BIOEFFICACY OF ORGANIC EXTRACTS OF FISH POISON BUSH (*GNIDIA GLAUCA*, FRESEN) AGAINST COWPEA WEAVIL (*CALLOSOBRUCHUS MACULATUS*, FABRICIUS)

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DECLARATION

I, Wilhelmy Marion Jebet, duly declare that the work presented in this thesis is my original work and has not been presented for a degree or any other award in any other university or any other institution.

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DEDICATION

In loving memory of my Mother, Hellen Jepkoech Berege. Her optimism, my inspiration.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
cm	Centimeter
CNS	Central Nervous System
DAERA	Department of Agriculture, Environment and Rural Affairs
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
ESAP	Ethiopian Society of Animal Production
FAO	Food and Agriculture Organization
FAOSTAT	FAO Statistical Database
g	Grams
GRAS	Generally Regarded As Safe
ha	Hectare
HPL	Post Harvest Losses
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
IGRs	Insect Growth Regulators
IITA	Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
IPM	Integrated Pest Management
IR	Inhibition Rate
m	Meter
ml	Milliliters
mm	Millimeter
OHLH	Orange Headed Leaf Hopper
ORCs	Olfactory Receptor Cells
PM	Percentage Mortality
PR	Percent Repulsion
SEM	Standard Error of Mean
SSA	Sub-Saharan Africa
t	Tonne

ABSTRACT

Cowpea weevil (*Callosobruchus maculatus*) is a major pests of stored cowpea in the tropical region of the world. In Kenya, the damage caused by *C. maculatus* impacts negatively on its economic and nutritional values and contributes to food insecurity. The widely adopted use of chemical pesticides is marred with health and environmental hazards. Global concern on synthetic chemicals has led to heightened restrictions and limitations on their use. This, therefore, has prompted the search for alternatives to synthetic pesticides. New studies are focusing on the use of botanicals as a novel approach to the management of pests. *Gnidia glauca* has been exploited by local people in control of post-harvest pests. However, no scientific research has been undertaken to evaluate its potential anti-insect properties. In this study, four organic leaf extracts of *G. glauca* (methanol, ethyl acetate, DCM and blend) were evaluated for contact toxicity, oviposition deterrence, inhibition of progeny emergence and repellency against cowpea weevil. The plant leaves were collected from Embu County, Kenya. The samples were prepared, extracted and investigation carried out under ambient laboratory conditions. The experimental design entailed five test concentrations (2g/100ml, 4g/100m, 6g/100ml, 8g/100ml, and 10g/100ml) of each extract, the untreated control, the solvent control and the positive control-Actellic. Each bioassay had four replications. Adult weevils (1-3 days old) were exposed to the extracts and mortality was monitored daily for the first four days. Subsequently, oviposition deterrence was assessed on the 15th day while inhibition of progeny emergence was evaluated on the 49th-day post-treatment. Extract repellency was assessed for the first 6 hours after treatment. Screening for plants phytochemicals was conducted using the standard recommended procedures. The results of this study revealed all *G. glauca* extracts, to a varied extent, induced mortality on *C. maculatus*. Mortality was concentration and exposure time dependent. Highest mortality of 89.74% was recorded with 10g/100ml ethyl acetate extract 96 hours post-treatment. The extracts significantly deterred oviposition with the 10g/100ml concentration of ethyl acetate, DCM and blend statistically ($p>0.05$) comparable to the activity of synthetic pesticide. All the extracts were found effective in inhibition of progeny emergence. Ethyl acetate extract at the test dose of 10g/100ml demonstrated the highest inhibition of 99.3% while the least inhibition of 9.03% was exhibited by 2g/100ml methanol extract. *G. glauca* extracts proved to be attractant of *C. maculatus* rather than repellant, none of the extract concentration attained repellency greater than 50%. Results also showed that the extracts had tannins, phenols, flavonoids, terpenoids, saponins, alkaloids, cardiac glycosides and steroids which have been associated with insect control properties. It was therefore concluded that the plant extracts, possess bioactivities against *Callosobruchus maculatus* on the tested parameters of contact toxicity, oviposition deterrence, inhibition of progeny emergence and repellency. Hence the studied extracts can further be purified and developed into the plant-derived bio-pesticides to control *C. maculatus.*

CHAPTER ONE

INTRODUCTION

1.1 Background information

Cowpea (*Vigna unguiculata* [L.] Walp.) is one of the ancient food plants known to man. It is commonly referred to as the black-eyed peas in English and '*kunde*' in Swahili. *Vigna unguiculata* is a dicotyledonous crop belonging to family Fabaceae. It is notably a drought tolerant, warm-weather legume that forms a deep taproot system. Therefore, it is adapted to the drier regions of the tropics where other legumes such as soybean and mung bean do not thrive (TJAI, 2010). Cowpea has the ability to fix atmospheric nitrogen via its root nodules and thrives in areas with poor soil. Kolawale *et al.* (2000) have shown that cowpea can thrive in soil consisting of more than 85% sand, organic material of less than 0.2% and low levels of phosphorous. These hallmarks make cowpea the ideal crop for sustainable subsistence farming in tropical and subtropical regions with low rainfall and infertile soil (FAO, 2004).

Although cowpea cultivation is now widespread across the world, Africa remains the predominant producer as reported in FAO report (2004). Langyintou *et al.* (2003) indicate that the area under cowpea cultivation in Africa is 87%, 10% is in America while Europe and Asia account for the rest. In the mid-1980s, FAO stopped publishing cowpea statistics. Therefore, there is no reliable source of data on cowpea production trends. However, using FAOSTAT data in correspondence with national programs, the approximation of the worldwide area under cowpea production in 2011 is 14 million hectares and the annual production is estimated to be larger than 4.5 million tonnes (Dias *et al.,* 2016). West and Central Africa produces more than 8 million tonnes with Nigeria being the largest producer and consumer of cowpea. It is estimated that the area under cultivation in Nigeria is 5 million hectares with an annual production of 2.4 million tonnes. Other cowpea producers include Niger, Mali, Burkina Faso, and Senegal. In Kenya, it is cultivated in the drylands of Eastern, Coast, and North Rift Valley regions where it is mainly intercropped with maize and sorghum (Frossard *et al.,* 2012). The cowpea varieties grown in these arid and semi-arid regions of Kenya include; *M66, K80,* and *KVU27-1* (KARI, 2008).

In sub-Saharan Africa (SSA), cowpea is a multifunctional crop. It serves as an important income earner to the farmers and grain traders. Cowpea also serves as a fodder crop for livestock and more important, it is food for man (Langyintou *et al.,* 2003; Cook *et al.,* 2005). It is a highly nutritious and palatable legume. Its young leaves, green pods, green seeds and even dry seed are consumed in different forms. Based on the dry weight, cowpea seeds have 25% crude protein, 65% carbohydrate, 1.9% fat and 6.3% fiber (FAO, 2012). It is also a source of minerals and vitamins such as thiamine, riboflavin and niacin. It is, therefore, a good source of protein, vitamins, and minerals (Nielsen *et al.,* 1997). As fodder crop, cowpea can be used as forage, silage and hay (FAO, 2012).

Despite the socio-economic importance of cowpea, a number of challenges still hinder its production from fields to stores. Insect pests cause the most predominant loss of cowpeas either at the fields or as the stored grain (Beck and Blumer, 2009). The losses caused by insect infestation is more severe in the harvested grains. In SSA, the annual losses due to post-harvest grain loss are estimated at US$ 4 billion (FAO, 2011). In addition, 50-90% of the germination losses as well as 10-15% losses of stored pulses and cereals are caused by insect attack (Adunga, 2006). Furthermore, World Bank (2011) identified post-harvest loss (PHL) as a major contributor of hunger and food insecurity in SSA. Obeng-Ofori (2010) further accentuates that food security in the developing nations of sub-Saharan Africa and Asia can only be attained by practicing sustainable good agricultural practices. Prevention and control of post-harvest losses by pests in fields and along the supply chain is imperative to combat the loss of grains hence realization of sustainable food security.

The cowpea weevil (*Callosobruchus maculatus* F.) is a major post-harvest insect pest that bores into the grains of cowpeas and causes degradation in the quality of cowpeas within two to three months. It is a cosmopolitan polyphagous pest that originated from Africa but is distributed all over the tropics and subtropics. Cowpea weevils do not only attack cowpea, but also beans of various species (CABI, 2017). Damage of cowpea grains by *C. maculatus* results in qualitative and quantitative losses. These losses are manifested by seed perforation and reductions in weight, decline in the nutritive and organoleptic properties of the grain and even loss of germination potential of seeds (Oluwafemi, 2012). Attack by *C. maculatus* on cowpea can be so severe such that 100% infestation is attained within few months. Such infestation can account for up to 60% loss of the grains (Kang *et al.*, 2013). These losses, prompted by insects' infestation, consequently affect the market price of the grain (Boxall, 2002).

The widely adopted and the most effective and efficient method used in protection of stored products is the use of synthetic pesticides. These synthetic insecticides, however, have serious drawbacks. Their misuse has resulted in the accidental poisoning of the insecticide users. Their indiscriminate use has led to tolerance and pest resurgence (Norris *et al.,* 2003). They also exhibits lethal effects on non-target and beneficial organisms in addition to adverse environmental and health hazards (Asawalam *et al.,* 2006). Moreover, a dearth of safe synthetic pesticides faces the developing nations. In addition to the consequences of use of synthetic insecticide, most farmers in the developing countries lack knowledge and proper facilities and equipment suitable for the effective conventional chemical control. Therefore, there is apparent need to find alternatives to conventional pesticides that are selective, biodegradable into non-toxic compounds, and can be utilized with the integrated pest management (IPM) program (Peshin and Dhawan, 2009).

Botanicals are greatly utilized in East Africa as part of the post-harvest pest control strategy. In Kenya, the commonly used plant species include *Tephrosia vogelis, Eucalyptus spp, Lantana camara* and *Azadirachta indica (*Obeng-Ofori, 2010). In Uganda, the stored cowpeas are protected from bruchid beetles by application of marigold extract (Kawuki *et al.*, 2005). African nutmeg (*Monodora myristica*), *Lantana camara* and Enuopiri (*Euphorbia lateriflora*) have been are bioactive against *Callosobruchus maculatus* (F.) and maize weevil, *Sitophilus zeamais* (Ogunsina *et al.*, 2011). The information available on the use of botanical extracts as an alternative method for controlling cowpea weevils is limited. Farmers in Eastern region of Kenya use various plant species for control of the stored product pest. Among these plants is *Gnidia glauca.* However, there is no scientifically validated data on the use of this plant against cowpea weevils. It is against this background that this study was designed to bio-screen the organic extracts of *Gnidia glauca* for its activity against cowpea weevil (*Callosobruchus maculatus* F.).

1.2 Statement of the problem and justification

Eradication of post-harvest losses (PHL) is imperative in the attainment of food security in developing nations of sub-Saharan Africa (SSA). Post-harvest losses of grains alone in SSA are estimated at US$ 4 billion annually (World Bank, 2011). Cowpea is greatly damaged by *C. maculatus*, cowpea weevil, during storage. Losses associated with cowpea weevil infestation include economic loss, nutritive, and germinative losses (Cambpell and Runnion, 2003). There are a number of conventional methods for managing weevils, for example, the use of synthetic chemicals. However, such methods are bedeviled by a number of economic and ecological shortcomings. These include residual toxicity, adverse effects on the beneficial and non-target organisms, the risk of user's contamination, pesticide resistance and high-cost of purchase (El-Kamali, 2009). Global concern about the use of these synthetic pesticides in the production of food crops has led to heightened restrictions and limitations on their use (Koul and Dhaliwal, 2003). The use of plants as a post-harvest protectant of grains is an ancient technique practiced in most parts of the world. However, their bio-efficacy has not been scientifically documented.

