

Title

Rearing performance of Black Soldier Fly (*Hermetia illucens*) on municipal biowaste in the outdoor ambient weather conditions of Pakistan and Indonesia

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Abstract

The availability and continuous supply of Black Soldier Fly larvae (BSFL) is crucial for
 efficient operation of a BSF biowaste recycling facility. Its rearing performance was for the
 first time investigated in Pakistan under outdoor ambient weather conditions. Comparison of
 the findings with the BSF rearing performance of Indonesia's facility highlights the life stages
 needing special attention. In Pakistan, mean BSF emergence, hatching and survival rate of
 58.8% (SD 15.2), 44.5% (SD 21.8) and 91.4% (SD 1.68) were achieved respectively. A
 positive significant correlation was found between the number of emerged flies and prepupae
 (R=0.75) and the number of eggs produced and hatched (R=0.92). On average BSF took 49.5
 days (SD 3.20) to complete one life cycle under ambient temperature and relative humidity RH
 between (22-35°C) and (24.7-89.3%) respectively. The mean duration of eclosion, pre-
 oviposition, egg hatching, larval feeding and pupation was 15.6 days (SD 1.6), 3.5 days (SD
 0.5), 3 days (SD 0.6), 22 days (SD 2.5), 3.8 days (SD 1.2) respectively. In Pakistan, the life
 cycle duration was longer with a smaller number of eggs/fly, lower BSF emergence and
 hatching rates as compared to Indonesia. BSF tolerated the semi-arid weather conditions of

Pakistan, successfully developed into all instars and completed all life cycles under observation. It is suggested to provide controlled environmental conditions at the nursery stage to improve BSFL rearing performance for sustainable biowaste management.

Key words: Black Soldier Fly; Rearing performance; Biowaste recycling; Sustainable solid waste management; *Hermetia illucens*; Larval development;

Introduction

Black Soldier fly *Hermetia illucens* L. (Diptera:Stratiomyidae) originally native to South America is an amazing non-pest and non-vector organism of warm and humid regions (Joyce et al., 2009; Cicková et al., 2015). Black soldier fly larvae (BSFL) are voracious feeders of varying types of biowaste and can reduce it by 80% weight with a 20% bioconversion to valuable market products in just two weeks consisting of 45% protein; 30% fat rich animal feed (the fattened larvae) and stable organic residue (the soil amendment) (Dortmans et al., 2017; Lohri et al., 2017;Gold et al., 2018; Fowles & Nansen 2020). BSFL waste recycling technology exhibits certain hygienic and environmental advantages such as waste sanitization, negligible greenhouse gas (GHGs) emissions, no leachate and methane gas production (Mertenat et al., 2019). The CO₂ gas emissions are 47 times lower in BSFL biowaste processing as compared to conventional composting (Mertenat et al., 2019). In another study a comparison of BSF technology with other biowaste recycling options such as composting, vermi-composting and anerobic digestion showed considerably lower global warming potential and higher waste reductions in lesser time (Lohri et al., 2017).

BSF records have been scientifically reported in up to 80% of the world regions including Indonesia, China, Africa, India and Taiwan due to its increasing commercial value

(Banks et al., 2014, Diener et al., 2011a., Nyakeri et al., 2016; Lohri et al., 2017, Rindhe et al., 2019). They act as “ecological engineers” for developing countries (Diener et al., 2009), like Pakistan facing poor waste management practices and lacking financial resources where 50-70% of municipal solid waste is organic with perceived lower economic value. Various studies have been conducted on use of BSFL to treat municipal biowastes such as kitchen and food waste from households, institutions and restaurants (Diener et al., 2011b; Dortmans, 2015; Lalander et al., 2019; Nguyen, Tomberlin, & Vanlaerhoven, 2015), animal manures (Rehman et al., 2017; Miranda, Cammack, & Tomberlin, 2019), agricultural waste (Liu, Wang, & Yao, 2019), human faeces (Lalander et al., 2013), digested and undigested sludge (Lalander et al., 2019).

