



The black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): Trapping and culturing of wild colonies in Ghana

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ABSTRACT

The larvae of the black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae), are promising candidates to be utilized in alternative organic waste management and for fish and livestock feed production. The scalability of this technology in Ghana will depend on a steady source of large numbers of BSF larvae. The objectives of this study were to identify the most attractive organic manure dumps or heaps in the study area for trapping wild BSF egg clutches and assess the effect of local environmental conditions on the trapping and laboratory rearing of BSF. The study compared the number of egg clutch trapped at different microhabitats including piggery, chicken and sheep waste dumps and on a compost heap. The piggery dump waste was the most suitable site for trapping BSF egg clutches. No egg clutch was deposited nearby poultry and sheep waste microhabitats. Results showed no differences in temperature between microhabitats during egg trapping but relative humidity differed between poultry, sheep and compost, however this did not have any effect on egg clutch trapping. No significant differences in temperature and humidity were observed during larval rearing. Significant differences in weight and length of larvae from both piggery and compost sites were observed on days 5 and 10 after egg hatch. A small scale laboratory colony rearing has been successfully established in Ghana. The design of the larval breeding system appears to be suitable for respective up-scaling that could provide sufficient larval quantities for composting organic waste and producing feed components for livestock and fish.

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Introduction

The black soldier fly (BSF), *Hermetia illucens*, is a valuable insect species whose larvae have enormous potentials for converting organic waste into compost, while the larval biomass generated could also be harvested for its protein and fatty acid content [1]. Large quantities of black soldier fly larvae have to be generated so that they can be used to inoculate the organic fraction of the municipal solid waste, to convert it to compost. Similarly, large quantities of the larvae must be generated to meet demands for its use as protein-source for animal feed formulation. In order to meet the increasing demands for the larvae, for use in bioconversion of organic waste and biomass generation at any point in time, it is important to have a black soldier fly colony readily available. Therefore, colonization and production methods must be developed.

Although there has been much research focusing on the use of BSF larvae to manage swine, chicken and cattle manure [7], as well as municipal organic waste [9], few reports have dealt with its progeny initiation from the wild [7]. Again, depending on the insect species, specific environmental requirements, for instance regarding temperature, humidity, feeding substrate need to be considered. Leppla [11], collected BSF eggs from the wild, and reared them to adults in 38 days at 29.3 °C, however, he was unable to establish a larval population over multiple generations. Sheppard et al. [17], collected eggs in an open-sided caged layer house and successfully maintained them in the laboratory, but was difficult to maintain suitable temperature essential for eliciting mating in the large greenhouse. The rearing of *H. illucens* designed for organic waste management, and also for fish and animal feed production has not received adequate attention, in spite of its immense prospects and economic potential. Much have been reported on the mass rearing of the BSFL in the developed countries [12,17], with varying degrees of success. Facilities and environmental conditions that prevail in the temperate countries differ considerably from that of the tropics. Moreover, in developing countries such as Ghana, suitable facilities for rearing insects do not exist as can be found and reported in the developed nations. Locally, easily adaptable method has to be designed for the mass rearing of this insect, taking into considerations the major environmental factors such as relative humidity, temperature, light and feed. Thus, the study hypothesized that there will be variation in wild *H. illucens* egg clutches trapped on organic manure dumps or heaps in the study area. The objectives of this study were to:

- 1) identify the most attractive organic manure dumps or heaps for trapping wild BSF egg clutches, and
- 2) assess putative differences in successfully establishing a laboratory colony of BSF from wild collections under local environmental conditions.

Materials and methods

Study location

The study was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra, Ghana. BNARI is located about 20 km north of Accra (5°40'36.6" N; 0°11'52.5" W, and 76 m above sea level).

Colony initiation

Study of the most suitable microhabitat or waste where BSF egg clutches can be easily harvested in the study area

Five egg-laying traps prepared according to Sheppard et al. [17] and consisting of five plies of cut corrugated cardboard that were held together with a rubber band. Traps were 8 cm long and were placed at equidistance (10 cm between and within rows) from each other and 2 cm above the microhabitat (piggery waste dumpsite, poultry waste dumpsite, sheep waste dumpsite and a compost heap) (Figs. 1 and 2). These microhabitats were of the same age (about three weeks old) but the piggery and sheep waste microhabitats received daily addition of waste from the pig and sheep staples. Egg collection was conducted every two weeks for 13 months (October, 2015–October, 2016).

