MORPHOMETRIC CHARACTERISTICS OF BLACK SOLDIER FLY AND THEIR GROWTH RATE ON DIFFERENT WASTE MATERIALS

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CERTIFICATE

This is to certify that thesis entitled, **"Morphometric characteristics of black soldier fly and their growth rate on different waste materials"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **Master of Science** in **Entomology,** embodies the result of a piece of Bona fede research work carried out by **Sunanda Sarkar Rumpa,** Registration **No. 15-06699** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: June, 2022
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DEDICATED TO
MY
MOTHER

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SAU, Dhaka **The Author**

Morphometric characteristics of black soldier fly and their growth rate on different waste materials

ABSTRACT

By

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A study was conducted in SAU Insectarium at Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh during the period of July to December, 2021 to study the morphometry and growth rate of black soldier fly in different waste materials.Two different experiments were conducted using CRD method. Four different type of waste materials with different level of nutrient content available for black soldier fly larvae were selected for the study namely WM_0: Control (Rice straw), WM_1: Kitchen waste, WM_2: Restaurant waste, WM_3: Fish garbage and WM_4: Leftover fruits, 4 replications were done to find out their efficacy on black soldier fly culture media.The life stages of black soldier fly was observed through the egg hatching (1-4 days), larval growth through six instars with five exuviate stages which took up to 13 to 18 days to complete. The last exuviate stage marks the transition from larva to prepupa, with ceasing of eating for further pupation (color: black). Male and female adult stage duration varied for 2 to 3 days although average adult stage duration was for 5 to 8 days for most of the black soldier flies. The mean larval period was observed as long as 15.50 days (±2.50). The mean egg to adult period for male black soldier fly was observed 45.10 days (±4.25), whereas for female black soldier fly the duration was recorded as 48.25 days (±4.13). Among the different waste materials Kitchen waste, Restaurant waste, Fish garbage and Leftover fruits considering black soldier fly culture, treatment WM_4: Leftover fruits showed better performance in completing larval period, egg to adult duration and fecundity (10.00, 46.67 and 528) and took minimum days for hatching (3.05) demonstrating rapid growth and development as well as best fecundity. Treatment WM_4: Leftover fruits showed better performance in egg to larval period and egg to adult growth rate (49.05% and 60.13%) resulting the most suitable culture media for black soldier fly commercial culture.

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LIST OF ABBREVIATION AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BARI	=	Bangladesh Agricultural Research Institute
BBS	=	Bangladesh Bureau of Statistics
FAO	=	Food and Agriculture Organization
et al.	=	And others
mg	=	Milligram
kg	=	Kilogram
SAU	=	Sher-e-Bangla Agricultural University
SRDI	=	Soil Resources Development Institute
g	=	Gram
cm	=	Centimeter
wt	=	Weight
LSD	=	Least Significant Difference
^{0}C	=	Degree Celsius
NS	=	Not significant
Max	=	Maximum
Min	=	Minimum
%	=	Percent
NPK	=	Nitrogen, Phosphorus and Potassium
CV%	=	Percentage of Coefficient of Variance
FY	=	Fiscal Year

CHAPTER I

INTRODUCTION

The black soldier fly (*Hermetia illucens*) is a common fly found in nature. Black soldier fly larvae are frequently found in manure in livestock operations and they feed on decomposing organic waste (Newton *et al.*, 2015). The black soldier fly, a harmless member of the Stratiomyidae family, has the potential to address two pressing issues in modern agriculture: providing an alternative source of protein for animal feed and properly managing organic wastes while also yielding a large amount of by-products and fertilizers (Taiwo and Otoo, 2013). The fly has a small lifetime of 6 to 10 days under mature settings and is similar in size to a honey bee, measuring between 13 and 20mm. Notably, it is not harmful or vector of any transformable contagious diseases (Tomberlin *et al.*, 2002). The insect is mostly found in tropical and sub-tropical warmer temperate countries (Diener *et al.*, 2011). However, the black soldier fly can tolerate extreme temperature throughout its life cycle, except during the oviposition period (Barry, 2004). The fly originated from North America, but climate change and human activities, animals, goods, foods, and fruit transportation worldwide facilitated its spread to other continents such as Europe, Asia, and Australia (Leek, 2017).

Black soldier flies are a highly effective waste control agent. Organic waste of either animal or plant origin, agricultural by-products, and other substrates are all edible to larvae. Due to the large decline in organic matter quantities brought on by this consumption, new waste treatment technologies utilizing insect bioconversion are now possible (De Marco *et al.,* 2015). The larvae also constitute a biomass that is rich in proteins and lipids and may be fed to animals, including fish and poultry (Cutrignelli *et al.*, 2018). There are several companies worldwide that produce black soldier flies larvae as feed (Kupferschmidt, 2015). Since black soldier flies larvae do not absorb pesticides or mycotoxins (Moula *et al.*, 2018), black soldier flies larvae are nowadays commercially raised on cereal by-products (Cutrignelli *et al.*, 2018). Nonetheless, apart from agricultural by-products, larvae could also be raised on animal manure and household organic waste. Although growth performance and meat quality of poultry fed on black soldier flie larvae raised on horse manure have been previously investigated (Schiavone *et al.,* 2017).

Scavengers like the larva of the black soldier fly are frequently utilized to hasten the decomposition of organic matter. According to Newton *et al.* (2015), black soldier fly larvae may effectively use organic resources including leftover food and garden trash. These substances are transformed by black soldier fly larvae into protein, keratin, fat, and other substances that may be used as high-quality feed in agriculture and aquaculture. This process can also be used as a sustainable way to manage garbage (Holmes *et al.,* 2016). Other benefits of black soldier fly larvae include the suppression of nuisance species like the house fly, *Musca domestica* L. (Diptera: Muscidae), the decrease of diseases and smells (Beskin *et al.,* 2018), and the elimination of pathogens, and antibiotics; and concerns with antibiotic-resistance in the associated microbial communities (Cai *et al.,* 2018). The black soldier fly larvae can, depending on the feeding medium, contain high concentrations of lipids (>30% of dry weight, dw) and protein (around 40% of dw) (Kroeckel *et al.,* 2012). Recently, interest in using black soldier fly larvae as a raw material for animal feed has increased, mainly due to its potential as a sustainable source of high-quality protein (Veldkamp *et al.,* 2012).

Many studies have investigated the use of biowaste and by-products, such as animal manure, brewery by-products, fish offal, and fruit and vegetable waste, for raising black soldier fly larvae (Meneguz *et al.,* 2018; Barroso *et al.,* 2019). These investigations have demonstrated that the larval rearing substrate significantly affects the composition and performance of the larvae. However, knowledge of the relationship between substrate and larval nutritional content is still sparse and conflicting. For instance, several research discovered that protein-rich substrates produced protein-rich larvae. (Meneguz *et al.,* 2018; Myers *et al.,* 2014) whereas another study found no such relation (Barragan-Fonseca *et al.,* 2018).

