ASSESSMENT OF THE CHEMICAL COMPOSITION AND PESTICIDAL PROPERTIES OF *Cassia didymobotrya* ASH ON *Myzus persicae* AFFECTING TOMATO CROPS IN ISHAKA

BY

ABAMAZIMA MARIA

BPH/0005/122/DU

SUPERVISED BY

MR. ONCHWERI ALBERT

MR. KHAUKHA JUDE TADEO NATTE

RESEARCH REPORT SUBMITTED TO THE SCHOOL OF PHARMACY OF KAMPALA INTERNATIONAL UNIVERSITY-WESTERN CAMPUS IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR DEGREE OF PHARMACY

JUNE, 2017.

DECLARATION

I, Abamazima Maria (BPH/0005/122/DU) hereby declare that this research report is my original work and not a duplication of similar work of any scholar for academic purpose as partial requirement of any institution or otherwise. It has therefore never been submitted to any institution of higher learning for the award of any qualification in any field of study.

I declare that all the material cited in this write up that are not mine have been duly acknowledged.

Signed...........................

Date..16th June..2017

This study report has been submitted with the approval of University supervisors,

Mr Onchweri Albert

Signed...........................

Date ...16th June, 2017...

Mr Khauka Jude Tadeo Natte

Signed...........................

Date ....16th | June | 2017.

TABLE OF CONTENTS

DEDICATION

This work is dedicated to my beloved parents Mr. Abundinabo David and Mrs. Busingye Jacinta and my entire family for their love and unfailing support toward my education. I would also like to dedicate this piece of work to my classmets and friends for their encouragement and coperation during my course of study.

ACKNOWLEDGEMENT

I sincerely appreciate all the people who have assisted me various ways to carry out this research and compiling this work.

I am particularly grateful to my supervisors, Mr. Onchweri Albert and Mr. Khauka Jude Tadeo Natte for the guidance provided in the course of this project.

I also appreciate the technical support provided by Mr. Ibanda Ivan during the intensive laboratory processes of this work.

I further thank my family for their unwavering financial support throughout this project.

ABSTRACT

Background: *Myzus persicae* (green peach aphids) are crop pests; they are among the most destructive insect pests on cultivated plants in temperate regions. Many of them are green in color, but others may be white wooly or black (McGravin,1993).Their damage to plants has made farming a complicated venture across the whole world. From the zoological standpoint, they are a highly successful group of organisms (Piper, 2007).

The study was conducted to evaluate the chemical composition and pesticidal effects of wood ash from *Cassia didymobotrya* on aphids affecting crops. DUDU® (Acelamectine) was used as a positive control in a concentration of 1ml/1L of water.

It was hypothesized that *Cassia didymobotrya* ash has pesticidal effects on aphids which increase with an increase in its concentration.

The study was carried out at KIU-WC Pharmacognosy laboratory. Ash obtained from a 1 year *Cassia didymobotrya* tree was exposed to aphids obtained from tomato plants in varying concentrations and also subjected to qualitative analysis.

Results:

From the results of the qualitative analysis of this ash, it contains chlorides, phosphates, sulphates and Calcium ions and all except Calcium are suspected causes of the death of the aphids. This is because,

- ***Chlorides;*** chloride ions are used in dichlorodiphenyltrichloroethane (DDT) a notorious pesticide which because of its toxicity from its organic component is being withdrawn from market (Lear and Linda, 2009).
- ***Sulphates;*** inorganic Sulphur (contained in sulphates) is a generally accepted pesticide that was used in earlier times to kill pests (Michael *et al.*, 2002).
- ***Phosphates:*** these are the main ingredients used from both natural and synthetic pesticides.

It was also discovered that th ash from *Cassia didymobotrya* is effective in killing Myzus persicae and that its efficacy at a concentration of 5mg in 30Ml of water is 98.64% while the efficacy at a concentration of 10mg in 30 Ml of water is 99.04%.

Conclusion:

Wood ash from *Cassia didymobotrya* ontains chlorides, sulphates, phosphorous and calcium ions because of these chemicals the acacia wood ash is effective against aphids.

The efficacy of this ash is dependent on the concentration of the ash; that is, the higher the concentration of the ash, the higher its efficacy, except for forming a paste at higher concentrations that would make its application difficult by blocking the pump nozzle.

There is need for more research in isolating the effective component of this ash that specifically causes death of the aphid

CHAPTER ONE

1.0 BACKGROUND

1.1 Introduction

Cassia didymobotrya is a species of flowering plant commonly known as African senna belonging to the family Fabaceae . Its leaves are used in various countries in Africa to treat malaria, pneumonia, gastrointestinal problems and also as purgative. (Mahadevan et al., 2002).

It is a hairy shrub usually growing up to about 5 meters tall but known to reach 9 meters at times. Its leaves are elongated up to a half a meter with pairs of leaflets, ovoid in shape up to 6 centimeters long. The plant is known to have a peanut butter scent or smell of burnt popcorn. It give racemes of yellow bright flowers that yield flat brown legume pods of up to 12 centimeters long containing 10 bean-like seeds of up to a centimeter long each.(Blundell, 1987).

Aphids are crop pests; they are among the most destructive insect pests on cultivated plants in temperate regions. Many of them are green in color, but others may be white wooly or black (McGravin, 1993).Their damage to plants has made faming a complicated venture across the whole world. From the zoological standpoint, they are a highly successful group of organisms (Piper, 2007).

They are majorly monophagous .However, the prevalent type of aphid in Ishaka; the green peach aphid *(Myzus persicae*), feeds on hundreds of plant species across many families including tomatoes, beans, peas, papaya, potatoes, sugarcanes and various other crops, and is a vector for more than 110 plant viruses. (McGravin, 1993).

They feed by sucking sap out of the phloem vessels in plants. (Spiller *et al.*, 1990).

Also, they secrete honey dew which is an attractant of fungi that cause additional damage to the crops.

Plants exhibiting aphid damage have a variety of signs which include decreased growth rates, mottled leaves, yellowing of leave, stunted growth, curling of leaves, browning, wilting, low yields and death. In commercial crop faming especially, the damage caused by aphids has resulted in large amounts of resources and efforts being spent attempting to control the activities of aphids. (McGravin,1993).