Temperature and relative humidity at three different sections of each microhabitat were taken on the day and at the time of trap set and their means recorded. Traps were checked for egg clutches 24 h after deployment and removed 48 h thereafter. This is because BSF eggs begin to hatch 3.5 days at 30 °C [22] or 4.3 days at 24 °C [17].

Incubation of harvested egg clutches

Egg clutches sired were immediately transferred to a clean, moist surface in order to prevent microbial contamination [15]. The number of egg clutches sired in the grooves of the cardboard traps were counted and their weight determined using an analytical balance. The traps with egg clutches harvested from different microhabitats were incubated separately under ambient conditions on 50 g of finely grounded moistened layer chicken meal placed in 250 mL incubation boxes (Fig. 3) with ventilated lids in the laboratory.

Larval feeding

Five days after egg clutch incubation, 1000 larvae from each microhabitat replicated three times were picked with the aid of disinfected forceps and transferred separately into 50 L plastic containers. These plastic containers had been cleaned with detergent and disinfested with 70% alcohol and dried under the sun for at least 6 h. The 50 L plastic containers had ventilated lids to promote aeration, reduce generation of excessive heat and also to prevent the escape of larvae (Fig. 4).



Fig. 1. Trapping of BSF egg clutches at different microhabitats (a) compost heap; (b) poultry waste dump.

Temperature and relative humidity were monitored at 10.00 >a.m. from three different locations of the larval cages using digital thermohydrographs. Larvae were fed every 3rd day till the fifth instar stage when feeding was stopped. The larvae were reared on layer meal moistened to 70% in the laboratory under 12:12 L: D photoperiod. Feed given was computed based on a formula adapted from Stamer (Personal Communications, 2015) as follows:

$$\text{Feed amount (g)} = \frac{\text{Number of larvae} \times 0.1 \text{ g}}{\text{MC}} \times 25 \times 70\% \quad (1)$$

Where MC = moisture content of diet,

0.1 = Amount of feed per larvae per day [7], adjusted every 3rd day,

25 = Estimated larval feeding duration (days) [4,23],

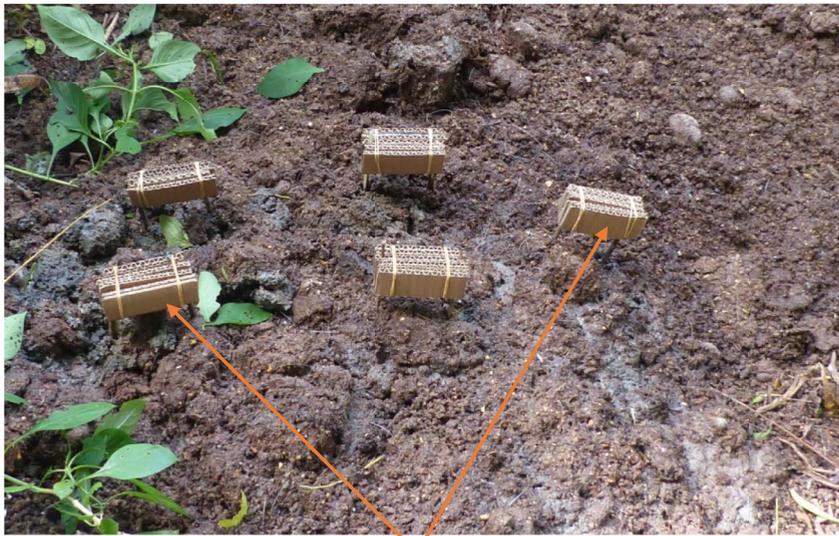
70% = Adjusted moisture content of diets.

Prepupae sorting

Feeding of the larvae was stopped and prepupae were picked with the aid of disinfected forceps, when about 50% of the larvae had turned into prepupae as indicated by change in color from beige to dark brown or black [8] in a particular larval cage from each trapping section and microhabitat. Five hundred prepupae replicated 3 times were weighed and kept in appropriate sized plastic eclosion containers with crushed shredded papers to induce pupation (Fig. 5(a)). To prevent laying



a



b

Fig. 2. Trapping of BSF egg clutches at various microhabitats (a) sheep waste dump and (b) piggery waste dump.