Due to losses incurred as a result of weevil infestation on cowpeas and the drawbacks associated with the use of conventional pesticides for controlling cowpea weevil infestation, it is critical to explore alternative control measures against weevils. Plants produce a diverse array of chemicals, some of which have potent pest-control properties (Koul *et al.*, 2008). Scott *et al.* (2003) perceive plant constituents as Generally Regarded As Safe (GRAS). This gives prospects of a source of safer, less expensive and easily processed insecticide against weevils after determination of their insecticidal properties (Viglianco *et al.,* 2008). Traditional use of *G. glauca* against *C. maculatus* needs to be evaluated and confirmed.

1.3 Hypotheses

i. Methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* have contact toxicity effects on *C. maculatus*.
ii. Methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* have oviposition deterrent effects on *C. maculatus*.
iii. Methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* have inhibition effects on the emergence of F1 progeny of *C. maculatus*.
iv. Methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* have repellent effects on *C. maculatus*.
v. Methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* have phytochemicals active against *C. maculatus*.

1.4 Objectives

1.4.1 Main objective

To determine the bio-efficacy of selected organic leaf extracts of *Gnidia glauca* against *Callosobruchus maculatus*.

1.4.2 Specific objectives

1. To determine the contact toxicities of methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* on *C. maculatus.*
2. To determine the oviposition deterrence of methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* on *C. maculatus.*
3. To determine the emergence of F1 progeny of *C. maculatus* from cowpea grains treated with methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca.*
4. To determine the repellent effects of methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* on *C. maculatus.*
5. To determine qualitative phytochemical composition of methanolic, ethyl acetate, DCM and blend leaf extracts of *G. glauca* on *C. maculatus.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Cowpea (*Vigna unguiculata*)

2.1.1 Description

Cowpea is an annual herbaceous legume grown in the drier tropical and subtropical regions of the world. It belongs the family Fabaceae and subfamily Faboideae. Cowpea can grow up to the height of 80cm and possesses an erect or sub-erect stem. It has a taproot system on to which nodules are attached. Cowpea leaves are tinged purple while its pods are curved, coiled or straight. The texture of the seeds could either be wrinkled or smooth. The seeds are of different colors including red, brown, black and white, and/or could be spotted, blotched, marbled or speckled (Duke. 1983).

Vigna unguiculata species is composed of ten perennial subspecies and one annual subspecies. The eleven cowpea subspecies further comprise the cultivated form (*var. unguiculata*) and the wild form (*var. spontanea*). *var. unguiculata* is further classified into five cultivar groups (cv-gr) based the phenotypic traits of pods and seeds. They include cv-gr. *Unguiculata*, cv-gr. *Melanophthalmus*, cv-gr. *Biflora*, cv-gr. *Sesquipedalis* and cv-gr *Textilis* (Pasquet, 1996).

Cowpea is a drought resistant and warm weather crop that is extensively farmed in the sub-Saharan Africa. It grows in soil with relatively low fertility and thrives in acidic and neutral soil. Cowpea is well adapted to sandy soil but is less tolerant to waterlogged soil

(Duke, 1983). Cowpea originated from Africa. PROTA (2006) shows that an array of wild types of cowpeas are distributed throughout the continent. The West African States possess a wider genetic diversity of the cultivated cowpea and also the most primitive forms of *V. unguiculata* (Maxted *et al.*, 2004). Cowpea is nowadays cultivated across the tropical and subtropical regions of southern USA, the Middle East and Central and South America. In Kenya, cowpea, is grown in the drier region of Eastern and the coastal regions. Figure 2.1 shows the dry grains, pods and leaves of cowpea, *Vigna unguiculata*.

Figure 2.1 Cowpea, *Vigna unguiculata* (L.)

Source: www.seedman.com

2.1.2 Nutritional and economic importance

Cowpea makes a substantial economic and nutritional contribution in the marginal regions of Africa where food insecurity and malnutrition are major challenges. Cowpea is consumed either as the dry seeds, green pods or leaves. The proximate nutritional composition of cowpea grains is 25% crude protein, 65% carbohydrate and 3.5% fats (FAO, 2004). The grains also contain vitamins and minerals such as iron and zinc. Its high protein content is important to the poor farmers characterized by protein deficiency in the diets (ESAP, 2005). Cowpea is a multi-purpose crop. Apart from its common use as food, cowpea serves as a fodder crop for livestock. After the crop is harvested, the hay left over is fed to livestock. It serves as a high-value nutritional forage (FAO, 2004). Intensive research is being done by research bodies such as IITA, ICRISAT, ILRI to boost crop-livestock system in the drier region of sub-Saharan Africa (Cowpea Stakeholders' Workshop, 2004).

Cowpea is also a significant source of income for many households. Women, particularly, are involved in the cowpea trade in West African countries (Okike, 2000). Freshly harvested green pods and the succulent leaves are sold directly to the consumer. Dried grains are commonly sold to the national granaries as the primary product. Secondary and cowpea derived products such as the flour are also available in the market (FAO, 2004). Further, cowpea can be intercropped with other crops. It also contributes to soil fertility in marginal lands. It fixes atmospheric nitrogen through its symbiotic association with the beneficial nitrogen-fixing bacterial in the soil. The broadleaf nature and the ground cover potential of cowpea minimizes soil erosion and preserves soil moisture. Its tap root system stabilizes the soil (DeBoer, 1997).

2.1.3 Production constraints

2.1.3.1 Abiotic factors

Various abiotic factors affect cowpea production at different developmental stages. These factors include extreme temperatures, drought and nutritional stresses. Cowpea is a warm-weathered crop. An optimum temperature of between 25-35°C is ideal for its growth. Extreme temperatures affect the growth and development of plants. A review by Barlow *et al.* (2015) showed that extreme cold temperature caused sterility and abortion of formed grains while intense heat results in the reduced development of the grains.

Drought adversely affects cowpea production in the fields. Cowpea is majorly produced under rain-fed agriculture particularly in Africa. Lack of water by the crops affects the leaf production and area, biomass production, grain production and consequently the yields (Rao, 2014). Extreme rainfalls also cause reduced yields of cowpea (Green Life Crop Protection Africa, 2017). The ideal minimal annual rainfall for cowpea is 300-650mm. Excessive rainfall during flowering causes flower abortion.

2.1.3.2 Biotic factors

The biotic factors that affect cowpea production and utilization include parasitic weeds, insect pests and diseases. Weeds greatly affect cowpea growth and yields (RUFORUM. 1995). Parasitic weeds cause approximately US$ 1.2 billion damages to the maize and cowpea crops in Kenya and Nigeria (IITA, 2011). Of major importance is the cowpea witchweed, *Striga gesnerioides*. *S. gesnerioides* has been reported to cause a significant loss of cowpea in West African countries including Togo, Nigeria, Benin, Chad and

Senegal. Other weeds of cowpea include *Amaranthus viridis, Chloris barbata, Cleome gynandra and Parthenium hysterophorus.* There are a number of strategies put in place for the management of weeds. They include cultural, mechanical and chemical weed control strategies (Hager, 2009).

Diseases also affect cowpea production at different developmental stages. They could be bacterial, fungal or viral (AATF, 2003). Fungal infections commonly induce pre and post seedling emergence mortality, stem, roots and foot rots and wilting of the cowpea. Fungal infections can also cause leaf diseases such as Cercospora leaf spot and pod diseases such as Lamb's tail pod rot and scab. Bacterial diseases affecting cowpea include bacterial blight and bacterial pustule. Cowpea (severe) mosaic, cowpea (yellow) mosaic, cowpea aphid-borne mosaic and cowpea golden mosaic are viral diseases affecting cowpea (Singh and Allen, 1979).

Insect pests cause a total annual loss of 20% in agricultural produce (Tnau Agritech, 2016). Insects attack cowpeas from fields to stores. Insect pests of cowpeas are classified into pre-flowering pests, post-flowering pests and storage pests. Pre-flowering insect pests include leafhoppers, aphids and foliage beetles. Flower thrips, mylabris and pod sucking bugs affect cowpeas at the flowering stage (Allen, 1979; Obeng –Ofori, 2010). Cowpea weevil (*Callosobruchus maculatus*) is the major storage insect pest of cowpea grains (Allen, 1979; Bagheri, 1996).

2.2 Cowpea weevil (*Callosobruchus maculatus*)

2.2.1 Description

Cowpea weevil (*Callosobruchus maculatus*) (Coleoptera: Bruchidae) is an invasive species of beetle that attacks stored legumes (Fabaceae). *Callosobruchus maculatus* is distributed throughout the tropical and subtropical regions of the world but it is dominant in Africa (CABI, 2017). Cowpea weevil belongs to the genus *Callosobruchus* in the subfamily Bruchinae and family Chrysomelidae (Kergoat *et al.,* 2007). *Callosobruchus maculatus* adults are 2.0-3.5 mm long and lack the snout of a true weevil. It is reddish-brown overall, with black and gray elytra marked with two central black spots (CABI, 2017). Two forms of this species exist; a dispersal (flying) form and a sedentary (flightless) form. The sex difference in flightless form is quite distinctive while the sexes in flying form are subtly dimorphic (Beck and Blumer, 2014). Figure 2.2 show male and female cowpea weevil.

Figure 2.2 Cowpea weevil (♂: male, ♀: female)

The images are of the same scale and the squares are 1mm.

Source: Beck and Blumer, (2014).

2.2.2 Life cycle

Upon infestation, the weevil lays a single fertilized egg on the surface of the grain. The egg is characteristically spindle-shaped, clear and shiny. It develops into a larva that burrows into the endosperm and attacks the grains from within. The developing larva of the weevil feeds entirely on the grains. After a series of molting, the larva is ready for pupation. It burrows to a location beneath the seed coat where it emerges as an adult insect. The emergence of the weevil leaves a hole in the grain. The adult weevil attains maturity 24 to 36 hours after emergence. The adult weevil has a lifespan of about 1 to 2 weeks. Adulthood of *C. maculatus* is wholly devoted to mating and egg-laying (Beck and Blumer, 2014). Under favorable conditions, the whole metamorphosis occurs within 45-48 days (Devi, 2014). Figure 2.3 shows different developmental stage of cowpea weevil during metamorphosis.

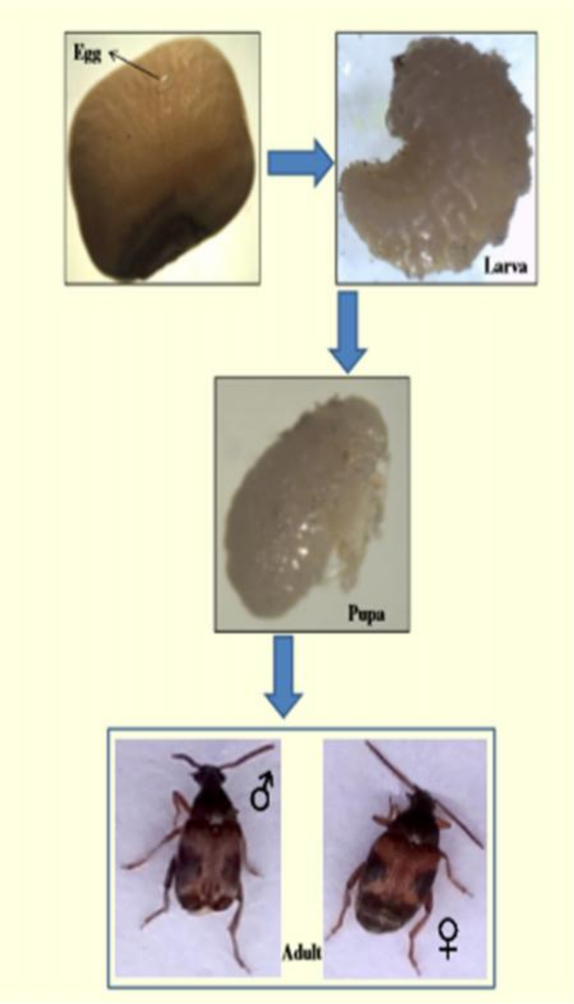

Figure 2.3 Developmental stages of *C. maculatus*.

