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Substrates most preferred for black soldier fly *Hermetia illucens* (L.) oviposition are not the most suitable for their larval development

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RESEARCH ARTICLE

Abstract

Larvae of black soldier fly (BSF), *Hermetia illucens* (L.1758) (Diptera: Stratiomyidae), are increasingly being used as animal feed ingredients. Larvae are usually produced by placing eggs, obtained from adult rearing on growing substrates but can also be obtained by exposing substrates to naturally occurring BSF females. In the latter system, the substrate needs not only to be nutritious for the larvae but also attractive to the females for oviposition. The 'preference-performance principle' suggests that female insects prefer to oviposit in substrates that maximise offspring fitness. In this study conducted in Ghana, six organic substrates known to be suitable for BSF production were evaluated for their suitability as oviposition attractants and larval development: pito mash (waste from a locally brewed sorghum drink), millet porridge mash, pig manure, chicken manure, fruit waste, and waste from roots and tubers. These were first exposed outdoors to measure the quantity of eggs laid on them by naturally occurring BSF females. In a second experiment, the quality of the substrates as larval rearing media was tested by placing a standard amount of young larvae to measure the individual and total weights of prepupae obtained, their number, and their development time. The nutritional profiles of both the prepupae and the substrates were determined. The substrate used significantly influenced the quantity of eggs laid and the development of the resulting prepupae, but the substrates most favourable for larval development were not the most favoured by gravid BSF for oviposition. In the oviposition tests, millet porridge mash was the most attractive substrate, whereas only a few eggs were recovered from the other substrates. All substrates allowed the successful development of larvae but pig manure was more productive than the others.

Keywords: fly oviposition, performance-preference, offspring fitness

1. Introduction

The larvae of the black soldier fly (BSF), *Hermetia illucens* (L. 1758) (Diptera: Stratiomyidae) are a readily available source of protein that can be utilised in animal feed to replace non-sustainable and expensive protein sources (Tomberlin and Van Huis, 2020; Van Huis *et al.*, 2020; Wang and Shelomi, 2017). In Africa, in particular, there is advocacy for poultry and fish farmers to include insects, particularly BSF, in their feed to improve the nutrition of their livestock and reduce production costs (Abro *et al.*, 2020; Chia *et al.*, 2019; Ssepuuya *et al.*, 2017), even though there is no data yet on BSF adoption rates by farmers (Abro *et al.*, 2020).

Production of BSF larvae can be achieved using a wide variety of organic waste streams. Several studies have tested the suitability of many organic waste streams on the growth, development, and proximate composition of BSF, with promising results (Banks *et al.*, 2014; Čičkova *et al.*, 2015; Danieli *et al.*, 2019; Miranda *et al.*, 2019). In most BSF production systems, adults are reared in cages to obtain eggs that are placed on the most suitable and available substrates (e.g. Caruso *et al.*, 2014; Diener *et al.*, 2009). However, egg production is a complicated technique that requires specific expertise. Therefore, small systems have been developed for individual farmers or hobby gardeners, consisting of exposing substrates to naturally occurring BSF females for laying eggs (Kenis *et al.*, 2018; Nyakeri *et al.*, 2017). In a simple system such as natural oviposition that relies on wild fly populations, it is crucial to select substrates that are suitable for stimulating oviposition and also meet the nutritional requirement for the development of the larvae. Egg-trapping efficiency is paramount to the effectiveness of such a BSF larvae production system (Sripontan *et al.*, 2017). The major determinant of egg-trapping is the baiting material used in luring gravid females.

The 'preference-performance principle' postulates that gravid female insects prefer to oviposit in substrates that maximise offspring fitness (Jaenike, 1978). Such behaviour is common among phytophagous species (Gripenberg *et al.*, 2010), and it has also been observed in detritivorous flies (Baleba *et al.*, 2019). In holometabolous insects such as BSF, where the juveniles are incapable of relocating after hatching and with no parental care, females should favour oviposition substrates that are most suitable for larval development. Based on this principle, the study focused on whether substrates preferred by wild BSF females for oviposition were the most suitable for optimum larval development.