The BSF life cycle can be engineered to a duration of 45 days under controlled environmental conditions to maximize its benefits in terms of biowaste treatment and product harvest (Dortmans et al., 2017). Life starts with eggs laid by female flies after mating and a copulation period of two days also known as “pre-oviposition”. The hatchlings produced after a period of 3-4 days are fed moist chicken feed mixture for the first 5 days (5 days old, 5-dol) and then transferred to waste treatment unit where they are fed biowaste for 12 days. Around 2-5% of harvested larvae is sent back to the rearing unit and the pupation period starts, during which the creamy yellowish 17-dol develop into the brownish black 6th instar called prepupa (Dortmans et al., 2017). After a two days transformation period the prepupa turn into immobile hook shaped pupa, resulting in the emergence of adult flies known as ‘eclosion period’ which may last for 12 days and the life cycle starts again (Dortmans, 2015). Previous studies reported a rate as high as 74-97% of fly emergence from prepupa and hatching rate of ~70% under suitable environmental conditions (Holmes et al., 2012; Dortmans et al., 2017, Jucker et al., 2017; Tomberlin, Sheppard & Joyce, 2002; Tomberlin et al., 2009).

Constant and abundant sunlight, humidity between 50-70% and warm temperature ranging from 25-30°C ensures successful mating. This resilient organism has developed special survival traits when it comes to food availability and can transform themselves to adult flies in just two weeks, provided sufficient quality and quantity of food (Dortmans, 2015; Sheppard et al., 1995). However, under food shortage or any unfavorable conditions such as extreme temperatures, toxicity risk or oxygen deficiency, they can reduce or abandon food intake (Diener et al., 2011a) and may prolong their life cycle. An economically viable BSF biowaste recycling facility requires sufficient production of young larvae at its rearing unit and smooth waste supply at the waste treatment unit (Lohri et al., 2017).

Considering the country's severe waste management challenges, BSFL was, for the first time, reared in this study under outdoor ambient weather conditions in Pakistan at pilot scale to minimize the need of maintaining sophisticated controlled environmental conditions. The reared larvae were utilized in recent studies on reducing the area of BSFL waste treatment and kitchen waste bioconversion at household level (Mahmood et al., 2021a, b) The system was kept low-cost to get a model that could be applied at larger levels in the field with minimum investment and resource utilization. All stages from the flies' emergence back to pupae formation and life cycle duration were monitored. The need for controlled conditions was determined by comparing the results with the performance of BSF reared in any other region under suitable climate conditions, such as Indonesia.

Materials and methods

Rearing Unit set up

BSF rearing in this study refers to the following life stages i) pupation ii) fly emergence iii) adult mating and oviposition iv) hatching v) larval feeding stage and vi) prepupae metamorphosis

The study was conducted at Pakistan's first BSF rearing and municipal waste treatment pilot scale research facility in Sahiwal established by The Urban Unit, a Punjab government organization. It is a semi-arid region with ambient temperature of 22-35°C and 24.7-89.3% relative humidity. The site consisted of a greenhouse rearing unit of 75 m² (Diener et al., 2011a; Nyakeri et al., 2017) with brick powder floor and the roof made up of mud and wood logs. The experiments were performed in a time period of 6 months from March to August. To keep the environment cool and humid, native plants were grown, jute cloth fixed on side walls was kept wet all time, the site was water sprayed three times a day to maintain humidity. BSF pupae were sourced from the FORWARD (From Organic Waste to Recycling for Development) project site of EAWAG, Switzerland in Sidoarjo, Indonesia. Fruit and vegetable waste was used as a feeding substrate sourced from a nearby market. After establishing a stabilized BSF population in Pakistan, the experiments were performed.

Dark cage operations

A thick black denim cloth 'dark cage' with a size of 158×76×150 cm, with a 30 cm wide tunnel on one side, was constructed to facilitate pupation and safe fly emergence (Dortmans et al., 2017). Plastic pupation boxes (36×25×13cm), containing 7-10 cm layer of moist compost and the prepupae, were stacked upon each other in a diagonal position inside the cage. The number of prepupae in each cage depended on the total number of prepupae harvested on the respective days.

Love cage operations

An experimental setup meant for flies mating and oviposition known as ‘love cage’ (158×76×150cm) was designed using a white net cloth of mesh size 3 mm and 27 cm wide tunnel on one side (Dortmans et al., 2017; Nakamura et al., 2016; Sheppard et al., 2002; Zhang et al., 2010). The cage was equipped with a drinking water box (Tomberlin et al., 2002), a plant pot for additional moisture (Sheppard et al., 2002), an attractant consisting of decaying fruits, fruit water, dead flies and some larvae for attracting adults to lay eggs nearby and a set of 4 to 5 wooden planks (20 cm long and 3 mm thick) with very thin spaces in-between known as ‘eggies’. They were stacked upon each other on the top of attractant box to facilitate fly oviposit (Nakamura et al., 2016; Nyakeri et al., 2017; Shumo et al., 2019; Sripontan et al., 2017). The cage was placed at a location receiving constant sunlight for 8 to 10 hours (Shumo et al., 2019). The emerged flies were shifted from dark cage into a pre-weighed love cage by connecting mouth of both tunnels. In this study total 13 BSF life cycles were conducted with one love and dark cage assigned to its each respective cycle.