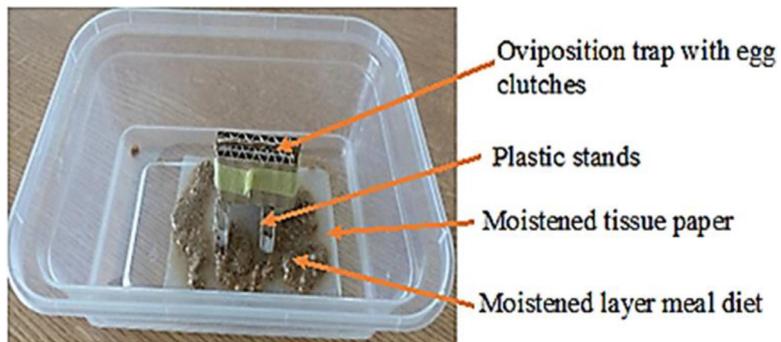


Fig. 3. Incubation of BSF egg clutches.



Fig. 4. 50 L larval box.



Fig. 5. Eclosion container with (a) prepupae and shredded; (b) muslin cloth to prevent parasitoids entry and adult escape.

of eggs by the parasitoid *Dirhinus giffardii* (Hymenoptera: Chalcididae) [6], in the prepupae, the eclosion containers were covered with fine-meshed muslin cloth secured in place with a rubber band (Fig. 5(b)).

Adult maintenance and egg collection to obtain F_1 generation

The eclosion containers were placed in adult wooden cages measuring $50 \times 50 \times 50$ cm (Fig. 6(a)), as soon as adults began to eclose from their pupae. The adult cages were placed in the adult rearing room to allow for more sunlight as sunlight is reported to encourage mating [13]. Moistened fresh layer feed were placed in transparent plastic containers ($8 \times 13.5 \times 5$ cm) part of whose covers have been cut and fitted with cut corrugated cardboards taped together with a masking tape. These setups served as egg harvesting devices (Fig. 6(b)) to entice female flies to lay eggs and were placed in the adult cages.

Environmental conditions (temperature and relative humidity) in the adult room were monitored with the aid of digital thermohydrograph. The egg harvesting devices were checked daily for the presence of sired egg clutches. Fresh water mist was sprayed from a sprayer onto the muslin cloth covering of the adult cages mid-morning and in the afternoon to provide water droplets [23] for maximum longevity of adults [10] and improve ambient humidity as the water evaporated [23].

Statistical analysis

Data on egg clutch trapping being positively skewed was square root transformed to conform to the normality assumptions before F -test (ANOVA) was performed. To establish possible relationships that may exist between temperature and relative humidity at the microhabitats where egg traps were set as well as larval length and weight, analysis of variance



Fig. 6. (a) BSF adult cage; (b) egg harvesting device.

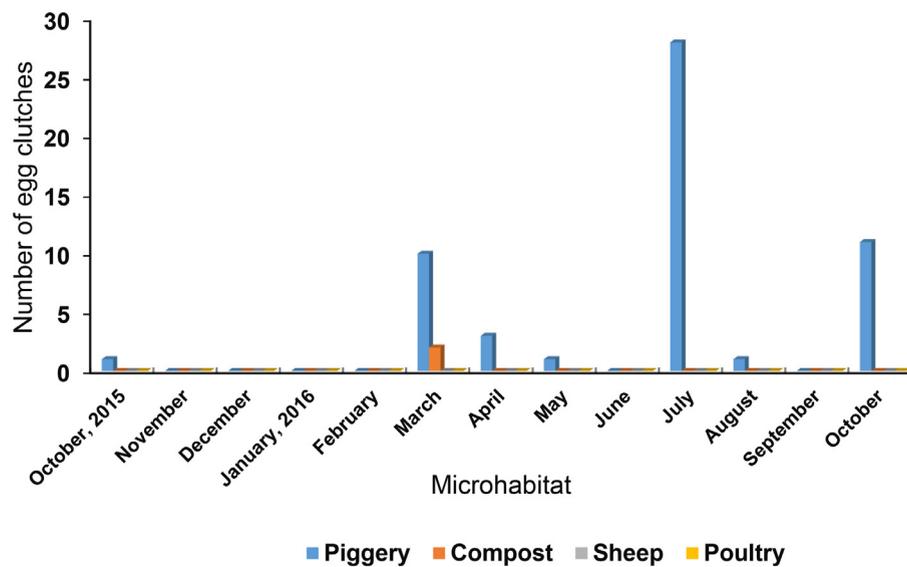


Fig. 7. Monthly egg clutch collections as affected by type of dumpsite.

