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Research

Development of an Optimum Diet for Mass Rearing of the Rice Moth, *Corcyra cephalonica* (Lepidoptera: Pyralidae), and Production of the Parasitoid, *Habrobracon hebetor* (Hymenoptera: Braconidae), for the Control of Pearl Millet Head Miner

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Abstract

The rice moth, *Corcyra cephalonica* Stainton, an alternate host for the production of the parasitoid, *Habrobracon hebetor* Say, was reared on different diets, including pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Poales: Poaceae) flour only, and in combinations of flours of sorghum [*Sorghum bicolor* (L.) Moench] (Poales: Poaceae), peanut (*Arachis hypogea* L.) (Fabales: Fabaceae), and cowpea [*Vigna unguiculata* (L.) Walp.] (Fabales: Fabaceae) to identify the optimal and economical proportion to be used under the conditions of Niger. The addition of cowpea or peanut to the pearl millet diet slightly increased *C. cephalonica* larval development time. Likewise, the addition of cowpea or peanut to cereal diets yielded a higher *C. cephalonica* larval survival. Female moths emerging from larvae fed on cereal and legume mixed diets produced higher eggs compared to the ones fed on sole and mixed cereals. Among legumes, cowpea addition is most interesting in terms of cost/production of *C. cephalonica* larvae. However, female moths emerging from larvae fed on different millet cowpea mix (5, 25, and 50%) laid significantly more eggs than those fed on sole pearl millet. Further, individual *C. cephalonica* larvae fed on 75% pearl millet + 25% cowpea produced significantly more *H. hebetor*. With an initial 25 *C. cephalonica* larvae kept for a 3-mo rearing period, the number of *H. hebetor* parasitoids produced will reach 2.68–10.07 million. In terms of cost/production ratio, the 75% pearl millet: 25% cowpea yielded better results.

Key words: *Habrobracon hebetor*, *Corcyra cephalonica*, millet head miner, pearl millet, cowpea

Niger is the third highest pearl millet [*Pennisetum glaucum* (L.) R. Br. (Poaceae)] producing country; most farmers in Niger rely on its grain as their primary source of food [Food and Agriculture Organization (FAO) 2016]. Pearl millet grows in poor soils under moisture stress and the millet head miner [*Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae)] causes major yield losses ranging from 40 to 85% (Krall et al. 1995). The biological control of the millet head miner was initiated in 2006 (Baoua et al. 2014) and the parasitoid, *Habrobracon hebetor* Say reared on rice moth *Corcyra cephalonica* Stainton was released at maturity stage mostly during August to September to control this pest (Ba et al. 2014, Baoua et al. 2018). The rice moth is one of the alternate hosts for mass production of egg and larval parasitoids of the hymenopteran families including Bethylidae, Braconidae, Platygastridae, and Trichogrammatidae (Parra 1997, Nathan et al. 2006, Deulkar et al. 2012, Hegade et al. 2014, Pomari-Fernandes et al. 2015, Singhamuni et al. 2015, Nasrin et al. 2016, Queiroz et al. 2017). To produce these parasitoids, *C. cephalonica* is reared on artificial and natural diets in the laboratory. The larger number and better quality of *C. cephalonica* eggs and larvae, and its higher production rate within a given time, are essential attributes, which enhance suitability of these eggs/larvae for mass production of parasitoids in biological control programs (Nathan et al. 2006, Nasrin et al. 2016).

For the past 20 y, scientists in the Sahelian countries in West Africa have been using larvae of *C. cephalonica* for mass production of the parasitoid *H. hebetor* for augmentative biological control of the millet head miner *H. albipunctella* (Payne et al. 2011; Ba et al. 2013, 2014; Baoua et al. 2014). Based on the effectiveness of *H. hebetor* causing more than 80% larval mortality of the pest, which resulted in 34% increase in millet yield in West Africa, a demand has been created for its supply to farmers in rural communities (Ba et al. 2013, Amadou et al. 2017). However, there have been challenges to produce an adequate number of parasitoids to meet farmers' demand. *Corcyra cephalonica* is mass cultured on a mixture of one-third of pearl millet flour and two-thirds of millet grains (Bal et al. 2002, Ba et al. 2013, Kabore et al. 2017) and *H. hebetor* reared from third- and fourth-instar larvae of *C. cephalonica* (Ba et al. 2014). The parasitoids are released in small jute bags in millet fields (Ba et al. 2014). Each bag contains 25 *C. cephalonica* larvae and two mated *H. hebetor* females. Subsequent generations of *H. hebetor* emerge and disperse in millet fields (Ba et al. 2014, Baoua et al. 2018).