2. Material and methods

Substrates

Six organic waste substances (substrates) collected from markets, livestock farms, and local food processing industries were tested for their suitability as oviposition and larval rearing media. The substrates used were pito mash (waste from a locally brewed sorghum drink), millet porridge mash, pig manure, chicken manure, fruit waste, and waste from roots and tubers. The pito mash used in the study was obtained from a pito processer in Ashaiman and the millet porridge mash from Kisseman (both suburbs in Accra). Fruit waste, roots and tubers were from the Madina market (the main foodstuff market in the La Nkwantanang district of the Greater Accra Region). Pig manure was obtained from the Council for Scientific and Industrial Research's (CSIR) Animal Research Institute and chicken manure was obtained from a private poultry farm in Ashaiman. All substrates were used in the state in which they were collected, except chicken manure. Chicken manure meal was prepared using six kilograms of chicken manure to nine litres of water. This measurement was determined through preliminary tests to obtain a moisture content of about 78%. Black soldier fly larvae are known to prefer diets with moisture content between 70-80% (Cheng *et al.*, 2017; Lalander *et al.*, 2020; Li *et al.*, 2011; Meneguz *et al.*, 2018). This was also done to match up the moisture contents of the other substrates which ranged between 59 and 80%. Fruit waste was an amalgamation of five fruits: pawpaw, pineapple, orange, watermelon, and banana in equal proportions. Millet porridge was composed of millet grains, chilli pepper, black peppercorns, cloves, and ginger milled together. The mash is the remaining residue after straining out water that has been added to the aforementioned mixture to collect the starchy liquid for making porridge. Samples of each substrate from all the experiments made between September and December 2018 were collected and refrigerated at 4 °C. The substrates were pooled at the end of the experiment and sampled for analysis to assess the nutritional profile of each substrate used. The samples were dried at 65 °C until a constant weight was obtained. The nutritional profile (crude protein, crude fibre, crude fat, ash, and dry matter were determined using the procedure of the Association of Official Analytical Chemists (AOAC, 2016).

Oviposition test

The study was carried out at the University of Ghana school farm in Accra between July to September 2018. The data were collected daily for seven weeks. A well-shaded area with mango trees and shrubs was chosen as BSF adults take rest in trees where they mate as well. Five litre plastic bowls were filled with 500 g of each of the substrates to be tested and provided with an oviposition medium made up of corrugated cardboard (Booth and Sheppard, 1984) cut into 10 cm ×6 cm pieces, and taped together to form bundles of three. Four bowls (n=4) per substrate (total of 24 bowls) were used. The bowls were arranged on a roofed stand in a completely randomised order, making sure that the position of the bowl did not influence oviposition. The oviposition media were placed on dried plantain leaves that were placed on the substrates in the bowls. The dried plantain leaves were to help keep the substrate moist and avoid wetting of the cardboard since BSF females do not lay eggs in wet substrates. Two cardboard bundles were placed on each bowl used in the test. The flutes of the cardboards and dried plantain leaves were checked daily for seven weeks for the presence of eggs. Substrates were replaced every five days because, as the days went by, other organisms colonised them. A very fine tip needle was used in detaching the egg masses from the cardboard for weighing. Egg masses collected daily from each bowl were weighed separately and weights recorded.

Larval development tests

Small plastic bowls (750 ml) were filled with ten grams of the substrates (four replicates of each substrate). Egg masses weighing 10 mg each were placed on 6 cm ×6 cm plastic plates and placed on the respective substrates in the 750 ml plastic bowls to allow eclosion. The bowls were placed in a screen cage made of muslin in the laboratory to prevent oviposition by other insects. Dates of eclosion for the substrates (bowl) were recorded and larvae were allowed to develop for four days. On day five, the young larvae were transferred into 10 l bowls filled with 500 g of the substrates. The bowls were covered with a muslin cloth and kept in a screen house to prevent oviposition by other insects. A sample of 100 prepupae was selected from each bowl (400 for each substrate) and individual weights were recorded. Prepupae were identified by their characteristic dark brown or black colour. In addition, the total weight of prepupae (prepupal yield) recovered from each bowl was taken, the total number of larvae developed, and the duration of development (date of first prepupae) for each bowl were recorded.