No studies have been conducted to date to test if black soldier fly larvae could be raised on household organic waste and then used as livestock feed due to several reasons, including restriction by sanitary laws and a lack of public acceptance (Moula *et al.,* 2018). Nonetheless, using black soldier fly raised on animal manure and household organic waste as animal feed could potentially offer a sustainable way to recycle untapped resources in waste (Marono *et al.,* 2017). The use of defatted black soldier flies larvae meal as feed for laying hens has been previously reported (Park *et al.,* 2017). Indeed, feeding defatted black soldier flies meal to laying hens was shown to either increase (Mwaniki *et al.,* 2018) or decrease (Ruhnke *et al.,* 2018) eggshell thickness and eggshell strength. Moreover, black soldier flies larvae meal has been found to contain chitin (Wa´sko *et al.,* 2016), an indigestible substance. When indigestible material reaches the cecum of laying hens it modulates gut microbiota and short chain fatty acids (SCFA) production (Borrelli *et al.,* 2016). In the context of Bangladesh very few studies were done on the morphometric characteristics and growth rate of 'Black soldier fly' on different waste materials. The present study was carried out to meet the following objectives:

1. To investigate the morphometric characteristics and life cycle of 'black soldier fly' on different waste materials.
2. To evaluate efficiency of different waste materials on 'black soldier fly' growth rate.

CHAPTER II

REVIEW OF LITERATURE

An attempt has been made to update our understanding of the morpho-physiology of black soldier flies, their seasonal distribution, and their rate of development on various waste sources. The best feasible summaries have been created after carefully examining all readily available and accessible sources of information. It should be mentioned that most of the material was gathered outside of Bangladesh. However, consideration was given to data that is pertinent to and potentially useful in the context of the morpho-physiology of black soldier flies and their rate of development on various waste materials.

2.1 Classification of Black soldier fly

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Stratiomyidae

Genus: *Hermetia*

Species: *Hermetia illucens*

2.2 Life cycle of Black soldier fly

The black soldier fly must complete different stages in its whole life cycle, i.e., undergo a complete transformation process. According to Newton *et al.* (2005), the black soldier fly insect has five distinct life cycle phases: egg, larvae, pre-pupae, pupae, and adult. The insect completes its full life cycle between 40 to 44 days, showed in Figure 01 (Fok, 2014). The fertilized eggs hatch between 102 to 105 hours at 24°C temperature (Li *et al.*, 2011).

Newly hatched black soldier fly larvae are creamy-white and actively crawl towards the substrate, where they vigorously feed on available food during this life phase. It takes approximately two weeks for the larvae to reach maturity (Sheppard *et al.*, 2002; Myers *et al.*, 2008).

The black soldier fly can convert organic waste into high-quality protein and fat biomass during the larval and pupal phases. In this bioconversion process, the wastes are reduced substantially, and larvae can consume harmful pathogens and minimize harmful effects (Erickson *et al.*, 2004).

During the pre-pupal stage, the mouthpart is turned into a hooklike structure for moving around called the 'wondering phase.' After their mouthpart has changed, they cannot accept feed and try to leave the substrate and position themselves for pupation. Their inherent behavior of not consuming food during pre-pupation can be utilized in mass-rearing of the insect and guide them for self-collection as pre-pupae (Diener *et al.*, 2011).

The larvae weigh about 0.2g and 25mm in length in the last stage and 6mm in diameter. Despite the small size, the larvae are tough and robust and can still survive extreme oxygen deprivation if need be (Sheppard *et al.*, 2002). Another five substages must pass within the larval stage (Hall and Gerhardt, 2001).

2.3 Environmental factors for growth

The past study reports that the black soldier fly is sensitive to abiotic factor of the environment (Mutafela, 2015). The required parameters may vary with the development stages. Therefore, to achieve successful breeding in confinement, the conditions need to be monitored and regulated to ensure the fly's requirements. The factors include temperature, relative humidity, light, diet, and pupation substrate.

2.4 Waste management by Black soldier fly

Essentially, waste treatment by the black soldier fly consists of feeding organic waste to black soldier fly larvae to produce energy-rich larvae and organic fertilizer. Several features of black soldier fly make the insect mostly eye-catching for valorizing municipal wastes. The black soldier fly larvae's voracious appetite for decaying organic matter enables efficient conversion of a wide range of organic waste materials. The shortness of the black soldier fly's lifecycle allows its frequent reproduction, therefore ensuring a steady source of larvae to convert the organic waste and a reliable supply of energy-rich larvae that can be used as animal feed. The black soldier fly's flexibility enables its rearing with any food waste and makes its utilization in garbage treatment with less effort. As the larvae after full-grown crawling out of the waste by themselves; therefore, prepupae can be easily harvested. An optimized waste reduction and biomass production require the manipulation of biological attributes of the black soldier fly to take advantage of the natural features of the black soldier fly in waste management. Besides, waste should be treated reliably and consistently to stabilize the treatment and production processes and facilitate operations (Zurbrügg *et al.*, 2018).

2.5 Beneficial culture of Black soldier fly

The bioconversion technology involves the artificially growing black soldier fly larvae on separated bio-wastes. The black soldier fly larvae grow on the waste piles from which they extract nutrients and reduce the waste mass. The larvae's polyphagous nature and robust digestive system enable them to feed on a wide range of decaying organic materials both of animal and plant origin. In contrast, the voracious appetite facilitates large amounts of organic waste consumption during their growth cycle (Mutafela, 2015). As a result, the environmental-friendly remediation potential through black soldier fly is described as one of the most encouraging and sustainable methods of handling organic waste (Zheng *et al.*, 2013).

A result of the process, high quality animal protein as black soldier fly larvae, is harvested and processed into animal feed and a human food supplement. The residual waste can also be processed further to soil enrichment to enhance microorganism-rich fertilizer. The purification of leftover feeds by the sterile and antifungal bustle of black soldier fly makes it harmless for crop production use (Banks *et al.*, 2013; Nguyen *et al.*, 2015).

2.5.1 Black soldier fly larvae as poultry feed

The black soldier fly larvae grown on pig waste or kitchen residues have been shown a satisfactory result as a feed supplement for juvenile chicks (Hale, 1973). Partial replacement of the soybean meal of 10-20% for broiler chicken showed the production performance, feed efficiency, mortality, and carcass traits like those fed on commercial feed (Cullere *et al.*, 2016).

Fifty percent or complete replacement of soybean meal with moderately de-oiled black soldier fly larval meal in the diet for egg laying chicken did not modify their laying performance nor feed efficacy than standard organic diets for layers (Maurer *et al.*, 2016).

The high apparent digestible energy and the amino acid apparent ideal digestibility coefficients of black soldier fly larval meal make it a valuable constituent for the formulation of broiler feeds (De Marco *et al.*, 2015). Moreover, Arango *et al.* (2005) recommended that the black soldier fly larvae have a moderate amount of mineral content for domestic birds' nutrition, according to their mineral requirements (NRC, 1993).

2.5.2 Black soldier fly larvae as fish feed

Protein replacement in fish diets has been investigated by several authors using black soldier fly meals, larvae and prepupae. The fish species which were used in the black soldier fly larvae meal-based research are Channel catfish (Ictalurus punctatus), Nile tilapia (Oreochromis aureus), Hybrid tilapia (Nile tilapia, Oreochromis niloticus) crossed with Sabaki tilapia (Oreochromis spilurus), Rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo Salar), Turbot (Psetta maxima), Walking catfish and Yellow catfish (Tachysurus fulvidraco) (Bondari and Sheppard, 1981; Furrer, 2011; Sealey *et al.*, 2011; Kroeckel *et al.*, 2012; Tanushri *et al.*, 2014; Zhang *et al.*, 2012; Lock *et al.*, 2015; Rana *et al.*, 2015).

Most of these research outcomes showed that only a low level of dietary black soldier fly larvae had shown a similar parallel performance to that of fish-fed traditional conventional feedstuff, which may be explained by high larval protein and minerals content (Zhang *et al.*, 2019). Fish feed in high inclusion level (>33%) knock-off fish growth, palatability of the diet, and fish's protein digestibility (Kroeckel *et al.*, 2012).

