Publication information
Journal of Insects as Food and Feed
ISSN 2352-4588 (online edition)

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Wageningen Academic Publishers

Journal of Insects as Food and Feed, 2019; 5(2): 59-67

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Potentials of animal, crop and agri-food wastes for the production of fly larvae

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Received: 26 October 2017 / Accepted: 17 December 2018

RESEARCH ARTICLE

Abstract

Fly larvae, in particular those of house fly (*Musca domestica*) and black soldier fly (*Hermetia illucens*), are increasingly considered for animal feed worldwide. A simple way to produce fly larvae is to expose suitable substrates to attract adult flies that will lay eggs in the substrates from where larvae will be subsequently extracted. This study aims to evaluate the potential of animal manures and agri-food wastes for maggot production and to identify the fly species developing in the substrates. Experiments were conducted in the Republic of Benin. Twenty-six substrates were left uncovered for 10 hours and maggots were harvested after four days. Fresh substrates were then added to residual substrates and left uncovered for another 10 hours for another production cycle. In total, three production cycles were monitored. In the first cycle, nearly 100% of the flies were house flies but black soldier flies appeared in the following cycles. The result showed that crop and agri-food substrates produced more larvae than manures. The highest yield at the first production cycle was obtained with the mixture of soybean bran and maize grain pericarp. Maize bran, pig manure and chicken manure also showed potential for maggot production. Other substrates such as cow and sheep/goat manure produced nearly no maggot when used alone but the amount of larvae substantially increased when attractants such as chicken offal were added, or when mixed with pig and chicken manure. Production decreased with the production cycles for the majority of substrates, except for mixtures based on spent grain and pineapple grain, which produced a large amount of larva at the second harvest. The activity of maggots led to temperature elevation and reduction of substrate biomass. This study showed that several substrates, in particular soybean bran, maize bran, pig manure and chicken manure show potential for maggot production.

Keywords: *Musca domestica*, *Hermetia illucens*, maggots yield, substrate reduction, Benin

1. Introduction

In most animal production systems in Africa, a major constraint to further development is the prohibitive costs of feed, which represent 60-70% of production costs (Aniebo *et al.*, 2008; Kenis *et al.*, 2014). The most expensive feed constituent is protein (Charlton *et al.*, 2015). This is due to the fact that the protein components, e.g. fishmeal, meat meal and vegetal alternatives such as soya bean and other oilcakes, are also used as food for humans or for other uses (Odesanya *et al.*, 2011). It is necessary to search for cheaper, readily available protein sources to partially or completely replace these expensive components. To meet the growing demand for protein, invertebrates such as earthworms and insects have been explored (Tiroesele and Moreki, 2012; Van Huis, 2015). The use of insects as an alternative source of protein in animal feed is becoming more globally appealing (Makkar *et al.*, 2014; Van Huis, 2013; Van Huis *et al.*, 2013). Among these novel sources of protein, fly larvae (maggots) appear to be the most suitable and used alternative (Kenis *et al.*, 2014; Pastor *et al.*, 2015).

The most commonly used fly species are the black soldier fly (*Hermetia illucens* L., Stratiomyidae) and the common house fly (*Musca domestica* L., Muscidae). These species receive increasing attention because they can decompose organic wastes and contain between 40 and 60% of protein on a dry matter basis. They are also rich in lipids, minerals and amino acids (Veldkamp *et al.*, 2012). Maggots can be produced on a large range of substrates and used as alternative sustainable protein-rich ingredient in pig, fish and poultry diets (Bouafou *et al.*, 2007; Kenis *et al.*, 2014). They can be produced in industrial systems involving an adult rearing unit to produce eggs that are then placed in a substrate suitable for larval development (Drew and Pieterse, 2015; Pastor *et al.*, 2015). However, larvae of both species can also be obtained more simply by exposing substrates to attract naturally occurring female flies that will lay eggs in the substrate (Koné *et al.*, 2017; Nyakeri *et al.*, 2017; Pomalégni *et al.*, 2017). Nearly 6% of the small poultry farmers us this technique in Benin to feed their poultry (Pomalégni *et al.*, 2017). In this 'natural oviposition systems', yield will greatly depend on the quality and attractiveness of the substrate. Various organic wastes have been cited in the literature as fly attractants, e.g. animal manure (pig, chicken, cow, etc.), rumen content, rotten fruits, food wastes, fish offal, viscera, bran, spent grain, etc. (Boire *et al.*, 1988; Bouafou *et al.*, 2006; Mpoame *et al.*, 2004; Ossey *et al.*, 2012). However, these substrates have rarely been yet compared for their performance in natural oviposition systems and the few studies available provided highly variable results. Koné *et al.* (2017) tested chicken manure and sheep manure mixed with blood for attracting house fly larvae and obtained between 124 and 144 g of fresh larvae per kg of dry substrate. Anene *et al.* (2013) exposed poultry droppings, pig dung, cattle dung and cattle blood and obtained 12.7, 3.0, 4.2 and 7.8 g of dry larvae, respectively. Djissou *et al.* (2015) observed that soybean oil cake produced more house fly larvae (4.2 g per 100 g of substrate in wet matter) than pig manure (2.62) and chicken viscera (3.2). Nyakeri *et al.* (2017) evaluated the performance of four mixtures of animal and vegetal wastes for obtaining black soldier flies. The best performance was observed with mashed maize grains (17.6 kg over a six-month period), followed by vegetable remains (13.2 kg), fish remains (10.1 kg) and lastly animal manures (5.0 kg).