In Niger, pearl millet is grown during the rainy season from June to September and pearl millet panicles emerge in early August, which also coincides with the emergence of adults of *H. albipunctella* from the diapausing population from the soil (Gahukar et al. 1986). However, the parasitoid population would not survive a 9-mo long off-season when the host is in diapause (Kabore et al. 2017), requiring annual release of parasitoids in early August. The alternate host and the parasitoid multiplication should begin in May in order to accumulate enough numbers of parasitoids to meet farmers' demand. However, the challenge is that the month of May is not favorable for insect multiplication due to maximum temperatures reaching up to 45°C. It is also difficult to operate a cooling system in the laboratory room because of erratic electric power supply and high cost in Niger. Therefore, the insect's multiplication should start from mid-June onwards to coincide with the beginning of the rainy season and the subsequent favorable drop in temperature. Hence the need to accelerate the current production process of *C. cephalonica* for multiplication and field release of *H. hebetor* in early August.

Several studies have been conducted to standardize the *C. cephalonica* diet with locally available ingredients and to increase the numbers and quality of larval and adult population (Deulkar et al. 2012, Rajkumari et al. 2014, Wadaskar et al. 2015, Nasrin et al. 2016, Ahmad et al. 2017, Bharti et al. 2017). Additional studies have been conducted to improve the nutritional quality of the alternate host for parasitoid production (Hunter 2003, Kumar and Shenhmar 2003, Nathan et al. 2006, Rimbing et al. 2013).

To optimize the current technique for rearing *C. cephalonica* (Bal et al. 2002, Ba et al. 2014) and to improve the production of the parasitoid *H. hebetor*, we conducted laboratory trials evaluating pearl millet, sorghum [*Sorghum bicolor* (L.) Moench (Poaceae)], peanut (*Arachis hypogea* L. Fabaceae), and cowpea [*Vigna unguiculata* (L.) Walp. (Fabaceae)] as diet sources because these are locally grown and commonly available in Niger. Pearl millet was tested alone as well as mixed with sorghum, cowpea, and peanut in the first experiment and with different portions of cowpea in the second experiment.

Materials and Methods

Study Environment

The study was conducted under uncontrolled climatic conditions with a photoperiod of 13:11 (L:D), fluctuating temperatures of 33–39°C, and relative humidity of 65–76% measured with indoor thermometer/hygrometer (TA 298, Pro Signal). The study was carried out on benches of the entomology laboratory of the Institut National de la Recherche Agronomiques du Niger (INRAN) from July to September in 2015 and 2016.

Source of Insects and Diet Ingredients

Both *C. cephalonica* and *H. hebetor* used in this study were obtained from a mass rearing facility at the INRAN laboratory in Maradi, Niger. *Corcyra cephalonica* larvae were reared on a mixture of pearl millet grains and flour diet using the Bal et al.'s (2002) modified method described by Ba et al. (2014).

Different larval feeding media were prepared with different combinations of pearl millet, sorghum, peanut, and cowpea grain and flour. Locally available varieties of these grains free of pesticides were purchased at harvest time from a trusted organic farmer in Maradi.

Effect of Diet on *C. cephalonica* Development and Subsequent Production of *H. hebetor*

