



Research article

Evaluation of black soldier fly *Hermetia illucens* (L.) larvae flour as larval diet for dengue vector mosquito *Aedes aegypti* (Diptera: Culicidae)

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Article Info

Article history:

Received 16 August 2023

Revised 19 October 2023

Accepted 20 October 2023

Available online 31 October 2023

Keywords:

Aedes aegypti,

Hermetia illucens,

Larval diet,

Mosquito rearing,

Sterile insect technique

Abstract

Importance of the work: A low-cost ingredient for an *Aedes aegypti* larval diet is desirable for mosquito birth control programs.

Objectives: To investigate flour made from black soldier fly (BSF; *Hermetia illucens*) larvae as an alternative diet for *Ae. aegypti* larvae.

Materials & Methods: Prepupal BSF aged 18–21 d and two commercial animal diets were processed into flour and administrated to *Ae. aegypti* larvae. The nutrient contents were characterized and the experimental parameters were observed: L1 to pupation developmental time, mortality, sex ratio, pupal weight and emergence rate.

Results: The BSF flour had the highest nutrient content compared to two commercial diets, based on proximate and amino acid analyses. Larvae reared on BSF flour were characterized by the slowest immature developmental time, lowest mortality and heaviest pupae compared to the other larval diets. In addition, larvae fed on BSF flour produced a significantly higher ratio of male-to-female pupae, while larvae fed on the other two diets were opposite owing to higher mortality of larvae.

Main finding: Although notably, the BSF larvae flour resulted in delayed immature growth, its nutrient content was sufficient for larval development. The use of BSF larvae flour as a larval diet may reduce the cost of male *Ae. aegypti* production in the sterile insect technique program.

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<https://doi.org/10.34044/j.anres.2023.57.5.14>

Introduction

Aedes aegypti is a mosquito vector transmitting dengue, chikungunya, yellow fever, and Zika (Staples et al., 2009; Bhatt et al., 2013; Sokal et al., 2017; Staples et al., 2017). The distribution of this vector encompasses tropical and subtropical regions in Asia, Africa, America and even the Mediterranean area in Europe, while in Indonesia, *Ae. aegypti* has been found in 33 provinces (Rogers et al., 2006; Staples et al., 2017).

Mosquito birth control strategies, especially the radiation-based sterile insect technique (SIT), rely for their success on rearing and release techniques (Hendrichs et al., 2007). Optimization of rearing techniques for *Ae. aegypti* can be done by evaluating the source of ingredients in the larval diet that can produce large numbers of the highly competitive males at the lowest-possible cost. Rearing cost efficiency can be achieved by substituting several components from the existing commercial diets with an ingredient that is relatively cheaper and locally available (Khan et al., 2013; Mamai et al., 2019; Somda et al., 2019). Generally, commercial products of animal diets (fish and dog feed), cow liver flour, tuna flour and shrimp flour are used in mosquito mass-rearing (Damiens et al., 2012; Puggioli et al., 2013; Yahouédo et al., 2014; Lang et al., 2018).

Insects provide a source of protein, fat, carbohydrates, vitamins and minerals that can be used as an animal diet (Rumpold and Schluter, 2013; Ruhnke et al., 2018). The black soldier fly (BSF; *Hermetia illucens* L.) grows quickly into a large population that can be reared easily (Makkar et al., 2014). The BSF larvae play a role as an essential decomposing agent in breaking down organic substrates and returning nutrients to the soil. The BSF larvae can be used for composting household food scraps and agricultural waste in a waste management unit. Specifically, BSF larvae have been reported as the main component of the animal diets for some freshwater and saltwater fish, Pacific white shrimp and domestic poultry (Cummins et al., 2017; Katya et al., 2017; Muin et al., 2017; Schiavone et al., 2017; Ruhnke et al., 2018).

