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Original Research

An Investigation and Trapping Wild Colonies of the Black Soldier Fly, Morphological Identification of Larval Stages Under a Controlled Environment in Pakistan

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Abstract

The black soldier fly larvae *Hermetia illucens* L. (Diptera: Stratiomyidae) are an ideal insect to produce compost and animal feed. The goal of this study was to use Kitchen waste 50 grams, Cow dung 50 grams, and Rotten fruits 50 grams attractants to gather wild *H. illucens* and their egg clutches from their natural microhabitat in dumping sites. The fly was trapped in a 1×1×1 feet trap net. The results indicate that humidity 42.5±50.5%, F = 1.754, P = .233 and temperature 27.975±37.05ºC, F = 1.126, P = .353) during the capture of *H. illucens* and egg clutch in a natural environment. The life cycle has included seven instars, a duration of 39 days, and the longest time period of the instar 6th recorded. We have optimized the temperature range to 24-30ºC and humidity to 60-95% in indoor rearing of the *H. illucens*. We recorded the varying ingredient contents level, highest Moisture at 38.5%, Crude protein at 55.13%, Crude fiber at 28.18%, Crude fat at 28.18%, and Total Ash at 15.5%. The *H. illucens* indoor farming is recommended for quality of protein production under a controlled environment which will boost the Pakistani economy and reduce threats to aquatic fauna.

Keywords: Ecology, wild *H. illucens*, eggs clutches, instars stage, indoor environment, Khyber Pakhtunkhwa, Pakistan

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Introduction

Naturally, black soldier fly (*H. illucens*) is found outdoors, in livestock, and around the decomposed organic matter. They feed on rotting material as it provides scents to attract (*H. illucens)* for mating and the fly lays eggs on rotten material because their nutritional demands are met/fulfilled through naturally occurring organic matter. [1, 2]. Due to increased urbanization, the habitat of black soldier fly *H. illucens* is continuously affected and is gradually shrinking [3-5].

Morphologically, *H. illucens* is a shiny black metallic-looking fly often confused with a honeybee. Adults of this fly are characterized by a pair of wings and a lack of stingers [1, 5]. *H. illucens* belongs to Order Diptera and the family *Stratiomyidae*. The species are abundant in the warmer regions of the world and are also native to the warm temperate zone of the Americas [6, 7]. In recent years, the species has been documented to spread northwards in Central Europe; Ssymank and Doczkal recorded it from Germany, and Roháček and Hora recorded it from the Czech Republic. In South Africa, they have seen the specimens collected as early as 1915 and from Malaysia, Hawaii, Solomon Islands, New Caledonia, Mariana Islands, Palau, and Guam in the 1940s. By the 1960s, the black soldier fly had spread all over the world, the range it still occupies today [8, 9].

Globally, black soldier flies have commonly reared in semi-indoor and indoor setups under control environment [10]. Light intensity has an important role in *H. illucens* mating [11, 12]. The life cycle of *H. illucens* comprises four stages: egg, larvae, pupae, and adult [13, 14]. The larvae pass through different stages to reach pupae [9, 15] reported 5 instars in Korea during the larval stage while [9] reported 6 instars, the developmental stages are completed in 14 days [16, 17]. The adults have spongy, siphon-like mouths which are used for sucking the fluid. Fats are stored in the body during larval stages, which are then used by the adult fly for their survival [13]. High protein and fat concentrations have been reported in *H. illucens* larvae, which is necessary for fish growth [18, 19].

The *H. illucens)* can convert a variety of organic waste into insect biomass and compost, which are used as feed sources in poultry and aquaculture industries and as a fertilizer, respectively [20, 21]. The recycling process is highly significant in minimizing harmful waste and releasing NH_3 and CO_2 into the atmosphere, controlling harmful pathogenic bacteria while yielding large quantities of qualitative proteins. [22-24]. This study reported for the first time from Khyber Pakhtunkhwa, Pakistan, that there is a variation in wild *H. illucens* egg clutches trapped on organic manure at dumping sites in nature. For the rearing of the wild *H. illucens* optimized and adaptable temperature (24-30ºC) and humidity (60-95%) in a control environment under standard conditions. This study reports for the very first time seven instars of *H. illucens* with the morphological character of each instar. Similarly, each instar possesses a different level of nutritional content in terms of chemical composition. Furthermore, our focus was to collect wild black soldier flies from their natural habitat, and morphological identification at larval stages under a control environment.

Our research is driven by a core objective: to advance the methods of collecting black soldier flies (*H. illucens*) from their natural environment, incorporating heightened safety measures, and facilitating easy morphological identification, particularly during the larval stages. The significance of this study lies in its potential contributions to both scientific understanding and practical applications in various fields.