Two sets of three experiments were conducted to evaluate different diets for production of *C. cephalonica*. The first experiment consisted of five treatments: 1) 100% pearl millet, 2) 50% pearl millet + 50% sorghum, 3) 50% pearl millet + 50% cowpea, 4) 50% pearl millet + 50% peanut, and 5) 25% pearl millet + 25% sorghum + 25% cowpea + 25% peanut. One hundred grams milled grains of each diet was placed in a plastic cylindrical box of 105cc capacity (Ø = 7 cm) and infested with 25 freshly hatched *C. cephalonica* larvae collected from the mass rearing facility. The boxes were closed with muslin cloth and secured with rubber bands. These boxes were kept on the laboratory bench at ambient temperature. The experimental design involved three batches of the five described treatments replicated ten times (3 × 5 × 10). In the first batch of boxes, *C. cephalonica* larvae were kept for 1 mo to collect data on the number of living and dead larvae, and number of *C. cephalonica* emerging moths. The emerging moths were confined individually in egg laying boxes and number of laid eggs counted. The second batch of boxes was held for 2 mo to collect data on *C. cephalonica* population growth. From the third batch of boxes, 10 *C. cephalonica* fourth- and fifth-instar larvae of the second generation were exposed for parasitism to a single *H. hebetor* mated female in a Petri dish and replicated 10 times. The host larvae and female parasitoids were kept in the Petri dishes for 48 h after that parasitoids were removed from the Petri dishes. Two weeks later, the number of emerging *H. hebetor* offspring was recorded.

The second experiment was set up based on the results of the first experiment. For this experiment, the diets were prepared from a combination of flours of pearl millet and cowpea with the following proportions: 1) 100% pearl millet, 2) 50% pearl millet + 50% cowpea, 3) 75% pearl millet + 25% cowpea, and 4) 95% pearl millet + 5% cowpea. Here also, 25 freshly hatched *C. cephalonica* larvae were kept in three batches of 10 boxes (same as in the first experiment) for each of the three treatments (120 boxes). The same sets of data were collected from this experiment as described in the first experiment.

Modeling Insect Population Reared on Different Diets

A model was developed to project the *C. cephalonica* larval population growth over three generations starting with an initial population of 25 larvae feeding on different combinations of flours of pearl millet and different proportion of cowpea. The population growth rate was calculated based on the population increase in the first generation. This growth rate was used to project the population estimates of subsequent generations. The survival rate is considered in order to take into consideration the larval death from limiting factors. The population growth rate of the first generation of *C. cephalonica* was used to extrapolate the subsequent population with the following formula:

$$\text{Growth rate} = \left(\frac{\text{New larval population} - \text{Initial population}}{\text{Initial population}}\right) \times 100$$

The *C. cephalonica* larval population from the third generation was used to project the number of *H. hebetor* parasitoids that could be produced using the number of *H. hebetor* progenies emerging from individual *C. cephalonica* larva. In fact, the number of *H. hebetor* produced on a single *C. cephalonica* larva is multiplied by the total number of larvae produced at third generation to compute the parasitoid population. The total diet needed for producing *C. cephalonica* larvae up to third generation has been cost using the market price of millet and cowpea at harvest time and the cost of parasitoids computed using the following formula:

$$\text{Cost of } 1,000 \text{ parasitoids} = \frac{\text{Cost of cumulative diet consumed by } C.\ cephalonica \text{ larvae}}{\text{Total number of produced parasitoids}} \times 1,000$$

Data Analysis

Data were subjected to analysis of variance (ANOVA) using GENSTAT 14. When ANOVAs were significant, means were separated by LSD tests at the 5% level.

Results

Corcyra cephalonica Developmental Parameters When Fed on Diets of Different Proportions of Pearl Millet Mixed With Sorghum, Cowpea, and Peanut and the Number of Eggs Produced by the Female Moth

Corcyra cephalonica larva developed in 28–30 d with significantly longer development time when cowpea or peanut flour were added to cereals diets (Table 1). Similarly, the addition of cowpea or peanut flour to cereals diets yielded a higher larval survival (Table 1). The females of *C. cephalonica* emerging from larvae fed on pearl millet combined with cowpea or peanut flour laid significantly more eggs than the ones fed on cereals alone (Table 1).

Corcyra cephalonica Developmental Parameters When Fed on Diets of Different Proportions of Pearl Millet and Cowpea and the Number of Parasitoids Produced by the Host Larva

In the second experiment, the *C. cephalonica* larvae fed with the mixture of 50% pearl millet + 50% cowpea recorded a longer larval development time compared to 100% pearl millet diet or 95% pearl millet + 5% cowpea diet (Table 2). However, the larval survival did not differ between the diets (Table 2). An addition of 5–50% cowpea to a pearl millet diet significantly increased the number of eggs laid by *C. cephalonica* over the 100% pearl millet diet (Table 2). When given larvae of *C. cephalonica*, significantly higher number of *H. hebetor* progenies emerged from individual *C. cephalonica* larva fed with the combination of 75% pearl millet + 25% cowpea and 50% pearl millet + 50% cowpea (Table 2).

