



Use of Black Soldier Fly (*Hermetia illucens*)  
in bioconversion and feed production

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**COVER ILLUSTRATION**

**BLACK SOLDIER FLY LARVAE FEASTING ON A WATERMELON**

**ILLUSTRATED BY YESIM PACAL©**

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## **USE OF BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) IN BIOCONVERSION AND FEED**

### **PRODUCTION**

#### **ABSTRACT**

Population and economic growth, rapid urbanization and shifts in dietary preferences towards consuming more animal-derived products are some of the major challenges facing sub-Saharan Africa (SSA) today. Consequently, large amounts of urban waste are generated, with organic waste accounting for almost 80% of the total. However, urban organic waste, a rather valuable resource, is often disposed in municipal landfills due to the lack of adequate infrastructure. At the same time in the developing world there is frequently a lack of local and regional waste disposal management plans. While around one third of the food produced around the world each year is either lost or wasted, current agricultural production systems are failing to address the increasing demand on animal-derived products, let alone feeding the ten billion people expected to live on our planet by the year 2050. Moreover, these production systems are often associated with unsustainable land and natural resource use and in addition contribute to climate change by emitting massive amounts of greenhouse gases (GHG).

Recently, insect species such as the Black Soldier Fly (BSF) (*Hermetia illucens*) have been recognized as innovative alternatives for the traditional protein sources used in the production of livestock feed due to their high nutritive value and the fact that they can feed on various organic waste streams. By employing a set of laboratory-based experiments, this dissertation, aims to explore the use of BSF as a tool in the bioconversion of urban organic waste streams and for the production of high-quality livestock feed within a developing world context. Specifically, this work seeks to assess the influence of commonly and readily available urban organic waste streams on the nutritive quality of BSF larvae (BSFL) reared on them. Therefore, in chapter two, a holistic comparison of the nutritive value of BSFL reared on three different organic

substrates, i.e. chicken manure (CM), brewers' spent grain (SG) and kitchen waste (KW) was conducted. The results of the comparison indicate that BSFL differed in terms of nutrient composition depending on the organic substrates they were reared on. Therefore, identifying organic waste streams of high nutritive content is crucial for the successful production of high quality BSFL-derived feed. Moreover, this work seeks to understand the compound influence of the rearing substrate and temperature. Therefore, in chapter three, a study on the influence of temperature on the development of BSFL reared on two different organic waste streams (SG and CD) was conducted. The results show that SG-fed BSFL were more efficient and tolerated a wider range of temperatures in comparison to the CD-fed ones. Hence there was an influence of both the rearing substrate and the temperature on the fitness of BSFL. Chapters two and three contribute to the knowledge needed in operating, standardizing and optimizing successful BSFL large scale production facilities in the developing world. The research in chapter 4 aims to provide an insight regarding the safety of BSFL reared on urban organic waste streams. Urban organic waste streams are environments where pathogens normally thrive. Therefore, understanding the influence of such streams on the microbial composition of the BSFL gut is needed when identifying safe rearing substrates or deciding suitable decontamination measures and treatments. Thus, research on selected bacterial species isolated from the gut of BSFL reared on KW and CM by using a culture-dependent molecular approach was conducted. The results highlight the potential influence of the rearing substrate on the gut microbial community of BSFL which show a high variability of bacterial species. Additionally, the results show the potential of BSFL to vertically transmit certain bacterial species. Overall, this dissertation confirms the possibility of taking advantage of the readily available urban organic waste streams in Nairobi, Kenya, and arguably elsewhere in the developing world, to produce nutrient-rich BSFL-derived feed. Future research should focus on the development of optimized technologies in terms of: (1) rearing conditions such as temperature, (2) feeding methods, and (3) substrate pre-treatment and decontamination techniques. Moreover, a unified regional legal framework should be developed in order to regulate

the quality and ensure the implementation of adequate hygiene and safety measures in industrial mass production systems of insects for feed.

# Nutzung der Schwarzen Soldatenfliege (*Hermetia illucens*) in der Biokonversion und Futtermittelproduktion

## KURZFASSUNG

Anhaltendes Bevölkerungs- und Wirtschaftswachstum, rasante Verstädterung und Verschiebung der Ernährungspräferenzen hin zum vermehrten Konsum von tierischen Produkten und Proteinen sind einige der größten Herausforderungen, mit denen Subsahara-Afrika (SSA) heute umgehen muss. Infolgedessen werden enorme Mengen an Siedlungsabfällen erzeugt, wovon organische Abfälle fast 80% ausmachen. Es handelt sich jedoch bei diesen städtischen organischen Abfällen um eine wertvolle Ressource, die in der Futtermittelproduktion zum Einsatz kommen könnte, die allerdings oftmals auf kommunalen Deponien entsorgt wird. Eine passende Infrastruktur, sowie lokale und regionale Managementstrategien und -pläne zur Nutzung dieser Ressource fehlen in Entwicklungsländern. Rund ein Drittel der produzierten Lebensmittel weltweit gehen entweder verloren oder werden verschwendet, während die derzeitigen landwirtschaftlichen Produktionssysteme die steigende Nachfrage nach tierischen Produkten nicht decken können. Bei einer zu erwartenden Weltbevölkerung von 10 Milliarden Menschen im Jahr 2050 werden sich diese Probleme zukünftig noch verschärfen. Darüber hinaus sind die heutigen Produktionssysteme oft mit einer nicht-nachhaltigen Nutzung von Land und natürlichen Ressourcen verbunden und tragen durch den Ausstoß massiver Mengen an Treibhausgasen zum Klimawandel bei.

In jüngster Zeit wurden unterschiedliche Insektenarten, wie z.B. die Schwarze Soldatenfliege (BSF) (*Hermetia illucens*), aufgrund ihrer hohen Nährwerte und der Tatsache, dass sie sich von verschiedenen organischen Abfallströmen ernähren können, als innovative Alternative zu traditionell in der Produktion von Tierfutter eingesetzten Proteinquellen erkannt. Durch den Einsatz einer Reihe von laborgestützten Experimenten untersucht diese Arbeit den Einsatz der BSF in der Biokonversion von städtischen organischen Abfallströmen zur Herstellung von

hochwertigem Tierfutter im Kontext von Entwicklungsländern. Insbesondere zielt diese Arbeit darauf ab, den Einfluss des Nährwerts von häufigen und leicht zugänglichen organischen städtischen Abfallströmen auf den Nährwert von darauf aufgezogenen Larven der Schwarzen Soldatenfliege (BSFL) zu bestimmen. Im zweiten Kapitel dieser Arbeit wurde daher ein ganzheitlicher Vergleich des Nährwerts der BSFL auf drei verschiedenen organischen Substraten, nämlich Hühnermist (CM), Brauerei- (SG) und Küchenabfall (KW), durchgeführt. Die Ergebnisse dieser Untersuchung deuten darauf hin, dass die Nährstoffzusammensetzung der Substratquellen einen starken Einfluss auf die Entwicklung der BSFL haben. Die Identifizierung organischer Abfallströme mit hohem Nährwertgehalt ist demnach entscheidend für die erfolgreiche Produktion von hochwertigen BSFL-basierten Futtermitteln. Darüber hinaus versucht diese Arbeit die Wechselwirkung zwischen Aufzuchtsubstrat und den äußeren Umweltbedingungen, d.h. der Temperatur, nachzuvollziehen. Im dritten Kapitel wurde daher eine Studie über den Einfluss der Temperatur auf die Entwicklung von BSFL, die auf organischen Abfallströmen (SG und CD) aufgezogen wurden, untersucht. Die Ergebnisse zeigen, dass auf SG aufgezogene BSFL effizienter waren und einen breiteren Temperaturbereich tolerierten als die auf CD aufgezogenen Insekten. Sowohl das Aufzuchtsubstrat als auch die Temperatur haben demnach einen Einfluss auf die Fitness der BSFL. Kapitel zwei und drei entwickeln hier die notwendigen Grundlagen für den Betrieb, die Standardisierung und die Optimierung erfolgreicher großer BSFL-Produktionsanlagen in Entwicklungsländern. Ein weiteres Ziel dieser Forschungsarbeit ist es etwaige Risiken, die mit der auf städtischen organischen Abfallströmen aufgezogenen BSFL im Zusammenhang stehen, zu identifizieren. Diverse Krankheitserreger können sich leicht auf organischen Siedlungsabfälle entwickeln. Daher ist es notwendig den Einfluss solcher Abfallströme auf die mikrobielle Zusammensetzung des BSFL-Darms zu untersuchen. Dies ist entscheidend für die sichere Nutzung solcher Zuchtsubstrate, bzw. die Entwicklung von geeigneten Dekontaminationsmaßnahmen und -behandlungen. So wurde in Kapitel vier eine Untersuchung ausgewählter Bakterienarten, die aus dem Darm der BSFL isoliert wurden, auf KW und CM unter Verwendung eines kulturabhängigen molekularen



Ansatzes durchgeführt. Die Ergebnisse zeigen den potenziellen Einfluss des Aufzuchtsubstrats auf die mikrobielle Darmgemeinschaft der BSFL, was sich in der variablen Zusammensetzung der isolierten Bakterienarten widerspiegelt. Zusätzlich zeigen die Ergebnisse, dass BSFL bestimmte Bakterienarten vertikal übertragen können. Insgesamt bestätigt diese Dissertation das Nutzungspotenzial leicht verfügbarer organischer städtischer Abfallströmen in Nairobi, Kenia und in anderen Entwicklungsländern für die Produktion von nährstoffreichem BSFL-Tierfutter. Zukünftige Forschungsansätze sollten sich auf die Entwicklung optimierter Technologien in Bezug auf: (1) Aufzuchtbedingungen wie Temperatur, (2) Fütterungsverfahren und (3) Substratbehandlungs- und Dekontaminierungstechniken konzentrieren. Darüber hinaus sollte ein einheitlicher regionaler Rechtsrahmen entwickelt werden, um die Qualität und die Umsetzung angemessener Hygiene- und Sicherheitsmaßnahmen in der industriellen Massenproduktionssystemen von Insekten sicherzustellen.

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## 1 GENERAL INTRODUCTION



### 1.1 Introduction to organic waste

Waste materials may be solid, liquid, gaseous and/or radioactive substances that result from human activities and have no value to users (Diaz et al. 1996).

According to the European Commission's (EC) Waste Framework Directive number 2008/98/EC, waste is defined as: *"Any substance or object which the holder discards or intends or is required to discard"* (Smith 2015). The Australian Environment Protection Act (EPA) defines waste as follows: *"Waste encompasses all discarded, rejected and abandoned to unwanted or surplus material, intended or not intended for either sale, recycling, reprocessing as well as for recovery or purification through a separate operation by which the matter was produced; or anything considered by regulation or environment protection policy as a waste, with or without value"* (Lewis and Wagner 2008).

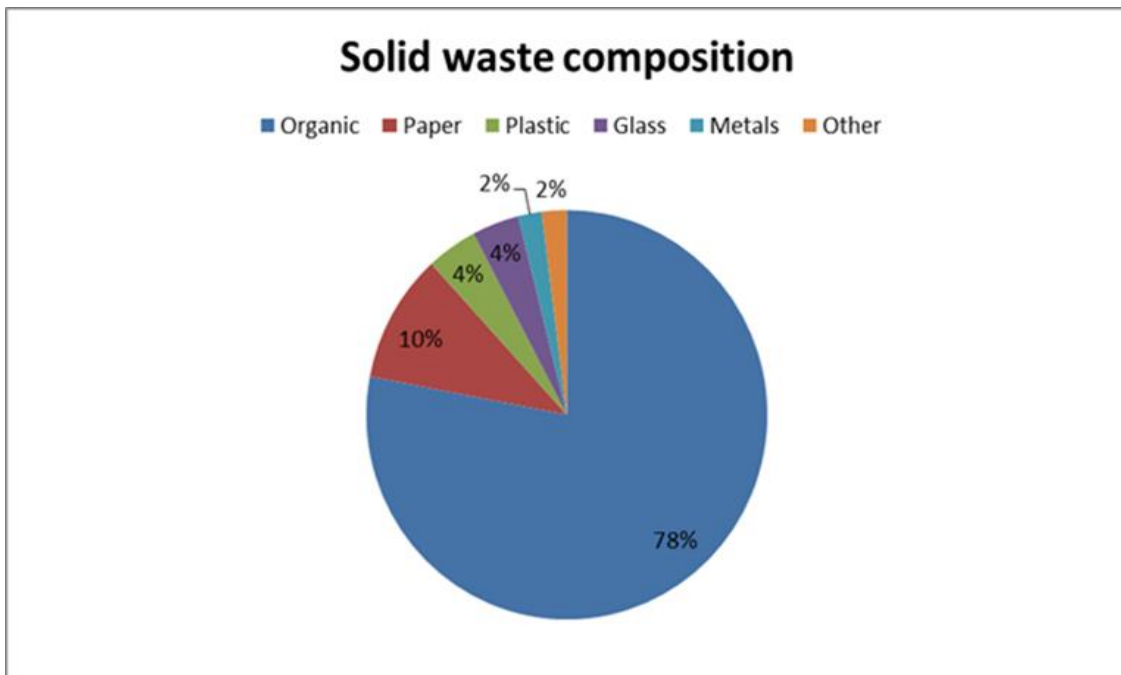
Organic waste includes all biodegradable waste from plants and animals, like paper and yard waste to wood, food, textiles or other organics or simply the garden and organic household wastes of the municipal waste stream (Njoroge et al. 2014). Like any other waste, organic waste is generated through human activities regardless of age and gender or location and monetary income (Noman and Zurbrugg 2002). This

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implies that the generation of organic waste is directly influenced by human consumption habits.

## 1.2 The status of organic waste in an “ever” developing world

Waste generation continues to rise with the worldwide increasing human population (Diaz et al. 1996). As of 2000, about 49% of the global population resided in urban areas and generated an average amount of daily waste of over 3 million metric tons which is projected to double by 2025 (Hoornweg et al. 2013). Njoroge et al. (2014) stated that organic waste constitutes the largest proportion of solid waste, accounting for more than 78% of the entire solid waste stream (Figure 1.1).



**Figure 1.1 Global solid waste composition in percentages where organic waste constitute 78% of all the solid waste, followed by 10% for paper, and 2% each for plastic, glass and metals (Njoroge et al. 2014).**

Among all constituents of organic waste streams, food waste remains to be a great challenge. According to the Food and Agriculture Organization of the United Nations (FAO), the global population generated food waste of approximately 1.6 billion tons in 2007 between production and consumption, which translated to about a third

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of the global food production (FAO 2014). Unfortunately, most of these wastes are dumped in landfills and consequently occupy a large space, resulting in the spread of pathogens and emission of noxious odors and act as a significant source of greenhouse gases (GHG) worldwide (FAO 2014).

The rising quantities as well as complexity of waste present a major environmental threat across Africa. According to the United Nations Environment Program (UNEP), about 1.7 to 1.9 billion metric tons of Municipal Solid Waste (MSW) is generated per year worldwide (Benn et al. 2011), with more than 40% of these wastes generated in African countries (Diaz et al. 1996). While giant economies like China and the USA were considered in the past as the leading producers of waste globally, Africa was always considered a region where solid waste is being mismanaged (Bhada-Tata and Hoornweg 2012). As a result, various initiatives have been undertaken with a view to scale up waste recycling and establish directives to facilitate waste management in developing countries like Kenya (Marshall and Farahbakhsh 2013).



**Figure 1.2** Garbage as far as the eye can see in Dandora, the main dumping ground in the Kenyan capital Nairobi (Mukoya/Reuters, 2018) (EJOLT 2015).

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### **1.3 Effects of organic waste on environment, human health and economies**

Waste generation impacts adversely on human health, the environment as well as on economic growth. By contrast, a well-run management system of solid waste is beneficial since it creates jobs and generates income for both the formal and informal sectors (Diener et al. 2009). Improper management of organic waste causes all types of pollution: water, solid and air. Indiscriminate dumping of organic wastes contaminates surface water while improperly dumped organic wastes create stagnant waters for breeding of insects and floods during rains (Gwo-fong 2011). Organic wastes also pollute water leading to waterborne and water related diseases such as cholera and typhoid (Srigirisetty et al. 2017). Research indicated that most diseases in Africa are caused by poor sanitation and hygiene, especially when there is improper organic waste management. Rodents and insect vectors like houseflies and mosquitos are attracted to waste, causing the spread of various diseases such as malaria (Lucas and Gilles 2003). Moreover, organic waste can also cause injuries, especially to waste handlers, while dust generated due to organic waste disposal can cause pollution, leading to additional health related problems (Jerie 2016). Additionally, decomposition of solid waste spread obnoxious odors and burning of it generates air polluting obnoxious fumes (Ferronato and Torretta 2019). UNEP reported that *“Every year, an estimated 11.2 billion tons of solid waste are collected worldwide and decay of the organic proportion of solid waste is contributing to about 5 per cent of global greenhouse gas emissions”* which in turn accelerates climate change (Benn et al. 2011).

Despite the high potential for recovery, monetary and employment benefits, the reuse and recycling of organic MSW in the developing world is still limited (Henry et al. 2006; Wilson et al. 2006; Ali et al. 2014). Therefore, most organic MSW is dumped in landfills leading to prolonged landfill life-spans, leachate pollution and the production of landfill biogas that is composed of 40 - 60% of methane in addition to CO<sub>2</sub> and trace amounts of other GHG (Giusti 2009). Thus, the situation calls for an immediate intervention in order to reduce the environmental pollution and public health threats associated with poor waste management in urban environments in the



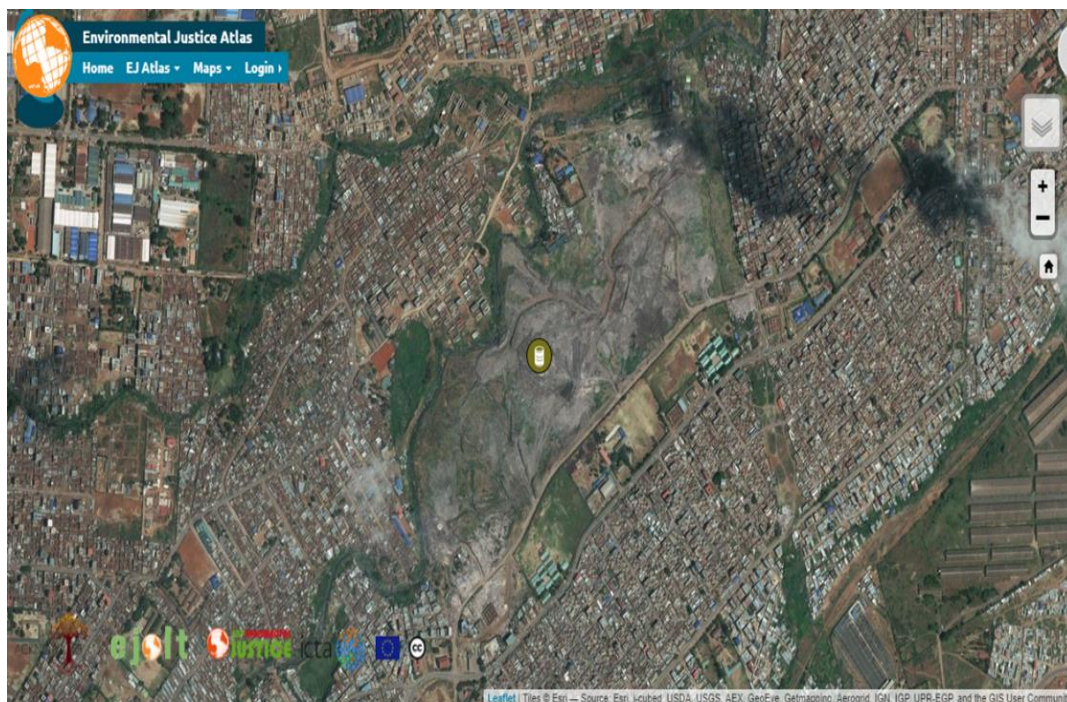
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developing world through adopting efficient and cost effective MSW management methods and strategies.

#### **1.4 Traditional organic waste management methods**

Many developing countries like Kenya employ poor municipal waste management methods with rates of waste collection falling below 70% despite the growing rate of waste production (Chalmin and Gaillochot 2011; Ali et al. 2014). Moreover, only 15% of the wastes collected is processed, mostly using unsafe and informal recycling methods, while above 50% of the wastes collected are usually disposed in uncontrolled landfills (Chalmin and Gaillochot 2011). Furthermore, waste management in countries like Kenya is poorly funded and affected by limited economic and technological opportunities which eventually lead to low management standards (Stuart 2009). Finally, a proper legal and administrative framework for efficient waste management is still lacking in most African countries (Stuart 2009).

The Dandora dump situated in the heart of the Nairobi slums of Korogocho, Baba Ndogo, Mathare and Dandora (Figures 1.2 and 1.3), which opened in the year 1975 with a financial support from the World Bank, is a good example of the poor status of waste management in Kenya (Barczak et al. 2015). Although declared full by the year 2001, the Dandora dump is still operating (Barczak et al. 2015). Epidemiological studies proved high rates of sickness and mortality in Dandora due to the unrestricted dumping of industrial, agricultural, domestic and medical wastes (Barczak et al. 2015).



**Figure 1.3** A world imagery map of the Dandora dump in Nairobi, showcasing its central location among urban settlements (EJOLT 2015).

### **1.5 Organic waste conversion techniques**

Currently, organic waste conversion techniques range from resource consumption reduction, composting, resources reuse and recycling, to anaerobic or aerobic digestion (Alvarez 2012). Composting constitutes the main option for converting organic wastes in landfills. However, its success is limited since it often lacks manual or mechanical heap mixing to maintain aerobic conditions. At the same time, it can deal with animal protein wastes, and requires planning and maintaining nutrient ratios in order to be successful (Alvarez 2012). Pathogenic organisms occurring in raw materials and composts are rapidly inactivated by the heat generated during the composting process (Ali et al. 2014; Wéry 2014). However, the main infection risks associated with composting are caused by the emission of bioaerosols and are attributable to opportunistic micro-organisms such as molds (Ali et al. 2014). Exposure to composting affects immune-compromised systems and may cause respiratory health conditions such as organic dust toxic syndrome, extrinsic allergic alveolitis

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(EAA), allergic rhinitis, asthma, upper air-way irritation together or mucous membrane irritations (Swan et al. 2003; Sykes et al. 2007; Ali et al. 2014).

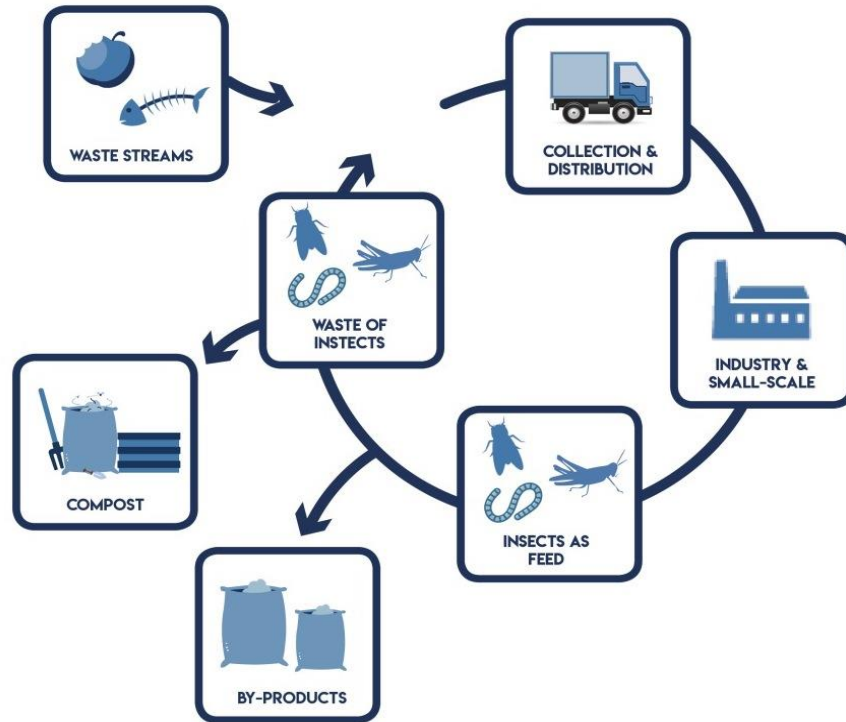
In addition to composting, resources reuse and recycling, anaerobic and aerobic digestion are methods that have always been used for waste conversion. Some countries apply the Advanced Thermal Conversion Technology (ATCT) which comprises of pyrolysis, i.e. thermally and anaerobically degrading biomass and organic solid wastes in order to generate synthesis gas, which is considered an essential source of energy that can be used for the general production of steam, electricity, hot water, and biofuels (Alvarez 2012). Organic solid wastes may also be subjected to fermentation in which methane is generated and used as a fuel (Alvarez 2012). Both aerobic and anaerobic digestion of organic waste can reduce it by more than 50% in volume and what remains is mainly the non-organic portion of it (Cunningham and Cunningham 2012). However, despite the current advanced technology, there is no wide use of modern waste treatment facilities in the developing world due to the high operational costs and the lack of human expertise associated with these techniques (Cunningham and Cunningham 2012).

