INFLUENCE OF AGRO-ECOLOGICAL ZONE ON AZADIRACHTIN CONCENTRATION AND EFFICACY OF NEEM LEAF POWDER AGAINST *SITOPHILUS ZEAMAIS* IN UGANDA

BY

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DECLARATION

I, Muleni Joseph, do hereby declare that, to the best of my knowledge, this work has never been submitted to any institution of higher learning for a ward of Masters of Science Degree in Crop Science or for any other academic purpose. Any similarity in the material, methodology or data is by coincidence. With exception of the work attributed to other scholars who are acknowledged, the material and information in this thesis is original and mine.

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SUPERVISORS' APPROVAL

This research entitled "**Influence of agro-ecological zone on Azadirachtin concentration and efficacy of neem leaf powder against maize weevil,** "*Sitophilus zeamais*" was carried out under our supervision and submitted with our approval as supervisors.

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AEZs:	Agro-Ecological Zones
ANOVA:	Analysis of Variance
AZA:	Azadirachtin
CONC.	Concentration
HPLC:	High Performance Liquid Chromatography
IPM:	Integrated Pest Management
LSD:	Least Significant Difference
NaCRRI:	National Crops Resources and Research Institute
NARL:	National Agricultural Research Laboratories
UV:	Ultra-Violet
RH:	Relative Humidity

ABSTRACT

Using synthetic insecticides to control *Sitophilus zeamais* has raised serious concern on the environment and human health. There is need to adopt a more affordable and eco-friendly option such as neem bio pesticide. Neem is reported to contain AZA active ingredient with insecticidal properties against *S.zeamais*. Neem leaf powders obtained from four AEZs were evaluated for AZA conc. and efficacy against *S. zeamais*. Extraction of AZA in neem leaf powders was done in two replicates using 100g of fine powder each obtained from the four AEZ while quantification was done using HPLC. Efficacy of neem leaf powder against *S. zeamais* was assessed using four doses: 0.5, 1.0, 1.5 and 2.0g. There were significant differences in conc. of AZA in neem leaf powders from the 4 different AEZs. Lake Victoria Crescent which experiences moderate climate with average rainfall had the highest AZA content (35.23 mg/g) while Karamoja dry land with hot climate had the lowest AZA (27.76 mg/g). Further, maize grain treated with neem leaf powders obtained from Lake Victoria Crescent with the highest conc. of AZA had the lowest mean weevil count (13/100g) with lowest mean number of damaged grains (4.8 /100g) while Karamoja dry land with the lowest AZA conc had the highest mean weevil number (25 /100gm) and the highest mean number of damaged maize grains (11.7/100g). Neem leaf powder dosage of 2.0g effectively reduced the mean grain damage to 10.0 compared to 20.0 when treated with 0.5g.When using neem leaf powder as an option, customized quantities should be used basing on the AEZ from which the neem leaf product is obtained and those using neem products from Karamoja dry land, should use more quantities compared to Lake Victoria Crescent AEZ.

CHAPTER ONE: INTRODUCTION

1.1 Background to the study

Maize, *Zea mays* belongs to the family of grasses (Poaceae) and it is considered to have originated from Central America and Mexico (Raj et al., 2022).Maize is cultivated throughout the world and is one of the major cereal crops produced worldwide for both as staple food and cash crop contributing to a stable household incomes in the whole world (Okparavero et al., 2022; Raj et al., 2022). The crop is cultivated in over 139 million hectare of land and around 600 million tons of maize is annually produced globally (Epule et al., 2017). Maize occupies the third position after rice and wheat in terms of area planted and production (Okparavero et al., 2022). World production of maize in the year 2018 was estimated to be 10.14 million tons produced from 4.8 million hectares of land (Getahun & Wondimu, 2020b). The United States of America is the chief producer of maize with over 30% production followed by China 21%, Brazil 7.9% and Africa contributing about 7.0% overall maize production in 2018 (Okparavero et al., 2022). In Sub-Saharan Africa, maize crop covers over 35 million hectares and over 70 million metric tons of maize grain were produced in the year 2019 (Gautam et al., 2021). In Uganda , maize is an important staple food for many households contributing to about 11% of the calorie's intake (Sserumaga et al., 2020). Uganda exported 215,000 tons of maize valued at U$53.9 million in 2018 (Sserumaga et al., 2020). And it is widely grown in eastern region which produces

over 50% of the annual production in the country (Mibulo & Kiggundu, 2018; Sserumaga et al., 2020).

Despite the global significance of maize, its production in Uganda has been constrained by many factors such as crop pests and diseases, low soil fertility, limited access to improved varieties, climatic changes, weeds such as *striga species*high cost of inputs (Okparavero et al., 2022). However, amongst all these factors, field and storage pests cause a greater risk to maize production globally (Awaad et al., 2021; Maier et al., 2017). Worldwide losses of maize grain during storage are reported to be majorly as a result of *S. zeamais* infestation which cause devastating loses of the maize crop (Jaya et al., 2020; Okparavero et al., 2022; Singh et al., 2020).

During storage, *S.zeamais* can cause about 80% loss of unprotected maize grain in only 6 months through feeding and contamination by excreta, thus causing reduction in grain weight and quality (Bhandari et al., 2022). The adult weevils bore holes on grain for feeding and lay eggs in the grain from which the larvae emerge out and become more veracious until maturity (Bhandari et al., 2022; Gautam et al., 2021).

Several measures have been adopted to manage the destructive activities of *S.zeamais* which include use of synthetic chemical compounds that kill, poison, attract or repel the *S.zeamais* (Halliru & Suleiman, 2022). Other measures include, cultural measures, mechanical / physical barriers such as tight polythene bags, silos, male sterilization, use of natural enemies and botanical pesticides (Ayalew, 2020a; Halliru & Suleiman, 2022).

The use of synthetic pesticides has been one of the effective pest control option to manage *S. zeamais* both in the field and storage (Gautam et al., 2021). However, there has been a major shift from synthetic chemicals to non-synthetic chemicals worldwide and this is due to the growing concern about the potential hazards to the ecosystem (Bhandari et al., 2022) and also leading to resistance, resurgence and residues that enter the food chain causing bio-accumulations and bio-magnification in addition to high costs (Bhandari et al., 2022; Gautam et al., 2021; Seye et al., 2022).

Neem tree has been reported to have natural pesticide which are less harmful, less persistent in the environment, less toxic to non-target organisms and less costly(Gupta, 2022; Rahardjo et al., 2020). Neem is also reported to contain azadirachtin active ingredient that makes it a potential bio pesticide against insect pests including maize weevil *S. zeamais*(Gupta, 2022; Rahardjo et al., 2020; Rashid, 2020; Seye et al., 2022).

Azadirachtin is reported to affect a broad spectrum of insects including *S. zeamais* through different ways such as acting as an anti-feedant ,insect growth regulator oviposition inhibitor and repellant effect against insect pests (Fernandes et al., 2019).

Research has showed that there is a wide variation in the azadirachtin concentration amongst neem trees growing in different habitats and agro-ecological zones(Intisar Elteraifi & Hassanali, 2011; Erenso & Berhe, 2016). The concentration and accumulation of azadirachtin like any other plant secondary metabolite is reported to be highly dependent on the environmental abiotic factors such rainfall, temperature, humidity, soil fertility and light among others (Ashokhan et al., 2020; Yeshi et al., 2022). Variations in the azadirachtin content in neem products is also attributed to the genetic variability of the individual neem tree species (Ashokhan et al., 2020; Cloyd, 2015; Tunca et al., 2020). This variation in azadirachtin concentration is reported to have a direct bearing on the efficacy of neem products against the target pests such as *S. zeamais* (Dawkar et al., 2019; Erenso & Berhe, 2016; Prakash et al., 2005). In Uganda, neem tree grows in varied agro-ecological zones with diverse soil types, temperatures and humidity. It is therefore against this background that this study was conducted to investigate the influence of agro-ecological zone on azadirachtin concentration in neem leaf powder and its efficacy against maize *S.zeamais* (Chombo et al., 2020; Paparu et al., 2020).

1.2 Problem statement

Despite the economic importance of maize grain in Uganda, its optimum supply in terms of quantity and quality has never been achieved majorly due to severe postharvest loses caused by *S.zeamais* (Ayalew, 2020b; Mbata & Toews, 2021). Several measures including mechanical, biological, physical and use of synthetic chemical insecticides have been adopted to minimize *S. zeamais* infestations in stored maize grain (Ayalew, 2020b). However, extensive use of non-degradable chemical pesticides is associated with increased costs, pollution, production of toxic substances in food among others (Wahedi et al., 2017). This makes these measures less attractive(Ayalew, 2020a; Mbata & Toews, 2021). This has necessitated urgent need to switch over to ecofriendly bio pesticide alternative which is readily biodegradable and less expensive to small scale farmers (Getahun & Wondimu, 2020b; Mbata & Toews, 2021). *Azasdirachta indica,* has been globally reported to contain plenty of different bioactive secondary metabolites including azadirachtin which is most efficacious with insecticidal and repellent properties against *S. zeamais* (Gebreegziabiher et al., 2017; Gupta, 2022). Studies have shown that the efficacy of the neem bio pesticide depends on concentration of azadirachtin in the neem material (Kaushik et al., 2007). However, neem plants exhibit great variations in content of plant secondary metabolites produced including azadirachtin (Jan et al., 2021; Yeshi et al., 2022). Variations in plant secondary metabolites such as azadirachtin are reported to be highly dependent on environmental abiotic factors such as rain fall,drought, heat stress,soil fertility(Yeshi et al., 2022).

Its further revealed that the concentration of azadirachtin in a neem plant is also influenced by climatic conditions that prevail in different habitats and agro-ecological zones(Gupta, 2022).With the evidence that neem trees from different agro-ecological zones may have varying concentration of azadirachtin and different efficacy levels, making uniform recommendation on quantity of neem plant materials to farmers without considering their geographical source may lead to failed storage grain protection and thus justification for this study.

Therefore, having knowledge on which agro-ecological zone in Uganda produces neem products with high concentrations of azadirachtin and which concentrations of azadirachtin are more efficacious in controlling *S. zeamais* in stored maize grain is very crucial in achieving effective protection of the stored maize grain.

1.3 Objectives of the study

1.3.1 General objective

This experimental study aimed at establishing the influence of agro-ecological zone on a zadirachtin concentration and its efficacy against *Sitophilus zeamais*.

1.3.2 Specific objectives

1. To investigate the influence of agro-ecological zone on azadirachtin concentration in neem leaf powder.
2. To determine the influence of agro-ecological zone on efficacy of neem leaf powder in protecting stored maize grain against *Sitophilus zeamais.*
3. To determine the effect of dose on the efficacy of neem leaf powder in reducing stored maize grain damages by *S. zeamais*.

1.4 Hypotheses of the study

i) There is no difference in azadirachtin concentration in neem leaf powder obtained from the four sampled agro-ecological zones in Uganda.

ii) Efficacy of neem leaf powder in inhibiting *S. zeamais* instored maize grain does not vary with agro-ecological zones.

iii) Efficacy of neem leaf powder against *S. zeamais* does not depend on dose of the leaf powders.

1.5 Conceptual framework

The azadirachtin concentration in the neem leaf powder is dependent on the agro-ecological zones which in turn are also influenced by the environmental factors (Figure 1.1).

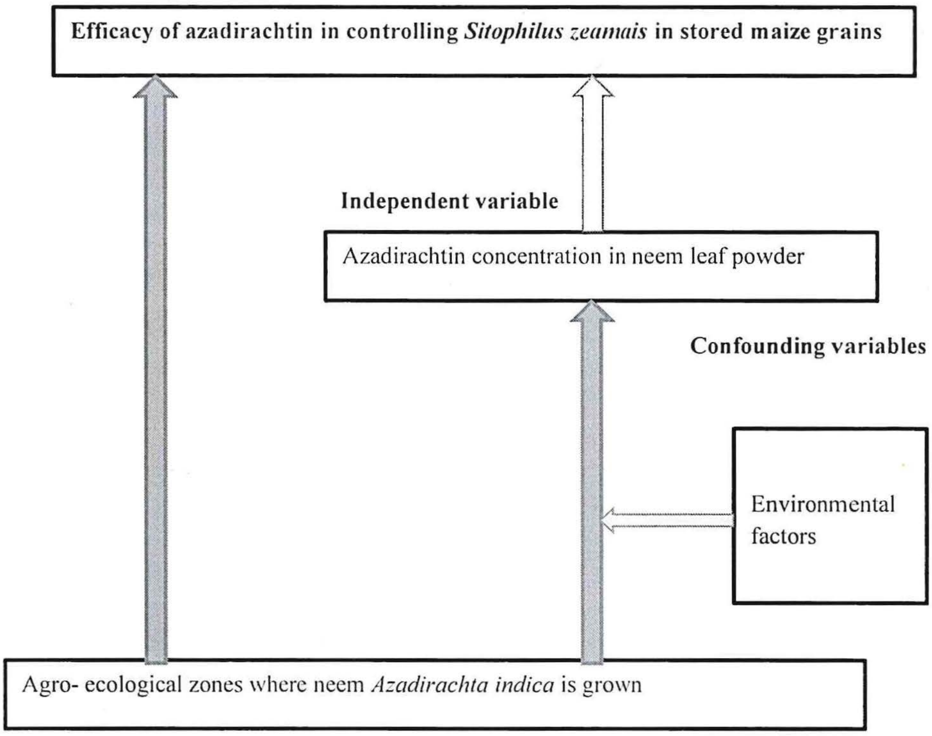


Figure 1.1: Conceptual framework

1.6 Significance of the study

i) The findings of this research study provide guidance to farmers and potential processors of natural bio pesticides on the agro-ecological zone(s) that yield neem leaf powders that contain highest amount of azadirachtin and most efficacious against *S.zeamais*.

ii) This study provides a basis for natural bio pesticide processors and farmers to determine customized quantities of the leaf powder from the different agro-ecological zones to be used per unit quantity of maize grain for protection against *S.zeamais*.

iii) The results of this study will provide a basis of knowledge for future research on neem products in pest management.

