EVALUATION OF GARLIC OIL EXTRACT AS POTENTIAL PYRETHRUM SYNERGIST AGAINST SIGNIFICANT POST HARVEST PESTS

BY

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DECLARATION

Declaration by the student

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DEDICATION

This thesis is especially dedicated to my parents Mr. and Mrs. Peter Lutta for standing with me spiritually, emotionally and materially throughout my education journey to MSc. level. I also dedicate to my family, brothers and sister your inspiration made it possible for me.

ABSTRACT

Post-harvest grain loss remains a major challenge facing farmers especially in developing countries. Pyrethrin is natural plant oil that forms the active ingredients of conventional insecticides combined with piperonyl butoxide as synergist. Garlic bulb is a plant that has been studied for various pharmacological properties. A study was done to evaluate the ability of natural *Allium* oil as a potential pyrethrin synergist against *Sitophilus zeamias* and *Prostephanus truncatus* significant post-harvest maize pests. The treatment solutions for bioassay tests was mixture of garlic oil extracted using hexane solvent and pyrethrin oil. The experimental design used for bioassay test was completely randomized design and was done in triplicate. The analysis of garlic oil extract using Fourier transform infrared spectroscopy confirmed the presence of amide, lipids and aromatic compounds linked with insecticidal activity The treatments used for bioassay were solutions containing pyrethrin with garlic oil in a ratio of 1:10 selected after preliminary tests. The highest concentration ratio of pyrethrin to garlic oil was 20 mg/ml: 200 mg/ml while the lowest was 14 mg/ml: 140 mg/ml. The insect's mortality rates were determined after 24, 48 and 72 hours intervals. There was significant change in percentage mortality rates of the insects with increase in concentration of test solutions and exposure time after the treatments. The treatments were found to be more potent on *Prostephanus truncates* compared to *Sitophilus zeamais*. The result showed piperonyl butoxide as the most effective pyrethrin synergist with mean mortality of 90% compared to garlic oil of equivalent concentration which caused 50% mortality. The standard convectional insecticide used 20 mg/ml actellic super dust recorded the highest mean mortality for both insects' species of 100% and 93%.The non-synergized pyrethrum containing 20 mg/ml pyrethrin only had a mean mortality of 29% which was lower compared to some treatments with less concentration of pyrethrin but synergized with garlic oil. The results were analysed using R CRAN software and value $p \leq 0.050$ was considered to be statistically significant at 95% confidence limit. The parameters used to evaluate efficacy were mortality rates relative to concentrations of the test solution and insect's exposure time. The study revealed that garlic oil can enhance the efficacy of pyrethrin against significant post-harvest pests. This can provide a benign solution to challenges associated with the current convectional pesticides. Garlic oil as a natural synergist may open prospect for farmers to start cultivating the plant as an insecticide cash crop.

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LIST OF ABBREVIATIONS

%	- Percentage
ANOVA	- Analysis of variance
CRD	- Complete Randomized Design
ESTs	- Esterases
$FeCl_3$	- Ferric Chloride
FTIR	- Fourier transform infrared spectroscopy
GABA	- Gamma-aminobutyric acid
Gm	- Gram
GSTs	- Glutathione-S-transferases
Hcl	- Hydrochloric Acid
KALRO	- Kenya Agriculture and Livestock Research Organization
LC_{50}	- Lethal concentration of 50% Mortality
MFOs	- Mixed function oxidases
Mg	- Milligram
Ml	- Milliliter
Pan	- Pannar
PBO	- Piperonyl butoxide
ppb	-Parts Per Billion
WHO	- World Health Organization

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CHAPTER ONE
INTRODUCTION

1.1 Background of study

Pyrethrum is a solvent extract from *Chrysanthemum cinerariafolium* with pyrethrin as an active ingredient. Pyrethrin consists of pyrethrin I ($C_{21}H_{28}O_3$), pyrethrin II ($C_{21}H_{28}O_5$), jasmolin I ($C_{21}H_{28}O_3$), jasmolin II ($C_{21}H_{30}O_3$), cinerin I ($C_{20}H_{28}O_3$), and cinerin I ($C_{20}H_{28}O_5$) esters which have insecticidal activities (Freemont *et al.,* 2016) . Maize weevil (*Sitophilus zeamias)* pest is one major threat of food insecurity in Africa due to post harvest damages it causes mainly to stored grain (Bhusal & Khanal, 2019). The larger grain borer is one of the most damaging pest that affects maize and other cereal crops common in the tropical region(Ognakossan *et al.,* 2013).

Garlic (*Allium sativum)* is a medicinal plant with insecticidal activity, its essential oil and powder have been applied in different parts of the world (Yang *et al* ., 2009) and (Plata-Rueda, *et al.,* 2017). Despite limitation due to lack of standards, natural botanical insecticides have shown potential of overcoming pesticide resistance by insects (Siegwart *et al*.,2015). A study conducted on insecticidal activity of some plants' powder against maize weevil (*Sitophilus zeamias*) revealed reduction on number of eggs laid depending with the concentrations of the treatments (Mahmoud & Zedan, 2018).

Maize is major staple food and is one of the leading cereal crop that is cultivated worldwide (Awika, 2011). Maize remains the main source of carbohydrates energy giving food which are obtained as calories directly from the grain or indirectly through its processed products (Slavin & Carlson, 2014). A large amount of maize produced globally is utilized for both human and animal consumption but in the United States of America more than one third of production is utilized in production of biofuel (Ranum *et al*.,2014)**.** The destruction of crops and stored grains by pests is a threat to food security and economic stability globally. The damages affect both the quantity and quality of harvested grains and consequently their products (Mutungi *et al.,* 2019).

The use of chemically synthesized pesticide has been linked with the rapid development of resistant strains in pest control and also environmental degradation due to pollution. This

has left the use of botanical insecticide as an alternative solution to these effects (Mkenda *et al.,* 2015). A recent research work revealed that continuous application of synthetic pesticides results in the decrease of earthworm (*Eudrilus eugeniae*) population which plays a significant role in improving soil fertility (Tiwari *et al.,* 2019).Some studies have shown correlation between decrease in soil quality and use of non-biodegradable pesticides as they inhibit enzymes that increase soil fertility (Campos *et al*., 2019).

The predominant insecticide resistance mechanisms are metabolic-based resistance and target site insensitivity. Metabolic resistance is associated with monooxygenase (MFOs), esterase (ESTs), and glutathione S-transferases (GSTs*)* (Zoh *et al.,* 2018).The target site insensitivity is due to alteration on genetic sequence and occurs in voltage-gated sodium channels, gamma-aminobutyric acid (GABA) receptors or acethylcholinesterases target site (Rivero *et al.,* 2010).

Pyrethrin induces toxic affect to insects by penetrating the cuticle and binding to sodium channels along the nerve cells. This leads to hyperexcitation of the nerve cell and death of the target pest as a result of shutting down of the central nervous system (Duke *et al.,* 2010). Synergists like piperonyl butoxide (PBO) are added to pyrethrin to inhibit activities of detoxification enzyme found in pests thus increasing the efficacy by enabling pyrethrin applied to reach its insecticidal potency. They also reduce the quantity of pyrethrin in the formulation of insecticides making them to be cost effective (Joffe *et al.,* 2015)**.**

The plant kingdom remains the most significant producers of bioorganic molecules that are utilized in defense mechanism against pests (Pino *et al.,* 2013).The botanical repellents have being traditionally used in house hold for many years and the practice has still being applied in some parts of the world as a preventive measure against insect pest has (Maia & Moore, 2011). Botanical plants insecticidal activities researched for several years have shown promising results as substitute to the current conventional pesticide (Mkindi *et al.,* 2017).

Goat weed *(Ageratum conyzoidesL*), a natural herb has been studied for pharmacological properties applicable to both animal and plants. The same herb has also been found to

possess potency against several insects including some post-harvest pest (Rioba & Stevenson, 2017). Safrole is the main bioactive molecule in sassafras found in *Sassafras albiduna* a plant member of *Lauraceae* family. It has numerous pharmacological properties and is a precursor of piperonyl butoxide that is added to pyrethrum as a synergist (Dewick, 2009).

The plant pesticides are easily biodegraded by microbes available in the ecosystem, this reduces environment pollutants as well as maintains biodiversity that has been affected by toxicity due to continuous usage of conventional pesticides (Dhir, 2016). Plant oil from genus *Ostericum* showed insect repellent properties when they were subjected to bioassay test against maize weevil (*Sitophilus zeamias*) and the red flour beetle *(Tribolium castaneum)* at different rates of application depending on concentration of test solution (Liu *et al.,* 2011).

The practice of using plant secondary metabolites extracts as preventive measures against pests began more than 3000 years ago (Pavela, 2016). Botanical pesticide products are currently being applied in the management of insects and diseases in agricultural sector (Abdelladi & Hartbauer, 2020).

1.2 Statement of problem

The damage of stored grains by pests remains one of the foremost contributors of post-harvest waste and the main cause of food shortage for small scale farmers in economically developing nations (Manandhar *et al.,* 2018). Maize weevil *Sitophilus zeamias* is one of the major pests that contribute to post harvest losses as a result of infesting stored cereals and destroying the grains (Nwosu,2018).

Research has also shown that metabolic products of maize weevil increases moisture content on the surface of grains which facilitates growth of *Aspergillus flavus* and production of a cancer agent aflatoxin moulds (Bhusa & Khanal,2019). Since its introduction from central America to West and East Africa before spreading to other countries in the African continent, the larger grain borer (*Prostephanus truncatus*) has become one of the most destructive pests that affects maize and other crops like cassava (Sosef *et al.,* 2017).

The use of pyrethroids in public health and agricultural sectors has led to rapid evolution of pyrethrum resistance reducing its effectiveness in management of insect pests (Ranson *et al.,* 2009).The other limitations associated with synthetic insecticides are high production cost, toxicity to non-target organism and environmental pollution due to their non-biodegradable nature (Donia & Alqasoumi, 2012).

This has made researchers to seek for pesticides that are effective ,environmental friendly and affordable as substitutes to current conventional ones that have been used in pest management (Stevenson *et al.,* 2017). Botanical insecticides have the potential of replacing synthetic pesticides in management of most insect pests because they are specific on target, biodegradable and are non-toxic to mammals (Ponsankar *et al.,* 2016)

Though pyrethrin is natural insecticide is used with piperonyl butoxide a synthetic synergist as pesticides limiting its applications in organic farming as PPO is non-biodegradable property and toxicity to non-target organisms (Jansen *et al.,* 2010).Thus they is a need to find a botanical synergist that will be non-toxic and biodegradable that will also increase the efficacy of pyrethrin.

Since the maize weevil and the larger grain borer are storage pest while pyrethrin toxic dose in insect has low toxicity to humans compared to PBO, thus garlic oil as an alternative will improve the efficacy of pyrethrin and make them less toxic green pesticide.

1.3 Justification

Biochemical components found in plant extracts have shown the potential of producing affordable natural insecticides with low toxic levels towards non target animals (Abreu-Villaça &Levin, 2017).The need for using botanical pesticides in farming is indispensable due to the rising cases of organic farming and degradation of the environment as a result of persistent use of synthetic insecticides in pest control (Pretali *et al*., 2016). Secondary metabolites like tannic acid present in garlic oil can be used to overcome photo instability of pyrethrin (Wanyika *et al.,* 2009). Plant oils as a natural synergist have been found to enhance the efficacy of pyrethrin against maize weevil (*Sitophilus zeamias*) (Kaguchia *et al*., 2018).

Garlic (*Allium sativum)* as an effective synergist will restore the confidence of farmers in using pyrethrum insecticides against maize weevil and the larger grain borer which had declined by changing the attitude of farmers towards conventional pesticides. This will also provide an alternative green pesticide that is affordable, biodegradable with minimum toxic effect to non–target organisms thus offering a solution to some challenges associated with current synthetic PBO use as pyrethrum synergist.

Garlic oil as an effective synergist will also result in the improvement on the quality of stored grains, increase food security and sustain economic stability in cereal growing regions. The outcome of the potential of garlic (*Allium sativum)* as an effective synergist of pyrethrum may also open employment opportunities for farmers to start cultivating the plant for commercial purposes which will have an economic impact in the lives of farmers and also stabilize the pyrethrum industry.

1.4 Study Objectives

1.4.1 General Objective

The general objective of this study was to investigate the effect of combining pyrethrin with garlic oil extract on insecticidal activity against the maize weevil (*Sitophilus zeamias)* and the larger grain borer (*Prostephanus truncatus*).

1.4.2 Specific objectives

1. To determine the phytochemical properties of pyrethrin combined garlic oil.
2. To determine *in vivo*, the capability of garlic oil to synergize pyrethrum against maize weevil (*Sitophilus zeamias)* and larger grain borer (*Prostephanus truncatus)*.
3. To compare the efficacies of pyrethrin combined with garlic oil with conventional Insecticide (actellic dust and pyrethrum).

1.5 Hypotheses

H_{a1}: Pyrethrin combined with garlic oil exhibits phytochemical properties.

H_{a2}: Garlic oil is capable of enhancing the efficacy of pyrethrin against maize weevil (*Sitophilus zeamias)* and grain borer (*Prostephanus truncatus)*.