In general, improper collection and treatment of MSW is associated with severe public health as well as environmental problems, the latter particularly related to global warming because organic waste emits significant amount of methane gas through open dumping or landfill disposal (Menikpura et al. 2013). Therefore, Premakumara et al. (2011) concluded that substantial conversion of organic wastes into beneficial resources holds a significant untapped potential for prolonging landfills' life, generating economic or environmental benefits, and pressure reduction on municipalities due to increasing complexity of MSW.

## **1.6 Bioconversion of organic waste into livestock feed**

Organic wastes may be effectively converted into animal feed through bioconversion. The literature defines bioconversion as a process in which organic materials are converted into useful energy by biological agents such as microorganisms and invertebrates (Elissen 2007). The idea is commendable since it involves the use of

sacrophages such as insect larvae to break down the organic waste biomass into reusable forms of byproducts (Figure 1.4).

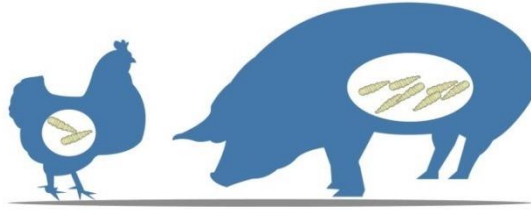


**Figure 1.4** An illustrated diagram that explains the process of converting organic waste streams into livestock feed and other by-products, including composts.

For instance, the use of the earthworm family Lumbriculidae (order Lumbriculida), such as the treatment of faecal sludge using the freshwater California Blackworm *Lumbriculus variegatus*, has been cited as potential options for nutrient production from organic waste (Elissen 2007; Edwards et al. 2010). Another option is the digestion of organic waste using insect species like the Common Housefly *Musca domestica* (Diptera: Muscidae), the larvae of the Mealworm Beetle *Tenebrio molitor* (Coleoptera: Tenebrionidae), crickets belonging to the Gryllidae family (order Orthoptera) and the Black Soldier Fly (BSF) *Hermetia illucens* (Diptera: Stratiomyidae) to produce protein for livestock and fish feed or human food with reduced environmental and human health effects (Makkar et al. 2014).

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## 1.7 Insects can meet the need for feed



A FAO report indicates that the global human population is likely to rise from the presently 7.3 billion people to 9.7 billion in 2050 (FAO 2009). This population increase threatens global food security and presents major problems with regard to protein supply (Merino et al. 2012; FAO et al. 2015). Ray and colleagues projected an increase in future shortage for food commodities like maize (67%), rice (42%), wheat (38%) and soybean (55%) (Ray et al. 2013). These estimates highlight the need for new affordable food and feed sources that have high nutrient content, including protein, fatty acids and micronutrients such as calcium, iron and zinc (Ritchie et al. 2018). Though the demand for feeds in Africa continues to increase and is estimated to possibly double by 2020, only 5% of the feeds imported to African countries come from other African countries (Thornton 2010; Brenton 2012).

Edible insects are novel sources for alternative protein for both human and animal consumption (Van Huis et al. 2013). They are common and globally distributed, have the potential to serve as a source for efficient food conversion, short breeding period and contain a high content of protein (Oonincx et al. 2015; van der Fels-Klerx et al. 2016). As a result, they can be developed and used to address problems of protein shortages in the food and/or feed industry (Nowak et al. 2016). There are over 2,000 edible insect species around the globe with adequate nutritional quality to address human malnutrition (Van Huis 2016). The nutritional contents in insects include high crude protein (CP) quantities, valuable crude fat (CF) that may substitute traditional protein sources (e.g. fish and soybean meals) in animal, poultry or aquaculture feed production industry (Makkar et al. 2014; Stamer 2015).

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However, many insect species lack the ability to convert various organic wastes and require specific nutrition in order to be effective bio-converters (Morgan and Eby 1975; Latsamy and Preston 2007; Morales and Wolff 2010; Zheng et al. 2012a). Besides, some of these insects are pests and/or vectors of human and/ or animal pathogens (Fotedar et al. 1992; De Jesús et al. 2004).

### **1.8 The study species Black Soldier Fly (BSF), *Hermetia illucens***

Black Soldier Fly (BSF), *Hermetia illucens* belongs to the kingdom Animalia, Phylum Arthropoda (Arthropods), Class Insecta (Insects), Order Diptera (Flies), Family Stratiomyidae (Soldier flies), Genus *Hermetia* and lastly Species *illucens* (Figure 1.5). The family Stratiomyidae comprises more than 260 species all over the world (Alvarez 2012).



**Figure 1.5 Black soldier fly adult on a rose, photographed by Didier Descouens in Fronton, Haute-Garonne, France (Descouens 2013).**

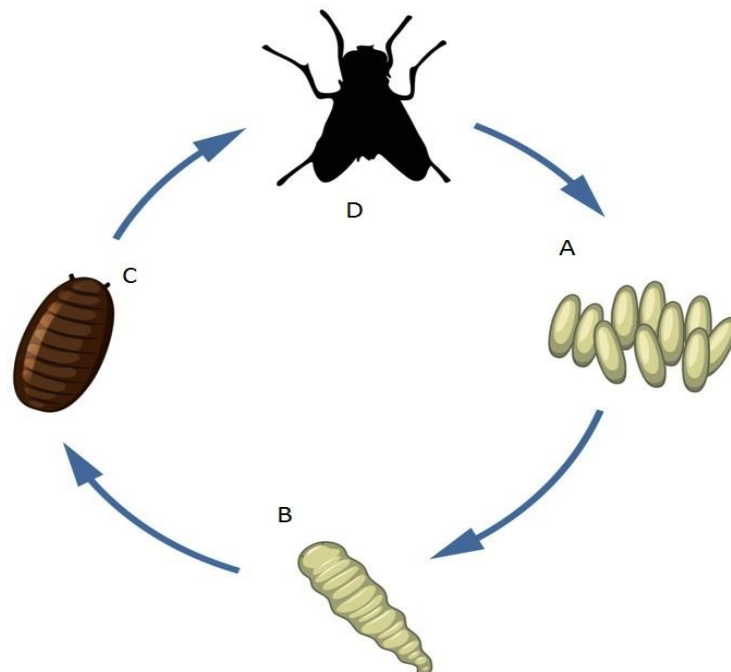
BSF has a life cycle (Figure 1.6) with egg, larva, pupa as well as an adult stage, and it needs about 40 - 43 days to develop from an egg to an adult fly, of which the eggs takes 4-6 days to hatch into the larva which lasts a minimum of 22 to 24 days before it changes into a prepupa and pupa and at least 14 days to an adult fly subject to various environmental factors such as temperature and humidity, the prevailing bacterial community, the pupation substrate and the strain (original location)

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(Tomberlin et al. 2002; Elissen 2007; Tomberlin et al. 2009; Li et al. 2011; Holmes et al. 2013a; Zhou et al. 2013). Bacteria, light intensity, as well as temperature and humidity influence the BSF oviposition preference together with the clutch size (Tomberlin et al. 2002, 2009; Holmes et al. 2013).

The prepupa migrates during the last larval stage to dry and suitable pupation sites from where they convert into pupa (Diener et al. 2011). This means that BSF prepupae may be self-harvested under favorable conditions (Tomberlin and Sheppard 2001). The adult flies feed on stored fat build-up during their larval stage and may require water (Banks et al. 2014). They are neither pests nor vectors of diseases (Sheppard et al. 2002; Čičková et al. 2015; Wang and Shelomi 2017). Black Soldier Fly larvae (BSFL) consume a range of organic wastes such as decaying fruits, vegetable waste, animal manure, municipal organic waste etc. (Li et al. 2011; Salomone et al. 2017; Rehman et al. 2017; Liu et al. 2017).

Adult BSFs exhibit a complex mating behavior which requires sunlight and flying space (Tomberlin and Sheppard 2001; Zhang et al. 2010).



**Figure 1.6** Diagram showing A) egg, B) larval, C) pupal and D) adult life stages of the Black Solider Fly (*Hermetia illucens*).

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In nature, BSF usually lay their eggs in moist organic material (Alvarez 2012). Livestock droppings usually offer great sites that attend to the individual reproductive needs of BSF (Sheppard et al. 2002). In conditions where such natural habitats have been eliminated, then BSF will always lay eggs in compost (Alvarez 2012). BSF larval diet influences traits of their life history (Tomberlin and Sheppard 2001; Nguyen et al. 2015). Laboratory BSF colonies are mainly cultured on Gainesville Housefly Diet, which comprises of 30%:50%:20% alfalfa meal, wheat bran and dry mass of corn meal, respectively, with 60-70% moisture (Hogsette 1992; Tomberlin et al. 2002; Sheppard et al. 2002). However, feral BSF are double the size of the laboratory-reared ones, indicating that laboratory diets may be at times are inadequate to maintain the insects' needs (Tomberlin et al. 2002).

### **1.9 The significance of the Black Soldier Fly *Hermetia illucens***

According to Van Huis et al. (2013), use of BSFL in bioconversion is more sustainable than other waste conversion methods because it addresses various economic, social, and environmental problems. BSFL are raised on wastes and once they feed on it, they reduce many health risks or environmental contaminations associated with wastes. In fact, these authors claim that BSFL bioconversion adds value to these wastes as they no longer negatively impact the environment but generate products that can be used by society.

The insects are able to transform organic waste streams to valuable products, as well as less toxic biomass, while producing little ammonia (Van Huis et al. 2013). Also, BSFL are able to decompose and convert various food wastes produced from organizations, institutions, as well as those that result from household activities (Banks et al. 2014). Various studies suggest that BSF is effective in reducing quantities of biomass, with estimates varying between 50 - 75% , illustrating its ability to significantly reduce waste materials (Barry 2004; Li et al. 2011; Barragan-Fonseca et al. 2017).

Unlike the adult BSF, the adult common housefly feeds on various wastes, including fresh human foods (Barry 2004). The common housefly is also a common



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vector of various human and animal pathogens (Issa 2019). Yet BSF presence deters oviposition by *M. domestica*, thereby considerably reducing their numbers (Furman et al. 1959; Sheppard 1983; Dobermann et al. 2019). Another beneficial aspect of BSFL use is the larvae ability to reduce odors because they act very fast on waste materials (Diener et al. 2011). Once the wastes are consumed, the quantity of odor produced is significantly reduced.

BSFs are used in organic leachate processing and treatment that is otherwise very expensive to treat. The organic leachate is very rich in nitrogen and carbon that is not suitable for marine life (Popa and Green 2012). Also, it is easy to clear a portion of leachate organic metabolites using BSFL compared to using any other means. Furthermore, BSFL are capable of reducing the pollution caused by wastes by 50 - 60% and sometimes even more (Van Huis et al. 2013).

Various products can be derived from the processed waste materials, including biodiesels, fertilizers, bioplastics and animal feeds (Tomberlin and Sheppard 2001; Barbi et al. 2019). Also, BSF reduces significantly the costs that are otherwise needed to collect and manage wastes (Banks et al. 2014). The technique also reduces the health hazards associated with wastes and cuts the budget used for purchasing various medications for the associated diseases.

Global energy reserves are continuously depleting due to high population growth as well as economic development (Zheng et al. 2012b). Currently, biodiesel is considered among the more appropriate alternatives, but the limiting factor for its use is the fact that it is often very expensive to produce (Zheng et al. 2012b). Another disadvantage is that biodiesel are often generated from edible oils that are as well consumed as food by human beings (Demirbas et al. 2016). Hence the use of BSF could be a meaningful alternative, among others, since their reproductive capacity is high, they have a short life cycle and hold the potential to aid in waste management (Li et al. 2011).

BSFL are very rich in proteins, calcium, lipids, as well as polysaccharides, giving them the potential to be utilized as feedstock and for biodiesel generation (Popa and Green 2012). The BSF prepupae contain about 30% and 40% of fat and protein,

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respectively (Jonathan Cammack and Jeffery Tomberlin 2017). This high protein content renders BSF favorable for use as feedstock for animals and as a protein source in pet and fish industries because its amino acid composition is similar to that of fish meals (Elwert et al. 2010; Shumo et al. 2019b). Also, the maggots contain crude fiber, extracts of free nitrogen, calcium, ash, and phosphorous (Barry 2004; Diener et al. 2011). The excretions from the maggots can be used as an alternative to peat moss. In fact, unlike peat moss, BSF prepupal excretions and casing can be used to replace swine, poultry, and daily feeds.

BSF prepupae are also used as feed in aquaculture (Bondari and Sheppard 1981; St-Hilaire et al. 2007b; Kroeckel et al. 2012; Stamer 2015). The aquaculture sector faces challenges of limited protein feeds as the overexploitation of marine fish stocks lead to a steady decrease in fish meal availability, coupled with a matching increase in their prices (Steinfeld et al. 2006; Worm 2016; FAO 2018). Since fish meal is not only supplied to the aquaculture sector, but also to various animal husbandry sectors like poultry and pigs, there is a need to look for other alternatives to supplement them.

### **1.10 Potential drawback of BSF**

Although there is sufficient evidence on the successful use of BSFL, there is so far no unequivocal evidence on the applicability of this technology on a large scale (Fléchet 2008). Sheppard et al. (1994) used a modified caged layer house with a concrete basin fitted beneath the cage batteries in order to allow easy harvesting and counting of the migrating prepupae though they could not regulate the feeding rates. In another study decomposition of feces from 12 pigs was achieved with the aid of an automated system that is composed of a dewatering belt that transported the feces to BSFL treatment beds, leading to a 56% feces reduction rate (Newton et al. 2005). Studies from Guinea and Indonesia provide good examples within the developing world by their leading efforts in palm kernel meal bioconversion into BSFL used as fish feed (Fléchet 2008; Hem et al. 2008). Notably, the above cited studies employed homogenous agricultural wastes as feedstock, suggesting that the use of

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inhomogeneous feeding sources, which is the focus of this research, remains poorly studied.

### **1.11 Problem statement**

Anthropogenic activities such as urbanization, industrial and agricultural activities continue to generate huge amounts of organic waste products which present a serious threat to environmental and human health (Giusti 2009). While various management methods to manage wastes like landfills have been used over decades, the approach has changed due to the very high amounts generated, the reduction of the number of those dumping sites and the associated environmental hazards (Giusti 2009). The use of incinerators is expensive and also cause air pollution (Jilani et al. 2007). Utilization of organic wastes as fertilizer is not only an attractive strategy but also cost effective since they provide plants with nutrients and supply the soil with organic matter for crop production (Smith 1995; Jilani et al. 2007). However, direct raw organic wastes application is not recommended for land use since they can harbor pathogens, toxic compounds, weed seeds and heavy metals, and cause foul odors (Kara and Asan 2007). Waste composting provides the most appropriate approach for addressing such constraints for use of organic solid wastes in agriculture (Goldstein 1980; Wolkowski 2003).

Various studies have focused on waste management implementation and their accomplishments (Lohri et al. 2016; Ouda et al. 2016; Sadeh et al. 2016; Sawadogo et al. 2018; Hettiarachchi et al. 2018). Others have explored differences in performance, development as well as outcomes and obstacles of waste governance in different countries (Zapata Campos and Hall 2013; Taherzadeh and Rajendran 2014). Some studies have demonstrated that BSFL may be used to manage and reuse waste in various systems such as in large-scale confined animal feeding practices and bioconversion of food waste (Sheppard et al. 2002; Barry 2004; Fléchet 2008; Diener et al. 2011). However, there are limited data on bioconversion waste management methods and the associated outcomes on the environment. The focus of the current study is to explore the role of biological friendly management methods of several organic waste streams using BSF in bioconversion and feed production in Kenya.

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### **1.12 Objectives**

- To analyze the nutritive value of BSFL reared on different organic waste streams in terms of crude protein, ether extracts, ash, acid detergent fiber, neutral detergent fiber, amino acids, fatty acids, vitamins, flavonoids, minerals and aflatoxins, while establishing if and how the type of organic waste stream influences the nutritive value of BSFL.
- To determine the effect of temperature on the development and survivorship of BSFL reared on two different organic waste streams, while establishing if and how the temperature, coupled with the type of rearing waste stream, influences the development and survivorship of BSF.
- To survey the bacterial community associated with BSFL reared on two different organic waste streams using morphological and molecular identification through the sequencing of the 16S rDNA gene, while establishing if and how the type of organic waste stream influences the gut microflora composition of the BSF.

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## 2 THE NUTRITIVE VALUE OF BLACK SOLDIER FLY LARVAE REARED ON COMMON ORGANIC WASTE STREAMS IN KENYA

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### 2.1 Abstract

In Africa, livestock production currently accounts for about 30% of the gross value of agricultural production. However, production is struggling to keep up with the demands of expanding human populations, the rise in urbanization and the associated shifts in diet habits. High costs of feed prevent the livestock sector from thriving and to meet the rising demand.

Insects have been identified as potential alternatives to the conventionally used protein sources in livestock feed due to their rich nutrients content and the fact that they can be reared on organic side streams. Substrates derived from organic by-products are suitable for industrial large-scale production of insect meal. Thus, a holistic comparison of the nutritive value of Black Soldier Fly larvae (BSFL) reared on three different organic substrates, i.e. chicken manure (CM), brewers' spent grain (SG) and kitchen waste (KW), was conducted. BSFL samples reared on every substrate were collected for chemical analysis after the feeding process. Five hundred (500) neonatal BSFL were placed in 23 x 15cm metallic trays on the respective substrates for a period of 3-4 weeks at  $28\pm 2^{\circ}\text{C}$  and  $65\pm 5\%$  relative humidity. The larvae were harvested when the prepupal stage was reached using a 5mm mesh size sieve. A sample of 200 grams

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prepupae was taken from each replicate and pooled for every substrate and then frozen at -20°C for chemical analysis. Samples of BSFL and substrates were analyzed for dry matter (DM), crude protein (CP), ether extracts (EE), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids (AA), fatty acids (FA), vitamins, flavonoids, minerals and aflatoxins. The data were then subjected to analysis of variance (ANOVA) using general linear model procedure. BSFL differed in terms of nutrient composition depending on the organic substrates they were reared on. CP, EE, minerals, amino acids, ADF and NDF but not vitamins were affected by the different rearing substrates. BSFL fed on different substrates exhibited different accumulation patterns of minerals, with CM resulting in the largest turnover of minerals. Low concentrations of heavy metals (cadmium and lead) were detected in the BSFL, but no traces of aflatoxins were found. In conclusion, it is possible to take advantage of the readily available organic waste streams in Kenya to produce nutrient-rich BSFL-derived feed.

## **2.2 Introduction**

The global food demand is expected to increase by 70% by the year 2050 in order to meet the demands of the 9.7 billion people who are forecasted to inhabit the globe by that time (Tilman et al. 2011). In the recent past already major shifts in diets have happened, favoring more animal-based foods, in particular milk, meat, fish and eggs, and these preferences are expected to increase with time (Makkar et al. 2014). These changes in dietarian pattern have been accelerated by economic growth, coupled with rapid migration from rural to urban areas, as well as an increasing awareness in nutritional needs. By the middle of the current century, cereal and meat production are expected to increase from 2.1 billion and 258 million tons produced per annum between 2005 and 2007 to 3.0 billion and 455 million tons, respectively, raising worldwide concerns regarding the status of food security (Alexandratos and Bruinsma 2012).

In the developing world, the livestock sector can act as a gateway towards alleviating poverty and enhancing food security (Thornton 2010; Otte et al. 2012).

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Kenyan poultry farming is a significant source of income, especially in rural areas, and contributes to more than quarter of the agricultural Gross Domestic Product (GDP) and accounts for 8% of the total GDP in Kenya (Omiti and Okuthe 2008). Yet feed costs make up more than 70% of the production costs (Craig and Helfrich 2009; Akinrotimi et al. 2011; Munguti et al. 2014), highlighting the important role economic feeds and their availability could play in successful poultry farming. Due to food-feed competition, feed constituents that are suitable for direct human consumption such as soybean and fish are expensive and collectively increase the costs of feeds (Munguti et al. 2014). In addition, global catches from the marine fish stocks have dwindled over the years due to overexploitation (Worm 2016). This increases the price of fishmeal, which is not only used in feeding livestock but rather is a major source of protein in farmed fish feed (Shepherd and Jackson 2013; FAO 2018). Moreover, the intensification of soybean production, especially in the tropics, resulted in land grabbing and deforestation in addition to other negative social and environmental consequences (Foley et al. 2011). For the reasons mentioned above, there is an urgent need to replace conventional feed ingredients such as soybean and fish with innovative, economically beneficial and environmentally sustainable ones (Tschirner and Kloas 2017).

Large-scale rearing of insects is a promising and innovative alternative as several insects' species can feed of various types of organic waste streams (Van Huis and Tomberlin 2017). In addition, insects are precious reservoirs of proteins, fatty acids, micronutrients and contain high amounts of energy (Rumpold and Schlüter 2013; Finke and Oonincx 2014; Nowak et al. 2016). The latter show a good profile of amino acids in general, and of the most-limiting essential ones like lysine, threonine and methionine, often lacking in plant-based protein sources for non-ruminants (Van Huis 2015).

The Black Soldier Fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae), the common house fly *Musca domestica* L. (Diptera: Muscidae) and the yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) are among the insect species that have been recognized as promising alternative sources of protein for animal feed (Hale

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1973; Stamer 2015; Hopley 2016). The first two naturally occur in animal droppings but also flourish on other organic waste substrates such as coffee bean pulp, vegetables residues, catering waste, municipal organic waste, straw, dried distillers grains with solubles (DDGS), and fish offal (Van Huis and Tomberlin 2017), and can add value by reducing organic waste biomass by 50-60% and turning them into high protein biomass (Sheppard et al. 1994). The yellow mealworm can be reared on vegetables and DDGS (Ramos-Elorduy et al. 2002; van Broekhoven et al. 2015). The dry weight of Black Soldier Fly larvae (BSFL) contain up to 50 % crude protein (CP), up to 35% lipids and have an amino acid profile that is similar to that of fishmeal (Elwert et al. 2010). They are recognized and utilized as alternative sources of protein for feed of poultry, pigs, and several species of fish and shrimp (van Huis 2018).

The adult fly can typically live for one to two weeks without the need to feed as it appears it can rely on fat body reserve that was acquired during larval stages and can even live longer when fed with water (Tomberlin et al. 2002). It does not carry diseases, and actively feeding BSFL secrete an info-chemical that keeps away other species of flies, thereby repelling potential insect pests and disease vectors such as *M. domestica* (Erickson et al. 2004). The same authors also reported that BSFL significantly influence the reduction of *Escherichia coli* and *Salmonella enterica* presence in cow dung while Liu and colleagues (Liu et al. 2017) reported the same influence on *Escherichia coli* in chicken manure. The economic feasibility of the use of insects as feed largely depends on cost effective and readily available organic waste streams, both in the developed and in particular in the developing world. So far, very few studies assessed the holistic nutritional contents of BSFL in terms of quality using experimental diets that were established within the means of the developing world. Unlike rationed diets, organic waste streams in the developing world are heterogeneous in nutritional composition and might be an environment where heavy metals can accumulate. Therefore, the current study sought to perform a comparative holistic analysis of the quality of the nutritional composition of BSFL reared on organic waste streams that are largely and readily available in urban areas of Kenya and the developing world in general. A comparative study that is essential when deciding



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which organic waste streams are potentially suitable for industrial large-scale BSFL production in Kenya.