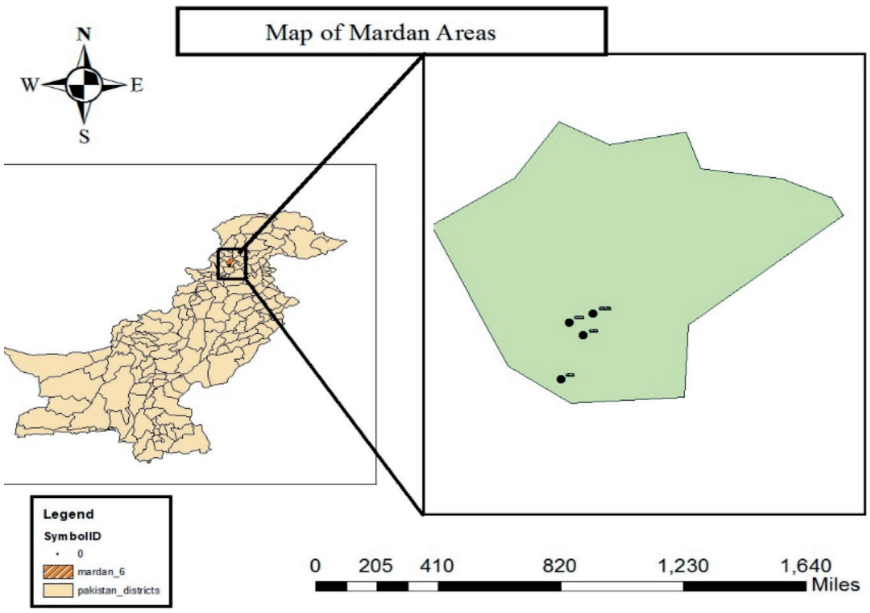

Fig. 1. Map of the study area in District Mardan (right side).

Material and Methods

Study Area

Sampling was done from 1st April 2021 till 30th April 2022 in district Mardan, Khyber Pakhtunkhwa Pakistan. Mardan region is connected to the district Malakand on the North, district Buner on the North-East, district Swabi on the East, district Charsadda on the West, and district Nowshera on the South. Mardan is located at 34 12'0N 721'60E with an altitude of 283 meters above sea level ArcGIS (version 10.5) was used for mapping of collection points (Fig. 1).

Trap Net

The trap net was designed from mosquito mesh using PVC (polyvinyl chloride) pipes with standard size (length 1 foot, width 1 foot, and height 1 foot). having an opening (8-inch ring) over the trap net for the entrance of the *H. illucens* fly (Fig. 2).

Attractant

Kitchen waste (50 grams), cow dung (50 grams), rotten fruits (50 grams), and chicken waste (50 grams) were used as an attractant [14]. All these attractants were kept in each trap net for four days at four different localities of district Mardan *i.e.* Sultan Abad, Rashaka, Baghdada, and Gul Maira. Temperature and humidity were recorded by using a thermohygrometer model, HTC − 2 at each site, early in the morning, in the afternoon, and late at night (Fig. 3 and **7**).

Eggies Design

The Eggies (Cotton corrugated channel to collect the insect egg) were designed from corrugated/ channeled cardboard (weighing 128 grams, having length and width of 8 cm and 25 cm respectively) that were gripped together with a rubber band and kept over each attractant inside the trap net so that *H. illucens* may lay eggs and will be collected then. The trap net was checked

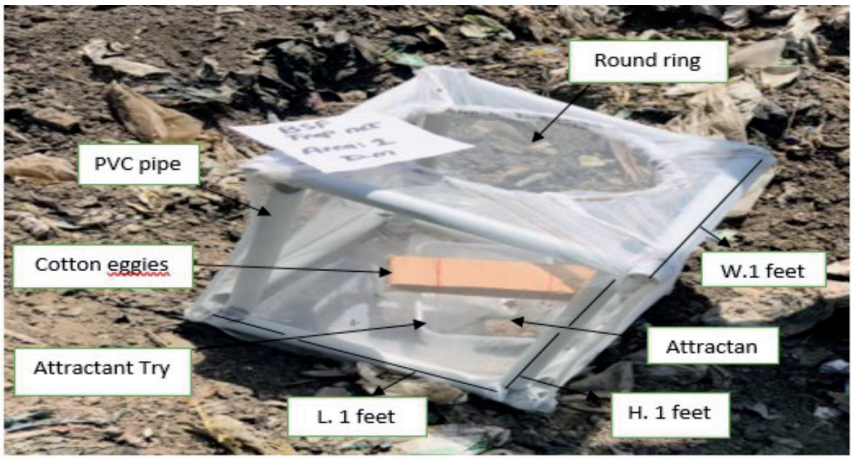

Fig. 2. Trap net.

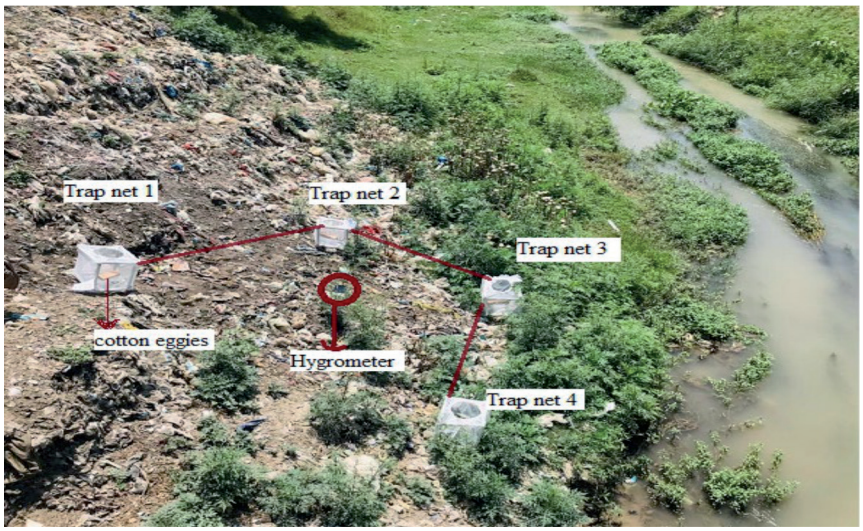

Fig. 3. *Hermetia illucens* collection from dumping site at District Mardan.

regularly after 12 hours for *H. illucens* confirmation and for the observation of their egg clutch in cardboard channels (Fig. 4).