Nursery operations

BSF eggs were weighed and carefully collected using a sterilized scraper in a pre-weighed container. They were placed onto a sieve with 16cm diameter, 4cm depth, 1.2 mm diameter dipped inside plastic nursery boxes dimensions (10×15×5cm) containing 1-2 kg chicken feed mixture (35% chicken feed; 65% water) (Diener et al., 2009; Nyakeri et al., 2017; Sheppard et al., 2002). The feed was sprinkled with wheat bran and the container was covered with a wet breathable cloth to retain moisture and protect eggs from predators (Dortmans et al., 2017; Sheppard et al., 2002). The hatchlings were fed on chicken feed for the first five days (Nyakeri et al., 2017; Shumo et al., 2019; Tomberlin et al., 2002; Tschirner & Simon, 2015) therefore named as 5-dol (5 days old larvae).

Enumeration of 5-dol and Larval waste feeding stage

The 5-dol (Tomberlin et al., 2002) were then manually sieved (mesh size 1.2 mm) from the feed residue and enumerated. A total 200 hand counted BSFL were fed with shredded and dewatered fruit-vegetable waste in sterilized plastic containers (23×17×8cm) labelled as ‘larveros’ covered with a moist breathable cloth to protect from houseflies and dry environment (Diener et al., 2011a; Dortmans et al., 2017; Jucker et al., 2017; Nyakeri et al., 2017, Shumo et al., 2019). Waste was provided in the amount consumable by larvae in 24hrs following the standard i.e., 650,000 larvae to treat 1 ton waste in 12 days according to FORWARD guidelines (Dortmans et al., 2017). The feeding continued until majority of the larvae transformed to their 6th instar i.e., prepupa (Shumo et al., 2019). Every time date of egg collection, hatching, first and last day of larval feeding was noted. This method was adopted to calculate duration of each BSF life cycle.

Transformation to pupae

Each larvero box was placed inside its pupation box (25×20×10cm) containing 8-10cm thick layer of moist compost. The inner walls were water sprayed to facilitate prepupae self-migration out of the waste source. Prepupae were allowed to rest and hide in pupation boxes until the majority of them metamorphose into immobile pupa and related dates were noted.

Determination of the rearing performance indicators

BSF rearing performance indicators were calculated and assessed on the basis of following measures i) number of emerged flies ii) amount of BSF eggs (g) iii) total number of BSF eggs in each cycle and iv) number of hatched BSFL counted on 5th day gives the approximate number of fertilized eggs.

BSF emergence rate (ER %) was calculated using (Eq.1) which is the ratio between number of BSF emerged from pupae and total number of pupae placed in dark cages (Tomberlin et al., 2002)

$$ER (\%) = (BSF_{emer} / P) \times 100 \quad (1)$$

Number of eggs/female fly was calculated by dividing the total number of eggs produced by the number of female flies (assuming 50%) emerged in each love cage

BSFL Hatching rate (HR%) was calculated using (Eq.2) which is the ratio between total number of BSFL hatched from fertilized eggs ($BSFL_{hatch}$) and total number of BSF eggs (E) produced (Nakamura et al., 2016). E was calculated by using the weight of 1 egg = 0.000029 grams

$$HR (\%) = (BSFL_{hatch} / E) \times 100 \quad (2)$$

Determination of BSF life cycle duration

The sum of durations (days) of BSF life stages in each cycle gives the average duration of BSF life cycle (LC) which was calculated using (Eq.3). It includes duration of (i) Eclosion (Ec), (ii) Mating and Egg laying (MEg), (iii) Egg Hatching (EH), (iv) Larval Waste feeding (LW), (v) Prepupae to Pupae transformation (PP) The first five days of young larvae feeding in nursery boxes was also added.

$$BSF \text{ LC (days)} = Ec + Meg + EH + LW + PP \quad (3)$$

Comparison with Indonesia data

A 6 month rearing performance data collection (unpublished) was obtained from Puspa Agro FORWARD project BSF site in Sidoarjo, Indonesia and averages were calculated. BSF rearing performance was assessed by comparing the values of performance indicators of both regions.