H_{a3}: The efficacy of pyrethrin combined with garlic oil against maize weevil (*Sitophilus zeamias)* and the larger grain borer (*Prostephanus truncatus)* is higher compared to actellic dust and pyrethrum synergized pesticides.

CHAPTER TWO
LITERATURE REVIEW

2.1 Pyrethrum

Pyrethrum is a solvent extract from *Chrysanthemum cinerariafolium* with pyrethrin as an active ingredient. Pyrethrin consists of pyrethrin I ($C_{21}H_{28}O_3$), pyrethrin II ($C_{21}H_{28}O_5$),

jasmolin I ($C_{21}H_{28}O_3$), jasmolin II ($C_{21}H_{30}O_3$), cinerin I ($C_{20}H_{28}O_3$), and cinerin I ($C_{20}H_{28}O_5$) esters that have insecticidal ability towards several insect pests (Kramp, 2010). Globally the main supply of pyrethrum in 1945 was Tasmania (Australia) that controlled over 50% of all supply but as time went they were overtaken by Kenya and Tanzania as world leading producers (Grdiša *et al.,* 2009).

Pyrethrum was cultivated in Japan in Tamari and Uyema as insecticides as early as 1880s (Matsuo,2019).Further research in early 1900's resulted in pyrethrum being used as a domestic pesticide and for production of pyrethroid (Fox *et al.,* 2008). Pyrethrum potent knockdown effects on target insects (Barnes, 2010).

Natural pyrethrin is preferred in pesticides formulations due to its broad spectrum effect on pests, low toxicity to non-target insects and low effective dose (Shawkat *et al.,* 2011). The time taken for original amount of pyrethrin to decompose by half is less than two hours, this makes it to be easily susceptible to natural biodegradation with no complexity and is therefore environmentally friendly (Antonious, 2004).

The toxicity of pyrethrin varies with the grade, pure form are slightly more toxic compared to the technical grade (El-Wakeil, 2013).The "shelf life" of pyrethrin is 2-5 years (Chrustek *et al.,* 2018) though a study on vegetable oil from plants like *Azadirachta indica A. Juss (neem tree), Thevetia peruviana* (yellow oleander) and *Gossypium hirsutum L.* (cotton) seeds confirmed they contain secondary metabolites like tannins that stabilize pyrethrin (Wanyika *et al.,* 2009).

2.2 Pyrethroids

Pyrethroids are synthetic insecticides with various structures which were designed from the natural pyrethrin. Pyrethroids unlike natural pyrethrin are affordable, photo stable and have greater insectidal bioactivity, but the major challenge is that they have been unified with the rapid evolution of insect resistance (Saha & Kaviraj, 2008). The toxicity due to pyrethroid towards mammals organs varies with generation where Type II for instance permethrin are more toxic than Type I the first ones like bioallethrin (Rehman *et al.,* 2014).

The three forms of pyrethroids which been found to be toxic to human being aredeltamethrin, permethrin and α-cypermethrin (Chrustek *et al.,*2018). Pyrethroids have also been found to

affect non target organisms, this includes high degree of toxicity to bees and towards aquatic life (Dubey *et al.,* 2010). Cyhalothrine, a type II used widely in pest control in agricultural industry was found to be detrimental to the renal and cerebral systems when tested in mice (Pawar *et al*., 2017). The contamination in aquatic ecosystem due to pyrethroid has been studied with aid biomarkers (Kaviraj & Gupta, 2014).

Recent studies on pesticide toxicology shows association between neurological disorders and pyrethroid (Mohammadi *et al.,* 2019). A research conducted recently showed the ability of using microbial biodegradation in eradication of pyrethroid accumulated in the aquatic environment (Bhatt *et al.*, 2019).

2.2.1 Mode of action of pyrethrum and pyrethroids

Pyrethrum and pyrethroids are both neurotoxin that affect peripheral cells of the central nervous systems by altering the voltage gated sodium channels. Their absorption results in continuous opening of voltage gated sodium channels in insect cell due to change in the membrane potential (Prusty *et al.*,2015). The amplitude of sodium ion current has been generated and are retained leading to continuous firing along the axons.

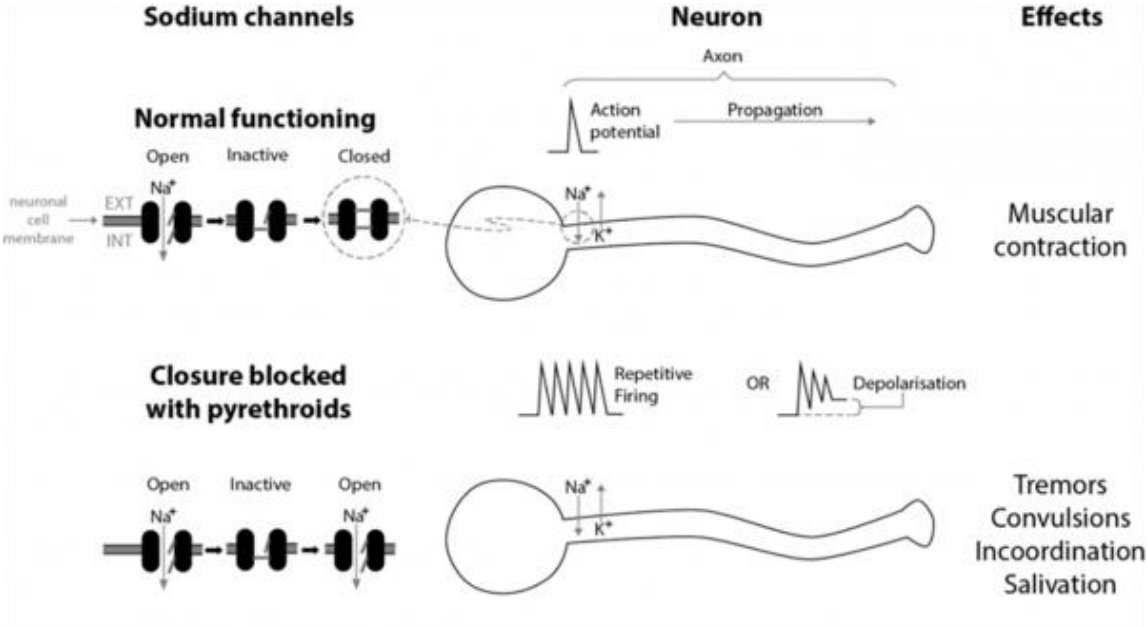

Figure 2.2: Illustration for the mechanism of action of pyrethrum and pyrethroid (Hénault-Ethier, 2016).

This results to restlessness, hyperactivity and paralysis of the nervous system leading to death of the insect (Chrustek *et al*., 2018).

2.3 Phytochemicals

Phytochemicals are biomolecules that are predominantly produced from plants and have numerous bioactivity including pharmacological properties (Nadia & Silva, 2018). The secondary metabolites form the biomolecular basis of medicinal properties of plants and have been successfully studied for insecticidal activities (Abbas *et al.*, 2012).

They are various class of phytochemicals which are distinguish base on chemical component present and structure like nitrogen and sulphur components among others referred us secondary metabolite (Yoo *et al*., 2018). Most of them contain heterocyclic bases examples are flavonoids and terpenoids which have been found to have diverse pharmacological properties (Achilonu *et al*., 2015).

Different solvents are used to separate biomolecules from plants, qualitative analysis is mainly done by identification where chemical reagent are added to phytochemicals react with biochemical components to produce characteristic colours due to formation of a complexes (Awaad *et al.*, 2017).

2.3.1Essential oils

Essential oils are variety of organic compounds containing fatty acids which are produced by botanical plants. They have exhibited insecticidal activity by varying mode of actions that have high efficacy with minimum toxicity towards non target organisms (Pavela & Benelli, 2016). The essential oils derived from *Lippia javanica spreng* have been studied for insecticidal bioactivity and found to be effective in control of *Sitophilus zeamais* (Kamanula *et al.*, 2017).

Though not all essential oils are bioactive some including eucalyptus, rosemary, among others have been successfully tested for pharmacological activity against storage grain pest (Hikal *et al.,* 2017). Some of the essential oils have also been successfully studied for anti-microbial properties as virucidals and parasiticidal (Bakkali *et al.,* 2008).

The essential oils of *Dysphania ambrosioides* were found to suppress the activities of *Alphitobius diaperinus* a beetle that affects birds by subjecting them to stress and facilitating the dispersal of lethal microbes in poultry (Arena *et al.,* 2018). Some aromatic plants oil produce biological molecules which can control insect pests by altering cellular physiological processes, cell development and behavioural modification which includes interfering with mating of insects (Sarwar, 2015).The plants essential oils as biopesticides can be prospective source of environmental friendly effective green pesticides (Mossa, 2016).

2.3.1 Mode of action of essential oil

The essential oils work by altering normal metabolism, physiological and behaviour processes in insects. They are inhaled, ingested or adsorbed when they come in contact with insect's tissue. Though the mechanisms of action of essential oils have not been well studied they have been found to affect various cellular processes including alteration of biomembrane permeability which interferes with mobility of metabolites involved in cellular transport system (Tripathi *et al.*, 2016).

Other plant essentials oil components have been found to retard growth and development of tissues in insects at distinct stage*s* (Dambolena *et al.,* 2016). Some have been assayed for insect potency and repellant activities with results demonstrating they are effective on some insects species at diverse concentrations (Zibaee & Khorram, 2015).

2.4 Garlic *(Allium sativum L.*)

Garlic plants are naturally grown in various parts of the world preferably in regions with mild climate. The bulb of the garlic plant has been discovered to have strong insecticidal activity. The essential oil and powder of garlic plant are applied in pest management in various parts of the global (Yang *et al.,* 2009). Allicin is the predominant active biochemical ingredient present in the extract of garlic plant (Shang *et al.,* 2019*).* Other

components include propenylallylthiosulfonate, ally methyl thiodulfonate, ajoene and y-L-glutamyl-s-alkyl –L-cysteine (Bayan *et al.,* 2014).

Garlic bulb biochemical ingredients have been investigated and found to have wide range of medicinal value with minimum side effects (Mikail *etal.,* 2010). Garlic extract in aqueous mixture of humic acids has been positively assayed for insects repellency a technique applied in pest management practices (Knueppel *et al.,* 2015). *Allium sativum* (Alliaceae) powder recorded highest mortality rate when tested along plant extracts against red flour beetle, *Tribolium castaneum* which attacks stored cereal grains (Ahmad *et al.,* 2019).

2.4.1 Allicin

Garlic cloves remains odour-free until they are crushed or injured and this activates enzyme allinase which acts on allin to release allicin unstable biomolecules with characteristic pungent smell (Omar & Al-Wabel, 2010). The biochemical pathway for this process involves conversion of allin to allicin, a reaction which occurs within seconds and is catalyzed by enzyme allinase found abundantly in garlic cloves (Prati *et al.,* 2014). Allicin has been investigated and discovered to possess antioxidant property in diverse studies (Bayan *et al.,* 2014) and (Elosta *et al*.,2017).

Allicin has also been found to inhibit various metabolic activities within cells in both simple and complex organisms. They have been established to possess antimicrobial properties against some significant drug resistant microbes (Borlinghaus *et al.,* 2014). A study on immunological properties of allicin shows itcan complement body immune system by amplifying phagocytosis and the activities natural of killer cells (El-Saber *et al* ., 2020).

They have also been found to inhibit growth of micro-organisms and cancerous cells (Arreola *et al.,* 2015). Naturally, the general biochemical composition of garlic bulbs is allin 1.7% and allinase 2.8% of dry weight but stress or injury to the plant tissues immensely elevates these amounts (Lawson & Hunsaker, 2018). A study performed on biochemical properties of allicin revealed that it is easily and safely absorbed by adults

stem cells due to its high rate of permeability across membrane without affecting the lipid bilayer forming acetone, ally methyl sulfide alongside other metabolites (Chan *et al.,* 2013).

2.4.2 Garlic oil

Majority of biomolecules that are present in garlic oil in high concentrations are sulphur base compounds that have been found to possess multiple biological activities related to medicine and health. The study on bioactivity of garlic oil against mealworm beetle (*Tenebrio molitor*) at high dose showed that it interferes with mobility of the insects due to paralysis as a result of induced muscle disorders (Plata-Rueda *et al.,* 2017).

The extract of garlic (*Allium sativum* Linn.) bulbs was successfully studied for repellency against tick *Hyalomma rufipes* (Acari: Ixodidae) (Nchu *et al.,* 2016)**.**The efficacy of garlic oil combined with mint oils were assayed and found to be effective against the black cutworm *Agrotis ipsilon* at all stages of the insect development (Sharaby & El-nujiban, 2015).

2.5 Post harvest pests

The two common significant post-harvest grains cereal pests are Maize weevil *(Sitophilus zeamias)* and the larger grain borer *(Prostephanus truncatus).* These two insect pests have also be found to attack crops while still in the field apart from the destructive effects they cause on stored grains (Borgemeister *e t al.,* 2003).

