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Activity of *Melia volkensii* extracts and isolation of its bioactive constituents against insect pests of economic importance

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ABSTRACT

Sweet potato weevil, *Cylas puncticollis,* fall armyworm, *Spodoptera frugiperda,* red flour beetle, *Tribolium castaneum* are insect pests of economic importance infesting sweet potato, maize and stored grain, respectively. Synthetic pesticides have been largely used by farmers to control these insect pests. However, the potential health and environmental risks associated with synthetic pesticides, coupled with their non-selectivity and pest resistance have led to renewed interest for safer alternative insect control products. Botanical pesticides have emerged as promising alternatives due to their non-persistence, high selectivity and low mammalian toxicity.

This study evaluated the potential of *Melia volkensii,* a tree species native to the drylands of East Africa, as a potential source of botanical pesticide. In vitro analysis of methanolic extracts of various plant parts of *M. volkensii* showed that the bark, leaves, nuts and pulp extracts all had antifeedant activity against the pest insect species tested. The nut and pulp extracts had higher antifeedant index (AFI) against *C. puncticollis* (nut; AFI=51%, pulp; AFI=51%)*, S. frugiperda* (nut; AFI=57%, pulp; AFI=58%) and *T. castaneum* (nut; AFI=71%, pulp; AFI=46%) at 20mg/mL. This study then investigated the effectiveness of nut and pulp extract in protecting sweet potato, maize crops and stored grains in practical conditions in greenhouse and simulated storage. Sweet potato plants in greenhouse treated with nut extracts showed the lowest tuber damage by *C. puncticollis* (18%) as compared to pulp extracts (30%), positive control (commercial insecticide; 33%) and negative control (76%). The nut extracts, pulp extracts and positive control significantly reduced maize leaf and whorl damage by *S. frugiperda* in the greenhouse as compared to the negative control ($p \leq 0.0001$). In simulated storage to evaluate protection of stored grains against *T. castaneum*, the nut powder and positive control (commercial insecticide) significantly reduced F1 progeny and larvae emergence after 42 days ($p \leq 0.0001$). The nut powder and positive control caused 66% and 96% mortality, respectively. On the other hand, the pulp powder did not offer protection of stored grains against *T. castaneum*. This study also employed a bioactivity-guided strategy to isolate and identify insect antifeedants from *M. volkensii*. Sequential fractionation of the crude extracts using n-hexane, dichloromethane, ethyl acetate and butanol showed that the dichloromethane fraction of nut and pulp had higher antifeedant activity. These fractions were purified by column chromatography, and in turn the selected bioactive fractions from the column were subjected to preparative-HPLC, preparative-TLC and recrystallization for further purification. This yielded three pure compounds which were elucidated and confirmed using NMR and LC-MS as reduced meliavolkenin, toosendanin and salanninolide. Reduced meliavolkenin did not show any antifeedant activity, while toosendanin showed strong activity against *T. castaneum* and *S. frugiperda* with a respective EC_{50} value of 0.4 mg/mL and 0.3 mg/mL Salanninolide also recorded high antifeedant activity against *T. castaneum* and *S. frugiperda* with a respective EC_{50} value of 1 mg/mL and 0.2 mg/mL. However, toosendanin and salanninolide had no activity against *C. puncticollis*.

In conclusion, this study confirmed the potential of *M. volkensii* as a source of botanical extracts for the control of pest insects based on laboratory and greenhouse experiments. In addition, it provided new leads which could be used in the formulation of new pesticides and shows that *M. volkensii* extracts could be incorporated in integrated pest management (IPM).

SAMENVATTING

De zoete aardappelkever, *Cylas puncticollis*, de trekrups, *Spodoptera frugiperda*, en de kastanjebruine rijstmeelkever, *Tribolium castaneum* zijn schadelijke insecten van groot economisch belang die omvangrijke schade kunnen veroorzaken in respectievelijk zoete aardappel, maïs en opgeslagen graan. Boeren gebruiken merendeels synthetische pesticiden om deze insectenplagen te bestrijden. De potentiële gezondheids- en milieurisico's die samenhangen met synthetische pesticiden, in combinatie met hun niet-selectiviteit en de ontwikkeling van insecticide-resistentie, hebben ertoe geleid dat er een hernieuwde belangstelling is voor veiliger, alternatieve insectenbestrijdingsproducten. Botanische pesticiden zijn naar voren gekomen als een veelbelovend alternatief vanwege hun niet-persistentie, hoge selectiviteit en lage toxiciteit voor zoogdieren.

Deze studie onderzocht het potentieel van *Melia volkensii*, een inheemse boomsoort in de droge gebieden van Oost-Afrika, als een potentiële bron van botanisch pesticide. In vitro biotoetsen met methanol extracten van verschillende plantendelen van *M. volkensii* toonden aan dat de schors, bladeren, noten en pulpextracten allemaal een anti-voedselactiviteit hadden tegen de geteste insecten. De noten- en pulpextracten hadden een sterkere activiteit tegen *C. puncticollis* (noot = 51%, pulp = 51%), *S. frugiperda* (noot = 57%, pulp = 58%) en *T. castaneum* (noot = 71%, pulp = 46%) bij een concentratie van 20 mg/mL. Deze studie onderzocht vervolgens de effectiviteit van noten- en pulpextracten bij het beschermen van zoete aardappel, maïsgewassen en opgeslagen granen in praktische omstandigheden in de serre en gesimuleerde opslag. De zoete aardappelplanten in de serre en behandeld met notenextracten vertoonden de laagste knolschade door *C. puncticollis* (18%) in vergelijking met pulpextracten (30%), de positieve controle (commercieel insecticide; 33%) en negatieve controle (76%). De notenextracten, pulpextracten en positieve controle verminderden significant de schade aan het maïsblad en de -kolf door *S. frugiperda* in vergelijking met de negatieve controle ($p \leq 0{,}0001$). Bij gesimuleerde opslag om de bescherming van opgeslagen granen tegen *T. castaneum* te evalueren, verminderden het notenpoeder en de positieve controle (commercieel insecticide) de opkomst van de F1-generatie en larven na 42 dagen ($p \leq 0{,}0001$). Het notenextract en de positieve controle veroorzaakten respectievelijk 66% en 96% afdoding. Anderzijds bood het pulppoeder geen bescherming van opgeslagen granen tegen *T. castaneum*. Deze studie maakte ook gebruik van een door bioactiviteit geleide strategie om de insecticidale componenten in de extracten van *M. volkensii* te isoleren en te identificeren. Sequentiële fractionering van de ruwe extracten met n-hexaan, dichloormethaan, ethylacetaat en butanol toonde aan dat de dichloormethaanfractie van noten en pulp een hogere anti-voedingsactiviteit had. Deze fracties werden gezuiverd met behulp van kolomchromatografie en op hun beurt werden de geselecteerde biologisch actieve fracties van de kolom onderworpen aan preparatieve HPLC, preparatieve TLC en herkristallisatie voor verdere zuivering. Dit leverde drie zuivere verbindingen op die met NMR en LC-MS werden opgehelderd en bevestigd als gereduceerd meliavolkenine, toosendanine en salanninolide. Gereduceerd meliavolkenine vertoonde geen anti-voedingsactiviteit, terwijl toosendanine een sterke activiteit vertoonde tegen *T. castaneum* en *S. frugiperda* met een respectievelijke EC_{50} waarde van 0,4 mg/mL en 0,3 mg/mL. Salanninolide had ook een hoge anti-voedingsactiviteit

tegen *T. castaneum* en *S. frugiperda* met een respectievelijke EC_{50} waarde van 1 mg/mL en 0,2 mg/mL. Toosendanin en salanninolide vertoonden echter geen activiteit tegen *C. puncticollis*.

In conclusie, deze studie bevestigde het potentieel van *M. volkensii* als bron van botanische extracten voor de bestrijding van plaaginsecten op basis van laboratorium- en serre-experimenten. Bovendien leverde het nieuwe aanwijzingen op die zouden kunnen worden gebruikt bij de formulering van nieuwe pesticiden en toonde het aan dat extracten van *M. volkensii* kunnen worden opgenomen in geïntegreerde plaagbestrijding (IPM).

MUHTASARI

Mdudu wa viazi vitamu, *Cylas puncticollis*, viwavi jeshi, *Spodoptera frugiperda* na mende mwekundu wa unga, *Tribolium castaneum* ni wadudu waharibifu wenye umuhimu kiuchumi wanaoambukiza viazi tamu, mahindi na nafaka iliyohifadhiwa mtawalia. Kemikali sumu zimetumiwa sana na wakulima kudhibiti wadudu hawa. Hata hivyo, hatari za kiafya na kimazingira zinazohusiana na viuatilifu sanisi, pamoja na kutobagua kwao na upinzani wa wadudu kumesababisha hamu mpya ya bidhaa salama za kudhibiti wadudu. Viuwa wadudu vya mimea vimeibuka kuwa suluhu mbadala kwa sababu ya kutodumu, ubaguzi wa juu na viwango vya sumu ilio chini kwa mamalia.

Utafiti huu ulitathmini uwezo wa *Melia volkensii*, aina ya miti asilia katika maeneo kavu ya Afrika Mashariki, kama chanzo cha uwezekano wa dawa ya mimea. Uchanganuzi wa ndani wa dondoo za methanoliki za sehemu mbalimbali za mmea wa *M. volkensii* ulionyesha kuwa dondoo kutoka kwa gome, majani, karanga za tunda na majimaji ya *M. volkensii* yote yalikuwa na sifa muhimu za kuzuia kula haswa kwa spishi za wadudu waliofanyiwa majaribio. Madondoo ya karanga za tunda na massa yalikuwa na kiashiria cha kutolisha (AFI) zaidi dhidi ya *C. puncticollis* (karanga za tunda; AFI=51%, massa; AFI=51%), *S. frugiperda* (karanga za tunda; AFI=57%, massa; AFI=58%) na *T. castaneum* (karanga za tunda; AFI=71%, massa; AFI=46%) katika kiwango cha ukolezi cha 20 mg/mL. Utafiti huu kisha ulichunguza ufanisi wa karanga za tunda na massa katika kulinda viazi tamu, mazao ya mahindi na nafaka zilizohifadhiwa katika hali tendo katika nyumba ya kijani na uhifadhi wa kuigiza. Mimea ya viazi tamu katika nyumba ya kijani iliyotibiwa kwa dondoo za karanga za tunda ilionyesha uharibifu mdogo wa kiazi kwa *C. puncticollis* (18%) ikilinganishwa na dondoo za massa (30%), udhibiti chanya (kiua wadudu cha kibiashara; 33%) na udhibiti hasi (76%). Madondoo ya karanga za tunda, massa na udhibiti chanya ulipunguza kwa kiasi kikubwa uharibifu wa majani ya mahindi na mkuyu unaofanywa na *S. frugiperda* kwenye nyumba ya kijani ikilinganishwa na udhibiti hasi ($p \leq 0.0001$). Katika hifadhi igiza ili kutathmini ulinzi wa nafaka zilizohifadhiwa dhidi ya *T. castaneum*, poda wa karanga za tunda na udhibiti chanya (kiua wadudu cha kibiashara) ulipunguza kwa kiasi kikubwa uzao wa kizazi chanya kwanza na kuibuka kwa mabuu baada ya siku 42 ($p \leq 0.0001$). Poda ya karanga za tunda na udhibiti chanya ulisababisha vifo vya 66% na 96%, mtawalia. Kwa upande mwingine, poda wa massa haukulinda nafaka zilizohifadhiwa dhidi ya *T. castaneum*. Utafiti huu pia ulitumia mkakati unaoongozwa na shughuli za kibayolojia kutenga na kutambua vizuia wadudu kutoka kwa *M. volkensii*. Ugawaji mfuatano wa dondoo ghafi kwa kutumia n-hexane, dichloromethane, ethyl acetate na butanol ulionyesha kuwa sehemu ya dichloromethane ya karanga za tunda na massa ilikuwa na shughuli ya juu ya kizuia kula kwa wadudu. Sehemu hizi zilisafishwa kwa kromatografia ya safu wima, na kisha sehemu kutoka kwa safu zilizo na nguvu dhidi ya wadudu ziliwekwa kwa preparative-HPLC, preparative-TLC na kusawazishwa upya kwa utakaso zaidi. Hii ilitoa misombo 3 safi ambayo ilifafanuliwa na kuthibitishwa kwa kutumia NMR na LC-MS kama reduced meliavolkenin, toosendanin na salanninolide. Reduced meliavolkenin haukuonyesha shughuli yoyote ya kuzuia kula kwa wadudu, wakati toosendanin ilionyesha shughuli kali dhidi ya *T. castaneum* na *S. frugiperda* ikiwa na EC_{50} ya 0.4 mg/mL na 0.3 mg/mL. Salanninolide pia ilirekodi shughuli ya juu

dhidi ya *T. castaneum* na *S. frugiperda* yenye EC_{50} ya 1 mg/mL na 0.2 mg/mL mtawalia. Toosendanin na salanninolide hazikuonyesha shughuli dhidi ya *C. puncticollis*.

Hatimaye, utafiti huu ulithibitisha uwezo wa dondoo za *M. volkensii* kama rasilimali yenye uwezo wa kudhibiti wadudu waharibifu kulingana na majaribio ya maabara na nyumba za kijani. Zaidi ya hayo, imetoa njia mpya ambazo zitaweza kutumika katika uundaji wa viuatilifu vipya na inaonyesha kuwa dondoo za *M. volkensii* zinaweza kujumuishwa katika usimamizi jumuishi wa wadudu (IPM).

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LIST OF ABBREVIATIONS

HPLC	-	High Performance Liquid Chromatography
TLC	-	Thin Layer Chromatography
LC-MS	-	Liquid Chromatography – Mass Spectrometry
NMR	-	Nuclear Magnetic Resonance
SEM	-	Standard Error of Mean
WHO	-	World Health Organization
DDT	-	Dichlorodiphenyltrichloroethane
POPs	-	Persistent organic pollutants
PBO	-	Piperonyl Butoxide
BST	-	Brine shrimp lethality test
MCF-7	-	Human breast carcinoma
H-29	-	Human colon adenocarcinoma
A-549	-	Human lung carcinoma
A-498	-	Human kidney carcinoma
PC-3	-	Prostate adenocarcinoma
PACA-2	-	Pancreatic carcinoma
MIC	-	Minimum inhibitory Concentration
FAW	-	Fall armyworm
GHS	-	Globally Harmonized System of Classification and Labelling of Chemicals
FAO	-	Food and Agriculture Organization
w/v	-	Weight by volume
AFI	-	Antifeedant index
CIP	-	Potato International Center
F1	-	First generation of offspring
IR	-	Inhibition rate
AM	-	Adjusted mortality
ppm	-	Parts per million
Ace	-	Acetone
MeOH	-	Methanol
$CHCl_3$	-	Chloroform
i.d	-	Internal diameter
$CDCl_3$	-	Deuterated chloroform
UV	-	Ultraviolet
MSD	-	Mass Sensitive Detector
COSY	-	Correlated Spectroscopy
HMBC	-	Heteronuclear Multiple Bond Correlation
ESI	-	Electrospray Ionization
^{1}H	-	Proton NMR
^{13}C	-	Carbon NMR
m/z	-	Mass to charge ratio
CD_3OD	-	Deuterated methanol
PPE	-	Personal protective equipment

DCM - Dichloromethane
EtOAc - Ethyl acetate
BuOH - Butanol

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CHAPTER 1

GENERAL INTRODUCTION

Partially adapted from:

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1.1 OVERVIEW OF SYNTHETIC PESTICIDES USE IN CROP PROTECTION

Pesticides are a key component in modern agriculture and have played important role in the management of crop insect pests as they help in reducing crop damage and eventual losses in produce and revenue [1–4]. There are currently more than 800 pesticide active ingredients in a wide range of commercial formulations registered for agricultural use [3]. These pesticides are vital in ensuring food security and economic development [2,5]. Even though over 98% of sprayed pesticides reach their intended target destination, pesticide drift occurs when pesticide particles are suspended in air and carried away by wind to other areas potentially causing pollution [3]. The indiscriminate use of chemical pesticides has led to contamination of water, soil and food sources, poisoning of non-target insects as well as emergence of insect resistance [6–9]. Where pesticides are applied, large-scale development of resistance and negative impacts on non-target organisms have emerged [8].

Excessive and unsafe use of these pesticides has been reported as a potential threat to human and the environment [2,6,9]. For example, deltamethrin and fenitrothion are classified by World Health Organization (WHO) as toxicity class II (moderately hazardous) pesticides, implying that non-safe application may result in health-related risks [2]. Safety measures are largely absent during pesticides application resulting in human poisoning by pesticides in developing regions [2,10]. It is reported that over 25 million farmers face slight poisoning especially in rural areas of developing countries annually resulting in about 180,000 deaths among agricultural workers every year [2,4,11]. Even though the use of appropriate personal protective equipment (PPE) during pesticide application is hardly used by farmers, it is postulated that this practice could reduce poisoning by about 44% [2,11]. Unsafe handling and application of pesticides are major threats to the sustainability of chemical pest control as well as farmers health and environment [11–13]. Pesticide use has also been reported to contribute to pollinators decline, reduced biodiversity, reduced nitrogen fixation and destruction of habitat, especially for birds [3].

Consumption of chemical pesticides even in trace quantities can cause serious and harmful health issues in humans. Organochlorines, organophosphates and carbamates have been reported to cause Alzheimer and Parkinson's diseases [13]. Pesticide exposure has also been reported to cause serious health problems associated with skin, eye, internal organs, congenital disorders, and overstimulation of neurotransmission system [4,5,11]. Respiratory problems, mental disorders, soft tissues sarcoma, multiple myeloma, non-Hodgkin lymphoma and abnormal cholinesterase levels and musculoskeletal disorders have also been reported in farmers exposed to pesticides [3,5,10]. The most harmful effects of chemical pesticides in humans are their carcinogenic effects as they can cause leukaemia, brain cancer, thyroid cancer, bladder cancer and prostate cancer [13,14]. The advent of Maximum Residue Limits (MRL) in food and horticultural products for export have stimulated intensive search for alternative insect control products which are safer and environmentally acceptable [8,12,15]. Moreover, some pesticides such as Aldrin, DDT, Endrin, Dieldrin, Heptachlor, Mirex and Chlordane are persistent organic pollutants (POPs) since they are non-biodegradable and accumulate in the environment [13].

The drawbacks described above led to the resurgence of botanical pesticides in the 1980s and 1990s as alternative source of insect control products [8,16–22]. While synthetic pesticides have a key role in crop protection as they contribute to crop damage reduction, they need to be judiciously applied by trained personnel especially in case of smallholder farmers to ensure human and environmental safety [1]. It is therefore imperative to incorporate other insect control agents into integrated pest management programs to reduce unnecessary usage of synthetic pesticides [1].

1.2 OVERVIEW OF BOTANICAL PESTICIDES USE IN CROP PROTECTION

The search for alternative compounds for insect control has been extensively directed to the plant kingdom [23]. Botanical pesticides are natural products that are effective against fungi, viruses, nematodes, bacteria and insect pests [1]. Recent research has evaluated several pesticidal plant species confirming that their use could result in comparable yield to when using commercial pesticides without severe environmental damage [24]. Plants synthesise secondary metabolites which act as defensive agents against insects, herbivores and plant pathogens and

plant extract formulations have emerged as alternative eco-friendly strategy for management of noxious insect pests [5,9,25]. This provides impetus for exploitation and development of botanical pesticides, especially in Africa, where there is abundance of pesticidal plants [9]. Botanical insecticides may not only offer effective pest control, but also serve as ecologically sound and economically feasible control option with low health risk to human and environment [26]. In fact, traditionally, farmers have used pest control products of plant origin to manage pests until the advent of synthetic chemicals [8,27].

Botanical pesticides have various modes of action including antifeedant, repellent, growth inhibition, development disruptor, oviposition deterrent, and toxicity against pests [1,7,9,27,28]. They also interfere with insect behaviour, biochemical processes, physiological activities, metabolic pathways and morphology [1]. The repellent effect of botanical pesticides promotes olfactory or other receptors to keep away the target insect pests from treated crops [7]. The antifeedant activity of the botanical pesticides renders treated crops unattractive or unpalatable to insects thereby preventing or disrupting feeding of the insects [7]. Most botanical pesticides do not pose acute toxicity but they exhibit sub-acute effects which limit the spread of insect population [28]. These activities have an overall effect in reducing crop damage by insect pests.

The use of crude plant based materials that are home-harvested and prepared using basic technology is still widespread in Africa [8]. Botanical pesticides are a potential source of insect control products and have proved effective against several insect pests even in crude form [1]. Using unrefined plant extracts generally prevents development of insect resistance due to the presence of multiple bioactive compounds [29]. The expected concentrations of plant toxins to which farmers are likely to be exposed in crude plant materials are typically low and the likelihood of acute toxicity from such materials is substantially lower compared to the risk from handling synthetic pesticides [8]. Even though plant extracts have showed activity in crude form, there is need to optimize pesticidal plants use by identifying their bioactive constituents that might provide models for new insect control products [8]. Natural products, particularly from plant origin, are potentially new insecticides for crop protection and chemical backbones for synthesis of new insecticides [30].

Some of the plant species that have reportedly been used to control insect pests are presented in table 1.1. These examples demonstrate that botanical pesticides can make significant contribution to insect pest management and control. Their activity against a range of insect pests and varied modes of action could limit crop yield losses by minimizing damages while safeguarding human health and the environment. Incorporation of botanical pesticides in integrated pest management (IPM) would likely reduce unnecessary use of synthetic pesticides [1].

Table 1.1: Some of the pesticidal plants against insect pests and their bioactive plant parts

Plant species	**Target insect pest**	**Bioactive plant part**	**Reference**
Fish bean (*Tephrosia vogelii),* bitter leaf (*Vernonia amygdalina),* fever tea (*Lippia javanica),* Mexican sunflower (*Tithonia diversifolia),* black jack (*Bidens pilosa),* Common lantana *(Lantana camara)*	Aphids, field bean pests	Leaves	[31]
Sugar apple (*Annona squamosa)*	Cabbage looper *(Trichoplusia ni)*	Seed	[9]
Thorn apple (*Solanum incanum)*	Termites	Fruit	[8]
White weed (*Ageratum conyzoides)*	Aphids, field bean pests	Flower, all parts	[8]
Wormwood (*Artemisia brevifolia)*	Cabbage butterfly *(Pieris brassicae)*	Not reported	[13]
Violet tree (*Securidaca longipendunculata)*	*Sitophilus spp.,* bruchid	Root bark	[8]
Golden shower tree (*Cassia fistula)*	Cabinet beetle *(Trogoderma granarium)*	Leaf	[28]
Garlic (*Allium sativum)*	Red flour beetle *(Tribolium castaneum)*, bean pod borer *(Maruca vitrata),* bean flower thrips *(Megalurothrips sjostedti),* cabbage looper *(Trichoplusia ni),* aphids, grasshoppers, thrips	Whole plant	[1,13]

Cinnamon (*Cinnamomum zeylanicum),* Chinese cinnamon (*Cinnamomum cassia)*	Thrips	Not reported	[1]
Wrinkled leaf Isodon (*Isodon rugosus),* Kheweshk *(Daphne mucronata)*	Pea aphid *(Acyrthosiphon pisum)*	Aerial part	[32]
Long pepper (*Piper rectofractum),* sugar apple (*Annona squamosa),* Chinese perfume plant *(Aglaia odorata)*	Cabbage cluster caterpillar *(Crocidolomia paronana),* Diamondback moth *(Plutella xylostella)*	Not reported	[1]
Neem tree *(Azadirachta indica)*	Bean pod borer *(Maruca vitrata),* bean flower thrips *(Megalurothrips sjostedti),* Diamondback moth *(Plutella xylostella),* cabbage butterfly *(Pieris brassicae)*	Seeds	[1,13]
Tobacco *(Nicotiana tabacum),* wild mustard *(Sinapsis arvensis),* whitetop *(Cardaria draba)*	Cabinet beetle *(Trogoderma granarium)*	Not reported	[1]
Globe amaranth *(Gomphrena globosa),* Siamese ginger *(Alphina galanga)*	Diamondback moth *(Plutella xylostella)*	Not reported	[13]
Syrian rue (*Pegaum harmala),* herb ivy (*Ajuga iva),* pipe vine *(Aristolochia baetica),* wild radish *(Raphanus raphanistrum),* turmeric *(Curcuma longa)*	Red flour beetle *(Tribolium castaneum)*	Whole plant	[1]
Chinaberry *(Melia azedarach)*	Leaf-miner fly (*Napomyza lateralis),* greenhouse whitefly *(Trialeurodes vaporariorum),* elm leaf beetle *(Xanthogaleruca luteola),* cabbage butterfly *(Pieris brassicae)*	Fruit	[13]
Velvet-fruit Zahna *(Zahna africana)*	Bruchids	Root bark	[8]
Thornapple *(Datura metel),* marvel of Peru *(Mirabilis jalapa),* Mexican marigold *(Tagetes minuta),* pignut *(Hyptis suaveolens),* Common lantana *(Lantana camara)*	Pink bollworm *(Pectinophora gossypiella)*, African pin stemborer *(Sesamia calamistis),* aphids, caterpillars, thrips, bruchid beetle	Not reported	[13]

Laurelwood *(Calophyllum inophyllum),* egg plant *(Solanum indicum),* cinnamon tree *(Cinnamomum verum),* brown mustard *(Brassica juncea),* neem tree *(Azadirachta indica),* butter tree *(Madhuca longifolia),* castor oil plant *(Ricinus communis),* citronella grass *(Cymbopogon nardus),* sesame *(Sesamum indicum)*	Cowpea weevil *(Callosobruchus maculatus)*	Not reported	[28]
Black seed *(Nigella sativa),* jatropha *(Jatropha curcas)*	Cotton bollworm *(Heliothis armigera)*	Not reported	[13]

1.3 CURRENT COMMERCIAL BOTANICAL PESTICIDES

Despite extensive research on efficacy of botanical pesticides against a wide range of insect pests, there are still only a handful in the pesticide market [1,7,33]. One of the major factors contributing to commercialization of botanical pesticides is availability of large quantities of feedstock and ease of cultivation of the source plants [1,7]. Cultivation of source plants requires large areas, thus posing potential competition with food production on arable agricultural land [7]. Botanical pesticides also face competition from synthetic pesticides which are easy to manufacture, have established production facilities, have a long shelf life, are easy to formulate and have ease of application [1]. Botanical pesticides formulation is also a challenging factor as one plant could have several active compounds that differ in chemical properties [1,7]. Even though botanicals have low mammalian toxicity, their regulatory and registration procedures are not different from conventional pesticides. The registration process is expensive and has several barriers making them rather unavailable in the market [1,33]. The botanicals' stability is dictated by weather conditions since they are easily degraded especially if applied in crude form making them having a short shelf life. The stability and quality of these pesticides are also dependent on the nature of plants used, temperature conditions, solvent system as well as storage medium [1]. Furthermore, extraction requires a range of organic solvents posing problems of pollution and disposal hence calling for better extraction methods. These challenges make most agrochemical companies unwilling to invest in botanical pesticides production [1].

While the use of botanical pesticides has increased in popularity in the recent years, it is important to consider their undesirable ecological and human health impacts. Their detrimental effects on pollinators, aquatic non-target organisms and beneficial arthropods such as parasitoids and predators should be studied [33–41]. Decrease in pollinators reduces agricultural productivity of crops and reduction in natural enemies could have serious consequences that may result in pest population dynamics including resurgence and eruption of secondary pests [37]. Relevant toxicological data of most studied botanical pesticides are not available and therefore their human toxicity, including skin irritations and allergic reaction must be considered for complete analysis of their impact [36,37].

The most commonly used commercial botanical compounds with insecticidal activity are pyrethrins, rotenone and azadirachtin while three others which have had limited use include nicotine, sabadilla and ryania [1,7,9,27,28,42]. Most commercial formulations of these botanicals contain synergists such as piperonyl butoxide (PBO), which prevents the insects from metabolizing the active principle and recovering from poisoning [27]. These commercial botanicals are described below.

1.3.1 PYRETHRINS

Pyrethrum, *Tanacetum cinerariifolium,* is the most predominant botanical accounting for 80% of botanical pesticides market and most of the world's pyrethrum is produced in Kenya [27,43]. Pyrethrins are derived from dried flowers of pyrethrum and its active ingredients are a mixture of 73% pyrethrin I and II, 19% cinerin I and II, and 8% jasmolin I and II as shown in figure 1.1 [13,43,44]. The flowers are ground to powder and extracted with hexane to yield an orange-coloured liquid containing the active principles, with the strongest bioactivity being contributed by pyrethrins I and II [43,44]. Pyrethrins exert toxic effect by disrupting sodium and potassium ion exchange processes in insect nerve fibre thereby interrupting the normal transmission of nerve impulses [3,27,45]. Pyrethrins are effective against most flying insects having a knock-down paralysis upon exposure, while in some insects, hyperactivity and convulsions are common [27,43]. They are also effective against house flies, flour beetles, mosquitoes, sawflies, caterpillars, leafhoppers and aphids [13,42]. The mammalian toxicity of natural pyrethrins is

infinitesimal [27,28]. Natural pyrethrins are unstable in light compared to synthetic derivatives (pyrethroids), a fact that has greatly limited their use outdoors [27]. Pyrethroids, developed in the 1970s and 1980s, are more stable in sunlight and represent one of the rare examples of synthetic pesticides based on a natural product model even though they differ from natural pyrethrins in structure and mechanism of action [46].

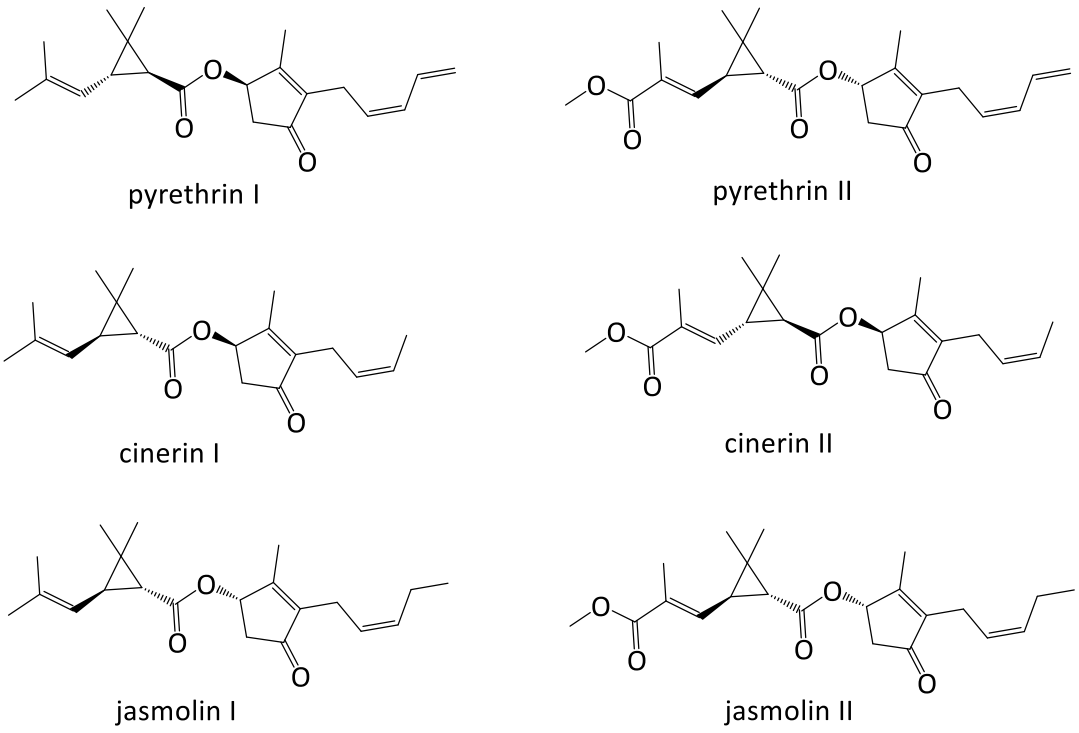


Figure 1.1: Chemical structures of pyrethrin I and II, cinerin I and II and jasmolin I and II

1.3.2 AZADIRACHTIN

Azadirachtin, presented in figure 1.2, is a well-known terpenoid from the neem tree and is used against a broad range of insect pests including stored product pests and cockroaches[13,28,44]. The neem tree was first reported to show insecticidal activity in Sudan when swarming desert locusts defoliated all local flora except for some introduced neem trees [46]. Azadirachtin is the most active principle in neem seeds and was first isolated based on its exceptional antifeedant activity against desert locusts from medium-polarity extracts of neem seeds residue after removal of the oil from the seeds [27]. The neem seed oil typically contains 0.2% - 0.6%

azadirachtin by weight [27,46]. Azadirachtin remains the most potent antifeedant against desert locust to date and has also shown efficacy against over 400 insect pests [3].

At physiological level, azadirachtin blocks synthesis and release of molting hormones (ecdysteroids) from the prothoracic gland leading to incomplete ecdysis in immature insects making an effective growth regulator [27]. In adult females, similar action leads to sterility. Moreover, azadirachtin is a potent antifeedant, repellent and oviposition deterrent to many insects [13,27,45]. Azadirachtin is nontoxic to mammals, fish and pollinators but like pyrethrins, it is rapidly degraded by sunlight [27,5]. It has systemic action in crops thereby greatly enhancing its efficacy and field persistence [27]. Azadirachtin is most effective against sucking insects such as white flies, jassid, mites among others [13,42].

Despite its strong antifeedant activity, the commercial success of azadirachtin has not lived up to its initial hype due to high cost of the refined product and relatively slow action on insect pests [27,45]. In fact, despite the years of research on azadirachtin, this botanical has never achieved registration in United Kingdom, Netherlands, Canada, Hungary, Philippines, Denmark South Africa and Australia [46]. It is however registered in New Zealand, India, Germany, Brazil, United States and Mexico.

