UNIVERSITY OF CAPE COAST

ESTABLISHMENT OF LABORATORY COLONY OF BLACK SOLDIER FLY, *Hermetia illucens* L. (DIPTERA: STRATIOMYIDAE)

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2019

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BY

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Thesis submitted to the Department of Conservation Biology and Entomology, School of Biological Sciences of the College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfillment of the requirements for award of Doctor of Philosophy degree in Entomology

JUNE, 2019

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ..... Date: .....

Name: Ebenezer Ato Ewusie

Supervisors' Declaration

I hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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ABSTRACT

Black soldier fly larvae are converters of organic waste into edible biomass and organic residue. This study investigated the feasibility of establishing indigenous black soldier fly, *Hermetia illucens* colony on different diets. Weighed oviposition traps were placed on five microhabitats to collect wild egg clutches which were separately incubated in the laboratory on layer meal diet. Thereafter, larvae were reared on layer meal-wheat bran mixtures and on market waste fractions. Five- day old larvae were reared in 80 litre barrels at different larval densities; 3,600, 4,800, 6,000 and 7,200 per 30kg dry weight of formulated organic market waste to study the effect of density on larval growth and biomass accumulation. Larvae of different ages; 4, 8 and 12 days old were exposed to different temperatures; 35, 40, 45 and 50°C and reared on layer meal diet to investigate the effects of temperature and age on larval growth and development. *H. illucens* colony was successfully established from 57 wild egg clutches collected. Market waste fractions as larval diet had significant effect on larval biomass and fecundity. Larval density of 4,800 produced significantly the highest growth in length and specific growth rate of larvae. Age and temperature significantly influenced larval development and fecundity. It was observed that larvae can withstand at least 4 hours of sustained high temperatures up to 45ºC and develop to adult. Wheat bran, layer meal-reduced diet mixtures, vegetable waste, fruit waste, uncooked food waste and their combinations were able to produce larval biomass and growth rates that were similar to layer meal and therefore could be substitutes for the standard layer meal diet, thus reducing cost of diet for mass rearing of black soldier fly.

ACKNOWLEDGEMENTS

I thank God Almighty for the gift of life and sound health that enabled me complete this work. My sincerest appreciation goes to my supervisors, Pastor Professor Peter Kofi Kwapong, Professor Godfred Kwabena Ofosu-Budu, and Dr. Christoph Sandrock for their forbearance, inexorable direction, exceptional suggestions, indefatigable assistance, critical reading of the thesis. I acknowledge with gratitude the sponsorship provided by the Swiss National Science Foundation and coordinated by Dr. Noah Adamtey and his team at the Research Institute of Organic Agriculture (FiBL) Switzerland.

I am highly indebted to Messrs; Eben Agbo Brown, Abdulia Suraji, Joesph Boakye-Ansah Karmer, Ernest Yeboah, Daniel Akuffu and Miss Shirley Boakye-Ansah for the technical support they offered. I am also grateful to faculty members of the Department of Conservation Biology and Entomology, School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, Ghana. I appreciate the assistance given by the Farm Hands at the Soil and Environmental Science Research Centre. I am again grateful to the Biotechnology and Nuclear Agriculture Research Institute and the Ghana Atomic Energy Commission. Miss Benedicta Essel deserves special thanks for her assistance in my experimental design and statistical analysis.

DEDICATION

I dedicate this work first to God Almighty and in memory of my grandmother, Madam Rosina Ama Owusuaa for her fortitude, foresight and upbringing.

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KEY WORDS

Black soldier fly

Microhabitat

Egg trap

Waste dump

Egg clutch

Feedstock

CHAPTER ONE

INTRODUCTION

The black soldier fly, *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae), is wasp-like and 15-20 mm long (Nguyen et al., 2015). It is a valuable non-pest tropical and warm temperate region insect which can possibly be used to uprade the 3 million tons of Ghana's low value organic waste streams per year (Samwine et al., 2017) into high-value protein for use as feed for livestock, poultry and aquaculture (Caruso et al., 2014). Thus, this insect could help address Millennium Development Goals #1 and 7 which have to do with eradication of extreme poverty and hunger as well as ensuring environmental sustainability. Attempts have been made to use the larvae of this insect in managing waste in Ghana on experimental basis (Bonso, 2013; Anankware, 2016), however, no colony of the insect has been established.

The objectives of this study include: 1) the establishment of an indigenous colony of the black soldier fly from the wild, and 2) develop a suitable organic substrate that could provide growth resources for commercial production of the larvae under laboratory conditions using different organic formulated diets, for bioconversion of organic waste to compost.

1.1 Background to the Study

The black soldier fly (BSF), *H. illucens* (L) is pervasive around the world from 49°N to 42°S (Lalander et al., 2013; Adeniyi & Folorunsho, 2015; Cammack & Tomberlin, 2017). BSF was first reported in 1930 in Hilo Sugar Company in Hawaain islands (Sharma et al., 2015). It is native to the

Americas, stretching from the southern tip of Argentina to Boston and Seattle and has spread to Europe, India, Asia and Australia during World War II (FAO, 2013). The species can be found in almost 80% of the world's continents including Africa (Olivier, 2009; Cammack & Tomberlin, 2017) through human-mediated dispersal (Cammack & Tomberlin, 2017). In Africa, particularly in Ghana and South Africa, the use of the BSF larvae in the bioconversion of organic waste has gained prominence in the past decade (Nyakeri et al., 2017).

An annotated benefits of the BSF larvae is shown in Fig. 1.1. The larvae valorise the organic waste by reducing both the volume and weight and convert it into high quality organic fertilizer. As the larvae colonize organic waste, their regular turning and movements in the waste aerate and reduce moisture and odour. The larvae are able to alter the micro flora of decomposing organic waste, reducing harmful bacteria such as *E. coli 0157:H7* and *Salmonella enteric* (Erickson et al., 2004). The larvae have a rich bacterial community in their intestine. As a consequence, they are able to produce bacteriostatic, bactericidal, and/or fungicidal compounds (Erickson et al., 2004). These compounds significantly reduce pathogens and thus eliminate hygienic and health problems. Western countries are using the BSF larvae to manage animal manure in commercial swine and poultry facilities (Gujarathi & Pejaver, 2013). In Ghana, however, this technique of waste management is not common despite its importance and the potential benefits it offers for the management of the mounting organic waste streams and its use as valuable protein source for animal feed.

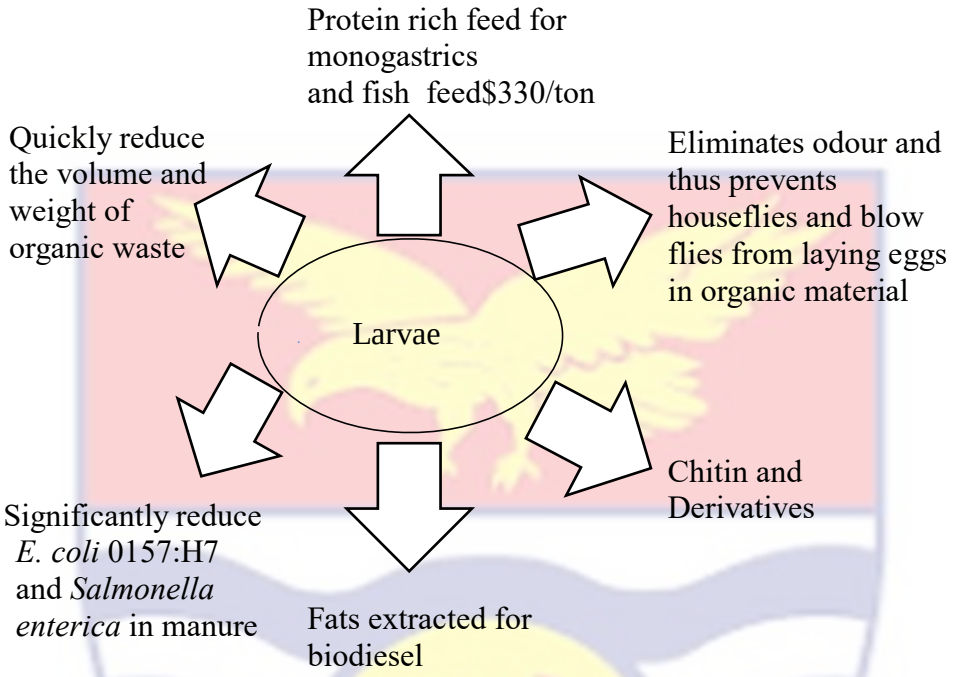


Figure 1. 1: Benefits of black soldier fly larvae modified from Sharma et al., 2015.

The conversion of organic waste streams into insect protein and organic fertilizer with the black soldier fly larvae, *H. illucens*, has been a novelty over the past decade (Makkar et al., 2014; UNEP, 2010). Large scale treatment facilities using the BSF larvae have been designed and built to treat up to 200 metric tonnes (MT) of organic material per day in countries such as the Netherlands, Canada, South Africa, China and USA (Diener et al., 2015). The larvae of this species can convert 50% of organic waste dry matter into insect biomass high in protein and fat (Sheppard et al., 1995), which can be used to develop feed industries (Makkar et al., 2014) for; poultry (Hale, 1973;

Schiarone et al., 2017), aquaculture (Bondari & Sheppard, 1987; St. Hilaire et al., 2007a; Magalháes et al., 2017) and livestock (Newton et al., 1977). The whole black soldier fly larva has been approved for use as a feed for farm-raised salmonid fish, by the Association of American Feed Control Officials representing the first approval of an insect being used for animal feed in the United States (AAFCO, 2016). The fat from the larvae has been extracted and used for biodiesel production (Li et al., 2011; Surendra et al., 2016), and the chitin and its derivatives have been used in medical and pharmaceutical applications (Park & Kim, 2010), as well as a number of industrial uses such as food processing and packaging, cosmetics, textiles and agriculture (Hamed et al., 2016).

A challenge facing the optimization and implementation of this system in developing countries like Ghana is the lack of reliable source of black soldier fly eggs and larvae (Sheppard et al., 2002). Colonies of insects are suitable for a wide range of investigations, from basic biological aspects to applied research. To facilitate insect rearing in the laboratory, insects must be sampled and collected in the field. Specific environmental requirements such as temperature, humidity, photoperiod (day/night length), sanitation, feed and population size are specific to each species and need to be regulated. Important information such as the lifespan, duration of life stages, feeding behaviour and preferences, reproduction can be obtained while rearing the insects.

1.2 Statement of the Problem

The black soldier fly (BSF), *Hermetia illucens*, is a common and widespread fly of the family Stratiomyidae. Black soldier fly larvae are edible and serve as food and feed (van Huis et al., 2013). The larvae are highly efficient in converting manure and organic waste to compost (Diener et al., 2011a; Banks, 2014), and matured larvae is composed of 42 – 45% crude protein, 31 – 35% fat (Sheppard et al., 1994; Newton et al., 2005b), as well as essential amino acids (Newton et al., 1977) suggesting its suitability as animal feed supplement for many livestock (St. Hillaire et al., 2007b; Makkar et al., 2014; Henry et al., 2015).

In tropical or subtropical climates, BSF adults might breed year-round, but in the temperate regions and during winter they must be provided with warmth from artificial lights (electrical bulbs) in order to survive and reproduce (Alvarez, 2012). The larvae on the other hand are quite hardy and can survive in more acidic conditions and higher temperatures than earthworms; *Eisenia fetida, Eudrilus eugeniae* and *Perionyx excavates* (Pandit et al., 2012).

The main difficulty is obtaining black soldier fly larvae or eggs to start or replenish a colony. Gravid adult females oviposit in dry cracks and crevices above and around moist decomposing organic matter (Gonzalez et al., 1963; Sheppard et al., 2002). With this natural inclination, Booth and Sheppard (1984), found that females readily sire their eggs in small openings (flutes) in the edges of corrugated cardboard held near attractive media. In some regions,

it is possible to start or maintain adequate larval colonies from native soldier flies; however, pest species such as houseflies and blowflies are also drawn to many of the foods used to attract soldier flies (such as fish offals, food and fruit waste, animal manure etc).

In order to generate sufficient quantities of black soldier fly larvae for use in feed formulation and organic waste management, it is important to produce and maintain large numbers of robust reproductive adult flies. It is thus important to develop production methods that will help develop or raise a colony (a group of organisms of the same type living or growing together) of this insect that can be used to convert the organic fraction of the municipal solid waste to valuable organic fertilizers for crop production, thus reducing the over-reliance on inorganic fertilizers. The use of BSF larvae in organic waste management represents an innovative approach to address challenges facing the ever-increasing global organic waste management problem and food supply chain. The use of this technology in organic waste management can provide environmentally friendly, nutritious ingredients for animal feed and a high quality organic fertilizer for crop production. However, the economic potential of this insect remains to be fully tapped in Ghana, a country with huge organic waste management problems and inadequate food and fish production.

Some studies have been done in Ghana to assess the potential of black soldier fly larvae to digest and degrade food waste (Bonso, 2013; Anankware, 2016), but these workers were not successful in the establishment of a thriving

BSF colony. Earlier attempts by Devic et al. (2013), to establish a colony was not successful, and the second attempt by Devic et al. (2014) was time consuming, costly and infested with housefly. There is lack of guidelines that can be followed to establish an indigenous BSF colony, from where robust larvae could be collected for research and other commercial purposes. There is therefore the need to undertake a study on how to trap and collect wild native BSF egg clutches and establish a thriving colony using locally available organic waste and to produce the larvae in large quantities for research purposes. This will ensure continious availability of significant number of adults that will produce egg clutches on a regular basis and hence produce robust number of larvae to degrade organic waste and for further research. To derive economic benefits of the fly it has to be available in large quantities through mass production. There is therefore the need to undertake a study on how to collect wild native egg clutches and establish a thriving mass laboratory rearing of this insect as a key component of several research projects.

1.3 Purpose of the Study

The purposes of the study were to:

- identify the most suitable microhabitat for harvesting wild BSF egg clutches,
- evaluate seasonal availability of egg clutch harvested from the wild,
- develop guidelines for mass rearing of indigenous black soldier fly, *H. illucens*
- identify cheaper sources of larval diet,
- explore organic market waste fractions as diet for BSF larvae.

1.4 Research Objectives

The objectives of the study included:

1. collection of egg clutches of BSF in the wild colonies and rear them in the laboratory
2. evaluation of the effect of selected larval diets on some life history traits of BSF
3. evaluation of the effects of feedstocks on the biology of BSF
4. assessment of the effects of organic waste combinations on the growth and development of the BSF
5. determine the effects of larval density on the growth and development of the BSF
6. effects of exposure of BSF larvae to high temperatures on their development and reproduction.

Research Questions

1. Are BSF adults available in the study area and which microhabitat is more attractive to trap and establish a BSF colony in a much easier and more efficient way?
2. Is the ambient temperature and humidity in the larval and adult cages suitable for breeding of BSF?
3. What will be the effects of the selected larval diets on the survival, development and reproduction of BSF?
4. What will be the effects of the feedstock type, temperature and humidity on the development of BSF larvae?

5. Will the different organic waste combinations as feedstock have any effect on BSF larval characteristics?
6. Will the addition of biochar as component of feedstock have any effect on BSFL growth, development and reproduction?
7. What will be the effect of high temperature on the growth, development and reproduction of BSF?

1.5 Significance of the Study

The results from this study could be used to reduce volumes of organic waste destined to the landfills and thus reduce greenhouse gases emissions and extend the lifespan of landfills, improve food and feed security thereby reducing food, meat and fish imports in Ghana. The results will be of benefit to goverments, farmers (Crop, animal and fish), waste management authorities or companies, entrepreneurs and academia.

Ghananian poultry and aquaculture farmers' grealty depend on the imports of protein resouces in formulating animal and fish feed. Soybean meal is the most important protein-rich ingredient for terrestrial animal feeds (Van Krimpen et al., 2013). However, cultivation of crops apportioned to animal feed, such as soy and grains, puts pressure on land obtainability, principally in tropical areas. As a result, these areas are exposed to deforestation, threatening tropical forests that are reservoirs of biodiversity and provide key ecosystem services (Foley et al., 2011). Furthermore, a large proportion of the world's fishery production is processed into fishmeal and fish oil to supplement these feeds (FAO, 2014). Such efforts have resulted in negative impacts on the

international fisheries worldwide due to overfishing. The growing demand for these ingredients has led to increased market prices over the last 5 years (Spranghers et al., 2017). Moreover, feed costs, represent 60–70% of total production costs (van Huis et al., 2013). Therefore, the need for alternative protein sources for livestock is becoming increasingly urgent. Such an alternative protein source can be provided by black soldier fly larvae. Moreover, they can be reared on organic waste streams and have a favourable feed conversion efficiency because they are cold-blooded (Premalatha et al., 2011).

Ghana's population is now estimated at 29.6 million up from the 24.5 million recorded during the 2010 Population and Housing Census (World Population Prospects, 2017 Revision) and generates about 3.0 million tonnes of solid waste per annum (Puopiel & Owusu-Ansah, 2014; Samwine et al., 2017) with a daily output of 13,000 MT of solid waste (Foray, 2012), majority of which is organic (KMA, 2012). Unfortunately, only a small fraction of the waste is recycled as normal heap comnposting takes a longer time and is labour intensive. A greater portion of the organic waste generated therefore ends up on landfill sites which reduce the life span of these facilities. In the intervening time, the unavailability and rising cost of land near urban areas and opposition by prospective neighbouring residents (Hoornweg & Thomas, 1999) have made dumps and landfills increasingly expensive and impractical (Ndegwa & Thompson, 2000). Agricultural production on the other hand has increasingly relied on enormous chemical and energy inputs, leaving soils depleted of soil nutrients and organic matter, leading to widespread surface

and groundwater contamination (DeLuca & DeLuca, 1997). Globally, organic matter anaerobically decomposing in landfills produces methane gas, a greenhouse gas associated with global warming, and landfill leachate, which may pollute groundwater over long periods of time (Goudie, 2000; Read et al., 2001). Solutions need to be researched and explored. Black soldier fly larvae can bioconvert these low value organic waste streams within a shorter period into high quality organic fertilizer for increased food production.

Delimitations

The growing population of Ghana, along with environmental degradation, climate change, declining sea, water and land resources are affecting food supply (crops, livestock, fisheries and aquaculture, and 'wild food') to feed its ever increasing population (Le et al., 2012). The expected negative effects of climate change resulting from greenhouse gas emissions and land use changes is generally projected to exacerbate the current and emerging problems with food security for many developing countries (Ingram et al., 2008). This may deprive many poor people of sustainable livelihood and health opportunities and consequently the possibility for improving their living conditions, quality of life and welfare (Olesen et al., 2013). The economy of Ghana is highly dependent on agriculture, employing about 60% of the workforce and contributing 32% to the country's Gross Domestic Product (GDP) (Diao, 2010), however, dependency on rainfall, coupled with poor soils and inappropriate soil fertility management strategies, requires new strategies for food production (Beddington et al., 2012).

This research work, focused on developing an efficient and easier way of establishing and maintaining a self-sustaining local or indigenous BSF colony and thus make the culture readily available for research and commercial purposes in Ghana. The scope of this research work thus, focused on attempts at mass rearing and keeping laboratory culture. Attempts were made to use wheat bran or layer meal – wheat bran mixtures as well as crop/market waste fractions as diet for BSF larvae. These were aimed at developing a low-cost larval diet whiles exploiting crop/market organic waste combinations for breeding BSF larvae from both waste management and animal feed production standpoint.

The studies were conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra, Ghana. BNARI is located about 20 km north of Accra (5º40′36.6″ N; 0º11′52.5″ W, 76 m above sea level). BSF microhabitats within the vicinity of BNARI was assessed for wild BSF egg clutches whiles larval diets was formulated from both standard BSF larval diet and a relatively cheaper wheat bran.

Limitations

This study was limited to the establishment of a thriving BSF colony using egg clutches obtained from the wild (indigenous adults) and the immediate environs of the Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission in Accra. Appropriate local larval diet was formulated and tested to ensure cost effectiveness. Trial bioconversion of

organic waste from markets within the Abokobi and Ga East Municipal Areas was carried out and the suitable waste combinations determined. Thus, the study did not consider faecal matter, liquid, e-waste, plastics and other hazardous wastes. It linked the possibility of integrating a novel composting programme into the waste management plans towards promoting environmentally friendly rural and urban agriculture.

Moreover, getting access to source-separated organic waste is essential to attaining clean compost and clean insect larvae. Attempting to persuade people and policy makers that waste separation is important for the environment and health were the greatest challenges that this work had to contend with. The researcher addressed this by motivating the local population, especially young people, as well as policy makers to support in this exercise.

DEFINITION OF TERMS

AMA	Accra Metropolitan Assembly
KMA	Kumasi Metropolitan Assembly
MSWM	Municipal Solid Waste Management
BSF	Black soldier fly
BSFL	Black soldier fly larvae
MOFA	Ministry of Food and Agriculture
USD	United States Dollars
MSW	Municipal Solid Waste
GSS	Ghana Statistical Service
MT	Metric Tonnes
PUA	Peri-Urban Agriculture
UN	United Nations
FAO	Food and Agriculture Organization
GHG	Green House Gas
GAEC	Ghana Atomic Energy Commission

Organization of the Study

The dissertation has been arranged in eight chapters. Chapter one introduces the research, provides background information, the objectives and describes the scope of work. Chapter two largely reviews and discusses the biology of BSF and attempts at mass rearing and keeping laboratory culture, challenges and solutions across the globe. Chapter three explores the possibility of establishing an indigenous stable colony of black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae) that will guarantee a robust population of BSF larvae for dependable inoculation of municipal solid waste. Chapter four, looks at the effects of different artificial diet formulations on the survival and reproductive performance of black soldier fly. In order to elucidate the suitability of the organic fraction of municipal waste for bioconversion by the BSF larvae, chapter 5 looks at selecting some market crop waste for decomposition by the BSF larvae to establish their effects on the growth rates of BSF and to help select and formulate optimal feedstock combination for bioconversion by BSF larvae. Larval stocking density may have significant impact on growth rate, developmental time and fecundity. Thus chapter six assess the effect of larval density on the growth and development of *Hermetia illucens.* During composting at the thermophilic stage, temperature within compost rises, chapter seven evaluates the effects of exposure of larvae to high temperature on the growth, development and reproduction of BSF. Finally, chapter eight presents the conclusions and recommendations gathered from the work.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The goal of this research was to investigate the concept that captive breeding and the initiation of rearing of black soldier flies (BSF), *Hermetia illucens* (L) (Diptera: Stratiomyidae) under controlled conditions is paramount in facilitating further research on the potential of this insect in Ghana. This research will provide high-quality larvae for waste management and production of nutrient rich protein for the formulation of animal and fish feed. This chapter is grounded largely on secondary data in the form of information obtained from published journal articles, books, conference proceedings and reports on related study from the internet and governmental documents. Again, this chapter reviews project documents from various sources such as Universities, student dissertations and thesis, Government and Departments to provide a comprehensive literature review. The review is to give insight into understanding the issues relating to BSF biology, attempts at mass rearing and keeping laboratory cultures, challenges and solutions across the globe. Additionally, the review provides information on the effects of temperature and organic waste fractions on BSF larval development. Personal views were also employed to guarantee thorough discussion of the various topics under consideration. In case of situations where there is limited previous studies to extract data for this study, information from similar studies using different organism have been used since such organisms are also arthropods with similar biology and economic importance. Moreover, knowledge gaps were identified which merit further investigation.

2.2 Theoretical Framework

The concept of mass rearing of black soldier fly, an insect whose larvae can play dual roles of transforming organic waste into high quality organic fertilizer and serve as alternative protein source for fish and animal feed to enhance food security formed the theoretical basis of this study. The mass production of insects is thought to have begun with the need of the rod-and-line fisherman for bait (Gardiner, 1968). Through the help of insect geneticists, insect nutritionists, and insect behaviourists, insects might be reared under conditions that will make them equally, more vigorous and more adaptable to the environment than the wild population (Knipling, 1979). Laboratory colonies are a vital component of scientific research on insects. When Thomas Hunt Morgan established colonies of the dipteran *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) in 1907, this species quickly became a model organism, revolutionizing our understanding of genes, heredity, and inheritance (Rubin & Lewis, 2000; Jennings, 2011). Other major scientific advances occurred in developmental biology, genomics, embryology, behavioural ecology, and disease biology with the development of laboratory colonies of nematode *Caenorhabditis elegans* (Maupas) (Rhabditida: Rhabditidae) (Ankeny, 2001), darkling beetles, *Alphitobius diaperinus* (Coleoptera: Tenebrionoidea) (Wang et al., 2013), zebrafish, *Danio rerio* (Cypriniformes: Cyorinidae) (Howe et al., 2013), and mice, *Mus musculus* (Rodentia: Muridae) (Phifer-Rixey & Nachman, 2015).

The aim of controlled insect rearing is to provide dependable, cheap sources of high-quality insects for their many important purposes (Leppla,

2009). Laboratory colonies offer disease-free organisms with a known rearing history, thus decreasing experimental unpredictability (Amanda et al., 2018). Additionally, by providing optimum environmental conditions suitable for the growth of the insects, laboratory colonies can provide research organisms throughout the year. Naturally, due to seasonal changes in weather conditions and its associated effects on food supply to insects, a greater number of insects are not easily available for research and other purposes. Specific life stages are also transient or unavailable for greater parts of the year, further limiting research prospects. Laboratory colonies are positioned to provide high-quality organisms that display consistent performance, assuming nutrition and diseases are effectively managed (Amanda et al., 2018). They facilitate and accelerate research and are integral to many facets of biological research. The greatest difficulty is to provide fresh host material or to develop a diet that is nutritionally complete and induces feeding, especially for predators or parasitoids (Leppla, 2009). Precautions must be taken to start colonies with an adequate number of clean specimens and maintain them with very limited levels of mortality (Amanda et al., 2018). These measures help to limit genetic bottlenecks caused by inbreeding, contamination with other species, and disease epizootics (Leppla, 2009).

Organic waste is biodegradable and could be generated from three different sources; agricultural, industrial and municipal. Organic waste should not be seen as a source of environmental pollution that has to be gotten rid of by putting it in landfills or burnt in incinerators, as this could cause other pollution problems. It should however be seen as a valuable resource that can

be transformed into marketable products, providing employment and profits (Muralikrishna & Manickam, 2017). Organic waste transformation means a process of reduction of organic waste fractions by volume and weight and recovering nutrients into a humus-like material used for soil conditioning applications. A number of nutrients are locked up in all three types of organic waste, which are urgently needed for the cultivation of crops. Fortunately, there are innovative ways of harnessing organic waste. One such solution is bioconversion, which involves the use of larvae of the black soldier fly to quickly convert these organic wastes into biofertilizer. In addition to removing organic wastes from our environment and landfills, organic fertilizer creates a much-needed product that revitalizes farmlands and helps the earth maintain its ability to feed its ever increasing population.

According to the results of the 2017 Revision, the world's population numbered nearly 7.6 billion as of mid-2017 (UN, 2017). With more space needed to accommodate this number, there is not much room left on planet earth for storing organic waste. Overflowing landfills and organically saturated soils which leach both nutrients and waste into local water supplies are only two of the many issues arising as we struggle to find creative solutions to handle the natural abundance of our organic waste. "Food Security" is one of the major elements of development and poverty alleviation and has been the goal of many international and national public organizations. The subject is of such significance viewed in the light of the fact that about 870 million people (out of which 852 million are from developing countries) are estimated to have been undernourished in the period 2010-12 (FAO,

2012). While "Food Security" is being used widely, the definition and concept of food security is elusive and undergoing refinement with time (Maxwell and Smith, 1992). “Food security is a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life” (FAO, 2002). Total global food production must be increased by 70% so as to be able to feed the world in 2050 (FAO, 2009). However, this will be exceedingly difficult as a result of the effect of climate change. In the next decade, crop and pasture yields are to be expected to decline in many places. For instance, parts of Brazil will experience a decline of about 14% in rice and wheat yields by 2050 whiles an average of 8% decline in yields for eight major food crops across Africa and South Asia has been projected (Vermeulen, 2014).

2.3 The Biology of the black soldier fly, *Hermetia illucens*

The black soldier fly (BSF), *H. illucens* (L) (Diptera: Stratiomyidae), is notable from other Stratiomyidae species by its large size (13 to 20 mm) and dappled pattern on its body (Nguyen et al., 2015). It is distributed throughout the sub-tropical and tropical regions of the world (McCallan, 1974, Üstüner et al., 2003, Diener et al., 2011b, Roháček & Hora, 2013) and is often found colonizing decomposing material, such as fruits, carrion (Dunn, 1916; James, 1935), and poultry manure (Leclerq, 1969, Axtell & Edwards, 1975). As described by Gujarathi and Pejaver (2013), black soldier fly adults do not have bristles and are wasp-like in appearance (Sheppard et al., 2002; Hardouin & Mahoux, 2003), their scutellum is noticeably developed with two translucent

"windows" located on the first abdominal segment (Sharma et al., 2015). They have particular vein system, with all the wing veins crowded near the costa with more pigmentation than the ones behind, while vein C, not surrounding the wing. Adults possess elongated antennae with three segments. White coloration appears near the end of each leg (Sharma et al., 2015).