The objective of this study was to evaluate, in natural oviposition systems, the potential of some animal manures and agri-food wastes for maggot production and to identify the fly species produced through substrate exposure.

2. Materials and methods

Substrates tested and experimental procedure

Experiments were conducted at the farm of the Faculty of Agricultural Sciences of the University of Abomey-Calavi between July and October 2016. The tests took place outdoors under a roof to avoid the effects of rain and direct sun.

The tested substrates included animal manures and agri-food wastes. The animal manures evaluated were: cow, sheep/goat, guinea-fowl, chicken and pig manures. The crop and agri-food wastes were: maize bran (residue from maize conversion to starch), soybean bran (residue from soybean conversion to tofu), brewers' spent grain and pineapple grain (residue from pineapple conversion to juice). To improve the yield of substrates with low productivity 1 g of dried maggot per 1 kg of dried substrate), mixtures of substrates were made between animal manure on the one hand and between the agri-food wastes on the other. Maize grain pericarp was used only in mixture with soybean bran to reduce moisture of this substrate. Poultry viscera were used as attractant to enhance cow and sheep/goat manures potential. A total of 26 substrates and substrates mixtures were tested (Table 1). The trial was conducted with six replications per substrate or mixture of substrates.

A quantity of 3 kg of fresh substrate and mixture of substrates was used for the production of maggots. Various quantities of water were added to the dried or not sufficiently moist substrates so as to have a moisture content of 70-80% for all substrates. Each tested substrate was put in a cylindrical plastic container (1,250 cm^3) placed under the roof. The substrates were exposed during ten hours (8 am tot 6 pm) to attract flies for oviposition. In order to avoid a new egg laying the second day and to ensure that larvae are of the same age, the substrates were covered with a mesh diameter of 1.2 mm after 10 hours of exposure.

Maggots were harvested the morning of the 5^{th} day after exposure, i.e. four days after the start of the experiment. After the first harvesting, 1 kg of fresh substrate or mixed substrate (at the same ratio) was added to the residual substrate and exposed during 10 hours for another production cycle. Maggots were then again harvested the 5^{th} day after exposure. The same procedure was repeated after the second harvest, so that three cycles of production were obtained. To evaluate the effect of the addition of 1 kg fresh substrate after the first and second harvests, two control treatments without addition of fresh substrate were set up. These control treatments were soybean bran + maize grain pericarp and maize bran.

Harvesting and processing of maggots

Harvesting was done using a broom, a shovel, a 4 mm sieve and the lucifugous behaviour of the maggots. Harvested maggots were cleaned by rinsing them with water and killed by plunging them during 20 minutes in hot water. Killed maggots were then taken to the laboratory and weighted before being dried in an oven at 60 °C for 72 hours to determine the dry matter.

Table 1. Nature and dry matter content of the tested substrates after addition of water.