## **2.3 Materials and Methods**

The study was carried in the laboratories of the International Centre for Insect Physiology and Ecology (*icipe*), in Nairobi, Kenya.

### **2.3.1 Stock colony**

*icipe* insectarium maintains a population of BSF adults which act as a stock colony. The population of the stock colony was originally collected from the surroundings of *icipe* in Nairobi and was maintained in the insectarium for 1 year before use in this study. Adult BSF are housed in an outdoors metal framed cage with screen mesh (1.8 x 1.8 x 1.8m with 1.5mm mesh) with strong access to daylight spectra and temperatures maintained at 28±5 °C to encourage mating to occur. The flies are supplied with water to prolong their life. Corrugated cardboard and some SG are placed within the cage to attract adult females for egg laying.

### **2.3.2 Preparation of substrate and larvae feeding**

The tested substrates, i.e. chicken manure (CM), kitchen waste (KW) and brewers' spent grain (SG), were all sourced locally. CM was collected from a broiler poultry farm in the greater Nairobi area and used one week after it was harvested. Though the use of manure in feeding farmed animals including insects is prohibited in the European Union (EU), feeding farmed insects with any type of substrate is not an issue as long as the end product is free of heavy metals, microbial and mycotoxins contaminants (Byrne 2017; IPIFF 2019). KW was a mixture of potato peeling, carrot remaining and peelings, rice and bread debris (contained no meat) collected from a local restaurant in Nairobi. SG was sourced from Tusker House, Kenya Breweries Ltd. in Nairobi after fermentation of barley in the beer production process. The substrates were chosen on the basis of their availability in Nairobi, with a view of their potential future use for large scale industrial BSFL production in Kenya and beyond in Africa.

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Metallic trays measuring 23 x 15cm were used to contain 1kg of the different substrates. Each substrate had six replications placed randomly in a wooden frame in a room. Five hundred (500) neonatal BSFL were placed carefully on top of the substrate in each of the trays. During the rearing process, the temperature was maintained at  $28\pm 2$  °C and relative humidity (r.h.) at  $65\pm 5\%$ . Distilled water was sprinkled on the substrate to ensure 65-70% moisture content. All substrates were replenished weekly with fresh ones. After reaching the prepupal stage, the insects were harvested, washed with tap water, oven dried at 60 °C for 48 hours, crushed using a laboratory blender and then stored for subsequent analyses in a refrigerator at -20 °C. A sample of the BSFL was collected from each replication, pooled and a 200g sample was used for the subsequent analyses.

### **2.3.3 Chemicals**

Aflatoxin B1, B2 G1 and G2, were purchased from Supelco (Bellefonte, Pennsylvania, USA), apigenin (>99%), luteolin (>97%), rutin hydrate (>94%), quercetin dehydrate (>98%), octadecanoic acid (>98.5%), glutamic acid, myristoleic acid, tetradecanoic acid, hexadecanoic acid, (Z)-9-hexadecenoic acid, linoleic acid, (Z)-9-octadecenoic acid, octadecanoic acid, (Z,Z)-9,12-Octadecadienoic acid, oleic acid, eicosanoic acid and amino acid standard solution (AAS 18) were purchased from Sigma-Aldrich (Chemie GmbH Munich, Germany).

### **2.3.4 Proximate analysis**

Prior to the proximate analysis and after reaching the prepupal stage, the insects were harvested, washed with tap water, oven dried at 60 °C for 48 hours, crushed using a laboratory blender and then stored for subsequent analyses in a refrigerator at -20 °C. Then, the BSFL samples were taken for proximate analysis using standard methods of the Association of Official Analytical Chemist (AOAC 1990). Dry matter was calculated by the weight difference between before and after drying the sample in the oven at 135 °C for two hours; ash was determined by heating the samples in a muffle furnace set at 600 °C for three hours while CP was determined

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using the Kjeldahl method and a nitrogen-to-protein conversion factor of 4.76 was used in the calculation of CP (Janssen et al. 2017). Diethyl ether was used as an extractant in the determination of crude fat using the Velp solvent extractor (SER 148/6). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed with a Velp fiber analyzer (FIWE 6) (VELP Scientifica, Usmate Velate, Italy) using reagents described by Van Soest and colleagues (van Soest et al. 1991). All the analyses were done in duplicates.

### **2.3.5 Amino acids**

The method for protein extraction was adopted from Hamilton and colleagues (Hamilton et al. 2012) and detailed in Musundire and colleagues (Musundire et al. 2016) Briefly, the BSFL samples were separately snap-frozen in liquid nitrogen and crushed into fine powder. The samples (2g each) were extracted for 1 hour in ice cold 5 v/w 100mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.2, 2mM dithiothreitol (DTT), 2.5% Polyvinylpyrrolidone (PVP), 0.5mM Ethylenediaminetetraacetic acid (EDTA), 1mM benzamide 0.1mM phenylmethanesulfonylfluoride (PMSF) in a magnetic stirrer. The samples were filtered through KERLIX™ Gauze Bandage Rolls Sterile Soft Pouch 5.7 × 2.7cm centrifuged at 8,000rpm for 30 min at 4°C to remove solid debris. Protein was precipitated between 45% and 80% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and the pellet recovered by centrifugation at 21,000rpm for 30 min at 4°C. The protein pellets were desalted in 20mM HEPES–NaOH pH 8 containing 2mMDTT using Sephadex G-25 gel filtration chromatography (PD-10 columns, GE Healthcare, Chicago, USA) to give 80.2mg and 77.9mg of proteins from processed and unprocessed insect samples, respectively. Ten (10) mg from each of the samples were separately transferred into a 5ml micro-reaction vial into which 2ml of 6N HCl were added and closed after careful introduction of nitrogen gas. The samples were hydrolyzed for 24 hours at 110°C. For tryptophan analysis, 10mg from each of the samples were separately transferred into a 5ml micro-reaction vial into which 2ml of 6N NaOH were added and then capped after careful introduction of nitrogen gas. The samples were hydrolyzed for 24 hours at 110°C. After the hydrolysis, the mixtures

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were evaporated to dryness under vacuum. The hydrolysates were reconstituted in 1ml 90:10 water: acetonitrile, vortexed for 30 s, sonicated for 30 min, and then centrifuged at 14,000rpm and the supernatant analyzed using a liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Qtof-MS) (Waters Corporation, Milford, USA). The analysis was replicated three times.

### **2.3.6 Mineral analysis**

After drying the prepupae and pre-experiment substrates, they were crushed using a laboratory blender and then ashed and digested in 6N HCl. An Atomic Absorption Spectrophotometer (Model AA6300, Shimadu, Japan) was used to analyze the following minerals: phosphorus, potassium, calcium, magnesium, sodium, iron, copper, manganese, cobalt and zinc.

### **2.3.7 Analysis of mycotoxins**

Samples of BSFL were analyzed for mycotoxins according to methods described by Cheng and Cappozzo (Cheng and Cappozzo 2008) and Musundire and colleagues (Musundire et al. 2016). Ten (10) g of every sample were snap frozen in liquid nitrogen, crushed into fine powder and extracted in 40mL acetonitrile-water (86:16, v/v) for 30 min while sonicating. Each mixture was allowed to settle for 30 min and then 6mL of each sample was filtered through a solid phase extraction cartridge Multisep® 228 AflaPat multifunctional columns (Roer Labs, USA). An aliquot (4ml) of each cleaned extract was evaporated to dryness in a stream of nitrogen gas. The dried samples were re-dissolved in 400µL methanol-water (20:80, v/v), vortexed for 1 min and then centrifuged at 14,000rpm for 5 min prior to analysis using a Waters liquid chromatography coupled to quadruple time of flight mass spectrometry (LC-QtoF-MS) (Waters Corporation, Milford, USA). Samples derived from different substrates were analyzed in five replicates.

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### 2.3.8 Analysis of fatty acids

BSFL samples reared on the three different substrates were snap frozen in liquid nitrogen and then ground into a fine powder and analyzed for fatty acids following the procedures described by Musundire and colleagues (Musundire et al. 2016). Briefly, a methyl esterification reaction was performed on 5g of each sample according to procedures outlined by Christie (Christie 1993). A solution of sodium methoxide in methanol was prepared to generate a concentration of 15mg/ml. An aliquot of the solution (500 $\mu$ L) was added to each ground insect sample, vortexed for 1 min and then sonicated for 5 min. The reaction mixture was incubated at 60 $^{\circ}$ C for 1 hour, thereafter, quenched by adding 100 $\mu$ L deionized water followed by vortexing for another 1 min. The resulting methyl esters were extracted using GC-grade hexane (Sigma-Aldrich, St. Louis, USA) and then centrifuged at 14,000rpm for 5 min. The supernatant was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and then analyzed using gas chromatography coupled to mass spectrometry (GC/MS) (Agilent Technologies, CA, USA). Fatty acids were identified as their methyl esters by comparison of GC retention times and fragmentation patterns with those authentic standards and reference spectra published by library-MS databases National Institute of Standards and Technology (NIST) 05, 08 and 11. Serial dilutions of the authentic standard octadecanoic acid (0.2-125ng/ $\mu$ g) were analyzed by GC/MS in full scan mode to generate a linear calibration curve (peak areas vs. concentration) with the following equation [ $y=7E+06x - 4E+07(R^2 = 0.9757)$ ], which was used for the external quantification of the different fatty acids.

### 2.3.9 Analyses of flavonoids

BSFL samples reared on the three different substrates were analyzed for flavonoids following the procedures described by Musundire and colleagues (Musundire et al. 2016) The samples were separately crushed into fine powder in liquid nitrogen. Two-and-a-half (2.5) g of each sample were independently extracted in 50mL methanol-water (80:20 v/v) by ultra-sonication in a sonication bath (Branson 2510, Danbury, USA) for 1 hour followed by filtration through a Whatman filter paper

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No. 32. The remaining residue was re-extracted twice, and the filtrate pooled separately. The extracting solvent was removed under reduced pressure at 40°C using a rotary evaporator (Laborata 4000, Heidolph Instrument, Germany) to give 60, 80, 100 and 120mg for KW, CM and SG, respectively. The extracts (5mg) from each of the samples were re-dissolved in 1mL water-methanol (95:5 v/v), centrifuged at 14,000rpm for 5 min and the supernatant analyzed using LC-Qtof-MS (Waters Corporation, Milford, USA). Five replicates were carried out with each replicate done on a different larvae sample.

### 2.3.10 Instruments' conditions

The instrument conditions were similar to those described by Musundire and colleagues (Musundire et al. 2016). LC-Qtof-MS and GC-MS instruments were used in the analysis. The instrument conditions included:

**LC-Qtof-MS.** The chromatographic separation was achieved on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) I-class system (Waters Corporation, Milford, USA). For amino acids analysis, the UPLC was fitted with an ACE C18 column (250mmx4.6 mm, 5 $\mu$  (Aberdeen, UK) with a heater turned off and an autosampler tray cooled to 5°C. Mobile phases of water (A) and acetonitrile (B), each containing 0.01% formic acid, was employed. The following gradients were used: 0 min, 5% B; 0-3 min, 5-30% B; 3-6 min, 30% B; 6-7.5 min, 30-80% B; 7.5-10.5 min, 80% B; 10.5-13.0 min, 80-100% B, 13-18 min, 100% B; 18-20 min, 100-5% B; and 20-22 min, 5% B. The flow rate was held constant at 0.7ml min<sup>-1</sup>. The injection volume was 1  $\mu$ L.

For analyses of flavonoids and aflatoxin, a UPLC was fitted to a Waters ACQUITY UPLC BEH C18 column (2.1mm x 50mm, 1.7 $\mu$ m particle size; Waters Corporation, Milford, USA) heated to 40 °C and an auto sampler tray cooled to 15 °C. Mobile phases of water (A) and methanol (B), each with 0.01% formic acid, were employed. The following gradients were used for i) flavonoids: 0-0.2 min, 10% B; 0.2-3 min, 10-60% B; 3-5 min, 60-80% B; 6-8 min, 80% B; 8-9 min, 100% B; 9-10 min, 100% B; 10-10.5 min 100-10% B; and 10.5-12 min 10% B; ii) aflatoxin: 0-0.2 min, 10% B; 0.2-3

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min, 10-90% B; 3-5 min, 90% B; 5-6 min, 90-10% B; and 6-7 min, 10%B. The flow rate was held constant at 0.4ml min<sup>-1</sup> for both analyses.

The UPLC system was interfaced with electrospray ionization (ESI) to a Waters Xevo Qtof-MS operated in full scan based on independent information acquisition (MS<sup>E</sup>) in positive mode. Data were acquired in resolution mode over the *m/z* range 100-1,200 for flavonoids: *m/z* 100-700 for amino acid analysis with a scan time of 1 s using a capillary voltage of 0.5kV, sampling cone voltage of 40V, source temperature 100 °C and desolvation temperature of 350 °C. The nitrogen desolvation flow rate was 500L/h. For the high-energy scan function, a collision energy ramp of 25-45eV was applied in the T-wave collision cell using ultrahigh purity argon (>99.999%) as the collision gas. A continuous lock spray reference compound (leucine enkephalin; [M+H]<sup>+</sup> = 556.2766) was sampled at 10 s intervals for centroid data mass correction. The MS was calibrated across the 50-1,200Da mass using a 0.5mM sodium formate solution prepared in 90: 10 2-propanol/water (v/v).

MassLynx version 4.1 SCN 712 (Waters Corporation, Milford, USA) was used for data acquisition and processing. The elemental composition was generated for every analyte. Potential assignments were calculated using mono-isotopic masses with a tolerance of 10ppm deviation and both odd- and even-electron states possible. The number and types of expected atoms was set as follows: carbon < 100; hydrogen < 100; oxygen < 50; nitrogen < 6; sulphur < 6 (Jared et al. 2016). The empirical formula generated was used to predict structures which were proposed based on the online database, fragmentation pattern, literature and confirmed using authentic standards.

Serial dilutions of authentic standards of aflatoxin B<sub>1</sub> (0.01–20 ng/μl), rutin, quercetin, luteolin, apigenin (1.8–181 ng/μl), and glutamic acid (0.01–10 ng/μl) were also analyzed by LC-Qtof-MS in MS<sup>E</sup> mode to generate linear calibration curves (peak area vs. concentration) with the following linear equations: aflatoxin B<sub>1</sub> [ $y = 13738x + 6611.5$  (R<sup>2</sup>= 0.9571)], rutin [ $y = 5578.4x - 39094$  (R<sup>2</sup>= 0.9960)], quercetin [ $y = 4372.4x + 79607$  (R<sup>2</sup>= 0.9854)], luteolin [ $y = 13433x - 23256$  (R<sup>2</sup>= 0.9994)] apigenin [ $y = 10288x - 11117$  (R<sup>2</sup>= 0.9995)] and glutamic acid [ $y = 40137x - 1353.1$  (R<sup>2</sup>= 0.9999)]

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which served as the basis for the external quantification of the aflatoxin, flavonoids and amino acids.

**GC/MS.** Fatty acid methyl esters (FAMES) were analyzed by GC/MS on a 7890A gas chromatograph (Agilent Technologies Inc., Santa Clara, USA) linked to a 5975C mass selective detector (Agilent Technologies Inc., Santa Clara, USA) by using the following conditions: inlet temperature 270 °C, transfer line temperature of 280 °C, and column oven temperature programmed from 35 to 285 °C with the initial temperature maintained for 5 min then 10 °C min<sup>-1</sup> to 280 °C, and held at this temperature for 20.4 min. The GC was fitted with a HP-5 MS low bleed capillary column (30m x 0.25mm i.d., 0.25 µm) (J & W, Folsom, USA). Helium at a flow rate of 1.25ml min<sup>-1</sup> served as the carrier gas. The mass selective detector was maintained at ion source temperature of 230 °C and quadrupole temperature of 180 °C. Electron impact (EI) mass spectra were obtained at the acceleration energy of 70eV. A 1.0µl aliquot of sample was injected in the splitless mode using an auto sampler 7683 (Agilent Technologies Inc., Beijing, China). Fragment ions were analyzed over 40-550m/z mass range in the full scan mode. The filament delay time was set at 3.3 min.

### **2.3.11 Statistical analysis**

Statistical Analysis System (SAS, version 9.1) was used for analyses. Collected data was subjected to Levene-test to test for normality, followed by one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure to test for significant differences among the means. In case of significant F-values, Bon-Tukey was used to separate the means at p<0.05.

## **2.4 Results**

### **2.4.1 Aflatoxins and Proximate Composition Analysis**

LC-Qtof-MS analysis for mycotoxins did not identify any traces of aflatoxin in the BSFL. There was an effect of substrate in all the proximate nutritional parameters (Table 1). The DM content of SG substrate was lower in comparison to the two other



rearing substrates (DF = 2; F = 23.23; R<sup>2</sup> = 0.939; p = 0.0149) (Table 1). The ash content of CM was higher while the KW and SG ash contents were comparable (DF = 2; F = 233.89; R<sup>2</sup> = 0.851; p = 0.0005). Organic matter was lower in KW while the values obtained from CM and SG were comparable (DF = 2; F = 115.42; R<sup>2</sup> = 0.987; p = 0.0015). Crude protein content was higher in KW in comparison to CM and SG (DF = 2; F = 169.8; R<sup>2</sup> = 0.991; p = 0.0008). Moreover, the values of NDF (DF = 2; F = 87.89; R<sup>2</sup> = 0.983; p = 0.0022) and ADF (DF = 2; F = 36.87; R<sup>2</sup> = 0.983; p = 0.0077) contents in SG were higher in comparison to the two other rearing substrates. The EE contents (DF = 2; F = 45.51; R<sup>2</sup> = 0.968; p = 0.0057) of the three substrates were rather low (2.7-7.2% DM) with CM the lowest.

**Table 2.1 Means (± standard error) of proximate composition (in % dry matter) of three common organic waste streams in Kenya.**

Parameters	CM	KW	SG	p-values
DM	93.3 <sup>a*</sup> ± 0.2	92.7 <sup>a</sup> ± 0.1	84.6 <sup>b</sup> ± 0.5	0.0149
Ash	20.2 <sup>a</sup> ± 0.3	7.2 <sup>b</sup> ± 0.3	6.2 <sup>b</sup> ± 0.8	0.0005
OM	86.6 <sup>a</sup> ± 0.8	80.4 <sup>b</sup> ± 0.4	92.0 <sup>a</sup> ± 0.1	0.0015
CP	15.3 <sup>a</sup> ± 0.0	20.0 <sup>b</sup> ± 0.5	12.2 <sup>a</sup> ± 0.2	0.0008
NDF	35.5 <sup>a</sup> ± 0.8	38.9 <sup>a</sup> ± 0.2	49.9 <sup>b</sup> ± 1.2	0.0022
ADF	18.3 <sup>a</sup> ± 0.9	25.2 <sup>a</sup> ± 1.3	38.6 <sup>b</sup> ± 2.5	0.0077
EE	2.7 <sup>a</sup> ± 0.6	7.2 <sup>b</sup> ± 0.3	7.2 <sup>b</sup> ± 0.2	0.0057

\*Means (n=2) in the same row followed with different superscripts are significantly different at p<0.05; DM, Dry Matter; OM, Organic Matter; CP, Crude Protein; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; EE, Ether Extract; CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain

There was an effect of substrate in all the proximate nutritional parameters of the BSFL except for ash (Table 2). The DM (DF = 2; F = 7.70; R<sup>2</sup> = 0.507; p = 0.0050) content of the BSFL was higher in KW fed ones. Crude protein (DF= 2; F= 38.48; R<sup>2</sup> = 0.838; p <0.0001) was high in the CM and SG fed larvae and lowest in the KW fed ones, though larvae fed on the latter substrate showed higher crude fat (DF = 2; F = 27.65; R<sup>2</sup> = 0.787; p <0.0001) content than the others. Organic matter (DF = 2; F = 306.09; R<sup>2</sup> =

0.976;  $p < 0.0001$ ) was lower in CM fed larvae as compared to larvae fed on the two other substrates. Both NDF (DF = 2;  $F = 32.91$ ;  $R^2 = 0.814$ ;  $p < 0.0001$ ) and ADF (DF = 2;  $F = 6.33$ ;  $R^2 = 0.456$ ;  $p = 0.0101$ ) contents were higher in SG fed BSF.

**Table 2.2 Means ( $\pm$  standard error) of proximate composition (in % dry matter) of Black Soldier Fly larvae reared on three different rearing substrates.**

Parameters	CM fed BSFL	KW fed BSFL	SG fed BSFL	p-values
DM	80.7 <sup>a*</sup> $\pm$ 1.2	87.7 <sup>b</sup> $\pm$ 1.0	83.1 <sup>a</sup> $\pm$ 1.6	0.0050
Ash	9.3 $\pm$ 1.8	9.6 $\pm$ 1.6	11.6 $\pm$ 0.5	0.4818
OM	59.8 <sup>a</sup> $\pm$ 0.4	90.4 <sup>b</sup> $\pm$ 1.6	88.4 <sup>b</sup> $\pm$ 0.5	<0.0001
CP	41.1 <sup>a</sup> $\pm$ 0.3	33.0 <sup>b</sup> $\pm$ 1.0	41.3 <sup>a</sup> $\pm$ 0.5	<0.0001
NDF	21.9 <sup>a</sup> $\pm$ 0.6	20.4 <sup>a</sup> $\pm$ 0.6	28.6 <sup>b</sup> $\pm$ 1.0	<0.0001
ADF	12.6 <sup>a</sup> $\pm$ 0.3	13.2 <sup>a</sup> $\pm$ 0.1	15.0 <sup>b</sup> $\pm$ 0.8	0.0101
EE	30.1 <sup>a</sup> $\pm$ 0.4	34.3 <sup>b</sup> $\pm$ 0.4	31 <sup>a</sup> $\pm$ 0.4	<0.0001

\*Means (n=2) in the same row followed with different superscripts are significantly different at  $p < 0.05$ ; DM, Dry Matter; OM, Organic Matter; CP, Crude Protein; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; EE, Ether Extract; CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain; BSFL, Black Soldier Fly larvae

There was a high correlation between DM content of the rearing substrates and those of the BSFL for CM (DF = 1;  $F = 34.34$ ;  $R^2 = 0.851$ ;  $p = 0.0011$ ) and KW (DF = 1;  $F = 8.14$ ;  $R^2 = 0.576$ ;  $p = 0.0290$ ). However, there was no high correlation between DM content of SG and that of the BSFL ( $p = 0.6391$ ). Moreover, there was a high correlation between OM content of the rearing substrates and those of the BSFL for CM (DF = 1;  $F = 1140.79$ ;  $R^2 = 0.994768$ ;  $p < 0.0001$ ), KW (DF = 1;  $F = 11.99$ ;  $R^2 = 0.666481$ ;  $p = 0.0134$ ;  $p = 0.0134$ ) and SG (DF = 1;  $F = 842.98$ ;  $R^2 = 0.992$ ;  $p < 0.0001$ ). Moreover, there was a high correlation between the ash content of CM and SG and that of the BSFL (DF = 1;  $F = 10.66$ ;  $R^2 = 0.640$ ;  $p = 0.0171$  for CM) (DF = 1;  $F = 23.93$ ;  $R^2 = 0.800$ ;  $p = 0.0027$  for SG) while there was no high correlation between the ash content KW and that of the BSFL ( $p = 0.4196$ ). There was a high correlation between CP content of two of the three rearing substrates and those of the BSFL ( $R^2 = 0.983$ ;  $p$

<0.0001 for CM,  $R^2 = 0.899$ ;  $p = 0.0003$  for KW and  $R^2 = 0.993$ ;  $p < 0.0001$  for SG). Moreover, a high correlation was observed between the EE contents of substrates and those of the BSFL (DF = 1;  $F = 1149.06$ ;  $R^2 = 0.995$ ;  $p < 0.0001$  for CM; DF = 1;  $F = 1413.99$ ;  $R^2 = 0.996$ ;  $p < 0.0001$  for KW; and DF = 1;  $F = 842.98$ ;  $R^2 = 0.992$ ;  $p < 0.0001$  for SG). There was a high correlation between the NDF content of the rearing substrates and those of the BSFL (DF = 1;  $F = 132.95$ ;  $R^2 = 0.956819$ ;  $p < 0.0001$  for CM; DF = 1;  $F = 32.85$ ;  $R^2 = 0.845562$ ;  $p = 0.0012$  for KW; DF = 1;  $F = 516.05$ ;  $R^2 = 0.988507$ ;  $p < 0.0001$  for SG). Likewise, there was a high correlation between the ADF contents of the rearing substrates and those of the BSFL for CM (DF = 1;  $F = 75.40$ ;  $R^2 = 0.926294$ ;  $p = 0.0001$ ), KW (DF = 1;  $F = 332.94$ ;  $R^2 = 0.982298$ ;  $p < 0.0001$ ) and SG (DF = 1;  $F = 159.60$ ;  $R^2 = 0.963769$ ;  $p < 0.0001$  for SG).