Statistical analysis for BSF rearing performance data

The statistically significant difference of rearing performance indicators among BSF life cycles was determined using two-way ANOVA without replication ($p < 0.05$) followed by Tukey's Honestly Significant Difference (HSD) Post Hoc Test. Pearson's Correlation analysis followed by Regression test was applied to determine the statistically significant relationship between the number of emerged flies and prepupae and the number of eggs produced and hatched ($p < 0.05$).

Results and discussion

BSF emergence and BSFL hatching rate

The mean BSF emergence rate of 58.8% (SD 15.2) was achieved under outdoor ambient temperature range of (23.0-33.2°C) and RH (24.7-65.3%) that did not differ significantly ANOVA ($P > 0.05$) among 13 BSF life cycles (Table 1, 3). A previous study also produced comparable results on similar rearing mediums (Jucker et al., 2017). However, it is higher than the emergence rate of 21-27% obtained on artificial diets under controlled environmental conditions (Tomberlin et al., 2002). Rearing BSFL on nutrient rich (esp protein) diet may result in higher BSF survival from prepupae (Chia et al., 2018; Mohamad et al., 2020). In this study fruit and vegetable waste was used as a feeding substrate which is high in carbohydrates and relatively low in proteins. The number of emerged flies showed a significant positive correlation with the number of prepupae for each life cycle, $R = 0.75$ ($df\ 11$) = $p < 0.05$ (Pearson correlation and Regression analysis, Fig. 1). In this study moist compost was used as a suitable pupation medium in all the life cycles (Dortmans et al., 2017; Holmes et al., 2013). However, a relatively higher emergence rate was reported for a similar pupation substrate in a past study under controlled conditions (Holmes et al., 2013).

222

223 Figure 1: Relation between number of prepupae and flies emerged

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225 One female BSF can lay on average 200-900 eggs (Holmes et al., 2012; Liu, Wang, &
226 Yao, 2019; Nakamura et al., 2016; Rindhe et al., 2019; Sheppard et al., 1995; Tomberlin
227 et al 2002). The number of eggs produced per fly (mean 48 eggs/fly) was very small as
228 compared to the figures cited in literature. This might be due to the insufficient storage
229 of nutrients in prepupae required for post development and performing necessary
230 activities such as mating and egg production. Another important factor is the health of
231 flies. The nutrient content of larval feeding substrates directly or indirectly determines
232 the health status at adult stage (Chia et al., 2018) and may affect oviposition. In another
233 study, BSF reared on fruits and vegetable waste even under controlled laboratory
234 conditions did not produce the optimum number of eggs (Jucker et al., 2017). It is
235 supported by the observation made during the study that many flies were not able to walk
236 and fly, which might have affected the number of mating. A similar observation was
237 reported in a past study (Chia et al., 2018).

238 Lower percentage of female flies relative to males may result in lesser amounts of eggs
239 (Jucker et al., 2017). A very weak correlation was found between the number of flies and
240 number of eggs produced ($R = 0.31$, $df = 11$; $p > 0.05$). It is possible that in this experiment
241 more male flies emerged from pupae than the number of female flies. Moreover, the
242 mean weight of fly 0.014g, SD 0.003 found in this study is also comparable with the
243 weight of male adults reared on fruits and vegetables in a previous study (Jucker et al.,
244 2017). Rearing larvae is a relatively easy process, however achieving successful mating
245 is challenging (Sheppard et al., 2002). Mating and egg laying occurred in all BSF life

cycles and tolerated a wide range of temperature and humidity. Similar observations were made in a past study (Sheppard et al., 2002)

The mean BSFL hatching rate of 44.5% (SD 21.8) was achieved under an outdoor ambient temperature range of (26.4-35.0 °C) and RH (35.7-69.9%) that showed no significant difference ANOVA ($p>0.05$) among 13 life cycles (Table 1, 3). The hatching rate achieved in this study is higher than the 39.5% rate obtained under controlled environmental conditions of (25°C T; 70% RH) in a small-scale rearing facility (Nakamura et al., 2016). Past studies reported egg hatching rates of as high as 70-86% under favorable and controlled environmental conditions (Chia et al., 2018, Dortmans et al., 2017; Holmes et al., 2012).

Table 1: Successful BSF emergence and BSFL hatching in each life cycle

Figure 2: Relation between number of eggs produced and hatched (survived) in each BSF LC under observation ($r=0.92$, $p<0.05$)

The number of hatched larvae may depend on the number of eggs produced. Higher the number of eggs higher could be the number of hatched larvae. The number of hatched larvae showed a significant positive correlation with the number of eggs produced in each life cycle, $0.92 (11) = p<0.05$ (Fig. 2).

