2024-08

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NM-AIST

https://doi.org/10.58694/20.500.12479/2739

VALORISATION OF SOLID SISAL LEAVES DECORTICATION WASTES USING BLACK SOLDIER FLY (*Hermetia illucens* L.) LARVAE

Aziza Athumani Konyo

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Environmental Science and Engineering of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

August, 2024

ABSTRACT

The amount of waste generated from sisal industries during sisal fiber production is huge and its management is still challenging and a menace to the environment. These wastes include sisal wastewater and sisal leaf decortication wastes (SLDW) which represent an opportunity for valorization into products. Nevertheless, the sisal leaf decortication wastes is suitable for resource recovery in order to reduce the impacts it causes by just being discarded in the environment without treatment. This study aimed at managing the SLDW using insect-based technology. Specifically, the study characterized the SLDW for its physical and chemical constituents, pretreatment of SLDW to render it suitable for the growth of the insects and optimization of the waste and other blends for production of livestock feed. The use of SLDW for various applications is limited due to its high acidic content and presence of saponin within it. This is the first study of its kind regarding the use the SLDW as a substrate for growth of BSFL. Pre-treatment was a necessary and challenging step done on the waste to meet minimum requirements for rearing of BSF. The resultant waste had Ca, P, K, Mn, Fe, Cu and Zn at varying levels which are all essential for animal growth. The SLDW contained 10 ± 0.01 percent of crude protein, 11 ± 0.02 moisture and energy (1615 kcal/g of Sisal de-corticated waste). The sun dried BSF larvae reared on SLDW contained 53 ± 0.005 percent crude protein, 4 ± 0.01 percent of crude fat, moisture content (10 ± 0.1) %, carbohydrate (43 ± 0.01) % and ash (37 ± 0.08) %. When rearing was done on SLDW, 3000 g of dried pre-treated waste yielded more wet BSF larvae (336 ± 41.3) g compared to 3000 g of fruit waste which yielded (244 ± 4.16) g of wet BSF larvae. Furthermore, the harvested BSFL after optimization process through blending of SLDW, RB and CDW improved growth rate of BSFL (2.2 gram per larvae) and contained sufficient nutritional value to feed poultry and fish, reducing the necessity to de-fatty the larvae as it is being practiced when market waste is being used. The SLDW is a promising feedstock for rearing BSFL because it has good reduction of the waste by 52%. This study eventually creates resource recovery sustainability in sisal industries.

DECLARATION

I, Aziza Athumani Konyo declare to the Senate of the Nelson Mandela African Institution of Science and Technology that, this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

22nd August 2024

Aziza Athumani Konyo   Date

The declaration is hereby confirmed by:

22nd August 2024

Prof. Anthony Manoni Mshandete   Date

22nd August 2024

Prof. Revocatus Lazaro Machunda   Date

22nd August 2024

Prof. Liliane Joseph Pasape   Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology a dissertation entitled: "*VALORISATION OF SOLID SISAL LEAVES DECORTICATION WASTES USING BLACK SOLDIER FLY (Hermetia illucens L.) LARVAE*" in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Environmental Science and Engineering of the Nelson Mandela African Institution of Science and Technology.

22nd August 2024

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22nd August 2024

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ACKNOWLEDGEMENTS

The successful completion of this dissertation was due to the supportive effort done by various people who were involved at various stages of this work.

I am therefore very grateful to my supervisor: Prof. Anthony Manoni Mshandete, Prof. Revocatus Lazaro Machunda and Prof. Liliane Joseph Pasape for their valuable inputs. The valuable comments on the early stage of proposal writing, guidance in the field sites being visited together and involvement in each stage of report writing made this study effectively completed.

I wish to appreciate the University of Dodoma for funding my PhD studies in Environmental Science and Engineering specialize in Environmental Science. I wish also to extend my deepest gratitude to all people collaborated with me at different stages of this dissertation as follows:

First, I wish to pass my sincere gratitude to Sisalana (T) Ltd. specifically Mwelya factory staff for assistance and getting permit to allow working with them during this study. Second, is to my colleagues under PhD in Environmental Sciences Engineering, cohort 10, for the full support in discussion and cooperation in all social and academic journey made this study successfully done.

I would like to express my sincere and heartfelt thanks to my parents: Mr. & Mrs. Athumani Konyo and my lovely sons Baraka, Brian and Braeden for their prayer and emotional support all the time when I was far away from them for the sake of this study.

Lastly, I would like to thank everyone in one way or another made this dissertation successful. May God bless you all.

DEDICATION

I am dedicating this study to my family.

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LIST OF ABBREVIATION AND SMBOLS

5DOL	Five days old larvae
ALL	Mixture of solid sisal waste, rice bran and cow dung manure
BSF	Black soldier fly
BSFL	Black soldier fly larvae
CDW	Cow dung manure waste
CHNS/O	Carbon, hydrogen, nitrogen, Sulphur/oxygen detecting machine
DO	Dissolved oxygen
HW	Hotel waste
LOD	Loss on dry method
NM-AIST	Nelson Mandela African Institution of Science and Technology
pH	Potential of hydrogen
RB	Rice bran
SLDW	Sisal leaves decortication waste
TSB	Tanzania sisal board
SLDW	Sisal leaves decortication waste
TVS	Total volatile solids
GWh	Gigawatt hours
AD	Anaerobic digestion
GC-MS	Gas chromatography-mass spectrometry
FW	Fruit waste
Mp	Mass of an empty, clean and dry porcelain dish
Mpds	Mass of the dish and sisal wastes ample
Mpa	The mass of the dish containing the ash (burned sisal waste sample)
(O_2)	Oxygen
SPSS	Statistical Package for the Social Sciences
(W1)	Weight of each bundle
(Ca)	Calcium
(Ni)	Nickel
(Zn)	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Wastes are undesired substances or materials resulting from the consumption or manufacturing of a certain product. Industries, agricultural operations, marketplaces, and households can all produce waste. Waste generation has become a global issue as a result of population growth, urbanization, and a rise in people's consumption needs (Ferdous *et al*., 2021; Ifeoluwa, 2019). Industries are the major global waste producers (Millati *et al*., 2019; Osorio *et al*., 2021) and this is due to lack of sufficient waste treatment technology (Khan *et al*., 2022; Zhang *et al*., 2019), resulted in improper waste disposal into the environment, which increased the amount of toxicity in the deposited areas (Geetha & Rajalakshmi, 2020; Kazi *et al*., 2005).

Waste generation can be categorized into residential, municipal, and industrial (Ogbonna *et al*., 2007; Oyelola & Babatunde, 2008). Moreover, the waste can be hazardous or non-hazardous (Millati *et al*., 2019). For the case of environmental sustainability, each category must be effectively managed to prevent negative impacts on the environment and human health (Ferronato & Torretta, 2019; Mrozik *et al*., 2021). In Tanzania there are different types of waste being generated such as solid waste, wastewater, e-waste, medical waste and industrial waste especially in urban areas. The waste category common in Tanzania is industrial wastes, where most of the industries have proper disposal channels of their waste but they opt not to use them to avoid costs and instead they channel the untreated wastes to the environment (Mihale, 2019; Mwatujobe, 2020). To manage various types of waste created, various waste treatment procedures have been used. Reducing, reusing, recycling and adding value to waste are some of the waste management practices employed in Tanzania (Kihila *et al*., 2021; Yhdego, 2021). Some of these approaches or technologies are costly and cannot be afforded by many. Others have engaged in sorting and selling to create income (Mushi *et al*., 2022). Urban residence in Tanzania, for example, used to collect and sort plastics before transporting them to collecting sites where they could be weighed and sold for further recycling procedures (Charles, 2021; Senzige *et al*., 2014). Sisal industries also generate a huge amount of sisal leaves decortication waste (SLDW), with only 2-6% being sisal fiber as the main product and the rest being waste (Kivaisi *et al*., 2010; Zhang *et al*., 2014) lack of technology to manage this waste being generated will continue to contribute to environmental pollution.

Valorization is another approach for reducing waste in the environment while also generating income (Roy *et al*., 2023). Valorization is a resource recovery technology that involves the process of transforming waste and making modification to make it useable, valuable again and ensure sustainable development (Mikucka & Zielińska, 2020). It can be accomplished chemically or biologically by utilizing living organisms (Jeong *et al*., 2021). The biological waste valorization approach is more attractive since it uses organisms that are capable of decomposing organic waste (Van Groenestijn & Kraakman, 2005). Different studies have been conducted on the aspect of waste management through valorization. Biological methods, such as resource and energy recovery from food waste and the degradation of keratinous material using assisted conventional protease are essential approaches (Guo *et al*., 2020; Sarangi *et al*., 2023). Moreover, one of the biological method or technology used to manage organic waste is through the use of insect called Black Soldier Fly (BSF).

The BSF is an insect belonging to the order of Dipterans, family of Stratomyidae, sub-family of Hermetinae (Hanson, 2022). The BSF is readily available in tropical, subtropical (Nyakeri, 2018) rather than in temperate regions in Eastern Europe and American continent (Nartshuk, 2009; Spinelli *et al*., 2019). However, BSF is now widespread in other parts of the world, including Tanzania (Banks *et al*., 2014). Adult BSF can be found in a variety of areas, including marshes, damp places in soil, under bark, animal dung, and decomposing organic materials. The use of black soldier fly larvae to decompose organic waste is a relatively low-cost, reliable means of addressing the problem of organic wastes accumulating in industries such as sisal leaves processing. This biological process quickly decomposes pollutants without emitting odors and on a wet weight basis, this method tends to remove a large volume of organic wastes by 50-78.9% or more (Diener *et al*., 2011). Moreover, BSF technology reduces the requirement for transportation of waste from the production site to the dumping place (Popov *et al*., 2021) and also, allowing valuable goods such as animal protein, liquid fertilizer and biogas to be produced.

Currently, the Tanzanian government has resumed the policy on sisal plantations to make it one of the leading foreign earning crops by 2025 (Reporter, 2021). This means that sisal plantation and production will expand, resulting in more sisal waste being produced in their factories. Previous studies have been reported that in sisal industries only 2-6% is considered as a useful commodity which is sisal fibre, while the other above 94% is a waste (Kivaisi *et al*., 2010; Sinha *et al*., 2015). As a result, the focus of this research will be on sisal decorticating waste as a resource for value addition. The use of Black soldier fly larvae to manage sisal decorticating waste can be a good approach as it can reduce waste and produce animal feed. Furthermore, waste reduction presents opportunities for individuals involved in managing sisal decortication waste. Those engaged in waste collection, sorting, transportation, and value addition can benefit from its commercial value from sisal decortication (Kumar *et al*., 2017; Sharma *et al*., 2021). Managing waste using BSF provides several opportunities. Individuals can generate income by establishing BSF rearing units, where they create systems to hatch and collect BSF eggs for commercial purposes. The use BSF larvae to convert sisal decortication waste led to waste reduction, which in turn decreases pollution and environmental effects.

Waste management using BSF creates a lot of opportunities as well, people generate the income through BSF rearing units whereby they create mechanisms to hatch and collect eggs of BSF for commercial use. After the conversion of sisal decortication waste using BSF larvae, the waste accumulation will be reduced, pollution and harm to the environment will be decreased. Moreover, most of the people produce BSF larvae in a commercial scale which used as a source of protein for poultry and fish (Edea *et al*., 2022; Nyakeri *et al*., 2017).

Various technologies have been applied to manage sisal decorticating waste and produce various products, aiming to minimize waste and maximize the utilization of sisal plants (Kivaisi & Mshandete, 2017; Muthangya *et al.*, 2013). According to Alexandropoulou *et al*. (2017) and Wagner *et al*. (2018), they used two fungal strains: CCHT-1 and *Trichordema reesei* to valorize sisal decorticating waste in biogas generation. The biogas technology deploying decortication waste was viable and the biogas generated was converted into electricity at Hale sisal factory (Colley *et al*., 2021). Moreover, sisal decorticating waste can be used in mushroom farming at household, small scale and commercial scale as well as for biofertilizer production. Apart from that, there has been limited research on the valorization of sisal decorticating waste, and among those that have, there is a rareness of information about the use of BSF larvae in the valorization of sisal decorticating waste. As a result, deliberate efforts to research has to continue and learn more about this technology that will complement other technological management of huge amount of SLDW being generated. Most of SLDW from sisal industries are just dumped into the environment without being treated (Yhdego, 2017). If this problem will not be addressed it will create environmental problems such as leaching of sisal wastewater into the nearby water bodies, plantations, private land etc, which can lead into conflicts. To address the problem, more appropriate and viable technology which reduce the volume of sisal waste being generated and convert it into biomass with financial incentive/commercial value has to be researched. Although black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomydiae) have been employed for treatment of various organic wastes such as excrement, kitchen waste, domestic waste, market waste, and some agricultural and industrial waste. Nevertheless, information on research on the detailed process and mechanism of conversion sisal wastes by the black soldier fly as an environmental protection insect with a large biomass, a high food conversion efficiency, and a high reproductive rate totally non-existent. Therefore, this study intended to manage sisal leaf decorticated waste (SLDW) and adding value on it using black soldier fly larvae BSFL to degrade SLDW and into BSFL for poultry feed and the unconsumed waste used as organic fertilizer.

