BINDURA UNIVERSITY OF SCIENCE EDUCATON

FACULTY OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

A LABORATORY EXPERIMENT ON THE EFFICACY OF NEEM EXTRACTS IN CONTROLLING ANTESTIA BUG (*ANTESTIOPSIS spp*) IN COFFEE

DEPARTMENT OF CROP SCIENCE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE BACHELOR OF AGRICULTURAL SCIENCE HONOURS DEGREE

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DECLARATION

I, Moses Mushonga declare that the dissertation, which I hereby submit for the degree in Crop Science at Bindura University of Science Education, is my own work and has not been previously submitted by me for a degree at any other tertiary institution.

Moses Mushonga:

Date: 08:10:2024

CERTIFICATION

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Date10/10/24…………………………

Chairperson… Mr P Mutsengi

…………………………………………………

Date…10/10/24…………………….

DEDICATION

I dedicate my project to my parents, knowing that every milestone is a testament to their love and support. Their love and sacrifices will forever be cherished and remembered.

ACKNOWLEDGEMENTS

My supervisor, Professor R. Mandaumbu, is deserving of recognition and gratitude for having enabled this study. I was able to complete every phase of writing my project with his help and counsel. I would like to thank my family and friends.

Lastly, I would like to express my gratitude to God for enabling me to complete this dissertation. My faith in Him will not waver.

ABSTRACT

The Antestia bug (Antestiopsis spp.) is a major pest that can inflict significant damage to coffee berries, leading to quality and yield losses for coffee producers. The use of synthetic insecticides to control this pest has raised concerns about environmental and health implications, prompting the exploration of alternative, more sustainable approaches. Neem (Azadirachta indica) and its derivatives have been identified as potential biocontrol agents against the Antestia bug due to their demonstrated insecticidal properties.This laboratory study aimed to evaluate the efficacy of a neem-based formulation in protecting coffee berries from Antestia bug infestation and damage.The experiment was conducted using a completely randomized design with six treatments (four neem, one control and one standard) and five replicates. Coffee berries were artificially infested with Antestia bugs and then treated with varying concentrations of a neem-based insecticide. Control treatments included an untreated control and a synthetic insecticide(Thunder) commonly used for Antestia bug management.The results showed that the neem-based insecticide reduced the number of Antestia bugs present on the coffee berries, with the highest concentration tested achieving a 40% reduction in bug population compared to the untreated control. Moreover, the neem treatment substantially decreased the level of feeding damage and berry drop caused by the Antestia bugs.These findings suggest that neem-based insecticides have the potential to be an effective and environmentally-friendly alternative for protecting coffee berries from Antestia bug infestation and damage. The results provide valuable insights for the development of integrated pest management strategies that incorporate neem-based approaches to mitigate the impact of this persistent coffee pest.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.0. BACKGROUND

Coffee (Coffea spp.) is a major crop globally, providing livelihoods for a lot of people and contributing a lot of dollars to the global economy (Organisation, 2023). Coffee is an important cash crop for many third world countries, and its production is endangered by different insect pests, including the Antestia bug. The Antestia bug is known to feed on coffee berries, causing significant damage and quality and quantity losses (Mondédji, 2014) . The antestia bug, a hemipteran insect native to Africa, is a serious pest of coffee in East and Central Africa, as well as in other coffee-producing regions (Ndungu, Owuor, & Otieno, 2012). It feeds on coffee berries by piercing the fruit and sucking out the juice, causing significant damage to the developing beans. This feeding behavior leads to several negative consequences for coffee production.

Pest management for the antestia bug in coffee has centraly relied on the use of synthetic insecticides, which can have adverse effects on the environment, human health, and the development of insecticide resistance (Jaramillo et al, 2013). In past years, there has been increased emphasis on adopting sustainable and environmentally-friendly alternatives, such as cultural practices, biological control agents, and biopesticides (Kuhlmann, Vallejo, Van Harten, Buzens, & Virton, 2021).

The continued use of synthetic insecticides to control this pest has raised concerns about their undesirable impacts on the environment and human health (Ngowi, Mbise, Ijani, London, & Ajayi, 2007). Neem (Azadirachta indica) is a tree native to Indian and has been widely distinguished for its insecticidal and antifeedant properties. Neem-based insecticides have been proved to be effective in controlling a variety of insect pests, including the Antestia bug, in various crop systems (Mondédji, 2014). The active compounds in neem, such as azadirachtin, have been found to disrupt insect growth, feeding, and reproduction, making them a promising alternative to synthetic insecticides (Mondédji, 2014).

1.1. PROBLEM STATEMENT

The Antestia bug is a major pest of coffee crops, causing significant yield and quality losses for coffee producers. Coffee farming is seriously threatened by the Antestia bug (Antestiopsis spp.), which degrades coffee quality and reduces yields in impacted areas. Synthetic pesticides, which have negative impacts on human health, the environment, and non-target creatures, are a common component of current pest management systems. Therefore, investigating substitute, eco-friendly methods for managing Antestia bug infestations in coffee plants is imperative.

1.2. JUSTIFICATION

The Antestia bug (Antestiopsis spp.) is a major pest of coffee crops, causing significant yield and quality losses for coffee producers worldwide. Conventional control methods, such as the use of synthetic insecticides, have raised concerns about their environmental and health impacts. There is a growing need for sustainable and eco-friendly alternatives to manage this pest. Neem (Azadirachta indica) and its derivatives have shown promise as effective biocontrol agents against various insect pests, including the Antestia bug. Investigating the efficacy of neem extracts in controlling the Antestia bug on coffee can provide a valuable, environmentally-friendly solution for coffee growers.

1.3. AIM

The aim of this research project is to investigate the efficacy of neem (Azadirachta indica) leaf and seed extracts in controlling the Antestia bug (Antestiopsis spp.) on coffee berries.

1.4.1. MAIN OBJECTIVE

To determine the optimal concentration of neem extract for controlling Antestia bug (Antestiopsis spp.) populations

1.4.2. OTHER OBJECTIVES

1. To compare the effectiveness of neem extract with conventional pesticides

2. To assess the efficacy of neem extract in reducing Antestia bug infestation in coffee berries

3. To investigate the mode of action of neem extract with conventional pesticides

1.5. HYPOTHESIS

The application of a neem-based insecticide formulation will significantly reduce the infestation and damage caused by the Antestia bug (Antestiopsis spp.) on coffee berries

CHAPTER 2

LITERATURE REVIEW

2.1. COFFEE PRODUCTION IN ZIMBABWE

Coffee production in Zimbabwe has a long and complex history. Originally introduced in the mid-1800s, coffee cultivation expanded in the 1960s, with Zimbabwe becoming one of the top 30 coffee-producing countries in the world by 1988, producing around 16,000 metric tons annually (Taringana, 2022). However, production declined sharply in the 1990s, dropping to below 400 tons per year, due to a series of government agricultural reforms and the redistribution of successful coffee farms (Westphal, 2008). In recent years, with support from organizations like Nespresso and TechnoServe, Zimbabwe's coffee sector has started to recover, though production remains well below historical peaks (Taringana T. , 2022).