(ANOVA) was used. The Tukey–Kramer procedure was used for mean comparison due to treatment effects. *t*-Test assuming equal variances was used to test for any relationship between larval length and weight instead of repeated measurement as selection of larvae was done randomly and not on the same subject. Additionally, *t*-test was used to test for any relationship between rearing conditions during the larval and adult rearing of egg clutches harvested from the piggery and compost microhabitats.

Results

Effect of type of organic waste dumpsite on egg clutch harvest

The distribution of BSF egg clutches trapped during the study is presented in Fig. 7. In all, fifty-five egg clutches were harvested from the piggery dump waste microhabitat from October, 2015 to October, 2016, while only 2 egg clutches were

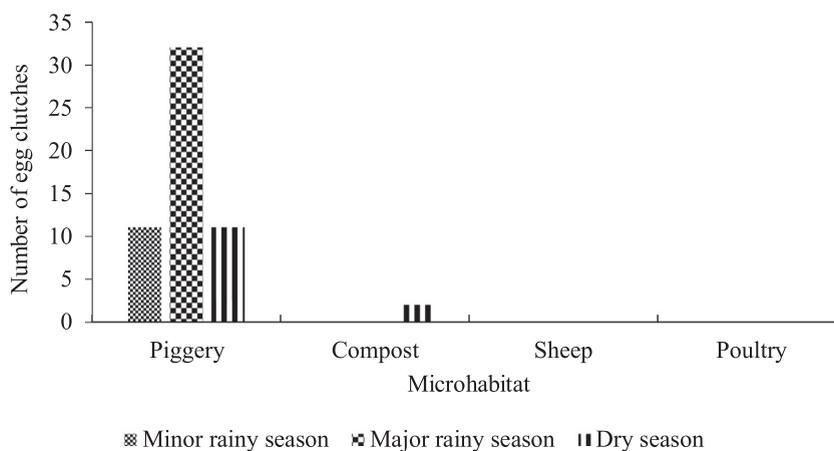


Fig. 8. Seasonal BSF egg clutch collections as affected by microhabitat.

harvested from the compost heap microhabitat within the same period. The average weights of all egg clutches harvested were 64 mg and 30 mg for the piggery waste dumpsite and the compost heap microhabitats respectively.

Seasonal variation in BSF egg harvesting

Seasons during the study did not influence number of egg clutches trapped ($df=2, 36, F = 0.40, p = .667$), but microhabitat did ($df=3, 36, F = 3.17, p = .036$). There was no interaction between season and microhabitat ($df 6, 36, F = 0.47, p = .824$). Further, no egg clutch was collected from both the poultry and sheep microhabitats in all the seasons (Fig. 8). On the average, 8.00 ± 6.69 , 3.00 ± 2.67 and 2.75 ± 2.42 egg clutches were trapped from the piggery microhabitat per month during the minor rainy, major rainy and dry seasons respectively. On the other hand, 0.5 ± 0.05 egg clutches per month were collected from the compost microhabitat in the dry season and none during the major and minor rainy seasons.

Climatic conditions of microhabitats

There were no significant differences in both temperature and relative humidity on the microhabitats during egg clutch harvest as determined by one-way ANOVA ($df=3, 48; F = 0.16, p = .920$) and ($df=3, 48; F = 0.24, p = .869$) for temperature and relative humidity respectively during egg trapping (Fig. 9(a) and (b)).

However, significant differences ($df=1, 309, F = 87.78, p < .0001$) were observed in both seasonal temperature and relative humidity on the microhabitats. Generally, microhabitat temperatures were high in the minor rainy season, October, 2015–December, 2015 ($31\text{--}33^\circ\text{C}$) whiles lower temperatures were recorded in the major rainy season, April–July, 2016 ($30\text{--}31^\circ\text{C}$) in the Southern part of Ghana. Similarly, percent relative humidity was 55–68 and 44–59% for the minor and major rainy seasons respectively in the southern part of Ghana.

Larval rearing

During larval rearing of egg clutches harvested from the piggery and compost microhabitats in separate larval boxes in the laboratory to evaluate larval growth and survival, significant difference was observed only in percent relative humidity (t -test, $p = .101$) but not temperature (t -test, $p = .010$), (Fig. 10(a) and (b)).