Currently, 10-16% of global crop production is lost due to pests. (http://www.exeter.ac.uk). In Uganda, they pose the greatest threat to the agriculture sector in terms of resultant losses. To avoid agricultural crop loses and enhance productivity, farmers employ agro-chemical pesticides. The production of these pesticides in Uganda however, is still as minimal as 2%, thus, 98% of are obtained through import. This increases their cost, making it hard for most local farmers to obtain them. (Thomson,2017).

The most commonly used pesticides on Uganda market are majorly class 2 according to WHO classification which signifies an increased level of toxicity; majorly cypermethrin and cypermethrin-profenos (Oesterlund et al., 2014).

Other pesticides used are shown in the table below, indicating their active ingredients and WHO toxicity classification. (Okonya,2015).

Number	Commercial pesticide name(s)	Active ingredient and concentration	WHO Toxicity class
1	Lava 100% EC	Dichlorvos 100%	1b
2	Bulldock 0.25 EC	Beta-Cyfluthrin 2.5%	2
3	Ambush	Permethrin 50%EC	2
4	Rocket 44 EC	Cypermethrin 40%+	2
5	Malataf 57 EC	Malathion 57%	3
6	Super ethoate Agrithoate 40 EC	Dimethoate 40%	2

The risk of pesticide toxicity from use of these pesticides is highly probable because most farmers use their teeth to open blocked nozzles of knapsack sprayers which is associated with a significantly high risk if acute poisoning (Oesterlund *et al.,* 2014). Additionally, the farmers are not accustomed to use of protective gear. Even unauthorized pesticides are being used by farmers in Uganda. (WHO,2009). Some times because of inappropriate labelling, wrong pesticides are used to spray aphids excessively (Morton *et al.*, 1994).

When used improperly, pesticides cause human poisoning, accumulate in food chain and the environment and lead to resistance in pests. (Ntow *et al.*, 2006).

The use of wood ash which is claimed to be effective against aphids (Haruna, 2012) would solve the great problems of aphid infestation, pesticide cost, hence improve nutrition and control environmental pollution because it is not an environmental pollutant.

Since the expense on pesticides used on market remarkably hinders tomato growth in Ishaka, local farmers have resorted to the use of affordable methods like use of wood ash in the control of these aphids.

Traditionally, in the evening after watering the crops, acacia wood ash is sprinkled on the crop leaves. The procedure is repeated after a fortnight. Also, across Uganda farmers utilize this method

The quality of the ash is affected by combustion conditions like temperature, and the type of tree from which the ash is obtained. Also, different plant parts yield ashes with differing chemical composition; plant foliage yields the highest quality ash.

1.2 Problem statement

While the demand for tomatoes increases with the increasing population, the rate of hindrance to tomato production caused by pests especially aphids, is on the rise, causing up to 10-16% global crop loss ((BBC, 2011). This has led to an increase in tomato production costs with a significant reduction in output and subsequently an increase in the prices of tomatoes for example, 1kg of tomatoes (10 tomatoes on average) cost 3500 Ugx in December 2016 from the 1000Ugx average price in 2015 as compared to the previous cost in 2014 which ranged btween 1000-1500 Ugx. (Taboola, 2016). Additionally, there is not a single garden of tomatoes that thrives to the production stage without being infested by aphids and therefore being sprayed using pesticidal chemicals which are pollutants to the environment and toxic to man, vegetation and aquatic life.

To worsen the problem of pesticide use alongside their pollutant and toxic properties, pesticides on market are losing their pesticidal effects on aphids due to development of resistance. This increases the need for discoveries of new pesticides of which wood ash would be lead.

Therefore, this study aims to establish a local, cheap and eco-friendly alternative method to control aphids as crop pests.

1.3 Objectives of the study

1.3.1 Broad objective

To assess the pesticidal effects of acacia wood ash on aphids affecting tomatoes crops in Ishaka, western Uganda to promote use of natural pesticides in aphid control.

1.3.2 Specific objectives

- To demonstrate method of obtaining pesticidal wood ash.
- To assess the chemical composition of acacia wood ash.
- To evaluate the pesticidal effects of acacia wood ash on aphids
- To discover if pesticidal effects of *cassia didymobotrya* vary with its concentration.

1.4 Study questions

- Does acacia wood ash have pesticidal effects on aphids?
- Does the pesticidal effect of wood ash vary with its concentration? If yes,
- What concentration of wood ash has highest pesticidal effect?
- What is the chemical composition of wood ash from *Cassia didymobotrya?*

1.5 Hypothesis

1.5.1 Null hypothesis

Acacia wood ash has pesticidal effects on aphids which increase with an increase in its concentration.

1.5.2 Alternative hypothesis

Acacia wood ash does not possess pesticidal effects on aphids.

1.6 Justification of the study

There has been great challenge to tomato growth in Bushenyi, caused by aphid infestation. This has resulted in low supply of tomatoes especially to the local population with low income, insufficient to meet their needs. Also, trials made by the local population with low income to grow tomatoes for personal consumption, this could be a great attribute to child malnutrition among Bushenyi children.

In addition to high costs met in purchase of pesticides, their chemical composition poses a great threat to the environment because they are potential environmental pollutants and when consumed on products, are also highly potential toxicants.

Also, when consumed on products, it is not as toxic as the chemical pesticides prevalent on market. Therefore, information obtained from this study has valuable in enhancing methods of pesticide control among farmers in Uganda and the rest of the world.

It has provided an evidence based publication to the promotion of wood ash use in the management of pests. Also, the knowledge obtained in this field is beneficial to the Department of Pharmacy in K.I.U western campus.

CHAPTER TWO

2.0 LITERATURE REVIEW

Tomatoes, also known *as Solanum lycopersicum* are perennial crops of the night shade family; Solanaceae. The crops typically grow up to 1-3 meters high. They have weak stem that prowls over the ground and veins over other plants. Tomato fruits are averaged 100grams. They are used as food for man. (Tomaat., 2010), tomato fruits are used as ingredients in salad, sources and drinks and can also be consumed raw (Parnell.,*et al* 2004) Different varieties of tomatoes grow in different conditions for example Gardener's Delight, Chadwick Cherry, Money Maker, Sweet Olive, and Alicante are tall growing tomatoes suitable for growth in gardens while bush/ Tambling varieties such as Tambling Tom, Tambler, and garden pealare suitable for growth in pots or hanging baskets. (Dauna, 2016).