2.5.1 Maize weevil

The maize weevil (*Sitophilus zeamias*) is a small weevil that belongs to order *Coleoptera* Family:*Curculionidae.* The body length size of mature insects grows up to 4.5mm and the width 1.5 mm (Serna-Saldivar, 2012).One of the unique features of the *Sitophilus zeamias* are the oval spots red, brown or yellow found in the forewings that are referred to as elytra. They are tough, situated along the abdomen and their main function is to protect the hind wings(Tefera *et al.,* 2010).

The female can lay up to 400 eggs in lifetime inside grain kernel which takes up to sixty days to hatch depending on prevailing environmental conditions. A minimum of thirty days is required for the eggs to transit to adult insect (Yaseen *et al.,* 2019).

Sitophilus zeamais remains a serious threat to food security in the developing countries and has significantly affected grains stored by small scale farmers (Dari *et al.,* 2010).This has greatly devastated the farmers economically most of who depend on maize farming as source of income and food.

The main method of managing *Sitophilus Zeamias* is by use of synthetic insecticides which are limited due to their contribution towards insect resistance and their toxicity towards non-target organism (Li *et al.*,2010). A study conducted on the aptitude of botanical powders extracted using different solutions to repel the maize weevil, *Sitophilus zeamais* on sorghum cereal grains showed variation on the decrease of repellency activities with time based on the polarity of the extract (Suleiman *et al.*,2018).

Another research done on similar species with essential oils from *Citrus aurantiifolia* and *Citrus recticulata* revealed that the insecticidal activity of the oils significantly vary depending on the types of enantiomers where *(R)*-limonene were found to be more potent relative to the *(S)*-limonene (Fouad &da Camara, 2017). One of the current development in pest management technique involves the use of radiography to detect grains infested by weevils though is expensive, it can be applied to monitor the extent of damage on the grains by insects (França-Silva *et al.*, 2019).

2.5.2 The larger grain borer (*Prostephanus truncatus)*

The larger grain borer (*Prostephanus truncatus)* belongs to order *Celeoptera* and family *Bestrchidae*. The general size of the body length of the adult is between 2.0 - 3.5 mm and width is 1.0 -1.5mm (Tefera, *et al.,* 2010). The female lays eggs in stored grains which under favourable environmental conditions takes from 24 days to develop to adults which have a lifespan of up to 100 days (Suma & Russo, 2005).

Prostephanus truncatus originated from American continent and was introduced in the of Africa continent through imported maize that came from Mexico to Tanzania in 1970s. This later spread to other parts of East Africa,causing serious post-harvest damages to maize and some non-cereal crops like cassava (Gueye *et al.,* 2008).

A study on social hormonal response of *Prostephanus truncatus* shows they use pheromones to spread and adapt in an habitat, thus the pheromones can be integrated with traps and applied in pest control methods (Jian,2019). Propolis, a compound with diverse medicinal value produced by bees and some plant extract has exhibited potential to abate damages on cereal grains by *Prostephanus truncatus* (Ete, 2018). The effect of post-harvest grain pests are overwhelming affecting both on quantity and quality of stored grains with their related products (Bakoye *et al.,* 2017).

2.6 Common methods of pest management

Due to the challenges associated with pest, scientists have come out with various techniques applied in pest control which have both disadvantages and advantages. They include cultural practices that are based on decreasing the intensity of pest in infestation by observing proper hygiene within grain stores. This involves cleaning, destroying by burning previously infested products and sorting to ensure grains that are free from pest are stored. Other cultural practices involve timely harvest to ensure quality of grains are maintained by preventing pests from attacking crops when they are still in the field (Kumar & Kalita, 2017). The cultural practices are effective preventive techniques though they are labour intensive thus become uneconomical when applied on large scale.

Biological control is a method that involves the use of other organisms to hinder the growth, development and multiplication of pests due to nutrients competition. This can be applied in pest control and management including in grain pests. The Combination of a fungus *entomopathogenic fungus* and *larval parasitoids* can be used as a biological agent in management of maize weevil in granary (Batta & Kavallieratos, 2018) . Main challenge of biological control is that the method is not viable on large scale application.

The adult insects can be selectively removed from grains using sieve of specific mesh size, this reduces the population of some pests though the method is tedious and not very effective. The grains can also be mixed with inert dry solid fine particles which control the population of insect pests as they reduce the amount of moisture in grains leading to death of adults pests for example, diatomaceous earth for the management of stored-product pests (Shah & Khan, 2014).The limitation of this technique is that the inert material might affect

the hygiene of the grains and also will require extra work to be removed from the grain before being used.

Insect pests can also be managed by controlling optimum climatic conditions that favour their multiplication, this involves reducing aerobic conditions and temperatures required for optimum growth of pests (Boardman *et al.,* 2011).Though this method can be effected by altering various environmental conditions challenges, are fluctuations of those parameters may affect the efficiency of this method. Another setback of this technique is that even at ideal handling conditions, it is not possible to eliminate the storage grain insect pest absolutely (Schmidt *et al*., 2018).

Planting genetically modified seeds though not common, can be applied as an effective method for pests control. This can be achieved by using varieties whose genomes have been genetically transformed from conventional types to resist specific insects pests (Dara, 2019). The method has been successfully applied on some crops magnificently for example, conventional cotton was genetically modified using microbe gene from *Bacillus thuringiensis* to resist bollworm *(Leptidoptera)* that was causing severe obliteration to the plant (Subramanian & Qaim, 2010). Though debate on genetically modified plant is still going on due to the risk of some organisms applied in biotechnology and fear of unknown long term effects, it remains a reliable technique for future application in pest control that will ensure food security (Carvalho, 2017).

Currently, air tight bags, a non-chemical technique is the most current method that has been adapted in control of post-harvest grain pests and kills insect by suffocation .The main challenge, however, is that it has been associated with growth of aflatoxin in the stored grains. This does not only contaminate farm produce but also aflatoxin mould has been proved to be a cancer agent when levels exceed 10ppb in grains and their products (Baoua *et al.,* 2014). The airtight bags have also been found to be easily perforated by some post-harvest insects as they mature reducing their effectiveness in pest control (De Groote *et al*., 2013).

While developing countries are seeking for alternative safe, effective and affordable insect pest control methods ,western countries are designing safe but exclusively complex methods based on altered extreme conditions which include use of radiations and electric

heaters among others (Mohapatra *et al.,* 2015). Secondary metabolites found in natural plants can be isolated easily in stable forms and have shown the potential of being used as botanical pesticides (Hikal *et al.,* 2017).

The application of nanoparticles and encapsulation techniques in pharmaceutical products have the potential of enhancing biopesiticide stability, efficacy and at the same time minimize toxicity of the active compounds to non-target organisms (Damalas & Koutroubas, 2018). The integration of nanotubes on insect protective food package was found to prolong the efficacy of clove oil against *Plodia interpunctella* commonly referred to as Indian meal moth (Kim *et al.,* 2019).The use of natural pesticide when optimized will offer solution to the limitations associated with the current conventional methods that are used in pest control.

2.7 Insect rearing

Insect rearing plays a significant role in bioassay test as it ensures the insects used are more or less of similar age, size and biological traits thus ensuring precision on the studies undertaken. The environmental conditions in the rearing container should be favourable for optimum growth of insects and at the same time prevent foreign ones from entering the colony (Cohen, 2018). Proper sanitation during rearing process is maintained by applying different aseptic techniques. Records of insect sources, rearing room conditions, grain source and colonies should be well kept (Sorensen *et al.,* 2012).The preferable moisture content of maize for insect rearing ranges is between 12-14% which can be achieved by moistening with water if it is low or drying at moderate temperatures of between 40 - 70°C in cases where the values are higher than recommended ones (Machingura, 2014).

2.8 Insecticide resistance

Insect resistance can be described as an adapted biochemical, physiological and behavioural change that reduces the sensitivity of a given species to an insecticide. It is manifested by persistent failure of an insecticide to achieve the expected level of efficacy when it is applied according to recommended toxic dose (Kaur & Garg, 2014). Pesticide resistance primarily is due to misuse of pesticide, lack of survey in monitoring and not alternating different types of chemicals ingredients used in formulation of insecticides

(Miller *et al*., 2010). The general outcome of resistance is that the targeted pests develop some defense mechanisms against insecticides that were previously lethal towards them (Hawkins *et al.,* 2019).The development of pesticide resistance has been primarily associated with the continuous use of chemicals synthesized insecticides in the control and management of arthropod pests (AL-Ahmadi, 2019).

2. 9 Synergists and synergism

Synergists are compounds which have low or are non-toxic effect to insect on their own but when used in combination with an insecticide they enhance the efficacy of the pesticide. Synergists work by inhibiting activities of metabolism resistance of enzymes and this ensures that pesticides achieve maximum response on target pest (Snoeck *et al.,* 2017). They are also applied in determination of specific resistance mechanism as they can selectively inhibit some specific metabolic pathways (Pasay *et al.,* 2009).

The phytochemical biomolecules can be used to enhance insecticide response and specificity to target site, with less toxic effects to environment (Rattan, 2010). Synergic effect of ethyl acetate extract of *Piper nigrum* L. (Black pepper) on pyrethrum against *Drosophila melanogaster* has been investigated at molecular level (Jensen *et al.,* 2006).

2.9.1 Piperonyl butoxide

Piperonyl butoxide (PBO) is commonly used as a synergist in pyrethrin formulation to enhance its insect's potency. It not only increases the efficacy but also reduces the amount of pyrethrin in the formulation of insecticide making it to be more cost effective (Gleave *et al.,*2017). Piperonyl butoxide acts by inhibiting biodegradation of pyrethrin and this ensures the desired amount of the original compound reaches and bind the target site in the pest (Mikaili *et al.,* 2013).

Piperonyl butoxide enhances the toxicity of pyrethrin either directly or as a synergist. This can be determined from the differences between lethal concentrations of piperonyl butoxide and pyrethrin that kills 50% (LC_{50}) of the target pest (Schleier & Peterson, 2010). The effective concentration ratio of pyrethrum with piperonyl butoxide(PBO) has been found to be 1:10 in a research that was conducted against maize weevil (Biebel *et al.,* 2003).

CHAPTER THREE
MATERIALS AND METHODOLOGY

3.1 Materials

3.1.1 Samples collection and identification

The garlic oil was obtained from the bulbs purchased from main market in Eldoret town in Uasin Gishu County as a bulb. Pyrethrum product 0.2% pyrethrin oil dissolved in extract dissolved in ethanol was obtained from pyrethrum board of Kenya and used as stock solution. Garlic bulbs were identified in biological department herbarium section of University of Eldoret. The culture for breeding insect specimen for Maize weevils *(Sitophilus zeamias)* were obtained from infested maize H614 while the larger grain borer *(Prostephanus truncatus)* from PAN 691 from local farmers which had been planted for domestic use and had no history of treatment with convectional insecticide prior to the study. The two insect specimens were identified in Zoology section in the department of biological science at University of Eldoret. The maize used for bioassay test was H6213 hybrid obtained from university of Eldoret farm department.

3.1.2 Experimental sites

The experiment was performed in University of Eldoret located in Eldoret town Uasin Gishu County located in the former expansive Rift valley province. The county is one of the leading maize producing region in the country contributing over 10% of the national input (Kamau *etal.,* 2014). The region has an area coverage of 3,345.2 km^2 and lies between longitude 34° 50’ East and 35 ° 37’ West and latitude of 0 °C 03’ South and 0 °C 55’ North (Akenga *et al.,* 2017).

The town is centre of commercial, educational, administrative, industrial and agricultural activities in Uasin Gishu County. The neighbouring counties to Uasin Gishu are Elgeiyo-Marakwet and Baringo to the East, Trans-Nzoia to the north, Kakamega in the West while Kericho to the south and Nandi South-west (Sorre *et al.,* 2013).

Uasin Gishu County is a water catchment region with an altitude range of between 1,250–1,850m (Gichuru *et al.,* 2019). The average annual rainfall is between 900 and 1200 mm with pattern varying across the year (Kibet *et al.,* 2011).

The research studies were conducted in laboratories of university of Eldoret in Uasin Gishu County between 2018 November and May 2019. The University is located in Chepkoilel area along Ziwa road which is around 13km from Eldoret town. The average day temperature of Eldoret town is about 24 °C while night can be as low as 10°C (Aura *et al.,* 2010).

3.1.3 Laboratory chemicals

The chemicals used for this study were of high purity analar grade which were obtained from the University of Eldoret Department of Chemistry and Biochemistry.

3.2. Methodology

3.2.1 Extraction of oil from the plant

The extraction of the garlic oil was done at department of Chemistry and Biochemistry research laboratory at University of Eldoret and performed as previously described (Tagoe *et al.,* 2010) and (Ali & Ibrahim, 2019) with few modifications, where 2.5 kg of fresh garlic bulb from Eldoret main market were used after the outer dry layers were peeled off and the remaining part cut into small pieces and air dried naturally in a room away from direct sunlight for two weeks . This was followed by grinding using a blender to obtain powder form, sieving and weighing.

The dry powder was transferred into 1 liter brown bottle and soaked with *n*-hexane for 3days at 25 °C and filtered using Whatman no.1filter paper. The filtrate was collected and concentrated using rotary evaporator under vacuum at 50 °C to remove *n*-hexane. The bulb was dried to remove aqueous solvent to increase the efficiency of *n*- hexane as a polar solvent in extraction of the garlic oil.