Nutritional composition

Samples of prepupae recovered and substrates were dried at 65 °C and weighed every 24 hours until a constant weight was obtained. The nutritional profile (crude protein, crude fibre, crude fat, ash, and dry matter) of the samples were determined using the procedure of AOAC (2016). The whole study included three consecutive experiments made between September 2018 and December 2018, with each trial taking a minimum of one month to complete. In total, twelve replicates per substrate were made. Three consecutive experiments were made to provide information on the effect of different periods on development.

3. Data analyses

All analyses were performed using the R environment for statistical computing (version 3.6.2) (R Core Team, 2018). The nutritional profile (crude protein, crude fibre, ash, fat, and moisture) of the substrates were subjected to a one-way analysis of variance (ANOVA) ($\alpha<0.05$). The effect of different factors (substrate and period of the experiment) on the weight of prepupae was examined using a linear mixed effects model (Pinheiro *et al.*, 2018). In this model, the fixed effect was the substrate while the random effect was the period of the experiment. The maximum likelihood (ML) was used to estimate the parameters of the model because the data on weight of prepupae were normally distributed. Similarly, the effect of different factors (substrate and week of the experiment) on the weight of eggs was examined using a linear mixed effects model (Pinheiro *et al.*, 2018). In this second model, the fixed effect was the substrate while the random effect was the week of the experiment. The penalised quasi-likelihood (PQL) was used to estimate the parameters of the model because data on the weight of eggs were not normally distributed. PQL is a flexible technique that can deal with non-normal data (Venables and Ripley, 2002). The linear mixed effect 'lme' function of package 'nlme' (Pinheiro *et al.*, 2018) was used for linear mixed effect model using maximum likelihood estimator and the 'glmmPQL' function of package 'MASS' (Venables and Ripley, 2002) was used for the linear mixed effect using penalised quasi-likelihood (PQL) estimator.

The means and confidence intervals of the weight of the prepupae and the weight of eggs were computed from the linear mixed effects models using the package 'effects' (John, 2003). A post hoc test was used for means structuration in the function of the treatment (substrate) for the weight of eggs and the weight of prepupae. The package 'emmeans' (Russell, 2020) was used to perform the post hoc tests.

The function 'ggplot' of the package ggplot2 (Wickham, 2016) was used to construct the evolution curve of the weight of eggs in the function of the substrates and the weeks. To determine the effect of substrates and the experimental periods (seasons) on the crude protein content of prepupae produced, a general linear model was used to compute a two-way ANOVA ($\alpha<0.05$). All the other parameters (fat, ash, and crude fibre) were pooled for proximate analyses due to insufficient sample quantities. The duration of development (egg to first prepupae), the total weight of prepupae (prepupal yields) recovered and the total number of individual prepupae recovered were subjected to a two-way ANOVA to show the effect of substrate on them ($\alpha<0.05$). In all ANOVAs, the Tukey HSD test was used as a post hoc test.

4. Results

Proximate analysis of substrates

The crude protein and the moisture content for all the six substrates were significantly different ($P<0.0001$). Pito mash had a significantly higher crude protein content when compared with the other substrates, except roots and tubers (Table 1). Fruit waste recorded the highest moisture content. Likewise, the ash, crude fibre, and fat contents of the different substrates were significantly ($P<0.0001$) different (Table 1). The ash content of millet porridge mash was not significantly different from pito mash, however, the other substrates had significantly different ash content. Chicken manure had higher ash content compared to the other substrates. A significantly different crude fibre content was obtained for roots and tubers. Millet porridge mash and fruit waste had significantly different crude fibre content from pito mash, pig manure, and chicken manure. The fat content of millet porridge mash, pito mash, and fruit waste were significantly different from those of pig manure, roots and tubers, and chicken manure (Table 1).

Oviposition test

The type of substrate significantly ($P<0.001$) influenced the quantity of eggs laid (Table 2). The estimate of the variance explained by the week of the experiment (random effect) is different from zero (5.06), meaning that the week of the experiment affected the weight of eggs. Only 14.74% of the total variance of the random effect is attributed to the week of the experiment effect.

Females laid significantly more eggs on millet porridge mash than on other substrates (Table 3 and Figure 1).