The type of substrate types on which black soldier fly larvae were reared brought up and the processing handling method might have affected their fishes' utilization. For example, a black soldier fly meal was included in the Atlantic salmon diet and replaced at least 50% in the Atlantic salmon diet without affecting the growth performance or fillet quality (Lock *et al.*, 2015).

The insect meal increases the amount of fat in fish muscle and changes the lipid nature of fishes considerably more significant than a fish meal (St-Hilaire *et al.*, 2007), hence, the taste of the fish fillets with a partial replacement of fish meal by insect meal (10-50%) in the diet of fishes does not affect the fatty acids (FA) profiles, aroma, and flavor significantly to the level that is perceived by the consumers (Makkar *et al.*, 2014). For instance, no difference in organoleptic properties was found in Atlantic salmon (Lock *et al.*, 2015) or rainbow trout (Sealey *et al.*, 2011) fed up with 50% black soldier fly meal.

2.5.3 Black soldier fly larvae as animal feed

Black soldier fly larval meal is a suitable feed ingredient in pig diets because of its high-level amino acids and calcium contents and good palatability. The live black soldier fly larvae and prepupae have been used to feed the animals like the alligators (*Alligator mississippiensis*) (Bodri and Cole, 2007) and highland frogs (*Leptodactylus fallax*) (Dierenfeld and King, 2008).

The complete replacement of formulated feed by live black soldier fly larvae to the young alligators resulted in lesser feeding and growth than the formulated feed. Besides, when black soldier fly larvae are fed to the Mountainous chicken frogs, poor nutritional assimilation results. It appears that whole black soldier fly larvae may be less beneficial for the animals that swallow their food entirely, as the frog and alligator. For instance, the digestibility of calcium rich black soldier fly larvae in mountain frogs was only 44% compared to 88% of black soldier fly larvae that had been used as "processed" (Dierenfeld and King, 2008).

On the contrary, the black soldier fly larvae have been successfully used in captive animals feeding and breeding for the lizards and amphibian species, mostly as the source of mineral contents (Dierenfeld and King, 2008). On the other hand, the frass of black soldier fly that reared on dried grains was assessed as feed for the Giant River prawn (*Macrobrachium rosenbergii*), resulted in similar performance as regular prawn feed with better economic outcomes (Tiu, 2012).

2.5.4 Bio-diesel production from Black soldier fly larvae

The biodiesel from black soldier fly larvae can be extracted and being explored. Several studies have been carried out on the fatty acids profile of black soldier fly larvae lipid and fat-fed on several feeds and wastes, such as food leftover (Surendra *et al.*, 2016; Zheng *et al.*, 2012), fruit waste (Leong *et al.*, 2016), sewage slurry (Leong *et al.*, 2016), domestic animals manure (Li *et al.*, 2011), palm canal cake (Leong *et al.*, 2016) and rice stubble (Zheng *et al.*, 2012), could be appropriate for biodiesel production.

Newton *et al.* (2005) reported that biodiesel's manufacture from the oil of black soldier fly larvae nurtured on swine dung would produce as much energy as anaerobic assimilation of a similar type of manure. Moreover, the biodiesel's energy properties produced from the oil of black soldier fly larvae fed on animal manure are equivalent to supplementary biodiesels of rapeseed oil-based biodiesel (Li *et al.*, 2011).

According to Zheng *et al.* (2012), the biodiesels manufactured from black soldier fly larvae developed on rice hay and hotel leftover food meet most European environmental standards. The oil properties of black soldier fly larvae-based fat are associated with those of rapeseed-oil-based biodiesel.

2.5.5 Chitin production from Black soldier fly larvae

The amino acid levels in black soldier fly larvae are high, even more, significant than the soymeal or the FAO Reference value of Protein (Makkar *et al.*, 2014).

Further, the high level of protein and lipid contents of black soldier fly larvae can be utilized to exploit animal nourishing and biodiesel production. Another valuable industrial product that could be extracted from black soldier fly larvae is chitin, a leading component of the larvae's exoskeleton. From the commercial point of view, chitin is an exciting compound because it shows high nitrogen content (6.9%) related to artificial cellulose (Diener, 2010; Caruso *et al.*, 2013).

The chitin is used as a chelating agent in medications, cosmetics, biotechnologies, phytosanitary, and other industrial products (Kumar, 2000; Caruso *et al.*, 2013; Younes and Rinaudo, 2015). Extraction of chitin from black soldier fly larvae and marketing in the markets could increase the profit derived from the maggots.

Nevertheless, the financial viability of the extraction of chitin from black soldier fly larvae has not yet been examined (Diener, 2010).

As the past research did not discuss the profit that could be projected from chitin production of black soldier fly larvae, it is hard to judge whether black soldier fly larvae constitute a reliable source of chitin, linked to crab and shrimp shells, that are so far the primary commercial sources of chitin (Younes and Rinaudo, 2015).

2.5.6 Organic fertilizer production from Black soldier fly larvae

Limited studies have explored the efficacy of black soldier fly waste residue, raw materials, or post-processed agricultural fertilizer for soil amendments. Choi *et al.* (2009) reported that the bioconversion of food waste produced promising results relating to the use of the waste residue (no information regarding the post-treatment) as an ancillary compound for conventional fertilizer. The researchers did not observe any significant difference between the black soldier fly waste residue's biochemical composition and an enhanced microbial fertilizer. Besides, scientists reported that the growth performances and chemical composition of Chinese cabbages grown on black soldier fly residue were like cabbages grown with commercial fertilizer. Likewise, agronomic experiments conducted in Ghana showed that the application of black soldier fly biofertilizer at the rate of 10 t/ha together with inorganic fertilizer could increase the crop production up to 55% compared to the application of inorganic fertilizer alone for various local short-cycle cash crops, especially shallots (onion) and maize. Furthermore, applying black soldier fly bio-fertilizer alone obtained better results than poultry manure combined with inorganic fertilizer (Adeku, 2015; Murray, 2016; Quilliam *et al.,* 2017).

On the other hand, Newton and his colleagues (2005) found poor growth performance of basil (*Ocimumb asilcum*) and sudangrass (*Sorghan sudanense*) grown on swine manure processed by black soldier fly larvae mixed with either mud or sand. The result could be attributed to the immaturity of the waste residue obtained from the black soldier fly larvae rearing process, as reported by Dortmans *et al.* (2017) and Lohri *et al.* (2017), which results in oxygen depletion in the soil following its application and inhibits plant growth (Brinton and Evans, 2001). Therefore, the waste must undergo a maturation phase (Dortmans, 2015; Lohri *et al.*, 2017; Dortmans *et al.*, 2017).

2.6 Financial benefit of Black soldier fly

Most of the financial benefits are associated with the black soldier fly larvae rearing skill because this handling method valorizes low-value biological waste into high-value protein and minerals at comparatively low cost (Spranghers *et al.*, 2017; Diener *et al.*, 2010). The significant economic benefits advised in the literature are the returns from the sales of the larvae and possibly the waste deposit and cost savings on controlling the organic waste due to bulk and noxious waste reduction (Barry, 2004; Amatya, 2008; Alvarez, 2012).