The species is not considered to be a disease vector or a nuisance to humans (Furman et al., 1959), however, some cases of intestinal myiasis after accidental ingestion of adults in the tropical and subtropical areas have been reported (Adler & Brancato, 1995; Lee et al., 1995; Gonzales & Oliva, 2009). Myiasis is the parasitic consumption of tissue by the larvae of a dipteran fly. The possibility of disease transfer from BSF to humans in a waste management facility is not known, however, with the use of adequate personal protective equipment, the incidence of such transfer is not anticipated to be pointedly worse than current vectors existent at a landfill (Alvarez, 2012).

2.3.1 Anatomy

The black soldier fly undergoes complete metamorphosisis (holometabolous insect) and passes through five stages in its lifecycle: egg, larva, prepupa, pupa and adult. The larval stage is further divided into phases called instars. An instar is defined as the period between each moulting of their skeleton (Alvarez, 2012). Larvae are saprophagous and photophobic (Canary, 2009), thus they live and feed on organic matter (animal or plant) in decomposition (James, 1935). Their head capsule is separate from their body, their strong mouthparts are not only for eating purposes but also contributes to their

locomotion (Caruso et al., 2014). The body of the larva consists of 11 segments with hairs and bristles covering it. Larval colour is creamy or light brown up until pupation, then it turns to dark brown. The larva can grow up to a maximum length of 27 mm, 6 nmm in width (Caruso et al., 2014) and weight of 220 mg in their last larval stage when they are dull and whitish in colour (Diclaro & Kaufman, 2009). All through larval stages and on daily basis, a larva feeds gluttonously from 25 to 500 mg of decaying organic materials such as rotting fruits and vegetables, grains, fish offal, particularly animal manure and human excreta (van Huis et al., 2013; Diener et al., 2011a; Hardouin & Mahoux, 2003).

Adults do not feed but need misting fresh water sprays onto the muslin cloth covering of the adult cage to provide drinking water in suitable particle size and improve ambient humidity as the water evaporated. Adults having access to water have been found to live up to 14 days but those deprived of water scarcely last more than 8 days (Tomberlin et al., 2002; Caruso et al., 2014). Males are smaller than females (Tomberlin, et al., 2002) and sexes are distinguished by an anatomic difference on the last abdominal segment. Females have a retractile tubular oviduct whereas males show an aedeagus (male insect reproductive organ) and a pair of hooks which assist it to clasp the female genital organ during copulation (Caruso et al, 2014).

2.3.2 Life Cycle

It has a complete life cycle with an egg, larva, pupa and adult stage. These are shown in Figure 2.1. Black soldier fly life begins with an egg that eventually

hatches into a tiny larva within 3 – 4 days at temperature of 27 – 36°C. The larva is nnn white, segmented and voraciously feeds on moist, decomposing organic wastes for about 4 weeks if feed and temperature are favourable, but when enough feed is not available, the larval stage can last up to four months (Hardouin & Mahoux, 2003).

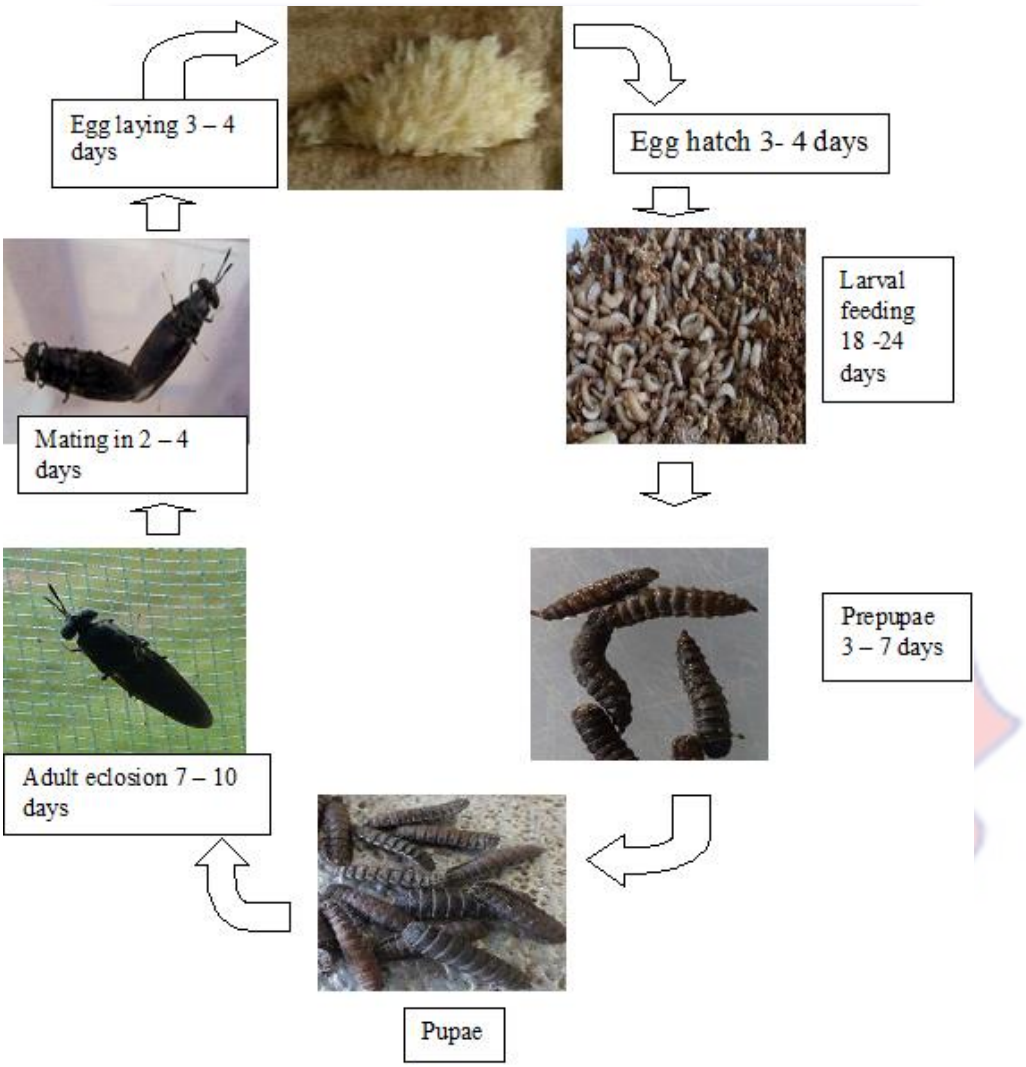


Figure 2. 1: Life cycle of the Black soldier fly. Source of pictures: Ebenezer Ato Ewusie

The adults are photophilic and requires light intensity of between 63 - 110 µmol $m^{-2}s^{-1}$ of visible light (400 - 700 nm) in addition to temperatures of

25 - 35°C and space for mating to occur (Tomberlin & Sheppard, 2002; Zhang et al., 2010). Adult males assemble in small numbers near sheltered bushes (Sheppard et al., 2002) and display lekking behaviour (Tomberlin & Sheppard, 2001) in order to find a mate. Two days after emergence, females mate once with one oviposition event in their lifetime and mated females selectively oviposit 500 – 1,200 eggs in dry crevices near a moist food resource two days after successful copulation (Tomberlin et al. 2002; Diener et al., 2011a; Banks et al., 2014). Ovipositing substrates make available moisture which protects the eggs from drying and aids hatching, as well as providing larvae with an immediate food source after hatching (Holmes et al., 2012). Eggs hatch within four days (102-105hrs at 27°C) and the neonate larvae begin feeding (Booth & Sheppard, 1984; Holmes et al., 2012). The larvae feed for between 4 weeks and 5 months (Caruso, et al., 2014) depending on food availability (Furman et al., 1959, Hardouin and Mahoux, 2003), and optimum temperature 20 - 30°C range (Tomberlin et al., 2009; Canary, 2009), storing enough resources to reach critical weight, while developing through six larval instars (L1-L6). The critical weight is the minimal mass at which pupation can occur in a normal time-course (8 - 14 days for BSF) (Badenhorst, 2017).

As soon as the critical weight was attained, larval growth ceases and ecdysteroids is secreted to stimulate a series of endocrine and physiological events (Davidowitz et al., 2003; Smykal et al., 2014; Noriega, 2014). For instance, naturally, Juvenile hormone (JH) inhibits the secretion of prothoracic hormone (PTTH) and ecdysteroids, however, once critical weight was reached, the level of JH decreases and change begins (Nijhout & Williams,

1974). The decrease in the amount of JH leads to the deactivation of the *corpora allata*, the glands which secrete JH. The ensuing secretion of PTTH at that point stimulates the secretion of ecdysteroids, which cause the larvae to cease feeding and commit to pupariation (Davidowitz et al., 2003).

In the terrestrial environment, the larval stage of *H. illucens* is threatened by the loss of body water. Moisture content of the air may potentially affect the physiology and, in turn, the development, longevity and oviposition of this insect (Gullan & Cranston, 2010). Like temperature, unfavourable humidity affects growth, resulting in problems when estimating development times. Laboratory studies in relative humidity with *H. illucens* range from 50 – 99% relative humidity (Bradley & Sheppard 1983; Booth & Sheppard 1984; Furman et al., 1959; Tingle et al., 1975).

The larvae consume voraciously, from 25 – 500 mg of fresh matter/larva/day of decaying organic waste such as rotting fruits and vegetables, coffee bean pulp, distillers' grains, fish offal and particularly animal manure and human excreta (Hardouin & Mahoux, 2003; Diener et al., 2011b; van Huis et al., 2013) breaking it down into fine components. BSFL is rich in protein, and are a good source of feed for fish and chicken in both backyard and commercial purpose (Zuidhof et al., 2003; Alvarez, 2012; Tran et al., 2015).

At the end of the larval stage (prepupa), the larva empties its digestive tract and stops feeding and moving (Hardouin & Mahoux, 2003). The

prepupae, the last immature stage, display a noticeable wandering habit and will self-harvest or crawl out of the organic waste in search of a dry and protected pupation site (Diener et al., 2011a). At this stage, the prepupae are at their maximum size, exhibiting large protein (36 – 48% and fat (31–35%) contents including favourable profiles of essential amino acids to sustain them through metamorphosis (Newton et al., 1977; Stamer, 2005; St- Hilaire et al., 2007a). The prepupa is morphologically different from the larval stage. For example, its labrum becomes curved down like the beak of an eagle (Diener et al., 2011a) and used as a hook to pull them to a suitable pupation substrate (Schremmer, 1986). The substrate may vary considerably as larvae have been found in decaying organic matter, including beeswax, catsup, decaying vegetables, potatoes, and manure (Drees & Jackman, 1998; James, 1935; Sheppard et al., 1995; Oldroyd, 1964).

Pupation usually lasts two weeks (Hall & Gerhardt, 2002) before eclosing as an adult, but is highly variable (Sheppard et al., 1995) and can last up to 5 months (Hardouin & Mahoux, 2003). After eclosion, the adult fly lives for only a few days or weeks, and does not bite or engage in pest-like behaviour. It does not seek to enter homes or restaurants, but lives its short adult life remote from humans, maturing and mating primarily in wooded areas (Sheppard & Newton, 2000). Adult flies vary in colour from black, metallic blue, green or purple, to brightly coloured black and yellow patterns (Drees & Jackman, 1998). The adults do not feed and rely on the fats stored during the larval stage (Diclaro & Kaufman, 2009), but have sponge-like mouthpart that allows them to lap up liquid (Oliveira et al., 2016). Adults

engage in an aerial mating process and females oviposit near suitable larval medium (Sheppard et al., 1995).

H. illucens are poikiltherms, as are most insects, and temperature directly affects growth and development (Gullan & Cranston 2010). Despite available food resources temperature may retard or escalate growth and development. Optimum temperatures for culturing and studying *H. illucens* range from 24 to 29.3° C (Sheppard & Newton, 2000; Furman et al., 1959; Bradley & Sheppard, 1983; Tingle et al., 1975; Booth & Sheppard, 1984). Tingle et al. (1975) found no adult emergence when pupae were held at 7.1°C and at 12.6°C, 27% of field-collected pupae emerged, suggesting a developmental (or growth) threshold between 7.1 and 12.6°C.

2.4 Environmental and Economic Concerns

Annually, Ghana generates about 3.0 million tons of solid waste (Puopiel, 2010) with a daily output of 13,000 MT of solid waste (Foray, 2012) but approximately, 60 – 70% of this waste is organic. Various Metropolitan Municipal and District Assemblies (MMDAs) are however only able to collect about 75% of these waste and dispose in unengineered landfills. The remainder is left in community dumps, open spaces, in water bodies, beaches and storm drainage channels causing flooding, pollution and severe hygienic and health problems (MOH, 2000; Government of Ghana, 2008). A more serious cause of concern is the emission of greenhouse gases (GHG), particularly methane (CH_4) and nitrous oxides (N_2O) which occur as a result of anaerobic fermentation of organic waste, either in the landfills or from their

management by conventional composting systems. Therefore, management options that are targeted at reduction of waste as well as treating waste as a resource are essential for a holistic and integrated sustainable waste management (Dortmans et al., 2017).

Ghana's population has grown quickly since 2000 at an annual rate of 2.5% and now stands at 24.2 million according to the 2010 Population and Housing census (GSS, 2012). This calls for an urgent increase in food production, however, due to inappropriate farming techniques, the soils have lost most of its fertility properties and some have been degraded. Only few farmers can afford the use of organic fertilizers to replenish the soil to boost crop production (Sampat, 2001). Using compost instead of the inorganic fertilizers not only will reduce contamination but also dramatically improve soil structure, the water holding capacity, microbial activity and hence crop yield. Unfortunately, the long composting period, and its resultant increased cost has not made composting of these organic resources attractive to farmers (Danso et al., 2006; Adamtey et al., 2009).

Ghana has a huge deficit in fish demand of 460,000 MT., which is addressed by imports at a cost of USD 200 million every year (Frimpong & Adwani, 2015). Aquaculture production in Ghana has been increasing in recent times (MOFA, 2012) but, the industry is constrained by the lack of good quality and affordable feed. The development of rich protein substitutes for fishmeal production is paramount in reducing the overall aquaculture production costs, and relieving pressure on fish farmers (McCallan, 1974),

because the production cost of imported commercial feed keeps on rising (FAO/NACA, 2012).

Unavailability and rising cost of land especially near urban areas have made dumpsites and landfills increasingly expensive and impractical (Ndegwa & Thompson, 2000). Although composting can transform organic wastes into bio-products which can be used as bio-fertilizers (Nakasaki & Marui, 2011), the economic benefits of conventional composting are often marginal due to the low value addition (Westerman & Bicudo, 2005). The composting process in Ghana is labour intensive, takes up to 4 months to mature, thereby affecting the nutritive value (nitrogen content) (Danso, et al., 2006; Adamtey et al., 2009). This has resulted in a low patronage of the technology and the use of the compost product in crop production (Danso et al., 2006). Thus there is a need to look for a faster, economical and efficient method of producing high quality compost (Adamtey et al., 2009).

2.5 Advantages of establishing mass rearing colony of black soldier fly in Ghana

The larvae of the black soldier fly (BSF), *Hermetia illucens* L., can play dual roles of recycling organic waste into high quality organic-fertilizers (compost) as well as exploitation of the larvae as a protein rich animal feed (Čičková et al., 2012). Bioconversion of organic waste using the larvae of the black soldier fly is cost-effective as the burrowing action of the larvae as they feed turns the compost, saving labour in turning of compost. In addition, pathogens are

digested by the larvae and their excreta and exuviae of *H. illucens* larvae enriches the compost, thus producing a high quality compost.

As a component of a complete diet BSF larvae have been found to support good growth of chicken (Hale, 1973), swine (Newton et al., 1977), rainbow trout (St-Hilaire et al., 2007b) and catfish (Newton et al., 2005). Additionally, BSF prepupae meal can replace at least 25% of the fish meal in a diet with no reduction in gain or feed conversion ratio in rainbow trout (*Oncorhynchus mykiss*) (St-Hilaire et al., 2007a, Stamer et al., 2014), the African catfish, (*Carias gariepinus)* (Adeniyi & Folorunsho, 2015), the Atlantic salmon (*Salmon salar*), the Nile tilapia, (*Oreochromis niloticus)* (Rana et al., 2015), and the blue tilapia (*Oreochromis aureus*) (Makkar et al., 2014). Bondari and Sheppard (1981), also revealed that there was no significant difference between diets using BSF larvae and commercial diets when blind taste tests with tilapia and channel catfish was conducted. Hem et al. (2008), successfully used the larvae of the black soldier fly that was fed on palm kernel meal as feedstuff in tilapia culture in the Republic of Guinea. It is an appropriate alternative to fishmeal in animal feed with a potential market value of 330 USD/ ton dry weight (Newton et al., 2005b).

2.6 Attempts at BSF Rearing

While much research focusing on the use of BSF larvae to manage animal manure (Newton et al., 2005b; Diener et al., 2009), and the organic fraction of the municipal solid waste (Halloran et al., 2014; Diener et al., 2009), information on mass rearing of the black soldier fly to achieve this target is relatively limited (Yushin, 2016). Tingle et al. (1975), reported rearing BSF eggs collected in the wild to adults within 38 days at 29.3^{o}C, but were not successful to establish a population over multiple generations. Sheppard et al. (2002), collected eggs in an open-sided caged layer house and successfully maintained them in the laboratory of the University of Georgia, Tifton, GA, but it was costly to maintain suitable temperatures essential for prompting mating in the large greenhouse using artificial lights. Nakamura et al. (2016), established a BSF culture using 9 females collected in Tsukuba, Japan (36°03′N, 140°04′E) in October 2013. The capture of wild BSF adults is possible but because of the fact that they might need some time to adjust to life in captivity together with the short life of the adult BSF (5 – 14 days), adults would have died without egg clutch production and hence cannot be relied on to establish a colony (Caruso et al., 2014).

Although some efforts have been made in Ghana to assess the potential of black soldier fly larvae to digest and degrade organic waste and also to produce fish and animal feed (Bonso, 2013; Maquart et al., 2015); Devic et al., 2013; Anankware, 2016), however, information on mass rearing of the black soldier fly is limited (Yushin, 2016). Devic et al. (2013), had their first colony in Ghana collapsed without offspring. A second attempt in 2014 yielded some

positive results, but was time consuming, costly and had competition with houseflies.

Anankware et al. (2013), trapped adult BSF that oviposited on chicken manure covered with banana leaves left in the open for two days. The adults were then kept in a metal cage measuring 80 x 80 x150 cm covered with a small mesh and got a small-sized colony, but there is no information on the continued existence of this colony. This may be due to the fact th at no guidelines or rearing protocols were developed during his study and again there might have been no succeesion plan for the colony to be maintained even after his studies.

Even though black soldier fly develops naturally in organic material, larvae and adults become inactive when environmental conditions are sub-optimal (Yushin, 2016). The optimum environmental conditions suitable for the continued sustenance of the BSF colony to provide ready source of materials for research and commercial purposes is important. There is currently a lack of guidelines that can provide information on the culturing of the fly in Ghana. For the above reasons, the development of efficient rearing systems for black soldier fly is imperative to deliver a reliable source of larvae that can be used for industrial purposes such as BSF bioconversion of the ever increasing organic waste volumes and an excellent animal and fish feed additive.

2.7 Methods used to establish BSF colony in the Laboratory.

The main difficulty in establishing a BSF colony is obtaining black soldier fly larvae or eggs to start or replenish the colony. Naturally, adult black soldier flies oviposit in dry cracks and crevices above and around moist decomposing organic matter (Gonzalez et al., 1963; Tomberlin et al., 2009). The source of BSF eggs for mass production is usually collected using egg-trapping technique. Sripontan et al. (2017), indicated two techniques for BSF egg-trapping which were baiting and trapping material. Baiting material is a major factor that affects the egg-trapping efficiency because BSF female may search for a specific food source for their offspring (Sripontan et al., 2017). Booth and Sheppard (1984), found that females readily sire egg clutches in the flutes of corrugated cardboard held near attractive larval media (such as fermented chicken feed). James (1935), showed that BSF female lay egg on various materials, such as fruit, carrion and manure (Tingle et al., 1975). Sripontan et al. (2017), compared the differences in egg-trapping efficiency of different baiting materials, including fruit waste, household food wastes, chicken manure, pig manure, and dairy manure and found out that fruit wastes are the most efficient for trapping BSF egg clutches. In thier work however, the egg clutch collection was low, 4.10 egg clutches per week and there was no indication as to whether they were able to raise a colony from that collection.

2.8 Housing for BSF Rearing

The central and most important requirement for sustainable BSF mass rearing is availability of well secured building having two physically distinct separate spacious rooms for a hatchery and an insectarium (Caruso et al., 2014). Egg

clutches are incubated and fed till maturity to provide pupae for the next generation in the hatchery whiles the insectarium maintains a population of adults which acts as a broodstock from where egg clutches are collected. The entire building must be free from pesticide contamination and larval trolley stands should either be smeared with a layer of grease or stand in dirty oil to prevent the entry of ants from climbing into larval rearing cages. Laboratory windows should be made of louver blades and with fine nylon net for ventilation.

Adult black soldier flies mate while in flight (aerial questing), hence adult cages should be spacious enough to allow for adult males to congregate (lekking areas) and "call" the females (Banks, 2014). However, successful mating has been reported in adult cages of 76cm x 114cm x 137cm (w x d x h) and larger (Tingle et al., 1975, Sheppard et al., 2002; Tomberlin et al., 2002; Nakamura et al., 2016) kept outdoors or under direct sunlight. Time of day and light intensity are significantly correlated with mating (Tomberlin & Sheppard, 2002), with sunlight promoting the most successful mating. However a quartz-iodine artificial light source produces a 61% mating rate compared to a sunlight control (Zhang et al., 2010), allowing indoor rearing of BSF. Adult cages can be made of wood or metal and apart from the bottom of the adult cage that should have a wooden or metal base, all sides should be provided with fine muslin cloth to keep ants and parasitoids out but allow for cross ventilation.

2.9 Diet for Larval Rearing

The larvae of the black soldier fly, *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae), is omnivorous and feeds on a wide range of plant (Newton et al., 2005) and animal waste (Tomberlin et al., 2002; Diener et al., 2011b). Nevertheless, respective nutritive values are supposed to affect survival and numerous life-history traits of the larvae, as well as adult flies. An important part in insect rearing is proper nutrition as it has a substantial impact on their production (Erens et al., 2012). Nutritional requirements of insect species differ, nonetheless, some dietary components such as carbohydrates, proteins, lipids, minerals, vitamins and water should be part of the diet (Erens et al., 2012). The Gainesville housefly diet consisting of 50% wheat bran, 30% alfalfa meal, and 20% corn meal (Hogsette, 1992; Tomberlin and Sheppard, 2001) or layer hen feed mixed with water (60 – 70% moisture) (Sheppard et al., 2002) were used in earlier rearing trials. These diets are however, either expensive or had to be imported with hard earned foreign currency. Identifying an inexpensive local substitute as diet in mass rearing of black soldier fly larvae in Ghana is of paramount importance.

2.10 Pupation Substrate

The prepupae of *H. illucens* search for suitable pupation substrates to bury themselves for protection from predators, desiccation and flooding while they undergo metamorphosis (Lima et al., 2009; Holmes et al., 2013). Such pupation sites should have an ambient temperature of 27 – 36^{o}C and relative humidity of 60 – 70% to prevent dehydration, minimize development of mould (Holmes, 2010; Sheppard et al., 2002; Alvarez, 2012) and to encourage

eclosion (Holmes, 2010; Sheppard et al., 2002). The pupation substrate itself should be porous enough and loose to allow for easy burrowing of wandering prepupae (Alvarez, 2012). The substrate should also provide adequate oxygen levels for the pupae to breathe. If the pupation medium is too fine the spiracles, can be clogged leading to death (Alvarez, 2012). The depth of the pupation medium should not be too deep, such that the emerging flies fail to reach the surface. On the other hand, it should be deep enough for the prepupae to deem it is adequate so that it does not rove around to get a more suitable place and deplete its fat. Sometimes, the prepupae might pupate when a suitable medium is not identified (Holmes, 2010). A depth of the medium of between 15 and 20 cm is considered ideal. Pupation can last 5 – 14 days depending on temperature and ambient humidity (Alvarez, 2012; Caruso et al., 2014).

2.11 Effects of Temperature on BSF Development

Temperature affects the growth and development of the black soldier fly. Being poikilotherms, temperature directly affects the growth and development of *H. illucens* (Gullan & Cranston, 2010). The ideal temperature ranges for survival, growth and reproduction for *H. illucens* is between 27 and 36°C (Tomberlin et al., 2009). BSF larvae also consume waste at low temperature conditions but not very quickly because their activity is generally slowed (Žáková & Borkovcová, 2013). During composting with BSF larvae, temperature could rise from 25°C to above 40°C within an hour, leading to a high larval mortality rate (Cheng & Lo, 2016). Food consumption rate of the

black soldier fly larvae declined with temperature and stopped at 15°C (Newby, 1997).

Tomberlin et al. (2009), reported that when black soldier fly larvae were reared at 27 and 30°C, the survival rate averages at 74 – 97%, yet, when the temperature is increased to 36°C, the survival rate of black soldier fly larvae was only 0.1%. The age of larvae before exposure to different temperatures is likely to influence variation in larval characteristics, duration and fecundity and needs to be investigated.

2.12 The use of BSFL to Produce Compost from Organic Waste

Composting of the organic fraction of household, municipal, and agricultural wastes can reduce the volume of wastes that are sent to landfill and provide a rich, organic material that can be used as a soil conditioner for crop production (Goyal et al., 2005; Levy & Taylor, 2003; Smith & Hughes, 2006). The type of feedstock used in composting has a major impact on the composting process and the compost quality.

The larvae of the black soldier fly have powerful mouthparts and efficient enzymatic activity of their digestive system and is able to feed on a gamut of organic substrates (St. Hilaire et al., 2007b; Hem et al., 2008; Martinez-Sanchez et al., 2011) reducing and transforming these organic waste materials (Diener et al., 2009). Enzymes such as amylases, proteases and lipases are found in their intestines and are most active. Other enzymes such as leucine arylamidase, α-galactosidase, β-galactosidase and α-mannosidase, are

strongly effective in BSF larvae (Kim et al., 2011). The black soldier fly larvae (BSF), *H. illucens* offer a promising opportunity for management of organic waste to a more valuable resource and as a source of income for small entrepreneurs. The larvae feed voraciously on decaying matter reducing significantly its dry matter content between 58 – 70% (Sheppard, 1983; Myers et al., 2008; Diener et al., 2011a), in a faster and more efficient than other known species (Diener et al., 2011a). Being a bacteriophage, it also feed on bacteria and other pathogens (Ericson et al., 2004; Sealey et al., 2011; Yu et al., 2011). The potential therefore exists for the use of the larvae to sanitize dairy manure, as it can control *Escherichia coli* in dairy manure (Liu et al., 2008), compost or fish components made from municipal solid wastes. The black soldier fly larvae can also eliminate breeding by house fly, an important vector in spreading of pathogens (Bradley & Sheppard, 1983).

BSFL is widely used in many parts of the world (Dieu et al., 2015) for the bioconversion of yard waste, domestic waste, animal manure and human excrement to produce compost. In addition, the larvae are harvested as feed for cattle and poultry (Newby, 1997; Newton et al., 2004). The use of BSFL for bioconversion of vegetable food waste and domestic solid waste as well as animal manures for the production of livestock feed and compost for crop production has also been studied severally in Vietnam (Dieu et al., 2015).

Information on the use of *H. illucens* larvae in composting of organic materials in Ghana is limited. Organic wastes contain considerable amounts of plant nutrients, such as nitrogen, phosphorus, potassium and micronutrients

(UN-HABITAT, 2010). If these nutrients are recycled through composting, these can be used in improving soil fertility for crop production. It is important to determine the chemical composition of the organic market waste stream so that its suitability for use by BSFL for growth and reproduction can be assessed. This will clarify the suitability of the waste fractions for bioconversion by BSF larvae.

Research Gaps

- In a tropical region such as Ghana, no data or records are available as to the most effective site to harvest BSF egg clutches neither are there records of guidelines for laboratory rearing of this valuable insect.
- The reference diet for feeding BSF larvae is the layer meal diet which is expensive in Ghana. Hence a cheaper source of larval diet needs to be investigated.
- The use of *H. illucens* larvae in organic market waste management and ensuring good sanitation in Ghana where there is low value addition to most of the aricultural produce.
- The effects of this market waste on the BSF larvae have not been investigated in Ghana.

CHAPTER THREE

THE BLACK SOLDIER FLY, *Hermetia illucens* (DIPTERA: STRATIOMYIDAE): TRAPPING AND CULTURING OF WILD COLONIES IN GHANA

3.1 Introduction

The black soldier fly (BSF), *Hermetia illucens*, is a valuable insect species whose larvae have enormous potentials for converting organic waste into compost, while the larval biomass generated could also be harvested for its protein and fatty acid content (Banks et al. 2014; Diener et al., 2009; Zheng et al., 2011). Large quantities of black soldier fly larvae have to be generated so that they can be used to inoculate the organic fraction of the municipal solid waste, to convert it to compost. Similarly, large quantities of the larvae must be generated to meet demands for its use as protein-source for animal feed formulation. In order to meet the increasing demands for the larvae, for use in bioconversion of organic waste and biomass generation at any point in time, it is important to have a black soldier fly colony readily available. Therefore, local colonization and production methods must be developed.