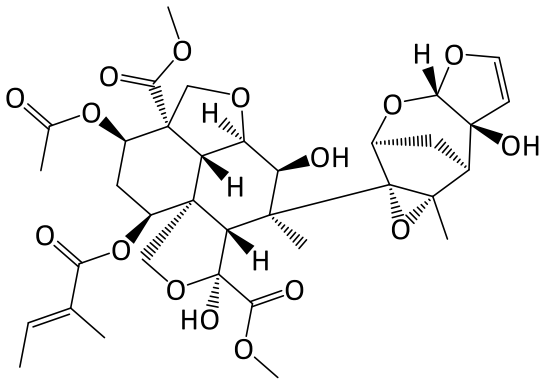


Figure 1.2: Chemical structure of azadirachtin

1.3.3 NICOTINE

Nicotine, shown in figure 1.3, is the oldest alkaloid used in agriculture and is one of the first compounds to be used as insecticide [3]. It is obtained from tobacco plants (*Nicotiana tabacum*) and is effective against various insects including leafhoppers, aphids, whiteflies and thrips [28, 44]. It is a synaptic poison that mimics the neurotransmitter acetylcholine causing symptoms of poisoning similar to those seen in carbamates and organophosphates [27,45]. It is an extremely fast-acting nerve toxin that disrupts nerve impulse activity causing failure of body systems that depend on nervous input for proper functioning [27,46]. Its usage has decreased due to toxicity of nicotine to vertebrates and its rapid dermal absorption in humans had made it to lose regulatory approval in many countries [28,43,47]. For example, Hungary restricts nicotine, while Germany, Denmark and The Netherlands do not permit its use [46]. There is however interest in preparing stable nicotine fatty acid soaps with reduced bioavailability and toxicity to human [27].

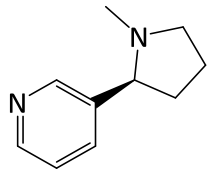


Figure 1.3: Chemical structure of nicotine

1.3.4 ROTENONE

Rotenone, whose chemical structure is presented in figure 1.4, is an isoflavonoid isolated from the roots or rhizomes of tropical leguminous plants *Derris* and *Lonchocarpus spp*, mostly grown in Venezuela and Peru [1,13,27,44]. Extraction of the plant root yields resins containing 45% rotenoids with major constituents being rotenone (45%) and deguelin (22%). It is commonly available as dust but liquid formulations are also available for organic agriculture. Rotenone dusts (containing 1-5% active ingredients) for home and garden use are effective against mites and lice on animals [28,43]. Liquid formulation sprays (containing 8% rotenone and 15% total rotenoids) have been used to control beetles, caterpillars and aphids in plants [43,46]. Rotenone causes malfunctioning of mitochondria thereby blocking the electron transport chain and preventing energy production [3,27,28,43]. In insects, it exerts toxicity on nerve and muscle cells causing

rapid cessation in feeding, and death occurs after several hours or days after exposure [27,45]. Rotenone also acts as contact insecticide, cellular respiratory enzyme inhibitor and stomach poison and its use as agricultural insecticide is limited to organic food production [13,27,43]. Its use as fish poison dates back to over 150 years in water management programs [28,43]. In fact, pure rotenone is comparable to DDT and other synthetic pesticides in terms of acute toxicity in mammals, although it is much less toxic at levels seen in formulated products [27]. Safety concerns have been raised about rotenone due to reports on residues in olive oil [27].

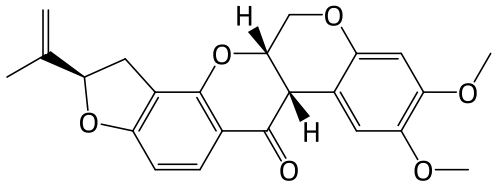


Figure 1.4: Chemical structure of rotenone

1.3.5 SABADILLA

Sabadilla is an alkaloid isolated from seeds of the South American lilly, *Schoenocaulon officinale* and its active principles being cevadine and veratridine (figure 1.5) which typically exist in 2:1 ratio and are collectively referred to as veratrine [3]. The chemical structure of this botanical has potent antifeedant and insecticidal activity against a range of insect pests such as stink bugs, leaf hoppers, caterpillars and harlequin bugs among others [27,42,44–46]. Sabadilla is neurotoxic, slows down sodium channels and disturbs membrane polarization causing paralysis and eventual death [3,27,45,46]. It is a contact and non-systemic pesticide readily degradable in sunlight and is non-hazardous to not-target organisms [3]. It is toxic to mammals, but commercial formulations contain less than 1% active ingredient thus providing a margin of safety [27,28]. Resistance of vegetable and fruit bugs and citrus thrips has become a concern which has, since seen, made application of this botanical pesticide become limited [3,27].

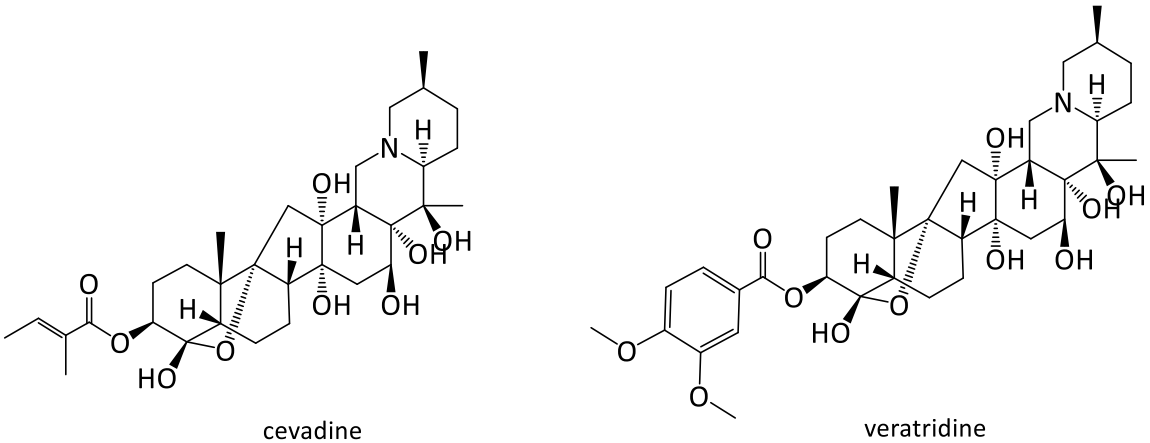


Figure 1.5: Chemical structures of cevadine and veratridine

1.3.6 RYANIA

Isolated from stem wood of the Caribbean shrub, *Ryania speciosa*, the alkaloid ryanodine acts as a muscle poison and also has toxic and growth inhibiting activity [43,46]. Ryania, containing ryanodine presented in figure 1.6, is another botanical in declining use which is effective in protecting field and fruit crops against caterpillars, thrips, moths, earworms, beetles, tobacco budworm and aphids [28,42,46]. The powdered wood contains <1% ryanodine that interferes with calcium release in muscle tissue in insects [27,45]. It is a slow acting stomach poison causing feeding cessation and is reported to be most active in hot weather [27]. Ryanodine also acts against insects by interfering with the nerve impulse at the calcium channel level provoking sustained contraction of the muscles and paralysis [3].

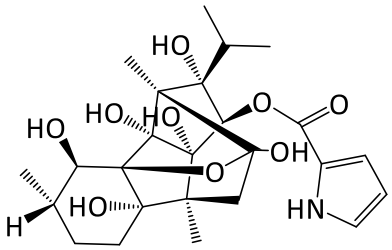


Figure 1.6: Chemical structure of ryanodine

1.4 *Melia volkensii*

Melia volkensii Gürke (Meliaceae) is a tree species that grows in the arid and semi-arid areas of Eastern Africa. This tree is the focus of insecticidal and phytochemical evaluation in this research. This section outlines the occurrence, uses, potential application of the tree in insect control as well as its phytochemical composition.

1.4.1 OCCURRENCE AND USES OF *MELIA VOLKENSII*

M. volkensii is an indigenous multipurpose subtropical deciduous tree (15-25 m), shown in figure 1.7, native to the dryland of Eastern Africa with natural distribution range in Kenya, Ethiopia, Tanzania and Somalia (figure 1.8) [48–51]. *M. volkensii* is a fast-growing tree producing fruits after 4-5 years. It is drought tolerant and is grown for its mahogany termite-resistant timber, firewood, shade, fodder, ornament, agroforestry and is also used by locals to treat a variety of human disorders like skin disorders, diarrhoea and eczema [47,50,52]. Tea prepared from *M. volkensii* bark is used by locals in folk medicine to alleviate pain and is said to be poisonous at high dose [53].

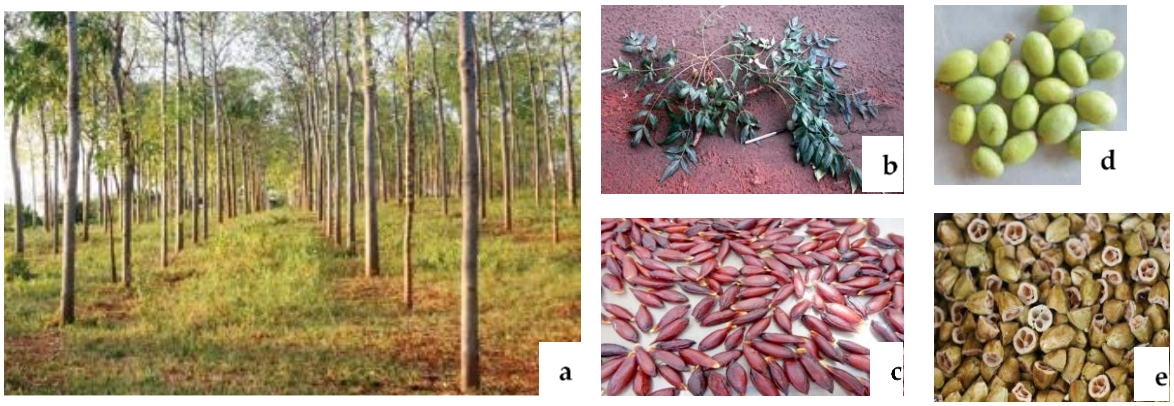


Figure 1.7: *Melia volkensii* and its various parts: (a) 10-year old *M. volkensii* plantation, (b) leaves, (c) seeds, (d) fruits and (e) nuts [50]

M. volkensii leaves and twigs are browsed by goats, cattle, Oryx and contribute to about 20% and 9% of dry and wet season browse respectively of giraffe in Tsavo National Park in Kenya [54]. The resultant cake after ethanolic extraction of *M. volkensii* fruits has proved to be more acceptable and palatable to goats when compared to wheat bran supplement [55]. Its use as fodder

underscores its safety in mammals [56]. The present study evaluates *M. volkensii* as a potential source of antifeedant agents against insect pests of economic importance in Africa.

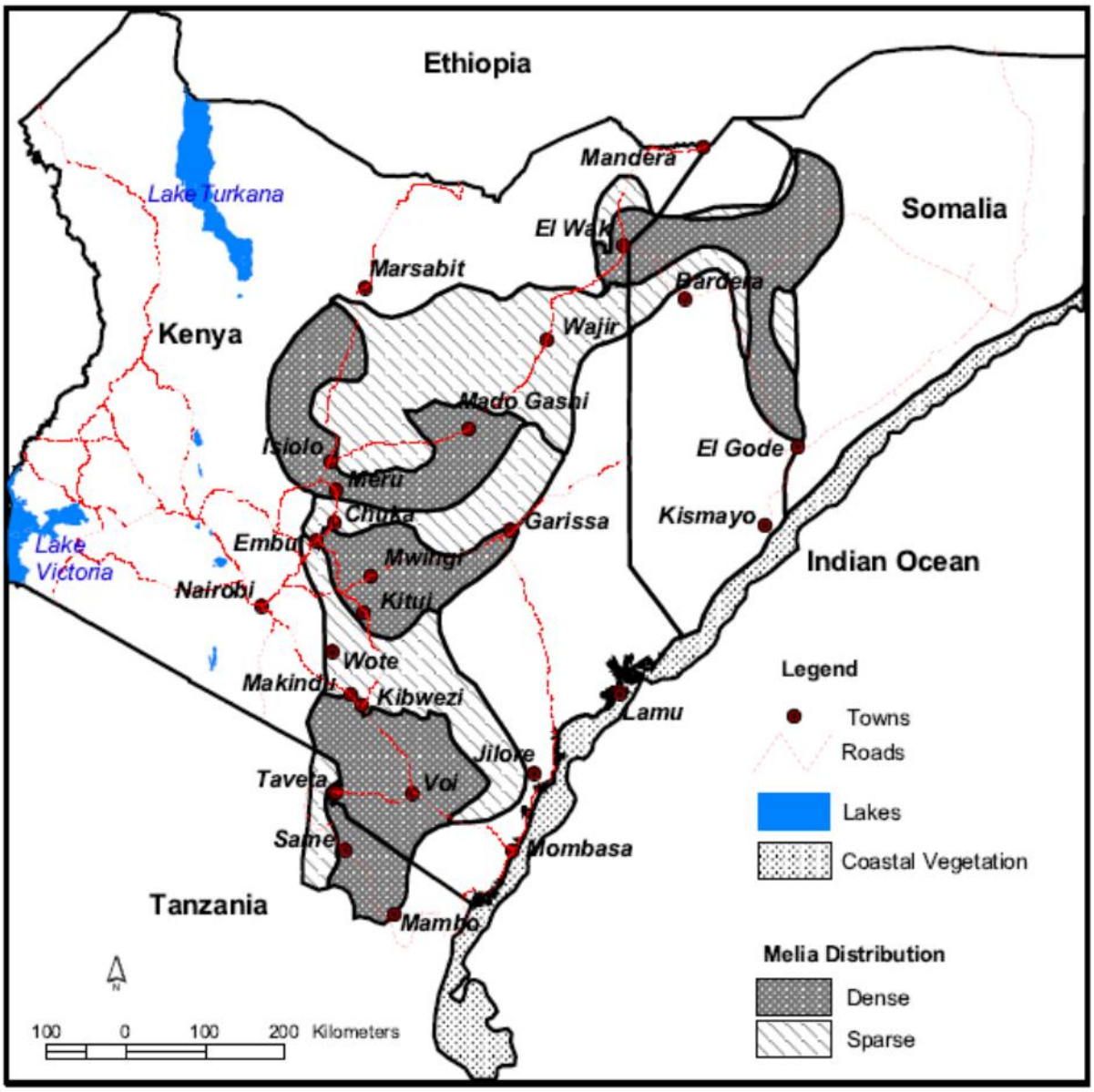


Figure 1.8: *Melia volkensii* distribution in Eastern Africa [50]

1.4.2 APPLICATION OF *MELIA VOLKENSII* IN INSECT CONTROL

Plant species belonging to Meliaceae family have traditionally been used to control insects and have attracted attention due to their range of biological activities against insects as well as their pharmacological benefits to human [55,56]. The Meliaceae plant family is a known source of compounds with insecticidal, growth-inhibition, antifeedant and insect-deforming properties. Six species have been subjected to botanical pesticide evaluation; these include *Azadirachta indica* A. Juss (Indian neem tree), *Azadirachta excelsa* Jack (Philippine neem tree), *Azadirachta siamens* Valeton (Siamese neem tree), *Melia azedarach* Linnaeus (chinaberry), *Melia toosendan* Siebold and Zucc., and *M. volkensii* [57]. Of about 300 known limonoids, about one third are from *A. indica* and *M. azedarach* implying that extensive search for new insecticidal compounds has concentrated in these two species [3,57]. This present study focuses on *Melia volkensii* which is less exploited compared to *A. indica* and *M. azedarach*.

M. volkensii was first reported to contain insect growth-regulating and antifeedant compounds on larvae and adult desert locust, *Schistocerca gregaria* in 1982 [56]. Reduced feeding, poor growth, prolonged intermoult periods and high mortality during ecdysis were observed when 2% of aqueous extract of *M. volkensii* fruit kernel extract was added to desert locust diet [58]. Application of *M. volkensii* extracts slowed down larval development, significantly delayed sexual maturity, reduced fitness, decreased mobility and reduced fecundity in *S. gregaria* [58,59]. Similar effects were observed when seed extract was applied to their preferred host plants mainly *Schouwia thebaica* Webb, *Fagonia olivieri* DC (fagonbush plant) and *Hyoscyamus muticus* Linnaeus (Egyptian henbane) in a field trial experiment [60]. As a result of the near complete loss of mobility and other sub-lethal effects, S. *gregaria* were not able to escape their predators especially birds, wasps, and carabids, while about 30% fell victims of cannibalism [60]. Although the mode of action of the extracts is still unknown, it is postulated that the active compounds in *M. volkensii* extracts could affect hormone levels in *S. gregaria* larvae [61].

Since the first report of insect antifeedant potential of *M. volkensii,* more studies have been done with extracts from fruit and seeds showing promising activity against a variety of insect pests as presented in table 1.2 [62]. In fact, crude fruit extracts from *M. volkensii* have been reported to

pose activity towards a broad range of insect orders including Diptera, Lepidoptera, Coleoptera among others [63].

Table 1.2: Activity of *Melia volkensii* against insect pests

Target Insect	**Biological Activity**	**Plant Part Used**	**Reference**
Desert locust *(Schistocerca gregaria)*	Antifeedant, repellence, growth inhibition, mortality	Fruit	[59,62,63]
Cabbage looper *(Trichoplusia ni)*	Antifeedant, growth inhibition, mortality	Fruit, seed	[63–66]
True armyworm *(Pseudaletia unipuncta)*	Antifeedant, growth inhibition	Fruit, seed	[8,63–65,67]
Diamondback moth *(Plutella xylostella)*	Antifeedant	Fruits	[65,67]
Stink bug *(Nezara viridula)*	Antifeedant, growth disruption, mortality	Fruit	[62]
Coranus arenaceus	Growth inhibition	Fruit	[62]
Mexican bean beetle *(Epilachna varivestis)*	Antifeedant, growth inhibition	Seed	[61,65]
Yellow fever mosquito *(Aedes aegypti)*	Growth inhibition, mortality	Fruit	[55,68]
Mosquito *(Anopheles arabiensis)*	Growth inhibition	Fruit kernel	[57]
Southern house mosquito *(Culex quinquefasciatus)*	Oviposition deterrence, mortality	Fruit	[23,68,69]
London underground mosquito *(Culex pipiens molestus)*	Growth inhibition, mortality	Seed	[69]

M. volkensii extracts have proved to be a feeding deterrent, growth disruptor, slow-acting contact insecticide and topical application causes malformations of wings, antennae, legs and pronotum in southern green stink bug, *Nezara viridula* Linnaeus, a polyphagous pest, which attacks a variety of crops, including nuts, corn, cotton, grains and tomatoes [62]. A delay of the imaginal moult was observed in immature *Coranus arenaceus* Walker even though there were no deformities in resultant adults after topical application of the *M. volkensii* extracts at 1, 5, and 10 µg/µL [62]. Even though ethanolic extracts from *M. volkensii* nut showed low toxicity against

N. viridula (L.) (LC_{50} of 35.23 µg/mL), disruption of the molting process led to eventual death of the insects [62].

Antifeedant and larval growth inhibitory effects of fruit extracts have been observed in *Trichoplusia ni* Hübner (cabbage looper) and *Pseudaletia unipuncta* Haworth (true armyworm) [63,64]. Crude seed extracts are also an effective growth inhibitor against *T. ni* (dietary EC_{50} = 7.6 ppm) and feeding deterrent (DC_{50} = 0.9 µg/cm^2) [66]. Prolonged exposure to *M. volkensii* extracts has been observed to lead to a decrease in antifeedant response when tested against *T. ni* implying that the insect could develop tolerance to the extracts [67]. However, when tested against *Plutella xylostella* Linnaeus (diamondback moth) and *P. unipuncta*, there was no significant decrease in feeding deterrent response to the extracts following continuous exposure [70]. It has been postulated that triterpenoids from seed kernels of *M. volkensii* are responsible for the insecticidal activity in *T. ni* [8].

Comparative efficacy has been observed with *M. volkensii* extracts, other Meliaceae plant extracts (*A. indica, A. excelsa, M. azedarach,* and *Trichilia americana* Sessé & Mocino) and commercial botanical insecticides (ryania, pyrethrum, rotenone and essential oils of rosemary and clover leaf) when tested against *T. ni* and *P. unipuncta* [43]. When applied to cabbage leaf disks in a choice bioassay, *M. volkensii* fruit extract showed potent antifeedant properties against *Epilachna varivestis* Mulsant (Mexican bean beetle) [62]. Growth inhibition has also been observed in *P. unipuncta* (dietary EC_{50} = 12.5 ppm) with refined seed extracts in a leaf disc bioassay [66]. The seed extracts also showed feeding deterrent effects on third-instar larvae of *P. unipuncta* and *P. xylostella,* and adults of *E. varivestis* (DC_{50} = 10.5, 20.7 and 2.3 µg/cm^2, respectively) [66]. In fact, *M. volkensii* seed extracts have been recorded to have stronger antifeedant activity compared to pure allelochemicals: digitoxin, cymarin, xanthotoxin, toosendanin, thymol and *trans*-anethole against *P. unipuncta, P. xylostella* and *E. varivestis* [66]. When applied to *Spodoptera litura* Fabricius, neem, rotenone, *M. volkensii* extract, toosendanin, *Annona squamosal* L. extract and pyrethrum at 1% concentration recorded larval growth (% relative to control) of 4, 98, 26, 48, 61, and 57%, respectively, after 96 h in a comparative study [71].

Fruit kernel extracts of *M. volkensii* have also showed growth inhibiting activity against vector mosquitoes such as *Aedes aegypti, Culex quinquefasciatus* and *Anopheles arabiensis* [23,54]. Investigation of *M. volkensii* fruit extracts against *Aedes aegypti* larvae concluded that *M. volkensii* has acute toxicity at high doses and growth-inhibiting activity at lower doses [72]. Earlier investigations indicated that bioactive compounds from *M. volkensii* could be more potent than azadirachtin, a commercially available botanical pesticide [50,55]. A column chromatography-purified fraction of *M. volkensii* fruit kernel extract showed growth-inhibiting activity at low concentrations while lethal effects were exhibited during ecdysis (LC_{50} of 5.4 µg/mL in 48 h) against *Anopheles arabiensis* Giles with an [57]. Mortality (LC_{50} of 34.72 µg/mL in 48 h) and oviposition deterrence was observed in second-instar larvae of *Culex quinquefasciatus* Say (Southern house mosquito) when treated with refined methanolic fruit extracts [73].

The granular formulation of *M. volkensii* fruit acetone extract showed S- and U-shaped postures and frequent stretching in *C. quinquefasciatus;* such postures and stretching are a characteristic of mosquito larvae reared in *M. volkensii* fruit extract [23]. The test granules also caused 86% mortality in third- and fourth-instar larvae of *C. quinquefasciatus* within 36 h [23]. Acetone extracts from *M. volkensii* seeds have recorded growth inhibitory effects and equal toxicity (LD_{50} of 30 µg/mL) for larvae and pupae of *C. pipiens* f. molestus Forskål (London underground mosquito) [69]. *M. azedarach* seed extracts recorded lower toxicity (LD_{50} of 40 µg/mL), while pure azadirachtin A recorded higher toxicity (LD_{50} of 1–5 µg/mL) against *C. pipiens* when compared with *M. volkensii* extracts [69]. The water solubility of the acetone seed extract from *M. volkensii* may indicate the presence of saponins as toxic principles, thus making it an interesting candidate for application against aquatic insects such as mosquitoes and other vectors of diseases [69]. Seed extracts of *M. volkensii* have also shown antiplasmodial inhibition against *Plasmodium falciparum* [52].

1.4.3 INSECT ANTIFEEDANT COMPOUNDS ISOLATED FROM *MELIA VOLKENSII*

Insect antifeedants have been found in major classes of secondary metabolites – alkaloids, phenolics, and terpenoids [74]. However, it is in the terpenoids that the greatest number and diversity of antifeedants, and the most potent, have been found. Most well-documented antifeedants are triterpenoids [74]. Effective antifeedant compounds have been isolated from *M. volkensii* with activity against a range of insect pests as shown in table 1.3 and figure 1.9. Some of the insect antifeedant compounds isolated from methanolic extracts of *M. volkensii* fruit include 1-cinnamoyltrichilinin, 1-tigloyltrichilinin, 1-acetyltrichilinin, salannin, volkensin, toosendanin and ohchinin-3-acetate [47,50,74–76].

Table 1.3: Insect antifeedants isolated from *Melia volkensii*

Compound	Plant part	Biological Activity	Reference
Volkensin	Seed, fruit	Antifeedant against fall armyworm *(Spodoptera frugiperda)*	[62,77]
Salannin	Seed, fruit	Antifeedant and weight reduction against stiped cucumber beetle *(Acalymma vittata),* house fly *(Musca domestica),* bean ladybeetle *(Epilachna varivestis),* tobacco budworm *(Heliothis virescens),* fall armyworms *(Spodoptera frugiperda),* Egyptian bollworm *(Earias insulana),* rice leafroller *(Cnaphalocrocis medinalis)* and cotton leafworm *(Spodoptera littoralis)*	[77–79]
Toosendanin	Root bark	Growth inhibitor and oviposition deterrent against European corn worm *(Ostrinia nubilalis),* cabbage butterfly *(Pieris brassicae),* cabbage looper *(Trichoplusia ni)*	[47,61]
Meliantriol	Not reported	Antifeedant against desert locust *(Schistocerca gregaria)*	[58]
1-cinnamoyltrichilinin	Not reported	Antifeedant against cotton leafworm *(Spodoptera littoralis)*	[79]
1-tigloyltrichilinin	Not reported	Antifeedant against southern armyworm *(Spodoptera eridania)*	[79]
Nimbolin B	Not reported	Antifeedant against *Spodoptera* species (*exigua, eridania* and *littoralis*)	[79]
Ohchinin-3-acetate	Fruit	Antifeedant	[75]

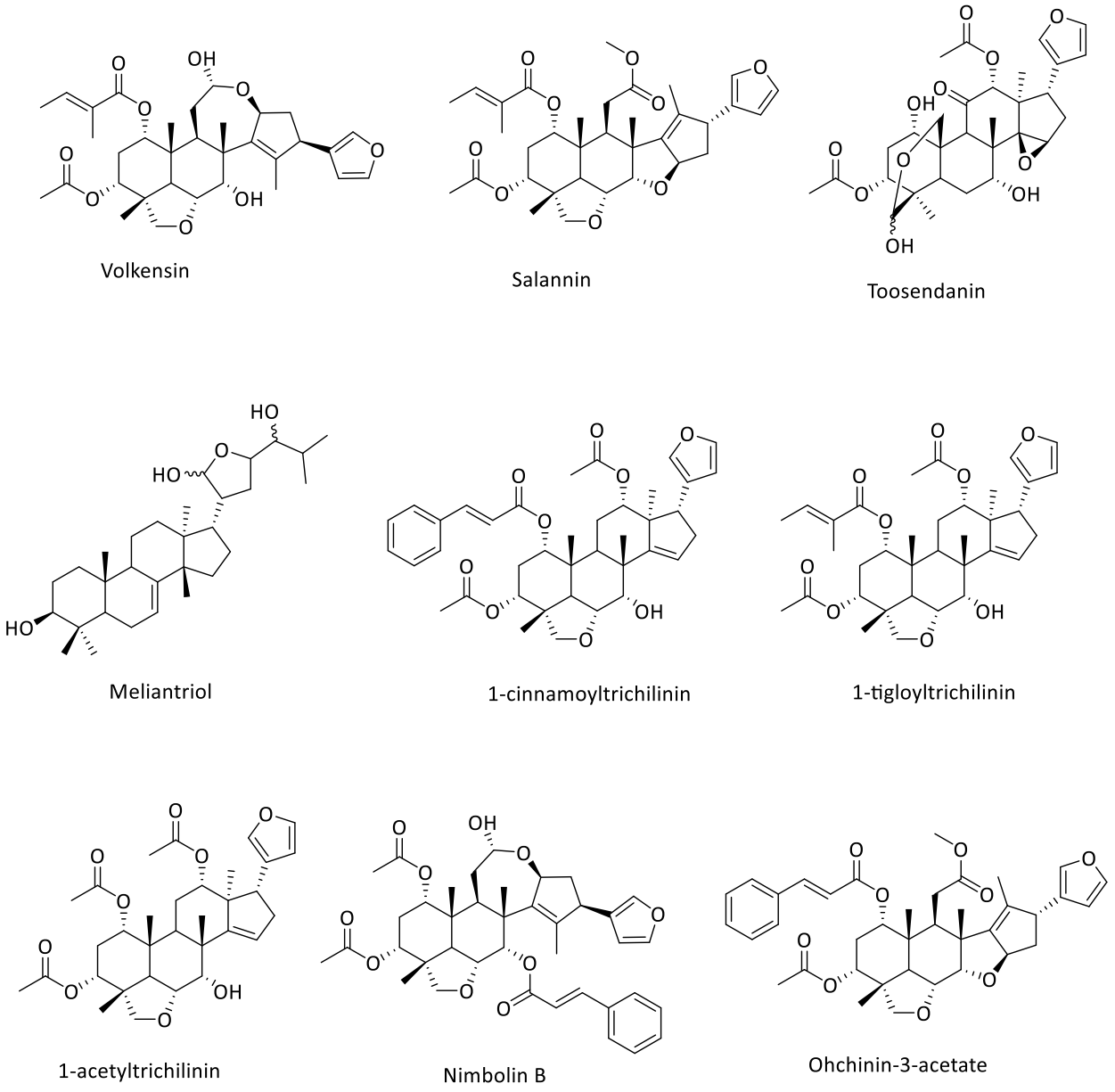


Figure 1.9: Chemical structures of compounds isolated from *Melia volkensii* with antifeedant and growth-inhibition activity against insects

Volkensin, isolated from whole fruits of *M. volkensii* has also shown high activity against *S. frugiperda* with 50% (ED_{50}) feeding inhibition at concentration of 3.5 µg/cm^2 of leaf surface [53]. Salannin has been found to have antifeedant activity against Colorado Potato Beetle, *Leptinotarsa decemlineata* and fall armyworm, *Spodoptera frugiperda* [34,38,41]. Salannin also showed a lower activity with ED_{50} of 13 µg/cm^2 against *S. frugiperda* [53]. Toosendanin, isolated

from root bark and stem bark of *M. volkensii* has also been reported to have antifeedant activity against insects [52]. Toosendanin has been commercialized in China and is an effective growth inhibitor against *Ostrinia nubilalis* (Hübner), a repellent against *Pieris brassicae* (L.) and an oviposition deterrent against *Trichoplusia ni* (Hübner) [62]. *M. volkensii* seed extracts, extracted in cold water, have been reported to contain unsaturated fatty acids (oleic acid, linoleic acid and gadoleic acid) and saturated fatty acids (palmitic acid, stearic acid and arachidic acid) [54]. Fatty acids with at least 18 carbon atoms have been found to synergistically enhance insecticidal activity of insecticides [80]. Oleic acid, linoleic acid, linolenic acid, and ricinoleic acid have enhanced insecticidal activity of organophosphates and carbamates when applied against sucking insects and defoliating insects [80].

1.4.4 PHARMACOLOGICAL ACTIVITY OF *MELIA VOLKENSII*

Further chemical investigations of *M. volkensii* have focused on pharmacological effects of the tree outlining its usefulness in folk medicine for antibacterial and cytotoxic activities [47]. The search for new anticancer compounds from the tree has resulted in isolation of meliavolin, meliavolen, meliavolkin, melianinone, 3-episapelin A, nimbolin B, meliavolkinin, meliavolkensins A and B, melianins A, B and C, 1,3-diacetylvilasinin and meliavolkenin, all of which are active against a range of human tumor cell lines [62,67]. Meliavolkensins A and B, isolated from root bark of *M. volkensii*, have demonstrated moderate activity in Brine Shrimp Lethality Test BST LC_{50} 107 µg/mL and 89 µg/mL, respectively, and comparative cytotoxicity against tumor cell lines MCF-7 (human breast carcinoma), A-549 (human lung carcinoma) and H-29 (human colon adenocarcinoma) [81].

Meliavolkenin, isolated from root bark of *M. volkensii* was reported to be significantly active in BST and showed moderate cytotoxicity against three human tumor cell lines [ED_{50} 10, 4.3 and 0.67 µg/mL in A-549, MCF-7 and HT-549, respectively [82]. (E)-volkendousin has also been isolated from *M. volkensii* and showed good cytotoxicity against six human tumor cell lines [49]. Bioactivity guided isolation of volkensinin from *M. volkensii* using BST showed weak toxicity against six human tumor cell lines [ED_{50} 28, 28, 34, 30, 8.4 and 29 µg/mL in A-498 (human kidney carcinoma), PC-3 (prostate adenocarcinoma), PACA-2 (pancreatic carcinoma), A-549, MCF-7 and

HT-29, respectively] [68]. Pure compounds toosendanin, kulactone and scopoletin have been isolated from root and stem bark. Toosendanin and kulactone recorded activity against *Escherichia coli* with minimum inhibitory concentration (MIC) of 12.5 µg/mL, while against *Aspergillus niger*, they recorded MIC of 6.25 µg/mL [52]. Crude methanolic extracts of *M. volkensii* seeds also demonstrated 99% inhibition at 100 µg/mL against *Mycobacterium tuberculosis* [76]. Radiorespirometry-guided bioassay against *M*. *tuberculosis* led to isolation of 12B-hydroxykulactone, 6B-hydroxykulactone and kulonate which had MIC values of 16, 4 and 16 µg/mL, respectively [76].

Meliavolkinin, melianin B, melianin C and 1,3-diacetylvilasinin, isolated from root bark of *M. volkensii* have all shown marginal cytotoxicity against certain human tumor cell lines [83]. Also isolated from root bark of *M. volkensii* are meliavolin, which showed cytotoxicity to human tumor cell lines (ED_{50} 11.25, 5.34, 0.95 µg/mL to A-549, MCF-7 and HT-29) and meliavolkin which exhibited potency to human cancer cell lines (ED_{50} 0.57, 0.26, 0.12 to A-549, MCF-7 and HT-29, respectively) [82]. Volkensinin, isolated from root bark has also showed weak cytotoxicity against six human tumor cell lines [ED_{50} 27.90, 28.56, 29.55, 8.43 and 28.52 µg/mL in A-498, PC-3, PACA-2, A-549, MCF-7 and HT-29, respectively] [68]. Compounds with cytotoxic ED_{50} values of 4 µg/mL are considered active in search of new anticancer drugs, however, borderline cytotoxicity could be indication of other useful bioactivity [84].

In this study, we aimed to evaluate the potential of *M. volkensii* extracts to effectively protect sweet potato, maize and stored grains against the African sweet potato weevil, *Cylas puncticollis* (Boheman) and the fall armyworm, *S. frugiperda* and red flour beetle, *Tribolium castaneum* Fabricius, respectively. These are major insect pests in sweet potato, maize production, and stored cereals in Africa.

1.5 INSECT PESTS OF ECONOMIC IMPORTANCE IN AFRICA

Food security in Africa is under threat from agricultural insect pests which cause qualitative and quantitative losses in crop yield. Some of the insect pests of economic importance include the fall armyworm, *Spodoptera frugiperda*, African sweet potato weevil, *Cylas puncticollis* (Boheman) and *Tribolium castaneum*, red flour beetle. These are major insect pests which cause significant damage in maize production, sweet potato and stored grains respectively in Africa. This section outlines the insect pests that were selected for this study.