Figure 3: Relation between number of eggs produced and hatched (survived) in each BSF life cycle under observation.

Hatching rate was calculated with the count of 5-dol once the feed looks sieve able and larvae achieved considerable size. Therefore, it is possible that many of them had died earlier due to any environmental stress such as low or high feed moisture, dry weather, overcrowding and overheating in nursery boxes. Successful BSF rearing requires optimum temperature, ranging between (25-35°C) and a relative humidity of 70-90% (Chia et al., 2018; Dortmans et al., 2017; Holmes et al., 2012; Liu et al., 2019; Lohri et al., 2017). Lower humidity levels can dehydrate the pupae's outer membranes, desiccate eggs and shrink young larvae ultimately causing death (Holmes et al., 2012). The study resulted in a high survival rate (young larvae to preupae) of 91.4% (SD 1.6) which is comparable to the survival rate of larvae fed on fruit and vegetable waste in past studies under controlled conditions (Jucker et al., 2017; Lalander et al., 2019; Gold et al., 2020).

BSF life cycle duration

On average one life cycle of BSF took 49.5 days (SD 2.8; range 44-53 days) under outdoor ambient temperature and RH ranging between (22-35°C) and (24.7-89.3%) respectively (Table 2, Fig. 3). The life cycle duration differed significantly (ANOVA $p < 0.05$) among 13 BSF cycles (mean difference $> \text{HSD } 4.18$, Table 3) and is shorter as compared to 71 days in a previous study conducted on similar type of rearing substrate (Jucker et al., 2017). However, in an engineered rearing setup BSF life cycle completes within 40-45 days (Dortmans et al., 2017; Holmes et al., 2012; Sheppard et al., 1995; Tomberlin et al., 2002, Tomberlin et al., 2009).

Development from Prepupae to Fly

The mean fly eclosion duration of 15.6 days (SD 1.6; range 13-18days) was achieved under outdoor ambient temperature range of (23.1-32.9 °C) and RH of (39.3-70.9%) and it varied significantly ANOVA ($p < 0.05$) among 13 BSF cycles (mean difference $> \text{HSD } 4.1$, Table 2, 3). The results are comparable with the fly development duration achieved

in a previous study conducted on similar type of rearing substrates under the controlled environment (Jucker et al., 2017; Shumo et al., 2019). Moreover, eclosion occurred in all life cycles and it tolerated outdoor ambient weather conditions. Under optimized conditions adult fly emerge approximately 10-17 days after the initiation of pupation stage and may even extend to 5 months if conditions become unfavorable (Dortmans et al., 2017; Rindhe et al., 2019; Tomberlin et al., 2009). Therefore, the findings of present study are in accordance with the literature.

Table 2: Total duration of BSF life cycle and its life stages in a semi-arid region of Pakistan

Table 3: Analysis of variance for BSF rearing performance indicators (n=13, p<0.05)

Mating and Egg laying

The pre-oviposition duration takes 4 days (2 days mating and 2 days egg laying) after adult emergence under controlled environmental conditions (Dortmans et al., 2017; Holmes et al; 2012; Liu et al., 2019; Nakamura et al., 2016; Tomberlin & Sheppard 2002; Tomberlin et al., 2009). In this study similar pre-oviposition period of average 3.5 days (SD 0.5; range 3-4 days) was achieved under outdoor ambient temperature range of (22.1-33.7°C) and RH (24.7-65.3%) with no significant differences among 13 BSF cycles; mean difference<Tukey HSD 4.18 (Table 2, 3). Similar pre-oviposition duration was noted in another study conducted under higher range of ambient temperature between 28-38°C and 40-60% humidity. It might be possible that fly had stored enough fat reserves in its body at pupation stage that prevents prolonging of the pre-oviposition period (Tomberlin et al., 2002).

Development from Egg to Young Larvae

The mean egg hatching duration of 3 days (SD 0.6; range 2-4 days) was achieved under outdoor ambient temperature ranging between (25.5-34.0°C) and RH (27.0-89.3%) with

no significant differences among 13 BSF cycles; mean difference < Tukey HSD 4.18 (Table 2, 3). The results are comparable with the egg hatching duration of 3-5 days under controlled conditions (Chia et al., 2018; Dortmans et al., 2017; Holmes et al., 2012; Diener et al., 2011a; Sheppard et al., 1995). The results are also comparable with the hatching duration of 4 days found in a previous study performed under higher temperature and RH ranging between 28-38°C and 40-60% respectively (Nyakeri et al., 2017).