CHAPTER TWO: LITERATURE REVIEW

2.1 Biology of maize weevil

The adult *S.zeamais* is generally small and brown in color with clearly marked reddish brown spots on the wing cover (Attia et al., 2020). The body length measures 2.5-4.0mm with its head pointed into a long thin snout which has clubbed, segmented, elbowed antennae and a pair of mandibles at the end (Attia et al., 2020; Ojo & Omoloye, 2016). The female deposits a small oval white egg and covers the hole as the ovipositor is removed, with a waxy secretion that creates a plug(Ojo & Omoloye, 2016). The plug quickly hardens and leaves a small raised area on the grain surface and it is this that provides the only visible evidence that the kernel is infested (Ojo & Omoloye, 2016). Its only one egg which is laid inside each maize grain with each single female laying up to 300 eggs during her life time and adults can live up to 5 months (Bhandari et al., 2022; Rashid, 2020).

The incubation period of eggs is 3 to 7 days and the emerging larvae feeds on the grain for about 3 weeks and then goes into pupation for 6 to 7 daysand it emerges out from the grain as an adult (Bhandari et al., 2022). The complete life cycle takes 36 days at 30°C and 75 to 76% RH (Bhandari et al., 2022). Temperatures between 15 and 34°C and RH of 40% are the optimum breeding conditions for *S. zeamais* (Ojo & Omoloye, 2016). When the adults emerge, the females move to the grain surface and release sex pheromones to attract the males (Ojo & Omoloye, 2016). The life span of the *S.zeamais*varies with temperature however, the life cycle is longer in cooler areas compared to warmer environments (Demis & Yenewa, 2022). For instance the life cycle of the maize weevil at 25°C is about 37 days and takes about 110 days at

18^{o}C though the life cycle can completed faster in warmer environmental conditions (Demis & Yenewa, 2022).

2.2 Economic importance of *Sitophilus zeamais*

S.zeamais is the serious damaging storage pests of maize causing significant reduction of 80% in both grain quality and quantity (Bhandari et al., 2022). It is one of the most destructive pests of stored maize grain in the tropics and temperate regions of the world(Manjhu et al., 2022). *S. zeamais* capable of penetrating and infesting intact kernels of grain in which immature stages can undergo development (Bhandari et al., 2022; Manjhu et al., 2022).

S. zeamais infestation in most cases start in a mature crop when the moisture content of maize falls to 18-20% (Bhandari et al., 2022). The grown-up female *S.zeamais* drills into the kernel and lays the eggs (Bhandari et al., 2022). The larvae and pupae eat up the inward pieces of the kernel bringing about damaged kernel and diminishing grain weight (Bhandari et al., 2022). The infestation causes loss in quantity of products, reduces nutritional quality, alters the chemical composition of grains, causes off odors in the end-use products and produces contaminants such as excreta, body fragments and chemical secretion which increase moisture content of maize grain (Gautam et al., 2021). *S. zeamais* causes significant losses in maize grain by reducing the grain nutritional value culminating in outright losses of 20-90% in untreated maize grains (Demis & Yenewa, 2022). Infestations can also elevate the temperature and moisture content of stored maize grain hence leading to fungal growth in maize grain (Demis & Yenewa, 2022). It has been revealed that *S.zeamais* is responsible for about 15-20% loses of stored maize grain every year resulting into quality and quantitative reduction which directly cause a big threat to food security worldwide (Getahun & Wondimu, 2020a; Nwosu, 2018).

2.3 Management of *Sitophilus zeamais*

2.3.1 Cultural control

Use of crop pest management practices that make the environment less favorable for the *S.zeamais*to develop into insect eggs, pupae and adult weevils have been reported to effectively reduce maize grain damages by *S.zeamais*and have therefore, been adopted as an alternative control option(Boddupalli et al., 2020; Damena et al., 2022).

Use of resistant varieties

In contrast to the use of destructive synthetic chemicals, planting of maize varieties that are resistant to *S. zeamais* is now a sustainable and economically viable option for minimizing postharvest losses of maize grain caused by *S.zeamais* (Nwosu, 2018). Many varieties of maize grain possess traits of grain and pericarp hardness which make such varieties less attractive to *S.zeamais* attack compared to the less susceptible varieties (Mamoon-Ur-rashid et al., 2021). Different varieties of maize have different levels of susceptibility to storage maize weevil *S. zeamais*. and therefore, identifying whether the variety is resistant or susceptible can help farmers to anticipate what type of control may be required (Suleiman & Rosentrater, 2015).

Use of resistant varieties of maize would be the best cultural approach in control of *S. zeamais* with 82.1% adult mortality compared to other cultural options however, breeding maize variety to improve resistance against *S. zeamais* is hindered by linkage of undesirable genes, lack of knowledge of genetic basis of resistance and limited inter-specific cross compatibility (Nwosu, 2018).

The laboratory experiment to assess the resistance of six open pollinated approved maize varieties revealed that, the yellow variety containing maximum grain hardness exhibited high resistance against *S. zeamais* during 90 days of storage compared to varieties containing minimum values of grain hardness (Mamoon-Ur-rashid et al., 2021). Hiruy & Getu (2018) reported that six out of the 21 maize varieties screened for resistance against *S. zeamais* showed 100 % resistant factor that confers resistance against *S. zeamais* attack. While the study to evaluate *S. zeamais* infestation in a choice and no choice test under laboratory conditions in four environments in Uganda revealed that hybrids G56 (L49/T2) and G58 (L51/T2) had the least *S. zeamais* damage and were rated as resistant variety to *S. zeamais* during storage compared to control which (Sserumaga et al., 2020).

Timely harvesting and sanitation

Harvesting of mature cobs of maize as soon as possible after it has reached maturity with 17-18% moisture content effectively reduced the chances of grain damage by *S.zeamais* compared to early or late harvested maize grain with high moisture content making it prone to *S.zeamais* attack (Nwosu, 2018). Harvesting on time means the maize weevil has less time to infest and reproduce on the standing maize crop (Nwosu, 2018). Often *S.zeamais* infests the edges the field before moving to the rest of the crop and harvesting the infested areas and storing that grain separately effectively reduces further infestation (Nwosu, 2018).

Good storage hygiene and Sanitation

The removal of infested residues of the previous seasons' harvest is essential and gives 62-68% mortality of adult *S.zeamais* compared to infestations resulting from the previous harvest season (Suleiman et al., 2016). Cleaning of all storage containers and ware houses such as granaries, bins, bags, areas around and within cracks, crevices, doors and windows using brooms, shovel and vacuum cleaners before storage of new products effectively reduced *S.zeamais* infestation of stored maize grain by 78.5% (Nwosu, 2018).

2.3.2 Physical and mechanical control

Use of force and manipulation of storage environment to provide unfavorable conditions such as use of direct heat, airtight storage, cold, inert dust, physical exclusion of air, sieving picking and physical disturbance are reported to effectively reduce the infestation of maize grain by *S. zeamais* (Ojo & Omoloye, 2016, 2021).

Temperature and moisture control

Temperature and moisture are reported to be effective strategies for managing *S. zeamais* in stored maize grain (Damena et al., 2022). Exposing maize grains to temperatures below 10°C and maintaining the temperature above 100°C at 11% RH for 6 months storage induced 90% mortality at all stages of *S. zeamais* (Damena et al., 2022).

Physical disturbance of stored maize grain

Physical disturbance of stored maize grain has been reported to significantly reduce storage pest population especially the *S. zeamais* of maize grain due to mechanical un rest of maize grain compared to un disturbed maize grain (Suleiman et al., 2016). Frequent disturbance of stored maize grain through turning the maize grain ultimately resulted into reduction of insects by starving and directly killing the insects that are outside the grains as insects were not able to completely bore holes into maize kernel to feed and to lay eggs thus causing 98% mortality (Suleiman et al., 2016).

Use of air tight containers

Air-tight container systems eliminate gas exchange between maize storage and external environment thus reducing oxygen levels to 5% below which increases a carbon dioxide accumulation in stored maize grain there by eliminating maize weevils by 80% (Brumm et al., 2021).

The study conducted on the effectiveness of controlled ambient aeration during storage of maize grain against *S. zeamais* found that there was over 60% mortality of adult maize weevil due to reduced aeration thereby reducing the number of*S.zeamais* compared with the weevil population levels in control (Maier et al., 2017). Carbon dioxide controlled atmosphere at concentration of 50% and 60% in stored maize grain resulted into 98% adult *S.zeamais* mortality at all stages after 10 days of exposure under laboratory conditions (Darfour, 2019).

Reduction of oxygen in maize grain storage ecosystem by use of hematic method causes carbon dioxide accumulation which 100% suffocates and dehydrates *S.zeamais* in storage maize (Yakubu et al., 2011). A study to determine effectiveness of hermetic post-harvest maize grain storage at 27°C (a non-chemical control strategy) against *S. zeamais* revealed 100% adult mortality in 6 days for both 16% moisture of maize grain and kernel density of 1.24g/cm3 compared to the control (Yakubu et al., 2011).

Physical and mechanical control of *S. zeamais* are considered to be simple and effective maize grain protecting option in most developing countries however, they require expensive equipment (Suleiman et al., 2016).

2.3.3 Biological control

This control measure involves purposeful utilization of introduced or resident living organisms usually natural enemies to suppress the activities and population of one or more storage maize pests including *S. zeamais* (Ghimire, 2021). And it is reported to be 100% effective if applied rightly (Ghimire, 2021). Use of biological control measures against *S. zeamais* include, use of predators, parasitoids and pathogens/ microorganisms decrease the population density of pest organisms including insects, mites or plant pathogens (Saleh et al., 2019).

Use of parasitoids

Natural enemies that lay eggs in or on other organism including insect pests (host) usually kill their host during their larval stage as they feed on it and develop (Atwa et al., 2018). The study on host-substrate preference of *Theocolax elegan* a larval parasitoid of maize weevil under laboratory condition revealed that *Theocolax elegans* parasitoid effectively parasitized *S.zeamais* larval on stored maize grain host–substrate causing 70% larval mortality after 90 days of storage (Sitthichaiyakul & Amornsak, 2017). Another study on biological control of *S.zeamais* in bagged maize grain with *Lariophagus distinguendus* and *Corcyra cephalonica* revealed that parasitoids are suitable for biological control of stored maize weevil are able to find their hosts in jute bags over large distances compared to maize grain bagged without parasitoids (Ghimire, 2021).

Use of bio controls in management of storage maize weevil has many advantages over the traditional, physical/mechanical and chemical control because of less danger caused to the environment and have high multiplication rate once released in storage facility(Alam et al., 2020; Ghimire, 2021) .

Predators

The organisms that prey and feed on other insect pests are reported to effectively reduce maize grain storage pests such as *S.zeamais* (Atwa et al., 2018) . Predators often feed on various stages of a particular pest such as eggs, larvae, pupae and adult. Each predator kills and feeds on a number of prey individuals during their development (larvae to adult) (Atwa et al., 2018). Predators tend to check the population of their prey for example predatory bugs, predatory wasps and spiders which prey on insect pests(Atwa et al., 2018). The predators such as Anthocorid bug, (*Xylocoris flavipes*) are reported to cause 84.64% of larval and adults mortality of *S. zeamais* in stored maize grain compared to 90% mortality by *Dorylus labiatus* the most efficient predatory ant species to suppress *S.zeamais*(Mantzoukas et al., 2022).

Use of microorganisms

Under favorable conditions, micro-organisms such as nematodes, bacteria, viruses and other microbes cause fatal diseases to arthropod pests including *S.zeamais* that causes damage to stored maize grain (Mantzoukas et al., 2022).

Use of fungi

Fungi produce spores that attach onto the cuticle of insect pests, germinate and receive cues from the cuticle surface thereby producing degenerating enzymes by germ tubes which breach the cuticles of insects which depletes the body nutrients and disruption of the circulatory system (Mantzoukas et al., 2022).These microorganisms are compatible with plant derived bio pesticides (Mantzoukas et al., 2022).*S. zeamais* for example,when exposed to the formulation of fungus *Beauveria bassiana* combined with *Chhromolaena odorata* leaf powder causes 98% mortality rate of maize weevil after 21 days of post-treatment of stored maize grain (Mantzoukas et al., 2022).

2.3.4 Chemical control

Protectant insecticides are applied with the intent of providing long lasting control against *S. zeamais* for many months of maize grain storage (Nwosu, 2018) while fumigants are gases / fumes that are short-lived but fast-acting and can be effective if applied reactively to maize grain against *S. zeamais* infestation. (Nwosu, 2018). Synthetic chemical pesticides such as weevil-cide 60%,aluminium phosfume 2 (pellets/ tablets), Phosphide 60%, Phostoxin 60%, phosphine, surfuryl fluoride and many others are used in management of *S. zeamais* especially in large scale stores (Darfour, 2019; Nwaubani et al., 2020).

Other synthetic insecticides that effectively manage *S. zeamais* in stored maize grain with 100% mortality include malathion, aluminium phosphide, phostoxin, temaphos, celphos, Bextoxin, agroxin tablets, pirimiphos, permethrin, detamethrin and fumigation of the grains with phosphine or methyl bromide which all effectively (up to 100%) controlled storage maize (Damena et al., 2022; Darfour, 2019) .