Source: Arjanbahi (2010).

2.3 Storage pests control strategies

2.3.1 Cultural and sanitary control

Cultural and sanitary strategies of insect pest control involve specific practices implemented to reduce the likelihood of insect infestation and damage. Sanitary control techniques entail crop hygiene procedures such as cleaning of storage vessel to remove debris, laid eggs, larvae and dead insects left after consumption of the grain. It also involves aeration of granaries before the next batch of grain is brought in for storage (Newman, 2008). Cultural method of pest control involves cultivation of resistant crop varieties, crop rotation and/or the use of trap crops to keep the pest away from the desirable crop (PPATM, 2015).

2.3.2 Physical control

Physical control of insect pests forms an integral part of IPM. This method plays a role in pest prevention, monitoring and control (Alder, 2010). To prevent an infestation, a uniform temperature above 55°C is applied to the grains for 60 minutes or 60°C for about one minute. Temperatures can also be lowered below 13°C to prevent insect development. Grains can also be stored in hermetic storage structures or packed in insect-proof packages (Riudavets *et al.*, 2007).

For the control of pests, extreme temperatures and mechanical methods are employed. The process involves the application of vacuum to the stored products in order to eliminate oxygen from the inter-granular spaces. Carbon dioxide can also be applied in pressure chambers at high pressures of up to 35 bars for few hours. In addition, high temperatures above 50°C can be applied to the structure. The stored product can also be cold treated at temperatures of about -20°C for 24 hours (Adler, 2006).

The advantage of using physical method of insect control is that the insects do not develop resistance. The quality of the product is not altered and is free from toxic residues.

However, some physical methods of insect pest control such as freezing may be costly (Alder, 2010).

2.3.3 Biological control

Biological control is an effective tool in pest management either at the fields or at the storage level. It involves strategies such as the use of cultivars resistant to pest, pheromone traps and the use of natural enemies to control or suppress insect pests (Hodges, 1998). Most biological agents used against insects include parasitoids, predators and pathogens (Futch, 2010). The use of parasitoids has been commercially exploited for the control of pyralid moths and beetles of stored products (Schöller *et al.,* 2006). The parasitoids used in the control of cowpea weevils are the larval parasitoids *Dirnamus basalus* and *Eupelmus ssp* and the egg parasitoid *Uscana lariophaga* (Stolk *et al.,* 1998).

The use of biological method possesses a number of advantages. It does not pose health and environmental hazards. The use of natural predators is specific to the target pest and do not harm the beneficial organisms. The demerits associated with biological control of pests is that it is a costly method of pest control. It rarely eliminates pest and therefore not the ideal method for the control of heavy infested grains (Scholler, 2010).

2.3.4 Chemical control

Chemical control of insect pests entails prevention and eradication of pests using insecticides. Insecticides could be synthetic or naturally occurring chemicals that kill or alter physiological functions of insects. These chemicals vary in chemical composition and structural arrangement of compounds. Commonly used classes of pesticides include organophosphates, organochlorides, carbamates and pyrethroids (BASF, 2013).

Insecticides use different routes of entry into the target site in the insect. Stomach poisons are ingested with food, contacts insecticides are absorbed via the cuticle and fumigants enter via the spiracles and tracheae. Insecticides employ different modes of action in the insect. They can be neuroactive agents, insect growth regulators, metabolic inhibitors and physical poisons. Organophosphate and carbamates are synaptic poisons that target the acetylcholinesterase (AChE). They inhibit AChE by phosphorylation (organophosphates) and carbomylation (carbamates). This inhibition results in accumulation of acetylcholine at the synaptic junctions. This causes tremors, uncoordinated movements, paralysis and eventual death of the insect (Das, 2013). Pyrethroids, the derivatives of pyrethrin, are axonal poisons. They target the voltage-gated sodium ion channels. These sodium channel modulators prolong the action potential of the nerves. Juvenile Hormone Analogue such as the hydropene and methopene; fenoxycarb and Pyriproxyfen mimic the juvenile hormone in the insects (BASF, 2013).

The use of synthetic chemicals is a popular method of pests control because they are effective, fast-acting and easy to use as compared with other control options (Futch, 2010). This method, however, is bedeviled by a number of economic and ecological shortcomings. These include residual toxicity, adverse effects on the beneficial and non-target organisms, the risk of user’s contamination, pesticide resistance and high-cost of purchase (El-Kamali, 2009). Aldrin and dieldrin caused the death of seed-eating game birds, raptors that preyed on the game birds and also the death of fish-eating birds (Gay, 2012). Pirimiphos-methyl (Actellic), an organophosphate pesticide, has been established to affect male rat reproductive performances (Ngoula *et al.,* 2007). The IPM program directs the use of chemical pesticides as the last resort in the control of pest; and it should be specific to the target (DAERA, 2016).

2.4 Insecticidal properties of plants

Plants are a rich source of bioactive compounds including the alkaloids, terpenoids, steroids, carotenoids, flavanoids, glycosides and a range of essential oils. These compounds are products of secondary metabolism in plants. They do not serve any physiological functions in plants and neither are they present in all plant species. Phytochemicals serve a defense function in plants. Their evolution is attributed to selective pressure exerted by plants in a process of self-defense against pests (Arnason *et al.,* 1989).

The existing literature indicates that these phytochemical constituents contain an array of properties including insecticidal, antioxidant, anti-diabetic and antibacterial among others. Phytochemicals are produced in larger quantities during pest invasion. They have varied effects on insects. They can kill the insect, repel or attract, alter their development and even affect oviposition (Chowański *et al.,* 2016). Various mechanisms of action are employed by these secondary metabolites. Some act on the nervous system. They target the acetylcholinesterase enzyme, the acetylcholine receptors, the gamma-aminobutyric acid receptors and the voltage-gated sodium ion channels. Some acts as growth hormone regulators (IGRs). They mimic the Juvenile hormone and ecdysone hormone in the insects altering their growth and development (Silva, 2014). The biocidal activity of phytochemicals occurs when they target the central nervous system (Silver *et al.,* 2014). Phytochemicals may also disrupt the basic metabolic functions of the cells.

Utilization of secondary metabolites as plant protectant dates back to about 400 BC during the ancient Rome (Dayan *et al.,* 2009). In the 17th century, nicotine derived from tobacco leaves was used to protect plum from beetles (Silva, 2014). Other botanicals that have been employed in the management of pest throughout history include sabadilla, rotenone, pyrethrum and neem (Grdisa and Grsic, 2013). Neem has been intensively exploited and is the only plant from which several commercial products have been developed (Obeng-Ofori, 2010). Botanical insecticides have been hailed as the attractive alternative to synthetic insecticides. They are accessible and cheaply available to the farmers. They rapidly degrade in the environment, therefore, reduces the opportunity for residual toxicity. In addition, the pleiotropy nature and employment of various modes of action of phytochemicals reduce the possibility of resistance development by the pests (Chowański *et al.,* 2016). Table 2.1 shows examples of plants with insecticidal activities.

Table 2.1: Examples of plants with insecticidal activities (FAO Corporate Document Repository)

Plant species	**Activity and tested insect**	**Treatment**	**Major constituents**
Nigella sativa	Contact insecticides against *B. chinensis*.	Seed extract	Volatile oil Fixed oil Amorphous glucoside
Citrus aurantifolia	Oviposition deterrence of *C. maculatus* Inhibition of progeny emergence on *C. rhodesianus*	Fruit peel extract	Limonene Monoterpenes Sesquiterpenes
Citrus limon	Fumigant toxicity against *S. oryzae* and *C. maculatus*.	Leaf powder	Limonene Pinene Terpinine
Limonia acidissima	Oviposition deterrence of *C. chinensis*.	Bark extract	Bergapten
Azadirachta indica	Repellency, oviposition and feeding deterrence Inhibition of progeny emergence	Leaf, bark extracts	Azadirachtin
Lycopersicon esculentum	Inhibition of progeny emergence on *S. granarius*	Leaf extract	Coumaroylputrescine Tomatidine Tomatine
Nicotiana tabacum	Antifeeding activity and larval mortality in *T. castaneum*	Leaf extract	Nicotine Nornicotine Anabasine
	Oviposition deterrence and inhibition of progeny emergence on *C. maculatus*	Leaf powder	

2.5 *Gnidia glauca*

2.5.1 Plant description and geographical distribution

Gnidia glauca (Fresen.) Gilg is commonly known as the fish poison bush in English and *'muthira'* by the Embu community of Central Kenya. It is a large, much-branched shrub growing up to 6 m tall or small tree up to 15 to 24 m tall. It is a flowering plant and semi-woody herb in the family Thymelaeacea. The trunk of this plant is brown and scaly when mature. The blaze is yellow and with fragrance. The young branches are pubescent and become glaborous as they mature. The leaves of *G. glauca* are either simple, alternate, spiral or clustered at the upper part of the branches. The flowers are yellow and located at the terminal heads. *G. glauca* bear indehiscent fruits each with a single black seed (Prabhu Kumar *et al.,* 2004).

G. glauca is widely distributed in tropical Africa, including Nigeria, Sudan, Ethiopia, Malawi, and Zambia. It is also found in southern India and Sri Lanka. *G. glauca* inhabits forest margins, woodland savannah, on rocky grassy slopes and around stream beds (Brink, 2009). In Kenya, it is found in the drier regions of Embu County. Figure 2.4 show the image of *Gnidia glauca* shrub with an actual height of 1.5m.

Figure 2.4 A photo of *Gnidia glauca*.

Source: Dinesh Valke (2014)

2.5.2 Medicinal and cultural uses

G. glauca plant has various uses. This plant displays a wide range of phytomedicinal properties. It is also utilized in agrochemical applications. *G. glauca* has been reported to contain anti-helminthic, antifungal and anti-diabetic properties (Nethravathi, 2010). It is also an insecticidal, molluscicidal, piscicidal and homicidal agent (Rao *et al.,* 2013). In Kenya, the boiled root is drunk for the treatment of indigestion and the bark is made into arrow poison. It is used in some communities to treat cancer, sore throat, abdominal pain, wounds, burns and snake bites. In Northwestern Ethiopia, the natives use this plant species as an antiviral agent for rabies treatment. The roots are crushed into powder, mixed with milk and consumed orally for a week (Teklehaymanot and Giday, 2007).

Research into some fish poison plant species in the genera *Derris* and *Lonchocarpus* confirmed the presence of constituents with anti-insect properties which led to the discovery of the largely utilized insecticide rotenone.

A study by Godghate *et al.* (2015) confirms the antibacterial activity of the organic leaf and flower extracts of *G. glauca*. Further, it has been scientifically established that methanolic leaf extract of *G. glauca* possess free radical scavenging activity and reducing power (Rao *et al.*, 2013). *G. glauca* has also been scientifically confirmed to be active against pests and pathogens of agricultural importance. Aqueous extracts of *G. glauca* exhibits inhibition activity against *Phytophthora* parasitica, a pathogenic fungi of pineapple. The organic extracts of dried and fresh bark of *G. glauca* has larvicidal potential against larvae of *Aedes eagypti* (Ghosh *et al.*, 2015). In addition, a study by Njoroge (2016) showed that the aqueous and dichloromethanolic leaf extract of *G. glauca* inhibited the acetylcholinesterase activity in stem borer (*Chilo partellus*).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and preparation of plant materials

Fresh leaves of *Gnidia glauca* were collected from Siakago division, Mbeere North Sub County, in Embu County, Kenya, (Latitude: 0° 34' 59.99" N, Longitude: 37° 37' 59.99" E). Figure 3.1 shows the map of Siakago, Embu County.