1.2 Statement of the problem

The BSF technology is an emerging green solution for organic waste management that could improve current practices for handling sisal decortication waste at the end of the production process of sisal fibres (Hu *et al*., 2024; Matheka *et al*., 2022). Sisal decortication wastes is envisaged an important source of biomass energy for BSF technology, however due to its physicochemical properties its utilization efficiency could be low and its processing can be challenging. Therefore, research of its bioconversion by BSF larvae, although is in very early stages it should involve studies of the mechanisms of the bioconversion and degradation by BSF larvae in combination with pretreatments /amendments procedures. Even though it is scientifically established that BSF larvae are versatile in their feed preferences and can consume a variety of organic waste if the available Nitrogen (N) and Total Volatile Solids (TVS) content are high to support the larval growth (Barragán-Fonseca *et al*., 2020; Matheka *et al*., 2022). Sisal decorticated waste being among the saprophagous material suits to be consumed by BSF larvae since they are commonly feed on the decomposing organic matter (Nyakeri *et al*., 2017). Nevertheless, natural ecological factors, such as parasites, microorganisms, hormone analogues, acid–base compounds, corrosive substances, salts, drought, food shortage or oxygen deficiency due to waste source turning anaerobic, temperatures reaching lethal values or elevated heavy metals concentrations exceeding a certain threshold level may prove fatal to larva population, affecting the growth and developmental, increase larval mortality, and reducing the rates of pupation and emerging. The presence of saponin in SLDW can affects the growth of BSFL since high concentrations of saponins reported to cause toxic to insects (Oakenfull & Sidhu, 2023; Zaynab *et al*., 2021) as it causes cell membrane disruption and reduction of ability to absorb food which leads to reduction of larval survival and growth rates (De Geyter, 2012; Mirhaghparast *et al*., 2022). Therefore, one the challenge sisal decortication wastes utilization for rearing BSF larvae would be inherent low pH coupled to saponins and other organic acids which needs to be addressed. On the other hand, one of the challenges applying industrial wastes such as sisal decortication wastes in rearing BSF larvae is its heterogeneous nutritional composition which dependent on the sisal processing employed. The variation in the nutrient content of the sisal decortication similarity impacts reared BSF larvae compositions. Therefore, it calls for research on formulations based on sisal decortication wastes as main substrate for production of targeted end BSF larvae compositions depending on the intended feeds formulation or other desired applications. This study conducted pretreatment methods on sisal decorticated waste so as to meet growth conditions of BSF larvae reared on it by improving their growth rate. The pretreatment methods involve neutralization process and blending of sisal decorticated waste with other waste in small ration to enrich it with nitrogen. The sisal decorticated wastes include solid as well as wastewater which channeled directly to the environment. This creates detrimental effects on both human health and in the environment. This study uses solid waste as substrate or raw material to feed the BSF larvae. Meanwhile, the use of BSF larvae in decomposing organic waste is a reliable method to combat the problem of organic waste from the industries. However, currently there is limited information based on the use of SLDW in rearing BSF larvae and produce valuable agricultural products such as industrial protein (fish and chicken feed) as well as organic fertilizer.

1.3 Rationale of the study

Valorization of solid sisal decorticating waste using BSF technology is a model that provides nutrient recycling, waste reduction and value addition in the form of harvested larva will be used as a protein source for poultry, pet and fish. Furthermore, it is a model which significantly contribute to economic viability, competitiveness and strategic development in environmental management and agriculture. The objectives formulation of this study was constructed in a such a way to gather information's related to proper growth of BSF in managing sisal waste. The interconnectedness of these aspects will contribute to the cooperation and collaboration among actors including political or government stakeholders, economic and society as well. This will influence the sustainability of sisal waste valorization and environmental wellbeing. Indeed a paradigm shifts towards a circular economy aiming to close the loop which is achievable through biowaste valorization, which is an important feature of sustainable development, nutrient metabolism and food economy.

1.4 Research objectives

1.4.1 General objective

Assessment of the valorization potential of sisal leaves decortication waste using BSF larvae technology.

1.4.2 Specific objectives

(i) To characterize the physical and chemical constituents of the sisal decorticated waste from sisal industries in Tanzania.

(ii) To pre-treated sisal leaves decortication waste on the growth and development of black soldier fly larvae.

(iii) To optimize the sisal leaf decortication waste to suit the production of black soldier fly larvae which is suitable for the production of livestock feeds.

1.5 Research questions

(i) What are the physical chemical characteristics of sisal decorticated waste?

(ii) How to pre-treat sisal leaves decortication waste to meet conditions for growth of black soldier fly?

(iii) What other nutrients are needed for a better formulation that will yield better livestock feeds through rearing of the black soldier fly?

1.6 Significance of the study

Through this study the SLDW generated from sisal industries was managed as it is used to feed BSF larvae. Apart from that the industrial protein was produced through harvesting the BSF larvae reared on SLDW. To ensure sustainability the products obtained was sold, this act change the economic status of the people involved in valorization of sisal decorticating waste and those using products formed after the process. Apart from animal feed and biofertilizer other valuable products such as protein powder, oil and cosmetic products can be generated using BSFL which eventually increases the economies of the people.

1.7 Delineation of the study

Despite the best efforts made to accomplish the objectives set which showed promising results, the further development of this technology from an experimental scale into a practicable full-scale plant has not yet been tested. This is constrained by time and finances. While the study aimed to scale up the production of BSF larvae and biofertilizer to a commercial level, organic waste management using *H. illucens*, as a strategies for upscaling this technology are scarce (Diener *et al*., 2011). So far, the BSF technology has been successfully tested in the small scale and showed a good result. However, it remains uncertain to what level the obtained result from small scale can perform to the large scale as an important step for the future industrial application of this technology (Singh & Kumari, 2019). Additionally, the systems for processing organic solid waste and producing larvae based on a batch process so as to have a continuation process based on the BSF life cycle (Guo *et al*., 2021; Yang-Jie *et al*., 2023). In this study, a continuous BSF composting system was not studied; the exploration of certain advanced technologies or the implementation of large-scale pilot projects was beyond the scope of our available resources.

Moreover, this study recognizes that further research and investment are necessary to fully leverage the findings obtained and realize the commercial potential of sisal waste management and product diversification. Future efforts could focus on securing additional funding, establishing strategic partnerships with industry stakeholders, and extending the research timeline to facilitate the scaling up of promising technologies identified in this study.

Apart from that, collaboration with governmental agencies, private enterprises, and research institutions may be crucial for overcoming the barriers to commercialization and ensuring the sustainable development of the sisal industry. By aligning the findings with ongoing initiatives and leveraging collective expertise and resources, the impact of this research can be maximized and contribute to the broader goal of advancing sustainable agricultural practices and rural development.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The sisal industries produce huge amount of waste (Oudshoorn *et al.,* 1995; Srinivasakumar *et al.,* 2013). The majority of those industries are not implementing the technologies that can handle the sisal waste created in during fiber production. This has caused a significant environmental hazard because the waste was not treated before being released into the environment (Colley *et al*., 2021; Singh *et al*., 2021). The act of throwing SLDW in the environment contributed to the emission of greenhouse gases in the atmosphere which has impact in climate change (Peter *et al*., 2019; Woodhouse *et al*., 2015). To ensure the environmental pollution caused SLDW has reduced or come to an end, different technology have been are still going on. In this study, literatures will be analyzed based on existing research for organic waste management around the world, as well as case studies for each of the research objectives being set.

2.2 Key concepts

2.2.1 Waste

This concept described as unwanted or unused substances that resulted from our daily activities and can be seen in our surrounding environment as unpleasant materials. Waste contain same characteristics of the substance accessible in the products generated from it (Amasuomo & Braird, 2016). Waste can be categorized into different groups depends on their characteristics can be, hazardous and non-hazardous waste (Brandl, 2021). Production of waste is huge in urban areas due to various economic activities. Most of the countries struggle to achieve economic growth and sustainable development which led them to engage in industrial activity. Industries are the main polluters if proper measures are not implemented (Saxena *et al*., 2020; Taqui *et al*., 2019). Its everyone role to be involved in waste reduction plans by changing the way of produce, consume wastes (Ferronato & Torretta, 2019). Therefore, even in sisal industries proper measures should be taken to eradicate both liquid and solid wastes being emitted during sisal fibre production.

2.2.2 Waste management

The process of reducing or eliminating the effects of waste, from its origin of production to the disposal centers, is essential towards achieving the managing the waste. In managing waste proper measures should be taken from the way it is collected, transported, treated and disposed by following the rules and regulations in managing each waste category which differs from country to another (Demirbas, 2011). An ecofriendly method should be applied to deal with the waste emission comes from its sources and channeled to the environment (Bharagava *et al*., 2020). This will eliminate the problem of polluting the environment and ensure environmental sustainability. In sisal industries in Tanzania few ecofriendly methods have been applied (Colley *et al.*, 2021; Govindasamy *et al.*, 2020). However, it operates in very few industries due to its cost and advanced technology (Payne *et al.*, 2019; Şafak & Çifçi, 2023). Therefore, there is a need to use other technology such as the use of black soldier fly which will reduce the SLDW as well as providing animal feed and biofertilizer. This technology is cost effectiveness and can be operated with any one at a very low skill.

2.2.3 Valorization of waste

Waste valorization is the process of converting waste materials to other useful products (Roy *et al.*, 2023). This act reduce the environmental impacts and enhance economic benefits due to resource recovery (Ginni *et al*., 2021; Talan *et al*., 2021). Various technologies have been used to valorize waste and obtain products that can be used as a new product (Meninno, 2020). The waste valorization can be applied in various kind of waste including agro waste, plastic, metal and glass. In sisal industries SLDW has been valorized and used to make various products including mushroom and biogas. However, there is limited information on valorizing SLDW using black soldier fly technology. Therefore, this study implements the BSF technology in valorizing SLDW and making animal feed and biofertilizer.

2.3 Theoretical Literature Review

Several existing theories or models can be used to provide a baseline for this study. Here are the theories or models applied in this study.

2.3.1 Bioconversion Theory

The bioconversion theory explains the way organic waste can be converted using biological processes and get valuable products (Bouwer & McCarty, 1984). The biological process used is done by microorganisms or other living organisms including black soldier fly larvae. This can study can apply bioconversion theory to evaluate and understand the proper way of using living organism such as black soldier fly larvae in managing sisal leaves decortication wastes to get the useful products such as animal feed that is protein-rich biomass and biofertilizer. Through bioconversion theory, this study examines BSF larvae in detail as living organisms responsible for converting organic waste. Issues such as the specific stage for active conversion, harvesting time, and biomass conversion efficiencies are closely observed.

2.3.2 Insect Bioconversion Model

The insect bioconversion model explores the key stages concerning the growth and development of insects including their favorable environmental conditions, feeding substrates, pest and disease as well as rearing systems. Moreover, through this model issues like black soldier fly feeding regimes, and strategies to scale up production. This study adopts the insect bioconversion model to ensure the required conditions of black soldier fly are well observed to achieve the intended objectives. The condition.

2.3.3 Socio-economic Model

The socio-economic model describes the interconnectedness between people’s economic activities, their social systems, and environmental factors within their society or region. This study uses the socio-economic model to assess the potential of black soldier fly technology in managing sisal decortication waste and use the products to benefit the community including livestock keepers, farmers, and sisal industries. Therefore, these theories and models, the current study can create a comprehensive understanding of the theoretical foundations, practical implications, and interdisciplinary relationships involved in valorizing sisal leaf decortication waste using BSF technology.

2.4 Inferential literature review

In this part of the inferential literature review various existing research related to this study were addressed to make inferences based on the effectiveness of using the black soldier fly technology in managing sisal leaves decortication waste, its challenges, and potential outcomes of using it as per objectives and research questions being set.

2.4.1 Characterization of sisal leaf decortications waste

Among the characteristics of fresh sisal waste are; fresh sisal waste contains 90% moisture and have a low pH of about four (Kategile, 1986). It also includes a substantial amount of oxalic acid, saponin as well as minor amounts of malic and citric acids. Saponin has toxic compounds used as insecticide (Ellen *et al*., 2007; Qasim *et al*., 2020). The presence of saponin in SLDW that is going to rear BSF larvae can affect its growth rate. Therefore, the afore mentioned qualities, sisal decorticating waste must be pre-treated for pH, acidity, and saponin in order to meet the requirements of BSF rearing. The pH of the sisal waste should be raised to meet the requirements for the BSFL to digest the fibrous waste and ensure its survival (Peguero, 2023). One of the important measures to raise the pH and satisfy the desired condition is to add sodium hydroxide to the fresh sisal decorticating waste (Kategile, 1986). However, biochar made from rice husk can be used to raise the pH (Karam *et al*., 2022; Masulili *et al*., 2010). For the case of saponin, studies suggest that diets with low saponin concentrations of about 0.1-1% can have adverse effects on certain insects (Qasim *et al*., 2020). Therefore, pretreatment should be done by washed water, alkaline treatment and fermentation so as to reduce or remove saponin in SLDW (Gil-Ramirez *et al.*, 2018; Wina *et al.*, 2005).

2.4.2 Pre-treatment of sisal leaf decortication waste

For effective outcomes in using SLDW in making various products pretreatment of it is important. There different methods of pretreating SLDW are: Physical, chemical and biological.

(i) Physical method

Physical pretreatment methods in the valorization of SLDW is vital since it modify the physical structure of the waste material. The method such as collection of SLDW and sorting to remove flume tow enhance its efficient and sustainable utilization.