Control Environment

Temperature (24-30ºC), humidity (60%-95%), and sodium light (100 watts) were maintained in the laboratory for the survival of *H. illucens* and to ensure higher yield in the lab.

The temperature was maintained by using an inverter while a humidifier model, 3L, HM300 was used for the maintenance of humidity in the required range.

Incubation of Harvested Egg Clutches and Larval Feeding

To prevent microbial contamination, the egg clutch and *H. illucens* were transferred to the rearing unit [24, 25]. The number of egg clutches sired in the grooves of the cardboard traps was counted initially and the final weight was determined using digital balance**.** The egg clutches harvested from different sources, for example, fruit waste (1000g), chicken waste (1000g), cow dung (1000g), and kitchen waste (1000g) were taken individually in plastic containers of standard size having length, width, and depth of 15 inches, 10 inches, and 5 inches respectively (Fig. 5). A similar method was used for all four sites. After 3 days, eggs were successfully hatched in each waste source.

Each larva was provided with 5 g feed daily *i.e.,* fruit waste, chicken waste, cow dung, and kitchen waste while after hatching the feed quantity was increased by 1 gm per larva each day up to the pre-pupal stage.

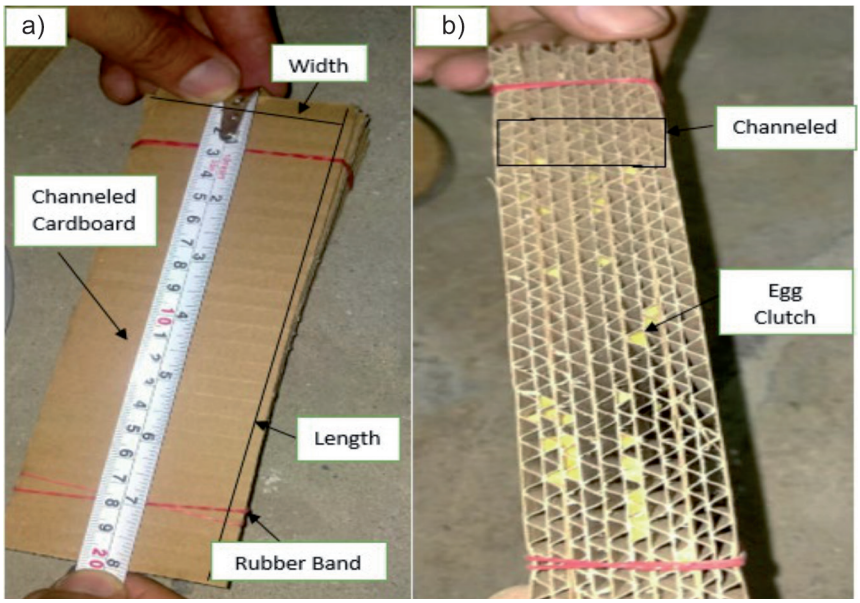

Fig. 4. a) Eggies from cardboard, b) Eggs clutch and channeled in cardboard.

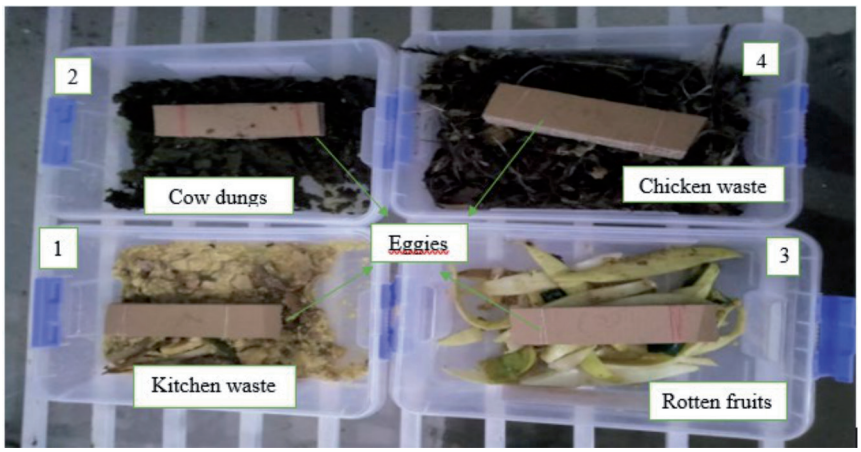

Fig. 5. *Hermetia illucens* eggs harvesting on different sources.

Determination of Larval Instar

After 2 days of egg hatchlings, the larvae were transferred to Petri dishes to examine the larval stages in detail. Development of all larval instar stages was examined microscopically by using an Olympus SD-30 microscope with an attached camera (6 megapixels).

Morphological Identification of Black Soldier Fly

The *H. illucens* was observed under a microscope and the body parts were examined in detail with the help of an attached camera. The *H. illucens* were identified by using identification keys [13].