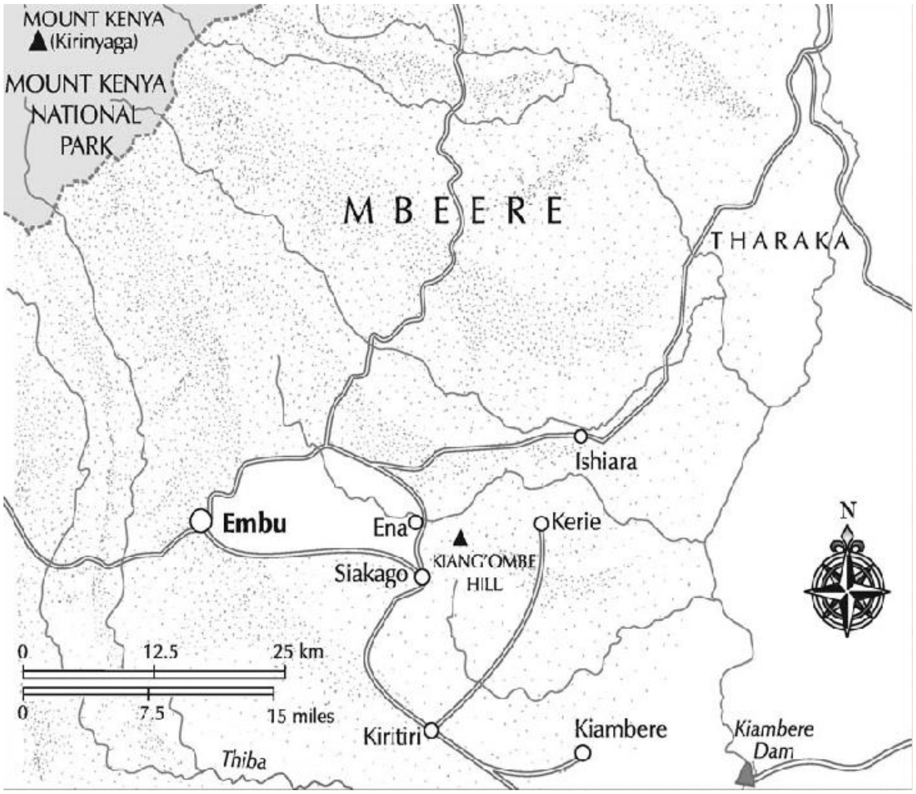

Figure 3.1 Map of Siakago, Embu County

Map source: www.brookiesway.co.za

The leaves were collected in the dry month of August, 2016. The collection of the plant was guided by ethnobotanical information from local farmers and herbalists. The identification and authentication of the plant were done by an acknowledged taxonomist and voucher specimens of the collected plant were deposited at Kenyatta University herbarium for future reference. The samples were cleaned to ensure they are free from any other compound and transported to the Biochemistry and Biotechnology laboratories at Kenyatta University (Latitude: 1°10'50.0"S, Longitude: 36°55'41.0"E) where this study was undertaken.

The leaves were air dried at room temperature. The dried leaves were then milled into powder by use of an electric mill to obtain a fine powder. The fine powder was stored in airtight containers at room temperatures awaiting extraction.

3.2 Organic extraction

Five hundred grams of *Gnidia glauca* powder were weighed into a conical flask. Seven hundred and fifty milliliters of methanol was added into the conical flask. The mixture was allowed to stand and regularly swirled for 72 hours. After 72 hours, the extract was decanted and filtered into a labeled conical flask using Whatman no.1 filter paper and funnel under vacuum. Two hundred and fifty milliliters of methanol was added to the remnant of the filtration and allowed to stand and regularly swirled for four hours. A second decantation and filtration was done (Handa *et al.*, 2008). A similar procedure was repeated for ethyl acetate and dichloromethane extraction. For blend extraction, methanol, ethyl acetate, and dichloromethane were mixed in the ratio 1:1:1 to make up one liter of blend solvent. The extraction procedure was similarly done for the blend extract.

The extracts were concentrated using rotary evaporator at 64.7°C for methanol, 77.1°C for ethyl acetate and 39.6°C for dichloromethane. Blend extract concentration was carried out by fractional concentration. The temperatures were consistently raised from 39.6°C to 77.1°C to entirely expel the three solvents. The extracts were separately placed in open beaker to allow any remaining solvent to evaporate until a sticky viscous paste was obtained. This sticky paste of each extract was weighted separately, and the percentage yield was calculated using the formula:

$$\%\,Yield = \left(\frac{WE}{WM}\right) * 100$$

Where; WE is the weight of the extract

WM is the weight of the plant material

The final extract was then kept as a stock solution (100%) and stored at 4° C until use in bioassay experiments (Kamruzzaman *et al.*, 2004).

3.3 Insect culture

Adult *Callosobruchus maculatus* were obtained from infested cowpea grains (*VITA-3*) at Githurai market, Nairobi, Kenya and its identification was done by an acknowledged entomologist. Whole cowpea grains (*VITA-3*) were first sorted out and disinfected by placing them in a deep freezer at -4°C for 3 days. The grains were then removed, air dried for 2 days to prevent moldiness and transferred into two plastic jars. Seventy pairs of

Callosobruchus maculatus were introduced into each of the jars and the container was covered with muslin cloth and held in place with the aid of rubber band for seven days to allow the insects to oviposit. After seven days, all the adult insects were all removed. The set up was the kept for forty nine days at moderate temperature of 26 ± 3°C, relative humidity of 70% and 12:12h (Light: Darkness) to allow emergence of progenies. The newly emerged adult *C. maculatus* were used for the subsequent study.

3.4 Preparation of extract concentration

Five different concentration of the four extracts were used in this study. These were 2g/100ml, 4g/100ml, 6g/100ml, 8g/100ml and 10g/100ml (extract weight in grams in 100ml of the extraction solvent). These concentrations (w/v) correspond to 2%, 4%, 6%, 8% and 10% respectively. The desired concentrations were attained by dilution of the stock solution (10g/100ml) which corresponded to 10%.

3.5 Determination of contact toxicity

Contact toxicity test was done as follows; 10g of cowpea seeds were put into each of the plastic vials. 2ml of each of the extract concentration (2g/100ml, 4g/100ml, 6g/100ml, 8g/100ml and 10g/100ml) were then added separately to the vials. The mixture was shaken for 5-10 minutes to ensure uniform mixing after which the extract liquid was filtered out.

The set up was air dried for 30 minutes to allow the traces of the solvent to evaporate. Ten adult *C. maculatus* (1-3 days old) were introduced into each vials and then covered with lid. Several tiny openings were made on the sides of plastic vials to ensure ventilation.

The control groups were also set. For the untreated group, 10g of cowpea seeds were put into the plastic vials after which ten adult *C. maculatus* were introduced. In the solvent control, 10g of cowpea seeds were put in the vial and 2ml of pure solvent was added and mixed together. The set up was left for 30 minutes to allow the solvent to evaporate. Ten adult *C. maculatus* were then introduced into the plastic vials and covered with lid. The positive control was designed using the same procedure as the solvent control except that the solvent was substituted by 0.2ml of the synthetic pesticide, Actellic. Four replicates were made for each treatment. (Taponjou *et al.,* 2002, Ogendo *et al.,* 2005).

The weevil mortality was assessed 6, 24, 48, 72, and 96 hours after the insects were introduced. Mortality assessment was done by probing the weevils with a sharp pin on the abdomen (Adedine *et al.,* 2011). Death was confirmed when there was no response to the probing by the insects. *C. maculatus* percentage mortality was assessed as:

$$\%\ Mortality = \left(\frac{DN}{TN}\right) * 100$$

Where; DN is the number of dead insects

TN is the total number of insects

The data on mortality was corrected using Abbott's formula (1925)

$$CM\% = \left(1 - \left(\frac{NT}{NC}\right)\right) * 100$$

Where; CM is the percentage corrected mortality

NT is insect population in treated vial

PC is insect population in control vial

3.6 Evaluation of oviposition deterrence

At the end of the fourth day, both dead and alive insects were removed from the vials and the set up left for 10 more days to check for oviposition deterrence. 20 cowpea seeds were randomly picked from each the groups and assessed for oviposition. The number of eggs laid on the treated and the control groups were counted and recorded and the percentage of oviposition deterrence calculated using the formula adopted by Arivoli and Tennyson (2013).

$$\% OD = \frac{EC - ET}{EC + ET} * 100$$

Where; EC is the number of eggs in control groups

ET is the number of eggs in the treated group

3.7 Evaluation of progeny emergence

For evaluation of progeny emergence, the set up was left for 35 more days. The number of adults that emerged was counted. Percentage Inhibition rate was calculated using the formula described by Tapandjuo *et al.* (2002).

$$\% IR = \frac{CN - CT}{CN} * 100$$

Where; CN is the number of emerged insects in the control

TN is the number of emerged insects in the treated container.

3.8 Determination of repellence activity

Repellency was assessed using the area preference method described by Obeng-Ofori *et al.* (1988). Test areas consisted of 10 cm Whatman Nº 1 filter papers cut in half. Each extract solution (1 mL) was applied to a half-filter-paper disc as uniformly as possible with a pipette. The other filter paper half was treated with the solvent alone. Extract-treated and solvent control half-discs were air-dried to evaporate the solvent completely. Full discs were then be re-made by attaching treated halves to untreated halves of the same dimensions with a cellotape. Each filter paper was then be placed in a Petri dish and ten adult weevils were released at the center of each filter paper disc and then covered. Each treatment was replicated 4 times. The number of insects present on treated (NT) and solvent control (NC) areas sides of the disc was counted six hours post treatment. For the positive and the untreated controls, one half of the filter paper was treated with the reference drug, Actellic (1ml). The other filter paper half was left untreated. Percent repellency (PR) values for the test was calculated as using Thien *et al.* (2013) formula:

$$PR = \frac{NC - NT}{NC + NT} * 100$$

For index of repellency, the formula described by (Mazzonetto, 2002) was employed:

$$IR = \frac{2G}{G + P}$$

Where; G is Treated group

P is control group

3.9 Qualitative phytochemical screening

The extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by Harbone (1998) and Kotake (2000). Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested for were flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, sterols, and terpenoids. These metabolites been have associated with potent anti-insect properties of various plants species.

3.9.1 Test for saponins (Froth test)

About 0.5g of each plant extract was put in a test tube. 3ml of sodium bicarbonate solution was added to the test tube and shaken vigorously. The mixture is then allowed to stand for about 20 minutes and froth indicates the presence of saponins and no froth indicates the absence of saponins.

3.9.2 Test for alkaloids

The extracts were tested for alkaloids by first acidifying 5 ml of each extract with 1M HCl. This acidic medium was heated and then treated with Dragendroff‘s reagent. The formation of an orange or reddish brown precipitate was regarded as positive for the presence of alkaloids (Harbone, 1998; Kotake, 2000).

3.9.3 Test for terpenoids (Salkowski test)

To 0.5 g of each of the extract was added 1 ml of ethyl acetate/petroleum ether and then mixed into 2 ml of chloroform. Three milliliters of concentrated sulphuric acid (H_2SO_2) was carefully added alongside to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids (Harbone, 1998; Kotake, 2000).

3.9.4 Test for flavonoids (Sodium hydroxide test)

Extracts were tested for flavonoids by mixing 2 ml of each extract with 2 ml of diluted sodium hydroxide (NaOH). An intense/golden yellow precipitate indicated positive results (Harbone, 1998; Kotake, 2000).

