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Comparison of the performance of different baiting attractants in the egg laying activity of the black soldier fly (*Hermetia illucens* L.)

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Abstract
The black soldier fly has become an important insect in bioconversion activity. However sustainable use of the insect is now dependent on the development of efficient artificial rearing systems. The concern however is on the scarcity of information on Black Soldier Fly rearing in general and on specific aspects of the production chain in particular such as use of baiting attractants for adult oviposition. The current study compared baiting efficiency of different attractants on the black soldier fly females in an effort to search for an optimal baiting material. Tested materials included cow manure, mixture of fruits (pineapples, bananas, watermelon and avocado), commercial sweet scent, fish and frass tea. The performance of a baiting material was evaluated in terms of mass of eggs laid on an oviposition medium. The study reports that for the strain of Black Soldier Fly (BSF) used in the study, fresh cow manure consistently attracted the highest oviposition of eggs followed by frass tea while sweet scent was the least effective. Results from this study can be used to improve the efficiency of BSF egg-trapping which is a necessity of successful black soldier fly production in captive environments.

Keywords: Performance, baiting attractants, egg laying activity, black soldier fly, *Hermetia illucens* L.

Introduction
Black soldier flies are small, harmless insects that have the potential to provide promising solutions to two growing global problems namely, the high cost of animal feed and the disposal of large amounts of organic waste through the process of bioconversion [1]. The black soldier fly combines the production of animal feed and waste reduction in one system in which organic waste is recycled into a sustainable animal protein source by the larvae stage [2]. The process involves the feeding of segregated bio-waste to the larvae stage of the insect until they mature after which they are harvested and post-processed into a suitable animal feed product [1]. Meanwhile, the feeding process reduces the contents of the waste into a residue which can also undergo further processing to produce organic fertilizer for sale or soil amendment, thereby enabling even environmentally hazardous wastes to become a source of income generation and employment creation [3]. In this way a black soldier fly technology not only manages organic waste but also adds value to it.
The black soldier fly develops naturally on organic material [4]. However under sub-optimal environmental conditions, productivity dwindles and this affects natural bioconversion process. To ensure sustainability and constancy of application of the technology, the development of artificial rearing systems for the insect to augment the natural population has become necessary [5, 6]. *Hermetia illucens*, L is an especially attractive option for commercial mass-rearing because of attributes which make it easier to comply with sanitary regulations. Among them is the fact that unlike other species such as the house fly (*Musca domestica*), blue bottle (*Calliphora vomitoria*) and the blow fly species (*Chrysomya spp.*), the adult fly does not come into contact with any degrading or fresh organic material [6, 7].
Black soldier fly production facilities are more adapted to the economic potential of developing countries because they can be developed and operated at low cost (low building and maintenance costs; independent from power supply) [8]. The concern however is on the scarcity of information on black soldier fly rearing [9]. Enterprises and small entrepreneurs who have invested significant amounts of money into this technology are interested in keeping a competitive edge over potential entrants [10].
Artificial production in captivity involves maintaining an adult colony in a greenhouse or an equivalent enclosure to provide a continuous supply of eggs, and therefore larvae for

bioconversion process. There is however contradictory information on the use of oviposition attractants. Whereas it is known that putrescence from composting organic matter attract and induce mated females to lay eggs, there is no agreement on the specific attractants themselves. Spoiled grain, with its gentle fermented smell [11], fermented oats, corn, and brewer's hops, carrion [4], manure [12] meat or fish [13] and frass from previous larvae feeding activity [4] have all been utilized as attractants in different studies, and are thought to provide conditions that meet adult black soldier fly females' reproductive needs [4]. However the focus of bioconversion is not solely rearing *Hermetia illucens*, but rearing them efficiently for optimal waste management and /or protein production [14]. This includes optimal production of eggs, which therefore necessitates the determination of the most suitable attractant for black soldier flies reared in captivity.

In this study, we compared baiting efficiency of different attractants on the black soldier fly females in an effort to search for an optimal baiting material. These baiting materials included cow manure, mixture of fruits (pineapples, bananas, watermelon and avocado). The performance of a baiting material was evaluated in terms of mass of eggs laid on an oviposition medium. Results from this study could be used to improve the efficiency on BSF egg-trapping, which is helpful for any experiment and application of BSF in bioconversion.

Materials and methods

Study site

The study was done at Enviro Flight LLC, Ohio in the United States of America. Enviro Flight LLC is a private enterprise involved in the large-scale production of Black Soldier Fly larvae for the production of animal feeds for the pet industry.

Source of baiting materials.

These frass used in the study was obtained from previous larvae remnants from the nursery which consisted of dried distillers' grains and cookies mixture. The fruits, fish and commercial sweet scent were purchased form nearby Fairborn town while cow manure was obtained from a farm within the vicinity. Obtaining the manure was relatively easy and cheap as no cost was involved except that for transportation.

