UNIVERSITY OF EDUCATION, WINNEBA

EFFECTS OF ETHANOLIC AND AQUEOUS LEAF EXTRACTS OF SOURSOP (*ANNONA MURICATA* L.) ON *SITOPHILUS ZEAMAIS* MOTSCH. AND *CALLOSOBRUCHUS MACULATUS* F.

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DECLARATION

Candidate's Declaration

I, **Elizabeth Owusuaa,** hereby declare that apart from materials reviewed as literature that have been duly acknowledged, this thesis is my own original work and that; this work has never been presented for any degree either in part or in whole to the university or elsewhere. Work done by other authors that served as useful source of information have been duly acknowledged by making reference to them.

Signature……………………….……………

Date…………………………………………

Supervisor's Declaration

I, **Mr. David Osam Acquaye,** hereby declare that the preparation and presentation of this work was supervised in accordance with the guidelines for supervision of thesis as laid down by the University of Education, Winneba.

Signature………………………………………

Date……………………………………..……

DEDICATION

I dedicate this work to my dearest husband, Mr. Oppong Appiah Felix. Throughout this challenging and rewarding academic journey, your support, love, and encouragement have been my guiding lights.

Thank you for being my anchor, my confidant, and my source of endless inspiration.

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ABSTRACT

Sitophilus zeamais Motsch. (maize weevil) and *Callosobruchus maculatus* F. (cowpea weevil) pose formidable challenges to agricultural productivity and food security, causing substantial post-harvest losses in grains and legumes. This study investigates the potential effects of ethanol and aqueous leaf extract of soursop (*Annona muricata*) on these two economically significant pest species. The study employed a comprehensive biochemical laboratory analysis, and evaluated the impact of four (4) varying concentrations of ethanolic and water extract of soursop leaf on the mortality, and reproductive ability of the two insect species. It further assesses the potential effect of the two soursop extracts on the quality of stored maize and cowpea. The study findings showed that ethanol was able to extract phytochemicals such as alkaloids, tannis, and triterpenoids which were absent in the water extract. The results of the study further revealed that both ethanol and water extract exhibited significant difference in insecticidal properties against cowpea weevils with varying degree of efficacy (*p-value<0.05*). Higher mortality in the insects was recorded in the treatment with water extract. On the contrary, there was no statistical difference (*p-value>0.05)* in various concentrations of the two soursop extract on the mortality of *S. zeamais* and *C. maculatus.* Moreover, comparatively to the control, there was less reduction in weight and the overall quality of the cowpea and maize grains with treatment application. Lastly, there was less increase in the progeny of *S. zeamias* and *C. maculatus* in the treatment as compared to the untreated. It is therefore imperative to conduct field trials in actual grain and legume storage facilities to validate the efficacy of soursop extract under real world conditions.

CHAPTER ONE

INTRODUCTION

1.0 Overview

This chapter considered the background to the study, statement of the problem, purpose and objectives of the study, and significance of the study, scope of the study, limitations of the study and the organization of the rest of the study.

1.1 Background to the Study

Cereals and legumes are considered staple food for a large number of people in Asia and Africa (Serna-Saldivar, Tellez-Giron, & Rooney, 1987; Murty & Kumar, 1995). Maize has been reported by Darfour & Kurt (2016) as the leading cereal grain food produced in Ghana as it accounts for over 50% of the total cereals produced annually. These food crops (cereals and legumes) are rich in carbohydrates, proteins, vitamin B complex, and minerals, and hence, provide a lot of health benefits to humans, and as such, are literally referred to as poor man's food (Kumari, 2017). However, the production and storage of cereals and legumes in our part of the world faces a lot of challenges caused by insect pest destruction.

The commonly known insect pests that cause reduction in the quality and quantity of cereals and legumes in Ghana includes but not limited *to Sitophilus zeamais* Motsch. (maize weevil) and *Callosobruchus maculatus* F. (cowpea weevil). It has been reported by numerous researchers including Throne (1994), Ress (2004), and Ojo & Ogunleye (2013)) that *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F. are common pests that cause both qualitative and quantitative damage to farm produce mainly grains, legumes and cereals on the farm and during storage in sub-Saharan Africa.

The female adult weevils bore holes into grains and legume kernels and lay their eggs and this result in a consequential reduction in the quality of the food. Farmers in their desperate attempt to protect their crops and investments have relied heavily on the use of synthetic insecticides. These synthetic and inorganic pesticides have surpassed their beneficial effects (Chen, Shen, Chen, & Wan, 2019). The uses of synthetic pesticide to kill pests and insects that attack crops have a serious repercussion on the environment and human health. Similarly, Bardin, Fargues, and Nicot (2008) reported that synthetic pesticide use inadvertently affects non-target plants and animal species and as such distort the ecosystem.

Due to these associated risk with the use of chemical and synthetic pesticides, their application for pest control have been banned in some developed countries (Emeasor, Uwalaka, & Naji, 2017). A new paradigm shift from the usual chemical and synthetic insecticide to the use of bio-pesticides which involves the use of plant extract is now underway. Modern day researchers are now interested in investigating the use of plant extract as a possible replacement for the synthetic insecticides as they are apparently safer and are more readily available and cost effective (Koomson, Darkwah, Miwornunyuie, & Puplampu, 2020).

Plants extract such as leaf, root, bark, flowers, and seed extract of soursop *(Annona muricata* L.*)* have been reported as effective herbal extract that is known for use as anti-inflammatory, antimalarial, anticancer and anti-inflammatory by Ministry of Health (2018), and a similar finding has been reported by Naspiah, Nashruhim, and Fitrani (2013).

Additionally, Ogbuehi and Onuh (2019) recounted how leaf extract of soursop was effective in the control of fleabettles (*Podagrica* spp.) and therefore had positive impact on the yield of okra (*Abelsmoschus esculentus* L. Moench).

Therefore, it is not out of context that this study sought to examine the biopesticide effect of ethanolic and aqueous leaf extracts of soursop (*Annona muricata* L.) in the control of *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F. in stored grains (maize and cowpea) in the laboratory.

1.2 Problem Statement

Agriculture faces a lot of challenges arising from pest damage during the initial stage of the cultivation of crops, through field management, harvesting of crops, to storage of farm produce.

The postharvest losses (PHL) of grains and legumes caused by insect pests tend to be heavy. According to Aulakh and Regmi, (2015) there is a significant reduction in both the quality and quantity of crop from the time of harvest to consumption caused by insect pests. Aphilis (2014) estimated that the PHL associated with maize is between 15-26%.

The greatest portion of these losses in legumes, grains, or cereals occurs whilst the un-harvested crops are still on fields as well as during storage and these is usually caused by insect infestations. Among the pests that cause these destructions to maize and cowpea grains are *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F. (Tefera, 2012), and these insects have routinely been controlled with the use of inorganic pesticide.

However, the increasing chemical pesticide use over the past decades poses serious health consequences on humans with the most vulnerable ones being infants, young children, agricultural farm workers, and pesticide applicators (Pesticide & Human Health, 2014). These effects may be immediate in nature or they may take months or years to manifest. The harmful effects of chemical pesticide use have been reported to be implicated in human studies of leaukemia, lymphoma, cancer of the brain, still birth, spontaneous abortion, sterility and infertility (Bardin *et al*., 2008).

It is against this backdrop that environmentalists and biologists have advocated for the use of bio-pesticides instead of inorganic or synthetic pesticides in the control of pests since they are less detrimental to the environment and less harmful to living things. Even so, little improvement has been made in the adoption of bio-pesticide in the control of pests in food crops and therefore has led to the new pathway in scientific enquiries. Researchers such as Amalia and Yusa (2018) have reported how extract of soursop is effective in the control of leaf caterpillar (*Plutella xylostella*) on rose apple plant. Yet, there is paucity of information on the efficacy of soursop in the control of *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F. in stored grains, legumes, and cereals.

It is in this regard that this study sought to ascertain the efficacy of soursop as a biopesticides in the control of *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F. in stored grains.

1.3 Purpose of the Study

To determine the effects of ethanolic and aqueous leaf extracts of soursop (*Annona muricata* L.) on the control of *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F. in stored grains (maize and cowpea).

1.4 Objectives of the Study

This study sought to:

1. determine the phytochemical constituents of the soursop leaf extracts of *Annona muricata* L. using ethanol and water.
2. determine the effect of minimum concentration of soursop leaf extracts of *Annona muricata* L. as a biopesticide against *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F.
3. compare the efficacy of the two soursop leaf extracts of *Annona muricata* L. on stored grains of maize and cowpea.
4. determine the effect of soursop leaf extracts of *Annona muricata* L. on reproductive ability of *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F.

1.5 Significance of the Study

Firstly, the outcome of this study will bring to light the phytochemicals present in both ethanolic and water extracts of soursop leaves *Annona muricata* L. Also, this study will help ascertain the biopesticide effect of ethanolic and aqueous leaf extract of soursop (*Annona muricata* L.) in the control of *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F. in the stored grains of maize and cowpea. Moreover, even though this study determines the biopesticide effect of ethanolic and water leaf extracts of soursop in stored grains under laboratory conditions, effectiveness of soursop leaves extract will be relevant to the storage of grains in silos and barns across the nation.

Furthermore, the outcome of this study could provide useful information to stakeholders such as the Ministry of Food and Agriculture in the establishment of an effective farmers' education program aimed at improving the adoption and utilization of biopesticide in the storage of food crops, such as cereals and legumes. Exploring the efficacy of soursop extract in the control of maize and cowpea weevils will inform agrochemical producing companies on the required concentration of soursop leaf extracts of *Annona muricata* L. to be used in the production of soursop biopesticide.

In addition, the findings of the study will provide valuable insight into the practical application soursop leaf extracts of *Annona muricata* L. in enhancing food security and reducing economic losses in agricultural communities. Lastly, this study will serve as a basis for future research, since it will serve as a useful source of information to researchers.

1.6 Scope of the Study

The study sought to find out the phytochemical constituents of both ethanolic and aqueous extract of soursop. Also, this study focused on using soursop leaf extracts of *Annona muricata* L. as a biopesticide for the control of *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F. in stored grains. Different concentrations of soursop extracts (ethanolic and water) were used to determine the most effective concentration needed to control insect pests of stored grains. Only extract from the leaf of the soursop of *Annona muricata* L. was used, and the other parts of the soursop plant was not used. Also, only fresh harvested dried grains of maize and cowpeas less than one (1) week old without any synthetic or any form of pesticide applied after cultivation were used in the study.

1.7 Limitations of the Study`

The outcome of the study may be affected by environmental variations such as temperature, humidity and light intensity as according to researchers such as Credland, Dick, Wright (1986). Life cycle of *S. zeamais* and *C. maculatus* tend to be reliant on these factors.

Also, the study was conducted on a small scale under controlled laboratory conditions, and hence, the findings may not represent real-world storage conditions and dynamics of larger scale storage facilities or field conditions.

In addition, the duration of the storage period in this study was relatively short, and long-term storage conditions may present different challenges and effects on the quality of maize and cowpea.

Moreover, the study should have been expanded to cover field crops of the maize and cowpea and other types of food crops as different crops respond differently to soursop extract, and their storage characteristics may vary. Thus, the findings of the study may not be generalized to other food crops outside the selected food crops and storage conditions. Therefore, generalization of the result should be done cautiously.

Additionally, acquiring grains which have never had any form of pesticide applied was really difficult, and hence, the results could have been altered by any form of pesticide that was applied on the crop during the cultivation period by farmers.

Lastly, the purity level of the soursop leaves may have interfered with the results of the study.

1.8 Organization of the Study

The study has been presented under five chapters. Chapter one focuses on the background to the study, statement of the problem, purpose of the study, research objectives, significance of the study, scope of the study, and some limitations to the study. The second chapter reviews related literature to the study. The third chapter discusses the methodology that was used for the study, with the fourth chapter dealing with the presentation and discussion of the results obtained. Finally, chapter five gives summary of findings, conclusions, and recommendations.

CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

This chapter has five major parts. The first part details with a general literature review on maize which encompasses a brief description of maize, production and trade in Ghana, importance and uses of maize, and post-harvest losses associated with maize production. The next part is also about the general description of cowpea which comprises a brief description of cowpea, production and trade, importance and uses of cowpea, and the post-harvest losses associated with cowpea production. The third part talks about overview of biopesticides, overview of phytochemicals, brief description of *Sitophilus zeamais* Motsch. (maize weevil), destructions caused by *Sitophilus zeamais* Motsch. (maize weevil), brief description of *Callosobruchus maculatus* F. (cowpea weevil), destruction caused by *Callosobruchus maculatus* F. (cowpea weevil), control of storage pests of Grains and Legumes. The fourth part consists of brief description and production of soursop, uses of soursop. The last part deals with the Empirical studies.

2.0.1 Overview of bio-pesticides

Biopesticides, a captivating category of pest control agents sourced from nature, including microorganisms, plants, and biochemicals, have garnered substantial recognition and interest recently. They are prized for their eco-friendly nature and potential to provide effective pest management while mitigating adverse impacts on ecosystems and non-target organisms. This comprehensive examination explores selected pivotal literature on biopesticides, offering insights into their multifaceted applications and contributions to sustainable agriculture.

Isman (2006) seminal work serves as a foundational reference for understanding the potential of botanical extracts and essential oils as biopesticides. These naturally occurring compounds have emerged as promising alternatives to synthetic chemicals, offering a more sustainable approach to pest control. As Isman highlights, botanical biopesticides offer advantages extending beyond pest control. They often come with appealing fragrances, making them more acceptable in applications where traditional pesticide odors raise concerns. Additionally, their biodegradability and low environmental persistence render them environmentally friendly choices for farmers seeking to reduce their chemical footprint in agriculture. Expanding upon this foundation, Kumar and Poehling (2006) delved deeper into the ecological benefits of biopesticides. Biopesticides are categorised into three primary groups: microbial pesticides, botanicals, and biochemicals. This systematic classification provides a comprehensive perspective on the diversity within the biopesticide domain. Microbial pesticides harness the power of microorganisms such as bacteria, fungi, and viruses to combat pests. Botanicals, conversely, utilize plant extracts or essential oils as active ingredients, often derived from aromatic plants like neem, pyrethrum, and rosemary. Biochemicals consist of naturally occurring compounds like pheromones and insect growth regulators, which disrupt pests' developmental processes. Chandel and Kaur (2019) review reinforces the ecological benefits of biopesticides by emphasizing their role in promoting sustainable agriculture. Biopesticides exhibit multiple modes of action against pests, making it challenging for them to develop resistance, a growing concern with synthetic pesticides. Furthermore, biopesticides have the potential to reduce pesticide residues in food crops, addressing food safety and consumer health concerns. These advantages align with the global shift towards sustainable agricultural practices and the growing demand for organically produced food.