A study to evaluate toxicity of permethrin synthetic and selected botanical insecticides under Laboratory conditions revealed 90% adult *S.zeamais* mortality with maize grain treated with permethrin after 21 days under laboratory condition compared to 18.26% with *Ageratum conyzoides and 6.14% with Cymbopogon nardus* (Emeasor et al., 2022). The study to evaluate the relative toxicity of some insecticides such as deltamethrin at 20ppm, emamectin benzoate at 5000 ppm, spinosad at 700ppm concentration revealed 100% mortality of adult maize weevils in comparison with organophosphate insecticides such as malathion at 16 ppm concentration (Parimala et al., 2011).

Synthetic insecticides used as grain protectant provide rapid lethal effect in stored maize grain that renders 100% mortality of adult *S. zeamais* (Chaudhary et al., 2017) .Damena et al., (2022) reported 100% adult weevil mortality with application of different insecticides such as emmamaectic benzoate 5.76 WDG, neem oil, chlorophyrifos 50%, cypermethrin 5% EC, malathion 505 EC at doses of 0.3,5,1.5,2 and 5ml per liter applied on maize grain compared with the control that had 0.0% mortality.

However, due to the adverse effects that include environmental pollution, Atmosphere ozone depletion, toxic wastes, the long residual period of toxicity and documented resistance and resurgence, safe and cheap alternative that involves use of Bio-pesticide have been (Chaudhary et al., 2017).

Poor storage facilities in developing countries renders use of synthetic chemicals ineffective and pest resistance to chemicals as most storage structures are prone to pest re-infection resulting to shorter pesticide re application intervals (Cosmas, 2018). Coupled with the inauspicious macro-economic environment in most developing countries, the resource constrained farmers cannot easily access the synthetic chemicals to preserve their grain amounting to huge storage maize grain losese (Cosmas, 2018). Use of synthetic chemicals is also associated with increased effects posed on human health, ecosystem, soil micro biota and water sources (Langsi et al., 2017). These harmful environmental implications of the chemicals have compelled researchers to search for alternative naturally occurring pest control agent referred to as bio pesticide (Langsi et al., 2017). Over 90% of the applied synthetic chemicals including pesticides reach water sources due to surface run-off thus exposing farmers who are the major consumers of agricultural produce to higher health risks and therefore, control measures of maize weevil with less risks have been deployed as an alternative (Nwaubani et al., 2020).

Use of Semiochemicals

Semio chemicals such as pheromones effectively reduced damages caused *S.zeamais* in storage maize grain (Stuhl & Romero, 2021). Chemicals produced by organisms as massagers affect the behavior of other individuals of insects and are reported to be effective against insect pests including *S. zeamais* (Stuhl & Romero, 2021) .

They are usually wind-borne but may be placed on soil, vegetation or various items and used to uphold communication disruption or mass trapping of insects by lures or attract (Stuhl & Romero,2021). They are usually produced by male and female insects are used for mating, aggregation, defense, host recognition and have also been isolated effectively for control of *S.zeamais* (Deb, 2019). These can be alarm pheromones, food trial or sex pheromones and can affect the behavior or physiology of insects for example parasitoid Theocolax elegans that has been found to be 70% effective inattacking adult *S. zeamais* (Deb, 2019; Stuhl & Romero, 2021). *S.zeamais* pheromone traps are used as sex attraction pheromones to lure adult maize weevils and as they crawl or fly into the holding tray, the thick catching oil holds them (Saleh et al., 2019).

2.3.5 Use of botanical pesticides

These are potential plant –based botanicals reported to contain insecticidal effects and are being used as botanical pesticides against insect pests including *S.zeamais*in stored maize grain (Srivastava et al., 2020). Botanical pesticides have been found to be affordable and most effective insecticides against storage grain pests including the *S.zeamais*(Fernandes et al., 2019; Srivastava et al., 2020). Studies on efficacy of most botanical pesticides have revealed that plant bio pesticides such as Neem

Azadirachta indica, E. camaldolensis,M. oleifera, A indica, N. tobacum, L .camara among others have proved to offer positive results in control of *S. zeamais* in stored maize grain (Halliru & Suleiman, 2022).

2.3.6 Use of neem as a bio pesticide

Neem has for long been known for its natural insecticidal properties (Fernandes et al., 2019). It is easily biodegradable, less toxic to beneficial organisms, least persistent in the environment and effective pesticide against many insect pests including the maize weevil (Fernandes et al., 2019; Gupta, 2022). A recent study by Erenso & Berhe (2016) reported 63.3%, 70.8% and 82.05% *S. zeamais* mortality on maize grain treated with 1%, 2% and 3% neem leaf powders for 90 days of storage compared to 0.0% maize grain with no neem leaf powder treatment. More recent studies have revealed that maize grain treated at with 1.0% concentration of neem leaf extract caused up to 100% maize weevil mortality compared 0.0% mortality in the untreated maize grain (Getahun & Wondimu, 2020); Rashid, 2020).

Neem contains azadirachtin the active ingredient that has for long been known to produce potential neem-based bio-pesticides used in suppressing *S. zeamais* (Reddy & Neelima, 2022). Azadirachtin is alimonoid insecticide produced by *Azadirachta indica* tree and it is a natural product made by plants that belong to Meliaceea and Rutaceae family (Dawkar et al., 2019; Gupta, 2022). Azadirachtin active ingredient is found in all parts of neem tree including the leaves, bark, fruits, seeds, roots and oil (Fernandes et al., 2019). However, neem seeds have been proved to contain higher concentration of azadirachtin(40-50%) compared to the leaves with 30-40%, stems 30-40% and roots 10-30% azaddirachtin (Ojo & Omoloye, 2016). Neem leaves are mostly preferred by majority farmers since they are easily obtained and can easily be processed though recommended to collect the neem leaves before flowering (Fernandes et al., 2019).

Nevertheless, Azadirachtin is the most predominant active compound in neem and accounts for the majority of bio-pesticide activities of the neem(Ojo & Omoloye, 2016). Azadirachtin is among the various compounds already isolated for its bio active properties against insect pests(Ojo & Omoloye, 2021). It is the most promising and effective botanical insecticides that exhibits significant growth inhibitory activities against agricultural pests (Chaudhary et al., 2017).It exhibits anti-feedant and oviposition deterrent activity which causes anti peristaltic movement in the alimentary canal of insects (Chaudhary et al., 2017; Srivastava et al., 2020). Besides azadirachtin, there are also other classes of active chemical compounds reported to have mortality, repellency, anti-feedant, anti-oviposition and insecticidal effects against majority of insect pests and they include, nimbin, nimbinin, salinin, glycoside, tannins though azadirachin is the most effective against *Sitophilus zeamais*(Chaudhary et al., 2017; Srivastava et al., 2020).

2.4 Production of Azadirachtin by plants

Azadirachtin is one of the secondary plant metabolites that often accumulate in a plant that is subjected to stress including various elicitors or signal molecules (Farjaminezhad & Garoosi, 2019). These metabolites usually play a major role in adaptation of plants to environment and in overcoming stress condition (Farjaminezhad & Garoosi, 2019).Plant secondary metabolites are natural products found in selected plants that are beneficial for plant survival and reproduction in the environment and are derived from building blocks synthesized in primary and intermediary metabolism (Ashokhan et al., 2020; Dawkar et al., 2019). Secondary plant metabolites are numerous chemical compounds produced by the plant cell through metabolic pathways derived from the primary metabolic pathways (Ashokhan et al., 2020).

Plants have evolved numerous mechanisms for accommodating changes arising in their fluctuating growth conditions to enable functional flexibility under the influence of environmental factors without affecting cellular and physiological processes (Isah, 2019). They are therefore, synthesized from primary metabolites (Carbohydrates, lipids and amino acids) needed in plant defense against herbivores and pathogens and offers protection against environmental stress due to their color and taste (Latif et al., 2020).

Plant secondary metabolites play a critical role in plant stress tolerance and adaptation and are known to be influenced by the environmental and climatic changes (Hodgson et al., 2019). Production of plant secondary metabolites by the plants is regarded as an adaptive capacity in coping up with stress constraints during the challenging and changing environment of growth (Hodgson et al., 2019).

This involves production of complex chemical types and interactions in the structural and functional stabilization through signaling process and path way (Hodgson et al., 2019). Drought alone had the strongest effects on phenolic concentrations and compositions with moderate effects elevated carbon dioxide and temperature(Hodgson et al., 2019).The concentration of these secondary metabolites however, increases under moderate drought (Ebifa-Othieno et al., 2020). It has been reported that plants that experience some form of stress while growing tend to accumulate increased levels of useful nutrients(Ebifa-Othieno et al., 2020). Production of secondary metabolites in most cases is an adaptive response employed by various plants taxonomic groups in coping up with stress and defensive stimuli (Latif et al., 2020).

Plant elicitors are molecules which trigger different plant morphological and physiological responses for enhanced production of defensive secondary metabolites in plants which play an important role in adaptations of plants to stressful conditions(Zaeem et al., 2022). They are chemical compounds from abiotic and biotic sources that can stimulate stress responses in plants leading to enhanced synthesis and accumulation of secondary metabolites (Zaeem et al., 2022). However, elicitors can be divided into abiotic and abiotic elicitors comprised of substances that are of non-biological origin and grouped in physical such as light, osmotic stress, salinity, drought thermal stress, chemical and hormonal factors such as potential of hydrogen, sucrose, ultra violet, gibberellic acid, silver, copper and cobalt and ozone (Dawkar et al., 2019). However, the type and concentration of secondary molecules produced by plants is determined by many factors such as species, genotype, physiology, developmental stage and the environmental factors during growth (Dawkar et al., 2019; Isah, 2019).

However, the concentration of secondary plant metabolites such as azadirachtin are strongly dependent on the growing conditions that have an impact on the metabolic pathways responsible for accumulation of related natural products (Li &

Ramakrishna, 2014). The production of secondary metabolites by plants is much influenced by the rate of photosynthesis which is also influenced by so many factors such as availability of minerals such as potassium, copper, magnesium, chloride which also regulate the photosynthetic process (Isah, 2019). Variations in genotypic factors and geographical conditions have are also reported to influence variations in the concentration of azadirachtin in neem tree *Azadirachta indica* obtained from different parts of *Azadirachtin india* (Prakash et al., 2005).

Drought stress, flooding, salinity, low temperature, water dehydration, binary stress in green houses, irrigation, nitrogen supply, anti-transpiration agents, water logging are the other factors that influence production of secondary metabolites in plants (Isah, 2019). The stress condition triggers the plant defense mechanism leading to a variety of biochemical signals which in turn induce the expression of genes related to secondary metabolite synthesis(Ashokhan et al., 2020). Abiotic stresses can affect the chemical composition and the amount of essential oils produced by plants (Elsadig et al., 2017; Muzira et al., 2020). The amount and quality of secondary metabolites produced by the plant also depends on external factors such as soil composition, presence of endopytic organisms, altitude, processing and storage (Ashokhan et al., 2020).

2.4.1 Mode of action of neem Azadirachtin

Azadirachtin is now known to affect a broad spectrum of over 200 insect species including aphids, mealy bugs, thrips, white flies, caterpillars (Tunca et al., 2020).It has an anti-feedant and can disrupt the normal insect growth, molting, repels larvae and adults, it sterilizes adults and deters egg laying insects (Tunca et al., 2020).

Azadirachtin affects insects in many different ways including, acting as an insect growth regulator, ant-feedant, repellant and oviposition inhibitor (Cloyd, 2015).

The azadirachtin hinders the action of ecdysones that prevents the insect larvae from shedding their exoskeleton thus azadirachtin alters their life cycle and inhibits the development of immature insects (Cloyd, 2015). It works as a stomach poison in which insects must ingest the active ingredient during feeding in order to be negatively affected which is better on chewing insects like *S.zeamais* than the sucking insects that is why azadirachtin is most effective against biting and chewing insects (Cloyd, 2015). It also acts as antagonist by inhibiting the synthesis or metabolism of the insect molting hormone ecdysone, consequently by inhibiting the molting process and subsequently metamorphosis (Cloyd, 2015).

Azadirachtin reduces the level of insect hormone Ecdysome by disrupting the insects' molting process so that the immature larvae cannot develop into adults(Cloyd, 2015). It has minimal contact activity against most insect parts and works best at a wormer temperature with reduced efficacy at lower temperatures (Fernandes et al., 2019). The activity of azadirachtin against insects is dependent on plant type and the PH of the growing medium with less systemic activity at pH greater than 7.0 alkali (Kharwar et al., 2020). However, after treatment with the neem-based pesticides, the insects will get crippled, gets distorted wings and the immature larvae and nymphs remain in an immature stage and die (Kharwar et al., 2020). However, some soft skinned insect larvae may be killed by direct contact with spray. Adults are not killed by growth regulating properties of azadirachtin but mating and other sexual communications may be disrupted which results into reduced fecundity (Ukoroije & Bobmanuel, 2019).

Azadirachtin also affects the biochemical level of an insect by impacting the insect's endogenous metabolites and interferes with the serotonin pools in the neuro endocrine system of insects(Zhou, Qin, Zhang, Chen, et al., 2020; Zhou, Qin, Zhang, Liu, et al., 2020). It suffocates and blocks the pores of an insect suppressing the population (Zhou et al., 2020). However, azadirachtin is more effective on the young life stages of insects than eggs and adults but slower-acting than with conventional insecticides which is primarily due to azadirachtin altering or modifying the behavior of insects (Cloyd, 2015). Studies show that the feeding behavior of insects depends upon both neural input from insect for example taste receptors on tarsi, mouth parts and oral cavity and the central nervous integration of the sensory code (Ukoroije & Bobmanuel, 2019). Azadirachtin therefore, stimulates specific deterrent cells in Chemo receptors and blocks the firing of sugar receptor cells which stimulate feeding (Ukoroije & Bobmanuel, 2019).

2.4.2 Agro-ecological zone variability and Azadirachtin concentration in neem tree

Both wild and cultivated plant species produce a remarkable diversity of plant secondary metabolites which accumulate conditionally in various parts of the plant (Yeshi et al., 2022) . They are synthesized to regulate defensive signaling pathways to safe guard the plant against stress and also synthesized for attraction, communication and mediating stress (Yeshi et al., 2022).