The percentage of oil extracted was calculated using the formular below;

Weight of Garlic bulb sample	***2.5 kg***
Weight of weighing bottle	0. 0502 kg
Weight of weighing bottle + oil extract	0.06052 kg
Weight of oil extract	0.01032 kg

$$Oil\ content(\%) = \frac{Weight\ of\ oil}{Weight\ of\ sample} \times 100$$

$$\frac{0.01032}{2.5} kg \times 100g = 0.4128\%$$

The remaining oil extract was weighed, and stored at 4 °C in a refrigerator awaiting further analysis.

3.2.2 Determination of physical properties of garlic oil

The physical properties of the oil extract was done as outlined by Rafe (2014).The colour of the extracted garlic oil was determined from observation while the odour detected from the characteristic smell, both experiments were repeated with tests independent from the ones previously done. The density of oil extract was determined by weighing a sample of known volume of the extract in a weighing bottle of known mass using an analytical balance and recorded. The mass of oil was calculated from the difference between mass of weighing bottle with garlic oil extract and that of empty weighing bottle .The density was calculated using the as shown below;

Weight of empty bottle (W_X)	*20 g*
Weight of empty bottle + 5 ml of oil extract (W_y)	24.5 g
Volume of equal of garlic (V_o) =5 ml	5ml

$$Density = \frac{Mass}{Volume} = \frac{W_y - W_x}{V_0}$$

$$\frac{(24.51 - 20.00)g}{5\ ml} = 0.902 g/ml$$

The refractive index of garlic was determined at 25°C using refractometer. The solubility of garlic oil in water was determine by weighing 0.5gm of extracted oil, dissolving in 1ml of distill water and observed if they were miscible

The garlic oil extracted was further assayed for biochemical composition by determining the presence of functional groups associated with insecticidal activity using Fourier transform infrared spectroscopy (FTIR). The analysis was performed as previously done by Kannan *et al.,* (2014) and Raju *et al .,* (2016) with few modifications using 1ml of garlic oil which was placed in infrared transparent glass sample cell and placed in the sample holder for analysis in FTIR spectroscope (Shimadzu, Japan) that had a scan range from 400 to 4800 cm^{-1} with a resolution of $cm^{-1.}$

3.2.3 Preparation of test solution

Pyrethrin oil was prepared from 0.2% pyrethrin oil extract dissolved in acetone and the standard pyrethrum for this experiment was prepared using pyrethrin oil and synthetic piperonyl butoxide ($C_{19}H_{30}O_5$) a methylenedioxyphenolic organic compound. The ratio of concentration of pyrethrin to garlic extract mixtures were prepared in a ratio of 1:10 test using acetone as a solvent. The concentration of prepared pyrethrin were 14 mg/ml, 16 mg/ml, 18 mg/ml and 20 mg/ml while that for garlic oil extract were 140 mg/ml, 160 mg/ml, 180 mg/ml and 200 mg/ml respectively. The two prepared solutions were mixed in ratio of 1:1 to make test solution and stored in 250 ml amber bottles. The optimum concentration ratios for the study were chosen from preliminary tests done using various concentration ranges of treatments containing pyrethrin: garlic oil between 2:20mg/ml to 25:250 mg/ml. The standard pyrethrum for this experiment was prepared by mixing equal volumes of 20 mg/ml pyrethrin oil and 200 mg/ml piperonyl butoxide while 20 mg/ml actellic super dust (0.3% permethrinand1.6% pirimiphos methyl) a pyrethroid served as standard for conventional insecticide. The test solutions were stored in a refrigerator at 4 °C after preparation and later assayed for insecticidal properties (Rowshan *et al.,*2013).

3.2.4 Phytochemical screening

Phytochemical screening of the test solution was done as explained by Manouze *et al.*, (2017) and Patil & Deshmukh,(2016) using standard procedures that are generally

applicable for natural plants extracts. The test was done parallel with a test tube containing phytochemical test reagents alone as a blank for comparative analysis. Tannins were determined by measuring 2 ml of extracts stock test solution and stirring in 3 ml of distilled water. This was followed by addition of drops of 5% $FeCl_3$ solution. The formation of a green precipitate indicated the presence of tannins. Saponins were determined by measuring 5ml of extracts test solution, shaking in 5 ml of distilled water in a test tube and warming, formation of stable foam was taken as a positive test for the saponins.

Test for flavonoid was performed by measuring 2ml of extracts of test solution and1ml of 10 % lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids. Anthraquinones were analysed by measuring 2 ml of test solution and boiling with 10% hydrochloric acid for 10 minutes on water bath, filtered and cooled. Same volume of chloroform was added to the filtrate followed by 3 ml of 10 % ammonia. The mixture was then heated and lack of formation of pink-rose colour was taken as negative test for anthraquinones derivatives (Gollo *et al.*,2020).

Test for terpenoids was done by measuring 2 ml of extracts test solution and dissolving in 2 ml of chloroform followed by evaporation to dryness.2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicated the presence of terpenoids. Tests for steroids were done by measuring 2 ml of extracts of test solutions , dissolving in 2 ml of chloroform and 2 ml concentrated sulphuric acid. Red colour produced on lower layer indicated the presence of steroids.

Alkaloids test was done by measuring 3 ml of extract test solution and stirring with 3 ml of 1% HCl on a steam bath. This was followed by addition of Mayer's (a solution of mercuric chloride and potassium iodide) and Wagner's reagents (solution of iodine in potassium iodide) formation of turbidity by precipitate was taken as positive test for alkaloids. Tests for glycosides were done by measuring 2 ml of extracts of test solutions, dissolving in 2 ml of chloroform and 2 ml ethanoic acid. Formation of blue greenish colour was taken as a positive test for glycosides (Akhtar *et al.*,2018).

3.2.5 Insect rearing

Mass rearing for both insects was done as described by Hategekimana *et al.,*(2017) and Athanassiou *et al.,* (2017) with some few modifications. The rearing was done in the department of biological science incubation room for 30 days using clean and disinfected plastic bottle. The day temperature ranges of the room during rearing period were between 25-29 °C, relative humidity of between 55-62 % and predominantly 12:12 light: dark photo period interval.

The insects with infested maize were separated from the whole consignment. The remaining grains were sorted, disinfested in oven for 1 hour at 60°Cand allowed to cool in desiccators. A 1.5kg of disinfested maize were weighed differently and transferred into two separate 2l disinfected plastic jars each of diameter 15cm and height 25cm.The lids for rearing jars had four small holes of diameter 2 cm with muslin cloth and were placed on top glass containers smeared with tangle foot oil to prevent foreign insects and mites from entering into the insect's colony.

A 100 pairs insects obtained from infested maize of each species were introduced separately in the two jars and left for two weeks. The adult weevils were removed from rearing jars after laying eggs by sieving using clean 5mm sieve that retained the grains containing the eggs as the adult insects passed down .The grains containing eggs were reintroduced back into the rearing plastic bottle container as second generation and left for 30 days for the eggs to develop producing new generation of adults.

3.2.6 Bioassay test

This was done as illustrated by Mulungu *et al.,* (2011) and Suleiman *et al.,* (2012) at University of Eldoret Biotechnology laboratory. The moisture content for maize H6213 hybrid use for bioassay was determined by weighing 20 g five samples from the maize consignment and transferring them into clean labelled crucibles dishes which had been dried in an oven set at 105 °C for 1hr, cooled and weighed.

The samples in crucibles were placed in oven set at 105 °C for 8 hrs, removed, cooled in the desiccators for 1hr and re-weighed. The percentage moisture content was calculated using the following formular.

The percentage of moisture contents of maize samples for bioassay test were calculated using the formular below;

$$Moisture\ content\ (M.C)\% = \frac{(Weight\ loss\ in\ sample\ taken)}{initial\ weight\ od\ sample} g \times 100$$

$$Average\ \%\ moisture\ contents = \frac{13.0 + 13.0 + 12.9 + 12.9 + 12.8}{5} = 12.90\%$$

The maize was disinfested further by storing in freezer at -20°C in a freezer for two weeks. They were removed on the same day the bioassay tests were performed and dried in oven at 70°C for 1hr and cooled in desiccators before being used.

The maize containing reared insects were poured into two combined sieve of pore sizes 5 mm and 1mm. Maize seed were retained on top, most insects on the second sieve while other smaller objects including some smaller insects passed through both sieves to collecting container. The insects that were retained on sieve of pore size 1mm were taken to be of the same size and used for bioassay test. For both insects the weight of one specimen could not be detected using 0.001 gm sensitive analytical balance and thus they were taken to be of negligible weight.

The experimental layout used for bioassay test was Complete Randomized Design (CRD) where treatments were placed in test tubes covered with aluminum foil supported by test tube rack while plastic container containing insects for bioassay test were arranged in a tray before the experiment. The experimental procedures involved were, 20 gm of the maize seeds which were weighed and transferred into 250 ml small plastic jar of diameter 7.5cm and height 15cm.The lid had four holes each having a diameter of 1cm and muslin cloth to ensure steady supply of air in the containers during the experiment. A 4 ml of each the test solution treatment was added into the 250 ml plastic jar mixed 20 gm of maize grain and left for 30 minutes for acetone to evaporate. The experiments were done in triplicate.

10 unsexed pairs of insects were randomly selected after sieving and transferred into each of the 250 ml plastic container containing maize treated with test solution and covered. The

plastic containers used for bioassay test had been previously assigned numerical codes based on concentration of treatment solutions. They were rearranged randomly after the substrate, treatment solutions and insects had been placed in the plastic containers on the bench waiting determination of mortality rate based on the experiment time intervals, and this was done to avoid predetermination of the result by assumption.

The experiment was performed at day temperature of between 25°C and 27°C, relative humidity of 63-69% and predominantly 12Light:12 Darkness hour's period. The insects were considered dead when they could not move their body or legs when touched with a fine thin brush while observing under magnifying lens. The dead insects were removed from the bioassay container after counting. The mortality rates were determined at time intervals of 24, 48 and 72 hrs. The parameters used to evaluate efficacy of treatment solutions were concentrations, exposure time and mortality rate.

3.2.7 Mortality assays

The number of dead insects in each plastic container was counted at 24, 48 and 72hrs intervals. The mortality was calculated as a percentage using the following formular;

$$\boldsymbol{Insect\ mortality\ rate} = \frac{\boldsymbol{number of\ dead\ insects}}{\boldsymbol{total\ number\ of\ insects}} \times \mathbf{100}$$

Since no mortality occurred in the untreated sample which was the control for the experiment, Abbot's formular shown below for corrected mortality (%) percentage was not applied.

Abbot's formula

$$\boldsymbol{Pr} = \frac{\boldsymbol{Po - Pc}}{\mathbf{100 - Pc}}$$

Where, $\mathbf{P_T}$**,** is the Corrected mortality (%); $\mathbf{P_O}$, is the observed mortality (%); $\mathbf{P_C}$, is the control mortality (Paramasivam & Selvi, 2017).

3.3 Data analysis

The data were analysed using R CRAN (comprehensive r archive network) software tool for statistical analysis. The one way analysis of variance (ANOVA) analysis were used to determine the relationships between mortalities rate, concentrations of test solutions and exposure time using Duncan's test. The percentage mortalities of test solutions were determined and the lethal concentration (LC_{50}) values for test solutions that killed both insects were obtained. The value $p \leq 0.05$ was considered to be statistically significant during data analysis.

CHAPTER FOUR

RESULTS

4.1 Physical properties

The % mean moisture content of maize used for bioassay was **12.90 %**

Table 4.1: Garlic oil extract

The percentage of garlic extracted was 0.4128 % and had the following physicochemical properties;

Property	Result
Density	0.902 g/ml
Refractive Index	1.450
Melting Point	12-13°C
Colour	Yellowish-green
Odour	Strong pungent
Solubility	Insoluble in water

Table 4.2: Table 4.2: FT-IR frequency range and functional groups

The following IR frequency range of were used in characterization and identification of functional groups with insecticidal activities present in garlic oil extract;

Peak no	*Wave number in cm^{-}*	*Functional group*	*Comment*
1	2916.42	C-H stretching Alkane	predominant for lipids
2	2848.91	C-H stretching Alkane	Predominantly for lipids
3	1735.96	C=O stretching Amide	mainly amide bond for protein
4	1462.07	C-H bending Alkane	CH_2 bending for lipids
5	1172.74	S=O stretching Sulfonate	presence of sulfur organic compound
6	719.46	C=C bending/aromatic Alkenes	presence of lipids

Table 4.3: Table 4.3: Phytochemical tests

The qualitative analysis was done for the test solution containing garlic oil with pyrethrin and following results obtained;

Phytochemicals	***Results***
Tannins	+
Saponins	+
Flavoniods	+
Anthraquinones	-
Terpenoids	+
Alkaloids	+
Steroids	+
Glycosides	+
+ Present	**- Absent**

Data analysis

Table 4. 4: Table 4.4: Maize weevil *(Sitophilus zeamias)* variation of % mortality with time.