Development from 5-dol to Prepupae

The young larvae grew and transformed into prepupae in almost 22 days which includes 5 days feeding on chicken feed; 17 days waste feeding (SD 2.5; range 19-26 days) under outdoor ambient temperature ranging between (26.7-33.7°C) and RH (42.2-66.4%) (Table 2). The process resulted in up to 85% dry weight waste reduction, leaving behind a small proportion i.e., 15% of residue which is a stable soil conditioner. Waste feeding duration differed significantly ($p < 0.05$; mean difference > HSD 4.18) in all life cycles and is comparatively longer than 13-18 days ideal duration under optimum conditions (Dortmans et al., 2017; Holmes et al., 2013; Rindhe et al., 2019). At this stage BSFL can survive high temperatures of up to 45°C and lower humidity of 25% provided right quality and quantity of food. It is possible that feeding substrate might have delayed developmental duration as also reported in past studies (Jucker et al., 2017, Nguyen et al., 2013; Lalander et al., 2019). In this study, young larvae successfully developed and transformed into the sixth instar in all the BSF life cycles and tolerated outdoor ambient weather conditions.

Development from Prepupae to Pupae

In this study prepupae successfully transformed into pupae stage in a shorter duration of 3.8 days (SD 1.2 range 2-6) as compared to 7-8 days reported in literature (Chia et al.,

2018; Holmes et al., 2013, Rindhe et al., 2019). Transformation occurred in all life cycles with no significant difference in mean durations and tolerated outdoor ambient temperature ranging between (24.9-33.9°C) and RH (38.3-75.5%).

Comparing BSF rearing performance of Pakistan and Indonesia project site

In Indonesia, BSF rearing was performed following an engineered approach under suitable outdoor ambient conditions. The only environmental condition being controlled was the air replacement in the nursery room. Rearing performance of BSF biowaste processing facilities of both countries were compared to highlight the life stages more likely to be affected by dry semi-arid weather requiring special attention in future. In Indonesia also fruit and vegetable market waste was used as larval feeding substrate.

Table 4: Mean values representing BSF rearing performance of Pakistan and Indonesia facility

The study conducted in Pakistan resulted in lower mean BSF emergence and BSFL hatching rates but higher survival rate as compared to those obtained at FORWARD BSF site in Indonesia (Table 4). The degree of decrease for both performance indicators is nearly similar. Considering 50% of female BSF population, average number of eggs per fly was very small i.e., 96 as compared to 370 eggs laid per female fly at BSF site in Indonesia (Table 4). It implies that mating and egg laying stage was more affected and sensitive to arid hot environment as compared to fly emergence and larvae hatching. This might be due to ability of larvae to survive and tolerate in variety of biowastes and environmental conditions as already discussed above.

Figure 3: Comparison of BSF LC duration in outdoor ambient environmental conditions of Pakistan and Indonesia

In Pakistan, BSF took longer i.e., 49 days to complete its life cycle under semi-arid weather conditions as compared to 44 days in Indonesia's warm humid environment (Fig.3). Longer larval feeding stage of 22 days in Pakistan might have delayed life cycle duration (Fig. 3). In Indonesia, larval development on waste was engineered to 12 days (Fig.3) and then 17 days old larvae (5 days chicken feed+ 12 days waste, 17-dol) were transferred to larvero boxes for harvesting the prepupae. In Pakistan, 5-dol were fed on waste until majority of them turned to prepupae that might have prolonged the waste feeding stage and fully grown larvae resulted in higher survival rates. Moreover, transformation of prepupae to pupae took 3.8 days as compared to 2 days in Indonesia (Fig. 3).

Another operational difference encountered was the connection of dark cage to love cage. In this study, dark cage was connected to love cage on the onset of first batch of flies. This method was adopted to ensure flies of same age. However, in Indonesia, the dark cage stage duration was engineered to 15 days (Fig. 3). Moreover, several dark cages were being connected to one love cage in order to ensure enough number of flies for successful mating and eggs. In Pakistan's study, one dark cage was emptied in one love cage in order to note the exact duration for BSF life cycle. It is possible that collection of flies in love cage on daily basis from one dark cage might not have built sufficient flies density required for successful mating and resulted in smaller number of eggs. Moreover, Indonesia's warm (25-32°C) and very humid (70-90%) weather is more suitable for BSF rearing than the semi-arid region of the study conducted in Pakistan.