The type of plant secondary metabolite produced can be due to genetic variability of plant species(Yeshi et al., 2022). However, variations in the concentration of secondary metabolites in plants is highly dependent on environmental abiotic factors (growth conditions) such as those that intensify climate change such as rainfall, drought, heat stress even if other factors remain constant (Jan et al., 2021). Any change in an individual plant environmental factors, for example light, temperature, soil water, soil fertility or salinity, can alter the concentration of the plant secondary metabolite(Jan et al., 2021). And this makes the plant species to exhibit great variation in the content of secondary plant metabolites produced including azadirachtin in neem tree (Srivastava et al., 2020; Yeshi et al., 2022).

Variability in agro-ecological attributes such as, rainfall, temperature, soil type, latitude and altitude therefore, influence the azadirachtin content in neem tree(IE Elteraifi & Hassanali, 2011; Kaushik et al., 2007). Neem trees growing in regions with moderate climate, average rainfall of 400 mm and high altitude over 470 m above sea level proved to be rich in azadirachtin content (Intisar Elteraifi & Hassanali, 2011). While trees growing in lower altitudes, alluvial or sandy soil, with hot climate reflected very low azadirachtin content (Elteraifi & Hassanali, 2011).

Rainfall is reported to be the major factor affecting the level of azadirachtin in neem seed kernels, and the optimal rainfall is reported to be 717 mm that gives a concentration of azadirachtin content (Biosci et al., 2019). Season alone, temperature and relative humidity had no direct effect on the azadirachtin content (Kaushik et al., 2007; Tomar & Kaushik, 2011).

Neem samples collected from different zones in Thailand revealed that the total azadirachtin A and B content had highly significant variation among the different provinces ranging from 89.15 to 2,028,06mg/ml (Biosci et al., 2019). Latitude is also reported to have a high negative influence on azadirachtin content(IE Elteraifi & Hassanali, 2011). The highest azadirachtin concentration (2,028.63mg/ml) was found in neem collected from tropical climate zone of Pattani province and so could be suitable source for neem insect bio pesticide compared to 1,478.2mg/ml obtained from hot sub-humid zone of Chachoengsao province and 1,078.3mg/ml azadirachtin obtained from the hot semi-arid zone in Khonkaen province (IE Elteraifi & Hassanali, 2011). Related studies also show that there are variation in azadirachtin concentration in different neem trees which is attributed to different conditions found in different habitats such as temperature, humidity and also due to genetic differences among the neem trees (Sidhu et al., 2003; Tunca et al., 2020).

The study conducted to quantify and evaluate the oil level and azadirachtin content of *A. indica* in seed kernels collected from trees growing in different habitats and to establish their relationship with the agro-ecological zones in Sudan revealed that neem trees growing in different habitats in different agro-ecological zones exhibit variations in azadirachtin content (IE Elteraifi & Hassanali, 2011). The study on neem biodiversity in India for bio resource revealed that there is annual variation of azadirachtin in neem obtained from agro-ecological zones of India (Kaushik et al., 2007). The concentration of azadirachtin varied from 200 to 1600 ppm (mg/ml) of the seed kernel. Azadirachtin content was found to be affected by climate and agro ecological habitat (Kaushik et al., 2007).

Further studies on performance of azadirachtin content basing on regional and geographical habitat indicate that azadirachtin content obtained from neem tree growing in plateaus ranked first with average concentration of 4743ppm (Elteraifi& Hassanali, 2011). The neem trees growing in plains and high lands ranked second with the average concentration of 2373 and 2427 ppm while neem trees growing in desert regions recorded the lowest azadirachtin content with an average of 1656 ppm(IE Elteraifi & Hassanali, 2011; Kaushik et al., 2007).

In a study to quantify the azadirachtin production in neem trees growing from different geographical conditions where azadirachtin samples were grouped into four distinct climatic classes, Hot-semi arid with mild winter, hot sub humid, hot arid and hot semi-arid with cold winter revealed that neem leaf powder samples from hot sub-humid, hot arid and hot semi-arid with cold winter type of climate were found to be statistically lower in terms of azadiractin synthesis compared with the samples from hot semi-arid with mild winter (Elteraifi & Hassanali, 2011; Kaushik et al., 2007).

It is therefore, important to note that hot semi-arid mild winter type of climate is prevalent in azadirachtin levels (Elteraifi& Hassanali, 2011).While the trees growing in hot semi-arid but with cold winter have lower average content thus moderate climatic conditions are found to be favorable for azadirachtin synthesis where as extreme climatic conditions are found to be un favorable for getting higher azadirachtin content (Elteraifi & Hassanali, 2011).

2.5 Agro-ecological zones in Uganda

Uganda is a land locked country in East Africa and its main economic activity is agriculture with nearly 34 million people engaged in agriculture production and trade(Chombo et al., 2020). And to better its agricultural potential, the country was divided into 10 major agro-ecological zones on basis of distinct soils, vegetation type and climatic conditions (Chombo et al., 2020).Besides Uganda being divided into regions such as central, south, north, eastern and west(Chombo et al., 2020). The country was also divided into political districts which were further divided into agro-ecological zonesbasing on the variations in altitude, mean annual precipitation, productivity, major vegetation type, landscape and the soils (Chombo et al., 2020).AEZs are land resource units defined in terms of temperature regime, rainfall and potential transpiration ratio and soil water holding capacity(Chombo et al., 2020).

Uganda is a large country with different agro-ecological zones as shown in Fig 3.1 and classified with climatic features resulting from its location and varying altitude(Chombo et al., 2020). However, it has been found that spatial patterns in rainfall seasons within the country are not similar within the different agro-ecological zones (Solanke & Yadav, 2019). Agro-ecological zones are largely determined by the amount of rainfall received which captures variability in altitude, soil productivity, and crop / livestock system and therefore, agro-ecological zones are curved out of climatic zones correlated with land reforms (Chombo et al., 2020).

Agro-ecological zones in Uganda were also be classified as L.victoria crescent, Mt. Elgon farm lands, North eastern central bush farm lands, South western highlands, Ssese Islands Sango plains, West Nile farmlands among others (Minai, 2015; Okonya et al., 2013). On the other hand, Uganda's agro-ecological zones were also classified as Western mid-altitude farm lands, L. Victoria crescent, South eastern L. Kyoga flood plains, Afro-mountain highlands, Northern moist farmlands and South western range lands among others (Kiwuka et al., 2012).However, large part of Uganda consists of plateaus, lying between 1000 and 2500 meters above sea level with moderate temperatures between 15 to 30°C with precipitation which varies from 750 to 1500mm(Abdalla et al., 2020). But due to climatic change, the onset of rainy season is increasingly unreliable and rainfall distribution is more uneven with erratic heavy rainfall event (Chemurot, 2017).

The central and western parts of Lake Victoria basin and Mount Elgon are areas with relatively high rainfall while the cattle corridor runs from Karamoja region in north east to Ankole region in south west together with the western rift valley area are distinct low rainfall areas (Chemurot, 2017). In southern Uganda, rainfall is bimodal with peak during the long rains from March to May and the short rains from September to December and this pattern allows growing of two different crops per year however, in the northern part of Karamoja, the pattern changes into one rainy season with longer dry periods at the end of the season (Kiwuka et al., 2012).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Neem leaf sample collection sites

Neem leaves used in this experiment were collected from each of the four selected homesteads of Kakomongole trading center (Nakapiripirit) 1.9044^{o}N,34.6552^{o}E, Kimanya catholic parish church (Masaka) 0’20’49.9’S 31^{o}44’10.4’E, Ayivu sub county headquarters(Arua) 3.0379^{o}N,30.9417^{o}E and Budadiri Health Center IV (Sironko) 1.1705^{o}N,34.3335^{o}E. The four sampled districts represented the four agro-ecological zones of Uganda namely the Lake Victoria crescent, Karamoja dry land, Eastern High lands and West Nile farm land respectively.

3.2 Description of AEZ of the sample collection sites

3.2.1 Lake Victoria crescent

Lake Victoria crescent agro-ecological zone covers areas of central Uganda around Lake Victoria including districts of Masaka, Luweero, Mukono, Wakiso, Mityana, Kalangala, Mpigi and Rakai and has average rain fall of 1200-1450mm. It has altitude ranging from 1000-1800m above the sea level and has hilly and flat areas and some wet lands and forests with moderately good soil (Chombo et al., 2020). Lake Victoria Crescent AEZ being near Lake Victoria has a bimodal type of rainfall pattern with two seasons (Saint Pierre et al., 2016).The average annual rainfall received is between 1100mm to1200mm with 100-110 rainy day (Saint Pierre et al., 2016). The average maximum temperature does not exceed 300^{o}C and the minimum is not below 100^{o}C.without almost equal length of day and nights throughout the year (Chemurot, 2017).However, the months of March and April receive very heavy and well distributed rains up to 1200mm (Chemurot, 2017).

3.2.2 Karamoja dry land

Karamoja agro-ecological zone is found in North Eastern Uganda and mostly bordering Kenya semi-arid area (Chemurot, 2017). This AEZ includes districts of Nakapiripirit, Moroto, Kotido ,Kabong and has annual average rainfall ranging between 300mm in pastoral regions to 1200mm in western Abim and Nakapiripirit (Chemurot, 2017). Karamoja dry land AEZ has annual temperature ranging from 16°C in highlands to 24°C in the rest of the region (Chemurot, 2017). The altitude of Karamoja dry land AEZ ranges from 999 to 2720m (3278-8924ft) resulting in climatic changes within the peak (Chemurot, 2017). Temperature usually drops by 6.5° C for every 1000m as one climbs high and there is only one wet season from April to August (Chemurot, 2017). Unlike, most parts of the country which receive two distinct rainy seasons, Karamoja dry land receives only a single long rain period usually between April and May with a break in June 20, 2023(Chemurot, 2017). The climate in Karamoja dry land AEZ is semi-arid with some years being completely dry. The daily temperature can even reach as high as 40°C / 104°F (Chemurot, 2017).

3.2.3 West Nile Farm Land

This is found in North Western and it is generally flat with underletting hills with average rain fall of 1340-1370mm and with altitude of 350-1340m above sea level and the soils range from moderate to good (Chemurot, 2017). The land scape in the West Nile farm land is marked by long slopes and wide often swampy valleys and ranges from high lands (±1500m) in South west, mid altitude(1200m) around Arua City to the low altitude (700m) towards the East and North East but rainfall decreases towards the East and North Eastern low lands (Hengsdijk et al., 2019). The West Nile Farm land AEZ has hot and overcast summers and winters which are long, humid wet and mostly cloudy over the course of the year (Hengsdijk et al., 2019). Temperatures vary from 62°F to 92°F and rarely below 57°F or above 97°F however, the minimum and maximum temperatures (23.7-30°C) are of modified equatorial type with the vegetation classified as wooded savanna (Hengsdijk et al., 2019). The rainfall varies between 1500 and 1700mm and less pronounced bimodal which mainly occur in March to June but August to November is usually associated with dry season (Hengsdijk et al., 2019). However, average annual rainfall is 1352 mm, with a clear dry period from December to March, when a minor rainy season starts until July (Hengsdijk et al., 2019).

The soil characteristics show spatial pattern from more clay soils in highlands, sandy loam soils in the mid altitude to gravelly soils towards a large part of the low lands (Hengsdijk et al., 2019). However, along the Nile, soils tend to contain more clay however, the major types of soil this AEZ include vertisoils, leptosoils, alluvial deposits and feral soils that are moderately fertile(Hengsdijk et al., 2019). The hot season lasts for 2 months from January to march with an average daily high temperature above 89°F(Hengsdijk et al., 2019). And the hottest month of the year is march with an average high temperature of 92°F while the coldest month is July with average low temperature of 62°F (Rubaire-Akiiki et al., 2006).

3.2.4 Eastern High land

This covers the ranges of Mount Elgon with rain fall over 1400mm and altitude of between 1300-3600m with mostly rich volcanic soils. Mount Elgon is characterized by low annual rainfall of 3000 mm (Chemurot, 2017).