No.	Substrates	Code	Quantities (kg)	Dry matter content (%)
1	Cow manure	CoM	3	40.23
2	Sheep/goat manure	SGM	3	48.31
3	Guinea-fowl manure	GFM	3	37.78
4	Chicken manure	ChM	3	29.89
5	Pig manure	PM	3	34.12
6	Cow + guinea-fowl manures	Co-GFM	1.5+1.5	34.405
7	Cow + chicken manures	Co-Chm	1.5+1.5	28.06
8	Cow + pig manures	Co-PM	1.5+1.5	28.94
9	Sheep/goat + guinea-fowl manures	SG-GFM	1.5+1.5	39.91
10	Sheep/goat + chicken manures	SG-ChM	1.5+1.5	37.92
11	Sheep/goat + pig manures	SG-PM	1.5+1.5	39.39
12	Sheep/goat manure + chicken viscera	SGM-ChV	2.7+0.3	47.2
13	Cow manure + chicken viscera	CoM-ChV	2.7+0.3	24.65
14	Spent grain (brewery waste)	SpG	3	17
15	Pineapple grain	PG	3	14.22
16	Maize bran	MB	3	27.82
17	Soybean bran	SB	3	10.4
18	Soybean bran + maize brans	SB-MB	1.5+1.5	17.22
19	Soybean bran + spent Grain	SB-SpG	1.5+1.5	20.52
20	Soybean bran + pineapple grain	SB-PG	1.5+1.5	15.27
21	Soybean bran + maize grain pericarp	SB-MT	2.25+0.75	14.17
22	Maize bran + spent grain	MB-SpG	1.5+1.5	16.74
23	Maize bran + pineapple grain	MB-PG	1.5+1.5	19.15
24	Spent grain + pineapple grains	SpG-PG	1.5+1.5	19.95
25	Soybean bran + maize grain pericarp control	SB-Mtcont	2.25+0.75	15.09
26	Maize bran control	Mbcont	3	17.29

Determination of fly species

Fly species were identified for four substrates that had showed high yields: poultry manure, pig manure, maize bran and soybean bran. The substrates were exposed as for the other experiments but, after one day, they were placed separately in cages. Five and ten days later, the larvae were harvested and put in plastic cups with a bit of substrate to allow them to complete their growth. The emerged adults were collected and preserved in 96% ethanol. The flies were identified with a stereomicroscope using identification keys (Delvare and Aberlenc, 1989; Zumbado, 2006).

Data collection

Maggots from each substrate and replicate were weighed when wet and then weighed again after drying. A sample of 200 g of each substrate and mixture of substrates was taken for dry matter percent determination before exposure to egg laying and after each harvest. This allowed calculating the dry matter of the substrates (Table 1) to express yields in dry weight of maggots per dry weigh of substrate, and to calculate the dry weight loss between exposure and harvest. Weights were measured using a digital balance with a precision of 0.01 g. The temperature of the substrates was also measured every day during the maggot production.

Data analysis

The productivity of the substrates was estimated using the formula:

$$\text{Productivity (g of maggot/kg of substrate in dry matter)} = \frac{\text{total quantity of maggot (g)}}{\text{total quantity of substrate (kg)}}$$

The total quantity of substrate in dry matter was calculated from the total fresh weight of the substrate and the amount of dry matter obtained with the fresh sample of 200 g following this formula:

$$\text{Total dry matter of substrate (kg)} = \frac{\text{total quantity of fresh substrate (kg)} \times \text{dry matter in a fresh sample of 200 g (g)}}{\text{200 g}}$$

The substrate reduction (%) was estimated by the difference between residual and initial substrate weight divided by the initial substrate weight, in dry matter.

$$\text{Substrate reduction (\%)} = \frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} \times 100$$

All the parameters: substrates productivity, substrates reduction, substrates temperature were statistically analysed by two-ways (substrate and production cycle) analysis of variance (ANOVA), using the GLM procedure of SAS (version 2.0; SAS, Cary, NC, USA) (α=0.05). Student Newman and Keuls test was used for comparison of means among treatment (*P*≤0.05 level of significance). All statistical analyses were carried out using SAS (version 2.0).

3. Results

Maggot production

There were strong statistical differences in yields among substrates (DF=25; F=35.10; *P*<0.0001), among production cycles (DF=2; F=126.96; *P*<0.0001) as well as in the substrate / production cycle interaction (DF=50; F=22.35; *P*<0.0001). In general, crop and agri-food substrates provided higher maggot productivity compared to animal substrates, in the three production cycles (Table 2). The more productive substrate at the first production cycle was the mixture of soybean bran and maize grain pericarp (67.767±4.879 g of maggot/kg of substrate) whereas cow manure showed the lowest productivity (0.005±0.004 g/kg). For the second and third cycles, maize bran and pineapple grain mixture (35.104±4.96 g/kg) and maize bran (10.43±3.542 g/kg)

Table 2. Productivity of the different substrates (means ± standard deviation) for the three production cycles.[1,2]