Conclusion

In this study BSF successfully developed into its six instars and completed all life cycles under observation, produced fertile eggs with high larval to pupae survival rates and tolerated outdoor ambient weather conditions of a semi-arid region in Pakistan. Information from this study is important for small scale ecopreneurs who cannot afford sophisticated and expensive facilities with controlled environmental conditions. Comparison of BSF rearing performance with Indonesia data concluded that mating and egg laying stages were more affected and sensitive to arid hot environment as compared to the flies' emergence and larvae hatching stage. The inability of flies to lay enough eggs may risk the continuity and completion of BSF life cycle unless enough amount of 6th instar (the prepupae) is available in stock. This stage can be improved by providing suitable environmental conditions and nutrient rich feeding substrate at larval development stage. Following recommendations may help in improving overall BSF rearing performance in Pakistan for future studies:

- Mixing fruit and vegetable waste with protein rich waste such as abattoir and/or kitchen waste at larva feeding stage
- Combined effect of substrate, temperature and humidity on BSF life cycle traits should be studied because it is very difficult to identify a single absolute factor affecting rearing performance.
- Monitoring of light intensity and analysing its effects on fly mating behaviour can help to improve BSF emergence rate in future studies.
- Higher hatching rates could be achieved by improving egg harvesting techniques.

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Table 1: Successful BSF emergence and BSFL hatching in each life cycle

Cycle no	BSF emergence rate	Total amount of eggs collected	No of eggs produced	No of hatched larvae	BSFL hatching rate	No of eggs/fly	Survival rate
	(%)	(g)			(%)		(%)
C1	55.7	2.6	89,655	29,520	32.9	9	90.3
C2	49.2	12.97	447,241	158,223	35.4	34	92.5
C3	78.2	3.76	129,655	52,442	40.4	11	91.2
C4	43.4	20.11	693,552	81,860	11.8	54	89.7
C5	54	8.65	298,276	28,714	9.6	27	93.6
C6	51.9	8.17	281,724	118,628	42.1	68	90.7
C7	82.4	2.89	99,655	20,165	20.2	10	88.9
C8	75	53.3	1,837,931	1,163,155	63.3	176	94.1
C9	68.7	19.86	684,828	135,424	19.8	103	92.6
C10	36.8	4.6	158,621	123,351	77.8	26	91.5
C11	64.6	5.9	203,448	113,181	55.6	28	93.4
C12	49.4	3.77	130,000	70,240	54	29	90.3
C13	82.3	20.44	704,828	470,028	66.7	46	89.6
SUM		167.02	5,759,414	2,564,931			
Mean	58.8				44.5	47.8	91.4

Table 2: Total duration of BSF life cycle and its life stages in a semi-arid region of Pakistan

Cycle no	Pupation to flies emergence	Mating+egg laying	Egg hatching	Waste feeding	Pp to pupae	BSF Eclosion	BSF life cycle
(days)							
C1	10	4	4	20a	5	15abc	48acdi
C2	14	4	3	22ab	3	17abc	51abdef
C3	12	3	3	25b	4	16abc	52aef
C4	13	3	3	19a	4	17abc	47bdi
C5	10	4	3	22ab	5	15abc	49abei
C6	13	4	2	26b	3	16abc	53ef
C7	10	3	3	20a	3	13b	44i
C8	12	3	3	23ab	6	18c	55f
C9	15	4	3	22ab	2	17abc	51adf
C10	11	3	3	25b	5	16abc	52af
C11	10	3	3	26b	3	13b	50adei
C12	14	4	2	19a	2	16abc	46ci
C13	10	3	2	22ab	4	14abc	46ci
Mean	11.85	3.46	2.85	22.38	3.77	15.62	49.54
SD	1.82	0.52	0.55	2.50	1.24	1.56	3.20

Note: Values with different alphabetical letters differed significantly from each other along the column (ANOVA $p < 0.05$, Tukey HSD test), values with no letters did not differ significantly

Table 3: Analysis of variance for BSF rearing performance indicators (n=13, $p < 0.05$)

BSF emergence and BSFL hatching rate						
Source of variation	SS	df	MS	F	P-value	F crit
Within the group	4340.99	12	361.74	1.04	0.47	2.68
Error	4166.508	12	347.2			
BSFL life cycle duration and its life stages						
Within the group	83.84	12	6.98	2.48	0.01	1.91
Error	168.76	60	2.81			

Table 4: Mean values representing rearing performance at Pakistan and Indonesia BSF

sites

BSF Rearing Performance Indicators	Pakistan (Urban unit, Sahiwal BSF site)	Indonesia (FORWARD project site-Sidoarjo)
BSF emergence rate (%)	58.8	82.4
Eggs/fly (#)	48	185
Eggs/female fly (assuming 50% male female population)	96	370
BSFL Hatching rate (%)	44.5	59.2
Survival rate (%)	70	91.4
BSF life cycle duration (days)	49.5	44