1.5.1 THE FALL ARMYWORM, *SPODOPTERA FRUGIPERDA*

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E Smith) is a lepidopteran insect that undergoes holometabolous metamorphosis and its life cycle includes egg, larvae, pupa and adult as presented in figure 1.10 [85]. The duration of egg stage is about two to three days followed by six instar larval stages of FAW [69,70]. Duration of larval stages is about 14 days in summer and 30 days in cooler conditions averaging about 3.3, 1.7, 1.5, 1.5, 2.0 and 3.7 days from instar 1 to 6, respectively, when reared at 25°C [86].

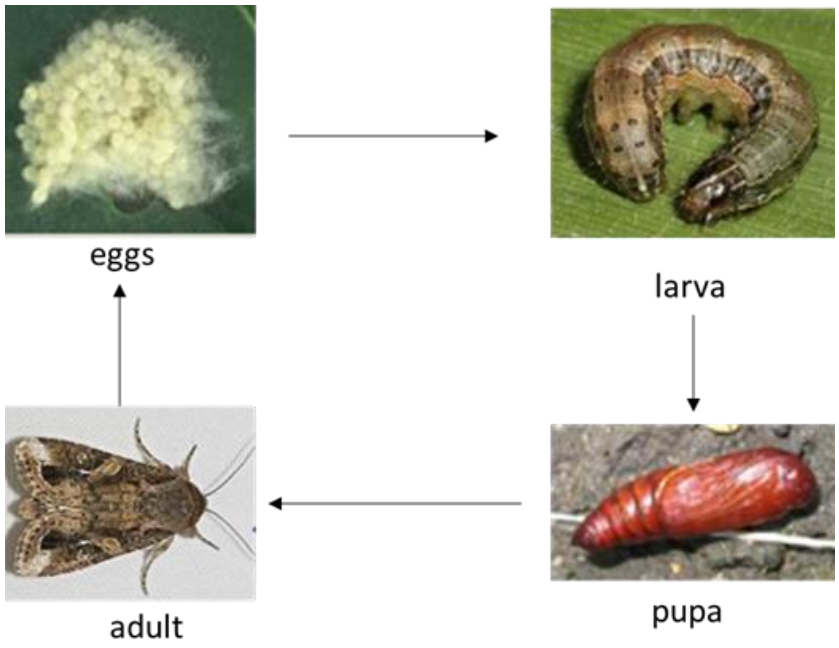


Figure 1.10: Life cycle of *Spodoptera frugiperda* [87]

Pupation then takes place in the soil at depth of 2 to 8 cm where the reddish brown pupa measuring 14 mm to 18 mm length and 4.5 mm width, takes about 8 to 9 days before the adult moth emerges [86]. The moths, having a wingspan of 32 to 40 mm, are nocturnal and are most active during warm, humid evenings and have a lifespan range of about 7 to 21 days [86]. In its lifetime, an adult female FAW averages about 1500 eggs demonstrating high fecundity [83,85]. FAW have a high migratory ability and the moths can cover over 100 km per night during which they can find a broad range of habitats [85]. Its larvae and adult are presented in figure 1.11.

Figure 1.11: Fall armyworm larvae and adult [86]

The FAW is invasive, polyphagous, migratory and causes severe economic damage in maize, millet, sugarcane, vegetable, sorghum, cotton, rice among several other crops in South America, USA, Asia and Africa [83,86,88,89]. It is native to tropical regions and is a key insect pest of maize which was first reported in West Africa in 2016 and has since spread rapidly throughout Sub-Saharan Africa and currently occurs in 44 African countries [89]. This pest feeds on virtually all parts of maize leading to considerable damage and sometimes results in total crop failure [90]. Maize is a primary staple food crop in Africa and recent FAW invasion threatens food security of millions of people in the region [89]. Left unmanaged and in absence of natural predators, its larvae can cause up to 100% yield loss in maize as they heavily feed on maize foliage, ear and tassels [24,85,90].

FAW feeding affects crop growth parameters such as plant height, leaf area, dry weight and FAW density as low as 0.2 - 0.8 larvae per plant during late whorl stage is sufficient to reduce yield by

5-20% [86]. FAW prefers to oviposit in plants at early whorl stage and at this development stage, the plants are less tolerant than plants infested at later stages [86]. These larvae mainly feed on epidermal leaf tissue making holes in the leaves as shown in figure 1.12. In young plants, they also feed on the whorl thereby causing deadheart symptoms, while in older plants, they feed on maize cobs and kernels reducing crop yield and quality [89].

Figure 1.12: Maize leaf damage caused by fall armyworm [86]

There have been deliberate efforts in some countries to promote an integrated approach in control of FAW [91]. The traditional integrated management strategies include monitoring and inspection for timely response, deep ploughing, early planting, planting early maturing varieties, intercropping, rotation with non-host crop, handpicking egg masses and larvae, applying sand, sawdust or soil in the whorl [83,85]. Even though these cultural control strategies have proved 'somewhat successful', there is still over-reliance on synthetic pesticides, which is the primary management strategy for FAW management [85,86,88]. An average of five insecticide sprays per maize cycle may be applied per season [92]. Biological control using natural predators and parasitoids like *Trichogramma pretiosum, Telenomus remus, Campoletis sonorensis, Chelonus insularis, Diapetimorpha introit* and *Ichneum promissorius* has also been reported [85]. Presence of insect predators for both egg and larvae keep FAW populations under control. Earwigs *Doru*

lineare, *Doru luteipes, Picromerus lewisi, Arma chinensis* and bugs such as *Eocanthecona furcellata and Andrallus spinidens* have been found to effectively prey on FAW eggs and larvae [85]. s

Dependence on insecticides has raised pest resistance concerns resulting in greater number of applications, increased product dosage, or use of more toxic alternative products which consequently comes at an increased cost and are harmful to the environment [93]. Carbamates, pyrethroids and organophosphates, which are majorly used by African farmers have faced resistance from the fall armyworms [24,93]. In fact, some of the chemicals approved for use against FAW are highly hazardous pesticides that are acknowledged to present high levels of acute or chronic hazards to human health or environment according to internationally accepted classification systems such as the World Health Organization (WHO), the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [85]. Furthermore, FAW larvae have also developed resistance to genetically modified maize (Bt maize), which was introduced as a resistant variety [83,88]. It has also been reported by some farmers that synthetic pesticides are not effective against FAW forcing them to use higher doses with frequent application and this causes bioaccumulation in the environment and hastens pesticide resistance [89]. Even though synthetic pesticides have played a role in managing FAW, because of safety concerns and resistance, it is imperative to explore an integrated pest management (IPM) system [89].

There is global focus on botanical insect control products that have proven to be effective and are less harmful to humans and the environment as alternative to synthetic pesticides [94]. African farmers have a long history of using plants with pesticidal properties and this could also be incorporated in management of FAW [24]. A recent review done in 2020 listed 69 plant species with potential for application in FAW management including *A. indica*, *Schinus molle*, *Ageratum conyzoides L, Dysphania ambrosioides, Corymbia citriodora* and *Phytolacca dodecandra* among others [83,95]. Extracts of these plants have shown mortality, growth regulating activity, antifeedant effects, oviposition deterrent, decreased pupa weight, reduced hatching rate, juvenomimetric activity and sublethal effects against FAW [90]. The use of effective botanical pesticides could reduce over-reliance on synthetic pesticides [90]. Given the importance of maize

in these regions, this pest has evolved into a more serious problem in the affected countries threatening the food security of millions of people [91,94]. The sustainable method for management of this invasive pest requires development of ecologically sound crop protection products and botanical pesticides present an interesting option in integrated management of FAW.

1.5.2 THE AFRICAN SWEET POTATO WEEVILS, *CYLAS PUNCTICOLLIS*

Sweet potato, *Ipomoea batatas* (L. Lamarck) is a primary source of carbohydrates and is ranked the seventh most important crop among all known edible crops [81–83]. It can be consumed while raw, boiled or fried and its roots are appreciated as high fibre food while as green vegetable, it has high levels of vitamin A, vitamin C, potassium and iron [99]. Sweet potatoes also supplement family income for some farmers in Africa [100]. Other components of the crop have been incorporated in formulation of a range of edible commodities like jam, sauce, pickles and confectionary [98]. It is planted by farmers in over 100 countries in the tropical and sub-tropical areas and produces good yield on marginal lands with little investment [101]. In Sub-Saharan Africa, it is an important subsistence food crop grown mainly in small-scale and used as a staple food and famine reserve food supply [98]. Despite the economic demand for sweet potato, its tubers are threatened by insect pests and if not well treated and adequately stored could lead to severe economic loss [102].

In Africa, significant crop damage and economic losses are caused by African sweet potato weevils, *Cylas puncticollis* Boheman, shown in figure 1.13 a, which oviposits and develops to adult stage inside the vines and tubers (figure 1.13 b) [98,102].

a b

Figure 1.13: Adult African sweet potato weevil, *Cylas puncticollis* (a) and sweet potato tuber damage (b) caused by the weevil [103,104].

The female creates small cavities with her mouthparts in the tuber or vines and deposits eggs. The eggs are then sealed within the oviposition cavity with a plug of faecal material, a brownish liquid [89,90]. The egg is ovoid, creamy white measuring about 0.7 mm in length and 0.5 mm width and this stage lasts about 2 days at 27°C. The female produces an average of 80, 117 and 150 eggs when reared at 20°C, 27°C and 30°C, respectively, during their lifespan [106]. When larvae emerge, they burrow directly into the tuber or vine. The larvae, which are up to four instars, are legless and white in colour and this stage last about 14 days at 27°C [103,107].

Mature larvae stops feeding and create a pupal chamber and pupation lasts average 4 days before the adult weevil emerges by chewing a hole through the exterior of the plant tissue [106]. The teneral adults remain in the storage roots for about 6 days before they leave the roots., but the adult could also remain for a considerable period and feeds within the tuber [103,104]. The average lifespan of the adult weevils is 71, 79 and 62 days at temperatures of 20°C, 27°C, and 30°C, respectively. The adults tend to hide in the shade of sweet potato foliage during the day while in dark, they move about and fly infrequently in short distances [106]. The adults also feign death when disturbed [108]. The life cycle of *C. puncticollis* is presented in figure 1.14.

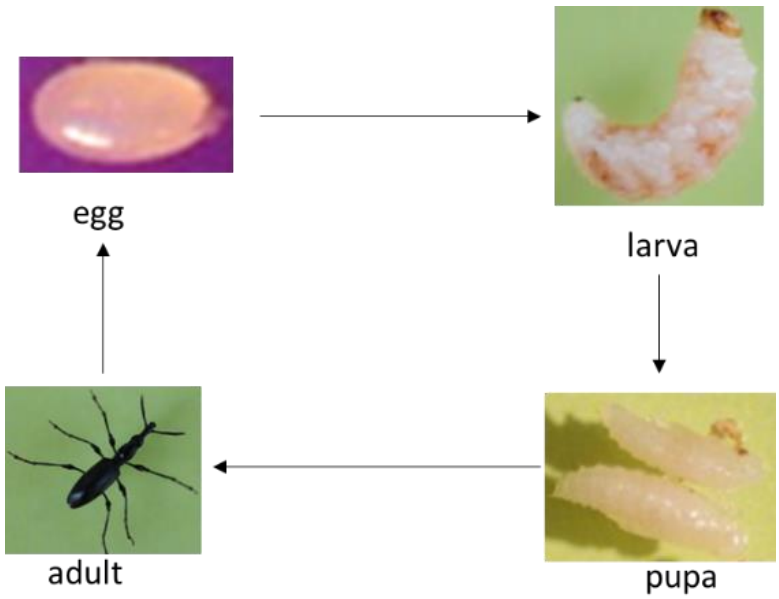


Figure 1.14: Life cycle of *Cylas puncticollis* [107]

C. puncticollis is one of the major threats to sweet potato production in Sub-Saharan Africa [106,109]. The weevil is considered monophagous since significant feeding, growth and reproduction only occur in sweet potatoes [110]. Infestation occurs during the dry season when high temperatures crack the soil surface thereby exposing the edible tubers; infestation can also occur through planting vines [111]. These weevils can cause up to 100% yield loss, especially during dry season [110–113] and the weevil damage continues to increase during storage [114].

Their larvae feed and tunnel in the tubers and stem causing extensive reduction in marketable yield and quality [115]. The tunnels are filled with frass which produces terpenes which cause bad odour and a bitter taste rendering the sweet potato tubers unpalatable and unmarketable [116,117]. These terpenes can affect mammalian liver and lungs [118]. Even though both larvae and adults feed on tubers in the field and in storage, it is the larvae that are considered to be the most devastating life stage [109,117]. Sweet potato weevils also infest the leaves and flower seeds [99]. Even small populations of sweet potato weevils can considerably result in loss of quality and economic value of the tubers [100,115].

It is difficult to control *C. puncticollis* using chemicals because of the cryptic nature of the larvae and the nocturnal activity of the adult weevils [111]. Application of parathion and chlorpyrifos insecticides have been used in overcoming weevil infestation on sweet potato, however pesticide residues have been reported [102]. In South Africa, insecticides based on deltamethrin, gamma BHC, tralomethrin and triazophos have been registered for control of these weevils. It has been recommended that a first application is executed when foliage damage is observed and treatment repeated every 2 – 3 weeks to maintain low weevil populations and to limit oviposition in the tubers [106]. Sweet potato vines can also be submerged in insecticide suspensions before planting to keep initial infestation low [106]. Chemical insecticides are generally used in developed countries in controlling these weevils but their use among poor farmers is generally low [102]. In fact, in Kenya where this study was based, there is no registered product for control and management of sweet potato weevils by the Pest Control Products Board, the regulatory body mandated to regulate use of pest control products. Endosulfan, fenthion and fenitrothion at 0.05% applied by drenching the soil at 50 and 80 days after planting have shown to be efficient and reduce tuber damage [102]. Even though it is unlikely that the use of chemical pesticides can be eliminated, the existing insecticides may have lost attrition due to insect resistance, microbial degradation or cancellation and timely replacements are unlikely [96,105]. It is therefore imperative to explore alternative sources of control products for sweet potato weevils, especially for the smallholder farmers in Africa.

Historically, control of these weevils has been primarily based on sanitation and cultural practices [102,105,88,92]. In Africa, efforts to control this pest are minimal and farmers rely mostly on cultural methods such as crop rotation, non-infested planting materials, hand picking of pests, weeding, mulching, banking (filling in of soil cracks), flooding weevil-infested fields after harvest, intercropping, destruction of crop residue to avoid residual infestation or brushing around areas [101,104,105,113,117]. Prevention of soil cracking, which exposes tubers to weevil infestation, by ridging the area around the potato crown during planting or frequent irrigation has also been reported to reduce weevil damage [119]. Other cultural practices that reduce weevil damage are: using deep-rooted cultivars, planting cuttings deep, prompt harvesting, selecting new planting

areas far from infested farms [119]. These cultural methods involve changing or modifying cultivation practices which directly or indirectly suppress pest population [119]. Even though these methods have shown possibility of reducing weevil populations, they are however labour intensive and could be ineffective if not strictly followed [117].

Application of plant-based substances in form of extracts, powders or essential oils could be explored as possible antifeedant, repellent or toxic agents and incorporated in management practices by local farmers in Africa. Research has reported some plant species used in control of these weevils. *Aframonum melegueta, Dennettia tripetalla* and *Xylopia aethiopica* have showed varying levels of activity against sweet potato weevils. The fruits and leaves of these plant species reduced tuber damage, exhibited toxicity, repellence effects and also inhibited progeny emergence and oviposition of the weevils [97]. The powders of *A. melegueta, D. tripetalla* and *X. aethiopica* have also demonstrated significant reduction in stored tuber damage when admixed with sweet potato in storage conditions [98]. At 3% and 5% (w/w), these plant powders also suppressed adult emergence of the weevils [98]. In storage, maintaining sweet potato tubers at 15°C has also been reported to increase larval mortality [106]. Other botanicals which have also been reported as effective candidates for control of sweet potato weevils include *Cymbopogon citratus, A. indica, Allium sativum* and *Capsicum fructescens* [120]. Biopesticides such as azadirachtin and Spinosad have also been evaluated for efficacy against the sweet potato weevils in the laboratory, but no studies have been reported on field trials [102].

Technologies aimed at managing sweet potato weevils would boost production and impact on livelihoods of millions of farmers in Africa. The need to develop effective and agro-ecologically sustainable products of plant origin for application in IPM underscores the overall goal of this research study.

1.5.3 THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

Tribolium castaneum (Herbst), the red flour beetle, presented in figure 1.15 is a polyphagous, cosmopolitan pest species belonging to the order of Coleoptera [121]. The insect was first reported in 1936 as the most important pest in wheat mills in Kansas [122].

Figure 1.15: *Tribolium castaneum* larvae and adults

It breeds in broken grain, grain dust, high-moisture wheat kernels or flours among other stored products, and its life cycle includes four stages: egg, larva, pupa and adult as presented in figure 1.16. Its microscopic eggs are ovoid and white and embryogenesis typically lasts 3.5 – 5 days in ideal laboratory conditions [123]. Its larvae are worm-like and yellowish in colour and this stage lasts about 2 – 3 weeks. The pupal stage may last 5 – 6 days and the pupae are either white or yellowish in colour. Typically, the life cycle from egg to adult is about 3 – 4 weeks in optimal conditions of food, temperature and humidity with temperature being the most important factor affecting development rate [122]. *T. castaneum* can develop in a temperature range of about 22°C – 35°C, however, optimal conditions have been reported as 35°C and 60% relative humidity while there is little development below 15°C and above 40°C [122]. Adult *T. castaneum* may live more than 3 years though in laboratory settings, the life span may be 1 – 6 months. A single female may lay 300 to 400 eggs, however, egg production decreases after 3 – 4 months [123].

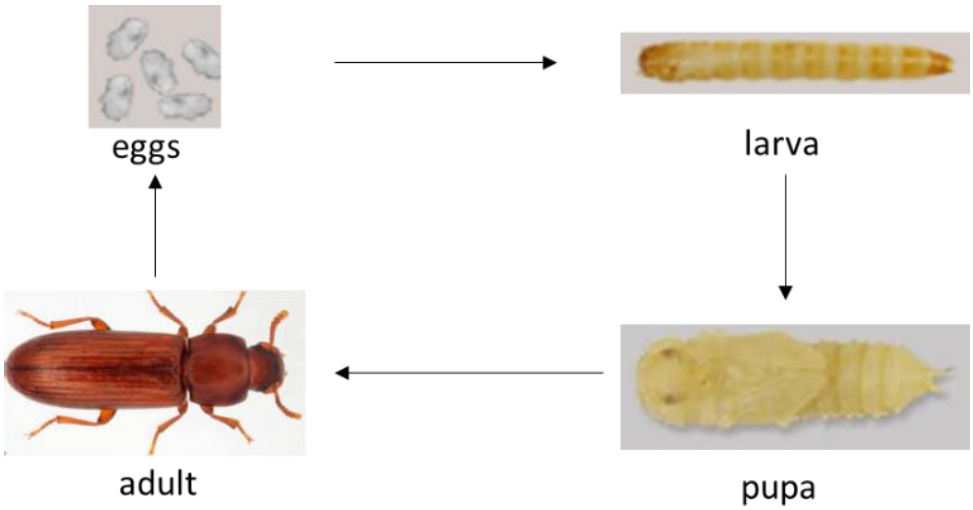


Figure 1.16: Life cycle of *Tribolium castaneum* [124]

T. castaneum has remained an important polyphagous insect of stored grains with a worldwide distribution and is generally found in granaries, grocery shops, warehouses, stored grains processing plants, grain elevators, flour mills, stores and grain barns [121,122,125]. It is a secondary pest whose larvae and adults attack broken kernel stored grains, milled grain products, nuts among other stored products and has been reported in 246 commodities [121,122]. This beetle can cause losses of 10 – 40% of total global products in storage [126].

The infestation causes physical damage and contamination by excrements, webbings, silk and insect body parts leading to decrease in quality, quantity and economic value of the grains [121,127]. The insect also attacks the germ part or the embryo of the grains [128]. In addition, *T. castaneum* is reported to secrete quinones in stored products posing a human health risk [126,129]. The two quinones, methyl-1,4-benzoquinone and ethyl-1,4-benzoquinone, produced by adults are considered carcinogenic and also cause allergenic diseases [130,131]. They also render the grains unpalatable and unmarketable due to discoloration of flour and musty odour [128,32]. Moreover, biochemical activity in contaminated grain could result in higher heat and humidity making the grains susceptible to bacteria and fungi contamination, moulds and toxigenic species [128,133,135].

The general approach to control this pest is using contact insecticides and fumigants such as phosphine and methyl bromide which have raised concerns on grounds of environmental safety, ozone depletion, carcinogenic effects and insect resistance [122,136,137]. The reliance on chemical pesticides is high, especially in countries producing large quantities of cereals for domestic consumption and export [138]. Phosphine fumigation has become increasingly limited in use because of its genotoxicity to occupationally exposed fumigators [135]. Methyl bromide is an ozone-depletor and is being phased out as agreed through the Montreal Protocol [30,139]. The insect has developed resistance to all classes of insecticides used against it [125]. There is also accumulation of residues in grains when synthetic pesticides are used leading to adverse health effects in humans [140]. There is a limited number of active ingredients worldwide used in treatment of stored grains and this has led to repeated use of restricted chemicals despite insect resistance [141].

Evaluation of plants as source of grain protectants to limit post-harvest losses could provide safer substitutes or at least supplement the conventional pesticides, especially in developing countries where there is availability of plant materials [142]. Apart from being eco-friendly and low mammalian [143] toxicity, botanicals have multiple modes of action and this minimizes development of resistance populations [127,133]. More than 120 plant species have been reported for effective control of stored grain pests [144]. Some of these plant species which have been investigated for activity against *T. castaneum* include *Jacaranda mimosifolia* (D. Don), *Ambrosia tenuifolia* (Spreng), *Brassica campestris* (L.), *Solanum sisymbriifolium* (Lam), *Achillea bierbersteinii, Rhododendron thymifolium* among others [129,142].

Further research has found that plant powders and essential oils could be effective in control and management of stored insect pests [125,140,143,144]. Plant essential oils have been considered as a kind of botanical pesticides and have shown antifeeding, repellent and growth inhibitory properties while their volatile nature can make them function as fumigants against storage pests [135,145,146]. Botanical plant powders have also been traditionally used as grain protectants and have shown contact toxicity, repellency, oviposition deterrent and antifeedant effects. Powdered leaves of *Carum copticum, Cuminum cyminum* and *Cassia sophera* have shown

potency as grain protectants [147–149]. Neem kernel and garlic powders have also been reported to be effective in controlling stored grain pests [149]. In fact, mixing neem leaves with grains has been a practice since early days and this is still followed in developing countries [149]. Other plant species whose powders have been reported as biocidal against *T. castaneum* also include *Zingiber officinale, Dracaena arborea, Telfairia occidentalis* and *Vitex grandifolia* [149]. With cereal grains making the majority of agricultural produce maintained in storage facilities and being an important component of the world food supply, it is imperative that this beetle is managed and controlled [133,148].

1.6 RESEARCH OBJECTIVES

The overarching objective of this study was to evaluate the potential of *M. volkensii* as a source of bioactive antifeedants in crop protection against the African sweet potato weevil, *C. puncticollis,* fall armyworm, *S. frugiperda* and the red flour beetle, *T. castaneum.* These are major insect pests of economic importance in sweet potato production, maize production and stored grains respectively, in Africa.

To achieve this aim, three specific objectives were defined for this research:

1. To evaluate the efficacy of *Melia volkensii* crude extracts (from the bark, leaves, nuts and pulp) for antifeedant activity against *C. puncticollis, S. frugiperda* and *T. castaneum* in laboratory conditions. This part of the research aimed to investigate the importance of various plant parts on activity against the insect pests. Herein, crude extracts were prepared and tested at different concentrations to select the plant parts with high antifeedant activity for further investigation.

2. To evaluate the efficacy of *Melia volkensii* crude extracts against *C. puncticollis, S. frugiperda* and *T. castaneum* in green house and simulated storage conditions. The major aim of this part was to validate the laboratory-based results in practical conditions and here, the tests were done in greenhouse and simulated storage conditions. In these tests, the effects of *M. volkensii* on the insect pests and most importantly on the reduction in crop damage was investigated. Consequently, to have an idea on the practical use of the

M. volkensii extracts, commercial insecticide was included as a reference in this investigation. We aimed that these data could support the use of *M. volkensii* extracts by farmers in the control of pest insects.

3. Our third research objective was to isolate and characterize compounds that carry insecticide activity against *C. puncticollis*, *S. frugiperda* and *T. castaneum*. Herein, different chemical analytical techniques were employed in a bioactivity-guided strategy to isolate and characterize bioactive compounds. This investigation aimed to provide more information on new leads in insecticide formulation using *M. volkensii.*

CHAPTER 2

Efficacy of *Melia volkensii* crude extracts for antifeedant activity against *Cylas puncticollis, Spodoptera frugiperda* and *Tribolium castaneum* in laboratory conditions

Partially adapted from:

V. Jaoko *et al.*, "Laboratory and Greenhouse Evaluation of *Melia volkensii* Extracts for Potency against African Sweet Potato Weevil, *Cylas puncticollis*, and Fall Armyworm, *Spodoptera frugiperda*," *Agronomy*, vol. 11, no. 1994, pp. 1–9, 2021.

2.0 INTRODUCTION

Insect pests not only affect food quality and quantity of affected crops, but also serve as vectors of plant diseases, especially in the tropical regions of Asia, Africa and Latin America [150,151]. Although synthetic pesticides have proved effective against insect pests, they have disruptive endpoint [152,153]. Their continued use has been reported to pose undesirable effects and negative consequences on the environment, human and other non-target organisms [150–152,154,155]. Moreover, synthetic pesticides are expensive and in many cases, only produced moderate results along with major ecological damage [156]. In contrast, low toxicity of botanical pesticides makes their processing inexpensive and in many cases, the feedstocks are locally available and affordable [156]. This has led to focus on viable pest control strategies with botanical pesticides emerging as potential alternatives because of the rich source of bioactive chemicals in plants [135,150,154,156,157].

Considerable efforts have been focused on the plant kingdom for safer alternatives and eco-friendly sources of pest control agents [150,158]. Botanical pesticides have proven to have diverse modes of action including antifeedant, oviposition inhibitor, repellent, toxicity and growth disruptor [156,159]. This array of modes of action acts concertedly on behavioural and physiological processes limiting chances of pest resistance, unlike in synthetic pesticides which are based on single active ingredient [160,161]. In this study*, Melia volkensii*, an indigenous tree of the family Meliaceae was evaluated for potential efficacy against sweet potato weevil (*Cylas puncticollis*), fall armyworm (*Spodoptera frugiperda*) and red flour beetle (*Tribolium castaneum*). These are insect pests of economic importance in Africa causing economic loss in sweet potato and maize production as well as stored grains [130,145,162–175]. *M. volkensii* has been reported to contain bioactive compounds with antifeedant activity against a wide range of insect pests [176–184].

Sweet potato is among the most vital food crops and is cultivated in over 100 developing countries [185]. It is a flexible crop which can adapt in soils with marginal nutrients, adverse

environmental and climatic conditions as well as irregular rainfall [185]. Sweet potato is high in energy, vitamin C, potassium, fibre, vitamin A, iron, minerals among other body nutrients [99], [185]. Moreover, it has pharmacological benefits such as antioxidant, anticancer, immunomodulatory and antimicrobial properties [185]. Traditionally, it serves as food security and steady source of income for low-income earning households in Africa with potential of yield improvement and all-year round availability [186]. This crop has been the focus of intense, coordinated global effort to realize its full potential as a source of food, processed products and income for millions of smallholder farmers and low-income consumers in Africa, Latin America and Asia [157].

Infestation by sweet potato weevil, *C. puncticollis* is one of the major constraints to sweet potato production in Africa reducing the crop yield by 25-75% [84,177–179]. These weevils infest all plant parts including the vine, foliage, flower seeds and extensive feeding on the tubers can lead to complete loss in sweet potato harvest [99,119,120,189–193]. Synthetic pesticides have been used to control these weevils, however, there have been reported health and environmental concerns [186]. In Ethiopia, crop rotation by smallholder farmers is the most commonly practiced method to control *C. puncticollis* infestation [188]. This strategy results in pest population decrease while also improving soil fertility and non-tuber crop plants such as maize, sorghum and vegetables could be potential candidates for crop rotation with sweet potato. However, crop rotation could have some bottlenecks which include investment on machinery for alternative crops, loss of income to farmers who invest in large-scale sweet potato farming as well as lack of crop specialization among farmers.

The use of botanical pesticides has been reported as alternative to synthetic pesticides in control of sweet potato weevils [106,128,194]. Some botanicals which have shown efficacy against *C. puncticollis* include *Carica papaya, Chromolaena odorata*, *Moringa oleifera* and *A. indica* [185]. *Beauvaria bassiana* has also been reported to effectively reduce *C. puncticollis* damage [185].

Fall armyworm, *S. frugiperda*, is an insect pest whose larvae feeds on more than 100 different crop species, causing heavy damage in cultivated cereals, legumes, vegetable crops, cotton, rice,

sugarcane among others [18,188,195–198]. Its preference is maize, a staple food for over 300 million smallholder farmers in Africa posing threat to food security, nutrition and livelihoods [188,196,199,200]. This pest is present in nearly all Sub-Saharan African countries with its larvae defoliating and causing extensive damage in maize fields [196,197]. The favourable climatic conditions in most African countries allows *S. frugiperda* to complete several generations every year resulting in yield losses in maize production [200]. *S. frugiperda* attacks all stages from seedling emergence to cob formation, reducing photosynthesis, affecting plant growth and causing significant losses in crop yield [155,200]. To manage against this pest, farmers use synthetic pesticides to mitigate against economic losses [195,200]. Although the use of chemical pesticides to control heavy infestation is justified, their frequent application is unsustainable as it leads to development of pesticide resistance, increased production cost, health hazards and negative environmental impacts [201–205]. This has necessitated the need for alternative low-risk methods for management of fall armyworm [206,207]. Furthermore, the Food and Agriculture Organization (FAO) has emphasized the use of botanicals among other biopesticide options for management of this pest in Africa [207]. It has also been demonstrated that use of botanical pesticides reduces application of synthetic pesticides resulting in lower level of resistance development among *S. frugiperda* populations because of their wide range of modes of action [208].

Red flour beetle, *T. castaneum,* is a destructive pest of stored products whose adults and larvae cause serious damage in a wide range of grains and cereals, spices and dried fruits but milled grain products such as flour is their preferred diet [150,201,209,210]. It is of Indo-Australian origin and is a secondary pest whose larvae or adults cannot cause damage on sound grains unless primary damage has been caused by other pests [211,212]. This pest also attacks the germ part of grains hence lowering the germination percentage of affected seeds [211,12]. Due to its high rate of infestation, chemical pesticides and fumigants have been used to control this pest for a long time [202,213]. However, drawbacks associated with application of synthetic pesticides such as toxicity to non-target organisms, pesticide induced resistance, pesticide residues and increased environmental concerns have been raised [201,203,209]. Phosphine, a synthetic

fumigant has been widely used to control this pest, however, its excessive use is becoming ineffective with time [152]. Moreover, frequent use of pyrethroids and organophosphates has resulted in pest resistance emergence depicting a major problem for management of stored product pests with traditional fumigants [152]. Concerns about adverse effects of these fumigant residues in food and environment have been raised [152]. This has necessitated the need for more effective and healthier alternatives [202,213]. Pesticides of plant origin are considered safe alternatives to the chemical pesticides and could be used in control of *T. castaneum* as they are environmentally safe, easily available and less hazardous to human [92,158,213]. Traditionally, farmers have mixed plant leaves, seeds, roots and barks with stored grains to protect against *T. castaneum* [213]. Sugarcane bagasse-based lignin has also been reported to contain toxic and repellent properties against *T. castaneum* [213]. Plant essential oils and powders have also been successfully used to control this pest [156,201].

This investigation sought to evaluate the potential antifeedant activity of *M. volkensii* leaf, pulp, nuts and bark extracts against sweet potato weevil (*Cylas puncticollis*), beet armyworm (*Spodoptera exigua* – used as substitute for *S. frugiperda*, which causes more damage in Africa) and red flour beetle (*Tribolium castaneum*) in a laboratory bioassay.

2.1 MATERIALS AND METHODS

2.1.1 COLLECTION AND PROCESSING OF PLANT MATERIALS

Fresh *M. volkensii* nuts, pulp, bark and leaves samples were collected from a plantation farm in Kiambere, Kenya. Kiambere is located approximately 187 km east of Nairobi with coordinates of 0°41'10" S 37°54'55" E and is about 720 m above sea level. The sampling was done from trees aged between 5 to 10 years old in September 2018, during the dry season. Fruit pulp and nut samples were collected from waste materials which had been generated after seed extraction while leaves and bark were freshly harvested. The whole plant twig was harvested and all the leaf material including old, mature and young leaves were used. The plant parts were dried in the shade for 21 days. The dried samples were then pulverized using an electric grinder with 850 µm pore size ready for extraction.

2.1.2 EXTRACTION OF PLANT MATERIALS

The resulting powder of each plant part (nut, pulp, bark and leaf) was macerated in methanol in the ratio of 1:5 (w/v) for 48 h at room temperature in an Erlenmeyer flask. The mixture was then filtered using filter paper (Whatman no. 1) in a Buchner funnel and the residue obtained was discarded while the filtrate was pre-concentrated in a rotary evaporator at 35°C until near dryness. High vacuum was then used to completely dry the crude extracts. The crude extracts were weighed to calculate the yield and stored at 4°C awaiting insect bioassay.

2.1.3 PREPARATION OF TREATMENTS AND WORKING SOLUTIONS

For each crude extract (nut, pulp, bark and leaf), 1 g was accurately weighed and dissolved in 95 % methanol in a 10 mL volumetric flask. This constituted 100 mg/mL concentration stock solution of crude extract, this was also used as the highest concentration of working solution. Serial dilutions were done to make 80, 60, 40 and 20 mg/mL concentrations of working solutions for bioassay. For negative control, methanol was used.

2.1.4 REARING OF INSECTS

2.1.4.1 SWEET POTATO WEEVIL, *CYLAS PUNCTICOLLIS*

African sweet potato weevils, *C. puncticollis,* used in the experiment were obtained from a continuous insect culture maintained in the Laboratory of Agrozoology at Ghent University, Belgium. The insects were reared using sweet potato tubers placed in a plastic container (10 x 20 x 30 cm) in a growth chamber set at 26 ± 2 ℃, 70% relative humidity and 16 h light:8 h dark photoperiod [116].