Successful tomato production is not easily achieved because it requires highly intensive management, marketing skills and significant investment. Tomato production per acre is high and yields are significantly affected by pests and environmental problems. Tomatoes are outdoor self-pollinating crops, favorably growing in warm environments of temperature ranges between 21.1◦C to 29.4◦C during day and between 18.3◦C and 21.1◦C at night. Most species require about 75 days from transplanting to first harvest (William and George, 2012).

In Uganda, tomatoes are better grown in rainy seasons to reduce the labor of watering them. However, tomato growth during rainy seasons is heavily affected by pests such as Aphids that inflict a heavy cost of pesticides on farmers. (Daily monitor February, 25th 2015). The attack of these pests reduces both the economical and nutritional value of tomatoes. Aphids are among the most common pests affecting tomatoes in western Uganda; mostly affecting young plants- bed tomatoes.

Although they singly cause minimal damage to plant foliage, aphids' severe damage results from their feeding on fruits or by spreading certain diseases. They are sap-sucking pests which cause leaf discoloration, leaf or fruit deformation or defoliation. Tomato seedlings are weakened, their leaves stained and in case of mature plants, leaves turn yellow and curly. They are soft bodied, pear-shaped insects with a pair of dark cornicles and a cauda protruding from the abdomen.

Green peach aphids- the most common aphids affecting tomatoes- are either wingless pale yellow to green adults of up to 24mm long or winged adults with a dark dorsal blotch on a yellow green body. Their nymphs have three characteristic dark lines on their bodies. The wingless forms are more common but can undergo adaptation modification to winged form under conditions of over congestion on one plant. They feed in dense colonies excreting honey dew on which sooty mold grows and also attract aunts which inflict an additional damage to the tomatoes; the fungal infection causes mottling of foliage and heavy discoloration. (Spiller, *et al.*, 1990).

Aphids have complicated life cycles. Females can reproduce with or without a mating female aphids may lay eggs or give birth to wingless nymphs. The female can produce 20-100 nymphs which grow quickly in one week and start to reproduce, hence a rapid destruction of crops. (Verela, 2016).

The wingless forms are more common but can undergo adaptation modification to winged form under conditions of over congestion on one plant. They feed in dense colonies excreting honey dew on which sooty mold grows and also attract aunts which inflict an additional damage to the tomatoes; the fungal infection causes mottling of foliage and heavy discoloration. (Spiller *et al.*, 1990).

One of the ways of preventing against aphid infestation is by avoiding excess nitrogen which encourages new leafy growth that aphids love to feed on; but this rather adversely affects the quality and quantity of the crop yield.

Additionally, Aphids can be controlled by use of insecticidal soap solution; however, soap spray may damage plant especially at high temperatures and in high concentrations. (Cranshaw, 2008).

Also, use of natural enemies particularly lady beetles and parasitic wasps can control aphids at an environmental-friendly cost; however, it is realistically impossible for a local farmer in western Uganda because these natural enemies tend to fly away within 48 hours of application after release without laying eggs. Thus, it requires repeated application of large numbers of about 1500 lady beetles applied at least twice to gain effectiveness. (Flint, 2013).

An alternative biological methods used in aphid control are:

1. Planting tall plants like maize around the perimeter of the garden before tomatoes are planted.(Cheryl, 2016).
2. Use of bell papper powder: where the strong bell paper powder selected is mixed in water in proportions of 0.05 Kg/L. the mixture is boiled at 100∘C for 10 minutes, cooled and used to spray the infested crops. The best time for spraying is between 10am and noon (Dawhirst, 2010).

However, research on the biological control of these pests demonstrates that the level of pest control was inadequate for suppressing the pest populations. (Adati *et al.*, 2008).

Insecticide use increases tomato production tenfold (Kamara *et al.*, 2010). However, pesticide use in aphid control (especially broad spectrum pesticides) contaminates all plants within the treated area hence causing harm to beneficial insects. Also, pesticides lead to resistant aphid populations. The higher the population of pesticide-resistant aphid populations, the worse the situation becomes. Many of the pesticides used in western Uganda kill beneficial predator insects making them counter-productive for aphid management. Also, pesticides used are toxic to humans. Exposure to them increases risks of both immediate toxicity and long-term effects like cancer and birth defects in developing foetus. Insecticides applied near water ways and ponds-which is always the most convenient land for tomato growth- can potentially poison fish and other aquatic organisms. Organophosphate pesticides for example Acephate carbaryl malathion (classified as carcinogenic by the Environmental Protection Agency (EPA) are toxic to the nervous system; especially in children and are highly toxic to humans at low concentrations. They are also moderately toxic to aquatic life, honey bees and other beneficial insects (Pesticide Research Institute, October 2016).

Wood ash, a residue of burnt wood is comprised mostly of Calcium, Potassium, Magnesium, and Phosphorous, but also contains trace amounts of Manganese, Sodium, Boron, Zinc, Copper and Molybdenum. It is capable of natural soil amending components that support new plant growth and thus has been used widely by gardeners and farmers to rejuvenate soil fertility. Wood ash has an N-P-K value of 0-1-3. In addition, wood ash produces good amounts of calcium carbonate which serves as a source of calcium to the garden which gives it an added advantage for use on tomatoes because tomatoes are calcium-loving plants. Because tomatoes are much more prone to potassium deficiencies, which in addition to aphid infestation cause curling of leaves, yellowing between veins brown sports, slow plant growth and reduced yield, they thus more so need wood ash which is an excellent source of Potassium.(Timothy, 2010).

In Uganda, farmers in Masindi, Mukono, Kapchorwa, Buikwe, and Nwoya district practice production of chemical-free fruits and vegetables by use of ash mixed with urine to control against pests especially aphids. In doing this, they save more than 40% of losses to pests and diseases. Additionaly, Potassium and sodium from the ash, and nitrogen from the urine add nutritive value to the soil. However, according to the food and agricultural organization's teca segment, the concentration of ions in urine can burn the plants and therefore should be diluted 2:1 (water to urine) prior to administration. Also, the urine is prone to loss of value on exposure because of the volatile nature of ammonia, the ingredient of choice in urine. (Christine, 2016).

Typically, 0.43-1.85% of burned wood results in ash. Conditions of combustion affect the amount and composition of the residue (Misra and Baker, 1993), for example high temperatures reduce ash yield (Etiegni and Campbell, 1991).

The quantity of Calcium carbonate as the major component of wood ash ranges between 25% (Lamer, 2000) and 45% . Less than 10% is Potash and less than 1% is phosphate. These numbers vary with temperatures (Misra *et al.*, 1993).