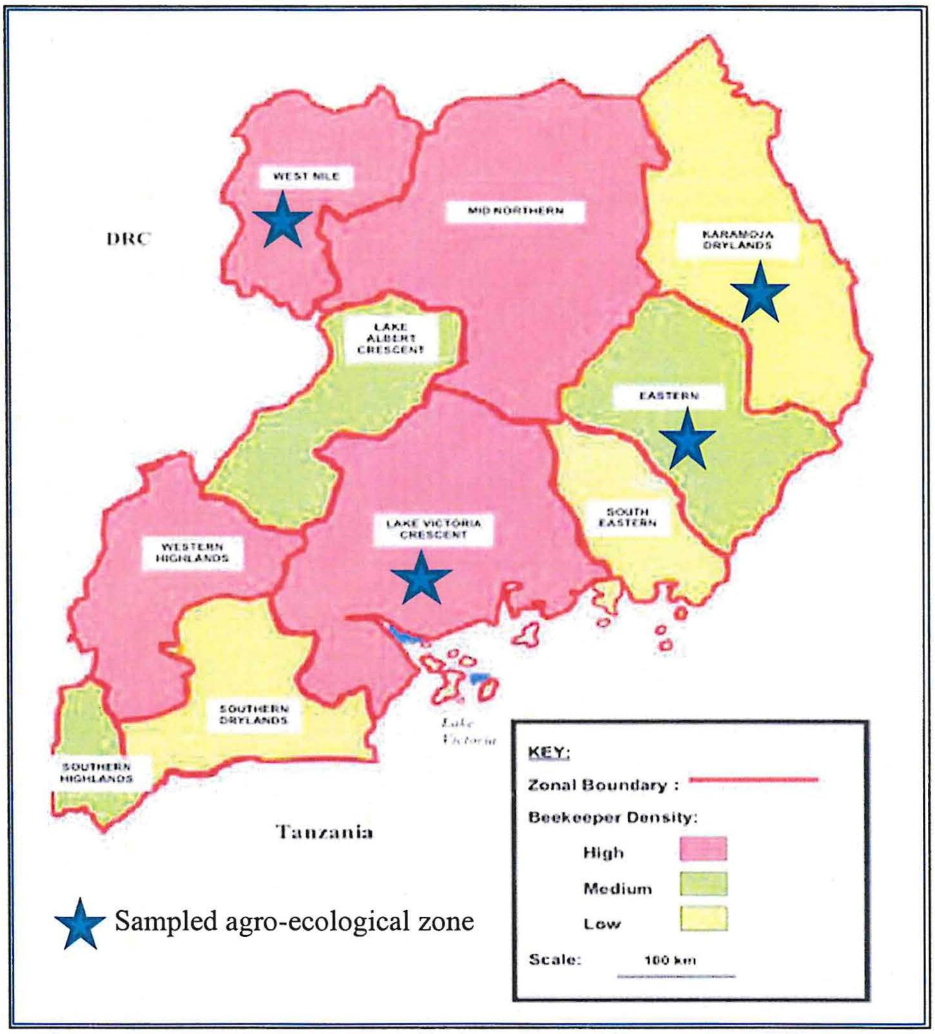


Figure 3.1: The ten Agro-ecological zones of Uganda. The sampled zones re marked with blue star

3.3 Neem leaf sample collection and processing

Fully fresh grown vibrant and healthy green leaves with smooth, glossy and sharp serrated edges and without any physical damage were harvested by destructively pulling the leaflets from the petiole and the leaf base attached to the stem. The spreading branches of the marked neem trees that were estimated to be in the age range of 10 to 16 years according to the heads of the four selected homesteads in each sampled districts were considered thus avoiding the tender young and the near-senescence yellowing as well as the already dry leaves. The fresh leaves collected from each of the sampled districts were bulked separately and put in 1kg packing bag. Each bulked sample of leaves were then packed in a separate cotton bag and later transferred to labeled laboratory bowls (18/10 steel, 6000ml, reversed rim ground HXD=120x305mm) each lined with a paper towel at the bottom to absorb excess moisture (Fig 3.2).

The laboratory bowls were then covered tightly with a lid and each with identity tags then transported to the Crop Science laboratory at the Department of Agriculture, Kyambogo University for drying and processing. The neem leaves were thoroughly washed with distilled water and each sample of leaves separately air-dried under shade in ventilated room with ambient conditions by displaying the leaves on the laboratory table for 10 days till constant weight of each sample was attained as 4,680g, 4,080g, 4,500g and 4,280g. The dried leaves were then ground by pounding using a laboratory mortar and pestle into fine powder as recommended by Kaushik (2007). The powder was then sieved using a laboratory metallic sieve of 2.5mm to obtain uniform sized particles of fine powder (Fig 3.3).

The sieved leaf powder samples were taken to NARL, Kawanda and tested for quality using the method described by Linus (2014) before use in the experiment. Two portions of each sample were kept, one for use in the determination of azadirachtin concentration and the other portion reserved for efficacy experiment against *S. zeamais.as* the leaf powder samples for the efficacy experiment were measured into weights of 0.5g / 100g of maize grai;,p[[n, 1.0g/ 100g of maize grain, 1.5g/100g of maize grain and 2.0g/100g of maize grain respectively and used as experimental treatments.

Figure 3.2: Sample of neem leaves in a laboratory bowl.

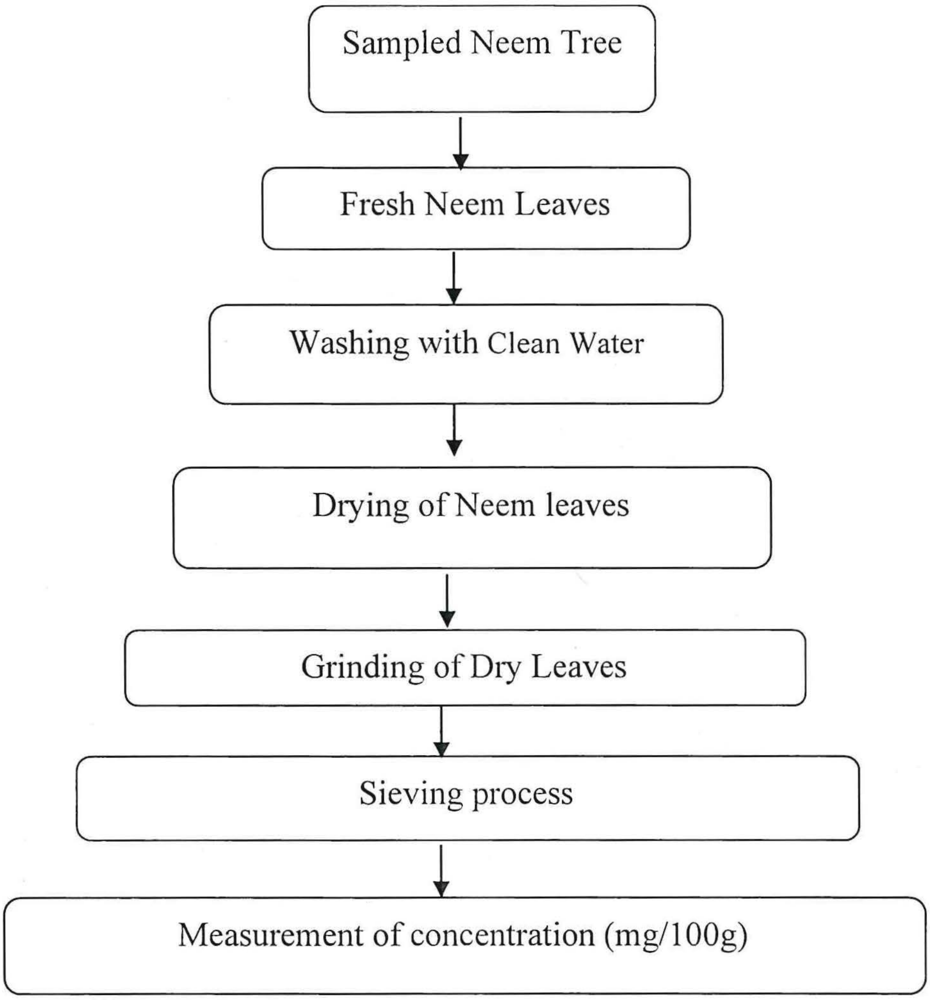


Figure 3.3: Steps involved in processing of neem leaf powder.

3.4 Preparation of maize grain

Freshly harvested clean, dry, undamaged, and untreated dry maize grains of variety Longe 10, a commonly grown maize variety in Uganda, was sourced from demonstration farmers around the National Agricultural Research Laboratories (NARL) Kawada and was graded manually and dirt, broken kernel and debris removed by winnowing as suggested by (Mandudzi & Edziwa, 2016). The grain was further cleaned using 4mm sieve mesh screen so as to remain with intact kernels as recommended by (Mandudzi & Edziwa, 2016). The maize grain was disinfected by freezing at $5^{o}C$ for a period of 7 days in order to kill any live insects as recommended by (Ognakossan et al., 2018).

Moisture content was determined using Dickey-John hand held moisture tester (Model-Mini GAC 2100) Brand–Dickey–John 2005, Weight-1.61bs to test the grain moisture content using a 500g sample and maintained the latter in the range of 12 - 13% during the experiment. The Test Weight (TW) was determined using a seed buro test weight scale as recommended by (Mandudzi & Edziwa, 2016). And a 250g sub-sample from which approximately 100g (144 grains) were obtained and arranged in 64 lots which were placed in 500 ml beaker each perforated with a lid with tiny openings to allow aeration.

Grain weight was determined with a seed buro model 9000 AG. Test weight computer grain grading scale (Seed buro: Inch. 2020) while broken maize grain and foreign material (BMFM) were determined by sieving a 250g representing a sub sample with a 4.76mm (12/64-Inch) round hole hand held screen with the weight passing through the screen plus a handpicked non-maize material on top and expressed as percentage weight as recommended by Mandudzi & Edziwa, (2016). Damage on maize grain was determined by visual inspection according to procedural protocols in the USDA grain inspection Hand book (FGIS 2013).

The quantity of damaged grains was expressed in percentage by dividing the total number of maize grain (n) by the number of maize grains with holes (damaged by the weevil infestation) and multiplied by 100%. The number of live and dead weevils in a grain sample was determined according to the methods develop and applied by Yakubu (2013). The containers were then inspected and pressurized using an air compressor to ensure that there is no breakage. However, the inside of each container was scrubbed with hot water, dish detergent and finally triple rinsed and dried.

3.5 Determination of Azadirachtin concentration in neem leaves

The experiment to determine the concentration of azadirachtin, the active ingredient in 4 samples of neem leaf powder obtained from the four selected AEZs of Uganda was conducted in the Biochemistry laboratory of the National Crops Resources and Research Institute (NaCRRI), Namulonge located approximately 30km north east of Kampala capital city of Uganda in Wakiso district with coordinates of 0031 30N, 32 3654E, latitude of 0.5250 and 32.6150 longitude.

3.5.1 Preparation of stock solution

Azadirachtin standard used in quantification of azadirachtin in neem leaf powder samples was bought from Sigma Aldrich (St Louis, MO, USA) and the azadirachtin standard was dissolved in High Performance Liquid Chromatography (HPLC) grade methanol (Merck) to make a stock concentration of 1000mg/g. The stock solution was diluted with HPLC grade methanol in series to make samples with concentrations of 250, 125, 62.5, 31.3, 15.6 and 7.8mg/g. Each concentration of standard was filtered through 0.22-nm nylon membrane filters (Millipore, Billerica, MA, USA) before High Performance Liquid Chromatography analysis. Azadirachtin standard used was dissolved in a methanol to give a concentration of 2mg/ml. The solution was prepared and stored in a refrigerator in amber glass bottles to protect it from light while stock and standard solution were all not filtered because of using the analytes that were previously assessed using the same filter samples.

3.5.2 Preparation of standard solutions

Azadirachtin was weighed to 10 mg and transferred into 10ml volumetric flask. The volume of the solution was made up to the mark (10ml) with methanol as recommended by Biosci et al., (2019). The volumetric flask was sonicated for 2-3 minutes to affect the complete dissolution of the compound. Suitable aliquots of standard were prepared by serial dilution of the standard solution by picking 1 ml of the stock. The prepared standards were then injected to HPLC to obtain concentration in the linearity range. A typical chromatogram obtained from the analysis of Azadirachtin, using the optimized chromatographic conditions at 210nm, was then generated.

3.5.3 Extraction of Azadirachtin from neem leaf powder samples.

A weight of 100g of neem leaf powder each obtained from four selected Agro-ecological zones was extracted in 500ml of methanol using a stirrer at 1,000 rpm soaked overnight and incubated for 24hrs in dim light under $4^{0}C$ room temperature as azadirachtin compounds are sensitive to light and heat. This was followed by sonification for 45mins and the mixtures were thereafter centrifuged using a refrigerated centrifuge at 5,000 rpm for 10mins.The supernatant was collected, and water was added at a ratio of 40:60 (water: methanol). The mixture was then partitioned against equal amounts of Di-chloro- methane in separating funnels. The solution was mixed thoroughly and left to separate into two immiscible solvents (Methanol +Water +Di-chloro methane). Following the separation, the upper water: methanol layer was discarded and the lower phase of the Di-chloro methane layer was collected and then evaporated to form dry matter. The retrieved dry matter was weighed and re–dissolved in High Performance Liquid Chromatography grade methanol at 100m/g concentration and was used a stock solution while the prepared extracts were filtered through a 0.22-mm nylon membrane filter prior to HPLC analysis using High Performance Liquid Chromatography Shimadzu system (Fig3.4).

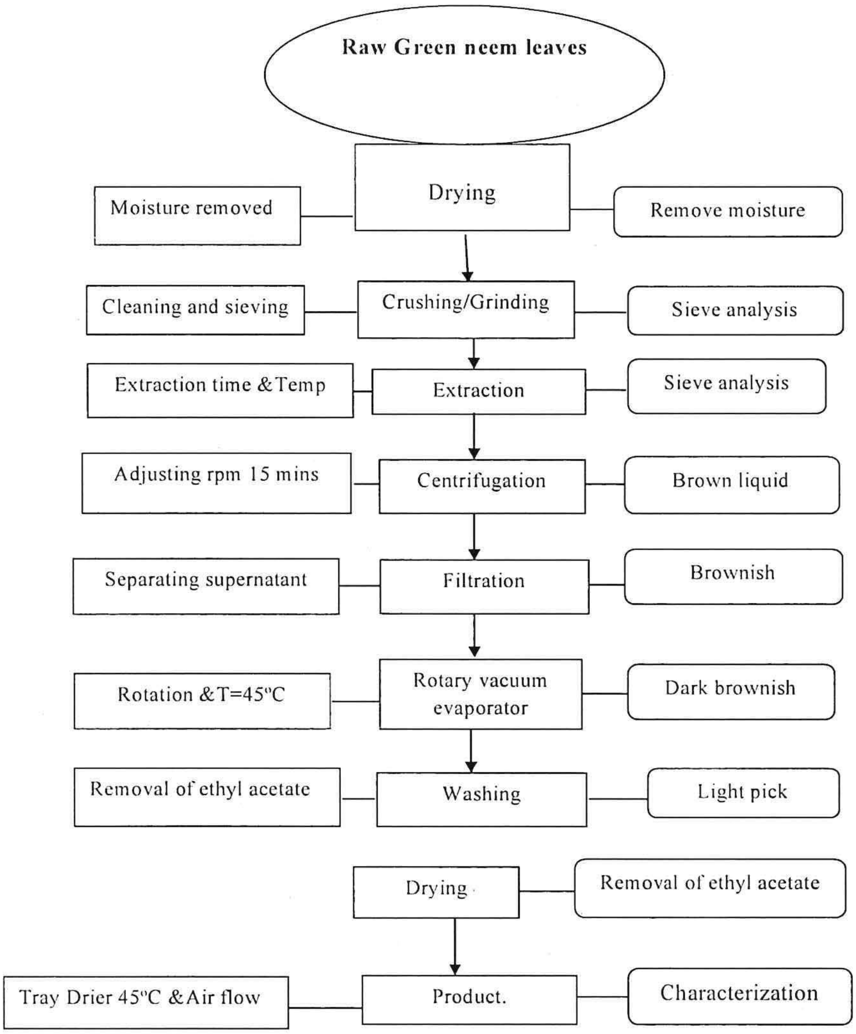


Figure 3.4: Stages of azadirachtin extraction from neem leaf powder

3.5.4 Quantification of Azadirachtin content in neem leaf powder

Quantitative estimation of Azadirachtin content in neem leaf powder was done using High Performance of Liquid Chromatography (HPLC) System (CBM-20A, SHIMADZU Co. Ltd, Japan) equipped with two gradient pump system, (LC-20AT, Shimadzu), UV-detector (SPD-10A, Shimadzu), The Azorba X SB-C18 end capped

4.6mm X 250mm reverse phase column was used and the mobile phase 90% methanol and 10% water at a flow rate of 0.5ml/min.