Although there has been much research focusing on the use of BSF larvae to manage of swine, chicken and cattle manure (Newton et al., 2005; Diener et al., 2009), as well as municipal organic waste (Halloran et al., 2014; Diener et al., 2009), few reports have dealt with its progeny initiation from the wild (Sheppard et al., 2002; Diener et al., 2009). Again, depending on the insect species, specific environmental requirements, for instance regarding temperature, humidity, feeding substrate need to be considered. Leppla (2002), collected BSF eggs from the wild, and reared them to adults in 38 days at 29.3^{o}C, however, he was unable to establish a larval population over multiple

generations. Sheppard et al. (2002), collected eggs in an open-sided caged layer house and successfully maintained them in the laboratory, but was difficult to maintain suitable temperature essential for eliciting mating in the large greenhouse. The rearing of *H. illucens* designed for organic waste management, and also for fish and animal feed production has not received adequate attention, in spite of its immense prospects and economic potential. Much have been reported on the mass rearing of the BSFL in the developed countries (Tingle et al., 1975; Sheppard et al., 2002; Nakamura, et al., 2016), with varying degrees of success. Facilities and environmental conditions that prevail in the temperate countries differ considerably from that of the tropics. Moreover, in developing countries such as Ghana, suitable facilities for rearing insects do not exist as can be found and reported in the developed nations. Locally, easily adaptable methods have to be designed for the mass rearing of the insects, taking into consideration the major environmental factors such as relative humidity, temperature, light and feed. Thus, the study hypothesised that there will be variation in wild *H. illucens* egg clutches trapped on organic manure dumps or heaps in the study area. The objectives of this study were to:

1) identify the most attractive organic manure dumps or heaps for trapping wild BSF egg clutches, and

2) assess putative differences in successfully establishing a laboratory colony of BSF from wild collections and to evaluate respective local environmental conditions for rearing of BSF.

3.2 Materials and Methods

3.2.1 Study Location

The study was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra, Ghana. BNARI is located about 20 km north of Accra (5º40′36.6″ N; 0º11′52.5″ W, and 76 m above sea level).

3.2.2 Colony Initiation

3.2.3 Study of the most Suitable Microhabitat or waste where BSF egg Clutches can be easily Harvested in the Study Area

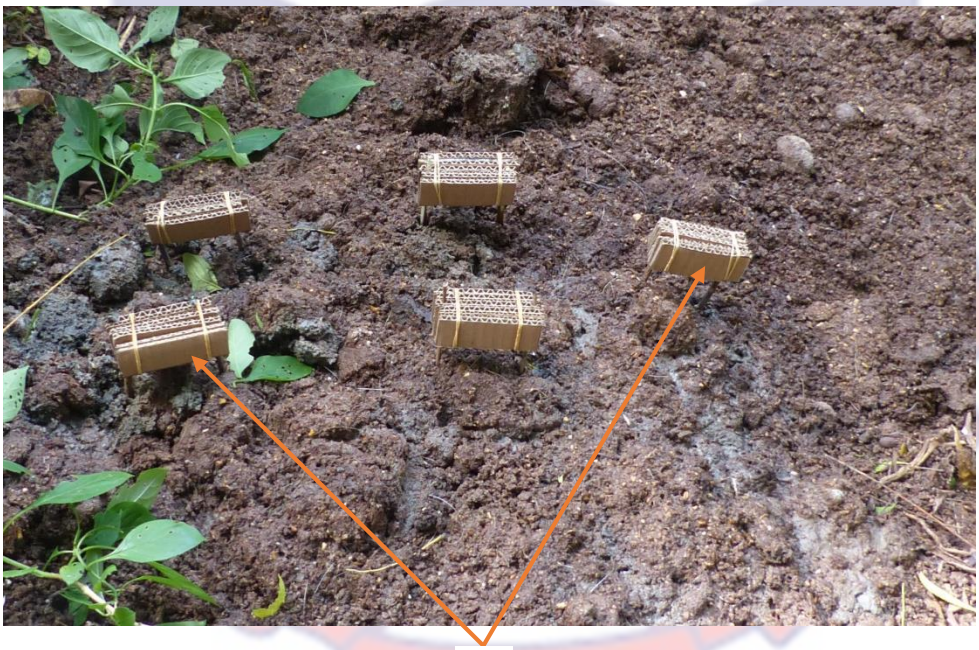
Five egg-laying traps prepared according to Sheppard et al. (2002), and consisting of five plys of cut corrugated cardboard that were held together with a rubber band. Traps were 8cm long and were placed at equidistance (10cm between and within rows) from each other and 2cm above the microhabitats (piggery manure dumpsite, poultry manure dumpsite, sheep manure dumpsite and a compost heap) (Figures 3.1 and 3.2). These microhabitats were of the same age (about three weeks old) but the piggery and sheep waste microhabitats received daily addition of waste from the pig and sheep styles. Egg collection was conducted two times in a month (at two weekly intervals) for 13 months (October, 2015 – October, 2016).

Temperature and relative humidity at three different sections of each microhabitat were taken on the day and at the time of trap set and their means recorded. Traps were checked for egg clutches 24 hrs after deployment and removed 48 hrs thereafter and inspected for egg clutch deposition. This is

because BSF eggs begin to hatch 3.5 days at 30°C (Tomberlin and Sheppard, 2002) or 4.3 days at 24°C (Sheppard et al., 2002).

Figure 3. 1: Trapping of BSF egg clutches at different microhabitats a) Compost heap; b) Poultry waste dump

a



b

Figure 3. 2: Trapping of BSF egg clutches at various microhabitats a) Sheep waste dump and b) Piggery waste dumpsites

3.2.4 Incubation of Harvested Egg Clutches

Egg clutches sired were immediately transferred into a clean, moist surface in order to prevent contamination by fungus (Shakil, 2014). The numbers of egg clutches sired in the grooves of the cardboard traps were counted and their weight determined using an analytical balance. The traps with egg clutches harvested from different microhabitats were incubated separately under ambient conditions on 50g of finely grounded moistened layer meal placed in 250 ml incubation boxes (Figure 3.3) with ventilated lids in the laboratory.

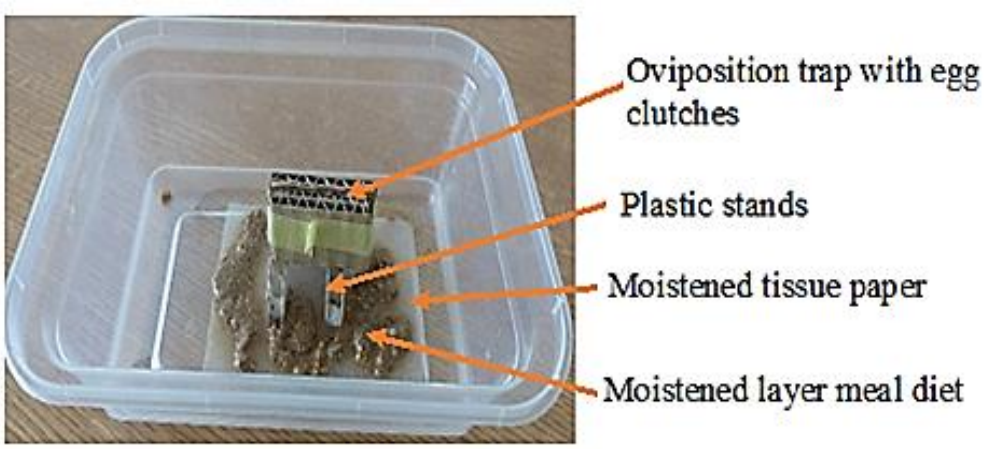


Figure 3. 3: Incubation of BSF egg clutches

3.2.5 Larval Feeding

Five days after egg clutch incubation, 1000 larvae from each microhabitat replicated three times were picked with the aid of disinfected forceps and transferred separately into 50 litre (L) plastic containers. These plastic containers had been cleaned with detergent and disinfested with 70% alcohol and dried under the sun for at least six hrs. The 50L plastic containers had ventilated lids to promote aeration, reduce generation of excessive heat and to prevent the escape of larvae (Figure 3.4).

Figure 3. 4: 50 L larval box

Temperature and relative humidity were monitored at 10.00 am from three different locations of the larval cages using digital thermohydrographs. The larvae were reared on layer meal diet moistened to 70% moisture content in the laboratory under 12:12 L: D photoperiod. Feed given was computed based on a formula adapted from Stamer (Personnal Communications, 2015) as follows:

$$Feed\ amount\ (g) = \frac{Number\ of\ larvae \times 0.1g}{MC} \times 25 \times 70\% \qquad (3.1)$$

Where MC = moisture content of diet

0.1 = Amount of feed per larvae per day (Diener et al. 2009)

25 = Estimated larval feeding duration (days) (Tomberlin et al. 2009; Caruso et al. 2014).

70% = Adjusted moisture content of diets

3.2.6 Prepupae Sorting

Feeding of the larvae was stopped and prepupae were hand-picked with the aid of disinfected forceps, when about 50% of the larvae had turned into prepupae as indicated by change in colour from beige to dark brown or black (Tomberlin et al., 2009; Caruso et al., 2014; Dortmans, 2015) in a particular larval cage from each trapping section and microhabitat. Five hundred prepupae replicated 3 times were weighed and kept in appropriate sized plastic eclosion containers with crushed shredded papers to induce pupation (Figure 3.5a). To prevent laying of eggs by the parasitoid *Dirhinus giffardii* (Hymenoptera: Chalcididae) (Devic and Maquart, 2015), in the prepupae, the eclosion containers were covered with fine-meshed muslin cloth secured in place with a rubber band (Figure 3.5b).

Figure 3. 5: Eclosion container with prepupae and shredded paper a); b) Muslin cloth to prevent parasitoids entry and adult escape.

3.2.7 Adult Maintenance and Egg Collection

The eclosion containers were placed in adult wooden cages measuring 50 x 50 x 50cm (Figure 3.6a), as soon as adults began to eclose from their pupae. The adult cages were placed in the adult rearing room to allow for more sunlight as sunlight is reported to encourage mating (Newton, et al., 2005b). Moistened fresh layer feed were placed in transparent plastic containers (8 x 13.5 x 5cm) part of whose covers have been cut and fitted with cut corrugated cardboards taped together with a masking tape. These setups served as egg harvesting devices (Figure 3.6b) to entice female flies to lay eggs and was placed in the adult cage.

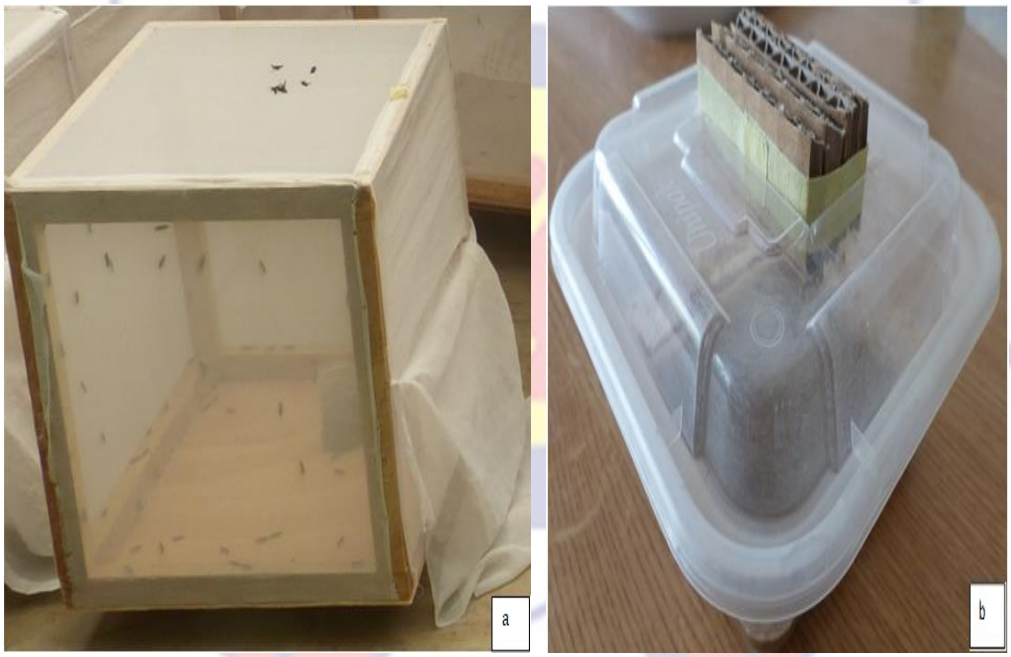


Figure 3. 6: (a) BSF Adult cage; b) Egg harvesting device

Environmental conditions (temperature and relative humidity) in the adult room monitored with the aid of digital thermohydrograph. The egg harvesting devices were checked daily for presence of sired eggs. Fresh water mist was sprayed from a sprayer onto the muslin cloth covering of the adult cage mid-

morning and in the afternoon to provide water droplets (Tomberlin et al., 2009) for maximum longevity of adults (Tomberlin et al., 2002; Myers et al., 2008; Holmes, 2010) and improve ambient humidity as the water evaporated (Tomberlin & Sheppard, 2002; Tomberlin et al., 2009).

3.3 Data Analyses

Data on egg clutch trapping being positively skewed were square root transformed to conform to the normality assumptions before F-test (ANOVA) was performed. To establish possible relationships that may exist between temperature and relative humidity at the microhabitats where egg traps were set as well as larval length and weight, analysis of variance (ANOVA) was used. The Tukey-Kramer procedure was used for mean comparison due to treatment effects. T-test was used to test for any relationship between rearing conditions during the larval and adult rearing of egg clutches harvested from the piggery and compost microhabitats.

3.4 Results

3.4.1 Effect of type of Organic Waste Dumpsite on egg Clutch Harvest

The distribution of BSF egg clutches trapped during the study is presented in Figure 3.7. In all, fifty-five egg clutches were harvested from the piggery dumpwaste microhabitat from October, 2015 to October, 2016, whiles only 2 egg cluctes were harvested from the compost heap microhabitat within the same period. The average weights of all egg clutches harvested were 64mg and 30mg for the piggery waste dumpsite and the compost heap microhabitats respectively.

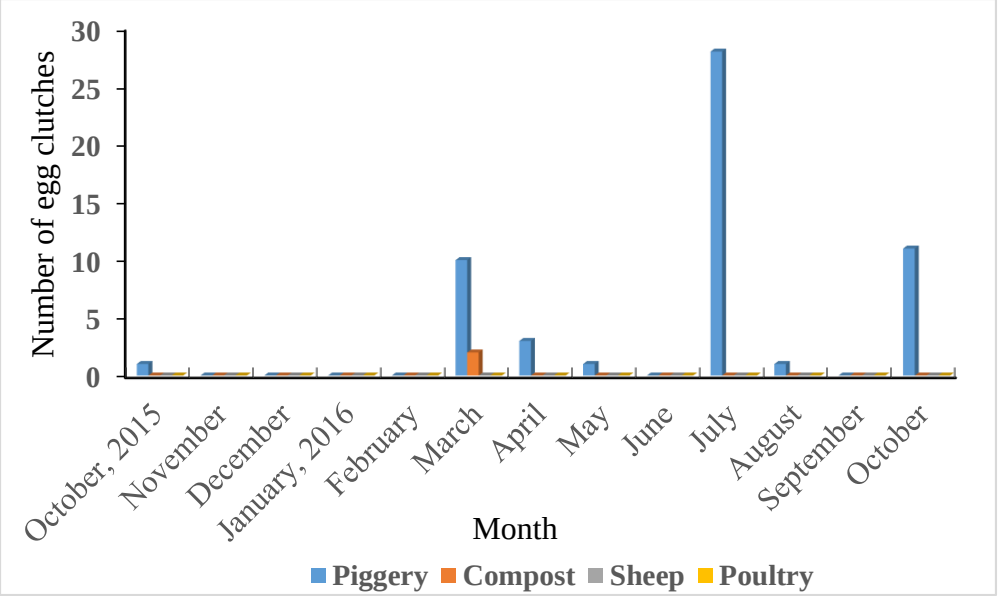


Figure 3. 7: Monthly egg clutch collections as affected by type of dumpsite

3.4.2 Seasonal Variation in BSF Egg Harvesting

No significant difference was observed in egg clutch harvested during the minor rainy (October – December, 2015) and the major rainy April – July, 2016 seasons respectively (df = 1, 11; F = 0.94; p = .353). Further, no egg clutch was collected from both the poultry and sheep microhabitats in both seasons (Figure 3.8). On the average, 7.50±6.83 and 2.80±1.49 egg clutches were collected from the piggery microhabitat per month during the major and minor rainy seasons respectively. On the other hand, 0.22±0.22 egg clutches per month were collected from the compost microhabitat in the minor rainy season and none during the major rainy season.

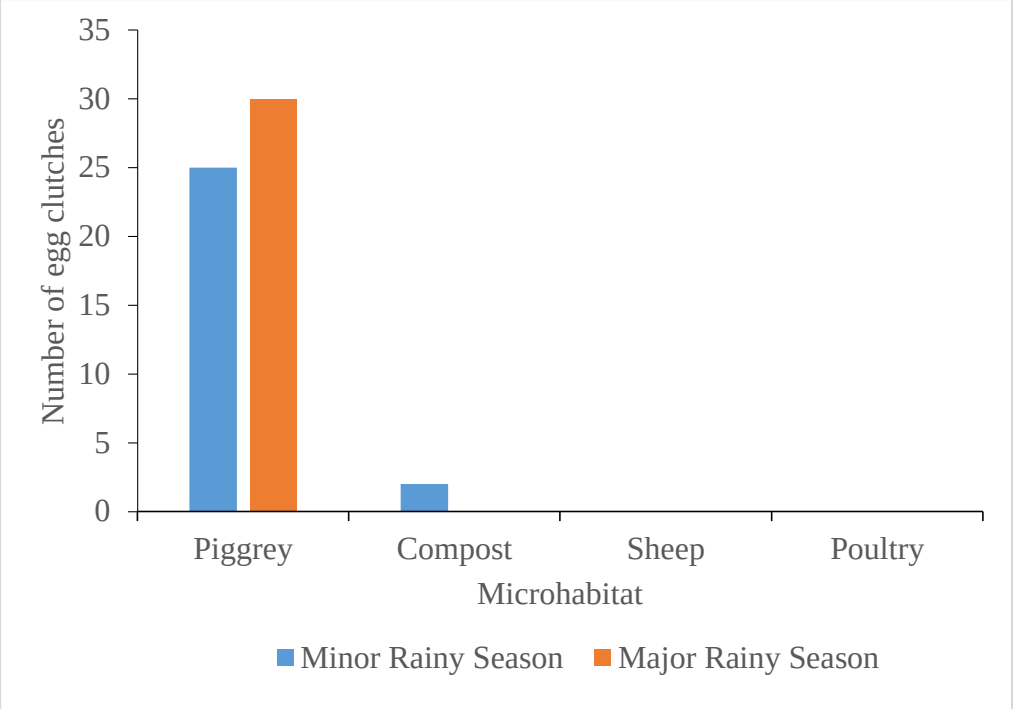


Figure 3. 8: Seasonal BSF egg clutch collections as affected by microhabitat

3.4.3 Climatic Conditions of Microhabitats

There were no significant differences in both temperature and relative humidity on the microhabitats during egg clutch harvest as determined by one-way ANOVA (df = 3, 48; F = 0.16, p = .920) and (df = 3, 48; F = 0.24, p = .869) for temperature and relative humidity respectively during egg trapping (Figure 3.9 a, b).

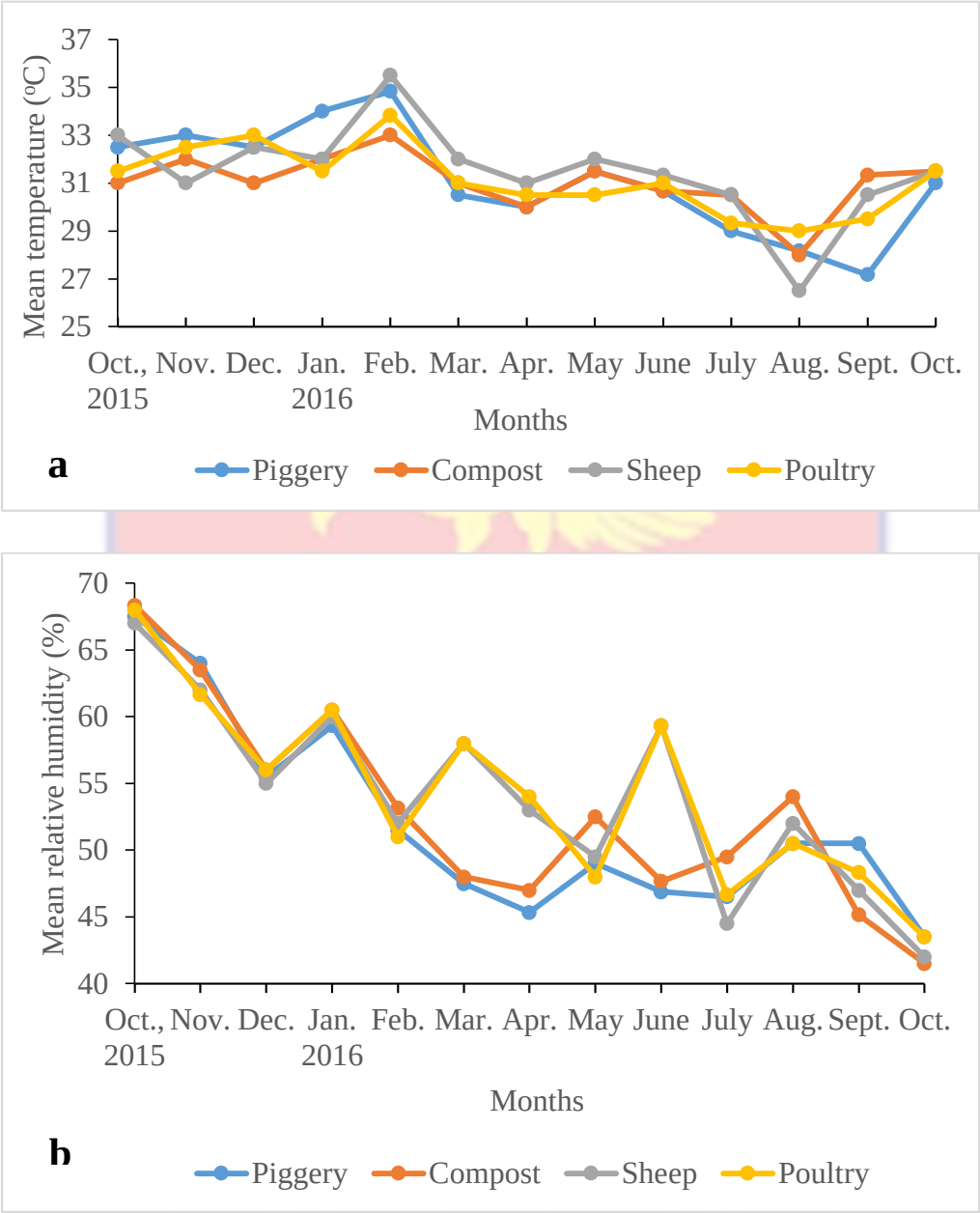


Figure 3. 9: Mean monthly microhabitat a) temperature b) relative humidity during BSF egg clutch collections

However, significant differences (df = 1, 309, F= 87.78, $p < .0001$) were observed in both seasonal temperature and relative humidity on the microhabitats. Generally, microhabitat temperatures were higher in the minor rainy season, October – December, 2015 (31 – 33°C) whiles lower

temperatures were recorded in the major rainy season April – July, 2016 (30 – 31^{o}C) in the Southern part of Ghana. Similarly, percent relative humidity was 55 – 68 and 44 – 59% for the minor and major rainy seasons respectively in the Southern part of Ghana. Within the same period percent relative humidity during egg harvesting was 46 – 68% and from May – August, 2016 was 41 – 59%.

3.4.4 Larval Rearing

During larval rearing of egg clutches harvested from the piggery and compost microhabitats in separate larval boxes in the laboratory to evaluate larval growth and survival, a significant difference was observed only in percent relative humidity (t-Test, p = .101) but not temperature (t-Test, p = .010), (Figures 3.10 and 3.11).

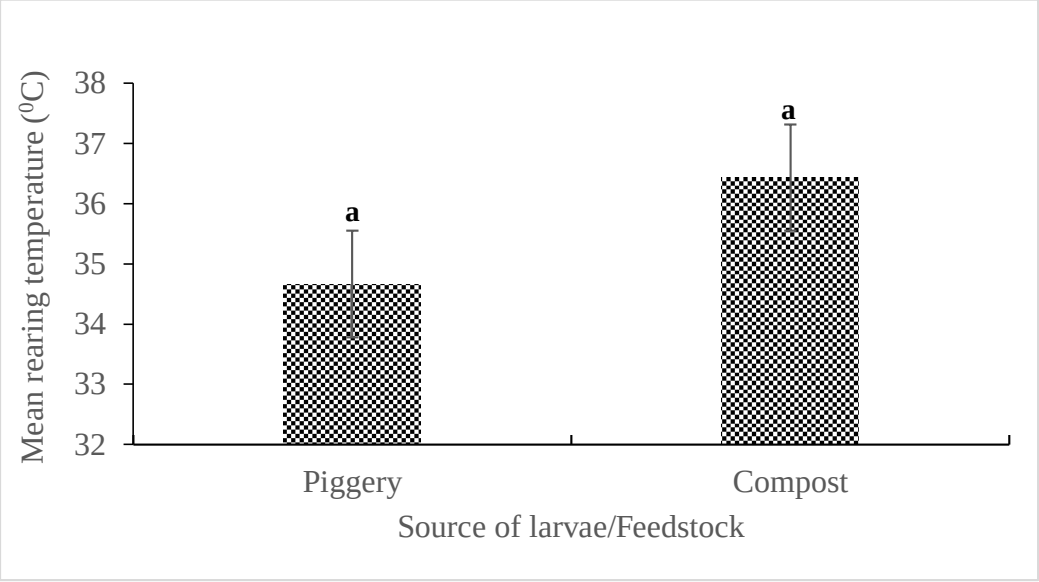


Figure 3. 10: Mean temperature in larval boxes during larval rearing

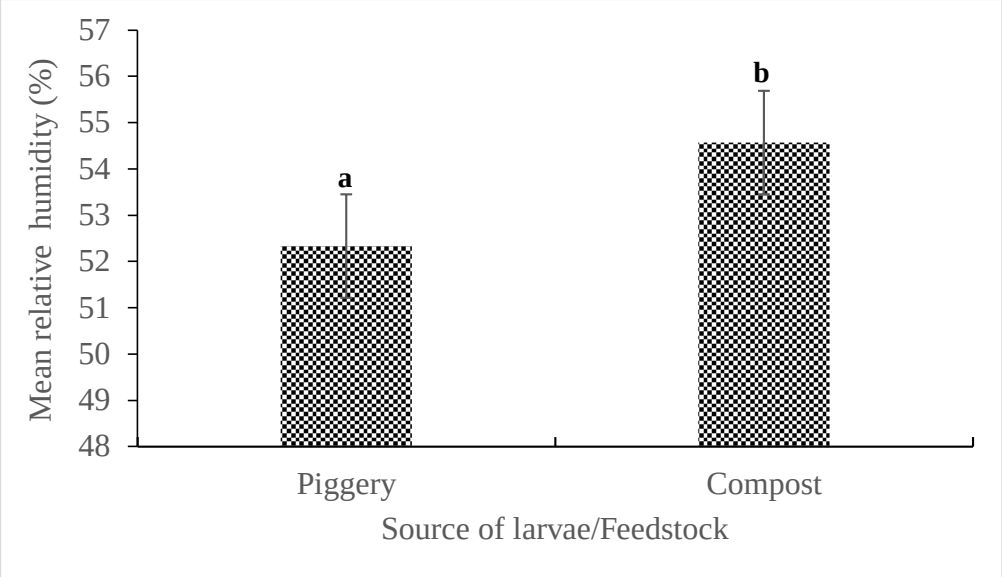


Figure 3. 11: Mean percent relative humidity in larval boxes during larval rearing

Significant differences in both weight and length of larvae were observed on day 5 after egg hatch and in length only on day 10 (Figures 3.12 and 3.13).