Substrate	Productivity (g of maggots/kg of substrate in dry matter)		
	1st production	2nd production	3rd production
CoM	0.005±0.004 fA	0±0 fA	0±0 cA
SGM	0.904±0.448 fA	0.09±0.043 fA	0.015±0.01 cA
GFM	0.383±0.008 fA	0.03±0.021 fB	0.045±0.023 cB
ChM	7.083±3.307 deA	3.717±2.268 efB	0.384±0.268 cB
PM	10.708±4.453 dA	0.043±0.031 fB	0±0 cB
Co-GFM	0.267±0.16 fA	0.045±0.044 fA	0.015±0.014 cA
Co-ChM	2.482±0.998 eA	1.436±0.937 fA	0.036±0.033 cB
Co-PM	0.528±0.456 fA	0.021±0.004 fA	0±0 cA
SG-GFM	0.323±0.089 fA	0.014±0.007 fB	0.032±0.021 cB
SG-ChM	3.593±1.462 eA	0.663±0.078 fB	0.006±0.005 cB
SG-PM	2.556±1.253 eA	0.553±0.536 fB	0±0 cB
SGM-ChV	4.515±1 eA	0.004±0.001fB	0±0 cB
CoM-ChV	5.279±0.946 eA	0.002±0.001 fB	0±0 cB
SpG	3.906±1.227 eB	13.377±3.286 cdeA	3.195±1.579 bB
PG	0.668±0.068 fA	0.002±0.001 fB	0±0 cB
MB	27.536±4.499 bA	18.814±5.218 bcdAB	10.43±3.542 aB
SB	21.654±6.631 bcA	8.071±2.604 edB	1.778±1.016 bB
SB-MB	16.28±3.806 cdA	21.727±4.305 bcA	4.456±2.055 bB
SB-SpG	29.367±3.425 bA	14.802±4.556 cdeB	2.706±1.087 bC
SB-PG	8.756±2.099 deA	4.707±1.669 eB	9.345±3.908 aA
SB-MT	67.767±4.879 aA	11.674±2.817 cdeB	1.55±0.811 bB
MB-SpG	9.249±3.84 deB	25.984±6.456 bA	1.413±0.901 bcB
MB-PG	0.327±0.279 fB	35.104±4.96 aA	9.654±3.901 aB
SpG-PG	0.781±0.379 fB	8.217±1.757 edA	3.742±1.608 bB
SB-Mtcont	66.585±5.528 aA	6.701±1.525 eB	0.107±0.097 cC
MBcont	30.248±1.715 bA	8.698±1.969 edB	0.318±0.223 cB
Fisher F	F=43.71	F=13.40	F=4.72
Probability	*P*<0.0001	*P*<0.0001	*P*<0.0001

[1] Similar lower case letters in the same column indicate no significant difference between substrates at 0.05 level.

[2] Similar capital letters in the same row indicate no difference between production levels at 0.05 level.

respectively showed the highest quantity of maggots harvested. In the third production cycle, most substrates did not produce anything. Maggot productivity decreased with the production cycle for the majority of substrates, with the notable exception of a few mixtures including spent grain (brewery wastes) and pineapple grain, which significantly increased between the first and the second production cycles (Table 2).

Effect of maggot activity on substrate reduction

Table 3 shows the substrate reductions registered after the activity of maggots. The substrate type (DF=25; F=20.11; $P<0.0001$) and production cycle (DF=2; F=161.21; $P<0.0001$) significantly affected substrate reduction. The interaction was also significant (DF=50; F=9.74; $P<0.0001$). The highest substrate reductions were obtained during the first production cycle with chicken and pig manures (81.94 and 83.71% respectively). The lowest rates of substrate reduction were obtained at the third cycle and were registered by the mixture of cow and chicken manures (0.26%), mixture of sheep/goat and guinea-fowl manures (0.29%) and mixture of cow and pig manures (0.38%).

As for the maggot production, substrate reduction significantly increased for a few mixtures based on spent grain and pineapple grain between the first and the second production cycles (Table 3). For all substrates, the reduction decreased between the second and third production cycles.

Table 3. Larval activity and substrate reduction in each of the three production cycle (means ± standard deviation).[1,2]