Consequently, to calculate the financial benefits of a black soldier fly handling skill for manure production, Amatya (2008) used two indicators, i.e., the bulk of waste reduction and the financial outcome of the larvae. Amatya (2008) also recommended evaluating the cost-effectiveness reduction of the putrescent waste, related to the black soldier fly rearing process, which is economical and improving the financial assessment, carried out by Newton *et al.* (2005).

2.7 Impact on environment by Black soldier fly

The most valuable protein-rich component for domestic animal feed is the soybean meal. Conversely, farming crops for livestock feeding puts stress on land and other resources, particularly in highly populated countries. Subsequently, these areas need to convert agricultural land or deforestation that threatens the tropical and sub-tropical forests, which are the harbours of biodiversity and supply necessary environmental facilities. Besides this harmful impact on the environment, conventional supply protein sources for humans are also not favourable from the financial perspective. On the other hand, waste management is most important for the environment as well as human health. As an alternative to animal feed products producing maggot meals from high-polluting waste piles or less cost food processing by-products, the black soldier fly larvae are nearly five times more environmentally friendly than manufacturing traditional crops-based feed products (Smetana *et al.*, 2016). In this process, the nutrient leakage reduction of the contamination-prone waste can be 50-60% (Newton *et al.*, 2005).

The black soldier fly larvae-based biodiesel production shows a higher conversion rate (460 Lt^{-1} of larvae, DW) and yields (30 to 50×106 L $ha^{-1}year^{-1}$) compared to typical crop-based biodiesel production (FAO 2008; Li *et al.*, 2011; Zheng *et al.*, 2013). Considering the odor reduction by black soldier fly larvae due to short waste processing period, less bacterial activity as larvae consume most of them, exposing and dehydrating the waste, scientists conclude that the black soldier fly larvae culture has an immense beneficial effect environment (Newton *et al.*, 2005).

2.8 Social impact of Black soldier fly

Conventional food production systems such as agriculture, aquaculture, and livestock rearing cannot cope with the current food demand (Foley *et al.*, 2011, Mekonnen and Hoekstra, 2012). As we tend towards 2050, food insecurity, shortage of protein, malnutrition, and sanitation inferiority are expected to be the major global dietary challenges (FAO, 2013). However, the situation will worsen in developing countries like Africa and Asia, where population growth is exponential and accounted for 98% of the people globally categorized as food insecure in 2011-2013 (FAO, 2013).

The overwhelming amounts of organic waste are generated by the rapidly increasing human population from diverse agricultural farms, municipal markets, households, supermarkets, industries, animal rearing and human settlements (VanHuis *et al.*, 2013).

The waste includes agricultural farm by-products, pre, and post-consumer food leftovers, expired manufactured foods, industrial waste streams, market waste, animal manures, and human faeces. In most developing countries, up to two-thirds of the generated organic waste is neither collected nor treated created nuisance and health hazards (Diener *et al.*, 2010; Taiwo and Otoo, 2013; Van Huis *et al.*, 2013). This vast proportion of wastes could be reused through black soldier fly bioaccumulation. In such venture, low earning poor people, as well as women, could be employed in black soldier fly farming. Gaining new, low-cost black soldier fly production facilities will definitely pave employment opportunities to the marginal population and increase economic and social safety.

2.9 Present status of Black soldier fly larvae farming

The reckless price hike of the conventional fish and poultry feeds and the vulnerability of the concerned industries have brought black soldier fly larvae culture under the shade of light for mass investment worldwide as an alternative feed source (Taiwo and Otoo, 2013).

The present study of black soldier fly larvae farming tries to complete the global black soldier fly market and demand various product types and applications. According to the market investigation of Meticulous Research (2019), the global black soldier fly market is segmented on product type (protein meal, bio-fertilizer, whole dried larvae, larvae oil, chitin/chitosan), application (animal feed, agricultural fertilizer, pet food, and others), and geography (North America, Europe, Asia-Pacific, Latin America, the Middle East, and Africa). Originally, Europe is likely to witness the rapid growth of black soldier fly farming during the prediction period. The fast growth of black soldier fly research in this region and attributed to the government approvals for using black soldier fly larvae as animal feed and pet food; sanction funding and investments in black soldier fly research and industry; and the presence of many entrepreneurs (Newton *et al.*, 2015).

There are some factors such as a large supply of organic wastes from agriculture, food and beverage sectors; high demand for protein-rich animal feed; also, supportive policies provided by the administration for black soldier fly farming, are positive people to do black soldier fly insect farming and their utilization in domestic animals and birds food, feed, and various industrial applications. Some of the progressive organizations working in the global black soldier market are Agri Protein (South Africa), Bioflytech (Spain), Enterra Feed Corporation (Canada), Entobel(Vietnam), Entofood (Malaysia), Entomo Farm (France), Enviro Flight (US), Hexafly (Ireland), F4F (Chile), Hermetia GmbH (Germany), Innova Feed (France), Protenga (Malaysia) and Protix (The Netherlands), etc. Notably, some pilot projects have been run in her-e-Bangla Agriculture University (SAU) and Bangladesh Agricultural University (BAU) to utilize black soldier fly in fish and poultry feed.

2.10. Future prospect of Black soldier fly farming

Larvae based animal feed and waste management The cost-beneficial effects of black soldier fly culture have drawn attention to people to utilize their wastes in a useful manner (Surendra *et al.*, 2016). The growing interest of people in black soldier fly culture is due to black soldier fly farming's profit without any investment. Nowadays, many farmers started their black soldier fly culture by using their wastage, mainly kitchen wastes. Moreover, people are more concerned about their health and the protection of the environment. For that, organic products from an eco-friendly culture system have a good market demand. From this point of view, we can forecast that for some days, we can see the transformation of Kitchen wastes and use it for black soldier fly culture (Matan, 2020).

The main attraction of this culture is using only home trash materials that we do not usually use. For that reason, it will be more popular with homemakers and agro-based people within a few days in countries like Bangladesh. On the other hand, environment specialists claim that aquafeed hampers the ecological balance and other environmental systems. Many fish are caught to produce the fish meal for fish and poultry feed production, which becomes a threat to fish biodiversity and caused many species extinction. To solve this problem by keeping a balanced environment, we should search for an alternative protein source for feed production, while black soldier fly larvae protein could be a potential replacement for fish meal (Belghit *et al.*, 2018).

CHAPTER III

MATERIALSAND METHODS

3.1 Location of the Experiment

The experiment was conducted in the Entomology laboratory, under the Department of Entomology at Sher-e-Bangla Agricultural University (SAU), Dhaka Bangladesh during the period of July to December, 2021 to study the morphometry, growth rate in different waste materials of black soldier fly.

3.2 Climate of the experiment area

The experiment was conducted in SAU Insectarium under required temperature (26-30 degree centigrade) and humidity (65-70%) at Sher-e-Bangla Agricultural University (SAU), Dhaka. The detailed meteorological data in respect of temperature, relative humidity and total rainfall recorded by the Weather Station of Bangladesh, Shere-Bangla Nagar, Dhaka during the period of study have been presented in Appendix II.

3.3 Selection of waste materials

Four different type of waste materials with different level of nutrient content available for black soldier fly larvae were selected for the study namely kitchen waste, restaurant waste, fish meal and leftover fruits, and control to find out their efficacy.

3.3.1 Preparation of waste materials

The waste materials consisted of different kitchen waste, restaurant waste, fish garbage and leftover fruits, collected from nearby market, student hall and residential areas of Sher-e-Bangla Agricultural University (SAU), Dhaka. Waste materials were collected in plastic bags during transport. At the insectarium, different waste materials and control medium according to the experimental design were set up in a fly net enclosed wooden case and the humidity and temperature was maintained for ensuring the suitable rearing and growth environment for black soldier fly larvae. Separation of wate materials were presented in Plate 01.