This method involves the process of separating waste out of the sisal fibre. The technique of extracting sisal fibre by removing the bark or outer layer of the sisal leaf is known as sisal decorticating (Arthanarieswaran *et al*., 2014). The extracted sisal fibre is acquired during the decorticating process when the leaf is crushed between rollers and then mechanically scraped, after which the obtained sisal fibre is washed and dried (Mwaikambo, 2006). The waste generated from the sisal decorticating process is termed as sisal decorticating waste. The waste obtained can further be chopped to reduce its size (Mshandete *et al*., 2006) for it to be easily used in biogas production. The cutting of sisal leaves is also applied in paper making (Qin *et al*., 2022). Physical methods applied to waste generated from sisal production can be utilized for mushroom cultivation, biogas production and even Black soldier fly larvae production for animal feeds (Muthangya *et al*., 2009). In this study physical methods such as sun drying the SLDW and sieving it was done to get the potential SLDW that used as a substrate to rear the BSF larvae.

(ii) Chemical method

The use of chemical method in pretreating SLDW enhance the usability of sisal leaf decortication waste in various applications. The type of chemical method depends on the product one wants to make from SLDW. For in preparation of activated carbon from SLDW different controlled conditions such temperature and time were keenly observed (Dizbay-Onat *et al*., 2017). Moreover, the studies that used chemical methods includes: extraction of saponins from SLDW with more hecogenin of about 460 mg. Kg-1 to be used for pharmaceutical industries (Santos & Branco, 2014), extraction of saponin by using solvents such as ethanol and pure water (Ribeiro *et al*., 2015) which was used in foods, pharmacy and cosmetics (Bezerra *et al*., 2018; Tamura *et al*., 2012). This study uses chemical method in pretreating SLDW including addition of ingredients that makes black soldier fly larvae to grow well in pretreated SLDW.

(iii) Biological method

The use of effective microorganisms as biological pretreatment in making silage under anaerobic condition is also a significant biological method (Kalyuzhnyi *et al.*, 2000). The equipment should be appropriately set in this process to achieve a satisfactory result of silage production, especially in terms of generating a healthy environment for microbial breakdown (Lindmark *et al*., 2012). The biological method can go further into the use of enzymes. In SLDW enzymes has used and show good performance in producing high value pectin (Yang *et al*., 2018). Other researchers have managed to pre-treat the (Muthangya *et al.*, 2013).

Apart from the opportunities that were managed by other researchers and succeeded in pre-treating the sisal decorticating waste and producing valuable products, some challenges were also aroused. According to Rajabu and Manyele (2015) found that the high retention time of substrate which is sisal decorticating residue, high investment costs, and low plant availability were among the challenges for biogas production from sisal decorticating waste. Furthermore, concerns such as waste sourcing and the market for waste-derived products were addressed as large-scale implementation challenges (Zurbrügg *et al*., 2018). Pretreatment of sisal decorticating waste has been carried out for the creation of a variety of goods, as detailed in the literature cited above. However, there is limited information on pre-treating SLDW to make it appropriate for consumption by Black soldier fly larvae.

2.5 Technologies of value addition on sisal waste

Sisal waste can be effectively utilized using various technologies to produce valuable products.

2.5.1 Pharmaceutical

According to Srinivasakumar *et al*. (2013) sisal leaves contain wax (0.38%) and hecogenin (0.10%). Due to the technology developed by the Indian Institute of Science (IISc), Bangalore has invented the technique that uses hecogenin from the sisal waste to produce pharmaceutical raw material for the manufacture of drug including cortisone. Another technology mentioned is the ultrasound-assisted extraction of pectin from sisal waste. The sisal waste was dried, crushed, and stored in a tight container during this procedure. Then, using continuous ultrasound waves at a frequency of 20 kHz and an ultrasonic generator probe that was directly submerged in the suspension, pectin was extracted from sisal waste. Pectin being extracted can be used in pharmaceutical, food, and detoxication (Endress, 1991; Thakur *et al*., 1997).

Production of fungicide and bioinsecticide from SLDW juice was also successful technology in value addition of sisal waste. From the technological process, two products were obtained, one from a juice centrifuge which shows effectiveness in treating *Candida albicans* and the other product was from an aqueous extract of dried decortication residue which also performs well in *Brevipalpus phoenicis* the red and black mite in its application it producing 100% mortality in 12 hours (Cantalino *et al*., 2015).

2.5.2 Biogas production

Sisal residue has been reported to form biogas through processes of anaerobic digestion (Mshandete *et al*., 2005) as a pretreatment method can produce electricity of about 102 GWh which is equivalent to 3% of current power that was produced in Tanzania in 2009 (Terrapon-Pfaff *et al*., 2012). Biogas that was manufactured from SLDW using anaerobic digestion (AD) technology was sufficient for consumption (Salum & Hodes, 2009). Other technology has been used to minimize the size of sisal waste in order to make it easily degradable and produce a significant amount of biogas energy. The sisal waste was dried for 5 days before being ground in a laboratory mill to a particle size of 2 mm, ready for use in biodigester reactors (Mshandete *et al*., 2006). Moreover, it has been observed that sisal waste mixed with fish waste in various proportions has significantly yielded biogas in the process of biogas production from sisal waste (Mshandete *et al*., 2004).

The utilization of SLDW in biogas production enables the sisal industries to have environmental and socio-economic benefits. Initially, the biogas production was only at one site but due to its efficiency it shows led to Tanzania sisal board (TSB) plan to increase production of biogas and electricity production to other sisal estates including include Gomba estate, Rudewa estate and Mkumbara estate. This has been done as there is huge amount of waste generated from sisal industries about 444 000 tons of sisal per year (Mshandete *et al*., 2004). Utilization of this tons of SLDW will add value in sisal production once used to produce valuable product from it.

2.5.3 Animal feed

Due to the increase in the number of livestock and scarcity of fodder created by climatic conditions such as drought and other geographical factors, SLDW has been used as fodder, especially in semi-arid countries (Cantalino *et al*., 2015). Sun-dried SLDW is used as feed for ruminants as it has more nutrients than barley straw. The formulation of sundried SLDW and cotton seed cake as animal feed tends to increase the live weight of sheep and goats (Gebremariam & Machin, 2008). Therefore, for the process of fattening goats and sheep countries like Eritrea and Ethiopia use SLDW as fodder. Apart from that, another technology being reported is ruminant feed manufacture, which appears to boost milk production in cows and sheep fattening (Frank, 1957).

The invention which uses industrial and agro waste such as sisal decorticating waste adds value to it and makes valuable agricultural products (Stratton & Rechcigl, 1998). The sisal waste has been proven to be used as a nutritious animal feed for goats and sheep (Srinivasakumar *et al.*, 2013). Moreover, fish and chicken feeds are also among the agricultural products that can be derived from organic waste using BSFL (Abd El-Hack *et al*., 2020; Barragan-Fonseca *et al*., 2017). The BSF larvae obtained in this process can be further optimized and make pellets that can be used as poultry feed and aqua feed. The BSF larvae can be optimized to contain high levels of protein. This can enhance animal health by attaining the specific nutritional requirements. In this study, SLDW will be pre-treated and further mixed with other supplements to nourish so as to produce the BSF larvae that have the essential nutrient content for fish and poultry.

2.5.4 Mushroom production

The SLDW once thrown in the industrial waste ponds and let to stay for sometimes the mushroom starts to emerge naturally from the decomposing SLDW. However, for more yield of mushroom reared on SLDW studies have been done and reported that SLDW should be supplemented with other materials such as cow dung manure (Raymond *et al*., 2012) and chicken manure (Magingo *et al*., 2004; Mwita *et al*., 2011). The other way of improving mushroom production is by using shredded leaf and dry fiber powder waste, mixed with wheat-bran and triturated charcoal and let it stay for eighteen days (Oliveira do Carmo *et al*., 2021). The mushroom cultivation using SLDW provide essential leads to the understanding of value addition of sisal crop and waste utilization forming valuable products.

Generally, various technologies for value addition of sisal waste have been reported to provide valuable goods such as medication, organic fertilizer, ruminant feed, and biogas. However, there is limited research on the use of Black soldier fly technology to degrade sisal waste by adding value to it and producing poultry and fish feed.

2.6 Optimization of value addition for sisal waste into livestock feeds using Black soldier fly

For commercial or large-scale production of BSF larvae that can meet the demand of the market in animal feed industry optimization is crucial. The process of optimizing can cover the whole process from egg collections to SLDW formulations. In obtaining BSF eggs can be done in the wild or by purchasing eggs from a particular rearing units or farms and allowing them to hatch and develop into mature Black soldier fly. If the capture is from the wild, the instrument should be set up correctly to encourage BSF to lay eggs in it. Regardless of the method used, whether wild collecting or purchasing eggs in the market, the conditions must be appropriate for the growth of Black soldier flies in order to maximize their numbers and make them successful in decomposing sisal waste. According to Sripontan *et al*. (2017), in order to capture BSF eggs in the wild, an egg trap must be set ahead of time, and it must include a rearing box (plastic box), as well as food sources such as vegetables, fruit, manure, or other organic substances. It has been noted that, BSF females prefer to lay eggs near fruit waste rather than animal manure.

The optimum conditions that Black soldier fly needs for them to grow well is a source of substrate as diet formulation, temperature, moisture, pH and sunlight for their proper growth (Chia *et al.*, 2018). The substrates such as fresh brewer's discarded grains (malt/corn starch) and brewer's yeast, both by-products of the brewing industry, can be used as a substrate, as well as chicken or cow dung manure mixed with organic household wastes such leftovers and rotting fruits.

Different organic wastes that treated biologically and used to rear BSF larvae can yield BSF larvae with different nutritional value as shown in the Table 1.

Table 1: Nutritional profile of BSF larvae reared in different organic waste

Substrate	**Energy (Kcal)**	**Nutritional profile (%)**				**Authors**
		Protein	**Fat**	**Ash**	**Dry matter**	
Banana peal	270.72	11.63	3.64	12.95	90.48	Puraikalan (2018)
Food waste from restaurants	375	20.41	19.58		73.01	Cammack and Tomberlin (2017), Carmona-Cabello *et al.* (2020)
Breweries waste	3543.52	18.58	6.75	2.96	21.31	Bravi *et al.* (2021), Rachwał *et al.* (2020), Senthilkumar *et al.* (2010)
Chicken manure	6905	34.50	0.05	26.20	60.27	Halim *et al.* (2017) and Singh *et al.* (2018)
Cow dung manure	4539.54	8.00		12.86	84.50	Maj *et al.* (2021) and Vijayaraghavan *et al.* (2012)
Rice bran	4855	19.25	17.20	4.64	94.60	Bhosale and Vijayalakshmi, (2015)
Pineapple peal	169.2	9.13	1.57	4.81	17.30	Huang *et al*. (2011)

Table 1 above shows different organic wastes that have been used to rear BSF larvae and give positive results in terms of nutritional content required by animal feed. However, research is needed for using SLDW to rear BSF larvae.

The conditions such as temperature, pH moisture content and light were keenly observed. The recommended temperature for BSF to grow varies from hatching of eggs to other developmental stages. According to Chia *et al*. (2018), egg viability and hatchability were detected between 15–40°C, with egg eclosion being very poor below 15°C and above 40°C.

As a result, successful high egg eclosion has been observed at temperatures of 19-35°C. When it comes to growing BSF larvae, it's been found that those raised at 27.6°C and 32.2°C developed successfully (Harnden & Tomberlin, 2016). The BSF larva can grow in the substrate moisture content ranges between 45% - 85% in this interval the maximum growth rate was highest at (0.73 mg day−1) (Bekker *et al*., 2021). Moreover, BSF larvae were unable to develop on diets at 40% moisture while BSF larvae fed diets at 70% moisture developed faster, grew bigger, and required minimal amount of food (Cammack & Tomberlin, 2017). Depending on the type of substrate provided, approximately 20 - 70 mg/larva. day will be consumed; if it is attractive to them, they will consume more (Joly, 2018)

2.7 Availability of sisal leaves decortication waste for Black soldier fly production

In sisal industries there is opportunity of getting enough SLDW for BSFL production throughout the year. Tanzania is among the countries which are world's largest sisal producer of sisal products up to 24 600 tons of sisal products per annum (Mshandete *et al*., 2013). This ensure availability of SLDW as a substrate to rear BSFL as each ton of sisal fiber generates 24 tonnes of solid SLDW (Colley *et al*., 2021).

2.8 Technical Gaps

In valorization of sisal leaves decortication waste various research has been done using different technologies. However, there is limited research on using black soldier fly to valorize sisal leaves decortication waste in the following context:

2.8.1 Substrate pretreatment

There was a lack of information on the specific additives needed to maximize the suitability of SLDW to be used as a substrate for BSF larvae. This includes removing the acidity and addition of ingredients such as potential nutrients to enrich SLDW-based diets for BSF larvae.