CHAPTER THREE

3.0 MATERIALS AND METHORDS.

3.1 Introduction

This chapter contains study design, study area, study population, sample size, inclusion and exclusion criteria, sampling techniques, and data collection procedures.

3.2 Study design

The study was experimental in nature, analyzing the chemical composition of wood ash and investigating its pesticidal effects on aphids. Each aphid sample was monitored for 24 hours and results from the experiment provided data for analysis.

The chemical composition of the ash was assessed using qualitative analytical methods.

3.3 Study area

The study was conducted in Ishaka-Bushenyi district, at the laboratories of KIU-WC school of pharmacy.

3.4 Study population

The experiments utilized aphids from one tomato garden in Ishaka.

3.4.1 Specimen identification

The acacia specie was identified by the botanist in Mbarara University of science and technology Apio Eunice as Cassia didymodotrya, and the aphids were identified by the entomologist in KIU-TH Mr Khaukha Jude as *Myzus persicae* (green peach aphids).

3.5 Inclusion and exclusion criteria

All the aphids were collected from the same garden of tomatoes in Ishaka; Bwegyiragye village.

3.6 Sample size

A total of 180 aphids were used. Each sample utilized 15 aphids (Lazzarotto *et al.*, 2011) subjected respectively to the negative and positive control and to the experimental ashes in different concentrations.

3.7 Sampling techniques

tomato stems and leaves infested with aphids were plucked from the garden and taken to the Laboratory for identification by an entomologist within 30 minutes; packed in a polyethene bag.

Fresh aphid specimens were picked randomly from a tomato garden while attached to the plants using gloved hands, brought to the laboratory within 30 minutes and were then extracted off the plant by beating method and collected on a tray underneath the tomato stems and leaves.

They were then exposed to positive and negative control and experimental ash suspensions in two concentrations

3.8 Data collection procedures

Data was collected by reporting observations of the pesticidal effectiveness of different concentrations of wood ash, and the standard chemical by taking direct record of the time taken for all aphids in the given experimental set to die

3.8.1. Wood ash preparation and collection.

3.8.1.1) method of ash collection

- ✓ *Cassia didymobotrya* tree of one year growth was cut from the lowest part of its main stem.
- ✓ The stem and foliage were cut into small pieces of about 4cm by 4cm and dried under the sun.
- ✓ Dry stem pieces were then placed in a metallic stove and burned until ashes formed, which were collected in the stove's lower apartment.
- ✓ Periodically, the ashes were scooped from the stove using a metallic ladle, placed in a metallic bucket and allowed to cool under room conditions of about 25°C

3.8.1.2) Reconstitution of the ash

- o Two different concentrations of wood ash were prepared.
- o The first was made to contain 5g of wood ash mixed in 30Ml of distilled water.
- o The second will contain 10g of wood ash mixed in 30Ml of distilled water.
- o From each suspension of wood ash 1Ml was picked and used to sprinkle a set of aphids in a petri dish.

- The time taken for all aphids in a given set to die was record in a table.

3.8.1.3) Testing chemical composition of wood ash

The ash was tested qualitatively for the presence of anions (bicarbonates/carbonates, chlorides, nitrates, phosphates, sulphates) and cations (Ammonium, Barium, Calcium, Ferric, Ferrous, Lead, Magnesium, Potassium, Silver, Zinc and Copper.

3.8.2 Method of analysis of the ash

10g of ash were weighed using an analytical weighing balance, placed in a beaker, and 100 Ml of distilled water added to it, thoroughly mixed using a metallic rod to make a suspension. Using filter paper and funnel, the suspension was filtered and the filtrate used as sample for the following procedures (in house procedure).

3.8.2.1 Test for bicarbonates/ carbonates

The sample solution was boiled in a test tube and the liberated gas passed through Calcium hydroxide solution in another test tube.

3.8.2.2 Test for chlorides

1 Ml of sample solution was acidified using1ml of Nitric acid and then treated with Silver nitrate solution. Then another 1 Ml of nitric acid was added.

3.8.2.3 Test for nitrates

To 2 ml of the sample solution in a test tube was added concentrated Sulphuric acid and the mixture heated. It was then cooled and the test tube inclined. To the inclined tube, one drop of ferrous sulphate solution was added.

3.8.2.4 Test for phosphates

2 ml of the aqueous sample solution were treated with 1 Ml of Silver nitrate solution, then the resultant solution was boiled.

3.8.2.5 Test for sulphates

2ml of the aqueous sample solution were acidified using 1Ml of Hydrochloric acid and then treated with 1 ml of Barium chloride solution. To test for sulphites/ dithionates, 2 drops of Iodine solution were added to the resultant precipitate solution.

3.8.2.6 Test for Calcium/Magnesium

To 1 Ml of solution was added 5 drops of 1M Sodium carbonate. To differentiate between the presence of Calcium and Magnesium, 10 mg of ash were placed on a watch glass, wetted with 10M Hydrochloric acid, and then put to flame. The flame was observed for color change.

Confirmation test for presence of Magnesium

2Ml of solution were made alkaline with 1Ml of Ammonia and then treated with 1Ml of sodium hydroxide solution. To the resultant solution was added 1Ml of Ammonium hydroxide solution followed by 1ml of Sodium Hydrogen phosphate solution.

3.8.2.7 Test for Potassium

A Platinum was annealed in a laboratory burner flame until the flame becomes colorless. Then the wire was dipped into the sample solution and put back into the flame. Then the flame was then observed for change in color (Flame test).

3.8.2.8 Test for Silver.

1ml of the sample solution was treated with Potassium chromate, then 1Ml of dilute Nitric acid was added.

3.8.2.9 Test for Copper

2 drops of 2M Ammonium hydroxide were added to 1ml of the sample solution followed by 3Ml of Ammonium hydroxide solution and observed for changes.

3.8.2.10 Test for Zinc

1Ml of sample solution was treated with a solution of Potassium ferrocyanide.

3.8.2.11 Test for Sodium

A Platinum wire was annealed in a laboratory Bunsen flame until the fame become colorless, then, the wire was dipped into the sample solution and put back to the fire flame.