Recent studies reported BSF larvae as the new potential diet for *Aedes* mosquito mass-rearing to support the SIT program (Mamai et al., 2019; Somda et al., 2019; Polidori et al., 2023). Hence, the present study aimed to evaluate whether dry-ground BSF larvae alone could support the larval development of *Ae. aegypti*. The BSF larvae flour was compared with two commercial diets that have been used to rear *Ae. aegypti* in other studies: dog biscuits (Pedigree®) and koi fish pellets

(Takari®) (Shetty et al., 2016; Sasmita et al., 2021; Ernawan et al., 2022). The dog biscuits, costing approximately USD 2.95/kg in local Indonesian markets, have ingredients of crude protein (minimum 18%), crude fat (minimum 8%) crude fiber (maximum 5%), moisture (maximum 12%), calcium (maximum 1.4%) and total phosphorus (maximum 1.3%), while, koi fish pellets, costing USD 3.30/kg, contain crude protein (minimum 30%), crude fat (minimum 3%), crude fiber (maximum 4%), crude ash (maximum 12%) and moisture content (maximum 12%). The impacts of the BSF larvae flour compared to two commercial diets were investigated based on: developmental time, mortality, weight of pupae and sex ratio of *Ae. aegypti*. The results of the present study should provide useful information regarding an alternative ingredient in the larval diet for *Aedes* mosquito mass-rearing in the SIT program.

Materials and Methods

Aedes aegypti strain

The *Ae. aegypti* strain used was originally collected from the field in South Tangerang City, Banten province, Indonesia in 2017 and was reared at the Research Center for Radiation Process Technology-National Research and Innovation Agency, Jakarta, Indonesia in a climate-controlled insectary with 26±2°C temperature, 75±5% relative humidity and a 12 hr: 12 hr daylight:darkness photoperiod. The rearing methods of this study were the same as those described by Ernawan et al. (2018, 2019, 2022).

Larval diet preparation

Three different diets were tested on the *Ae. aegypti* larvae in the laboratory, consisting of BSF larvae flour and two commercial diets. The two commercial diets were dog biscuit (Pedigree®; Mars Petcare Co. Ltd.; Bangkok, Thailand), and koi fish pellet (Takari®; CPPETINDO; Sidoarjo, Indonesia). The BSF larvae used in this study were obtained from the Waste Management Unit of Depok City, West Java province, Indonesia. A sample of 500 g of BSF larvae aged 18–21 d (prepupal) were collected on a metal tray and placed inside a freezer (model AB-600-T-X; GEA; Shanghai, China) at 0–2°C for 24 hr. Then, the BSF larvae were stored at room temperature for 1 hr and quickly washed with clean water followed by hot, boiled water until the larvae had transformed into a chewy texture. Then, the BSF larvae were dried in an

oven (model B 5042; Heraeus; Frankfurt, Germany) at 70°C for 72 hr. The dried BSF larvae were ground into flour. Samples of 500 g each of both the commercial diets (dog biscuits and koi fish pellets) were dried and ground using the same procedure as for the BSF larvae flour.

Proximate and amino acid analysis of larval diet

All three *Ae. aegypti* larval diets were analyzed for their proximate and amino acid contents, according to Association of Official Analytical Chemists (2005) in the analytical laboratory of Bogor Agricultural University, Indonesia.

Larval diets administration

Samples of 600 first instar larvae of *Ae. aegypti* were collected from the colony culture and separated evenly onto three plastic trays (29 cm × 20.5 cm × 7.5 cm) each holding 1 L of water, with a density of 200 larvae/tray. The larvae in each tray were fed with one of the BSF larvae flour, koi fish diet flour or dog diet flour at a dose of 0.53 mg/larva/day according to Puggioli et al. (2016). All tested diets were administered in flour form. All treatments were repeated three times. The study parameters investigated were: developmental time (pupation time), larval and pupal mortality, male-to-female ratio, weight of male and weight of female pupae, and emergence rate.

Developmental time, mortality, and productivity of *Aedes aegypti*

The developmental time was determined using the duration from the first instar larvae until pupation (Puggioli et al., 2013). The number of surviving larvae was counted when the first pupae were observed. Thereafter, the numbers of dead larvae and pupae were counted daily. Mortality was determined based on Equation (1):

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae or pupae}}{\text{Initial number of larvae or pupae}} \times 100\% \quad (1)$$

The productivity parameters of sex ratio (male:female) at 4 d pupation, pupal weight and the percentage of adults that emerged were assessed following the protocols described by Steinwascher (1982) and Ernawan et al. (2017, 2022). Briefly, the sex ratio was determined as the total number of males:females in each treatment. For pupal weight, 20 male and 20 female pupae were taken randomly in each treatment

and then weighed individually. The adult emergence rate was determined by the number of pupae that successfully emerged into the adult stage.