Chemical Composition of *H. Illucens*

The *H. illucens* products such as Pre pupa 21 g, Pupa 28 g, Shell 24 g, and die Adult *H. illucens* 53 g were washed in tap water and then dried in a micro-oven at 90ºC for 8 minutes. These four products were powdered in a grinding machine and stored in a sterilized plastic box at room temperature for further analysis.

Small-Scale H. illucens Industry

In the picturesque landscapes of Khyber Pakhtunkhwa, small-scale Black Soldier Fly *H. illucens* farms have emerged as silent heroes in the realm of sustainable agriculture. These modest yet impactful initiatives play a crucial role in waste management, livestock feed production, and environmental conservation.

Results and Discussion

The egg clutches were trapped (Fig. 4) from dump waste micro habitat through different attractants from 1st April 2021 to 30 April 2022. The average weight of all egg clutches harvested was 1g, 1g, 0.5g, and 0.9 g respectively (Fig. 6). Moreover, this study provided four important results: (1) The *H. illucens* spp. were more attracted to the chicken waste dumpsite, and more egg clutches were harvested as a result. Therefore, chicken wastes are suggested as the most suitable habitat for the collection of wild *H. illucens spp.* and to harvest egg clutches (2) Successful rearing of wild *H. illucens spp.* in a controlled environment rather than nature. (3) Confirmation of wild (*H. illucens spp.*) larval instar stages with identical characteristics and of the same timing during their developmental stages. (4) Confirmation of external morphology of wild *H. illucens* spp.

Climatic Conditions in Nature

There were no significant differences in both temperature and relative humidity on the microhabitats during *H. illucens* collection and egg clutch harvest as determined by One-way ANOVA for temperature ($df = 3$, $F = 1.126$, $P = .353$) as shown in Fig. 7, and relative humidity during egg trapping ($df = 3$, $F = 1.754$, $P = .233$) as shown in Fig. 8. The attractants are used to attract female flies towards themselves, whereby the adult female *H. illucens* lays eggs over the waste. Sripontan *et al.* have reported similar results to our study, the presence of *H. illucens* in a humid and warm area in nearby locations to its natural habitat, which provides a more suitable habitat for the survival and offspring feeding of the *H. illucens* [26]. Lewylle et al. have reported that certain environmental conditions like humidity, temperature, and feeding substrate should be taken into consideration during *H. illucens* rearing [27]. The current study is in accordance with the above statement, which indicates that the habitat and life cycle of *H. illucens* is completely dependent upon these conditions (*i.e.,* humidity, temperature, and feeding substrate).

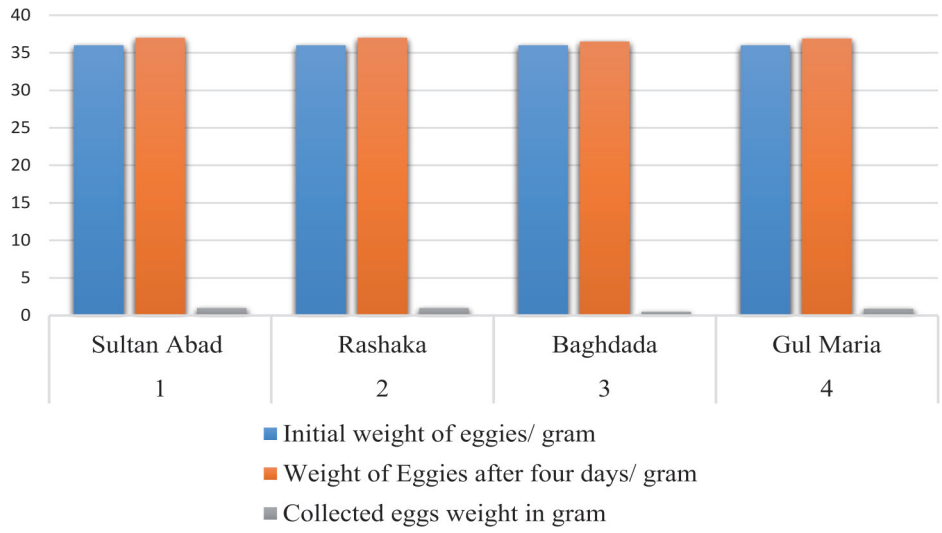

Fig. 6. Collection of *Hermetia illucens* eggs clutch from the target dumping site.

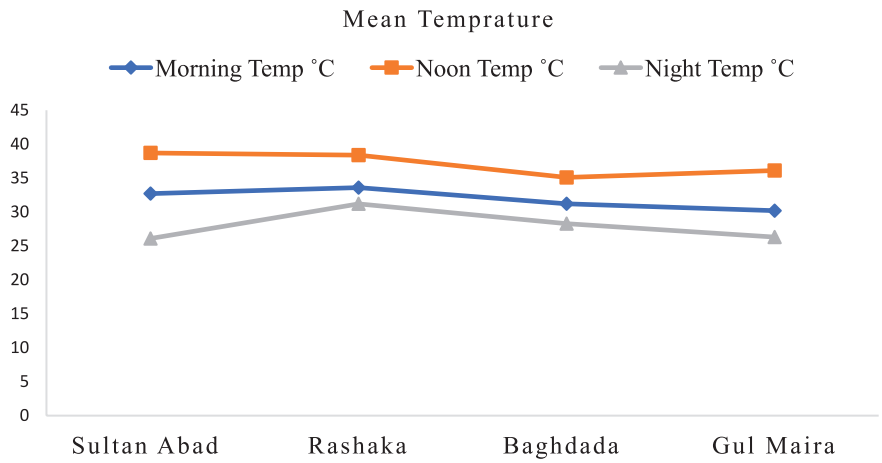

Fig. 7. Mean temperature during *Hermetia illucens* eggs clutch collections.

