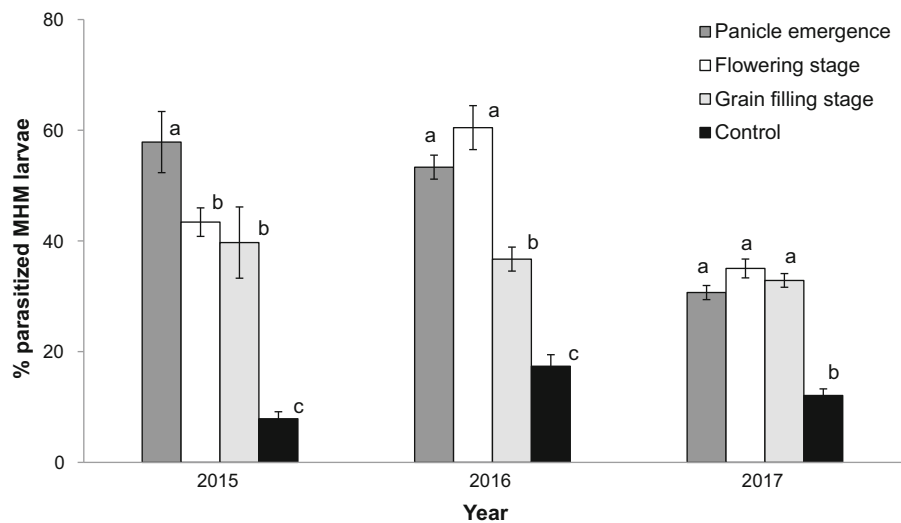
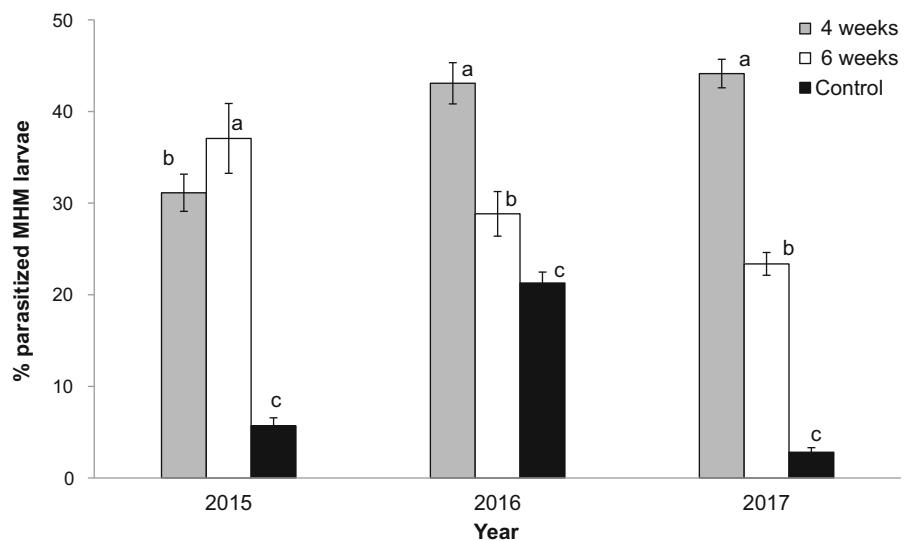


Fig. 2 Parasitized larvae of millet head miner (MHM), *H. albipunctella* (% mean \pm SE) due to *H. hebetor* following releases of the parasitoid at different weeks after first sight of MHM eggs in 2015, 2016 and 2017. For each year, bars bearing different letters were significantly different (Student–Newman–Keuls test, $\alpha = 0.05$)