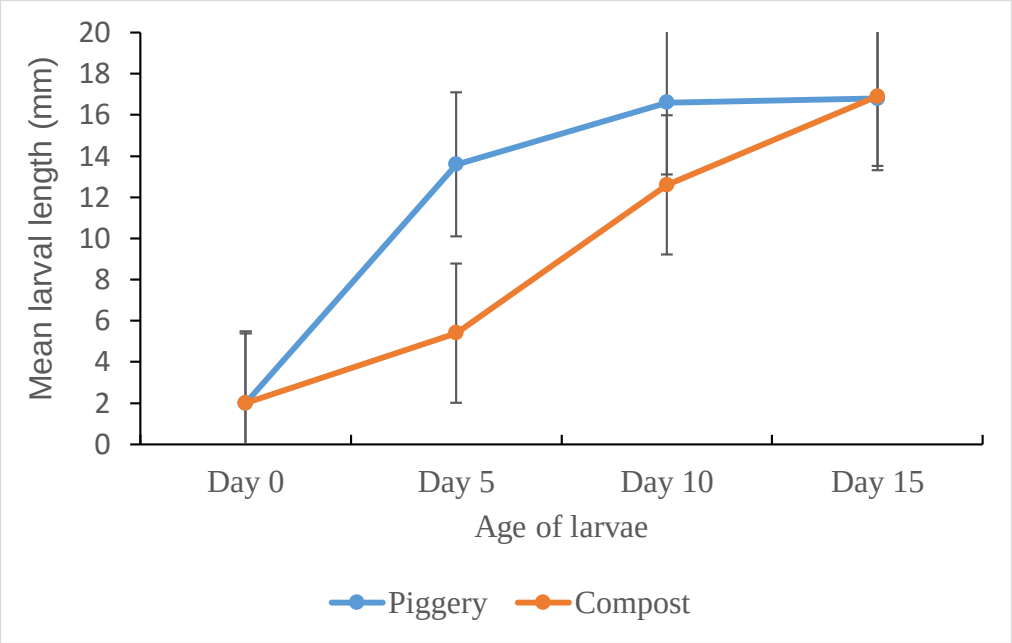


Figure 3. 12: Effect of age on wild black soldier larval length

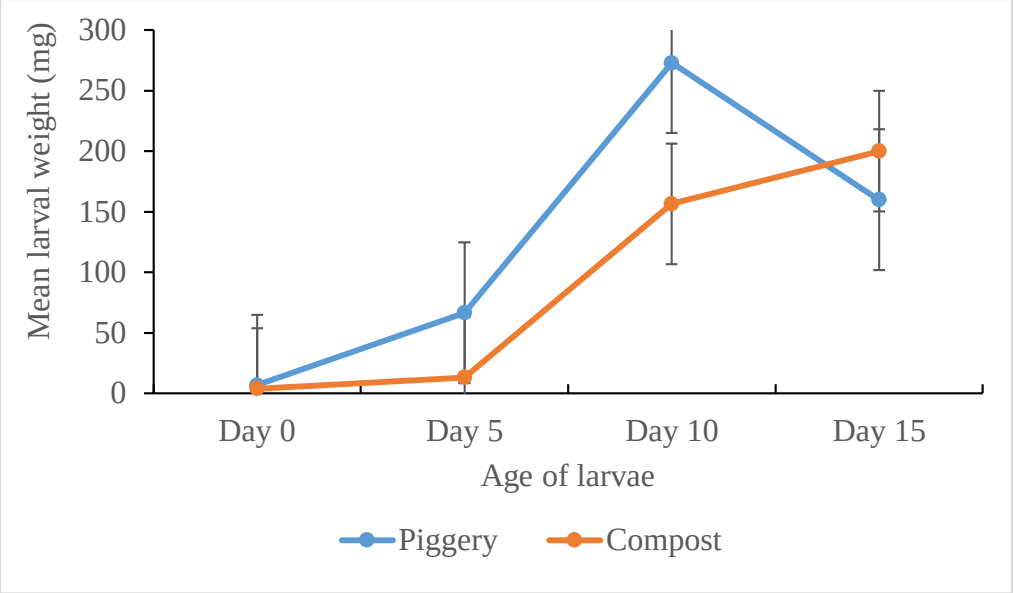


Figure 3. 13: Effect of age on wild black soldier larval weight

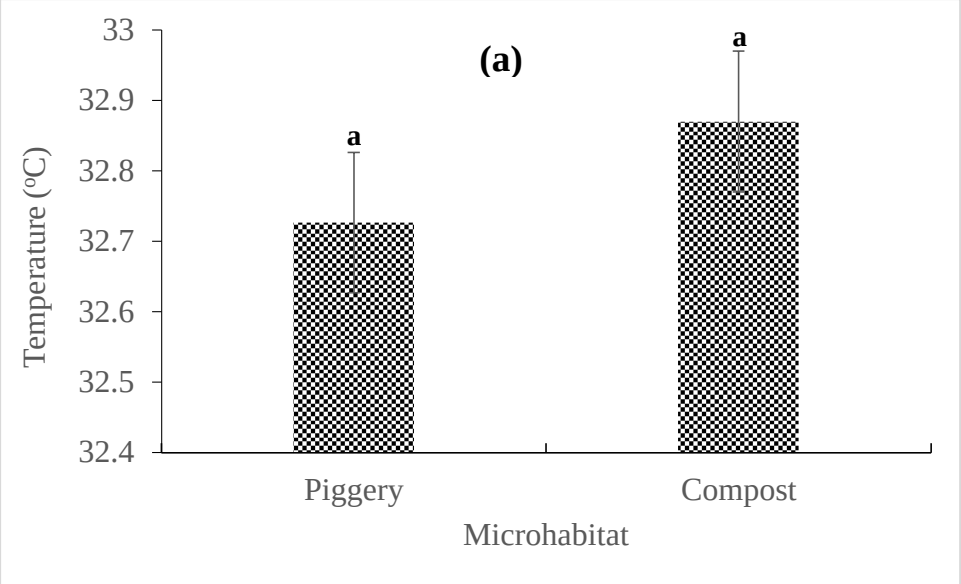
3.4.5 Pupal Development and Adult Rearing

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

No significant differences in temperature (t-Test, p = .211) and percent relative humidity (t-Test, p = .074) were observed during adult rearing (Figures 3.14*a* and *b*). Temperature was 32.72°C±0.06 and 32.87±0.06°C whiles percent relative humidity was 71.10±0.36% RH and 73.82±0.94% for adults' eclosing from prepupae from the piggery and compost microhabitats respectively.

Table 3. 1: Mean (± SE) Larval and pupal periods, percent eclosion, sex ratio and egg clutch of prepupae from the piggery and compost microhabitats (*$p < .05$) N = 500

Microhabitat	Larval period (Days)	Pupal period (Days)	Eclosion (%)	Sex Ratio		No. of egg clutches/female
				Male	Female	
Piggery	22±0.58	8±0.14	54.66±2.74	1.17±0.02:	0.96±0.08	0.96±0.02*
Compost	19±0.29	10±0.60	65.00±6.66	1.23±0.12	1.30±0.12	0.68±0.02*
P-value	0.370	0.107	0.730			0.026



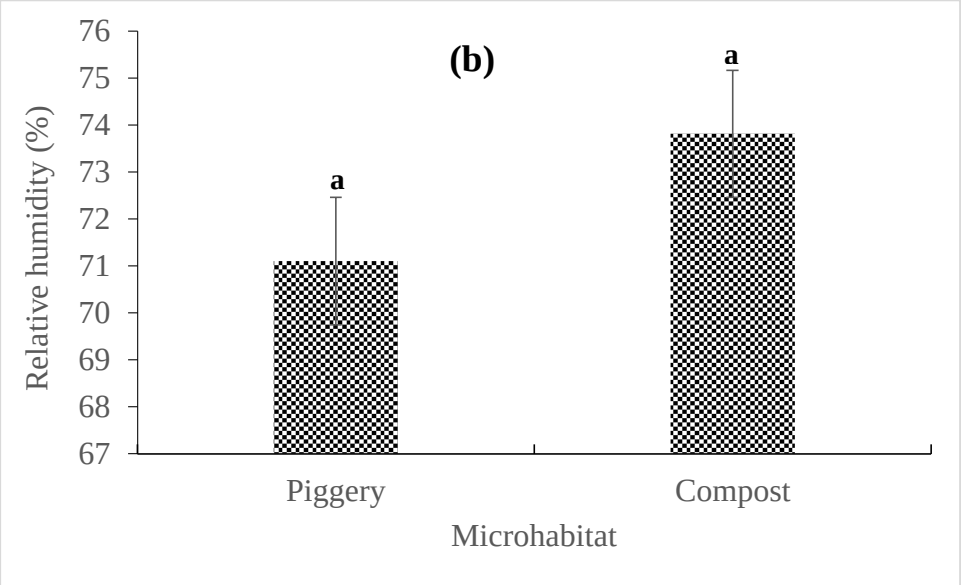


Figure 3. 14: Mean a) temperature b) percent relative humidity in adult cages during larval rearing

3.4.6 F_1 Egg Clutch Production

F_1 adult female flies eclosing from prepupae recovered from incubated egg clutches harvested from both the piggery and compost microhabitats sired egg clutches which were statistically different from each other (t-Test, $p <. 05$) (Table 3.1). These egg clutches were supplied to the colony to improve the

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

3.5 Discussion

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

The outcomes obtained from the egg clutch harvest showed the importance of organic waste dumpsite microhabitats in the collection of *H. illucens* egg clutches (Sheppard et al., 2002; Collavo et al., 2005; Tomberlin et al., 2009). Oviposition traps placed a few centimeters above piggery waste dumpsite and compost heap microhabitats enticed gravid *H. illucens* females to sire egg clutches. Organic wastes release several volatiles such as alcohol, aldehydes, and organic acids during decomposition that attract insects (Caruso et al., 2014; Sharma et al., 2015). Depending on the type of organic waste involved, the volatiles released would be different and would therefore attract insects differently. In addition, the age of the organic waste will also affect the rate and type of volatiles released. Caruso et al. (2014), showed that

maintaining the RH of oviposition substrate at 60 – 70% help release volatile attractive substances. Experience shows that it is necessary to maintain the substrate humidity around 60–70%, as it helps in releasing volatile attractive substances. It is further recommended to renew baits regularly, approximately every 10 days. However, olfactometric experiments conducted by Tomberlin, (2001) did not identify the specific molecules or group that act as attractants for BSF. For instance, females of *Drosophila melanogaster* use their gustatory abilities to assess the quality of oviposition sites (Yang et al., 2008). It is however probable that, the oviposition substrate is chosen by the female according to its nutritional value for its offspring. Additionally, adults require bacteria to attract females for oviposition (Zheng et al., 2013) while males need lekking sites to establish successful mating (Sripontan et al., 2017) and correct lighting conditions (Zhang et al., 2010) to reproduce and oviposit efficiently.

The absence of egg clutch harvest from the poultry and sheep waste dumpsites may possibly be due to the depletion of nutrients particularly nitrogen (Zhang et al., 2010) in these wastes by microorganisms. The low egg clutch harvest from the compost heap microhabitat could be due to temperature build-up during the thermophilic stage in the compost which might have been detrimental to egg hatch and larval development (Sripontan et al., 2017). Caruso et al. (2014), showed that males assemble in small numbers near sheltered vegetation growing around decomposing organic matter (BSF microhabitats) and engage in competitive display that attract females in order to find a mate (Tomberlin & Sheppard, 2002). Additionally,

the different vegetation growing around decomposing organic matter (BSF microhabitats) provide adult resting places, heat, and protection from rain (Caruso et al., 2014) and assure the adults of droplets of water. The compost heap did not have any vegetation close by and so might explain the very low egg clutch trapped as there were no resting places for adult flies and thus depriving the BSF adults' areas to congregate for lekking behaviour which could lead to mating and subsequent oviposition (Banks et al., 2014).

The insect-substrate interaction is a complex phenomenon that depends on; environmental factors, substrate availability and physicochemical characteristics, and the presence of either conspecific immature insects or competing species (Caruso et al., 2014). These factors will therefore account for the differences in the number of sired egg clutches observed in the organic waste examined (Caruso et al., 2014), as the insect has to be attracted to the site before it can lay the eggs. *H. illucens* gravid females naturally oviposit eggs around waste which is ample and most consumed by its larvae in its immediate environment (Sripontan et al., 2017) and thus piggery waste seems to be the only food source for field populations of BSF in the study area and which may provide a more suitable habitat for survival and feeding of the offspring of the BSF (Caruso et al., 2014).

Number of egg clutch harvested did not vary between rainy seasons (t-Test, p = .353) indicating an eight months in the year possibility of harvesting BSF egg clutches from especially the piggery microhabitat to establish or revamp a colony. Sheppard et al. (2002), reported that adult black soldier flies

typically mated and oviposited at temperatures of 24 – 40ºC. In this study, a temperature range of (28 – 37ºC) was recorded during the entire harvesting period. This temperature range is in accord with the reported view of *H. illucens'* greater prevalence to tropical and subtropical habitats (Bondari & Sheppard, 1981; St-Hilaire et al., 2007a).

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

The source of egg clutch influenced larval weight and larval length gain within the first 10 days (4^{th} larval stage). *H. illucens* typically has six larval instars with developmental commitment to metamorphosis occuring early in the 5^{th} instar. Larvae obtained from the egg clutches harvested from the compost heap microhabitat increased rapidly in weight and length between days 5 and 10 such that, by day 15, no significant differences in growth rate was observed between the two sources of larvae. The reason may be due to the fact that larvae hatched from egg clutches harvested from the compost had higher or better efficiency of conversion of ingested food (Diener, et al., 2009). This involves homeostatic adjustment of consumption rates and efficiency parameters such that an insect can approach its "ideal" growth rate

even with foods of different quality in various environments (Xue et al., 2010).

It was found in this study that piggery manure dumps or compost heaps are important for BSF egg deposition and that there were no significant differences in life history traits of egg clutches from different microhabitats. A total of 694 egg clutches were sired by F_1 female flies eclosing from both the piggery and compost microhabitats and these egg clutches helped as a basis for colony establishment and has provided numerous BSF larvae. The results of this study indicate that the materials and methods adopted for laboratory rearing of target insect are efficient and mass rearing of BSF is feasible in Ghana. Layer meal was used in establishing this colony just as has been used by other researchers (Tingle et al., 1935; Sheppard et al., 2002), however, larvae that are intended for waste treatment and feed production, could be reared on wastes from restaurant, vegetable or fruit waste and even market waste, just to reduce cost of colony maintenance (Nguyen et al., 2013). However, further studies may be needed for establishing a colony fed solely with any of the above wastes mentioned.

3.6 Conclusion

- Egg traps prepared according to Booth and Sheppard 1984, could be placed 2cm above piggery dumpsite and on compost heap.
- To increase egg hatching success and neonate survival, BSF eggs should not come into direct contact with liquids, which can be achieved by placing the egg traps on stands e.g. stone marbles.

- Larval feed must not be too thick (about 4 – 5 cm) in thickness to allow for complete digestion; thus feed given should be just enough for complete assimilation within a day.
- Larval cages should be cleaned regularly with detergent, disinfested with 70% alcohol and sun dried for about 6hrs before reuse.
- Prepupae should be protected from the parasitoid *Dirhinus giffardii* (Hymenoptera: Chalcididae) by the use of very fine muslin cloth in covering larval, pupal and adult cages.
- Provision of misting water at least twice a day to adult flies is essential and this water spray should not wet the floor of the adult cage.
- Dead adults should be swept off the floor of the adult cages twice a week and the adult cage thoroughly washed once every three months.
- BSF egg trapping was successful on the piggery and compost microhabitas during March to October and not possible from November to February on all microhabitats.

CHAPTER FOUR

EFFECTS OF DIFFERENT ARTIFICIAL DIET FORMULATIONS ON THE SURVIVAL AND REPRODUCTIVE PERFORMANCE OF BLACK SOLDIER FLY, *Hermetia illucens*, (DIPTERA: STRATIOMYIDAE).

4.1 Introduction

Artificial diet formulation is the process of selecting the kinds and amounts of ingredients (including vitamin and mineral supplements) to be used in the production of a diet containing planned concentrations of nutrients (Cohen, 2004). Artificial diets are used for the domestication, colonization, mass production and maintenance of a large number of animal species important for human welfare. For instance, fishes (Ruohonen et al., 2003), crustaceans (Catacutan, 2002), molluscs (García et al., 2011), echinoderms (Basurco et al., 2015), pork (Woyengo et al., 2014), poultry (Basurco et al., 2015) and insects (Cohen, 2004) are reared and maintained on different types of artificial diets. Artificial diets must fulfil sensory requirements and be nutritious for animals within a context of economic practicability (Cohen, 2004). The production of artificial diets is one of the most substantial direct input costs in animal breeding (Chaudhury and Skoda, 2007; Woyengo et al., 2014).

Nutrition is an important contributory factor to life span, rate of aging, and reproductive potential (Weindruch & Walford, 1982; Chippindale et al., 1993; Good & Tatar, 2001; Walker et al., 2005; Fontana et al., 2010) of animals. Insects feed on particular diets to obtain the required mixture of nutrients that are necessary for their survival, growth, and fecundity (Behmer, 2009). However, proteins and carbohydrates are two significant

macronutrients that provide the essential amino acids and energy, that influence their development, growth, and fecundity (Karasov & del Rio, 2007; Simpson & Raubenheimer, 2012). Studies have shown that insects such as grasshoppers, crickets, ants, various caterpillars, etc., have the ability to regulate their intake of protein and carbohydrate independently when provided the opportunity (Simpson & Raubenheimer, 1993, Simpson et al., 2006; Lee et al., 2008; Dussutour & Simpson, 2009; Altaye et al., 2010; Cease et al., 2012). The life history traits of mostly phytophagous insects are influenced by resource availability (Awmack & Leather, 2002). For instance, the relative intake of the two main nutritional resources, protein and carbohydrate which serve as tissue building-blocks and an energy source respectively, have significant impact on an individual's growth, development, survival and fecundity (Joern & Behmer, 1997; Naya et al., 2007; van Huis et al., 2008).

Kaspi et al. (2002), demonstrated that adult fly size and development time of Mediterranean fruit flies were related to the amounts of protein and sugar in larval diet. Gobbi et al. (2013), fed 600 BSF first instar larvae on hen meal, hen meal+meat meal and meat meal and found out that meat meal was the worst of the three diets in terms of percentage mortality and duration of larval and pupal stages. Food ingested by the BSFL determines both physiological and morphological development of the adults. Larvae reared on equal proportions of protein and carbohydrates (21%: 21%) at 70% moisture content developed the fastest and on the least amount of food and had the greatest survivorship to prepupal stage (Cammack & Tomberlin, 2017).

The larvae of *H. illucens* feed on a variety of organic matter such as kitchen waste and spoiled food (Newton et al., 2005), fruits, vegetables, animal remains and animal manure (Newton et al., 2005), and can reduce manure accumulation by up to 56% (Sheppard, 1983). The larvae of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), can be used as an ecological engineer for treating organic waste into usable products such as compost and also as component in fish or animal feed formulation. To achieve some of these practical uses of the larvae, it is important to identify methods that can be used to rear the larvae in large quantities. One of the required methods is to establish the least expensive type of artificial feed/diet that can be used to feed the larvae to increase in biomass. It is also essential to economically mass rear this important insect in order to study their life history, behaviour, feeding habits, and applicability in waste management and as a component of animal feed. Larval diet is one of the critical and costly aspects in mass rearing of insects. A careful consideration in the selection and formulation of the larval diet could reduce the cost significantly, while achieving the same effects on survival, growth and reproduction. Thus, the careful selection of diet composition can significantly reduce the cost but achieve the same effect on larval growth.

Good nutrition has significant impact on insect rearing and reproduction (Erens et al., 2012). Nutritional requirements for growth differ among insect species, however, dietary components such as carbohydrates, proteins, lipids, minerals, and vitamins are always important constituents (Erens et al., 2012). These important nutrients can be sourced from different

diets but the cost could differ. Earlier reports of rearing black soldier fly larvae used the Gainesville housefly diet (Hogsette, 1992; Tomberlin, 2001) or layer hen feed mixed with water (60 – 70% moisture) (Sheppard et al., 2002). These diets are not available locally, or had to be imported with hard-earned foreign currency. The availability and accessibility of sources of larval diet that are locally availabile for sustained laboratory colonization of *H. illucens* could affect the cost and availability (Sy & Campos, 2008; Nestel & Nemny–Lavy, 2008; Puggioli et al., 2013). It is therefore necessary to conduct research to identify locally available sources of diet formulations and their effect on BSF larval development and reproduction. This study was conducted to evaluate the growth and reproductive performance of *H. illucens* larvae reared on reduced layer meal mixtures. The hypothesis tested was that wheat bran or layer meal-wheat bran mixtures will be suitable substitutes to the standard (layer meal) *H. illucens* larval diet. The aim of this study was to identify suitable larval diet mixtures that will reduce cost and will not affect the quality of the larvae that are reared in large quantities in Ghana.

4.2 Materials and Methods

4.2.1 Study Location and Source of Black Soldier Fly Eggs.

The study was conducted at the Black Soldier Fly Laboratory of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) in Atomic, Accra, Ghana. Atomic is located about 20 km north of Accra and lies between latitude 5º40′36.6″ N and longitude 0º11′52.5″ W, with an elevation of 76 m above sea level (Ewusie et al., 2010). The vegetation is Coastal Savannah, and the area is

characterized by a bimodal rainfall pattern with the major rainy season falling between April and July, and a minor rainy season between October and December. Mean annual rainfall is 810mm distributed over less than 80 days, and temperatures are moderate with maxima rarely exceeding 32ºC while the minimum does not fall below 17ºC. The average humidity is about 60 – 70% (Ofori et al., 2014).

4.2.2 Proximate Analysis of Diets

Hundred grams of each diet was used to determine the proximate composition of each diet (AOAC, 2001- 2009) mainly moisture, protein, fat, fibre, ash and carbohydrate contents. Total nitrogen was determined by the micro- Kjeldahl method (AOAC, 2001) and crude protein was obtained by using a Nitrogen Protein conversion factor of 6.2. Moisture content was determined by oven-drying at 105 ºC overnight, fat was determined by the ether extraction method, using a Soxhlet system, ash by heating for 5hrs at 550ºC in a muffle furnace and crude fibre was determined using acid-base hydrolysis (extraction with 0.5 M H_2SO4 and 0.5 M NaOH), (AOAC, 2009). Carbohydrate was computed using the formula (100 – (% moisture +% ash +% crude protein +% crude fat +% crude fibre)) (AOAC, 2009). Results were expressed as percent total dry weight.

4.2.3 Experimental Set Up

Egg clutches were obtained from a colony of black soldier fly that was established from wild collected egg clutches from both piggery and compost microhabitats in 2015, which has been maintained for over ten generations. *H.*

illucens egg clutches were incubated on bread crumbs mixed with water at 25% moisture content for 4 days. Uniformly sized one hundred larvae, which were 5-days old, were then randomly assigned to 15 plastic containers (20 × 15 × 7cm) with a ventilated lid (Figure 4.1).

Figure 4. 1: Small black soldier fly larval cages

Five different formulated artificial diets at 70% moisture content (Table 4.1), replicated three times were given to the larvae in the cages above.

Table 4. 1: Artificial diet formulations for rearing black soldier fly, *Hermetia illucens* used for the study (200 grams each)

Diet	Constituent	Percentage combination (%)	Quantity
1	Wheat bran	100	200
2	Layer meal (Control)	100	200
3	Wheat bran+Layer meal	25:75	50:150
4	Wheat bran+Layer meal	75:25	150:50
5	Wheat bran+Layer meal	50:50	100:100

Twenty larvae, representing 20% of the larvae in each treatment were randomly picked with the aid of forceps and used to determine larval weight and length at five days intervals, until all larvae turned to prepupae. Larval period was determined as the interval between egg hatch and the day at which a larva turned prepupae, whiles the interval between prepupae and eclosion served as the pupal period.

4.2.4 Quantitative Nutrition and Efficiency of Conversion of Digested Food

Quantitative nutritional aspects determination were based on the terminology used in Scriber and Slansky (1981); B = (I–F)–M and ECD = B/ (I–F), where B = assimilated food used for growth (measured as prepupal biomass), I = total food offered during the experiment, F = residue in experimental boxes (undigested food + excretory products), M = assimilated food metabolized (calculated by mass balance). ECD = efficiency of conversion of digested

food. High ECD values signify high food/diet conversion efficiency (Diener et al. 2011a).

4.2.5 Prepupae Sorting

In a particular larval cage, larvae that had turned into prepupae (as indicated by colour change from beige to dark brown or black Tomberlin et al. 2009; Caruso et al. 2014; Dortmans, 2015) were hand-picked with the aid of forceps from each diet treatment and their total weight determined. Thereafter, the prepupae were kept in cylindrical plastic containers (8 x 10cm) and provided shredded papers to induce pupation (Figure 3.4a).

Percent prepupae recovered from each diet treatment was computed after all prepupae had been removed. Dead larvae were counted and percent larval mortality in each diet treatment were calculated. The diets left over after all prepupae and dead larvae had been removed from each treatment were individually weighed and percent diet reduction computed. The eclosion containers were covered with fine-meshed muslin cloth secured in place with a rubber band (Figure 3.4b) in order to prevent laying of eggs by the parasitoid, *Dirhinus giffardii* (Hymenoptera: Chalcididae) (Devic & Maquart, 2015).

4.2.6 Adult Maintenance and Egg Collection

As adults' eclosed from the prepupae, the time interval between prepupae set up and adult eclosion was noted and this served as the pupal period. Eclosing adults were transferred into wooden cages (45 x 45 x 50cm) that had muslin cloth covering all sides except the base that was made of plywood (Figure

3.6a) in the adult rearing room at the laboratory under ambient conditions. The roof of the rearing room was constructed with transparent corrugated sheets. Moistened fresh larval diet were placed in transparent plastic containers (8 x 13.5 x 5cm) part of whose covers had been cut and fitted with cut corrugated cardboards taped together with a masking tape. These setups served as egg harvesting devices (Figure 3.6b) to entice female flies to lay eggs and were placed in the adult cages. Fresh water was sprayed onto the muslin cloth covering the adult cages to provide drinking water and improve on the humidity. Egg harvesting devices were inspected daily and egg clutches sired were counted and recorded. All dead adults were recorded, sexed and percent eclosion computed at the end of the study. Egg clutch per female was computed by dividing the number of egg clutches sired by females eclosing from each treatment, this is because ISO (Isolated) female technique could not be used as no mating or egg collections occurs in smaller cages (53 x 91 x 53cm and 38 x 46 x 38cm) (Sheppard et al., 2002). Additionally, BSF adult males assemble in small numbers and display lekking behaviour (Tomberlin & Sheppard, 2001) in order to find a mate.

4.3 Data Analyses

The data were analyzed using GenStat Release12.1 (2009) and complimented by Excel. Data collected were tested for normality using the Shapiro–Wilk test and, where necessary, log-transformed to meet the assumptions of normality and homogeneity of variances before F-test was performed. Analysis of variance (ANOVA) was used to test for differences in development time with regard to larval and pupal periods, larval weight and length as well as any

relationship between the proximate compositions of the formulated diets. The level of significance was set at $p < .05$. Pairwise comparisons were performed using Tukey-Kramer procedure.

4.4 Results

4.4.1 Proximate Analysis of Larval Diets

Carbohydrate and protein levels were significantly lower ($p < .001$) in the 100% wheat bran (WB) diet than those from the other diets (Table 4.2). In contrast, crude fibre and moisture contents were significantly higher ($p < .001$) in the 100% WB. The 100% layer meal (LM) and the 75% LM: 25% WB diets contained more protein and carbohydrates respectively than all the other diets. No significant difference was found in carbohydrate contents in 75% LM: 25% WB and the 50% LM: 50% WB diets.

Table 4. 2: Proximate analysis (mean ±SE) of formulated artificial larval diets used for the study (Dry weight).

Larval diet	Composition (%)	Moisture (%)	Crude proteins (%)	Crude fat (%)	Crude ash (%)	Crude fibre (%)	Carbohydrate (%)
Layer meal	100	6.02±0.16^{a}	16.98±0.03^{e}	4.04±0.04^{e}	7.56±0.00^{d}	4.32±0.00^{d}	61.09±0.04^{c}
Wheat bran	100	11.29±0.03^{e}	14.53±0.02^{a}	3.24±0.00^{a}	5.52±0.00^{b}	5.72±0.00^{e}	59.69±0.02^{a}
Layer meal:Wheat bran	75:25	9.93±0.03^{d}	15.12±0.00^{b}	3.43±0.00^{b}	5.37±0.02^{a}	3.86±0.00^{a}	62.28±0.04^{d}
Wheat bran:Layer meal	25:75	7.30±0.02^{b}	16.35±0.00^{d}	3.83±0.00^{d}	8.04±0.00^{e}	4.15±0.00^{c}	60.33±0.04^{b}
Layer meal:Wheat bran	50:50	8.65±0.01^{c}	15.71±0.01^{c}	3.65±0.00^{c}	5.85± 0.00^{c}	4.02±0.00^{b}	62.12±0.01^{d}
Lsd(0.05)		0.06	0.04	0.07	0.00	0.02	0.12

Within columns values with different superscripts are significantly different (p<.05).

4.4.2 Larval Growth, Prepupae Recovery and Mortality

No significant differences were observed in larval growth (weight and length), in response to the five diets at the end of the larval growth period (Table 4.3). Although 100% wheat bran diet recorded the mean largest larval weight (253.33 mg), it was not statistically significantly from the other treatments. However, no significant difference was observed in the mean number of prepupae harvested from all the formulated diets $F(4, 10) = 3.748$, $p = .354$ (Table 4.3). Prepupae recovered ranged between 95 and 99%. The comparisons indicated that the highest mean prepupae recovery was observed in the 75% LM: 25% WB formulated diet (99.33±0.67%) whiles the lowest was recorded in 50% LM: 50% WB (95.00±2.52%), (Table 4.3). Both the least mortality and percentage eclosion were recorded in the 25% LM: 75% WB diet, for which the values were 1.66±1.66 and 36.29±22.46% respectively (Tables 4.4 and 4.5).

Table 4. 3: Effect of larval diet on mean (±SE) larval weight, length and % prepupae recovered (*$p < .05$)

Treatment	Larval weight (mg)			Larval length (mm)			% prepupae
	Day			Day			recovered
	5	10	15	5	10	15	17
100% Layer meal (LM)	51.33±0.02	230.00±0.02	240.00±0.00	6.90±0.24	18.70±0.06	20.90±0.05	95.00±2.08
100% Wheat bran (WB)	43.33±0.02	196.33±0.00	253.33±0.02	7.00±0.21	18.20±0.33	20.20±0.06	95.33±1.85
75%LM: 25% WB	40.00±0.01	260.00±0.01*	250.00±0.00	6.30±0.20	18.50±0.08	20.60±0.03	99.33±0.67
25%LM: 75%WB	40.00±0.01	200.00±0.01	230.00±0.00	6.50±0.19	18.30±0.05	20.60±0.04	98.33±1.67
50%LM: 50%WB	40.00±0.02	220.00±0.02	240.00±0.00	6.80±0.25	18.40±0.03	20.60±0.05	95.00±2.52
Lsd (0.05)	14.72	44.75	44.69	0.86	1.29	1.26	6.07

Table 4. 4: Effect of larval diets on (mean ± SE) larval period, larval mortality, percent diet reduction and percent efficiency of conversion of digested food (% ECD).