The azadirachtin was detected at 210nm and the chromatographic peaks of the analytes were determined by comparing the retention time of each of the neem leaf powder samples with an azadirachtin standard (95%) through a filter using Value stage vacuum pump. A mobile phase was pumped at a flow rate of 1 ml/min at room temperature and the azadirachtin compound was detected by chromatography with standards and by elucidation of their spectral characteristics using a photo-diode array detector. And the UV-Vis spectra detection were recorded at 210 nm while Chromatograms were recorded at 280nm. The overall Azadirachtin content in each of the neem leaf powder samples was based on injections of known quantities of external standard samples of azadirachtin and by comparing their relative measurements as revealed by integrated HPLC peak arrears and estimated using standard HPLC method.

3.6 Efficacy of neem leaf powder against *Sitophilus zeamais*

Experiments to assess the efficacy of neem leaf powder against *Sitophilus zeamais* were conducted under ambient conditions in the Laboratory at National Agricultural Research Laboratories, Kawanda(0° -25 N, 32°-51 'E) located 12km north of Kampala, Uganda. Kawanda is characterized by average daily temperatures in the range of 16 and 29°C.

Four treatments of neem leaf powders obtained from the four selected agro-ecological zones each arranged in four different doses of 0.5 g, 1.0g, 1.5g, and 2.0g, and each replicated four times and the control had no neem leaf powder.Maize grain of was adjusted to 13% moisture content were separately mixed with 0.5g, 1.0g, 1.5g and 2.0g of neem leaf powder in glass jars (12cm height x 6.5 diameter) and the admixtures were shaken manually for 5 minutes and then tumbled for 15 minutes in a mechanical tumbler. The control had no neem leaf powder replicated 4 times while Skana super grain dust at a recommended rate of 0.5g/100g of maize grain was used as a positive control. The Experimental units were laid out in a completely randomized design.

The maize grain lots were left undisturbed for 1 hour and thereafter, data were recorded on live weevil counts at 0, 30, 60, and 90 days after treatment application the level of weevil infestation. A clean white plain paper was used to cover the platform from where the counting was conducted. This was done in order to ease visualization of *S. zeamai*s.

Data were recorded on damaged maize grains (grains with holes), undamaged maize grain (grain with no holes) and these were expressed as percentage infested and uninfected maize grain, respectively and data were also recorded on number of dead and live weevils which were counted at 0, 30, 60, and 90 days after treatment.

(a) **(b)**

Figures 3.5: (a) Clean and dry undamaged maize grains. (b) Infested maize grains.

3.7 Data analysis

The linear effect model was fitted to the data of weevil counts and damaged maize grains. The linear model was $y_{ij} = \mu + t + \varepsilon$. **where:** y_{ij} = Observed effect, μ = Overall **mean and ε = Random error.**

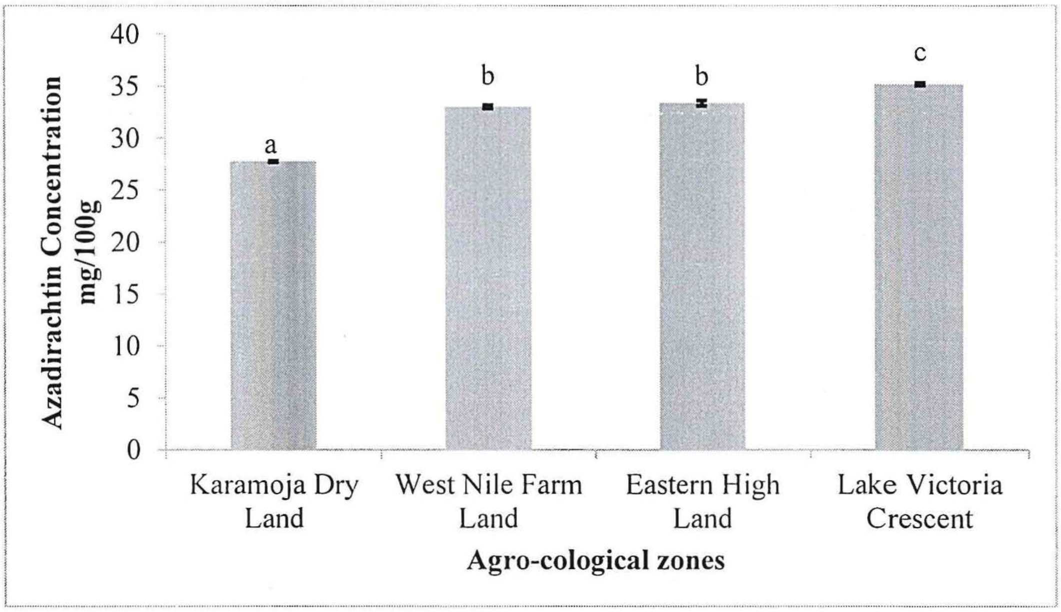
Prior to statistical analysis by Analysis of Variance (ANOVA), the data were checked for normality and equality of variances using Shapiro-**wilk and bartlett's** tests respectively.

The effect of Agro-ecological zone on the concentration of Azadirachtin and the effect of neem leaf powder doses on weevil maize grain infestation over time weresubjected to analysis of variance at the alpha level of 0.05, using GenStat version 15. When the ANOVA test was significant; a Tukey post hoc test was used to separate the means. Thereafter, the means and their respective standard errors were plotted onto a histogram and line graphs using Microsoft Excel.

CHAPTER FOUR: PRESENTION OF RESULTS

4.1. Azadirachtin concentration in neem leaves from different agro-ecological zones of Uganda

The findings of this study show significant differences in the concentration of Azadirachtin ($P<0.001$) in neem leaf powders obtained from the 4-selected agro-ecological zones of Uganda. The Lake Victoria crescent recorded the highest concentration of azadirachtin (35.23mg/g), Eastern high land , West Nile farm land while Karamoja dry land (Nakapiripirit) had the lowest (27.76 mg/g) (Figure 4.1).



Mean (±) with different lower case letters across the columns are significantly different ($P<0.001$) at 0.05% level of significance.

Figure 4.1: Azadirachtin concentration in neem leaves from 4-selected agro-ecological zones.

4.1.2. Hplc chromatograms

The agro-ecological zones from where the neem leaf powders were obtained, the retention time and their interaction significantly stimulated the azadirachtin concentration (Figure 4.2).

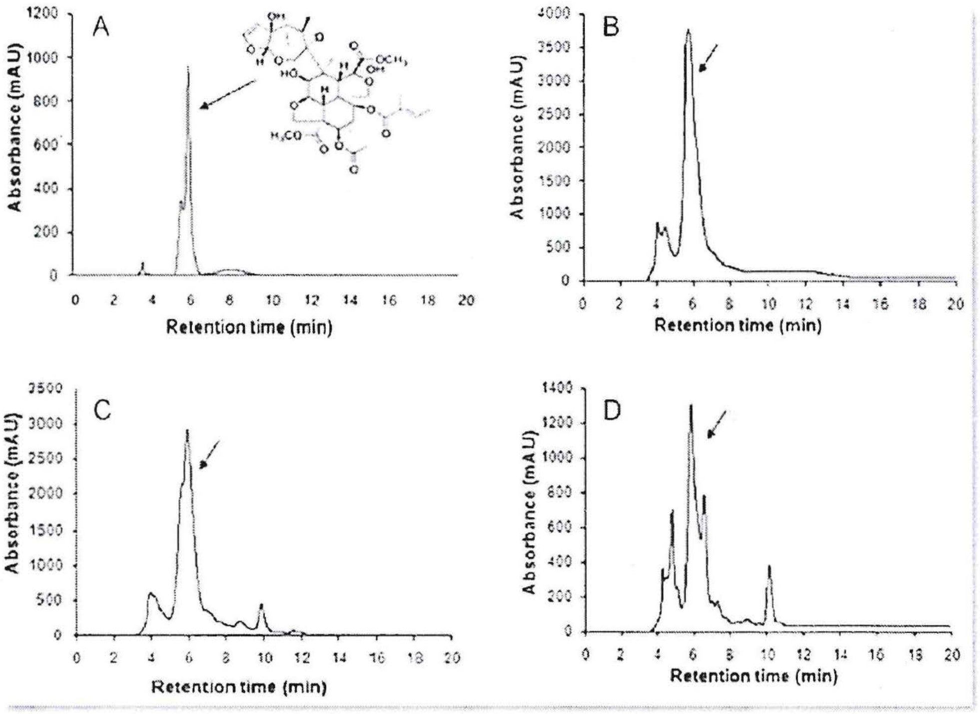


Figure 4.2: Hplc Chromatograms of Azadirachtin standard and retention time for neem leaf samples; A) Karamoja dry land, B) L. Victoria crescent, C) Eastern high land, and D) Western Nile farm land

4.2 Efficacy of neem leaf powders obtained from the four selected Agro-Ecological Zones against *Sitophilus zeamais* in maize grain

From 30 days to 90 days of experimental time, there were significant differences ($P < 0.001$) in *S. zeamais* counts across the four selected agro-ecological zones in all the treatments except in control with no neem leaf powder. And maize grains treated with neem leaf powder obtained from Lake Victoria Crescent had a significantly lower *S. zeamais* infestation compared to Eastern high lands, West Nile farm land, Karamoja dry land and the control (Table 4.1). After 60 days of experiment, a relatively lower rate of *S. zeamais* infestation was recorded in samples treated with neem leaf powders obtained from Lake Victoria crescent and Eastern high land compared to the maize grain treated with neem leaf powders from West Nile farm land and Karamoja dry land which had a relatively higher *S.zeamais* infestation.

Table 4.1: Mean number of weevils from maize grain treated with 2g of neem leaf powder from different Agro-ecological zones over time.

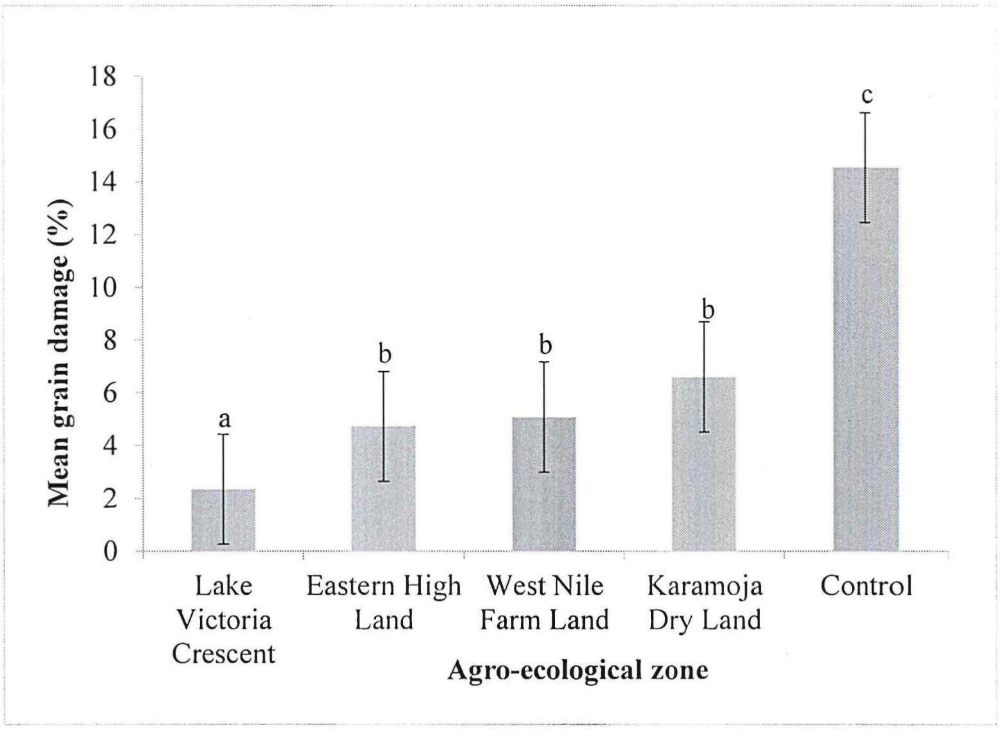
Time	**Agro-ecological zone**				
(days)	Lake Victoria Crescent	Eastern High Land	West Nile Farm	Karamoja Dry Land	Control
30	1.6±0.6a	3.5±0.3a	3.5±0.3a	3.1±0.4a	8.5±0.8b
60	2.8±0.6a	6.1±0.5ab	6.6±0.7b	6.9±0.3b	18.6±1.4c
90	5.0±0.9a	9.4±0.4ab	10.2±0.8ab	12.3±0.2b	30.9±3.1c
L.S.D	1.30	1.05	1.29	0.87	0.02
C.V%	34.6	13.9	15.8	9.80	0.00

Means (±) followed by the same lower case letters across the raw are not significantly different from each other according to Tukey HsD test.