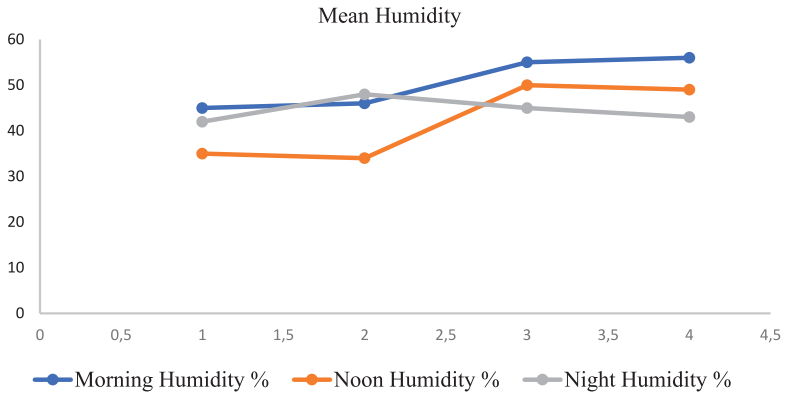

Fig. 8. Mean relative humidity during *Hermetia illucens* egg clutch collections.

Larval Rearing

The larva was reared in fruit waste, cow dung, kitchen waste, and chicken waste in separate larval boxes under standard conditions in a controlled environment. To evaluate morphological differentiation, survival, and growth response of each instar to different feed i.e. fruit waste, cow dung, chicken waste, and kitchen waste were recorded. It has been reported in certain research papers that *H. illucens* larvae are used to manage municipal organic wastes, chicken, swine, and cattle manure with its descendent initiation from the wild. [28, 29]. In the current research investigation, it has been documented that *H. illucens* are attracted by organic wastes, fruit

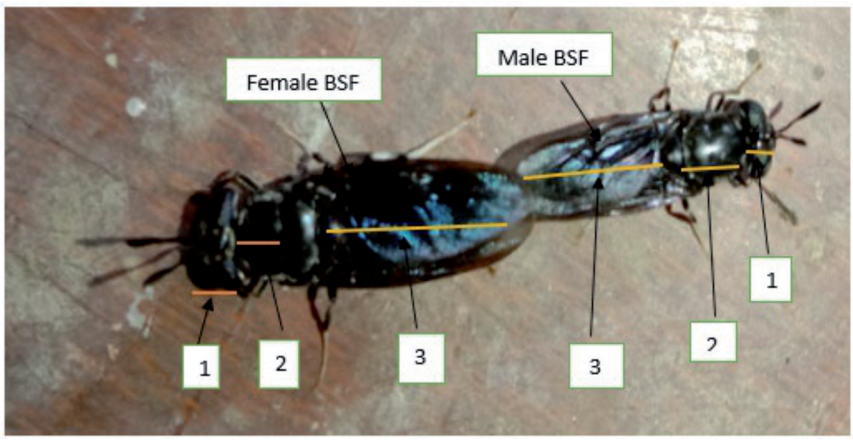

Fig. 9. 1: Head, 2: Thorax, 3: Abdomen. Of *Hermetia illucens.*

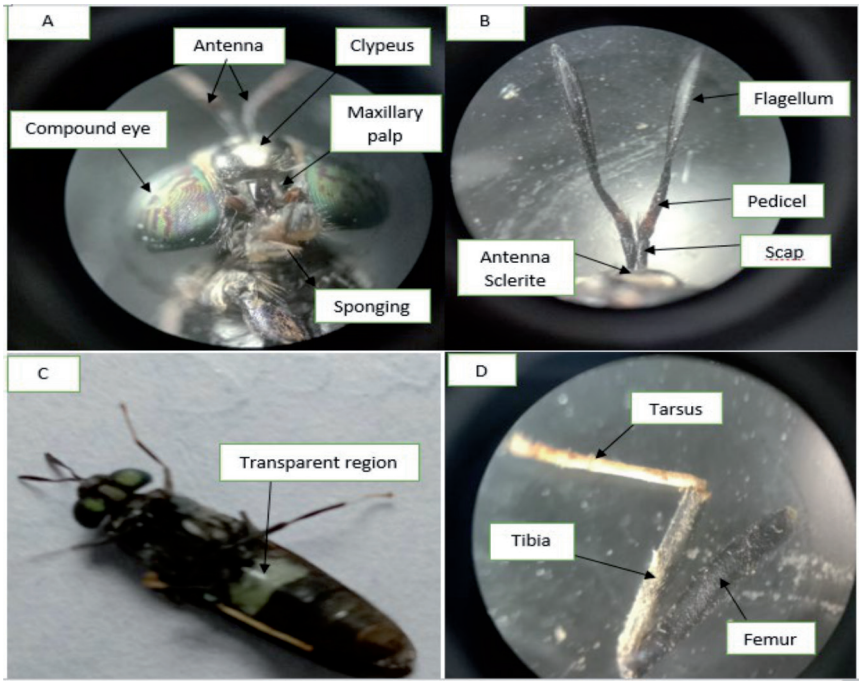

Fig. 10. A: Head region, B: Antenna, C: Transparent region, D: Legs.