Egg collection

Two cages with peak adult emergence were selected from the insectarium in the greenhouse and two compartments not adjacent to each other were selected within the cages to avoid interference of scent of baiting attractants. Five attractants namely cow manure, fruits mix (pineapples, bananas, watermelon and avocado), a commercial sweet smelling liquid, rotten fish and frass tea were selected for the trial. Each of the baiting attractant was put into a separate small plastic containers to the half mark level (Figure 1). The containers were then covered with a nylon net that allows the spread of odor but prevent the insects seeing and touching the trapping material within [15]. A lid with twenty equidistantly perforated holes was then used to secure the nylon material to the plastic container (Figure 1). To trap the eggs of black soldier fly, a block of wood pieces stuck together by rubber bands was used (Figure 2). All of the experiments in this study used the same egg trap. Each day at 4pm, an attractant pair was put into a cage in separate compartments and the block of wood pieces bound together by rubber bands was put on top of each attractant containing vessel (Figure 1).

After 24 hours, the attractant and the block of wood with laid eggs were removed, and the eggs scratched from the woods with a pen knife. The eggs were weighed using a weighing scale (model). This was repeated until all the attractant pairs had been tried to give a total of eight treatments per attractant and 40 results in total. Care was taken to ensure uniform distribution of the attractants both within the cages and the compartments.

Fig 1: Set up of the experiment: Rubber bound wooden pieces on top of plastic box containing an attractant inside a chamber of a cage**.**

Fig 2: Rubber bound wooden blocks sued as oviposition sites by the black soldier fly**.**

Results

The performance of the baiting attractants in terms of weight of laid eggs was is indicated in Table 1 below. Cow dung manure emerged as the attractant that stimulated the highest weight of laid eggs followed by frass tea, fruits and fish. Again, cow dung manure emerged as the most consistent stimulant for egg laying in black soldier flies among all the tried substrates. However, its egg laying stimulating effect decreased with increase in time of usage. The same trend was observed with fish (Table 1). For frass tea, the number of eggs laid increased with increase in the number of days provided the bait attractant remained wet. For fruits, no particular pattern was established whereas commercial sweet scent emerged as the least egg laying stimulant. The results show wide variability within and between the attractants (Figure 3).

Table 1: Daily egg collections per attractant**.**

Substrate	Amount of eggs laid per day							
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
Manure	6.605	4.888	4.906	2.6868	7.892	6.38	5.959	1.458
Scent	0.142	0.178	0.274	0.035	0.085	0.065	0.175	0.043
Fish	4.179	4.117	0.594	0.198	1.206	0.111	0.082	0.056
Frass	3.71	4.902	4.034	2.098	4.753	7.584	4.753	1.0272
Fruits	2.914	1.073	1.203	1.150	3.128	1.84	2.831	1.79