4.2.1 Potential of neem leaf powder to protect maize grains against *Sitophilus zeamais*

The level of treatment, source of neem and time ($P < 0.001$) significantly influenced the percentage of the damaged maize grains. The highest mean percentage (14.5%) of damaged maize grains was recorded in the untreated control. The lowest number of *S. zeamais* (2.0%) was observed in the maize grains treated with neem leaf powder from Lake Victoria crescent agro-ecological zone (Figure 4.3a).

The number of weevils and the number of damaged maize grains decreased with every unit increase in the neem dose implying that neem leaf powder samples from different agro-ecological zones had a significant effect on the number of weevil and the percentage of damaged maize grains. The number of maize weevil infestation and damage of grains significantly decreased ($P < 0.05$) with increase in dose of neem leaf powder (Figure 4.3b).



Means with different letters are significantly different (P<0.01) at 0.005% level of significance.

Figure 4.3a: Mean grain damage from maize grain treated with of neem leaf powder obtained from sampled agro-ecological zones of Uganda.

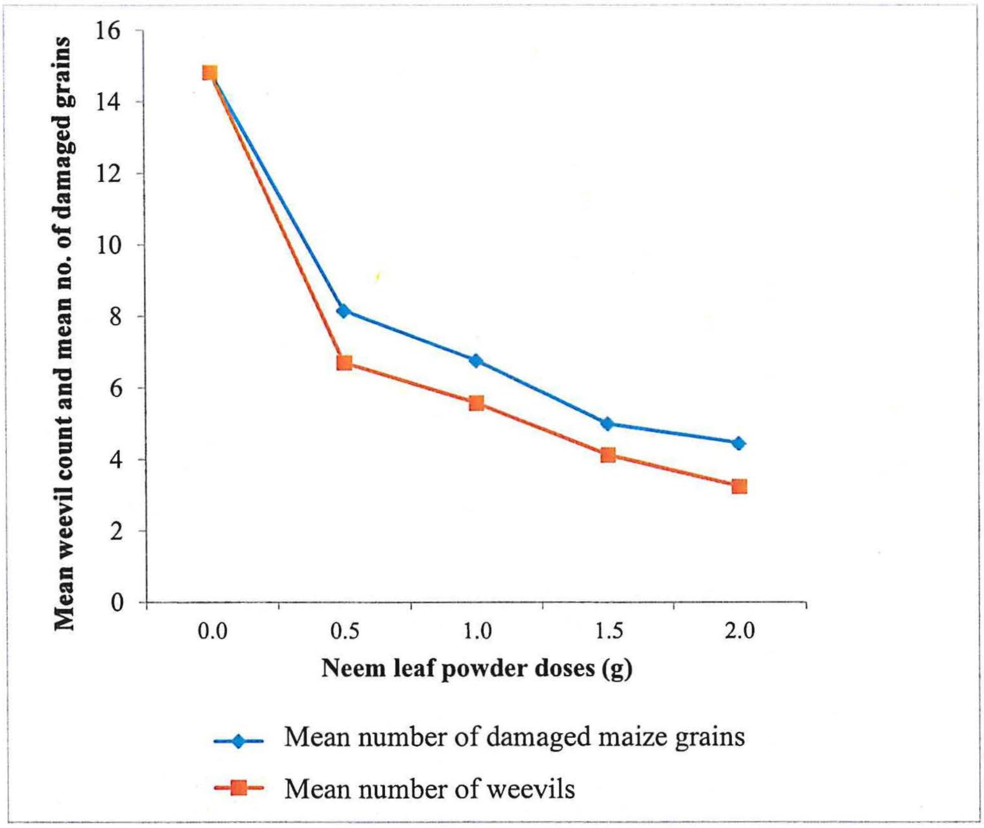
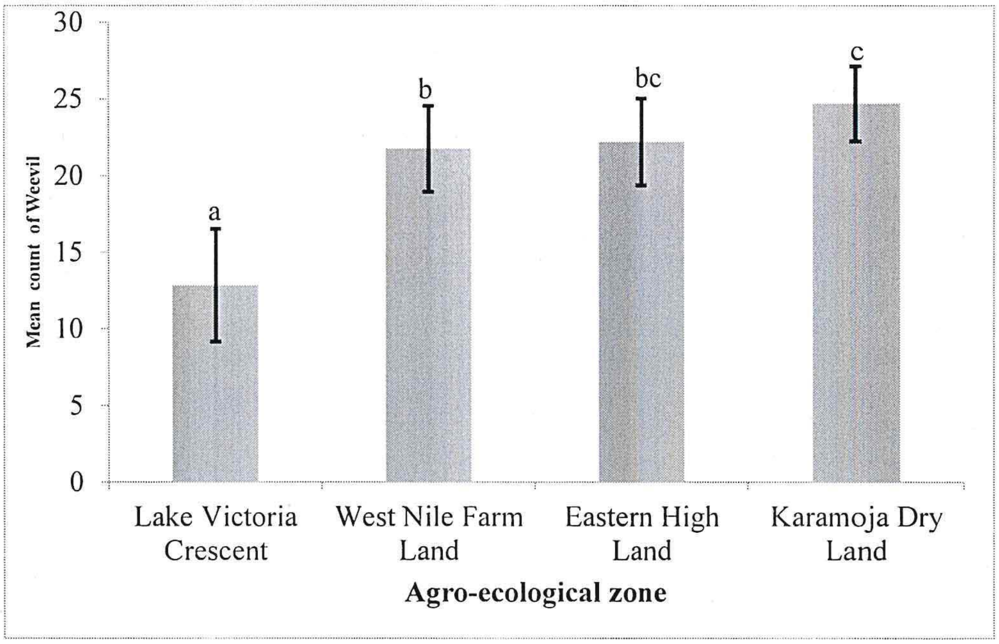


Figure 4.3b: Number of weevils and number of damaged grains recorded with varying quantity of neem leaf powder

4.2.2 Mean *S.zeamais* count from maize grains after 90 days post treatment with neem leaf powder from different Agro-ecological zones of Uganda

Treatment of maize grain with neem leaf powders from the four selected Agro-ecological zones significantly suppressed weevil infestation. Efficacy of neem leaf powders was significantly ($p < 0.05$) different showing significant differences in *S.zeamais* counts cumulatively recorded in maize grains. Lake Victoria crescent had the lowest S.zeamais accumulative count while karamoja dry land had the highest *S.zeamais* cumulative counts (Figure 4.4).


30
25
20
15
10
5
0
Mean count of Weevil
a
b
bc
c
Lake Victoria Crescent
West Nile Farm Land
Eastern High Land
Karamoja Dry Land
Agro-ecological zone

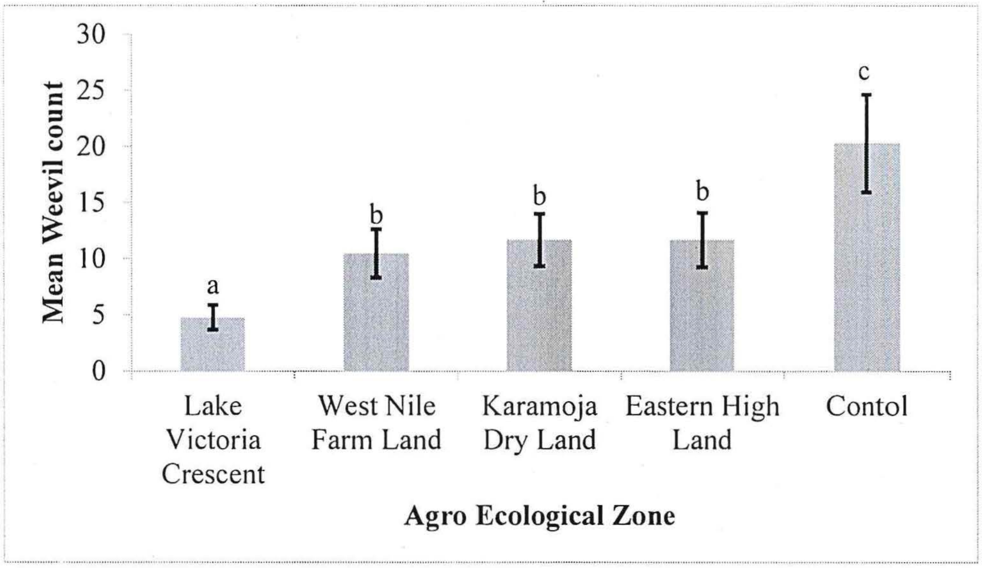
Means with different lowercase letters are significantly different (P<001) at 0.05% level of significance.

Figure 4.4: Mean *S.zeamais* count from maize grains at 90 days post treatment with neem leaf powder obtained from different Agro-ecological zones in Uganda.

4.2.3 Mean weevil count from the maize grain treated with neem leaf powders obtained from sampled agro-ecological zones

Treatment of maize grain with neem leaf powders from different Agro-ecological zones significantly reduced weevil infestation across all the four Agro-ecological zones ($p < 0.05$) compared to untreated grains in control experiment. However, after 90 days of experiment, the highest number of weevil count was seen in the untreated control followed by maize grain treated with neem leaf powder from Karamoja dry land Agro-ecological zone while the lowest weevil count was recorded in maize grain treated with neem leaf powders from Lake Victoria Crescent. Infestation was significantly reduced with increase in doses of neem leaf powders and the time of application ($p < 0.01$).

Highest weevil infestation represented by weevil count was significantly higher in maize grain with no neem leaf powder followed by maize grain treated with neem leaf powder from Karamoja dry land while Lake Victoria Crescent recorded the (Figure 4.5).

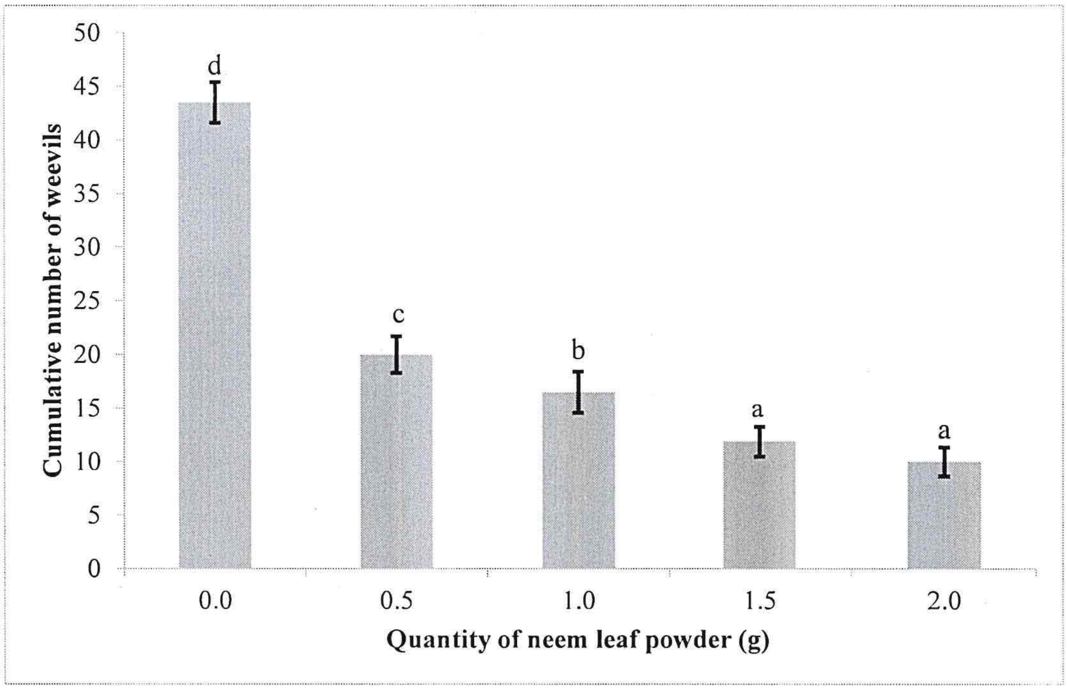


Means with different lower case letters are significantly different (P<0.001)at 0.05 level of significance.

Figure 4.5: Mean S. zeamais count from maize grain treated with neem leaf powders obtained from the four agro-ecological zones of Uganda.

4.3.1 Cumulative number of *Sitophilus zeamais* from maize grain after 90 days of treatment with varying quantities of neem leaf powder

When maize grains were treated with varying doses of neem leaf powder, i.e. 0.0, 0.5, 1.0, 1.5 and 2.0g, the number of weevil count were significantly higher ($P < 0.001$) in untreated maize grain (control) than in all the samples treated with any dose of neem leaf powder. However, doses of neem leaf powder differed significantly ($P < 0.001$) from one another with exception of 1.5 and 2g doses (Figure 4.6).



Means with different lower case letters are significantly different at $P<0.05$ level of significance

Figure 4.6: Cumulative number of weevilsfrom maize grains after 90 days of treatment with varying quantities of neem leaf powder obtained from different Agro-ecological zones of Uganda

4.3.2 Mean weevil count across four agro-ecological zones obtained by treating maize grain varying doses of neem leaf powders after 90 Days

There was a significant difference in number of weevils in maize grains treated with 0.5g of neem leaf powder across all the Agro-ecological zone except in the maize grain with no treatment. However, subsequent increase in neem leaf powder doses up to 2.0g had significant reduction of weevil counts across the four Agro-ecological zones with neem leaf powder from Lake Victoria crescent exhibiting a significant difference from the rest of doses.