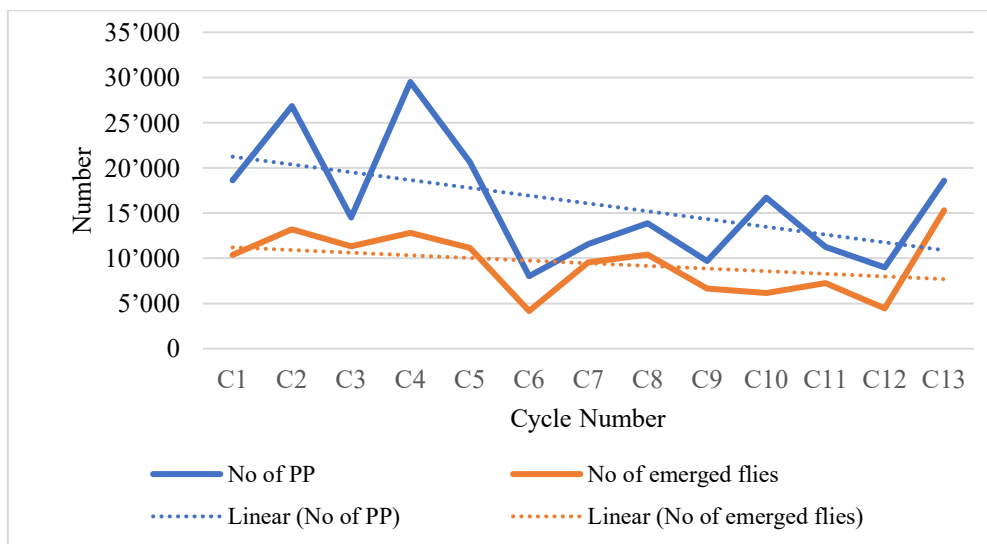


Figure 1: Relation between number of prepupae and flies emerged ($r=0.75$, $p<0.05$)

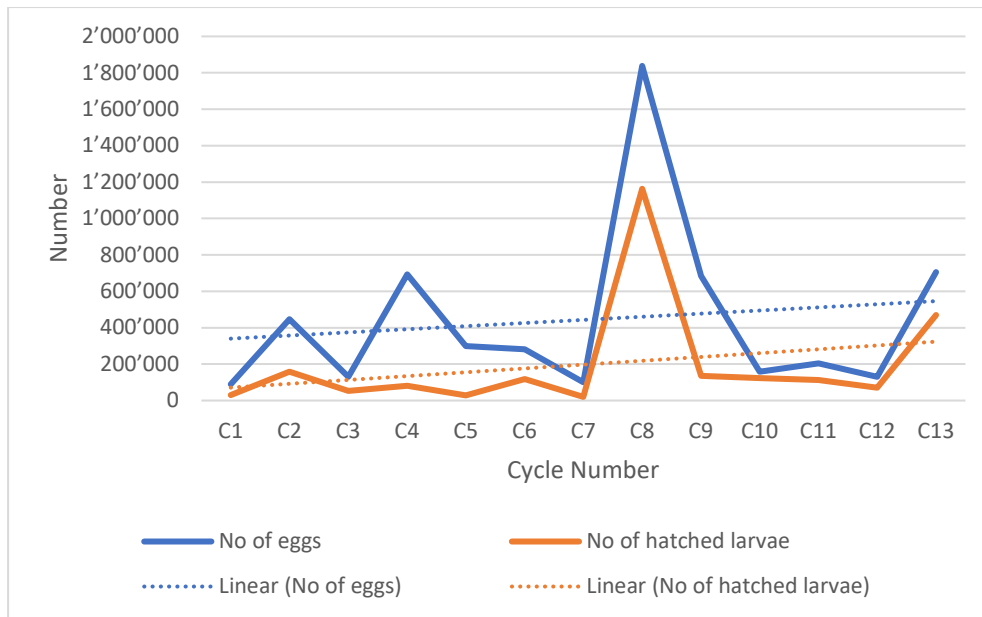


Figure 2: Relation between number of eggs produced and hatched (survived) in each BSF LC under observation ($r=0.92$, $p<0.05$)



Figure 3: Comparison of BSF life cycle duration in outdoor ambient environmental conditions of Pakistan and Indonesia