Diet	Larval period (d)	Larval mortality (%)	Diet redction (%)	ECD (%)
100% Layer meal	14.00±0.00	5.00±2.08	80.90±0.86	0.24±0.00
100% Wheat bran	17.00±0.00	4.66±1.86	90.13±0.56*	0.22±0.02
75%LM: 25% WB	15.00±1.00	3.00±0.66	83.59±1.67*	0.18±0.01
25%LM: 75%WB	16.00±1.00	1.66±1.66	87.94±0.58*	0.22±0.02
50%LM: 50%WB	16.00±1.00	5.00±2.52	86.43±0.96*	0.22±0.11
Lsd (0.05)	2.27	6.07	2.60	0.03

**Significantly different at p<.05.*

Table 4. 5: Effect of (mean ± SE) larval diets on prepupal weight, pupal period, percent eclosion and egg clutch per female, (p > .05).

Diet	Prepupal weight (mg)	Pupal period (d)	Eclosion (%)	No. of egg clutches per female
100% Layer meal	188.10±0.93	11.00±1.53	50.05±10.91	1.17±0.37
100% Wheat bran	156.80±1.96	10.00±0.58	74.70±12.14	1.16±0.37
75%LM: 25% WB	184.80±1.77	10.33±1.20	77.74±9.76	0.93±0.21
25%LM: 75%WB	191.50±0.83	9.33±0.88	36.29±22.46	3.58±1.71
50%LM: 50%WB	185.10±0.83	10.33±0.88	90.82±5.83	1.03±0.33
Lsd (0.05)	25.27	3.09	46.85	0.84

4.4.3 Prepupal Development and Percent Waste Reduction

The formulated diets did not have significant difference F (4, 10) = 3.478, $p > .05$ on the prepupal weight, pupal and larval periods, percent eclosion, larval mortality and egg clutch per female production (Table 4.5). Statistically significant differences in diet reduction F (4, 10) = 3.478, $p < .001$ were observed in the study. Larval diet reduction ranged between 80 and 90% (Table 4.4). The least larval diet reduction of 80.90±0.86% was observed in 100% LM and this was not statistically different from the 25% LM: 75% WB and the 50% LM: 50% WB diets. A significantly higher diet reduction of 90.13±0.56 was observed in the 100% WB diet (Table 4.4). The efficiency of conversion of digested food (ECD) ranged between 0.18 and 0.24% (Table 4.4). The complete wheat bran (WB 100%) diet recorded the least ECD (0.18 %) but this was statistically not significant (Table 4.4).

4.5 Discussion

The nutrients an organism absorbs from the diet taken are critical for growth and development, and regulate how organisms can maximize their fitness (Stearns, 1992). Proteins, carbohydrates, fats and crude fibre are the four major nutritive components of diet that contribute to development of insect larvae (Nash & Chapman, 2014). Proteins provide essential amino acids (Dadd, 1985), necessary for cell growth and also serve as major components of eggs.

The chemical composition analysis of larval diets used (Table 4.2), showed that the moisture content of the diets varied significantly F (4, 10) =

3.478, $p < .001$. The moisture contents of all diets used in this study were very low, (6 – 11%). It was thus essential to moisten each of them to 70% and so push the moisture content within the optimum range (60 – 90%) for BSF larval feeding (Myers et al., 2008). Larval diets did not influence larval characteristics measured. This observation may be due to the fact that all diets had the minimum nutritive contents for BSF larval development. All the diets have approximately 15 – 17% crude protein and 60 – 62% carbohydrate, the two significant macronutrients providing essential amino acids and energy respectively (Simpson & Raubenheimer, 1993), and equally supported the growth and development of *H. illucens* larvae. The fat and protein contents of the layer meal diet were significantly higher compared to that in wheat bran, nonetheless, diets formulated from these two sources supported the growth and development of *H. illucens* larvae.

The formulated diet that yielded the largest number of prepupae in the current study was the 75% LM: 25% WB (Table 4.3). Whole wheat bran diet recorded the heaviest and shortest larvae, but these were not statistically different from the other diets (Table 4.3). Larval development time for all diets was not significantly different and ranged from 14 – 17 days. This result agrees with that of Gobbi et al. (2013), but better than that reported by Sheppard, et al. (2002), (14 – 21 days) when they fed BSF larvae with either layer meal or Gainesville housefly diet which had a crude protein content of 15 to 22% as well as an excellent source of vitamins and minerals (Tkáčová et al., 2015). These findings may be because insects are very adaptable and can adjust their metabolism so as to develop even on sub-optimal diets (Kwang

and Roh, 2010). Thus with other factors such as temperature and relative humidity being optimal, insects will develop normally on artificial diets regardless of the source of nutrition (Davis, 1972). Adult eclosion, ranged from 36.29±22.46 – 77.74±9.76 with the least observed in 25 % LM: 75% WB and the highest in 50% LM: 50% WB, far higher than the 27.2% reported by Tomberlin et al, (2002). Diet reduction of between 80 and 90% recorded in this study was higher than the 26 and 43% reported by Diener et al. (2011a). Further research should adopt the layer meal-reduced diet on an experimental scale at mass rearing facilities to confirm the suitability of these new artificial diets for rearing *H. illucens* for use in waste management and animal feed production.

CHAPTER FIVE

REARING OF BLACK SOLDIER FLY, *Hermetia illucens* (DIPTERA: STRATIOMYIDAE) ON SELECTED ORGANIC MARKET WASTES IN ACCRA

5.1 Introduction

The management of solid waste is a major challenge facing municipal authorities worldwide, mainly due to increasing waste generation associated with growing global population, urbanization and economic growth, combined with varying production and consumption behaviour (Patel et al., 2010; Karak et al., 2012; Rachel & Marshall, 2013). Ghana, a low middle-income country is facing difficulties in the management of its municipal solid waste as a result of financial, infrastructural and technical challenges (Anastasi et al., 2005; Adeniyi & Folorunsho, 2015). The effect of poor environmental sanitation and solid waste in cities and communities in Ghana affect the realization of the Millennium Development Goal 7 (Ensuring Environmental Sustainability). Annually, Ghana generates about 3.0 million MT of solid waste (Puopiel, 2010; Samwine et al., 2017) with a daily output of 13,000 tonnes of solid waste (Foray, 2012). The organic fraction (vegetables, food, fruits, and greens) constitutes around 44 – 65% of the total solid waste generated (KMA, 2012). Only a small fraction of solid waste generated in Ghana is recycled mostly by the informal sector and a few private firms. Large quantites of organic wastes are left uncollected on streets, choked gutters and neighbourhoods causing insanitary conditions. These organic wastes serve as substrates for pathogens that cause diseases such as dysentery, cholera and also flooding. There is also an overwhelming dependence on landfilling, the least preferred waste

management option on the waste management hierarchy (Diener et al., 2009). Thus, there is little reuse of the organic fraction and hence, a large portion of the organic waste end up on landfill sites which reduces the life span of these facilities.

Recycling the huge organic fraction component from the waste stream will thus reduce the volume of waste directed to dump sites, and will enhance the collection and storage of waste (Sheppard & Newton, 2000). A universally accepted method of reducing organic waste volumes adopted by most countries is composting which involves the use of microorganisms, worms, and bio starters (Snyman & Vorster, 2010). Composting presents a viable long-term option for the management of organic waste. There are clear environmental benefits in composting, such as the reduction of waste to landfill, reduction in greenhouse gases, conservation of resources and potential economic benefits (Ewusie et al., 2018). Composting is a preferred and environmentally sound method whereby organic waste is reduced to organic fertilizer and soil conditioners through biological processes (Gautam et al., 2010; Alexander, 1999). The high organic carbon content and biological activity of compost make it effective for applications such as erosion control and revegetation (Anastasi et al., 2005).

The moisture content of the feedstock intended for bioconversion by BSF is important as it affects larval development (Alvarez, 2012). Regan et al. (1973), and Murwira et al. (1990), reported that the optimum moisture content for compost is between 50 and 70% which is close to the optimum moisture

range of diet of BSF larvae (60 – 90%). Moisture content of feedstock below 40% and above 90% slows down larval feeding (Fatchurochim et al. 1989) and might even cause them to leave the feedstock thereby slowing down decomposition, hinders aeration, nutrients are leached out, and emission of odour as a results of anaerobic decomposition. Thus it is imperative that the moisture of any BSF larval feedstock should be monitored to ensure optimum degradation as well as larval growth and development.

Growth rate of BSF larvae varies significantly depending on rearing substrate's nutritional quality (protein, lipid, carbohydrate and water content of diet) as well as its physical properties (particle size and physical properties of cellulosic bulking agent) (Tomberlin et al., 2002; Hunter & McNeil, 2000; De Haas et al., 2006) and positively correlates with larval length and percentage survival (De Haas et al., 2006). Earlier studies investigating the life history traits of *H. illucens* larvae and adults used artificial diets such as Chemical Specialties Manufactures' Association (CSMA) standard fly larval diet (Furman et al., 1959) and Gainesville diets (Hogsette, 1992), which were developed for raising house flies, and a 15% protein layer ration (Tomberlin et al., 2002). May (1961), also examined the development of the black soldier fly but failed to define the diet composition implemented. The appropriate market waste fractions for decomposition by black soldier fly larvae were not known. Diener et al., 2009) used BSF larvae to bioconvert thoroughly mixed organic waste in Costa Rica, but they did not indicate the various feedstock and their amounts used. That study nonetheless, showed that the technology

can be used to manage organic waste in Low and Middle-Income developing countries as well as deriving additional product (animal feed).

Temperature and humidity are important conditions for general living, growth and activities of insects and is also one of the key indicators in composting. Changes in temperature are normally used as a measure of microbiological activity during composting as well as determining the stability of organic material (Fogarty & Tuovinen, 1991). Temperature in a compost heap characteristically follows a pattern of rapid increase to 49- 60^{o}C within 24 - 72 hours of heap formation and is maintained for several weeks. This is the thermophilic stage of composting and involves the degradation of easily degradable compounds under aerobic conditions by organic refuse converters such as worms, microorganisms, houseflies and black soldier fly larvae (Barnard et al., 1998; Beard & Sands, 1973; El Boushy, 1991; Elissen et al., 2006; Ramos-Elorduy et al., 2002). The increased temperature kills pathogens, weed seeds, and phytotoxins. During this phase, oxygen must be supplied by either mixing, forced aeration, or turning the compost pile. As the active composting phase subsides, temperature gradually declines to around 38^{o}C. Mesophilic organisms recolonize and the curing phase begins. However, these temperatures are far above the optimum temperature range for BSF development which had been predicted to be 27 – 36^{o}C (Tomberlin et al., 2009). It would thus be noteworthy to monitor temperature fluctuations during composting with *H. illucens* larvae.

The objective of the study was to evaluate the suitability of different market organic waste fractions in the Ghanaian markets for breeding BSFL and the effect of these fractions on the growth rates of BSFL. The study hypothesised that market crop waste fractions will serve as *H. illucens* larval diet. The specific objectives were to assess the effect of type of market organic waste fractions on BSF:

(a) co-composting temperature and humidity

(b) larval characteristics

(c) percent eclosion, and

(d) adult egg clutch production.

In particular, this study focused on the identification of suitable waste fractions for breeding BSF larvae and for future production of high quality compost for use in the urban and peri-urban farming to improve soil fertility as well as harvesting of larvae as a protein component for animal and fish feed.

5.2 Materials and Methods

5.2.1 Study Location

The study was conducted at the Ghana Atomic Energy Commission's Biotechnology and Nuclear Agriculture Research Institute farm in Accra, Ghana. The study site was located about 20 km north of Accra (05º 40' N and 0º 13' E), with an elevation of 76 m above sea level. Ghana is situated in West Africa, just above the Equator.

5.2.2 Black Soldier Fly Colony

The experiments were carried out at the Biotechnology and Nuclear Agriculture Research Institute, Ghana. The BSF larvae used were from a colony raised from wild collected eggs which has been breed over 10 generations at the BSF laboratory of the Soil and Environmental Science Research Centre, Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission. Larvae were reared at temperature range of 30-33ºC, relative humidity of 58 – 71%, and 12:12 hour day: night period.

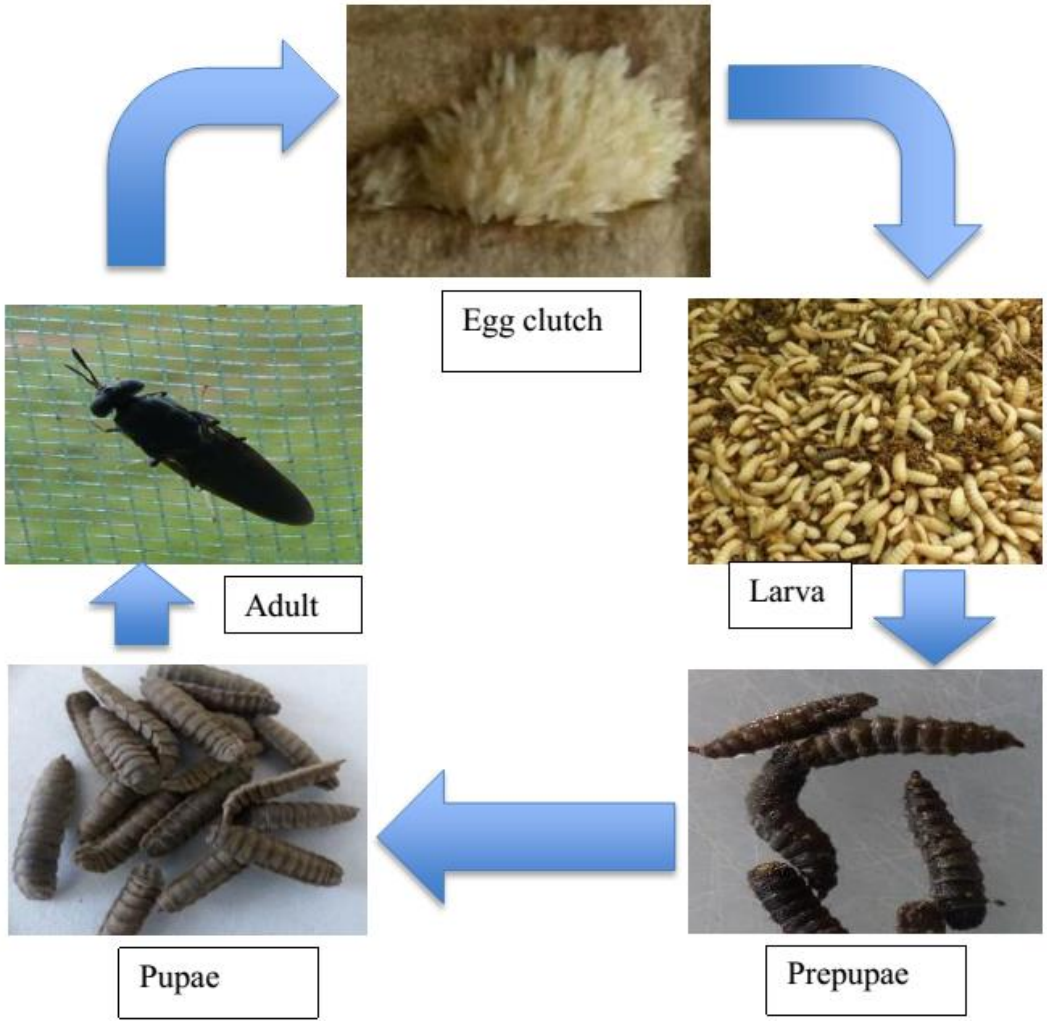


Figure 5. 1: Life stages of *Hermetia illucens*

5.2.3 Market Waste Collection

Market wastes were procured from the Madina and Dome Markets which are in two different municipalities in Accra, Ghana. These wastes were source separated and sorted into vegetables, fruits, uncooked food waste (cassava, plantain) and materials with high Carbon to Nitrogen (C/N) ratio (Corn husk and plantain peduncle) (Figure 5.2a). They were chopped (Figure 5.2b) and sieved to pass through a 10mm mesh to increase the surface area for easier consumption by the larvae. The feedstocks were formulated based on the ratio in Table 5.1.

Figure 5. 2: Organic market waste: (a) freshly collected market waste (b) sorted waste being chopped to 10mm particle size.

Table 5. 1: Feedstock and ratio of combination for BSF composting

Treatment	Feedstock combination	Ratio of combination (kg)
1	Materials with high C/N ratio (Corn husk +Plantain peduncle)	0.6: 1.4
2	Vegetable waste (*Kontomire +Cabbage +Carrot)	0.8: 0.6: 0.6
3	Fruit waste (Watermelon+ Orange+ pineapple)	1: 0.5: 0.5
4	Uncooked food waste (Cassava +Plantain)	1.4: 0.6
5	Corn husk +Plantain peduncle + Kontomire +Cabbage +Carrot	0.3:0.7:0.4:+0.3+ 0.3
6	Corn husk +Plantain peduncle + Watermelon +Orange +Pineapple	0.3:+0.7:+0.5 +0.25 +0.25
7	Corn husk +Plantain peduncle + Cassava +plantain	0.6+1.4+1.4+0.6
8	Kontomire+Cabbage+Carrot+Watermelon+Orange+Pineapple	4:0.3:0.3+0.5+ 0.25+0.25
9	Kontomire + Cabbage +Carrot + Cassava +plantain	0.4:0.3:0.3+0.7+0.3
10	Watermelon +Orange +Pineapple +Cassava +plantain	0.5 +0.25+0.25 +0.7 +0.3
11	Corn husk +Plantain peduncle +Kontomire +Cabbage +Carrot + Watermelon +Oranges +Pineapple +Cassava + Plantain	0.15 +0.35 + 0.2 +0.15 + 0.15 +0.25 +0.125+0.125 +0.35 + 0.15

*Cocoyam leaves

5.2.4 Experimental Design

The experiments were conducted in a 60 L waste bins covered with a sewn muslin cloth shower caps with an elastic band (Figure 5.3). Two hundred and forty (240) hand counted 5-day old BSF larvae were inoculated into a 2 kg of specific type of organic market waste which has been chopped. The experiment used one time feeding (lump feeding) of eleven feedstock and their combinations as shown in Table 5.1. Equal quantities of the above feedstock without larvae served as control. The experiments were replicated 3 times in a mosquito-netted composting yard under ambient conditions. The arrangement of the containers followed a Randomized Complete Block Design (RCBD), replicated three times. Blocking was done to cater for any variability in the directions of both wind and sun in the wire-meshed composting area. The study was conducted from August to October, 2015.

Figure 5. 3: Arrangement of composting bins in a mosquito-netted composting yard.

5.2.5 Temperature, Relative Humidity and Moisture Content Measurements

Temperature and relative humidity at three different sections of each of the composting media were monitored daily at 10.00am using digital thermohydrographs and compost thermometer. Moisture content of the composting media were monitored every fifth day. A total of 10 g each of the eleven composting feedstock without larvae were picked from different sections of each composing feedstock into already weighed aluminium cans and oven dried for 24 hours at 105°C, (Beune et al., 2008) after which the samples were removed into a desiccator and the final weight determined. The weights of the aluminium containers were subtracted from both the initial weight and the final weight of the compost and moisture content determined using equation 5.1

$$Moisture\ Content\ (\%) = \frac{(Wet\ weight - Dry\ weight)\ \times 100\ \%}{Wet\ weight} \quad (5.1)$$

5.2.6 Larval Measurements

One hundred larvae from each of the three replicated treatments were hand-picked with the aid of forceps for the determination of larval length and weight. Larval length was taken with dividers and a ruler whiles larval weight was determined with a microbalance. Measurements were carried on day 5, 10 and 15 after BSF larval feeding. All larvae were returned to their respective treatments after the measurements.

5.2.7 Prepupae Collection and Setting

On the 16th day after larval inoculation when more than 50% of prepupae were observed in all the composting feedstocks, they were picked with the aid of forceps, counted and weighed after which they were kept in ventilated plastic eclosion containers with crushed shredded papers as covering to induce pupation (Figure 3.5a). Thereafter prepupae were removed each day. All prepupae from same feedstock were pooled and 100 prepupae from each feedstock were kept in ventilated eclosion containers with shredded papers and covered with muslin cloth to prevent parasitoids entry and adult escaping (Figure 3.5b). These 100 prepupae were thus set aside for adult characteristics study.

5.2.8 Adult Maintenance

As soon as adults' began to eclose, the eclosion containers were placed in wooden adult cages measuring 80 x 80 x 80 cm (Figure 3.6a). The adult cages were themselves placed in a room roofed with transparent corrugated sheets to allow for indirect sunlight in order to encourage mating (Sheppard et al., 2002). An egg harvesting device (Figure 3.6b) was also placed in each adult cage to harvest egg clutches. Temperature and relative humidity were monitored within the adult cages using digital thermohydrographs. Fresh water was sprayed from a sprayer onto the muslin cloth covering of the adult cages to provide drinking water in suitable particle size. This helped to improve ambient reative humidity as the water evaporated. Adult mortality was checked daily and dead ones were sorted according to their sex, counted and recorded.

5.2.9 Egg Clutch Collection

Fifty grams moistened fresh layer meal diet were placed in transparent plastic containers (8 x 13.5 x 5cm) part of whose covers have been cut and fitted with cut corrugated cardboards taped together with a masking tape. This setup served as egg harvesting device (Figure 3.6b) to entice female flies to lay eggs and were placed in the adult cages (Figure 3.6a) and partly covered with cardboard to provide shade or hiding place. The egg harvesting device was checked daily and egg clutches sired were counted and recorded till all flies were dead in any particular adult cage. The number of egg clutches counted and recorded was divided by the total number of dead female in a cage to estimate female fecundity.

5.2.10 Feedstock Characteristics.

Some macro nutrients and chemical characteristics of the feedstocks used for the experiment are shown in Table 5.2. Moisture contents were determined gravimetrically by heating to constant weight at 105°C in an oven for 24 hrs (Bueno et al., 2008). Electrical conductivity and pH were analysed in a 1:10 and 1:5 de-ionised water soluble extract by conductivity meter and pH meter respectively (TMECC, 2002). Total carbon (C) content of the feedstocks was determined by dry combustion using Thermo Finnigan EA112 Elemental Analyzer. Total nitrogen was analysed using Kjedahl method (Okalebo et al., 2002). In order to determine the P and K, samples were digested with nitric acid and then analyzed using inductively coupled plasma mass spectrometry (ICPMS) (Stoffella & Kahn, 2001).

Table 5. 2: Some chemical characteristics of organic market waste used in study

Treatment	Phosphorus ($mgkg^{-1}$)	Potassium (gkg^{-1})	Organic Carbon (gkg^{-1})	Nitrogen (gkg^{-1})	pH
CH	0.36a	8.33i	63.36i	0.62b	9.41
VW	0.72j	8.63j	45.54b	2.00l	6.22
FW	0.69i	5.86e	58.40f	1.32g	5.52
UCFW	0.62f	3.10a	60.65g	0.59b	6.82
CH+VW	0.48b	7.96h	50.11d	0.98e	7.87
CH+FW	0.56e	7.33g	49.90d	0.86d	7.43
CH+UCFW	0.66h	5.06d	57.08e	0.46a	9.15
VW+FW	0.51c	6.06f	44.37a	1.72h	5.96
VW+UCFW	0.63g	4.43c	62.03h	1.12f	6.41
FW+UCFW	0.52d	3.83b	63.36i	0.76c	6.11
CH+VW+FW+UCFW	0.69i	6.10f	47.61c	0.88d	6.88
Lsd (0.05)	0.15	0.45	0.43	0.04	

Means with same alphabets are not significantly different ($p < .05$).

CH – Corn husk+Plantain peduncle, VW - Vegetable waste, FW – Fruit waste, UCFW – Uncooked food waste.

5.2.11 Data Analyses

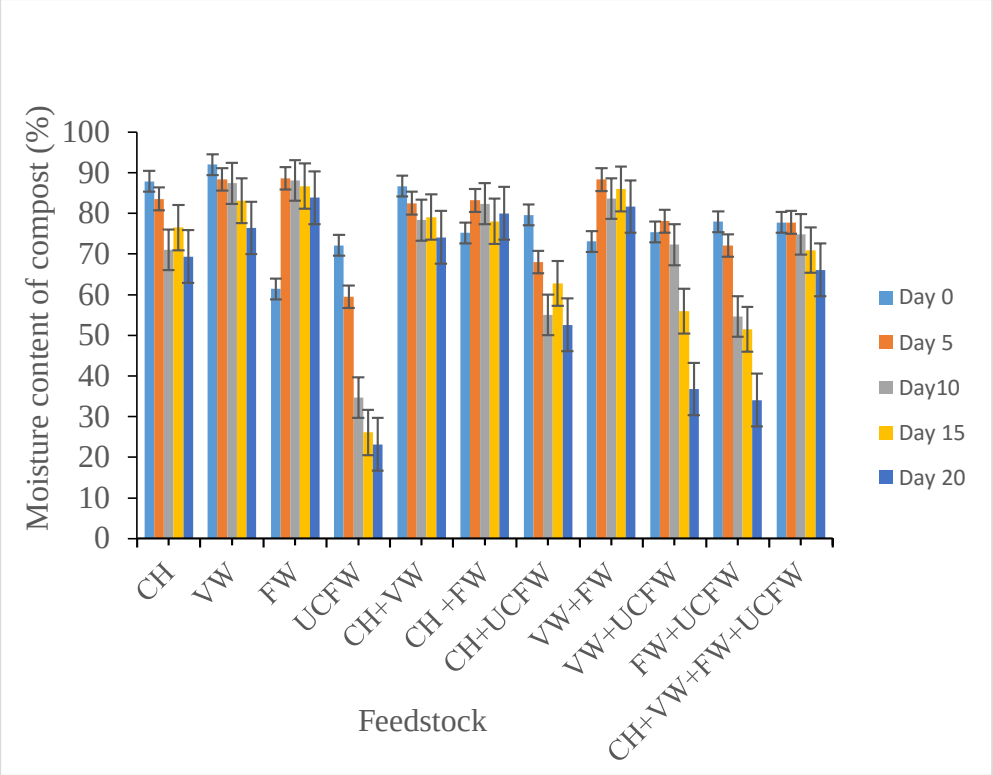
All statistical analysis was done with both Microsoft Excel and GenStat software version 12. Data were tested for homogeneity before being subjected to a single - factor ANOVA for statistical significance (α= 0.05) followed by

Tukey-Kramer procedure to clarify which of the means were significantly different from each other.

5.3 Results

5.3.1. Moisture Content

Moisture is necessary to support both feeding and metabolic processes of BSF larvae as well as microbes that might be in composting feedstock. Figure 5.4 shows the moisture content variations during the 20 days' larval bioconversion process all of which were statistically significant ($p < .001$). The initial moisture content of the feedstock ranged between 61 and 90% which was within the range reported by Myers et al., (2008). Mostly, the moisture content for the feedstocks decreased towards the end of the process except for fruit waste, corn husk+ fruit waste and vegetable waste + fruit waste. Whereas there was a gradual decrease in the initial moisture content of most of the feedstocks from day 5 till day 20, in the fruit waste only, and the mixtures of corn husk + fruit waste, vegetable waste + fruit waste, vegetable waste + uncooked food waste showed increases on day 5 and even at the end of the study, which was day 20. These feedstocks had still higher moisture contents than their initial.



CH – Corn husk+Plantain peduncle, VW - Vegetable waste, FW – Fruit waste, UCFW – Uncooked food waste.

Figure 5. 4: Effect of age on moisture contents of decomposing feedstock

5.3.2 Temperature and Relative Humidity

Table 5.3 shows the mean temperature patterns recorded in the selected market wastes undergoing decomposition by larvae of the black soldier fly. The highest temperature of 39.66±0.19^{o}C and 38.55±0.44^{o}C were recorded in vegetable waste + fruit waste and all the feedstocks combined respectively only at the beginning of the study. Significant differences in temperature, (df =10, 22; F = 15.73, p < .001 and df =10, 22; F = 2.83; p = .02) were observed at 0 and 10 days respectively during the study. The lowest temperature during the bioconversion process was 28.33^{o}C and was recorded in vegetable waste on day 20.