Larval development

Substrates significantly ($P<0.001$) influenced the individual weight of prepupae (Table 4). The estimate of the variance explained by the period of the experiment (random effect) is different from zero (150.06), meaning that the period of the experiment influenced the weight of the prepupae. Only 29.62% of the total variance of the random effect is attributed to the week of the experiment effect. The detailed result of the linear mixed effects model (Table 4) showed that all substrates were significantly different. The substrate pig manure had the highest performance of prepupae production (Table 5).

Furthermore, there was a significant effect ($P<0.001$) of the substrate used on the total weight of prepupae recovered (total prepupal yield). The total prepupal yield of pig manure was significantly higher in comparison to the other substrates, while chicken manure and fruit waste had comparable prepupal yields (Table 6). Millet porridge mash was also significantly higher in total prepupal yield when compared to chicken manure, fruit waste, and roots and tubers. The development time and the total number of individuals that survived to prepupae were also

Table 1. The nutritional composition of the various substrates tested.[1,2]

Substrate	Crude protein ± SE (%)	Ash ± SE (%)	Fat ± SE (%)	Crude fibre ± SE (%)	Moisture ± SE (%)
Millet porridge	20.8±0.4^{b}	1.8±0.1^{e}	13.8±0.1^{a}	20. 0±0.7^{b}	73.9±0.1^{d}
Pito mash	30.6±0.3^{a}	3.2±0.2^{de}	12.0±0.2^{a}	31.8±0.3^{a}	79.9±0.1^{b}
Fruit waste	8.1±0.4^{e}	10.4±0.6^{c}	12.4±1.2^{a}	15.7±1.0^{b}	87.1±0.4^{a}
Pig manure	16.1±0.2^{c}	16.3±0.1^{b}	6.7±0.9^{b}	35.1±2.3^{a}	70.8±0.3^{e}
Roots and tubers	3.3±0.1^{f}	4.3±0.1^{d}	8.1±0.1^{b}	1.9±0.2^{c}	59.1±0.1^{f}
Chicken manure	13.0±0.3^{d}	23.8±0.7^{a}	7.7±0.5^{b}	35.7±1.3a	78.0±0.4^{c}
Df	5	5	5	5	5
F-value	1,028	503.6	14.82	126.1	1,381
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] Means in the same column followed by the same letter are not significantly different (Tukey's HSD, $P<0.05$).
[2] Df = degree of freedom; SE = standard error.

Table 2. Effect of the substrate on weight of eggs: detailed results of the linear mixed effects model.[1]

Source of variation	Estimate	SE	Df	*t*-value	*P*-value
(Intercept)	2.842857	2.649670	156	1.072910	0.285
Fruit waste	-0.110714	3.541258	156	-0.031264	0.975
Millet porridge mash	28.796429	3.541258	156	8.131694	<0.001
Pig manure	1.346429	3.541258	156	0.380212	0.704
Pito mash	-1.700000	3.541258	156	-0.480055	0.632
Roots and tubers	-0.453571	3.541258	156	-0.128082	0.898

[1] Df = degree of freedom; SE = standard error.

Table 3. Estimate of the weekly mean weight of eggs laid and confidence intervals (CI).[1,2]

Substrates	Weekly mean weight of eggs ± SE (mg)	CI_lower	CI_upper
Chicken manure	2.8±1.3^{b}	-2.3	8.0
Fruit waste	2.7±1.37^{b}	-2.4	7.9
Millet porridge mash	31.6±4.9^{a}	26.5	36.8
Pig manure	4.2±2.5^{b}	-0.9	9.3
Pito mash	1.1±0.7^{b}	-3.9	6.3
Roots and tubers	2.4±2.1^{b}	-2.7	7.5

[1] Means in the same column followed by the same letter are not significantly different (Tukey's HSD, *P*<0.05).
[2] SE = standard error.