Statistical analysis

The data on developmental time, mortality, male-to-female ratio, weight of pupae and adult emergence rate were subjected to analysis of variance followed by a Tukey's post hoc test. The data that did not satisfy normality and homogeneity tests were analyzed using the nonparametric independent sample Kruskal-Wallis test. Sex ratio was analyzed using a nonparametric chi-square test. All tests were considered significant at $p < 0.05$. All data analyses were performed using the Statistical Package for the Social Sciences (SPSS; IBM SPSS® version 22 for Windows; Armonk, NY, USA).

Results

Nutritional composition of diets

The nutritional composition of the *Ae. aegypti* larval diets are presented in Table 1. The BSF larvae flour had the highest nutritional value compared to koi fish and dog diets, except for nitrogen-free extract. The percentage of crude protein in the BSF larvae flour was highest compared to the other two commercial diets. The results were supported by amino acid analysis (Table 1).

Effects of diets on developmental time, mortality and productivity

The mean times to pupation are presented in Fig. 1. The larval diets significantly affected the developmental time from the L1 to the pupal stage. Larvae fed with BSF larvae flour took the longest time to pupate compared to the koi fish and dog diets, for both male and female specimens. In particular, the larvae reared on BSF larvae flour required 8.95±0.90 d (males) and 8.78±0.90 d (females) for pupation. In general, compared to the other larval diets, the dog diet resulted in the fastest growth rates in the larval stages, while the koi fish diet was the second-fastest.

The BSF larvae flour had the least effect on larval and pupal mortality. Only 2.66±0.29% of the larvae fed on the BSF larvae flour and none of the pupae from the same cohort died. These results were significantly lower than for the koi fish diet (larval stage: 8.33±2.46%; pupal stage: 3.31±2.79%) and dog diet (larval stage: 8.66±0.57%; pupal stage: 3.78 ± 1.49%).

Table 1 Proximate and amino acid analysis results of *Ae. aegypti* larval diets

Ingredient	BSF larvae flour (%)	Koi fish diet (%)	Dog diet (%)
Moisture	1.72±1.48	2.09±1.58	2.91±0.44
Crude ash	9.25±0.87 ^b	9.15±0.25 ^b	5.86±0.14 ^a
Crude protein	31.10±1.43 ^c	18.45±0.72 ^a	26.29±0.61 ^b
Crude fiber	5.48±0.63 ^b	0.32±0.03 ^a	0.94±0.01 ^a
Ether extract	36.31±0.95 ^c	1.93±0.06 ^a	6.64±0.38 ^b
Nitrogen-free extract	16.14±0.68 ^a	68.06±0.98 ^c	57.36±0.75 ^b
Aspartic acid	2.83±0.11 ^b	1.49±0.07 ^a	1.55±0.03 ^a
Glutamic acid	4.93±0.60 ^b	3.41±0.06 ^a	2.86±0.03 ^a
Serine	1.74±0.06 ^c	0.49±0.05 ^a	0.80±0.03 ^b
Glycine	2.10±0.19 ^c	1.74±0.04 ^b	1.34±0.06 ^a
Histidine	0.59±0.07 ^b	0.43±0.04 ^a	0.32±0.04 ^a
Arginine	1.73±0.07 ^c	1.23±0.04 ^b	1.06±0.07 ^a
Threonine	1.19±0.05 ^b	0.97±0.03 ^a	1.12±0.04 ^b
Alanine	0.97±0.02 ^b	0.66±0.06 ^a	0.59±0.02 ^a
Proline	2.34±0.09 ^c	1.71±0.04 ^b	1.08±0.06 ^a
Tyrosine	1.57±0.10 ^c	0.88±0.06 ^a	1.13±0.04 ^b
Valine	0.75±0.06 ^c	0.53±0.03 ^b	0.33±0.05 ^a
Methionine	0.63±0.03 ^b	0.43±0.05 ^a	0.57±0.05 ^b
Cysteine	0.59±0.03 ^b	0.33±0.05 ^a	0.61±0.04 ^b
Isoleucine	1.05±0.08 ^c	0.76±0.04 ^b	0.54±0.04 ^a
Leucine	1.84±0.04 ^c	1.29±0.06 ^b	1.13±0.06 ^a
Phenylalanine	1.15±0.06 ^c	0.83±0.04 ^b	0.64±0.05 ^a
Lysine	1.68±0.06 ^c	1.15±0.05 ^b	0.97±0.05 ^a

BSF = black soldier fly

Mean ± SD in each row superscripted with different lowercase letters are significantly ($p < 0.05$) different based on one-way analysis of variance and a *post hoc* Tukey test.