Plate 01. Separation of wate materials

3.3.2 Rearing of black soldier fly larvae

Feeding medium was added daily until the control group reached harvest size (individual larvae weight ~100 mg, total feeding period was 8 days). The larvae were kept in complete darkness during the experiment, only while feed was added light was present. Feeding media was added depending on how much uneaten media was left in the crates at feeding and according to the size of the larvae. Total feed added per crate was recorded daily. At the end of the trial, total weight of larvae and waste material were recorded per crate. The larvae were grown at 30˚C with 65% relative humidity. At the end of the eight-day growth period the larvae were separated from the feeding media by manual sieving (Plate 02).

Plate 02. Egg collection and hatching.

3.4 Trapping of Black soldier flies through attractant

Black soldier flies that are adults can be found in nature. Establishing some basic natural black soldier fly attractants, such as fruit scraps in a plastic bowl, will encourage female black soldier fly to deposit an egg. This is one approach of employing natural attractants. Water, overripe bananas or mangos, rice bran, and a plastic bowl are the key ingredients for constructing an attractant. In order to generate a soft dough-like consistency, water was added to 500g of rice bran and straw dust in a plastic dish. This mixture was then stored in a cold, dark location for three days until it began to emit an offensive odor. Then 3-4 banana were blended and mixed with the mould. The mixture was placed in a plastic bowl and setup using wooden structure made out of four pair of wood plank for egg laying as shown in Plate 03.

Plate 03. Attractant for black soldier fly

3.5 Design of the experiment

Two different experiments were conducted using CRD method with 4 replications.

Experiment I: Identification and morphometric characteristics study of the collected specimen

Experiment II: Growth rate evaluation in 4 different waste materials

WM_1: Kitchen waste (Leftover parts of vegetables)

WM_2: Restaurant waste (Organic portion of restaurant waste)

WM_3: Fish garbage (Fecals, scales etc)

WM_4: Leftover fruits (Different wasted fruits parts)

WM_5: Control (Dried Rice straw in chipped form)

Total treatments combination for the experiment were (4×5) 20.

3.6 Identification and study of the collected specimen

The specimens were kept in a dry state until observation. After the observation, the sample were washed and preserved with ethanol. Morphological identification was made under a stereoscopic microscope. For observation of the terminalia, male 20 abdomens were treated with 10% KOH and transferred to distilled water for dissection as needed. They were placed on double-sided tape and examined under a stereoscopic microscope. Wings were mounted on prepared slides using gum-chloral mounting media and the length was measured under stereoscopic a microscope.

3.7 Data Collection

Data were collected on the following parameters under stereo microscope:

i. Head
ii. Thorax and
iii. Abdomen

Head, thorax, abdomen and wing were observed under stereomicroscope and photograph of individual sample was captured.

For growth rate study data were collected on the following parameters:

i. Incubation period
ii. Larval period
iii. Pupal period
iv. Egg to adult
v. Adult longevity
vi. Total life span

3.8 Statistical analysis

The data obtained for different characters were statistically analysed to find out the significance of found results. Percent growth rate data were analysed using computer based Statistix-10 program for CRD and means separation was done by the Least Significant Difference Test (LSD).

CHAPTER IV

RESULT AND DISCUSSION

The study of morphology is a common means of biological grouping and classification. The morphometric analysis was done at Entomology lab of central laboratory, Sher-e-Bangla Agricultural University. The black soldier flies were identified using conventional taxonomy on most morphological characters in adult stages which were the most important stage of detection of any pest for successful management.

4.1 Morphometric characteristics and life stages of black soldier fly

The different population attributes resulted from the experiments were carried out to study the morpho-physiology and life stages of black soldier fly under laboratory condition.

4.1.2 Development of black soldier fly

The egg length was observed 1.02 mm (±0.25 S.E.). After hatching the length of larvae was found 20.50 mm (±2.15 S.E.) and weight was measured 0.25g (±0.05 S.E.). Completion of larval period pupal length and weight was measured and found length of pupa 18.33 mm (±2.50 S.E.) and weight was measured 0.10g (±0.03 S.E.). The larva diet can affect the size of adult black soldier fly (Newton *et al.*, 2015). This finding means that the artificial feed with different waste materials used in this study can provide adequate nutrition for black soldier fly growth and is beneficial for their breeding. The body and wing lengths of female adults are slightly larger than those of male adults for male adult black soldier fly length was measured 12.33 mm (±4.15 S.E.) and weight was measured 9.5g (±3.25 S.E.). considering female adult length was found 13.50 mm (±5.10 S.E.) and weight was measured 10.33g (±2.67 S.E.), and these results are consistent with those of a previous study (Cai *et al.*, 2018). This may be because female bodies need to prepare for future spawning. The details of external morphological structure of female and male adult black soldier fly are characterized and show no statistically significant difference. All external morphological structures conform to the characteristics of *H. illucens.*

Table 01. Body length (mm) and weight (g) at different stages of black soldier fly

Attributes	**black soldier fly**	
	Mean	**S.E.**
Egg		
Length (mm)	1.05	±0.25
Larvae		
Length (mm)	20.50	±2.15
Weight (g)	0.25	±0.05
Pupa		
Length (mm)	18.33	±2.50
Weight (g)	0.10	±0.03
Male		
Length (mm)	12.33	±4.15
Weight (g)	9.05	±3.25
Female		
Length (mm)	13.50	±5.10
Weight (g)	10.33	±2.67

Major stage of black soldier fly both for future culture and commercial feed alternatives larva and its growth is the measured in five different durations. Significant growth was observed considering the body length. For 1-2 day of larval growth body length was observed 1.5-2.0 mm (±0.50 S.E.), 4-5 days body length increased and measured at 3.5-4.0 mm (±1.00 S.E.), at 6-7 days the length was 6.5-8.0 mm (±1.00 S.E.) and at the final stage of larval development 10-12 days old larva was measured at 10.0-15.0 mm (±2.50 S.E.) of body length (Table 02). Similar changes in larval length was also observed by Cutrignelli *et al.*, (2018).

Table 02. Larval growth of black soldier fly

Larval age (Days)	Body length (mm)
1-2	1.5-2.0 (±0.50)
4-5	3.5-4.0 (±1.00)
6-7	6.5-8.0 (±1.00)
10-12	10.0-15.0 (±2.50)
18-30	15.0-20.0 (±3.00)