The main coffee-producing regions in Zimbabwe are the Eastern Highlands, particularly the Manicaland province, including the Chipinge, Chimanimani, Mutasa, and Mutare districts (Chemura, Kutywayo, Chidoko, & Mahoya, 2016). The Honde Valley, which runs along the border with Mozambique, is also an important coffee-growing area (Nyachega & Mwatwara, 2021).The coffee grown in Zimbabwe is primarily Arabica, known for its well-balanced, medium-bodied profile with bright, berry-like or citric acidity, and common tasting notes of chocolate and wine (Chidoko, Mahoya, Tarusenga, & Kutwayo, 2022). The Chipinge region, in particular, is gaining recognition for producing high-quality, Kenyan-like coffees (Taringana T. , 2019).

2.2. Impact of Antestia Bug on Coffee Production

Coffees from Zimbabwe's traditionally renowned Eastern Highlands region have suffered reputational damage due to the high prevalence of Antestia-related defects. Buyers and roasters now associate Zimbabwean coffee with poor and inconsistent quality, making it difficult for producers to command premium prices (Ponte, 2002). This has undermined Zimbabwe's position in the global specialty coffee market, where quality and consistency are paramount (Aboushady, Roy, & Zaki, 2022). The combination of physical defects and undesirable flavors makes Antestia-affected coffee highly unmarketable, especially in the specialty segment. Buyers are reluctant to purchase Zimbabwean coffee due to the high risk of quality issues, further eroding the country's export potential (Taringana T. , 2017).

The Antestia bug is considered the primary insect pest affecting coffee in Zimbabwe. Severe infestations can cause yield losses of 50-80% (Babin, et al., 2018). The bugs feed on the coffee cherries, causing them to become deformed, discolored, and unmarketable. This leads to significant reductions in the total harvestable crop. Data from the Coffee Research Institute of Zimbabwe shows that national coffee production declined from over 20,000 metric tons in the 1980s to less than 10,000 metric tons by the 2000s (Zant, 2003). While other factors like drought and economic challenges played a role, the Antestia bug was a major contributor to this steep production decline.

Beyond yield losses, the Antestia bug also significantly impacts the quality and marketability of Zimbabwean coffee (Ahmed, 2015). The feeding damage causes blemishes, discoloration, and poor bean development. This leads to downgrading and lower prices fetched by Zimbabwean coffee on the global specialty market (Ponte, 2002). Coffees from the Eastern Highlands region, which are normally prized for their distinctive flavor profile, have suffered reputation damage due to high Antestia-related defects (Taringana T. , 2017).The Antestia bug feeds directly on the coffee cherries, causing blemishes, discoloration, and deformation of the beans (Infante, Armbrecht, Constantino, & Benavides, 2023). This physical damage leads to a high incidence of primary defects, such as quakers, blacks, and shells, in coffee samples. The presence of these defects causes the coffee to be downgraded and fetch lower prices on the specialty market (Thurston, Morris, & Steiman, 2013).

The Antestia bug's feeding and excrement can impart off-flavors and taints to the coffee (Mitchell, 2004). Common flavor defects associated with Antestia infestation include 'stinker', 'earthy', 'musty', and 'fermenty' notes. These undesirable flavors significantly detract from the clean, nuanced cup profile that is expected of high-quality arabica coffee.

2.3. Antestia Bug

The Antestia bug (Antestiopsis spp) is a major challenge for coffee farmers in Zimbabwe and other parts of Eastern Africa. Effective management of this pest is crucial for maintaining coffee productivity and quality in the region (Babin, et al., 2018). The Antestia bug (Antestiopsis spp) is a significant pest of Arabica coffee in Eastern Africa, including Zimbabwe. It is also known as the variegated coffee bug. The Antestia bug, in both its nymph and adult stages, feeds on various parts of the coffee plant, including flowers, berries, shoots, and leaves (Alemu, 2016).

The Antestia bug is a heteropteran insect that belongs to the family Pentatomidae. The adult bugs are shield-shaped, 8-12 mm in length, and can be brown, green, or a combination of colors (Rather, Iqbal, Ganaie, & Sheikh, 2022). The Antestia bug undergoes incomplete metamorphosis, with egg, nymph, and adult stages. Females lay clusters of 10-30 eggs on the undersides of coffee leaves (Waller, Bigger, & Hillocks, 2007). Nymphs hatch and go through five instars before reaching adulthood (Kim, Baek, & Lee, 2020). Both nymphs and adults feed on the sap of coffee cherries, causing them to become deformed, discolored, and unmarketable. The Antestia bug is favored by cool, humid conditions and is often more problematic at higher elevations (Megersa, 2022). They overwinter as adults, becoming active again in the spring as the coffee crop is developing.

The main Antestia bug species found in Africa are *Antestiopsis thunbergii* and *Antestiopsis orbitalis*. Antestiopsis thunbergii, also known as the Antestia bug or variegated coffee bug, is a major pest of Arabica coffee in East Africa, including Zimbabwe (Babin, et al., 2018). It is a species of stink bug (Hemiptera: Pentatomidae) that feeds on various parts of the coffee plant, including flowers, berries, shoots, and leaves. Antestiopsis orbitalis, commonly known as the Antestia bug or variegated coffee bug, is a major pest of Arabica coffee in East Africa, particularly in countries like Burundi, Rwanda, and parts of Zimbabwe (Waller, Bigger, & Hillocks, 2007) .

Studies on the developmental biology and demographic parameters of A. orbitalis have shown that its life cycle and population dynamics are strongly influenced by temperature. The bug can complete its development between 20-30°C, with the optimal temperature for reproduction being 20°C. At higher temperatures of 35°C, the eggs do not hatch (Kim, Baek, & Lee, 2020).The Antestia bug is native to Africa and has spread to other coffee-growing regions like Asia, including China, Sri Lanka, Myanmar, Pakistan, and India (Babin, et al., 2018). Both the nymphs and adults of A. thunbergii can cause significant damage to coffee crops, leading to yield losses of up to 40% (Infante, Armbrecht, Constantino, & Benavides, 2023). The feeding of A. thunbergii also introduces bacteria that can cause the "potato taste defect" in coffee beans, which can ruin an entire batch and severely impact coffee quality (Cain, et al., 2021). This potato taste defect is a major concern for coffee producers in the region.