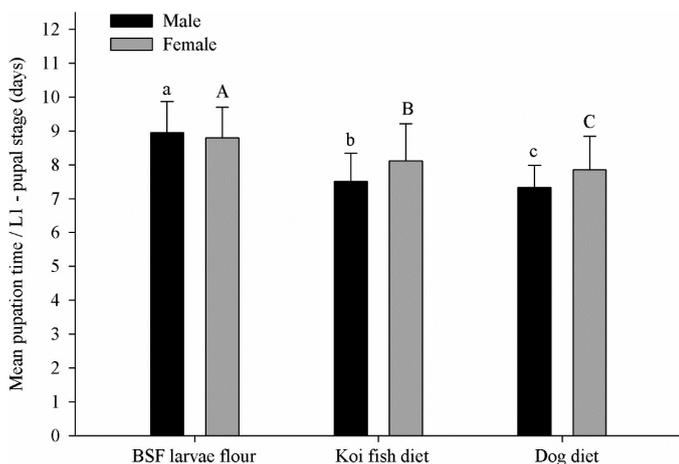


Fig. 1 Pupation time of *Ae. aegypti* larvae fed on various larval diets, where columns and error bars represent mean and SD, respectively. Significant ($p < 0.05$) differences within sex are indicated by different lowercase (males) or uppercase letters (females) based on one-way analysis of variance *post hoc* Tukey test. BSF = black soldier fly

Table 2 Mortality rate of *Ae. aegypti* larvae fed on various larval diets

Larval diet	Mortality (%)	
	Larval stage	Pupal stage
BSF larvae flour	2.67±0.29 ^a	0.00±0.00
Koi fish diet	8.33±2.47 ^b	3.31±2.79
Dog diet	8.67±0.58 ^b	3.78±1.50

BSF = black soldier fly

Mean ± SD in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different based on one-way analysis of variance and a *post hoc* Tukey test.

At 4 d, the sex ratio based on the surviving larvae had a significant male bias for the pupae from the BSF group and a significant female bias for the pupae from the other two tested diets. The number of male pupae from the BSF group was 2.5 times higher than for female pupae (Table 3, Fig. 2).

The pupal weight was significantly affected by the larval diet. Male, female and the sum of male and female pupae reared on the BSF larvae flour were significantly heavier than for the other two tested commercial diets (Table 4). There was no significant difference in the adult emergence rate among diets. All pupae from all experimental diets emerged into the adult stage.

Table 3 Adult sex ratio of *Ae. aegypti* from larvae fed on various larval diets

Larval diet	Number of pupae (% total)		Ratio (male: female)	Statistical values (chi squared; degrees of freedom; significance)
	Male	Female		
BSF larvae flour	434 (71.86%)	170 (28.14%)	2.55	115.391; 1; $p < 0.0001$
Koi fish diet	224 (43.33%)	293 (56.67%)	0.76	9.209; 1; $p = 0.002$
Dog diet	228 (42.46%)	309 (57.54%)	0.74	12.218; 1; $p < 0.0001$