B) Materials used

i) Acacia tree
ii) Machete
iii) Metallic stove
iv) Metallic ladle
v) Metallic bucket
vi) Petri dishes

3.8.3 Positive control (formulation and use)

3.8.3.1) Method

- DUDU® (Acelamectin) was used as the experiment's positive control.
- It was purchased from one of the agro-veterinary shops in Ishaka town.
- 1ml of DUDU®(-Acelamectin) was measured using a calibrated test tube, diluted with 5 ml of distilled water and put in a 5L plastic sprayer. The test tube used was then rinsed using another 5ml of distilled water and the rinse put in the sprayer to which will be added more distilled water to top it to the 1L mark.
- The solution was used within 10 minutes after preparation.
- 2ml of the chemical mixture were sprayed on the aphids in a petri dish.
- Time taken for all aphids to die was taken using stop watch and recorded in the table below.

3.8.3.2) Materials used

i) DUDU® (standard pesticide)
ii) Distilled water
iii) 1Ml pipette
iv) 5L plastic sprayer
v) Petri dishes

3.8.4 Negative control

3.8.4.1) Method

- Distilled water was obtained from KIU-WC pharmaceutical chemistry laboratory and used as the negative control.
- 1L of distilled water was put in a 5L plastic sprayer and about 2Ml of the water sprayed on the aphids on a petri dish.
- Time taken for all aphids to die was taken using stop watch and recorded in a table.

3.8.4.2) Materials used

i) Distilled water

ii) Plastic can

iii) Petri dishes

3.8.5 Exposure experiment.

- The already prepared acacia wood ash suspensions was used in the exposure experiment.
- 2Ml of each of the suspensions from 3.8.1 A2 were used to spray aphids in their respective petri dishes and observed for how long they took to die.
- The time taken for the aphids to die was then recorded in a table.

3.9 Methods of data presentation

Data was presented in form of tables, graphs, pictures and descriptive statements.

3.10 Data Analysis Procedures

- A bar graph showing mortality time of aphids in negative control was plotted (or adapted from 3.8.4).
- This was compared against the bar graph for the results from exposure experiments (3.8.3).
- 5 The results were interpreted along the difference in the graphs obtained by the mortality in both of ash suspensions and control experiments.
- Pesticidal effectiveness {E (%)} was calculated from the equation below as adopted and modified from (Augustus *et al*, 2009).

  E (%) = <u>(mortality time in negative control – mortality time in exposure sample)</u> x100

Mortality time in negative control

Where: E (%) = percentage of pesticidal effectiveness of sample in relation to the control;

- A mortality of 25% was considered significant.

3.11 Ethical consideration

- A letter of introduction from school of pharmacy carry out experiments in KIU-WC laboratories was obtained.
- The experiments were carried out in triplicates in order to minimise error and ensure consistency.

CHAPTER FOUR

4.0 RESULTS

4.1 CHEMICAL COMPOSITION ASH FROM *Cassia didymobotrya*

4.1.1 Test for bicarbonates/ carbonates

The sample solution was boiled and the liberated gas passed through Calcium hydroxide solution. Formation of a white precipitate would indicate presence of carbonates and/or bicarbonates. Therefore the sample solution did NOT contain carbonates or bicarbonates.

4.1.2 Test for chlorides

1 Ml of sample solution was acidified with Nitric acid and treated with Silver nitrate solution. A cudy white precipitate was formed, insoluble in Nitric acid soluble in Ammonium solution but reappeared on addition of Nitric acid solution which indicated presence of chlorides.

4.1.3 Test for nitrates

The sample solution was heated with concentrated Sulphuric acid and cooled. To the inclined tube, one drop of Ferrous sulphate solution was added. There was no brown color forming at the interface of the two solutions indicated absence of nitrates.

4.1.4 Test for phosphates

The aqueous sample solution was treated with silver nitrate solution. A light yellow precipitate of orthophosphate formed, which did not change color on boiling, but dissolved in 10% dilute ammonia, this showed presence of phosphates.

4.1.5 Test for sulphates

The aqueous sample solution was acidified with Hydrochloric acid and treated with Barium chloride solution. A white precipitate formed, which indicated presence of sulphates. To test for sulphites/ dithionates, 2 drops of iodine solution were added to the resultant precipitate solution. The yellow solution persisted , which indicated absence of sulphites and dithionates.

4.1.6 Test for Calcium/Magnesium

To 1 Ml of solution was added 5 drops of 1M sodium carbonate. White precipitate formed which indicated presence of either Magnesium/Calcium ions. To differentiate between the presence of Calcium and Magnesium, 10 mg of ash were placed on a watch glass, with 10M Hydrochloric acid, and then put to flame. The flame turned dull red which indicated presence of Calcium.

4.1.7 Test for test for the presence of Magnesium

The solution was made alkaline with Ammonia and treated with Sodium hydroxide solution. The solution remained clear. This indicated the absence of Magnesium.

4.1.8 Test for potassium

The flame test performed on the sample was negative for Potassium because the flame did not turn violet in color.

4.1.9 Test for Silver

1Ml of the sample solution was treated with Potassium chromate , then 1Ml of dilute Nitric acid was added and the solution observed for formation of a red precipitate which did not appear. therefore Silver ions were absent.

4.1.10 Test for Copper

2 drops of 2M Ammonium hydroxide were added to 1ml of the sample solution followed by 3Ml of Ammonium hydroxide solution and observed for formation of a blue precipitate which did not form therefore, Copper was absent.

4.1.11 Test for Zinc

When 1Ml of sample solution was treated with a solution of Potassium ferrocyanide, there was no precipitate formed hence absence of Zinc.

4.1.12 Test for Sodium

The flame test performed on the sample was negative for Sodium because the flame did not turn yellow in color.

Bicarbonates/ carbonates	✗
Chlorides	✓
Nitrates	✗
phosphates	✓
sulphates	✓
Calcium	✓
magnesium	✗
potasium	✗
Silver	✗
Copper	✗
Zinc	✗
Sodium	✗

Table 1 showing summary of the chemical composition of wood ash

4.2 EFFECT OF WOOD ASH ON APHIDS

4.2.1 Table 2 shows the time taken for aphids to die.

	Exposure experiment 1 (5g/ 30ml) [min]	Exposure experiment 2 (10g/ 30ml) [min]	Positive control (Dudu®) (1ml /1L of H_2O [min	Negative control (H_2O) [Min]
Time taken for all aphids to die in 1st experiment	19	18	4	840
Time taken for all aphids to die in 2nd experiment	24	15	7	2400
Time taken for all aphids to die in 3rd experiment	27	16	5	1890
Average time	23.33	16.33	5.33	1710