#### 2.4.2 Mineral Composition Analysis

The three rearing substrates exhibited different accumulation patterns of minerals (Table 3). Five major minerals, i.e. required in amounts greater >100 mg/day, were detected. Of those, phosphorus (DF = 2;  $F = 82.18$ ;  $R^2 = 0.982$ ;  $p = 0.0024$ ), magnesium (DF = 2;  $F = 63.38$ ;  $R^2 = 0.934$ ;  $p < 0.0001$ ) and sodium (DF = 2;  $F = 237.67$ ;  $R^2 = 0.981$ ;  $p < 0.0001$ ) significantly differed among the tested BSFL whereas potassium and calcium did not.

**Table 2.3 Means ( $\pm$  standard error) of mineral composition (g/kg DM) of three common organic waste streams in Kenya.**

Parameters	CM fed BSFL	KW fed BSFL	SG fed BSFL	p-values
Phosphorus	1.0 <sup>a*</sup> $\pm$ 2.74	2.0 <sup>a</sup> $\pm$ 0.58	4.8 <sup>b</sup> $\pm$ 1.37	0.0024
Potassium	1.7 $\pm$ 5.07	3.6 $\pm$ 1.01	1.3 $\pm$ 0.36	0.0688
Calcium	1.94 $\pm$ 1.90	1.93 $\pm$ 0.42	3.5 $\pm$ 0.62	0.0528
Magnesium	1.0 <sup>a</sup> $\pm$ 0.23	1.3 <sup>a</sup> $\pm$ 0.14	2.2 <sup>b</sup> $\pm$ 0.01	<0.0001
Sodium	3.3 <sup>a</sup> $\pm$ 0.05	1.3 <sup>b</sup> $\pm$ 0.14	0.8 <sup>c</sup> $\pm$ 1.71	<0.0001
Iron	2.1 $\pm$ 1.43	0.9 $\pm$ 0.15	0.4 $\pm$ 0.05	0.3662
Copper	0.6 $\pm$ 0.20	0.6 $\pm$ 0.19	0.9 $\pm$ 0.31	0.5311
Manganese	0.3 $\pm$ 0.09	0.2 $\pm$ 0.07	0.3 $\pm$ 0.09	0.5980

<b>Cobalt</b>	2.5 ± 0.76	1.1 ± 0.29	3.2 ± 1.00	0.1601
<b>Zinc</b>	0.9 ± 2.93	0.5 ± 1.45	1.0 ± 3.32	0.2131

\*Means (n=2) in the same row followed with different superscripts are significantly different at p<0.05; CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain; BSFL, Black Soldier Fly larvae

BSFL fed on the different substrates exhibited different accumulation patterns of minerals (Table 4). Five major minerals, i.e. required in amounts greater >100 mg/day, were detected. Of those, potassium (DF = 2; F =13.41; R<sup>2</sup> = 0.641; p = 0.0005), calcium (DF = 2; F = 68.58; R<sup>2</sup> = 0.901; p <0.0001) and magnesium (DF = 2; F = 3.65; R<sup>2</sup> = 0.328; p = 0.051) significantly differed among the tested BSFL whereas sodium did not.

**Table 2.4 Means (± standard error) of mineral composition (g/kg DM) of Black Soldier Fly larvae reared on three different rearing substrates.**

<b>Parameters</b>	<b>CM fed BSFL</b>	<b>KW fed BSFL</b>	<b>SG fed BSFL</b>	<b>p-values</b>
<b>Phosphorus</b>	3.9 ± 0.31	4.1 ± 0.33	4.6 ± 0.56	0.3446
<b>Potassium</b>	4.9 <sup>a*</sup> ± 0.08	5.7 <sup>b</sup> ± 0.04	4.4 <sup>a</sup> ± 0.01	0.0005
<b>Calcium</b>	3.2 <sup>a</sup> ± 2.32	2. <sup>b</sup> ± 1.41	1.7 <sup>c</sup> ± 0.51	<0.0001
<b>Magnesium</b>	4.0 <sup>a</sup> ± 0.34	3.3 <sup>b</sup> ± 0.06	3.5 <sup>ab</sup> ± 0.09	0.0510
<b>Sodium</b>	2.4 ± 0.12	2.0 ± 0.09	2.6 ± 0.07	0.1371
<b>Iron</b>	0.6 <sup>a</sup> ± 0.43	2.2 <sup>b</sup> ± 0.00	0.3 <sup>a</sup> ± 0.00	0.0045
<b>Copper</b>	0.4 <sup>a</sup> ± 0.00	0.2 <sup>a</sup> ± 0.00	0.5 <sup>b</sup> ± 0.00	0.0006
<b>Manganese</b>	1.4 <sup>a</sup> ± 0.01	0.9 <sup>b</sup> ± 0.01	1.1 <sup>a</sup> ± 0.01	0.0050
<b>Cobalt</b>	4.6 <sup>a</sup> ± 0.01	2.6 <sup>b</sup> ± 0.01	6.5 <sup>c</sup> ± 0.02	<0.0001
<b>Zinc</b>	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.02	0.1831
<b>Ca: P (ratio)</b>	8.3	5.2	3.7	

\*Means (n=2) in the same row followed with different superscripts are significantly different at p<0.05; CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain; BSFL, Black Soldier Fly larvae

There was no significant correlation between phosphorus concentrations of the rearing substrates and those of the BSFL (p = 0.0354 for CM; p = 0.1591 for KW; and p = 0.8562). Moreover, there was no significant correlation between potassium concentration of KW rearing substrate and that of the BSFL (p =0.0153). However, there

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was a high correlation between potassium concentrations of CM and SG rearing substrates and those of the BSFL (DF = 1; F =74.15; R<sup>2</sup> =0.902618; p <0.0001 for CM and DF = 1; F =84.50; R<sup>2</sup> =0.913509; p <0.0001 for SG). For calcium concentrations, there was a high correlation between the concentrations in two of the three rearing substrates tested i.e. CM and SG and those of the BSFL (DF = 1; F =45.77; R<sup>2</sup> =0.851213; p =0.0001 for CM, p =0.1453 for KW and DF = 1; F =13.07; R<sup>2</sup> =0.620257; p =0.0068 for SG). Similarly, there was a high correlation between sodium concentrations in the three rearing substrates tested (DF = 1; F = 22.72; R<sup>2</sup> = 0.739595; p = 0.0014 for CM, DF = 1; F = 27.13; R<sup>2</sup> = 0.772271; p = 0.0008 for KW and DF = 1; F = 22.00; R<sup>2</sup> = 0.733312; p = 0.0015 for SG). Moreover, there was a high correlation between magnesium concentrations of the three rearing substrates tested and those of the BSFL (DF = 1; F = 74.66; R<sup>2</sup> = 0.903213; p <0.0001 for CM, DF = 1; F = 262.88; R<sup>2</sup> = 0.970466; p <0.0001 for KW DF = 1; F = 762.03; R<sup>2</sup> = 0.989611; p <0.0001 for SG).

#### **2.4.3 Amino Acids Composition Analysis**

Both limiting and non-limiting amino acids were detected in the BSFL, with the choice of substrate significantly affecting their concentrations (Table 5). Yet, in general and for the most limiting amino acids like lysine, methionine, isoleucine and tyrosine no significant substrate effect was found. Tryptophan was not detected in the analysis, as it might have been destroyed during the acid hydrolysis process. However, no significant substrate effect was detected in the BSFL for the most important amino acids in animal nutrition, especially for non-ruminants, i.e. methionine (p = 0.2891), lysine (p = 0.9296), isoleucine (p = 0.7181) and leucine (p = 0.342). KW reared larvae showed significantly higher levels of the non-essential amino acids' proline (DF = 2; F = 59.69; R<sup>2</sup> = 0.888; p <0.0001), hydro-proline (DF = 2; F = 4.48; R<sup>2</sup> = 0.374; p = 0.0298) and tyrosine (DF = 2; F = 11.27; R<sup>2</sup> = 0.600; p = 0.001) compared to the larvae fed on the other tested substrates. Glutamine was detected in larvae reared on KW. Glutamic acid (DF = 1; F = 28.97; R<sup>2</sup> = 0.743; p = 0.0003) was only detected in CM fed BSFL, while KW fed BSFL in most cases showed the highest concentrations of the different amino acids especially in the Phenylalanine (DF = 2; F = 20.07; R<sup>2</sup> = 0.727; p <0.0001) content, followed by SG and CM fed BSFL (Table 3).

Table 2.5 Means ( $\pm$  standard error) of amino acids concentration (mg/g) in Black Soldier Fly larvae reared on three different rearing substrates.

Parameter	CM fed BSFL	KW fed BSFL	SG fed BSFL	p-values
Histidine†	3.5 $\pm$ 0.3	3.3 $\pm$ 1.6	4.7 $\pm$ 0.5	0.6138
Arginine†	1.1 <sup>b*</sup> $\pm$ 1.8	5.0 <sup>c</sup> $\pm$ 4.3	2.5 <sup>a</sup> $\pm$ 2.2	<0.0001
Lysine†	4.1 $\pm$ 0.6	4.7 $\pm$ 0.5	4.7 $\pm$ 1.6	0.9296
Glutamine	0	8.1 $\pm$ 0.8	0	0.0229
Glutamic acid	0	6.1 <sup>b</sup> $\pm$ 0.5	3 <sup>a</sup> $\pm$ 0.2	0.0003
Proline	1.5 <sup>a</sup> $\pm$ 1.8	5.1 <sup>b</sup> $\pm$ 3.5	2.4 <sup>a</sup> $\pm$ 1.6	<0.0001
Valine†	7.2 $\pm$ 0.8	1.2 $\pm$ 2.2	9.3 $\pm$ 0.8	0.0729
Methionine†	6.1 $\pm$ 0.8	7.9 $\pm$ 0.8	7.4 $\pm$ 0.8	0.2891
Tyrosine	2.2 <sup>a</sup> $\pm$ 3.4	4.6 <sup>b</sup> $\pm$ 3.8	3.0 <sup>a</sup> $\pm$ 3.5	0.0010
Isoleucine†	1.6 $\pm$ 1.5	2.6 $\pm$ 4.5	1.8 $\pm$ 1.4	0.7181
Leucine†	3.0 $\pm$ 5.2	2.9 $\pm$ 5.2	3.7 $\pm$ 4.8	0.3420
Hydro-proline	7.7 <sup>a</sup> $\pm$ 4.7	2.5 <sup>b</sup> $\pm$ 4.5	8.7 <sup>a</sup> $\pm$ 2.5	0.0298
Phenylalanine†	1.9 <sup>a</sup> $\pm$ 2.4	4.6 <sup>b</sup> $\pm$ 4.7	2.4 <sup>a</sup> $\pm$ 1.7	<0.0001

\*Means (n=2) in the same row followed with different superscripts are significantly different at  $p < 0.05$ ; CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain; BSF, Black Soldier Fly; † indicates essential amino acids; BSFL, Black Soldier Fly larvae

#### 2.4.4 Flavonoid Composition Analysis

A total of five flavonoids were detected, with two of them showing a significant substrate effect in their concentrations (Table 6). For both apigenin (DF = 2; F = 6.78;  $R^2 = 0.492$ ;  $p = 0.0087$ ) and kaempferol (DF = 2; F = 5.40;  $R^2 = 0.454$ ;  $p = 0.0196$ ) lower concentrations were recorded in KW fed BSFL as compared to the other substrates.

Table 2.6 Means ( $\pm$  standard error) of concentration of flavonoids (mg/g) in Black Soldier Fly larvae reared on three different rearing substrates.

Substrate	CM fed BSFL	KW fed BSFL	SG fed BSFL	p-values
Luteolin	8.2 $\pm$ 0.7	9.1 $\pm$ 3.6	9.5 $\pm$ 7.3	0.8752
Apegenin	8.3 <sup>a*</sup> $\pm$ 1.1	3.7 <sup>b</sup> $\pm$ 6.2	8.2 <sup>a</sup> $\pm$ 1.1	0.0087
Quercetin	4.6 $\pm$ 1.0	7.9 $\pm$ 3.9	5 $\pm$ 8.2	0.142
Rutin	8.1 $\pm$ 2.8	4.1 $\pm$ 0.7	5.1 $\pm$ 2.7	0.4538
Kaempferol	24.7 <sup>a</sup> $\pm$ 4.6	4.2 <sup>b</sup> $\pm$ 0.6	17.4 <sup>a</sup> $\pm$ 6.6	0.0196

\*Means (n=2) in the same row followed with different superscripts are significantly different at  $p < 0.05$ ; CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain; BSFL, Black Soldier Fly larvae

#### 2.4.5 Vitamins Composition Analysis

Table 2.7 Means ( $\pm$  standard error) of concentrations ( $\mu\text{g/g}$ ) of vitamins detected in Black Soldier Fly larvae reared on three different rearing substrates.

Parameter	Retention time (min)	CM fed BSFL	KW fed BSFL	SG fed BSFL	p-value
Gamma tocopherol	34.3	2.3 $\pm$ 0.2	2.0 $\pm$ 0.2	1.7 $\pm$ 0.1	0.3767
Alpha tocopherol	34.8	7.3 $\pm$ 1.3	9.9 $\pm$ 0.7	17.6 $\pm$ 2.5	0.0713
Provitamin D3	35.3	1.3 $\pm$ 0.1	1.5 $\pm$ 0.1	1.6 $\pm$ 0.1	0.1399

CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain; BSFL, Black Soldier Fly larvae

Three vitamins were detected though the substrate had no significant effects on their concentrations, i.e. Gamma tocopherol ( $p = 0.3767$ ), Alpha tocopherol ( $p = 0.0713$ ) and Provitamin D3 ( $p = 0.1399$ ) (Table 7).

## 2.4.6 Fatty Acids Composition Analysis

Table 2.8 Means ( $\pm$  standard error) of fatty acids concentrations ( $\mu\text{g/g}$ ) in Black Soldier Fly larvae reared on three different rearing substrates.

Parameter	Retention Time(min)	CM fed BSFL	KW fed BSFL	SG fed BSFL	p-values
Lauric acid	19.3	7.1 <sup>a*</sup> $\pm$ 1.0	7.4 <sup>a</sup> $\pm$ 9.0	11.0 <sup>b</sup> $\pm$ 1.1	0.0098
Myristic acid	19.6	6.8 $\pm$ 0.2	6.9 $\pm$ 11.1	7 $\pm$ 0.3	0.872
Palmitic acid	19.8	6.5 $\pm$ 0.2	0	6.1 $\pm$ 0.3	0.3448
Stearic acid	26.1	6.9 <sup>a</sup> $\pm$ 0.1	5.6 <sup>a</sup> $\pm$ 0.4	9.6 <sup>b</sup> $\pm$ 1.6	0.001
Arachidic acid	26.9	6.9 <sup>a</sup> $\pm$ 0.4	6.1 <sup>a</sup> $\pm$ 0.5	8.5 <sup>b</sup> $\pm$ 0.6	0.0061
Palmitoleic acid	23.05	4.4 $\pm$ 5.6	4.2 $\pm$ 3.8	5.1 $\pm$ 3.9	0.3641
Oleic acid	25.0	8.0 $\pm$ 0.2	7.2 $\pm$ 0.1	8 $\pm$ 11.9	0.3348
Linoleic acid	25.3	5.8 $\pm$ 0.3	7.5 $\pm$ 0.1	0	0.0766
g-Linolenic acid	25.0	5.6 <sup>a</sup> $\pm$ 0.1	5.5 <sup>ab</sup> $\pm$ 0.5	7.4 <sup>b</sup> $\pm$ 0.4	0.0009
Arachidonic acid	26.4	6.7 <sup>a</sup> $\pm$ 0.3	5.7 <sup>b</sup> $\pm$ 0.1	0 $\pm$ 0.1	0.0018
Eicosapentaenoic acid	26.4	5.6 <sup>a</sup> $\pm$ 0.3	5.9 <sup>a</sup> $\pm$ 0.1	6.8 <sup>b</sup> $\pm$ 0.1	0.0106

\* Means (n=2) in the same row followed with different superscripts are significantly different at  $p < 0.05$ ; CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain; BSFL, Black Soldier Fly larvae

In total, eleven fatty acids were detected in BSFL (Table 8). Except for linoleic and arachidonic acid, concentrations of fatty acids in SG fed BSFL were always higher, and in some cases even significantly higher than in BSFL fed on the two other substrates (Table 8).

## 2.5 Discussion

With the rapid rise in urban populations in Kenya and elsewhere in Africa, the problem of waste management increases. Organic waste accounts for more than 78 % of the entire solid waste stream in developing countries (Bhada-Tata and Hoornweg 2012), waste in Africa and beyond in the Global South is often dumped in landfills without prior separation of organic waste streams leading to the loss of valuable



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organic resources that could otherwise be reclaimed (Njoroge et al.). Centralized waste management systems that are widely applied in the developed world require sophisticated infrastructures and are often economically beyond the means of most developing countries. Therefore, there is a need to establish alternative waste management systems that can valorize and recycle organic waste streams yet within the economic means of the developing world. In addition, rapid population growth in Africa and Asia, coupled with urbanization and changes in consumer preferences lead to an increasing demand for food, particularly in terms of animal protein (Thornton 2010). Because of the ecological and economic shortfalls of common protein sources like fish and soy meal in most animal feed (Tidwell and Allan 2001), sustainable and yet nutritionally promising alternative sources of protein are urgently needed to ascertain food security today and in the future.

In this study, we measured the potential of recycling three readily available organic waste streams in Nairobi, Kenya, and arguably also in other megacities in the developing world, and their influence on the nutritional quality of BSFL as a proposed alternative protein source for livestock feed. As the quality of livestock feed is mainly measured in the concentrations of CP present in the feed's dry matter, we conducted a proximate analysis applying the standard methods described by the Association of Official Analytical Chemist (AOAC 1990) using a nitrogen-to-protein conversion factor of 4.76 (Janssen et al. 2017) and obtained significantly high values of CP, surpassing those of soybean meal and other commonly used plant proteins in livestock feeds such as canola, cottonseed and sunflower meal (Willis 2003). The CP values we obtained in our study ranged between 33 and 41 % which is slightly lower than the range of values (39 to 43%) reported by Spranghers and colleagues (Spranghers et al. 2017) for BSFL reared on various organic waste streams. Moreover, Nguyen and colleagues (Nguyen et al. 2015) reported a CP value of 39 % for BSFL reared on fruits and vegetables waste while Sheppard and colleagues reported 42% CP for BSFL reared on chicken manure. However, the previously mentioned CP values were obtained using the Kjeldahl standard nitrogen-to-protein conversion factor of 6.25 while the values we reported were obtained using the conversion factor of 4.76 (Sheppard et al. 1994). The total

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nitrogen content in insects in general and BSF in specific contain nitrogen originating from both protein and non-protein sources such as chitin. Hence, separating non protein from protein nitrogen was necessary in order to obtain accurate crude protein content and to avoid over estimated values that were previously reported using the standard conversion factor (Janssen et al. 2017; Caligiani et al. 2018). When calculated using the standard nitrogen-to-protein conversion factor of 6.25, the range of CP values we obtained from this study (39 to 54 %) surpassed the range of values previously reported in literature ( Nguyen et al. 2015; Spranghers et al. 2017). Our results were closer to that obtained by Caligiani and colleagues (Caligiani et al. 2018) and Janssen and colleagues (Janssen et al. 2017) who both used a nitrogen-to-protein conversion factor of 4.76. Similarly, the EE content we found in BSFL was higher than those reported from full fat soybean meal (Willis 2003) and commercially available fishmeal (Moghaddam et al. 2007). Newton and colleagues and St-Hilaire and colleagues reported even higher EE values in BSFL than we found in our study (Newton et al. 2005; St-Hilaire et al. 2007). Previous studies reported an influence of the rearing substrate on the EE content. For instance, Nguyen and colleagues (Nguyen et al. 2015) reported higher EE content for larvae reared on fish and liver in comparison to chicken feed. However, there was no apparent influence of the rearing substrates on the EE content of BSFL in our study.

In addition to CP, other nutritional components of livestock feed can greatly enhance the quality of animal production, including several minerals and vitamins including calcium, magnesium, phosphorus, copper, cobalt and vitamins A or D. For example, calcium and phosphorous play important roles in x physiological functions of animals including muscle mass reductions, neuro-signaling, enzymatic activity, metabolic reactions, construction of proteins, maintenance of osmotic and acidic-alkaline equilibria, construction of membranes etc. (Crenshaw 2001; Ewing and Charlton 2007). When referring to bone and egg formation in layer hens, calcium in particular plays a crucial role as it contributes to more than 90% of the mineral matrix in the bones (Hafeez et al. 2015). Therefore, deficiencies in calcium and phosphorus can result in bone loss, growth retardation and abnormal posture (Hafeez et al. 2015).

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The range of calcium concentrations reported in this study (1.7 to 3.2 %) was lower than that reported in literature (2.4 to 5.8 %) (Newton et al. 2005; Dierenfeld and King 2008; Finke 2013). Previous studies already established an influence of the rearing substrate on the mineral content of BSFL. Moreover, Newton and colleagues (Newton et al. 2005) reported higher calcium concentration for BSFL reared on poultry manure in comparison to the concentration we obtained from BSFL reared on a similar substrate. Such a variability could be further justified by the fact that the outer layer of the larvae's skin releases a deposit of calcium carbonate ( $\text{CaCO}_3$ ) which may lead to the high calcium and ash content (Newton et al. 1977; Barragan-Fonseca et al. 2017). However, while the BSFL calcium levels we detected varied among the three organic waste streams tested, phosphorus levels remained unaffected in our study. Then again, Newton and colleagues (Newton et al. 2005) reported higher levels of phosphorus in BSFL reared on poultry manure than those reared on swine manure. This variability could also be attributed to the influence of the rearing substrate on the mineral content of BSFL.

Animal feed needs to contain sufficient quantities of vitamins to facilitate the development of proper and healthy body functions (Weber and Windisch 2017). Vitamins contribute essentially to the development of the immune system in animals while also helping in the digestion of other nutrients for energy production (Weber and Windisch 2017). We did not observe any influence of the rearing substrates on vitamins in the BSFL. Though gamma and alpha tocopherol and provitamin D3 were detected in all BSFL samples in our study, Finke and colleagues (Finke 2013) and St-Hillarie and colleagues reported relatively higher levels of alpha tocopherol and provitamin D3. In addition, they found more vitamins including biotin, folic acid, vitamin A, and niacin in their studies with BSFL (St-Hilaire et al. 2007).