2.1.4.2 BEET ARMYWORM, *SPODOPTERA EXIGUA*

In this study, beet armyworm, *Spodoptera exigua* (Hübner) was used in the laboratory bioassays as a substitute for *S. frugiperda* (more destructive to cereals in Africa) which was ultimately used in the greenhouse experiments in Kenya. *S. exigua* larvae were obtained from a continuous insect culture maintained in the Laboratory of Agrozoology at Ghent University, Belgium for over 4 years. *S. exigua* larvae were reared using artificial diet described by Christians et al., 2018 [205] and after emergence, the adults were fed on honey solution. The colony was kept in a growth chamber at 26 ± 2 °C with 75% relative humidity and 16 h light:8 h dark photoperiod.

2.1.4.3 RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

An insect culture of the red flour beetle, *Tribolium castaneum,* established in Laboratory of Agrozoology at Ghent University, Belgium was used to rear the insects using the method described by Jbilou et al., 2006 [202]. The insects were reared on wheat flour mixed with yeast (10:1, w/w) kept in 300 ml plastic jars covered with muslin cloth held with rubber bands for ventilation. The jars were kept in growth chamber maintained at 28±2 °C, humidity of 70±5 % and 14 h light:10 h dark photoperiod.

2.1.5 LABORATORY ANTIFEEDANT BIOASSAYS

2.1.5.1 Antifeedant bioassay against *Cylas puncticollis*

A choice bioassay was used to investigate the antifeedant efficacy of *M. volkensii* crude extracts against *C. puncticollis*. Sweet potato tubers were sliced into square shapes, 1 cm thick. 100 µl of the respective plant part extracts (nut, pulp, leaf and bark) at concentrations of 100, 80, 60, 40 and 20 mg/mL was discharged and evenly spread on the sweet potato slices. For negative control, 100 µl of methanol was discharged onto the potato slice. The treated potato slices were placed in the laminar flow for 30 minutes to evaporate methanol. 9 cm diameter Petri dishes were partitioned into 2 halves and one half labelled as treatment while the other half as control. The sweet potato slices were placed in the respective partitions. 10 adult *C. puncticollis* aged 3-5 days old, that had previously been starved for 12 h, were introduced into the Petri dish.

The Petri dishes were placed in a climate-controlled incubator at 26 ± 2°C, 70% relative humidity and 16 h light:8 h dark photoperiod. The tests were done in five replicates with three independent biological repetitions per concentration of each crude extract. Antifeedant index against *C. puncticollis* was calculated using the formula described by Liyun et al., 2020 [214] with slight modification whereby determination of the number of feeding holes in the tubers was replaced by determination of the weights of treated and untreated tubers after 24 hours. The weight of potato tubers was measured after 24 h and antifeedant index calculated using the equation below:

$$\text{Antifeedant index} = \frac{\text{C} - \text{T}}{\text{C} + \text{T}}\ x\ 100$$

C – weight of potato consumed in negative control diet;

T – weight of potato consumed in treated diet

2.1.5.2 Antifeedant bioassay against *Spodoptera exigua*

Leaf discs were used in a choice bioassay to investigate antifeedant activity of *M. volkensii* crude extracts against *S. exigua*. The tests were performed on a 9 cm diameter Petri dish in which two leaf discs of Chinese cabbage (*Brassica rapa*, subspecies *pekinensis*) leaves, 9 cm^2 were distributed. 100 µL of crude extracts from each plant part (nut, pulp, leaf and bark) at

concentrations of 100, 80, 60, 40 and 20 mg/mL was discharged and spread evenly on the leaf discs. For negative control, 100 µL of methanol was discharged on the leaf discs. The treated leaf discs were placed in the laminar flow for 30 minutes to evaporate methanol. In the centre of the Petri dish, five third instar-larvae that had previously been starved for 3 h were released.

The Petri dishes were placed in a climate-controlled incubator at 26 ± 2 °C with 75% relative humidity and 16 h light:8 h dark photoperiod. The tests were done in five replicates with three independent biological repetitions per concentration of each crude extract (leaf, nut, pulp and bark). The leaf area of both control and treated diet was calculated after 8 h. The leaf area calculation was done by placing the leaf on a 1 mm graph paper and counting the feeding holes on the leaf [214,215]. Antifeedant index against *S. exigua* was calculated using the formula described by Akhtar and Isman, 2004 [70] below. *S. exigua* larvae were used in the laboratory bioassays in Ghent University, Belgium, as a model for *S. frugiperda*, which were later tested in greenhouse conditions in Nairobi, Kenya (where *S. frugiperda* causes significant damage in maize production).

$$\text{Antifeedant index} = \frac{\text{C} - \text{T}}{\text{C} + \text{T}}\ x\ 100$$

C – leaf area consumed in negative control diet

T – leaf area consumed in treated diet

2.1.5.3 Antifeedant bioassay against *Tribolium castaneum*

Flour disc was used in a no-choice bioassay to determine antifeedant effect of *M. volkensii* extracts (nut, pulp, leaf and bark) against *T. castaneum*. The flour discs were prepared using the method described by [203]. This was done by mixing water and wheat flour in the ratio 3:1 and vortexing for 1 minute. Aliquots of 50 µl of the flour suspension were discharged into 96-well plate lid using digital pipette. Using a micro-pipette, 10 µl of respective crude extract at various concentrations (100, 80, 60, 40 and 20 mg/mL) was added on the discharged flour suspension as treatments. For control, 10 µl of methanol was added to the flour mixture. The flour mixture was placed under laminar flow for 8 hours to form flour disc. The flour discs for the respective treatments were placed in separate 6-well plates containing 10 adult insects, that had previously

been starved for 12 hours. The weight of flour discs in control and treatment was determined after 48 hours and antifeedant index calculated using the formula below. There were 5 replicates and 3 independent biological repetitions per treatment.

$$\text{Antifeedant index} = \frac{\text{C} - \text{T}}{\text{C} + \text{T}}\ x\ 100$$

C – weight of flour disc consumed in control;

T – weight of flour disc consumed in treatment

2.1.6 STATISTICAL ANALYSIS

All the statistical analyses were conducted using GraphPad Prism version 6.01. Antifeedant indices within each treatment were checked for normality (Shapiro-Wilk test) to assess if data followed a normal distribution [216]. Not all the data for antifeedant indices were normally distributed, and non-parametric tests were performed. Antifeedant indices from laboratory bioassays were therefore subjected to a Kruskal-Wallis test to perform analysis of variance followed by Dunn's test for multiple comparisons between means [125]. To determine significant difference between the different means, Bonferroni correction was applied and means were considered significantly different if their *p*-value was greater than the corrected Bonferroni *p*-value [217].

2.2 RESULTS AND DISCUSSION

2.2.1 LABORATORY BIOASSAY OF *M. VOLKENSII* CRUDE EXTRACTS AGAINST *C. PUNCTICOLLIS*

M. volkensii nut, pulp, bark, leaf extracts all showed varying antifeedant activity against adult *C. puncticollis* at 100, 80, 60, 40 and 20 mg/mL concentrations as presented in figure 2.1. The antifeedant activity was dose-dependent for all the plant parts (nut, pulp, bark and leaf), with 100 mg/mL concentration giving the strongest activity against *C. puncticollis*. In the nut extracts, all the tested concentrations recorded over 50% antifeedant activity with 51.1%, 53.3%. 51.9%, 54.2% and 61.9% antifeedant indices recorded for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively. It was also observed in the nut extracts that there was no significant difference in antifeedant activity at all concentrations.

In the pulp extracts, all the concentrations tested recorded antifeedant activity above 50% with indices of 50.8%, 54.4%, 59.4%, 64.5% and 63.1% observed in 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively. There was also no significant difference in activity at concentrations of 40, 60, 80 and 100 mg/mL of pulp extracts. Antifeedant activity of pulp extracts at 20 mg/mL concentration was significantly different from 80 mg/mL and 100 mg/mL; while no significant difference was observed between 20 mg/mL, 40 mg/mL and 6 mg/mL concentrations.

When bark extracts were tested against *C. puncticollis*, antifeedant indices of 42.9%, 50.1%, 56.20%, 57.9% and 61.8% were recorded for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively. Antifeedant activity of bark extracts at 20 mg/mL concentration was significantly different from 80 mg/mL and 100 mg/mL; while there was no significant difference between 20 mg/mL, 40 mg/mL and 60 mg/mL concentrations as presented in figure 2.1.

In the leaf extracts, there was no significant difference in antifeedant activity at all the concentrations tested. Antifeedant indices of 44.5%, 46.1%, 47.% 54.1% and 54.0% were observed for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively in the leaf extracts.

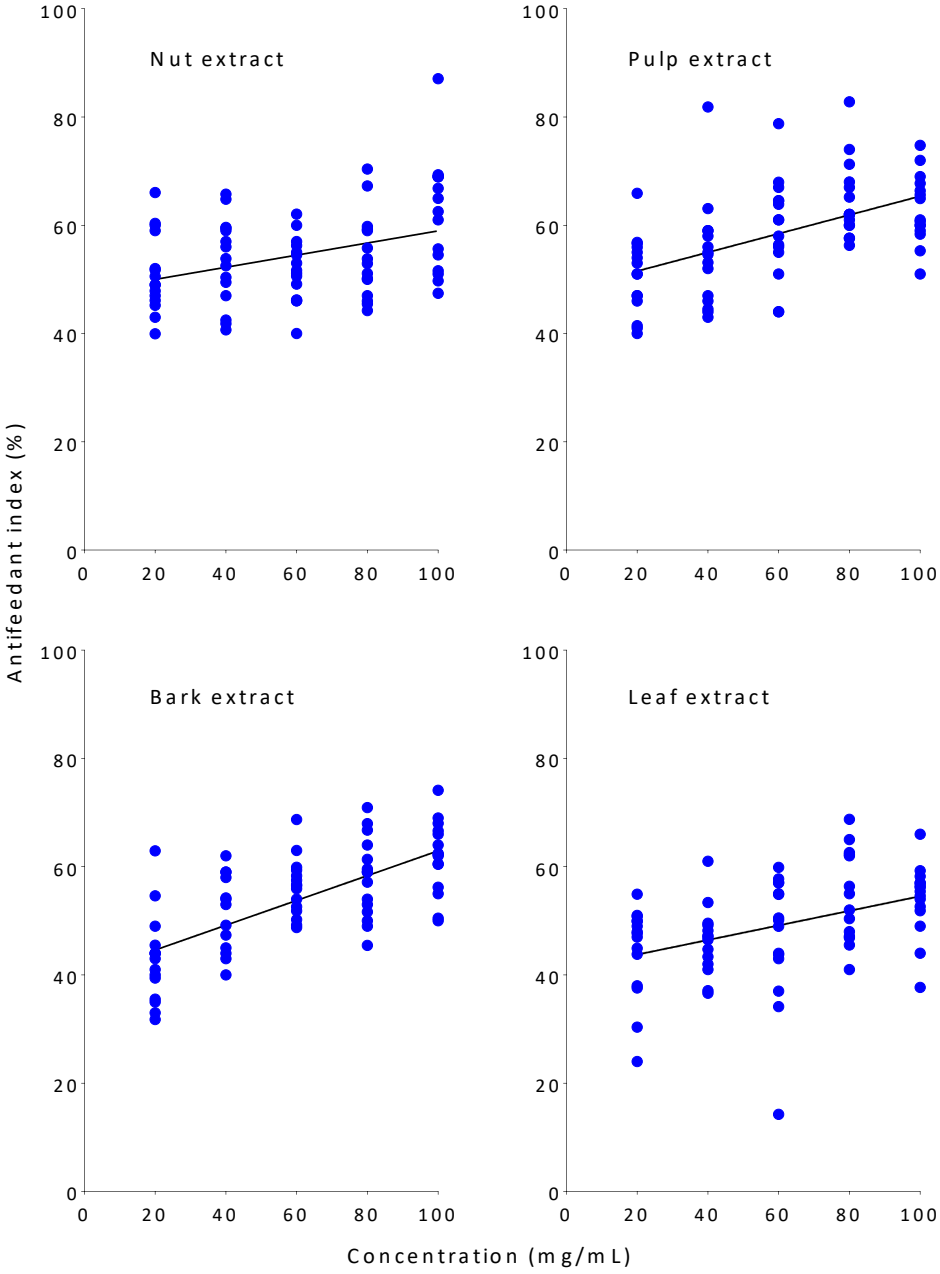


Figure 2.1: Graphical representation of antifeedant index (AFI) of crude extracts from various parts (nut, pulp, bark, leaf) of *Melia volkensii* against *Cylas puncticollis* at different concentrations. AFI values are presented as individual data points based on 3 independent biological repeats, each consisting of 5 technical repeats.

When antifeedant activity of different plant parts was compared at 20 mg/mL concentration, there was no significant difference in the activity between the different plant parts as presented in table 2.1. However, the nut and pulp extracts showed higher mean antifeedant indices of 51.1% and 50.8% respectively compared to 42.9% and 44.5% antifeedant indices for bark and leaf extracts respectively against *C. puncticollis.*

Table 2.1: Antifeedant indices of crude extracts, at 20 mg/mL concentration, from various parts of *M. volkensii* against *C. puncticollis*. Means followed by same superscript are not significantly different.

Plant part	Leaf extract	Bark extract	Pulp extract	Nut extract
Antifeedant index	44.5 ± 2.2^{a}	42.9 ± 2.1^{a}	50.8 ± 1.8^{a}	51.1 ± 1.9^{a}

In related studies, laboratory bioassays of pure botanical insecticides pyrethrins, toosendanin, veratrine, matrine, stemonine, rotenone, nicotine showed AFI indices of 97.9%, 97.9%, 59.4%, 33.9%, 69.3%, 35.4%, 39.9% respectively against *Cylas formicarius* at 20 mg/mL concentration [214]. Results from the present investigation show crude nut, pulp, leaf and bark extracts at 20 mg/mL concentration, exhibited higher antifeedant activity than some of pure botanical pesticides veratrine, nicotine and rotenone (tested at 2 g/L concentration).

The laboratory bioassay results presented here show that the bark, nuts, pulp and leaves of *M. volkensii* contain antifeedant compounds against *C. puncticollis*. The antifeedant activity observed could be due to the presence of bitter limonoids in *M. volkensii* that makes the diet unpalatable [218]. Some of the limonoids with insect control potential which have reportedly been isolated from *M. volkensii* include 1-acetyltrichilinin, salannin, nimbolin B, volkensin among others [51,78].

Even though all the plant parts tested showed activity against the insects, the nut and pulp recorded higher activity against *C. puncticollis*. Previous studies have reported that *M. volkensii* fruits contain most of the reported antifeedant compounds from the tree [62]. This could be the reason for higher activity observed in the nut and pulp since they form part of *M. volkensii* fruit.

These results could be a useful indication in the search for new and potent antifeedant compounds, especially in the nut and pulp of *M. volkensii* against *C. puncticollis.*

2.2.2 LABORATORY BIOASSAY OF *M. VOLKENSII* CRUDE EXTRACTS AGAINST *S. EXIGUA*

Leaf disc bioassay was used to evaluate the antifeedant activity of *M. volkensii* crude extracts against *S. exigua* in the laboratory. *M. volkensii* nut, pulp, bark, leaf extracts all showed varying antifeedant activity against adult *S. exigua* at 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL, 100 mg/mL concentrations as presented in figure 2.2. The antifeedant activity was observed to increase with increase in concentration for all the plant parts with 100 mg/mL concentration giving the strongest activity.

For the nut extracts, all tested concentrations recorded over 50% antifeedant activity with indices of 56.6%, 70.0%, 74.4%, 80.5% and 83.1% recorded for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL, 100 mg/mL concentrations respectively. The activity of nut extracts at 20 mg/mL concentration was significantly different from 60 mg/mL, 80 mg/mL and 100 mg/mL; while no significant difference was observed between 20 mg/mL and 40 mg/mL concentrations. In the pulp extracts, antifeedant indices of 57.8%, 60.2%, 62.5%, 68.2% and 73.9% were recorded for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively. There was no significant difference in activity at 20 mg/mL, 40 mg/mL, 60 mg/mL and 80 mg/mL concentrations in the pulp extracts. However, activity at 20 mg/mL and 40 mg/mL concentrations were significantly different from activity observed at 100 mg/mL concentration.

For *M. volkensii* bark extract, the antifeedant activity was above 50% for all the concentrations tested against *S. exigua* with antifeedant indices of 50.5%, 52.6%, 60.7%, 66.85% and 67.8% for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL, 100 mg/mL concentrations respectively. There was no significant difference in activity at 20 mg/mL, 40 mg/mL, 60 mg/mL and 80 mg/mL concentrations of bark extracts. However, activity at 20 mg/mL concentrations was significantly different from activity observed at 100 mg/mL concentration. In the leaf extract, antifeedant indices of 48.7%, 53.8%, 57.8%, 64.4% and 66.4% were observed in 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL, 100 mg/mL concentrations respectively. There was no significant difference

in activity at 20 mg/mL, 40 mg/mL, 60 mg/mL and 80 mg/mL concentrations of leaf extracts. Antifeedant activity at 20 mg/mL concentrations was significantly different from activity observed at 100 mg/mL concentration of leaf extracts.

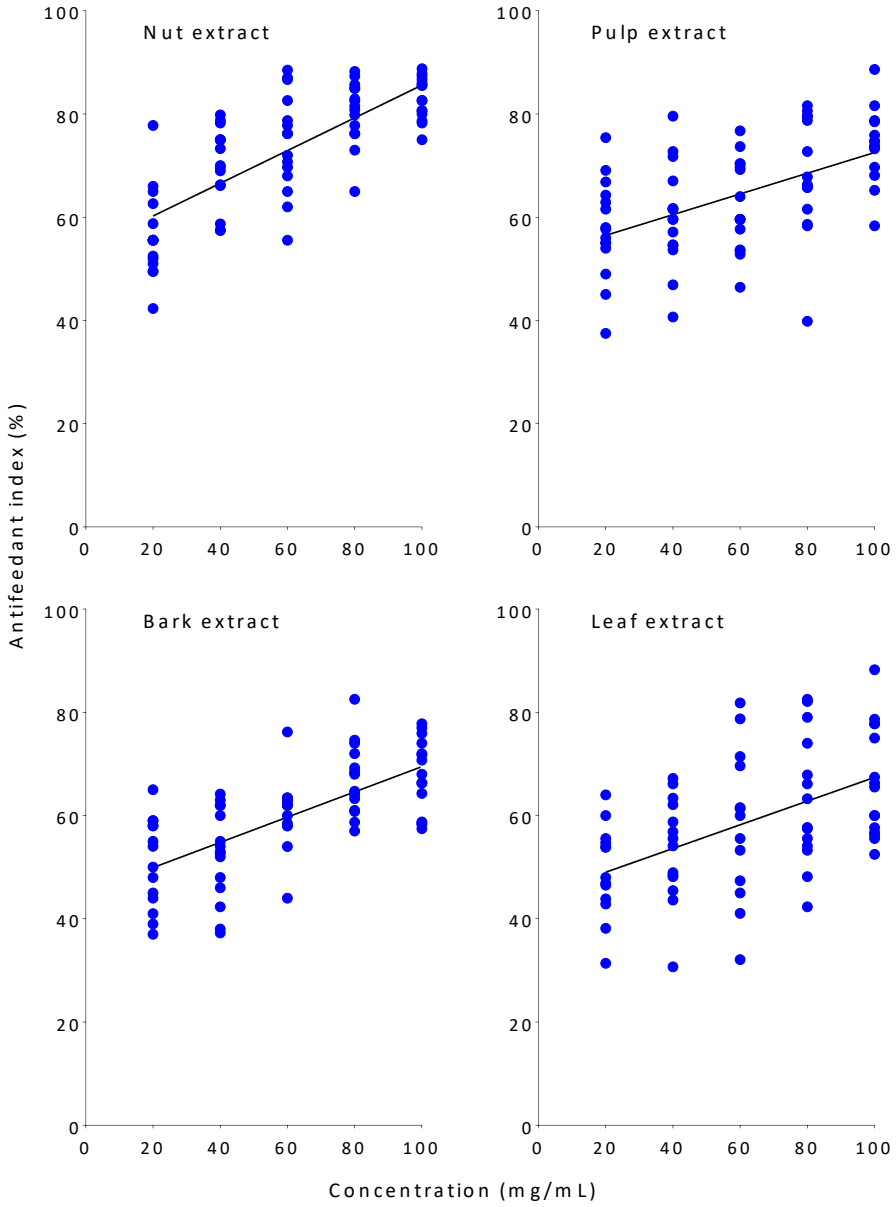


Figure 2.2: Graphical representation of antifeedant index (AFI) of crude extracts from various parts (nut, pulp, bark, leaf) of *Melia volkensii* against *Spodoptera exigua* at different concentrations. AFI values are presented as individual data points based on 3 independent biological repeats, each consisting of 5 technical repeats.

When antifeedant activity of different plant parts was compared at 20 mg/mL concentration against *S. exigua*, there was no significant difference in activity for all the plant parts as presented in table 2.2. The nut and pulp extracts showed higher mean antifeedant indices of 56.6% and 57.8% compared to 50.5% and 48.7% for bark and leaf extracts, respectively.

Table 2.2: Antifeedant indices of crude extracts, at 20 mg/mL concentration, from various parts of *M. volkensii* against *S. exigua*. Means followed by same superscript are not significantly different.

Plant part	Leaf extract	Bark extract	Pulp extract	Nut extract
Antifeedant index	48.7 ± 2.2[a]	50.47 ± 2.2[a]	57.8 ± 2.4[a]	56.6 ±2.2[a]

Caterpillars in early instar stages are susceptible to secondary metabolites from plants when exposed through direct contact or ingestion during feeding [219]. In related studies, clove oil showed antifeedant activity of 56.9% at 1% concentration against *S. frugiperda* larvae [220]. It was also observed that powdered and aqueous extracts of *Peumus boldus* (Molina) recorded of 39.6% and 38.4% activity respectively at 20 mg/mL concentration against *S. frugiperda* [221,[222]. Aqueous plant extracts of *Calotropis gigantea* and *Cresscentia cujete,* at 2.5% concentration, showed antifeedant activity of 9.9% and 8.6% respectively against *S. frugiperda* larvae [195]. Hexane extracts of *Blumeamollis*, tested at 5% concentration also exhibited antifeedant index of 46.8% while the same concentration of *Hygrophila aurigullata* ethyl acetate extracts caused 68.5% antifeeding against *Spodoptera litura* in a no-choice bioassay [223].

Antifeedant indices of 95% and 45% have been reported by *Maytenus senegalensis* and *Solenostemma argel* extracts respectively against 3rd instar larvae of *Spodoptera littoralis* after 48 hours of a choice assay [224]. Dichloromethane extracts of *Duguetia lanceolata* stem have also showed potency against *S. frugiperda* exhibiting LC_{50} of 946.5 µg/mL against first instar of *S. frugiperda* [225]. Methanolic neem leaf extracts when applied at 3% (v/v) in leaf disc laboratory bioassay have recorded 80% larval mortality in *S. frugiperda* after 6 hours [196].

Further botanical evaluations report that methanolic extracts of *Momordica charantia, Tectona grandis, Tamarindus indica* and *Madhuca indica* all showed over 80% antifeedant activity against third instar larvae of *Spodoptera litura* in a leaf disc bioassay when applied at dosage of 100 mg/21 cm^2 [182]. Results of the present investigation have shown that *M. volkensii* methanolic extracts exhibit comparable antifeedant activity against *S. exigua* larvae when compared with other botanical extracts in related investigations. *M. volkensii* nuts and pulp extracts have also demonstrated higher potency against *S. exigua* than the leaf and bark extracts.

2.2.3 LABORATORY BIOASSAY OF *M. VOLKENSII* CRUDE EXTRACTS AGAINST *T. CASTANEUM*

Crude extracts from *M. volkensii* nut, pulp, bark and leaves generally showed certain degree of antifeedant effect against *T. castaneum* as presented in figure 2.3. Similar to *C. puncticollis* and *S. exigua*, the antifeedant activity against *T. castaneum* was observed to be dose-dependent for all the plant parts (nut, pulp, bark and leaf), with 100 mg/mL concentration giving the strongest activity. The nut extracts showed strong antifeedant activity against *T. castaneum* with antifeedant activity of 70.7%, 74.7%, 85.6%, 89.5% and 97.0% for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively. There was no significant difference in activity at 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations of nut extracts while activity at 20 mg/mL concentration was significantly different from activity at 80 mg/mL and 100 mg/mL concentrations (p<0.0001). There was also no significant difference between activity at 20 mg/mL and 40 mg/mL concentrations (p>0.9999).

The pulp extracts showed antifeedant indices of 46.2%, 53.3%, 59.7%, 70.4% and 71.7% for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively. There was no significant difference in activity at 20 mg/mL, 40 mg/mL and 60 mg/mL concentrations of pulp extracts against *T. castaneum*. However, antifeedant activity at 20 mg/mL and 40 mg/mL concentrations were significantly different from activity observed at 80 mg/mL and 100 mg/mL concentration of pulp extracts. The bark extracts recorded lower antifeedant activity compared to the nut and pulp extracts. Antifeedant indices of 34.2%, 38.9%, 39.9%, 41.2% and 46.9% were recorded for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively against *T. castaneum*. There was no significant difference in activity at 20 mg/mL, 40

mg/mL, 60 mg/mL and 80 mg/mL concentration of bark extract. There was, however, a significant difference in activity at 20 mg/mL and 100 mg/mL concentration (p<0.0001). The leaf extracts recorded the lowest mean antifeedant activity with antifeedant indices of with 21.4%, 24.5%, 30.4%, 40.6% and 50.4% for 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations respectively.

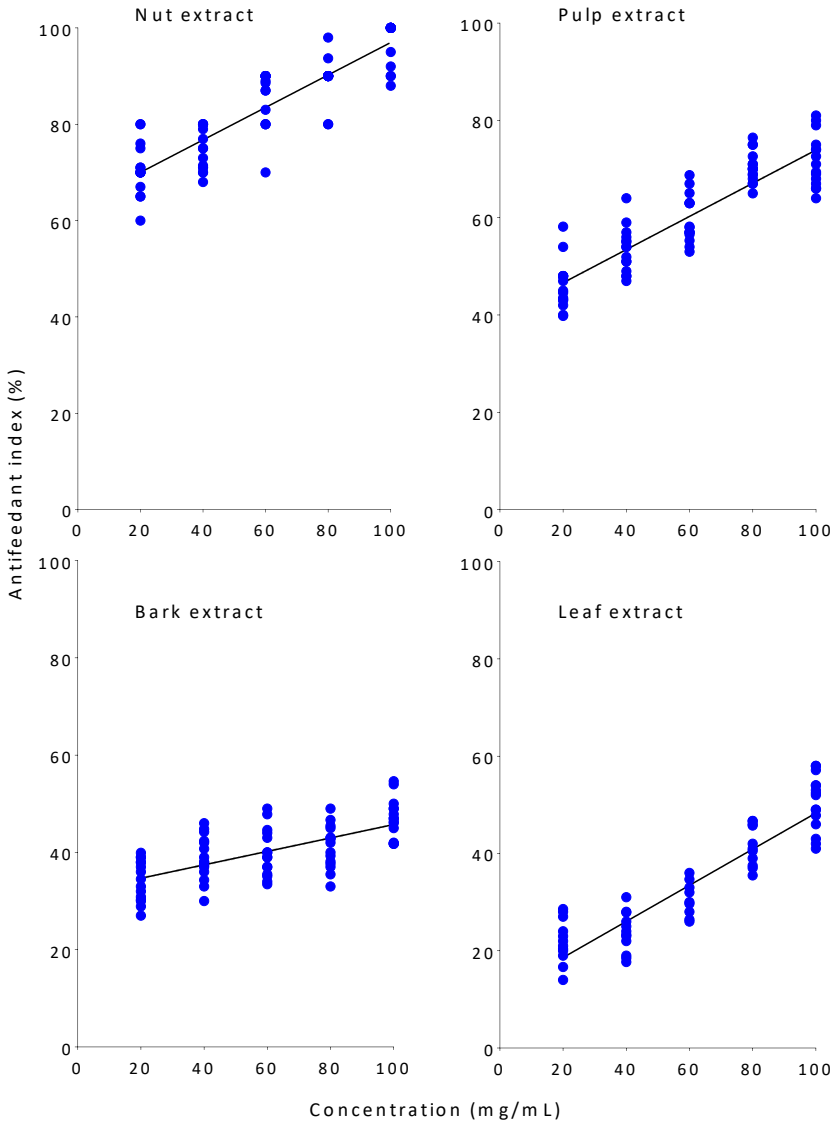


Figure 2.3: Graphical representation of antifeedant index (AFI) of crude extracts from various parts (nut, pulp, bark, leaf) of *Melia volkensii* against *Tribolium castaneum* at different concentrations. AFI values are presented as individual data points based on 3 independent biological repeats, each consisting of 5 technical repeats.

When antifeedant activity of different plant parts was compared at 20 mg/mL concentration against *T. castaneum*, there was a significant difference in activity for the various plant parts as presented in table 2.3. The nut showed highest mean antifeedant index of 70.7% while pulp extract recorded second highest activity at 46.2% against *T. castaneum*.

Table 2.3: Antifeedant indices of crude extracts, at 20 mg/mL concentration, from various parts of *M. volkensii* against *T. castaneum*. Means followed by same superscript are not significantly different.

Plant part	Leaf extract	Bark extract	Pulp extract	Nut extract
Antifeedant index	21.4 ±1.1^{a}	34.2 ±1.1^{ac}	46.2 ±1.3^{bc}	70.7 ± 1.4^{b}

In previous studies, *Datura stramonium* extracts have been reported to be effective against *T. castaneum* especially at higher concentrations with feeding deterrent index of 97.2% at 0.3% (w/v) in a flour disc bioassay [203]. *Mentha piperita* essential oil and its main components (menthone and menthol) all recorded 100% feeding deterrent index against adult *T. castaneum* when tested at 100 µL/L [153]. Aqueous extracts from *Genipa americana* have shown 63% food deterrence against *T. castaneum* when tested at 500 mg/g of wheat flour [226].

Other plants species have shown toxicity against *T. castaneum* including *Ageratum conyzoides*, *Alternanthera nodiflora*, *Ambrosia maritime*, *Cardiospermum halicacabum*, *Eclipta prostrate*, *Polygonum glabrum*, *Pulicariaundulata*, *Solanum dubium*, *Sonchus cornatus* and *Sonchus oleraceus* with LC_{50} values of 10, 20, 20, 200, 10, 10, 20, 100, 20 and 20 mg/mL respectively [155]. Ethanolic extract of *P. hydropiper* also reported an overall repellence of 80.3% against *T. castaneum* at 500 mg/mL concentration [213]. Plant essential oils *Tagetes lucida, Lepechinia betonicifolia, Lippia alba, Cananga odorate* and *Rosmarinus officinalis* have caused 90%, 92%, 96%, 98% and 75% repellence effect respectively at 0.2 µL/cm^2 after 4 hours of exposure against *T. castaneum* [157]. Similar studies using botanical materials report 70%, 64% and 67% repellence *in N. sativa, S. aromaticum* and *T. ammi* respectively against *T. castaneum* after 72 hours at 15% concentration [211].

Piper nigrum oil has also demonstrated toxic effect against 4th instar larvae of *T. castaneum* due to vapor action in a filter paper bioassay with LC_{50} value of 14.0 µL/1cm^2 of filter paper strip. *P. nigrum* oil also inhibited adult emergence recording EC_{50} of 6.9 µL/1cm^2 of filter paper strip after exposure of 4th instar larvae in Petri dish containing different concentrations of the oil (in µL/1cm^2 of filter paper strip) [204]. Seed extracts of neem tree have reported 52.5% mortality against adult *T. castaneum* when topically applied in a laboratory bioassay [210]. Leaf extracts of *Artemisia vulgaris* and *Aphanamixis polystachya* have also been reported to be toxic against *T. castaneum* [213].

Crude extracts from plants often consist of complex mixtures of compounds and this could be advantageous as natural mixtures may act synergistically against insect pests [227]. Mortality against *T. castaneum* has been observed in *Polygonum hydropiper* ethanol extract with LD_{50} value of 14.85 mg/cm^2. Crude extracts may show greater overall bioactivity compared to individual constituents and insect resistance is less likely to develop due to their varied modes of action [227]. These reasons support the use of crude, chemically unrefined plant extracts which are simpler and cheaper to prepare if the plant materials are locally available [227]. The results of this study provide an indication that *M. volkensii* crude extracts, especially the nut and pulp extracts, may be a potential grain protectant due to their strong antifeedant activity against *T. castaneum.*

2.3 Conclusion

In the present investigation, *M. volkensii* nut, leaf, bark and pulp extracts have all showed certain extent of antifeedant activity against *C. puncticollis, S. exigua* and *T. castaneum*. This implies that antifeedant compounds could be present in these parts of *M. volkensii* tree. The nut and pulp extracts showed mean higher antifeedant activity as compared to the leaf and bark extracts and this could be an indication that more potent compounds could be present in the nut and pulp. This study serves as basis for future studies on *C. puncticollis, S. frugiperda* and *T. castaneum* that aim to reduce application of synthetic chemicals and damage caused by these pests without negative environmental and health impact. The use of botanicals in control of these insect pests can be embraced under an integrated pest management approach and *M. volkensii* extracts could be combined with other control methods to achieve more effective pest management results. The antifeedant activity observed in this study suggests that *M. volkensii* materials could be further exploited as potential source of insect control products with minimal environmental impact. The availability of *M. volkensii* renewable resources such as nuts, pulp, bark and leaves make this plant a potential candidate for insect control with minimal interference on the plant. In this regard, *M. volkensii* could be further exploited as a source of natural insecticide.

CHAPTER 3

Efficacy of *Melia volkensii* extracts and powders against *Cylas puncticollis, Spodoptera frugiperda* and *Tribolium castaneum* in green house and simulated storage

Partially adapted from:

V. Jaoko *et al.*, "Laboratory and Greenhouse Evaluation of *Melia volkensii* Extracts for Potency against African Sweet Potato Weevil, *Cylas puncticollis*, and Fall Armyworm, *Spodoptera frugiperda*," *Agronomy*, vol. 11, no. 1994, pp. 1–9, 2021.