Lacey and Shapiro-Ilan (2008) review shifts attention towards microbial biopesticides, providing insights into their practical application. In orchard systems and integrated pest management (IPM) programs, microbial biopesticides have emerged as valuable tools. Entomopathogenic nematodes, for example, are microscopic worms that parasitize and eliminate insect pests. Bacteria such as *Bacillus thuringiensis* produce toxins lethal to specific pests, offering targeted and environmentally friendly pest control solutions.

Isman (2020) review delves into the commercial aspects of biopesticides, providing a forward-looking perspective. The commercial development of plant essential oils and their constituents as active ingredients in biopesticide products has gained momentum. However, this development comes with its share of challenges, including issues related to production scalability and cost-effectiveness. The review underscores the need for continued research and innovation to overcome these hurdles and unlock the full potential of botanical biopesticides in mainstream agriculture.

Thakur and Geetanjali (2016) review revisited the current status and future prospects of biopesticides, particularly in the context of sustainable farming practices. Their review recognizes the pivotal role of biopesticides in achieving sustainability goals by reducing chemical inputs, minimizing environmental harm, and conserving beneficial insects and pollinators.

Dara and Dara (2019) complement this perspective by providing a comprehensive overview of the opportunities and challenges surrounding biopesticides in sustainable agriculture. They emphasize the multifaceted nature of biopesticides, which not only control pests but also offer benefits such as improved soil health and reduced pesticide residues in harvested crops. However, challenges such as regulatory hurdles and the need for farmer education and training remain to be addressed for widespread adoption.

Kumar, Mishra, and Malik (2018) contribute to the expanding body of literature by delving into the exciting realm of botanical insecticides. These biopesticides leverage the natural defensive properties of plants, which have evolved over millennia to protect themselves from insect pests. The authors highlight the growing body of evidence supporting the efficacy of botanical insecticides in pest management strategies, showcasing their potential as effective alternatives to synthetic chemicals.

Furthermore, Kumar and Poehling (2017) present a compelling case for the integration of biopesticides into integrated pest management (IPM) approaches. This integrated approach combines multiple pest control strategies to optimize efficacy while minimizing environmental impact. Biopesticides, with their compatibility with other IPM tools like biological control agents and cultural practices, fit seamlessly into this holistic approach to pest management.

Lastly, in 2008, Koul and Dhaliwal offered a unique perspective by examining the utilization of biopesticides within the context of Indian agriculture. India's diverse agroclimatic regions and high population density make it particularly vulnerable to pest-related challenges. The review sheds light on the specific challenges and opportunities associated with biopesticides in addressing these issues. It emphasizes the need for tailored solutions and increased awareness among farmers about the benefits of biopesticides in reducing yield losses and enhancing food security.

These comprehensive reviews collectively highlight the remarkable significance of biopesticides in modern agriculture. They spotlight the potential of biopesticides to provide effective pest control solutions while minimizing harm to the environment and non-target organisms. As the world continues to seek sustainable pest management practices, biopesticides stand out as a promising and environmentally responsible choice. Their continued development and adoption hold the key to a more sustainable and eco-conscious agricultural future.

2.0.2 Overview of phytochemicals

Phytochemicals, bioactive compounds found in plants, have captivated researchers and health enthusiasts alike due to their potential health benefits. These compounds are abundant in fruits, vegetables, herbs, and whole grains, and their diverse chemical structures contribute to various physiological effects in the human body. This review delves into a selection of key literature on phytochemicals, shedding light on their multifaceted roles and contributions to human health.

Dr. Watson Ross Ronald's seminal work in 2008 offers a foundational understanding of phytochemicals. He defined phytochemicals as non-nutritive compounds found in plants that possess health-promoting properties. These compounds have gained attention for their antioxidant, anti-inflammatory, and anticancer properties. They are divided into several classes, including flavonoids, polyphenols, carotenoids, and glucosinolates, each with unique health benefits. Expanding upon this foundation, Fraga (2005) review delves deeper into the antioxidant properties of phytochemicals. Antioxidants play a critical role in neutralizing harmful free radicals in the body, thereby reducing oxidative stress and its associated diseases. Phytochemical-rich diets have been linked to a lower risk of chronic diseases such as heart disease and cancer, partly due to their antioxidant content.

Hannan, Dash, and Sohag (2020) examined the potential of phytochemicals in mitigating the risk of metabolic syndrome. Metabolic syndrome is a cluster of conditions, including obesity, high blood pressure, and insulin resistance, that significantly increase the risk of heart disease and type 2 diabetes. Phytochemicals, particularly those found in fruits and vegetables, have been shown to improve various components of metabolic syndrome, offering a natural and holistic approach to managing these conditions.

The immune-boosting properties of phytochemicals come under the spotlight in various studies. Meydani and Ha (2018) study discusses how phytochemicals, such as flavonoids and polyphenols, can modulate immune function. These compounds have been shown to enhance the body's defense mechanisms, making it more resilient to infections and chronic diseases.

Baer-Dubowska's in 2003 provided insights into the chemo-preventive potential of phytochemicals. Chemoprevention involves using natural compounds to inhibit the development of cancer. Phytochemicals like sulforaphane, found in cruciferous vegetables, have demonstrated promising anti-cancer properties by influencing various cellular processes involved in tumor growth.

Furthermore, the role of phytochemicals in promoting cardiovascular health is explored in a review by Mattes (2010). He discusses how phytochemicals, particularly those found in nuts and berries, can contribute to reduced risk factors for heart disease, including improved cholesterol levels and blood pressure regulation.

Singh and Singh (2017) researched into the epigenetic effects of phytochemicals. Epigenetics refers to changes in gene expression that do not involve alterations to the DNA sequence. Phytochemicals like curcumin and resveratrol have been shown to influence epigenetic modifications, potentially affecting the development and progression of diseases like cancer.

Lastly, Seeram (2018) explored the emerging field of phytochemicals as neuroprotective agents. These compounds have shown promise in supporting brain health and potentially delaying cognitive decline. Polyphenols found in foods like berries and green tea, for example, have been associated with improved cognitive function and a reduced risk of neurodegenerative diseases.

These comprehensive reviews together highlight the remarkable significance of phytochemicals in human health. They focus the potential of phytochemicals, abundant in a wide variety of plant-based foods, to provide multifaceted health benefits. As the field of phytochemical research continues to expand, their roles in preventing chronic diseases, supporting the immune system, and promoting overall well-being become increasingly evident.

2.1 Brief Description of Maize

Maize (*Zea mais* L.), often referred to as corn, stands as a pivotal agricultural crop with a rich history, intricate botanical features, and global significance. Its journey from its wild ancestor, teosinte (*Zea mexicana*), is a subject of continuous debate among experts, some positing that maize is a domesticated version of teosinte (Galinat, 1988).

From a botanical perspective, maize belongs to the Zea genus within the family Gramineae (Poaceae), commonly known as the grass family. This monoecious, tall annual grass exhibits several distinctive characteristics. Its leaves are characterized by overlapping sheaths and broad, conspicuously distichous leaf blades. Maize plants develop staminate spikelets that are arranged in long, spike-like racemes, eventually forming large, spreading terminal panicles known as tassels. In addition, pistillate inflorescences are nestled in the leaf axils, where spikelets are neatly organized in 8 to

16 rows along an axis approximately 30cm long. This axis, often referred to as the cob, possesses a thickened and almost woody texture (Wuana & Okieimen, 2010). The entire structure, known as the ear, is enveloped in numerous large foliaceous bracts. Moreover, a striking mass of long styles, termed silks, extends from the tip as a cluster of silky threads (Hitchcock & Chase, 1971).

Maize seeds, commonly known as corn seeds, are small, hard grains (Figure 1) that serve as both a dietary staple and a crucial component in various industries, including food processing, animal feed production, and ethanol manufacturing.

One of maize's remarkable traits is its reproductive mechanism. Being a wind-pollinated plant, it exhibits adaptability by engaging in both self-pollination and cross-pollination, depending on environmental conditions. This flexibility contributes to the genetic diversity of maize varieties, aiding their resilience to changing climates and pests. The global significance of maize is profound. It serves as a primary food source for numerous communities worldwide, providing essential carbohydrates and nutrients. Furthermore, maize-derived products, such as cornstarch, corn syrup, and corn oil, find extensive usage in the food and beverage sector.

Notably, the genus Zea, which includes maize, has not been associated with significant native toxins (International Food Biotechnology Council, 1990). This underscores the safety and importance of maize as a reliable and valuable food resource, sustaining populations across the globe.

Figure 1: Grains of maize *(Zea mais*)

2.1.1 Maize production and trade in Ghana

Maize (Fig. 1), also known as corn, is a cornerstone of both agriculture and nutrition in Ghana, as well as across the broader African continent. It serves as a major source of food nutrients for a substantial portion of the Ghanaian population and has significant implications for food security. However, it's crucial to note that the majority of those engaged in maize farming in Ghana are smallholders and individuals with lower socio-economic statuses. Furthermore, maize cultivation is primarily rain-fed, posing challenges due to the country's variable climatic conditions.

Ghana's geographical diversity allows maize to flourish in various ecological zones, including the northern regions, although the central to southern parts of the country are the primary maize-producing areas. Interestingly, more than 50% of rural households are involved in maize cultivation, while 16% of urban households also participate in its production, indicating its significance in both rural and urban diets (Quinones & Diao, 2011).

On a global scale, maize is one of the critical crops contributing to human nutrition. Shockingly, about 90% of the world's caloric requirement is met by only 30 crops, with wheat, rice, and maize alone providing approximately half of the calories (Carpenter, Pingali, Bennett, & Zurek, 2005). In Ghana, maize has undergone a significant transformation, becoming the primary source of calories and gradually replacing traditional staple crops like sorghum and pearl millet, which were once the most consumed food crops in the northern regions (SRID-MoFA, 2011).

Maize is predominantly used at the domestic or household level rather than on an industrial or commercial scale. It serves as a fundamental component of the diet, while also finding applications in poultry and livestock feeds, as well as the brewing industry, thus highlighting its versatility in various sectors (SRID-MoFA, 2011).

Understanding the planting and harvesting seasons is crucial in Ghana's maize production. The major maize planting season typically spans from April to May, with harvesting occurring in August or September. Interestingly, a substantial quantity of maize remains within the households of producers, primarily serving as their staple food (Gage, Bangnikon, Abeka-Afari, Hanif, Addaquay, Antwi, & Hale, 2012). Only a relatively small percentage, approximately 20% to 25%, of the total maize production is used for industrial processing and other purposes. The wholesale price of maize is influenced by factors such as proximity to markets, transportation costs, and seasonal variations, with prices generally rising during off-seasons (Amanor-Boadu, 2012).

Maize's significance in Ghana's agricultural landscape is further underscored by the fact that it accounts for over 50% of total grain output, even though yearly yields have seen modest growth at just 1.1% (Rondon & Ashitey, 2011). Ghana's maize production reaches impressive figures, with one million metric tons of maize being marketed annually in the country. The per capita consumption of maize in Ghana was estimated at 42.5 kg in 2000 (Seed Registration and Certification Institute, Ministry of Food and Agriculture [SRID-MoFA], 2000), and the nation consumed an estimated 943,000 metric tons in 2006 (SRID-MoFA, 2007). Between 2007 and 2010, the average maize production in Ghana stood at 1.5 million metric tons (Rondon & Ashitey, 2011). However, it's important to note that in 2012, Ghana recorded a maize yield of 1.2-1.8 metric tons per hectare, which was significantly less than the yield of 4-6 metric tons per hectare obtained in on-station trials (IFPRI, 2014).

Despite its critical role, maize production in Ghana faces several limiting factors. These challenges encompass drought during critical early stages of crop growth, low levels of essential soil nutrients, such as nitrogen and phosphorus, and the ever-present threats of pests and diseases (Obour, Kwamena, & Owusu, 2020). Additionally, poor management practices, including low plant populations, improper planting times, ineffective weed control, limited use of inputs (especially fertilizers and improved seeds), and untimely application of adequate quantities of fertilizers, contribute to yield limitations. Furthermore, inadequate drying and storage facilities result in high postharvest losses, while poor market access exacerbates the challenges faced by maize producers (Adu, Abdulai, Alidu, Nutsugah, Buah, Kombiok, Obeng-Agu, Paulinus, & Okolie, 2014).

Maize is a fundamental crop in Ghana, providing essential nutrients to a significant portion of the population and contributing significantly to both rural and urban diets. However, the industry faces a range of challenges, from climatic factors to issues with management practices and infrastructure. Addressing these challenges is critical for ensuring the continued growth and sustainability of maize production in Ghana.

2.1.2 Importance of maize

Maize, commonly referred to as corn, stands as an agricultural cornerstone in the vibrant tapestry of Sub-Saharan Africa (SSA). Its profound importance transcends its role as a mere crop; it serves as a powerful catalyst in the battle against poverty and a bulwark against food insecurity for countless marginalized families (Zuma, Kolanisi, & Modi, 2018). Notably, maize assumes the mantle of being the primary staple food crop for a staggering population of over 300 million Africans (Mathenge, Smale, & Olwande, 2014). Its versatility in culinary applications is equally awe-inspiring, as Africans ingeniously integrate maize into an extensive array of dishes, including porridges, pastes, grits, and even the production of traditional beer.

But the significance of maize doesn't end with its role as a dietary cornerstone. Fresh maize, known as "green maize" when still on the cob, plays a pivotal role in bridging the hunger gap that looms large after the arid dry season. Furthermore, its nutritional prowess is remarkable, offering an invaluable source of essential nutrients. Maize stands tall as a rich reservoir of carbohydrates, protein, iron, vitamin B, and an array of vital minerals that contribute holistically to health and well-being (Galani, Orfila & Gong, 2020). A precise breakdown reveals maize grains boasting a composition of 72% starch, 10% protein, 4.8% oil, 8.5% fiber, 3.0% sugar, and 1.7% ash (Chaudhary, 1983).

Within the realm of agriculture, *Zea mais*, the scientific name for maize, is unquestionably the paramount cereal fodder and grain crop, thriving under both irrigated and rainfed agricultural systems in the semi-arid and arid tropics (Hussan, Haqqani, & Shafeeq, 2003). To underscore its omnipresence, consider the per capita consumption of maize in Ghana, estimated at 42.5 kilograms in the year 2000 (SRID-MoFA, 2000). This figure serves as a stark testament to maize's centrality in the daily diets of Ghanaians. Furthermore, in 2006, the nation witnessed an estimated national consumption of a staggering 943,000 metric tons of maize, emphasizing its pivotal role in meeting the nutritional and food security needs of the entire nation (SRID-MoFA, 2007).