Male Female

Figure 01. Adult black soldier fly

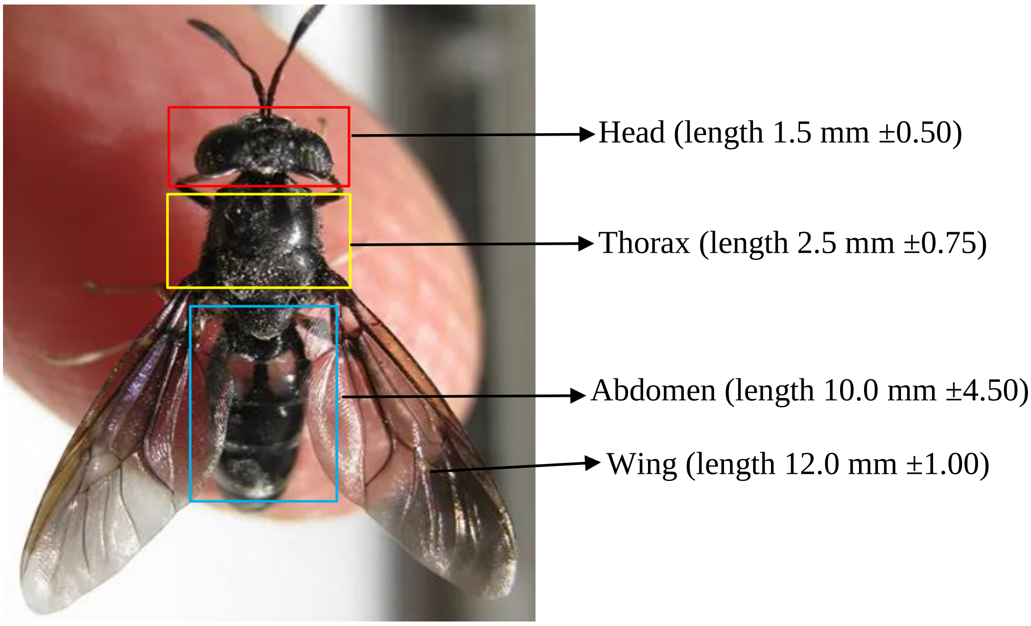

Figure 02. Abdomen, head and wing of adult black soldier fly

4.2.1 Life stages of black soldier fly

Life stages of black soldier fly was observed for the egg hatching (1-4 days), the black soldier fly larvae grow through six instars with five exuviate stages taken up to 13 to 18 days to complete, and this phenomenon is consistent with previous research (Miranda *et al.,* 2019). Results show that the first four exuviate stages of the black soldier fly larvae occur at the rapid growth stage (1–12 days), and each exuviate tends to be associated with a doubling of body size. The last exuviate stage marks the transition from larva to prepupa, with ceasing of eating for further pupating (color: black). The black soldier fly larvae development time, which also affects the exuviate time, is influenced by diet, temperature, toxic compounds, and water content (Miranda *et al.,* 2019). The pre pupal stage as the last larval development stage took 8 to 10 days to complete, and pupal duration was observed for 10 to 30 days. Male female adult stage duration varied for 2 to 3 days although average adult stage duration was for 5 to 8 days for most of the black soldier fly (Figure 3 and Table 3).

Table 03. Life stages of black soldier fly

Growth stage	**Egg**	**1st instar larva**	**2nd instar larva**	**3rd instar larvae**	**4th instar larva**	**5th instar larva**	**Pre-pupa (6th instar larva)**	**Pupa**	**Adult lifespan**
Duration	4±0.75 days	3.25 ±0.25 days	3.45 ±0.75 days	4±0.25 days	3.90 ±0.35 days	4.20 ±0.20 days	6±0.75 days	10±0.75 days	7±0.85 days

[Temperature: 26℃; RH: 70%-80%]

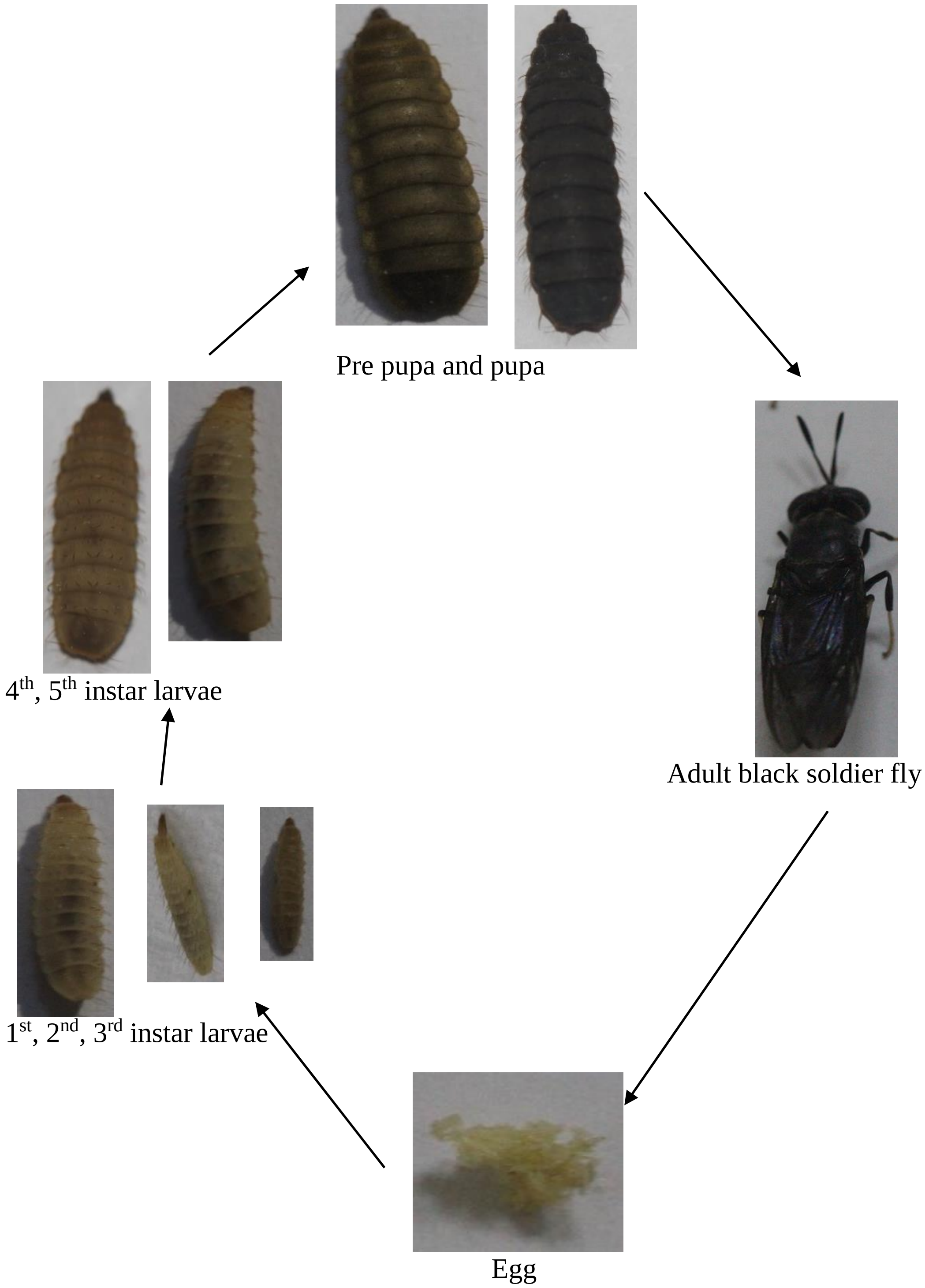

Figure 03. Life stages of black soldier fly

4.2. Growth and development of black soldier fly

Variations amongst the growth and developmental periods of black soldier fly were observed during the study.

4.2.1. Incubation period of black soldier fly

The mean incubation period was 4.33 days (±0.25).

4.2.2. Larval period of black soldier fly

The mean larval period was observed as long as 15.50 days (±2.50).

4.2.3. Pupal period of black soldier fly

Mean pupal period was required 15.00 days (±2.33).

4.2.4. Egg to adult period of black soldier fly

The mean egg to adult period for male black soldier fly was observed 45.10 days (±4.25), whereas for female black soldier fly the duration was recorded as 48.25 days (±4.13).