Larval Population at Second Generation and Projected Numbers of *C. cephalonica* Larvae and *H. hebetor* Produced in Third Generation From Combination of Different Proportions of Pearl Millet and Cowpea

Starting with an initial number of 25 larvae of *C. cephalonica* by the third generation, about 1.6 million and 2.7 million larvae were produced on 75% pearl millet + 25% cowpea and 50% pearl millet + 50% cowpea, respectively (Table 3). With this number of *C. cephalonica* larvae, about 7.2 and 10 million *H. hebetor*, respectively, could be produced (Table 3). However, when considering total diet required for *C. cephalonica* larval production, the 75% pearl millet + 25% cowpea yielded the best cost/production ratio for *H. hebetor* production (Table 3).

Discussion

Several studies were conducted with a combination of locally available diet ingredients in an effort to enhance production of *C. cephalonica* culture in the laboratories. The post-embryonic development ranged from 28 to 30 d. Other studies reported 35–40 d using diets including sorghum, pearl millet, rice, wheat, and groundnut (Shazali 1986, Prakash and Senthilkumar 2005, Rajkumari et al. 2014, Wadaskar et al. 2015). The differences could be attributed to different experimental conditions (i.e., temperature and humidity), but they included duration from egg to adult emergence, while in this study, duration of eggs was not included. The larval survival was higher on the mixed cereal + legumes than on sole cereal diet. Similar results were reported in other settings (Osman 1984, Chaudhuri and Senapati 2017). In our case, both cowpea and peanut gave similar results and we selected cowpea as the additive to pearl millet diet over peanut due to lower cost and availability.

The survival of *C. cephalonica* larvae was not affected by addition of different proportions of cowpea (5–50%) to millet diet. However, female moths emerging from larvae fed on different millet and cowpea mix (5–50%) laid 2.4–2.6 times more eggs than those fed on sole millet. Rajkumari et al. (2014) and Ahmad et al. (2017) recorded highest fecundity with females of *C. cephalonica* emerging

Table 1. *Corcyra cephalonica* developmental parameters when fed on diets of different proportions of pearl millet mixed with sorghum, cowpea, and peanut and the number of eggs produced by the female moth

Treatments	Larval and pupal development time (days ± SE)	Larval survival (%)	No. of eggs per female (mean ± SE)	No. of *H. hebetor* adults emerging from individual *C. cephalonica* larva (mean ± SE)
100% pearl millet	28.20 ± 0.36b	88.32 ± 5.28b	38.42 ± 15.51c	3.10±0.25
50% pearl millet + 50% sorghum	28.60 ± 0.34b	82.29 ± 3.72b	40.42 ± 10.14c	2.84±0.20
50% pearl millet + 50% cowpea	30.00 ± 0.26a	98.68 ± 1.08a	94.17 ± 24.14ab	3.29±0.33
50% pearl millet + 50% peanut	30.20 ± 0.20a	99.70 ± 0.20a	88.25 ± 22.81ab	2.49±0.31
25% pearl millet +25% sorghum + 25% cowpea +25% peanut	29.20 ± 0.25a	99.08 ± 0.85a	112.33 ± 14.09a	4.10±0.42
ANOVA	$F = 9.05$; df = 4, 44; $P < 0.001$	$F = 7.03$; df = 4, 44; $P < 0.001$	$F = 3.40$; df = 4, 234; $P = 0.01$	$F = 3.64$; df = 3, 55; $P = 0.01$

Table 2. *Corcyra cephalonica* developmental parameters when fed on diets of different proportions of pearl millet and cowpea and the number of parasitoids produced by the host larva