Maize is not just a crop; it's a lifeline for millions across Sub-Saharan Africa. It provides sustenance, nutrition, and a pathway out of poverty, embodying the resilience and resourcefulness of the communities it serves.

2.1.3 Post harvest losses of maize

Post-harvest losses of maize and other grains in sub-Saharan Africa (SSA), particularly in countries like Ghana, represent a persistent and significant challenge that has far-reaching economic and food security implications. According to a report by the World Bank in 2011, these losses have been estimated to have a value of USD 4 billion for grains alone, an astonishing figure that is equivalent to the annual caloric requirement of approximately 48 million people in the region. These losses not only affect the livelihoods of farmers but also hinder efforts to combat hunger and improve nutrition.

The scale of post-harvest losses is staggering. The FAO and World Bank approximated that up to 47% of the USD 940 billion needed to eradicate hunger in SSA by 2050 will be required in the post-harvest sector (FAO-World Bank, 2010). Furthermore, Prusky (2011) estimated that about 30% of the world's produced food is lost or wasted, amounting to a staggering 1.3 billion tons per year. These losses occur in a world where over 870 million people go hungry (Gustavsson, Cederberg, Sonesson, van Otterdijk, & Meybeck, 2011).

One alarming aspect of these losses is their variability, with post-harvest loss (PHL) rates ranging from 10% to as high as 70% (FAO-World Bank, 2010; Prusky, 2011). These fluctuations are often attributed to various factors, including inadequate storage facilities, poor transportation infrastructure, pests, spoilage, and contamination during storage and processing.

The implications of such substantial losses extend beyond food security concerns. They translate into economic costs as well. Post-harvest losses increase the cost of produce and, consequently, reduce consumers' purchasing power. They also divert income away from farmers' pockets, thereby impacting their livelihoods and perpetuating poverty in rural areas (Opit, 2014).

To address this issue, experts have been advocating for investment in post-harvest loss reduction to enhance food security (GIZ-Deutsche, Gesellschaft, Zusammenarbeit, 2013). As the global population is expected to exceed 9.1 billion people by the year 2050 (Parfitt et al., 2010), the importance of reducing food loss and waste becomes even more critical. The World Food Conference of 1974 aimed to achieve a 50% reduction in PHL by 1985 (Parfitt et al., 2010), a target that has not been fully met, especially in SSA, where limited success has been achieved so far (World Bank, 2011).

One of the significant challenges in addressing post-harvest losses in SSA is the lack of comprehensive data. Reports and studies consistently point to major data gaps in quantifying PHL in the region (Gustavsson et al., 2011; Parfitt et al., 2010; Prusky, 2011). Without accurate data, it is difficult to develop targeted interventions and policies to reduce these losses effectively.

In the case of Ghana, the marketing practices have been identified as a contributing factor to PHL (Darfour & Rosentrator, 2016). Many producers in Ghana are not integrated into the formal marketing chain and tend to sell their grains at the farm gate to traders who then transport them to urban markets (Rondon & Ashitey, 2011). This disconnected approach can lead to inefficient handling and transportation, contributing to losses.

Efforts to mitigate post-harvest losses are evident in Ghana, with the establishment of the National Food Buffer Stock Company (NAFCO) as part of the government's strategy (Rondon & Ashitey, 2011). NAFCO, a state-owned enterprise, was created to purchase, preserve, store, sell, and distribute excess grains across the country. This initiative aims to reduce losses, ensure price stability, and establish emergency grain reserves.

Addressing post-harvest losses of maize and other grains in sub-Saharan Africa, including Ghana, is imperative for achieving food security, reducing poverty, and improving nutrition. These losses represent a substantial economic cost and hinder efforts to combat hunger in the region. While there have been efforts and initiatives, such as the establishment of NAFCO in Ghana, to address this issue, there is still much work to be done. Data collection and analysis are crucial for developing effective strategies, and it is essential for governments, organizations, and farmers to collaborate and invest in solutions that can reduce post-harvest losses and contribute to global food security.

2.2 Brief Description of Cowpea

Cowpea (Figure 2), scientifically known as *Vigna unguiculata* (L.), is a versatile legume primarily cultivated for its edible seeds. These seeds exhibit a diverse range of colors, including cream or beige, light brown, dark brown, reddish brown, and black

(Kabas et al., 2007). Its journey began in Africa, particularly near Ethiopia, where it originated and underwent domestication. Subsequently, cowpea's cultivation and development became prominent in the farms of the African Savannah (Duke, 1990, cited by University of California, 2006). While it has now spread its roots across the globe, adapting to various climates and environments, Africa remains the dominant producer of this valuable legume (Summerfield, Minchin, & Neves, 1980, as cited in Smith, Korsten, & Aveling, 1999).

Cowpea holds a significant position in agriculture and nutrition, as it ranks among the five most crucial legumes in tropical regions. Its importance lies in its dual role, providing a source of protein for many people and enriching the soil with nitrogen (Duke, 1990). This nitrogen-fixing ability benefits the ecosystem and surrounding crops.

In recent years, there has been growing interest in cowpea due to its nutritional benefits, resilience in the face of adverse environmental conditions, and potential for enhancing food security in regions prone to drought and high temperatures. Researchers have also focused on breeding improved cowpea varieties with higher yields, pest resistance, and nutritional value to further enhance its contribution to global agriculture (Smith, Korsten, & Aveling, 1999).

Cowpea, *Vigna unguiculata* (L.), is a vital legume with a rich history in Africa and a promising future on the global agricultural stage, contributing to both food security and soil health. Its diverse seed colors and adaptability make it a valuable crop with a wide range of applications in cuisine and agriculture.

Figure 2: Cowpea *(Vigna unguiculata* L.)

2.2.1 Importance of cowpea

Cowpea *(Vigna unguiculata* L.) (Fig. 2), often referred to as the "black-eyed pea," holds a prominent place in African agriculture and nutrition. Its significance spans several critical aspects of livelihood, food security, and cultural heritage across the continent, particularly in West and Central Africa.

Cowpea serves as the most economically important indigenous African legume crop (Langyintuoa, Lowenberg-DeBoerb, Fayec, Lambert, Ibrod, Moussad, Kergnae, Ushwahaf, Musaf, & Ntoukamg, 2003). This crop contributes significantly to the livelihoods of millions of people, especially in rural areas. Farming communities rely on cowpea cultivation not only for subsistence but also as a source of income through its sale in local markets.

The nutritional value of cowpea cannot be overstated. It boasts an impressive protein content of approximately 24.8% and a substantial carbohydrate composition of up to 63.6% (Davis, Giller, Kroschel, & Wery, 1991). In regions where protein deficiency is a concern, cowpea plays a pivotal role in providing essential nutrients.

In many African regions, including Ghana, cowpea serves as a crucial buffer against food scarcity. Its drought tolerance and adaptability to varying climatic conditions make it a dependable source of sustenance during periods of crop failure or food shortages.

One of the remarkable ecological benefits of cowpea is its ability to fix nitrogen in the soil. This biological process enhances soil fertility, benefiting not only cowpea crops but also other plants in crop rotation systems. It exemplifies sustainable agricultural practices in action.

Beyond its agronomic importance, cowpea holds a special place in the hearts of African communities. Various traditional dishes and seasonings are prepared from cowpea, contributing to the rich tapestry of African cuisine. Homemade weaning foods, in particular, demonstrate the adaptability of cowpea in catering to diverse dietary needs (Lambeth, 2002).

Cowpea is a linchpin of African agriculture and nutrition, embodying resilience, sustenance, and cultural heritage. Its economic contributions to rural communities, nutritional value, role in food security, soil enrichment, and culinary versatility underscore its enduring significance. As Africa continues to grapple with food security challenges and sustainable agriculture, cowpea remains a beacon of hope and a testament to the power of indigenous crops.

2.2.2 Cowpea production and trade across the globe

Cowpea production and trade are pivotal aspects of the agricultural landscape, with a particular emphasis on West Africa and, notably, Ghana. According to the 2003 report from the International Institute of Tropical Agriculture (IITA), the Food and Agriculture Organization (FAO) estimated that global cowpea dry grain production had reached a substantial 3.3 million tonnes by the year 2000. This data underscores the significant scale of cowpea cultivation worldwide (IITA, 2003).

West Africa stands out as a primary hub for cowpea cultivation, with approximately 9.3 million hectares of agricultural land dedicated to its growth. In East Africa, cowpea traditionally finds its place on small farms, often intercropped with cereals such as millet and sorghum. This intercropping strategy effectively maximizes the utilization of available agricultural resources (Muyinza, Komurembe, Lugoloobi, Musitalla, & Aguti, 2003). In Ghana, cowpea holds a special agricultural significance. The northern region of the country is recognized for having the highest production of cowpea. Here, farmers heavily depend on cowpea as both a source of sustenance and green vegetables. Cowpea cultivation in Ghana primarily takes place at the smallholder level, typically on plots of land that are less than an acre in size. The versatility of cowpea is evident in its consumption patterns; it is commonly consumed alongside other staple foods and serves dual purposes as a food grain and a vegetable, particularly among smallholder communities (Muyinza et al., 2003).

The cultivation of cowpea not only contributes to food security but also offers livelihood opportunities for many small-scale farmers. Furthermore, it plays an essential role in diversifying crops, thereby enhancing the resilience of farming systems in regions like West Africa.

Cowpea production and trade hold significant global importance, with West Africa and Ghana serving as key players in its cultivation. The adaptability, nutritional value, and contributions to smallholder farming make cowpea an indispensable component of the agricultural landscape in these regions, ensuring food security and economic sustainability for local communities.

2.2.3 Post harvest losses of cowpea

The cowpea seed beetle, scientifically known as *Callosobruchus maculatus* (Fab.), is a significant threat to cowpea storage in tropical regions, leading to notable post-harvest losses. Although the exact extent of these losses remains uncertain, historical estimates have typically been expressed in percentages. For instance, research conducted by Caswell in 1976 revealed a 50% loss of cowpea in Ibadan and slightly over 30% loss in Zaria due to this pest (Caswell, 1976). Additionally, Caswell's work in 1980 documented that the damage inflicted by *C. maculatus* begins at approximately 10% in January and escalates to a striking 50% by July (Caswell, 1980).

Throughout tropical Africa, it is believed that *C. maculatus* is responsible for consuming between 50% and 90% of stored cowpea annually (IITA, 1989). These estimations highlight the substantial impact of this pest on cowpea post-harvest losses. It's important to note, however, that these figures are rough estimates based on historical observations and may not provide precise measurements of the issue.

To gain a more accurate understanding of cowpea losses attributable to *C. maculatus*, future research should focus on obtaining up-to-date and precise data regarding the economic and nutritional implications of these losses. This, in turn, can aid in the development of more effective pest management strategies, ultimately contributing to ensuring food security in regions heavily dependent on cowpea cultivation.

2.3 Brief Description of *Sitophilus zeamais* Motsch. (Maize Weevil)

The maize weevil (Figure 3), *Sitophilus zeamais* Motsch. is an intriguing insect belonging to the family Curculionidae and sub-family Calandrinae (Hill, 1983). This small but noteworthy creature typically measures between 2.4 to 2.5 millimeters in length, making it easily overlooked, yet its characteristics are of significant interest to entomologists and agriculturists.

One of the most distinguishing features of the maize weevil is its elongated snout, scientifically referred to as a rostrum (Hill, 1983). This rostrum is a key adaptation for its feeding and survival. Equipped with specialized chewing mouthparts, the weevil efficiently gnaws through grains and seeds, posing a significant threat to stored crops.

In terms of its physical appearance, the maize weevil exhibits a range of colors, typically varying from light brown to black. This adaptive coloration enables it to blend seamlessly into its surroundings, providing effective camouflage (Hill, 1983). Its elytra, the hardened wing covers, are adorned with four reddish-orange elongated spots and thoracic punctures, adding to its distinctive appearance.

The maize weevil's antennae are noteworthy as well, consisting of eight segments, and they are often extended during locomotion (Dobie et al., 1974). This extension serves multiple purposes, including aiding in navigation and sensory perception, which are crucial for its survival in its environment.

Notably, the maize weevil possesses metathoracic wings, allowing it to engage in flight (Dobie et al., 1974). However, its flight capabilities are limited, and it predominantly relies on crawling for movement.

Differentiating between male and female maize weevils is possible through a specific characteristic-the rostrum. In females, the rostrum is smoother, longer, and more curved compared to males, a feature that aids in gender identification (Southgate, Howe, & Brett,1957).

The understanding of the morphology and behavior of the maize weevil holds paramount importance in agriculture due to its notorious reputation for infesting stored grains, particularly maize, leading to substantial economic losses (Dobie, Haines, & Hodges, 1974). Therefore, comprehensive knowledge of this species is essential for the development of effective pest management strategies, ensuring the protection of grain stores and agricultural crops.

The maize weevil, *Sitophilus zeamais* Motsch. (Fig. 3), is a small yet captivating insect with a remarkable set of features, including its long snout, specialized mouthparts, and distinct coloration. Its significance in agricultural ecosystems underlines the necessity of in-depth study and management to safeguard grain stores and crops.

Figure 3: Maize weevil

***2.3.1Biology of* S. zeamais**

The biology of *S. zeamais*, commonly known as the maize weevil, is a fascinating subject that exhibits significant variations based on environmental conditions. Understanding these variations is crucial for effective pest management in stored agricultural products.

The impact of temperature on the life cycle of *S. zeamais* is particularly noteworthy. In cooler areas, the life cycle tends to be considerably longer compared to warmer environments (Cash, 2011). For instance, at a temperature of 25°C, the maize weevil completes its life cycle in a relatively short span of 37 days. However, in a cooler climate, such as 18°C, the life cycle extends to a lengthy 110 days. This stark contrast underscores the sensitivity of this species to temperature variations.

Intriguingly, the distribution of Sitophilus spp. eggs on grains of stored agricultural products presents an interesting pattern. Research by Smith (1986), and Nardon, Grenier, and Chessel (1988) has revealed that these eggs can exhibit both random and aggregated distributions. This means that eggs may be laid haphazardly across the grains or, conversely, clumped together, potentially affecting the survival and development of the larvae.