Amino acids are essential for quality livestock production, especially their ability to break down other proteins and to produce energy (Henchion et al. 2017). Though the amino acid profile of soymeal is generally of a better quality than that of other plant-based feeds, it is still deficient in lysine, methionine, threonine and valine when compared to an animal based protein source (Henchion et al. 2017). Previous

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studies established that the amino acid profile of several edible insects including the yellow mealworm, common housefly, and BSF is comparable to that of soybean meal with methionine or methionine and cysteine and sometimes arginine as the most limiting essential amino acids for growing swine and broilers (Veldkamp and Bosch 2015). We could show that BSFL reared on the three tested organic waste streams had a higher quality amino acid profile than the FAO (FAO 2002) standard amino acid profiles reported for soybean and sunflower meal. The BSFL methionine levels in our study even surpassed that of fishmeal reported by FAO (FAO 2002) and corresponded with the recommended range for broiler chickens as per the standards of the National Research Council (NRC) (National Research Council 1994). Similarly, the range of methionine levels (6.4 to 7.9%) in BSFL detected in our study surpassed those of methionine (0.7 to 0.9%) of BSFL reared on various organic waste streams and previously reported in literature (Barragan-Fonseca et al. 2017). Moreover, we detected higher lysine levels in BSFL than commonly found in soybean and sunflower meals (Makkar et al. 2014) and ours were only slightly lower than in fish meal (Makkar et al. 2014). Moreover, lysine levels detected in our study (4.1%) were higher than the levels previously detected by Arango Gutiérrez and colleagues (Arango Gutiérrez et al. 2004), viz. 2.1% for BSFL reared on chicken manure. Thus, rearing BSFL on the three tested organic waste streams resulted in terms of amino acids profile in a high quality protein source that would be in this respect nearly at par with fish meal and clearly surpass many plant-based protein sources in livestock feed.

FAs are usually subdivided into either saturated fatty acids (SFA) or unsaturated fatty acids (USFA). High levels of SFAs in diets such as palmitic and myristic acid are not favorable because they raise the level of low density lipoproteins (LDLs) by suppressing the expression of LDL receptors (Sacks and Willett 1991; Medicine 2006). USFAs are important for human growth, skin protection and can decrease the possibility of thrombosis formation (Asif 2015). Insect fat is abundant in USFAs and resembles chicken and fish in their level of unsaturation (DeFoliart 1992); yet, FAs profiles considerably vary among different insect species (Akullo et al. 2017). Such variability may be associated with environmental conditions such as the insect's age,

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sex or size and its digestion and enzymatic activities (Oranut et al. 2010). We obtained higher USFA in comparison to SFA contents confirming the high quality of the BSFL FA profile. Like Makkar and colleagues (Makkar et al. 2014) we observed that the FA profile is influenced by the rearing substrate, with SG in general resulting in higher FA levels than the other two tested organic waste stream materials. BSFL FA profiles were predominantly composed of lauric, oleic and stearic acids. This corresponds to previous studies done by Leong and colleagues (Leong et al. 2015) and Zheng and colleagues (Zheng et al. 2012b) though their values of lauric acid were relatively high compared to our results. The total amount of USFA in BSFL is close to those in olive, or soya oils (Berezina 2017). In particular, the concentration of lauric acid in BSFL-derived oil is considerably higher than in those derived from soybean, sunflower, and oil palm (Masson et al. 2015). Lauric acid is known to react against lipid enveloped viruses, and many pathogenic bacteria and protozoa (Lieberman et al. 2006; Ramos et al. 2009; Fortuoso et al. 2019).

Flavonoids occur naturally in vegetables, fruits and medicinal plants. Flavonoids occur naturally in vegetables, fruits and medicinal plants. They perform significant biological actions by acting as antioxidants, anti-carcinogens, anti-allergens, anti-pathogens and growth promoters in different animal species (Saeed et al. 2017). Studies on insects like edible stink bugs confirmed the presence of alkaloids, flavonoids and steroids among other bioactive compounds (Musundire et al. 2014; Cheseto et al. 2015). Flavonoid rich feed are sometimes associated with pharmacological effects (Kelemu et al. 2015). In our BSFL we found rutin, aepiginin and luteolin, belonging to the flavone class, usually associated with fruit skins, red wine, buckwheat, red pepper and tomato skin (Hara et al. 1995; Kreft et al. 1999), and kaemferol and quercetin belonging to the flavonol class that are more prevalent in onion, red wine, olive oil, berries and grapefruits (Stewart et al. 2000). The presence of these flavonoids in the insects can probably be traced back to the different rearing substrates and were acquired through feeding.

Contrary to previous studies which showed that insects are prone to accumulate toxins or heavy metals ingested through contaminated feed or water

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(e.g.(Marone 2016)) we did not detect any aflatoxins or other mycotoxins in our BSFL. Mycotoxins are metabolic products of fungi with harmful poisoning effects to animals and humans. Monogastrics like pigs and poultry are highly susceptible to such contaminated feed (Tola and Kebede 2016). Thus, to further promote insect-derived feeds, routine and rigorous testing schemes for toxins and heavy metals, both in the rearing substrates as well in the harvested BSFL, have to be implemented. Purschke and colleagues (Purschke et al. 2017) reported that BSFL accumulated heavy metals but not mycotoxins when fed with contaminated substrates, though their concentrations in BSFL tissues -apart from Cd and Pb- remained below the initial substrate concentrations. Therefore, in order to ensure feed and food safety from farm to fork, screening of both the substrates and the BSFL-derived feeds as for contaminants such as Cd and Pb is a necessity. In addition to toxins, microbiological contamination might be a matter of concern when it comes to the utilization of insects in feedstock production. Indeed, insects are often affiliated to various microbes such as bacteria, fungi and viruses which are part of their defense repertoire (Belluco et al. 2013; Rumpold and Schlüter 2013). However, BSF have shown an extreme resistance to various environmental conditions through their ability to reduce and possibly suppress possible bacterial and fungal contaminations aided by chemical and biological agents they produce (Jeon et al. 2011; Choi et al. 2012). It is suggested that contact with wild insects and other sources of contamination should be avoided in order for properly maintained insect mass rearing facilities to remain free from pathogenic hazards (Belluco et al. 2013). Moreover, the possibility of insects to harbor parasites can be limited and contained through introducing and maintaining environmental conditions that are unfavorable to parasitic existence (Belluco et al. 2013). Therefore and on the basis of our current knowledge, biological risks associated with insect consumption can be minimized using simple hygienic measures such as appropriate processing methods, i.e. heating or freezing similar to measures that are applied during processing of poultry, pork and fish (Belluco et al. 2013).

Comparing the nutritional composition and quality of BSFL we found in our study with previous research indicates considerable variability. This might be due to

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differences in methodological set-up, environmental conditions, rearing substrates, time of harvest, as well as harvesting and processing methods (Newton et al. 2005; St-Hilaire et al. 2007a; Diener et al. 2009; Kroeckel et al. 2012; Finke and Oonincx 2014; Van Huis and Tomberlin 2017; Liu et al. 2017). Yet we found the differences between our results and those by Newton and colleagues (Newton et al. 2005) and St-Hilaire and colleagues (St-Hilaire et al. 2007) puzzling, considering that these authors tested similar organic waste streams and processed and harvested the BSFL more or less the same as we did. Hence, we hypothesize that genetic heterogeneity of BSFs is an additional factor that needs to be considered and should thus be looked at.

## **2.6 Conclusion**

We could show that commonly available organic waste streams in urban environments of the developing world can be successfully used to produce high quality BSFL that have the potential to substitute other animal- or plant-derived protein sources in commercial livestock feed. Wide-scale application of this approach would greatly reduce the ecological and economic footprint of feed, thereby contributing to more sustainable animal husbandry systems. Moreover, it can provide valuable ecosystem services through the bioconversion of municipal and organic waste streams into bio compost. To achieve this, the next step must be the development of appropriate and cost-effective BSF mass-rearing technologies. Following the footsteps of Kenya and Uganda where dried insect products were recently approved for use in all animal and fish feed (Byrne 2017), a regional African insect feed policy aiming to ensure safety of production within adequate hygiene standards should be introduced.

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### **3 INFLUENCE OF TEMPERATURE ON SELECTED LIFE-HISTORY TRAITS OF BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) REARED ON TWO COMMON URBAN ORGANIC WASTE STREAMS IN KENYA**

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#### **3.1.1 Abstract**

In sub-Saharan Africa, urban populations are projected to increase by 115% in the coming 15 years. In addition, economic growth and dietary shifts towards animal source foods have put high pressure and demand on agricultural production. The high ecological footprint of meat and dairy production, as well as high feed costs, prevent the livestock sector from meeting the increasing demand in a sustainable manner. Insects such as the black soldier fly (BSF) have been identified as potential alternatives to the conventionally used protein sources in livestock feed due to their rich nutrient content and the fact that they can be reared on organic side streams. Substrates derived from organic byproducts are suitable for industrial large-scale production of insect meal. Although efficient in waste management and in feed production, BSF larvae are very sensitive to the external environment such as temperature and rearing medium. Therefore, we studied the effect of temperature and substrate type, i.e., brewers' spent grain (SG) and cow dung (CD), on the development and survival of BSF larvae. Both organic substrates were readily available in Nairobi, Kenya, the location of the experiments. In our experiment, 100 3–5-day-old BSF larvae were placed into containers that contained either SG or CD and further treated at temperatures of 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C. The duration of larval development was recorded, and the prepupae were removed, weighed, and placed individually in separate, labeled, 35-mL plastic cups filled with moist sawdust. After emergence, 10 2-day-old adults (5 males and 5 females) from every replica per substrate were transferred into a cage (40 × 40 × 40 cm) and allowed to mate for 24 h at their respective temperatures. The laid egg batches were collected and counted, and the adult flies' longevity was recorded. The data were subjected to a two-way analysis of variance (ANOVA) using



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the general linear model procedure. BSF larvae reared on SG developed faster than those reared on CD; the former also favored higher temperatures for their larval development and emergence into adults. The optimum range was 25–30 °C. With increasing temperatures, the longevity of adult BSF decreased, while the fecundity of females increased. Thus, it is possible to take advantage of the readily available SG waste streams in the urban environments of Kenya to produce BSF larvae-derived livestock feed within a short duration of time and at relatively high temperatures.

### **3.1.2 Keywords**

Organic waste bioconversion; black soldier fly (BSF); rearing temperature; development; growth; longevity; fecundity

### **3.2 Introduction**

In 2014, 54% of the world population resided in urban areas, while in 1950 this was only 30%; by 2050 two thirds of the world population will live in urban areas (UN 2014). In particular, urban populations in sub-Saharan Africa (SSA) are projected to increase by 115% from today's 170 to 360 million in the coming 15 years (UN 2014). This adds up to estimates that the global food supply will need to increase by 60% in order to meet the demand of the 10 billion people expected to live by the year 2050 (FAO 2009). The rapid urbanization and growing human population are coupled with continued economic growth in addition to shifts in dietary preferences, favoring more animal source foods (ASFs) (Crosson and Anderson 1994; van der Zijpp 1999; Delgado 2003; Ndambi et al. 2007). Therefore, it is not surprising that both the production and consumption of ASFs in the developing world is forecasted to increase sharply (FAO 2009). However, this increase represents a major challenge due to the high ecological footprint associated with the production of meat and dairy products (Steinfeld et al. 2006; de Vries and de Boer 2010; Alexandratos and Bruinsma 2012; Mekonnen and Hoekstra 2012; Gerber et al. 2013). In addition, the level of productivity of many agricultural systems in the developing world is still quite low in terms of the efficiency of land and water resources use (Bruinsma 2003). On the other hand, the level of

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malnutrition associated with insufficient protein consumption in developing countries is still very high (Ayele and Peacock 2003; Neumann et al. 2003; Randolph et al. 2007; Narrod et al. 2012; FAO et al. 2015). Moreover, costs of livestock production such as poultry farming in the developing world are increasing mainly because of the high feed costs, now more than 70% of the production costs (Craig and Helfrich 2009; Akinrotimi et al. 2011; Munguti et al. 2014). The use of food ingredients in livestock feed production that are also directly consumed by humans, such as fish and soybean, create a food-feed competition leading to further increases in ingredient costs and consequently to high feed costs (Munguti et al. 2014). Moreover, the massive expansion of soybean cultivation has put pressure on land availability, especially in the tropics, often leading to deforestation and other negative effects for the environment (Foley et al. 2011). Therefore, access to affordable feed is significant for more profitable and affordable poultry production.

The current combination of inefficient production and unsustainable consumption patterns points to the need to adopt cost effective production systems where alternative protein sources for animal feed with lower ecological footprints are used in order to achieve a more sustainable agricultural production and improved food security while safeguarding the already fragile ecosystems and natural resources in the developing world (Poppy et al. 2014; Tschirner and Kloas 2017). Mass-produced insects emerge as some of the promising alternatives as some species can be reared on various types of organic waste including poultry, pig and cattle manure as well as on coffee bean pulp, vegetable residues, catering waste, municipal organic waste, straw, dried distillers' grains with solubles (DDGS) and fish offal (Nguyen et al. 2015; Leong et al. 2016; Van Huis and Tomberlin 2017; Meneguz et al. 2018). Among the insect species identified as alternative ingredients for animal feed are the black soldier fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae), the common house fly *Musca domestica* L. (Diptera: Muscidae) and the yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Rumpold and Schlüter 2013; Makkar et al. 2014; Stamer 2015; Hopley 2016). In addition, insects contain high amounts of energy, fatty acids, micronutrients and especially proteins (Sheppard et al. 1994; Finke and Oonincx 2014;

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Nowak et al. 2016). For instance, BSF larvae which have been used as an accepted feed ingredient for poultry, pigs and a number of fish and shrimp species contain about 35-49% crude protein (CP), 29-35% fat and have an amino acid pattern comparable to fishmeal (Renna et al. 2017; Spranghers et al. 2018; Dabbou et al. 2018; Gasco et al. 2019).

Insects are known to inhabit a wide variety of environments, including extreme ones, due to their adaptive behavioral and physiological mechanisms (Dixon et al. 2009). However, these tolerance mechanisms are not well understood (Dixon et al. 2009). Moreover, insects along with other ectotherms depend largely on ambient temperatures to regulate their metabolism and development rates (Jarošík et al. 2004). Forecast modelling suggests that due to climate change, insects inhabiting more temperature versatile geographic regions will survive elevated temperatures while those inhabiting regions where little temperature variances occur will experience a decline in their populations as global warming proceeds (Addo-Bediako et al. 2000; Deutsch et al. 2008). BSF, originally traced back to the Americas, is currently known to be found in tropical as well as temperate regions across the globe (Sheppard et al. 1994). Various studies looked into the effects of different diets on laboratory reared BSF as well as the influence of temperature on development and survival of BSF larvae using laboratory prepared diets (Tomberlin et al. 2009). Other studies investigated the influence of organic waste streams as rearing substrates on the development and survival of BSF larvae (Holmes et al. 2013; Oonincx et al. 2015; Nguyen et al. 2015; Tinder et al. 2017). Yet, most of these studies were carried out with the aim of understanding and developing BSF larvae large-scale production systems in the developed world where indoors climate-controlled facilities can be easily established. However, to the best of our knowledge no study so far has investigated the combined influence of urban organic waste streams-based diets and temperature on the development and survival of BSF in developing world context.

Therefore, this study sought to investigate the influence of temperature on selected life-history traits of BSF reared on two different and readily available urban organic waste streams in the urban environment of a large city in SSA. This comparison

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allowed us to determine which of the two organic waste streams performs best along with the accompanying optimum temperatures. Information from this study is important for improving rearing methods in SSA, as well as in creating cost effective and environmentally sustainable alternative livestock feeds that can buffer the impact of climate change especially for small-scale livestock producers who are not connected to international feed markets and local feed producers that neither can afford and implement sophisticated climate-controlled production facilities.

### **3.3 Materials and Methods**

#### **3.3.1 Study location**

The study was carried in the laboratories of the International Centre for Insect Physiology and Ecology (icipe), in Nairobi, Kenya.

#### **3.3.2 Preparation of substrates**

The tested substrates, cow dung (CD) and brewers' spent grain (SG), were both sourced locally. The fresh CD was collected from Farmers Choice slaughterhouse in Kahawa West in Nairobi; the bovines originated from different ranches in Kenya where they had been raised on natural grassland. SG was sourced from Tusker House, Kenya Breweries Ltd. off Thika Road in Nairobi after fermentation of barley in the beer production process. The substrates were chosen based on their availability in Nairobi with a view of their potential future use for large scale industrial BSF larvae production.

#### **3.3.3 Stock colony**

The stock population of BSF populations was maintained at the insectary in icipe. Adult BSF were housed in outdoor metal framed cage with 1.5 mm screen mesh (1.8 x 1.8 x 1.8m) with direct access to daylight to encourage mating. The flies were supplied with water to prolong their life. Corrugated cardboard and some SG are placed within the cage to attract adult females for egg laying. The colony was maintained in the insectary for over 8 generations before use in this experiment.

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### **3.3.4 Experimental setup**

Ten batches of eggs were collected from the stock colony and placed into smaller containers (15 x 9.4cm) containing an oviposition substrate of moist-to-liquefied SG (100 g). Each setup was closely monitored three times a day to ensure egg hatching. After hatching, one hundred 3-5 days larval instars were transferred into different clear plastic 500mL containers with the two test substrates, CD and SG. The experiment was conducted in incubators (MIR-554-PE, Sanyo/Panasonic cooled incubators, Japan) with air humidity of 70% and photoperiod of 12L: 12D. Each substrate was subjected to different temperature treatments of 15°C, 20°C, 25°C, 30°C and 35°C. Each substrate-temperature treatment was replicated five times and was aerated daily to ensure that the substrate is thoroughly turned and well moisturized.

Each treatment was monitored daily and the duration of the larval development was recorded. The prepupae were removed, weighed and placed individually in separate, labelled, 35-ml plastic cups filled with moist sawdust. Each cup with the prepupae was covered with a breathable lid and returned to their respective temperature regimes for daily monitoring, and subsequently recorded for the numbers of puparia formed. Further adult emergence was monitored daily. Upon emergence, ten 2-days old adults (5 males and 5 females) from every replicate per substrate-temperature treatment were transferred into a cage (40 x 40 x 40 cm) and allowed to mate for 24 hours at their respective temperatures. Thereafter, an oviposition device with a small bowl of moist chicken manure and 2-3 cardboards were placed in each cage to provide sites for oviposition. A 10% sugar solution in water was provided daily in a vial through a filter paper inserted into the vial's lid. The laid egg batches were recorded and the numbers of eggs per batch counted under a microscope. The adult flies' longevity was recorded daily until all the caged flies were dead.

### **3.3.5 Statistical analysis**

R Statistics (version R 3.3.3) and Stata (version 15.1) were used for analyses. Collected data was subjected to Levene-test for normality, followed by a two-way

analysis of variance (ANOVA) using the general linear model (GLM) procedure. Where significant differences existed Tukey or LSD HSD post hoc was used to separate the means at the  $p < 0.05$  level.

### 3.4 Results

#### 3.4.1 Development of BSF larvae

Both temperature and substrate type significantly influenced BSF larval development, with SG fed BSF larvae needed significantly less time to reach prepupal stage in temperature treatments tested. Time needed for larval development decreased gradually with increasing temperatures and was the shortest at 30°C for SG fed larvae and 35°C for CD fed larvae (Table 1).

**Table 3.1 Mean values ( $\pm$  SD) of the duration of development (in days) for black soldier fly larvae reared on two different organic substrates at five different temperature regimes**

Temperature (°C)	CD	SG
15	238.8 $\pm$ 0.45 <sup>aA</sup>	206.2 $\pm$ 5.02 <sup>bA</sup>
20	180.4 $\pm$ 32.81 <sup>aA</sup>	96.3 $\pm$ 1.51 <sup>bB</sup>
25	86.8 $\pm$ 2.17 <sup>aB</sup>	32.8 $\pm$ 2.06 <sup>bC</sup>
30	85.2 $\pm$ 3.96 <sup>aB</sup>	24.7 $\pm$ 3.59 <sup>bC</sup>
35	83.4 $\pm$ 4.04 <sup>aB</sup>	29.6 $\pm$ 1.40 <sup>bC</sup>

\*Means (n=5) in the same row followed with different lowercase and in the same column with different uppercase are significantly different at  $p < 0.05$ ; CD, cow dung; SG, spent grain

Prepupal weight was significantly influenced by both temperature and substrate type, with SG fed BSF larvae weighing heavier than those fed with CD. Prepupal weights increased with increasing temperatures, with prepupae reared on CD substrate weighing heaviest at 30°C, while those reared on CG were heaviest when reared at 25 and 30°C (Table 2).

**Table 3.2 Mean values ( $\pm$  SD) of prepupal weight (in grams) for black soldier fly reared on two different organic substrates at five different temperature regimes**

Temperature ( $^{\circ}$ C)	CD	SG
15	0.076 $\pm$ 0.005 <sup>aB</sup>	0.128 $\pm$ 0.004 <sup>bB</sup>
20	0.082 $\pm$ 0.004 <sup>aB</sup>	0.132 $\pm$ 0.010 <sup>bB</sup>
25	0.088 $\pm$ 0.004 <sup>aB</sup>	0.153 $\pm$ 0.013 <sup>bA</sup>
30	0.106 $\pm$ 0.027 <sup>aA</sup>	0.156 $\pm$ 0.011 <sup>bA</sup>
35	0.086 $\pm$ 0.005 <sup>aB</sup>	0.137 $\pm$ 0.013 <sup>bB</sup>

\*Means (n=5) in the same row followed with different lowercase and in the same column with different uppercase are significantly different at  $p < 0.05$ ; CD, cow dung; SG, spent grain

### 3.4.2 Development of BSF pupae

Pupal developmental time differed significantly across different temperatures for BSF pupae previously reared on both substrates. Pupal developmental time decreased gradually with increasing temperatures and was the shortest at 35 $^{\circ}$ C for prepupae reared on both substrates (Table 3). Pupae reared on SG needed significantly less time to emerge as adults than those reared on CD.

**Table 3.3 Mean values ( $\pm$  SD) of the pupal developmental time (in days) for black soldier fly reared on two different organic substrates at five different temperature regimes**

Temperature ( $^{\circ}$ C)	CD	SG
15	182.91 $\pm$ 29.68 <sup>aA</sup>	150.3 $\pm$ 32.26 <sup>bA</sup>
20	131.1 $\pm$ 36.67 <sup>aA</sup>	61.5 $\pm$ 16.24 <sup>bB</sup>
25	54.4 $\pm$ 15.14 <sup>aB</sup>	22.3 $\pm$ 3.81 <sup>bC</sup>
30	51.8 $\pm$ 17.69 <sup>aB</sup>	13.0 $\pm$ 2.11 <sup>bC</sup>
35	48.3 $\pm$ 15.49 <sup>aB</sup>	17.5 $\pm$ 3.69 <sup>bC</sup>

\*Means (n=5) in the same row followed with different lowercase and in the same column with different uppercase are significantly different at  $p < 0.05$ ; CD, cow dung; SG, spent grain

### 3.4.3 3.3. Longevity and fecundity of BSF adults

Longevity of BSF adult flies was significantly influenced by both temperature and substrate type, with BSF adults previously reared as larvae on SG living significantly longer than those previously reared on CD (Table 4). Longevity decreased with increasing temperatures, with all BSF adults irrespective of their larval rearing substrate living the longest at 15 °C.

**Table 3.4 Mean values ( $\pm$  SD) of the longevity (in days) of adult black soldier fly reared on two different organic substrates at five different temperature regimes**

Temperature (°C)	CD	SG
15	13.2 $\pm$ 1.304 <sup>aA</sup>	14.2 $\pm$ 0.447 <sup>bA</sup>
20	9.8 $\pm$ 2.683 <sup>aB</sup>	12.6 $\pm$ 2.503 <sup>bB</sup>
25	9.6 $\pm$ 0.548 <sup>aB</sup>	10.2 $\pm$ 0.616 <sup>bC</sup>
30	8.4 $\pm$ 0.518 <sup>aB</sup>	9.2 $\pm$ 1.373 <sup>bD</sup>
35	7.1 $\pm$ 0.316 <sup>aC</sup>	7.4 $\pm$ 0.699 <sup>bE</sup>

\*Means (n=5) in the same row followed with different lowercase and in the same column with different uppercase are significantly different at  $p < 0.05$ ; CD, cow dung; SG, spent grain

There was a significant effect of both temperature and substrate type on the number of eggs produced for BSF previously reared on both CD and SG (Table 5). BSF adults previously reared on both CD and SG produced more eggs at higher temperatures and most eggs at 30 °C. However, at 35 °C egg production declined. The eggs produced by flies derived from the SG substrate produced more eggs than those derived from the CD substrate.