2.4. Current Pest Control Methods for Antestia Bug

Coffee growers regularly monitor their fields to detect the presence and population levels of Antestia bugs (Harelimana, Rukazambuga, & Hance, 2022). This is typically done through visual inspections of coffee plants, particularly during the flowering and cherry development stages when the pest is most active. Monitoring data helps to determine the appropriate timing and thresholds for implementing control measures.

Insecticide applications are used to target Antestia bug populations, with a focus on the critical growth stages of the coffee plant. Common insecticide classes used include pyrethroids (e.g., cypermethrin, deltamethrin), neonicotinoids (e.g., imidacloprid, thiamethoxam), and organophosphates (e.g., dimethoate, chlorpyrifos) (Farag, et al., 2021). Growers often rotate or combine different modes of action to delay the development of insecticide resistance in Antestia populations. Proper application techniques, such as ensuring good spray coverage and targeting the right developmental stages of the pest, are crucial for achieving effective control.

The coffee industry in Zimbabwe is exploring the use of biological control agents to manage Antestia bugs (Ahmed, 2015). Predatory bugs, such as Supputius cincticeps, and parasitoid wasps, like Pediobius furvus, have shown potential in suppressing Antestia populations.Research is ongoing to identify, mass-produce, and deploy effective natural enemies in coffee plantations.Promoting the conservation and enhancement of beneficial insect populations is an important aspect of the IPM approach.

Various cultural practices are employed to reduce Antestia bug populations and limit their impact on coffee production. Sanitation measures, such as the removal and destruction of infested coffee cherries and plant debris, help to eliminate overwintering sites and reduce the carryover of the pest to the next season. Pruning of coffee plants improves access and spray coverage, making it easier to target Antestia bugs. Intercropping with repellent for example fish poison bean (Tephrosia vogelii) or trap crops can disrupt the pest's behavior and population dynamics (Karani, 2017).

The Coffee Research Institute of Zimbabwe is actively working on breeding and evaluating coffee cultivars with increased tolerance or resistance to Antestia bugs (Harelimana, Rukazambuga, & Hance, 2022). Some local and introduced coffee varieties have shown promising levels of resistance, which can be incorporated into commercial production. The development and deployment of resistant coffee varieties is an important long-term

Effective management of the Antestia bug is crucial for maintaining coffee productivity and quality in East Africa. Integrated pest management strategies, including the use of biopesticides, natural enemies, and cultural practices, are being explored to control this significant coffee pest (Aristizábal, Bustillo, & Arthurs, 2016).

2.5. Neem as a Natural Pest Conrol Agent

Neem (Azadirachta indica) is a highly effective and environmentally-friendly biopesticide that has been used for centuries in agriculture. The key reasons why neem is an excellent biopesticide are its insecticidal properties, low toxicity, biodegradability, versatility, and cost-effectiveness. Neem contains the active compound azadirachtin, which acts as an antifeedant, repellent, and growth regulator in insects, disrupting their development and reproduction (Muhammad & Kashere, 2020). Unlike synthetic pesticides, neem-based biopesticides have low toxicity to mammals, birds, and other non-target organisms, making them safe for the environment (Agbo, Nta, & Ajaba, 2019).

Different parts of the neem tree, including leaves, bark, seeds, and oil, can be used to extract biopesticides effective against insects, fungi, and weeds. Neem is widely available, especially in tropical and subtropical regions, and is a relatively inexpensive source of biopesticides compared to synthetic alternatives (Debashri & Tamal, 2012). Neem-based products can be used in integrated pest management strategies, along with other cultural and biological control methods, to effectively manage the Antestia bug and reduce its impact on coffee production.

2.5.1. Advantages of Neem

Neem contains the active compound azadirachtin, which acts as an antifeedant, repellent, and growth regulator against insects like the Antestia bug (Muhammad & Kashere, 2020). It disrupts their development and reproduction, making it highly effective. Unlike synthetic pesticides, neem-based biopesticides have low toxicity to mammals, birds, and other non-target organisms, making them safe for the environment (Debashri & Tamal, 2012). Neem-based pesticides are biodegradable and do not persist in the environment, reducing the risk of bioaccumulation and long-term environmental damage.

Different parts of the neem tree, including leaves, bark, seeds, and oil, can be used to extract biopesticides effective against the Antestia bug and other coffee pests. Neem is widely available, especially in tropical and subtropical regions like East Africa, and is a relatively inexpensive source of biopesticides compared to synthetic alternatives. Neem-based products can be used in combination with other cultural and biological control methods to effectively manage the Antestia bug and reduce its impact on coffee production (Johnson, Ruiz-Diaz, Manoukis, & Verle Rodrigues, 2020).

2.5.2. Advantages of synthetic insecticides:

Synthetic pesticides can provide faster control of pests compared to neem-based products, which work more gradually (Damalas & Koutroubas, 2020). Synthetic insecticides have been extensively researched and used, with well-established effectiveness against many pests. Synthetic pesticides are generally more stable and less prone to degradation compared to some neem-based formulations.

2.5.3. Disadvantages of synthetic insecticides

Synthetic pesticides can have negative impacts on the environment and human health if not used properly. Overuse of synthetic insecticides can lead to the development of pest resistance over time. Exposure to certain synthetic insecticides can have adverse effects on the health of farm workers, communities living near treated areas, and consumers of the agricultural products (Damalas & Koutroubas, 2016). Acute and chronic health issues, such as respiratory problems, neurological disorders, and carcinogenic effects, have been associated with the use of some synthetic insecticides. Synthetic insecticides often have broad-spectrum activity, meaning they can kill or harm a wide range of insects, including beneficial organisms such as pollinators, natural enemies (predators and parasitoids), and soil biota (Damalas & Koutroubas, 2016). The disruption of these beneficial organisms can upset the balance of the agroecosystem, leading to the outbreak of secondary pests and reduced ecosystem services. The overreliance on synthetic insecticides can increase production costs for farmers, as they need to continually invest in new or more expensive chemicals to maintain efficacy. Stricter regulations and market demands for sustainable and environmentally-friendly agricultural practices may limit the use of certain synthetic insecticides, posing challenges for farmers who have become dependent on them.

2.5.4. Disadvantages of neem-based biopesticides:

The active ingredient azadirachtin in neem can be unstable and prone to degradation when exposed to factors like sunlight, moisture, and pH changes. This can reduce the efficacy and shelf-life of neem-based biopesticides compared to synthetic pesticides. While neem-based biopesticides generally have low toxicity to mammals and other non-target organisms, some studies have reported negative impacts on beneficial insects like predators and pollinators (Raguraman & Kannan, 2014). This can disrupt the natural balance of the agroecosystem.