Pupal development and adult rearing

Prepupae harvested from egg clutches obtained from the piggery and compost microhabitats showed no significant differences on larval period (t -test, $p = .370$), pupal period (t -test, $p = .107$) and percent eclosion (t -test, $p = .730$). However, source of egg clutch had significant effect on F_1 adult egg clutch production (t -test, $p = .026$).

No significant differences in temperature (t -test, $p = .211$) and percent relative humidity (t -test, $p = .074$) were observed during adult rearing. Temperature was $32.72^\circ\text{C} \pm 0.06$ and $32.87 \pm 0.06^\circ\text{C}$ whiles percent relative humidity was $71.10 \pm 0.36\%$ RH and $73.82 \pm 0.94\%$ for adults' eclosing from prepupae from the piggery and compost microhabitats respectively (Figs. 11 and 12).

F_1 egg clutch production

F_1 adult female flies eclosing from prepupae recovered from incubated egg clutches harvested from both the piggery and compost microhabitats sired egg clutches which were statistically different from each other (t -test, $p < .05$) (Table 1).

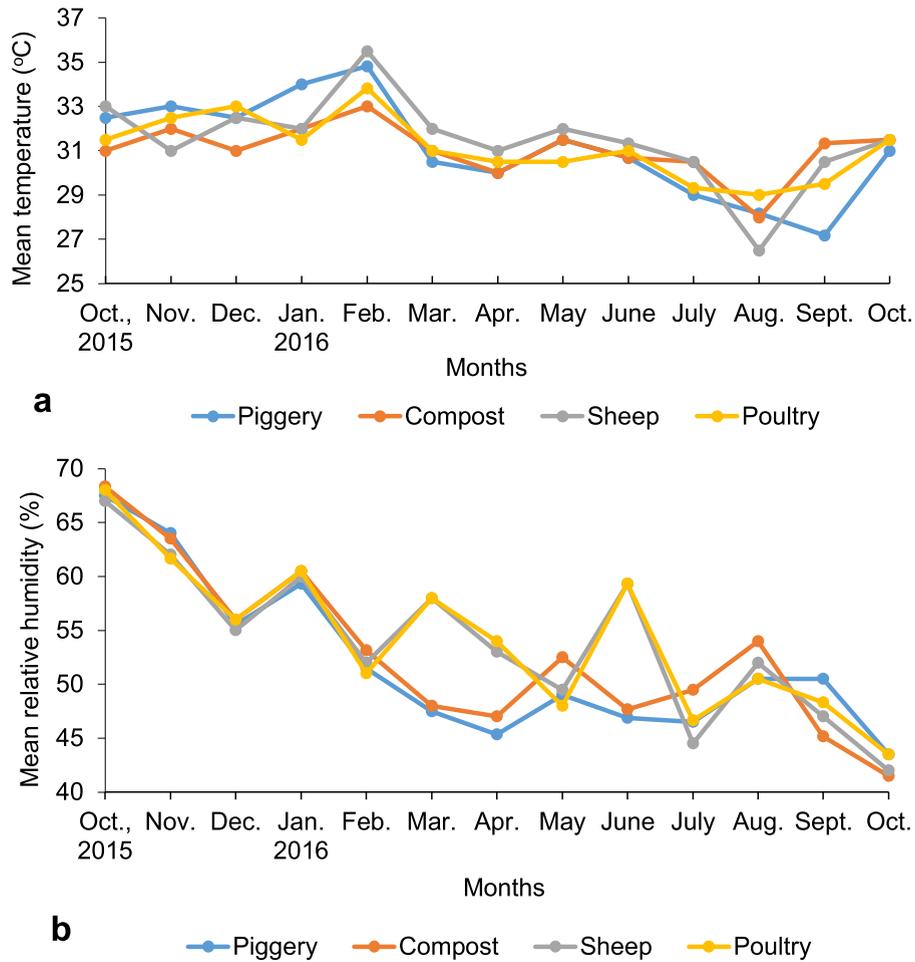


Fig. 9. Mean monthly microhabitat (a) temperature (b) relative humidity during BSF egg clutch collections.

Table 1

Mean (\pm SE) larval and pupal periods, percent eclosion, sex ratio and egg clutch of prepupae from the piggery and compost microhabitats ($*p \leq .05$) $N = 500$.