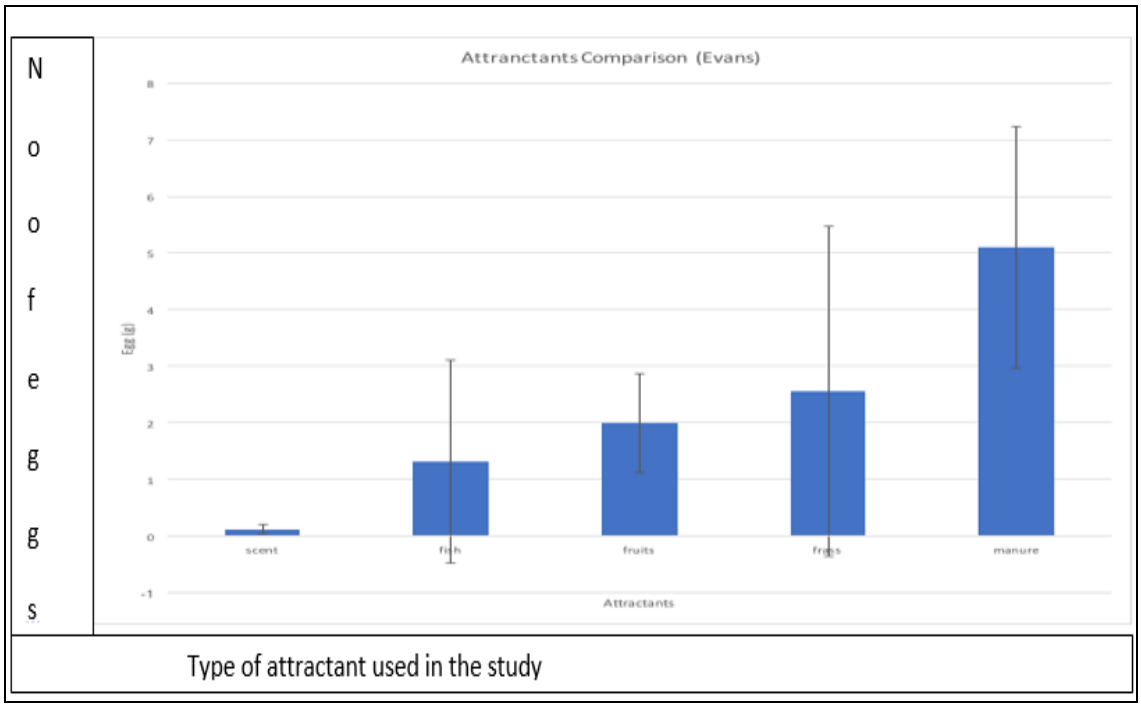

Fig 3: Graph of average weight of eggs in grams per attractant used in the study

Discussion

The source of BSF eggs for mass production is usually collected from egg-trapping technique. Two major components in egg-trapping technique are baiting and trapping material. Though both egg baiting and trapping efficiency determines the efficiency of BSF production, baiting material is a major factor as it affects the egg-trapping efficiency because BSF female may search for a specific food source for their offspring [15]. An egg trap is designed to test the trapping efficiency of odors from different materials [16, 17]. The results indicate that cow manure and frass tea were more attractive to BSF and stimulated more egg-laying activity in the black soldier fly compared to the rest of the used bait attractants (Table 1). This finding is supported by other studies. Among the substrates used by Bonso [13] (fish, chicken feed, human faeces and rat meat carcass), rat meat and human faeces emerged as the better attractants compared to fish and chicken feed. This shows that certain scents attract the females better than others. However important to note that only rotting substrates attracted appreciable egg laying activity while the commercial sweet smell elicited little oviposition (Table 1). This shows that sweet smells are not attractive to black soldier fly laying females. This is in contrast to insects such as bees (*Apis mellifera*) which are attracted by sweet sugary smells.

Previous studies have found that rotting food emits volatile compounds into the air which are detected by mated black soldier fly females [15]. For example, the fermentation of fruits has been found to release compounds such as ethanol, acetic acid, ethyl acetate, and acetaldehyde [20; 19]. These compounds attract the adults of vinegar fly [20].

The inducement of more laying activity by fresh cow manure compared to aging manure can be attributed to either of two possible reasons which are related. One, the amount of scent produced and two, the wetness of the substitute. Fresh manure produced more smell which could be detected by many flies within the cage compared to aging manure whose smell progressively reduced as did the number of eggs laid (Table 1). Therefore not only is the type of scent important as explained above but also the amount of smell. Added to this is the fact that fresh cow manure was wet compared to aging manure. This made the fresh manure more attractive as the wetness contributed to production of more odour. The amount of odour is an attractant for BSF to lay their eggs nearby as it is indicative of a feeding opportunity for the fresh larvae when the eggs hatch. Old manure however is significantly less attractive to flies [21]. The finding is not surprising as manure is the principal food of many insects in nature including larvae of the black soldier fly [22].

For frass tea, the reverse situation was observed in this study. Generally in this study, moist aged frass tea stimulated more eggs to be laid compared to fresh frass tea (Table 1). This can be explained by gradual increase of the smell of the attractant with time due to fermentation process. The necessity for fermentation was also reported in the study of Sripontan *et al.* [15]. In the study, the use of thoroughly wet chicken feed proposed by Banks *et al.* [23] and successfully used by Diener *et al.* [24] proved relatively slow in attracting ovipositing adults black soldier flies probably because the fermentation process was slow and took long to generate the right kind of smell [15]. The efficiency of frass may be also due to the pheromones left by feeding larvae. The pheromones are recognized by conspecific females which are then attracted to lay eggs. This is in line with the findings Stankus [25] who reported that the best attractant is an already existing colony probably because of the pheromone effect.

Though the current study established no particular pattern for fruit mixes, the study of Sripontan *et al.* [15] reported that odour of fruits was more efficient at attracting Black Soldier Flies compared with attractants such as household food wastes, chicken manure, pig manure, and dairy manure. Other studies have reported rotting carcass meat to be a good attractant of oviposting female adults of the black soldier fly. However the stench generated was a public nuisance and therefore necessitated the location of the production facility away from human habitats and wearing of nose masks [13].

The variation in the stimulating effect of different attractants shows that different geographically differentiated strains of the black soldier flies may be responsive to and attracted by different attractants akin to findings of studies of other

insects. The feeding preference of Diptera has been reported to be affected by prior exposure of food. Oliveira *et al*.[16] Reported that the larval stage diet affected the feeding preference of adult of vinegar fly *Drosophila melanogaster*. Considering that induction of feeding preference is possible, BSF are more likely to lay eggs on waste which is abundant and was most utilized by the field BSF population in the environment. This hypothesis explains why BSF adults have varied attractant preferences in different studies [21].

Conclusion

In conclusion, most of the attractants in the study proved useful in baiting egg laying adult Black Soldier Fly females. However cow manure and frass were more effective in attracting the strain of BSF used in the study. Both cow manure and frass are freely available and therefore can be sustainably used in captive Black Soldier Fly production systems.

Conflict of interest

The authors declare no conflict of interest.

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