3.0 INTRODUCTION

Sweet potato weevils, *C. puncticollis*, are considered the most important sweet potato pest in Africa [158]. The weevil infestation could range from between 20% to 100% depending on sweet potato variety, season and storage conditions [158]. Its larvae is the most destructive development stage feeding and tunnelling in the sweet potato tubers and mature vines giving unpleasant odour and bitter taste leading to reduction in market value [158]. Research on *C. puncticollis* management has not been extensively explored worldwide, especially in the developing world [158]. Synthetic pesticides and cultural methods have been used in management of *C. puncticollis* [158]. However, continuous application of synthetic pesticides has been associated with negative impacts on human, livestock and environment [137,181]. This has created an impetus for investment in alternative means of insect control that are safer and environmentally friendly [137,181]. Botanicals have therefore been proposed as alternative source of insect control products because of their high specificity, little adverse impact on environment and non-toxicity to humans when used at recommended doses [137,181].

Fall armyworm, *S. frugiperda*, is a phytophagous pest with a wide range of host plants and a long-range migratory pest capable of flying over 100 km in a single night [194]. It feeds on maize whorls, ears and tassel causing substantial damage with occasional total yield loss [188,208]. *S. frugiperda* larvae cause crop damage while the adults ensure rapid spread of the pest by flying across long distance and rapidly reproducing [18]. *S. frugiperda* is an important economic pest of maize in Africa whose management is key to maize cultivation [197,208]. Synthetic pesticides used to control this pest have been also associated with residual toxicity, photo toxicity and vertebrate toxicity [224]. An alternative approach is therefore necessary to avoid overreliance on synthetic pesticides [223,228,229]. Bioactive plant materials have demonstrated efficacy as botanical pesticides for control of *S. frugiperda* [188,208]. Such plant-derived materials are readily and cheaply produced by smallholder farmers as crude extracts [224]. They are also biodegradable and have low mammalian toxicity [18].

The red flour beetle, *T. castaneum*, is a major insect pest of stored grains worldwide [157,230–232]. The use of plant materials to control *T. castaneum* is an ancient practice and typically involves mixing of grains with herbal mixture of the identified plant species [210,233]. Management of stored grain pests using plant materials has received a lot of attention and plants like *Calotropis procera*, *A. indica*, *Cassia fistula*, *Chrysanthemum coronarium* and *Lantana camara* have been evaluated for efficacy against stored grain pests [234,235]. These botanicals are environmentally safe, less toxic, target specific as compared to synthetic pesticides and they are also readily available [22,212,219,225,236–239].

In the previous laboratory bioassays, *M. volkensii* nut and pulp crude extracts presented the highest antifeedant activity against *C. puncticollis, S. exigua* and *T. castaneum.* For this reason, the nut and pulp were selected for further investigation. The current investigation aimed to evaluate the potential of *M. volkensii* nut and pulp crude extracts to protect sweet potato, maize and stored grains against damage by *C. puncticollis, S. frugiperda* and *T. castaneum* in practical conditions. To achieve this objective, greenhouse trials were conducted to determine the efficacy of 20 mg/mL concentration of *M. volkensii* nuts and pulp crude extracts in protection of cultivated maize and sweet potato against *S. frugiperda* and *C. puncticollis* in greenhouse conditions. Simulated storage trials were also conducted to determine the efficacy of 20 mg/mL concentration of *M. volkensii* nuts and pulp powders against *T. castaneum* in a simulated storage.

3.1 MATERIALS AND METHODS

3.1.1 INSECT REARING

3.1.1.1 Sweet potato weevil, *C. puncticollis*

African sweet potato weevils, *C. puncticollis,* were obtained from an insect culture maintained in the entomology laboratory at Kenya Forestry Research Institute in Nairobi, Kenya. The insects were reared using sweet potato tubers placed in a plastic container (10 x 20 x 30 cm) in a growth chamber set at 26 ± 2 ℃, 70% relative humidity and 16 h light:8 h dark photoperiod [116].

3.1.1.2 Fall armyworm, *S. frugiperda*

S. frugiperda larvae were obtained from a continuous insect culture maintained in the entomology laboratory at Kenya Forestry Research Institute in Nairobi, Kenya. *S. frugiperda* larvae were reared using young maize leaves and after emergence, the adults were fed on honey solution. The colony was kept in a growth chamber at 26 ± 2 °C with 75% relative humidity and 16 h light:8 h dark photoperiod.

3.1.1.3 Red flour beetle, *T. castaneum*

An insect culture of the red flour beetle, *T. castaneum,* established in the entomology laboratory at Kenya Forestry Research Institute in Nairobi, Kenya was used to rear the insects using the method described by Jbilou et al., 2006 [202]. The insects were reared on wheat flour mixed with yeast (10:1, w/w) kept in 300 ml plastic jars covered with muslin cloth held with rubber bands for ventilation. The jars were kept in a growth chamber maintained at 28 ± 2 °C, humidity of 70 ± 5 % and 14 h light:10 h dark photoperiod.

3.1.2 PREPARATION OF TREATMENTS

M. volkensii nut and pulp crude extracts, at 20 mg/mL concentrations, were used in the greenhouse trials. The respective crude extracts were first dissolved in methanol and topped up with water (96% of total volume). For negative control 4% methanol in water (*v*/*v*) was applied. The respective treatments were exposed to laminar flow to evaporate as much methanol as possible. The positive control used in the greenhouse trial was HABLE 5 WG, a synthetic pesticide

which contains emamectin benzoate 50 g/kg as active ingredient. It is used locally by farmers to manage *S. frugiperda* and *C. puncticollis* infestation in Kenya. The pesticide was applied at manufacturers' recommended dose of 5 g/20 litres of water.

3.1.3 EFFICACY OF *M. VOLKENSII* EXTRACTS AGAINST *S. FRUGIPERDA* IN GREENHOUSE CONDITIONS

'Grade H 512' maize cultivar from Kenya Seed Company was purchased from a local Agrovet shop in Nairobi, Kenya. Six maize seeds were sown in 3 lit plastic planting pots in a greenhouse (temperature: minimum of 18°C at night and maximum of 28°C during daytime; relative humidity of 70 – 80%; natural light). After seedling emergence and establishment, two plants were thinned out leaving four plants in each planting pot. All agronomic practices including hand weeding and watering were consistently done.

When the maize crops attained knee-height (6 weeks after planting), the pots were placed in transparent insect cages (60 x 60 x 90 cm) purchased from Vermandel, Belgium. Five newly hatched larvae were introduced on each plant. The larvae were placed at the whorl of the plants, the feeding site. As soon as feeding was noted (2 days after infestation), the following treatments were applied: (a) 20 mg/mL concentration of *M. volkensii* crude nut extract, (b) 20 mg/mL concentration of *M. volkensii* crude pulp extract, (c) commercial pesticide (HABLE with active ingredient being emamectin benzoate) as positive control, (d) negative control (4% methanol in water). 120 ml of each treatment was sprayed using 1-L hand sprayer in each planting pot until run-off at 2 days after infestation and repeat spraying was done 5 days after infestation. Treatment was directed at the whorl of the maize plant and on top of the leaf. The planting was replicated 4 times per treatment with 3 independent biological repeats.

Baseline data on leaf damage was collected two days after infestation (just before application of the treatments) to establish the extent of damage before treatment. The percentage of whorls infested was recorded and leaf damage was evaluated for individual plants 7 days after treatment using visual rating scale of 1-9 as presented in table 3.1 [240] . Where 0 = no visible damage, 9 = most leaves with long lesions and complete damage.

Table 3.1: Visual rating scale for maize leaf damage assessment [209]

Rating scale	Description
0	No visible leaf damage;
1	Only pin-hole damage on leaves
2	Pinholes, shot hole damage to leaf
3	Small elongated lesions (5-10 mm) on 1-3 leaves
4	Midsized lesions (10-30 mm) on 4-7 leaves
5	Large elongated lesions (>30 mm) or small portions eaten on 3-5 leaves
6	Elongated lesions (>30 mm) and large portions eaten on 3-4 leaves
7	Elongated lesions (>30 mm) and 50% of leaves eaten
8	Elongated lesions (30 mm) and large portions eaten on 70% of leaves
9	Most leaf with long lesions and complete defoliation observed

3.1.4 EFFICACY OF *M. VOLKENSII* EXTRACTS AGAINST *C. PUNCTICOLLIS* IN GREENHOUSE CONDITIONS

'Kabode' sweet potato cultivar vines, a fast-maturing variety and susceptible to sweet potato weevils, was purchased from Potato International Centre (CIP), Nairobi. Three sweet potato vines were planted in 20 L plastic planting pots in the greenhouse (temperature: minimum of 18°C at night and maximum of 28°C during daytime; relative humidity of 70 – 80%; natural light). The planting pots were perforated at the base with holes 1 cm wide to avoid water stagnation. After establishment, weaker vines were thinned out, leaving two vines in each planting pot.

Four months after planting (period of tuber formation), the potato crown was not watered for 5 days (to allow cracking of soil for easy entry of the weevils into the soil). 20 adult sweet potato weevils aged 4 - 7 days were artificially introduced to each pot and placed on the potato crown. The planting pots were then put in transparent insect cages (60 x 60 x 90 cm; Vermandel, Belgium). 3 days after infestation, the following treatments were applied: (a) 20 mg/mL concentration of *M. volkensii* crude nut extract, (b) 20 mg/mL concentration of *M. volkensii* crude pulp extract, (c) commercial pesticide (HABLE with active ingredient being emamectin benzoate) as positive control, (d) negative control (4% methanol in water). 1 L of respective treatment was drenched over the potato crown using a watering can. Repeat treatment was 7 days later.

Five and half months after planting, we harvested the sweet potatoes and determined the percentage tuber damage, vine perforation and tuber perforation indices as described by Nta and Oku, 2019 [97]. The planting was replicated 4 times per treatment with 3 independent biological repeats. Testing of major plant nutrients to evaluate phytotoxicity of crude extracts against the sweet potato plants was done as per protocol developed by Okalebo et al., 2002 [241].

3.1.5 EFFICACY OF *M. VOLKENSII* POWDERS AGAINST *T. CASTANEUM* IN SIMULATED STORAGE CONDITIONS

Melia volkensii nut and pulp were dried and ground into fine powder. 1 g of respective nut and pulp powders was thoroughly mixed with 50 g of cracked maize grains with aid of glass rod to ensure thorough admixture of each plant powder with grains in a 250 ml plastic jar, to mimic grain storage conditions. Maize used in this study was cracked because *T. castaneum* is a secondary insect pest and attacks maize after primary infestation has taken place. For positive control, actellic dust (active ingredient; pirimiphos-methyl & thiamethoxam) was used while in negative control no treatment was applied.

Each jar lid was perforated to allow ventilation and 20 adult red flour beetles (5 to 7-day old) were introduced and the jars kept in growth incubator at 32℃ for 42 days (approximate period from egg to adult emergence of *T. castaneum*). There were 5 replications per treatment and 2 independent biological repeats. After 42 days, % reduction in adult emergence or inhibition rate (IR) was calculated as below [242]:

$$\% \text{ IR} = \frac{\text{no of F1 progeny in negative control } - \text{ no of F1 progeny in treatment}}{\text{no of F1 progeny in negative control}} \, x \, 100$$

The number of larvae (all larval stages combined) in each treatment was recorded and % larvae inhibition rate (IR) calculated as below:

$$\% \text{ IR} = \frac{\text{no of larvae in negative control } - \text{ no of larvae in treatment}}{\text{no of larvae in negative control}} \, x \, 100$$

The adjusted mortality (% AM) of adult *T. castaneum* was calculated using the Abbot's formula below and the insects were considered dead when no movement was observed after touching them carefully with entomological forceps.

$$\%\ \mathrm{AM} = \frac{\%\ \text{mortality in treatment} - \%\ \text{mortality in negative control}}{100 - \%\ \text{mortality in negative control}}\ x\ 100$$

3.1.6 STATISTICAL ANALYSIS

Graphical representations were generated using GraphPad Prism version 6.01. First, normality test was done using Shapiro-Wilk test. Based on the analysis, non-parametric analysis was done. In the greenhouse and simulated storage investigations, non-parametric Mann-Whitney test was performed to compare significance between the various treatments and negative control [243]. Adjusted mortality was corrected using Abbott's formula [160].

3.2 RESULTS AND DISCUSSION

3.2.1 EFFICACY OF *M. VOLKENSII* CRUDE EXTRACTS AGAINST *S. FRUGIPERDA* IN GREENHOUSE CONDITIONS

Fall armyworm larvae prefer to feed on young maize leaf and whorls [244]. In the current investigation, maize leaf and whorl damage were characterized by mass of holes on leaves, ragged edges and larval frass. Highest leaf damage rating of 7.6 was observed in negative control as presented in figure 3.1. There was significant reduction in leaf damage in maize treated with the nut extracts, pulp extracts and positive control with leaf damage ratings of 4.5, 5.0 and 4.6 respectively when compared with negative control ($p < 0.0001$, N=12).

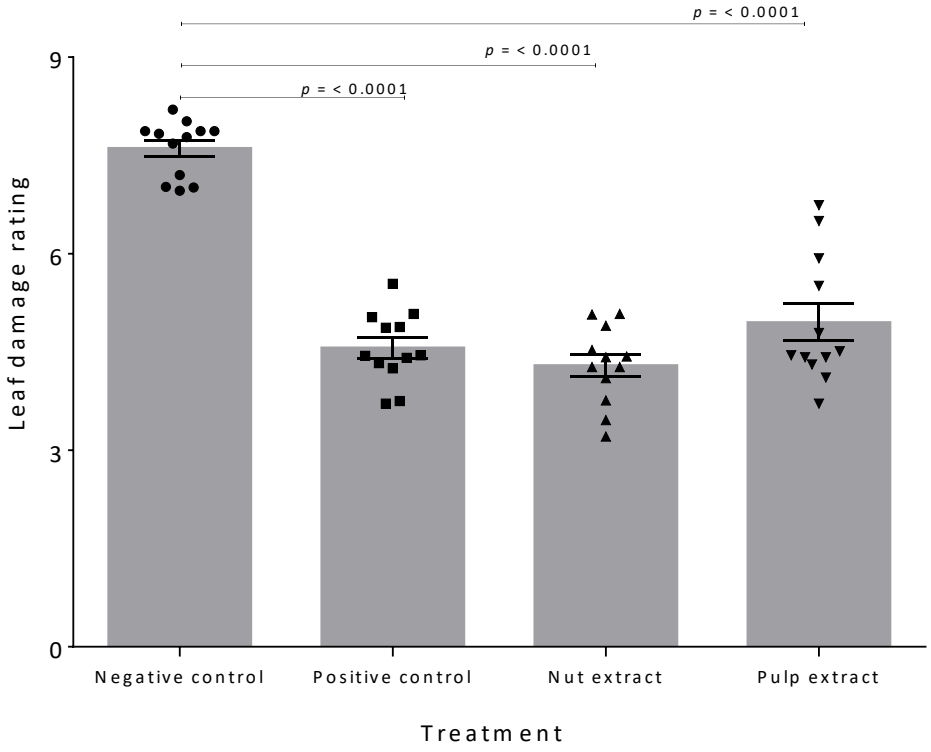


Figure 3.1: Scatter plot representation of maize leaf damage by *Spodoptera frugiperda* in various treatments. Points are leaf damage ratings ± SEM with 4 technical repeats and 3 independent biological repeats per treatment. *p*-values, to indicate significant difference, were determined by Mann-Whitney test

The results of this study highlight the effectiveness of the *M. volkensii* nuts and pulp extracts as alternative measure against *S. frugiperda*. Interestingly, the nut extracts showed almost equal

protection against maize leaf damage from *S. frugiperda* as the synthetic pesticide, positive control with leaf damage rating of 4.5 and 4.6 respectively. The extent of leaf damage of various treatments was also observed visually where there was intensive feeding by *S. frugiperda* larvae on maize leaf in the negative control as compared the feeding in the nut, pulp and positive control.

Maize whorl is the growing part of the plant and is the preferred feeding site for FAW larvae. In this study, the highest whorl damage of 96% was observed in the negative control as presented in figure 3.2. There was significant reduction in whorl damage in nut extract, pulp extract and positive control with just 17%, 35% and 5% whorl damage respectively when compared to negative control ($p < 0.0001$, N=12).

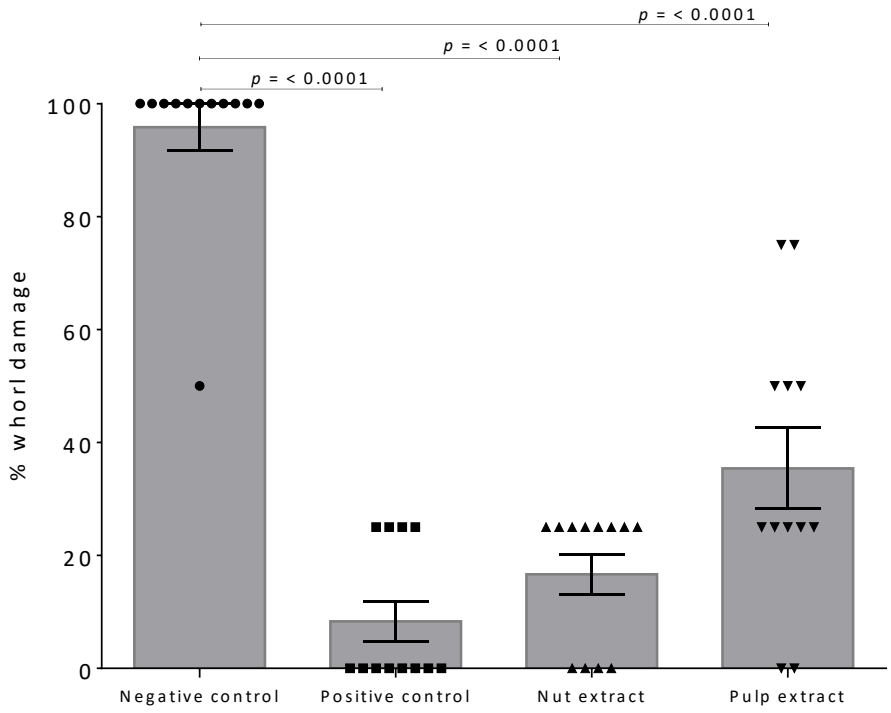


Figure 3.2: Scatter plot representation of maize whorl damage by *Spodoptera frugiperda* in various treatments. Points are % whorl damage ± SEM with 4 technical repeats and 3 independent biological repeats per treatment. *p*-values, to indicate significant difference, were determined by Mann-Whitney test.

The observed reduction of leaf and whorl damage caused by fall armyworm could be a result of antifeedant compounds in *M. volkensii* nut and pulp extracts which interfere with growth and development of the insects probably through food deterrence [219]. Plant-based products begin to affect development of fall armyworm a few days after leaf spraying, usually seven days after application [219]. In previous studies to determine effectiveness of botanicals in maize protection against *S. frugiperda*, aqueous extracts of *Melia azedarach, Allium sativa, A. indica* recorded leaf damage ratings of 2.6, 2.4 and 2.7 respectively while synthetic pesticide, cypermethrin recorded leaf damage rating of 2.3 [18]. In sorghum field trial, synthetic pesticides chlorantraniliprole, cyantraniliprole, flubendiamide, lambda-cyhalothrin, methoxyfenozide and novaluron recorded whorl infestations of 10%, 12.5%, 32.5%, 45.0%, 40.0% and 15% respectively [245].

Environmental safety of an insecticide is paramount and insecticides do not necessarily have to cause mortality on target organism to be acceptable [156]. Antifeedants render plants unattractive and unpalatable thereby reducing plant damage by insect pests even without killing the insect pest [246]. In the long run, decrease in food consumption leads to insect population reduction through disrupted metamorphosis or reduced fecundity and this could make antifeedants an important tool in integrated pest management [156].

This study shows that crude nut and pulp extracts can provide a degree of protection to maize by significantly reducing maize whorl damage and significantly minimizing maize leaf damage. Extensive leaf damage by *S. frugiperda* can lead to poor plant health and ultimately reduced maize yield at harvest. Feeding on the whorls of young maize plants could also damage the growing point of the maize leading to reduced yield, stunted growth or even death of the maize plant. Moreover, severe maize infestation by *S. frugiperda* could likely reduce photosynthetic carbon fixation and consequently reduce plant growth and productivity [18]. Going by the results of this investigation, *M. volkensii* nut and pulp extracts could provide protection of cultivated maize against *S. frugiperda* infestation, however, the nut extract shows higher potency against the insect.

3.2.2 EFFICACY OF *M. VOLKENSII* CRUDE EXTRACTS AGAINST *C. PUNCTICOLLIS* IN GREENHOUSE CONDITIONS

The highest sweet potato tuber damage was observed in the negative control with 76% tuber damage as presented in figure 3.3. There was a significant reduction in tuber damage (18%) in the nut treatment compared with negative control (p=<0.0001, N=12). Also observed was a significant reduction in tuber damage with pulp extract (30% tuber damage; p=0.0087, N=12) and positive control (33% tuber damage; p=0.0033, N=12) when compared with negative control.

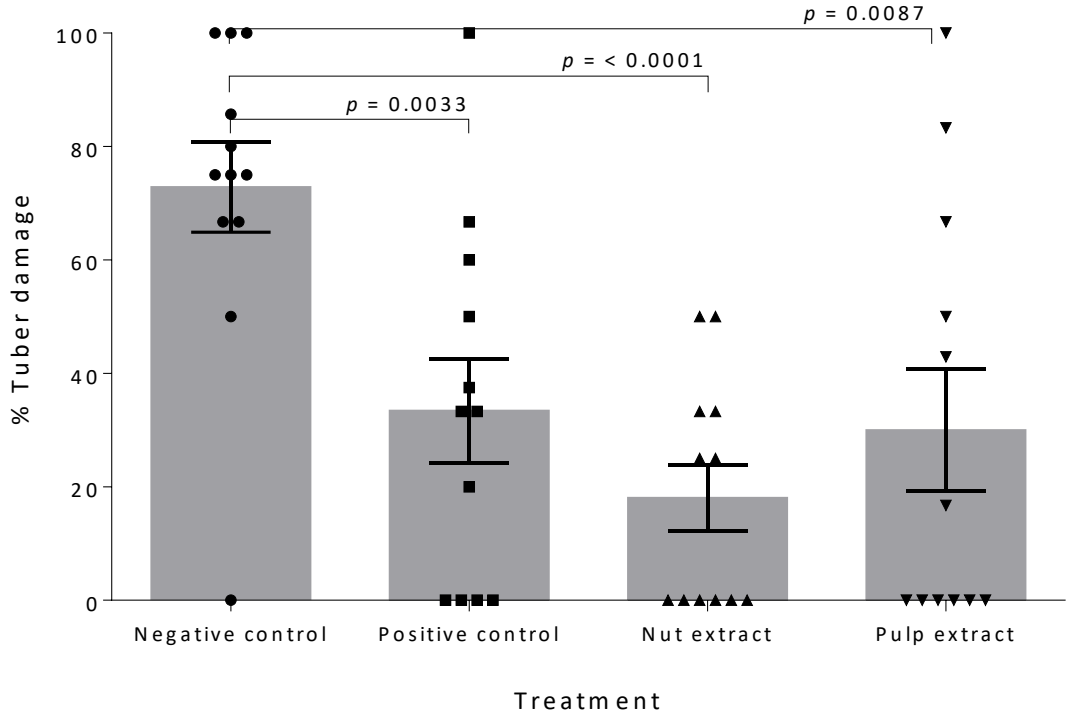


Figure 3.3: Scatter plot representation of sweet potato tuber damage by *Cylas puncticollis* in various treatments ± SEM with 4 technical repeats and 3 independent biological repeats per treatment. *p*-values, to indicate significant difference, were determined by Mann-Whitney test.

Previous studies done to evaluate effectiveness of botanicals in control of *C. puncticollis* in field conditions reported reduction in tuber damage by 25.3%, 6%, 26.1% and 22.4% for *Carica papaya, Chromolaena odorata*, *Moringa olifeira* and *Azadirachta indica* respectively [185]. These studies were done with 10% (w/v) of aqueous extracts of the respective plant where low weevil infestation was also reported as a result of the botanical treatments [185]. The same study reported 12.9% tuber damage when cypermetrin was applied in a field experiment. Botanical crude extracts from *Aframomum melegueta, Dennettia tripetalla* and *Xylopia aethiopica* fruits have shown protection of sweet potato tuber from damage by *C. puncticollis* with 11.5%, 15.1%, 10.6% tuber damage respectively [98].

In a separate study, neem seed oil, applied at concentration of 2.5% (v/v) recorded sweet potato weevil infestation of 5.6% while diazinon recorded 9.9% infestation when applied at 0.3% (v/v) in a field experiment [99]. Neem extracts have been reported to contain antifeedant, repellent and insect sterilization properties which prevents *C. puncticollis* from defoliating sweet potato [185]. *Lantana camara* leaf extracts have also shown efficacy in reducing sweet potato weevil severity recording an average of 6 damaged tubers per potato crown, while *M. azedarach* and malathion recorded damage severity of 9 and 6 tubers respectively [188]. It was also reported that less sweet potato damage occurs under irrigation scheme than when no irrigation is done [188]. In a storage experiment, neem oil reported complete protection of sweet potato tubers from attack by *C. puncticollis* while aqueous extracts from neem leaf exhibited 10.5% damage after 8 weeks of storage [187]**.**

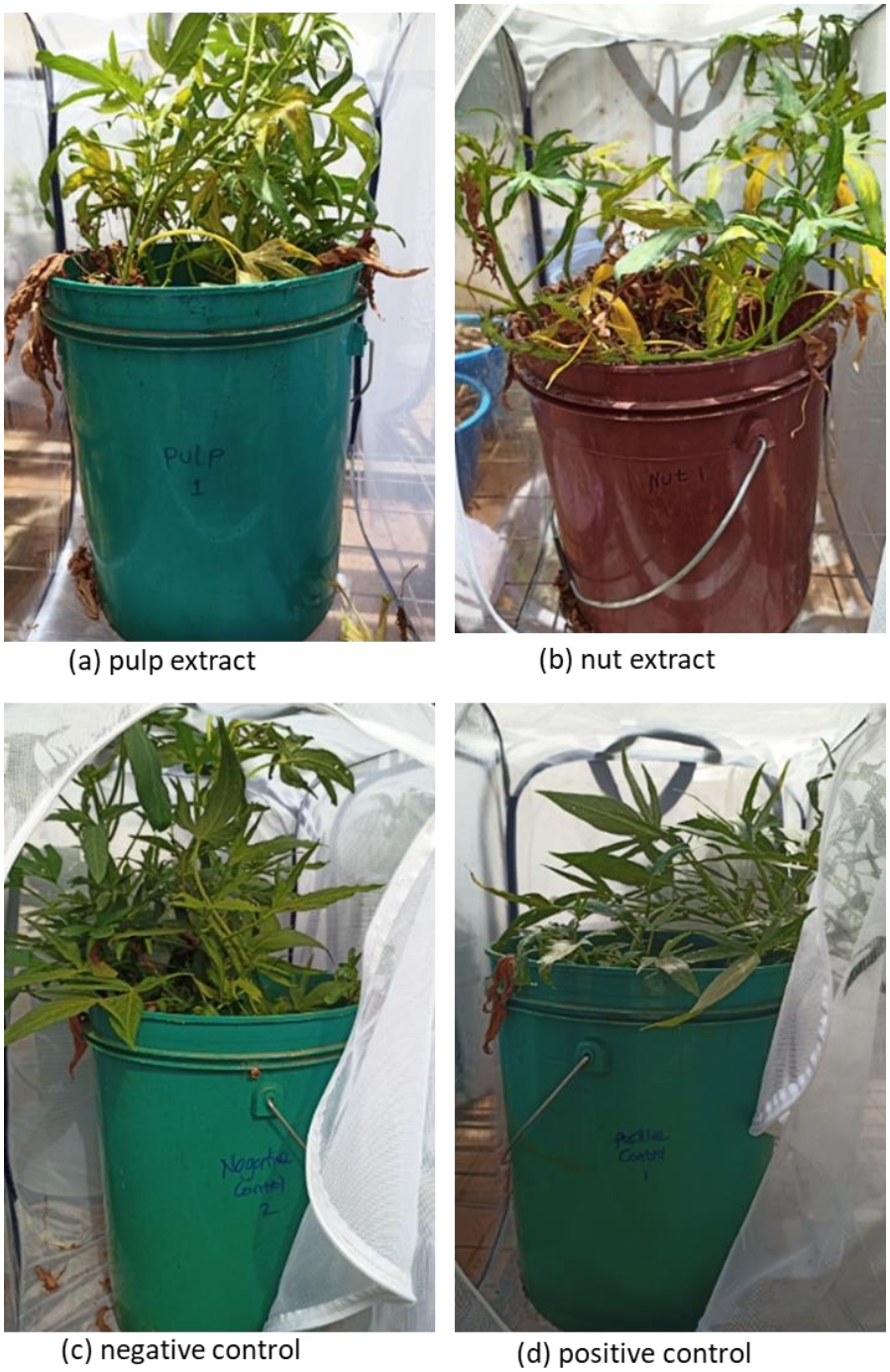
While some botanical pesticides are not as effective as synthetic pesticides, their use generally carries lower risk to human, environment, non-target organisms and further minimizes emergence of pest resistance [226]. Moreover, the ease and lower cost of preparing plant extracts makes them viable alternatives in pest management [226]. The concept of using antifeedants to protect crops without directly killing insect pests is an attractive one which has received considerable attention [223]. In the current evaluation of sweet potato protection, the commercial pesticide (HABLE) offered certain degree of protection (33% damage); however, it was not as effective as the nut extracts (18% damage). In Kenya, where this study was based,

there is no registered insecticide specific to *C. puncticollis* and farmers use other broad-spectrum insecticides like emamectin benzoate (HABLE 5wg) to manage *C. puncticollis* populations. Therefore, *M. volkensii* nut extracts could potentially be an important component in management of FAW and *C. puncticollis*. It is important to note that repeated exposure of pests to botanical extracts could potentially lead to habituation or loss of efficacy in pest management. [66]. This highlights the importance of combining different control strategies in an IPM approach to prevent pest resistance emergence.

In Kenya, *M. volkensii* nuts, pulp, bark and leaves are readily available and are part of waste generated during seed extraction, timber processing and pruning. These waste materials could be a useful raw material for the formulation of botanical pesticides, as they contain potential insecticidal compounds. The use of *M. volkensii* extracts, especially nuts and pulp, in management of *C. puncticollis* could relieve smallholder farmers from the negative effects associated with the use of synthetic pesticides.

3.2.3 PHYTOTOXICITY OF VARIOUS TREATMENTS ON SWEET POTATO

Temporary phytotoxicity was observed in sweet potato crowns treated with *M. volkensii* nut and pulp extract. This was through visual observation of the aerial parts of the potato crown. The following features were observed: wrinkled leaves, scorching, general chlorosis, recurved leaf edges and twisted growing tips as presented in figure 3.4. The phytotoxic effect was observed 5 days after treatment and lasted for 7 days after which the leaves and vines regained their original green coloration. Analysis of major elemental nutrients: nitrogen, potassium, phosphorous in the plant tissues (leaves and vines) and the soil in each of the planting pots revealed varying degree of nutrient presence in various treatments as presented in table 3.2.



(a) pulp extract (b) nut extract

(c) negative control (d) positive control

Figure 3.4: Physical appearance of sweet potato crowns treated with *M. volkensii* crude pulp extracts (a), nut extracts (b), negative control (c) and positive control (d). Phytotoxicity was visually observed through wrinkled leaves, scorching, general chlorosis, recurved leaf edges and twisted growing tips in the sweet potato crowns treated with nut and pulp extracts.

Table 3.2: Concentration levels of major plant nutrients, nitrogen, phosphorous and potassium, in sweet potato tissues (vines and leaves) and in the soils from respective treatments to evaluate phytotoxicity.

Treatment	Nitrogen (%)	Phosphorous (ppm)	Potassium (ppm)
Pulp extract sweet potato tissues	1.4	754.4	12484.9
Nut extract sweet potato tissues	1.7	693.6	11389.9
Positive control sweet potato tissues	3.6	1212.5	23682.8
Negative control sweet potato tissues	2.7	1152.6	24492.5
Soil from pulp extract	0.5	71.2	465.8
Soil from nut extract	0.6	73.4	453.7
Soil from positive control	0.6	79.2	465.6
Soil from negative control	0.6	80.1	465.6

Lower nitrogen levels were found in sweet potato vines and leaves in nut (1.7%) and pulp (1.4%) treatments while there were higher levels of nitrogen in the positive control (3.6%) and negative control (2.7%). There was, however, little variation in the concentrations of nitrogen in the soils from the planting pots in the respective treatments with 0.5%, 0.6%, 0.6% and 0.6% concentrations of nitrogen in the soils from pulp extract, nut extract, positive and negative control respectively.

Phosphorous concentration was lower in the nut (693.6 ppm) and pulp (754.4) treatments while positive and negative control has 1212.5 ppm and 1152.6 ppm respectively. The phosphorous levels in the soils from the various planting pots were comparable with 71.2 ppm, 73.4 ppm, 79.2 ppm and 80.1 ppm of phosphorous in soils from the pulp, nut, positive and negative control planting pots respectively.

Potassium levels were also lower in pulp extract treatment (12484.9 ppm) and nut extracts (11389.9 ppm) while positive and negative control had higher levels of potassium with concentrations of 23682.8 ppm and 24492.5 ppm respectively. The potassium concentration in the soils from the various planting pots was comparable at 465.8 ppm, 453.7 ppm, 465.6 ppm

and 465.6 ppm concentrations of potassium in soils obtained from pulp, nut, positive and negative control respectively.

The overall observation was that there are lower concentration levels of the major plant nutrients (nitrogen, phosphorous and potassium) in the sweet potato tissues treated with nut and pulp extracts as compared to the positive and negative control. However, there were almost equal concentrations of these major plant nutrients in the soils taken from the respective planting pots in all the treatments. This could imply that crude extracts did not affect nutrients bioavailability and presence in the soil. The concentrations of the plant nutrients in the plant tissues could have been affected by the *M. volkensii* nut and pulp extracts leading to the visual phytotoxicity observed in the respective treatments.

Several factors could be attributed to the phytotoxicity observed in sweet potato treated with the nut and pulp extracts. The low levels of major plant nutrients (N, P and K) could be due to blockage of cambium, phloem or xylem in the sweet potato roots by the crude extracts thereby minimizing translocation of the nutrients from the soil. This could have led to systemic blockage of uptake of major plant nutrients by the crops thereby affecting their bioavailability in the plant tissues. Another reason for the phytotoxicity could have been due to burning effect of crude extracts observed through leaf chlorosis and scorching. This could have led to breakdown in photosynthesis resulting in nutrient deficiency recorded. Moreover, it has been reported that spraying plant extracts at higher concentrations could result in possible phytotoxicity as observed when 10% of *Caryocar brasiliense* Camb. aqueous extract was sprayed in a maize field experiment [219]. This could be similar to the phytotoxicity observed in this study against sweet potato where 20 mg/mL of *M. volkensii* nut and pulp crude extracts were applied. The temporary phytotoxicity lasted for 7 days implying that affected crops could regenerate without impacting the overall development of cultivated sweet potato.