Microhabitat	Larval period (days)	Pupal period (days)	Eclosion (%)	Sex ratio		No. of egg clutches/female
				Male	Female	
Piggery	22 \pm 0.58	8.00 \pm 0.14	54.66 \pm 2.74	1.17 \pm 0.02:	0.96 \pm 0.08	0.96 \pm 0.02*
Compost	19 \pm 0.29	10.00 \pm 0.60	65.00 \pm 6.66	1.23 \pm 0.12	1.30 \pm 0.12	0.68 \pm 0.02*
p-Value	.370	.107	.730			.026

These egg clutches were supplied to the colony to improve the genetic variability in the existing population needed for increased larval production for fish feed formulation and compost production. In this study, the life cycle (eggs – adults) were 29.16 \pm 1.35 and 28.63 \pm 1.06 days for eggs from piggery and compost microhabitats respectively, not statistically different ($p > .05$).

Discussion

Three important results emerged from this study; (1) more *H. illucens* egg clutches were harvested from the piggery waste dumpsite, suggesting it is the most suitable microhabitat to harvest wild *H. illucens* egg clutches in the study area; (2) season of the year did not influence number of BSF egg clutch harvested; (3) source of egg clutch influenced larval weight and larval length gain within the first 10 days (4th larval stage).

The outcomes obtained from the egg clutch harvest showed the importance of organic waste dumpsite microhabitats in the collection of *H. illucens* egg clutches [3,5,17,23]. Oviposition traps placed a few centimeters above piggery waste dump-

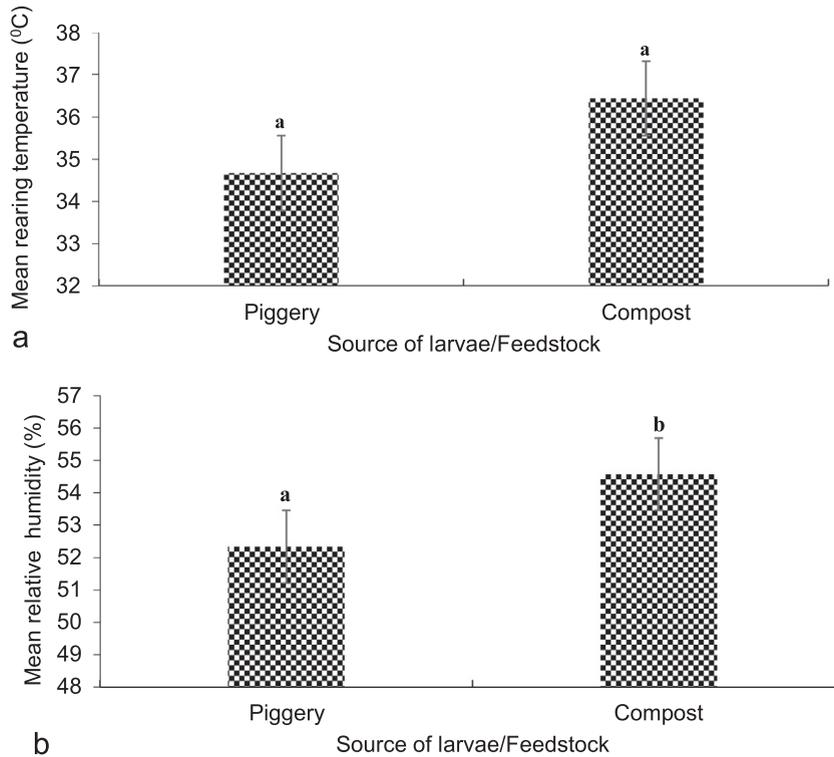


Fig. 10. (a) Mean temperature in larval boxes during larval rearing. (b) Mean percent relative humidity in larval boxes during larval rearing.

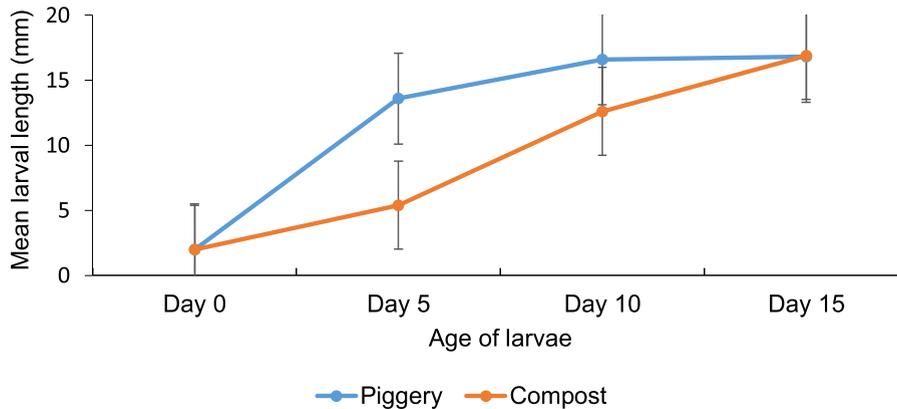


Fig. 11. Effect of age on wild black soldier larval length.