The developmental period of *S. zeamais*, from egg to the adult stage, is further influenced by temperature. Arbogast (1991) notes that this period can range from 25 days at a temperature of 29.1°C to an average of 35 days at 27°C. Notably, at a temperature as low as 18.2°C, the developmental period can extend to a staggering 94 days when only one larva is present in the kernel. This duration decreases to 110 days at the same temperature when three eggs are oviposited into a single kernel (Birch, 1945). This discrepancy in developmental time may be attributed to the competition for limited food resources within the kernel.

Another factor that impacts the developmental period of *S. zeamais* is grain moisture content. Arbogast (1991) reports that lower grain moisture content, approximately 11%, can add four to five days to the normal developmental period. This highlights the importance of moisture regulation in stored grains to mitigate infestations.

The life stages of *S. zeamais* are characterized by four larval instars, each lasting approximately five days, followed by a pupal period of five days. After emerging from the kernel, adult maize weevils may remain inside for up to three days before emerging to infest other grains. On average, these adults have a lifespan of about three months, although some individuals have been known to live for over a year.

The biology of *S. zeamais* is intricately linked to environmental factors, particularly temperature, egg distribution, and grain moisture content. Understanding these factors is essential for effective pest management strategies in the storage of agricultural products (Cash, 2011; Smith, 1986; Nardon et al., 1988; Arbogast, 1991; Birch, 1945). The ability to adapt to varying conditions is a testament to the resilience and adaptability of this insect species.

2.3.2 Damage of maize by sitophilus zeamais Motsch. (maize weevil)

The maize weevil, scientifically known as *Sitophilus zeamais* Motsch., belongs to the Coleoptera family Curculionidae (Doe, 2023). It holds a notorious reputation as one of the most destructive pests of grains, cereals, and various stored products in the regions of sub-Saharan Africa (Throne, 1994). Its impact is profound, posing a significant threat to both agricultural production and food security.

In the field, the maize weevil inflicts damage by penetrating grains and laying its eggs within small cavities it creates on the grain's surface. This initial infestation can occur even before the harvested crop is stored, setting the stage for further destruction. Once these infested grains are transferred to storage facilities, the developing larvae continue their destructive activities, further exacerbating the losses (Haines, 1991).

The consequences of *S. zeamais* infestations are twofold, encompassing both qualitative and quantitative damage to stored products. In untreated stored maize, grain weight losses can range dramatically, spanning from 20% to a staggering 90% (Muzemu, Chitamba, & Mutetwa, 2013). These variations in the extent of damage are influenced by an array of factors, including the type of storage structures employed and the physical and chemical properties of the grains themselves.

The heavy presence of both adult weevils and their larvae has emerged as a pressing concern in the realm of storage entomology (Markham, Bosque-Perez, Borgemeister, & Meikle, 1994). These infestations lead to substantial postharvest losses, jeopardizing the availability of food resources and, in turn, food security in tropical regions. Effective measure s and strategies for pest control and grain protection are imperative to mitigate these losses and ensure a stable food supply for the population.

The maize weevil, *Sitophilus zeamais* Motsch., is a formidable adversary in the realm of agricultural storage. Its capacity to cause extensive damage to grains and stored products highlights the importance of proactive pest management strategies to safeguard food security in sub-Saharan Africa and beyond.

2.3.3 Brief description of Callossobruchus maculatus F. (cowpea weevil)

C. maculatus Fab. (Coleoptera: Bruhidae) is a major pest of economically important leguminous grains such as cowpea, lentil, green gram, and black gram (Haines, 1991). It belongs to the family Bruchidae; super family Chrysomeloidea and subfamily Bruchinae (Hill, 1983). The body size ranges from 2.0mm to 3.5mm and it is brown in colour with a pair of emarginate eyes (Hill, 1983). Cowpea weevils do not have long snouts. It has strait elytra that are not able to cover the posterior end of the abdomen completely. The most distinguishing feature is the presence of a pair of parallel ridges (outer and inner) on the ventral sides of each hind femur, each of which bears a tooth near the apical end. These teeth are roughly equal in length (Haines, 1991).

Male and female cowpea weevils are easily distinguished by their general appearance. The distinguishing feature is the colour of the plate covering the end of the abdomen. While the female has an enlarged and dark colour plate on both sides, the male has a smaller plate and lacks stripes. In some cases, females are larger in size than males and also, females are black while the male are brown in colour (Figure 4). The females often have strong markings on the elytra, which consist of two large marginal dark patches midway along the elytra and smaller patches at the anterior and posterior ends leaving a slight grey-brown cross shaped area covering the rest. The male are much less distinctly marked (Anon, 1991). Their antennae have ten (10) segments which are slightly serrated and are incapable of being flexed backwards when walking (Dobie, 1974).

Figure 4: Male and Female Cowpea weevils

2.3.4 Biology of C. maculatus

The biology of *C. maculatus*, commonly known as the cowpea weevil, is intriguing and multifaceted, influenced by various environmental conditions, with temperature and humidity playing significant roles. Specifically, under conditions of 27°C and 70% relative humidity (RH), the developmental period from egg to adult takes approximately 30 days (Credland, Dick, & Wright, 1986).

One of the fascinating aspects of *Callosobruchus spp.*, to *which C. maculatus* belongs, is their utilization of oviposition marker pheromones. These chemical signals are pivotal in regulating the distribution of eggs on seeds. This behavior is of paramount importance as it effectively prevents the overcrowding of eggs on a few seeds. Left unchecked, such overcrowding would lead to intense intraspecific competition among the developing larvae (Messina & Renwick, 1985; Messina, Bloxham, & Seargent, 2007; Credland & Wright, 1990; MBata, 1992).

Understanding the intricacies of the biology and behavior of *C. maculatus* is of paramount importance, especially in the context of agriculture and pest control. These insights are instrumental in devising strategies to manage and mitigate infestations of this insect, particularly in crops like cowpeas, where it can cause substantial damage. Such knowledge empowers agriculturalists to implement effective measures to safeguard their harvests from the potentially devastating effects of *C. maculatus* infestations.

2.3.5 Damage of cowpea by Callossobruchus maculatus f. (cowpea weevil)

Callossobruchus maculatus F., commonly known as the cowpea weevil, is a notorious pest that poses a significant threat to cowpea crops, both in the field and during storage (Agona & Muyinza, 2003). This pest belongs to the Coleoptera bruchidae family and has a well-defined lifecycle that can wreak havoc on cowpea yields.

The lifecycle of *Callossobruchus maculatus* F. begins with the infestation of cowpea pods before harvest. Female weevils are known to be particularly adept at finding mature, ripening cowpea pods for oviposition (Agona & Muyinza, 2003). Once the eggs are laid, the developing larvae penetrate the cowpea seeds. These larvae are the most destructive stage of the pest, as they reside within the seeds, consuming their contents.

As the larvae grow and mature, they cause a substantial reduction in both the quantity and quality of the infested cowpea seeds (Agona & Muyinza, 2003). Farmers can incur significant losses due to the diminished market value of damaged seeds. After completing their larval stage, the weevils pupate within the seeds. Following this stage, adult weevils emerge from the seeds, leaving behind characteristic emergence holes on the grain. This emergence of adult weevils can mark the end of one infestation cycle.

The entire lifecycle *of Callossobruchus maculatus* F. typically lasts between 20 to 30 days, with the duration influenced by environmental factors such as temperature and humidity (Agona & Muyinza, 2003). This rapid lifecycle allows for multiple generations of weevils within a single growing season, exacerbating the potential for damage to cowpea crops.

Studies have conservatively estimated that losses attributed to *Callossobruchus maculatus* F. infestations can range from 5% to 15% within a storage duration of 3 to 6 months (Agona & Muyinza, 2003). These losses not only impact the livelihoods of farmers but also food security, as cowpea is a vital source of nutrition in many regions.

To mitigate these losses, effective pest management strategies and proper storage practices are crucial. Integrated pest management, including the use of safe and environmentally friendly control methods, can help safeguard cowpea crops from the destructive impact of the Cowpea weevil (Agona & Muyinza, 2003).

2.3.6 Methods for controlling storage pests of grains and legumes

Controlling storage pests such as maize and cowpea weevils is a critical challenge for maintaining the quality and safety of grain and legume crops (Phillips & Throne, 2010). Historically, the primary approach to address these pests has been the use of chemical or synthetic pesticides, which were highly effective. However, over time, the use of synthetic pesticides has raised significant environmental and health concerns. These chemicals have been linked to the development of pesticide-resistant pests and have often burdened farmers with high maintenance costs due to their expensive nature (Bardin, Fargues, & Nicot, 2008; Araar, Caboni, Simeone, & Cavoski, 2009).

One of the most alarming consequences of the extensive use of chemical pesticides is their adverse impact on the environment, including their toxic effects on ecosystems, humans, and livestock (Salaki, Paendong, & Pelealu, 2012). The realization of these drawbacks has led to a growing shift towards adopting more sustainable and eco-friendly pest control methods.

An increasingly popular and ecologically responsible alternative is the use of biopesticides, which represent a biological control technique offering both safety and environmental friendliness. Biopesticides are derived from natural sources, including plants, microbes, or other biological organisms. They operate by disrupting the pests' life cycles or behaviors, reducing the necessity for chemical interventions. Although biopesticides have gained recognition as an integral component of integrated pest and disease control strategies, their popularity still lags behind that of synthetic pesticides. Some may associate biopesticides primarily with organic farming practices, even though the necessary technology and knowledge are widely accessible (Sumartini, 2017).

An exemplary biopesticide is acetogenin, a naturally occurring compound found in soursop leaves and seeds. Acetogenin exhibits a range of biological activities when applied to insects, including inhibiting or deterring feeding, disrupting growth and development, and causing mortality (Prado et al., 2014). This makes it a promising solution for controlling pests like maize and cowpea weevils. What makes biopesticides like acetogenin particularly appealing is their specificity. They effectively target pests while causing minimal harm to non-target organisms, humans, and the environment. This precision contrasts sharply with the broad-spectrum nature of chemical pesticides, which often harm beneficial insects and disrupt ecosystem balances.

The shift towards biopesticides presents a promising approach to controlling storage pests in grains and legumes while safeguarding the environment and human health. Embracing biopesticides aligns with the principles of sustainable agriculture and represents a positive step toward more ecologically balanced farming systems. It is imperative that farmers and policymakers continue to explore and promote the use of biopesticides to ensure the long-term health and resilience of our agricultural ecosystems.

2.4 Economic Importance and Uses of Soursop

Soursop, *Annona muricata* L. (Annonaceae), is native to the tropical regions of the Americas and the Caribbean, and is considered the most important species of the family. Mexico is the principal producer of soursop in the world. Production currently is about 23,715 metric tons per year, with a commercial value of around 159,856 million pesos (US $8,295,632). Most of the production is concentrated in the state of Nayarit, with a planted area of 1,990 ha, distributed in the municipalities of

Compostela (1,912ha), San Blas (52.4 ha), Bahía de Banderas (12 ha), Tepic (7.16 ha), and Xalisco (6.54 ha) (SIAP, 2016).

The major use of this fruit is fresh consumption; however, it has a variety of medicinal and industrial uses (Coto & Saunders 2001; Jimenez, Garcia, & Rodriguez, 2017). Soursop fruit are eaten fresh or made into pulp, drinks, jelly, custards, syrup and ice cream (Barbeau, 1998).

Some under-exploited tropical fruits such as soursop *(Annona muricata* L) can be utilized in the development of exotic processed products, thus adding value to tropical fruits, reducing import substitution and increasing foreign exchange (Mary, Badrie & Comissiong, 2000).

In Ghana, one of the medicinal plants that have been used for a long time is soursop. The fresh fruit (Figure 5), bark, flowers, root, seed and leaf have been used to treat several diseases (Mardiana & Adeanne, 2015). This plant is reported very useful in various disease treatment such as preventing and treating cancer, treating hemorrhoid, reducing cholesterol, eliminating acne (Ministry of Health, 2018) fever, respiratory illness, malaria, liver, heart and kidney infection (Naspiah, Nashruhim & Fitrani, 2013). Various studies have revealed about pharmacological activity of *A. muricata* L. such as antimicrobial, antiprotozoan, insecticide, larvicide, selective cytotoxicity to tumoral cells, anxiolytic, anti-stress, anti-ulceric, wound healing, anti-icteric, hepato-protective, hypoglycemic and antioxidant (Naspiah, et al., 2013). Ethanol, water and n-hexane extract of soursop leaf show antioxidant properties by neutralizing free radicals using the DPPH method (Lawal, Hamid, Shehu, Ajibade, Subair, Ogheneovo, Mukadam, & Adebayo, 2017). The benefits of *A. muricata* L. leaf extract are reported as an antioxidant and correlate with secondary metabolites (Agu, Paulinus & Okolie, 2017). This is supported by research from phytochemical screening conducted, where the ethanol extract of *A. muricata* L. leaf contain compounds alkaloid, saponin, terpenoid, flavonoid, coumarin, lactone, anthraquinon, phenol, and phytosterol (Gavamukulya, Fred, & Hany, 2014). Other reports suggest that soursop plants have a powerful phytochemical called annonaceous acetogenins which are found only in the annonaceae family. These chemicals in general have been documented with antitumor, antiparasitic, and insecticidal. These acetogeneins are strong inhibitors of enzyme processes that are found only in membrane of cancerous tumour cells. The antioxidant activity is also related with their ability to quench reactive oxygen species such as singlet molecular oxygen and peroxyl radicals, thus acting as deactivators of excited molecules or as chain breaking agents respectively (Agu, Paulinus & Okolie, 2017).

Figure 5: One Full and One Longitudinal Half of Soursop fruit

2.5 Empirical Studies on Bio-pesticides

Bio-pesticides, a class of pest control agents derived from natural sources such as plants, microorganisms, and minerals, have garnered increasing attention in recent years. The rationale behind this growing interest lies in their potential to address the environmental and health concerns associated with conventional chemical pesticides. Bio-pesticides offer viable alternatives for effective pest management.

One of the key advantages of bio-pesticides is their eco-friendliness. Unlike synthetic chemicals, bio-pesticides are typically less harmful to non-target organisms, including beneficial insects, wildlife, and humans. This attribute is particularly crucial in the context of sustainable agriculture, where the preservation of biodiversity and ecosystem health is paramount. The use of plant extracts, as demonstrated in the studies aligns perfectly with this eco-friendly approach.