Table 4. Effect of the substrate on weight of prepupae: detailed results of the linear mixed effects model.[1]

Source of variation	Estimate	SE	Df	*t*-value	*P*-value
Fruit waste	38.9	1.2	7,201	32.71452	<0.001
Millet porridge mash	54.0	1.2	7,201	45.40229	<0.001
Pig manure	123.4	1.2	7,201	103.91715	<0.001
Pito mash	113.0	1.2	7,201	95.11055	<0.001
Roots and tubers	21.9	1.2	7,201	18.46500	<0.001

[1] Df = degree of freedom; SE = standard error.

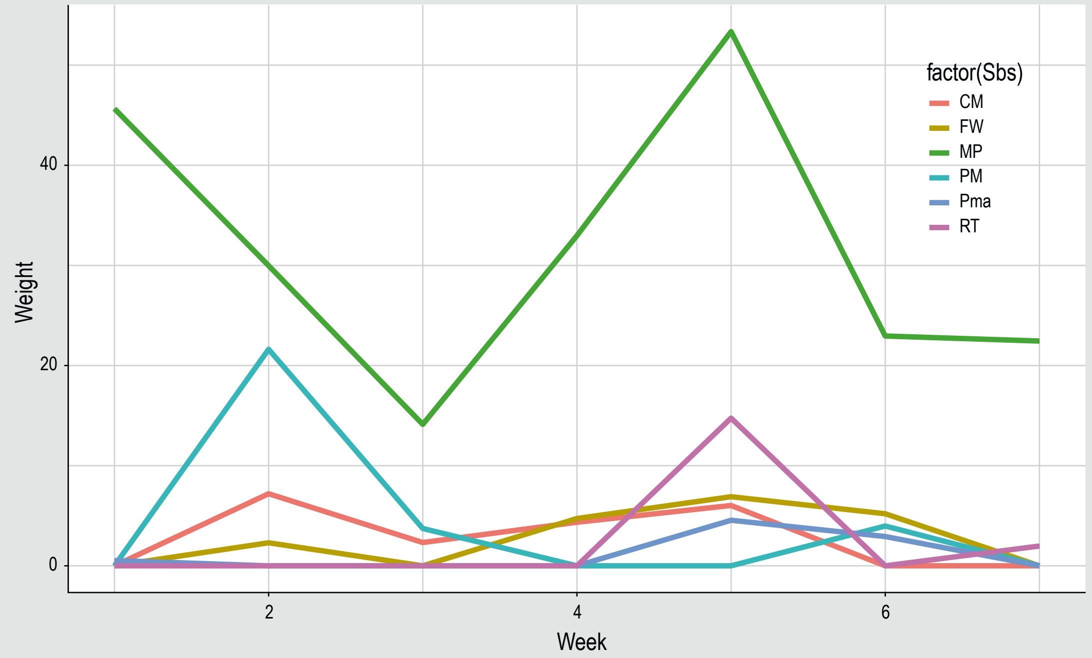

Figure 1. Evolution curve of the weight of eggs in function of the substrate and the week. CM = chicken manure; FW = fruit waste; MP = millet porridge; PM = pig manure; Pma = pito mash; RT = roots and tubers.

Table 5. Estimate of the means and confidence intervals (CI) of the weight of individual prepupae.[1,2]

Substrate	Means ± SE (mg)	CI_lower	CI_upper
Chicken manure	81.4±12.1^f	69.9	92.9
Fruit waste	120.3±10.3^d	108.9	131.7
Millet porridge mash	135.4±8.9^c	123.9	146.8
Pig manure	204.9±2.4^a	193.4	216.3
Pito mash	194.5±9.6^b	183.0	205.9
Roots and tubers	103.4±15.1^e	91.9	114.8

[1] Means in the same column followed by the same letter are not significantly different (Tukey's HSD, $P<0.05$).
[2] SE = standard error.