Table 5. 3: Mean (±SE) temperature of feedstock during bioconversion by BSFL

Treatment	Temperature (°C) Age (days)				
	0	5	10	15	20
CH	35.55±0.22de	30.88±1.22a	30.32±1.45abcd	30.44±0.62a	30.99±0.69a
VW	36.22±0.78cde	32.44±1.47a	32.55±1.28ab	30.11±1.11a	28.33±0.19a
FW	32.66±0.33f	32.22±1.36a	28.66±0.19d	30.66±0.88a	30.99±1.20a
UCFW	34.66±0.33e	30.99±0.38a	32.44±1.72abc	30.88±0.62a	30.11±0.67a
CH+VW	37.33±0.33bc	30.56±0.11a	29.66±0.66bcd	29.66±0.00a	32.66±0.33a
CH+FW	37.55±0.22bc	30.66±0.58a	30.88±0.72bcd	29.44±0.40a	29.77±1.23a
CH+UCFW	36.77±0.11cd	30.44±0.40a	29.99±0.33ab	30.55±0.39a	30.78±1.46a
VW+FW	39.66±0.19a	31.33±1.02a	32.88±0.58ab	28.88±0.22a	29.14±0.48a
VW+UCFW	35.22±0.80de	31.66±1.34a	33.33±0.50a	30.44±0.67a	29.44±0.86a
FW+UCFW	36.22±0.80de	32.78±1.74a	31.00±1.15abcd	29.11±0.48a	30.66±0.66a
CH+VW+FW +UCFW	38.55±0.44ab	33.44±1.42a	29.22±0.22cd	30.66±1.02a	31.77±0.73a
Lsd (0.05)	1.47	3.48	2.89	2.02	2.32

Means with same alphabets are not significantly different ($p < .05$).

CH – Corn husk+Plantain peduncle, VW - Vegetable waste, FW – Fruit waste, UCFW – Uncooked food waste.

Table 5.4 also shows the relative humidity in the composting medium with time. Highly significant differences in humidity (df = 10, 22; F = 5.74; $p <$ .001) were observed on the first day of composting only. It was observed that

relative humidity was higher (71.44 – 81.88%) at the end of the study in all feedstocks (Day 20).

Table 5. 4: Mean relative humidity of feedstock during bioconversion by BSFL

Treatment	Relative humidity (%)				
	Age (days)				
	0	5	10	15	20
CH	64.66±0.19ab	64.55±2.68a	69.22±0.91a	72.10±0.78a	79.10±0.62a
VW	69.33±1.20b	64.55±2.74a	66.32±2.18a	73.99±1.86a	81.88±0.98a
FW	65.11±0.11ab	63.10±1.74a	67.22±1.84a	74.10±1.18a	77.33±0.76a
UCFW	64.33±2.00ab	61.88±3.72a	68.78±0.96a	71.99±0.84a	73.44±5.11a
CH+VW	60.78±0.22a	65.10±3.82a	68.99±3.24a	75.99±1.20a	72.55±2.45a
CH+FW	64.66±0.19ab	59.78±0.78a	66.55±2.99a	75.00±1.20a	76.44±0.56a
CH+UCFW	67.44±0.62ab	65.44±5.16a	70.33±1.45a	73.33±2.08a	76.77±1.49a
VW+FW	62.66±1.50ab	60.77±.58a	69.10±2.07a	74.99±0.19a	77.66±1.20a
VW+UCFW	63.44±0.89ab	66.77±4.00a	71.77±0.48a	70.88±1.09a	76.44±2.72a
FW+UCFW	63.44±1.06ab	66.99±5.23a	71.77±0.48a	71.99±1.02a	71.44±2.92a
CH+VW+FW+UCFW	65.99±0.33ab	60.55±2.48a	65.66±2.52a	73.00±1.00a	76.66±2.87a
Lsd (0.05)	2.92	6.53	5.28	3.39	6.53

Means with same alphabets are not significantly different ($p < .05$).

CH – Corn husk+Plantain peduncle, VW - Vegetable waste, FW – Fruit waste, UCFW – Uncooked food waste.

5.3.3 Larval Characteristics

The effect of the different market waste feedstock on larval weights and lengths are presented in Tables 5.5 and 5.6. No significant differences in both larval weight and length were observed on the day of larval inoculation (Day 0). However, significant differences, (p < .001) were observed from day 5 to 15 for both larval weight and length. All larvae attained their maximum weight on day 10 after inoculation and at that time the maximum larval weight was observed in larvae fed with a mixture of vegetable waste and uncooked food waste (233.33mg), which had a hgih C:N ratio, but this was not significantly different from that of vegetable waste, fruit waste and uncooked food waste mixture as well as that of all the feedstock combined (Corn husk +Vegetable waste + fruit waste + uncooked food waste) all with lower C:N ratio. However, when larvae were just about entering the 5th instar stage, in this case at day 15, they empty their digestive tract, and thus reduce in weight, hence at that stage, larvae reared on the feedstock composed of all the combination of feedstocks (Vegetable waste + fruit waste + corn husk + uncooked food waste), lower C:N ratio, were the heaviest (176.66mg). Vegetable and fruit mixture feedstock produced the longest larvae on the 10th day (1.80cm), but on the 15th day after larval inoculation, the longest larvae were recorded in the fruit waste and uncooked food waste mixture (1.76cm). The larvae reared on corn husk feedstock were the least heavy and shortest. Thus feedstock type affected the larval weight during the study. Feedstock type significantly influenced larval duration (df = 10, 22; F =812.34; p < .001(Table 5.7), with the shortest larval duration observed in uncooked food waste feedstock substrate.

Table 5. 5: Mean weight (mg) of BSF larvae reared on selected market waste

Treatment	Day 0	Day 5	Day10	Day 15
CH	6.8	103.33±14.52bcd	110.00±26.45a	100.00±0.00a
VW	6.8	116.67±3.33de	216.66±31.79e	143.33±12.02bcd
FW	6.8	76.66±8.82a	156.66±12.02bc	153.33±6.66cd
UCFW	6.8	110.00±5.77cd	150.00±5.77bc	100.00±5.77a
CH+VW	6.8	90.00±5.77abc	136.66±3.33ab	103.33±3.33ab
CH +FW	6.8	103.33±6.66bcd	140.00±0.00ab	103.33±3.33b
CH+UCFW	6.8	103.33±6.66bcd	170.00±10.00bcd	103.33±3.33ab
VW+FW	6.8	86.66±a14.52b	146.66±13.33abc	123.33±3.33abc
VW+UCFW	6.8	140.00±0.00f	233.33±6.66e	160.00±0.00cd
FW+UCFW	6.8	153.33±6.66f	210.00±17.32de	153.33±6.66cd
CH+VW+FW+UCFW	6.8	133.33±6.66ef	190.00±5.77cde	176.66±8.82d
Lsd (0.05)		22.27	44.48	17.96

Means with same alphabets are not significantly different ($p < .05$).

CH – Corn husk+Plantain peduncle, VW - Vegetable waste, FW – Fruit waste, UCFW – Uncooked food waste.

Table 5. 6: Mean length (mm) of BSF larvae reared on selected market waste

Treatment	Day 0	Day 5	Day10	Day 15
CH	0.02	11.76±0.48ab	14.3±1.05a	14.33±0.53a
VW	0.02	12.56±0.14ab	16.2±0.12ab	17.47±0.38b
FW	0.02	11.67±0.39ab	16.97±0.38ab	16.9±0.06ab
UCFW	0.02	13.06±0.22ab	15.43±0.44ab	15.07±0.78ab
CH+VW	0.02	10.93±0.06a	15.57±0.69ab	14.97±0.20ab
CH +FW	0.02	11.77±0.28ab	15.57±0.26ab	15.07±0.45ab
CH+UCFW	0.02	12.53±0.73ab	16.37±0.27ab	15.7±0.45ab
VW+FW	0.02	12.27±0.73ab	15.2±0.30ab	15.47±0.73ab
VW+UCFW	0.02	13.27±0.34ab	18.07±0.29b	16.8±0.00ab
FW+UCFW	0.02	14.2±0.46b	17.57±0.14ab	17.67±0.13b
CH+VW+FW+UCFW	0.02	13.7±0.43b	17.77±0.62b	17.4±0.28b
Lsd (0.05)		1.18	1.44	1.29

Means with same alphabets are not significantly different ($p < .05$).

CH – Corn husk+Plantain peduncle, VW - Vegetable waste, FW – Fruit waste, UCFW – Uncooked food waste.

5.3.4 Prepupal, Pupal and Adult Characteristics

Among the eleven feedstock tested, the weightiest prepupae were obtained from the corn husk (20±0.28), whilst the most weightless were observed in the corn husk + fruit waste (11.58±0.16mg) (Table 5.7). Similarly, the feedstock type significantly affected the percent adult eclosion, with the highest adult eclosion observed in both the fruit waste + uncooked food waste (65.74%) and the mixture of all the waste (65.28%) (Table 5.7). A very low adult eclosion was obtained from the mixture of vegetable waste + fruit waste mixture (18.75

%). Even though, adult eclosion was least with prepupae obtained from the vegetable waste + fruit waste mixture, its females were the most fecund producing 2.28±0.39 egg clutches per female whilst the least fecund females were those from the uncooked food waste that gave 0.41±0.02 egg clutch per female. This could be explained by the fact that fruits and vegetables are known to be rich in carbohydrates, fibre, high in potassium and an array of amino acids (Vicente et al., 2009). These nutrients and minerals are required for growth and particularly needed by female insects to mature oocytes for egg production (Khattak et al., 2006). However, given the low eclosion rate of vegetable waste + fruit waste diet, it may be necessary to reduce its percentage composition when formulating BSF diet from organic waste fractions.

Table 5. 7: Effect of composting feedstock on prepupal weight, larval duration, percent eclosion and egg clutch per female. N = 100

Feedstock	Prepupal weight (mg)	Larval duration (Days)	Eclosion (%)	No. of egg clutches/female
CH	20.00±0.28^{d}	18.33±1.20^{a}	28.47±0.40^{b}	0.50±0.00^{a}
VW	15.18±0.18^{bc}	17.00±0.58^{a}	46.52±0.40^{c}	0.71±0.04^{a}
FW	15.61±0.04^{c}	17.00±0.58^{a}	63.88±0.80^{ef}	0.96±0.20^{a}
UCFW	13.08±1.66^{abc}	18.33±1.20^{a}	43.75±1.20^{c}	0.41±0.02^{a}
CH + VW	11.83±0.18^{ab}	18.00±1.54^{a}	62.50±0.80^{ef}	0.46±0.01^{a}
CH + FW	11.58±0.04^{a}	17.00±0.58^{a}	51.38±1.60^{d}	0.62±0.02^{a}
CH + UCFW	16.63±0.03^{cd}	18.00±1.52^{a}	59.72±1.60^{e}	0.71±0.00^{a}
VW + FW	14.66±0.16^{abc}	19.00±1.52^{a}	18.75±1.20^{a}	2.28±0.39^{b}
VW + UCFW	15.62±0.04^{c}	18.00±1.15^{a}	46.52±0.40^{c}	0.48±0.03^{a}
FW + UCFW	16.12±0.04^{c}	17.33±0.88^{a}	65.28±0.80^{f}	1.89±0.02^{b}
CH + UCFW +VW + FW	16.15±0.02^{c}	17.00±0.58^{a}	65.74±1.66^{f}	0.99±0.01^{a}
Lsd (0.05)	1.54	1.11	1.88	0.34

Means followed by the same superscripts in a column are not significantly different ($p<.05$)

CH – Corn husk+Plantain peduncle, VW - Vegetable waste, FW – Fruit waste, UCFW – Uncooked food waste.

5.4 Discussion

This study investigated how different organic waste fractions in Ghanaian markets affect BSFL composting in terms of larval growth rates (final larval weight and length), co-composting temperature and humidity, percent adult

eclosion and egg clutch production. The compostion of the organic waste fractions had differential effects on all the parameters measured.

5.4.1 Moisture Content

Average percentage moisture of all feedstock in this study was 71%. BSF larvae and even microorganisms can make use of organic molecules if they were dissolved in water. Regan et al. (1973) and Murwira et al. (1990), reported that the optimum moisture content for compost was between 50 and 70% which is close to the optimum moisture range of diet of BSF larvae (60 - 90%) as reported by Myers et al. (2008). Moisture content of feedstock below 40% and above 90% slows down larval feeding (Fatchurochim et al., 1989), and might even cause them to leave the feeding medium or substrate, thereby slowing down decomposition, reducing aeration, nutrients are leached out and emission of odour increases as a result of anaerobic decomposition. There was gradual reduction of moisture contents of feedstocks every 5th day possibly due to evaporation during the mixing of the feedstock by the larvae. However, in treatments such as fruit waste only, the mixture of fruit waste and corn husk or vegetable waste with or without BSF larvae there was a marginal increase in moisture content as a result of the soft textured nature of fruits and vegetables with high moisture contents. There was rapid release of moisture from these feedstocks that led to significant quantities of moisture being accumulated in the composting bins. Thus, further modifications have to be made to the composting bins to ensure that leachate drains out from the bins into a collection bowl and possibly hasten compost maturity.

5.4.2 Temperature and Relative Humidity

Temperature and humidity are important conditions for general living, growth and activities of insects. The ideal temperature ranges for survival, growth and reproduction of *Hermetia illucens* is between 27 and 36°C which guarantees 74 – 97% survival (Tomberlin et. al., 2009). *H. illucens* larvae also consume waste in low temperature conditions but not very quickly because their behaviour is generally slower (Žáková & Borkovcová, 2013). Again, temperature is one of the key indicators in composting. Changes in temperature are normally used as a measure of microbiological activity during composting as well as determining the stability of organic material (Fogarty & Tuovine, 1991). Temperature in a compost heap characteristically follows a pattern of rapid increase to 49 – 60^{o}C within 24 – 72 hours of heap formation and is maintained for several weeks. This is the thermophilic stage of composting and involves the degradation of easily degradable compounds under aerobic conditions by organic refuse converters such as worms, microorganisms, houseflies and black soldier fly larvae (Barnard et al., 1998; Beard & Sands, 1973; El Boushy, 1991; Elissen et al., 2006; Ramos-Elorduy et al., 2002). Increased temperature kills pathogens, weed seeds, and phytotoxins. During this phase, oxygen must be supplied by either mixing, forced aeration, or turning the compost pile. As the active composting phase subsides, temperature gradually declines to around 38^{o}C. Mesophilic organisms recolonize and the curing phase begins. In our study, composting temperature never reached the thermophilic stage, with the highest being 39^{o}C in all feedstocks combined, giving an indication of increased microbial action (Rasapoor et al., 2009) only on the commencement of the study.

This finding is significant, as the optimum temperature range for BSF development has been predicted to be 27 – 36^{o}C and that temperatures above 36^{o}C are lethal to the survival of *H. illucens* larvae (Diener et al., 2009). Thus the composting pile not reaching the thermophilic stage was ideal for larval survival and development, giving an indication of the suitability of *H. illucens* larvae for composting. Although temperature has been associated with different stages of the composting process, this study found that compost temperature decreased without notable differentiation between stages, and this may be attributed to the feeding behaviour of *H. illucens* whereby they burrow and thus aerate the feeding medium (Erickson et al., 2004; Sharma et al., 2015) and reduction in moisture and odour. This comes from their ability to modify the micro flora of the compost, reducing the harmful bacteria such as of E. coli 0157:H7 and *Salmonella enteric* through production of bacteriostatic, bactericidal, and/or fungicidal compounds (Holmes et al., 2012).

Just as temperature, relative humidity is an important abiotic factor affecting insect development; however, unlike temperature, the effect relative humidity has on insect development is rarely studied (Holmes, 2010). The present study showed that market waste bioconversion by black soldier fly larvae resulted in higher relative humidity of the composting medium with time. However, these increasing relative humidties were within the optimum for larval development.

5.4.3 Larval Characteristics

Increase in larval weight and length at the end of larval feeding were significantly affected by feedstock composition (df = 10, 32, F = 20.07, p < .001) and (df = 10, 32, F = 8.013, p < .001) respectively. The heaviest larvae were recorded in larvae reared on a mixture of all the organic market waste used, whilst the longest larvae were observed in larvae reared on a mixture of fruit waste + uncooked food waste. Larvae reared on corn husk had significantly smaller mean weights and lengths, p < .0001. Larval duration was not significantly altered by feedstock compostion (df 10, 32, F = 0.470, p = .891) and ranged between 17 and 19 days, compared to 14 and 15days for larvae fed on high protein standard layer feed (Diener et al., 2011b; Supriyatna et al., 2016).

Feedstock composition also had significant effect on prepupal wet weight (df = 10, 32, F = 21.053, p < .0001) and number of egg clutch sired (df = 10, 32, F = 26.268, p < .0001). Interestingly, total prepupae weight was significantly larger for larvae reared on corn husk. These results indicate that market waste identified were able to support at varying levels, the growth and reproduction of the larvae of *Hermetia illucens*. This could be due to the differences in protein levels, amino acids, non fibre carbohydrate levels of these substrates. The results confirm that laboratory reared *H. illucens* retain the ability to survive development through a wide range of fluctuations in the nutritional environment.

Holometabolous insects inherently have vigorous mechanisms to guarantee that development is successful in the environment in which their larvae develop, and that the nutritional target is obtained (Nash & Chapman, 2014). The relationship between larval growth rate, critical weight and the endocrinological control of larval development offers the possibility of significant plasticity in the determination of adult size and energy stores (Nijhout, 1999; Edgar, 2006). Critical weight is a point reached during the exponential growth rate of the final larval instar, which determines when the process of pupation can begin (Nash & Chapman, 2014). This critical weight is influenced by diet quality and is relatively insensitive to external environmental factors (Davidowitz et al., 2003; Davidowitz & Nijhout, 2004). Critical weight thus allows an insect to adapt the rate of its development to diverse nutritional environments in order to optimise key adult traits, such as body size (Andersson, 1994).

Even though larval duration in all feedstock treatments (17 – 19 days) were a little bit longer than those fed on high protein standard layer feed (15 days) (Diener et al., 2011b; Supriyatna et al., 2016), they were similar to being fed on meat and hen feed (19days) (Gobbi et al., 2013) or high protein and high lipid materials (Oonincx et al., 2015). It is probable that all the feedstocks could provide the necessary proteins and carbohydrates needed for BSF larval development (Simpson et al., 2006; Suzuki et al., 2013). There was a decrease in larval weight for all feedstock during the study after day 10 as the larvae reached the prepupal stage. This observation has been made by other researchers and has been attributed to; cessation of feeding, use of reserves for

metamorphosis (Caruso et al., 2014), and empting of larval digestive tract (Hardouin & Mahoux, 2003) in preparation for pupation.

Little is known about the mineral requirements of insects, but inferring from the known nutritive composition of insects (Barman & Rajan, 2011), it is reasonable to surmise that sodium, potassium, calcium, magnesium; chloride and phosphorus are essential minerals for the physiology of insects (Nation, 2001). Nation (2001), showed that many phytophagous insects require quite large amounts of potassium and only trace amounts of sodium for their metabolism and growth (Williams et al., 2016). It was found that feedstock with over 60% of carbon and pH near neutral enhanced BSF larval development. Trace amounts of other minerals may be necessary, but studies on mineral requirements for black soldier fly larval survival and development are lacking.

5.3.4 Prepupal, Pupal and Adult Characteristics

The type of feedstock significantly influenced prepupal weight, pupal period, percent eclosion and number of egg clutch produced by a female adult. All earlier studies investigating the life history traits of *H. illucens* larvae and adults used artificial diets. Chemical Specialties Manufactures' Association (CSMA) (Furman et al., 1959) and Gainesville diets (Hogsette, 1992), which were developed for raising house flies, and a 15% protein layer ration (Tomberlin et al., 2002), have been used to raise black soldier flies. May (1961), also examined the development of the black soldier fly but failed to define the diet composition implemented. Before this study, the suitable

market waste fractions for decomposition by black soldier fly larvae were not known. Diener et al. (2009), used BSF larvae to bioconvert thoroughly mixed organic waste in Costa Rica, but they did not indicate the various waste fractions (feedstock) and the quantities used. That study nonetheless, showed that the technology can be used to manage municipal organic waste fractions in Low and Middle-Income developing countries as well as deriving additional product (animal feed). The results confirm that *H. illucens* can develop successfully on a wide range of different protein and carbohydrate sources, including market waste and has thus confirmed the practicability of the BSF larvae surviving and developing on various municipal organic waste fractions.

This study is expected to serve as an impetus to government, municipal and district assemblies as well as entrepreneurs to heavily invest in this technology for sustainable organic waste management in Ghana. Exploration of the potential use of black soldier flies as an agent for waste management on a large-scale system should continue.

5.5 Conclusion

By reason of their generalist nature and high nutritional content larvae of the black soldier fly is seen as a good candidate for addressing organic waste management, and serving as an alternate protein source for animal and fish feed production, two of the most important global issues of our time. This study is the first to investigate which of the organic waste fractions in Ghanaian society as diet at different moisture levels impacts the life-history of the black soldier fly. Additionally, summaries were made of some past studies

and compared to the results generated in this study. These data provide valuable insights into the generalist nature of the black soldier fly larvae and its ability to utilize a variety of resources for larval development. A lot of the previous research work on the black soldier fly centred on the use of its larvae as a biological control agent of house flies and manure management agent in animal rearing facilities. Our study provides additional information on the life history of black soldier flies reared on organic market waste fractions. This information is necessary for developing its potential as an ecological engineer in managing municipal solid waste. However, because of the large differences between feedstock, further research is needed to perfect and improve larval rearing for mass production of BSF larvae for animal and fish feed production.

In this study, *H. illucens* larvae consumed various decaying material. The largest larvae were obtained after consuming vegetable waste only, vegetable waste + uncooked food waste, fruit waste +uncooked food waste and corn husk + uncooked food waste mixtures. These waste materials are suitable for consumption by *H. illucens* larvae.

CHAPTER SIX

EFFECT OF LARVAL DENSITY ON THE GROWTH AND DEVELOPMENT OF BLACK SOLDIER FLY, *Hermetia illucens* (DIPTERA: STRATIOMYIDAE)

6.1 Introduction

The black soldier fly (BSF), (Linnaeus,1978) *Hermetia illucens* (Diptera: Stratiomyidae), is a large (13 to 20mm), wasp-like fly (Tomberlin et al. 2002) that is distributed in the tropical and warmer temperate regions of the world between about 45°N and 40°S (McCallan, 1974). The larvae are polyphagous, with powerful mouthparts and efficient enzymatic activity of their digestive system to drastically reduce large quantities of varied organic matter faster and more efficient than other known species (Diener et al., 2011a). Significantly larger larval numbers of BSF is acknowledged to reduce house fly, *Musca domestica* L., production by 94 – 100% (Bradley and Sheppard, 1983), manure dry matter by 58% (Myers et al., 2008) and that of municipal organic waste by 70% (Diener et al., 2011a) compared with similar age uninhabited manure. High quality organic-fertilizers produced with BSF larvae from municipal waste recycling play an important role in increasing crop water use efficiency, nutrient uptake, soil organic matter content and crop yield as compared to conventional fertilizer (van Huis et al., 2013).

An emerging approach to decompose waste within the shortest possible time is to inundate organic waste with larvae of bioconverters such as the black soldier fly (BSF), *Hermetia illucens* Linnaeus, common housefly *Musca domestica* Linnaeus, yellow mealworm *Tenebrio molitor* Linnaeus

(FAO, 2011; Veldkamp et al., 2012). This also necessitates a mass rearing programme of bioconverters (Peters & Barbosa, 1972).

However, this requires a cost effective mass rearing of the target species and management of space in the rearing facility. One of the ways to saving space is to maximize insect density in rearing containers, without adversely affecting the quality of reared individuals (Riddick & Wu, 2015**)**. Rearing different densities of the larvae in the same container can cause a change in the behavior of individuals, and potentially promulgate harmful effects on survival, development, and growth (Peters & Barbosa, 1972). Additionally, the stocking density of larvae may have a significant impact on specific growth rate, developmental time and fecundity of insects (Riddick & Wu, 2015). The study hypothesised that rearing different densities of *H. illucens* larvae in the same sized containers will not affect growth parameters and selected life history traits of the larvae. The significance of this study was to determine the effects of high densities of black soldier fly larvae on Specific Growth Rate (SGR) and larval development and to make the effort to establish BSF larval density suitable for bio converted compost production technology in Ghana.

6.2 Materials and Methods

6.2.1 Study Location

The study was conducted at the Black Soldier Fly Laboratory of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) in Atomic, Accra, Ghana. GAEC

is located about 20 km north of Accra and lies between latitude 5º40′36.6″ N and longitude 0º11′52.5″ W, having an elevation of 76 m above sea level (Ewusie et al., 2010). The vegetation falls within the coastal savannah zone of Ghana, and the area is characterized by a bimodal rainfall pattern with the major rainy season falling between March and June, and a minor rainy season between October and December. The mean annual rainfall is 810mm distributed over less than 80 days, and temperatures are moderate with maxima rarely exceeding 32ºC while the minimum does not fall below 17ºC. The average humidity is about 60 – 76% (Ofori et al., 2014).

6.2.2 Black Soldier Fly Colony

The experiments were carried out at BNARI/GAEC. The BSF larvae used were from a colony raised from wild collected eggs which has been breed over 24 generations at the BSF laboratory (Figure 6.1) of the Soil and Environmental Science Centre, BNARI.

Figure 6. 1: BSF Laboratory showing the hatchery

6.2.3 Design of Composting Barrel and Stands

Wooden stands were constructed in such a way that there was an inclination at one end, into which 150L barrels were placed in them. Each of the barrels had a 2cm diameter hole drilled at the tilted bottom end to which a 15cm piece of 2cm diameter PVC pipe covered with a muslin cloth was fitted to provide channel for the draining of leachate from the feedstock which is collected into a container. An opening (gate) of 30cm x 45cm and held together with hinges were cut in one side and covered with muslin cloth as shown in Figure 6.2 to facilitate loading with feedstock and also to reduce heat generation during larval feeding.

6.2.4 Market Waste Collection

Market wastes were procured from the Madina and Dome Markets which are in two different municipal districts in Greater Accra, Region, Ghana. The wastes were source separated and sorted into vegetable, fruit, uncooked food waste and material with high C/N ratio. They were chopped and sieved to pass through a 10mm mesh to increase the surface area and for easier consumption by the larvae.

Figure 6. 2: Organic waste sorted into various food fractions on arrival at the composting platform

Figure 6. 3: Hand shredded food waste fractions

6.2.5 Addition of Biochar as a Component of Feedstock

Biochar is a carbon-rich charcoal-like product obtained when biological wastes, crop residues, animal manure, or any type of organic waste material, are heated at relatively low temperatures (<700°C) in a closed container with little or no oxygen (Lehmann & Joseph, 2009). Biochar is gradually being acknowledged by researchers for its immense role in carbon sequestration, reducing greenhouse gas emissions, renewable energy, waste mitigation, and soil improvement (Kookana et al., 2011), which alone or in combination have either a communal or economic benefit or both (Lehmann & Joseph, 2009). Biochar hastens compost maturity, adsorbs ammonia (Steiner et al., 2010) and reduce nitrogen loss during and after composting, improves soil structure and enhances microbial activity (Clough et al., 2013).

The initial stage of composting is characterized by low pH due to increase production of organic acids which suppresses microbial activity and growth (Beck-Friis et al., 2003). Additions of biochar to composting feedstock at the beginning of composting increase the pH and eliminate acid inhibition to microbes (Zimmerman et al., 2011). It is anticipated that the addition of biochar as a component of feedstock for black soldier larval co-composting, will hasten the breakdown of organic material, increase its surface area and act as bulking agent that adsorbs moisture thus aerating the medium particularly when feedstock with high moisture contents are used.

6.2.6 Experimental Design

One hundred and fifty litre capacity barrels were mounted on specially constructed wooden boxes at the composting yard and filled with 30kg dry weight of formulated market waste (Table 6.1). Larvae were added to these wastes at a density of 3,600; 4,800; 6,000 and 7,200. Each treatment was replicated three times under ambient conditions. The composting yard has a concrete floor 1.5 m above ground and the upper part made of wood interspaced with mosquito netting to guarantee adequate ventilation and prevention of insects particularly houseflies (*Musca domestica*) and blow flies (Calliproidae spp.). The roof has three slopes and was made of corrugated aluminium sheets. Equal quantities of the above feedstock without larvae served as control. The arrangement of the containers followed a Randomized Complete Block Design (RCBD). Blocking was done to cater for any variability in the directions of both wind and sun in the mosquito-netted composting shed.

Table 6. 1: Composition, fraction, moisture content and fresh weight of organic waste used in formulating a 30kg dry weight feedstock for bioconversion by *H. illucens*

Composition	Feedstock	Fraction	Moisture content	Fresh Weight (kg)
10 % Biochar	Biochar (rice)	10	1.2	0.30
45 % VW	Cocoyam leaves	50	60	16.88
	Cabbage	40	62	14.21
	Carrot	10	35	2.07
40 % FW	Watermelon	50	85	40.00
	Pineapple	30	60	9.00
	Orange	20	55	5.33
5 % UCFW	Cassava	50	20	0.94
	Plantain	50	20	0.94

VW = Vegetable waste, FW = Fruit waste, UCFW = Uncooked food waste

Figure 6. 4: Set up of larval density experiment (Arrangement of composting barrels in mosquito-netted composting shed)

6.2.7 Larval Measurements

Measurements were carried out on 100 randomly sampled larvae from each treatment. This was replicated three times on day 5, 10 and 15 after BSF larval feeding to determine growth in length and specific growth rate. The larval length was measured with dividers and a ruler whiles their weights were taken using microbalance and the growth in length was calculated for larvae according to a modified formula given by Gambel et al. (1985):

$$Growth\ in\ Length = \frac{Final\ Mean\ larval\ length - Initial\ Mean\ larval\ length}{Duration\ of\ the\ experiment\ in\ days} \quad (6.1)$$

Mean increase in body weight was calculated within the culture period of 15 days. The larval growth rate was calculated as Specific Growth Rate (SGR). The SGR was measured in the larval stage with the following formula by Ikhwanuddin et al. (2012):

$$SGR\,(\%) = \frac{Final\ body\ weight\ (mg) - Initial\ body\ weight\ (mg)}{Culture\ period\ (day)} \times 100\% \quad (6.2)$$

6.2.8 Prepupae Collection and Setting

Three hundred prepupae were randomly hand-picked with the aid of forceps from each treatment 15 days after inoculation. These prepupae were weighed and kept in ventilated plastic eclosion containers with crushed shredded papers as covering to induce pupation (Figure 3.5a). The eclosion containers were then covered with a muslin cloth to prevent adult escape after eclosion as well as laying of eggs in the prepupae by parasitoids (Figure 3.5b).