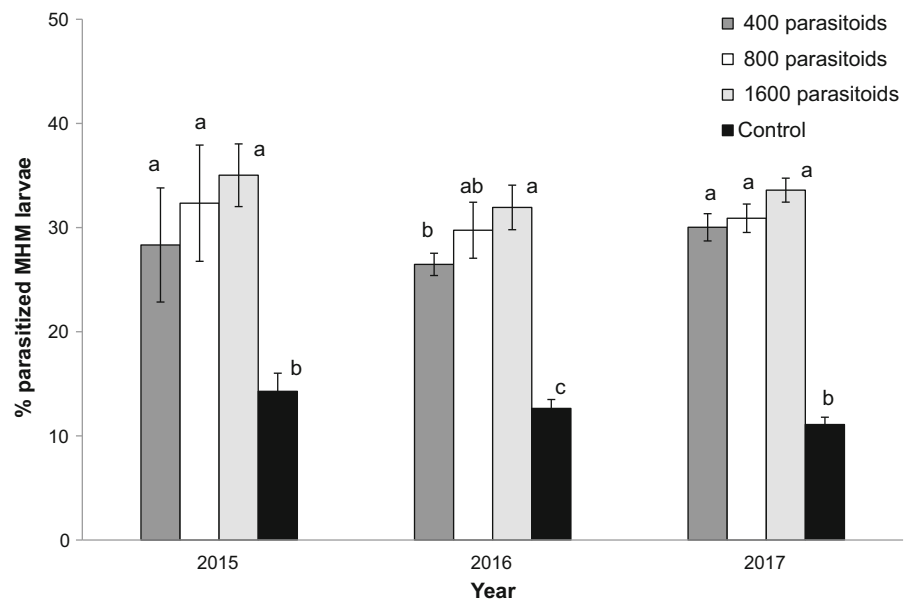
Discussion

In the current study, we found the natural parasitism of the MHM due to *H. hebetor* in the field to vary between 8 and 12% from 2015 to 2017, indicating the need for augmentative biological control as previously reported (Baoua et al. 2014; Ba et al. 2014). Indeed, from the different experiments, releases of parasitoids led to significantly higher parasitism of the MHM compared to control villages that did not receive parasitoids, confirming previous findings (Ba et al. 2014; Baoua et al. 2014; Amadou et al. 2017).

Moreover, our findings suggest that the best timing for deployment of parasitoid bags is either at pearl

millet panicle emergence/flowering stage or four - weeks after first sight of MHM eggs. As reported earlier, *H. hebetor* usually preferred parasitizing late instar larvae of its different host species (Amir-Maafi and Chi 2006; Akinkulore et al. 2009; Ghimire and Phillips 2010; Saxena et al. 2012). It is then crucial that releases of parasitoids coincide with the period when late instar larvae of MHM are available in the field. Typically, the MHM moth lays eggs on emerging panicles (Gahukar et al. 1986) and it takes two weeks for the parasitoid progeny to disperse from bags after deployment (Baoua et al. 2018). This timing coincides with the growth of MHM into third and

Fig. 3 Parasitized larvae of millet head miner (MHM), *H. albipunctella* (% mean \pm SE) due to *H. hebetor* following releases of different numbers of the parasitoid in 2015, 2016 and 2017. For each year, bars bearing different letters were significantly different (Student–Newman–Keuls test, $\alpha = 0.05$)



fourth instar larvae of the MHM (Kadi-Kadi 1999; Green et al. 2004).

Though the ability of farmers to describe crop insect pests (Ochou et al. 1998; Tefera, 2004; Poubom et al. 2005; Abteu et al. 2016), including pearl millet insects and the MHM life cycle (Tanzubil and Yakubu 1997; Ba et al. 2013), has been well documented in Africa, egg scouting by farmers would require some specific training (Silvie et al. 2001; Gautam et al. 2017). Also, scouting for eggs could be time consuming. Therefore, it will obviously be much easier to use the millet phenology stage as reference for releases of parasitoids. Using plant phenology stages has also been suggested for releases of the parasitoid *Telenomus remus* (Nixon) (Hymenoptera: Platygasteridae) against fall armyworm in maize, cotton and soybean in Brazil (Pomari et al. 2013). Given that farmers usually have different planting dates and use different varieties of different flowering time, the releases of parasitoids will require some coordination among farmers. Indeed releases of parasitoid could start in farms where pearl millet flowered early. The parasitoid will multiply and subsequent generations will disperse to other farms.