4.2.5. Adult longevity of black soldier fly

The adult longevity for both male and female black soldier fly was recorded and found female adult longevity significantly higher compared with male adult black soldier fly and these findings also support the results of (Miranda *et al.*, 2019). From the present study, the duration for adult longevity for male black soldier fly was recorded 9.45 days (±1.50), whereas for female black soldier fly the duration was 10.67 days (±1.25).

Table 04. Variations of growth and developmental period (days) of black soldier fly

Developmental period	Developmental period (days)	
	Mean	**S.E.**
Incubation	4.33	±0.25
Larvae	15.50	±2.50
Pupa	15.00	±2.33
Egg to adult (male)	45.10	±4.25
Egg to adult (Female)	48.25	±4.13
Adult longevity (male)	9.45	±1.50
Adult longevity (Female)	10.67	±1.25

4.2 Growth and development of black soldier fly in different waste materials

4.2.1 Growth rate (days) of black soldier fly

Four different waste materials was considered for the following study to find out the most suitable growing environment and dietary requirements for black soldier fly larvae. Kitchen waste, Restaurant waste, Fish meal and Leftover fruits was considered and black soldier fly culture was set up in plastic container with these following waste materials. The duration (days) required for hatching, larval period, egg to adult duration and fecundity of black soldier fly female was measured. Among these waste materials WM_4: Leftover fruits showed better performance in larval period, pupal period egg to adult duration and fecundity (10.00, 7.15, 46.67 and 528) and took minimum days for egg to larval growth (3.05) demonstrating rapid growth and development as well as best fecundity. On the other hand, maximum egg to larval development duration was observed in WM_0: Control treatment (4.15). WM_0: Control also caused longest larval period, pupal period, egg to adult duration and fecundity (18.50, 8.57, 60.33 and 502) making it unsuitable for commercial black soldier fly culture (Table 5). Similar findings were also observed by (Matan, 2020).

Table 05. Growth and development of black soldier fly in different waste materials

Treatments	Growth rate (days) of black soldier fly				
	Egg to larval	Larval period	Pupal period	Egg to adult	Fecundity
WM_0	4.15a	18.50a	8.57a	60.33a	502d
WM_1	3.95bc	14.15b	8.00b	50.13bc	513c
WM_2	4.10ab	10.28cd	7.33cd	48.50bc	525ab
WM_3	4.03ab	11.33c	7.50c	52.10b	522b
WM_4	3.05c	10.00d	7.15d	46.67c	528a
LSD (0.05)	0.64	1.22	0.31	4.05	4.18
CV (%)	8.91	7.10	5.89	7.35	6.52

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by LSD at 5% level of probability.

[Note: WM_0: Control (Rice straw), WM_1: Kitchen waste, WM_2: Restaurant waste, WM_3: Fish garbage and WM_4: Leftover fruits]

4.2.2 Growth rate (%) of black soldier fly

Among the different waste materials Kitchen waste, Restaurant waste, Fish meal and Leftover fruits considered and black soldier fly culture The egg to larval growth and egg to adult growth (%) was measured. Among these waste materials WM_4: Leftover fruits showed better performance in egg to larval period and egg to adult growth rate (49.05% and 60.13%) resulting the most suitable nutritional requirement for black soldier fly culture. on the other hand, lowest egg to larval period and egg to adult growth rate was observed in WM_0: Control treatment (34.33% and 41.21%). Similar findings was also observed by (Matan, 2020).

Table 06. Growth rate (%) of black soldier fly in different waste materials

Treatments	Growth rate (%) of black soldier fly	
	Egg to larval	Egg to adult
WM_0	34.33e	41.21d
WM_1	37.67d	46.33c
WM_2	44.19b	54.90b
WM_3	41.50c	51.58bc
WM_4	49.05a	60.13a
LSD (0.05)	2.95	4.93
CV (%)	10.03	8.19

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by LSD at 5% level of probability.

[Note: WM_0: Control (Rice straw), WM_1: Kitchen waste, WM_2: Restaurant waste, WM_3: Fish garbage and WM_4: Leftover fruits]

4.2.3. Total life span of (Egg to adult)

Considering selected waste materials, Kitchen waste, Restaurant waste, Fish garbage and Leftover fruits for black soldier fly culture in the study, the total life span (days) was measured. Among these waste materials WM_4: Leftover fruits showed better performance (48.33 days ±3.15 S.E.) resulting the most suitable culture media for black soldier fly commercial culture. On the other hand, highest total life span was observed in WM_0: Control treatment (64.20 days ±6.00 S.E.) (Table 6).

Table 07. Total life span of black soldier fly in different waste materials

Treatments	Total life span (days) of black soldier fly	
	Mean	**(±) S.E.**
WM_0	64.20a	6.00
WM_1	51.13cd	3.08
WM_2	56.95b	5.60
WM_3	53.50bc	5.25
WM_4	48.33d	3.15
LSD (0.05)	4.22	--
CV (%)	6.31	--

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by LSD at 5% level of probability.

[Note: WM_0: Control (Rice straw), WM_1: Kitchen waste, WM_2: Restaurant waste, WM_3: Fish garbage and WM_4: Leftover fruits]

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted in SAU Insectarium at Sher-e-Bangla Agricultural University (SAU), Dhaka Bangladesh during the period of July to December, 2021 to study the morphometry, growth rate in different waste materials of black soldier fly two different experiments were conducted using CRD method. Four different type of waste materials with different level of nutrient content available for black soldier fly larvae were selected for the study namely WM_0: Control (Rice straw), WM_1: Kitchen waste, WM_2: Restaurant waste, WM_3: Fish garbage and WM_4: Leftover fruits, 4 replications were used to find out their efficacy for black soldier fly culture media.

The egg length was observed 1.02 mm (±0.25 S.E.). After hatching the length of larvae was found 20.50 mm (±2.15 S.E.) and weight was measured 0.25g (±0.05 S.E.). Completion of larval period pupal length and weight was measured and found length of pupa 18.33 mm (±2.50 S.E.) and weight was measured 0.10g (±0.03 S.E.). The body and wing lengths of female adults are slightly larger than those of male adults for male adult black soldier fly length was measured 12.33 mm (±4.15 S.E.) and weight was measured 9.5g (±3.25 S.E.). considering female adult length was found 13.50 mm (±5.10 S.E.) and weight was measured 10.33g (±2.67 S.E.). Major stage of black soldier fly both for future culture and commercial feed alternatives larva and its growth is the measured in five different durations. Significant growth was observed considering the body length. For 1-2 day of larval growth body length was observed 1.5-2.0 mm (±0.50 S.E.), 4-5 days body length increased and measured at 3.5-4.0 mm (±1.00 S.E.), at 6-7 days the length was 6.5-8.0 mm (±1.00 S.E.) and at the final stage of larval development 10-12 days old larva was measured at 10.0-15.0 mm (±2.50 S.E.) of body length. Life stages of black soldier fly was observed for the egg hatching (1-4 days), the black soldier fly larvae grow through six instars with five exuviate stages taken up to 13 to 18 days to complete. Results show that the first four exuviate stages of the black soldier fly larvae occur at the rapid growth stage (1–12 days), and each exuviate tends to be associated with a doubling of body size. The last exuviate stage marks the transition from larva to prepupa, with ceasing of eating for further pupating (color: black). The pre pupal stage as the last larval development stage took 8 to 10 days to complete, and pupal duration was observed for 10 to 30 days. Male female adult stage duration varied for 2 to 3 days although average adult stage duration was for 5 to 8 days for most of the black soldier fly.