6.2.9 Adult Maintenance

As soon as adults began to eclose, the containers were placed in adult wooden cages (50 x 50 x 50cm) (Figure 3.6a) placed in the adult rearing room which had greater part (¾) of the roofing made with transparent corrugated sheets to allow for indirect sunlight. Thus, adults were kept under natural light. Temperature and relative humidity were monitored within adult cages using digital thermohydrographs. Fresh water was sprayed from a sprayer onto the muslin cloth covering of the adult cages to provide drinking water in suitable

particle size and improve ambient humidity as the water evaporated. Adult mortality was checked daily and dead ones were sorted according to their sex, counted and recorded.

6.2.10 Egg Clutch Collection

Moistened fresh larval diet were placed in transparent plastic containers (8 x 13.5 x 5 cm) part of whose covers have been cut and fitted with cut corrugated cardboards taped together with a masking tape. This setups served as egg harvesting devices (Figure 3.6b) to entice female flies to lay eggs were placed in the adult cages (Figure 3.6a) and partly covered with cardboard to provide shade or hiding place.

Egg harvesting devices were checked daily and egg clutches sired were counted and recorded till all flies were dead in any particular adult cage. The numbers of egg clutches counted and recorded were divided by the total number of dead female in a cage to obtain number of egg clutches laid per female.

6.2.11 Data Analyses

The data were analysed using GenStat Release12.1 (2009) and assisted by Excel. All data were analysed following a completely randomized design. The one-way analysis of variance (1-way ANOVA), using Standard Least Squares with *F* statistic, tested the effects of rearing density on growth in length, final body weight, Specific Growth Rate, larval and pupal periods as well as egg clutches per female, percent eclosion, number of females and males with the

level of significance set at $p < .05$. Tukey-Kramer procedure was used to test for differences in pairs of means.

6.3 Results

6.3.1 Specific Growth Rate

There were highly significant differences ($p < .001$) between all treatments in relation to both larval weight (LW) and specific growth rate (SGR) (Table 6.2). Larvae in the 4,800 treatment exhibited the largest weight and specific growth rate.

Table 6. 2: Mean growth in length, larval weight and Specific Growth Rate of black soldier fly larvae

Stocking density Number of larvae	Growth in length per day (mm)	Final mean larval weight on day 15 (mg)	SGR (%)
3, 600	1.38±0.00^{b}	226.7±3.33^{b}	1506±22.22^{b}
4, 800	1.45±0.00^{d}	240.0±0.00^{c}	1595±0.00^{c}
6, 000	1.36±0.00^{a}	200.0±0.00^{a}	1328±0.00^{a}
7, 200	1.44±0.00^{c}	230.0±0.00^{b}	1528±0.00^{b}
Lsd (0.05)	0.007	5.767	38.45

Within columns values with different superscripts are significantly different (p<.05).

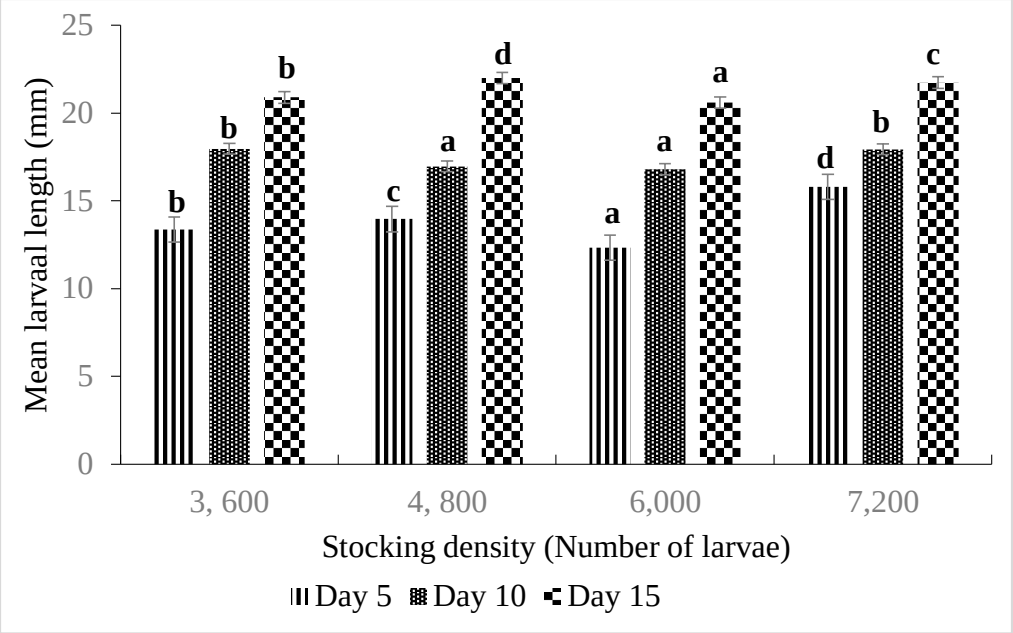


Figure 6. 5: Effect of stocking density on larval length with age of compost

Significant differences in larval length were observed across treatments throughout the 15 days of larval measurements with the longest larvae (22.00±0.00) observed with the 4,800 larval density treatment (Figure 6.5). Additionally, the 4,800 larval density treatment recorded the highest daily growth in length (Table 6.3).

Table 6. 3: Effect of larval densities on mean pupal weight, pupal period, and percent eclosion, number of eclosed females and males and egg clutch per female oviposited

Stocking density	Prepupal weight (mg)	Pupal period (days)	Eclosion (%)	No. of Females	No. of males	No. of clutches per female
3, 600	131.00±1.00[a]	10.33±1.20[a]	55.11±7.90[a]	69.33±14.38[a]	96.00±17.58[a]	0.77±0.20[a]
4, 800	158.30±0.88[b]	11.67±1.33[a]	69.66±7.09[a]	95.33±3.18[a]	113.70±23.92[a]	0.56±0.15[a]
6, 000	127.70±1.45[a]	10.67±0.88[a]	61.22±2.76[a]	75.33±9.26[a]	108.30±2.60[a]	0.72±0.17[a]
7, 200	158.30±2.33[b]	11.67±0.88[a]	68.78±0.29[a]	52.00±4.00[a]	154.30±3.92[a]	1.10±0.40[a]
Lsd (0.05)	5.461	4.037	17.71	30.44	56.50	0.823

Within columns values with different superscripts are significantly different (p<.05).

The above results demonstrate that larval density had significant effect only on mean prepupal weight. Pupal period (Time from prepupae to teneral adult) was unaffected by rearing density F (3, 8) = 0.39, p = .76 (Table 4.4). Rearing density had no effect on number of eclosed adults, number of females and males that eclosed and number of egg clutch sired per female, $p > .05$.

6.4 Discussion

The development of a technique to quickly degrade organic solid waste using black soldier fly larvae *H. illucens* constitutes a promising alternative in waste management as it generates several products of added value (animal feed, larval compost, biofuels). One purpose of this study was to establish a suitable rearing density for *H. illucens*. This will form the perspective of developing space-efficient loading capacity the system can support, while taking into account removable resources such as feed and non-removables such as living space (Mihelcic, 2008). The aforementioned are of increased relevance when one expects the business community to take up the process to a commercial scale (Riddick & Wu, 2015).

The hypothesis that a low to high rearing density has limited or no effects on *H. illucens* larval growth and subsequent development was partially confirmed in this study. The results revealed that larval density influenced larval weight and hence the SGR in *H. illucens*. In this study, the heaviest larvae with the highest SGR was achieved in treatment with 4,800 larvae whilst the least was observed in treatment with 6,000 larvae. Increased density is generally acknowledged to considerably affect fitness components,

behaviour and metabolism in insects (Applebaum & Heifetz 1999; Lazarević et al., 2004). These phase changes are initiated by aggregation pheromones and are hormonally regulated (Applebaum & Heifetz, 1999). It may well reduce (Tammaru et al., 2000) or prolong insect development (Lord, 1998; Roberts, 1998) while survival, body weight and fecundity are generally reduced at high density (Hooper et al., 2003, Lazarević et al., 2004). Negative density effects may be attributed to starvation, accumulation of toxic waste products and/or mechanical interference (Roberts, 1998; Lazarević et al., 2004). However, in the present study, pupal period, percentage eclosion, number of females and males eclosed and fecundity were not influenced by larval density, further suggesting that moderate to high rearing densities (3, 600, 4,800, 6,000 and 7, 200 larvae) had only minor, negative effects on *H. illucens* growth and development.

Weight of larvae is typically reduced in response to high density (Tammaru et al., 2000). Again, Tammaru et al. (2000), found that crowding during early larval development strongly delayed larval growth in *Epirrita autumnata* and reduced pupal weight. However, the results in this study showed that the highest density (7,200) produced the second heavier larvae. This may perhaps be due to the fact that larvae under crowded conditions might grow quicker than less dense larvae because of competitive need to feed more actively (Leonard, 1968).

The length of larvae was clearly influenced by the density conditions. This effect of crowding on larval development is found in some other species

of insects such as *Diploptera punctate* (Eschscholtz) (Blattodea: Blaberidae), and *Mythimna separate* (Walker) (Lepidoptera: Noctuidae) (Riddick et al., 2014). Applebaum and Heifetz (1999), avered that food consumption, metabolic rate and general activity were enhanced by crowded conditions in insects that show density-dependent responses. This finding shows that Dipterans such as *H. illucens* also exhibit density dependent response.

In this study, the highest fecundity was observed with females' eclosing from the highest larval density treatment but there was no clear effect of larval density on oviposition in *H. illucens*. Javois et al. (2004), reported no clear effect of larval density on oviposition in *Yponomeuta evonymellus* L. They explained that this could be linked to difficulties in possessing adult "memory" of the larval stage during metamorphosis in holometabolous insects (Fantinou et al., 2008). However, a two factor ANOVA results of the effect of larval density on both weight and the number of eggs sired per female, showed that there were highly significant interaction effects of larval density on larval weight and egg clutches sired by a female ($F_{3,3} = 7.18$, $p < .001$. This showed that the effect of density on the number of egg clutches sired by *H. illucens* females was mediated by larval weight. Thus, it is clear that the negative effect of crowding on fecundity was confined mostly to females that were exposed to crowded conditions as larvae and this is in agreement with Kazimírová, (1996) who also found out that fecundity in *Mamestra brassicae L.* is influenced by larval rearing density. This could be linked to physiological restraints that affect the apportionment of reserves carried over from the larval

stage into adulthood (Moreau et al., 2006) or concomitant with reduced obtainability of food at high densities.

The results show that larval stocking density might lead to a restriction in the availability of food during larval development and may influence life history traits. It can be concluded that higher/lower stocking density affected larval weight and SGR of *H. illucens* larvae. Higher densities other than 4,800 larvae per 30kg in a 150L container are not suitable for culture conditions. Further work is needed to clarify the differences in fitness of both sexes, as well as quantify the relationships among larval survival, adult size and successful mating.

CHAPTER SEVEN

LARVAL GROWTH RATE OF THE BLACK SOLDIER FLY, *Hermetia illucens* (DIPTERA: STRATIOMYIDAE) IN TEMPERATURE GRADIENTS

7.1 Introduction

The black soldier fly is economically important in organic waste management and their larvae have been found to reduce animal and municipal organic waste by 42 – 70 % (Tomberlin et al. 2009, Myers et al., 2008; Diener et al., 2011a). House flies, *Musca domestica* L. (Diptera: Muscidae), and bacteria such as *Salmonella spp*. and *Escherichia coli* (Erickson et al., 2004; Liu et al., 2008) are suppressed when organic waste is colonized by black soldier larvae. Matured larvae and or prepupae of black soldier flies are high in protein and fat and can be harvested and processed as protein supplementation for use as feed for livestock, poultry, and aquaculture (Bondari & Sheppard 1981; Newton et al., 1977).

Temperature is one of the most important environmental factors that affect the rate of insect growth and development (Taylor, 1981), physiology and behaviour (Ratte, 1985). There is ample scientific evidence that lends credence to the fact that there is a positive relationship between temperature increases and increased rates of development for insects (Angilletta et al., 2004). Again, it is established that high temperatures have effect on body size of insects. Insects such as *Drosophila melanogaster* raised in low temperatures tend to be larger and develop slowly, whereas insects raised in high temperatures have a tendency to be smaller but develop more quickly

(Angilletta et al., 2004). The optimum temperature range for *H. illucens* larval feeding is 20 - 30°C range (Tomberlin et al., 2009; Canary, 2009). The rate of development has generally been used to quantify the effect of temperature. Studies by other researchers have shown that temperature controls seasonal and daily cycles, and as a consequence indirectly impacts a number of aspects of insect biology, such as sex ratio (Zheng et al., 2008), adult life span, survival and fecundity (Yang et al., 1994). Thus, temperature greatly affects colonization, distribution, abundance, behaviour, life history, and fitness of insects (Hoffman et al., 2003).

The primary objective of this study was to determine the upper/lethal temperature threshold for *H. illucens* larval growth and subsequent development and thus to inform breeders of the highest operating temperatures for a fully functional BSF co-composting operations in Ghana. During windrow composting, a temperature of 50 – 68^{o}C over a period of 3 – 14 days is needed to deactivate pathogens and destroy weed seed (Adamtey, 2010; Nema, 2009), but these temperatures are far above the optimum temperature range for BSF development which have been suggeted to be in the range of 27 – 36^{o}C (Tomberlin et al., 2009). Thus, the study hypothesised that *H. illucens* larvae exposed to higher temperature will not have any effect on life history traits. The specific objective was to investigate larval growth and subsequent development of *H. illucens* across a range of age and temperature. A temperature of 35°C was chosen to reflect an estimate for the lower limit of temperature and 50°C as the maximum that could be generated when co-

composting with BSF larvae and which may ensure their growth and development.

7.2 Materials and Methods

7.2.1 Source of BSF Larvae

The BSF larvae used were obtained from a colony maintained year-round at the BSF laboratory of the Soil and Environmental Science Centre, Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission, which were raised from wild collected eggs harvested from piggery waste dump and compost heaps in 2014.

7.2.2 Experimental Design

One hundred larvae which were 4, 8 and 12 -days old were randomly assigned to plastic containers (20 × 15 × 7 cm) with a ventilated lid and exposed to 35, 40 and 45°C in an incubator for 4 hours and afterwards reared on layer meal in an ambient regime until pupation in the laboratory. Same number of larvae replicated three times but kept in the laboratory at ambient temperature served as control. On every fifth day, 20% of larvae were selected randomly, their lengths taken with a ruler and weighed on an electronic balance to the nearest 0.01 mg after which they were returned to their respective containers. Days to reach prepupae were recorded.

7.2.3 Adult Maintenance and Egg Collection

Prepupae were kept in cylindrical plastic containers (8 x 10 cm) and provided with shredded paper as pupation substrate. These containers were covered

with muslin cloth held in place with a rubber band. Eclosing adults were transferred into wooden cages (60 x 60 x 60 cm) that had muslin cloth covering all sides except the base that was made of plywood (Figure 9b) and kept in the adult rearing room which had greater part (3/4) of roofing made with transparent sheets. This allowed for the adults to be exposed to indirect sunshine to facilitate mating. Moistened fresh larval diet were placed in transparent plastic containers (8 x 13.5 x 5 cm) part of whose covers have been cut and fitted with cut corrugated cardboards taped together with a masking tape. This setup which served as egg harvesting device to entice female flies to lay eggs, were placed in the adult cages and partly covered with cardboard to provide shade or hiding place. Fresh water was sprayed from a sprayer onto the muslin cloth covering of the adult cages to provide drinking water in suitable particle size and improve ambient humidity as the water evaporated. Period to adult emergence, percent eclosion, sex and fecundity of eclosed female adults were recorded for each treatment.

7.2.4 Data Analyses

A 3×3 factorial design was used to examine the effects of age and temperature on larval period, prepupal development, percentage emergence, sex and egg clutch production. Development times of larvae and pupae, larval and prepupal weights and lengths were subjected to analysis of variance (ANOVA), with age and temperature as main effects, replication as a random factor. Because all larvae (900) subjected to 50^{o}C died within 3 days after exposure, this temperature treatment was not included in the data analysis. If the interactions of age and temperature were significant, pairwise comparisons

were conducted using Scheffé test. All statistical analyses were performed using both Microsoft Excel and GenStat, 12th version.

7.3 Results

7.3.1 Larval Weight and Length

The effects of age and temperature on mean *H. illucens* larval weight and length are shown in Figures 7.1 and 7.2 respectively. Analysis of variance showed that larval weight differed highly significantly across age ($F = 21.41$; $p < .001$) and temperature ($F = 21.41$; $p < .001$). The interaction of age and temperature with regard to larval weight was also significant ($F= 30.29$; $p < .001$). Larvae of all ages exposed to 35ºC were the heaviest. Overall, larvae of ages 4, 8 and 12 exposed to 35ºC were on the average 9 – 19% heavier than similar ages exposed to 45 and 40ºC. There was a consistent marked reduction in weight of 8-day old larvae exposed to all the temperature treatments (Figure 7.1).

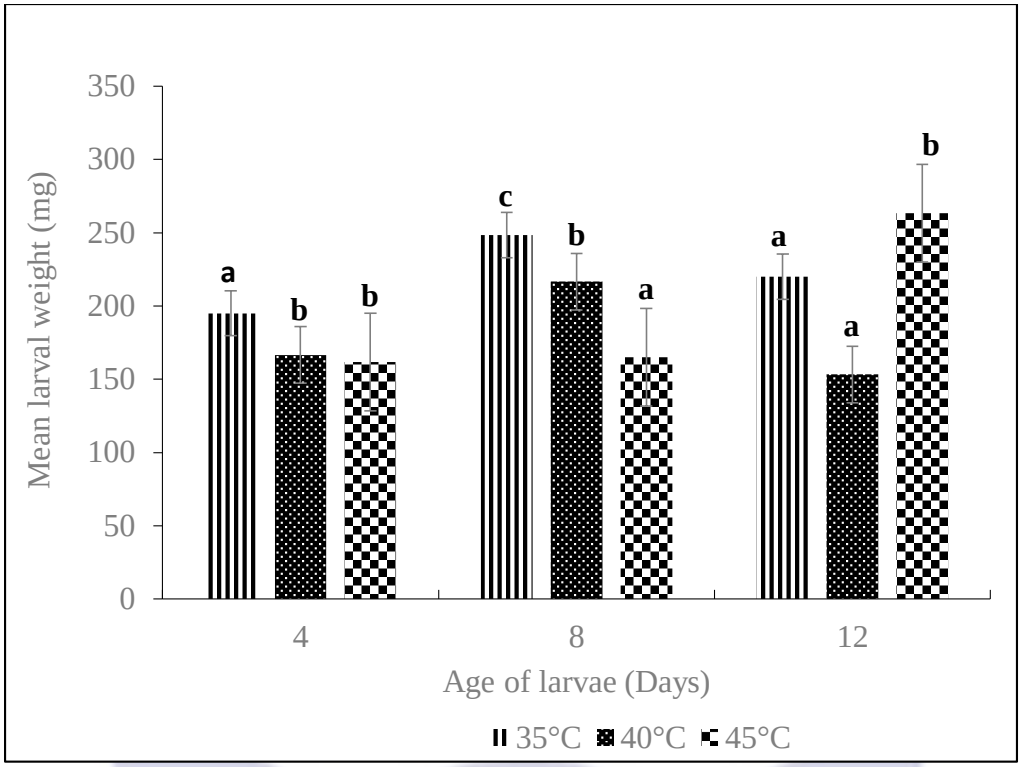


Figure 7. 1: Effect of age and temperature on larval weight (mg)

The effects of age ($F = 8.19$; $p < .001$) and temperature ($F = 85.98$; $p < .001$) significantly influenced larval length just as the interaction of age and temperature ($F = 15.98$; $p < .001$). Larvae of all ages exposed to 40^{o}C had virtually the same length and were on the average the shortest, whilst 12 - day old larvae exposed to 45^{o}C were the longest. Twelve day old larvae exposed to 35^{o}C and 45^{o}C experienced a decrease in length (Fig. 7.2).

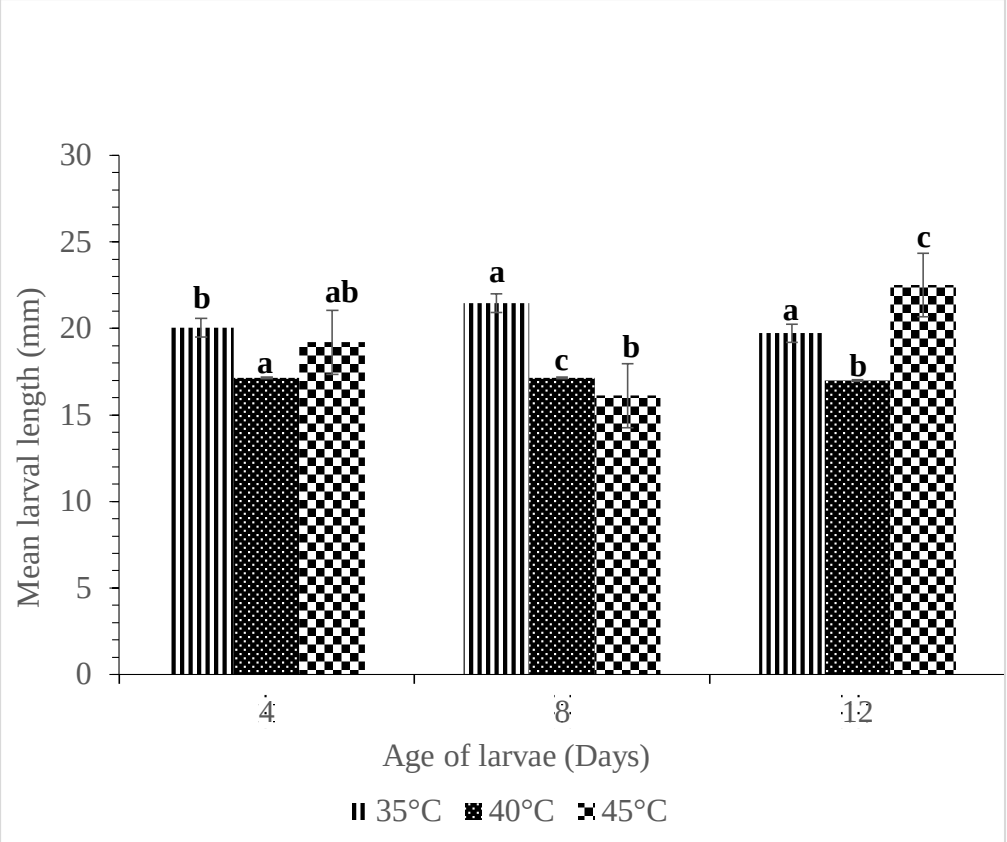


Figure 7. 2: Effect of age and temperature on larval length (mm)

7.3.2 Larval Period

Larval period significantly differed across age (F= 34.35, $p < .001$), and temperature (F= 44.66, $p < .001$) as well as interaction of age and temperature (F= 4.06, $p = .006$). Larval period decreased with increased exposure temperature, whiles it largely increased with age. Thus, larvae exposed to 45^{o}C for 4 hours required approximately 2 – 4 days longer to reach prepupal stage than those exposed to 40 and 35^{o}C respectively for the same exposure time (Table 7.1).

Table 7. 1: Life history data for laboratory-reared black soldier flies at three different ages and temperatures

Age (days)	Temp. (ºC)	Larval period (days)	Prepupae wt (mg)	Pupal period (days)	Emergence (%)
4	35	20.00±0.00	162.15±3.92	14.66±0.56	38.13±2.65
	40	16.16±0.16	200.38±2.08	15.66±0.56	44.84±9.24
	45	14.33±0.21	153.36±0.99	9.33±1.74	48.02±9.40
8	35	20.00±0.00	245.03±5.16	14.16±0.16	61.52±11.96
	40	19.33±0.42	220.33±7.68	13.66±0.49	43.16±10.74
	45	18.66±0.42	170.98±6.87	12.66±0.56	64.92±4.60
12	35	22.50±1.14	143.02±7.14	14.50±0.56	65.82±2.26
	40	20.50±1.06	146.64±1.56	12.33±0.33	56.36±8.21
	45	17.50±0.22	147.63±1.29	12.83±0.48	62.53±4.76
		***p*< 0.05**	***p*< 0.05**	***p*< 0.05**	***p*>0.05**

7.3.3 Prepupal Weight

Prepupal weight differed significantly across age (F= 144.38; $p < .001$), temperature (F= 37.15; $p < .001$) and there was a significant interaction of age and temperature (F= 25.66; $p < .001$). The average prepupal weight of larvae of all the ages treatments exposed to 40ºC were nearly 3 – 20% heavier than those exposed to 35ºC and 45ºC respectively (Table 7.1).

7.3.4 Pupal period

Pupal development time differed significantly across age ($F = 18.79$; $p < .001$) but not, temperature ($F = 0.46$; $p = .80$). Highly significant interaction of age and temperature ($F = 9.30$; $p < .001$) was observed. On the average, pupal period for 12 day old larvae exposed to any of the temperature treatments was nearly 2 – 3 days less than that for ages 8 and 4 respectively.

7.3.5 Percent Emergence

The main effects of age ($F= 1.36$; $p = .26$) did not influence the number of adults eclosing from harvested prepupae, whiles temperature did ($F= 4.14$; $p = .02$). The interaction of age and temperature was not significant ($F = 0.82$; $p = .52$). Survival of 4, 8 and 12 day-old larvae to adults averaged 44, 57 and 62% for 35, 40 and 45 ^{o}C respectively (Table 7.1).

7.3.6 Egg Clutches per Female

Egg clutch sired per female differed significantly across age ($F =3.66$; $p = .03$) and temperature ($F = 5.69$; $p = .006$) but not across the interaction of age and temperature ($F = 1.59$; $p = .19$). The maximum as well as the minimum fecundity were found in female flies' eclosing from 12 day old larvae exposed to 35 and 45^{o}C respectively (Figure 7.3).

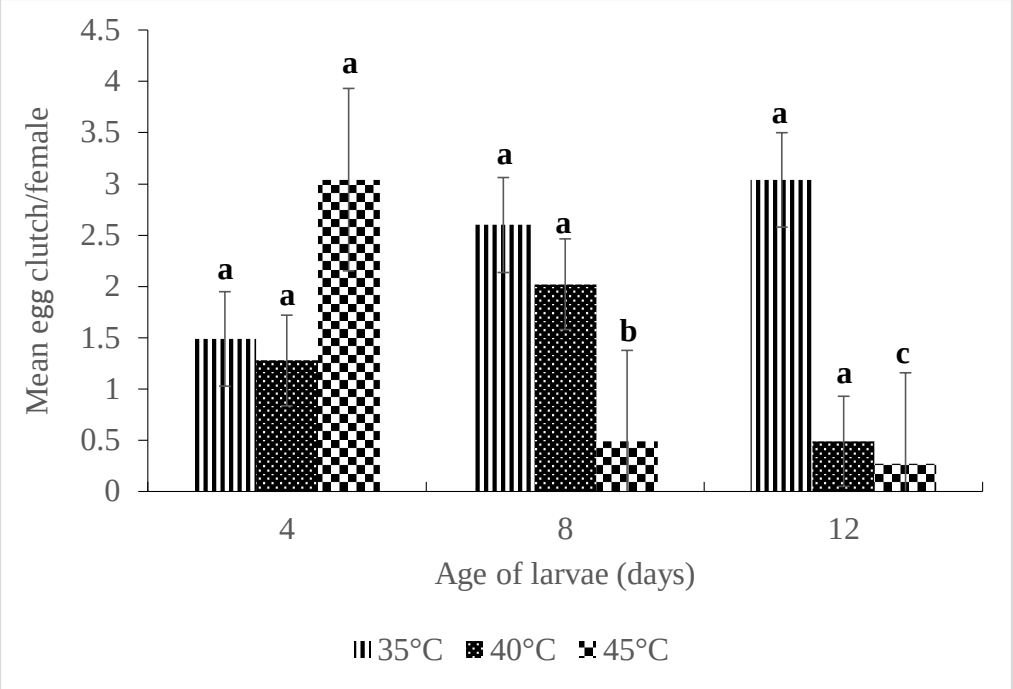


Figure 7. 3: Effect of age and temperature on fecundity

7.4 Discussion

This study considered the impacts of higher temperatures on differently aged *H. illucens* larvae and their effect on subsequent larval development. At 50ºC, all larvae of all ages were dead by day 3 after heat exposure and hence no development parameters were obtained. This indicates that exposure of larvae to four hours at 50ºC at any age is detrimental for the growth and development of *H. illucens* larvae. Thus if they are to be used for organic waste bioconversion, temperature build up in the composting medium should be monitored so that it does not exceed 50ºC to ensure increased waste conversion.