2.8.2 Black soldier fly larvae production using sisal leaves decortication waste scale-up and commercialization challenges

Most of the studies have demonstrated the use of BSF technology to valorize other organic waste such as market waste, domestic waste and chicken waste at small scale and large scale. However, there is limited research on both low and large-scale production of BSF larvae using SLDW. Moreover, for this study to be beneficial, it should be scaled up to meet industrial or commercial needs. Technical challenges related to scaling up larval rearing units, optimizing SLDW processes, and ensuring consistent product quality should be considered.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research framework

In this study, different methodologies were undertaken to accomplish the intended objectives. The framework shown in Fig. 1 describes the various procedures used to achieve the results. First, the construction of the Black soldier fly-rearing unit was done, to ensure a safe environment for the survival of the BSF and proper growth of their larva. The BSF eggs were collected at the Muriet dumping site in Arusha where the trap was set. During the capture of the BSF egg, plastic containers with fermented fruit waste and eggs made with wood were used to attract female BSF to come and lay eggs. The collected eggs were transported in a hatching media with a temperature of 30°C and a moisture content of 75%. Meanwhile, the sisal waste was pre-treated to make it useful for BSF larvae to consume. One of the pre-treated measures is aerobic fermentation for it to decompose and make it easily edible by the BSF larvae. The pre-treated waste was valorized by adding essential nutrients such as rice bran, and cow dung manure to increase the nutrient value in it. The BSFL that fed on pre-treated sisal waste, produces an edible material that can be utilized as animal feed. This study has adopted the method of wild mining to obtain the eggs of the Black soldier fly. The wild BSF attracted residues mixed with fermented fruit waste to lay eggs which then hatched and larvae emerged for large-scale production. The valorized sisal waste will then be saved as the substrate for the growth of BSF larva. After 14 days the BSFL was harvested manually, washed in hot water at 65°C for two minutes then sun-dried. Once the moisture content was eleven the sundried BSFL were packed in the paper bags ready for laboratory analysis.

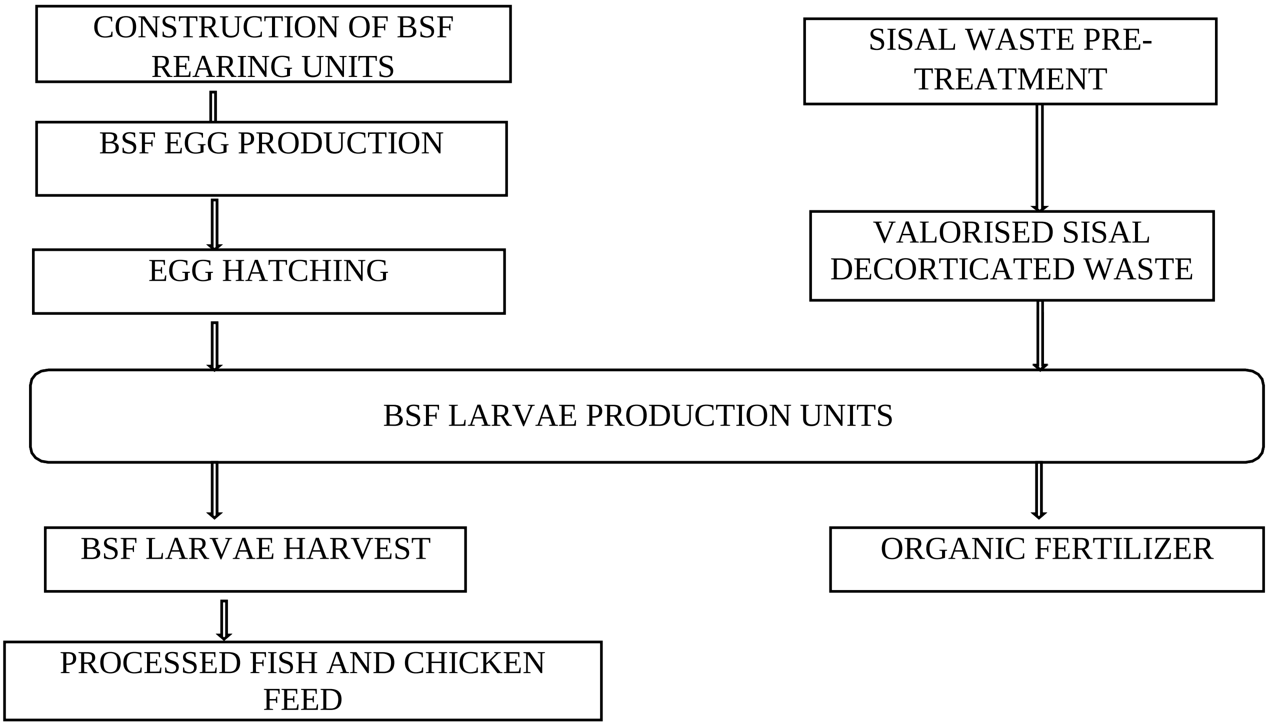

Figure 1: Research framework

3.2 Description of study area

This study was conducted at Mwelya Sisal estate located in the Korogwe district in the Tanga region at latitude 4°88'0"S and longitude 38°28'0" E as shown in Fig. 1. The selection of the study area is due to the availability of sisal waste which is discharged to the environment without being pre-treated. The sample of the sisal waste was collected immediately after the sisal leaf decortication. The sample was sun-dried for five days in the factory's open space. The dried SLDW was packed into the sacs and transported to the Nelson Mandela premises for further process and analysis.

This study was carried out from February 2022 to September 2022 involving visiting the Mwelya sisal estate and intensive study of sisal waste. Then the collection of sisal decorticating waste was done ready for the treatment, data collection, and data analysis at Nelson Mandela Institution of Science and Technology premises.

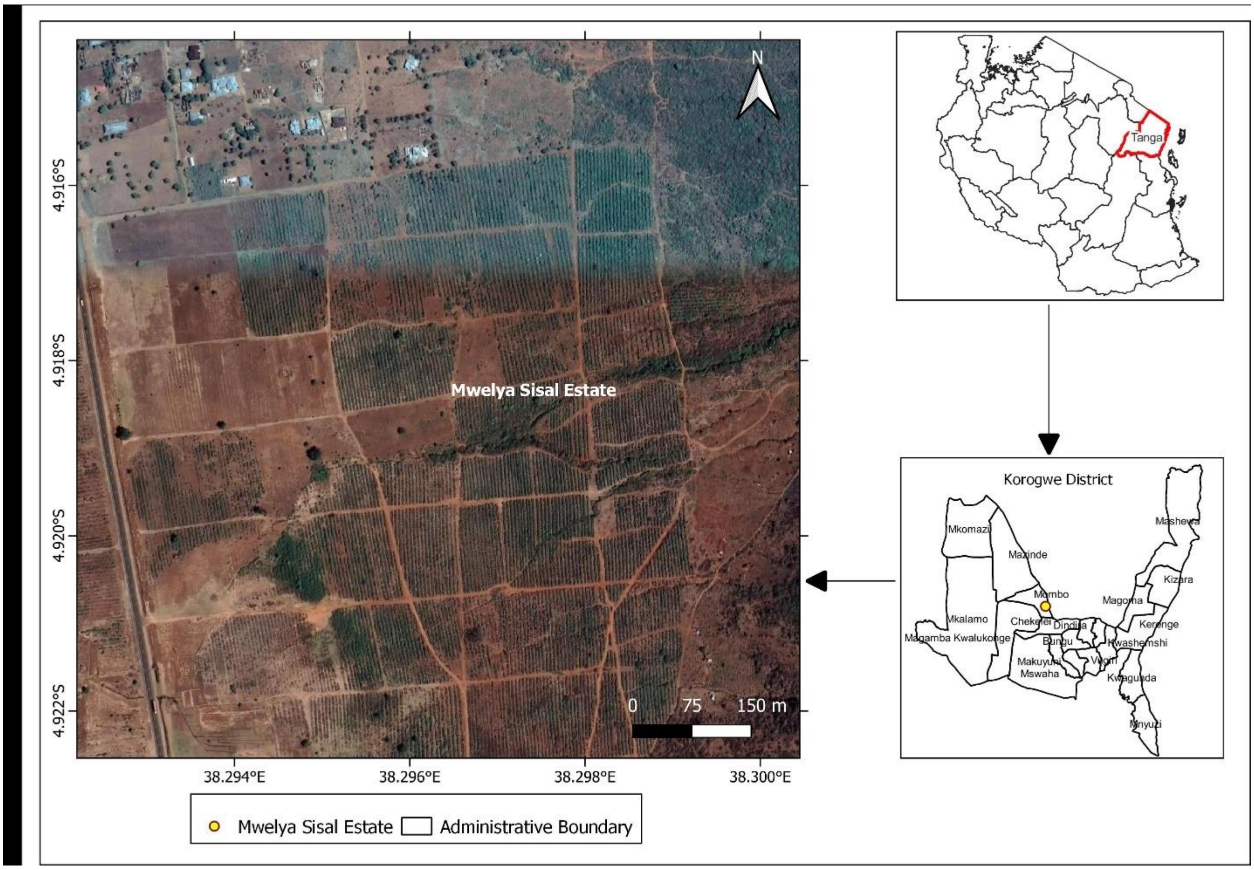

Figure 2: The map showing Mwelya sisal estates

3.3 Black soldier fly-rearing unit

The rearing unit (Plate 1) was constructed at the premises of the Nelson Mandela African Institute of Science and Technology (NM-AIST). Egg-laying traps were prepared consisting of cut corrugated cardboard that were held together with a rubber band. Traps 8 cm long were placed at equidistance (10 cm between and within rows) from each other and 2 cm from a food source (organic waste) set as an attractant. This setup was taken to the Muriet damp site in Arusha and waited to stay for 48 hours to collect BSF eggs. The eggs were put in the box and transported from Muriet to NM-AIST where were incubated at a temperature of 30°C. The young BSF larvae start hatching after three days. The young larvae fed on maize bran for five days before being transported to the SLDW. The rearing unit is constructed in such a way that a cage containing trays with pretreated SLDW was placed inside the roof as shown in Fig. 3 to avoid rain affecting the production of BSFL using SLDW.

Plate 1: A Black soldier fly larvae rearing unit set at NM-AIST with a vertical rack inside the cage

3.4 Sampling strategy, criteria, and procedures

This study involves a generic purposive sampling strategy. The reason for this choice is that generic purposive sampling is a non-probability sampling technique in which the researcher involves a variety of criteria based on research objectives and questions when planning to select a sample population (Casteel & Bridier, 2021; Miyahara, 2019). Therefore, in this study, the sampling strategy, criteria, and procedures were reflecting the objectives being set. The decision to choose the Mwelya factory is due to the sisal production being conducted throughout the year this made the availability of sisal waste to be used in my study all year round. Also, at the Mwelya factory waste is just discharged into the environment without being treated which creates environmental challenges. The second study area is at the Nelson Mandela Institution of Science and Technology premises the choice of this site is due to the availability of open space to construct and set up a BSF rearing unit and also the availability of a laboratory that fits the analysis of the sample worked on.

The sampling of the sisal waste was fresh from the sisal decorticating machine; this enhanced the study to pretreat the fresh decorticated waste that has not been contaminated by other impurities once damped in the environment. Moreover, since the harvesting time is conducted throughout the year and at a cycle of six months the study will collect and analyze data from young sisal leaves of three years and those that have been aged six years since they have been planted and regularly harvested. This will enable us to assess the correlation of waste being generated based on the age of sisal leaves.

Selection criteria for additives such as rice bran and cow dung manure that were added to the SLDW to improve yield of larvae reared in pretreated SLDW was all being the agro waste which could be easily available and at low cost. The amount of rice bran and cow dung manure as described in Table 3.

3.5 Methods of data collection

The methods of data collection were done based on the requirement of each specific objective being formulated.

3.5.1 Characterization of sisal leaves decorticating waste

In this objective, the quantification of the amount of waste generated was done to identify the actual amount of waste being discharged to the environment and which can be suitable to be used to feed BSFL. Moreover, the analysis of chemical nutrient composition such as moisture, dry matter, organic matter, crude protein, crude fiber, total fats, calcium, phosphorus, pH value, and energy (MJ/Kg) in SLDW was done.

(i) Waste generated

To quantify the sisal waste generated (Plate 2), the weight of each bundle comprising 30 sisal leaves was measured using a measuring scale before being fed into the decorticator machine. To produce sisal fiber while the wastes were thrown into the damping place as shown in Fig. 4 (Surjit *et al*., 2022).