Table 4.2: Mean weevil count across four agro-ecological zones obtained by treating maize grain varying doses of neem leaf powders after 90 days.

Dosage (g)	**Agro-ecological zone**			
	Lake Victoria Crescent	Eastern High Land	West Nile Farm	Karamoja Dry Land
0.0	44c	44d	44d	44c
0.5	10b	24c	22c	24b
1.0	5ab	20bc	19bc	23ab
1.5	4ab	12ab	13ab	18ab
2.0	2a	11a	11a	16a
LSD (5%)	6.7	7.7	4.7	7.0
CV%	34.4	21.5	32.8	18.9

Means followed by different lower case letters along the column are significantly different at P<0.05

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion of Results

5.1.1 Variation in Azadirachtin Level between Agro-ecological Zones

The results of the study show that neem trees growing in different Agro-ecological zones of Uganda exhibit significantly varying concentrations of azadirachtin, the active ingredient in neem leaf powder with the highest concentration (35.23 mg/g) detected in the sample collected from Lake Victoria Crescent agro-ecological zone while Karamoja semi-arid in north eastern had the lowest concentration of Azadirachtin 27.65 mg/g. This is in line with the findings of Tomar & Kaushik (2011) which show that the azadirachtin content in neem seeds collected from the four agro-ecological zones of Gujarat state of India significantly varied from 200 to 16,000ppm (mg/g) of the neem powder (Kaushik et al., 2007).

The azadirachtin concentrations in this study were slightly lower than those reported by Tomar & Kaushik, 2011 and this could be because of the varying climatic conditions in the neem growing areas, the neem tree growing habitat and also the part of neem tree plant being used. It is also revealed that the neem tree leaves used in this study contain quite less concentration of azadirachtin compared with seeds that were used in the study conducted by Tomar & Kaushik (2011).

Variability in climatic attributes of the different agro-ecological zones of Uganda such as rainfall, temperature, soil type, latitude, humidity and altitude could be the major factor causing variations in azadirachtin concentration in the neem leaves (Sserumaga et al., 2020).

And that explains why the neem trees growing in agro-ecological zones with moderate climate, average rainfall of 400 mm, and high altitude of > 470 m above sea level proved to be rich in azadirachtin content agreeing with IE Elteraifi & Hassanali (2011) while trees growing in lower altitudes, alluvial or sandy soil, with hot climate reflected very low azadirachtin content which is in line with the report of Elteraifi & Hassanali (2011).

It is evident from this study that Lake Victoria crescent agro-ecological zone experiences moderate climate with average rainfall and high altitude(Sserumaga et al., 2020). And this perhaps explains the highest levels of azadirachtin content in neem leaf powder from Lake Victoria crescent compared to other AEZs in the study such as Karamoja dry land and West Nile farm land that is in lower altitudes and experience hot climate and with sand soils.

This is in line with the findings of (Biosci et al., 2019) and Elteraifi& Hassanali (2011) that agro-ecological zones and habitat from which the neem tree grows has influence on concentration of azadirachtin. For instance, it is reported that rainfall was the major factor affecting the level of azadirachtin in neem tree even when other factors remained constant (Biosci et al., 2019; Jan et al., 2021).

The variations in terms of Azadirachtin concentration across the selected Agro-ecological zones of Uganda are in line with findings from a laboratory study reported by Kaushik et al. (2007) that grouped azadirachtin samples into four distinct climatic classes namely Hot-semi arid with mild winter, Hot sub-humid , Hot arid, and Hot semi-arid with cold winter.

The study revealed that samples from hot sub-humid, hot arid and hot semi-arid with cold winter type of climate were found to be statistically lower in terms of azadirachtin synthesis compared with the samples from hot semi-arid with mild winter (Intisar Elteraifi & Hassanali, 2011).

This study therefore, suggests that the .differences in Uganda's agro-ecological zone attributes could have resulted into variability in azadirachtin levels in the neem leaf powder obtained from the four selected agro-ecological zones of Uganda. These results are in line with the previous studies that showed that neem trees growing in regions with moderate climate, average rainfall of 400mm and altitude of greater than 470m above the sea level proved to be rich in azadirachtin content while trees growing in lower altitude ,alluvial or sandy soils with hot climate reflected very low azadirachtin concentration (IE Elteraifi & Hassanali, 2011; Kaushik et al., 2007).

5.1.2 Efficacy of *Azadirachta indica* leaf powder

The findings of this study showed that treating maize grains with *A. indica* leaf powder adequately reduced the level of weevil infestation in maize grains and the number of damaged maize grains by *S.zeamais*. This is in conformity with the previous study by Rashid (2020) which revealed that extracts and powders from*A. indica* are toxic against *S.zeamais* and this toxicity could be due to presence of azadirachtin active compound in neem that exhibits insecticidal effects against a number of insects including maize weevil *S.zeamais* (Ukoroije & Bobmanuel, 2019)

5.1.3 Effect of dosage of neem leaf powder on infestation of the *S. zeamais* in stored maize grains

All doses of neem leaf powder had a significant effect on the weevil counts compared to the control, but there was no significant difference between them. The results of this study revealed that the neem leaves are efficacious against *S. zeamais*. The activity of the different dozes of neem are attributed to the potency of azadirachtin, the active ingredient that was found present in the neem leaves from the four sampled agro-ecological zones of Uganda.

5.2 Conclusions

This Research study has revealed that neem leaf powders obtained from neem trees growing in different agro-ecological zones in Uganda had varying concentrations of azadirachtin with Lake Victoria crescent agro-ecological zone having the highest concentrations followed by Eastern highland, West Nile farmland and Karamoja dry land having the least concentration.

The study results also revealed that neem leaf powders from all the agro-ecological zones proved to control *S. zeamais* in stored maize grain and can therefore, be used as an alternative pesticide against *S. zeamais* in stored maize grain. However, the best results areattainable when one uses neem leaf powders from Lake Victoria crescent and Eastern high lands agro-ecological zones. This therefore, means that the agro-ecological zone from where neem leaf is obtained, increasing neem leaf powder dosage to optimum and time interval played a pivotal role in efficacy of neem leaf powders against *S. zeamais*.

5.3 Recommendations

Owing to the varying concentrations of azadirachtin in neem leaf powders across the different Agro- ecological zones in Uganda, farmers and industrial users should preferably use neem tree materials from Lake Victoria crescent, followed by Eastern high lands & Western farm land, basing on the level of azadirachtin concentration. However, if neem materials from agro-ecological zones with lower azadirachtin concentration such as Karamoja dry land are to be used for maize grain protection against *S .zeamais*, then higher doses of neem powders should be used in order to achieve the highest efficacy.

The fact that all doses of neem leaf powder were not significant from each other, and yet all were significant from the control, implies that the quantities used were very close to each other and therefore, the differences could not be determined, which would have provided the optimum dose of neem leaf powder for the control of *S. zeamais*. There is therefore, need to experiment further to establish the most ideal and effective dosage for every neem leaf powder sample obtained from the selected agro-ecological zone.

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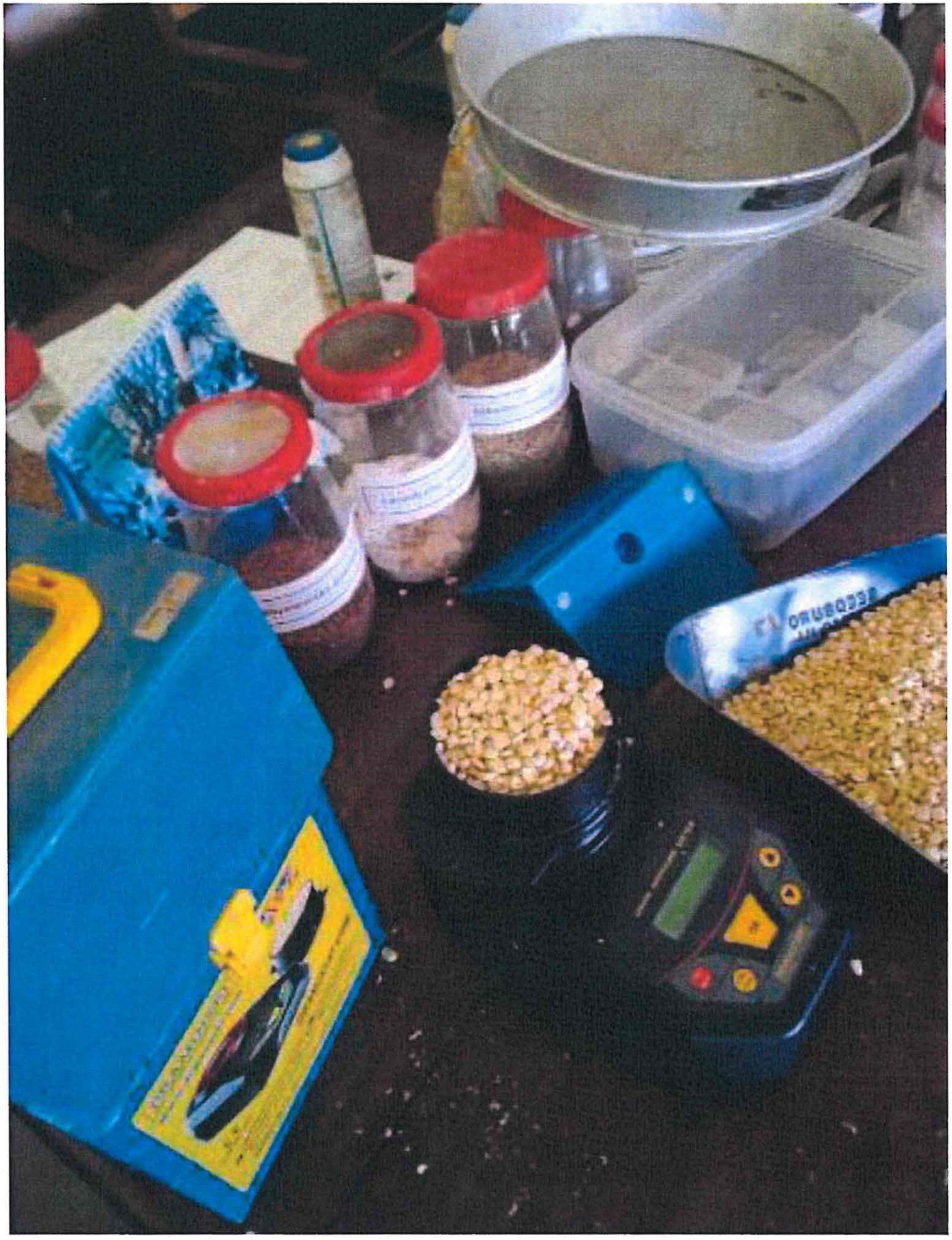
APPENDICES

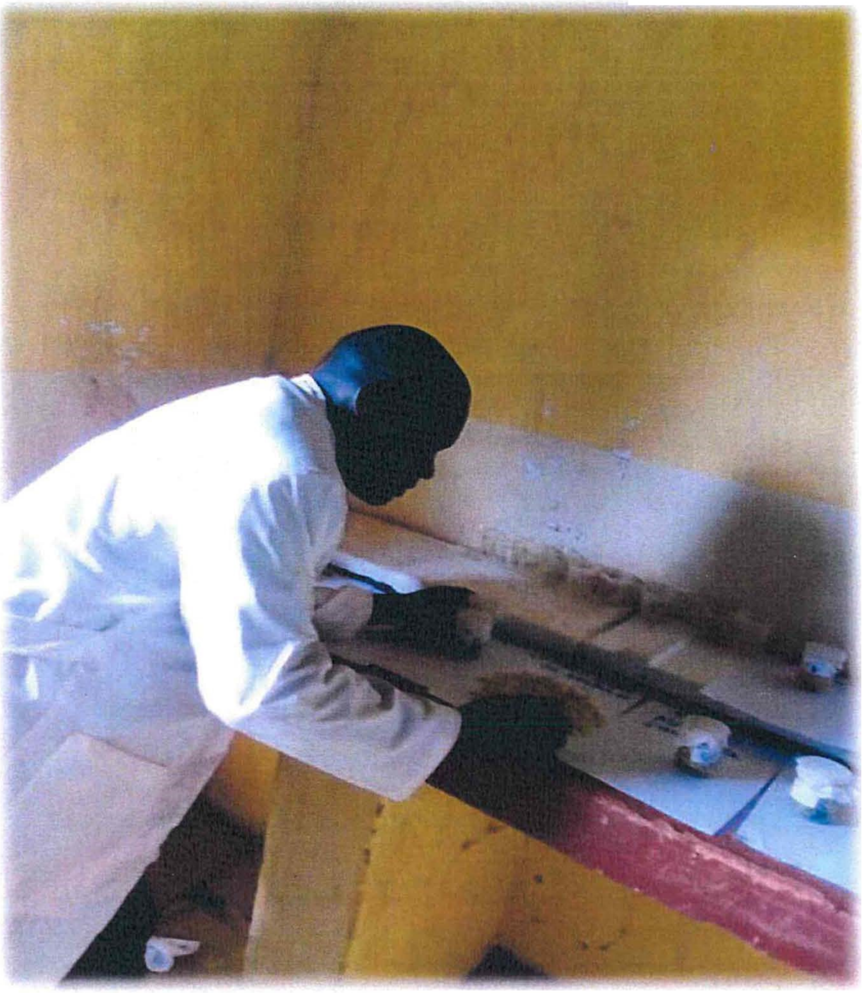
Appendix 1: Taking moisture content of Maize grain samples



Appendix 2: Drying of neem leaves

Appendix 3: Experimental layout

Appendix 4: Data collection



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