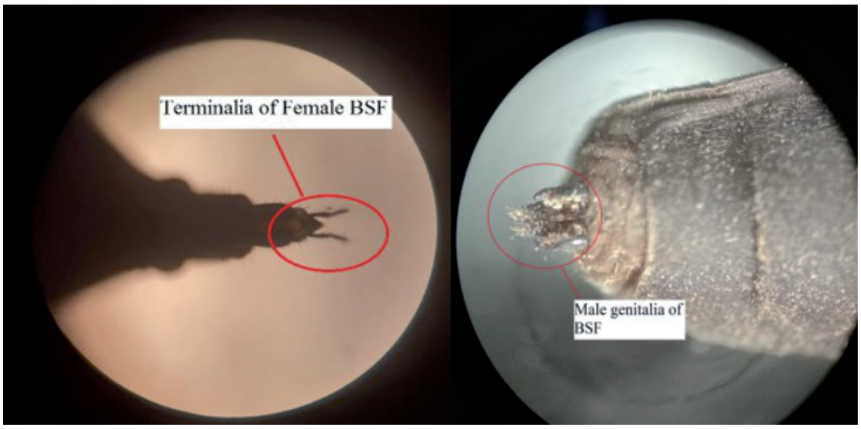

Fig. 11. Sexual dimorphism of *Hermetia illucens.*

waste, chicken waste, cow dung, and kitchen waste in the wild.

Adult

The *H. illucens* body has three regions: head, thorax, and abdomen (Fig. 9). The head consists of a pair of antennae, compound eyes that are bluish and yellowish green, a spongy mouth, and one pair of maxillary palp, spherical, and clypeus. The antenna is differentiated into three parts; scape, pedicle, and flagellum (long terminal segment) abdominal region of *H. illucens* is transparent, with three pairs of legs, and a pair of wings. Legs have visible and distinct three parts; the femur dorsal is black and the upper part is covered with tiny hairs while the lower part bears no hair, ventral is white. The tibia is black with white spots while the tarsus is white (Fig. 10).

There is no obvious external sexual dimorphism except genitalia. The male genitalia organ is short, looks like a flower, and is protractible, with two pairs of posterior lobes (Table 1). Female genitalia are fork-shaped and protractible and the genitalia are entirely covered by microscopic hairs in both males and females (Fig. 11).

Table 1. The size difference in male and female *Hermetia illucens.*

	Eye Length in mm	Thorax Length in mm	Abdomen Length in mm
Male	2.71	2.246	3.132
Female	2.997	2.333	3.132

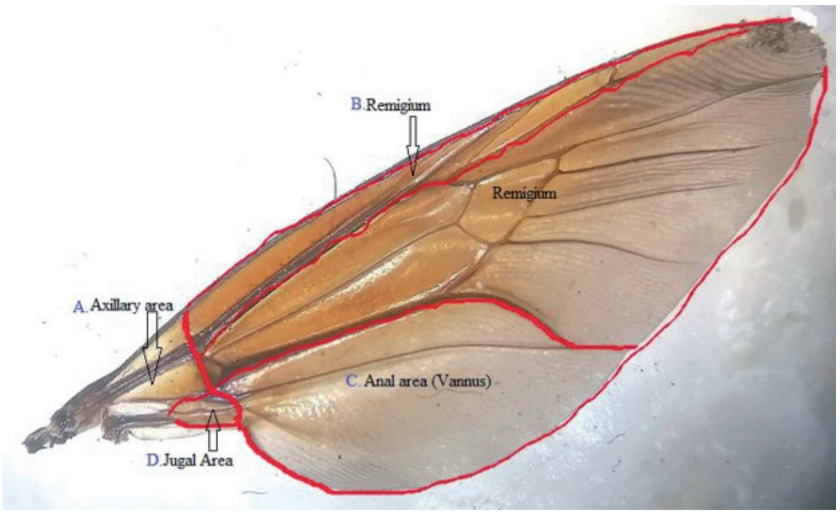

Fig. 12. Wings of *Hermetia illucens.*

Wings are shiny and clustered, bearing a thin and delicate vein that provides passage of hemolymph, which is essential for the proper functioning of wings and various sense organs found on the wings. Wing venation plays an important role in the morphological identification of insects and is an important characteristic in taxonomy (Fig. 12). A mature adult of black soldier fly survives only for about 7 days.

Eggs

A mature *H. illucens* adult lays about 870 eggs individually during its lifetime (about 7 days) Hatching period is 3 days and is dependent on the source. The eggs are laid in colonial clusters, shape structure having a length and width of about 0.9 mm and 0.2 mm respectively bear pale yellow or creamy white color with a black spot over the egg. After hatching, the egg clutch should be transferred to a clean and moist surface immediately to prevent microbial contamination (Fig. 13).