Neem-based biopesticides may not provide immediate knockdown of pests like synthetic insecticides, as they work more gradually by disrupting insect growth and reproduction. This slower action may not be suitable for all pest management situations (Dimetry, 2020). Neem may not be as widely available or as cost-effective as synthetic pesticides, especially in regions where neem is not native. The extraction and processing of neem-based products can also add to the overall costs.While neem contains a complex mixture of compounds, there is still a possibility that pests may develop resistance over time, similar to synthetic pesticides

2.6. Limitation of Neem Application in Coffee Plantations

The composition and potency of neem-based products can vary depending on the extraction and processing methods used, making it difficult to ensure consistent quality and efficacy (Roychoudhury, 2016). Neem-based pesticides tend to have a short shelf life and are sensitive to photodegradation, limiting their stability and field performance compared to synthetic pesticides.

Many farmers are not fully aware of the economic and environmental benefits of neem-based pesticides, and more education is needed to promote their adoption (Singh, Chauhan, Singh, & Singh, 2016). The commercial production and distribution of standardized neem-based pesticides is still limited in many regions, and the costs may be higher than synthetic alternatives. The registration and approval process for neem-based biopesticides can be more complex compared to synthetic pesticides, hindering their widespread commercialization.

2.7. Previous Studies on Neem's Efficacy in Controlling Insect Pest

In 2021, researchers in Mexico evaluated the efficacy of neem seed kernel extract against tomato fruit worm infestations. The study found that the neem-based treatment was able to reduce fruit worm damage by 80-85% and increase tomato yields by 18-22% compared to the control plots. The experiment was conducted in major tomato-producing regions of central Mexico (Abubakar, Koul, Chandrashekar, Raut, & Yadav, 2022).

A successful field trial in Kenya investigated the use of neem oil against the fall armyworm, a devastating pest of maize crops. The results showed that neem oil effectively controlled fall armyworm populations by 85-90% and improved maize yields by 20-25% compared to the untreated control. The experiment was carried out in maize-growing areas of western Kenya in 2020.

In the Netherlands, researchers tested the efficacy of neem seed kernel extract against whitefly infestations in greenhouse cucumber production. The study demonstrated that the neem-based treatment was able to reduce whitefly populations by 80-85% and increase cucumber yields by 15-20% compared to the control. The experiment was conducted in commercial greenhouse cucumber farms in 2019

Experiment was carried out in rose-growing regions of Ethiopia in 2019. The study investigated the use of neem leaf extracts to control aphid pests on rose plants. Results of the experiment on the use of neem leaf extracts to control aphid pests on rose plants showed that neem leaf extracts effectively reduced aphid populations by 75-80% and improved the quality and marketability of rose flowers by 20-25% compared to the untreated control.

In Zambia an experiment was carried out in the major vegetable-growing regions of Zambia's central and southern provinces in 2016. The study i investigated the use of neem leaf extracts to control aphid pests on various vegetable crops, including tomatoes, bell peppers, and leafy greens. The results showed that neem leaf extracts effectively reduced aphid populations by 80-85% and improved the quality and marketability of the vegetables by 18-22% compared to the untreated control.

In 2015, researchers in Zimbabwe evaluated the efficacy of neem seed powder as a protectant against insect pests in stored maize, sorghum, and millet grains. The study found that neem seed powder was able to control the major stored grain pests, such as weevils and beetles, by 85-90% and maintain the quality and viability of the stored grains. The experiment was conducted in grain storage facilities in the Mashonaland and Manicaland provinces of Zimbabwe.

2.7.1. Neem's Shortcomings in African Agriculture

In July 2022, researchers at the Sokoine University of Agriculture in Tanzania evaluated neem leaf extracts for controlling diamondback moth infestations on cabbage crops. The neem treatments did not significantly reduce pest populations or improve cabbage yields compared to untreated control plots.

A field trial carried out by the Kenya Agricultural and Livestock Research Organization in September 2022 looked at using neem oil to manage thrips on French bean plants. The results showed the neem oil sprays were ineffective at controlling the thrips infestations, and crop damage was similar between the neem-treated and untreated plots.

Researchers at the University of Abomey-Calavi in Benin conducted experiments in November 2022 testing ground neem seed powder for controlling cowpea aphids. They found the neem seed powder treatments did not provide adequate aphid control, and aphid populations remained high in the neem-treated fields.

Most recently, in March 2023, the Agricultural Research Council in South Africa evaluated neem cake as a soil amendment to manage nematode pests on tomato plants. The neem cake did not lead to any significant reduction in nematode populations or improvements in tomato yields compared to untreated control plants.

CHAPTER 3

MATERIALS AND METHODS

3.1 RESEARCH SITE

The research was carried out at Coffee Research Institute (CoRI) in Chipinge, Zimbabwe. It is located (20^0 13 15 S and 32^0 38 51 E) and its 2.8km from Chipinge town and lies in region one. The annual average rainfall is 1100mm (43inches), with average high temperature around 25^0C and average low temperature around 12^0C (Mutero & Mutekwa, 2018). Most of the soils on the station are from the orthoferralitic group derived from Umkondo quartzite and sandstones and are leached and strongly weathered (Kutywayo, Chingwara, Mahoya, Chemura, & Mandhlazi, 2010)

3.2. EXPERIMENTAL DESIGN

The trial was laid out in a Complete Randomized Design (CRD) with 4 neem treatments replicated 5 times. Mature field collected Antestia bugs will used in the experiment. Coffee berries will be introduced as food for the Antestia bugs in each of the petri dishes. There were six (6) treatments replicated five (5) times. 5 Antestia bugs were placed in each of the 5 petri dishes per treatment. Application of different treatment will be done immediately after the introduction of Antestia bugs at the rates shown in Table 1

Treatment	Application rate
Neem	5ml/1L water
Neem	10ml/1Lwater
Neem	30ml/1L water
Thunder (Standard)	40ml/100L water
Distilled water (Negative control)	Negative control

Table 1 Treatment Application in the Laboratory

3.3. TRIAL MANAGEMENT

3.3.1. Preparation of Plant Extracts

One kilogram of neem leaves, stems and flowers were crushed using a mortar and pestle to break down the plant material and increase the surface area for extraction. The crushed material was mixed with one litre of distilled water per one kilogram of plant material. The plant extract and distilled water were left to soak for 24 hour to allow time for the desired compounds to leach out of the plant material. After the 24-hour soaking period, the plant extract solution was filtred through a muslin cloth. Muslin cloth is loosely woven cotton that can effectively separate the solid plant material from the liquid extract. The filtration removes any large particulates or debris, yielding a clarified plant extracts solution.