Substrate	Substrate reduction (%)		
	1st production	2nd production	3rd production
CoM	1.56±0.16 hA	0.79±0.2 dB	0.72±0.12 dC
SGM	6.37±0.15 fghA	1.1±0.99 dB	0.84±0.08 dC
GFM	16.27±2.22 cdefghA	3.67±0.52 dB	1.18±0.10 dC
ChM	81.94±14.24 aA	18.14±1.36 cdB	4.58±1.00 cdB
PM	83.71±13.42 aA	12.28±8.13 cdB	1.62±0.31 dB
Co-GFM	11.59±0.53 cdefghA	2.63±0.11 dB	0.56±0.04 dC
Co-ChM	31.6±2.89 bcdefA	7.52±0.33 dB	1.63±0.18 dC
Co-PM	9.84±0.88 efghA	2.27±0.21 dB	0.38±0.13 dC
SG-GFM	11.15±1.83 cdefghA	1.95±0.09 dB	0.29±0.05 dB
SG-ChM	22.53±0.93 bcdefghA	4.66±0.46 dB	1.01±0.07 dC
SG-PM	14.92±0.24 cdefghA	6.27±0.74 dB	0.81±0.13 dC
SGM-ChV	10.74±0.74 defghA	0.81±0.18 dB	0.98±0.56 dB
CoM-ChV	3.41±0.86 ghA	1.07±0.47 dB	0.26±0.11 dB
SpG	36.21±2.83 bcdA	29.8±4.16 abcA	7.47±1.08 bcB
PG	13.61±3.54 defghB	44.99±3.11 aA	10.94±2.57 abcB
MB	21.84±2.76 bcdefghA	18.64±4.56 cdA	8.05±2.54 bcB
SB	34.41±3.11 bcdeA	13.31±3.63 cdB	9.03±1.71 abcB
SB-MB	34.02±8.48 bcdeA	26.58±7.00 bcAB	8.93±2.58 abcB
SB-SpG	28.64±7.48 bcdefgA	21.09±7.34 cdA	15.93±6.72 abA
SB-PG	16.06±2.97 cdefghB	44.97±4.02 aA	17.98±4.62aB
SB-MT	22.7±5.31 bcdefghA	12.99±4.31 cdB	11.44±2.91 abcB
MB-SpG	14.41±2.45 defghA	18.25±6.64 cdA	5.44±1.94 cdB
MB-PG	44.5±1.22 bA	41.43±8.74 abA	14.49±3.81 abcB
SpG-PG	31.95±2.81 bcdefA	29.11±3.27 abcA	7.19±1.35 bcB
SB-Mtcont	39.73±4.95 bcA	16.72±5.2 cdB	7.62±1.86 bcB
MBcont	20.59±6.83 bcdefghA	16.65±2.56 cdA	6.28±1.57 bcB
Fisher F	F=16.60	F=11.49	F=5.88
Probability	$P<0.0001$	$P<0.0001$	$P<0.0001$

[1] Similar lower case letters in the same column indicate no significant difference between substrates at 0.05 level.

[2] Similar capital letters in the same row indicate no difference between production levels at 0.05 level.

Effect of maggot activity on substrates temperature

The evolution of substrate temperatures during the three production cycles is shown in Table 4. For the three cycles, the highest temperatures were recorded 24 hours after substrates exposure (44.83 °C with soybean bran and maize grain pericarp mixture; 42.08 °C with maize bran and spent grain mixture; 41.92 °C with spent and pineapple grains mixture respectively for 1st, 2nd and 3rd production cycles). At the same time, cow and sheep/goat manures showed the lowest temperature. Substrates' temperatures generally increased in the first 24 hours after exposure, before decreasing between 48 and 72 hours after exposure.

The effect of larval activity on temperature of substrates depended on the type of substrate and the production cycle as shown by the significant interactions between type of substrate and production cycle (DF=50; $4.41 \leq F \leq 7.52$; $P<0.0001$). Chicken manures presented the highest fresh substrate temperature (37.58 °C) (Table 4). This temperature was statistically higher than those obtained with sheep/goat manure and chicken viscera mixture (28.5 °C), maize bran (29 °C), soybean and maize brans mixture (28.83 °C), mixture of soybean bran and spent grain (29.33 °C) and soybean bran and maize grain pericarp mixture (29.42 °C) which recorded the lowest initial temperatures. Regardless of the cycle of production, the temperatures of crops and agri-food wastes were statistically higher than those achieved for animal substrates, 48 and 72 hours after exposure.

Identification of fly species

Flies emerged from four substrates are presented in Table 5. They belonged to three different families (Muscidae, Calliphoridae and Stratiomyidae). The four substrates had attracted all the families identified. *M. domestica* was by the most abundant species in all substrates, representing almost 100% of the flies in the first cycle. *H. illucens* was found only in the second and third cycle, probably as a result of eggs laid in the first cycle. It was more abundant in maize and soybean brans than in chicken and pig manures. Finally, some Calliphoridae of the genus *Chrysomyia* were found in the four substrates in very low quantities (<1%).