site and compost heap microhabitats enticed gravid *H. illucens* females to sire egg clutches. Organic wastes release several volatiles such as alcohol, aldehydes, and organic acids during decomposition that attract insects [4,16]. Depending on the type of organic waste involved, the volatiles released would be different and would therefore attract insects differently. In addition, the age of the organic waste will also affect the rate and type of volatiles released. Caruso et al. [4], showed that maintaining the RH of oviposition substrate at 60–70% help release volatile attractive substances. It is further recommended to renew baits regularly, approximately every 10 days. However, olfactometric experiments conducted by Tomberlin [21] did not identify the specific molecules or group that act as attractants for BSF. For instance, females of *Drosophila melanogaster* use their gustatory abilities to assess the quality of oviposition sites [25]. It is however probable that, the oviposition substrate is chosen by the female according to its nutritional value for its offspring. Additionally, adults require bacteria to attract females for oviposition [27] while males need lekking sites to establish successful mating [18] and correct lighting conditions [26] to reproduce and oviposit efficiently.

The absence of egg clutch harvest from the poultry and sheep waste dumpsites may possibly be due to the depletion of nutrients particularly nitrogen [26] in these wastes by microorganisms. The low egg clutch harvest from the compost heap

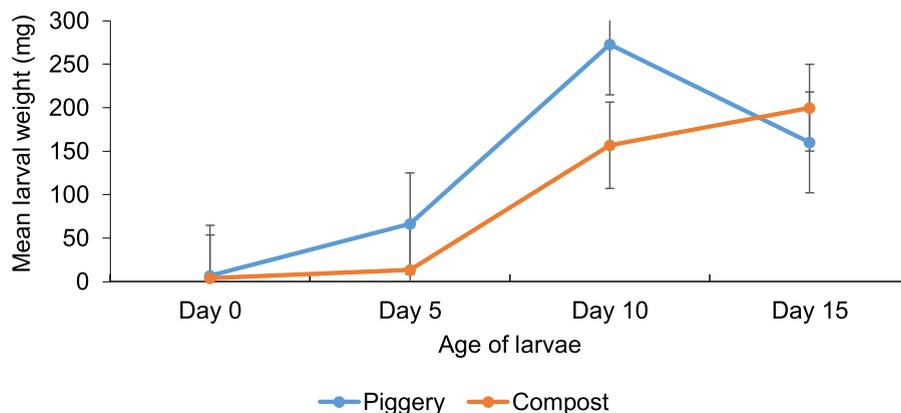


Fig. 12. Effect of age on wild black soldier larval weight.

microhabitat could be due to temperature build-up during the thermophilic stage in the compost which might have been detrimental to egg hatch and larval development [18]. Caruso et al. [4], showed that males assemble in small numbers near sheltered vegetation growing around decomposing organic matter (BSF microhabitats) and engage in competitive display that attract females in order to find a mate [22]. Additionally, the different vegetation growing around decomposing organic matter (BSF microhabitats) provide the adults resting places and protection from both heat and rain [4] and assure the adults of droplets of water. The compost heap did not have any vegetation close by and so might explain the very low egg clutch trapped as there were no resting places for adult flies and thus depriving the BSF adults areas to congregate for lekking behavior which could lead to mating and subsequent oviposition [1].

The insect-substrate interaction is a complex phenomenon that depends on; environmental factors, substrate availability and physicochemical characteristics, and the presence of either conspecific immature insects or competing species [4]. These factors will therefore account for the differences in the number of sired egg clutches observed in the organic wastes examined [4], as the insect has to be attracted to the site before it can lay the eggs. *H. illucens* gravid females naturally oviposit eggs around waste which is ample and most consumed by its larvae in its immediate environment [18] and thus the piggery waste seems to be the only food source for field populations of BSF in the study area and which may provide a more suitable habitat for survival and feeding of the offspring of the BSF [4].