The application of plant extracts to inhibit the growth of pests has been done in numerous research such as garlic plant extracts to cope with snail pests (Rusdy, 2010); papaya leaf extract to inhibit plant pests (Julaily & Setyawati, 2013), inhibit larvae of *Plutella xylostella* (Siahaya & Rumthe, 2014); ethanol extract of melinjo leaves (Moniharapon & Moniharapon, 2014); dan jengkol rind extract (Ambarningrum, Widyastuti, & Setyowati, 2011).

Garlic plant extracts, as examined by Rusdy (2010), represent a prime example of the bio-pesticide potential of botanical sources. Garlic contains sulfur compounds that act as natural insect repellents. These compounds deter pests such as snails while leaving beneficial insects unharmed. By harnessing the power of garlic, farmers can protect their crops without resorting to chemical pesticides that may have adverse effects on the environment and human health.

Papaya leaf extract, as explored by Julaily and Setyawati (2013), extends the range of bio-pesticide applications. The active compounds in papaya leaves, such as alkaloids and flavonoids, possess insecticidal properties. This not only helps in combating plant pests but also provides an opportunity for utilizing a common tropical plant resource in pest management strategies.

The study conducted by Siahaya and Rumthe (2014) on the ethanol extract of melinjo leaves highlights the importance of exploring indigenous plants for pest control. Melinjo, native to Southeast Asia, has traditionally been used for various purposes, and its pest control potential adds another layer of value to this plant species. It underscores the importance of preserving and harnessing local biodiversity for sustainable agriculture.

Ambarningrum et al. (2011) brought attention to jengkol rind extract, demonstrating that plant parts often overlooked can hold valuable properties for pest management. The discovery of pest-repelling compounds in jengkol rind reinforces the idea that nature provides us with a myriad of solutions waiting to be explored.

In addition to these studies, neem-based bio-pesticides, as investigated by Sharma et al. (2018), have gained global recognition. Neem, a versatile tree native to the Indian subcontinent, has a long history of use in traditional medicine and agriculture. Its active compound, azadirachtin, disrupts the growth and development of a wide range of pests. This natural bio-pesticide has proven effective against aphids, caterpillars, and leafhoppers, offering an eco-friendly alternative to chemical insecticides.

Marigold extracts, as studied by Wang, Wang, and Cheng (2017), provide another avenue for bio-pesticide development. Marigolds contain terpenoids, natural compounds known for their pesticidal properties. These extracts show promise in repelling nematodes and soil-borne pathogens, making them valuable components of crop rotation systems aimed at maintaining soil health.

Bautista-Banos and Barrera-Necha (2002) evaluated the in-vitro and in-vivo antifungal activity of aqueous extracts of leaves and stems of *Achras sapota*, *Annona reticulata*, *Bromelia hemisphaerica*, *Carica papaya*, *Citrus limon*, *Chrysophylum* *cainito*, *Dyospiros ebenaster*, *Mangifera indica*, *Persea americana*, *Pouteria sapota*, *Spondias purpurea*, and *Tamarindus indicus* from the state of Morelos, Mexico against *C. gloeosporioides* in mango and papaya fruits in postharvest handling. The researchers reported that the aqueous leaf extract of *C. limon* and *P. americana* completely inhibited the *in vitro* development of *C. gloeosporioides. In vivo* results showed that the leaf and stem extracts of *D. ebenaster* had fungicidal effects on mango fruits, and the leaf extract of *C. papaya* completely inhibited decay in papaya fruits.

Also, Ogbuehi and Onuh (2019) carried out an experiment to determine the effects of different concentration (0% concentration (control), 25% concentration, 50% concentration, and 75% concentration) of leaf extract of soursop have on the control of Flea beetles (*Podagrica spp.*) and yield of okro plants *(Abelnusclus esculentus* (L. Moench) at the Teaching and Research Farm of Imo State University, Owerri Nigeria. It was found out from the study outcome that the numbers of damaged leaves, number of infected plants significantly reduced by all the treatments compared to the control with a massive reduction occurring in treatment of higher concentration (75%) of soursop. It was however concluded that soursop leaf extract possess insecticidal potential in controlling flea beetles.

Moreover, Abdullah and Sina (2003) undertook and experimental studies at Syarif Hidayatullah State Islamic University in Malaysia to ascertain the potential of soursop seed extract as a bio-pesticide against aphids on Chilly. In the experiment, aphids were subjected to a toxicity test against 80,000ppm, 60,000ppm, 40,000ppm, 20,000ppm, 10,000ppm, and 1,000ppm of seed extract of *Annona muricata* with Diathion 1,000ppm and water used as control. It was found out that, 59% aphids mortality was reached within 24 hours for the 80,000ppm concentration whereas 50%

mortality occurred only 48 hours after application for the 60,000ppm concentration. For the 40,000 and 20,000ppm concentrations, 48% and 52% mortality were reached after 72 hours and 120 hours after application respectively.

In conclusion, the research findings and additional insights into bio-pesticides emphasize their pivotal role in sustainable pest management. These natural solutions not only protect crops but also safeguard ecosystems and human well-being. As the global agricultural community continues to seek environmentally friendly and safe pest control methods, bio-pesticides derived from plant extracts and other natural sources offer a compelling path forward. By tapping into the vast reservoir of nature's solutions, we can create a more sustainable and resilient agricultural future.

CHAPTER THREE

METHODOLOGY

3.0 Overview

This chapter covers the materials and methods used for the extraction of soursop leaf. It also discusses treatment procedures, data collection, and evaluation of treatment effects.

3.1 Source of Insect and Insect Culture

Initial stocks of insects used for the experiment were obtained from maize and cowpea grains that were bought from the Winneba main market. The organisms were accurately identified using the descriptions given by Southgate *et al*. (1957) and Hill (1983). The maize and cowpea grains were put in different jars, and adult *S. zeamais* and *C. maculatus* were introduced into the jars containing maize and cowpea seeds respectively. The jars and its' contents were covered with muslin cloth. The jars were kept at room temperature at the Biology Education Department laboratory, Winneba for the insects to breed and multiply under favourable laboratory conditions (temperature of 30±2°C, and relative humidity of 70±5% (Owusu, 2001)). After three weeks of oviposition, the parent insects were sieved out after oviposition. The grains were kept in the laboratory for adult emergence while the emerging generation of same age insects re-cultured under laboratory conditions. The F1 generations were used for the experiment.

3.2 Source of Reagents, Solvents, and Other Materials Used

Ethanol that was used for the extraction was purchased from Mina Chemical shop in Accra. Rotary evaporator (Brand: LABTECH) used for concentrating the extract was obtained from the Chemistry Education laboratory of University of Education, Winneba. Distilled water, reagents such as $FeCl_3$, HCl and all other materials such as pestle and mortar, weighing scale (Brand: RADWAG), flat bottom flask, tray, rotary, and atomizer (Brand: TEEJET) used were obtained from Biology Education laboratory of University of Education, Winneba. The blender used was also a mini max blender (Brand: MINMAX 3IN1).

3.3 Sources of Soursop Leaf and Preparation of Crude Extracts

The leaves of *Anonna muricata (*soursop) were collected from a soursop plant at the backyard of a residence in Kintampo. The leaves were washed thoroughly under tap water to remove any foreign material and dirt, air dried under shade away from moisture for about three weeks and crushed into powder with a mini max electric blender (MINMAX 3 IN 1). Weighed 300g of the powdered leaves were introduced into 2400ml each of 70% ethanol and distilled water. The two separate mixtures were shook at 4 hours intervals for 2 days. The mixtures were filtered using Whatman filter paper (No. 10) and the extracts were concentrated by removing the solvent using a rotary evaporator at 3-6 rpm for 3 hours.

3.4 Source of Maize and Cowpea and their Sterilization

Grains of maize and cowpea used for the study were purchased directly from farmers immediately after harvesting from the field. They were dried under direct sunlight for one week to reduce the moisture content. The grains were then disinfected in an oven at the temperature of 60°C for 2 hours as used by Damena et al. (2022) to destroy eggs larvae, and insects that might have infested the grains. The grains were allowed to cool for the experimental setup.

3.5 Determination of Phytochemical Compounds Present in Crude Extracts (Water and Ethanol)

Water and ethanol crude extracts were tested for the presence of the following phytochemical compounds by using the procedure and reagents as utilized by Shaikh and Patil (2020); alkaloids, phenolic, tannins, glycosides, saponins, flavonoids, triterpenoids and physterols.

3.5.1 Alkaloid test

About 0.05g solvent-free extract was mixed with few ml of dilute HCl and filtered. 2 drops of wagner's reagent was added to the filtrate along the side of the test tube. The presence of alkaloids was indicated by a reddish brown precipitate.

3.5.2 Phenolic compound test

Ferric chloride test: 250µl of the crude extract was diluted with 750µl of distilled water. 5 drops of 5% $FeCl_3$ solution was added and the presence of phenolic compound was indicated by dark green precipitate.

3.5.3 Tannin test

Gelatin test: 500µl of the crude extract was added with 500µl of 1% gelatin solution, again 100µl of 10% NaCl and the formation of white precipitate indicated the presence of tannin.

3.5.4 Glycoside test

Keller Kiliani test: 500µl of the crude extract was added to 750µl of glacial acetic acid. 1 drop of 5% $FeCl_3$ and 750µl of H_2SO_4 were added. The formation of a violet/purple/ brown layer signified the presence of glycosides.

3.5.5 Saponins test

500µl of the crude extract was diluted with 500µl of distilled water and the mixture was vigorously shaken. The presence of saponins was identified by a stable, persistent froth.

3.5.6 Flavonoids test

Ferric chloride test: 250µl of the crude extract was diluted with 750µl of distilled water. 5 drops of 10% $FeCl_3$ solution was added. The presence of flavonoids was spelled out by a green precipitate.

3.5.7 Triterpenoides and phytosterols test

Salkowski's test: For each crude water and crude ethanol extract, 500µl of the extract was added to 500µl of chlorofoam followed by the addition of a few drops of concentrated H_2SO_4. The mixture was vigorously shaken and allowed to standstill. The presence of golden yellow layer at the button indicated the presence of triterpinoides. Red colour in the lower layer indicated the presence of phytosterols.

3.6 Determination of Minimum Concentration of Soursop Extract Effective for Use as Bio-pesticide against *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F.

Into separate clear plastic containers, 20g grains of maize were weighed and introduced. The maize were coated with 1 ml of either concentration (0.02g/ml, 0.04g/ml, 0.08g/ml, and 0.16g/ml) of ethanol or water extract. The coated grains were air-dried for 30 minutes after which 10 randomly selected species each of *Sitophilus zeamais* Motsch. were placed in the separate plastic containers containing the coated grains of maize. The plastic and its constituents were covered with muslin cloth and tied with a rubber band to secure it and was observed for a 2-week period. The set up was replicated in each of the three experimental treatments.

At the end of the two weeks, the number of dead insects in each of the four treatment setups was counted to help determine the average number of dead insects in each of the treatment (T). The average number of dead insects in control (C) setup of three (3) replicates was also determined for comparative analysis with the treatment. The treatment with the highest number of mortality of insects was regarded as the treatment with the concentration that is most effective for use as a biopesticide against the organism in the treatment.

Similar method was replicated using grains of cowpea, and *Callosobruchus maculatus* F.

Average number of dead insects in treatment (T) was calculated using the formula by American Psychological Association (2020).

$$T = \frac{number\ of\ dead\ in\ setup\ 1 + setup\ 2 + setup\ 3}{3}$$

3.7 Determining the Effect of the Two Extracts (Water and Ethanol) on the Quality of Stored Grain (Maize and Cowpea)

The concentrations of soursop extracts (0.16g/ml of water and 0.16g/ml of ethanol) which had the greatest effect on the mortality of insects (maize weevils) after the two weeks period were used in this part of the test.

In this experiment, 50g of maize grains were put into separate clear plastic containers and were coated with 2ml of 0.16g/ml concentration of either ethanol or water extract of soursop leaves. The coated maize grains were allowed to air dry for 30 minutes. 20 randomly selected insect species of maize weevils were introduced into each of the containers with its constituents. The lid of the containers were covered with muslin cloth and secured with a rubber band. Each of the experimental setup (water and ethanol extract coating) with maize was triplicated and was carried out for a 60-day period. Three controls were setup where the grains of maize were not coated with extract. Using same method as with the maize and maize weevil, a similar setup was done with grains of cowpea where cowpea weevils were introduced.

At the end of the 60 days treatment, the grains were reweighed to determine the change in weight (reduction). Also, each grain was hand-picked and assessed to see whether a hole had been bored on it by the insects or not. The number of intact grains (grains without holes) were counted and similar was done for the grains with the holes (bored). The total number of grains (Intact and Bored) was determined, and a percentage analysis was carried out to determine the percentage of intact grains and the percentage of bored grains. A comparative analysis was drawn between the treatment setups and the control. The treatment with the least reduction in weight and least number of bored grains in each of the treatment set up was ascribed as the treatment with the highest quality, and the treatment with the greatest reduction in weight with highest number of bored grains was noted as the treatment with least quality.

Percentage of final intact grains

$$= \frac{\text{Number of final intact grains}}{\text{Total number of grains (Intact } + \text{bored grains)}}\ x\ 100$$

Percentage of bored grains

$$= \frac{\text{Number of bored grains}}{\text{Total number of grains (Intact } + \text{bored grains)}}\ x\ 100$$

$$\text{Percentage of final weight } = \frac{\text{Final weight}}{\text{Initial weight}}\ x\ 100$$

Percentage reduction in weight

$$= \frac{\text{Change in weight (Initial weight} - \text{Final weight)}}{\text{Initial weight}} \; x\; 100$$

3.8 Determining the Effect of Soursop Leaf Extracts on Reproductive Ability of *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* F.

With respect to this objective, the number of both alive and dead weevil in each of the treatment setup to compare the effects of the two extract (water and ethanol) on stored grain (maize and cowpea) was determined. This helped establish the number of new offsprings produced at the end of the 60 days treatment period as 20 species of insects were introduced in each of the setups. The treatment with the highest number of organisms (both living and dead) was labeled as the treatment with the least negative effect on reproductive ability of the insect species. On the other hand, the treatment with the least number of insects (both living and dead) was considered as the treatment (water or ethanol extract) with the most positive effect on limiting reproduction in the said organism species.