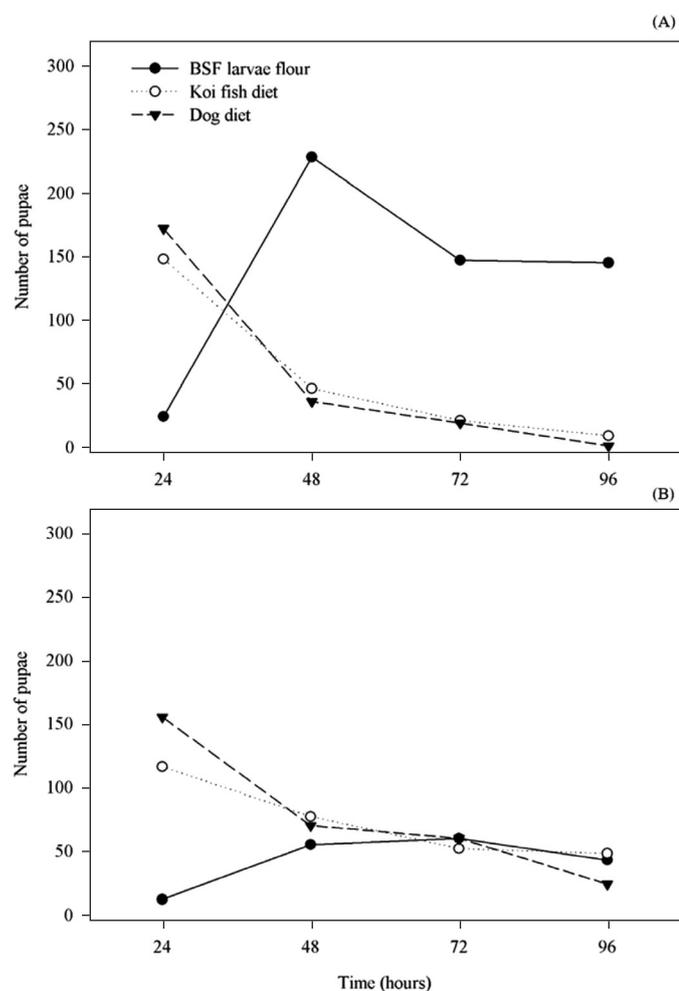
BSF = black soldier fly

Table 4 Weight of pupae and adult emergence of *Ae. aegypti* larvae fed on various larval diets

Larval diet	Weight of pupae (mg)			Adult emergence rate (%)
	Male	Female	Male+Female	
BSF larvae flour	0.47±0.028 ^a	0.73±0.005 ^a	1.20±0.027 ^a	100±0.00
Koi fish diet	0.43±0.007 ^b	0.64±0.007 ^b	1.07±0.009 ^b	100±0.00
Dog diet	0.44±0.008 ^b	0.64±0.005 ^b	1.08±0.009 ^b	100±0.00

BSF = black soldier fly

Mean ± SD in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different based on nonparametric independent sample Kruskal-Wallis (weight of pupae) and one-way analysis of variance *post hoc* Tukey tests (adult emergence rate).

**Fig. 2** Numbers of pupae from larvae fed on various larval diets at four time points: (A) male; (B) female, where BSF = black soldier fly

Discussion

The availability of insects as a nutrient supply makes them a potential food source in the livestock industry for dietary protein (Jonas-Levi and Martinez, 2017; Berggren et al., 2019). Protein is the basic component in producing animal food which plays an important role in the developmental process (Patel et al., 2019). The high protein content in the BSF larvae flour was presumably caused by the success of organic compound conversion by the larvae, combined with the optimal larvae age that was used in this study. The BSF larvae are able to convert organic matter, including protein, and to assimilate energy in the form of nitrogen, phosphorus, calcium and other trace elements from organic waste into their biomass that can then be used in the developmental process (Makkar et al., 2014; Gold et al., 2018; Mertenat et al., 2019). BSF larvae aged approximately 18–21 d are known to reach maximum size as they grow to the final stage/instar of the larvae. This allows more biomass to be obtained. In addition, another study reported that in the final stage/instar, the BSF larvae contained the highest protein (Lalander et al., 2019). External factors, such as the drying process, potentially affected the protein content inside the BSF larvae flour in the present study. Aniebo and Owen (2015) reported that drying using an oven increased the protein content by up to 4% compared to drying under sunlight.

The high protein content inside the BSF larvae flour was also reflected in the amino acid analysis result (Table 1). Generally, the amino acid content in the BSF larvae flour was the highest compared to the other diets. Amino acids in the BSF larvae flour can be categorized as a complete composition, containing both essential and nonessential parts in this flour. The presence of essential amino acids could be expected to ensure the protein quality of the BSF larvae flour that can be utilized as an animal diet. Ruhnke et al. (2018) mentioned that BSF larvae flour has good quality protein for animal feed. This is supported by it containing essential amino acids, such as lysine and methionine, which have been used to replace up to 33.30% of the protein source in fish diet and domestic poultry diet manufacture (Bondari and Sheppard, 1981; Sealey et al., 2011). However, the proportion of amino acids is necessary to be assessed in future studies to develop the optimal nutrient requirement for a mosquito larval diet.