3.9.5 Test for steroids

To test for steroids, 0.5 g of each of the extract was dissolved in 2 ml of chloroform. Three milliliters of concentrated H_2SO_4 was carefully added by the sides of the test tube to form a layer. A reddish brown color at the interface indicated the presence of the steroidal ring (Harbone, 1998; Kotake, 2000).

3.9.6 Test for phenols

The extracts were screened for phenols by adding 1 ml of ferric chloride solution to 2 ml of each extract. Formation of blue to the green color indicated the presence of phenolics (Harbone, 1998; Kotake, 2000).

3.9.7 Test for cardiac glycosides (Keller-Kilian test)

To test for cardiac glycosides, 0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 2 drops of 10% ferric chloride ($FeCl_3$) solution. This was under-layered with 1 ml of concentrated sulphuric acid. A brown, violet or greenish ring at the interphase indicates the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Harbone, 1998; Kotake, 2000).

3.10 Data management and statistical analysis

Quantitative data on repellency, contact toxicity, oviposition deterrence and inhibition of progeny emergence were entered into a spreadsheet program and subjected to descriptive statistics. It was expressed as mean ± SEM. For inference statistics, the data were analyzed by one-way ANOVA at 5% significance level followed by Tukey's post-hoc test for pairwise separation and comparison of mean among groups (treatment and controls). A $P \leq 0.05$ was considered statistically significant. Data were analyzed using Minitab version 17.0 statistical software. The quantitative and qualitative data of the study were expressed in tables.

CHAPTER FOUR

RESULTS

4.1 The percentage yield of organic leaf extracts of *G. glauca*

The results of the percentage yields of *G. glauca* organic leaf extracts are presented in Table 4.1. The methanolic extract had the highest yield of 9% while yields by blend (6.4%) and dichloromethane (3%) followed respectively. Ethyl acetate extract had the least yield of 2.50%.

Table 4.1 Percentage yields of *G. glauca* leaf extract of four organic solvents

Extract	Weight of the extract (g)	% yield of the extract
Methanolic	45.10	9.02
Ethyl Acetate	12.50	2.50
Dichloromethane	15.00	3.00
Blend	32.00	6.40

4.2 Contact toxicities of the selected organic leaf extracts of *G. glauca* against *C. maculatus*

Results of contact toxicity of methanolic leaf extract of *G. glauca* against *C. maculatus* are presented in Table 4.2. Six hours post-treatment, the effects of the extracts at all the tested concentrations were not significantly different from the effect of the solvent and the untreated controls ($P>0.05$; Table 4.2). However, as table 4.2 shows, the effect of the extracts, 6 hours post exposure, was significantly lower than the effect of the standard drug (Actellic) ($P<0.05$) at all the tested concentrations. However, after 24 hours of exposure, the plant extract concentrations of 6g/100ml, 8g/100ml, and 10g/100ml caused comparable contact toxicities on *C. maculatus* ($P>0.05$; Table 4.2) but their effects were significantly lower than the reference insecticide ($P \leq 0.05$).The contact toxicities recorded by 2g/100ml (2.50%) and 4g/100ml (7.50%), 48 hours post exposure, were comparable but significantly lower than those of extract concentrations of 8g/100ml and 10g/100ml (Table 4.2).

The methanolic extract continued exerting contact toxicities on *C. maculatus* 72 hours after treatment. At this hour, the concentrations of 2g/100ml and 4g/100ml recorded mortality rates of 7.50% and 12.5% respectively. These effects were comparable to the effects of the untreated and the solvents controls ($P > 0.05$). However, these effects were significantly lower than the effects of extract concentrations of 6g/100ml, 8g/100ml, and 10g/100ml ($P \leq 0.05$; Table 4.2). At 96 hours after treatment, the dose of 8g/100ml exerted a higher contact toxicity effect (72.50%) than the dose of 10g/100ml (70.00%). However, these effects were statistically comparable ($P > 0.05$) but significantly lower than the effect of the reference pesticide, Actellic ($P \leq 0.05$; Table 4.2).

Table 4.2: Contact toxicity effects of methanolic leaf extract of *G. glauca* on *C. maculatus*

Extract Conc. g/100ml	Mean % of corrected mortality ± SEM with exposure period (hours)				
	6hr	24hr	48hr	72hr	96hr
Untreated Control	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
Solvent Control	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
2g	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	2.50 ± 2.50^{d}	7.50 ± 2.50^{d}	10.00 ± 0.00^{de}
4g	2.50 ± 2.50^{b}	5.00 ± 2.89^{c}	7.50 ± 4.79^{d}	12.50 ± 4.79^{d}	15.00 ± 2.89^{d}
6g	5.00 ± 5.00^{b}	27.50 ± 6.29^{b}	35.00 ± 6.45^{c}	42.50 ± 6.29^{c}	52.50 ± 2.50^{c}
8g	5.00 ± 2.89^{b}	32.50 ± 6.29^{b}	52.50 ± 4.79^{b}	62.50 ± 4.79^{b}	72.50 ± 4.79^{b}
10g	7.50 ± 4.79^{b}	35.00 ± 5.00^{b}	52.50 ± 2.50^{b}	57.50 ± 2.50^{bc}	70.00 ± 4.08^{b}
Actellic	40.00 ± 7.07^{a}	92.50 ± 2.50^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}

Values expressed as Mean ± SEM (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (P>0.05) followed by Tukey’s post hoc test.

The ethyl acetate leaf extract of *G. glauca* generally demonstrated a remarkable contact toxicity against *C. maculatus* (Table 4.3). At six hours post-treatment, the extract concentration of 10g/100ml caused a contact toxicity effect comparable to the reference pesticide, Actellic (P>0.05). After 24 hours, the effects of extract concentrations of 2g/100ml, 4g/100ml and 6g/100ml had no significant difference (P>0.05) but were significantly lower than those of the concentrations of 8g/100ml and 10g/100ml (P<0.05; Table 4.3).

After 72 hours of exposure, there was a steady increase in mortality rate with an increase in extract concentration. The concentration of 10g/100ml recorded the highest mortality of 72.50% while the concentration of 2g/100ml recorded the least mortality of 15.0% by this hour (Table 4.3). At 96 hours after treatment, the contact toxicities exhibited by the test concentrations of 2g/100ml and 4g/100ml were not significantly different (P>0.05) from each other. However, they were significantly lower than the effects of the tested concentrations of 6g/100ml and 8g/100ml (P<0.05). The extract concentration of 10g/100ml caused the highest contact toxicity (90%) on *C. maculatus* 96 hours post-treatment. This effect was comparable to the effect of the reference insecticide, Actellic (P>0.05; Table 4.3).

Table 4.3: Contact toxicity effects of ethyl acetate leaf extract of *G. glauca* on *C. maculatus*

Extract Conc. g/100ml	Mean % of corrected mortality ± SEM with exposure period (hours)				
	6hr	24hr	48hr	72hr	96hr
Untreated Control	$0.00±0.00^{c}$	$0.00±0.00^{d}$	$0.00±0.00^{e}$	$0.00±0.00^{f}$	$0.00±0.00^{e}$
Solvent Control	$0.00±0.00^{c}$	$0.00±0.00^{d}$	$0.00±0.00^{e}$	$0.00±0.00^{f}$	$0.00±0.00^{e}$
2g	$0.00±0.00^{c}$	$2.50±2.50^{cd}$	$5.00±2.89^{de}$	$15.00±2.89^{ef}$	$15.00±2.89^{d}$
4g	$5.00±2.89^{bc}$	$10.00±4.08^{cd}$	$17.50±2.50^{cde}$	$22.50±2.50^{de}$	$22.50±2.50^{d}$
6g	$5.00±5.00^{bc}$	$5.00±5.00^{cd}$	$10.00±4.08^{cd}$	$35.00±6.45^{cd}$	$40.00±4.08^{c}$
8g	$10.00±5.77^{bc}$	$17.50±4.79^{bc}$	$25.00±6.45^{bc}$	$47.50±2.50^{c}$	$72.50±2.50^{b}$
10g	$25.00±6.45^{ab}$	$30.00±4.08^{b}$	$52.50±4.79^{b}$	$72.50±4.79^{b}$	$90.00±4.08^{a}$
Actellic	$40.00±7.07^{a}$	$92.50±2.50^{a}$	$100.00±0.00^{a}$	$100.00±0.00^{a}$	$100.00±0.00^{a}$

Values expressed as Mean ± SEM (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (P>0.05) followed by Tukey’s post hoc test.

The dichloromethanolic leaf extract of *G. glauca* caused varied contact toxicity effects on *C. maculatus*. Table 4.4 shows that all the tested concentrations caused comparable contact toxicity effects 6 hours post-treatment. These effects had no significant differences with the effects of the solvent and the untreated controls (P>0.05). A similar observation was made in all the tested concentrations, 24 hours post exposure (Table 4.4). At 48 hours post exposure, the tested concentrations of 6g/100ml, 8g/100ml and 10g/100ml induced insignificantly different mortality on *C. maculatus* (P>0.05; Table 4.4).

The tested doses of 4g/100ml, 6g/100ml and 8g/100ml caused comparable contact toxicities on the adult cowpea weevil, 72 hours after treatment. However, these effects were significantly different from the effects of tested concentrations of 2g/100ml and 10g/100ml at the same time (P<0.05). At 96 hours post-treatment, the test doses of 2g/100ml, 4g/100ml, 6g/100ml and 8g/100ml achieved mortality rates of less than 50%. The extract concentration of 10g/100ml attained the highest contact toxicity effect of 62.50% by this time (Table 4.4). However, this effect was significantly lower than the effect of the positive control (P<0.05).

Table 4.4: Contact toxicity effects of dichloromethanolic leaf extract of *G. glauca* on *C. maculatus*

Extract Conc. g/100ml	Mean % of corrected mortality ± SEM with exposure period (hours)				
	6hr	24hr	48hr	72hr	96hr
Untreated Control	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{f}
Solvent Control	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{f}
2g	0.00±0.00^{b}	5.00±5.00^{b}	7.50±4.79^{cd}	7.50±4.79^{d}	15.00±2.89^{e}
4g	0.00±0.00^{b}	10.00±0.00^{b}	15.00±2.89^{cd}	22.50±2.50^{c}	25.00±2.89^{de}
6g	5.00±2.89^{b}	5.00±2.89^{b}	17.50±4.79^{bc}	22.50±2.50^{c}	30.00±4.08^{cd}
8g	5.00±2.89^{b}	7.50±2.50^{b}	17.50±2.50^{bc}	20.00±0.00^{c}	42.50±4.79^{c}
10g	7.50±4.79^{b}	10.00±4.08^{b}	32.50±4.79^{b}	45.00±2.89^{b}	62.50±4.79^{b}
Actellic	40.00±7.07^{a}	92.50±2.50^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Values expressed as Mean ± SEM (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (P>0.05) followed by Tukey’s post hoc test.

The blend leaf extract of *G. glauca* demonstrated low contact toxicity effects against *C. maculatus* (Table 4.5). No death was recorded by the extract concentrations of 2g/100ml, 4g/100ml, 6g/100ml and 8g/100ml by the sixth hour. At 24 hours after treatment, all the tested concentrations caused comparable contact toxicity effects ($P>0.05$). These effects were not significantly different from the effects induced by the untreated and the solvent controls ($P>0.05$; Table 4.5). At 48 and 72 hours post-treatment, the highest mortalities of 22.50% and 30% respectively, were recorded by the treatment concentration of 10g/100ml (Table 4.5).

None of the tested concentrations achieved mortality rate greater than 50% by the 96th hour of exposure. The highest mortality rate of 45% was recorded by 10g/100ml extract concentration followed by 35% mortality rate induced by 8g/100ml extract concentration within 96 hours. The extract concentrations of 2g/100ml, 4g/100ml and 6g/100ml had low contact toxicity effects of 15%, 20%, and 27.50% respectively within this time period (Table 4.5). Moreover, there was a gradual increase in mortality with time for every test concentration of this extract. However, the mortality rates in all the treatments were significantly lower than the standard drug ($P<0.05$; Table 4.5).