There is ample scientific evidence that lends credence to the fact that there is a positive relationship between temperature increase and increased

rates of development for insects (Angilletta et. al. 2004). Again, it is established that high temperatures have effect on body size of insects (Jaworski & Hilszczański, 2013). Insects reared at low temperatures tend to be larger and develop slowly, whereas insects reared at high temperatures have a propensity to be smaller but develop more quickly (Angilletta et al., 2004). In this study, larvae exposed to 35ºC were on the average 9 – 19% heavier than those treated with 45 and 40ºC respectively, confirming the results of Angilletta et al. (2004).

At higher temperatures, it takes a shorter development time for egg, larva and pupa (Jaworski & Hilszczański, 2013) and this results were confirmed in the case of larvae in this study, where consistently higher temperatures led to a shortening of the mean larval period of *H. illucens*. Larvae exposed to 35ºC required on the average 3 – 4 days longer to reach prepupal stage than those exposed to 40ºC and 45ºC respectively. Park et al. (2010), reported of similar observation where the egg-to-adult mean development times of melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae), at different temperatures on cucumber were 40.8, 20.5, 12.7, and 9.8 days at 15, 20, 25, and 30°C, respectively.

Pupal period on the other hand averagely decreased with age. On the average, 12 days old larvae exposed to any of the other three temperature treatments required nearly 2 – 3 days less to complete the pupal stage than that for ages 8 and 4. This finding, agrees with Chidawanyika et al. (2017), working with Parthenium Beetle *Zygogramma bicolorata* (Coleoptera:

Chrysomelidae). The upper temperature limit for successful development of the black soldier fly has been found to be in the range of 30 – 36°C and that persistent temperatures of 36°C are beyond the optimal range of development (Tomberlin et al., 2009). It was observed in this study however, that soldier fly larvae can withstand at least 4 hours of sustained temperatures up to 45°C and still be able to develop into adults and sire egg clutches.

Temperature and age influenced fecundity, $p < 0.05$. In this study, the number of egg clutches per female was highest in female's eclosing from 12-day old larvae exposed to 35°C (3.04±0.54 egg clutches per female). All studies reviewed showed that female *H. illucens* adults were likely to sire an egg clutch in its short adult life (Tomberlin et al., 2002; Nakamura et al., 2016). Tomberlin et al. (2002), exposed 4 – 6 day old larvae to consistent temperature of 27, 30 and 36°C, but they did not report on the number of egg clutches sired per female. In this study, however, more than an egg clutch per female was recorded with the average number of egg clutches sired per female reaching their highest values at 35°C (2.38 egg clutches per female). Female adults were able to sire egg clutches at all temperatures (35 – 45°C) even though at 45°C, an amazingly low fecundity was observed. This phenomenon has been recorded in various other insect species, where increases in culturing temperatures often resulted in noticeable decreases in female productivity (Vasicek et al., 2002; Mehrparvar & Hatami, 2007). This result indicates that 45°C may well be the upper lethal temperature for *H. illucens* larvae. Insects are known to live in a wide range of thermal climates, but there is very little variability in the maximum temperature (40 – 50°C) which they can survive

(Heinrich, 1981). Temperature influences metabolism and growth in ectotherms (Williams et al., 2016) and temperature had persistent effects on the development and metamorphic traits of *H. illucens*. Temperature is the most important abiotic factor affecting insect growth, development rate, and survival of *H. illucens* and a temperature difference of even 3°C have been found to produce significant fitness trade-offs' for males and females, influencing life history attributes (Tomberlin et al., 2009).

Survival of 4, 8 and 12 day-old larvae to adults averaged 44, 57 and 62% for 35, 40 and 45°C respectively. The results indicated that larval exposure to high temperatures of 40 and 45°C seems to have a positive relationship with larval survival to adulthood. This result is at variance to earlier studies (Bayoh & Lindsay, 2003; Depinay et al., 2004). Although larvae reared at 45°C had a high probability (67%) of eclosing into adulthood, mean fecundity declined drastically, probably implying that 45°C might be the upper lethal temperature (ULT).

Results in this study have demonstrated that *H. illucens* larvae are capable of withstanding 4 hours of sustained high temperature treatments and are able to grow to adulthood. These results provide basic information on how brief exposure of *H. illucens* larvae to high temperature affects their survival and reproductive performance especially when it is expected to be used in bioconversion of organic waste. During organic decomposition, temperature could rise significantly due to microbial activities, which is dependent on the type of organic waste and volume/size and moisture content (Liang et al.,

2003). However, this is the first study to investigate the effect of brief higher temperature on *H. illucens* larvae in Ghana. Thus, more studies are required to (i) improve on egg clutch production per female after brief exposure to higher temperature, (ii) undertake percentage hatchability trials of eggs sired under these conditions and their subsequent development into adults, and (iii) test the suitability of such larvae in valorisation of organic waste and or manure.

CHAPTER EIGHT

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The black soldier fly (BSF) *(H. illucens*), could be one of the important means of solving the organic waste management challenges facing municipal and local government authorities in Ghana, whiles at the same time yielding nutritive animal feed ingredients and quality organic fertilizer for improved food production. BSF thrives in tropical regions like Ghana, and is not a nuisance nor a vector of diseases unlike other insects. It is known to be voracious and can degrade organic waste and other decaying materials. However, scientific culturing of this valuable insect is lacking and very little information was known of the effects of diets other than the standard layer meal on life history traits of BSF in Ghana.

8.1 Summary

In this research the best microhabitat for trapping BSF egg clutches was found to be the piggery waste dumpsite. There were no significant difference in life history traits of egg clutches obtained from different microhabitats (piggery and compost dumpsites). Again, there was no variation in the trapping of BSF egg clutches during the major and minor rainy seasons, though it was higher in the major than the minor rainy seasons.

This research tested five formulated BSF larval diets from layer meal and wheat bran and found out that wheat bran only or layer meal-reduced diet mixtures could be suitable substitutes for the standard larval diet without

affecting their growth, developmental time and reproductive performance, thus improving the cost-effectiveness of diet for black soldier fly mass rearing.

Five-day old larvae were fed on eleven different market waste fractions in composting bins. Temperature in the composting bins never reached the thermophilic stage (49 – 60^{o}C**),** possibly due to high moisture content of the feedstock. Results demonstrated that the larvae can be propagated successfully on almost all the organic market waste fractions. The results again showed that feedstocks with higher percent carbon, total nitrogen, moderate phosphorous and minimum potassium were the most suitable.

The effect of larval density on the growth and development of black soldier fly, *H. illucens* on 30kg dry weight of formulated organic market waste fractions was evaluated. Rearing density had a significant effect on larval weight, growth in length and specific growth rate. Overall, this study showed that *H. illucens* larvae can be reared successfully at a density of a larva/78cm^{3} in containers filled with formulated organic market waste fractions. Higher densities other than 4,800 larvae per 30kg dry weight in 150L (378,956.14cm^{3}) containers were not suitable for BSF larval culturing.

This research also assessed the effects of larval age and exposure to higher temperatures. Results showed that both larval age and exposure to higher temperatures (30 to 45^{o}C) had significant effects on larval weight, length, larval period and prepupal weight. It was observed that black soldier

fly larvae can withstand at least 4 hours of sustained high temperatures up to 45^{o}C and still be able to progress to adulthood and sire egg clutches.

8.2 Conclusions

Studies were done to establishment a laboratory colony of black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae) in Ghana. The major findings were:

1. The piggery manure dump microhabitat is the most suitable environment for trapping BSF egg clutches from the wild.
2. Rainy season of the year did not influence BSF egg clutch collection, but was better in the major rainy season.
3. The temperature and relative humidity in the study area fell within the optimum range for breeding *H. illucens*.
4. Larval cages, prepupal containers and adult cages should have very fine-meshed muslin cloth covering to as much as possible prevent entry of the parasitoid, *Dirhinus giffardii* (Hymenoptera: Chalcididae).
5. Larval cages and composting containers should be constructed in such a way as to ensure sufficient ventilation so as not generate heat above 50^{o}C which could force out larvae or cause very significant larval mortality.
6. From the larval diet formulation studies, it was observed that wheat bran only or layer meal-reduced mixtures could serve as cheaper alternate substitute for the standard larval diet without affecting their growth, developmental time and reproductive performance.
7. Larval diet influenced percent eclosion and egg clutch production but not larval developmental time.

8. The temperature in a BSF bioconversion process never reached the thermophilic stage. Probably this could be due to the type of the organic waste quality, size and moisture content. This is a good omen, as temperatures could not build up so much so as to kill BSF larvae if used in bioconversion efforts.
9. BSF larvae developed fairly well on almost all the organic market waste streams. These results suggest that most of the organic waste fractions could be used in feeding the BSF larvae, which could lead to both reduced cost in maintaining the larvae and volume of waste to be sent to landfills/dump fills.
10. Larval density did not influence life history traits but significantly influenced growth parameters studied.
11. Growth of larvae in densely populated waste formulations may be diminished and hence higher densities other than 4,800 larvae per 30kg in 150L containers are not suitable for bioconversion of organic market waste fractions.
12. After particle size reduction of organic waste fractions, the feedstock for larval biodegradation should be placed in baskets with openings to drain off significant moisture before the introduction of larvae.
13. Bio composting bins should be fitted with an outlet for leachates from the compost to drain out to avoid increase in compost moisture which will create anaerobic conditions that might force larvae out of the composting medium or may delay compost maturity.
14. The highly lignified organic waste fractions (corn husk, plantain peduncle) could be harmer- milled before adding to feedstock formulation.
15. Source-separation of waste should be encouraged at both the household and waste assembly points to facilitated easy application of this technology as a waste management option.

8.3 Recommendations

This work provided additional information to support the assertion that it is technically feasible to raise a BSF colony with egg clutches trapped from the wild and that the larvae could successfully be bred on reduced layer meal formulations and most organic market waste fractions in Ghana.

1. The highly lignified fractions (corn husk) could be harmer- milled before feedstock formulation.
2. The egg clutches obtained from adults that eclosed from the feed formulation and market waste bioconversion trials were just transferred to the laboratory colony and thus were not followed up to even the first generation. Thus following them through at least the third generation would provide more insight into egg hatchability and percent surviving to adult.
3. Two alternative larval diets were determined; however, the prospects of their suitability in mass rearing operations were not assessed. As a result, it is proposed that a study to evaluate these diets on an experimental scale to confirm the suitability of these new artificial diets for rearing *H. illucens* for use in waste management and animal feed production. This should include the examination of critical quality control parameters of flies such as adult, egg, larval and prepupae quality, across various generations.
4. Different aged larvae were subjected to brief period of high temperatures and quite surprisingly, egg clutches were sired but the hatchability and subsequent development of such egg clutches were not studied. The fertility of such egg clutches sired after larvae were exposed to brief higher temperatures and their eventual development into adults merit further study.

5. Additional effort should be made to design equipment for harvesting both larvae and prepupae from feedstock for more efficient rearing techniques of this insect as the methods described herein are somewhat backbreaking.
6. It is envisage that authorities at the Municipal and District Assemblies would take advantage of the technology to ensure an environmental-friendly, and economically-sound waste management practice. This is because waste destined for landfill sites could potentially serve as a suitable diet, potentially reducing greenhouse gas emissions and extending the lifespan of landfills. The compost resulting from the bioconversion process would improve agricultural productivity in a sustainable way to meet the growing domestic food demand and as a result provide livelihood opportunities in both rural and urban areas.
7. The research and scientific community as well as the media should join forces to stem interest in rearing this beneficial insect for addressing the challenging effects of waste management and improvement in food production in Ghana.
8. The larvae of *H. illucens* is proclaimed by many researchers as one of the best candidates as fish feed ingredients in partial or complete substitution for fish meal, with regard to their nutritional attributes, ease of rearing, and biomass production. Thus entrepreneurs could take advantage and invest in its breeding and utilization not only for the animal and fish feed market, but for the processing of the lipid content of the larvae into biodiesel, or extracting the chitosan from larval exuviae or from BSF prepupae.
9. The quality of the prepupae as affected by the type of organic waste should be studied

10. The maximum quantity of organic waste used in this study was 30kg. To be more commercially efficient, greater quantities of the waste and larval stocking density should be evaluated.
11. Studies should be conducted to determine the bioconversion ratio (i.e. the ratio between the quantity of organic market waste used and the quantity that is turned to compost) by the BSF larvae depending on the type of market organic waste.

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Nationality: Ghanaian.

Marital Status: Married with 2 children.

Residential Address: Flat C3, Atomic Residential Area, Atomic Energy, Atomic – Accra. P. O. Box AE 1, Atomic Energy, Atomic, Accra, Ghana.

PROFESSIONAL ACTIVITY

Oct. 2008 to date	**Scientific Officer**, Ghana Atomic Energy Commission.
05/2003 – 09/2008	**Assistant Scientific Officer,**
01/1996 – 04/2003	**Senior Technician,**
09/1992 – 12/1995	**Technician,**
10/1990 – 08/1992	**Service Personnel,** Adu Gyamfi Secondary School, Teaching General Science, Physics and Biology (At forms 1, 3, 4 and 5).

01/1985 – 10/1987 **Pupil Teacher,** Akokoaso Day Secondary School, Akokoaso, Ghana. Teaching General Science and Agricultural Science (Forms 1 to 3).

EDUCATION/QUALIFICATION

- M. S. Degree (With Research) in Entomology, Texas Tech University, Lubbock, Texas, USA, 2006 – 2008.
- BSc. (Zoology/Botany), University of Cape Coast, Ghana, 2001 – 2003.
- Diploma (Laboratory Technician), University of Cape Coast, Ghana, 1987 – 1990.
- GCE "A" Level Certificate, University Practice Secondary School, Cape Coast, Ghana, 1981 – 1983.
- GEC "O" Level Certificate, St. Martin's Secondary School, Nsawam, Ghana. 1976 – 1981.

FELLOWSHIP/TRAINING PRORAMMES ATTENDED

- Two-Day Training Programme for Directors, Deputy Directors, Centre Managers and Heads of Departments/Sections at the SNAS Conference Room, 24th – 25th January, 2019.
- TEEAL/AGORA Train-the-Trainer Course, School of Nuclear and Allied Science, Atomic Campus, Atomic Energy, Atomic. 24 – 26 September, 2014.

- National Workshop on the "Logical Framework Approach for TC Project Design", 12th – 16th November, 2012, Ghana Atomic Energy Commission, Accra – Ghana.
- Web 2.0 Learning Opportunity, CSIR-INSTI, Accra, 9th – 13th August, 2010.
- Sensitization workshop on the Use of the Smart Toolkit for Evaluating Information Projects, Product and Services (Part of the CTA Information and Knowledge for Development (InK4DEV) Week of Activities) CSIR-INSTI, Accra, 18th – 19th October, 2010.
- FAO/IAEA Regional Training Course on principles of integrated area – wide tsetse/trypanosomosis control/eradication with emphasis on the sterile insect technique, 17th November – 12th December 1997, TTRI, Tanga, United Republic of Tanzania.
- Fellowship Training at the IAEA Seibersdorf Laboratory, Vienna, Austria, in the Handling, Rearing and Sterilization of tsetse flies, 3rd October, 1993 – 4th February, 1994.

TEACHING AND EXAMINATION EXPERIENCES

2018 – Date Lecturer, Graduate School of Nuclear and Allied Sciences (SNAS), Atomic, Accra. Teaching; General Entomology (NARP 661), Stored Products Entomology (NARP 663), Radioisotopes and Radiation Techniques in Entomology (NARP 673), Applied Entomology (NARP 662), Integrated Insect Pest and Vector Management (664) and Genetic

Control of Insect Pests Using Sterile Insect technique (668).

2014 – 2016 Part – Time Lecturer, Baldwin College, Osu, Accra. Teaching Haematology

2011 – 2014 Part - Time Lecturer, Radford University College, Accra Ghana. Teaching; Haematology, Clinical Chemistry, Medical Laboratory Techniques, Physician Office Laboratory procedures, Basic Medical Laboratory Technology and preparing remedial students in WASSCE Integrated Science

1990 – 1992 Science Tutor, Adu Gyamfi Secondary School, Jamasi, Ashant, Ghana. Teaching; General Science, Physics and Biology (At forms 1, 3, 4 and 5) (National Service).

1985 – 1987 Science Tutor, Akokoaso Day Secondary School, Akokoaso, Ghana. Teaching; General Science and Agricultural Science (Forms 1 to 3).

LIST OF PUBLICATIONS AND SCIENTIFIC PAPERS

i. PUBLICATIONS IN REFEREED JOURNALS

1. Osae, Michael, Wilson, D., Adabie-Gomez, D. A., Annoh, C. E., **Ewusie, E.A.,** Kluvitse, F. (2005). The effect of gamma irradiation on the biology of the Cigarette Beetle, Lasioderma Serricorne (Coleoptera: Anobiidae) *Journal of the Ghana Science Association*. Vol. 8 (1) 40 - 45.
2. Afful, S., Dogbe, S. A., Ahmed, K, **Ewusie, E. A.** (2008). Thin Layer Chromatographic Analyses of a Soil Ecosystem. *West Africa Journal of Applied Ecology* Vol. 14, 2008.

3. **Ewusie, E. A.,** Nkumsah, A. K., Alimatu, S., Osae, M. Y. (2010). Preliminary studies on the potential of inherited sterility for the control of diamondback moth (Plutella xylostella) (Lepidoptera: Plutellidae) of crucifers in Ghana. *Agricultural and Food Science Journal of Ghana.* Vol. 8: 631 – 641
4. **Ewusie, E. A.,** Parajulee, M. N., Adabie-Gomez, D. A., Wester, D. (2010). Strip cropping: A potential ipm tool for reducing whitefly, Bemisia tabaci Gennadius (Homoptera: Aleyrodidae) infestations in cassava. *West African Journal of Applied Ecology* Vol. 17:109 – 119
5. S. Afful, E. Enimil, B. Blewu, G. Adjei Mantey and **E. A. Ewusie.** (2010). Gas Chromatographic Methodology for the Determination of Some Halogenated Pesticides. *Research Journal of Applied Sciences, Engineering and Technology 2*(6): 592-595.
6. E. S. K. Ofori, S. Yeboah, J. Nunoo, E. K. Quartey, W. Torgby-Tetteh, E. K. Gasu & **E. A. Ewusie** (2014). Preliminary Studies of Insect Diversity and Abundance on Twelve Accessions of Tomato, Solanum lycopersicon L. Grown in a Coastal Savannah Agro Ecological Zone. *Journal of Agricultural Science;* Vol. 6, No. 8; 72 – 82.
7. Joseph Nunoo, Enoch Selorm Kofi Ofori, Emmanuel Kwatei Quartey, Emmanuel Kwame Gasu, **Ebenezer Ato Ewusie,** Bernard Tawiah Odai, Wellington Torgby-Tetteh and Wisdom Selorm Kofi Agbemavor (2014). Characterisation of Some Physico-chemical Properties of F5 Breeding Lines of Tomatoes. *British Journal of Applied Science & Technology*, *4*(27): 3967-3975.
8. Charles. E. Annoh, **Ebenezer. A. Ewusie**, Millicent. A. Cobblah, Michael. Y. Osae, Bernard. A. Boateng, Peter. K. Kwapong, Kwame. Aidoo, Paul. P. Bosu

(2017). Status and trends of monitoring insect pollinators in mango ecosystem in southern Ghana. *Journal of Applied Management Science.* Volume-3, Issue-1, Paper-3, 34 – 48.

9. **E. A. Ewusie,** P. K. Kwapong, G. Ofosu-Budu, C. Sandrock, A. Akumah, E. Nartey, C. Teye-Gaga, S. K. Agyarkwah and N. Adamtey (2018). Development of Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae) in Selected Organic Market Waste Fractions in Accra, Ghana. *Asian Journal of Biotechnology and Bioresource Technology 4*(1): 1-16.

ii. TECHNICAL PAPERS

1. Annoh, C. E., Adabie-Gomez, D. A., Osae, M, **Ewusie, E. A.,** and Timpo, S. (2004). Rapid appraisal of public awareness on the importance of pollinators for their conservation.
2. **Ewusie, E. A.,** Parajulee, M. N., Adabie-Gomez, D. A., Wester, D. (2008). Reduced whitefly (Bemisia tabaci) Gennadius (Homoptera: Aleyrodidae) infestations in cassava using strip Cropping.
3. **Ewusie, E. A.,** Annoh, C. E., Osae, Y. M., Nkumsah, A.K., (2009). Population dynamics of Bactrocera invadens (Diptera: Tephritidae) and environmental factors influencing populations in a mango plantation at Kwabenya.
4. Ofori-Ayeh, E., Asare. K. D., Annoh, C. E., **Ewusie, E. A.** (2009). Integrated pest management: Habitat manipulation strategy for the control of maize stemborers in Ghana.
5. **Ewusie, E. A**. (2010) Preliminary rearing of the African invader fruit fly Bactrocera invadens (Diptera: Tephritidae) in the laboratory.

6. S. Afful, E. Enimil and **E. A. Ewusie** (2010). A Review of Chromatographic Techniques for Determination of Pesticide Residues.
7. **Ewusie, E. A.,** Billah, M., Ofori, S. E., Egyir-Yawson, A. (2011). Reproductive assessment from different mating ratios of male and female Bactrocera invadens (Diptera: Tephritidae) to maintain a laboratory colony for subsequent sterile insect technique control programme in Ghana.
8. **Ewusie, E. A.,** Billah, M., Adabie-Gomez, D. A., Annoh, C. E., Aggrey-Korsah, R. (2012). Effect of parental ageing on fecundity and pupal weight of laboratory reared Bactrocera invadens (Diptera: Tephritidae).
9. **Ewusie, E. A.,** Yeboah, S., Ofori, E. S. K., Osae, M. Y, Egyir-Yawson A., Marri, D., Tetteh, A. E. (2013). Preliminary studies into extraction of proteolytic enzyme, papain enzyme for the production of local protein bait.
10. S. Afful, **E. A. Ewusie** (2013). Current trends in liquid-liquid micro extraction for analysis of pesticide residues in food and water.
11. **Ewusie, E. A.**, Kwapong, P. K., Ofosu-Budu, G. Adamtey, N., Stamer, A., Akumah, A.M., Nertey, E. Tetegaga, C., Agyarkwa, S. (2016). The black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): trapping and culture of wild colonies in Ghana.
12. **Ewusie, E. A.**, Kwapong, P. K., Ofosu-Budu, G. Adamtey, N., Stamer, A., Akumah, A.M., Nertey, E. Tetegaga, C., Agyarkwa, S. (2016). Laboratory evaluation of various larval diets on life – history traits of black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae).
13. **Ewusie, E. A.**, Kwapong, P. K., Ofosu-Budu, G. Adamtey, N., Stamer, A., Akumah, A.M., Nertey, E. Tetegaga, C., Agyarkwa, S. (2017). Effects of

different artificial diet formulations on the survival and reproductive performance of black soldier fly, *Hermetia illucens*, (Diptera: Stratiomyidae).

PUBLICATIONS IN PREPARATION

1. **Ewusie Ebenezer, A.,** Kwapong Peter, K., Ofosu-Budu, G., Andreas Stamer, Akumah Asiwome, M, Eric Nertey, Christopher Tetegaga, Agyarkwa Seth, K., Sandrock Christoph, Adamtey Noah (2019). Effect of larval density on the growth and development of black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Current Journal of Applied Science and Technology* ***(In press).***
2. **Ewusie Ebenezer, A.,** Kwapong Peter, K., Ofosu-Budu, G., Andreas Stamer, Akumah Asiwome, M, Eric Nertey, Christopher Tetegaga, Agyarkwa Seth, K., Sandrock Christoph, Adamtey Noah (2018). Effects of artificial diet formulations on the survival and reproductive performance of black soldier fly, *Hermetia illucens*, (Diptera: Stratiomyidae).
3. **Ewusie, E. A.**, Kwapong, P. K., Ofosu-Budu, G. Adamtey, N., Stamer, A., Akumah, A.M., Nertey, E. Tetegaga, C., Agyarkwa, S. (2019). The black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): trapping and culture of wild colonies in Ghana. ***Scientific African Journal*** **(Submitted).**
4. Asiwome M Akumah, Eric K. Nartey, Godfred K. Ofosu-Budu, **Ebenezer A Ewusie,** Christopher Teye-Gaga, Noah Adamtey. (2018). Characterization of Market Waste in Ghana for use as Feedstock in Composting.
5. Teye-Gaga, Christopher, Ofori-Danson, Patrick K., Agyakwah, Seth K., Ocloo, Fidelis C. K., **Ewusie, Ebenezer A.,** Akumah, Asiwome M., Ofosu-Budu, Godfred K. Adamtey, Noah. (2018). Evaluation of Larval Meal Diet

of Black Soldier Fly (*Hermetia illucens*: L. 1758) on Fingerlings Culture of Nile tilapia (*Oreochromis niloticus:* L).

6. **Ewusie Ebenezer Ato,** Samuel Kofi Agbeve. (2018). Pesticide usage in the production of watermelon by farmers in the Ada-West District of Ghana.
7. Samuel Kofi Agbeve, **Ebenezer Ato Ewusie**, Samuel Afful. (2018). Organochlorine pesticide residue levels in parts of watermelon grown in the Ada-West District.

POSTERS

1. Delphina A. Adabie-Gomez, C. E. Annoh, C. I. Mahama, E.A. Ewusie. (2005). FAO/IAEA International Conference on Area-wide Control of Insect Pests: Integrating the Sterile Insect and related Nuclear and other Techniques, Vienna, Austria, 9 – 13 May, 2005.
2. C. A. Annoh, M. A. Cobblah, E. A. Ewusie, B. A. Boateng, P. K. Kwapong. (2013). Harnessing pollinators: A natural resource to boost the mango industry in Ghana. Poster presented at the Ghana Science Association 28th Biennial Conference, University of Ghana, Legon, 14th – 19th July, 2013.

HANDBOOK/MANUALS

1. Charles, E. Annoh, Millicent A. Cobblah, Ebenezer A. Ewusie, Bernard A. Boateng (2014). GEF/UNEP/FAO-GPP GH. Study Field Guide For Mango Pollinator-Friendly Practices (Dodowa/Somanya STEP SITE).

SEMINARS AND CONFERENCES PAPERS

1. **Ewusie, E. A**. (1994). Quality control of blood and subsequent for feeding tsetse flies. Ghana Atomic Energy Commission.
2. Adabie-Gomez, D. A., Annoh, C. E., **Ewusie, E. A**. (2005) Experiences in Management of Area- Wide Control of Glossina palpalis palpalis (Diptera: Glossinidae) in Ghana using Bait Technology and Releases of Sterile Males. FAO/IAEA International Conference on Area Wide Control of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques, Vienna, Austria, 9 – 13 May, 2005 (Poster Presentation: IAEA-CN-131/P96).
3. **Ewusie, E. A**. (2006). Food Security in Africa – The way forward. International Agronomic Development class, Texas Tech University, Lubbock, Texas, USA.
4. **Ewusie, E. A**. (2007). Livestock grazing system for mutton production in the Ga District of Ghana. Forages and livestock management class. Texas Tech University, Lubbock, Texas, USA.
5. **Ewusie, E. A**. (2009). Successful poster presentation. Biotechnology and Nuclear Agriculture Research Institutional seminar.
6. Annoh, C. E., Cobblah, M. A., **Ewusie, E. A.,** Boateng, B. A., Kwapong, P. K. (2013). Harnessing Pollinators: A natural resource to boost the mango production in Ghana. Ghana Science Association 28th biennial conference on "harnessing our natural resources for national development: The role of science and Technology", University of Ghana, Legon, Accra, Ghana. 15th – 18th July, 2013.
7. Marri, D., Wilson, D. D., Gomez, D. A. A., **Ewusie, E. A.,** Osae, M. Y. (2013) Evaluation of the efficacy of entomopathogenic fungus *Beauveria bassiana*

for the control of the invasive fly *Bactrocera invadens* (*Diptera: Tephritidae).* Ghana Science Association 28th biennial conference on "harnessing our natural resources for national development: The role of science and Technology", University of Ghana, Legon, Accra, Ghana. 15th – 18th July, 2013.

JOURNAL PAPERS REVIEWED/EXAMINED

1. Economics of small holder agriculture in rural Nigeria: Ekiti State experience with cassava production. Manuscript No.IJAS-12-212.
2. In vitro evaluation of the ovicidal and larvicidal efficacies of aqueous and ethanolic extracts of stem bark of *Piliostigma thonningii* (Fabaceae) on *Heligmosomoides bakeri* (Nematode, Heligmosomatidae). Manuscript No.IJMPR-12-069.

SUPERVISION OF UNDER - GRADUATE RESEARCH

External Supervisor of Mr. Stanley Akwesi Acquah of the Radiation Technology Centre, Ghana Atomic Energy Commission, for his BSc. Dissertation titled "Evaluating drying methods for black soldier larvae (*Hermetia illucens)* and its use as fish and mono-gastric feed in Ghana" at Accra Technical University. (2016).

REFEREES

1. Prof. Megha N. Parajulee,

   AgriLife Research, Texas A&M System,

   1102 East FM, 1294, Lubbock, TX 79403, USA.

2. Dr. (Mrs). Delphina Adabie – Gomez,

   School of Nuclear and Allied Science,

   University of Ghana Atomic Campus,

   Atomic Energy, Atomic, Accra. Ghana.

3. Pastor Prof. Peter Kofi Kwapong,

   Department of Conservation Biology and Entomology,

   School of Biological Sciences, College of Agriculture and Natural Sciences,

   University of Cape Coast, Cape Coast. Ghana.