4. Discussion

The best productivity with single substrates, in the first cycle, was obtained by maize bran, soybean bran, pig manure and chicken manure. Cow manure, guinea-fowl manure, pineapple grain and sheep/goat manure produced the lowest yield. These results can be explained by many factors among which physical condition, nutritional value, nitrogen content, smell and the presence or absence of an oviposition attractant (Anene *et al.*, 2013). Pig manure and chicken manure have a naturally pungent smell, whereas maize and soybean bran need to be fermented. After fermentation, they become very attractive to flies. These results confirm the finding of Djissou *et al.* (2015) who observed that soybean oil cake produced more maggots (4.18 g/100 g of substrate in wet matter) than pig manure (2.62) and chicken viscera (3.20).

Cow and sheep/goat manures are characterized by a pungent smell and nitrogen content and high carbon/nitrogen ratio (Larraín and Salas, 2008). These characteristics were not favourable for egg laying and larval development. Mpoame *et al.* (2004), when evaluating maggot production of cow and chicken manures, also showed that cow manure presented a low productivity (0.79 g/kg of substrate) compared to chicken manure (2.3 g/kg). The low yield obtained with guinea-fowl manure compared to chicken manure can be linked to its quality. The guinea-fowl manure used in the experiment had been obtained from a stock that had been previously moistened a week to two earlier and decomposition process had been already initiated with losses of nitrogen, in particular ammonium (Pastor *et al.*, 2011). This may explain the low attractiveness of this substrate to flies. Koné *et al.* (2017) recommend that, in a house fly production system based on natural oviposition, poultry manure should contain a sufficient amount of cellulose (from litter), not have been moistened before the addition of water the day of exposure. Similarly, Larraín and Salas (2008) showed that composted swine manure did not allow the development of the house fly larvae because of the reduced moisture and the low nutritive value of the compost. Moon *et al.* (2001) and Wortman *et al.* (2006) established that moisture levels of substrates below 30-40% do not permit the development of the house fly larvae; the optimum moisture being in the range of 50 to 80% during the production cycle.

The combination of low productivity substrates with highly nutritious attractants or favourable substrates strongly increased the yield. For example, adding chicken viscera to cow and sheep/goat manures, strongly enhanced the substrates' productivity. Similarly, mixing sheep/goat and chicken manures at 1:1 ratio increased sheep/goat manure productivity by 297.45%. Cow and sheep/goat manures are characterized by low smell and nutritive elements content; the addition of chicken viscera and manure, enhance their attractiveness and the conditions for larvae development. Similar observations were made by Bouafou *et al.* (2006) who reported that the maggot productivity of peels and pieces of yam and banana was enhanced by adding fish and rabbit viscera.

Maggots yield with soybean bran tripled when this substrate was mixed with maize grain pericarp. Indeed, the original moisture content of soybean bran is so high that the substrate during the production process becomes pasty, making the environment unfavourable to larval development and complicating harvesting. The addition

Table 4. Variation of substrate temperature during the three production cycles.