The table below the variation of % percentage mortality rate of *Sitophilus zeamia*s with varying concentration of tests solutions and exposure time;

Treatment ***(concentration in mg/ml*) p ≤ 0.050**	***Variation in Mean % mortality with time***			
	24 hrs	**48 hrs**	**72 hrs**	**groups**
20 mg/ml actellic	83.33	96.67	100.00	a
20 mg/ml + piperonyl butoxide 200 mg/ml	73.33	96.67	100.00	a
pyrethrin 20 mg/ml+ garlic oil 200 mg/ml	43.33	50.00	56.67	b
pyrethrin 18mg/ml + garlic oil 180 mg/ml	43.33	50.00	53.33	b
pyrethrin 16mg/ml + garlic oil 160 mg//ml	26.67	33.33	36.67	c
pyrethrin 14 mg/ml+ garlic oil 140 mg/ml	23.33	33.33	36.67	c
pyrethrin 20 mg/ml	20.00	26.67	40.00	c
acetone(blank)	0.00	0.00	3.33	d
Untreated	00.00	00.00	00.00	d

Table 4.5: Mean % mortality of maize weevil (*Sitophilus zeamias*) at varying treatments

The table below the variation of % percentage mortality rate of *Sitophilus zeamia*s with varying concentration of treatments.

Groups **p ≤ 0.050**	***% Mortality***	***Groups***
20 mg/ml actellic dust	93.33	a
pyrethrin 20 mg/ml+ piperonyl butoxide 200 mg/ml	90.00	a
pyrethrin 20 mg/ml+ garlic oil 200 mg/ml	50.00	b
pyrethrin 18 mg/ml+ garlic oil 180 mg/ml	48.89	b
pyrethrin 16 mg/ml + garlic oil 160 mg/ml	32.22	c
pyrethrin 14 mg/ml + garlic oil 140 mg/ml	31.11	c
pyrethrin 20 mg/ml	28.89	c
acetone(blank)	1.11	d
Untreated	0.00	d

Table 4.6: Table 4.6: Mean % mortality of maize weevil *(Sitophilus zeamias)* at varying time of exposure.

The variation of % mortality of maize weevil (*Sitophilus zeamias)* with treatments at different exposure times are shown below:

Time **p ≤ 0.050**	***% Mortality***	***groups***
Dead after 24 hrs	34.81	a
Dead after 48 hrs	42.96	b
Dead after 72 hrs	47.41	c

Table 4.7: The Larger grain borer (Prostephanus truncatus) variation of % mortality with time

The table shows below the variation of % percentage mortality rate of Prostephanus truncatus with varying concentration of tests solutions and exposure time;

Treatment(concentration in mg/ml)	**Variation in Mean % mortality with time $p \leq 0.050$**			
	24 hrs	**48 hrs**	**72hrs**	**Groups**
20 mg/ml actellic dust	100.00	100.00	100.00	A
pyrethrin 20 mg/ml + piperonyl butoxide 200 mg/ml	93.33	100.00	100.00	A
pyrethrin 20 mg/ml + garlic oil 200mg/ml	53.33	60.00	66.67	B
pyrethrin 18 mg/ml + garlic oil 180mg/ml	43.33	60.00	63.33	B
pyrethrin 16 mg/ml + garlic oil 160 mg/ml	36.67	46.67	53.33	C
pyrethrin 14 mg/ml + garlic oil 140mg/ml	33.33	43.33	46.67	C
pyrethrin 20 mg/ml	26.67	43.33	53.33	C
acetone(blank)	0.00	3.33	6.67	D
Untreated	0.00	0.00	0.00	D

Table 4.8: Mean of % mortality of larger grain borer (Prostephanus truncatus) at varying treatments

The table shows below the variation of % percentage mortality rate of *Prostephanus truncatus* with varying concentration of tests solutions (treatments).

$Groups **p ≤ 0.050**	***% Mortality***	***Groups***
20 mg/ml actellic dust	100.00	a
pyrethrin 20 mg/ml + piperonyl butoxide 200 mg/ml	97.78	a
pyrethrin 20 mg/ml + garlic oil 200 mg/ml	60.00	b
pyrethrin 18 mg/ml + garlic oil 180 mg/ml	55.56	b
pyrethrin 16 mg/ml + garlic oil 160 mg/ml	45.56	c
pyrethrin 14 mg/ml + garlic oil 140 mg/ml	41.12	c
pyrethrin 20 mg/ml	41.11	c
acetone(blank)	3.33	d
Untreated	0.00	d

Table 4.9: Mean % mortality of the large grain borer (Prostephanus truncatus) at varying time of exposure

The variation of % mortality of *Prostephanus truncatus* with treatments at different exposure times are shown below:

Time **p ≤ 0.050**	***% Mortality***	***Groups***
dead after 24 hrs	43.00	A
dead after 48 hrs	50.74	B
dead after 72 hrs	54.44	C

CHAPTER SIX

DISCUSSION

5.1.1 Physical properties

The percentage of oil extracted from the sample was 0.4128 % (Table 4.1).The results for density, colour ,odour (Tables 4.2 and table 4.3) and refractive index of the extract showed consistent to the findings on work done by Rafe, (2014) making the properties of the garlic oil extract appropriate to be used for the study. The results for the percentage moisture content of maize samples used for bioassay test (appendix III) were found to be within the recommended ranges that are suitable for insect rearing (Machingura, 2014).

5.1.2 Fourier transform infrared spectroscopy (FT-IR) analysis

The results from Fourier transform infrared spectroscopy(FT-IR) were correlated with standard wave numbers from Sigma aldrich IR Spectrum Table and Coates, (2006).The infra-red(IR) spectrum (appendix VI) had some frequency range with close to those obtained by Nagarajan & Ramesh,(2017) from ethanolic extract of garlic powder. The interpreted results for FT-IR spectrum (Table 4.2) show presence of aromatic compounds, methylene, amides, sulphonates and other hydrocarbons functional groups associated with plants essential oils.

The functional groups that were established to be present in the IR spectrum of garlic oil have been verified to exhibit bioactivities related pharmaceutical properties (Chowański *et al.*, 2016). Sulphur compounds which are vital for the bioactivity in garlic oil are represented by S=O stretch in the FTIR spectrum agreeing with studies of Satyal *et al*., (2017). Sulphur also plays a significant role in the development and sustaining of metabolites including cofactors that are involved in enzymatic biochemical reactions in the living cells (Dunbar *et al*., 2017).

The hydrocarbons in the FTIR spectrum can be interrelated to essentials oils which contain terpenes that have been found to impede bioactivities in insects (Beg, 2017).The insecticidal ability of monoterpenes studied against *Sitophilus oryzae* revealed they inhibit the actions of acetycholinesterase and adenosine triphosphatases (Mona *et al.,* 2018).The

essential oils have also been found to repel, distract desire for food and interfere with reproduction in insects (Brari & Kumar, 2019).

5.1.3 Phytochemical Analysis

The test solution for phytochemical screening contained equivalent amount of pyrethrin with garlic oil extract, the results show some connection to the findings of Mikail, (2010) and Pandey *et al*., (2018). The phytochemical results (Table 4.3) confirmed the presences of specific secondary metabolites like alkaloids, steroids, glycosides etc. in the test solution that have been ascertained to possess insecticidal activities. The pyrethrin with garlic oil which was used to prepare treatment solutions tested positive for tannins, saponins, flavonoids, steroids, terpenes, glycosides, steroids and alkaloids compounds. This also corresponds to the work of Rajam & Saranya, (2013) and Singh & Kumar, (2017) on the whole plant extracts of *Allium Sativum.*

The insecticidal activity of the treatment solutions may have been due to presence of phytochemicals which that has been confirmed in various studies. A study on garlic plant extract revealed that they possess broad spectrum pharmacological activities that act by binding cell receptors and altering biochemical pathways of several metabolic functions in the living cells (Wink, 2015).

A study conducted using flavan glycoside, a secondary metabolite produced by plant for defense mechanism shows it inhibits digestive enzymes and discourage feeding leading to retarded growth on insects (War *et al.,* 2012).This concurs with a study done on some stored products pests that showed that phytochemicals impede feeding of insects (Nawrot & Harmantha, 2012). Saponins have also been found to halt growth and moulting in insects due to their biochemical interaction with cholesterol that down regulates synthesis of steroids (Ikbal, 2010).

Other plants secondary metabolites like alkaloids have also been found to act as inhibitors for the biosynthesis of steroid hormones which play a significant role in biochemical processes in insects (Ileke *et al* ., 2016). Several studies on phytochemicals have produced promising results that they can be applied in insecticide formulation and having potential

of being utilized as a source of lead molecule for designing pesticides (Campos *et al.*, 2019).

5.1.4 Bioassay of treatment solutions against Maize weevils *(Sitophilus zeamias)*

The results for mortality of *Sitophilus zeamias* showed increase in rates of percentage mortality with increasing concentration of treatments solutions at 95% confidence limit. They was also significant variation at between mortality rates between concentrations of treatment solutions and time (Table 4.4).The mean percentage mortalities for varying concentrations of pyrethrin with garlic oil were between 50% and 31%. This outcome shows analogous traits to studies of Vedovatto *et al.,* (2015) and Pangnakorn and Chuenchooklin, (2018).

The pyrethrin with garlic oil mixture that recorded the highest mortality against *Sitophilus zeamias* was 20 mg/ml pyrethrin and 200 mg/ml garlic oil which had mean of 50% for the three replicates. This was followed by pyrethrin 18 mg/ml and 180 mg/ml garlic oil with mean of 49% for the three time intervals. Though the mean values for percentage mortality of 16 mg/ml pyrethrin and 160 mg/ml garlic oil and 14 mg/ml pyrethrin and 140 mg/ml garlic oil recorded lower than the lethal concentration (LC_{50}) with means of 32% and 31% respectively, this values were higher compared to non-synergized pyrethrum that contained 20 mg/ml pyrethrin oil alone and had mean mortality of 29% for three replicates. This agreed with findings on related studies done on pyrethroid by (Norris *et al.,* 2019).

The percentage means mortality of *Sitophilus zeamias* at varying time of death was significantly different. There was increase in rate of percentage mortality with increase of exposure time of the insects towards the treatment solutions (Table 4.6).The mean value for percentage mortality after 72 hours was the highest with 47% while the lowest was 35% after 24 hours .The mean value for percentage mortality after 48 hours was 43%.

5.1.5 Bioassay of treatment solutions against the larger grain borer (*Prostephanus truncatus).*

The bioassay results for *Prostephanus truncatus* showed similar trends with those of *Sitophilus zeamais* of increase in mortality with concentration of treatment solutions and exposure time (Tables 4.4 and 4.7) at 95% confidence limit. The mean percentage mortalities for varying concentration of pyrethrin with garlic oil were between 60% and 41%. The tables also depicted variations in the percentage of mean mortalities with treatments and exposure time.

The treatments were more potent on *Prostephanus truncatus* with treatment containing concentration of 16 mg pyrethrin oil +160 mg garlic oil recording mortality rate of 53.33% after 72hrs compared in contrast to *Sitophilus zeamias* which 36.67 % at the same interval (Tables 4.6 and 4.9). The only treatment that had mortality less than 50% (LC_{50}) for *Prostephanus truncatus* after 72 hour exposure time was 14 mg/ml pyrethrin + 140 mg/ml garlic oil while for *Sitophilus zeamias* were those with concentration less than 16 mg/ml +160 mg/ml.

The mean value for percentage mortality at varying time after 72 hours was the highest with 54% while the lowest was 43 % after 24 hours (Table 4.12).The percentage mortality after 48 hours were significantly different with mean value of 51 %. The difference in mortality rates between the insects species show correlation to work done on the same insect pests when they were tested against pyrethroids (Giga & Canhão, 1991). This may be as a result of species variability like body size which generally *Prostephanus truncatus* are smaller compared *Sitophilus zeamais.* .The discrepancy in mortality rates between the two species may also be attributed to the uniqueness of the cuticles between the two insects which have been established to affect the rates at which treatments penetrate into insect tissues (Tak & Isman, 2015).

5.1.6 Bioassay of conventional insecticides, blank and control against both insect species

The actellic dust which served as standard for conventional grain insecticide and pyrethrum standard (pyrethrin 20 mg/ml + 200 mg/ml piperonyl butoxide) had the highest mean mortalities with both having 100% after 72 hours (Tables 4.4 and 4.7) compared with

treatment with highest concentrations (pyrethrin 20 mg/ml + 200 mg/ml garlic oil) that recorded 56.67 % for *Sitophilus zeamias* and 66.67% mortality during same period at 95% confidence limit. This shows relations to studies done by Mulungu *et al.,* (2011) and Joffe *et al.,* (2015) where both serve as standards.

Acetone which was the solvent for test solution and served as blank for bioassay test had highest mean mortality of 3.3% which occurred in *Prostephanus truncatus*, this may have been caused by natural factors (Ngwej *et al.,* 2019). Since the mortality rate due to acetone in *Prostephanus truncatus* was not significantly different (Table 4.5 and 4.8) with a mean value of less than 5% in all the tests conducted, the values were taken as non-substantial with reference to the experimental data (Denlinger *et al.,* 2015).Since no mortality was observed on the untreated sample which acted as control for both insects Abbott corrected mortality formular was not applied (WHO, 2013).