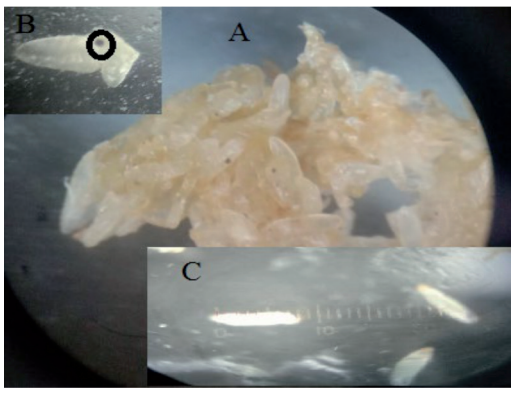

Fig. 13. A (eggs colonial cluster), B (Black spot over egg), C (length of egg).

Larva: (1st instar)

1st instar larvae were kept under observation after 3 days of hatching. Special morphometric characteristics are distinguished, color yellow-white, time 4 days, average length 2.10 mm, average width 0.5 mm, average

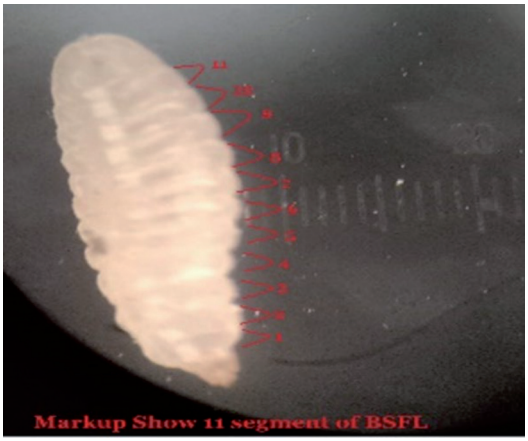

Fig. 14. Morphology of instar 1.

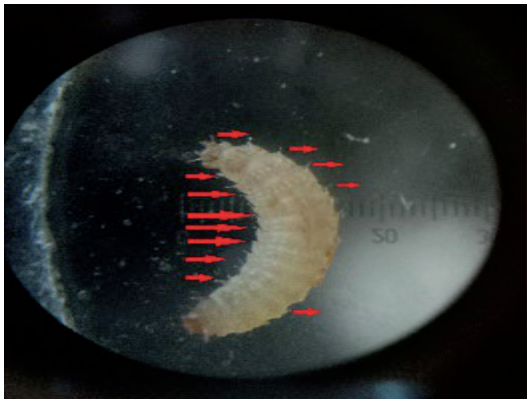

Fig. 15. Morphology of instar 2.

weight 0.03 g, segment 11, and brisks of hair are not present over the body (Fig. 14).

Instar 2nd

Morphometric character; time 4 days, color light brown, average length 4.04 mm, average width 1.06 mm, average weight 0.06 g, segments 11 and brick of hair are visible over the body (Fig. 15).

Instar 3rd

Recorded gradual changes occurred in coloration. The color was recorded as dark brown, average length of 8 mm, average width of 2.77 mm, average weight of 0.09 grams, time of 5 days. The number of segment 11 and bricks of hair are visible (Fig. 16).

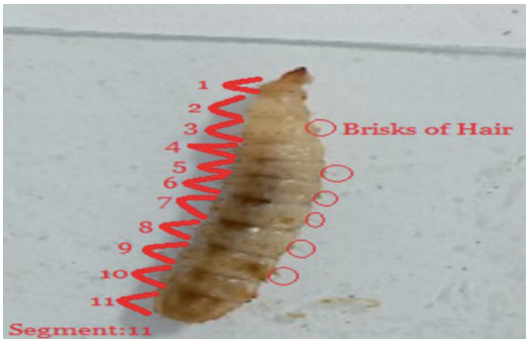

Fig. 16. Morphology of instar 3.

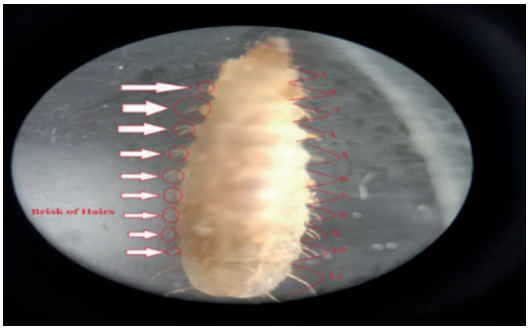

Fig. 17. Morphology of instar 4.

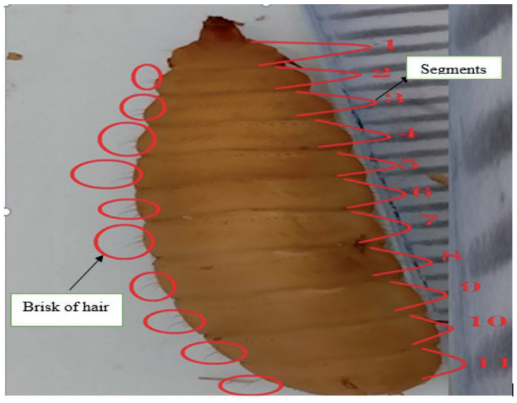

Fig. 18. Morphology of instar 5.

Instar 4th

Morphometric was recorded; Average length 16mm, Average width 2.96mm, average weight 0.1 grams, time 2 days, color light brown, Segments 11 and hair are visible (Fig. 17).

Instar 5th

The Color is dark brown, the Average length is 2 cm, the average width is 0.4cm, the average weight is 0.20 grams, Segment 11, the time is 7 days, and the hair size of each segment is large and visible (Fig. 18).