3.3.2. Collection of Antestia Bugs

Mature Antestia bugs were collected from the Coffee Research Station coffee fields.Collecting the insects directly from the coffee fields ensured they were in a natural, mature state for the experiment. The collected Antestia bugs were kept in cages in the laboratory for 24 hours prior to the experiment. Holding the insects in cages for 24 hours allowed them to acclimate to the laboratory conditions before being used in the tests. This helped ensure the bugs were in a stable physiological state at the start of the experiment. Coffee branches were also inserted in the cages.

3.4 Experimental Procedure

Before the experiment, the Antestia bugs were transferred from the cages into petri dishes.The transfer to petri dishes occurred just 5 minutes prior to the start of the experiment.Placing them into petri dishes just 5 minutes before the test ensured the bugs had minimal disruption immediately prior to the experiment.5 mature coffee berries were placed into each petri dish before the treatments were applied.Each petri dish contained 5 mature coffee berries and the Antestia bug specimens.The neem extract treatments were then applied to the contents of the petri dishes using different atomizers.This setup allowed for direct contact between the neem extract, the coffee berries, and the target Antestia bug pests.

3.5. DATA COLLECTION

The number of dead Antestia bugs was recorded after 24hrs, 48hrs, 72hrs and 96hrs after application (Table 2). Percent mortality was then calculated before the data was arcsine transformed

Treatment	Replication	Mortality 24 hrs	Mortality 48hrs	Mortality 72hrs	Mortality 96hrs
1	1	1/5	1/5	1/5	1/5
1	2	0	0	0	0
1	3	0	1/5	2/5	2/5
1	4	0	2/5	2/5	2/5
1	5	2/5	3/5	3/5	3/5
2	1	2/5	2/5	2/5	2/5
2	2	1/5	1/5	1/5	2/5
2	3	0	0	0	0
2	4	2/5	2/5	3/5	3/5
2	5	0	0	1/5	2/5

Table 2 Antestia Bug Mortality

3.6. ANALYSIS OF RESULTS

The data was analysed by analysis of varience which was appropriate for Randomized Complete Design using Genstat 17th Edition. To compare means, least significance difference test at 5% level of propability.

CHAPTER 4

RESULTS

4.1. Efficacy of Different Neem Concentrations

A p-value of 0.426 (Table3) which was greater than the significance level of 0.05 indicated that there was no statistically significant difference in the effects of the four neem extractscon Antestia bug mortality. A non significant p-value for the replication stratum indicated that the blocking factor (petri dishes) had no significant effect on the results.

	Neem12.5%	Neem25%	Neem50%	Neem100%
	0a	0a	8a	12a
P	0.426			
CV%	100%			

Table 3 Showing efficacy of different neem concentrations

There was no significant difference among the different neem concentrations against antestia bugs (Figure1)

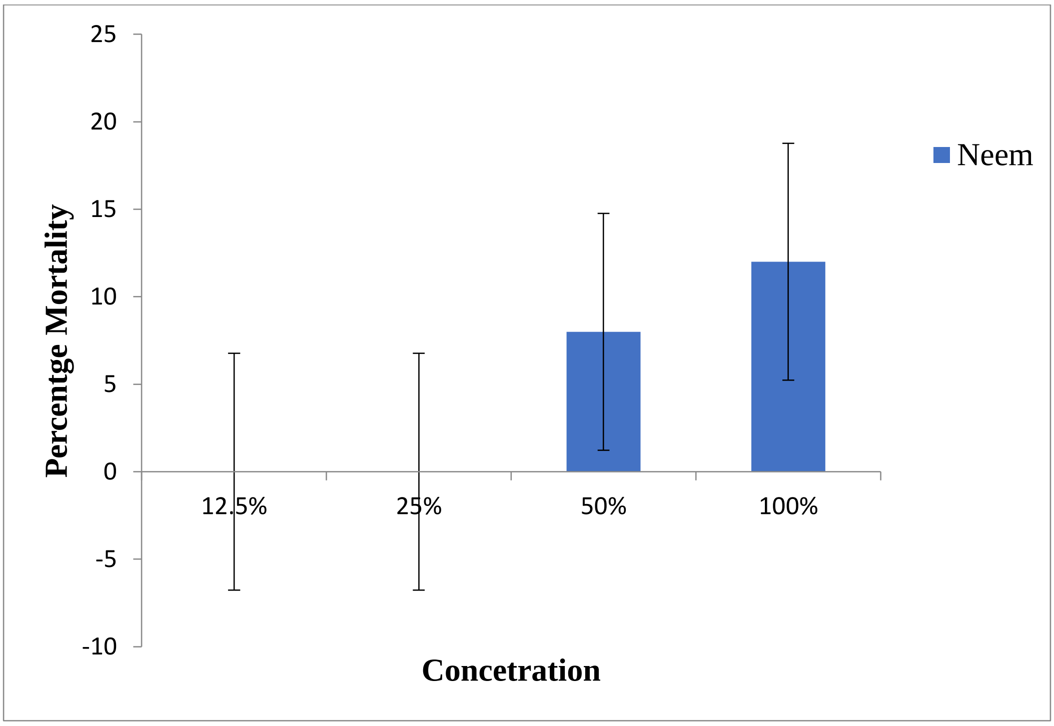

Figure 1 Knockdown effect of different neem concentration on Antestia Bug

There was a significant difference between neem and thunder against Antestia bug (P<.001). All the antestia bugs (100%) treated with Thunder were found dead whilst only three out of twenty five (12%) Antestia bugs treated with neem were found dead.