Treatments	Larval and pupal development time (days ± SE)	Larval survival (%)	No. of eggs laid per female (mean ± SE)	No. of *H. hebetor* adults emerging from individual *C. cephalonica* larva (mean ± SE)
100% pearl millet	29.4 ± 0.27b	81.33 ± 3.31a	43.80 ± 6.16b	2.77 ± 0.37b
50% pearl millet + 50% cowpea	30.41 ± 0.14a	82.00 ± 3.99a	111.4 ± 5.60a	3.76 ± 0.50ab
75% pearl millet + 25% cowpea	29.91 ±0.19ab	84.89 ± 3.82a	113.4 ± 11.70a	4.90 ± 0.78a
95% pearl millet + 5% cowpea	29.56 ± 0.25b	70.67 ± 5.51a	104.8 ± 15.16a	2.90 ± 0.43b
ANOVA	$F = 3.96$; df = 3,75; $P = 0.01$	$F = 2.16$; df = 3; 70; $P = 0.1$	$F = 10.13$; df = 3, 36; $P < 0.001$	$F = 3.51$; df = 3, 32; $P = 0.03$

Means bearing different letters were significantly different (Student–Newman–Keuls test, α= 0.05).

Table 3. Larval population at second generation and projected numbers of *C. cephalonica* larvae produced in third generation when fed on a combination of different proportions of pearl millet and cowpea

Treatments	Larval population at second generation (mean ± SE)	Larval population at third generation	No. of *H. hebetor* produced with third generation *C. cephalonica* larvae	Cost of production for 1,000 parasitoids in U.S. dollar
100% pearl millet	818.17 ± 69.97	2,596,822	7,193,196	0.53
50% pearl millet + 50% cowpea	831.17 ± 72.55	2,680,257	10,077,766	0.58
75% pearl millet + 25% cowpea	651.33 ± 135.51	1,631,790	7,995,771	0.37
95% pearl millet + 5% cowpea	687.00 ± 148.86	1,819,176	5,275,610	0.53

Means bearing different letters were significantly different (Student–Newman–Keuls test, α= 0.05).

from larvae fed on mixtures of cereals fortified with chickpea flour. Mehendale et al. (2014) reported that a sole sorghum diet produced adults with low fecundity.

Individual *C. cephalonica* larvae fed on pearl millet diet supplemented with 25–50% cowpea are more suitable for *H. hebetor* development. In fact, the parasitoid *H. hebetor* produced more progenies per larva when given host larvae reared on 50% pearl millet + 50% cowpea or 75% pearl millet + 25% cowpea. This situation may be due to protein fortification as pearl millet contains only 11.8% protein while cowpea contains about 25% (Davis et al. 1991). Scriber and Slansky (1981) and Ahmad et al. (2017) also reported that the growth, development, and fecundity of insects are strongly dependent upon the quality and quantity of food ingested.

Even though the larval population increases in second and third generations of *C. cephalonica* reared on sole pearl millet and combination of pearl millet and cowpea are somewhat similar, the *H. hebetor* production from the third generation of *C. cephalonica* reared on 50% pearl millet and 50% cowpea was higher than other treatments. With an initial 25 *C. cephalonica* larvae kept for a 3-mo rearing period, the number of *H. hebetor* parasitoids will reach 2.68–10.07 million. However, in terms of cost/production ratio, the 75% pearl millet + 25% cowpea yielded better results.

The timely production of quantity and quality of alternate host larvae is necessary for the multiplication of parasitoids in the laboratory and for field release. It is critical to have a steady supply of *C. cephalonica* larvae at the beginning of the cropping season in early June, at the end of summer in the Sahel, to provide cultures of the parasitoid to the farmers' cooperatives. In turn, the cooperatives need to multiply sufficient parasitoids by early August to meet the demands of the farmers for field release. Starting this process, with an initial population of 25 larvae, the farmers' cooperatives will be able to produce sufficient *C. cephalonica* larvae within 3 mo. The cooperatives should start rearing of *C. cephalonica* in early June and release the parasitoids in August. The predicted number of *H. hebetor* from the 75% pearl millet + 25% cowpea diet will be sufficient to cover pearl millet fields in villages of southern and northern parts of the country during the cropping season.

Conclusion

The head miner is the key constraint for pearl millet production in Niger. Augmentative biological control using the local larval parasitoid *H. hebetor* is the best currently available option to control this pest. *C. cephalonica* is one of the alternate hosts for raising the parasitoid *H. hebetor*. A mixture of 75% pearl millet and 25% cowpea is the best diet for mass production of healthy *C. cephalonica* larvae for cost-effective production of *H. hebetor* in the laboratory and by the farmers' cooperatives for filling jute bags for field release of the parasitoid.

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