Instar 6th

Pre-pupae are similar and look like mature larvae, the color is blackish, average length 2.5 cm, average width 0.4 cm, average weight of 0.30 grams, and segments 11 with hair-like structure known as bent hooks used for locomotion (Fig. 19).

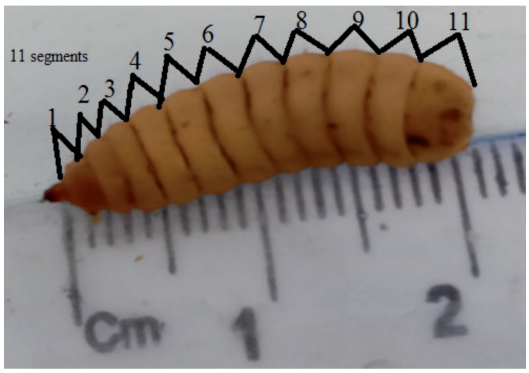

Fig. 19. Morphology of instar 6.

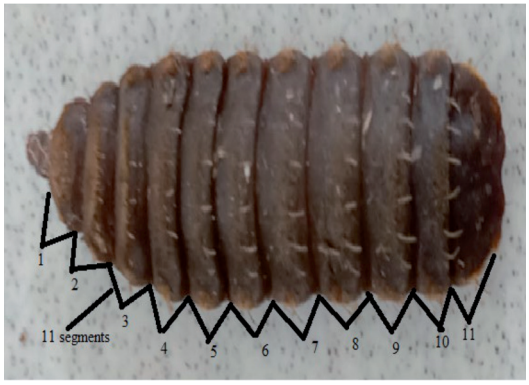

Fig. 20. Shown pupa morphology.

Table 2. Chemical composition analysis of *Hermetia illucens* products.

	Name of Samples			
Name of Parameters	Adult *H. illucens*	Pre-pupa	Pupa	*H. illucens* Larvae- Shell
Moisture%	38.5	17.4	28.1	9.2
Crude-Protein %	52	55.13	45.13	51.13
Crude-Fat %	17	28.8	24.4	13.3
Crude-Fiber %	10.5	15.6	17.5	21.5
Total Ash %	4	12	11	15.5

Pupa 7th

Pre-pupa changed to pupa, the color is fully black, and the remaining character was similar to pre-pupa. It starts movements in a dark, dry place and stops feeding (Fig. 20).

After seven days the pupa starts molting in a dark and suitable environment. The shell remains and raises a complete fly. Fly starts flying toward light for mating and starts the next cycle.

Proximate Chemical Composition of (*H. Illucens)* Products

The four products of (*H. illucens)* were analyzed at (Feed & Water Testing Laboratory Poultry Research Institute, Murree Road Rawalpindi) (Table 2).

The highest moisture was recorded at 38.5% in dead *H. illucens*, crude protein at 55.13%, crude fat at 28.8% in pre-pupa, crude fiber at 21.5%, and total ash at 15.5% in *H. illucens* larvae shells.

In another study, [30] showed suitable temperature (24-40ºC) for mating of the adult *H. illucens* which is in accordance with our study during the morning, afternoon, and night time which was 31.925ºC, 37.05ºC and 27.975°C, respectively. Average humidity recorded in the wild was 50.5%, 42%, and 44.5% during morning, noon, and evening, respectively. Temperature was maintained between 23ºC and 33°C during the harvesting period in the control environment but 25ºC was found to be more suitable for *H. illucens* mating and oviposit along with other standard conditions. Our results show the highest recorded humidity (48%) and lowest humidity level (42%), which is in accordance with the humidity level (44-68%) as reported by [8, 31]. although the humidity was maintained in the range of 60%-96% during indoor rearing which showed the best results. Kim et al. reported in a subsequent study, the Larval stages up to 5 have and 6 [32, 33].

The life cycle of *H. illucens* is 45 days in total. They spend two stages of their life in the growing media eggs (4 days) and larva stage (18 days). Only when they are transforming from pre-pupa (14 days) to adults (9 days), they complete the metamorphosis [34]. In this study, we found the life cycle of *H. illucens* a total 39 days. Egg hatching time is 2 days, 1st instar 4 days, 2nd instar 4 days, 3rd instar 5 days, 4th instar 2 days, 5th instar 5 days, 6th instar 6 days, 7 instar 3 days and adluts live for 8 days to complete their cycle.

Conclusions

Here, our study provides evidence of the high number of *H. illucens*, and their egg clutches were trapped through our proposed attractants *i.e.,* chicken intestine, kitchen waste, Cow dung, and rotten fruits. The ideal conditions, such as humidity and temperature significantly enhance *H. illucens* egg clutch in natural environment as well as in rearing units. Following the growth, the waste consumption is standard which we provided yields huge insect protein biomass. Our finding also suggests the larval stages as the best source of protein, fats, ash, and fibers for feeding. Furthermore, we recommend this larva of *H. illucens* as a rich content of protein for the aquaculture and livestock feed sector, similarly, the compost is a fertilizer for agricultural purposes as a prime choice. Though the study is limited to providing production on a large scale, however, it may also help in building a plant for large-scale production.

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Conflict of Interest

We declare that there is no conflict of interest.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Consent for Publication

All authors have provided their consent to publish this paper.

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