5.1.7 Efficacy of pyrethrin with garlic oil as biopesticides

This study established that by varying the concentrations of garlic oil extract and pyrethrin oil in the ratio of 1:10 shows significant increase of mortality rates of insect's specimen with concentrations and exposure time at 95% confidence limit. This can be attributed to the inherent properties from the pyrethrin combined with garlic oil extracts. The percentage mean mortality rate after 72 hours for 20 mg/ml pyrethrin, unsynergized pyrethrum was 40% for *Sitophilus zeamias* and 53.33 % for *Prostephanus truncatus* contrast to 20 mg/ml pyrethrin + 200mg/ml garlic oil that had 56.67% and 66.67 respectively at 95% confidence limit.

Some lower concentration of pyrethrin after 72hrs that contain garlic oil extract example 18 mg/ml pyrethrin + garlic oil 180 mg/ml (Table 4.4 and Table 4.7) had higher mortality of 53.33 % for *Sitophilus zeamias* and 63.33% for *Prostephanus truncatus* which were slightly compared to 20 mg/ml unsynergized pyrethrin that had 40% and 53.33 % respectively during same period at 95 % confidence limit. This denotes that some plant oils have insecticidal activity that can augment the efficacy of insecticide which is in accord

without come of study done using some different botanical pesticides on the same species by Mulungu *et al.*, (2007).

The increase in mortality rates of combined pyrethrin with garlic can be attributed to enhanced penetration of insecticides through the cuticles by extracted oils which act as surfactants (Tak & Isman, 2017). This may also due to aromatic compounds that are found in secondary metabolites like flavonoid present in plants that have been confirmed to have synergistic and antagonistic effects (Pavela, 2014).The aromatic compounds in the garlic oil are proven with C-H "oops" strong bonds stretch at 719 cm^{-1} frequency in FT-IR spectrum (Table 4 .2 and appendix 11).

5.1.8 Comparison on Safety of conventional insecticide with pyrethrin with garlic oil

Despite actellic dust and piperonyl butoxide showing superior efficacy, the main short coming associated with their continuous application is that they are non-biodegradable and toxic to non-target organisms affecting ecosystem (Oso, 2015). Piperonyl butoxide has also been found to impede molecular signals and also are associated with retarded development of the animals cells (Wang *et al.,* 2012).

A study conducted on rabbits revealed 20 mg/ml actellic affects metabolism of some important biomolecules found in blood cells (Omoyakhi *etal.*, 2008). The other challenge with actellic dust is that its effectiveness is limited to not more than four months after which it requires to be applied again (Mutambuki *et al,* 2019). Pyrethrum with garlic oil as effective green pesticide can be a solution to most of the challenges facing of conventional pesticides.

Since time immemorial garlic plant has been used as food spice with current studies indicating it is safe and contains medicinal values that have multiple benefits to human cells (Sethi *et al.,* 2014). The concentrations of garlic oil extract used are within the range considered to be safe as with reference to studies done using animal model, a member of *Canis* species found that administering garlic extract dose of 90 mg/kg daily for three months did not manifest any harmful effects on the body tissue (Yamato *et al* ., 2018).

The pyrethrin toxic doses levels for insects cannot have harmful effects on mammals because of their complex metabolic detoxification system (Ensley, 2007).The values of

treatment solutions are safe for use and can also be optimized further when formulating the green pesticide to be more effective.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The FT-IR analysis showed hexane extract of garlic contains functional groups linked to plant essential oils. The results from phytochemicals analysis established pyrethrin combined with garlic oil contain plant secondary metabolites which possess biopesticides activities. The pyrerthrin with garlic oil extracts contains flavonoids, glycosides, alkaloids and terpenoids secondary metabolites that have insecticidal bioactivity. The findings from this study scientifically confirmed that garlic oil has the ability of enhancing the efficacy of pyrethrin oil against *Sitophilus zeamias* and *Prostephanus truncatus.*

The treatments of same concentration were found to be more potent towards *Prostephanus truncatus* compared to *Sitophilus zeamias* on bioassay tests done under same conditions. The unsynergized pyrethrin had lower mortality rate compared with pyrethrin of less concentration combined with garlic oil. The efficacy of actellic dust, a conventional insecticide was superior compared to both pyrethrin combined with garlic oil and pyrethrum standard.

The mortality rates due to standard pyrethrum which contained piperonyl butoxide (PBO) as conventional synergist was higher compared to pyrethrin combined with garlic oil extract. Thus despite its toxicity and non-biodegradable nature PBO still remain the most effective pyrethrum synergist though the sides effects prevail over this.

6.2 Recommendations

1. The findings from this study can further be optimized to lead commercial to production of economically and environmentally friendly natural pyrethrum pesticide that can be applied in other insect pests apart from the two studied.
2. Further research should be carried to ascertain the specific lead molecule in garlic oil with synergistic properties, formulation for the green pesticide with suitable inert material or carrier and compound mode of application.

3. The effects of combining pyrethrin with garlic oil on the activities of metabolic resistance enzymes can be studied to determine the specific biochemical pathways they inhibit.

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APPENDICES

APPENDIX I: LIST OF PLATES

Plate 1: *Chrysanthemum cinerariafolium* plant (Source: Author, 2020)

Plate2: Garlic bulb *(Allium sativum L.*) (Source: Author, 2020)

Plate 3: *Sitophilus Zeamias*- (Source: Author, 2020)

Plate 4: *Prostephanus truncatus* (Source: Author, 2020)

Plate 5: Rearing *Prostephanus truncatus* and S*Sitophilus zeamias*

(Source: Author, 2019)

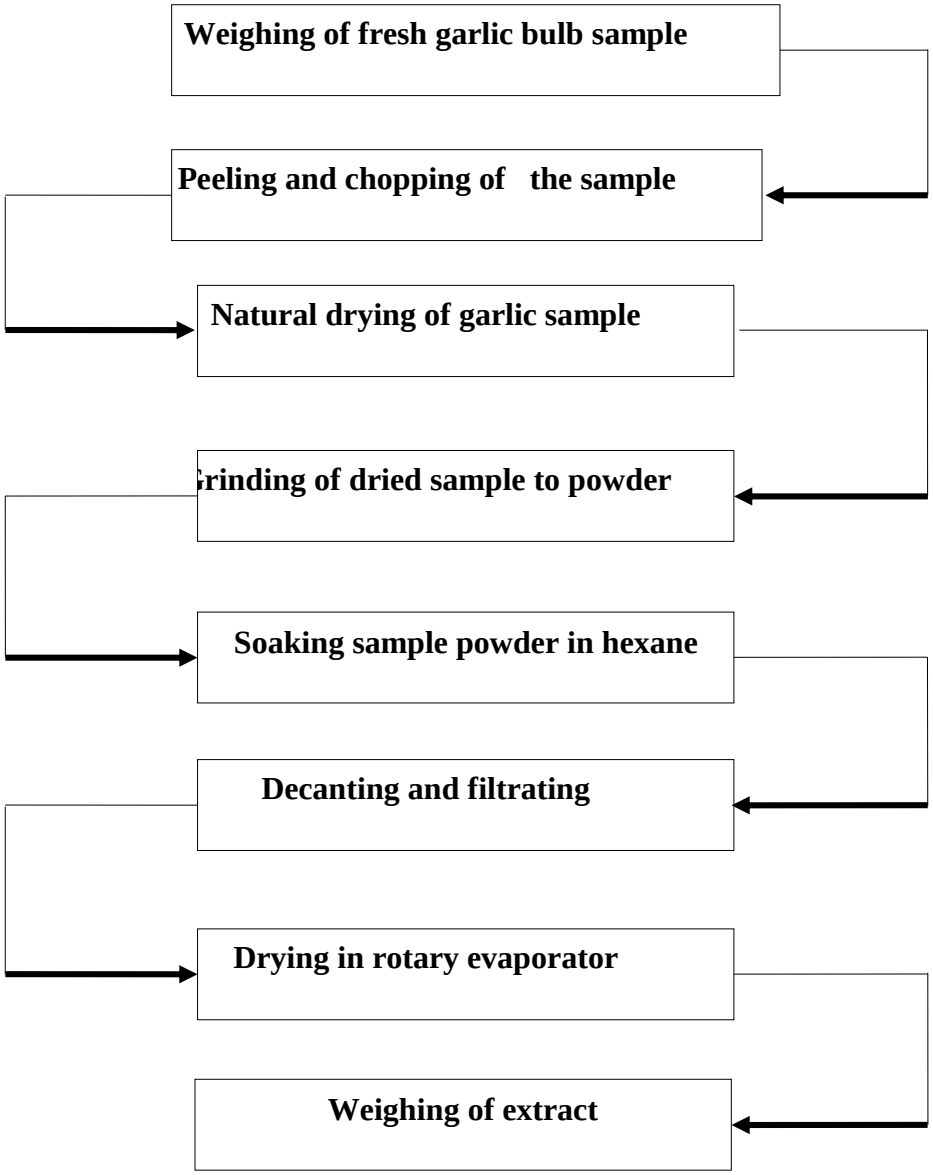
Plate 6: Preparation of materials and solutions for bioassay tests.

(Source: Author, 2019)

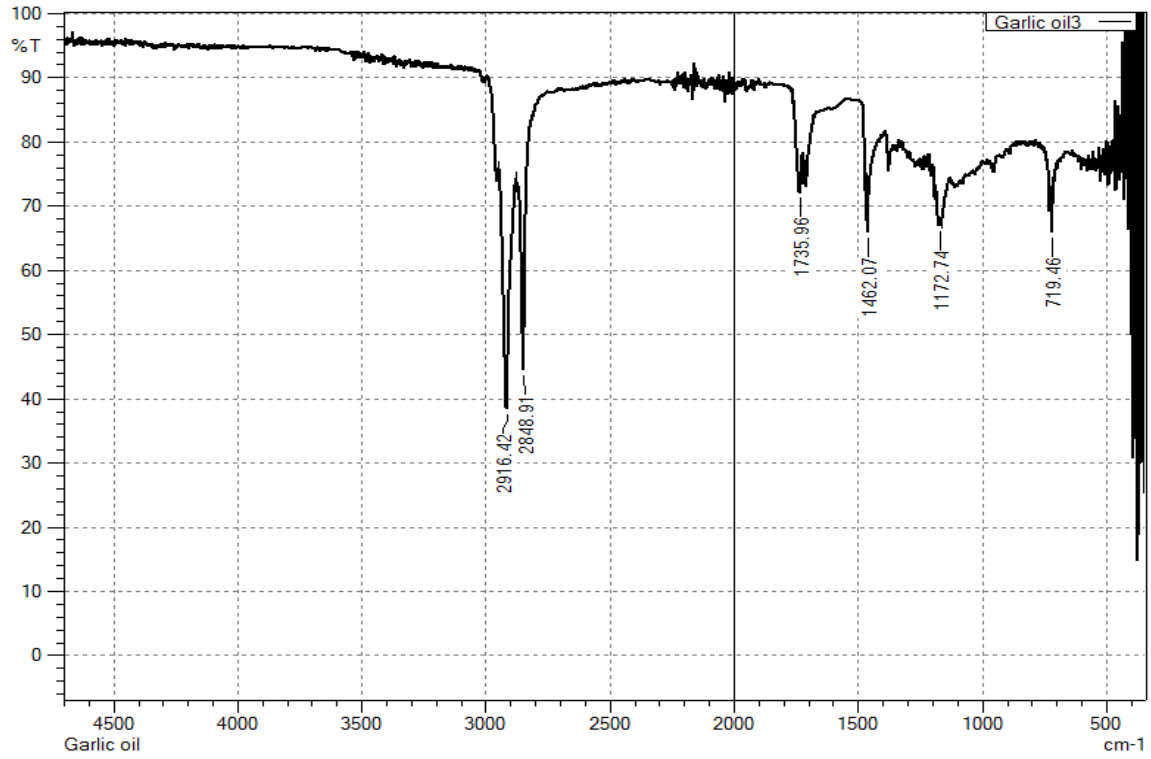
Plate 7: Determination of insect mortality during bioassay tests

(Source: Author, 2020)

APPENDIX II: FLOW DIAGRAM FOR EXTRACTION GARLIC OIL



APPENDIX III: FT-IR SPECTRUM FOR GARLIC OIL EXTRACT



APPENDIX IV: MOISTURE CONTENT FOR MAIZE FOR BIOASSAY TEST

Samples	$W_{1\,(g)}$	$W_{2(g)}$	$W_{3(g)}$	*% M.C*
1	20	17.40	2.60	13.0
2	20	17.40	2.60	13.0
3	20	17.42	2.58	12.9
4	20	17.42	2.58	12.9
5	20	17.44	2.56	12.8

APPENDIX V: PREPARATION OF SOLUTION

Solution	Method used
20mg/ml Actellic (Pirimiphos-methyl 16g/kg +Permethrin 3g/kg)	Prepared by dissolving 2g of actellic dust in acetone to make 100ml solution.
10% ammonia solution	Prepared by dissolving 10ml of ammonia solution in distilled water to make 100ml solution
5% ferric solution	Prepared by dissolving 5g of ferric chloride in distill water to make 100ml solution.
Garlic oil 1gm/l stock solution	This was done by weighing 1g garlic oil and dissolving in acetone to make 1000ml stock solution. Other test solutions were prepared from stock solution using the formular $C_1V_1=C_2V_2$where C_1 =concentration of stock solution, V_1 =volume of stock solution C_2 = concentration of test solution and V_2 =volume of stock solution.
10% Hydrochloric acid solution	Prepared by dissolving 10% concentrated hydrochloric acid in distill water to make 100ml solution.
Meyer solution	Prepared by dissolving 1.35g of mercuric chloride and 50g of potassium iodine in 100ml of water.
Pyrethrin oil 1gm/ l stock solution (1000ppm)	Prepared by weighing 0.5g of pyrethrin oil and dissolving in 500ml of acetone. Other test solutions were prepared from stock solution using the formular $C_1V_1=C_2V_2$where C_1 =concentration of stock solution, V_1 =volume of stock solution C_2 = concentration of test solution andV_2 =volume of stock solution.
Piperonyl butoxide 20mg/ml	Prepared by weighing 1g m of piperonyl butoxide and dissolving in acetone to make 50ml solution.
Wayner reagent	Prepared by weighing 30g grams of potassium iodide and dissolving in distill water to form 50ml solution.