3.2.4 EFFICACY OF *M. VOLKENSII* CRUDE EXTRACTS AGAINST *T. CASTANEUM* IN SIMULATED STORAGE

With the nut and pulp crude extracts showing higher antifeedant activity against *T. castaneum* in the laboratory bioassays, simulated storage trials were conducted using these two plant parts to demonstrate efficacy against *T. castaneum* in practical set up. The emergence of new adult *T. castaneum* (F1 progeny) after 42 days was compared in all the treatments.

The negative control and *M. volkensii* pulp powder saw an average of 22 and 48 new insects emerge from the stored grains after 42 days. The positive control (actellic dust) completely inhibited emergence of F1 progeny while average of only 2 adult insects emerged from the grains treated with *M. volkensii* nut powder as presented in figure 3.5.

Nut powder and positive control therefore significantly reduced emergence of adult *T. castaneum* with 92.3% and 99.1% inhibition rate ($p < 0.0001$, N = 10) respectively as presented in table 3.3. The pulp powder, however, significantly stimulated adult emergence with inhibition rate of -116.9% ($p < 0.0001$, N = 10).

F1 progeny production and adult emergence in the negative control indicated that the insects were capable of effective reproduction and that prevention of progeny emergence was exclusively due to the treatments applied.

Table 3.3: Inhibition rates of *T. castaneum* adult and larvae in various treatments after 42 days

Treatment	Dose (g/50g) grains	Adult inhibition rate	Larvae inhibition rate
Negative control	0.00	0.0	0.0
Actellic dust	0.03	99.1	97.1
Nut powder	1.00	92.3	78.3
Pulp powder	1.00	-116.9	-19.7

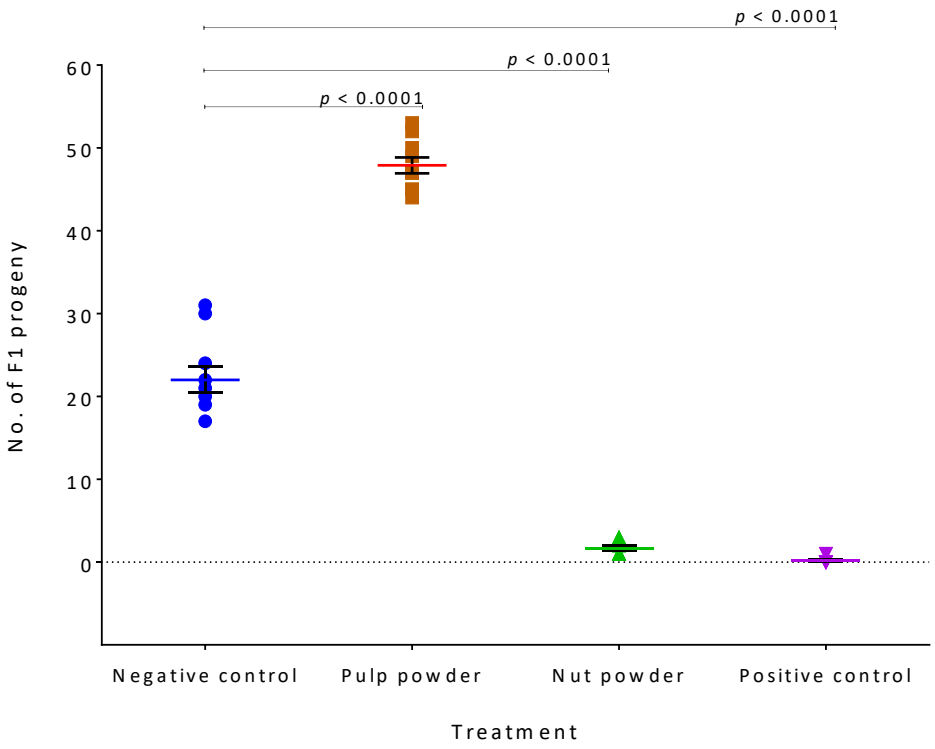


Figure 3.5: Scatter plot representation number of *Tribolium castaneum* F1 progeny emergence in various treatments after 42 days. The points are number of F1 progeny ± SEM with 5 technical repeats and 2 independent biological repeats per treatment. p-values, to indicate significant difference, were determined by Mann-Whitney test.

Botanical extracts from *P. aquilinum* and *C. erythraea,* at 10% concentration, have been reported to reduce adult emergence by 81% and 34% respectively at 7 weeks after treatment [151]. F1 progeny production of *T. castaneum* has also been suppressed using extracts of *Peganum harmala, Ajuga iva* and *Aristolochia Baetica* with an average of 0 new emergence after 32 days [202]. However, it was noted in the same study that larval emergence was 92.8%, 98.5% and 98.6% for *P. harmala, A. iva and A. Baetica* respectively [202]. Ethanol extract of *P. hydropiper* has also reportedly inhibited population growth of *T. castaneum* by causing 52.3% inhibition when applied at 500 mg/10g.

There was significant reduction in larval emergence and fewer larvae emerged from positive control (4 larvae; $p < 0.0001$, N = 10) and nut powder (29 larvae; $p < 0.0001$, N = 10). The negative control recorded 134 larvae while the pulp powder significantly stimulated larvae emergence and recorded 160 new larvae ($p < 0.0246$, N = 10) as presented in figure 3.6. The larvae emergence inhibition in the nut powder and positive control were 78.3% and 97.1% inhibition rates respectively as presented in table 3.3. The pulp powder, however, stimulated larvae emergence with larval inhibition of -19.7%.

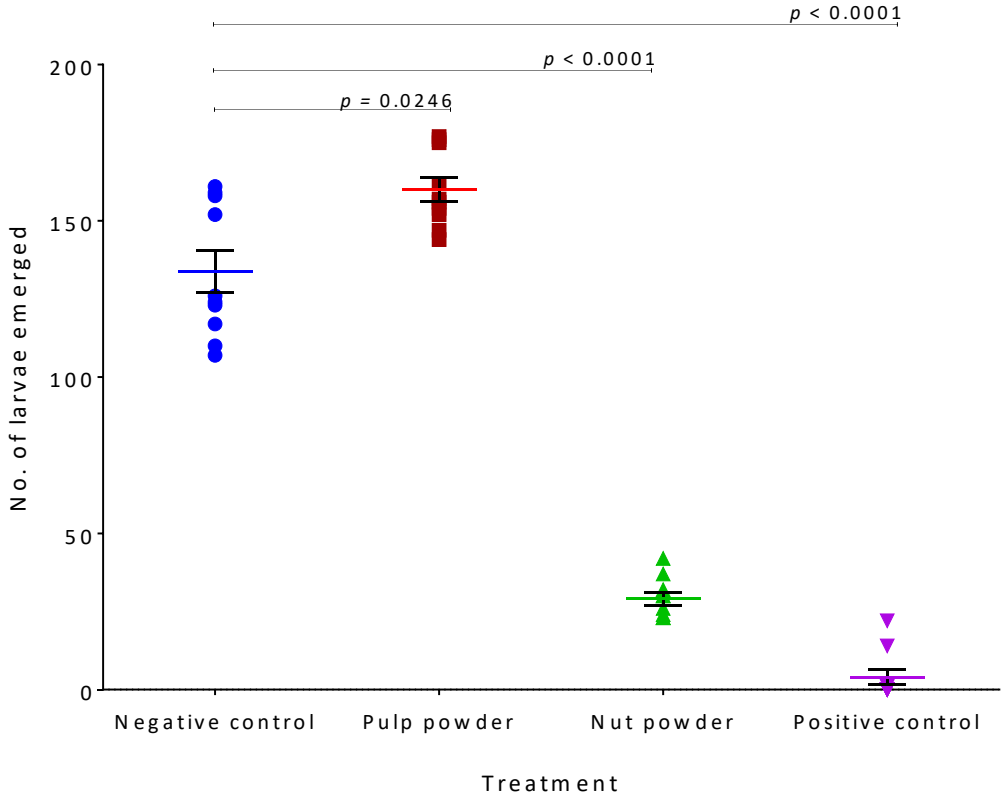


Figure 3.6: Scatter plot representation number of *Tribolium castaneum* larvae emergence in various treatments after 42 days. The points are number of larvae ± SEM with 5 technical repeats and 2 independent biological repeats per treatment. p-values, to indicate significant difference, were determined by Mann-Whitney test.

In this study, despite having an average of 29 larvae in grains treated with nut powder, there was only an average of 2 F1 progeny. This implies that *M. volkensii* nut powder could have affected morphological development of *T. castaneum* and probably the larvae lifecycle could have been unusually prolonged thus translating into low rate of larvae transiting to adult. Previous studies have reported that plant extracts prolong pupation and larval instars duration in insects [202]. Nut powder could therefore be a growth inhibitor against *T. castaneum.* The low number of larvae in the nut powder treated grains could be attributed to the antifeedant effect of the *M. volkensii* nut powders against *T. castaneum.* This has an overall effect in reducing future population of *T. castaneum* thereby enhancing crop protection.

Mortality, presented as population decline, of *T. castaneum* in the various treatments is presented in figure 3.7. The highest mortality of 96.1% was recorded in the grains treated with actellic dust while 66.0% mortality was recorded in the nut powder treated grains. The pulp powder did not show mortality against *T. castaneum* after 42 days. Actellic dust used a positive control has a contact toxicity and hence the high mortality of 96.1% of adult insects observed. The nut powder does not possess contact toxicity and therefore the 66.0% mortality recorded in the nut powders could be due to the starvation of the insects over time caused by antifeedant effect of the nut powder against *T. castaneum*. This is an interesting finding given that *T. castaneum* is among the least susceptible stored-product insect pests and is difficult to kill [247].

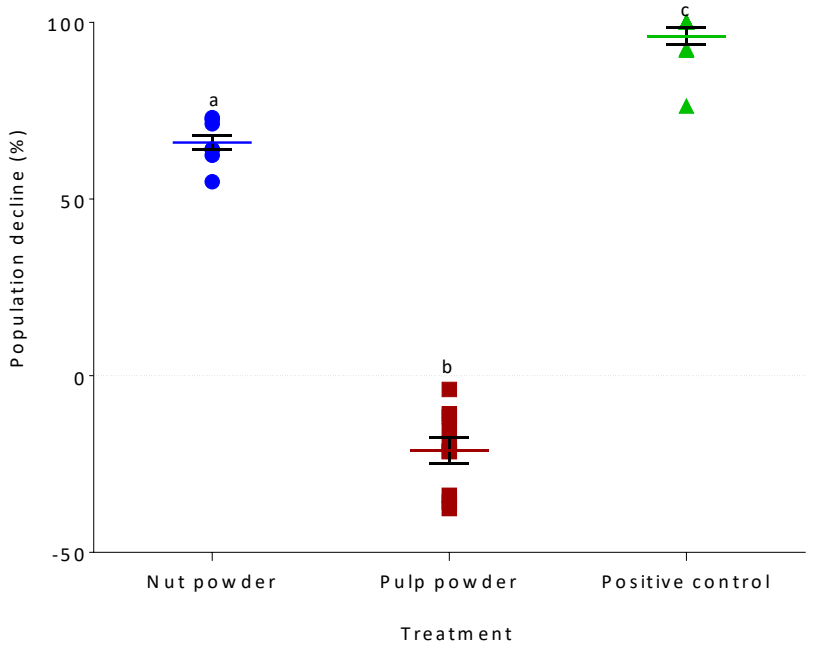


Figure 3.7: Scatter plot representing percentage population decline of *Tribolium castaneum* in various treatments after 42 days. The points are adjusted mortality ± SEM with 5 technical repeats and 2 independent biological repeats per plant part). Significant differences were calculated by Dunns' multiple comparison test followed by Bonferroni test (p<0.05). Means followed by same letter are not significantly different.

Botanical plant powders have been traditionally used as grain protectants either for contact toxicity, antifeedant, repellent or oviposition deterrent effects against *T. castaneum* [149]. Antifeedant action is a valuable weapon against grain-feeding insects and could reduce post-harvest losses in stores. Plant powders from neem seeds, peppermint, *Eucalyptus camaldulensis*, have been found to have 69.4%, 35.2% and 38.8% mortality respectively after 7 days when mixed with grains, at a concentration of 0.9 g/60 g of wheat flour [201]. Black pepper recorded 44.4% mortality when 0.6 g of powder was mixed with 60 g of wheat flour while lemon grass powder showed 24.6% mortality at concentration of 0.3 g/60 g of wheat flour [201]. Ground leaves, bark and seeds of *A. polystrachya* also provided protection against *T. castaneum* by reducing F1 progeny thereby reducing population growth [213]. Application of aqueous extracts, at 1000 ppm, of *Viola arvensis, Matricaria chamomilla, Brassica campestris* and *Jacaranda mimosifolia*

extracts to grains led to 68%, 57%, 56% and 49% mortality respectively against adult *T. castaneum* [137]. Hexane extracts of *Cucumis sativus, Tamarindas indica* and *Psidium guajava* showed toxicity against *T. castaneum* with 80%, 73% and 50% mortality recorded after 96 hours using film residue method [159].

Mortalities of 92%, 45%, 32% and 15% have been reported in *P. harmala, A. iva, A. baetica* and *R. raphinstrum* respectively against *T. castaneum* 32 days after treatment [202]. In a fumigation experiment using plant essential oils, *N. tabacum, C. procera, A. indica, D. stramonium* and *Eucalyptus camaldulensis* oils showed varying toxicity against *T. castaneum* with 65%, 40%, 27%, 27% and 18% mortality respectively after 72 hours of application when 15% concentration of the oils is applied [152]. Phosphine gas caused 80% mortality in adult *T. castaneum* at 500 ppm after 72 hours of fumigation [152]. *N. tabacum, C. procera, A. indica, D. stramonium* and *Eucalyptus camaldulensis* oils also significantly reduced progeny emergence 30 days after treatment relative to negative control [152]. *Rosmarinus officinalis, Melissa officinalis* and *Mentha piperita* have shown 58.7%, 42.7% and 40.0% mortality against adult *T. castaneum* in a simulated storage of wheat [212].

From the observations in this study, *M. volkensii* nut powders, when mixed with grains during storage, have the potential to slow down damage on grains by acting as antifeedant, growth inhibitors, oviposition deterrent and could cause mortality against *T. castaneum*. By inhibiting emergence of larvae and new generation of *T. castaneum* which feed on stored grains, *M. volkensii* nut powder could offer an alternative option as grain protectant. The nuts can potentially be vital for disruption of larval and pupal development effectively reducing adult populations in *T. castaneum*.

The pulp powder, however, has shown stimulant effect and has proved ineffective in protecting stored grains as it promoted emergence of *T. castaneum* adult and larvae. Previous studies have reported that diet affects development of *T. castaneum* and that more adults emerge on high protein content diet and that high carbohydrate content is not preferred (few adult emergences with longer development period) [248,249]. Protein is essential in egg production thereby stimulating insect development [250]. In this study, the protein and carbohydrate levels in *M.*

volkensii nut and pulp powders were not different and thereby the probable reason for the stimulating effect of *M. volkensii* pulp powder on *T. castaneum* is still unknown.

It is however important to note that since *M. volkensii* powders are directly admixed with the grains, toxicity studies on the effect of the powders needs to be undertaken to guarantee safety. In general, these results show that nut powder has comparable effect as the commonly used Actellic dust, a synthetic pesticide, in grain protection against *T. castaneum.* The LD_{50} values for oral toxicity in rats for the active ingredients of actellic dust, *i.e.* pirimiphos-methyl and thiamethoxam, are 1180-2050 mg/kg and 1563 mg/kg, respectively [251]. Even though they are easily excreted out of body through urine and fecal matter, consumption of higher concentrations could lead to bioaccumulation in humans. The grains treated with actellic dust should be consumed at least 14 days after application according to manufacturers' recommendation. The *M. volkensii* nuts showed consistent results in the laboratory and in the simulated trials and has potential for protection of stored grains against *T. castaneum*. The nut powders applied during grain storage could be removed from the stored grains through winnowing or fanning before consumption. This would minimize human exposure to the *M. volkensii* nut powders through ingestion.

3.3 CONCLUSION

In conclusion, the findings of this investigation warrant the basis for including *M. volkensii* nut extracts as an option in the control of *C. puncticollis*, *S. frugiperda* and *T. castaneum* in practical conditions. Opting for the *M. volkensii* extracts would not only be cost effective to the farmers, but also a milestone in environmental protection. Moreover, the synthetic pesticides used, Hable and Actellic dust, have not proved to be more efficacious than *M. volkensii* nut extracts. *M. volkensii* has no reported adverse effect on the environment or mammals, making it a potential botanical pesticide for biosafe application in integrated pest management.

The findings in this study provides an innovative approach in use of plant-based pesticides for grain protection in Kenya. This method shows extension potential and can be easily adopted by farmers because of its ease in application, practicability, availability of raw materials and

sustainability. It presents a less expensive method that can be prepared easily by local farmers, small scale traders and industries as economically valuable and safe replacement for conventional pesticides.

M. volkensii nuts and pulp could be a potential source of insecticidal compounds that have promoted antifeeding effects on *C. puncticollis, S. frugiperda* and *T. castaneum* in the present investigation. Moreover, growth disruption effects have also been observed for nut powder against *T. castaneum* in this study. *M. volkensii* therefore presents a high potential agent in integrated pest management, especially considering the interest in products with non-toxic mechanisms against insect pests.

CHAPTER 4

Isolation and characterization of bioactive antifeedants from *Melia volkensii* and their activity against *Cylas puncticollis, Spodoptera frugiperda* and *Tribolium castaneum*

4.0 INTRODUCTION

The search for plant-derived pesticides often begins with screening of the plant extracts for activity against selected insect pests [252]. Such bioassays are usually conducted with relevant evaluation parameters against insect pests such as antifeedant, survival, fertility among others [231]. Crude plant extracts consist of complex mixtures of active compounds and may even show greater overall bioactivity than the individual constituents [182]. The extracts show bioactivity in several ways including oviposition inhibition, antifeedant, growth regulatory, repellence and toxicity [180,246,253]. This makes plant-derived substances to become very promising in insect control as they can be lethal to insects or cause sublethal effects [231]. Secondary metabolites contribute to the defence system of plants and studies of such allelochemicals with activity against pest insects may provide new leads for the development of insecticides [255,256]. Plant antifeedants occur in all groups of chemical compounds, however, the most effective antifeedants are found in terpenoids, alkaloids, saponins and polyphenols [227,248,249,258]. When tasted by insects, these antifeedants lead to temporary or permanent interruption of food intake, depending on potency [156]. The search for pest control products with minimal detrimental effects has led to continuous evaluation of botanical resources and exploitation of new plant materials with high pesticidal activity [151,199,211]. Plant materials, with insecticidal properties, are one of the most important locally available and inexpensive method for control of insect pests [237,253,257–262]. Moreover, the use of plant materials to control pests is sustainable as they can be continuously propagated all year round, are biodegradable and they do not have adverse environmental effects [216,263–267].

This study investigated the insect control potential of *M. volkensii,* an indigenous tree species native to the drylands of Eastern Africa. Phytochemical studies of *Melia volkensii* reveal a range of compounds including limonoids, which are known to be strong insect antifeedants against a broad range of insects including dipterans, lepidopterans and coleopterans [266,268]. Some of the limonoids with insect control potential which have reportedly been isolated from *M. volkensii* include 1-cinnamoyltrichilinin, 1-tigloyltrichilinin, 1-acetyltrichilinin, salannin, 2',3'-

dihydrosalannin, ohchinin-3-acetate, nimbolin B among others [47,84]. It is also reported that insect antifeedants usually have a more oxidized or unsaturated structure and the molecular size, shape and functional stereochemistry also affect antifeedant activity [248].

During seed extraction from the plant, fruit is de-pulped and the nut that houses the seed is also removed [50]. This generates a large amount of waste which is usually discarded because the economic value of the nuts and pulp is not known. Other waste generated during timber processing and pruning are the bark and leaves. Interestingly, *M. volkensii* nuts and pulp have been reported to contain secondary metabolites which possess antifeedant activity against insect pests [167,266,267,269,270]. The use of extracts from *M. volkensii* nut and pulp for controlling insect pests could be an interesting solution since it would reduce the waste generation and could also be used in combination with other pest control methods in an integrated pest management system.

Considering the possibility of this practical use of *M. volkensii* extracts, this study evaluated the bioactive components with antifeedant activity against the African sweet potato weevil (*C. puncticollis*), fall armyworm (*S. frugiperda*) and red flour beetle (*T. castaneum*). These are insect pests of economic importance whose management is key to production of sweet potato, maize and stored grains [184,187,199,206,211,236,245,271,272]. This investigation reports activity-guided isolation of bioactive compounds from various plant parts of *M. volkensii* and their antifeedant activity against *C. puncticollis, S. frugiperda* and *T. castaneum.*

4.1 MATERIALS AND METHODS

4.1.1 INSTRUMENTATION

Column chromatography was performed in a glass column (L x i.d.; 1 m x 6 cm) with a 2 lit capacity. Silica gel 60 – 120 (0.015 – 0.040 mm; Merck) was used as stationary phase for column chromatography. Thin Layer Chromatography (TLC) analysis was carried out on silica gel plates (KG60-F254; Merck). Separation over preparative-TLC was performed over 100 mm x 200 mm glass plates precoated with 0.5 mm layer of silica gel 60. Purification of compounds by means of automatic preparative-HPLC were conducted using an Agilent 1100 Series system with UV

detector equipped with a Supelco Ascentis C18 column (I.D. x L 21.2 mm x 150 mm, 5 µm particle size). All ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100.6 MHz, respectively, on a Bruker Avance III, equipped with 1H/BB z-gradient probe (BBO, 5 mm). All spectra were processed using TOPSPIN 3.2. Types of spectra were acquired through the standard sequences available in the Bruker pulse program library. Custom settings were used for all other types of spectra. LC-MS analysis was done using Agilent 1200 Series HPLC equipped with a Supelco Ascentic Express C18 column (3 cm x 4.6 mm, 2.7 µm fused-core particles, 90 Å), Phenomenex Guard column (Security Guard Standard) and a UV-DAD detector. The HPLC was coupled to an Agilent 1100 Series MS with electrospray ionisation with a single quadrupole detector.

4.1.2 COLLECTION AND PREPARATION OF PLANT MATERIALS

Fresh *M. volkensii* nuts, pulp, bark and leaf samples were collected from a plantation farm in Kiambere, Kenya. Kiambere is located approximately 187 km east of Nairobi with coordinates of 0°41'10" S 37°54'55" E and is about 720 m above sea level. The plant parts were dried in the shade for 21 days. The dried samples were then pulverized using an electric grinder with 850 µm pore size to make the samples ready for extraction.

4.1.3 EXTRACTION OF PLANT MATERIALS

Plant powders from *M. volkensii* nut, pulp, bark and leaf were separately macerated in methanol at a ratio of 1:5 (w/v) for 48 h at room temperature in an Erlenmeyer flask. The mixture was then filtered using filter paper (Whatman no. 1) in a Buchner funnel and the residue obtained was discarded, while the filtrate was preconcentrated in a rotary evaporator at 35°C until near dryness. Subsequently, the crude extracts were completely dried using evaporation under high vacuum conditions. The respective crude extracts were weighed to calculate the yield and stored at 4 °C awaiting insect bioassay.

4.1.4 FRACTIONATION OF *M. VOLKENSII* CRUDE EXTRACTS

The crude extracts of *M. volkensii* nut, pulp, bark and leaf were subjected to liquid-liquid extraction. The 10 g of respective crude extract was dissolved in 50 ml of water and successively extracted with solvents of increasing polarity, starting with hexane (4x150 ml), dichloromethane

(DCM) (4x150 ml), ethyl acetate (EtOAc) (4x150 ml) and n-butanol (BuOH) (4x150 ml) using a separating funnel. This yielded 5 fractions namely: hexane, dichloromethane, ethyl acetate, butanol and the residual aqueous fraction.

Each organic fraction was dried with magnesium sulphate and filtered under vacuum with a Buchner funnel. The fractions were then concentrated in a rotary evaporator at 35 °C and later under high vacuum to complete dryness. All the fractions were screened for their antifeedant activity against *C. puncticollis, S. frugiperda* and *T. castaneum* at 20 mg/mL concentration. The dichloromethane fraction from the nut and pulp were further purified to identify the active principles. The other fractions from the nut and pulp (hexane, ethyl acetate, butanol, and residue) were not investigated further. The fractions from the leaf and bark of *M. volkensii* were also not further investigated because of lower antifeedant activity recorded.

4.1.5 BIOACTIVITY-GUIDED PURIFICATION OF *M. VOLKENSII* NUT EXTRACTS

The dichloromethane fraction of the nut extract was first subjected to column chromatography for further purification. The column was packed with silica (60 - 120 mesh size) using slurry method with hexane/ethyl acetate solvent ratio of 9:1 (the first elution solvent system). 80 g of DCM fraction sample was mixed with 160 g silica gel and dissolved in methanol; the solvent was evaporated to dryness using rotary evaporator. The dried silica/sample mixture was introduced on top of the packed column. Elution was done using the solvent system program presented in table 4.1 with increasing polarity.

Table 4.1: Program for purification of dichloromethane fraction of *M. volkensii* nut by column chromatography over silica gel

Mobile phase	Gradient elution	Fractions eluted
Ethyl acetate/hexane	10:90	1 – 5
Ethyl acetate/hexane	15:85	6 – 12
Ethyl acetate/hexane	20:80	13 – 14
Ethyl acetate/hexane	30:70	25 – 40
Ethyl acetate/hexane	50:50	41 – 50
Ethyl acetate/hexane	75:25	51 – 65
Ethyl acetate	100	66 – 93
Ethyl acetate/methanol	95:5	94 – 104

Ethyl acetate/methanol	90:10	105 – 125

A total of 125 fractions were collected from the column chromatography. Based on thin layer chromatography profiles (visualized within UV 254 nm and iodine staining), the 125 fractions were combined into 19 fractions which were later evaporated to dryness in a rotary evaporator at 35 °C. The combined fractions presented in table 4.2.

Table 4.2: Combined fractions after column chromatography purification of dichloromethane fraction of *M. volkensii* nut

Combined fractions	**Yield (g)**	**Appearance**
Fractions 1-4	4.91	Yellowish oil
Fractions 5-9	0.5	Yellowish oil
Fractions 10-15	2.34	Yellowish oil
Fractions 16-19	0.95	Brown oil
Fractions 20-22	0.33	Yellowish oil
Fractions 23-33	1.41	Brown oil
Fractions 34-40	3.44	Brown powder
Fractions 41-45	1.68	Brown oil
Fractions 46-48	2.74	Brown powder
Fractions 49-50	3.89	Green powder
Fractions 51-56	6.4	Brown powder
Fractions 57-61	2.14	Yellow powder
Fractions 62-65	1.07	Yellow powder
Fractions 66-81	4.37	Yellow powder
Fractions 82-93	1.8	Yellow powder
Fractions 94-101	2.12	Yellow powder
Fractions 102-108	0.64	Yellow powder
Fractions 109-117	1.1	Yellow powder
Fractions 118-125	0.51	Yellowish oil

The obtained fractions were screened for activity against *C. puncticollis, S. frugiperda* and *T. castaneum* and the most active fractions (49-50, 51-56, 57-61, 62-65 and 66-81) were subjected to subsequent purification to identify the bioactive principle compound.

Fraction 62-65, the fraction with the highest mean antifeedant index, was further purified by column chromatography using chloroform/acetone as solvent system. The column was packed with 50 g silica using chloroform/acetone (8:2) mixture, being the solvent for first elution. 350

mg of the fraction 62-65 was then dissolved in minimal amount of chloroform ($CHCl_3$) and liquid injection method was used to introduce the sample into the column using pipette. Column elution was done with chloroform/acetone solvent system in increasing polarity and column clean up done using with acetone/MeOH following the elution program in table 4.3.

Table 4.3: Program for separation of *M. volkensii* nut fraction 62–65 by column chromatography over silica gel

Mobile phase	**Gradient elution**
Chloroform/acetone	8:2
Chloroform/acetone	7:3
Chloroform/acetone	1:1
Acetone	100
Acetone/methanol	9:1

A total of 55 fractions were obtained after the column chromatography. The eluted fractions were monitored using TLC plates developed in $CHCl_3$/acetone (7:3). Based on the fractions' TLC profiles visualised in UV 254 nm and iodine, the 55 fractions were combined into 4 sub-fractions as indicated in table 4.4.

Table 4.4: Combined sub-fractions after column chromatography purification of *M. volkensii* nut fraction 62–65

Combined fractions	**Yield (mg)**	**Appearance**
Fractions 1-5	50	Brown powder
Fractions 6-17	60	Brown powder
Fractions 18-33	50	Pale cream powder
Fractions 34-55	40	Thick brownish oil

Fraction 18-33, the most abundant spot in TLC, was subjected to preparative-HPLC for further purification using the program in table 4.5.

Table 4.5: Preparative-HPLC conditions for *M. volkensii* fractions

Run conditions	
Column	C18 column (I.D. x L 21.2 mm x 150 mm, 5 µm particle size)
UV wavelength	222 nm
Injection volume	100 µL
Run time per injection	25 minutes
Solvent A	Water
Solvent B	Acetonitrile
Flow rate	4 mL/min

The preparative-HPLC isolation was achieved using automatic fraction collector with peak-based mode with gradient indicated in table 4.6. Based on the prep-HPLC profiles, 4 fractions were obtained. Fraction 4 eluted as a single peak from the prep-HPLC and was dried in a rotary evaporator and vacuum to yield a pure **compound 1.**

Table 4.6: Preparative-HPLC gradient method for purification of *M. volkensii* fractions

Gradient method	
Time (min)	%B
0.00	25.0
2.40	25.0
19.20	100.0
20.00	25.0

Using LC-MS and NMR spectral data, together with comparison with data available in literature, **compound 1** was elucidated as a reduced form of meliavolkenin.

LC-MS analysis was done by dissolving 1 mg of the sample in 1 ml MeOH and filtering it with a microfilter before transferring it into a sample vial and introducing it into the LC-MS. The LC-MS conditions are listed in Table 4.7, and the elution gradient for LC-MS is presented in Table 4.8. NMR analysis was done by dissolving 10 mg of sample in 0.5 mL of deuterated chloroform ($CDCl_3$) and then transferring into NMR tube before introducing into the NMR instrument.

Table 4.7: LC - MS conditions for the analysis of *M. volkensii* fractions, sub-fractions, and pure compounds

Run conditions	
Column	C18 column (3 cm x 4.6 mm, 2.7 µm fused-core particles, 90 Å)
UV1 wavelength	220 nm
UV2 wavelength	280 nm
UV3 wavelength	254 nm
Injection volume	2 µL
Run time	4.8 minutes
Solvent A	Water
Solvent B	Acetonitrile
Flow rate	1 mL/min
MSD mode	ESI (70 eV)

Table 4.8: LC-MS gradient method for the analysis of *M. volkensii* fractions, sub-fractions, and pure compounds

Gradient method	
Time (min)	%B
0.00	30.0
0.60	30.0
3.00	100
3.60	100
4.20	30.0
4.80	30.0

When tested for antifeedant activity against *C. puncticollis, S. frugiperda* and *T. castaneum,* **compound 1** was not bioactive.

The other bioactive fractions 49-50, 51-56, 57-61 and 66-81 from *M. volkensii* nut were further purified to isolate and identify the active principle. Each of the respective fractions was subjected to preparative-HPLC for further purification using the run program in Table 4.5 and the gradient in Table 4.6. 25 mg of each fraction was dissolved in 1 mL MeOH and filtered prior to injection into preparative-HPLC. After elution, the collected fractions and their elution times are presented in a tree diagram in Figure 4.1.

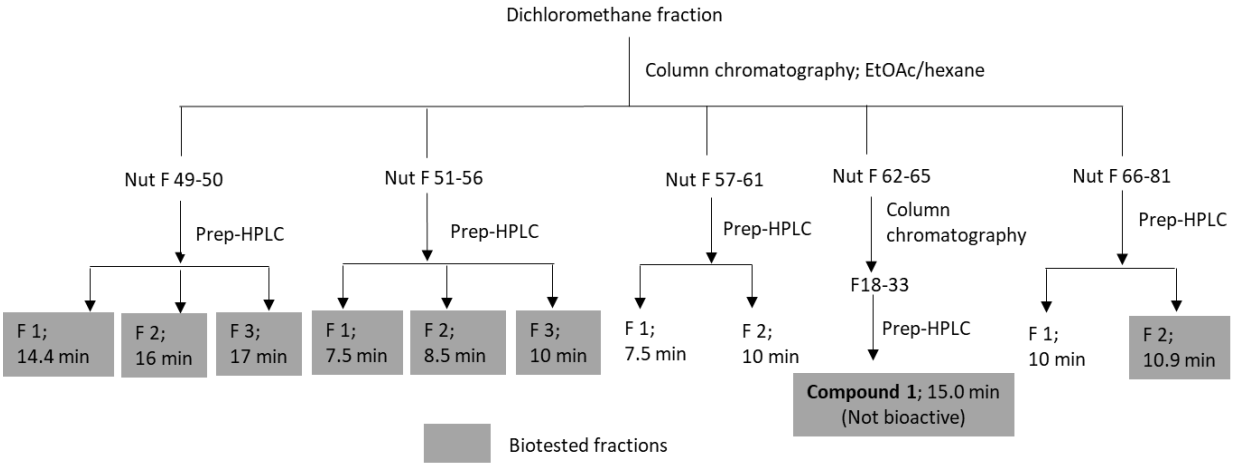


Figure 4.1: Scheme showing the obtained sub-fractions and their respective retention times after preparative-HPLC purification of bioactive fractions 49-50, 51-56, 57-61 and 66-81 of *M. volkensii* nut.

After preparative-HPLC purification of the bioactive fractions 49-50, 51-56, 57-61 and 66-81, a total of 10 sub-fractions were obtained as presented in Figure 4.1. Based on the retention times of preparative HPLC and LC-MS spectral data, sub-fractions with similar profiles were considered identical (for example, sub-fraction F3 from nut F 51-56, sub-fraction F2 from nut 57-61 and sub-fraction F1 from nut F 66-81 each had retention time of 10 minutes and hence were considered identical) as shown in figure 4.1. Consequently, only sub-fractions with different profiles were biotested for further investigation. The fractions that were tested for antifeedant activity against *C. puncticollis, S. frugiperda*, and *T. castaneum* are therefore listed in Table 4.9.