4.2.2 Calculations for Pesticidal effectiveness {E (%)}(adopted from 3.10)

$$E(\%) = \frac{\text{mortality time in negative control} - \text{mortality time in exposure sample}}{\text{Mortality time in negative control}} \times 100$$

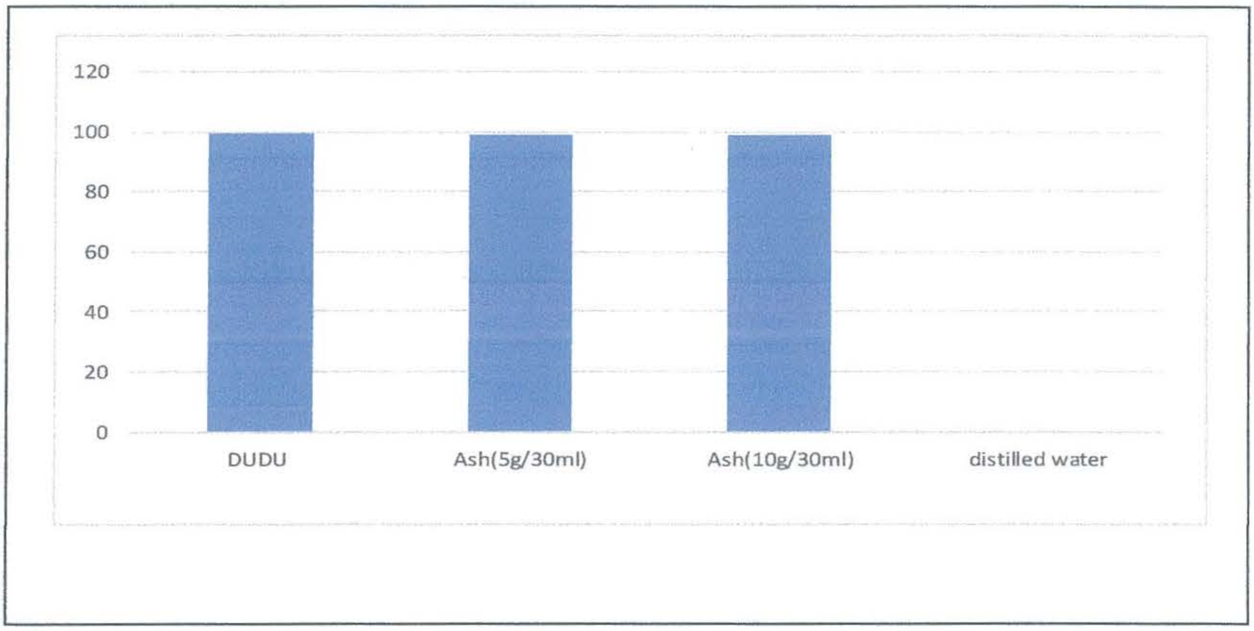
4.2.2.1 Calculations

Dudu: $E(\%) = \frac{(1710-5.33)}{1710} \times 100 = 99.69\%$

Ash (5g/ 30ml): $E(\%) = \frac{(1710-23.33)}{1710} \times 100 = 98.64\%$

Ash (10g/30ml): $E(\%) = \frac{(1710-16.33)}{1710} \times 100 = 99.04\%$

Distilled water: $E(\%) = \frac{(1710-1710)}{1710} \times 100 = 0\%$



Graph1 showing percentage efficacy E(%)

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMENDATION

5.1 Discussion

According to the results above, the aphids sprayed using acacia wood ash died in a time much shorter than those sprayed using a negative control (distilled water). This correlates with the hypothesis of the study (ash from *Cassia didymobotrya* has pesticidal effects against aphids).

Also, acacia wood ash in smaller concentrations (5g of ash mixed in 30 ml of distilled water) killed the aphids in 23.26 min giving it an efficacy of 98.84% as compared to the ash at a higher concentration which killed the aphids in 16.21 minutes giving it 99.19% efficacy in accordance with Augustus *et al*, 2009 analytical methords.

From the results of the qualitative analysis of this ash, it contains chlorides, phosphates, sulphates and Calcium ions and all except Calcium are suspected causes of the death of the aphids. This is because,

i. Chlorides; chloride ions are used in dichlorodiphenyltrichloroethane (DDT) a notorious pesticide which because of its toxicity from its organic component is being withdrawn from market (Lear and Linda, 2009).
ii. Sulphates; inorganic Sulphur (contained in sulphates) is a generally accepted pesticide that was used in earlier times to kill pests (Michael *et al.*, 2002).
iii. Phosphates: these are the main ingredients used from both natural and synthetic pesticides.

5.2 Conclusion

- Wood ash from *Cassia didymobotrya* chlorides, sulphates, phosphorous and Calcium ions.
- *Cassia didymobotrya* contains ash is effective against *Myzus persicae* (green peach aphids).
- The efficacy of *Cassia didymodotrya* ash is dependent on the concentration of the ash; that is, the higher the concentration of the ash, the higher its efficacy, except for forming a paste at higher concentrations that would make its application difficult by blocking the pump nozzle.

5.3 Recommendations

- The institution should procure an Atomic Absorption Spectrophotometer which will aid in perfecting future researches in quantifying different components of samples, reducing errors. and drawing conclusions.
- Farmers should take up use of Acacia wood ash in fighting pests such as aphids because it is effective, cheap and friendly to both the environment, plants and animals.
- More of cassia didymobotra should be grown.
- Further research should be conducted to investigate which component of the ash is actually responsible for killing of the aphids in order to come up with an economic and health friendly pesticide.

CHAPTER SIX

6.0 TIME FRAME AND WORK SCHEDULE FOLLOWED

The following working schedule was followed for the study

Month/activity	Dec 2016	Jan 2017	Feb 2017	March – May 2017	June 2017
Proposal writing					
Proposal presentation					
Data collection					
Data analysis					
Project presentation					

CHAPTER SEVEN

7.0 PROJECT BUDGET

The following was the budget for the study

NO	ITEM	TOTAL COST (Ugx)
1	Labour (bucket filling, tomato planting and watering)	50,000
2	Glass ware(conical flasks), latex gloves, nose masks, buckets	25,000
3	DUDU-ACELAMECTIN	5,000
4	Printing of proposal	40,000
5	Printing of project	30,000
6	Transport	100,000
7	Tomato seeds	50,000
8	Pipetes and petri dishes	10000
9	1L plastic spray pumps (2)	10000
10	Miscellaneous	50,000
11	Reagents	100,000
12	GRAND TOTAL	460,000

REFERENCES

Adati T, Manuele Tamo, S.R Yusuf and W.Hammond.,2008. Integrated pest management for cowpea-cereal cropping systems in the West African savannah. International journal of tropical insect science.