Season did not influence number of BSF egg clutches harvested indicating all season possibility of harvesting BSF egg clutches from especially the piggery microhabitat to establish or revamp a colony. Sheppard et al. [17], reported that adult black soldier flies typically mated and oviposited at temperatures of 24–40 °C. In this study, a temperature range of (28–37 °C) was recorded during the entire harvesting period. This temperature range is in accord with the reported view of *H. illucens*' greater prevalence to tropical and subtropical habitats [2,19].

The average weight of egg clutches obtained from the piggery waste dumpsite microhabitat was higher than that from the compost heap microhabitat. This was so, mainly due to the lower frequency of egg clutch harvest from the compost heap microhabitat. Out of a total of 27 weeks of egg clutch harvest, it was only in one week that the compost heap microhabitat yielded 2 egg clutches compared to 55 egg clutches obtained from the piggery waste dumpsite microhabitat during the harvesting period.

The source of egg clutch influenced larval length gain within the first 10 days (4th larval stage). *H. illucens* typically has six larval instars with developmental commitment to metamorphosis occurring early in the 5th instar. Larvae obtained from the egg clutches harvested from the compost heap microhabitat grew rapidly in weight and length between days 5 and 10 such that, by 15, no significant differences in growth rate was observed between the two sources of larvae. The reason may be due to the fact that larvae hatched from egg clutches harvested from the compost had higher or better efficiency of conversion of ingested food [7]. This involves homeostatic adjustment of consumption rates and efficiency parameters such that an insect can approach its "ideal" growth rate even with foods of different quality in various environments [24].

It was found in this study that piggery manure dumps or compost heaps are important for BSF egg deposition and that there were no significant differences in life history traits of egg clutches from different microhabitats. A total of 694 egg clutches were sired by F_1 female flies eclosing from both the piggery and compost microhabitats and these egg clutches have helped as a basis for colony establishment and has provided numerous BSF larvae. The results of this study indicate that the materials and methods adopted for laboratory rearing of target insect are efficient and mass rearing of BSF is feasible in Ghana. Layer meal was used in establishing this colony just as has been used by other researchers [17,20], however, larvae that are intended for waste treatment and feed production, could be reared on wastes such as restaurant, vegetable or fruit waste and even market waste, just to reduce cost of colony maintenance [14]. However, further studies may be needed for establishing a colony fed solely with any of the above waste mentioned.

Conclusions

In this study, more BSF egg clutches were trapped at the piggery dump site, a result which differed from what was reported by Sripontan et al. [18], but was consistent with egg trapping techniques for mass rearing of BSF. In addition, the source from which BSF egg clutches were trapped had no significant effect on life history traits. Further, temperature and relative humidity of the microhabitats as well as seasons did not influence egg clutch trapping. This study provided additional information to support the assertion that it is technically feasible to raise an *H. illucens* colony with egg clutches trapped from the wild.

Recommendations

- Larval cages, prepupal containers and adult cages should have very fine- meshed muslin cloth covering to as much as possible prevent entry of the parasitoid, *Dirhinus giffardii* (Hymenoptera: Chalcididae). This will also ensure sufficient ventilation so as not generate heat above 50 °C which could force out larvae or cause very significant larval mortality.
- To increase egg hatching success and neonate survival, BSF eggs should not come into direct contact with liquids, which can be achieved by placing the egg traps on stands e.g. stone marbles.
- Larval feed must not be too thick (about 4 to 5 cm) in thickness to allow for complete digestion, thus feed given should be just enough for complete assimilation within three days.
- Larval cages should be cleaned regularly with detergent, disinfested with 70% alcohol and sun dried for about 6 h before reuse.
- Provision of misting water at least twice a day to adult flies is essential and this water spray should not wet the floor of the adult cage.
- Dead adults should be swept off the floor of the adult cages twice a week and the adult cage thoroughly washed at least once every three months.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

E.A. Ewusie: Formal analysis, Writing - original draft, Writing - review & editing. **P.K. Kwapong:** Writing - review & editing. **G. Ofosu-Budu:** Writing - review & editing. **C. Sandrock:** Writing - review & editing. **A.M. Akumah:** Formal analysis. **C. Tetegaga:** Formal analysis. **S.K. Agyakwah:** Formal analysis.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.sciaf.2019.e00134](#).

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