Table 6. Effect of substrates on total prepupal yields, total number of individuals surviving to prepupae and development time from egg to prepupal stage.[1,2]

Substrates	Total prepupal yields ± SE (grams)	Total number of individuals surviving ± SE	Development time ± SE (days)
Chicken manure	23.4±3.4^d	262.6±10.5^b	17.1±0.5^c
Fruit waste	30.4±2.1^d	313.5±26.0^{ab}	22.6±0.8^a
Millet porridge	46.4±3.4^b	353.3±14.3^a	15.8±0.2^c
Pig manure	59.8±4.5^a	348.9±34.6^a	16.7±0.3^c
Pito mash	44.0±4.0^{bc}	252.7±21.2^b	16.3±0.2^c
Roots and tubers	31.9±2.4^{cd}	303.8±20.4a^b	18.9±0.9^b
Df	5	5	5
F-value	20.19	33.57	4.57
P-value	<0.001	<0.001	0.001

[1] Means in the same column followed by the same letter are not significantly different (Tukey's HSD, $P<0.05$).
[2] SE = standard error.

significantly ($P<0.001$ and $P=0.001$, respectively) affected by the substrate type. Prepupae reared on fruit waste and roots and tubers had the longest development time of 23 and 19 days, respectively when compared to the others (Table 6). The development time was not significantly different for prepupae reared on millet porridge mash, pig manure and pito mash, recording a lower number of days to reach prepupae. The total number of individuals surviving to prepupae on millet porridge mash and pig manure were comparable but significantly higher than those reared on pito mash and chicken manure. Fruit waste and roots and tubers were similar in the total number of individuals surviving to the prepupal stage.

There were significant interactions between the time the experiment was conducted and the substrate type on the total number of individuals surviving to the prepupal stage (F=5.35, df=5, $P<0.0001$) and also on the duration of development (F=7.08, df=5, $P<0.0001$) from egg to prepupae. However, there was no significant interaction between the time the experiment was conducted and the substrate type on total prepupal yields (F=1.17, df=5, $P=0.336$).

Proximate composition of prepupae

Furthermore, the substrate type had a significant (F=30.63, df=5, $P<0.001$) influence on the crude protein accumulated by the developing larvae. The crude protein content of the prepupae reared on pito mash was highest, but not significantly different from roots and tubers (Table 7). Prepupae reared on fruit waste had a higher crude protein than the prepupae reared on pig manure, while the prepupae reared on pig manure and chicken manure were similar (Table 7). The ranges of crude ash, fat, crude fibre, and moisture of the prepupae reared on the different substrates were 7.72-19.79%, 25.59-41.32%, 9.95-12.26%, and 57.50-61.83%, respectively (Table 7).

Table 7. The nutritional composition of black soldier fly prepupae reared on different substrates.^{1,2}

Substrate	Parameter (mean ± SE)				
	Crude protein ± SE (%)	Ash ± SE (%)	Fat ± SE (%)	Crude fibre ± SE (%)	Moisture ± SE (%)
Millet porridge mash	40.7±0.9^{bc}	7.7±1.8a	32.0±2.4a	12.3±0.8a	61.0±0.5a
Pito mash	43.4±1.5^{a}	11.5±1.5a	41.3±0.2a	10.2±1.4a	60.2±0.2a
Fruit waste	39.4±1.37^{c}	8.8±0.4a	38.1±4.0a	11.4±0.2a	60.6±0.5a
Pig manure	34.7± 0.5^{d}	15.1±1.5a	31.8±0.8a	9.9±0.3a	57.5±1.3a
Roots and tubers	42.6±1.8^{ab}	12.4±1.1a	40.6±1.1a	10.4±0.3a	59.3±1.3a
Chicken manure	36.2±2.6^{d}	19.8±3.8a	25.6± 2.2a	10.4±0.3a	61.8±3.0a

[1] Means in the same column followed by the same letter are not significantly different (Tukey's HSD, *P*<0.05).
[2] SE = standard error.