**Table 3.5 Mean values ( $\pm$  SD) of the number of eggs produced by black soldier flies reared on two different organic substrates at five different temperature regimes**

Temperature (°C)	CD	SG
15	0.0 <sup>aE</sup>	238.8 $\pm$ 50.50 <sup>bD</sup>
20	169.9 $\pm$ 90.75 <sup>aD</sup>	422 $\pm$ 4.24 <sup>bC</sup>
25	472.9 $\pm$ 79.56 <sup>aC</sup>	503.8 $\pm$ 68.028 <sup>bC</sup>
30	916.1 $\pm$ 125.11 <sup>aA</sup>	1,230.4 $\pm$ 242.51 <sup>bA</sup>
35	669.1 $\pm$ 25.26 <sup>aB</sup>	751.8 $\pm$ 114.96 <sup>bB</sup>

\*Means (n=5) in the same row followed with different lowercase and in the same column with different uppercase are significantly different at  $p < 0.05$ ; CD, cow dung; SG, spent grain



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### 3.5 Discussion

Temperature has proven to be a key factor in the development and survival of insects (Chaudhry 1956) Moreover, it is well established that BSF larvae are sensitive to their external environments and that temperatures influence their development and survival (Tomberlin et al. 2009; Park 2016). On the other hand, temperature and nutrition interact to affect key life-history traits in insects such as maturity, development rate, reproduction and survival (Clissold and Simpson 2015). Several studies looked into the influence of either laboratory reared diets at a constant temperature or organic side streams as feeding substrates on life-history traits of BSF larvae (St-Hilaire et al. 2007; Myers et al. 2008; Tomberlin et al. 2009; Kroeckel et al. 2012; Banks et al. 2014) . However, no previous study investigated the combined influence of temperature and urban organic waste material as rearing substrates in a developing world context. Those waste streams, cow dung and spent grain, were readily available in Nairobi, Kenya and arguably also in other megacities in the developing world. We measured the influence of five different temperatures and two organic waste streams on the fitness of BSF larvae as a proposed alternative protein source for livestock feed. We measured the duration of development of immature BSF larvae as well as BSF prepupae weights. We recorded significantly faster durations for BSF larvae and heavier weights for BSF prepupae reared on SG compared to those on CD even at the low temperatures of 15°C and 20°C. Development times of BSF immatures reared on both substrates decreased with increasing temperatures. The weights of BSF prepupae increased with increasing temperatures and were the heaviest at 25 and 30°C.

Several factors may have contributed to the differential development observed between the two rearing substrates. The most contributing factor was the difference in quality of the nutritional content between the rearing substrates. Several studies emphasized the importance of nutritional components such as proteins and carbohydrates in the development of insect larvae (Nijhout 2003; Lee et al. 2004; Simpson et al. 2006). Therefore, we assume that SG better provided the BSF

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immatures with the nutritional resources and energy required to complete their development stage faster. This observation is supported by findings of Harnden and Tomberlin (Harnden and Tomberlin 2016) who recorded faster development for BSF larvae reared on a grain-based diet in comparison to an animal tissue one at 32.2 °C. Meneguze *et al.* (Meneguz *et al.* 2018) also reared BSF larvae on SG but recorded faster durations in comparison to what we report in this study. Yet, on the other hand we noted heavier weights for BSF prepupae reared on SG than Tomberlin *et al.* (Tomberlin *et al.* 2002) in a similar study. The main reasons for these discrepancies are differences in methodologies and experimental set-ups as well as varying temperatures at which the BSF larvae were kept.

Food availability and access to nutritional resources are other crucial factors affecting larval and adult life history traits (Giberson and Rosenberg 1992; Hunter and McNeil 2000). For instance, the weight of BSF prepupae reared on CD in our study were lighter than those recorded by Myers *et al.* (Myers *et al.* 2008) for prepupae reared on the same type of substrate. While, Myers *et al.* (Myers *et al.* 2008) provided the larvae with fresh increments of CD on a daily basis, we opted for a lump sum amount of CD at the start of our experiment. Unlike fresh incremental diets, materials in lump sum diets age with time, leading to reduced amounts of nutritional components such as proteins and carbohydrates that are crucial for the development of insect larvae (Nijhout 2003; Lee *et al.* 2004; Simpson *et al.* 2006). Facing such reductions in nutritional components, larvae refer to compensatory feeding, leading to faster development times and reduced weight gains (Raubenheimer and Simpson 1997; Berner *et al.* 2005). This is also corroborated by Sheppard (Sheppard 1983) who observed an optimal development of BSF reared on fresh CD provided at low increments. The consistency and physical texture of the rearing substrates used in our study may have also affected the results. Although, we did not specifically test the consistency and physical texture of the rearing substrates, it was visually evident that CD was quite thick and therefore may have limited BSF immatures mobility and access to the little amount of nutrients available, consequently affecting their life table traits. Most importantly, the chemical composition of cow dung has been extensively

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summarized by Azevedo and Stout (Azevedo and Stout 1974) and Graber (Graber 1973) with high fiber ratio of about 27% and proportionately lower percentage of protein. A complex set of factors influence the extent to which the fibre will be digested by BSF, including the physical state of cow dung, the level of intake, and the amount of readily fermented nutrients (i.e. carbohydrate and protein) in the ration. Given that cow dung consists of largely non-nutritive elements and the ability of BSF to break down fibre, this might explain the considerable variation observed using the two substrates though subjected at the same temperature. On the other hand, brewer's spent grain has been found to contain several essential nutrients, which are crucial for BSF growth. Couch (Couch 2008) reported a proximate constituent of over 20 % crude protein, about 6 % ether extract, over 15 % crude fibre and 4 % Ash. This is further supported by NRC (National Research Council 1994), that reported that spent grains contains 25.3% crude protein (CP), 6.3% crude fat and around 2080 Kcal/Kg metabolisable energy and is also a good source of B vitamins, thus rendering them a potential substrate in BSF's production. The use of spent grain as BSF diet compared to cow dung might be the reason for the improvement in body weight gain of BSF prepupae which will translate to increased profit margin.

Pupation, a complex process involved with significant morphological and physiological transformations, is essential for holometabolous insects (Telles-Romero et al. 2011). Therefore, we additionally measured the duration of pupation as well as the adult longevity and fecundity as affected by the previously experienced temperature and substrate regimes. Adult emergence took longer at lower temperatures and was significantly shortest at 25 to 35°C and shorter for BSF previously kept on SG than those kept on CD at those temperatures. The relationship between temperature and adult emergence observed in our study is not uncommon in insects. For instance, Telles-Romero *et al.* (Telles-Romero et al. 2011) studied the effects of four temperature regimes (18, 20, 25 and 30°C) on the West Indian fruit fly *Anastrepha obliqua* and found a decrease in the duration until adult emergence with increasing temperatures. Moreover, moist sawdust, the pupation substrate used in our study, may have also collectively accelerated the developmental time of pupae to

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adult emergence. Our results is further supported by Holmes and colleagues (Holmes et al. 2013) who also observed low pupal mortality and a higher proportion of adult emergence and increased adult longevity when using wood shavings and concluded that such a pupation substrate significantly enhances BSF development. The reason for this is most likely the high moisture content (70%) and low compaction density of the wood shavings that facilitated pupation and emergence of BSF (Holmes et al. 2013).

Adult longevity significantly decreased with increasing temperatures, with BSF adults derived from larvae previously fed on SG recording higher longevity. This confirms our previously stated assumption regarding the influence of the nutritional content of the rearing substrate as well as access to nutritional resources on the larval and adult life history traits and explains why we observed - even at a similar temperature range (25-30°C) - shorter adult longevity in comparison to Myers and colleagues (Myers et al. 2008) who also reared BSF on CD. However, they noted greater longevity in adults that were previously fed with higher increments of fresh CD as BSF larvae. Moreover, since adult BSF do not feed and only consume water, exposing them to high temperatures will cause dehydration leading to increased mortality rate and reduced lifespan (Kroeckel et al. 2012). BSF fecundity was highest at 30°C and was significantly affected by the type of substrate fed to the larvae. The higher weight gain recorded at the prepupal stage is a good indicator of body size (Berger et al. 2008), which is likely to translate to larger adult body size in both males and females. Several studies have reported that larger sized females have higher energy reserves to lay more eggs (Berger et al. 2008). Although we did not measure the size of the BSF females, the fact that females that emerged from larvae reared on SG, had significantly higher fecundity than females that originated from those whose larvae were fed on CD.

Another factor that may have influenced the overall development of BSF may be related to its phenotypic plasticity. The stock colony from where we obtained the BSF eggs was housed in an outdoor insectarium subjected to light cycles and temperature regimes reflective of the seasonality in Nairobi. A study by Zhou and colleagues (Zhou et al. 2013) on BSF strains collected from three different climatic

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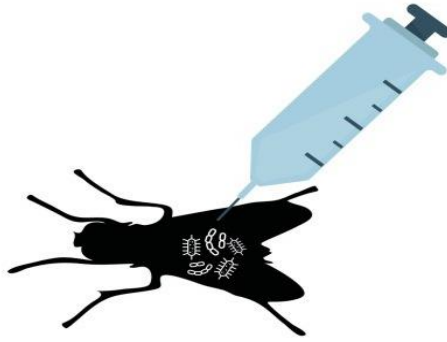
regions in the USA and China reared under identical conditions revealed different BSF life-history traits. The authors attributed this differential development to the phenotypic plasticity of BSF. Further studies are needed to verify whether phenotypic plasticity in BSF is exclusively genetically determined or may be also influenced by the environment.

### **3.6 Conclusions**

An increasing food-feed competition coupled with rising costs of and increased demands for feed sources raise the need for the production of more sustainable and cost-effective alternative feeds. Moreover, the greater demand for sustainable food production in a world with a changing climate requires the livestock sector to develop more environmentally friendly and affordable production systems. One such approach could be the utilization of mass-reared insects as alternative protein source in feed stock. Our results show that BSF larvae can be successfully reared on substrates derived from commonly available organic waste streams in urban environments in Africa and at temperatures that would not necessitate any energy-intensive and thus expensive heating or cooling systems for their mass production. The availability of such a production system would considerably lower the cost of livestock feeds and consequently would make animal protein more affordable for the growing urban populations in SSA, thereby improving food security and nutrition, especially for women, children and other vulnerable groups of society. Hence future research should focus on the development of adapted technologies for small- to medium-scale industrial mass production systems of insects like BSF where commonly available urban organic waste streams can be fed into.

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4      **A CULTURE-DEPENDENT MOLECULAR SURVEY OF VIABLE BACTERIAL SPECIES OCCURRING IN THE GUTS OF BLACK SOLDIER FLY LARVAE (*HERMETIA ILLUCENS*) REARED ON TWO COMMON URBAN ORGANIC WASTE STREAMS IN KENYA**



**4.1 Abstract**

In Africa, livestock production is challenged by the staggering costs of feed, which has limited the expansion of the livestock sector. The utilization of Black Soldier Fly larvae (BSFL) meal as potential alternative protein ingredients to substitute the expensive use of fishmeal and soybean in livestock feed has been widely documented. Although, BSFL voraciously feeds and grow in various organic waste streams, research on their microbial safety is very scarce in general and almost none existing in Africa. Thus, in the present study, a culture-dependent sequence-based survey was performed in order to isolate and identify viable bacterial species associated with the gut of BSFL reared on chicken manure (CM) and kitchen waste (KW). One hundred (100) 5-days neonatal BSFL were placed in 23 x 15cm metallic trays on the respective substrates (500 g) for a period of 3-4 weeks at  $28 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity. Fifty (50) prepupae from each substrate were harvested and the gut of each individual extracted. Aliquots of 0.1 mL from each extracted gut were spread onto conventional agar plates containing selective and nonselective media and incubated at  $37^\circ\text{C}$  for 48 h. Selected discrete bacterial strains were then aseptically removed and pure cultures established. The isolates were identified using morphological and molecular methods. Our results revealed that *Providencia* spp. was the most dominant bacterial species

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detected in the guts of BSFL reared on both CM and KW. *Morganella* spp. and *Brevibacterium* spp. were both detected in CM fed larvae, while *Staphylococcus* spp. and *Bordetella* spp. were specific to KW fed larvae. The variability in the bacterial species composition isolated in this study highlights the potential influence of the rearing substrate on the gut microbial community of BSFL. The dominant presence of *Providencia* spp. in the gut of larvae reared on both substrates highlights the potential ability of the larvae to vertically transmit certain bacterial species to animals, if pre-harvest measures are not re-enforced to ensure safety. Understanding the dynamics of bacterial communities associated with the guts of BSFL will be helpful in the selection of safe organic waste streams as well as in the application of proper substrate management. These strategies are key to produce safe insect-derived feed.

#### **4.2 Keywords**

Organic waste treatment; black soldier fly (BSF); gut microbiota; insect rearing; feed safety; food security

#### **4.3 Introduction**

The expanding world population, rapid urbanization, and growing welfare will cause an increase in the demand for meat. However, the inclusion of more animal-based products constitute a major challenge for our global food production system in terms of sustainability due to the high ecological footprint associated with the production of meat and dairy (Crosson and Anderson 1994; van der Zijpp 1999; Delgado 2003; Steinfeld et al. 2006; Ndambi et al. 2007; de Vries and de Boer 2010; Alexandratos and Bruinsma 2012; Mekonnen and Hoekstra 2012; Gerber et al. 2013). However, regardless of the increased appetite towards more animal-based products, levels of malnutrition associated with protein consumption deficiency rise steeply in many parts of the developing world (Ayele and Peacock 2003; Neumann et al. 2003; Randolph et al. 2007; Narrod et al. 2012; FAO et al. 2015). Moreover, the level of productivity of agricultural systems in the developing world is still relatively low due to the inefficient employment of natural resources (Bruinsma 2003). Additionally, high

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feed costs currently account for > 70% of livestock production costs in Africa, consequentially leading to an increase in the costs for the entire industry (Craig and Helfrich 2009; Akinrotimi et al. 2011; Munguti et al. 2014). High feed costs are mainly driven by the food-feed competition created by the consumption of raw materials as feed ingredients that are suitable for direct human consumption like fish and soybean (Munguti et al. 2014). Therefore, access to affordable and innovative feed is a prerequisite to establish profitable and sustainable livestock production systems and to ensure food security in the developing world.

Recently, protein-rich edible insects have been recognized as innovative protein alternatives due to their ability in decomposing and valorizing different organic waste streams (Nowak et al. 2016; Van Huis and Tomberlin 2017). Moreover, insects are rich in micronutrients, energy and fatty acids (Sheppard et al. 1994b; Finke 2013; Nowak et al. 2016). For instance, the black soldier fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) has been identified as a promising feed ingredient for poultry, pigs, and aquaculture (Rumpold and Schlüter 2013; Makkar et al. 2014; Stamer 2015; Hopley 2016). BSF, originally traced to the Americas, is present in most tropical and temperate regions of the globe (Sheppard et al. 1994). Crude protein constitutes about 35 to 49% of the total dry weight of BSL larvae (BSFL) while fat accounts for about 29-35% of their total dry weight and their amino acid profile is of a similar quality to that of fishmeal (Renna et al. 2017; Spranghers et al. 2018; Dabbou et al. 2018; Gasco et al. 2019). Though naturally occurring in chicken, pigs and cow manure, BSFL were successfully reared on other organic waste streams such as catering waste, urban municipal organic waste, fish viscera, vegetable remains, coffee bean pulp, straw and dried distillers grains with solubles (DDGS) (Nguyen et al. 2015; Leong et al. 2016; Van Huis and Tomberlin 2017; Meneguz et al. 2018).

BSF are not pests neither disease vectors, and their immature stages release chemicals that change the habitat such that it becomes less suitable for the common house fly (Furman et al. 1959). Moreover, previous studies have reported on BSFL's potential in reduction of *Escherichia coli* and *Salmonella enterica* loads in chicken manure and cow dung (Erickson et al. 2004; Lalander et al. 2013, 2015; Čičková et al.



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2015; Nguyen et al. 2015). However, several studies revealed a considerable influence of the rearing substrate on the gut microflora of insects including BSF (Dillon and Dillon 2004; Jeon et al. 2011; Engel and Moran 2013a; EFSA Scientific Committee 2015; Boccazzi et al. 2017; Klammsteiner et al. 2018). Therefore, the guts of BSFL may uptake pathogens present in rearing substrates, caused by inappropriate processing or storage methods. This may subsequently cause diseases in animals fed with BSFL-derived feed, highlighting the importance to select safe rearing substrates (Erickson et al. 2004; Čičková et al. 2015; Wang and Shelomi 2017).

Little is known about the safety or security of BSFL-derived feeds. Few studies investigated the influence of rearing substrates on the dynamics of BSFL gut microflora during laboratory rearing or in larger production facilities (Jeon et al. 2011; Zheng et al. 2013; Boccazzi et al. 2017; Bruno et al. 2019; Wynants et al. 2019). Moreover, these studies were carried out in developed countries where urban organic waste streams tend to be more homogenous than those in most developing countries, while insect production facilities operate under stricter hygienic regimes. Therefore, the current study sought to isolate and identify viable bacterial species associated with the guts of BSFL. The study was performed using a culture-dependent sequence-based approach. Two organic waste streams were used which are largely and readily available in many urban areas in the developing world: chicken manure (CM) and kitchen waste (KW). Although those waste streams are not allowed in the European Union (IPIFF 2019), their suitability should be investigated (van Huis 2019) as they fit in a circular economy policy, declared as a priority by most governments. This research can contribute in decisions to select potentially safe organic waste streams or to decide which rearing, processing and decontamination methods are required for large-scale BSFL production.

#### **4.4 Materials and Methods**

The study was undertaken at the International Centre for Insect Physiology and Ecology (*icipe*), in Nairobi, Kenya.

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#### **4.4.1 Stock colony**

The insect colony was previously described in our published articles (Shumo et al. 2019a, b). The *icipe* insectary maintains a population of BSF adults which acted as a stock colony for this study as well as the previous studies cited above. Adult BSF are housed in metal framed cages with screen mesh (1.8 x 1.8 x 1.8m with 1.5mm mesh) with strong access to daylight spectra; temperatures are maintained at  $28 \pm 5^{\circ}\text{C}$  to encourage mating. Flies are supplied with water in order to prolong their life. Corrugated cardboard and some spent grain (SG) are placed within the cage in order to stimulate oviposition.

#### **4.4.2 Preparation of substrate and larvae feeding**

The two feeding substrates, CM and KW, were both sourced locally. CM was collected from a broiler poultry farm in the outskirts of Nairobi and used one week after it was harvested. KW was a mixture of potato peelings, carrot peelings, rice and bread debris collected from a local restaurant in Nairobi. The substrates were chosen based on their availability in Nairobi with a potential for future large-scale industrial production of the BSFL.

One hundred (100) 5-days neonatal BSFL obtained from *icipe's* stock colony were placed carefully in 33cm x 23cm x 15cm plastic containers containing 500g of the substrates. During the rearing process, the temperature was maintained at  $28 \pm 2^{\circ}\text{C}$  and relative humidity at  $65 \pm 5\%$ . Distilled water was sprinkled on the substrate to ensure 65-70% moisture content. All substrates were replenished weekly with fresh ones. After reaching the prepupae stage, the insects were harvested and stored at  $-20^{\circ}\text{C}$  to avoid any changes in the BSF microflora until further analysis.

#### **4.4.3 Isolation and morphological characterization of bacterial cultures**

The isolation of the prepupal guts was performed following aseptic techniques and under a closed sterile microbiological hood. The exterior of each BSF was washed once in 70% ethanol and once 0.9% sterile phosphate-buffered saline (PBS). The entire prepupal gut was extracted with fine-tipped forceps and

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homogenized in a test tube containing 1.5mL of sterile 0.9% PBS. Guts of a total of 50 CM fed and 50 KW fed BSFL were successfully extracted. In order to isolate bacterial strains, aliquots of 0.1mL from each extracted gut were spread onto conventional agar plates containing Nutrient Agar, MacConkey Agar or Violet Red Bile Agar and incubated at 37°C for 48h. Three replicates were prepared for each medium type. Selected discrete bacterial strains were then aseptically removed by a sterile inoculation loop and pure cultured 3 to 4 times on the same agar medium for 48 h at 37°C. The isolates were identified using traditional bacterial methods: handbooks, identification keys based on colony characteristics (size, shape, color, margin and elevation) and microscopic morphology (Betsy and Keogh 2005; Cullimore 2010; Lacey 2012; Goldman and Green 2015).

#### **4.4.4 Molecular characterization of bacterial cultures**

##### **Extraction and amplification of 16S rDNA**

Bacterial isolates were aseptically harvested by scraping discrete bacterial colonies off the surface of cultures with a sterile inoculation loop. The genomic DNA was extracted using ISOLATE II Genomic DNA Kit (Bioline, London, UK). Extracted DNA was quantified using NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The 16S rDNA gene of each bacterial isolate was amplified in 30µL volume PCR mix containing 10X PCR buffer (GenScript USA Inc, New Jersey, USA), 0.5 pmol µl<sup>-1</sup> of each primer (27F 5-' AGAGTTTGATCMTGGCTCAG -3' (Lane 1991) and 1492R 5-' GGTTACCTTGTTACGACTT -3' (Turner et al. 1999) , 0.25mM MgCl<sub>2</sub>, 0.0625 U µl<sup>-1</sup> Taq DNA polymerase (GenScript USA Inc, New Jersey, USA) and 20ng µl<sup>-1</sup> of DNA template. PCR reactions were set up in a PTC 100 thermocycler (MJ Research, Gaithersburg, MD, USA). The cycling conditions involved an initial denaturation step at 95°C for 10 min, 35 cycles of a denaturation step at 94°C for 1 min, an annealing step of 52°C for 1 min and an extension step at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The expected product size was 1500 bp.

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### **DNA purification and sequencing**

The PCR products were resolved through 1% agarose gel for 1 h at 70 V (Bio-Rad model 200/2-0 power supply and wide mini-sub cell GT horizontal electrophoresis system, Bio-Rad laboratories, Inc., Hercules, California, USA). The DNA was then visualized under UV-illumination. A KODAK Gel Logic 200 Imaging System software (Raytest GmbH, Straubenhardt, Germany) was used to photograph, analyze and document the gel. Following the manufacturer's instructions, the QuickClean 5M Gel Extraction Kit II from GenScript (GenScript Corporation, Piscataway, New Jersey, USA) was used to purify the PCR products which were then sent to Macrogen Europe BV for bi-directional sequencing.

### **Morphological data analysis**

Stata (version R 15.1) was used for analyses. Bacterial isolates occurrence was expressed as a percentage of the total number of dissected BSF prepupae. A two-sample test of proportions (Z-test) for non-parametric data was used to compare the occurrence of bacterial isolates obtained from prepupal guts of BSF previously reared on CM and KW substrates.

### **Molecular data analysis**

The sequences were assembled and edited using Chromas version 2.13 (Technelysium Pty Ltd, Queensland, Australia). Consensus sequences from both the forward and reverse strands were generated and were then queried through BLASTN in the GenBank database provided by the National Center of Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>) for identification purposes and to check for similarity with organisms already identified. Any isolate exhibiting 97-100% sequence similarity to NCBI strains were considered as the correct species for that isolate. Moreover, the consensus sequences were aligned using ClustalX version 1.81. These alignments were used for phylogenetic and molecular evolutionary analyses that were conducted using MEGA version 6. A Neighbor-joining tree was constructed with bootstrapping and using the Kimura 2 distance matrix.

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## 4.5 Results

### 4.5.1 Morphological identification of bacterial isolates

A total of five bacteria isolates belonging to the genera *Providencia*, *Morganella*, *Brevibacterium*, *Staphylococcus* and *Bordetella* were obtained from the 50 dissected CM and KW fed BSF using morphological features. *Providencia* was the most abundant genus in the guts of both CM and KW fed BSF occurring at 59.5% [95% CI 49.0% – 70.0%] and 51.2% [95% CI 40.5% – 61.9%], respectively. Both *Morganella* and *Brevibacterium* occurred only in CM fed BSF at 27.4% [95% CI 17.9% – 36.9%] and 13.1% [95% CI 5.9% – 20.3%], respectively. Moreover, *Staphylococcus* and *Bordetella* both occurred only in KW fed BSF at 30.9% [95% CI 21.1% – 40.8%] and 17.9% [95% CI 9.7% – 26.1%], respectively (Figure 1). We found a difference in the occurrence of *Providencia* obtained from CM fed BSF in comparison to both *Morganella* ( $p = 0.001$ ) and *Brevibacterium* ( $p < 0.001$ ). Moreover, there was a difference between the occurrence of *Morganella* and *Brevibacterium* ( $p = 0.035$ ). In addition, the occurrence of *Providencia* obtained from KW fed BSF in comparison to both *Staphylococcus* ( $p = 0.036$ ) and *Bordetella* ( $p < 0.001$ ) differed. On the other hand, no significant difference was found between the occurrence of *Staphylococcus* and *Bordetella* ( $p = 0.080$ ).