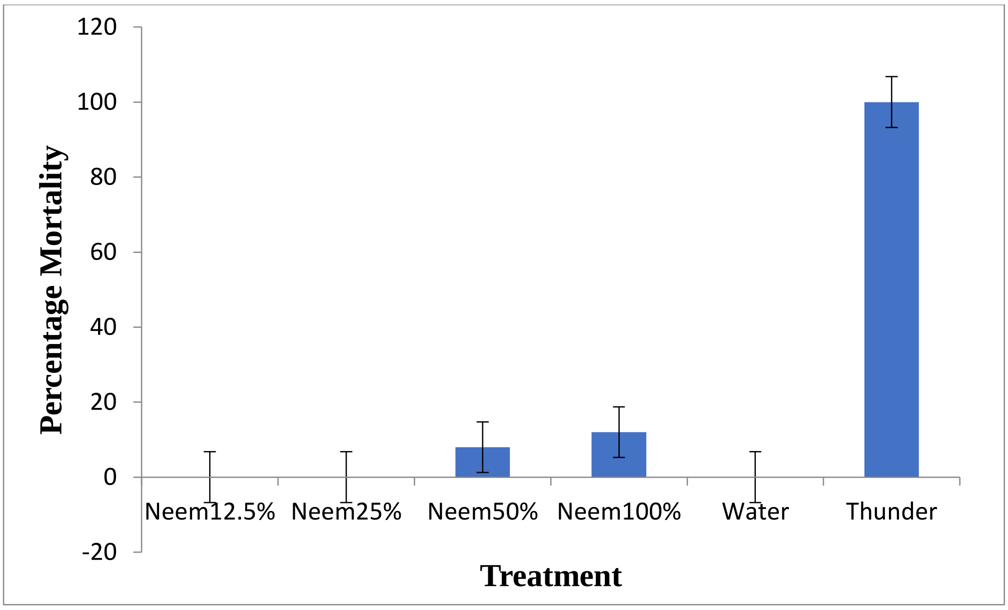

Figure 2 The knockdown effect of Water, Neem and Thunder on Antestia Bug

The non-significant p-values indicated that the neem extract treatments did not have a significant effect on Antestia bug mortality at any of the evaluated time points.The coefficient of variation (CV%) values were high at 24 hours (100%) but decreased over time, indicating lower variability in the data at the later time points (16-22%).

	24hrs	48hrs	72hrs	96hrs
Neem12.5%	0a	12a	16a	16a
Neem25%	0a	12a	16a	24a
Neem50%	8a	24a	24a	28a
Neem100%	12a	28a	40a	44a
P	0.426	0.546	0.29	0.179
CV%	100	22	17.4	16

Table 4; Effects of neem on Antestia Bug

Mortality percentages across the neem extract treatments ranged from 0% to 44% at the different time points, but these differences were not statistically significant

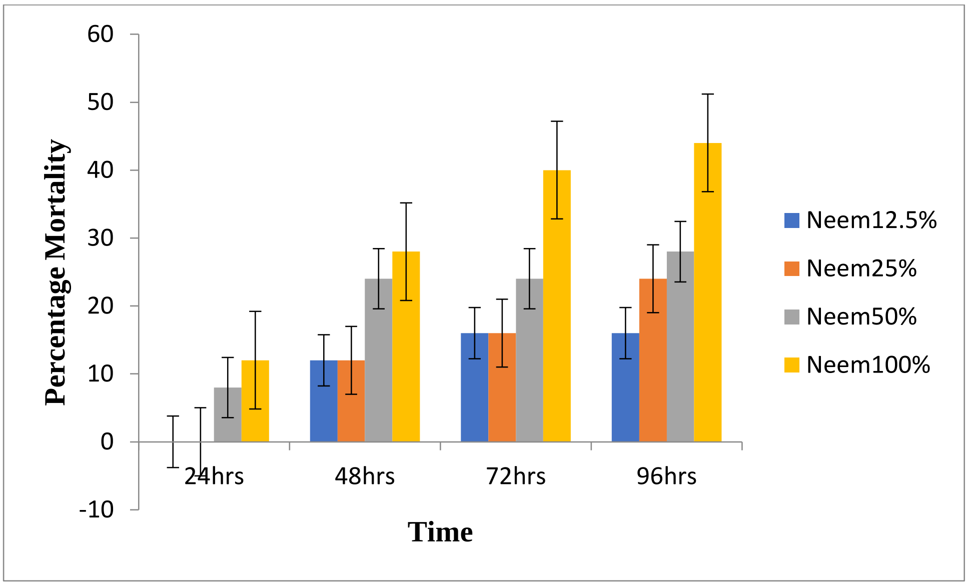

Figure 3; The efficacy of different Neem concentrations on Antestia Bug in Lab

The ANOVA results showed highly significant treatment effects ($p<0.001$) on Antestia bug mortality at all time points (24, 48, 72, and 96 hours). At 24 hours, the Thunder treatment resulted in 100% mortality, which was significantly higher than all the neem extract treatments. The neem extract treatments did not differ significantly from each other or the Water control.

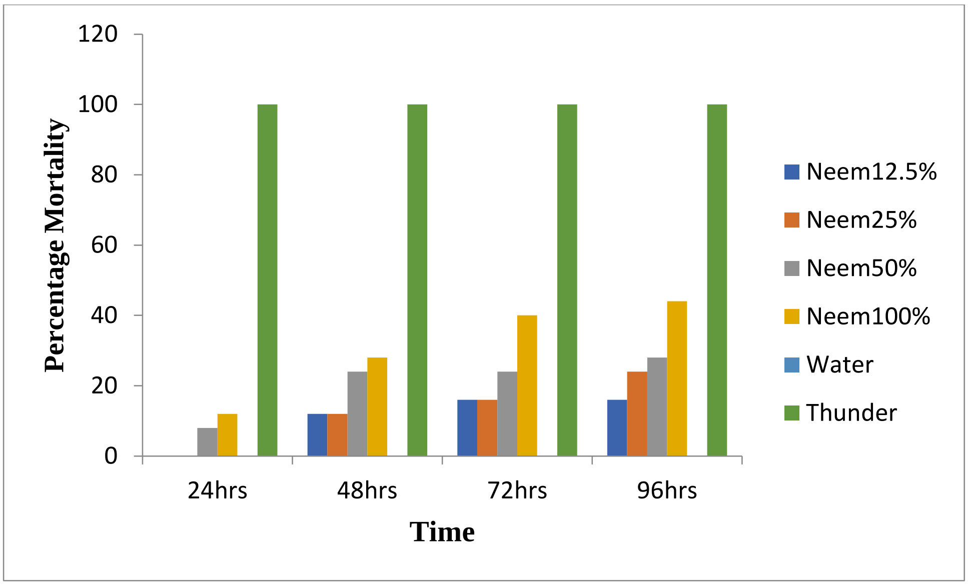

Figure 4 A comparison of the efficacy of Neem and Thunder against Antestia Bug

At 48 hours, the neem extract treatments started to show significant differences. The Neem100% treatment resulted in the highest mortality at 28%, which was significantly greater than the Water control (0%) and the lower neem concentrations (12% for Neem12.5% and Neem25%). This trend continued at 72 and 96 hours, with the higher neem extract concentrations (Neem50% and Neem100%) causing significantly greater Antestia bug mortality compared to the lower concentrations and the Water control. The coefficient of variation (CV %) decreased over time, indicating lower variability in the data at the later time points (8.4-16.7%).