Substrate	First production cycle				Second production cycle				Third production cycle			
	Initial	24 hours	48 hours	72 hours	Initial	24 hours	48 hours	72 hours	Initial	24 hours	48 hours	72 hours
CoM	30.39±0.3	31.33±0.22	30.96±0.37	30.87±0.29	30.87±0.18	31.46±0.43	31.37±0.71	31.46±0.52	31.72±0.26	29.29±0.92	31.37±0.58	29.87±1
SGM	31.72±0.37	32.72±0.23	31.91±0.33	33.25±0.44	31.79±0.1	32.08±0.65	31.83±0.7	32.21±0.56	32.1±0.28	29.92±0.99	32.21±0.77	30.33±0.91
GFM	31.42±0.33	33.75±0.6	32.46±1.35	33.67±0.77	32.22±1.18	32.75±0.36	32.58±0.2	31.42±0.24	32.31±0.18	31±0.81	32.08±0.15	30.83±0.92
ChM	37.58±1.29	34.83±0.48	37.17±0.6	36.08±0.45	34.75±1.04	35.75±0.67	35.08±1.04	35±0.99	35.17±0.31	32.33±0.76	34.62±0.47	32.12±1.36
PM	31.92±0.33	33.42±1.04	37.58±1.59	39±1.51	33.83±1.38	32.58±0.89	34.35±0.45	31.08±0.65	30.96±1.11	32.83±0.71	31.33±0.99	31.5±0.62
Co-GFM	30.33±0.17	32.75±0.21	30.83±0.45	32.58±0.2	31±0.98	31.75±0.17	31.52±0.49	31.02±0.21	31.96±0.31	30.42±1.04	31.67±0.4	30.5±0.91
Co-Chm	31.33±0.40	32.92±0.61	33.08±0.96	33.62±1.07	33.33±0.52	33.5±0.76	33.5±1.06	33.87±0.88	33.25±0.42	31.17±1.23	33.41±0.65	31.12±1.12
Co-PM	30.75±0.36	33.42±0.98	31.92±0.66	33.08±0.55	31.7±0.82	33.42±0.57	33.22±0.71	31.65±0.3	33.23±0.26	31.33±0.92	32.75±0.33	31.33±0.94
SG-GFM	30.65±0.34	33.72±0.24	31.25±0.83	33.5±0.22	31.48±0.81	32.92±0.51	32.72±0.64	31.82±0.2	32.12±0.29	30.75±1.03	32±0.26	30.58±1.02
SG-ChM	32.58±0.49	34.79±0.77	36.08±1.16	35.92±0.73	33.25±0.47	34.71±0.66	33.71±0.68	34.46±1.03	33.87±0.49	31.67±0.92	34.21±1.1	31.17±0.98
SG-PM	30.08±0.27	34.21±0.49	34.83±1.2	35.06±1.34	31.99±0.7	33.92±0.49	33.01±0.61	31.88±0.4	33.08±0.37	31.67±0.82	32.58±0.45	31.17±0.69
SGM-ChV	28.50±0.26	29.08±0.15	28.92±0.24	29.25±0.81	29±0.59	31.25±0.95	30.17±0.64	28.75±0.51	28±0.34	30.25±0.38	30±0.56	28.08±0.37
CoM-ChV	30.25±0.11	31.75±0.21	32.58±0.35	31.5±0.18	30.83±0.36	32.67±0.25	32.17±0.33	30.25±0.25	29.17±0.48	31.17±0.49	31.5±0.22	28.92±0.51
SpG	30.75±0.33	40.08±1.14	40.95±1.03	40.83±1.13	38.04±0.75	43.13±0.79	36.92±1.26	34.83±0.71	35.42±2.38	35.08±0.93	32.92±0.62	30.33±0.17
PG	33.00±0.41	35.42±0.27	35.33±0.31	31.58±0.33	30.5±0	31.83±0.31	30.58±0.2	31.25±0.57	28.33±0.17	34.91±1.07	30.17±0.24	29.08±0.23
MB	29.00±0.00	39.5±0.84	39.58±0.72	42.08±0.42	40.73±0.51	41.17±1.1	37.25±0.93	33.33±0.98	35.58±2.09	39.75±0.98	36.58±1.02	32.67±1.18
SB	29.83±0.17	33.92±0.7	39.83±2.07	41.71±1.91	32.58±1.11	36.67±1.08	33±0.91	32.25±0.91	28.92±0.24	33.92±0.51	32.25±0.38	30.33±0.42
SB-MB	28.83±0.17	38.21±0.56	40.04±1.13	40.75±1.8	36.75±0.87	39.08±1.27	37±1.09	33.08±0.73	33.42±1.71	37.5±2.04	35.5±1.5	31.75±0.8
SB-SpG	29.33±0.44	36.92±2	43.83±1.2	42.25±1.84	36.58±1.36	41.67±2.12	37.25±1.74	33.42±0.87	33.33±1.67	35.58±0.82	33.58±0.64	31.25±0.5
SB-PG	31.33±0.36	39.17±0.49	37.92±0.4	40.58±0.57	33.75±0.44	38.58±0.83	34.92±0.52	33.75±0.44	31.33±0.48	39.67±0.51	34.67±0.51	32.83±0.48
SB-MT	29.42±0.24	44.83±0.77	42.83±1.22	40.17±1.93	36.83±0.49	38.08±0.93	37.29±0.32	34.55±0.53	31.33±0.51	38.83±0.53	36.75±0.66	33.42±0.83
MB-SpG	29.92±0.27	41.07±1.28	40.88±0.83	42.25±1.2	36.33±0.48	42.08±0.87	36.5±0.89	36.08±0.81	34.71±2.01	34.17±0.9	33±0.53	30.5±0.22
MB-PG	31.67±0.31	33.67±0.4	38.58±0.84	37.25±0.63	32.25±0.28	39.83±0.28	35.67±0.57	33.8±0.66	29.83±0.17	38.17±0.7	34.08±0.66	31.17±0.38
SpG-PG	31.33±0.17	41.75±0.17	37.92±0.89	38.25±0.5	34.5±0.13	39.58±0.66	37.25±0.5	33.17±0.6	30.17±0.42	41.92±0.52	33.83±0.33	32.17±0.49
SB-Mtcont	30.5±0.55	39.58±0.64	41.25±0.42	38.58±1.05	35.83±0.95	35.92±1.21	41.03±1.47	35.33±0.99	32.33±1.31	35.33±0.6	39.17±1.52	33.67±0.79
Mbcont	31.08±0.44	41.72±0.83	39.92±1.06	34.5±0.98	41.25±1.37	40.08±0.76	36.5±0.66	33.67±0.33	32.08±0.9	36.17±0.54	34.75±0.48	31.83±0.4
Fisher F	F=18.54	F=27.13	F=19.43	F=15.17	F=15.27	F=19.94	F=10.14	F=6.86	F=4.47	F=14.35	F=9.48	F=2.89
Probability	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001