APPENDIX VI: RESULTS OF BIOASSAY TEST FOR *SITOPHILUS ZEAMIAS.*

Code	*Treatment*	*Number exposed*	*24 hr Alive*	*Dead*	*Alive*	*48 hrs Dead*	*72hrs Alive*	*Dead*
M1	Untreated	10	10	0	10	0	10	0
		10	10	0	10	0	10	0
		10	10	0	10	0	10	0
M2	Blank(acetone)	10	10	0	10	0	10	0
		10	10	0	10	0	9	1
		10	10	0	10	0	10	0
M3	20 mg/ml Actellic dust	10	2	8	1	9	0	10
		10	1	9	0	10	0	10
		10	2	8	0	10	0	10
M4	Pyrethrin oil 20 mg/ml	10	8	2	7	3	5	5
		10	9	1	8	2	7	3
		10	7	3	7	3	6	4
M5	Pyrethrin 20mg/ml	10	2	8	0	10	0	10
	Piperonyl butoxide 200	10	3	7	0	10	0	10
	mg/ml	10	3	7	1	9	0	10
M6	Pyrethrin 20mg/ml	10	5	5	4	6	4	6
	Garlic oil 200 mg/ml	10	6	4	6	4	4	6
		10	6	4	5	5	5	5
M7	Pyrethrin 18mg/ml	10	5	5	5	5	4	6
	Garlic oil180 mg/ml	10	6	4	5	5	5	5
		10	6	4	5	5	5	5
M8	Pyrethrin 16 mg/ml	10	7	3	6	4	6	4
	Garlic oil 160 mg/ml	10	8	2	7	3	7	3
		10	7	3	7	3	6	4
M9	Pyrethrin 14 mg/ml	10	8	2	7	3	6	4
	Garlic oil 140 mg/ml	10	7	3	6	4	6	4
		10	8	2	7	3	7	3

APPENDIX VII: BIOASSAY RESULTS FOR THE PROSTEPHANUS *TRUNCATUS*

Bioassay results for the larger grain borer (*Prostephanus truncatus*)

Code	**Treatment**	**Number**	**24hrs**		**48hrs**		**72hrs**	
L1	Untreated	Exposed	Alive	Dead	Alive	Dead	Alive	Dead
		10	10	0	10	0	10	0
		10	10	0	10	0	10	0
		10	10	0	10	0	10	0
L2	acetone(blank)	10	10	0	10	0	10	0
		10	10	0	10	0	9	1
		10	10	0	9	1	9	1
L3	20 mg/ml actellic	10	0	10	0	10	0	10
		10	0	10	0	10	0	10
		10	0	10	0	10	0	10
L4	Pyrethrin oil 20	10	7	3	6	4	5	5
	mg/ml	10	7	3	5	5	4	6
		10	8	2	6	4	5	5
L5	Pyrethrin 20 mg/ml	10	1	9	0	10	0	10
	Piperonyl butoxide	10	0	10	0	10	0	10
	200 mg/ml	10	1	9	0	10	0	10
L6	Pyrethrin 20 mg/ml	10	5	5	4	6	3	7
	Garlic oil 200	10	5	5	5	5	4	6
	mg/ml	10	4	6	3	7	3	7
L7	Pyrethrin 18 mg/ml	10	6	4	3	7	4	6
	Garlic oil 180	10	6	4	5	5	4	6
	mg/ml	10	5	5	4	6	3	7
L8	Pyrethrin 16 mg/ml	10	6	4	5	5	5	5
	Garlic oil 160	10	7	3	6	4	5	5
	mg/ml	10	6	4	5	5	4	6
L9	Pyrethrin 14 mg/ml	10	7	3	6	4	6	4
	Garlic oil 140	10	7	3	6	4	5	5
	mg/ml	10	6	4	5	5	5	5

APPENDIX VIII: DATA ANALYSIS

P ≤0.05

LARGE GRAIN BORER

ANOVA

```
> LARGE.GR.B.CSV_AOV <- aov(% MORTALITY~(TREATMENT+DEAD.OVER.TIME)^2,data = LARGE.GR.B.CSV)
> anova(LARGE.GR.B.CSV_AOV)
Analysis of Variance Table

Response: % MORTALITY
                         Df Sum Sq Mean Sq  F value    Pr(>F)
TREATMENT                 8  87891 10986.4 404.5000 < 2.2e-16 ***
DEAD.OVER.TIME            2   1854   927.2  34.1364 2.611e-10 ***
TREATMENT:DEAD.OVER.TIME 16   1057    66.0   2.4318  0.007769 **
Residuals                54   1467    27.2
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

MODEL TABLES

```
> model.tables (LARGE.GR.B.CSV_AOV,"means",digits=3)
Tables of means
Grand mean

49.38272

TREATMENT
                20mg/ml actellic dust                          acetone (blank)
                               100.00                                     3.33
 Pyrethrin 14mg/ml+Garlic oil 140mg/ml Pyrethrin 16mg/ml+Garlic oil  160mg/ml
                                41.11                                    45.56
 Pyrethrin 18mg/ml+Garlic oil 180mg/ml                       pyrethrin 20mg/ml
                                55.56                                    41.11
  Pyrethrin 20mg/ml+Garlic oil 200mg/ml         Pyrethrin 20mg/ml+PPO 200mg/ml
                                60.00                                    97.78
                             untreated
                                 0.00
DEAD.OVER.TIME
DEAD.OVER.TIME
DEAD  after 24 hrs  DEAD  after 48 hrs  DEAD  after 72 hrs
              42.96               50.74               54.44

 TREATMENT:DEAD.OVER.TIME
                                          DEAD.OVER.TIME
TREATMENT                                  DEAD  after 24 hrs  DEAD  after
48 hrs
  20mg/ml actellic dust                   100.00              100.00
  acetone(blank)                            0.00                3.33
  Pyrethrin 14mg/ml+Garlic oil 140mg/ml    33.33               43.33
  Pyrethrin 16mg/ml+Garlic oil  160mg/ml   36.67               46.67
  Pyrethrin 18mg/ml+Garlic oil 180mg/ml    43.33               60.00
  pyrethrin 20mg/ml                        26.67               43.33
  Pyrethrin 20mg/ml+Garlic oil 200mg/ml     53.33               60.00
  Pyrethrin 20mg/ml+PPO 200mg/ml           93.33              100.00
  untreated                                 0.00                0.00
                                          DEAD.OVER.TIME
TREATMENT                                  DEAD  after 72 hrs
  20mg/ml actellic dust                   100.00
  acetone(blank)                            6.67
  Pyrethrin 14mg/ml+Garlic oil 140mg/ml    46.67
```

```
  Pyrethrin 16mg/ml+Garlic oil  160mg/ml  53.33
  Pyrethrin 18mg/ml+Garlic oil 180mg/ml   63.33
  pyrethrin 20mg/ml                       53.33
  Pyrethrin 20mg/ml+Garlic oil 200mg/ml    66.67
  Pyrethrin 20mg/ml+PPO 200mg/ml         100.00
  untreated                                0.00
```

SUMMARY

```
> tgc_LGRB_1 <- summarySE(LARGE.GR.B.CSV, measurevar="% MORTALITY",
groupvars=c("TREATMENT","DEAD.OVER.TIME"))
> tgc_LGRB_1
```

	TREATMENT	DEAD.OVER.TIME	N	% MORTALITY	sd	se	Ci
1	20mg/ml actellic dust	DEAD after 24 hrs	3	100.000000	0.000000	0.000000	0.00000
2	20mg/ml actellic dust	DEAD after 48 hrs	3	100.000000	0.000000	0.000000	0.00000
3	20mg/ml actellic dust	DEAD after 72 hrs	3	100.000000	0.000000	0.000000	0.00000
4	acetone(blank)	DEAD after 24 hrs	3	0.000000	0.000000	0.000000	0.00000
5	acetone(blank)	DEAD after 48 hrs	3	3.333333	5.773503	3.333333	14.34218
6	acetone(blank)	DEAD after 72 hrs	3	6.666667	5.773503	3.333333	14.34218
7	Pyrethrin 14mg/ml+Garlic oil 140mg/ml	DEAD after 24 hrs	3	33.333333	5.773503	3.333333	14.34218
8	Pyrethrin 14mg/ml+Garlic oil 140mg/ml	DEAD after 48 hrs	3	43.333333	5.773503	3.333333	14.34218
9	Pyrethrin 14mg/ml+Garlic oil 140mg/ml	DEAD after 72 hrs	3	46.666667	5.773503	3.333333	14.34218
10	Pyrethrin 16mg/ml+Garlic oil 160mg/ml	DEAD after 24 hrs	3	36.666667	5.773503	3.333333	14.34218
11	Pyrethrin 16mg/ml+Garlic oil 160mg/ml	DEAD after 48 hrs	3	46.666667	5.773503	3.333333	14.34218
12	Pyrethrin 16mg/ml+Garlic oil 160mg/ml	DEAD after 72 hrs	3	53.333333	5.773503	3.333333	14.34218
13	Pyrethrin 18mg/ml+Garlic oil 180mg/ml	DEAD after 24 hrs	3	43.333333	5.773503	3.333333	14.34218
14	Pyrethrin 18mg/ml+Garlic oil 180mg/ml	DEAD after 48 hrs	3	60.000000	10.000000	5.773503	24.84138
15	Pyrethrin 18mg/ml+Garlic oil 180mg/ml	DEAD after 72 hrs	3	63.333333	5.773503	3.333333	14.34218
16	pyrethrin 20mg/ml	DEAD after 24 hrs	3	26.666667	5.773503	3.333333	14.34218
17	pyrethrin 20mg/ml	DEAD after 48 hrs	3	43.333333	5.773503	3.333333	14.34218
18	pyrethrin 20mg/ml	DEAD after 72 hrs	3	53.333333	5.773503	3.333333	14.34218
19	Pyrethrin 20mg/ml+Garlic oil 200mg/ml	DEAD after 24 hrs	3	53.333333	5.773503	3.333333	14.34218
20	Pyrethrin 20mg/ml+Garlic oil 200mg/ml	DEAD after 48 hrs	3	60.000000	10.000000	5.773503	24.84138
21	Pyrethrin 20mg/ml+Garlic oil 200mg/ml	DEAD after 72 hrs	3	66.666667	5.773503	3.333333	14.34218
22	Pyrethrin 20mg/ml+PPO 200mg/ml	DEAD after 24 hrs	3	93.333333	5.773503	3.333333	14.34218
23	Pyrethrin 20mg/ml+PPO 200mg/ml	DEAD after 48 hrs	3	100.000000	0.000000	0.000000	0.00000
24	Pyrethrin 20mg/ml+PPO 200mg/ml	DEAD after 72 hrs	3	100.000000	0.000000	0.000000	0.00000
25	untreated	DEAD after 24 hrs	3	0.000000	0.000000	0.000000	0.00000
26	untreated	DEAD after 48 hrs	3	0.000000	0.000000	0.000000	0.00000
27	untreated	DEAD after 72 hrs	3	0.000000	0.000000	0.000000	0.00000

DUNCAN'S MULTIPLE RANGE TEST

```
> duncan.test(LARGE.GR.B.CSV_AOV,"% MORTALITY",alpha = 0.05,console =
TRUE)

Study: LARGE.GR.B.CSV_AOV ~ "% MORTALITY"

Duncan's new multiple range test
for % MORTALITY

Mean Square Error:  27.16049
```

```
% MORTALITY,  means
    % MORTALITY std  r Min Max
0                0   0 15   0   0
10              10   0  3  10  10
20              20   NA 1  20  20
30              30   0  5  30  30
40              40   0 11  40  40
50              50   0 15  50  50
60              60   0  8  60  60
70              70   0  5  70  70
90              90   0  2  90  90
100            100   0 16 100 100

Groups according to probability of means differences and alpha level( 0.05 )

Means with the same letter are not significantly different.
```

% MORTALITY	groups
100	A
90	b
70	C
60	d
50	e
40	F
30	G
20	h
10	I
0	J