Percentage of final weevils

$$= \frac{\text{Total number of final alive weevils (dead + alive)}}{\text{Number of initial weevils introduced}} \; x\; 100$$

Percentage of final alive weevil

$$= \frac{\text{Number of final alive weevils}}{\text{Total number of final weevil (dead + alive)}} \; x\; 100$$

Percentage of final dead weevils

$$= \frac{\text{Number of final dead weevil}}{\text{Total number of final weevil(dead + alive)}} \; x\; 100$$

3.9 Statistical Analysis

Data obtained was subjected to analysis of variance (ANOVA), and concentration-responds. The ANOVA was performed with SPSS 16.0 software (SPSS, 2007), and Graphpad Prism software (Version 8) was used to determine IC50 for the various soursop extracts.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Overview

This chapter presents the results obtained for the various measurement and determination. The experimentation strategy employed involved testing for the presence of different phytochemicals in leaf extracts using the procedure described by Shaikh and Patil (2020) with slight modifications. Additionally, randomized control experiments were used to ascertain the optimal concentration of soursop leaf extracts and their impact on the preservation of grain quality as well as to reduce the ability of maize and cowpea weevils to reproduce.

4.1 Results

4.1.1 Phytochemicals present in ethanol and water extracts of soursop leaf

As part of the objectives of this study, the research sought to determine the presence of various phytochemicals (alkaloids, phenolic compounds, tannins, glycosides, saponins, flavonoids, triterpenoids, and phytosterol) in the two extracts (ethanol and water) of soursop leaf.

With the exception of phytosterols, which were not present in the ethanolic extract of soursop leaves, all of the phytochemical compounds tested (alkaloids, phenolic compounds, tannins, glycosides, saponins, flavonoids, and triterpenoids) were present in the ethanolic extract of soursop leaves. On the other hand, only phenolic compounds, glycosides, saponins, and flavonoids were found to be present in water extract of sousop leaves, whereas alkaloids, tannins, triterpenoids, and phytosterols were all lacking.

The results obtained are illustrated in Table 1 below.

Table 1: Phytochemicals Present in Ethanol and Water Extracts of Soursop Leaf

Phytochemicals	Ethanol Extract	Water Extract
Alkaloids	+ve	-ve
Phenolic Compounds	+ve	+ve
Tannins	+ve	-ve
Glycosides	+ve	+ve
Saponins	+ve	+ve
Flavonoids	+ve	+ve
Triterpenoids	+ve	-ve
Phytosterols	-ve	-ve

-ve represents not detected while +ve represents stands for present

4.1.2 Effects of concentration of soursop leaf extracts on mortality of Callosobruchus maculatus F.

Various concentrations of the two soursop extracts (ethanol and water) exhibited varying results on the mortality of *Callosobruchus maculatus* F.

The effects of different concentrations of ethanolic and aqueous leaf extracts of soursop on the mortality of bean weevils (*Callosobruchus maculatus* F.) were statistically different ($p < 0.05$). Increased in mortality was directly related to increase in concentration. Mortality (N=92) was greater in water extract. Ethanol extract had had an IC50 of 0.0410g/ml and that of the water extract been 0.0340g/ml. The control setup had an average death rate of 50.0%, which was lower than the rates for the treatments using extracts of ethanol (63.3%) and water (76.5%).

Table 2: Effects of Soursop Ethanol and Water Extracts at Various Concentrations on Mortality of *Callosobruchus maculatus* F after 14 Days

Concentrations (g/ml)	Ethanol extract mean mortality (n (%))	Water extract mean mortality (n (%))	Control mean mortality (n (%))
0.00			5.0(50.0)
0.02	3.3 (33.0)	5.0 (50.0)	
0.04	5.7 (57.0)	8.3 (83.0)	
0.08	8.0 (80.0)	8.3 (83.0)	
0.16	8.3 (83.0)	9.0 (90.0)	
IC50	0.0410g/ml	0.0340g/ml	

Field data, 2022

4.1.3 Effects of concentration of soursop leaf extracts on mortality of sitophilus zeamais motsch

Similarly, the study sought to determine the effect that various concentrations of the different extracts of soursop leaf have on the mortality of *Sitophilus zeamais* Motsch. In comparison to the control, the different extracts (ethanol and water) had little to no effect on the death of maize weevils; the mortality rate was nearly the same (the average death was around 10.0%). Additionally, there was no statistical difference in the mortality of maize weevils between water and ethanol extracts in terms of treatments (p>0.05) (Appendices 3 and 4). However, the treatment with water extract application had a significantly greater death rate (total death = 14) than the treatment with ethanol extract administration (total death = 13). The IC50 of ethanol extract on the mortality of *S. zeamais* was 0.1229g/ml, whilst that of water extract was 0.0620g/ml. The outcomes are represented in the Table 3 below.

Table 3: Effects of Soursop Ethanol and Water Extracts at Various Concentrations on Mortality of *Sitophilus zeamais* Motsch. after 14 Days

Concentrations (g/ml)	Ethanol extract mean mortality (n (%))	Water extract mean mortality (n (%))	Control mean mortality (n (%))
0.00			0.7(7.0)
0.02	1.0 (10.0)	0.7 (7.0)	
0.04	1.0 (10.0)	1.0 (10.0)	
0.08	1.0 (10.0)	1.3 (13.0)	
0.16	1.3 (13.0)	1.7 (17.0)	
IC50	0.1229g/ml	0.0620g/ml	

Field data, 2022

*4.1.4 Effects of soursop extracts on the quality of stored grains (maize), and on the reproductive ability of maize weevils (*sitophilus zeamais *motsch.*)

With respect to the effect of soursop extracts on the quality of stored grain (maize), after the 60 days application of 0.16g/ml concentration of soursop extracts of water and ethanol in separate treatments, it was noted that the overall weight of the stored grain decreased noticeably. The maize with the ethanol extract applied reduced by 2.98g (5.96%) in weight and the maize with water extract reduced in weight by 3.1g (6.20%). Also, in the control setup, the weight of the maize reduced by 21.88%.

Also, significant quantity of the maize grains were destroyed (bored by weevils) at the end of the period in all the setups with a higher destruction noticed in the control. 79.50% of the maize in the control were destroyed by the weevils, with the destructions in the treatments been 22.80% and 28 .92% in the ethanol and water extract respectively.

Considering the effect of the extracts on the reproductive ability of maize weevils, the average number of weevils (dead and alive) in the control rose dramatically from the original 20 weevils introduced to 188(940.00%). In the same vein, the numbers multiplied to just 56 (280.00%) in the set-ups with the ethanol extract, and to 71 (355.00%) in the setups with the water extracts.

Table 4: Effects of Soursop Extracts on the Quality of Stored Grains (Maize), and on Reproductive ability of Maize Weevils (*Sitophilus zeamais* Motsch.)

Variables	Ethanol Extract	Water Extract	Control
Initial weight of maize; (g)	50	50	50
Number of weevils introduced; (n)	20	20	20
Total number of initial grains introduced; (n)	180	166	171
Number of whole grains at the end of the period; n (%)	139 (77.20)	118 (71.08)	35 (20.5)
Number of grains bored by weevils at the end of the experiment; n (%)	41 (22.80)	48 (28.92)	136 (79.50)
Final weight of grains; g (%)	47.02 (94.00)	46.90 (93.80)	39.06 (78.12)
Reduction in weight at the end of experimental period; g (%)	2.98 (5.96)	3.10 (6.20)	10.94 (21.88)
Total number of weevils at the end of the experimental period; n (%)	56 (280.00)	71 (355.00)	188 (940.00)
Total number of alive weevils at the end of the experimental period; n (%)	42 (75.00)	49 (69.01)	162 (86.20)
Total number of dead weevils at the end of the experiment; n (%)	14, (25.00)	22, (30.99)	26 (13.80)

Field Data, 2022

4.1.5 Effects of soursop extract on the quality of stored legume (cowpea) and reproductive ability of cowpea weevils (Callosobruchus maculatus F.)

From Table 5 below, there was substantial reduction in weight in the control setup (36.36% reduction in weight) as compared to the 23.80% and 27.22% obtained for the treatments with ethanol and water extract respectively. Additionally, the total number of cowpea bored with holes in each setup was as follows: control (242; 79.08%), ethanol extract (193; 63.91%), and water extract (208; 67.31%).

The table also shows that after 60 days, there was an increase in *Callosobruchus maculatus* F. populations across all setups from the initial 20 insects. However, a larger percentage increase was seen in the control setup compared to the treatments (applied water and ethanol extracts). The final number of weevils in the control setup increased from the 20 weevils introduced to 348 (1740% increased), and those in the ethanol and water extract treatments had risen to 210 (1050%) and 249(1245%) respectively.

Table 5: Effects of Soursop Extracts on the Quality of Stored Legume (Cowpea), and on Reproductive ability of Cowpea Weevils (*Callosobruchus maculatus* F.)

Variables	Ethanol Extract	Water Extract	Control
Initial weight of cowpea grains (g)	50	50	50
Number of weevils introduced (n)	20	20	20
Total number of initial grains introduced (n)	302	309	306
Number of whole grains at the end of the period; n (%)	109 (36.09)	101 (32.69)	64 (20.09)
Number of grains bored by weevils at the end of the experiment; n (%)	193 (63.91)	208 (67.31)	242 (79.08)
Final weight of grains; g (%)	38.10 (76.2)	36.39 (72.78)	31.68 (63.36)
Reduction in weight at the end of experimental period; g (%)	11.90 (23.80)	13.61 (27.22)	18.32 (36.64)
Total number of weevils at the end of the experimental period; n (%)	210 (1050)	249 (1245)	348 (1740)
Total number of alive weevils at the end of the experimental period; n (%)	28 (13.33)	33 (13.25)	216 (62.07)
Total number of dead weevils at the end of the experiment; n (%)	182 (86.67)	216 (86.74)	13 (37.93)

Field Data, 2022

4.2 Discussion

4.2.1 Objective 1: Phytochemicals constituents of soursop leaf extracts (ethanol and water extract)

Phytochemicals are naturally occurring compounds found in plants. The use of bioactive compounds from plants and other biological sources as functional food, biofertility, biopesticides, and medicine to diseases, and to control insect pests has been documented over the years (Kumar, Singh, Sharma, Kaur, & Singh 2020).

Soursop contains several bioactive compounds including acetogenins, alkaloids, flavonoids, and phenols (Ana, Efigenia, Elhadi, & Eva 2015) which contribute to its medicinal properties and potential insecticidal effects. These phytochemicals are present in various parts of soursop plant including the leaves, bark, roots, fruits and so on. Soursop has potent anti-inflammatory, antimalarial, and anticancer properties (Ministry of Health, 2018), and has traditionally been used for the treatment of diseases.

In fact, a number of researchers have reported that eating soursop fruit and other plants that have certain amount of phytochemical have the propensity to reduce cancer risk by 40% (Naspiah, Nashruhim, & Fitrani, 2013).

Annonacin, a phytochemical in soursop is known to damage insects' neurological systems, resulting in their paralysis and eventual death (Lopes, dos Santos, and Silva, 2018). Also, phytochemical like flavonoids possess strong odor or taste and hence may act as natural repellents thereby deterring insects from approaching or feeding on the plants or plant produce (Ohmura, Doi, Aoyama, & Ohara, 2000). Moreover, phytosterols in soursop can disrupt the growth and development of insect pests by interfering with insect hormone regulation leading to abnormal growth and molting.

As part of the objectives of this present study, the researcher sought to determine the phytochemicals present in the two extracts (water and ethanol) of soursop leaves. It was observed that, phenolic compounds, glycoside, saponins, and flavonoids were present in both extracts. However, bioactive ingredients including alkaloids, tannins, and triterpenoids which were detected in ethanolic extract of soursop leaf were absent in the water extract.

According to Turkmen, Sari, and Velioglu (2006), the quantity and quality of extraction yield is strongly affected by many factors such as extraction method, extraction time, composition of the phytochemicals, the part of the plant been used for the extraction, and solvent used. Although ethanol and water are both polar solvent, ethanol is able to extract a broader range of phytochemicals including lipophilic (fat soluble) compounds and moderately polar substance. In contrast, water is more efficient at extracting hydrophilic (water soluble) compounds. Also, volatile phytochemicals such as essential oil may be lost or altered during extraction process, and ethanol is better at preserving these compounds than water. Taking these into consideration, since different solvents (ethanol and water were used) were used in separate extract preparation, they may have accounted for the discrepancies in the phytochemicals detected.

According to Vergara, Miranda, and Valdez (2018), extract of soursop using ethanol and water yielded phytochemical such as phenolics, saponins, flavonoids, and phytosterols. This is similar to findings in the current study with the exception of phytosterols which were not identified in this investigation. This disparity in the two study findings may have occurred with reason that the fruit of soursop plant was used in the study by Vergara et al. whilst the leaves of the plant were used in this present study. And phytosterols may have been concentrated much in the fruit of the soursop than in leaves therefore contributing to the difference.

4.2.2 Objective 2: Concentration of soursop leaf extracts (ethanol and water) effective for use as a bio-pesticide against Sitophilus zeamais motsch. and Callosobruchus maculatus F.

The effective concentration of ethanol and water leaf extracts of soursop for use against maize weevil and cowpea weevil can vary depending on a number of factors. They include the plant species, extraction method, and the specific bioactive compound present in the extracts.

It was identified from the study outcome that with the exception of 0.02g/ml of ethanol extract which had an average mortality of 33.0%, all the other concentrations of ethanol soursop extract used had a significant impact on the death of *Callosobruchus maculatus* F. (mean mortality>50.0%) compared to the case of the control which had an average of death of 50.0%. Also, it is further evident from the study results that, there was a significant difference ($P<0.05$) in the number of deaths between the treatment and the control. As the concentration of soursop extract increased, there was a corresponding increase in mortality rate of *Callosobruchus maculatus* F.

Similarly to the effect of various concentrations of ethanoic extract on *Callosobruchus maculatus* F. is the effect of various concentration of water extract. With the exception of 0.02g/ml of water extract, all the other concentrations (0.04g/ml, 0.08g/ml, and 0.16g/ml) had an average death rate higher than the in the control (mean mortality=50.0%).

Expectedly, it was realized that increasing the concentration of both ethanolic and water extracts resulted in increasing mortality of the weevils. This is in line with the report by Arivoli, Tennyson, and Jesudoss (2011) that each plant that contains toxin has different effective concentrations, that the higher the level, the more amount of toxin getting in contact with the skin of insects to inhibit growth and subsequently cause more death of the insects. Equally, Acharya, Mir, and Nayak (2017) stated that the higher the concentration of a biopesticide, the more amount of the poison that comes into contact with the insects' skin so that death rate will be higher.

The increase in amount of toxins or compounds such as annonacin with insecticidal properties confers enhanced inhibitory potential on the nervous systems as well as causing their death (Champy, Ho¨ glinger, Feger, Gleye, Hocquemiller, Laurens, & Ruberg, 2005; Lannuzel, Hoˆ glinger, Champy, Michel, Hirsch, & Ruberg, 2006).