Anna H Oesterlund, Jane F Thomsen, Deogratias K Sekimpi, James Maziina, Apio Racheal, and Erik Jors., 2014. "Pesticide knowledge practice and attitude and how it affects the health of small scale farmers in Uganda": a crosssectional study.

Cheryl Long,. may 23rd , 2016. Http:/www.rodalesorganiclife.com.

Christine Mwaura., 2016. "Urine-wood ash mix is farmer's cost-free pesticide" [farm bliz Africa].

Collaborative international cost of living index., 2016. http://www. Expatistan.com.
Dailymonitor.,February,25th2015.'Ntegetomatofarmer'
http://www.monitor.Ug/magazines/farming.

Dauna,.(Eden project, Bodelva-UK.

Dawhirst SY, Pickett JA, Hardie J., 2010. http:/ www. Ncbi.nlm.nih.gov/ pubmed/ 20831961.

Elwell and Maas., 1995, natural pest and disease control . Natural farming network., Zimbabwe. http://www.infonet-biovision.com.

Etiegni L and Campbel AG., 1991. "Physical and chemical characteristics of wood ash". Bio resources and Technology.

FAO,2010. Corporate Document Repository, Forestry department. "A glimpse of local technology knowledge for comprehensive watershed management".

George C. McGravin., 1993. Bugs of the world.

GRIN-link and Blundell,M ., 1987. "wild flowers of east Africa).

Haruna., 2012. Teca.fao.org/ discussion/contro of aphids.

Hume E., 2006. "wood ashes: how to use them in the garden." Ed Hume Seeds.

Joshua Sikhu Okonya and Jürgen Kroschel., 2015. "A crosssectional study of pesticise use and knowledge of small holder potato farmers in Uganda".

Kamara A.Y, D.Chikoye, F.E keleme, L.O. Omoigui and I.Y Dugje., 2010. "International journal of pest management".

Lamer BR., 2008. "Wood ash in the garden". Purdue University, department of horticulture and landscape architecture.

Lazzarotto CM., Lazari SMN., Penteado SRC.,2017. " Feeding Behaviour of Two Exotic Aphid Species on Their Original hosts in a New Invaded Area".

Lear and Linder., April 1st, 2009. 'Witness for nature' edited by Rachel Carson.

Marshall Brandley., 2009. " Rodales ultimate encyclopedia of Organic Gardening".

Michael W. Stimmann BruceF. Edridge .,2002. "pesticide application and safety training for aplicators of public health pesticides".

Misra MK and Baker AJ., 1993. "Wood ash composition as a function of fumase temperature [PDF]. Biomas and bio energy.

M.L Flint., (July 2013) Aphid Integrated Pest Management for Home Gardens and Landscape Professionals ; , University of California Davis.

Mustafa Haruna.,2012; "controlling aphids on tomatoes using wood ash". http/:www.teca.fao.org/discussion/controlling aphids on tomatoes using wood ash .

N.J.Spiller; L.Koenders; W.F. Tjallingii.,1990. 'Xylem ingestion by aphids- a strategy for maimtaining water ballace'. Entomologia experimentalis et applicata.

Ntow WJ, Gijzen HJ, Kelderman P, Drechsel P., (2006) farmer perceptions and pesticide use practices in vegetable production in Ghana. Pest Manag Sci.

Parnell, Tracy L.:Suslow, Trevor V.;Harris, Linda J .,March 2014." Tomatoes: Safe Methods to Store, Preserve, and Enjoy"

Rose,. Pesticide Research Institute: update 19th October 2016:

Rose Piper., 2007. Extraordinary animals: an Encyclopedia of Curious and unusual animals. Greenwood press. Pp.6-9.

Timothy S. Graffin., 2006. " use of wood ash on your garden".

Tomaat, September 2010, RZ seeds and services.

WHO, 2009. "The Recommended Classification of Pesticides by Hazard". In:World Health Organization, ED Stuttgart, Germany: Wisssenchaftliche Verlagsgesellschaft mbH)

William TK and George B., 2012. "Geogia Vegetable Team, Commercial Tomato Production Handbook "(B1312)

W.S. Cranshaw.,2008. "Insect control": soaps and Detergents University of Colorado. www.pesticideresearch.com and www.aphidsonworldsplants.info/ Methords of collecting, preserving and making slide preperations of aphids.