In this study, the only unfavorable effect of BSF larvae flour was that it resulted in the slowest immature developmental time among the three tested diets. The larvae in each larval stage must achieve a minimum larval mass before progressing to the next stage (Chambers and Klowden, 1990). The longer the mean pupation time of a male compared to a female individual fed on BSF larvae flour is shown in Fig. 1 and Fig. 2, with the male individuals fed on BSF larvae flour predominating relative to the other tested diets at 48 hr, 72 hr and 96 hr after the first pupae appeared. Generally, male *Ae. aegypti* pupate earlier than females, which was also true in this study (see the first 24 hr in Fig. 2). However, the presence of male pupae was prolonged until the next two 24-hr windows. To produce sterile males for release trials in the SIT, only the first and second days of pupation are taken, considering the quantity and size overlap between males and females on the third and fourth days of pupation. Even though BSF larvae flour contained the highest nutrient level, apparently the process of acquiring and metabolizing the nutrients was slower than for the other two diets tested. This was probably due to the BSF larvae having the lowest nitrogen-free extract which represents the nonstructural carbohydrates and serves as a source of energy for metabolism processes (Traughber et al., 2021). In addition, the BSF larvae flour had a denser texture and tended to agglomerate compared to the koi fish and dog diets. This condition was presumably because of the high crude ether content of the BSF larvae flour (Table 1). This texture leads to uneven distribution of the BSF flour on the water surface and so that it tends to accumulate in one area. The uneven distribution of the BSF larvae flour allegedly reduced the potential for scum formation which can

lead to mortality of *Ae. aegypti* larvae and pupae (Yahouédo et al., 2014). This was supported by the low mortality rates for the *Ae. aegypti* larvae and pupae (Table 2). Scum formation on the water surface needs to be considered in the larval maintenance process to avoid disruption of oxygen intake by *Ae. aegypti* larvae.

Larvae fed with BSF larvae flour resulted in significantly heavier pupae compared to the two commercial diets. However, based on the pupal weight, both male and female pupae fed on any of the larval diets were categorized as small (weight < 2.40 mg), according to Steinwascher (1982). The use of the BSF larvae flour also resulted in a significantly higher male-to-female ratio compared to the koi fish diet or the dog diet. This phenomenon may be an indication of secondary sex manipulation due to the considerably higher mortality of larvae and pupae. As much as 8.33% and 8.66% of larval mortality could be accounted for by the significant loss of male specimens, causing female-biased pupae in the koi fish diet and dog diet groups, respectively. A significant male bias was also found in *Ae. aegypti* larvae fed on Khan's diet at 28°C and 32°C (Farjana et al., 2012; Bond et al., 2017; Sasmita et al., 2019). The high production of male pupae compared to female pupae was expected to increase the sterile male productivity in the mass-rearing facility.

Based on the present results, the use of BSF (*H. illucens*) larvae flour as the *Ae. aegypti* larval diet had several beneficial effects, such as low immature mortality and a high male-to-female ratio. These results were consistent with the studies conducted by Somda et al. (2019) and Mamai et al. (2019), which demonstrated that the BSF larvae flour had positive effects on *Ae. aegypti* and *Ae. albopictus* in the mass-rearing facility. The administration of the BSF larvae flour reduced mortality and increased productivity without extending the rearing time of *Ae. aegypti* compared to the two other commercial diets. The BSF larvae flour had a complete range of nutrient ingredients that could be used in the diet for *Ae. aegypti* mass-rearing in the SIT program. From the economic perspective, the prices of the BSF larvae, dog biscuit and koi fish diets in the local market were approximately USD 1.55/kg, USD 2.95/kg and USD 3.30/kg, respectively. By using the BSF larvae flour, the estimated diet cost-savings were approximately 48% and 53% compared to the dog and koi fish diets, respectively. Further investigations are needed to assess the nutrient proportion to develop an optimal formulation of the BSF-based mosquito larval diet. Furthermore, investigation is required of the larval diet effects on the adult male quality parameters (longevity, flight ability and mating competitiveness), as these parameters can play an important role in the success of the SIT program.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The Research Center for Radiation Process Technology-National Research and Innovation Agency provided support through the SIT program in Indonesia. The International Atomic Energy Agency (IAEA) through the RAS 5082 and RAS5095 programs provided supervision. Heriyanto from the Waste Management Unit of Depok City, West Java province, Indonesia provided the BSF larvae and Dr Teguh Wahyono provided assistance in the proximate analysis.

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