However, when comparing the average fatality of the two treatment settings (treatment with ethanol extract (63.0%) and treatment with water extracts (77.0%), it can be seen that more fatalities were recorded in the treatment with water extract application. In addition, the ethanol displayed an IC50 value of 0.04g/ml indicating that a relatively higher concentration of the extract is necessary to achieve a 50% mortality rate among cowpea weevils. In contrast, the water extract exhibited a lower IC50 value of 0.0340g /ml, implying that lower concentration of water extract is required to cause 50% mortality rate among cowpea weevils. This findings then suggest that water extract possesses stronger insecticidal properties and more efficient at inducing cowpea weevil mortality. This result may have been caused by the presence of specific phytochemicals in the water extract that may not have been present in the ethanol extract but which had a major impact on the death of *Callosobruchus maculatus* F. Some phytochemicals or bioactive substances in the soursop water extract may also be more effective than those in the ethanol extract for short-term treatment or preservation.

With respect to the effect of extracts of various forms (ethanol and water) on *S. zeamais*, similar trends of mortality were recorded in all the various setups as little to no deaths were recorded in each case. The treatment setup which had water extract application had an average death of 12.0%, and that of the ethanol extract to be 11.0% compared with the untreated or control which had an average mortality of 7.0%. Also, regarding mortality difference in *S. zeamais* in relation to the changes in the concentration of the extracts applied, no statistical difference ($p>0.05$) was observed in both the treatment with the water and ethanol extract.

Further to the two extracts concentrations on the mortality of *S. zeamais*, ethanol extract displayed a higher IC50 value of 0.1229g/ml and that of water conversely exhibited a lower IC50 value of 0.0620g/ml. This finding signifies that a lower concentration of water extract is required to achieve a 50% inhibition of maize weevil activity.

Generally, as stated by Astuti and Widyastuti, (2016); and Latumahina, Hartika, and Wahyuni (2015), for a biopesticide to be effective against a particular group of insects, it should be able to kill more than 90% of that insect species. With the exception of water extract with a concentration of 0.16g/ml which had an average mortality of 90.0% on bean weevil, it can be stated that none of the individual concentrations of the various extracts is effective as a bio-pesticide against any of the two insect species as they all recorded an average death of less than 90.0%. Nevertheless, it was shown that the death rate in the various treatments increased with an increase in the concentration of all the different extracts, suggesting that raising the concentration of the various extracts could have a significant impact on the insect species' mortality.

4.2.3 Objective 3: Comparing the effect of soursop extracts (ethanol and water) on the quality of stored grains (maize and cowpea)

Soursop leaf extract can help maintain the quality of stored grains and legumes by serving as a natural insecticide, protecting them from infestation by pests such as weevils and other storage insects. By preventing or reducing insect damage, the extract can help preserve the quality of the stored crop.

Both ethanol and water extracts of soursop leaves were found to have effectively reduced the number of damaged cowpea and maize after the 60 days period compared to the control where no soursop extracts were applied. The majority of the insects were seen climbing up the walls of the plastic containers used for treatment, and some of the insects managed to attach themselves to the muslin cloth used to cover the containers. However, almost all of the insects in the control settings were found in the grains, where they infested and caused significant damage. As a result, the insects in the control setups damaged the majority of the grains.

According to Ogbuehi and Onuh (2019), soursop extract contains an active ingredient called acetogenins, and this bioactive compound is capable of repelling and inhibiting the activities of insect species. Additionally, acetogenins contained in annonaceae is poisonous to insect species, so insects try to avoid coming into contact with this compound.

This explanation may have led to the observation in the treatment setups where the insect tried to avoid coming into contact with the coated grains with extract. When comparing the effectiveness of the two extracts in preserving the quality of dried grains and legumes, it was found that the ethanol extract was better able to maintain the quality of the dried grain (maize) over the treatment period than the water extract because it caused a smaller weight loss (weight reduced by 5.96% vs. 6.20%).

Additionally, only 41 (22.8%) of the maize grains coated with ethanol were bored by weevils, compared to 48 (28.92%) of the grains smeared with water extract.

Similarly, there was a smaller weight loss in the stored cowpeas coated with ethanol extract (11.9g total weight reduced by 23.80% at the end of the experiment) than in the cowpeas coated with water extract (13.61g total weight decreased by 27.22% at the end of the experiment). Further evidence came from the study's findings, which showed that the amount of cowpea grains that the insects bore was higher in the treatment with water extract application (67.31% of the total number of cowpea seeds were bored) than in the treatment with ethanol extract application (63.91% of the total number of cowpea seeds).

The results of the two experimental treatments (treatments of maize and cowpea with both ethanol and soursop leaf extract) showed that the quality of both maize and cowpea could be preserved over time more effectively with the ethanol soursop leaf extract than the water extract. This may have been the case with reasons that ethanol was able to extract a wide range of phytochemicals such as alkaloids, tannins, and triterpenoids that water could not extract. And these phytochemicals may have influenced the longevity and pesticidal activity of ethanol soursop extracts.

4.2.4 Objective 4: Effect of soursop leaf extracts (ethanol and water) on reproductive ability of Sitophilus zeamais motsch. and Callosobruchus maculatus F.

Soursop extract, particularly its bioactive compounds such as alkaloid and acetogenins (Ana, Efigenia, Elhadi, and Eva, 2016) may interfere with the reproductive process of weevils and this could potentially lead to reduced fertility in adult weevils, affecting their ability to lay viable eggs. Also, soursop extract may act as a repellent to weevils, deterring them from approaching potential mates or breeding sites. And by disrupting the mating process, the extract could hinder successful reproduction and decrease the number of viable offsprings.

Costa, Silva, and Fiuza (2004) stated that bioactive compounds in plants can interfere with the larvae's alimentation and can influence the number of ovaries, and therefore, reduce egg production without causing death.

Studies by Peres, Sobreiro, Couto, Silva, Pereira, Heredia-Vieira, Cardoso, Mauad, Scalon, and Verza et al. (2017) reported the biological impact of flavonoid on the fertility and survival of *P. xylostella* eggs. Similarly, alkaloid another phytochemical in most plants was identified by Tavares, Cruz, Petacci, Freitas, Serratilde, and Zanuncio (2011) as effective in reducing the number of hatched eggs in *Spodoptera frugiperda.*

Although equal number of insect (20 each) were introduced into each of the setups, at the end of the experimental period, the final average number of maize weevil (both dead and alive) in the control setups had increased more than nine fold (from 20 to 188 (940% increment)). In contrast, the insect numbers in both treatment setups (ethanol and water) were just around threefold (280.0% and 355.0% respectively in the treatments with ethanol and water extracts).

Even though the number of maize weevils rose in each configuration, the treatment setup showed a much less rise than the control setup, indicating that the extracts used in the treatment had a more inhibitory effect on the rate of reproduction in the treatment than the control.

However, there was a small variation in the average final insect species (dead and living) when comparing the treatment setups with ethanol and water soursop extracts administration. In comparison to the treatment with the ethanol extract (280.0% rise in number of maize weevils), it was found that the rate of reproduction was higher in the water extract treatment (355.0% increased in number of maize weevils).

Similarly, to the observation made in the number of maize weevils, there was a remarkably higher numbers of cowpea weevil (final average number of cowpea weevil) in the control as compared to the case in the treatments with soursop extract (rate of increments were 1740.0%, 1050.0% and 1245.0% in the control, treatments with ethanol extract and water extract respectively).

These disparities in observations made between the controls and the treatments in the insect species (maize weevil and cowpea weevil) numbers may have occurred due to the presence of flavonoid and alkaloids as identified by Peres et al. (2017) and Tavares et al. (2011) in the extracts. These phytochemicals (flavonoid and alkaloids) can be some of the reasons behind the fertility, the amount of eggs that survived, the number of eggs hatched from the soursop extracts.

Furthermore, although both extracts significantly hindered reproduction in the two insect species, there was a relative higher rate of reproduction in the weevils (both maize and cowpea weevil) in the case of treatments with water extracts applied as compared to the insects in the setup with ethanol extracts application. In this regard, it can be stated that ethanol soursop leave extract was able to reduced reproduction in both maize and cowpea weevils than water soursop extract.

This difference in rate of reproduction in the treatments (ethanol and water extract treatments) may have resulted as some bioactive compounds such as alkaloid present in the ethanol extract which were absent in the water extract tend to hinder the reproductive ability of the two weevils (maize and cowpea weevils) (Saxena, and Tikku, 1998).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.0 Conclusions

Through a comprehensive investigation using various analytical techniques, this study was able to identify a diverse array of phytochemicals in both ethanol and water extract of soursop leaves. This finding underscores the rich insecticidal and medicinal potential of soursop leaves and highlights the significance of utilizing different solvents to uncover a broader spectrum of bioactive compounds. Ethanol extracted phytochemicals such as alkaloids, tannins, and triterpenoids which were absent in the water extract of the soursop leaves. These phytochemicals are notable for their medicinal, hepatoprotective, wound healing, defensive, and antioxidant effects.

The result of the study further revealed that, both ethanol and water extract exhibited insecticidal properties against both cowpea and maize weevils with varying degree of efficacy. Both extracts demonstrated considerable insecticidal activities at higher concentrations, however, a relatively higher mortality was observed in insects with ethanol extract application. This finding therefore suggests that the choice of solvent for extraction significantly impact the bioactivity of the plant compounds.

Moreover, the results obtained from the study provide valuable insight into the efficacy of soursop leaves extracts as a natural and friendly option for maintaining the quality of stored grains maize and weevils. Both ethanol and water extracts of soursop leaves demonstrated a remarkable potential in preserving the weight of stored maize and cowpea. The application of soursop leaves extracts acted as natural insect repellents, thereby effectively deterring insects from infesting the cowpea and maize grains. The treated grains experienced a minimal weight loss compared to the untreated samples, indicating the application of soursop extracts helped minimized overall deterioration by insects during storage.

Furthermore, the treated weevils experienced a substantial reduction in reproductive ability, resulting in a decreased number offsprings compared to the untreated control group. This remarkable effect on the reproductive capacity of both maize and cowpea weevils highlights the potential of soursop leaves extracts as efficient means of population control, thereby reducing the potential damage caused by these pests to cowpea and maize.

5.1 Recommendations

- The persistence and residual activity of both water and ethanol extracts should be investigated. Understanding the longevity and its insecticidal properties will help determine appropriate application frequencies in real-world storage conditions.
- Investigate the selectivity and potential toxicity of soursop extracts on non-target organisms, such as beneficial insects and other organisms present in the grains storage environment. It is crucial to ensure that the use of soursop extract does not harm beneficial species or disrupt ecological balance.
- Conduct field trials in actual grain and legume storage facilities to validate the efficacy of soursop extract under practical conditions.
- Perform a cost-effectiveness analysis comparing soursop extract treatment with conventional synthetic pesticides. Determining the economic feasibility of using soursop extract will be essential for its potential adoption by farmers and storage managers.
- Conduct comprehensive evaluations to assess the potential risk associated with handling and using soursop extract. Safety assessments should include dermal

and respiratory exposure scenarios to ensure the safety of agricultural workers and consumers.

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APPENDICES

APPENDIX A

EXPERIMENTAL OBSERVATIONS

Table 5.2.1: *Callosobruchus maculatus* F. Mortality with Soursop Ethanol Extract at Various Concentrations after 14 Days

Treatments	No. of Mortality			Total Mortality	Mean Mortality
	Setup 1	Setup 2	Setup 3		
T1	3	4	3	10	3.3
T2	6	6	5	17	5.7
T3	7	9	8	24	8.0
T4	8	9	8	25	8.3
Total	24	28	24	76	25.3
Average	6.0	7.0	6.0	19	6.3

Keys

T1=20grams of beans+10species of *Callosobruchus maculatus* F.+2ml of 0.02g/ml of ethanoic soursop extract

T2=20grams of beans+10species of *Callosobruchus maculatus* F.+2ml of 0.04g/ml of ethanoic soursop extract

T3=20grams of beans+10species of *Callosobruchus maculatus* F.+2ml of 0.08g/ml of ethanoic soursop extract

T4=20grams of beans+10species of *Callosobruchus maculatus* F.+2ml of 0.16g/ml of ethanoic soursop extract

Table 5.2.2: *Callosobruchus maculatus* F. Mortality with Soursop Water Extract at Various Concentrations after 14 Days

Treatment	No. of Mortality			Total Mortality	Mean Mortality
	Setup 1	Setup 2	Setup 3		
T1	4	6	5	15	5.0
T2	8	9	8	25	8.3
T3	9	8	8	25	8.3
T4	8	10	9	27	9.0
Total	**29**	**33**	**30**	**92**	**30.6**
Average	**7.3**	**8.3**	**7.5**	**23.1**	**7.7**

Keys

T1=20grams of beans+10species of *Callobruchus maculate*+2ml of 0.02g/ml of water soursop extract

T2=20grams of beans+10species of *Callobruchus maculate*+2ml of 0.04g/ml of water soursop extract

T3=20grams of beans+10species of *Callobruchus maculate*+2ml of 0.08g/ml of water soursop extract

T4=20grams of beans+10species of *Callobruchus maculate*+2ml of 0.16g/ml of water soursop extract

Table 5.2.3: *Callosobruchus maculatus* F. Mortality with no Soursop Extract after 14 Days

	No. of Mortality			Total Mortality	Mean Mortality
	Setup 1	Setup 2	Setup 3		
C	6	5	4	15	5.0

Key

C=20grams of beans+10species of *Callosobruchus maculatus* F.