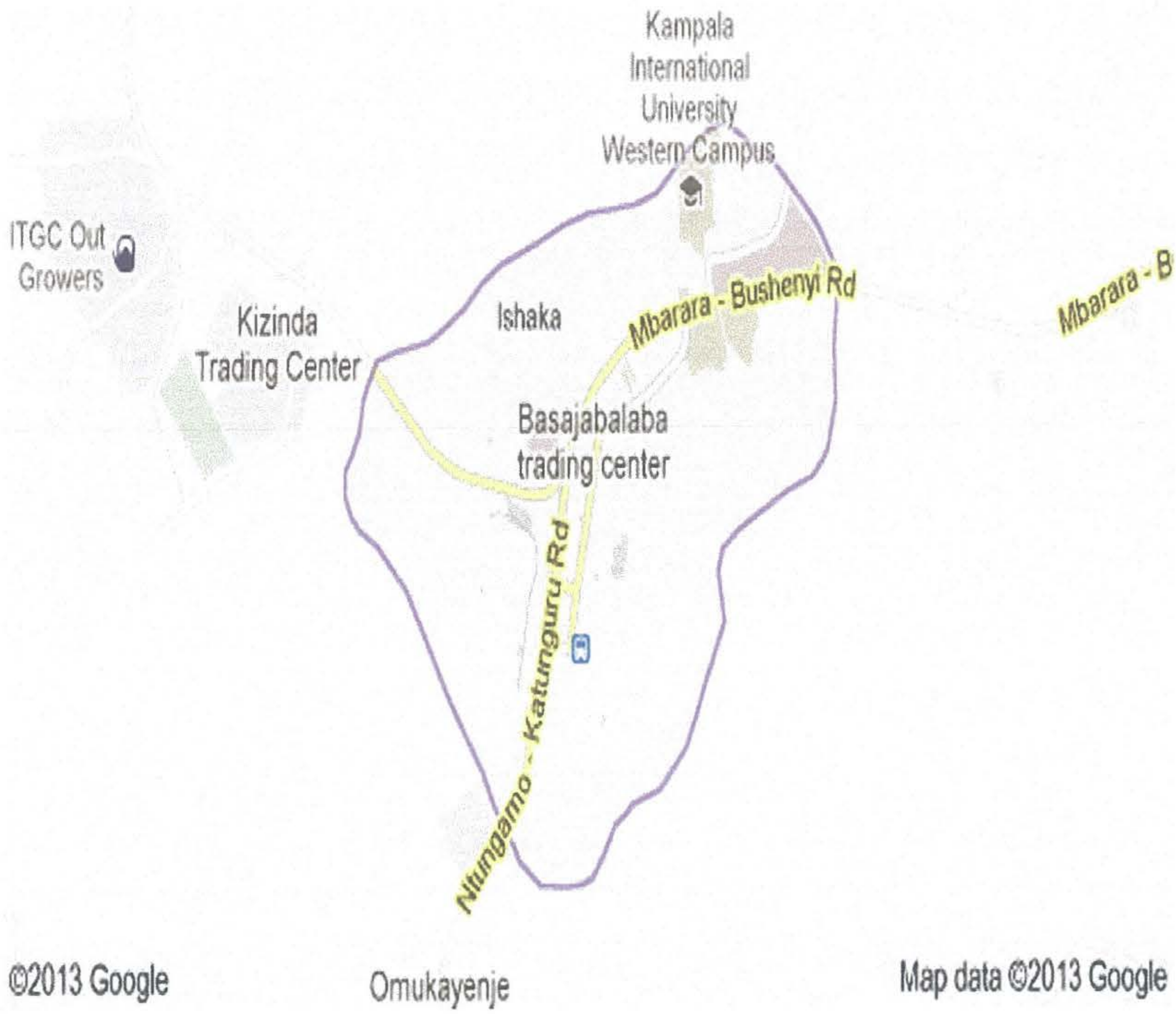
APPENDICES

APPENDIX I: THE MAP OF UGANDA

APPENDIX II: MAP OF BUSHENYI DISTRICT

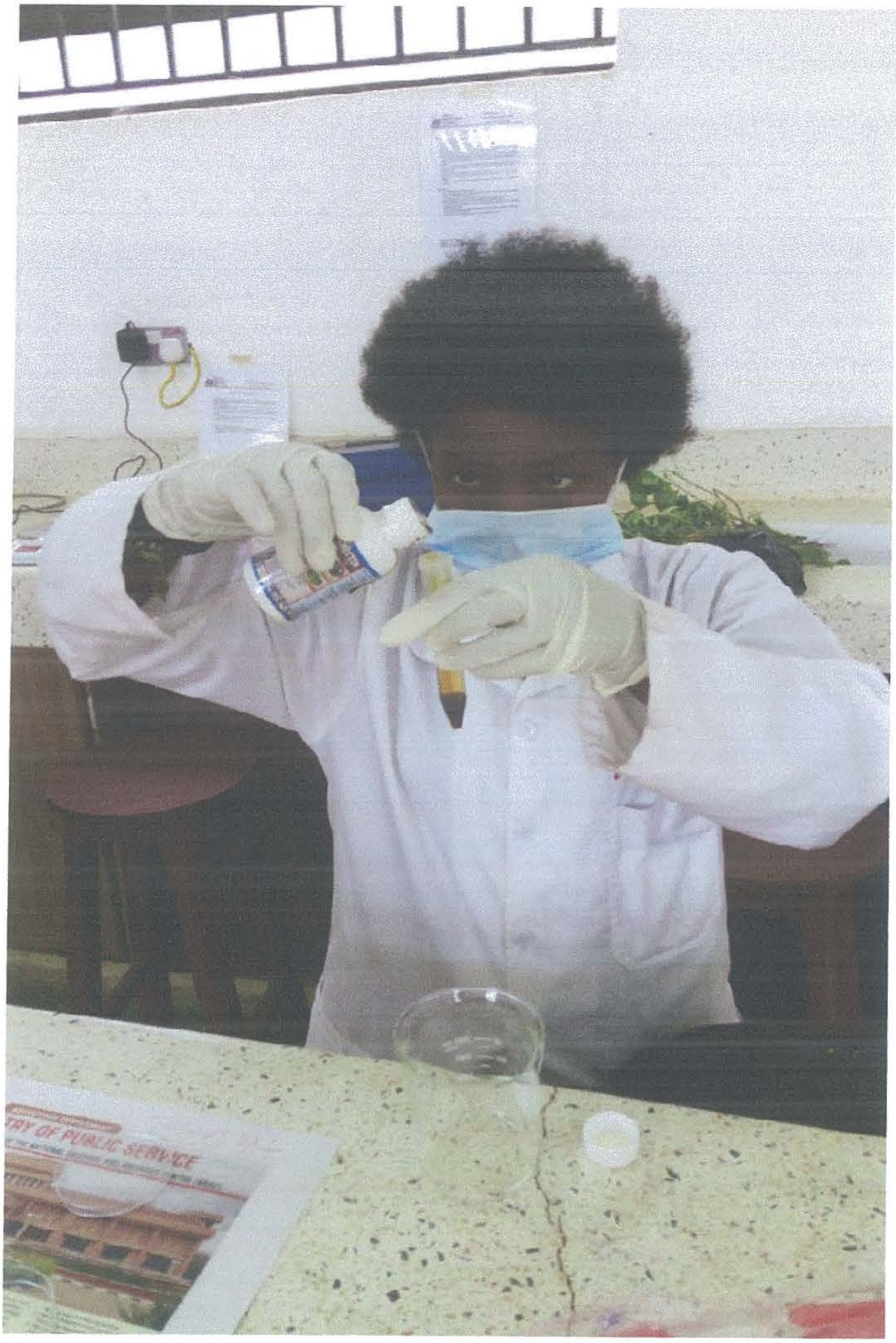
APPENDIX IV : PICTURE OF Cassia didymobotrya

APPENDIX III: MAP OF ISHAKA SHOWING KIU-WC



APPENDIX V: PICTURE SHOWING *Myzus persicae* ON STEM

APPENDIX VI: RESEARCHER RECONSTITUTING POSITIVE CONTOL(DUDU®)



APENDIX VII: RESEARCHER PLYING Cassia didymobotrya ASH ON Myzus Persicae