```
>  out_GR.B_1  <-  duncan.test(LARGE.GR.B.CSV_AOV,"DEAD.OVER.TIME",main ="Maize grain borer varying treatments")
> out_GR.B_1
$statistics
   MSerror Df     Mean       CV
  27.16049 54 49.38272 10.55344

$parameters
    test         name.t ntr alpha
  Duncan DEAD.OVER.TIME   3  0.05

$duncan
     Table CriticalRange
2 2.835327      2.843742
3 2.982372      2.991223

$means
                     % MORTALITY      std  r Min Max Q25 Q50 Q75
DEAD  after 24 hrs      42.96296 34.17318 27   0 100  25  40  55
DEAD  after 48 hrs      50.74074 34.29801 27   0 100  40  50  70
DEAD  after 72 hrs      54.44444 33.66502 27   0 100  45  60  70

$comparison
NULL
```

	% Mortality	groups
DEAD after 72 hrs	54.44444	A
DEAD after 48 hrs	50.74074	B
DEAD after 24 hrs	42.96296	C

```
> out_GR.B_2 <- duncan.test(LARGE.GR.B.CSV_AOV,"TREATMENT",main ="Maize
grain borer varying treatments")
> out_GR.B_2
$statistics
   MSerror Df     Mean       CV
  27.16049 54 49.38272 10.55344

$parameters
    test    name.t ntr alpha
  Duncan TREATMENT   9  0.05

$duncan
     Table CriticalRange
2 2.835327      4.925505
3 2.982372      5.180950
4 3.079201      5.349160
5 3.149503      5.471288
6 3.203559      5.565194
7 3.246726      5.640184
8 3.282135      5.701695
9 3.311764      5.753167

$means
                                        % MORTALITY        std r Min Max Q25 Q50
Q75
20mg/ml actellic dust                 100.000000  0.000000 9 100 100 100 100 100
acetone(blank)                          3.333333  5.000000 9   0  10   0   0  10
Pyrethrin 14mg/ml+Garlic oil 140mg/ml  41.111111  7.817360 9  30  50  40  40  50
Pyrethrin 16mg/ml+Garlic oil  160mg/ml 45.555556  8.819171 9  30  60  40  50  50
Pyrethrin 18mg/ml+Garlic oil 180mg/ml  55.555556 11.303883 9  40  70  50  60  60
pyrethrin 20mg/ml                      41.111111 12.692955 9  20  60  30  40  50
Pyrethrin 20mg/ml+Garlic oil 200mg/ml  60.000000  8.660254 9  50  70  50  60  70
Pyrethrin 20mg/ml+PPO 200mg/ml         97.777778  4.409586 9  90 100 100 100 100
untreated                               0.000000  0.000000 9   0   0   0   0   0

$comparison
NULL
```

$groups	% MORTALITY	groups
20mg/ml actellic dust	100.000000	A
Pyrethrin 20mg/ml+PPO 200mg/ml	97.777778	A
Pyrethrin 20mg/ml+Garlic oil 200mg/ml	60.000000	B
Pyrethrin 18mg/ml+Garlic oil 180mg/ml	55.555556	B
Pyrethrin 16mg/ml+Garlic oil 160mg/ml	45.555556	C
Pyrethrin 14mg/ml+Garlic oil 140mg/ml	41.111111	C
pyrethrin 20mg/ml	41.111111	C
acetone(blank)	3.333333	D
Untreated	0.000000	D

MAIZE WEEVILS

```
> MAIZE.WV..CSV.AOV <- aov(% MORTALITY~(TREATMENT+DEAD.OVER.TIME)^2,data
= MAIZE.WV..CSV)
> anova(MAIZE.WV..CSV.AOV)
Analysis of Variance Table

Response: % MORTALITY
                          Df Sum Sq Mean Sq F value    Pr(>F)
TREATMENT                  8  79847  9980.9  323.38 < 2.2e-16 ***
DEAD.OVER.TIME             2   2202  1101.2   35.68 1.331e-10 ***
TREATMENT:DEAD.OVER.TIME  16   1042    65.1    2.11   0.02136 *
Residuals                 54   1667    30.9
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLES OF SUMMARY OF MEANS OF MAIZE WEEVIL

```
> model.tables(MAIZE.WV..CSV.AOV,"means",digits=3)
Tables of means
Grand mean

41.7284
TREATMENT
TREATMENT
                20mg/ml actellic dust                          acetone(blank)
                                93.33                                    1.11
 Pyrethrin 14mg/ml+Garlic oil 140mg/ml Pyrethrin 16mg/ml+Garlic oil  160mg/ml
                                31.11                                   32.22
 Pyrethrin 18mg/ml+Garlic oil 180mg/ml                      pyrethrin 20mg/ml
                                48.89                                   28.89
 Pyrethrin 20mg/ml+Garlic oil 200mg/ml         Pyrethrin 20mg/ml+PPO 200mg/ml
                                50.00                                   90.00
                             untreated
                                 0.00
DEAD.OVER.TIME
DEAD After 24 hours    DEAD after 48 hrs    DEAD after 72 hrs
             34.81                42.96                47.41
```

TREATMENT:DEAD.OVER.TIME

	DEAD.OVER.TIME		
TREATMENT	DEAD After 24 hrs	DEAD after 48 hrs	DEAD after 72 hrs
20mg/ml actellic dust	83.33	96.67	100.00
acetone(blank)	0.00	0.00	3.33
Pyrethrin 14mg/ml+Garlic oil 140mg/ml	23.33	33.33	36.67
Pyrethrin 16mg/ml+Garlic oil 160mg/ml	26.67	33.33	36.67
Pyrethrin 18mg/ml+Garlic oil 180mg/ml	43.33	50.00	53.33
pyrethrin 20mg/ml	20.00	26.67	40.00
Pyrethrin 20mg/ml+Garlic oil 200mg/ml	43.33	50.00	56.67
Pyrethrin 20mg/ml+PPO 200mg/ml	73.33	96.67	100.00
Untreated	0.00	0.00	0.00

DMRT

```
>out_mw_2<-
with(MAIZE.WEEVILS._CSV,duncan.test(%MORTALITY,DEAD.OVER.TIME,df,MSerro
r =30.8642,group = TRUE))
> out_mw_2
$statistics
MSerror     Df          Mean        CV
30.8642     54          41.7284     13.31361

$parameters test
```

```
name.t ntr alpha Duncan DEAD.OVER.TIME   3  0.05

$duncan
Table CriticalRange
2 2.835327      3.031439
3 2.982372      3.188654

$means                  % MORTALITY  std  r   Min Max Q25 Q50 Q75
DEAD After 24 hrs       34.81481 28.47001 27   0  90  15  30  50
DEAD after 48 hrs       42.96296 34.28554 27   0 100  25  40  55
DEAD after 72 hrs       47.40741 34.48440 27   0 100  30  40  60
/
$comparison NULL$groups
```

	% MORTALITY	groups
DEAD after 72 hrs	47.40741	a
DEAD after 48 hrs	42.96296	b
DEAD After 24 hrs	34.81481	c

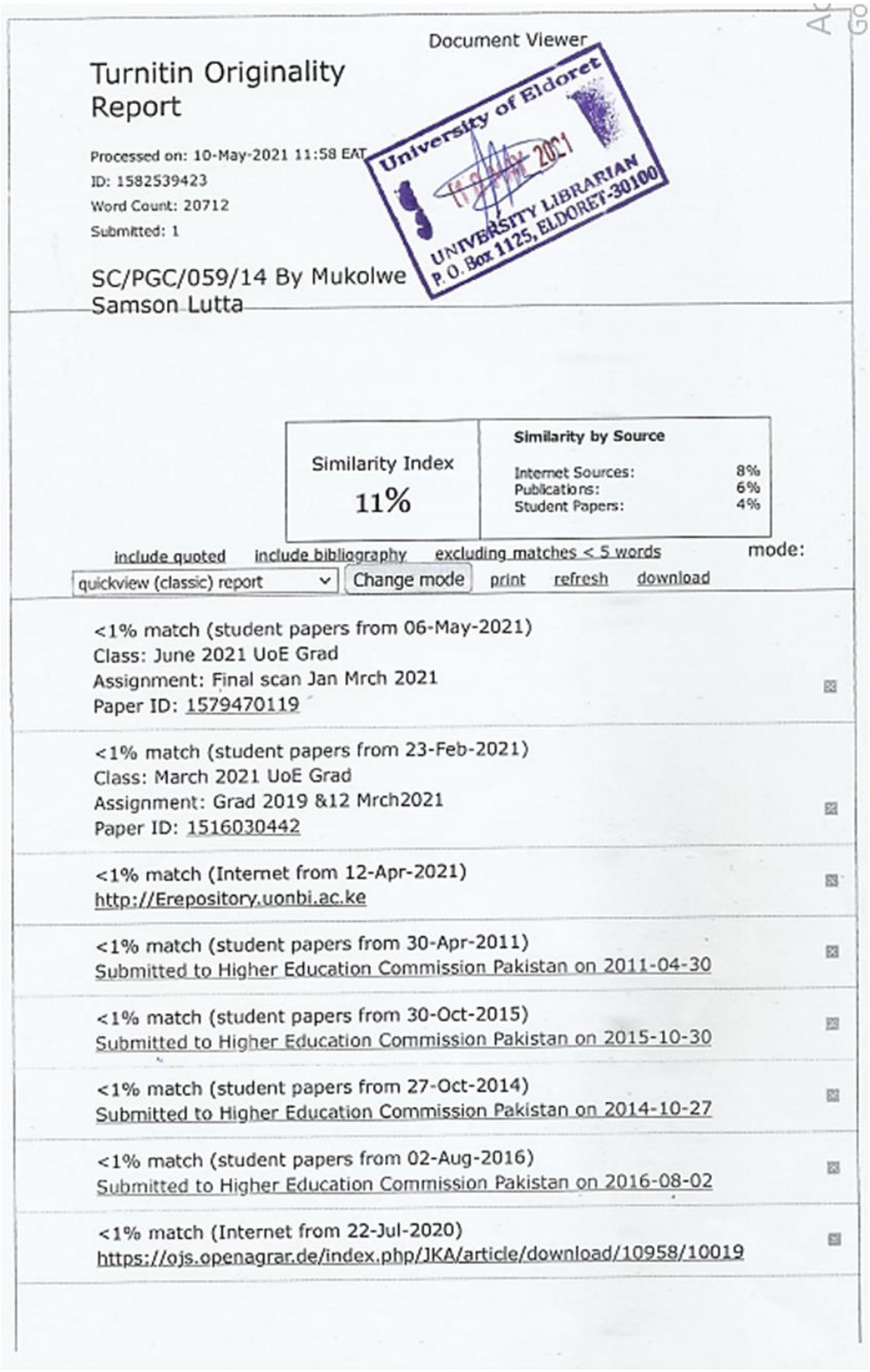
```
>out_MW.1<with(MAIZE.WV..CSV,duncan.test(%MORTALITY,TREATMENT,df,MSerro
r =30.8642,group = TRUE)) > out_MW.1
$statistics
MSerror Df    Mean       CV
30.8642 54 41.7284 13.31361

$parameters
test    name.t ntr alpha
Duncan TREATMENT   9  0.05

$duncan
     Table CriticalRange
2 2.835327      5.250607
3 2.982372      5.522911
4 3.079201      5.702224
5 3.149503      5.832413
6 3.203559      5.932517
7 3.246726      6.012456
8 3.282135      6.078028
9 3.311764      6.132897

$means
                                        % MORTALITY  std r Min Max Q25 Q50 Q75
20mg/ml actellic dust                  93.333333  8.660254 9  80 100  90 100 100
acetone(blank)                          1.111111  3.333333 9   0  10   0   0   0
Pyrethrin 14mg/ml+Garlic oil 140mg/ml  31.111111  7.817360 9  20  40  30  30  40
Pyrethrin 16mg/ml+Garlic oil 160mg/ml  32.222222  6.666667 9  20  40  30  30  40
Pyrethrin 18mg/ml+Garlic oil 180mg/ml  48.888889  6.009252 9  40  60  50  50  50
pyrethrin 20mg/ml                      28.888889 11.666667 9  10  50  20  30  30
Pyrethrin 20mg/ml+Garlic oil 200mg/ml  50.000000  8.660254 9  40  60  40  50  60
Pyrethrin 20mg/ml+PPO 200mg/ml         90.000000 13.228757 9  70 100  80 100 100
untreated                               0.000000  0.000000 9   0   0   0   0   0

$comparison NULL
```

$groups	% MORTALITY	groups
20mg/ml actellic dust	93.333333	a
Pyrethrin 20mg/ml+PPO 200mg/ml	90.000000	a
Pyrethrin 20mg/ml+Garlic oil 200mg/ml	50.000000	b
Pyrethrin 18mg/ml+Garlic oil 180mg/ml	48.888889	b
Pyrethrin 16mg/ml+Garlic oil 160mg/ml	32.222222	c
Pyrethrin 14mg/ml+Garlic oil 140mg/ml	31.111111	c
pyrethrin 20mg/ml	28.888889	c

acetone(blank)	1.111111	d
Untreated	0.000000	d

```
attr(,"class") [1] "group"
```

APPENDIX IX: SIMILARITY REPORT



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