Table 4.5: Contact toxicity effects of blend leaf extract of *G. glauca* on *C. maculatus*

Extract Conc. g/100ml	Mean % of corrected mortality ± SEM with exposure period (hours)				
	6hr	24hr	48hr	72hr	96hr
Untreated Control	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{e}
Solvent Control	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{e}
2g	0.00±0.00^{b}	2.50±2.50^{b}	7.50±4.79^{bc}	10.00±5.77^{cd}	15.00±2.89^{d}
4g	0.00±0.00^{b}	7.50±2.50^{b}	12.50±9.46^{bc}	17.50±4.79^{bc}	20.00±4.08^{d}
6g	0.00±0.00^{b}	7.50±4.79^{b}	20.00±4.08^{bc}	22.50±2.50^{bc}	27.50±4.79^{cd}
8g	0.00±0.00^{b}	7.50±2.50^{b}	12.50±2.50^{bc}	20.00±4.08^{bc}	35.00±2.89^{bc}
10g	5.00±2.89^{b}	10.00±4.08^{b}	22.50±4.79^{b}	30.00±4.08^{b}	45.00±2.89^{b}
Actellic	40.00±7.07^{a}	92.50±2.50^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Values expressed as Mean ± SEM (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (P>0.05) followed by Tukey’s post hoc test.

The contact toxicity effects of the four organic extracts of *G. glauca* were compared in this study. At six hours post-treatment, the four organic extracts caused comparable effects at the tested concentrations of 2g/100ml, 4g/100ml, 6g/100ml and 8g/100ml ($P>0.05$). The ethyl acetate extract tested concentration of 10g/100ml induced the highest contact toxicity at this time. This effect is significantly higher than the effects of methanolic, dichloromethanolic and blend extracts at the extract concentration of 10g/100ml ($P\leq0.05$).

The effects of the four organic extracts, 24 hours post exposure, were not significantly different from each other ($P>0.05$) at the tested concentrations of 2g/100ml and 4g/100ml. At 6g/100ml and 8g/100ml concentrations, the methanolic extract caused higher contact toxicity effects than the effects of ethyl acetate, dichloromethanolic and blend extracts. The contact toxicities of dichloromethanolic and blend extract were comparable at the test concentration of 10g/100ml ($P>0.05$). However, these effects were significantly lower than the effects of methanolic and ethyl acetate extracts ($P\leq0.05$).

At 48 hours post-treatment, the highest recorded mortalities by each of the four extracts were at the extract concentrations of 10g/100ml. However, the contact toxicities caused by the methanolic and the ethyl acetate extracts were significantly higher ($P\leq0.05$) than the contact toxicities of the dichloromethane and the blend extracts at this concentration (10g/100ml). In addition, the contact toxicities of the methanolic and the ethyl acetate extracts at this concentration of 10g/100ml were comparable (P>0.05). At the test concentrations of 6g/100ml and 8g/100ml, methanolic extract demonstrated high contact toxicity effects among the four organic extracts.

At 72 hours post exposure, each of the four *G. glauca* extracts at test concentrations of 2g/100ml, 4g/100ml, and 6g/100ml caused comparable contact toxicities (P>0.05). At the test concentration of 8g/100ml, the dichloromethanolic and the blend extracts contact toxicity effects were statistically similar (P>0.05). However, these effects were significantly lower than the effects of methanolic and the ethyl acetate extracts at this concentration (8g/100ml) (P≤0.05). At this time, ethyl acetate extract recorded the highest mortality among the four extracts at test concentration of 10g/100ml.

The effects of each of the four organic extracts, 96 hours post exposure, were not significantly different from each other (P>0.05) at the tested concentrations of 2g/100ml and 4g/100ml. At the test dose of 8g/100ml, the contact toxicities of methanolic and ethyl acetate extracts had no significant differences from each other (P>0.05). In addition, dichloromethanolic and blend extracts induced comparable effects at this tested concentration of 8g/100ml (P>0.05). At this hour, the five tested concentration of dichloromethanolic and the blend extracts recorded statistically comparable contact toxicities (P>0.05). The ethyl acetate extract, at test dose of 10g/100ml, recorded the highest mortality rate among the four *G. glauca* organic leaf extracts at this hour.

4.3 Oviposition deterrence of selected organic leaf extracts of G. *glauca* against C. *maculatus*

All the four organic leaf extracts of *G. glauca* reduced oviposition by *C. maculatus* (Table 4.6). The methanolic extract demonstrated low oviposition deterrence of the cowpea weevil. The mean percent deterrence of the tested concentrations ranged from 4.37% to 56.54% (Table 4.6). The effect caused by test dose of 2g/100ml of this extract was comparable to the effects caused by the solvent and the untreated controls ($P>0.05$). The oviposition deterrent effects induced by test concentrations of 4g/100ml, 6g/100ml and 10g/100ml of the methanolic extracts had no significant differences from each other ($P>0.05$). However, these effects were significantly lower than the effects of the test concentration of 8g/100ml ($P\leq0.05$).

The ethyl acetate extract demonstrated the highest oviposition deterrence among the four extracts. A maximum deterrence of 99.02% was observed at the extract concentration of 10g/100ml while the least oviposition deterrence of 46.78% was evoked by the test concentration of 2g/100ml (Table 4.6). The oviposition deterrent effects at test concentrations of 6g/100ml, 8g/100ml and 10g/100ml were not significantly different from each other ($P>0.05$). In addition, the effects of the three test doses (6g/100ml, 8g/100ml and 10g/100ml) of ethyl acetate extract were statistically comparable to the reference drug, Actellic ($P>0.05$, Table 4.6).

The dichloromethanolic leaf extract of *G. glauca* also manifested oviposition deterrent effects against *C. maculatus* (Table 4.6). This extract recorded the highest oviposition deterrence of 95.21% at test dose of 10g/100ml and the least oviposition deterrence of 58.36% at the concentration of 2g/100ml (Table 4.6). The tested concentrations of 6g/100ml, 8g/100ml and 10g/100ml induced statistically comparable oviposition deterrent effects ($P>0.05$). These effects were not significantly different from the effect of the positive control ($P>0.05$).

The blend leaf extracts caused oviposition deterrence of *C. maculatus* as shown in Table 4.6. Generally, there was an increase in oviposition deterrence with an increase in the extract concentration. The test dose of 2g/100ml elicited the least oviposition deterrent effect of 22.8% while the test dose of 10g/100ml evoked the highest effect of 81.13% (Table 4.6).

Table 4.6: Oviposition deterrence of four organic leaf extracts of *G. glauca* against *C. maculatus*

Conc (g/100ml)	Methanolic Extract	Ethyl Acetate extract	Dichloromethane extract	Blend Extract
Untreated Control	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{f}
Solvent Control	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{f}
2g	4.37±2.70^{d}	46.78±3.540^{c}	58.36 ±3.75^{b}	22.80 ±1.97^{e}
4g	43.46±2.14^{c}	75.79±4.54^{b}	58.83±2.30^{b}	38.29±3.93^{d}
6g	44.52±2.38^{c}	97.05±0.98^{a}	90.94±5.40^{a}	59.73±4.42^{c}
8g	56.54±3.93^{b}	96.12±1.57^{a}	90.94±5.40^{a}	78.69±2.61^{b}
10g	44.94±0.85^{c}	99.02±0.98^{a}	95.21±2.36^{a}	81.13 ±2.83^{b}
Actellic	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Values expressed as Mean ± SEM (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (P>0.05) followed by Tukey’s post hoc test.

Comparison of the deterrent effects of the four organic leaf extracts used in this study showed that ethyl acetate extract elicited the highest oviposition deterrence among the four extracts at the tested concentrations of 4g/100ml, 6g/100ml, 8g/100ml and 10g/100ml (Figure 4.6). The oviposition deterrent effects at extract concentrations of 6g/100ml, 8g/100ml and 10g/100ml were statistically similar for methanolic and ethyl acetate extracts (P>0.05). The methanolic extract demonstrated the least oviposition deterrence in all the

4.4 Inhibition of F1 progeny emergence activities of selected organic leaf extracts of *G. glauca* against *C. maculatus*

Table 4.7 shows the percentage inhibition of F1 progeny emergence activities of four organic leaf extracts of *G. glauca* against *C. maculatus*, 49 days post-treatment. The methanolic extract demonstrated the least inhibition activity among the four extracts. The mean percent inhibition of F1 progeny emergence activities of the tested concentrations ranged from 9.03% to 69.44% (Table 4.7). The effect induced by the test concentration of 2g/100ml was comparable to the effects of the untreated and the solvent controls (P>0.05). The test dose of 8g/100ml achieved a greater effect (69.44%) than the dose of 10g/100ml (54.86%) (Table 4.7). The ethyl acetate extract generally demonstrated remarkable inhibition of F1 progeny emergence activity (Table 4.7). The tested concentrations of 6g/100ml, 8g/100ml and 10g/100ml caused effects statistically similar to the effects of the reference drug, Actellic (P>0.05). All the five tested concentrations of this extract elicited effects significantly higher than the effects of the untreated and the solvent controls (P<0.05).

Generally, there was a steady increase in the activity of the dichloromethanolic extract with increase in concentration of the extract (Table 4.7). The test dose of 2g/100ml induced the least effect (34.03%) while the test dose of 10g/100ml caused the highest inhibition of F1 progeny emergence (97.22%). The effects caused by the test concentrations of 8g/100ml and 10g/100ml were statistically comparable ($P>0.05$). In addition, the effects induced by the concentrations of 8g/100ml and 10g/100ml were not significantly different with effects of the positive control ($P>0.05$; Table 4.7).

The effects of the five tested concentrations of the blend extract of *G. glauca* were significantly different from each other ($P<0.05$; Table 4.7). The highest inhibition of F1 progeny emergence activity (84.03%) was evoked by the concentration of 8g/100ml. The test concentration of 2g/100ml caused the least inhibition of F1 progeny emergence (15.97%) against *C. maculatus* (Table 4.7). The effects of the five test concentrations of the blend extract were significantly higher than the effects of the untreated and the solvent controls ($P<0.05$). In addition, none of the tested concentration of this extract evoked effects comparable to the effect of the positive control ($P<0.05$; Table 4.7).

Table 4.7: Percentage inhibition of F1 progeny emergence activity of four organic leaf extracts of *G. glauca* against *C. maculatus*

Conc. g/100ml	Methanolic extract	Ethyl Acetate extract	DCM extract	Blend extract
Untreated Control	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}
Solvent Control	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}
2g	9.03 ± 1.33^{f}	29.17 ± 2.66^{c}	34.03 ± 1.33^{d}	15.97 ± 2.37^{f}
4g	28.47 ± 1.33^{e}	75.00 ± 3.40^{b}	61.11 ± 2.54^{c}	37.50 ± 4.17^{e}
6g	40.97 ± 4.86^{d}	97.22 ± 1.60^{a}	76.39 ± 2.89^{b}	54.86 ± 4.86^{d}
8g	69.44 ± 3.76^{b}	99.30 ± 0.69^{a}	93.06 ± 2.66^{a}	84.03 ± 1.33^{b}
10g	54.86 ± 2.08^{c}	99.30 ± 0.69^{a}	97.22 ± 1.60^{a}	70.83 ± 2.66^{c}
Positive Control	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}

Values expressed as Mean ± SEM (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (P>0.05) followed by Tukey's post hoc test.

The inhibition of F1 progeny emergence activities of the four organic leaf extracts of *G. glauca* against *C. maculatus* were compared in this study. Among the four organic extracts, the methanolic extract recorded the least effects in all the tested concentrations. The highest activity was caused by ethyl acetate extract at test concentration of 10g/100ml. This effect had no significant difference with the effect of dichloromethanolic extract at test concentration of 10g/100ml ($P>0.05$).