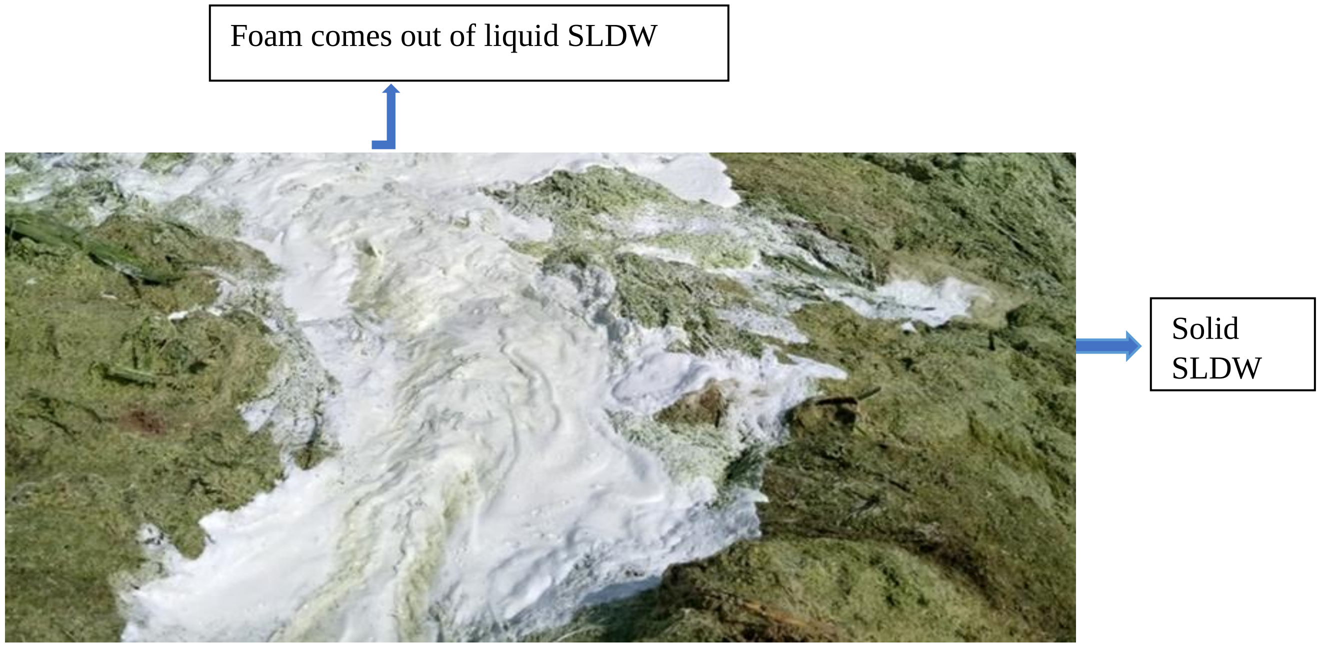

Plate 2: A dumping site of sisal wastes

Wet sisal waste was collected, weighed, and sent to the drying yard. The dried sisal wastes were sieved to separate flume tow and sisal waste residue. The weight of flume tows and sorted SLDW to be used as a substrate to feed BSFL was measured and recorded.

(ii) Physiochemical parameters of fresh and dried sisal decorticated waste

The pH, dissolved oxygen (DO), and conductivity of sisal waste were determined using a Hach 40 HQD Multiparameter meter. These physiochemical characteristics were measured on the site (in an industrial area) immediately after the decortication procedure. Using a 250 mL beaker, the 200 g of wet solid sisal wastes taken from a specific bundle immediately after being decorticated were squeezed to extract sisal waste juice. The multi-parameter probe was placed into the beaker containing sisal waste juice to get five readings from each sample. After cleaning the probe and analyzing the pH, dissolved oxygen (DO), and conductivity the same procedures were conducted to get more five replicates of data as shown in Plate 3.

Plate 3: Measuring physiochemical parameters of sisal leaves decortication waste

Later, pretreated sisal waste prepared as the substrate to rear BSFL was undergoing the process for measuring physiochemical parameters was repeated at the NM-AIST laboratory. The parameters measured after pretreatment were pH by squeezing the liquid comes from pretreated SLDW and put a probe of multiparameter in it. Nutrients in pretreated SLDW including protein, carbohydrate and fat were measured using the methods described in Table 2, concentration of saponin was measured using gas chromatography-mass spectrometry (GC-MS) where the sample was derivatize and inserted into a GC-MS system. The obtained spectra are analyzed to quantify the saponins and elemental profile. The aim was to observe the changes obtained after the pretreatment procedures.

(iii) Elemental profile of sisal decorticated waste

The sample of sisal decorticated waste was sun dried for five days and grounded to course powder using motor and pestle. About 5 g of the powdered sample was processed to make pellets using automatic pelletizer. The pellets being made was analyzed using X-ray fluorescence spectrometry (Mudroch & Mudroch, 1977; Odunlami *et al*., 2022). The parameters analyzed were elemental profile in SLDW. The model of the machine was SPECTRO XEPOS, manufactured by Specro analytical instruments Boschstrasse 10 D-47533 Kleve in German.

3.5.2 Pre-treatment of sisal decorticated waste to meet growth conditions of black soldier fly larvae

The sample of the sisal waste was collected immediately after decortications and fermented aerobically to produce the final product. The series of steps involved includes among the followings:

(i) Sun drying

The wet sisal decorticated waste was collected, weighed, and recorded. Furthermore, the wet sisal decorticated wastes were sun dried for five days until no change in weight (Plate 4). This procedure was done to sort easily the flume tow out of SLDW, required conditions for the rearing of BSF. Sisal decorticated wastes are acidic with a pH of 4 (Terrapon-Pfaff *et al*., 2012) where oxalic acid is reported to be the main (Kavishe *et al*., 2017) and succinic acids being minor (Santos *et al*., 2017).


Plate 4: Drying the SLDW at the yard of the sisal industries

(ii) Sieving

The dried sisal waste was separated from the flume tow by hand and later sieved by a sieve plate of 2 mm diameter (Plate 5) to get the fine sisal waste that can be easily consumed by BSF larvae.


Plate 5: Sieving of sun-dried sisal wastes to get potential waste for BSF consumption

(iii) Aerobic fermentation and neutralization

The preparation process for the substrate involved mixing sun-dried and sieved sisal wastes with water at a ratio of 1:2 and allowing aerobic fermentation at a temperature of 27°C for six days. To enhance the suitability of the substrate for Black Soldier Fly (BSF) larvae, a neutralizing agent was incorporated to adjust the pH (Liou & Liou, 2021), as sisal waste typically possesses a low pH unsuitable for BSF rearing. Additionally, a 100 g wood chip as a bulking agent was introduced to regulate moisture levels within the substrate also to create aeration in the BSFL substrate, ensuring an optimal environment for the growth and development of the BSF larvae (Beesigamukama *et al*., 2021). The biochar made from rice husks was used as a neutralization agent. Following these adjustments, the prepared substrate was ready for the introduction of five-day-old BSF larvae, facilitating their consumption and utilization of the substrate as a nutrient source.

Once the BSFL attained a pre-pupae phase were harvested manually. The pre-pupae (mature larvae) BSFL are collected for immediate use or further processing including drying, milling, and pelleting for use as animal feed. The remaining frass is collected for use as a biofertiliser. The BSF larvae harvested to be used as animal feed were analyzed as shown in Table 2. Fruit waste (FW), containing a mixture of avocado, watermelon, mangoes, and rotten banana was used as a control during the rearing of BSFL using pretreated SLDW.

3.5.3 Optimize the value addition of sisal waste into livestock feeds using Black soldier fly

The act of blending SLDW with other substrates improved the nutritional value of the products (larva of Black soldier fly). Normally, BSFL what they eat is what they will produce, if the substrate is rich in amino acids or omega 3 also the larva will contain the same nutrients (Fuso *et al*., 2021). In this study, since the aim was to produce poultry and fish feed from sisal decorticating waste, the substrate for BSFL was the result of mixing SLDW, rice bran (RB) and cow dung manure (CDW). This combination added value to sisal waste and made the substrate palatable for BSFL to consume. Moreover, the added nutrient which in turn was consumed by BSF larvae and then harvested has a lot of advantage in the growth of poultry and fish (Hu *et al*., 2002). The sampling of BSFL were taken counted into 100, weighed, and recorded. This enabled to assess the gram per larvae reared in RB, CDW. The selection of cow dung manure, rice bran and rice husks as biochar as an additional ingredient to the sisal waste adheres to their availability and affordability in the local environment.

3.5.4 Data handling

The data were collected from the field sites; Mwelya sisal estate, NM-AIST rearing unit and in harvesting unit. The data from both sites were collected, recorded and handled appropriately so that could be easily analyzed (Julita *et al*., 2020). Those that were obtained from laboratory analysis were also recorded in Excel form for analysis.

3.5.5 Laboratory analysis

The analysis comprises of physiochemical parameters of fresh and pretreated SLDW, the nutritional profile of substrate as well as the BSF larvae, and the microbiological profile of BSFL reared in SLDW.

(i) Analysis of the nutrient profile of BSF larvae

The SLDW sample was analyzed to check its content based on moisture, organic matter, crude protein, crude fat, calcium, and energy (MJ/kg). Moreover, the nutrition profile of BSFL reared in SDLW was also analyzed. Different instruments were used as shown in Table 2.

Table 2: Description of data collection methods

S/N	Content	Description
1	Moisture	The moisture content of the sisal waste was measured using the loss on drying method (LOD). • The sample of was taken from the dried SLDW. The aluminum cup was dried at 105^{o}C for 2 hours in the drying oven; measure the constant weight (A gram). • 10g of sample was weighed and put in the dried aluminum cup and measure the weight (B gram). • The sisal waste sample in the cup was dried in the oven at 120^{o}C for 30 minutes. • The cup from the drying oven was taken out, cool it in a desiccator, and weigh it (C gram). • Moisture content of sisal waste (%) = $\left[\frac{(B-C)}{(B-A)}\right]\times 100$ (Mmelikam *et al*., 2021).
2	Organic matter	From the sample that was used to determine moisture, the organic matter was analyzed. • Weigh and record the mass of an empty, clean and dry porcelain dish (Mp). • The entire oven-dried test sample from the moisture content experiment was taken in the porcelain dish, determine and record the mass of the dish and sisal wastes ample (Mpds). • The dish contained sample was placed in a muffle furnace and gradually increase the temperature in the furnace to 440^{o}C. • The sample was left in the furnace overnight. • The porcelain dish was removed carefully using the tongs, and allow it to cool to room temperature. • Weigh and record the mass of the dish containing the ash (burned sisal waste sample) (Mpa). Calculation: i. Determine the mass of the dry sisal waste sample $MD = M_{pds} - M_p$ ii. Determine the mass of the ashed (burned) sample $MA = M_{pa} - M_p$ iii. Determine the mass of organic matter $MO = MD - MA$ iv. Determine the organic matter (content) (Ball,1964). $OM = \frac{MO}{MD} X\ 100$
3	Crude protein	The protein content was analyzed using CHNS / O machine manufactured by Hanna-Kunath-Str.1 128 199 Bremen, German, model flash 2000 3 g of sisal waste sample that was dried and grinded then placed in a sample tray and inserted in the analyzer machine. Leave it for 12 minutes and read the value of Nitrogen (N). Calculation:

S/N	Content	Description
		Protein content=N x protein factor (6.25) (Ayuba, 2004).
4	Crude fat	• The glass ware was rinsed with petroleum spirit, drain, and dry in an oven at 102 º C for 30 minutes and cooled in a desiccator. • A piece of cotton wool was placed in the bottom of a 100 mLbeaker.Putaplug of cotton wool in the bottom of an extraction thimble and stand the thimble in the beaker. • 5 g of sisal waste sample was weighed into the thimble and adding 1.5 g of sand, then mixed the sand sample with a glass rod. • The glass rod was wiped with a piece of cotton wool and place cotton wool in the top of the thimble. • Dry the sample in an oven at 102ºC for 5 hours and allow the sample to cool in a desiccator. • The piece of cotton wool was taken from the bottom of the beaker and place it in the top of the thimble. • The thimble was inserted in a Soxhlet liquid. • Accurately weigh a clean, dry 150 mL round bottom flash and put about 90 mL of normal heptane. The heating of the flask was slowly done to vaporize the heptane. The boiling point of heptane is around 98°C, so maintain the temperature to ensure efficient distillation without decomposing the fat. • As the heptane vaporizes, it passed through the condenser and being collected in the receiver. • The distillation process continued until all the heptane is collected and only the fat remains in the distillation flask. • The distillation apparatus disconnected once distillation was completed, ensuring no heptane vapors are remained. • The distillation flask allowed to cool down to room temperature. • An empty flask was weighed and record the mass. This weight was used to calculate the mass of the recovered fat. • The recovered fat from the distillation poured in the empty pre-weighed flask. • The flask with the recovered fat was weighed and record the mass • The mass of the recovered fat was determined by subtracting the mass of the empty flask from the mass of the flask containing the recovered fat: • Mass of recovered fat=Mass of flask with fat−Mass of empty flask Percentage Yield= $\frac{\text{(Initial mass of fat in sample)}}{\text{Mass of recovered fat}}$ x 100%
5	Calcium	The sample of sisal waste will be dried and grounded to course powder using motor and pestle. The 5 g of the powdered sample was processed to make pellets using automatic pelletizer. The pellets being made was analyzed using X-ray fluorescence spectrometry (Mudroch & Mudroch, 1977).
6	Energy (MJ/kg)	The calorific value of the sisal waste sample was determined using the Adiabatic Bomb Calorimeter manufactured by Parr Instrument Company, in United States and its model is Parr 6200 Isoperibol Calorimeter. • The sisal waste sample was dried, grinded and sieved to get the fine

S/N	Content	Description
		sample. 0.2 g of sample was completely combusted under 3000 kPa pressure and calculations was done to obtain the energy (Musa & Nuruddin, 2015).

(ii) Analysis of microbiological profile of BSF larvae

Since BSF larvae are going to be used as animal feed. This study finds it worth analyzing the harvested larvae to ensure the product is safe and recommended for animal consumptions. The microbes such as *Listeria monocytogenes, Salmonella spp, Escherichia coli, Enterobacter sakazakii, Staphylococcus aureus* which normally affect the animals once are in their feed were analyzed.

For *Escherichia coli* testing, the sample of sundried BSF larvae was collected and seeded on cMacConkey agar at 37°C for 1.5 h The presence or absence of bacterial strains in BSF larvae samples was confirmed using the API-20E system (bioMérieux) (Pławińska-Czarnak *et al*., 2021).