5. Discussion and conclusions

The results indicate that the type of substrate used in BSF production systems can have a significant influence on egg-laying and larval growth indicators such as prepupal yields, final individual prepupal weight, larval survival, development time, and crude protein content of recovered larvae. The most efficient substrate acting as a bait to lure gravid female BSF to oviposit was by far millet porridge mash. In contrast, a study by Sripontan *et al.* (2017) showed that fruit waste was most efficient in attracting gravid BSF females for oviposition, while a study by Ewusie *et al.* (2019) reported pig manure as the most efficient substrate. It is worth mentioning that Sripontan *et al.* (2017) and Ewusie *et al.* (2019) did not include millet porridge mash. In addition, the field populations of BSF in the study site were pre-exposed to fruit waste and pig manure, respectively, and therefore, possibly accounting for their preference as oviposition substrate among the substrates. Jaenike (1983) reported that prior exposure by dipterans to a food source may subsequently influence their preference as an oviposition medium/site. Perhaps, the success of millet porridge mash as a lure in this study is due to pre-exposure of the field populations to chilli, an ingredient in millet porridge mash. In the location where the study was conducted, farmworkers usually cultivate chilli pepper and extract the seeds for planting. The remaining mesocarp after seed extraction is sun-dried and milled into powder for use in culinary activities. During sun-drying of the mesocarp, larvae of BSF are usually found colonising the chilli (personal observation; communication by the farmers). However, the low results obtained for the other substrates, including pig manure and fruit waste, which were considered by Sripontan *et al.* (2017) and Ewusie *et al.* (2019) as successful lures, may be because the substrates were tested simultaneously and millet porridge mash may have been too attractive, hiding the attractiveness of the other substrates. The fruit mix used by Sripontan *et al.* (2017) differed from what we used in this study by the exclusion of orange, but the inclusion of apple. Since testing was not done for the individual fruits in their efficiency on trapping eggs, it may be premature to conclude that the exclusion of apples and the inclusion of oranges accounted for the observed differences between the two studies. Few eggs were found on chicken manure. Booth and Sheppard (1984) observed that, although chicken manure was not the most attractive substrate, nonetheless, some quantity of eggs was laid on it. No eggs were, however, deposited on chicken manure as reported by Ewusie *et al.* (2019) and Sripontan *et al.* (2017). In another study, Ganda *et al.* (2019) exposed a high number of substrates to houseflies and BSF, although the eggs laid on each substrate were not counted, the quantity (weight) of larvae collected from the substrates provides a good indication of their attractiveness, however, results of this study showed that differences among substrates are much lower in larval performances than in oviposition rates. Ganda *et al.* (2019) obtained much more BSF larvae from agri-food wastes such as maize bran and soybean bran than from pig or chicken manure, which corroborates the results of this study. Like millet porridge mash, pito mash and roots and tubers seem not to have been reported as attractants used in collecting BSF eggs. Pito mash, in the presence of the other substrates, had the lowest luring ability for BSF oviposition.

Although all substrates were suitable for larval growth, the most efficient substrate was clearly pig manure. Pig manure recorded the highest individual prepupal weight, while chicken manure recorded the least. Prepupae that had grown on pig manure were twice as heavy as those reared on chicken manure. The total prepupae and the total number of prepupae surviving on pig manure were 25 and 59% more than on chicken manure. However, the development time was similar on both substrates. Both manures have been reported as suitable substrates for rearing BSF larvae (Miranda *et al.*, 2019; Oonincx *et al.*, 2015a; Zhou *et al.*, 2013). Oonincx *et al.* (2015a) reported better survival of BSF on pig manure than on chicken manure, while Miranda *et al.* (2019) reported the contrary. In addition, Zhou *et al.* (2013), recorded the highest larvae weight reared on chicken manure among the different substrates tested. The differences are attributable to the differences in the type of chicken and pig manures used. In particular, the quality of chicken manure to produce fly larvae may depend on whether or not it contains litter, and which type of litter. The chicken manure used in this study was from a deep litter house whereas, Zhou *et al.* (2013) used manure from a battery cage. Manure from a deep litter house has a mixture of wood shavings, chicken dropping, feathers, and feed leftovers, while manure from a battery cage is mainly made up of pure chicken dropping due to the nature and design of battery cages. Manure from the deep litter system was produced by broilers while manure from the battery cage system is from layers. The differences in the manure used could account for the differences observed in the two studies. In particular, the wood shaving contained in the manure from the deep litter system is probably a low-quality additive compared to straw litter (Koné *et al.*, 2017) or rice bran (Sanou *et al.*, 2018).

The results obtained on fruit waste were moderate but the development time was longer on this substrate. The weight and development time for the prepupae recovered from fruit waste (120 mg and 22.3 days, respectively) were similar to the values (120 mg and 22.0 days, respectively) reported by Meneguz *et al.* (2018).