Table 4.9: Fractions tested for antifeedant activity against *C. puncticollis*, *S. frugiperda* and *T. castaneum* after preparative-HPLC purification of fractions 49-50, 51-56, 57-61 and 66-81.

Fraction	**Sub-fractions obtained**	**Sub-fraction tested (after prep-HPLC)**
F 49 – 50	F1, F2, F3	**F1, F2, F3**
F 51 – 56	F1, F2, F3	**F1, F2, F3**
F 57 - 61	F1, F2	**None** (both sub-fractions were similar to sub-fractions from F1 and F3 from F 51 – 56)
F 66 - 81	F1, F2	**F2,** (sub-fraction F1 was similar to sub-fraction F3 from F 51 – 56)

Based on the bioassay results, sub-fraction F3 of fraction 51-56 was subjected to preparative-TLC for further purification. 30 mg of the fraction was dissolved in minimal chloroform/dichloromethane (1:9). The sample was loaded on the preparative-TLC plate and placed in the development chamber. Elution was done using chloroform/dichloromethane (1:9) solvent system. The preparative-TLC plate was dried and the selected silica scraped off, washed with chloroform/dichloromethane (1:9) and pre-concentrated in a rotary evaporator. This yielded **compound 2** (white powder) which was elucidated using LC-MS and NMR as toosendanin using the spectral data, together with comparison with data available in literature. The pure compounds were tested to find effective concentrations (EC_{50} values), required to cause 50% antifeeding effect against the pest insects. The complete isolation of pure compounds **1** and **2** from *M. volkensii* nut is presented in figure 4.2.

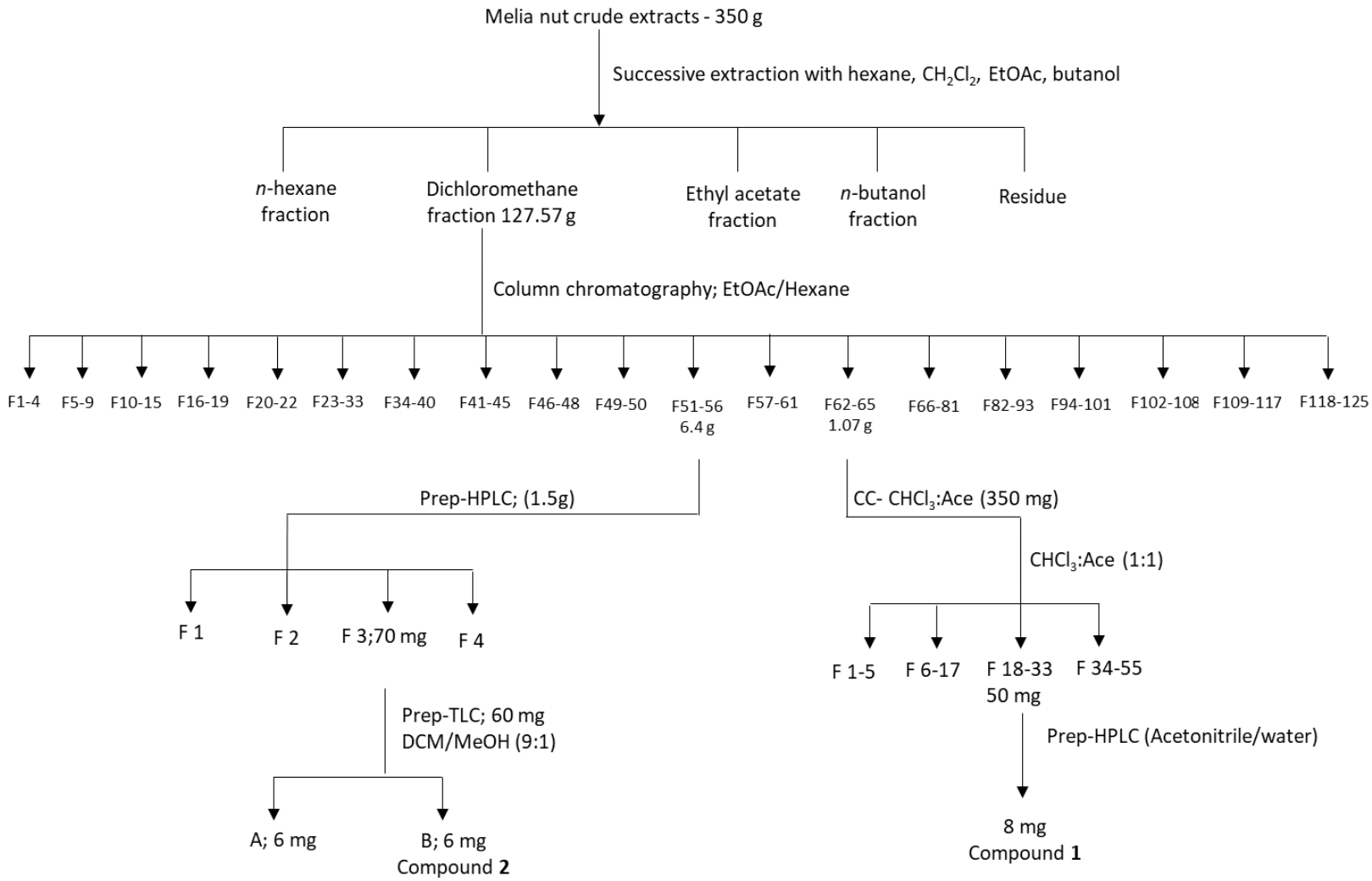


Figure 4.2: Complete isolation of **compound 1** and **2**, toosendanin and reduced meliavolkenin, from *M. volkensii* nut

4.1.6 Bioactivity-guided purification of *M. volkensii* pulp extracts

The dichloromethane fraction of the pulp extract was subjected to column chromatography for further purification. The column was packed with silica (60-120 mesh size) using slurry method with hexane/ethyl acetate 90:10, the first solvent system for elution. 80 g of the sample was mixed with 160 g silica gel and dissolved in methanol, the solvent was evaporated to dryness using rotary evaporator and introduced on top of the packed column. Elution was done using a mixture of hexane/ethyl acetate with a gradient of increasing polarity as presented in table 4.10.

Table 4.10: Program for separation of dichloromethane fraction of *M. volkensii* pulp extracts by column chromatography over silica gel

Mobile phase	Gradient elution	Fractions eluted
Ethyl acetate/hexane	10:90	1 – 11
Ethyl acetate/hexane	15:85	12 – 21
Ethyl acetate/hexane	20:80	22 – 32
Ethyl acetate/hexane	30:70	33 – 44
Ethyl acetate/hexane	50:50	45 – 54
Ethyl acetate/hexane	75:25	55 – 64
Ethyl acetate	100	65 – 75
Ethyl acetate/methanol	95:5	76 – 85
Ethyl acetate/methanol	90:10	86 – 95

A total of 95 fractions were collected from the column chromatography. Based on thin layer chromatography profiles visualized in UV 254 nm and iodine, the 95 fractions were combined into 14 fractions which were later evaporated to dryness in a rotary evaporator at 35 °C and presented in table 4.11.

Table 4.11: Combined fractions after column chromatography purification of dichloromethane fraction of *M. volkensii* pulp extract

Combined fractions	**Weight (g)**	**Appearance**
Fractions 1-3	9.29	Yellowish oil
Fractions 4-11	4.03	Yellowish oil
Fractions 12-21	1.01	Brownish oil
Fractions 22-23	2.22	Yellowish oil
Fractions 24-27	1.15	Brown oil
Fractions 28-32	0.91	Greenish oil
Fractions 33-41	4.21	Green waxy oil
Fractions 42-44	2.41	Brown waxy oil
Fractions 45-47	4.20	Brown powder
Fractions 48-54	4.32	Green powder
Fractions 55-64	9.88	Brown powder
Fractions 65-75	6.82	Brown powder
Fractions 75-85	4.71	Yellow powder
Fractions 86-95	2.23	Dark brown waxy oil

The combined fractions were screened for activity against *C. puncticollis, S. frugiperda* and *T. castaneum*. Based on the results, fraction 55 – 64 was subjected to subsequent purification to identify the principal compound. 3 g of fraction 55-64 was recrystallized in methanol at room temperature, yielding 136 mg of pure **compound 3**. Analysis of LC-MS and NMR spectral data together with comparison with data from the literature allowed the identification of the structure of **compound 3** as salanninolide. The complete isolation process is shown in Figure 4.3.

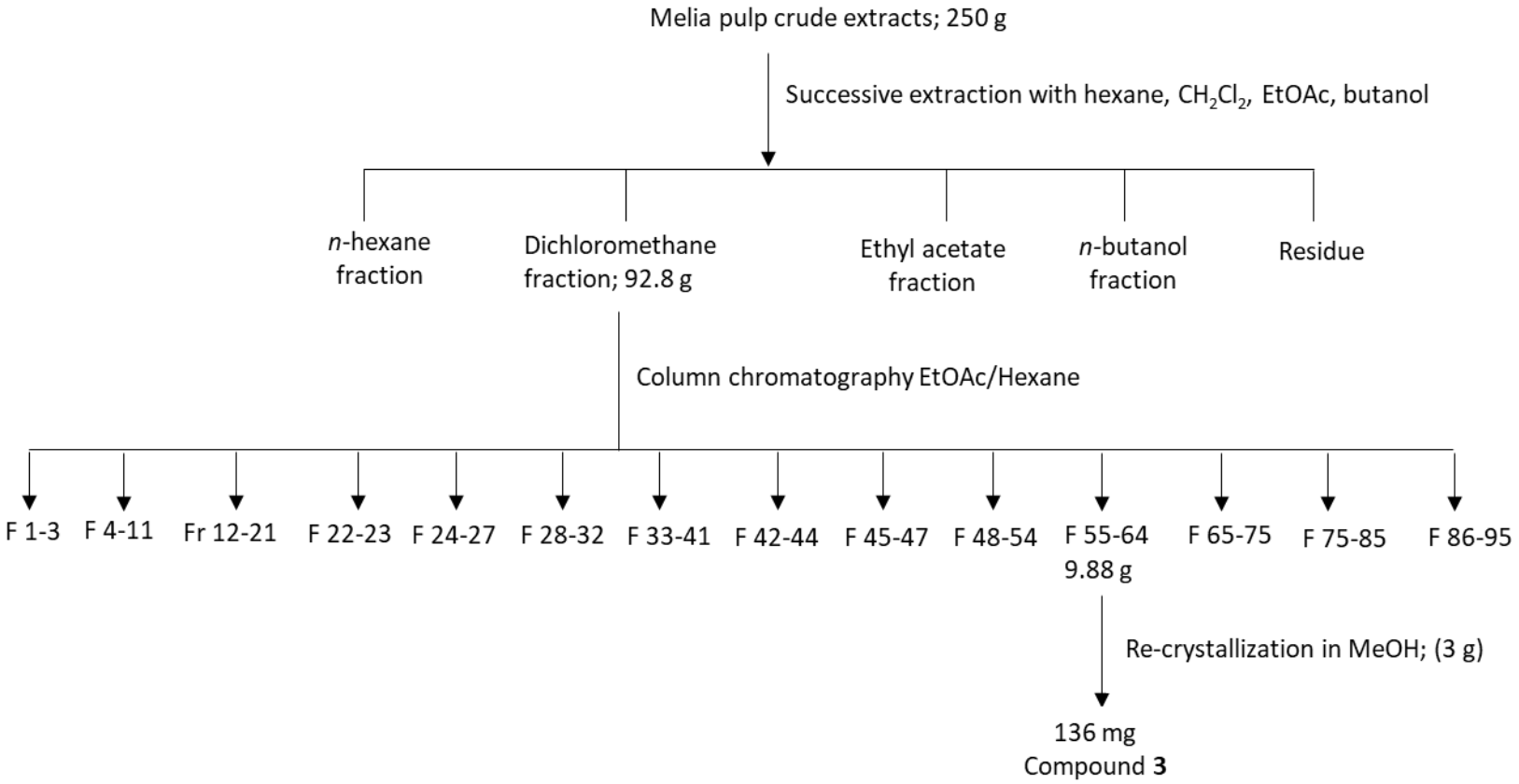


Figure 4.3: Complete isolation of **compound 3**, salanninolide, from *M. volkensii* pulp

4.1.7 Statistical analysis

All statistical analyses were conducted using GraphPad Prism version 6.01. Antifeedant indices within each treatment were checked for normality (Shapiro-Wilk test) to assess if data followed a normal distribution [216]. Not all data for the antifeedant indices were normally distributed, and nonparametric tests were performed. Therefore, the antifeedant indices from the bioassays in the laboratory were subjected to a Kruskal-Wallis test to perform analysis of variance followed by Dunn's test for multiple comparisons between means [125]. To determine significant differences between the different means, Bonferroni correction was applied and means were considered significantly different if their *p*-value was greater than the corrected Bonferroni *p*-value [217].

EC_{50} values (concentration causing 50% antifeeding effect compared with the control) were calculated using regression of the line of best fit [273,274]. Linear regression analysis was performed for dose-response experiment and EC_{50} values were determined by substituting the value of x (EC_{50}) in the regression equation ($Y = bx + a$) where b is the slope of the line, x is the independent variable and a stands for the Y-intercept [66]. The concentrations of the pure compounds were first converted to log concentrations before analysis [275–277].

4.2 RESULTS AND DISCUSSION

4.2.1 EXTRACTION AND FRACTIONATION YIELDS OF *M. VOLKENSII* CRUDE EXTRACTS

Extraction yield is a measure of solvent and methods' efficacy to extract out specific phytochemicals from plant matrix [207]. The presence of various phytochemical compounds with different chemical characteristics and polarity varies according to their solubility in a particular solvent [207]. The extraction and fractionation yields were calculated using the method applied by Tulashie et al., 2021 [196]. *M. volkensii* leaves and pulp gave higher methanolic extraction yields of 10.2% and 10.0% respectively. The nuts gave 7.0% yield while *M. volkensii* bark had the lowest yield of 5.3% as presented in table 4.12.

This could be an indication that there is a higher concentration of soluble organic compounds in the leaves and pulp than in the bark and nuts of *M. volkensii*. It is therefore imperative to state that phytochemicals in *M. volkensii* are soluble in methanol and that there is a higher amount of these phytochemicals in the leaves and pulp than in the bark and nut when extracted with methanol.

Table 4.12: Yields obtained after extraction and fractionation of various plant parts of *M. volkensii*

Plant part	**Crude extract yield (%)**	**Fraction yield (%)**				
		n-hexane fraction	Dichloromethane fraction	Ethyl acetate fraction	*n*-butanol fraction	Residue fraction
Bark	5.3	15.1	25.9	6.9	12.3	34.8
Leaf	10.2	14.9	16.0	16.4	23.9	19.3
Pulp	10.0	2.1	37.4	3.7	10.4	25.4
Nut	7.0	2.1	37.6	7.0	13.9	21.1

When fractionated in different solvents with increasing polarity, the dichloromethane fraction consistently recorded the highest yield at 37.4% and 37.6% in the pulp and nut extracts, respectively. Hexane fraction recorded the lowest yield of 2.1% when the pulp and nut extracts were fractionated. For *M. volkensii* bark, the residual fraction had the highest yield of 34.8%, while the ethyl acetate fraction had the lowest yield of 6.9%. For leaf extract, the butanol fraction yielded the highest yield of 23.9%, while the hexane extract had the lowest yield of 14.9% among the leaf fractions.

4.2.2 ANTIFEEDANT ACTIVITY OF FRACTIONS OBTAINED AFTER FRACTIONATION OF CRUDE EXTRACTS OF VARIOUS *M. VOLKENSII* PLANT PARTS

This section provides a summary of antifeedant activity of fractions obtained after liquid-liquid partitioning of crude extracts from various parts of *M. volkensii* (pulp, nut, bark and leaf). This was done to identify the fractions with highest activity against *C. puncticollis, T. castaneum* and *S. exigua* (used as substitute for *S. frugiperda*) for subsequent purification.

4.2.2.1 Antifeedant activity of *M. volkensii* pulp fractions

The pulp crude extract was fractionated into *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol fractions. All these fractions, together with the residual fraction, were screened for their antifeedant activity against *C. puncticollis, T. castaneum* and *S. exigua* as presented in figure 4.4. The most active fraction was further examined for antifeedant activity and subsequent isolation of the active ingredient.

When the fractions were tested against *C. puncticollis*, the mean highest activity of 61.2% was observed in the dichloromethane fraction. Ethyl acetate fraction, butanol fraction and hexane fraction had antifeedant indices of 44.1%, 39.5% and 38.2%, respectively. The residue did not show any activity with antifeedant index of 0.5% observed. Even though dichloromethane fraction had the highest mean antifeedant index, there was no significant difference in activity between dichloromethane and ethyl acetate fraction. There was, however, a significant difference in activity of the dichloromethane fraction when compared to hexane fraction, butanol fraction and the residue.

Against *T. castaneum*, the dichloromethane fraction showed the highest mean antifeedant index of 27.4% while hexane, ethyl acetate and butanol fractions had 11.2%, 16.4% and -0.3% antifeedant indices respectively. The residue had antifeedant index of 3.7% against *T. castaneum.* Similar observation was made against *S. exigua* where dichloromethane fraction had the highest mean antifeedant index of 69.7%. Hexane, ethyl acetate and butanol fraction had antifeedant indices of 10.6%, 49.5% and 22.6% respectively against *S. exigua*. The residue had low antifeedant index of 3.1% against *S. exigua.*

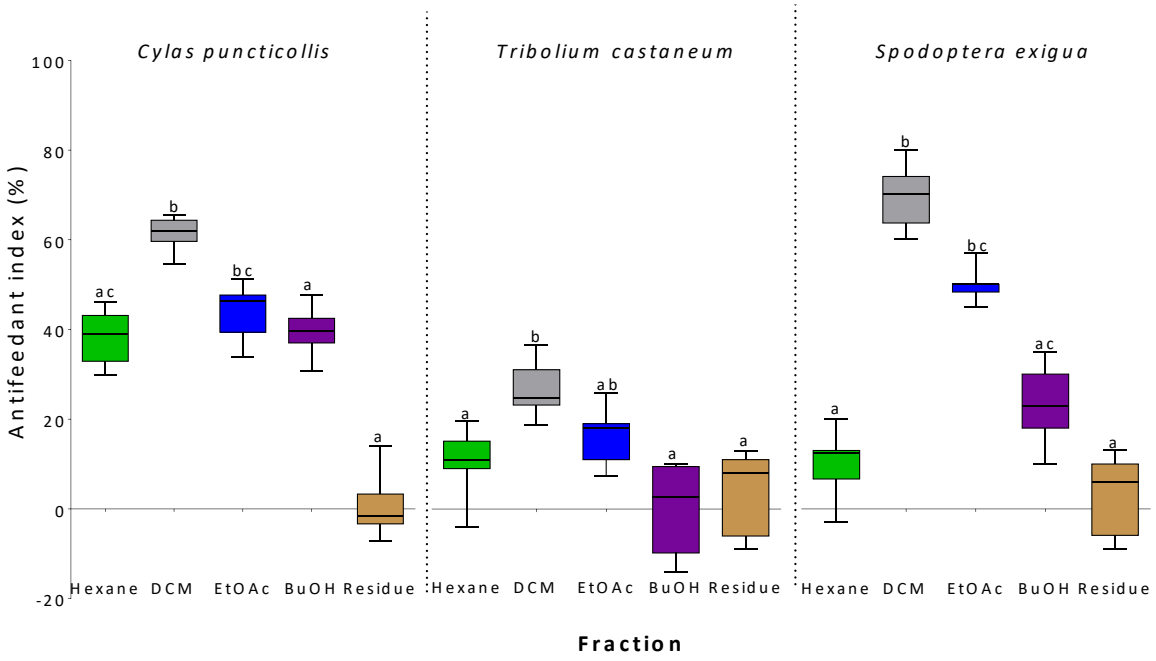


Figure 4.4: Box plot representation of the antifeedant index (AFI) of fractions obtained after liquid-liquid extraction of *Melia volkensii* pulp extract, against various insect pests. AFI were obtained after 3 independent biological repeats each consisting of 5 technical repeats and per fraction. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction. (DCM – dichloromethane; EtOAc – ethyl acetate; BuOH – butanol)

In this study, the antifeedant activity was retained in the dichloromethane fraction of the pulp suggesting that the bioactive compounds could be of medium polarity. It was also interesting to note that the dichloromethane fraction also had the highest yield at 37% compared to hexane, ethyl acetate and butanol fractions. This fraction was further purified for identification of bioactive principles. The hexane fraction, ethyl acetate fraction, butanol fraction and the residue were not further investigated due to their low antifeedant activity against *C. puncticollis, T. castaneum* and *S. exigua.*

4.2.2.2 Antifeedant activity of *M. volkensii* nut fractions

The nut crude extract was also fractionated into *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. These fractions, together with the residual fraction, were screened for antifeedant activity against *C. puncticollis, T. castaneum* and *S. exigua.* When tested against *C. puncticollis*, the mean highest activity of 62.3% was recorded in the dichloromethane fraction as presented in figure 4.5. This was not significantly different with activity of hexane and ethyl acetate fraction which recorded antifeedant indices of 38.4% and 34.6% respectively. Butanol fraction and the residue had no activity with antifeedant indices of -6.8% and 0.6% respectively against *C. puncticollis*. Against *T. castaneum*, the dichloromethane fraction of the nut extracts had the highest mean antifeedant activity at 60.5%. The activity of dichloromethane fraction (60.5%) was not significantly different with activity of ethyl acetate fraction (43.9%). Hexane, butanol and residue fractions did not show antifeedant activity with antifeedant indices of 5.2%, 8.5% and 0.6% respectively against *T. castaneum*.

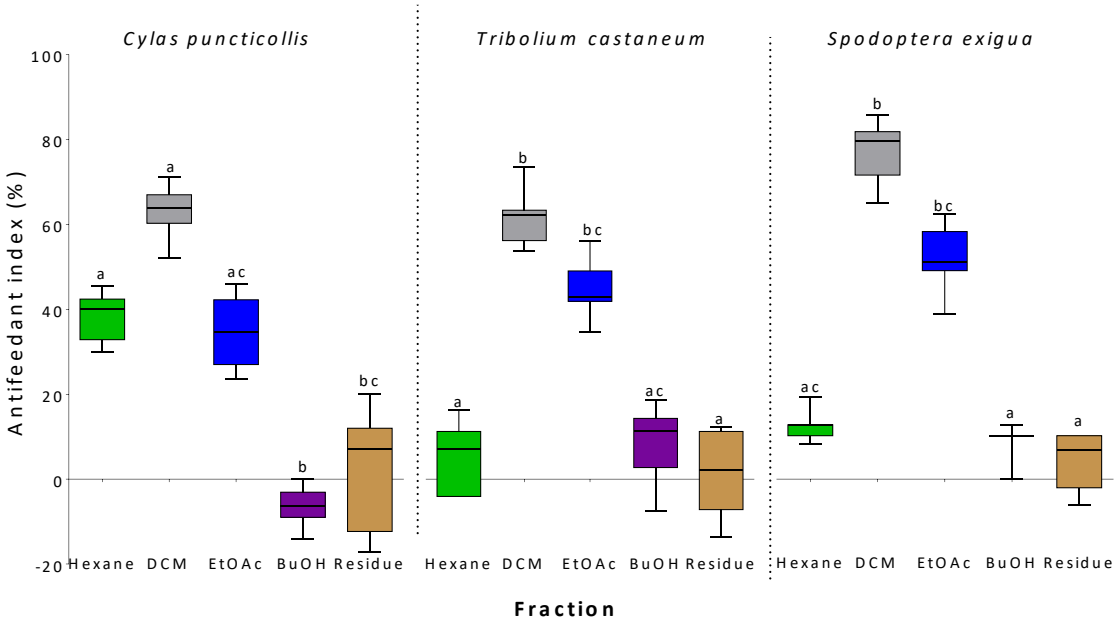


Figure 4.5: Box plot representation of antifeedant index (AFI) of fractions, obtained after liquid-liquid extraction of *Melia volkensii* nut extract, against various insect pests. AFI were obtained after 3 independent biological repeats each consisting of 5 technical repeats and per fraction. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction. (DCM – dichloromethane; EtOAc – ethyl acetate; BuOH – butanol).

The dichloromethane fraction also showed the highest mean antifeedant activity of 75.1% against *S. exigua*. The ethyl acetate fraction recorded an antifeedant index of 51.4%, while hexane, butanol and residue fractions had antifeedant indices of 11.7%, 9.8% and 3.8% respectively against *S. exigua*. Dichloromethane fraction consistently showed highest mean antifeedant activity against all the three insect pests tested. Interestingly, this fraction also had the highest recovery of 37%. The dichloromethane fraction was therefore further isolated to identify the active compounds against the insects. The hexane, ethyl acetate and butanol fractions were not further investigated.

4.2.2.3 Antifeedant activity of *M. volkensii* bark fractions

As presented in figure 4.6, the activity of hexane fraction of *M. volkensii* bark gave the highest mean antifeedant index of 57.0% against *C. puncticollis.* This is possibly an indication that the bioactive compounds in *M. volkensii* bark against *C. puncticollis* are non-polar. The ethyl acetate fraction, dichloromethane fraction, butanol fraction and the residue had antifeedant activity of 42.8%, 33.6%, 20.0% and -4.8% respectively. There was, however, no significant difference in activity between the hexane and ethyl acetate fractions.

When the bark fractions were tested against *T. castaneum,* dichloromethane fraction had mean highest antifeedant index of 43.1%. Ethyl acetate fraction, hexane fraction, butanol fraction and the residue had 41.1%, 4.5%, 22.4% and 7.1% antifeedant indices respectively. There was no difference in activity between dichloromethane, ethyl acetate and butanol fractions. Against *S. exigua,* the dichloromethane fraction had highest mean antifeedant index of 41.8% while ethyl acetate fraction had 34.1%. Hexane fraction, butanol fraction and the residue did not show antifeedant activity with -3.7%, 14.1% and 4.4% antifeedant indices respectively.

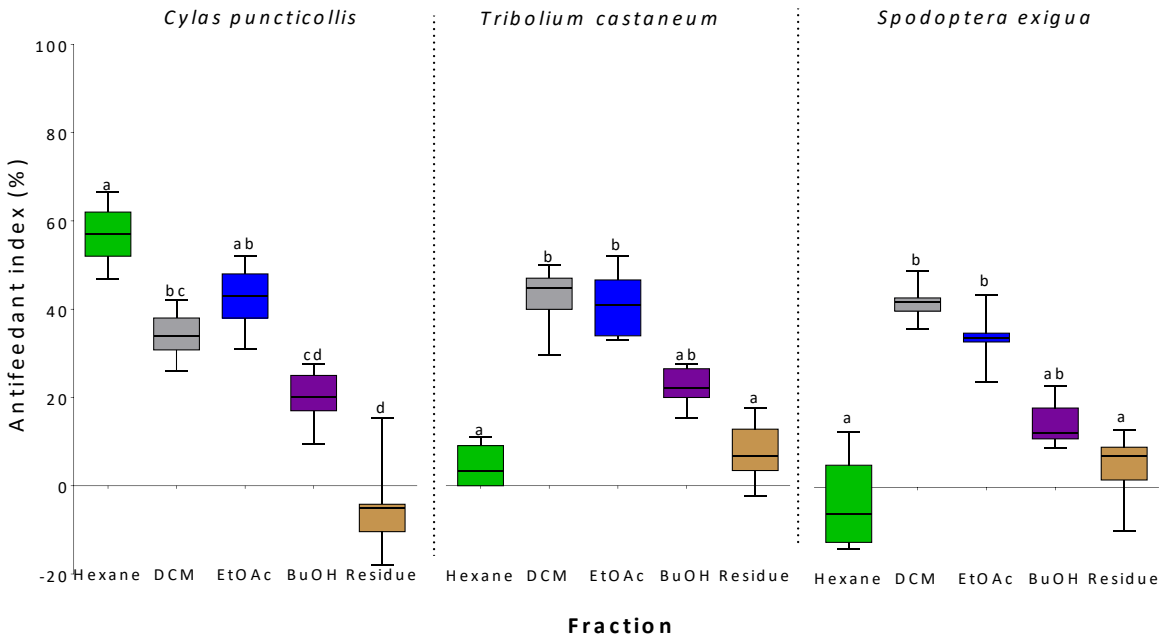


Figure 4.6: Box plot representation of antifeedant index (AFI) of fractions, obtained after liquid-liquid extraction of *Melia volkensii* bark extract, against various insect pests. AFI were obtained after 3 independent biological repeats each consisting of 5 technical repeats and per fraction. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction. (DCM – dichloromethane; EtOAc – ethyl acetate; BuOH – butanol).

Overall, the activity in the *M. volkensii* bark extracts were retained in the hexane and dichloromethane fractions after fractionation. The antifeedant activity of these active fractions was, however, lower than the activity of nut and pulp fractions against *C. puncticollis, T. castaneum* and *S. exigua.* For this reason, the fractions from the bark were not investigated further.

4.2.2.4 Antifeedant activity of *M. volkensii* leaf fractions

As presented in figure 4.7, the activity of hexane fraction of *M. volkensii* leaves gave consistently highest mean antifeedant activity against *C. puncticollis, T. castaneum* and *S. exigua.* In tests against *C. puncticollis,* the hexane extract had the highest antifeedant index of 45.8%. There was low activity in dichloromethane fraction, ethyl acetate fraction, butanol fraction and the residue exhibiting 12.8%, 2.7%, 5.6% and 6.4% antifeedant activity respectively. Against *T. castaneum,* the hexane fraction had antifeedant index of 15.5% while

dichloromethane, ethyl acetate, butanol fraction and the residue each had less than 2% antifeedant index. Hexane fraction also had mean highest antifeedant activity of 40.5% against *S. exigua* while dichloromethane, ethyl acetate, butanol and residue fractions exhibited antifeedant activity of 11.9%, 21.0%, -0.5% and 8.5% respectively. Even though the hexane extract of the leaf extract showed slightly over 40% antifeedant index against *C. puncticollis* and *S. exigua,* this was lower activity compared to the activity in the dichloromethane fraction of the pulp and nut extracts. In fact, the leaf fractions showed the lowest mean antifeedant activity against the insect pests when compared to the activity of the fractions from the other plant parts. Therefore, these fractions were not investigated further.

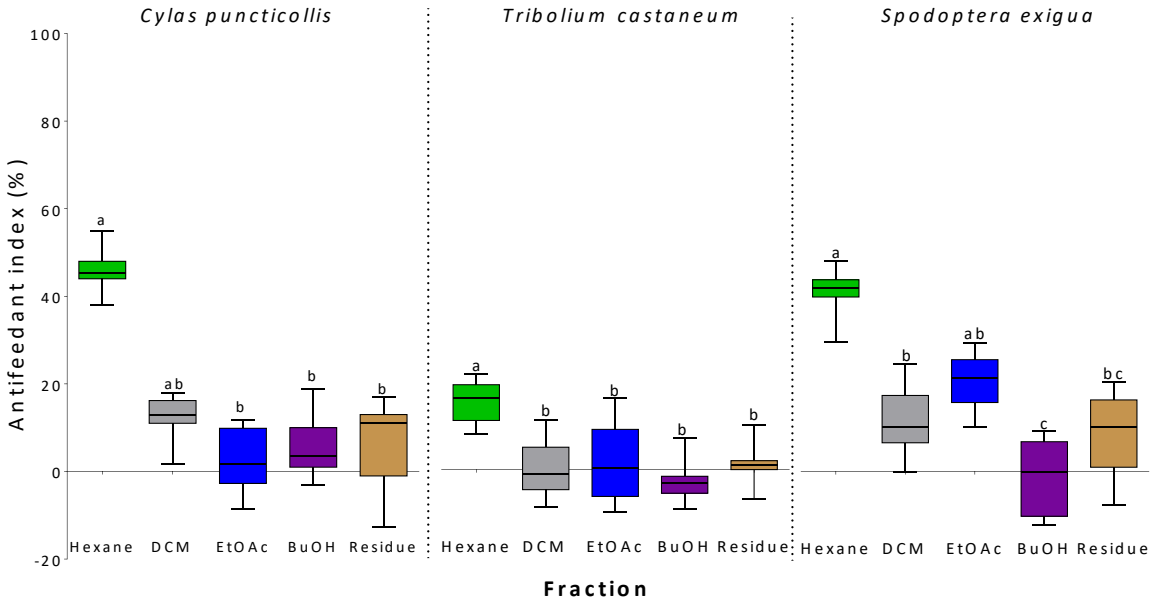


Figure 4.7: Box plot representation of the antifeedant index (AFI) of fractions obtained after liquid-liquid extraction of *Melia volkensii* leaf extract, against various insect pests. AFI were obtained after 3 independent biological repeats each consisting of 5 technical repeats and per fraction. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction. (DCM – dichloromethane; EtOAc – ethyl acetate; BuOH – butanol).

4.2.3 ANTIFEEDANT ACTIVITY OF COLUMN CHROMATOGRAPHY FRACTIONS OF *M. VOLKENSII* NUT

The fractions obtained after column chromatography purification of *M. volkensii* nut were screened for activity against *C. puncticollis, T. castaneum* and *S. frugiperda* to identify the most active fraction for subsequent purification.

4.2.3.1 Antifeedant activity of nut column chromatography fractions against *C. puncticollis*

When tested against *C. puncticollis,* antifeedant activity was highest in fractions 49-50, 51-56, 57-61, 62-65, and 66-81, with antifeedant indices of 63.7%, 60.3%, 66.0%, 67.3% and 39.0% respectively as presented in figure 4.8. There was no significant difference in activity of these fractions against *C. puncticollis*. Fraction 49-50 was eluted from the column with ethyl acetate/hexane 50:50 solvent system while fractions 51-56, 57-61 and 62-65 were eluted with ethyl acetate/hexane 75:25 from the column. This implies that the bioactive compounds could have moderate polarity. Because of their high activity against *C. puncticollis,* fractions 49-50, 51-56, 57-61, 62-65, and 66-81 were subjected to further purification processes to identify the bioactive compounds. The remaining fractions were not investigated further.