Table 5. Fly species inventory and abundance from the different substrates after one day egg laying.[1]

Substrates	*Musca domestica*	*Hermetia illucens*	*Chrysomyia* spp.
Maize bran	+++	++	+
Soybean bran	+++	++	+
Chicken manure	+++	+	+
Pig manure	+++	+	+

[1] +++: abundant; ++: low abundance; +: very low abundance.

of maize grain pericarp absorbs the excess of moisture, making the substrate more favourable to the development of the maggots.

The maggot yield during the first production cycle was generally superior to the second and third ones, when substrates were added to the residues. Most substrates produced less than 0.7 g/kg of substrate at the second and third harvests. The productivity of the control, i.e. maize and the mixture of soybean and maize grain pericarp which did not receive additional fresh substrate after the first and second harvest, was significantly lower than the productivity of the same substrates which received complements. The low maggots yield at the two last harvests was most probably due to the reduction of the quantity and availability of nutrients contained in the substrates. A similar observation was made by Bouafou *et al.* (2006). However, a few mixtures based on spent grain and pineapple grain increased their yield between the first and the second production cycles. This increase is entirely due to the appearance of black soldier fly larvae that probably appreciate these substrates and whose eggs and small larvae were not detected during the first harvest.

Larval activities significantly contributed to reduce the substrate mass. The substrate reduction varied between 0.26 and 83.71%. House fly larvae are known to reduce manure mass, moisture content and manure dry matter content by 75, 89 and 35%, respectively, within a week-long developmental cycle (Barnard *et al.*, 1998; Moon *et al.*, 2001). Wang *et al.* (2013) obtained a reduction of pig manure weight varying from 67.2 to 70% under house fly larvae action. The results also showed that the lowest levels of substrate reduction were recorded with low productivity substrates as cow and sheep/goat manures. Similar results were found by Tschirner and Simon (2015) when they evaluated the influence of different growing substrates on the production of black soldier fly. They concluded that productivity of the substrate was correlated with substrate reduction. As expected, substrate reduction was reduced in the second and third production cycles, when less larvae were present in the substrate.

The highest temperature in the substrates (42.08 °C) was recorded with the mixture of soybean bran and maize grain pericarp which also presented the highest maggots yield. This high temperature was surely due to the energy produced by larval density and activity. Indeed, the lowest temperature were recorded in cow and sheep/goat manures, i.e. the least productive substrates. Results have also shown that substrate temperature generally increased during larval growth before decreasing at maturation. Warburton and Hallman (2002) also measured temperatures up to 42 °C in substrates with high densities of black soldier flies.

5. Conclusions

This study has evaluated the potential of 26 substrates and mixtures of substrates to produce maggots. The results revealed that soybean bran, maize bran, pig manure and chicken manure produced the highest yield. The physical state of the substrate, in particular moisture, nutrients contents and smell must be considered to evaluate the productivity of substrates. In general, crop and agri-food wastes (e.g. soybean and maize brans) were more productive than animal manures. Some of them also showed the possibility to be used in two successive maggot production cycles when fresh substrate is added to the residues. In further studies, the nutrient content of the substrates and the chemical composition of maggots reared from these substrates will be investigated in order to better understand the characteristics of suitable substrates and identify the most suitable ones for maggot production. Finally, the safety of the production technique for the animals, the producer and the consumer will need to be ascertained before further dissemination.

Acknowledgements

This study was carried out as part of the project IFWA – Sustainable use of insects to improve livestock production and food security in smallholder farms in West Africa, funded by the Swiss Agency for Development and Cooperation and the Swiss National Science Foundation, in the framework of the Swiss Program for Research on Global Issues for Development (R4D).

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