Regarding the needed numbers of *H. hebetor* adults to be released, our results indicate that the release of either 400, 800, or 1600 parasitoids per village led to at least twice more parasitism of MHM larvae than in control villages that did not receive any parasitoids.

The dose of 800 parasitoids was as effective as the 1600 parasitoids. Given the prohibitive cost for producing the parasitoid (Amadou et al. 2019) one could recommend the use of 800 parasitoids. As suggested by Baoua et al. (2018) the release of 900–1000 parasitoids can disperse over a distance of 3 km from the release point within 2–3 weeks. This could be seen low as compared to numbers involved in augmentative releases of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae)—several hundred thousand per hectare—against corn borer in maize (Bigler 1986; Wang et al. 2014), or augmentative releases of *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae)—20 to 60 thousand per hectare—against Tephritidae fruit flies in orchards (Sivinski et al. 1996; Montoya et al. 2000). However, differences could be due to the nature of the parasitoid species; the pest cycle; the architecture of the crop plant, and the environment (Thorpe 1985; Cloyd and Sadof 2000; Pomari et al. 2013).

Given the nature of the crop (annual), the nature of the pest (one generation per year), the short period of time when the target pest is present (pearl millet reproduction stage), and the low possibilities for released parasitoids to survive the long dry season (Kabore et al. 2017), there is no reason to release more parasitoids than what is really needed. Apart from the economic implication, the release of excessive numbers of parasitoids per unit of area could lead to the

reduction in their efficiency (Knipling 1977). Moreover, the release of excessive numbers of parasitoids could lead to superparasitism, and thus decrease the number of parasitized hosts (Cave 2000; Martel and Boivin 2004; Reay-Jones et al. 2006). In fact superparasitism due to excess numbers of parasitoid has been reported also for *H. hebetor* (Strand and Godfray 1989).

Therefore, the recommended dose of 800 *H. hebetor* needs further investigation, as like most parasitoid species, *H. hebetor*'s reaction to host is density-dependent (Singh et al. 2016). In the current study, as low as 16% natural infestation of MHM was recorded in some of the experimental fields where different numbers of *H. hebetor* were released. This is lower than the typical infestation rate observed in the region (Baoua et al. 2014, 2018; Amadou et al. 2017). As a consequence, the releases of *H. hebetor* lead to only 35% parasitism of MHM larvae compared with over 70% to 90% parasitism reported in previous studies (Ba et al. 2014; Baoua et al. 2014, 2018). This could be due to reduced parasitoid searching efficiency caused by low host density, observed in other settings (Sivinski et al. 1996; Montoya et al. 2000). For this reason, the density of parasitoids to be released should be based on the ratio of numbers of parasitoids per number of host instead of the acreage of the crop (Parra and Zucchi 2004; Bueno et al. 2012; Pomari et al. 2013). Farmers will therefore need some training for the assessment of actual pest infestations before identifying the numbers of parasitoids needed for releases. Such an approach could be challenging especially in Africa where the use threshold intervention levels for pest control has been difficult to implement in the past (Silvie et al. 2013; Togbé et al. 2015).

However based on the above experiments, we can recommend the release of 800 parasitoids per 3 km radius in the early panicle stage of the crop to obtain maximum percentage of parasitism and control of MHM. If parasitoids are to be released using the jute bag technique (Ba et al. 2014), the 800 parasitoids correspond to 12 parasitoid bags. This will reduce the current numbers by 20%. Given the current price of \$3.34 per bag (Guerci et al. 2018) a saving of \$10 is expected per each release. Further investigations will be needed to confirm the effectiveness of the 800 parasitoids dose under higher MHM infestation for consistency.

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