The mean incubation period was 4.33 days (±0.25). The mean larval period was observed as long as 15.50 days (±2.50). Mean pupal period was required 15.00 days (±2.33). The mean egg to adult period for male black soldier fly was observed 45.10 days (±4.25), whereas for female black soldier fly the duration was recorded as 48.25 days (±4.13). From the present study, the duration for adult longevity for male black soldier fly was recorded 9.45 days (±1.50), whereas for female black soldier fly the duration was 10.67 days (±1.25). The black soldier fly samples were collected at different locations of SAU campus using pheromone trap (methyl-eugenol) at different waste dumping zones thought-out the experimental duration and number of black soldier fly was counted. Each trapping period was 48 hours and per days number of black soldier fly was collected and recorded from all the locations. The number found significantly higher in the months when warm and humid weather was available (July to September) but the number of black soldier fly drastically reduced in relatively colder months (November and December).

The duration (days) required for hatching, larval period, egg to adult duration and fecundity of black soldier fly female was measured. Among these waste materials WM_4: Leftover fruits showed better performance in larval period, pupal period egg to adult duration and fecundity (10.00, 7.15, 46.67 and 528) and took minimum days for egg to larval growth (3.05) demonstrating rapid growth and development as well as best fecundity. On the other hand, maximum egg to larval development duration was observed in WM_0: Control treatment (4.15). WM_0: Control also causes longest larval period, pupal period, egg to adult duration and fecundity (18.50, 8.57, 60.33 and 502) making it unsuitable for commercial black soldier fly culture. Among the different waste materials Kitchen waste, Restaurant waste, Fish meal and Leftover fruits considered and black soldier fly culture The egg to larval growth and egg to adult growth (%) was measured. Among these waste materials WM_4: Leftover fruits showed better performance in egg to larval period and egg to adult growth rate

(49.05% and 60.13%) resulting the most suitable nutritional requirement for black soldier fly culture. on the other hand, lowest egg to larval period and egg to adult growth rate was observed in WM_0: Control treatment (34.33% and 41.21%). Considering selected waste materials Kitchen waste, Restaurant waste, Fish meal and Leftover fruits for black soldier fly culture in the study, the total life span (%) was measured. Among these waste materials WM_4: Leftover fruits showed better performance (48.33 days ±3.15 S.E.) resulting the most suitable culture media for black soldier fly commercial culture. on the other hand, highest total life span was observed in WM_0: Control treatment (64.20 days ±6.00 S.E.).

In conclusion among the different waste materials Kitchen waste, Restaurant waste, Fish garbage and Leftover fruits considered and black soldier fly culture, WM_4: Leftover fruits showed better performance in larval period, egg to adult duration and fecundity (10.00, 46.67 and 528) and took minimum days for egg to larval growth (3.05) demonstrating rapid growth and development as well as best fecundity. WM_4: Leftover fruits showed better performance in egg to larval period and egg to adult growth rate (49.05% and 60.13%) resulting the most suitable culture media for black soldier fly commercial culture among these four treatments.

CHAPTER VI

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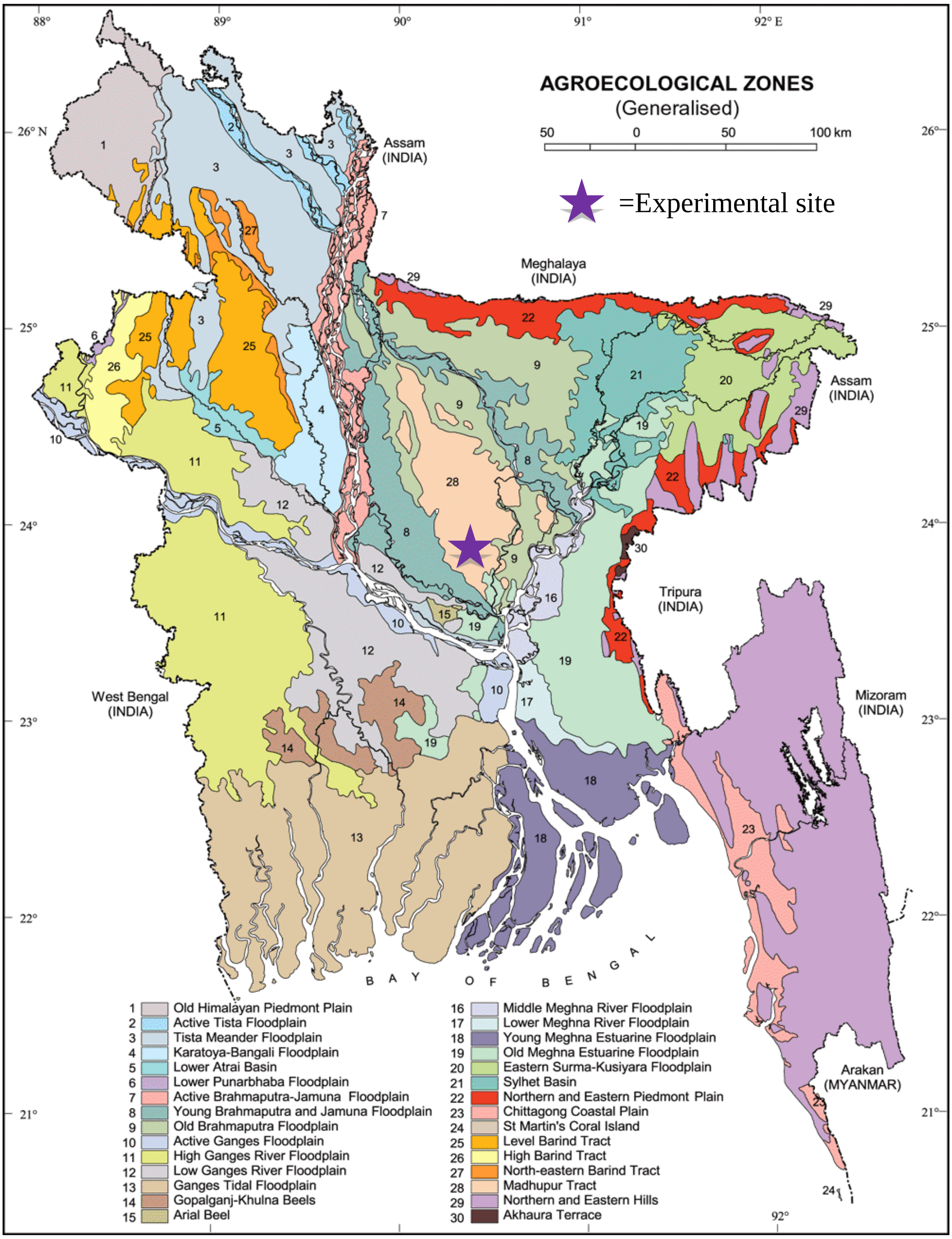
APPENDICES

Appendix I. Map showing the experimental site under study



Appendix II. Monthly meteorological information during the period from July to December, 2021

Year	Month	Air temperature (^0C)		Relative humidity (%)	Total rainfall (mm)
		Maximum	Minimum		
2021	July	25.20	12.80	69.00	00
	August	27.30	16.90	66.00	39
	September	31.70	19.20	57.00	23
	October	33.50	25.90	64.50	119
	November	28.10	11.83	58.18	47
	December	25.00	9.46	69.53	00

Source : Meterological Centre, Agargaon, Dhaka (Climate Division)