Among the substrates tested, pito mash had the highest crude protein content (over 30%) and this was translated into the crude protein content (43.4%) of the final prepupae obtained. High dietary protein is reported to be important in the growth and development of BSF larvae (Cammack and Tomberlin, 2017; Meneguz *et al.*, 2018; Oonincx *et al.*, 2015b). In addition, pito mash had a high moisture content, crude fibre and fat content. Furthermore, the second highest mean individual prepupal weight was recorded on pito mash following pig manure. The third highest total prepupal yield and development duration were recorded in pito mash. There is no previous documentation on the use of pito mash in the production of BSF larvae.

A shorter development time for BSF has been attributed to high dietary protein and fat contents (Oonincx *et al.*, 2015b). Similar findings were documented for yellow mealworms when fed on a high protein and a high fat diet, (Oonincx *et al.*, 2015b). High dietary moisture content has been reported to be necessary for growth. Cammack and Tomberlin (2017) demonstrated that moisture impacted larval development and adult life history more than the protein and carbohydrate contents of the diet. The requirement for high dietary moisture has been attributed to the morphology of the mouthpart of BSFL (Kim *et al.*, 2010; Purkayastha *et al.*, 2017), increased dietary moisture making it easy for scraping off food from feeding surfaces (Banks, 2014). Larvae of BSF may have performed well on pito mash due to the aforementioned qualities, although it was not successful as a lure for egg trapping of BSF.

Although generally, waste from roots and tubers had the lowest protein, moisture, fibre, and fat content, BSF performance was satisfactory. The mean prepupal weight, total prepupae yield and the total number of individuals were moderate. In addition, the proximate profile of the resulting prepupae was good, especially the crude protein content (42.6%). However, the development time was significantly longer than all substrates except for fruit waste. Food quality is known to affect the rate of development and survival in insects (De Haas *et al.*, 2006). A longer development time of BSF larvae was observed when fed low protein vegetable diets than when larvae were fed high protein diets (Nguyen *et al.*, 2013; Oonincx *et al.*, 2015b). Green *et al.* (2003) and Barragan-Fonseca *et al.* (2018) made similar observations in *Phormia regina* (Meigen, 1826) and *H. illucens,* respectively. All these studies confirm the possible reason accounting for the longer development time of BSF larvae reared on waste from roots and tubers and fruits.

Millet porridge mash, the preferred substrate for oviposition by wild populations of BSF, generally ranked among the best three in growth, development and nutrient composition of the prepupae recovered. It resulted in the third highest individual prepupal wet weight but far from pig manure and pito mash, the second highest total prepupae yield, and the highest survival rate. It was also in these three substrates that BSF larvae developed the fastest. Several authors reported that high protein and fat diets supported a higher growth rate in BSF and faster development than low-protein and low-fat diets (Nguyen *et al.*, 2013; Oonincx *et al.*, 2015b; Tschirner and Simon, 2015). Conversely, Ujvari *et al.* (2009) reported that very high crude fat (20-60%) may be detrimental to larval survival. A diet balanced in amounts of crude protein, fat, and calories seems to be more essential for short development duration and higher larval weight (Nguyen *et al.*, 2013).

Based on the preference-performance principle (Baleba *et al.*, 2019; Gripenberg *et al.*, 2010; Jaenike *et al.*, 1978), the most efficient egg trapping substrate should record the best growth and development of larvae, but this was not the case. Millet porridge mash was by far the most attractive substrate for oviposition, however, its performance as a larval substrate was clearly lower than pig manure, especially when individual prepupal weight is considered. Interestingly, the high survival rate of larvae in millet porridge mash partly compensated for the small size of the prepupae.

While most substrates were very poor in attracting females for oviposition, all allowed the successful development of larvae. Differences in larval performance among substrates were rather comparatively marginal. This suggests that BSF larvae production systems based on adult rearing for egg production can run with a large variety of substrates, thus the selection of the substrates to be used will largely depend on their availability and cost. In contrast, in systems based on natural oviposition, the choice of the substrate is much more critical and the addition of attractants should be considered, as practised in house fly larvae production systems (Koné *et al.,* 2017; Ganda *et al.*, 2019).

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Conflict of interest

The authors declare no conflict of interest.

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