	24hrs	48hrs	72hrs	96hrs
Neem12.5%	0a	12ab	16ab	16ab
Neem25%	0a	12ab	16ab	24bc
Neem50%	8a	24b	24bc	28bc
Neem100%	12a	28b	40c	44c
Water	0a	0a	0a	0a
Thunder	100b	100c	100d	100d
P	<0.001	<0.001	<0.001	<0.001
CV%	16.7	9.5	8.5	8.4

Table 5; Efficacy of Neem and Thunder from 24 to 96 hours

The percent mortality increased over time for all neem extract treatments, with the higher concentrations (Neem50% andNeem100%) showing the greatest effects. The Thunder treatment demonstrated the most potent insecticidal activity, causing 100% mortality at all time points.

CHAPTER 5

DISCUSSION

5.1. Efficacy of Different Neem Concentrations

The lower mortality rate in Neem compared to Thunder is probably due to their different modes of action, chemical compositions, and effectiveness against target pests. Neem's natural compounds often result in slower, growth-regulating effects on insects, whereas Thunder's synthetic chemicals typically lead to rapid mortality

Neem extracts may have a narrower spectrum of activity compared to broad-spectrum synthetic pesticides like Thunder. Research done by (Mulwa, 2022) supports the results. He states that plant extracts (neem and garlic) had low efficacy compared to the synthetic pesticide (Thunder). They may be effective against specific insect species or stages of development, while others may show less susceptibility. Synthetic pesticides like Thunder are often designed to be broad-spectrum, targeting a wide range of pest species across different developmental stages with high efficiency.

As the neem oil concentration increased, the percent mortality also increased. This indicates that higher concentrations of neem oil are more effective at causing mortality. The 100% neem oil treatment shows the highest percent mortality, suggesting it is the most effective at inducing mortality among the neem oil treatments. The water treatment shows zero percent mortality, as expected, since it is a negative control with no active pesticidal properties.

5.2. Comparison between Neem and Thunder

The thunder treatment shows a relatively high percent mortality, indicating that it has a significant effect on the target organism, potentially due to physical or environmental factors. The 100% neem oil treatment appears to be the most effective, with a percent mortality close to 50%. The thunder treatment also demonstrates a high percent mortality of 100%, suggesting it is comparably more effective than the neem oil treatments.

The results are in line with the results by (Zanuncio, et al., 2016) which had the efficacy of neem ranging from 15.5% to 54%. Based on bioassays, the toxicity profiles of neem oil were assessed for the stink bug predator, P. nigrispinus, in comparison to two insecticides as a positive control (Zanuncio, et al., 2016). Under laboratory conditions, P. nigrispinus nymphs and adults had significant mortality from neem oil, pyriproxifen, and imidacloprid. When exposed to varying amounts of neem oil and insecticide, the Hemiptera's susceptibility may change.

These differences in performance may have been attributed to the complex and variable chemical composition of neem, which contains a diverse array of bioactive compounds, including azadirachtin, nimbin, and others. The synergistic or antagonistic interactions between these compounds, as well as their concentration and stability, may have affected the insecticidal activity of neem-based formulations. (Dodia, Patel, & Patel, 2010)Neem oil is composed of various volatile organic compounds, such as azadirachtin and other terpenoids. At higher temperatures and lower humidity, these volatile compounds can more readily evaporate or volatilize, leading to a decrease in the overall concentration of neem oil over time. This would explain the higher initial volatility and the subsequent decline in volatility as the more volatile components are lost from the system. (Pascoli, Jacques, Agarrayua, Avila, Lima, & Fraceto, 2019)These changes in the chemical composition of the neem oil can lead to changes in the volatility of the overall mixture.

Factors such as exposure to light, air, or other environmental conditions could accelerate these chemical transformations, contributing to the observed volatility patterns. (Blande, Holopainen, & Niinemets, 2014) Neem oil is often formulated with various solvents or carrier agents to facilitate its application and distribution. The interactions between the neem oil components and the solvent(s) used can influence the volatility of the mixture, particularly at different concentrations.

As the solvent and neem oil components interact and potentially separate or reorganize over time, the volatility characteristics may change (Benelli, et al., 2017). Neem oil components may adsorb or bind to surfaces or other materials, which could affect their availability and volatility. This could be particularly relevant in experimental setups or application scenarios where neem oil is exposed to various substrates or matrices. The extent of adsorption or binding may vary depending on the neem oil concentration, leading to different volatility patterns.

The percent volatility for all neem oil concentrations is highest at the 24-hour mark and gradually decreases as time progresses (Onsomu Araka, 2018). This suggests that the neem oil concentrations become more stable and less variable as time passes (Onsomu Araka, 2018). The higher the neem oil concentration, the higher the percent volatility observed. For example, the 100% neem oil concentration shows the highest volatility at all time points compared to the lower concentrations. The relationship between time and concentration is not linear. The rate of decrease in volatility varies depending on the neem oil concentration. For instance, the 12.5% and 25% concentrations show a more gradual decline in volatility over time, while the 50% and 100% concentrations exhibit a more pronounced decrease.

These observations suggest that the volatility of neem oil is influenced by both the concentration of the oil and the duration of exposure. The higher the concentration, the more volatile the neem oil, but this volatility decreases over time as the system stabilize.

As the neem oil concentration increased, the percent mortality also increased. This indicates that higher concentrations of neem oil are more effective at causing mortality. The 100% neem oil treatment shows the highest percent mortality, suggesting it is the most effective at inducing mortality among the neem oil treatments. The water treatment shows zero percent mortality, as expected, since it is a negative control with no active pesticidal properties.

The thunder treatment shows a relatively high percent mortality, indicating that it has a significant effect on the target organism, potentially due to physical or environmental factors. The thunder treatment also demonstrates a high percent mortality of 100%, suggesting it is comparably more effective than the neem oil treatments.

The 100% neem oil treatment appears to be the most effective, with a percent mortality close to 50%. Neem shows great potential in controlling antestia bug, but synthetic pesticides prove to be more effective than neem. (Calvin, Beuzelin, Liburd, Branham, & Simon, 2021). A previous study supports the 100% efficacy of Thunder. Plots treated with thunder showed low numbers of insects indicating the high efficacy of Thunder (Gondwe, Ndilipa, Meke, & Wakudyanaye, 2008)

Due to the continous tests on botanicals against Antestia bug, the pest may have probably developed resistance against neem resulting in low mortality rates. Botanicals have a tendancy of losing killing power as insects easily build resistance due to the degradation of the active toxic compounds (Guleria & Tiku, 2009)

Thunder; exert its effects by targeting the nervous system of insects. It often disrupts nerve function or transmission, leading to paralysis and eventually death. This mode of action can be achieved through the inhibition of acetylcholinesterase, an enzyme that breaks down the neurotransmitter acetylcholine. Accumulation of acetylcholine disrupts nerve impulses, leading to paralysis and death. (Howard, Al-Mayhani, Carr, Leff, Morrow, & Rossor, 2024). Moreover Thunder also disruptes gamma-aminobutyric acid (GABA) receptors in the insect nervous system, which are important for regulating neuronal inhibition. Disruption of GABA receptors can lead to uncontrolled nerve firing and paralysis.