The other test was to analyze for *Listeria monocytogenes* this is a pathogenic bacteria and once it is present can cause food poisoning to animals. The culture of *L. monocytogenes* was conducted using TSYEA at 37 ± 1°C for 24 h (Kurpas *et al*., 2020; Lachtara *et al*., 2021). The loopful of bacteria was transported into 100 µl of TRIS buffer (A & A Biotechnology, Poland) (Lachtara *et al*., 2021). The extraction of DNA was done by the Genomic Mini protocol (A & A Biotechnology) with the modification and addition of 15 µl of lysozyme (10 mg/ml; Sigma-Aldrich, United States) for about 30 min at 37°C (Fleming *et al*., 2021).

Staphylococcus aureus was isolated using gram staining. The strains were tested to check if there is antimicrobial using broth microdilution method-based analysis, Muller Hinton agar (Thermo Fisher Scientific, Oxoid Ltd., Hampshire, UK) (Tomičić *et al*., 2022) and a disk with gentamicin (CN, 30 µg; range tested: 2–16 µg/mL) and enrofloxacin (ENR, 5 µg; range tested: 0.25–16 µg/mL) (Thermo Fisher Scientific, Oxoid Ltd., Hampshire, UK. The testing used a bacterial suspension with 1.5 × 108 CFU/mL (Dégi *et al*., 2022).

Cronobacter sakazakii was tested using culture-based methods, where samples were incubated in buffered peptone water as a non-selective medium. The mixture was enriched with Lauryl Sulfate Broth, to support the growth of Cronobacter while suppressing other bacteria. The isolation process used chromogenic *Cronobacter sakazakii* agar to separate the colonies though were not present. The confirmation of the absence of *Cronobacter sakazakii* in BSFL was done using α-methyl-D-glucoside.

(iii) Analysis of biofertilizer (frass)

Various methods including; potentiometric, gravimetric, Walkley black, dumas, and spectroscopy were used to analyze different parameters found in biofertilizers collected after harvesting the BSF larvae. In analyzing pH and EC, the potentiometric methods were used. This method is based on the measurement of the activity of hydrogen ions in an acid-base substance (Risch *et al*., 2015; Subirats *et al*., 2015). In measuring pH was determined in the ratio of acidic or basic in a compound (Subirats *et al*., 2015). In this study, the pH and EC of unconsumed feed after harvesting the BSF larvae which will be used as a biofertilizer were analyzed using potentiometric. The other method used in this study to analyze the dry matter was gravimetric method. This is the method used to analyze the amount of an analyte found on the mass of a solid (Chen *et al*., 2007; Pilaniya *et al*., 2010). Walkley black was also used in this study to analyze the organic carbon present in the biofertilizer produced (De Vos *et al*., 2007). It uses the certain amount of volume of acidic dichromate solution and then let to react with an amount of soil/frass being analyzed in order to oxidize the organic carbon. After the oxidation the titration of dichromate solution with ferrous sulfate which produce the volume of ferrous sulfate in mL was followed. Finally the organic carbon is calculated using the difference between the total volume of dichromate added and the volume titrated after reaction (Gelman *et al*., 2012).

In addition, determination of the total nitrogen in biofertilizer produced dumas method was used. The 100 g of sample was grounded to a fine, homogeneous powder to ensure accurate measurement. The measured sample was combusted in high-purity oxygen. This is typically provided from a compressed gas cylinder connected to the analyzer In this method the sample of the biofertilizer produced is mixed with copper and then heat is applied up at reasonably high temperatures of 900°C for five minutes while the oxygen (O_2) is being added (Chiam *et al*., 2021). After that the gas mixture obtained is added with copper and oxidizes it into the water, carbon dioxide and nitrogen oxides as by products. Furthermore, the nitrogen oxides are reduced to elemental nitrogen through copper wire. The nitrogen oxide then passes through a potassium hydroxide solution and is collected in a graduated cylinder. After this process, the nitrogen content obtained in the sample can be calculated usually after reading the nitrogen content (Houben *et al*., 2020). To convert detector reading to nitrogen percentage the formular used was Nitrogen (%) = (Mass of Sample (mg)/ Mass of Nitrogen (mg)) ×100.

For determination of phosphorus, potassium, calcium, magnesium, sulphur, manganese, iron, zinc, copper, boron and sodium. The spectroscopy is used to study the interaction of substance with light radiation when waves moves through the sample (Theophanides, 2012).

3.5.6 Optimize the value addition of sisal waste into livestock feeds using black soldier fly larvae

The optimization process was done to consider the addition of waste that can increase nutrients in the substrate of BSF larvae. The carbon to nitrogen ratio is essential on optimizing growth rate of BSFL to be used as animal feed and biofertilizer. The carbon to nitrogen ratio ranged from 16:1–18:1 reported to improve growth rate of the BSFL (Lu *et al*., 2021; Pang *et al*., 2020). The BSFL substrate should have high nitrogen content obtained from food waste, animal manure, and protein-rich materials are high in nitrogen including legume plants that can promote growth of BSFL. The blending of BSFL substrate with carbon-rich materials such as straw, sawdust, rice bran and rice husks will also contribute to the BSFL growth rate.

This study used the formula shown in Table 3 to enrich BSFL substrate with essential nutrients by adding cow dung manure and rice bran as per described ratio:

Table 3: Description of percentage composition used to blend SLDW

Substrate	Percentage composition (%)		
	SLDW	CDW	RB
SLDW	100	-	-
CDW	80	20	-
RB	80	-	20
ALL	60	20	20

The blending of SLDW described in Table 3, enabled to attain a carbon to nitrogen ratio of 16:1 that reported to support the growth of BSFL.

3.5.7 Statistical analysis

The analysis focused on comparing the nutrient content between the substrate before consumption by Black Soldier Fly (BSF) larvae and the harvested larvae themselves. Variables such as pH, moisture content, organic matter, protein content, calcium, fat, and energy were measured to assess the impact of the pretreatment of sisal waste on the nutritional profile. The data obtained were expressed in terms of mean values and standard deviations to provide a clear understanding of the central tendency and variability within the dataset (Mishra *et al*., 2019; Rietveld & Van Hout, 2017). This presentation format allowed for easy comparison between the different nutrient parameters before and after the pretreatment process. To determine significance difference independent samples student t-test was used to compare the means of two independent groups using SPSS (Statistical Package for the Social Sciences). Since, the number of samples were less than 30.

Overall, this analytical approach provided valuable insights into the effectiveness of the sisal waste pretreatment process in enhancing the nutritional quality of the substrate for BSF larvae, as well as the extent to which the larvae utilized these nutrients during their growth and development.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Characterization of sisal leaf decorticating waste

4.1.1 Waste generated

The sisal leaves decortication waste (SLDW) generated during sisal fiber production was analyzed in relation to the age of the sisal plants being decorticated. The results were as follows:

As observed in Table 4, the weight of each bundle (W1) varied even though there was an equal number of sisal leaves. This variation was caused by the age of the sisal plants. The first harvest occurs when the sisal plants are aged three. The harvest is repeated every six months if the farm is effectively maintained and after nine to 12 months if the farm is not well managed (Srinivasakumar *et al.*, 2013). The weeds and other grasses along the rows are removed as part of the management. This study found that older sisal leaves weigh more than younger ones as shown in Table 4.

Table 4: Amount of sisal decorticated waste generated

S/N	Plant Age (Years)	Weight of Bundle W1 (Kg)	Weight of Wet Waste W2 (Kg)	Weight of Dry Waste W3 (Kg)	%Weight of Wet Waste	Mean ± SD (%/*w*) of Wet	%Weight of Dry Waste	Mean ± SD (%/*w*) of Dry Waste
1	3	46.6	33.8	8.7	72.53	68.78 ±	18.67	
2		93.2	68.1	16.31	73.07	6.96	17.5	17.76 ± 0.81
3		47.9	29.7	8.2	60.75		17.12	
4	6	104.3	45.1	17.3	43.37	36.32 ±	16.59	16.41
5		137.6	42	20.1	30.52	6.52	14.61	±1.71
6		143.7	50.4	25.9	35.07		18.02	

According to the results obtained and presented in Table 4, the older sisal leaves produce fewer amounts of waste than younger ones (first harvest) when decorticated. This is evident when the weight of each bundle is compared to the weight of sisal waste generated before decortication. The mean average (68.78 ± 6.96; n=3) of the wet SLDW of 3 years showed a significant difference with the wet SLDW (36.32 ± 6.52; n=3) of 6 years at ($t < 0.05$). Moreover, the mean averages of dry SLDW at 3 years and 6 years were (17.76 ±0.81; n=3) and (16.41 ±1.71; n=3), respectively. Such averages do not show a significant difference at ($t < 0.05$). The obtained data correlated with the previous studies, implying that the amount of SLDW being generated is large amount of SLDW (Dos Santos *et al.*, 2016; Terrapon-Pfaff *et al.*, 2012) enough to fulfill the demand of the substrate needed for BSF rearing.

4.1.2 Physiochemical parameters of fresh sisal decorticated waste

The obtained findings for sisal waste physiochemical parameters are presented in Table 5.

Table 5: Physiochemical parameters of fresh sisal decorticated waste

Physiochemical parameters	Value (x̄ ± SD)
pH	4.5 ± 0.06
Conductivity (µS/m)	1523 ± 0.03

The pH of the fresh sisal decorticated waste as shown in Table 5 was 4.52 ± 0.06 (n=5), which implies that SLDW is acidic. Previous studies reported that SLDW has a pH 4.6 (Zwane *et al*., 2019) and pH of 4.48 (Lima *et al*., 2017). Therefore, this justifies that fresh sisal waste is acidic in nature. This reduce the quality of SLDW to be used as a substrate for BSFL as can inhibit the growth of beneficial microbes, slowing down the decomposition process and reducing nutrient availability. Furthermore, the study found the sisal waste had a conductivity of 1523 ±0.03 µS/m. This indicates that sisal waste has ions and specifically cations (Peter *et al*., 2019). Moreover, those cations are also essential elements for poultry and fish feed.

4.1.3 Elemental profile of sisal leaves decorticated waste

Table 6 shows the essential elements that are found in sisal decorticated waste.

Table 6: Elemental profile of sisal leaves decorticated waste

Elements	Na	Mg	Al	Si	P	S	K
SLDW (ppm)	2378	23 280	1537	5957	15 050	3052	23 283
FW (ppm)	1610	1800	264	915	5447	1801	1373
Elements	Ca	Mn	Fe	Ni	Cu	Zn	Se
SLDW (ppm)	130 800	120	2053	2.7	49	135	0.77
FW (ppm)	17 500	82	566	0.5	13	94	0.4

A total of 14 elements were present in the sisal decorticated waste that was analyzed. Calcium (Ca) was among the elements with a high value of 130 800 ± 0.117 ppm, whereas nickel (Ni) had the least value of 2.7 ± 0.02 ppm (n=4). This elemental profile proves that sisal waste has essential elements that can be fed by BSF larvae and later on produce products required by poultry and fish. According to previous studies, the essential elements that are required for feeding poultry and fish include calcium, iron, magnesium, potassium, sodium, and zinc (Saha & Pathak, 2021). Therefore, sisal waste is effective in the rearing of BSF larvae as it comprises essential elements that are required by poultry and fish (Pliantiangtam *et al*., 2021).

4.2 To pre-treat the sisal leaf decorticating waste to meet conditions for growth of BSF larvae

4.2.1 Sun drying

The sisal leaf decorticated waste that was collected from each bundle was dried and weighed. This enabled to get the potential SLDW with an appropriate moisture content of 11%. The obtained moisture enabled SLDW to be stored for further use without being distorted.

4.2.2 Sieving

The SLDW collected when a single bundle of sisal leaves entered in decorticator machine containing the flume tow which was hard to be digested by the BSF larvae. During pretreatment, the flume tow has to be sorted out of potential SLDW which is required to feed BSFL. Therefore, to get the potential waste that can feed BSF larvae sieving was done as shown in Table 7.

Table 7: The potential weight of SLDW obtained after sieving

S/N	Wet SLDW weight (Kg)	Dried SLDW weight (Kg)	Potential waste weight (Kg)	Flume tow (Kg)
1	48.1	17.3	12.7	4.6
2	37.9	14.8	11.9	2.9
3	56.8	17.5	13.2	4.3
4	22.7	10.7	9.5	1.2
5	68.1	33.3	25.7	7.6
6	59.1	24.3	19.2	5.1
7	78.7	32.6	26.4	6.2
8	55	22.4	17	5.4
9	93.2	47.7	35.9	11.8
10	44.3	30.2	21	9.2

4.2.3 Aerobic fermentation and neutralization

The substrate for rearing BSF larvae should have favorable conditions including the pH the SLDW after undergoing pretreatment procedures the pH was measured in all treatments including SLDW, CDW, RB, and ALL, and the results obtained were as follows:

Table 8: pH of substrates

S/N	Pretreated substrate	pH before pretreatment	pH after treatment
1	SLDW	4.5	7.6
2	CDW	7.71	8
3	RB	6.82	8.1
4	ALL	7.95	7.3

The obtained pH suits the growth condition of BSF larvae in the sense that the weight (gram/larvae) of the harvested BSF larvae was generally good. The study findings show that the sundried, aerobic fermentation and addition of rice husk in the sisal decorticated waste enabled the pH to rise from 4.52 ± 0.06, which was measured in fresh sisal decorticated waste, to 7.57 ± 0.06 of the pre-treated sisal wastes. Other parameters of pretreated SLDW are shown in Table 9.