4.5 Repellent activities of selected organic leaf extracts of *G. glauca* against *C. maculatus*

Table 4.8 shows the repellent activities of the four organic leaf extracts of *G. glauca* against *C. maculatus* 6 hours post treatment. Generally, there was a decrease in repellence with increase in extract concentration. The methanolic extract caused the highest repellency (50%) at the test concentration of 2g/100ml and the lowest repellency (5%) at the test concentration of 10g/100ml (Table 4.8). The tested extract concentrations of 2g/100ml and 4g/100ml induced comparable repellent effects on *C. maculatus* ($P>0.05$). These effects were significantly higher than the effects induced by the tested concentrations of 6g/100ml, 8g/100ml and 10g/100ml ($P<0.05$; Table 4.8).

The ethyl acetate extract demonstrated repellence activity on *C. maculatus* at different concentrations (Table 4.8). However, none of the tested concentrations achieved repellence activity greater than 50%. The highest repellent effect of 42.50% was caused by the extract concentration of 2g/100ml. As the extract concentrations increased, the repellence activities decreased. The least repellence effect of 30% was achieved by the tested concentrations of 8g/100ml and 10g/100ml (Table 4.8). All the tested concentrations of ethyl acetate extract induced comparable repellent effects on *C. maculatus* ($P>0.05$; Table 4.8).

The dichloromethanolic extract of *G. glauca* recorded repellence activities of 50% and below at all the tested concentrations (Table 4.8). The maximum repellence activity (50%) induced by the test concentration of 2g/100ml was significantly higher than the least repellence activity (35%) caused by the test concentration of 8g/100ml ($P<0.05$). None of the tested concentrations achieved repellent effect comparable to the effect of the reference drug, Actellic ($P<0.05$; Table 4.8).

The blend extract exhibited repellency against *C. maculatus* at different concentrations (Table 4.8). The highest repellent effect of 45% was caused by the test dose of 2g/100ml. In addition, the repellence activities caused by all the tested concentrations of the blend extract had no significant differences from each other ($P>0.05$; Table 4.8).

Table 4.8: Percentage repellent activities of four organic leaf extracts of *G. glauca* on *C. maculatus*

Conc. (g/100ml)	Methanolic extract	Ethyl acetate extract	Dichloromethanolic extract	Blend extract
Untreated Control	00.00±00^{e}	00.00±00^{c}	00.00±00^{d}	00.00±00^{c}
2g	50.00±4.08^{b}	42.50±2.50^{b}	50.00±4.08^{b}	45.00±2.89^{b}
4g	47.50±2.50^{b}	35.00±2.89^{b}	40.00±4.08^{bc}	40.00±4.08^{b}
6g	35.00±2.89^{c}	32.50±4.79^{b}	37.50±2.50^{bc}	37.50±4.79^{b}
8g	22.50±2.50^{d}	30.00±0.00^{b}	35.00±2.89^{c}	37.50±2.50^{b}
10g	5.00±2.89^{e}	30.00±4.08^{b}	37.50±4.79^{bc}	37.50±4.79^{b}
Actellic	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Values expressed as Mean ± SEM (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (P>0.05) followed by Tukey’s post hoc test.

Repellency index for the four organic leaf extracts of *G. glauca* were also evaluated in this study (Table 4.9). The methanolic and dichloromethanolic extracts at the test concentration of 2g/100ml had no repellent nor attractant effects on *C. maculatus*. All the test concentrations of ethyl acetate and blend extracts served as attractants to *C. maculatus* (Table 4.9).

Table 4.9: Repellency index values of four organic leaf extracts of *G. glauca* against *C. maculatus*

Conc. (g/100ml)	Methanolic extract	Ethyl acetate extract	DCM extract	Blend extract
2g	1	1.15	1	1.1
4g	1.05	1.3	1.2	1.2
6g	1.3	1.35	1.25	1.25
8g	1.55	1.4	1.3	1.25
10g	1.9	1.4	1.25	1.25

IR: <1 repellent; 1 neutral; >1 attractant

This study also compared the repellent activities of the four organic leaf extracts of *G. glauca* on *C. maculatus* at different concentrations. At each of the test concentrations of 2g/100ml, 4g/100ml and 6g/100ml, all the four extracts induced statistically similar repellent effects (P>0.05). The repellence activity of methanolic extract was significantly lower than the repellence activities of the ethyl acetate, dichloromethanolic and blend extracts at the test concentrations of 10g/100ml (P≤0.05).

4.6 Qualitative phytochemical screening

All the four organic leaf extracts were qualitatively screened for phytochemicals. The methanolic extracts of *G. glauca* had tannins, phenols, flavonoids, terpenoids, saponins, alkaloids and steroids but cardiac glycosides were absent. All the tested compounds were present in ethyl acetate extract. The phytochemicals present in DCM leaf extract of *G. glauca* were tannins, phenols, terpenoids, cardiac glycosides, alkaloids, and steroids but flavonoids, saponins and phenols were absent. The phytochemical composition of the blend extract was similar to the composition in DCM extract except that tannins were absent in the blend extract.

Table 4.10: Qualitative phytochemical screening of *G. glauca* organic extracts

Phytochemicals	**Extract**			
	Methanol	**Ethyl Acetate**	**DCM**	**Blend**
Saponins	+	+	-	-
Flavonoids	+	+	-	-
Phenols	+	+	-	-
Cardiac glycosides	-	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	-
Alkaloids	+	+	+	+

KEY: + = Present - = Absent

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Introduction

Identification and characterization of novel plant toxins with anti-insect potential have been emphasized as crucial in the development of ecologically safe pesticides. Phytochemicals play a critical role in insect-plant interactions. They form an intricate and dynamic chemical defense system against insect pest attack. These allelochemicals possess lethal and sublethal behavioral and physiological impacts on the different developmental stages of insects (Varma and Dubey, 1999; Chowański *et al.,* 2016). This study was designed to assess the effects of the four organic leaf extracts of *G. glauca* against cowpea weevil (*Callosobruchus maculatus*). It was apparent that this plant possesses properties against cowpea weevil.

5.1.2 Contact toxicities of organic leaf extracts of *G. glauca* on *C. maculatus*

The extracts exhibited considerably diverse contact toxicity effects which were dose and exposure duration dependent. At the end of the 96 hour period, the highest mortality of 89.74% was demonstrated by ethyl acetate extract. Subsequently, the methanolic extract followed with a mortality rate of 71.79% while the DCM extract achieved mortality of 61.54%. The blend extract elicited the least mortality of 43.59% among the four extracts. The variance in mortality caused by the extracts can be attributed to the mother solvent used in the extraction process. The content and the activity of the extracted phytochemicals depends on the polarity of the solvents and the solubility of that compound in the solvent (Dai and Mumper, 2010; Dehkharghanian *et al.*, 2010). Previous studies show that methanol extracts polar compounds such as amino acids, sugars and glycosides (Houghton and Raman, 1998). Yu *et al.* (2009) further inform that methanol can also dissolve molecules with medium polarity and low molecular weight such as phenols. Non-polar organic solvents extract non-polar compounds such as terpenoids and sterols (Cowan, 1999). Medium-polar solvents such as ethyl acetate effectively extract flavonoids, terpenoids, and sterols (Cowan, 1999), alkaloids, aglycons, and glycosides (Houghton and Raman, 1998). Therefore, the variation in toxicity effect of the extracts in this study can be attributed to the varying phytochemical composition of the extracts.

The variation in mortality of the extracts can also be ascribed to the mode of entry of the active components into the target site in the insect system. In this study, the mode of entry of the biocidal components of the extracts into the insect is thought to be via the integument system. The toxins penetrate the lipophilic and hydrophilic cuticle systems into the target sites where they employ various mechanism to exert their actions (Yu, 2015). Yu (2015) further shows that the hydrophilic-hydrophobic nature of the cuticle affects the entry of insecticide hence its efficacy. The chemical characteristic and polarity of the active component affects its movement through the cuticle. The outermost lipophilic phase of the cuticle favors the movement of nonpolar molecules. Therefore, only the toxins in the extracts with favored polarity and chemical nature made way to the target site, hence the diversity of mortality effects by the four extracts.

The high mortality rate (90%) exhibited by the ethyl acetate extract at test concentration of 10g/100ml indicates that this organic solvent extracted more active compounds with insecticidal activities. The high activity of this extract could also be linked to its high potential in extracting the phytochemicals of *G. glauca*. The qualitative analysis screening in this study showed that the ethyl acetate extract yielded positive results on all the tested phytochemicals. Further, the phytochemicals with insecticidal properties in this extract could be acting in synergy thus potentiating its toxicity effects.

The evaluation of the toxicity effects of the *G. glauca* leaf extracts generally shows that the bioactivities of ethyl acetate, DCM and blend extracts were directly proportional to the extract concentrations. The higher the dosage, the more potent the extract. This could be due to the increase in bioactive components as the concentration of the extract increases. In addition, the extracts manifested a higher mortality with an increased exposure time of the weevils to the treated cowpea seeds. The increase in exposure time permits an increase in uptake of active constituents. It also allows for more contact time with the target site hence the observed higher mortality with longer exposure span.

In this study, the possible mechanism of action of toxicity is inhibition of acetylcholinesterase enzyme in the cowpea weevil by the *G. glauca* phytochemicals. Many phytochemicals affect neurotransmission and signal transduction in organisms (Wink, 1993). Njoroge *et al.* (2016) show that the aqueous and DCM leaf extracts of *G. glauca* leaves possess acetylcholinesterase enzyme inhibitory activity in *Chilo partellus* larvae at concentrations of 0.25, 5 and 7 mg/ml. A similar study conducted by Friedman and McDonad (1997) reports that potato glycoalkaloid, α-chaconine, holds inhibitory activity against acetylcholinesterase enzyme in Colorado potato beetle. Binding of these antagonists to the acetylcholinesterase receptors cause physiological and biochemical disturbances and blockage. The subsequently recorded effects include symptoms such as restlessness of the weevil, lack of coordination, induction of unconsciousness and eventual death of the insect (Ofuya and Osadahun, 2005) as observed in this study. It could also be that the active constituents in these extracts targeted voltage-gated sodium channels. These channels are vital for electrical signaling in most excitable cells. Voltage-gated sodium channels have been targets for several plant toxins including pyrethrins from *Tanacetum cinerariaefolium* and sabadilla alkaloids (Silver *et al.,* 2014). Binding of these toxins to specific receptors in the sodium channels alters their functions. They could block the channel pore or alter its gating. This results in the cell being re-excited leading to inhibition of action potential generation and consequently, paralysis followed by death (BASF, 2013).

Other probable mechanisms that caused the death of *C. maculatus* in this study could include DNA intercalation, interference of protein biosynthesis and disruption of membrane stability in the insect by the plant's allelochemicals. A study by Wink *et al.* (1998) showed that there is a positive correlation between the degree of DNA intercalation and inhibition of DNA polymerase I, reverse transcriptase and translation at the molecular level and with toxicity against insects at organismic level. Alkaloids can also affect the anti-oxidant system in insects (Chowanski *et al.,* 2016). They generate reactive oxygen species (ROS) which induce metabolic stress in cells. Consequently, ROS cause processes such as the peroxidation of membrane lipids, protein damage or the disruption of mitochondrial membrane potential. Potatoes contain steroidal glycoalkaloids (α-solanine and α-chaconine) that act as phytoalexins. They protect potatoes against insect attack, fungi and phytopathogens (Omayio *et al.*, 2016). Adamski *et al.* (2014) evaluated and confirmed oxidative damage by α-solanine from fresh potato leaves on *Galleria mellonella* (L.).