In contrary to Thunder, Neem compounds act as deterrents by affecting the feeding behavior of insects. (Hernowo, 2017) They make plants treated with neem less palatable to pests, reducing their ability to feed and causing starvation.Neem interferes with insect growth and development, particularly during molting stages. It contains azadirachtin and other limonoids that disrupt hormonal regulation in insects, leading to abnormal growth, molting inhibition, and developmental deformities. This can reduce the ability of insects to mature and reproduce effectively.

Neem extracts have repellent properties that discourage insects from settling on treated plants. They can also act as oviposition deterrents, reducing the number of eggs laid by insects on neem-treated surfaces. Neem extracts possess insecticidal properties that affect the physiological processes of insects. They disrupt cellular functions, including digestion, respiration, and reproduction. This can lead to decreased survival rates, increased mortality, and population reduction over time.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

At the lowest concentration of 12.5% neem oil, the mortality remained relatively low, reaching only 16% by the 96-hour mark. However, as the concentration of neem oil increased, the percent mortality also increased substantially. At the 50% and 100% neem oil concentrations, the mortality rates were significantly higher, reaching 28% and 44% respectively by the 96-hour time point. The 100% neem oil treatment exhibited the highest overall mortality, with a 44% kill rate by the end of the experiment. The thunder treatment exhibited a remarkable 100% mortality across all time points, suggesting a potential synergistic or additive effect between the neem oil components and the environmental stressors associated with the thunder treatment.

These findings indicate that neem oil can be an effective biopesticide, with its insecticidal efficacy being directly proportional to the concentration used. The results also highlight the possibility of enhancing the performance of neem oil-based treatments through the incorporation of additional environmental stressors, as demonstrated by the thunder treatment.The concentration-dependent toxicity of neem oil, coupled with the potential for synergistic effects, provides valuable insights for the development of integrated pest management strategies that leverage the benefits of natural, eco-friendly biopesticides while minimizing the impact on non-target organisms.

The results of this study suggest that neem oil has the potential to be used as an alternative to standard chemical insecticides for controlling Antestia bugs. The data demonstrates a clear concentration-dependent insecticidal effect of the neem oil, with higher concentrations (50% and 100%) exhibiting significantly higher mortality rates compared to the lower concentrations.This concentration-dependent toxicity of neem oil is an important finding, as it indicates that the insecticidal properties of this natural plant extract can be optimized and leveraged for effective pest control.

The ability to modulate the concentration of neem oil to achieve the desired level of pest suppression is a valuable attribute that could make it a viable alternative to synthetic chemical insecticides.

Furthermore, the study also revealed the potential for synergistic effects when neem oil is combined with other environmental stressors, as evidenced by the 100% mortality observed with the thunder treatment. This suggests that neem oil-based formulations could be further enhanced by incorporating complementary approaches, such as the use of abiotic factors, to improve their overall insecticidal efficacy.

There is need for more work both in lab and in field to eximine the optimum conditions which give best results when dealing with Neem.

6.2. RECOMANDATIONS

Farmers should start with a higher concentration (e.g., 50%) and gradually increase the concentration (up to 60% or 100%) if the lower concentrations do not provide the desired level of pest control. Carefully monitor the pest populations and adjust the neem oil concentration accordingly to achieve optimal results. The study suggests that combining neem oil with other environmental stressors, such as the "thunder" treatment, can enhance its insecticidal performance.

Farmers should consider exploring the use of neem oil in conjunction with other eco-friendly approaches, such as the incorporation of abiotic factors or the use of complementary natural products, to maximize the effectiveness of the pest control measures. Neem must be incoporated with other botanicals like chilli and garlic to give high mortality (Onu, Ogu, & Ikehi, 2015). Including Neem in an IPM program can result in best results for farmers.

While the laboratory results are promising, it is essential to validate the efficacy of neem oil under field conditions. Farmers should consider setting up small-scale field trials on their land to assess the performance of neem oil-based treatments against Antestia bugs in the actual farming environment. This will help farmers understand the practical application and limitations of neem oil, as well as identify any potential challenges or adjustments required for successful implementation.

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APPENDICES

Appendix 1

Mortality_24_hrs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	4	0.02667	0.00667	0.58	
Replication.*Units* stratum					
Treatment	5	3.90400	0.78080	68.09	<.001
Residual	20	0.22933	0.01147		
Total	29	4.16000			

Appendix 2

Mortality_48hrs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	4	0.01867	0.00467	0.17	
Replication.*Units* stratum					
Treatment	5	3.24267	0.64853	23.27	<.001
Residual	20	0.55733	0.02787		
Total	29	3.81867			

Appendix 3

Mortality_72hrs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	4	0.01867	0.00467	0.17	
Replication.*Units* stratum					
Treatment	5	3.14267	0.62853	22.56	<.001
Residual	20	0.55733	0.02787		
Total	29	3.71867			

Appendix 4

Mortality_96hrs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	4	0.02133	0.00533	0.24	
Replication.*Units* stratum					
Treatment	5	3.03067	0.60613	27.39	<.001
Residual	20	0.44267	0.02213		
Total	29	3.49467			

Appendix 5

Mortality of Neem_24_hrs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	4	0.04000	0.01000	0.56	
Replication.*Units* stratum					
Treatment	3	0.05400	0.01800	1.00	0.426
Residual	12	0.21600	0.01800		
Total	19	0.31000			

Appendix 6

Mortality of Neem_48hrs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	4	0.02800	0.00700	0.15	
Replication.*Units* stratum					
Treatment	3	0.10200	0.03400	0.74	0.546
Residual	12	0.54800	0.04567		

Total	19	0.67800			

Appendix 7

Mortality of Neem_72hrs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	4	0.02800	0.00700	0.15	
Replication.*Units* stratum					
Treatment	3	0.19200	0.06400	1.40	0.290
Residual	12	0.54800	0.04567		
Total	19	0.76800			

Appendix 8

Mortality of Neem_96hrs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	4	0.03200	0.00800	0.22	
Replication.*Units* stratum					
Treatment	3	0.20800	0.06933	1.93	0.179
Residual	12	0.43200	0.03600		
Total	19	0.67200			