Table 8 shows that the pH increased compared to that of fresh sisal decorticated waste. This was because it was mixed with rice husk which was reported to raise the pH (Shi *et al*., 2019) at a ratio of 1:30 of biochar to SLDW. The obtained pH also suits the rearing of BSF, as it was reported to grow well at a pH between six and 10. Therefore, sisal decorticated waste should be sundried and mixed with water to be decomposed. The rice husk as a biochar was also added to the sisal decorticated waste to raise pH before being used as a substrate for BSF larvae to yield significant products. This aspect has enabled the utilization of SLDW in the rearing of the BSF larva, thus enabling the once-thought-unfit biomass to be used for the production of larvae with high yields.

Table 9: Parameters of the pretreated SLDW

Parameter	**Mean ± SD**
pH	7.5 ± 0.06
Moisture content (%)	11 ± 0.02
Organic matter (%)	71 ± 0.01
Protein (%)	10 ± 0.01
Energy (kcal)	1615 ± 0.58

The harvested BSF larvae obtained from FW and SLDW are shown in Table 10.

Table 10: Weight of wet BSF larvae

Sample	**Weight of substrate (Kg)**	**Weight of harvested wet BSF larvae (Kg) (Mean ± SD)**
FW	3000	244 ± 4.16
SLDW	3000	336 ± 41.3

The 3000 g of substrate that contained the pre-treated sisal decorticated waste yielded fresh BSF larvae of about 336 g, which was more than the 3000 g of fruit waste that yielded 244 g, as shown in Table 10. Fruit waste (FW) was set as the control since it is organic waste, which is mostly used to feed BSF larvae production (Dzepe *et al*., 2021; Fischer & Romano, 2021). This study proves that pre-treated sisal decorticated waste can yield more BSF larvae compared to fruit waste. The mean weight of FW (244 ±4.16) showed no significant difference with SLDW (381± 41.33) at ($t < 0.05$) and n=3). This implies that SLDW can also be used as a substrate and produce the required output even if the FW is commonly used as a substrate in the rearing of BSF.

4.3 To optimize the solid decortication waste through addition of other nutrients to meet conditions for BSFL and ensure suitability for livestock feeds

The pretreated SLDW has proved to rear BSF larvae (Konyo *et al*., 2022; Varela González, 2017). However, to enrich nutrients obtained from the BSF larvae raised in SLDW, pretreatment and supplement addition were done to be considered as high feed value (Peguero *et al*., 2021). The act of optimizing SLDW before being fed to the BSF larvae has been shown to improve the nutrition value of the substrate which contains a lot of nutrients (Fuso *et al*., 2021). The purpose of this objective was to determine the efficiency of optimized SLDW as a substrate to feed BSF with the expectation that they will produce the desired results in terms of size and weight of the larvae and that the BSF larvae reared on them contain essential nutrients suitable for poultry and fish feed. The decision to use BSF larvae raised on optimized SLDW is based on improving the economic status of livestock keepers by providing BSF larvae as feed for their animals at a low cost (Dewi, 2022; Handayani *et al*., 2021). Furthermore, the use of optimized SLDW as other organic wastes in BSF larvae rearing is environmentally friendly, as it reduces waste by 52–80% (Humairo & Aisah, 2022; Rohmanna & Maharani, 2022). This optimization would enable farmers to substitute costly poultry and fish feed with low-cost BSF larvae as an alternative protein source for local poultry and aquaculture.

4.3.1 Waste reduction

In all substrates (SLDW and FW), BSF larvae managed to consume the waste and grow. Having been harvested, BSF larvae left uneaten substrate which was used to determine the reduced waste in comparison with SLDW and FW trays treated the same, but BSF larvae were not introduced to it. The waste reduced after BSF larvae consumed the pre-treated sisal decorticated waste and fruit waste, as shown in Table 11.

Table 11: Percentage of waste reduced

Samples	Initial substrate weight (g)	Mean ± SD		Authors
		Final weight (g)	%Waste reduction	
FW	3000	646.6 ± 0.26	78	
SLDW	3000	1434 ± 0.05	52	
Kitchen waste			89.6	Mahmood *et al*. (2021)
Fruit waste			72.78	Dzepe *et al*. (2021)
Breweries waste			42.4	Meneguz *et al*. (2018)

BSF larvae managed to reduce dried sisal decorticated waste by 52%, and dried fruit waste as the control was reduced by 78%. The obtained results in Table 11 show a positive correlation with previous studies, which indicates that waste reduction by BSF ranges from 58 to 70% (Mallory *et al.*, 2022), 53 to 80% (Ferronato & Torretta, 2019; Nana *et al.*, 2018), between 50 and 75%depending on the nature of the substrate (Isibika *et al.*, 2021). Therefore, due to these findings, pre-treated sisal decorticated waste can be effectively managed using BSF larvae, since the percentage reductions fall within the range being reported. Moreover, dry matter reduction was also emphasized by Nyakeri *et al*. (2017) whereby reduction of the dry matter ranged from 50.3-81.8% with corresponding bioconversion and feed conversion rates which were between 14.9-20.8%, respectively depending on the substrates being used.

4.3.2 Growth rate of BSF larvae

This study aimed to optimize SLDW so that once will be used to rear BSF larvae it will improve their growth rate as well as nutrients. The improvement of the growth rate enables to harvest of larger BSF larvae and increases the yield of the larvae. This study has found that blending more than two substrates performs better in the growth rate of BSF larvae reared. The mixing of feed improves the efficiency of BSF larvae in consuming the substrate and hence improves their growth rate (Isibika *et al*., 2023).

The results obtained from this study show that the growth rate was higher in ALL which contains 80% SLDW mixed with RB and CDW each with 10% inclusion. An average weight of 0.2 g per larvae was obtained in trays with ALL substrate during the harvested day conducted on day 11. The average weight of BSF larvae reared into other substrates was 0.137 g, 0.122 g, and 0.145 g in CDW, HW, and RB, respectively. The obtained results correlate with other studies that BSF larvae had fed with different substrates of restaurant food waste (0.101 g) (Nyakeri *et al*., 2017). Moreover, the impact of feed formulation has been emphasized by Barragán-Fonseca *et al*. (2018) that BSF larvae reared on well-formulated feed with lots of nutrients perform better in their growth per day. Figure 8, shows how optimized SLDW performed for the growth of BSF larvae. The HW which was set as a control performed low in the end compared to the other substrates. This means that the SLDW can effectively yield better results compared with HW that mainly used as a substrate for BSF larvae rearing.

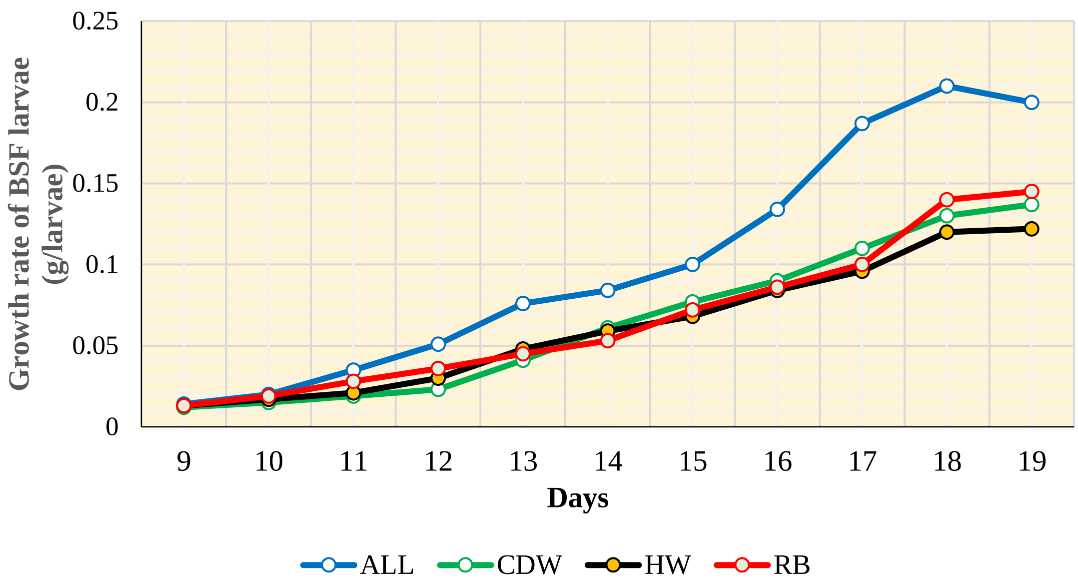

Figure 3: **The growth rate of Black soldier fly larvae reared in optimized sisal leaves decortication waste**

4.3.3 Nutrition value of Black soldier fly larvae fed with optimized sisal leaves decortication waste

The nutritional value of any product determines its quality for it to be recommended as animal feed. This study found the range of nutrients in BSF larvae raised using different substrates. Table 12 shows the nutrients of BSF larvae.

Table 12: **Nutrition value of BSF fed with optimized SLDW**

Nutrients	Substrates			
	ALL	**CDW**	**HW**	**RB**
Crude protein (%)	54.45	44.94	37.08	50.46
Carbohydrate (%)	43.59	40.36	38.17	36.83
Fat content (%)	4.11	4.72	24.91	4.55
Moisture content (%)	10.13	9.87	10.4	9.91
Ash (%)	29.43	25.81	19.42	25.13

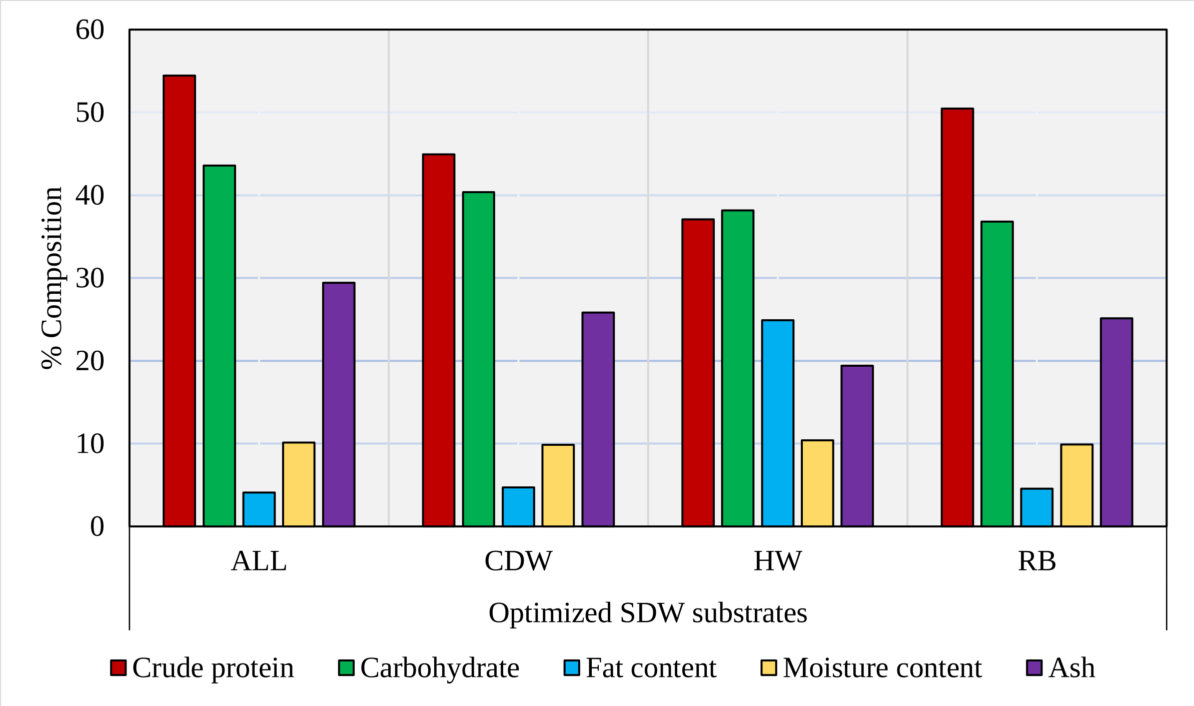

Figure 4: Percentage composition of nutrient content of BSF larvae reared in different substrate

From Table 12, the substrate-HW mainly used to rear BSF, in this study shows a low crude protein of 37.08% while substrate-ALL shows a higher crude protein of 54.45%. The obtained range of crude protein in this study was from 37.08 - 54.45% correlating with other findings such as 43.6% (Nairuti *et al*., 2021), depending on the type of feed given to BSF larvae. In addition, the variation of the percentage composition of the nutrient content of BSFL reared in different substrates was represented in Fig. 9.

The fat content of optimized SLDW was 4.11%, 4.72%, and 4.55% for ALL, CDW, and RB respectively. The obtained findings were also within the range as reported by Abd El-Hack *et al*. (2020). The low fat obtained in optimized SLDW suits it to be the best animal feed (Remillard & Crane, 2010) as it is used to reduce cholesterol in animals, also improve poultry growth rate (Ogbe & Affiku, 2011).