Table 5.2.4: *Sitophilus zeamais* Motsch. Mortality with Soursop Ethanol Extract at Various Concentrations after 14 Days

Treatment	No. of Mortality			Total Mortality	Mean Mortality
	Setup 1	Setup 2	Setup 3		
T1	1	2	0	3	1.0
T2	1	1	1	3	1.0
T3	2	1	0	3	1.0
T4	2	1	1	4	1.3
Total	**6**	**5**	**2**	**13**	**4.3**
Average	**1.5**	**1.3**	**0.5**	**3.3**	**1.1**

Keys

T1=20grams of maize+10species of *Sitophilus zeamais* Motsch.+2ml of 0.02g/ml of ethanol soursop extract

T2=20grams of maize+10species of *Sitophilus zeamais* Motsch. +2ml of 0.04g/ml of ethanol soursop extract

T3=20grams of maize+10species of *Sitophilus zeamais* Motsch. +2ml of 0.08g/ml of ethanol soursop extract

T4=20grams of maize+10species of *Sitophilus zeamais* Motsch. +2ml of 0.16g/ml of ethanol soursop extract

Table 5.2.5: *Sitophilus zeamais* Motsch. Mortality with Soursop Water Extract at Various Concentrations after 14 Days

Treatment	No. of Mortality			Total Mortality	Mean Mortality
	Setup 1	Setup 2	Setup 3		
T1	0	0	2	2	0.7
T2	2	1	0	3	1.0
T3	2	2	0	4	1.3
T4	1	1	3	5	1.7
Total	**5**	**4**	**5**	**14**	**4.7**
Average	**1.3**	**1.0**	**1.3**	**3.5**	**1.2**

Keys

T1=20grams of maize+10species of *Sitophilus zeamais* Motsch. +2ml of 0.02g/ml of water soursop extract

T2=20grams of maize+10species of *Sitophilus zeamais* Motsch. +2ml of 0.04g/ml of water soursop extract

T3=20grams of maize+10species of *Sitophilus zeamais* Motsch. +2ml of 0.08g/ml of water soursop extract

T4=20grams of maize+10secies of *Sitophilus zeamais* Motsch. +2ml of 0.16g/ml of water soursop extract

Table 5.2.6: *Sitophilus zeamais* Motsch. Mortality with no Soursop Extract after 14 Days

	No. of Mortality			Total Mortality	Mean Mortality
	Setup 1	Setup 2	Setup 3		
C	1	1	0	2	0.7

Field Data, 2022

Key

C=20grams of maize+10species of *Sitophilus zeamais* Motsch.

APPENDIX B

ANOVA ANALYSIS

Table 5.3.1: ANOVA Analysis for Difference between Various Treatments of Ethanol Extract of Soursop Leaves on *Callosobruchus maculatus* F.

Groups		Mean Mortality	SS	F	MS	P-value
Setup	1	6.0	2.667	6	1.333	0.037
	2	7.0				
	3	6.0				
Treatment	1	3.3	48.667	73	16.222	4.11E-05
	2	5.7				
	3	8.0				
	4	8.3				

Table 5.3.2: ANOVA Analysis for Difference between Various Treatments of Water Extract of Soursop Leaves on *Callosobruchus maculatus* F.

Groups		Mean Mortality	SS	F	MS	P-value
Setup	1	7.3	2.167	2.053	2.052	0.209
	2	8.3				
	3	7.5				
Treatment	1	5.0	29.333	18.526	9.778	0.002
	2	8.3				
	3	8.3				
	4	9.0				

Table 5.3.3: ANOVA Analysis for Difference between Various Treatment of Ethanol Extract of Soursop Leaves on *Sitophilus zeamais* Motsch.

Groups		Mean Mortality	SS	F	MS	P-value
Setup	1	1.5	2.167	2.60	1.083	0.153
	2	1.3				
	3	0.5				
Treatment	1	1.0	0.250	0.20	0.083	0.892
	2	1.0				
	3	1.0				
	4	1.3				

Table 5.3.4: ANOVA Analysis for Difference between Various Treatment of Water Extract of Soursop Leaves on *Sitophilus zeamais* Motsch.

Groups		Mean Mortality	SS	F	MS	P-value
Setup	1	1.3	0.167	0.051	0.083	0.951
	2	1.0				
	3	1.3				
Treatment	1	0.7	1.667	0.339	0.556	0.798
	2	1.7				
	3	1.0				
	4	1.3				

APPENDIX C

PLATES

Plate 1: Dry leaves of *Annona muricata*

Plate 2: Powdered leaves of *Annona muricata*

Plate 3: Researcher filtering ethanolic crude extract

Plate 4: Evaporating solvent from crude extract of *Annona muricata* leaves

Plate 5: Ethanolic extract of *Annona muricata* leaves

Plate 6: Water extract of *Annona muricata* leaves

Plate 7: Researcher handpicking bored cowpea

Plate 8: Sterilized cowpea

Plate 9: Maize setup

Plate 10: Cowpea setup

Plate 11: Researcher testing for the phytochemicals present in *Annona muricata* leaves

Plate 12: Display of tested phytochemical compounds in ethanolic and aqueous crude extracts of *Annona muricata* leaves

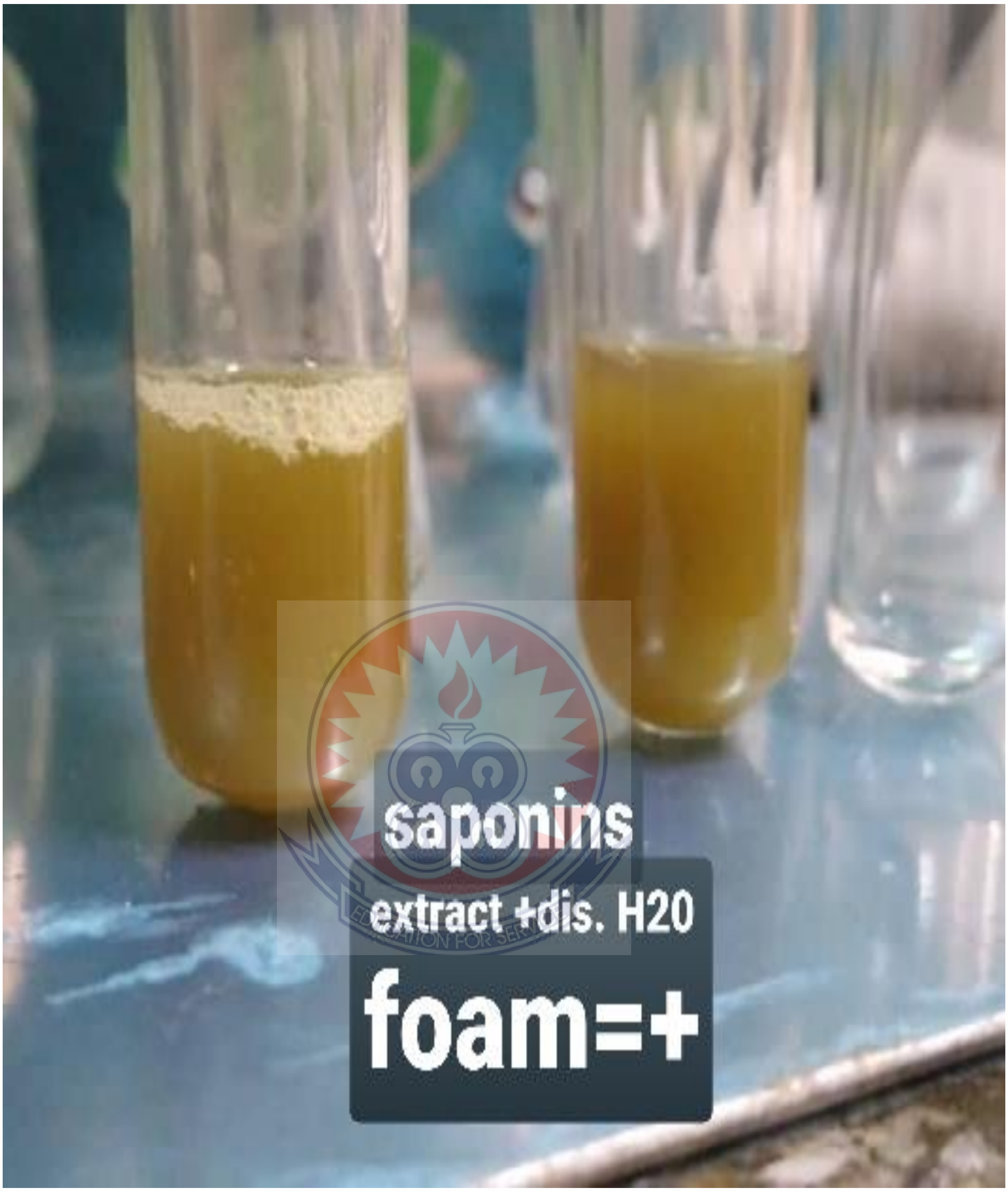

Plate 13: A test displaying a present of saponins

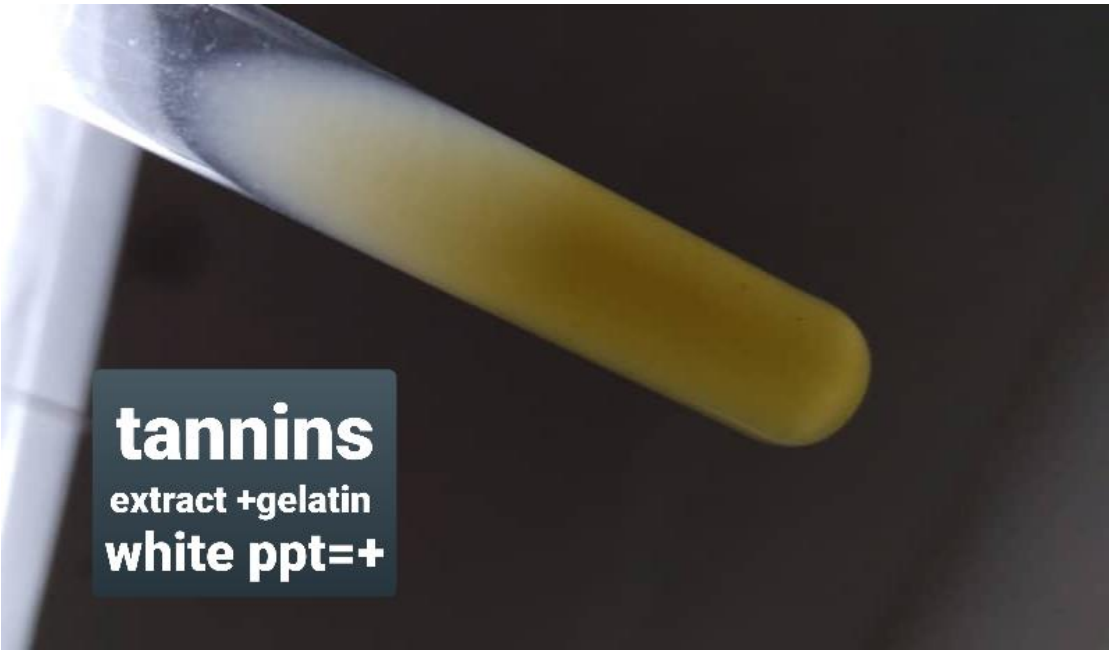

Plate 14: A test displaying a present of tannins

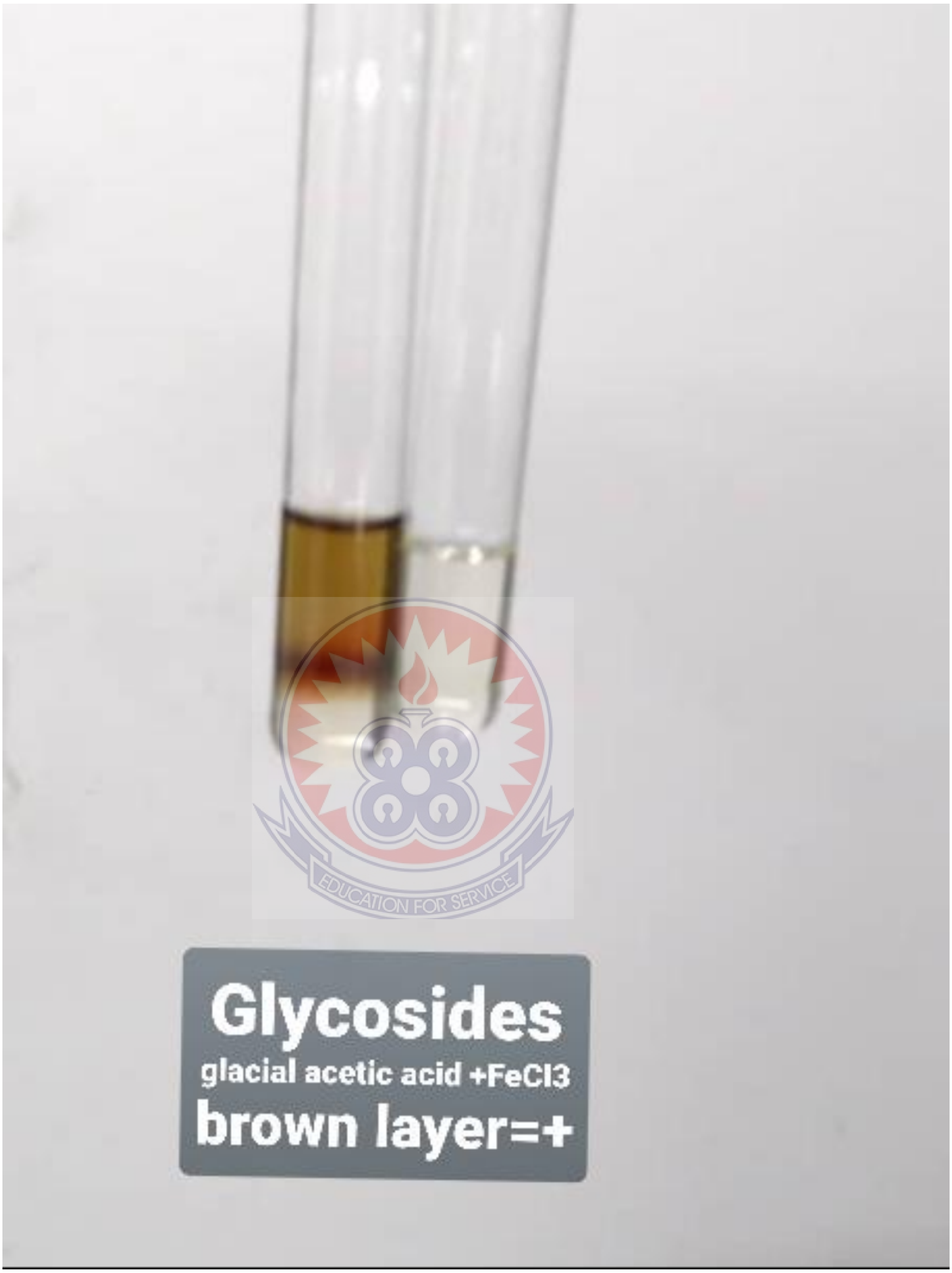

Plate 15: A test displaying a present of glycosides

Plate 16: Damage of cowpea by *Callossobruchus maculatus* F.

Plate 17: Damage of maize by *Sitophilus zeamais* Motsch.