5.1.3 Oviposition deterrence of organic leaf extracts of *G. glauca* on *C. maculatus*

All the four *G. glauca* leaf extracts caused a significant reduction in oviposition by *C. maculatus*. The egg-laying suppression varied depending on the extract and concentration. Oviposition deterrence caused by all extracts except the methanolic extract was concentration dependent. The higher concentration of 10g/100ml was effective in suppressing oviposition as compared to lower concentrations. This trend was in agreement with previous studies by Adedire and Lajide (1999), Adedire and Akinneye (2004) and Emeasor *et al.* (2005), who showed that there was a strong correlation between extract dosage and oviposition deterrence in bruchids on legumes.

In this study, the ethyl acetate extract at test doses of 6g/100ml, 8g/100ml and 10g/100ml were the most effective *G. glauca* extracts in deterring *C. maculatus* egg laying. The effectiveness of this extract in deterring oviposition could be linked to its significant ability to induce mortality on the adult *C. maculatus*. On that account, the high mortality of this extract affected the number of adult female weevils capable of laying eggs. In addition, exposure of extracts to adult *C. maculatus* could have weakened the insects and hindered maximum copulation. Consequently, there was a reduction in oviposition by the female weevils. This operative mechanism was postulated by Ofuya (1990).

The effectiveness of DCM extract at test concentrations of 6g/100ml, 8g/100ml and 10g/100ml as ovipositional deterrents could be attributed to the application of these extracts as contact insecticides. The consistency and texture (sticky nature) of these extracts probably inhibited movement of the weevils in the study. The stifling effect of the extracts could have affected sexual communication among the insects. This eventually could have led to the disruption of mating activities hence low production of eggs by *C. maculatus.* Previous studies in harmony with this finding include studies by Adedire and Lajide (1999), Adedire (2002), Emeasor *et al.* (2005) and Ileke *et al.* (2013). In addition, it could be speculated that the ovipositional deterrence in this study could be due to the effects of the extracts that reduced mating competition of male weevils for females. The extracts could also reduce the receptiveness of females for males. These lead to reduced mating by weevils hence decrease in egg production. A study by Ofuya and Osadahun (2005) reports that *E. aromatic* lessened both the receptiveness of female *C. maculatus* for male and the mating competition of male for female in this weevils.

5.1.4 Inhibition of F1 progeny emergence activities of organic leaf extracts of *G. glauca* on *C. maculatus*

The ethyl acetate and DCM extracts demonstrated the highest inhibition of progeny emergence on *C. maculatus*. The low concentrations of 4g/100ml of these two extracts achieved inhibition rate greater than 50%. The blend extract also exhibited a high inhibition of 84.03% at its highest dosage of 10g/100ml. The significant suppression of progeny development by these *G. glauca* extracts could be ascribed to a number of factors. The leaf extract of *G. glauca* could possess larvicidal as well as ovicidal properties. The eggs of the weevils have a short funnel-like structure at their posterior ends (Credland, 1992). Consequently, the metabolically toxic *G. glauca* extracts may have penetrated the eggs and chemically induced death of the egg or the first instar larvae of *C. maculatus* (Don-Pedro, 1990). Further, the extracts could have physically occluded this funnel-like feature on the weevils' eggs. Such occlusion cuts off the supply of gas to the larvae. The death of the developing embryo, therefore, may have occurred due to asphyxiation (Credland, 1992). A study conducted by Nethravathi *et al.* (2009) to assess the phytochemicals present and larvicidal capability of methanolic, chloroform, ethyl acetate, acetone and petroleum ether extracts of *Gnidia glauca* showed that this plant species possessed larvicidal potential against *Aedes aegypti*.

Another possible mechanism of progeny suppression is that *G. glauca* extracts could have disrupted the hormonal and biochemical processes hence interference with the normal embryonic and post-embryonic development of the weevil. Phytochemicals from different plant species have been reported to hold significant effects on insects molting process (Ge *et al.*, 2015). Ecdysis is a complex physiological process that occurs periodically in insects under tight hormonal regulation (Nation, 2008). A delicate balance of molting hormones (prothoracicotropic hormone, molting hormone, and juvenile hormone) must be sustained for proper metamorphosis and insect development (Cui, 2008). A compromise in molting hormones synthesis and secretion may affect insect transition to adulthood. Some plant toxins can act as Juvenile Hormone mimics, inhibitors of chitin biosynthesis, ecdysone receptors agonists/antagonist (Dinan *et al.,* 2001) and even inhibitors of Acetyl CoA carboxylase, thereby, interfering with proper insect metamorphosis. As a consequence, pupation and adult emergence is altered or inhibited, resulting in progeny suppression as observed in this study.

A study conducted by Ge *et al.* (2015) demonstrated effects of alkaloids (antofine N-oxide and tylophorin) from *Cynanchum mongolicum* on the molting hormone of *S.litura* larvae. Higher dosages of alkaloids resulted in higher mortality and greater disruption of development, 72 hours post-treatment. Treatment of *S. litura* with 800mg/L of *C. mongolicum* extract resulted in more than half of the pupae not molting into adults. Sun *et al.* (2012) further inform that treatment of larvae with alkaloids elongated the insect development time from the 3rd instar to emergence.

5.1.5 Repellence activities of organic leaf extracts of *G. glauca* on *C. maculatus*

The four organic leaf extracts of *G. glauca* demonstrated repellence activities on *C. maculatus.* The concentrations of the four extracts were inversely proportional the repulsion of the insects. An increase in extract concentration resulted in a decrease in the repulsion of *C. maculatus*. None of the concentrations in the four extracts attained a percentage repulsion greater than 50%, except for methanolic extract at 2g/100ml. The low repulsive activity of these four extracts is an indication of low extraction of phytochemicals with repellent activity by the extract solvents. In this study, *G. glauca* extracts served as attractants to the weevils as indicated by the repellency index. The organic extracts of *G. glauca* could be rich in phytochemicals that are natural attractants to *C. maculatus*. Terpenes from ponderosa pine bark have been characterized as attractants to bark beetle (*Ips confusus*) and iso-thiocyanates from the seeds of crucifera are attractants to insects seeking food and site for oviposition (Mahulikar and Chavan, 2007).

The phytochemicals responsible for attraction or repulsion of insects act by stimulating olfactory receptors (Rajashekar, 2012). They act as allomones, kairomones, or synomones during the plant-insect interaction interface (Metcalf and Kogan, 1987). Insects have a coded pattern of activity of the quality and quantity of semiochemical in complex mixtures present in the environment in multiple olfactory receptor cells (ORCs) in their antennae. In response to an emission of an odor substance, the chemical message is decoded and integrated into the olfactory centers of the central nervous system (CNS). This encounter ultimately causes olfactory induced changes in the behavior of the insects (Eisner and Meinwald, 1995). The attraction of the *C. maculatus* to *G. glauca* leaf extracts in this study could have employed this mechanism of action.

The findings of this study mirrored the observation by Prabu Seenivasan *et al.* (2004), who reported that ethyl acetate extract of *Citrullus colocynthis* exhibited a higher repellent activity against cowpea weevil (*C. maculatus*) at lower concentrations. Another research by Kosini and Nukenine (2017) on evaluation of insecticidal activity of *Gnidia kaussiana* (Thymeleaceae) against *Callosobruchus maculatus* in stored *Vigna subterranean*, showed that hexane extract of this plant demonstrated average repellency against *C. maculatus*.

5.1.6 Qualitative phytochemical screening of organic leaf extracts of *G. glauca*

In this study, the qualitative phytochemical analysis showed the presence of secondary metabolites in the four organic extracts of *G. glauca*. These phytochemicals included phenols, cardiac glycosides, terpenoids, flavonoids, tannins, saponins and alkaloids.

Plants secondary metabolites have previously been implicated in the insecticidal properties of plants. The anti-insect properties of phytochemicals are attributed to their multiple modes of action and to the synergistic activities among constituents. Saponins act as insect growth regulators. They alter insect growth and development at various stages. Fabaceae saponins have been reported to cause a reduction in the rate of adult emergence in *Callosobruchus chinensis* (Weissenberg *et al.,* 1998). Saponins also possess cytotoxic activities against insects. Aginoside, a steroidal saponin, caused mortality on the larvae of *Acrolepiosis assectella* while saponins of *Quillaja saponaria* possess larvicidal activities against the larvae of *Aede*s *aegypti* and *Culex pipiens* (Chaieb, 2010).

Terpenes are primary constituents of essential oils. They are linked to the insect repellent or attractant. Monoterpenes such as eugenol, limonene, camphor and thymol, commonly found in basil, have strong repellent activities against insects (Yang *et al.,* 2004). Further, Celangulin-V, sesquiterpenes from the root bark of Chinese bittersweet, *Celastrus angulatus* Max, possess toxicity effects against *Mythimna separate* (Zhang *et al.,* 2011). Flavonoids have also been associated with anti-insects properties. Rotenone is an insecticidal isoflavonoid extracted from the roots, seeds, stem, and leaves of

Leguminosae plants *Derris, Tephrosia* and *Lonchocarpus*. Rotenone and its derivatives are used against bean beetles, apple and pea aphids, corn borer and household pests (Kuol and Dhaliwal, 2001).

Alkaloids, phenols and cardiac glycosides have also been reported to possess insecticidal activities. Alkaloids from extracts of *Pergularia tomentosa* exhibited larvicidal effects against fifth instar larvae of migratory locust, *Locusta migratoria* (Acheuk, 2013). In addition, a study by Wójcicka (2010) shows that cereal phenolic compounds of Triticale (*Triticosecale Wittmack*) possess anti-insect activities against cereal aphids. The total phenols and o-dihydroxyphenols present in triticale caused a reduction in fecundity of the aphid. Other studies have also shown phenolic compounds of basil to have ovicidal, larvicidal and nymphicidal properties (Belong *et al.,* 2013). Therefore, the four determined bio-activities; contact toxicity, oviposition deterrence, inhibition of progeny emergence and repellence of *C. maculatus* by the four organic leaf extracts of *G. glauca* could have occurred due to the actions of these phytochemicals. It is, therefore, not a wonder that *G. glauca* is used against cowpea weevil.

5.2 Conclusions

The findings of this research study revealed that:

i. The four selected organic leaf extracts of *G. glauca* demonstrated contact toxicity activities against *C. maculatus* with ethyl acetate extract at test concentration of 10g/100ml eliciting the highest activity.

ii. The dichloromethanolic and ethyl acetate extracts at the test doses of 6g/100ml, 8g/100ml and 10g/100ml demonstrated the most effective oviposition deterrent activities on *C. maculatus*.
iii. The ethyl acetate extracts (6g/100ml, 8g/100ml and 10g/100ml) and DCM extracts (8g/100ml and 10g/100ml) demonstrated the highest inhibition of progeny emergence on *C. maculatus*.
iv. The four organic leaf extracts of *G. glauca* demonstrated attraction potential to *C. maculatus.*
v. The extracts possessed phytochemicals that have previously been implicated in the anti-insect properties of plants.

5.3 Recommendations

i. The use of the four *G. glauca* organic extracts in the control of *C. maculatus*.
ii. The use of concentrations higher than 10g/100ml of *G. glauca* organic extracts for effective control of *C. maculatus*.
iii. The use of the four *G. glauca* organic extracts as insect attractants rather than repellents.

5.4 Suggestions for further studies

i. Evaluation of possible mechanisms of contact toxicity, oviposition deterrence and inhibition of F1 progeny emergence by the organic leaf extracts of *G. glauca*.
ii. Bioassay-guided isolation and purification of active phytochemicals of leaf extracts of *G. glauca* with the aim of identifying which specific phytochemicals have potential anti-insect properties.

iii. *In vivo* assessment of the toxicological potential of the four organic leaf extracts of *G. glauca*.
iv. Determination of the effects of *G. glauca* extracts on seed quality, viability and organoleptic properties are recommended.

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