Table 13: The p value of protein content of BSF larvae reared on optimized SLDW

Nutrients	**Substrates (Mean x̄)**				***P*-value**		
	ALL	**CDW**	**HW**	**RB**	**(ALL&HW)**	**(CDW&HW)**	**(RB&HW)**
Crude protein (%)	54.45	44.94	37.08	50.46	0.0013	0.09	0.036

Table 14: t-Test of the protein content of BSF larvae reared on optimized

Substrate	t-Stat	t Critical
ALL	3.936	1.812
CDW	1.411	1.812
RB	2.010	1.812

This study has found that there is a significant difference in the growth rate of BSF larvae reared in ALL and RB when compared with the one reared in HW as a control. The t-statics show that t Stat obtained is 3.936 and 2.011 in ALL and RB, respectively which are higher than the t Critical 1.812 obtained as shown in Table 14. This implies that there is a significant difference between the growth rate of larvae reared in ALL and HW as a control and also a significant difference observed in BSF larvae reared in RB and HW. However, this study shows that in CDW the t Stat 1.412 and t Critical 1.812. This indicates that there is no significant difference in the growth rate of larvae reared in CDW and HW. Therefore, in the choice of additives to optimize SLDW the best ones are ALL and RB which give positive results.

4.3.4 Elemental profile of Black soldier fly larvae fed with optimized sisal leaves decortication waste

The microminerals that are essential in chicken feeds include copper, iodine, iron, manganese, selenium, and zinc. Studies have reported that zinc (Zn) in broiler chicken nutrition is important due to its role in several enzymes and metabolic functions (Huang *et al*., 2019; Ogbuewu *et al*., 2022). The reported essential microelements correlate with the results obtained in this study. Table 15, shows the BSF larvae reared in optimized SLDW have more Calcium, Potassium, Phosphorus, and Magnesium. All these elements show more than 1% in the obtained findings. However, there are other microelements as shown in Table 15.

Table 15: Elemental profile of BSF larvae fed with optimized sisal leaves decortication waste

Substrates	Elemental profile (%)									
	Na	Mg	Al	Ca	Mn	Fe	Cu	Zn	P	K
ALL	0.26	1.74	0.32	12.18	0.02	0.37	0.002	0.014	1.49	2.25
CDW	0.20	2.14	0.22	12.74	0.01	0.22	0.007	0.013	1.47	2.33
RB	0.14	2.34	0.1	12.2	0.02	0.21	0.001	0.018	1.56	2.32

The obtained finding shown in Table 15, correlates with previous studies in some elements. For instance, potassium obtained in BSF larvae reared in poultry was reported to be 1.51% which is related to the potassium obtained in this study (Gärttling & Schulz, 2022).

4.3.5 Analysis of microbiological profile of Black soldier fly larvae

The BSF larvae intend to be used as an alternative source of protein for animals. The BSF larvae were analyzed to see if there is a presence of microbiology organisms as shown in Table 16 including *Listeria monocytogenes, Salmonella spp, Escherichia coli, Enterobacter sakazakii, Staphylococcus aureus.* The presence of these microbes in animal feed may harm the health of the animal consuming it (Liu *et al*., 2022; Wason *et al*., 2021). The results of the safety testing of BSF larvae were as follows:

Table 16: Microbiology profile of BSF larvae

Microbes tested	Unit	Result	ISO limits
Listeria monocytogenes	cfu/g	Not detected	Absent
Salmonella spp	cfu/g	Not detected	Absent
Escherichia coli	cfu/g	Not detected	10 Max
Enterobacter sakazakii	cfu/g	Not detected	Absent
Staphylococcus Aureus	cfu/g	Not detected	Less than 10
Yeast and mould	cfu/g	20	100 max

4.3.6 Socio-economic and ecological benefits of optimized sisal decorticated waste

(i) Edible

The BSF larvae reared on optimized SLDW are harmless and have many advantages to be used as animal feed. They proved to be edible for animals such as poultry and fish (Bessa *et al*., 2020). They are highly nutritious and can used as alternative protein feed source (Bußler *et al*., 2016). Moreover can be processed into different forms such as dried powder or pellets which improves their shelf life (Kamau *et al.,* 2018).

(ii) Nutritive Value

The BSF larvae harvested in a SDW which has been pre-treated and formulated contains important nutrient that are suitable for fish and poultry feeds. This study has found that crude protein ranges from 44.94 - 54.44%, Fat 2.12 – 4.72%, Carbohydrates 36.83 – 43.59%, Moisture content 9.93 – 10.13% and Ash content, 25.81 – 37.42%. The obtained findings correlate with other studies which shows the nutrient content required for animal feed. The fat observed to be very low 2.12 – 4.72%, this is because SLDW used to rear BSFL has limited fat content though the obtained results correlated with other studies which reported the fat content can range from 1.6–24.9% (Finke, 2002). This shows that BSF larvae reared on pre-treated SLDW contain greater nutrient value therefore it is suitable to be used in fish and poultry feed.

(iii) Sustainable protein source at low cost

The use of BSF larvae reared on optimized SLDW will enable farmers to substitute costly animal feed with low-cost BSF larvae as an alternative protein source for local poultry and aquaculture (Siva-Raman *et al*., 2022). There are currently a variety of alternative sources of animal protein for poultry and fish; however, the majorities are prohibitively expensive due to their scarcity and high cost of obtaining raw materials, as well as their lengthy preparation time. This study has found that optimized SLDW has low costs because the raw materials are readily available and can be obtained freely in sisal factories, both small and large, because they are simply thrown into a dumpsite near the factory area.

Moreover, the time required to pre-treat and optimize SLDW is minimal. The SLDW were sun-dried for five days and aerobically fermented for seven days. Therefore, a total of 12 is enough to prepare SLDW to be used as a substrate to feed BSF larvae. Furthermore, the low cost is achieved not only through the short preparation time but also through the use of solar energy as a source of drying energy. Thus, the technology used is also affordable to anyone who wants to do it.

(iv) Environmentally friendly

This study employed a resource recovery technique whereby the waste generated during the production of sisal fibers, instead of being disposed of in the environment, was utilized to rear Black Soldier Fly (BSF) larvae. This approach transformed what would have been a potential source of environmental harm into an opportunity to produce an alternative source of protein for animals. Through rearing BSF larvae on the sisal waste, the study effectively converted this waste material into a valuable resource. The larvae can serve as a high-protein feed source for animals such as poultry, fish, and pets, thereby contributing to sustainable livestock management practices and reducing reliance on traditional protein sources. This resource recovery technique not only mitigates environmental pollution by diverting waste from disposal but also creates a valuable product that can contribute to food security and agricultural sustainability. Overall, the study demonstrates the potential for innovative solutions to address environmental challenges while simultaneously generating economic and ecological benefits

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Based on the first objective which involves characterization of SLDW, the results obtained reveal that SLDW bears characteristics that can be used in the rearing of BSF. The analysis indicates that SLDW has essential nutrients such as crude protein, fats, energy, and microelements that are required in the substrate that is used to feed BSF larvae. However, it was also found that it has some parameters like low pH that hinder sustainable growth and yield of BSF larvae. The results shows the pH of SLDW is 4.5 ± 0.06 which correlates with the previous studies that had a pH of about 5 (Brandon, 1949). Also the same results has been emphasized by other study which reported an SLDW having a pH of 4.48 ± 0.01(Lima *et al*., 2017). Among other parameters obtained after characterization which hinder growth of BSF larvae.

The second objective was to pre-treat sisal leaf decorticated waste so as to meet the growth conditions for BSF larvae. Initially the survival rate of the BSF larvae reared in SLDW was low and even the yield obtained after harvesting was not promising. After pretreating the SLDW so as to improve the quality of it. This act enabled the BSF larvae to consume the SLDW and reduce it by 52%. The obtained waste reduction percent is a little bit lower than the one obtained in Fruit waste 78% which was set as a control and the substrate that commonly used to rear BSF larvae. The waste reduction correlates with other findings by slight differences as follows; waste reduction in swine manure is 56% (Lalander *et al*., 2019) and in food waste is 52.3% (Ermolaev *et al*., 2019) and even lower reduction compared to the SLDW has been than the one reported to be 46.3% in abattoir waste (Siddiqui *et al*., 2022) and 13.2% in digested sludge (Lalander *et al*., 2019). Therefore, this study found that retreatment leads to get parameters requires for growth of BSF larvae and produce BSFL biomass also reduced SLDW by 52%.

The results obtained in the third objective of this study reports that optimized SLDW improves growth rate of BSF larvae and makes it contain suitable nutrients for the formulation of livestock feeds. At day 10 in the optimized SLDW and day eighteen since hatching day the larvae in the substrate named ALL (blended SLDW with rice bran and cow dung manure), its weight was averagely 0.22 g per larvae while those reared in substrate named HW was averagely 0.14 g per larvae. This indicates that optimization of SLDW increases yield of BSF larvae. Apart from that, the analysis explains that optimized SLDW has essential nutrients such as crude protein, fats, energy and microelements that are required in the substrate of BSF larvae. The use of optimized SLDW improves economic status of those engaged in poultry and fish farming as the BSF larvae contains essential nutrients to be used as animal feed obtained at reasonable low cost. Therefore, blending of substrate at genuine ratio of SLDW: CDW: RB improves the quality and quantity of BSF larvae the production for poultry and fish feed.

5.2 Recommendations

The first objective which involves characterization of SLDW, proves that SLDW bears characteristics that can be used in the rearing of BSF sisal industries should adopt BSF technology as their way to manage sisal leaves decortication waste being generated in their industries. This could include constructing BSF rearing units nearby their sisal fiber production sites. This initiative will diversify the income not only from sisal fiber but also from animal feed and biofertilizer produced using BSF technology. Moreover, during characterization this study identify the pharmaceutical components. Therefore, before using SLDW as a substrate for Black Soldier Fly (BSF) larvae, the extraction of pharmaceutical raw materials from the waste can be done. This additional step would enhance the value chain of SLDW utilization, providing opportunities for multiple value-added products derived from a single waste stream.

From the second objective that was to pre-treat sisal leaves decorticated waste so as to meet the growth conditions for BSF larvae this study observes the significant amount of flume tow that was just discarded. However, if this flume tow can be repurposed for the construction of gypsum and other building materials, it could substantially increase the value of the SLDW by generating additional products from it. To ensure effective value addition to SLDW, further research can be conducted to explore the potential for extracting valuable products from this waste material.

Based on objective three that intends to optimize the value addition of sisal leaves decortication waste into livestock feeds using black soldier fly larvae left the biofertilizer (frass) untouched. Therefore, frass obtained during this study can be researched and characterized.

In general, the utilization of agro-industrial wastes, such as sisal decorticating waste, to manufacture various useful goods is highly encouraged. By repurposing these wastes into valuable products, we not only minimize environmental pollution but also contribute to sustainable resource management and the circular economy.

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APPENDICES

Appendix 1: Patent certificate on the invention of this study

THE UNITED REPUBLIC OF TANZANIA

Form No 12

UHURU NA UMOJA

THE PATENTS (REGISTRATION) ACT CAP 217 R.E. 2002

CERTIFICATE OF GRANT OF PATENT

Regulations 29(3)

In accordance with Sections 28(1) of the Patents (Registration) Act, Cap. 217 R.E. 2002, it is hereby certified that a Patent having the number TZ/P/ 2023/00069 has been granted to:

Name of the owner: Nelson Mandela African Institution of Science and Technology
Address of owner: P.O Box 447, Kikwe, 23311, Nduruma village, Nelson Mandela Road, Plot no. 138/1/E, Block Nduruma, Arumeru, Arusha, Tanzania

Name of the inventor: AZIZA ATHUMANI KONYO; ANTHONY MANONI MSHANDETE; REVOCATUS LAZARO MACHUNDA and LILIANE JOSEPH PASAPE.
Address of the inventor: Kikwe, 23311, Nambala, Arumeru, Arusha; Kikwe, 23311, Nambala Arumeru, Arusha; Maji ya chai 23302, Arumeru, Arusha and Moshono, 23118, Arusha CBD, Arusha, Tanzania

On September 18, 2023

In respect of an invention described in an application for that Patent Certificate having a

Date of Filing of May 15, 2023

Being an invention for: A METHOD FOR REARING BLACK SOLDIER FLY LARVAE ON SISAL LEAF DECORTICATION WASTE

REGISTRAR OF PATENTS ★ TANZANIA ★

S. Kasera
DEPUTY REGISTRAR OF PATENTS

September 18, 2023

Appendix 2: prototype obtained from this study: Animal feed and bio fertilizer

Appendix 3: Prototype obtained from this study - animal feed and biofertilizer

Appendix 4: Product exhibition in Sahara venture sparks as one of the winners in the University project

Appendix 5: Attending the nanenane exhibition to showcase the BSF technology which produce animal feed and biofertilizer

RESEARCH OUTPUTS

(i) Publication

Konyo, A. A., Machunda, R., Pasape, L., & Mshandete, A. (2022). The Potential of Valorized Sisal Decorticated Waste in Rearing of Black Soldier Fly. *Recycling*, 8(1), 1-12.

(ii) Patent

A Method for Rearing Black Soldier Fly Larvae on Sisal Leaf Decortication Waste from registrar of patent Tanzania having the number TZ/P/2023/0069

(iii) Viable agricultural products

a. Animal feed rich in protein
b. Biofertilizer

(iv) Poster presentation