GenBank accession numbers provided for the nucleotide sequences of the bacterial isolates are as follows: *Providencia* spp. MSB6 = MK276967, *Providencia* spp. MSB9 = MK276968, *Providencia* spp. MSB12 = MK276969, *Providencia* spp. MSB22 = MK276974, *Morganella* spp. MSB27 = MK276976, *Brevibacterium* spp. MSB14 = MK276970, *Staphylococcus* spp. MSB18 = MK276972, *Bordetella* spp. MSB17 = MK276971, *Bordetella* spp. MSB21 = MK276973, *Bordetella* spp. MSB24 = MK276975.

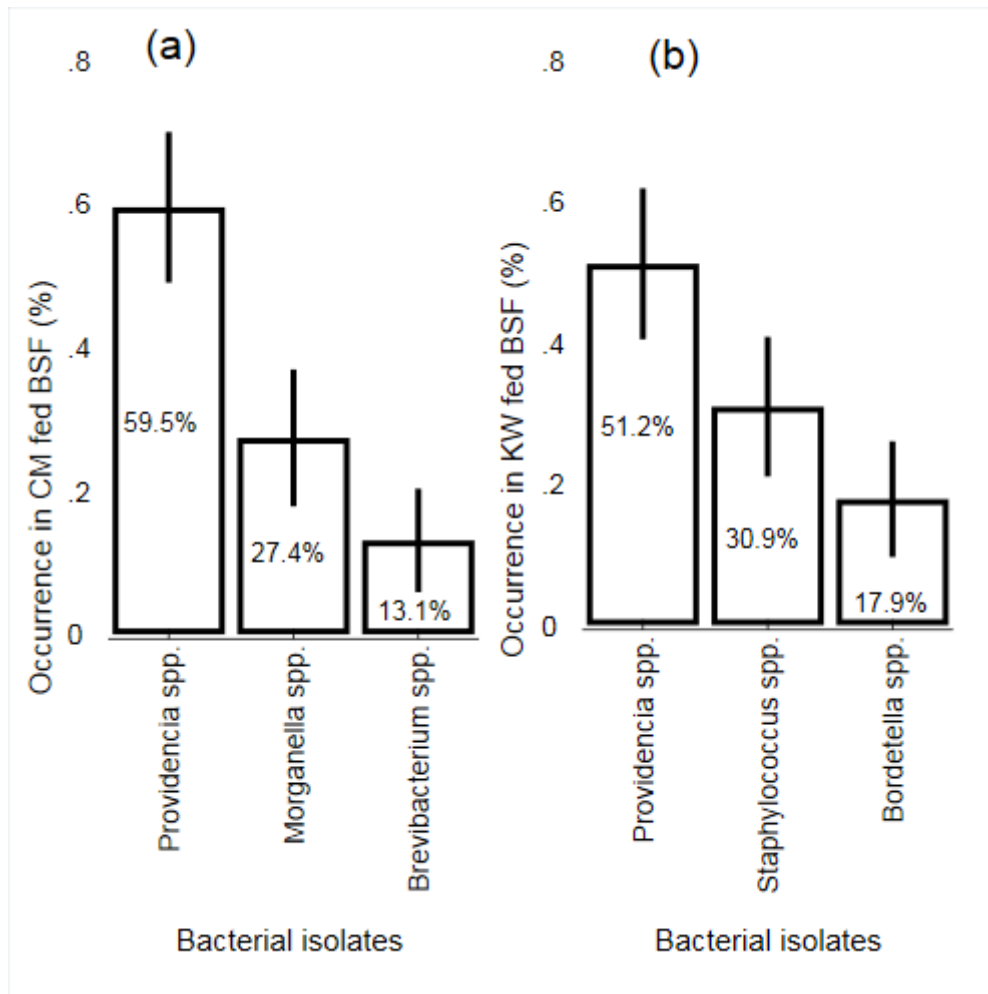


Figure 4.1 Mean bacterial isolates occurrence (in %) in the gut of (a) chicken manure (CM) and (b) kitchen waste (KW) fed Black Soldier Fly (BSF) prepupae. Error bars represent 95% confidence intervals. Note: It was not possible to italicize the Latin names of bacterial isolates *Providencia* spp., *Morganella* spp., *Brevibacterium* spp., *Staphylococcus* spp. and *Bordetella* spp. due to statistical software package limitations

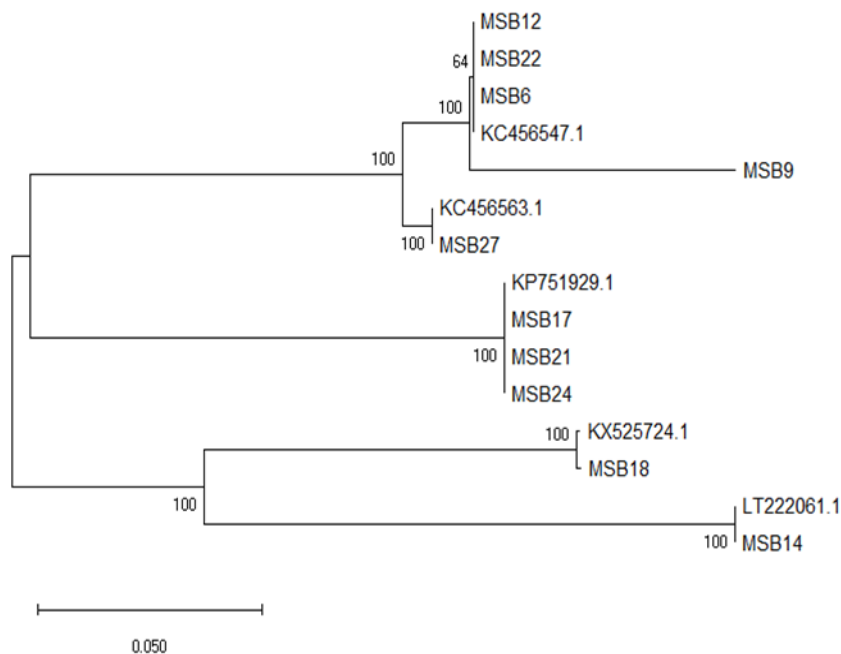
#### 4.5.2 Molecular characterization of bacterial isolates

The molecular identification of the isolated bacterial species depicting isolate species identities with 97-98% similarity and 0.0 E values after sequencing is consolidated (Table 4.1). Like the morphological identification, the molecular characterization also yielded five isolate species identities: *Providencia rettgeri* (isolates MSB6, MSB9, MSB12 and MSB22), *Morganella morgani* (isolate MSB27), *Brevibacterium luteolum* (isolate MSB14), *Staphylococcus* spp. (isolate MSB18) and

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*Bordetella* spp. (isolates MSB17, MSB21, and MSB24) (Table 4.2). Thus the molecular identification of the bacterial isolates corroborates that of the morphological study (Figure 4.1, Table 4.1).

The evolutionary history of the bacterial isolates was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 0.52129017 is presented in Figure 4.3. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree (Figure 4.3). The analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 966 positions in the final dataset. Five groups resulted from this analysis (Figure 4.3). The first group consisted of the *Providencia* spp. isolates where each of the samples MSB12, MSB22, MSB6 and MSB9 isolates branched separately. Furthermore, all the *Providencia* isolates linked to *Providencia rettgeri* of accession number KC456547.1 during blasting (Table 4.1). The second group consisted of *Morganella* spp. isolate MSB27 which linked to *Morganella morganii* of accession number KC456563.1 during blasting. The third group consisted of *Brevibacterium* spp. isolate MSB14 which linked to *Brevibacterium luteolum* of LT222061.1 during blasting. The last two clusters of the phylogenetic tree consisted of *Staphylococcus* spp. isolate MSB18 and *Bordetella* spp. isolates MSB17, MSB21 and MSB24, respectively (Figure 4.3). The *Staphylococcus* spp. isolate linked to *Staphylococcus* spp. accession number KX525724.1 while all the *Bordetella* isolates linked to *Bordetella* spp. of accession number KP751929.1 during blasting (Table 4.1).



**Figure 4.2 Phylogenetic tree showing the evolutionary relationships of bacterial isolates from chicken manure (CM) and kitchen waste (KW) fed Black Soldier Fly (BSF) prepupae.**

The genetic distances between the isolates were also interpreted using Kimura 2-parameter model. The estimates of evolutionary divergence between sequences of the bacterial isolates ranged between 0.001 and 0.281 (Table 4.2). Comparison of *Providencia* spp. isolate MSB12 isolated from CM fed BSF prepupae and *Providencia* spp. isolate MSB22 isolated from KW fed BSF with KC456547.1 gave a square distance of 0.001 and 0.003, respectively. Similarly, comparison of *Morganella* spp. isolate MSB27 isolated from CM fed BSF with KC456563.1, and *Bordetella* spp. isolate MSB17 isolated from KW fed BSF with KP751929.1 gave a square distance of 0.001 (Table 4.2). A principal component plot was generated (Table 4.3). In the Principal Coordinate Analysis (PCoA) plot, the 1st two axes explained 77.3% of the variation (the 1st axis 44.3% and the second axis 33.0%) (Figure 4.2). The PCoA separated the ten isolates into four distinct clusters. Each cluster was occupied by the isolates belonging to the different genera, i.e. *Providencia*, *Morganella*, *Bordetella*, *Brevibacterium* and *Staphylococcus*, respectively (Figure 4.2).



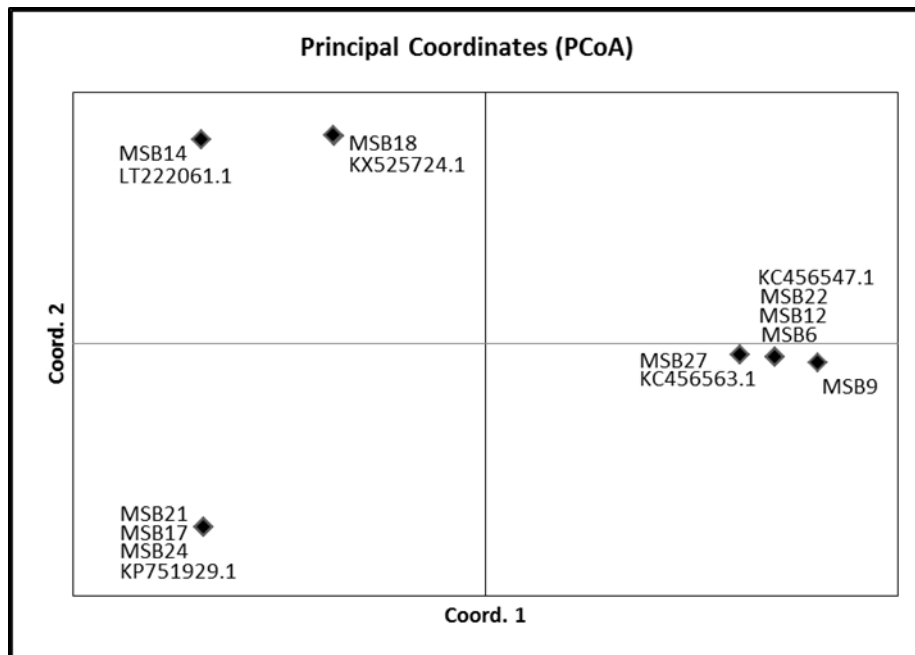


Figure 4.3 Plot of principal coordinate analysis (PCoA) via the covariance matrix with data standardization calculated using GenAlEx for the various bacterial species isolated from chicken manure (CM) and kitchen waste (KW) fed Black Soldier Fly (BSF) (PC1 = 44.3% and PC2 = 33.0%).

**Table 4.1 Identified bacterial species using 27 F and 1492 R primers**

Bacterial isolates codes	Bacterial isolates sources	GenBank Accession No.	ID from GeneBank	E Value	ID%	Species GenBank Accession No.
MSB6	BSF reared on CM	MK276967	<i>Providencia rettgeri</i> strain ALK417 16S ribosomal RNA gene, partial sequence	0	97	KC456547.1
MSB9	BSF reared on CM	MK276968	<i>Providencia rettgeri</i> strain ALK417 16S ribosomal RNA gene, partial sequence	0	97	KC456547.1
MSB12	BSF reared on CM	MK276969	<i>Providencia rettgeri</i> strain ALK417 16S ribosomal RNA gene, partial sequence	0	98	KC456547.1
MSB22	BSF reared on KW	MK276974	<i>Providencia rettgeri</i> strain ALK417 16S ribosomal RNA gene, partial sequence	0	98	KC456547.1
MSB27	BSF reared on CM	MK276976	<i>Morganella morganii</i> subsp. <i>morganii</i> strain ALK057 16S ribosomal RNA gene, partial sequence	0	97	KC456563.1
MSB14	BSF reared on CM	MK276970	<i>Brevibacterium luteolum</i> partial 16S rRNA gene, isolate BLKr1	0	97	LT222061.1
MSB18	BSF reared on KW	MK276972	<i>Staphylococcus</i> sp. strain 82584 16S ribosomal RNA gene, partial sequence	0	98	KX525724.1
MSB17	BSF reared on KW	MK276971	<i>Bordetella</i> sp. BAB-4401 16S ribosomal RNA gene, partial sequence	0	98	KP751929.1
MSB21	BSF reared on KW	MK276973	<i>Bordetella</i> sp. BAB-4401 16S ribosomal RNA gene, partial sequence	0	97	KP751929.1
MSB24	BSF reared on KW	MK276975	<i>Bordetella</i> sp. BAB-4401 16S ribosomal RNA gene, partial sequence	0	98	KP751929.1

**Table 4.2 Estimates of evolutionary divergence between sequences using 27 F and 1492 R primers**

Bacterial isolates	MSB6	MSB9	MSB12	MSB22	KC45 6547.1	MSB27	KC45 6563.1	MSB14	LT22 2061.1	MSB18	KX52 5724.1	MSB17	MSB21	MSB24	KP75 1929.1
<b>MSB6</b>															
<b>MSB9</b>	0.053														
<b>MSB12</b>	0.005	0.055													
<b>MSB22</b>	0.007	0.055	0.004												
<b>KC456547.1</b>	0.004	0.053	0.001	0.003											
<b>MSB27</b>	0.028	0.073	0.023	0.027	0.024										
<b>KC456563.1</b>	0.027	0.071	0.024	0.026	0.023	0.001									
<b>MSB14</b>	0.281	0.326	0.279	0.276	0.278	0.264	0.262								
<b>LT222061.1</b>	0.273	0.323	0.272	0.272	0.270	0.257	0.255	0.007							
<b>MSB18</b>	0.236	0.282	0.233	0.236	0.231	0.219	0.217	0.225	0.218						
<b>KX525724.1</b>	0.234	0.282	0.233	0.236	0.231	0.219	0.217	0.223	0.216	0.002					
<b>MSB17</b>	0.200	0.255	0.196	0.202	0.197	0.194	0.195	0.272	0.265	0.259	0.258				
<b>MSB21</b>	0.204	0.259	0.203	0.206	0.204	0.200	0.202	0.275	0.272	0.265	0.264	0.005			
<b>MSB24</b>	0.203	0.255	0.199	0.204	0.200	0.196	0.198	0.275	0.268	0.261	0.259	0.002	0.005		
<b>KP751929.1</b>	0.199	0.253	0.195	0.200	0.196	0.192	0.194	0.271	0.263	0.261	0.260	0.001	0.006	0.003	

#### 4.6 Discussion

Rapid population growth, urbanization and changes in consumer dietary preferences in the developing world result in an ever growing demand for food in general and for animal protein in particular (Thornton 2010). There are economic and ecological concerns regarding the use of fish meal and soymeal in livestock diets (Dicke 2018) and therefore innovative and sustainable alternatives sources of protein (Finke and Oonincx 2014) are crucially needed in order to ascertain global food security. Insects were recently recognized as alternative sources of protein for food and feed (Van Huis and Tomberlin 2017). In particular, the BSF stand out as a very promising species due to its larval ability to feed on and to valorize a wide range of organic substrates (Spranghers et al. 2018; Dabbou et al. 2018; Gasco et al. 2019). Several studies conducted in developed countries looked into the rearing under a number of environmental conditions of BSF, using a number of different diets . The safety of BSFL-derived feeds is a crucial matter as the larvae are often reared on organic waste streams and since that they are processed with their intestinal content (EFSA Scientific Committee 2015). Recently, the European Union (EU) created a legal framework that regulates and governs the production and consumption of insects for food and feed in order to ensure both nutritional quality and safety (The European Commission 2017). Yet, in the absence of such a framework, the microbial safety of BSFL in the developing world remains unclear.

Therefore, in this study we conducted a survey of the bacterial species associated with the gut of BSFL reared on two common organic waste streams in Nairobi. The survey was performed using a culture-dependent sequence-based approach. The objective was to study whether the resulting insects would be safe to use as an alternative protein source for livestock feed.

In our study, the bacterial isolate *Providencia* spp. (phylum Proteobacteria), was the dominant and detected in the guts of both CM and KW fed BSFL. Bacteria of the genus *Providencia* are Gram-negative opportunistic pathogens that have been isolated from a wide variety of environments and organisms ranging from humans to insects, sea turtles and shark mouths (O'Hara et al. 2000; Dillon and Dillon 2004; Behar

et al. 2005, 2008; Manos and Belas 2006; Foti et al. 2009; Ami et al. 2010; Interaminense et al. 2010; Hamden et al. 2013; Augustinos et al. 2015). In addition, *Providencia* spp. are associated with a wide range of human infections, and they show harmful pathogenic effects on their hosts. As such they can have an economic impact on the food safety industry (Linton and Hinton 1988). *Morganella* spp. belonging to the phylum Proteobacteria and *Brevibacterium* spp. belonging to the phylum Actinobacteria were both detected only in CM fed larvae. In contrast, *Staphylococcus* spp. (phylum Firmicutes) and *Bordetella* spp. (phylum Proteobacteria) were only detected in KW fed larvae. *Staphylococcus* spp. are Gram-positive bacteria and are commonly found as symbionts in the guts of different insect species like the common fruit fly *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), the southern house mosquito *Culex quinquesfasciatus* Say (Diptera: Culicidae), *Analeptes trifasciata* Fab. (Coleoptera: Cerambycidae) and the drosophila parasitoid wasp *Asobara tabida* Förster (Hymenoptera: Braconidae) (Zouache et al. 2009; Oyedokun and Adeniyi 2016). *Staphylococci* spp. are well known for developing antibiotic resistance as well as causing food-borne diseases and nosocomial infections (Kadariya et al. 2014). The variability among the bacterial isolates associated with the BSFL gut in our study may be attributed to the rearing substrates. In a previous study on the microflora of BSF higher bacterial diversity was found in food waste fed larvae in comparison to ones reared on cooked rice or calf forage fed ones. The bacterial variability was attributed to the influence of the rearing substrates (Jeon et al. 2011).

Environmental conditions probably play an important role in shaping the microbial communities in the guts of BSF. For instance, oxygen variability can influence the conditions and processes within the gut which in return influences the microbial communities inhabiting the gut (Hoback and Stanley 2001; Ke et al. 2010). The pH content of the rearing substrates possibly also facilitates the presence of certain bacterial species and inhibits the growth of others. Erickson and colleagues (Erickson et al. 2004) observed a reduction in pathogenic bacterial populations in alkaline BSF rearing substrates such as CM in comparison to acidic ones like cow or hog manure.

Moreover, temperature may have an influence on the bacterial composition of the rearing substrates and consequently on the BSF gut microflora. Studies on the potential of BSFL in the reduction of *Escherichia coli* and *Salmonella* spp. showed that both the nature of the rearing substrate as well as the temperature may influence the effectiveness of such a reduction. For instance, Erickson et al. (2004) reported that BSF reduced *Escherichia coli* and *Salmonella* spp. more at temperatures of 27°C and 32°C than at 23°C. Likewise, Liu and colleagues (Liu et al. 2008) demonstrated an increase in the effectiveness of BSF in the reduction of *Escherichia coli* and *Salmonella* spp. in cow manure at higher temperatures, although at 35° C all BSFL died. However, they observed a similar increase in the reduction of bacterial counts in cow manure controls not containing BSFL at the same temperature. Yet, Shumo et al. (2019a) found that BSFL survived at temperatures up to 35°C, indicating that phenotypic plasticity might influence their fitness regardless of substantial environmental variability. Phenotypic plasticity is the ability of an organism to modify either its phenotype or developmental events in response to changes in environmental conditions. (Moczek 2010; Kelly et al. 2012). Further studies are needed to verify whether phenotypic plasticity influences the dynamics of microbial communities associated with the guts of BSF.

#### **4.7 Conclusions and outlook**

This study demonstrated the influence of rearing substrates on the microbial community in the gut of BSFL as shown by the variability in the composition of the bacterial species isolated. This indicates the potential of BSFL to up-take, among others, pathogens from contaminated rearing substrates. Moreover, the dominant presence of *Providencia* spp. in the guts of BSFL reared on both substrates highlights the insect's ability to vertically transmit certain bacterial species and therefore to harbor and disseminate bacteria. While the methodology we used was limited to bacterial species that can be cultivated on the selected growth media, our aim was only to identify viable bacteria (EFSA Scientific Committee 2015). A purely molecular-based approach like PCR of DNA extracts from direct BSF samples is more sensitive, yet

it cannot distinguish viable from nonviable bacteria (FAO 2009). Future studies should employ both selective and nonselective media, as this allows for a more extensive survey of viable bacterial species. Moreover, sampling at different development stages of BSF and at various time intervals during the rearing process could elucidate the dynamics of the associated microbial communities. What remains to be seen is whether and how industrial BSF production systems utilizing similar or different rearing substrates, influence microbial communities. Selecting safe organic waste streams as well as applying proper substrate decontamination and management strategies are key aspects for the production of safe insect-derived feed. To achieve this, a continental African insect feed policy aiming to regulate production safety by setting appropriate hygiene and quality control standards needs to be developed.

## 5 SYNTHESIS

In the developing world, large amounts of waste are generated as a consequence of population and economic growth as well as rapid urbanization (Diaz et al. 1996; Bhada-Tata and Hoornweg 2012). Most often local and communal governments lack proper waste management plans and infrastructure that are required to address the increasing waste challenge (Chalmin and Gaillochet 2011). Organic waste, which accounts for almost 80% of the total urban waste generated in the developing world, is often disposed in municipal landfills without either prior separation nor treatment leading to extensive losses of an otherwise a valuable resource (FAO 2014; Njoroge et al. 2014). According to the Intergovernmental Panel on Climate Change (IPCC) of the United Nations (UN) fourth assessment report entitled “Mitigation of global greenhouse gas emissions from waste: conclusions and strategies”, the waste sector contributes to about 5% of global greenhouse gas (GHG) emissions with landfill methane (CH<sub>4</sub>) as the major GHG emitted (Bogner et al. 2008). Such methane emissions also occur during waste transport, treatment process and leakages and continues for several years after disposal in the landfills (Bogner et al. 2008). Methane emissions in the developed world are stabilizing as a result of increased methane recovery, decreased landfilling, waste generation and recycling. This can be achieved by changing local economic and policy conditions (Bogner et al. 2008). However in the developing world, GHG emissions from waste, especially methane from landfills, are increasing due to rapid population growth and urbanization (Bogner et al. 2008). Moreover, the latest IPCC report entitled “Climate Change and Land” (IPCC 2019) revealed that human land use contributes to almost a quarter of current GHG emissions and that land-use factors contribute greatly to the difference between GHG emissions scenarios that would lead to 1.5 or 2°C warming and 3°C (IPCC 2019). According to the report, one way to limit climate change would be to reduce food loss and waste related GHG emissions. Food waste, which accounts for one quarter of the total amount of food produced globally, creates a huge carbon footprint (IPCC 2019). Hence, there is an urgent need for the developing world to introduce innovative, sustainable, cost effective and locally feasible organic waste



management and reclamation methods other than the sophisticated and costly ones currently applied in the Global North. Such waste management methods would also contribute considerably to mitigating climate change by reducing GHG emissions, especially those of methane.

Population and economic growth, compounded by the growing urbanization, greatly affects dietary preferences in the Global South, leading to an increased demand for animal protein (Crosson and Anderson 1994; van der Zijpp 1999; Delgado 2003; Ndambi et al. 2007). The production of meat and dairy is required to double by the year 2050 in order to put up with such a demand (Alexandratos and Bruinsma 2012). The IPCC stated in its latest report entitled “Climate Change and Land” that the status of food security will increasingly suffer by future climate change (IPCC 2019). The report explained how climate change affects the four pillars of food security, i.e. availability, accessibility, utilization and stability of food. Future intensive rainfall will increase the risk of erosion of croplands soil leading to yield declines, increased prices, reduced nutrient quality, and disruptions in supply chains, especially in low-income countries (IPCC 2019). Moreover, drylands that are subjected to desertification, will suffer from extreme conditions such as drought, heatwaves and dust storms. While natural land processes absorb carbon dioxide (CO<sub>2</sub>) equivalent to almost a third of emissions from fossil fuels and industry, agriculture, among other types of land use, account for 23% of human GHG emissions (IPCC 2019). According to the report, the 23% of land-use related GHG emissions account for 13% of global CO<sub>2</sub>, 44% of CH<sub>4</sub> and 82% of nitrous oxide (N<sub>2</sub>O) emissions. The increase in the numbers of ruminants, specifically cattle, in addition to the intensification of rice production, are responsible for the increased methane emissions (IPCC 2019). The increased N<sub>2</sub>O emissions are caused by the increased use of fertilizers. When farmers apply more fertilizers than the plants can up-take, certain microbes use the N present in the fertilizers instead of O<sub>2</sub> for respiration and release N<sub>2</sub>O in return. Moreover, livestock manure also contributes to the increased emissions of N<sub>2</sub>O as it contains high levels of N that are increasingly deposited in pastures and rangelands (IPCC 2019). Therefore, reducing GHG emissions from agriculture, as well as other types of land use, is essential if global temperature

increases are to be kept between 1.5 and 2 °C (IPCC 2019). According to the report, meat-biased diets, among others, through the excessive use of arable land for grazing and feed production, significantly contribute to globally rising temperatures and increasingly become unsustainable (IPCC 2019). Thus, considering climate change, the current scarcity in natural resources, as well as the high ecological and economic footprints of current livestock production systems, alternative and innovative animal protein sources are urgently needed (Crosson and Anderson 1994; van der Zijpp 1999; Delgado 2003; Steinfeld et al. 2006; Ndambi et al. 2007; de Vries and de Boer 2010; Alexandratos and Bruinsma 2012; Mekonnen and Hoekstra 2012; Gerber et al. 2013).

The Black Soldier Fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) was recently recognized as an innovative and alternative source of protein for poultry, pigs and aquaculture feed due to its ability to thrive on various types of organic side streams, its high quality nutritional profile and its cosmopolitan distribution (Sheppard et al. 1994; Rumpold and Schlüter 2013; Makkar et al. 2014; Nguyen et al. 2015; Stamer 2015; Hopley 2016; Leong et al. 2016; van Huis and Tomberlin 2017; Renna et al. 2017; Spranghers et al. 2018; Dabbou et al. 2018; Meneguz et al. 2018; Gasco et al. 2019). Therefore, this thesis investigated the potential of BSF in valorizing urban organic waste streams, as well as its potential role in improving nutrition through the production of sustainable and cost-effective insect-derived livestock feeds, particularly in developing countries like Kenya.

The ecological and economic sustainability of BSF-derived feeds largely depends on the cost effectiveness and availability of the rearing substrates for the insects (Barragan-Fonseca et al. 2017; Smetana et al. 2019). Moreover, the nutritional quality of the rearing substrate is a key factor in the quality of the BSF larvae (BSFL) produced (Nguyen et al. 2015). Therefore, identifying nutritionally valuable, readily available organic waste streams in enough quantities is a prerequisite for success of BSFL mass rearing in the developing world. So far, few studies assessed the quality of the nutritive value of BSFL, using either experimental diets or organic side streams collected in the developed world (Barragan-Fonseca et al. 2017). Unlike artificially prepared diets, organic waste streams in the developing world are heterogeneous in

their nutritional composition and are often disposed in uncontrolled and unhygienic conditions (Diaz et al. 1996; Bhada-Tata and Hoornweg 2012). Thus, in chapter 2, I conducted a holistic chemical analysis on the nutritional quality of three readily available organic side streams in Nairobi, and arguably as well in other megacities in the developing world, and their influence as rearing substrates on the nutritional quality of BSFL. Such a comparative study is essential when deciding which organic waste streams are potentially suitable for BSFL mass rearing in Kenya and elsewhere in the developing world. The results demonstrated that the tested organic waste streams can be successfully used to produce high quality BSFL that have the potential to substitute other animal- or plant-derived protein sources in commercial livestock feed. However, comparing the results from chapter 2 to those reported in previous studies that used similar organic waste streams indicates the presence of additional factors such as differences in methodological set-up, environmental conditions, rearing substrates, time of harvest, harvesting and processing methods as well as genetic heterogeneity of BSFs (Newton et al. 2005; St-Hilaire et al. 2007a; Diener et al. 2009; Kroeckel et al. 2012; Finke and Oonincx 2014; van Huis and Tomberlin 2017; Liu et al. 2017). These factors influence the nutritional quality of BSFL in addition to the rearing substrate (Newton et al. 2005; St-Hilaire et al. 2007).

Earlier studies looked into the influence of individual factors, such as nutritional quality or temperature, on the development and survival of the insects (Atkinson 1995; Atkinson and Sibly 1997; Simpson et al. 2004; Bradshaw and Holzapfel 2008; Roeder and Behmer 2014; Rodrigues et al. 2015; Matavelli et al. 2015; Silva-Soares et al. 2017; Gray et al. 2018). Yet, Clissold and Simpson (2015) demonstrated an influence of the interaction between temperature and nutrition on the development rate, maturity, fecundity and longevity of insects. BSF are known to be sensitive to their external environments (Tomberlin et al. 2009; Park 2016). Therefore, it is expected that abiotic factors such as temperatures and their interactions with the rearing substrates could influence the quality of BSFL by affecting their development and fitness. Previous studies on fitness of BSF focused on the influence of either artificially prepared diets or organic waste streams as rearing substrates at constant

temperatures (St-Hilaire et al. 2007; Myers et al. 2008; Tomberlin et al. 2009; Kroeckel et al. 2012; Banks et al. 2014). Moreover, little is known on the effect of the interaction between temperature and urban organic waste material as rearing substrates in a developing world setting. Thus, in chapter 3, I sought to fill this knowledge gap by investigating the combined influence of temperature and commonly available organic waste streams on the duration of development of BSF immatures as well as the weight of the prepupae. The results demonstrated the individual effects of temperature and rearing substrate on the development, longevity, and fecundity of BSF. BSFL and BSF pupae developed faster at higher temperatures while adults' longevity increased at lower temperatures. Likewise, BSFL reared on the organic side stream with the higher nutritional quality, i.e. spent grain (SG), outperformed the ones reared on the organic side stream with the lower nutritional quality, i.e. cow dung, by surpassing them in pupal weight and requiring less time for larval development. However, Meneguz et al. (Meneguz et al. 2018) who also reared BSF larvae on SG recorded faster durations in comparison to what I reported in chapter 3. Yet, on the other hand I reported in chapter 3 heavier weights for BSF prepupae reared on SG than Tomberlin et al. (Tomberlin et al. 2002) in a similar study. The main reasons for these discrepancies are differences in methodologies and experimental set-ups as well as varying temperatures at which the BSFL were kept. Therefore, the knowledge obtained from chapters 2 and 3 is important when identifying and assessing the organic side streams suitable for BSFL mass rearing, and for looking beyond the nutritional quality of the rearing substrates and into substrates features such as texture, moisture content and substrate density in order to optimize BSFL industrial production systems.

Little is known on the safety of BSFL-derived feeds. On one hand, BSF is known to release chemicals during their larval stages that deter the common house fly *Musca domestica* L. (Diptera: Muscidae) (Furman et al. 1959). Moreover, BSF are considered neither pests nor disease vectors (Furman et al. 1959). However, a recent publication by Tomberlin and Van Huis (2020) pointed out that early BSF was considered a pest due to it producing myiasis in humans and pets. Additionally, they have the ability to reduce the presence of *Escherichia coli* and *Salmonella enterica* in

poultry manure and cow dung, respectively (Erickson et al. 2004; Lalander et al. 2013, 2015; Čičková et al. 2015; Nguyen et al. 2015). On the other hand, several studies revealed a considerable influence of the rearing substrate on the gut microflora of insects including BSF (Dillon and Dillon 2004; Jeon et al. 2011; Engel and Moran 2013b; EFSA Scientific Committee 2015; Boccazzi et al. 2017; Klammsteiner et al. 2018). Such an influence may result in the uptake of pathogens which might lead to the transmission of diseases to animals fed with BSFL-derived feeds. This highlights the importance of selecting appropriate rearing substrates for the production of safe feed (Erickson et al. 2004; Čičková et al. 2015; Wang and Shelomi 2017). Some studies investigated the influence of rearing substrates on the dynamics of BSFL gut microflora in the laboratory or in mass rearing facilities (Jeon et al. 2011; Zheng et al. 2013; Boccazzi et al. 2017; Bruno et al. 2019; Wynants et al. 2019). Yet most of them were carried out in developed countries where urban organic waste streams tend to be more homogenous and insect rearing facilities function under stricter hygiene conditions unlike the case in most developing countries. Assessing the safety of BSFL-derived feeds reared on organic waste streams contribute to decision making about which organic waste streams are potentially feasible or which rearing, processing and decontamination methods are required for large-scale BSFL production. Therefore, in chapter 4, I sought to isolate and identify viable bacterial species associated with the guts of BSFL reared in a culture-dependent, sequence-based approach on two different organic waste streams, i.e. kitchen waste and cow dung. While both rearing substrates are not permitted to be used in the European Union (EU) in commercial insect mass rearing systems, I investigated their suitability as they fit into the concept of a circular economy policy, declared nowadays as a priority by many governments (IPIFF 2019; van Huis 2019). I could show that that the rearing substrates considerably influences the composition of the gut microbial community of BSFL as demonstrated in the variability of the bacterial species isolated. This indicates the ability of BSFL to up-take pathogens from contaminated organic waste streams and to vertically transmit certain bacterial species.

## **6 LIMITATIONS OF THE STUDY**

The nutritional quality of the organic waste streams assessed in this research may have been comprised as a result of opting for a lump sum feeding approach. The quality of lump sum diets deteriorate with time, leading to a reduction in the amounts of vitally important nutritional components such as protein and carbohydrates essential for the development of insects (Nijhout 2003; Lee et al. 2004; Simpson et al. 2006). As a coping mechanism to the reduction in the amounts of such components, larvae compensate by developing faster and consequently weigh less compared to a regular diet (Raubenheimer and Simpson 1997; Berner et al. 2005). Therefore, using fresh and frequent supply of the rearing substrates to feed the larvae may have increased the quality of the BSFL produced, as demonstrated in several previous studies (Sheppard 1983; Myers et al. 2008). However, I opted for a lump sum feeding approach in order to maintain the consistency of the nutritional composition of the rearing substrates. I personally collected the substrates used in this study from an urban poultry farm, a restaurant, a slaughterhouse and a brewery production facility. For logistical reasons, a continuous collection of homogenous rearing substrates was not possible. However, opting for a lump sum feeding method in an industrial production may be more cost effective in comparison to an incremental diet in terms of reducing the amount of manual labor needed, as well as the costs and means of the organic waste streams transportation. However, ensuring the freshness of the rearing substrates in industrial settings is crucial in order to not compromise the nutritional quality of the larvae produced and this can be achieved by optimizing the storage and rearing facilities.

Furthermore, the consistency and physical texture of the organic waste streams used in this research may have influenced the development and behavior of the BSFL. Though, I did not actually test the consistency and physical texture of the studied organic waste streams, it was visually clear that the thickness of the rearing substrate minimized the mobility of BSFL and hindered their access to the substrate which consequently affected their development.

I applied a culture-dependent sequence-based approach while isolating and identifying the bacterial species associated with the gut of BSFL. Though culture-independent molecular approaches offer the possibility of recovering both viable and nonviable microbes present in the samples, and therefore provide a more detailed view of the species present in the microbial community, my objective was to isolate and identify the viable bacterial species only (Özoğul 2004; Gupta et al. 2012). Yet, this allowed me to only capture a fraction of the entire bacterial species associated with the sample (Staley and Konopka 1985; Amann et al. 1995). Moreover, the culture-dependent approach is restricted by the artificial media utilized and conditions adopted (Gupta et al. 2012). In future studies cultures should be grown at several temperatures and on different types of selective and non-selective media as this allows for a more extensive survey of viable bacterial species. Moreover, sampling at various time intervals during the rearing process, thereby capturing different development stages of BSF, could better elucidate the dynamics of the gut microbial communities. Thus, using both approaches, i.e. culture-dependent sequence-based and culture-independent molecular approaches, might lead to a better understanding and a more detailed identification of BSFs' gut microbial diversity. The increasing use of molecular tools, however, does not change the fact that cultivation and isolation are still integral parts of diversity studies because they allow investigation of the specific properties and requirements of the isolated strains, information that cannot be retrieved from molecular data alone (Gupta et al. 2012).

## 7 OUTLOOK FOR FUTURE RESEARCH AND POLICY



The outcomes and limitations of the research undertaken for this thesis have highlighted several topics that would merit further research. Furthermore, this thesis contributes to the knowledge required by policy makers in order to adopt and implement appropriate policies, as well as by the industry in order to optimize their production facilities and to ensure quality products.

- **Flies are more than what they eat**

Future studies on the nutritional quality of BSFL should not only investigate the quality of the rearing substrates but also their interactions with the abiotic factors surrounding the rearing process. Though the combined influence of temperature and rearing substrate was addressed in chapter 3, other factors such as moisture content, oxygen and pH levels should be researched as well. Understanding the role of such factors and their interactions on the nutritional quality of BSFL will help to optimize the industrial mass rearing of BSFL.

- **Phenotypic or genetic responses?**

Presently we know little to nothing on the causatives behind phenotypic plasticity in BSF. When it would be triggered by genetic factors and passed on from one generation to another (as suggested in chapters 2 and 3), it would increase the



ability of utilizing more competent strains of BSF that are unique to certain habitats or climatic zones. This could either be done by using strains from different regions (e.g. Zhou et al. 2013) or by using forms of genetic selection. Moreover, investigating the role phenotypic plasticity plays in the dynamics of microbial communities associated with the guts of BSF, as suggested in chapter 4, will increase our ability of utilizing more pathogen- and disease-resistant or tolerant BSF strains.

- **Replicating laboratory experiments in real-time industrial production systems**

Future research should examine the influence of the organic waste streams, such as those used in this thesis or others, on the nutritional quality and the fitness of BSFL in industrial BSF production systems. Moreover, the influence of the rearing substrates on the microbial communities associated with the gut of BSFL in such systems should be investigated. Assessing the quality and the performance of BSFL in industrial production systems is crucial for optimizing the production process in terms of determining the most suitable density of the rearing substrate and the most efficient feeding method as well as the optimal time of harvest.

- **Finding a balanced and a unified legal framework for large-scale insect farming**

Ensuring the safety of livestock feeds is a necessity as it guarantees the welfare and health of animals and humans. Therefore, the EU applies strict policies and legislations to regulate the utilization of insects as livestock feeds. For instance, it established restrictions on the feed that may be given to farmed insects that mirror those used for other farmed animals (IPIFF 2019). So far, only plant and plant-derived products are allowed to be employed as insects feeding substrates, with exceptions to some animal products such as fishmeal, milk and milk-derived products, egg and egg-derived products as well as honey (Silva-Soares et al. 2017). On the other hand, the use of manure, slaughterhouse and rendering derived products or catering waste as insects feed is strictly prohibited (IPIFF 2019). Moreover, the EU Animal Health Law – Regulation (EU) No 2016/429 on transmissible animal diseases obligates insect

producers to ensure the safety of their farmed insects in order to prevent the spread of diseases within their production facilities (IPIFF 2019).

So far, most countries in Africa lack a unified legal framework that sets guidelines and standards to ensure the quality and safety of insects' mass production. Aiming for a similar version of the very strict and one would argue at times overcautious EU legislations and regulations regarding insect farming might prevent these countries from finding innovative and economic solutions to valorize the enormous amounts of organic waste generated on the continent. Therefore, a unified yet balanced legal framework catering, reflecting Africa's abilities and needs, should be developed in order to ensure the selection of suitable and safe rearing substrates and the use of proper decontamination, hygiene and safety measures in order to provide the African markets with sustainable and economic insect-derived quality protein products.

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## 9 NAIROBI FIELD RESEARCH PHOTO JOURNAL

A Picture is worth a thousand words and in this section of my thesis, I reflect - through photographers- on my field visit and research stay in Nairobi, Kenya.



Figure 9.1 Trying to identify suitable organic waste streams while exploring the fastest growing city in East Africa. I was thrilled by the contrasts of this megacity observing a worker at Kenya Breweries Limited in Tusker house in Ruaraka, Nairobi while the background of the photograph is made up of slums and shantytowns.



Figure 9.2 I took this photo while visiting my local chicken manure supplier. My local supplier was a mother and a cleaner during the day hours and an entrepreneur afterwards. Every time I walked in her within home chicken farm to collect my share of chicken droppings, I would find out that her chicken farm is growing, and her business is expanding.



**Figure 9.3 Collecting cow dung from slaughter houses was rather very intense.**



**Figure 9.4 Rearing insects on a vegetarian diet. Here is a photo documenting how I utilized our kitchen waste to the extreme.**



**Figure 9.5 With time, it became evident to me that these flies were in love with the kitchen remains.**



Figure 9.6 Plastic containers filled with different organic substrates. I had to try several types of organic waste streams before settling to the ones I used for my research.



Figure 9.7 With the aid of a delicate camel-hair brush, I spent long hours patiently looking for the larvae that were comfortably buried within the rearing substrates.



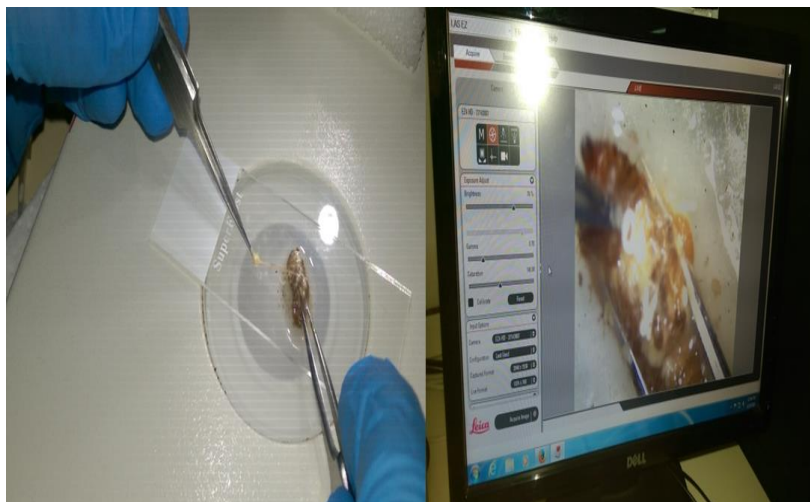
Figure 9.8 Ready for harvest. Here are samples of Black Soldier Fly as well as the rearing substrates stored in plastic bags and ready to undergo nutritional analysis.



**Figure 9.9** I used liquid nitrogen to dry the insect samples and a heavy-duty mixer/blender to crush them into powder prior to starting the nutritional analysis. Though the procedure was practical and handy, the liquid nitrogen was too costly, and I soon had to use oven heat instead.



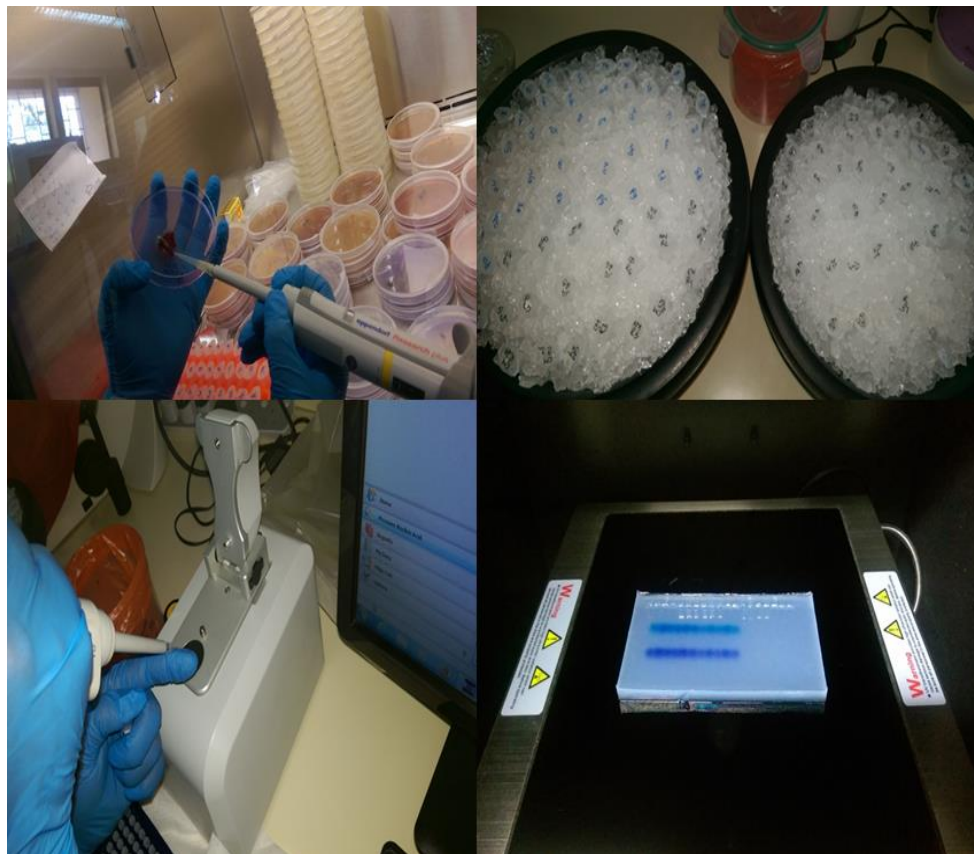
**Figure 9.10 Drying and manually crushing the black soldier flies was tiresome and time consuming, however much more cost effective in comparison to liquid nitrogen.**



**Figure 9.11 I often wondered while dissecting the entire guts of the black soldier fly, using fine forceps, if they were in pain or not?**



**Figure 9.12** A common method for identifying bacterial populations is the traditional morphological identification using a compound microscope and identification key handbooks.



**Figure 9.13** The extraction of DNA samples of the pre-cultured microbial populations is a rather modern approach and a reconfirmation to the traditional morphological observations.



## 10 LIST OF PEER-REVIEWED PUBLICATIONS

- Shumo M (2016) Black Soldier Fly: A Bio Tool for Converting Food Waste into Livestock Feed. *The Solutions Journal* 7:4: 36-39.
- Shumo M, Khamis F, Tanga C, et al (2019a) Influence of Temperature on Selected Life-History Traits of Black Soldier Fly (*Hermetia illucens*) Reared on Two Common Urban Organic Waste Streams in Kenya. *Animals* 9:79. <https://doi.org/10.3390/ani9030079>
- Shumo M, Osuga IM, Khamis FM, et al (2019b) The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Scientific Reports* 9:10110. <https://doi.org/10.1038/s41598-019-46603-z>

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