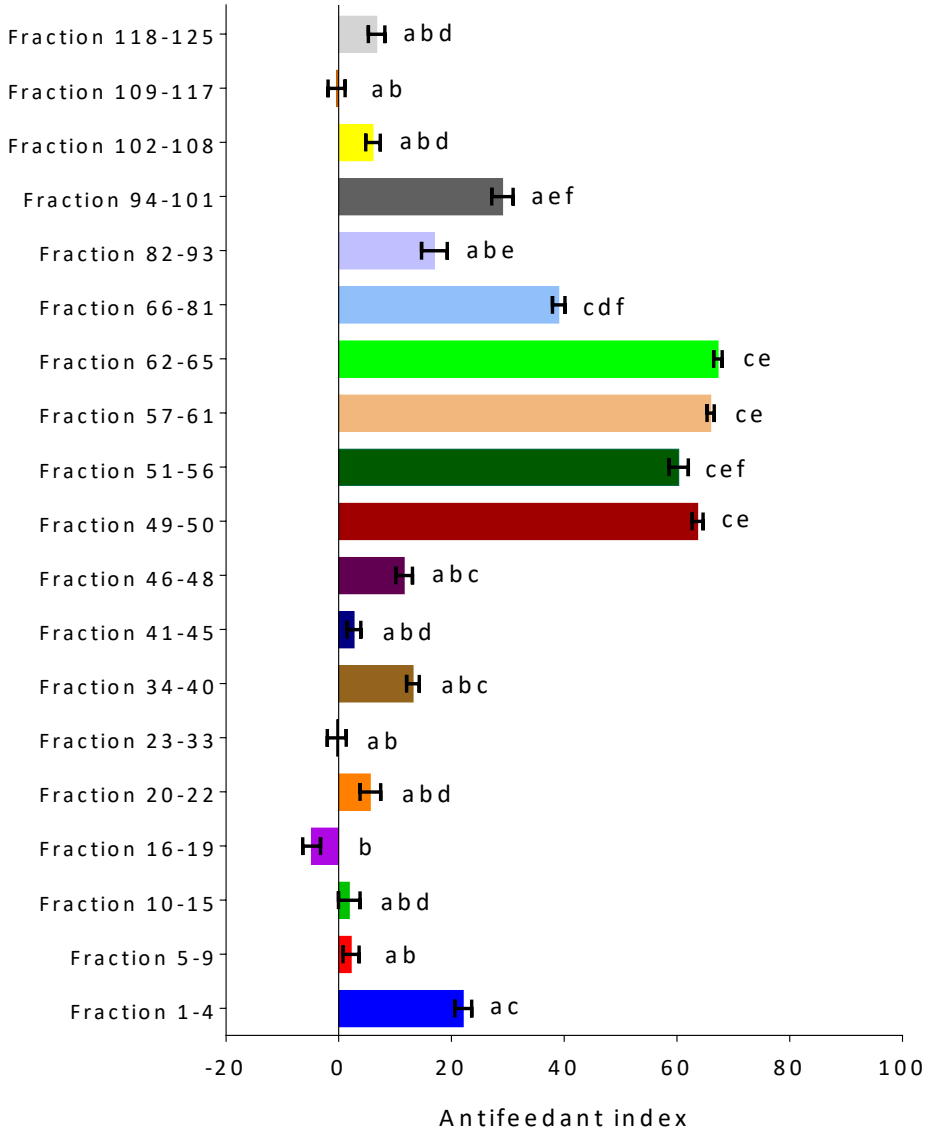


Figure 4.8: Graphical representation of the antifeedant index (AFI) of fractions obtained from *M. volkensii* nut after column chromatography, against *Cylas puncticollis*. AFI values are mean ± SEM after 5 technical repeats and 3 independent biological repeats. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction.

4.2.3.2 Antifeedant activity of nut column chromatography fractions against *T. castaneum*

Figure 4.9 presents the antifeedant activity, against *T. castaneum*, of the fractions obtained after column chromatography purification of *M. volkensii* nut. High antifeedant indices of 90.4% and 74.5% were recorded in fractions 62-65 and 66-81 respectively. There was however no significant difference in activity between these fractions and the activity of

fraction 49-50, 51-56 and 57-61 which recorded antifeedant activity of 44.3%, 45.8% and 54.3% respectively. Fractions 49-50, 51-56 and 57-61 had also previously showed antifeedant activity against *C. puncticollis*. The fractions which were identified for subsequent purification therefore were fractions 49-50, 51-56, 57-61, 62-65 and 66-81. The remaining fractions were not investigated further. It is again interesting to note that antifeedant activity was retained in the fraction with medium polarity.

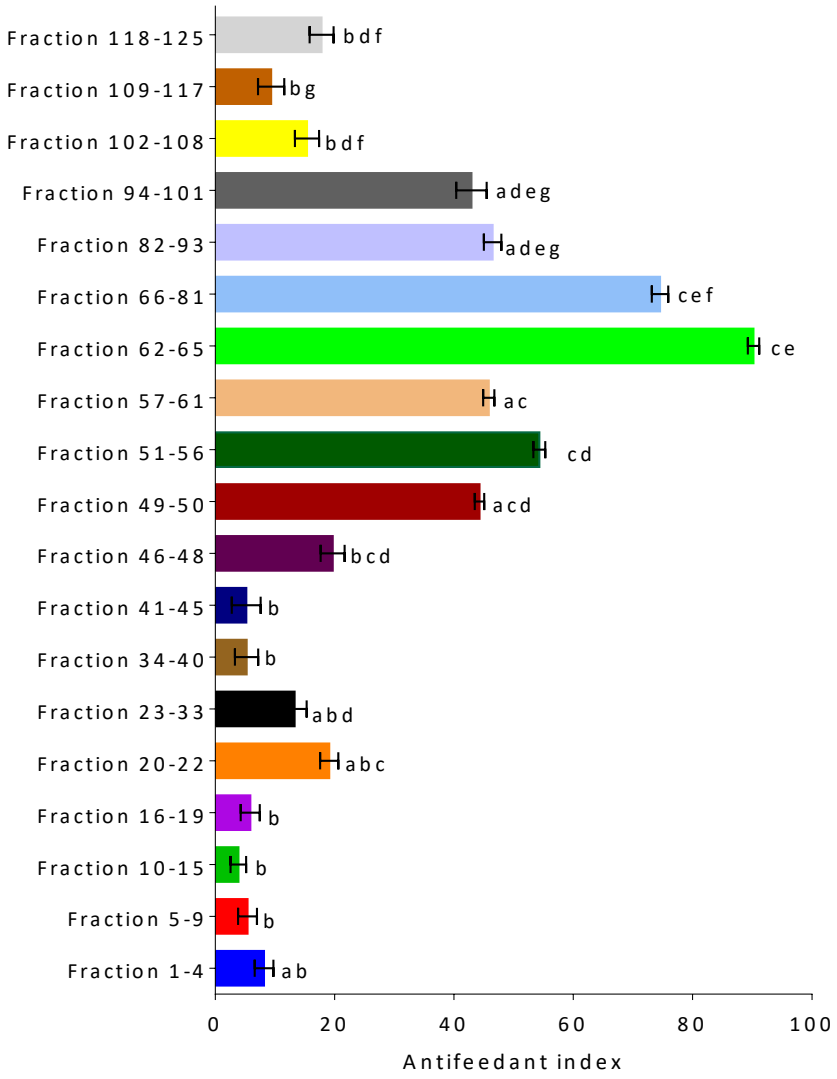


Figure 4.9: Graphical representation of the antifeedant index (AFI) of fractions obtained from *M. volkensii* nut after column chromatography against *Tribolium castaneum*. AFI values are mean ± SEM after 5 technical repeats and 3 independent biological repeats. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction.

4.2.3.3 Antifeedant activity of nut column chromatography fractions against *S. frugiperda*

The antifeedant activity of *M. volkensii* nut fractions obtained after column chromatography against *S. frugiperda* is presented in figure 4.10. Fractions 46-48, 49-50, 51-56, 57-61, 62-65, 66-81, 82-93, 94-101, 102-108 and 109-117 had strong antifeedant activity of 80.4%, 89.6%, 94.8%, 93.4%, 98.6%, 94.8%, 94.6%, 89.5%, 91.7% and 83.1% respectively. There was no significant difference on antifeedant activity against *S. frugiperda* in all these fractions. Fractions 49-50, 51-56, 57-61, 62-65 and 66-81 were selected for subsequent purification to identify the bioactive compounds. This is because these fractions had also previously shown consistent activity against *C. puncticollis* and *T. castaneum.* The remaining fractions were therefore not investigated further.

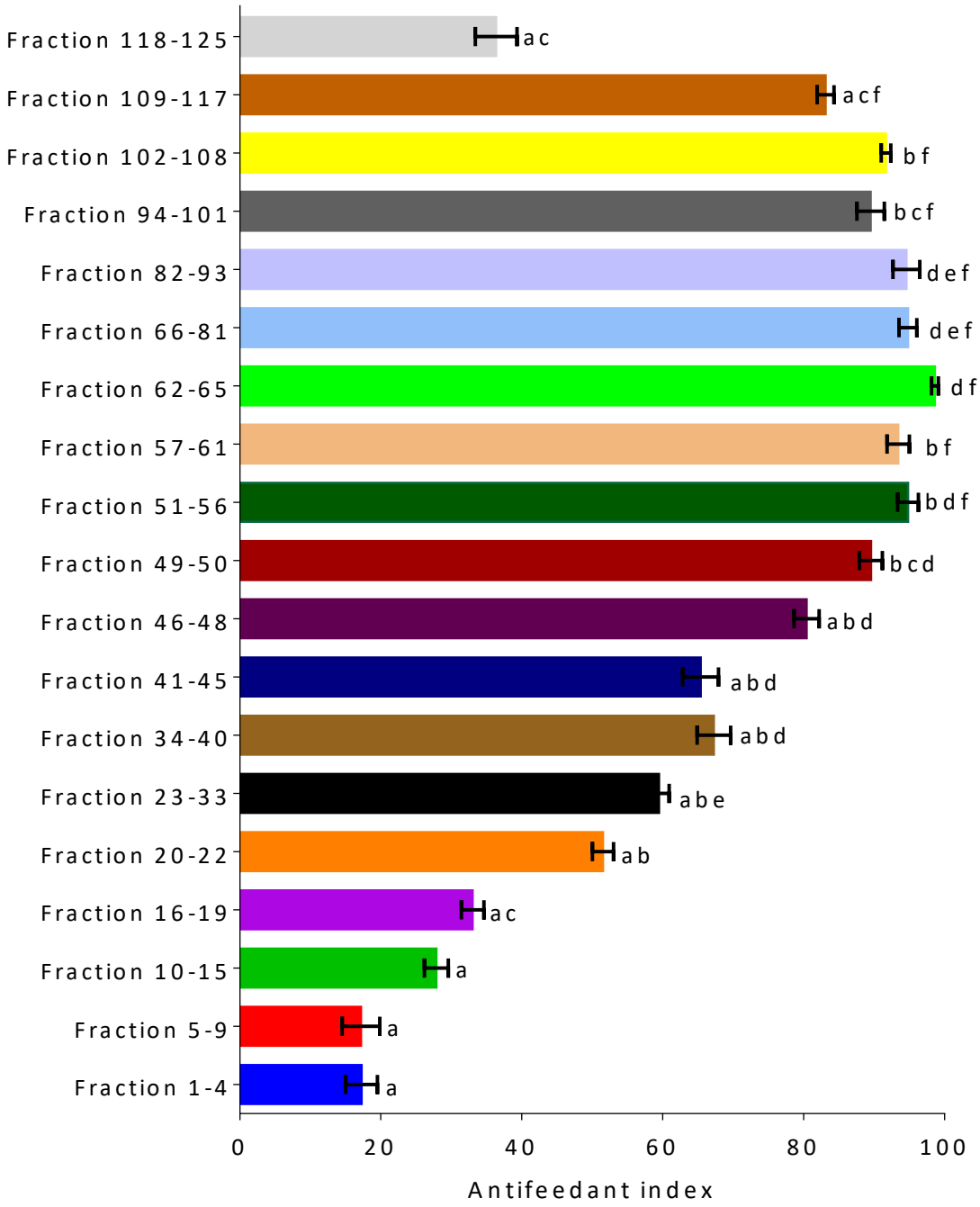


Figure 4.10: Graphical representation of the antifeedant index (AFI) of fractions obtained from *M. volkensii* nut after column chromatography against *Spodoptera frugiperda*. AFI values are mean ± SEM after 5 technical repeats and 3 independent biological repeats. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction.

4.2.3.4 Antifeedant activity of *M. volkensii* nut sub-fractions after preparative-HPLC

The bioactive nut fractions were further purified using prep-HPLC. The sub-fractions obtained from the prep-HPLC were biotested against *S. frugiperda, T. castaneum* and *C. puncticollis* as presented in figure 4.11. Against *S. frugiperda*, sub-fraction 66 – 81 fr 2 had the highest mean antifeedant index of 55.3%. This was not significantly different with sub-fraction 51-56 fr 3 and sub-fraction 51-56 fr 1 which showed antifeedant activity of 49.9% and 41.8% respectively. Lower antifeedant activities were recorded in sub-fractions 49-50 fr 1, 49-50 fr 2, 49-50 fr 3 and 51-56 fr 2 with antifeedant indices of 33.2%, 33.4%, 5.5% and 22.7% respectively.

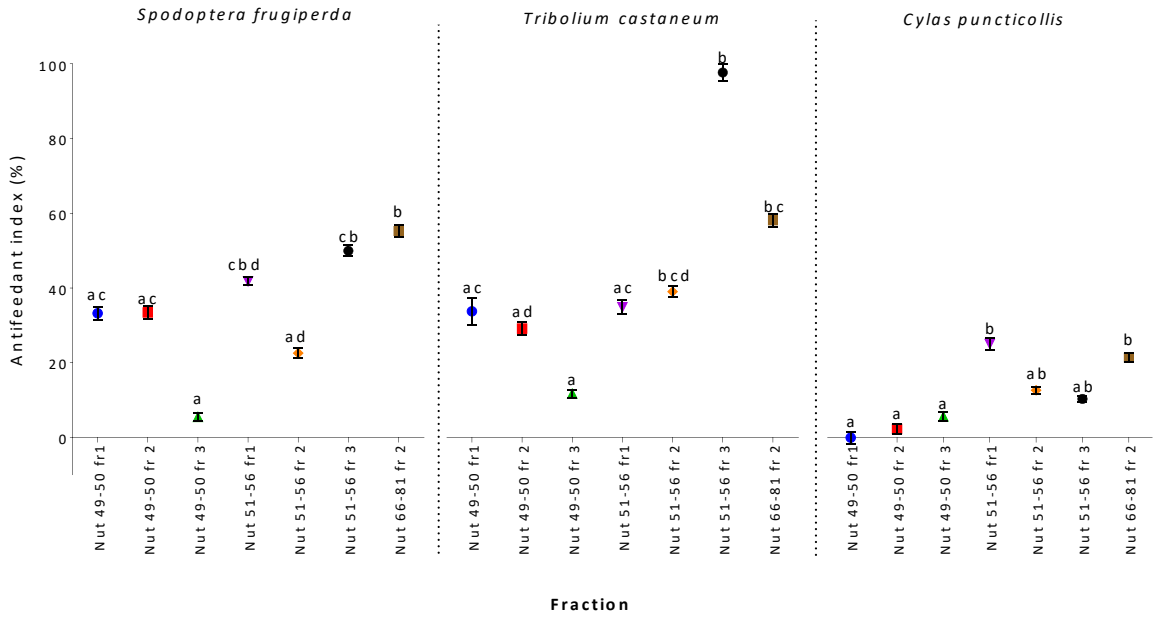


Figure 4.11: Symbol plot representation of antifeedant index (AFI) of sub-fractions obtained after preparative-HPLC purification of bioactive nut fractions, against various insect pests. The symbol is mean AFI ± SEM after 5 technical repeats and 3 independent biological repeats. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction.

Against *T. castaneum*, sub-fraction 51-56 fr 3 had the strongest antifeedant index of 97.7% as presented in figure 4.11. This was almost complete protection from feeding. Sub-fraction 66-81 fr 2 had antifeedant index of 58.1% while sub-fraction 51-56 fr 2 had 39.0% antifeedant

activity against *T. castaneum*. Sub-fractions 49-50 fr 1, 49-50 fr 2, 49-50 fr 3, 51-56 fr 1 recorded antifeedant activity of 33.8%, 29.2%, 11.7% and 34.9% respectively.

There was low antifeedant activity against *C. puncticollis* in all the sub-fractions. This could be an indication that crude extract acts synergistically against *C. puncticollis* and that the bioactivity could have been lost by isolating some components of the extracts. The sub-fractions 49-50 fr 1, 49-50 fr 2, 49-50 fr 3, 51-56 fr 1, 51-56 fr 2, 51-56 fr 3 and 66-81 fr 2 recorded antifeedant activities of -0.1, 2.5%, 6.1%, 27.1%, 13.7%, 11.2% and 23.2%, respectively against *C. puncticollis*.

Overall, sub-fraction 51-56 fr 3 gave strong antifeedant activity against *T. castaneum* (97.7%) and moderate activity (49.9%) against *S. frugiperda*. This sub-fraction certainly presented interesting activity and was further purified using preparative-TLC to yield pure **compound 2**, which was identified as toosendanin. Sub-fraction 66-81 fr 2 also had antifeedant activity against *S. frugiperda* (55.3%) and *T. castaneum* (58.1%). Comparison of the LC-MS spectral data showed that this sub-fraction was similar to compound 3, which was isolated from *M. volkensii* pulp. The remaining sub-fractions 49-50 fr 1, 49-50 fr 2, 49-50 fr 3, 51-56 fr 1 and 51-56 fr 2 were not further investigated.

4.2.4 ANTIFEEDANT ACTIVITY OF COLUMN CHROMATOGRAPHY FRACTIONS OF *M. VOLKENSII* PULP

The fractions obtained after column chromatography purification of *M. volkensii* pulp were screened for activity against *C. puncticollis, T. castaneum* and *S. frugiperda* to identify the most active fraction for subsequent purification.

4.2.4.1 Antifeedant activity of pulp column chromatography fractions against *C. puncticollis*

When tested against *C. puncticollis*, the highest activity was observed in fraction 55-64 with an antifeedant activity of 60.2%, as presented in figure 4.12. This was not significantly different from the activity of fractions 48-54 and 42-44 which recorded antifeedant indices of 44.7% and 30.8% respectively. Fraction 55-64 was subjected to further isolation to identify the bioactive compound, due to its high activity against *C. puncticollis*.

Fractions 55-64 were eluted with ethyl acetate/hexane 75:25 solvent system from the column implying that the bioactive compounds may be of moderate polarity. This is similar to *M. volkensii* nut, where most of the bioactive fractions were also eluted with ethyl acetate/hexane 75:25 solvent system. The remaining fractions were not investigated further.

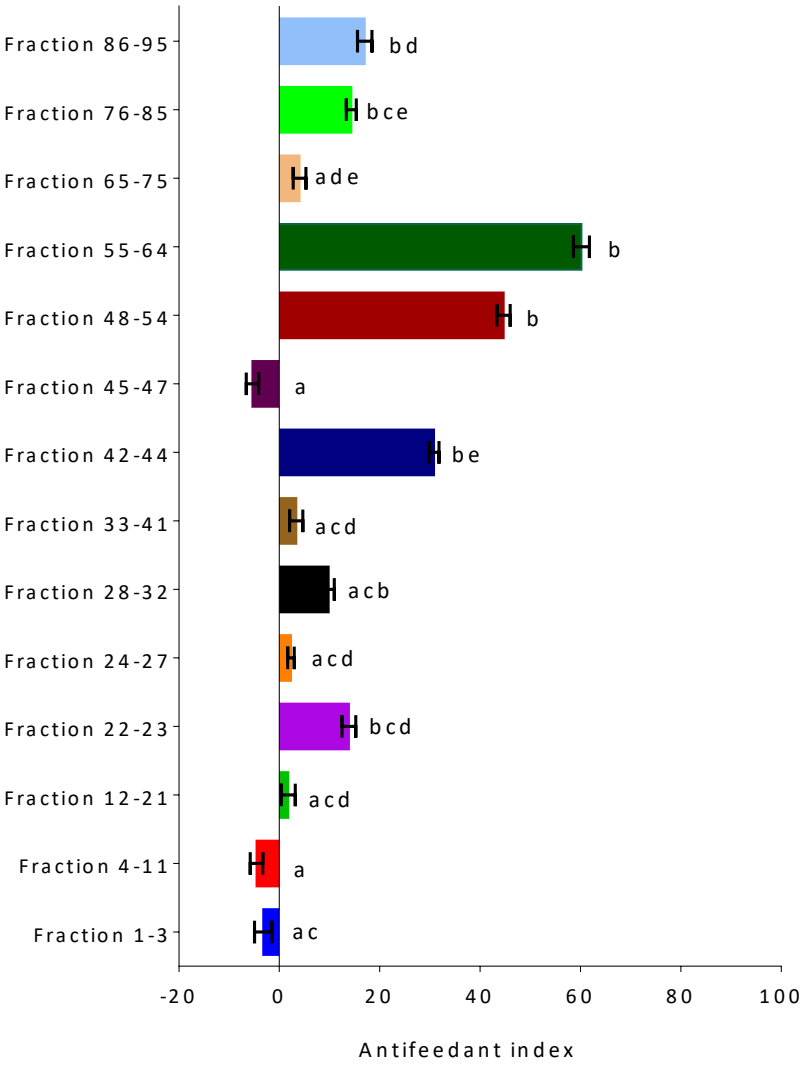


Figure 4.12: Graphical representation of the antifeedant index (AFI) of fractions obtained from *Melia volkensii* pulp after column chromatography against *Cylas puncticollis*. AFI values are mean ± SEM after 5 technical repeats and 3 independent biological repeats. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction.

4.2.4.2 Antifeedant activity of pulp column chromatography fractions against *T. castaneum*

When the pulp fractions were tested against *T. castaneum*, fraction 55-64 had the strongest activity of 44.6% as presented in figure 4.13. This was however, not significantly different from fractions 45-47, 48-54 and 76-85 which showed antifeedant activity of 12.9%, 11.4% and 10.3%, respectively. Fraction 55-64 was further purified because it showed the strongest

activity against *T. castaneum* and also activity against *C. puncticollis* and *S. frugiperda*. The remaining fractions were not investigated further.

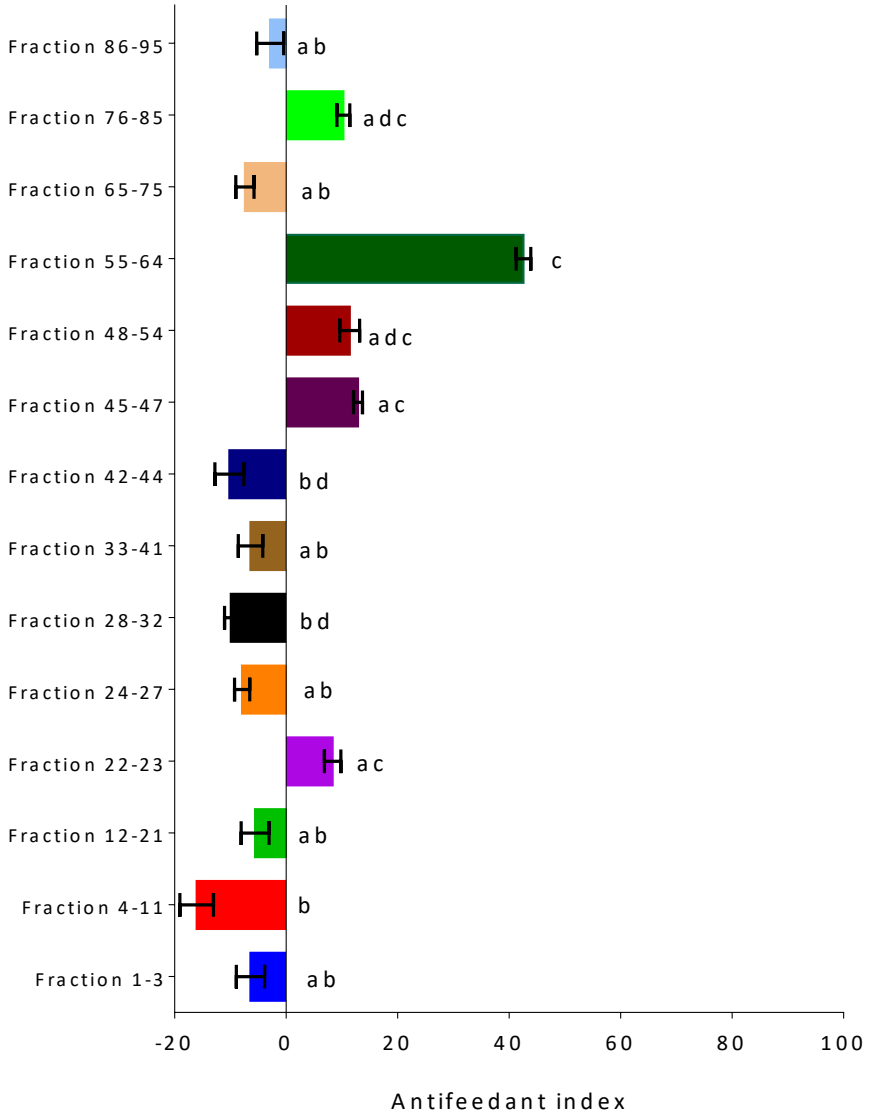


Figure 4.13: Graphical representation of the antifeedant index (AFI) of fractions obtained from *Melia volkensii* pulp after column chromatography against *Tribolium castaneum*. AFI values are mean ± SEM after 5 technical repeats and 3 independent biological repeats. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction.

4.2.4.3 Antifeedant activity of pulp column chromatography fractions against *S. frugiperda*

The bioactive fractions against *S. frugiperda* were 4-11, 33-41, 42-44, 45-47, 48-54, 55-64, 65-75, 76-85 and 86-95 which recorded antifeedant indices of 82.1%, 75.2%, 64.9%, 92.7%, 91.5%, 92.6%, 85.1%, 86.4% and 85.8% respectively as presented in figure 4.14. There were also no significant differences in the activity of these fractions.

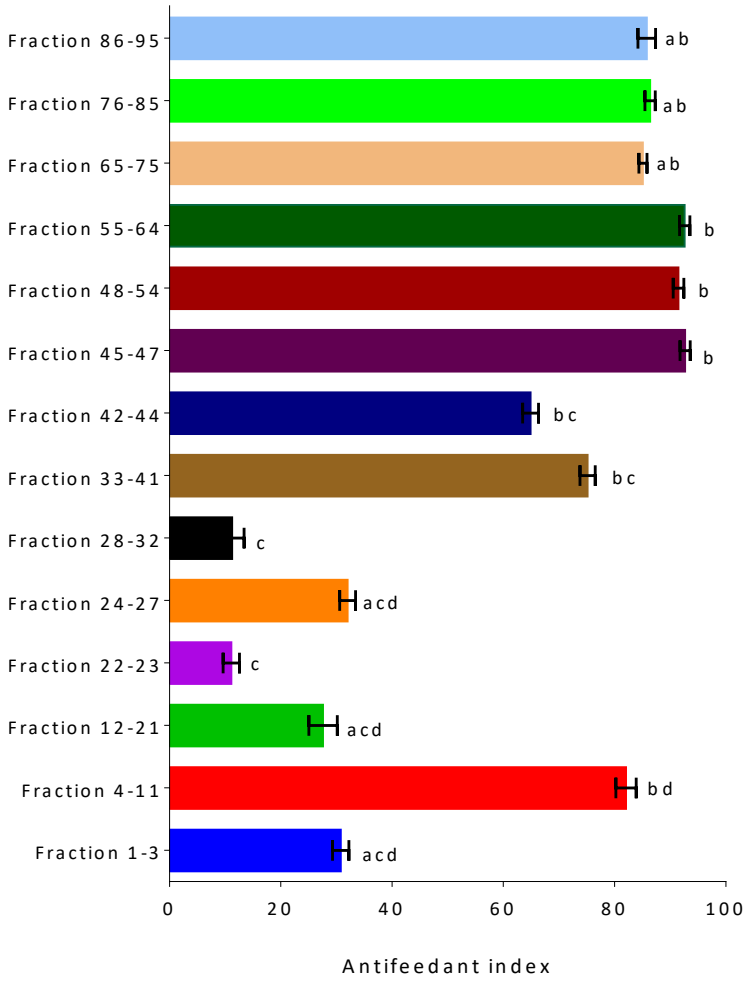


Figure 4.14: Graphical representation of the antifeedant index (AFI) of fractions obtained from *M. volkensii* pulp after column chromatography against *Spodoptera frugiperda*. AFI values are mean ± SEM after 5 technical repeats and 3 independent biological repeats. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction.

Fraction 55-64 showed consistently high activity against *C. puncticollis, C. puncticollis* and *T. castaneum* and therefore this fraction was further purified to identify the active compound. The remaining fractions were not investigated further.

4.2.5 ANTIFEEDANT ACTIVITY OF ISOLATED PURE COMPOUNDS

In this study, three pure compounds were isolated from *M. volkensii.* Compound **1** (reduced meliavolkenin) and compound **2** (toosendanin) were isolated from the nuts while compound **3** (salanninolide) was isolated from the pulp. Biotesting the pure compounds at 1% concentration against the insect pests showed varying results as presented in figure 4.15.

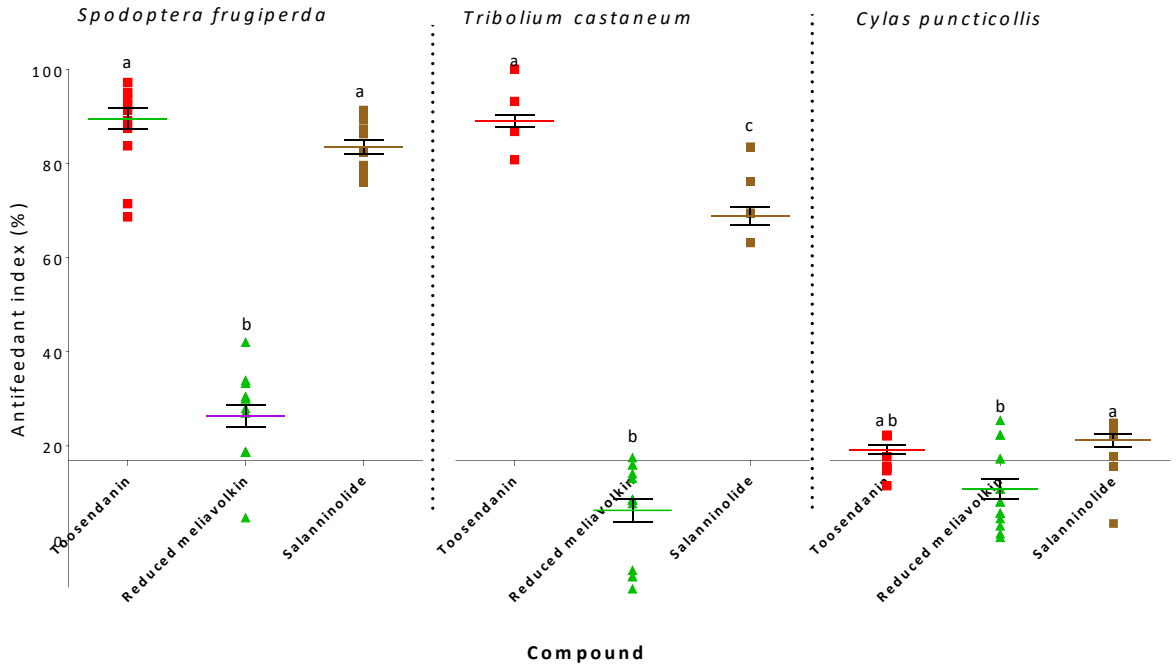


Figure 4.15: Aligned dot plot representation of the antifeedant index (AFI) of pure compounds isolated from *Melia volkensii* against various insect pests. The values are AFI ± SEM after 5 technical repeats and 3 independent biological repeats. Means followed by a different letter are significantly different (p<0.05) according to Dunns' multiple comparison test followed by Bonferroni correction.

Toosendanin had antifeedant activity of 89.5% against *S. frugiperda*, 89.2% activity against *T. castaneum* while low activity of 21.2% was observed against *C. puncticollis*. This is an interesting finding, particularly for *T. castaneum*, given that there is a limited number of active ingredients worldwide for the treatment of cereals against storage pests [141]. Toosendanin has been found in the bark of *M. toosendan* and *M. azedarach* and has been the subject of botanical pesticide research and has been marketed in China [240,272,273,248,278–283]. Toosendanin has also been isolated from the root bark of *M. volkensii* [52].

It is used in traditional Chinese medicine to kill gastrointestinal worms in humans [283]. Toosendanin is also used in clinical medicine against several human cancer cells in addition to its antifeedant, stomach poisoning and growth inhibition activity against insect pests [259,278–286]. Toosendanin has also exhibited pharmacological activities including anti-inflammatory, anti-oxidant, antibotulismic, anti-viral and anti-infective properties [287], [288]. It has also been reported to promote ceramide production and has potential for formulation of human skin products [289].

Toosendanin has been reported to cause 51.7% mortality against oriental armyworm, *Mythimna separata* (Walker) after 35 days when applied at 1% concentration [289,290]. Toosendanin has also shown antifeedant and toxicity effects against *Pieris rapae* [269]. 0.5% emulsifiable concentrate of toosendanin has shown high control effect on pests in vegetables, tea, fruits, flowers, tobacco and other economic crops [279]. Extracts based on toosendanin obtained from *M. toosendan* have shown that toosendanin caused significant mortality and inhibition of adult emergence against thee storage pests: *T. castaneum, Cryptolestes ferrugineus* and *Sitophilus oryzae* [283]. Toosendanin is considered as a good potential pesticide for controlling rotifers in microalgae mass culture [19,291].

Toosendanin is a digestive agent that negatively affects the digestive system which plays a considerable role in feeding, digestion and nutrient absorption in insects [292]. It targets the microvilli of midgut cells causing destruction of midgut epithelial cells leading to regurgitation, paralysis and death of insects [292]. It has been reported that most botanical pesticides such as azadirachtin significantly interfere with the digestive system causing insect malnutrition, growth inhibition and even death [292]. Toosendanin has shown to modulate sensory code underlying feeding behaviour via several different peripheral sensory mechanisms like

stimulating deterrent receptor cell located in the maxillary sensillum styconicum and inhibition of both sugar and glycosinolate receptor cells [248]. It is also postulated that steroid containing compounds in plant extracts inhibit protein by blocking sterol carrier proteins so that insects grow abnormally [195]. Toosendanin has very favourable mammalian toxicity (LD_{50} mice = 10 g/kg), is non-persistent and easily biodegradable [293]. While there are no specific biodegradation products of the pure phytochemicals reported, exposure of botanical pesticides to air, sunlight, moisture and high temperatures could break down their constituents. In addition, soil microorganisms produce enzymes that could modify the botanical pesticides with introduction of breakable groups giving metabolites which are less toxic than the parent product, and then render those metabolites biologically unavailable and non-toxic [294].

The reduced meliavolkenin had low antifeedant activity of 26.3%, 7.9% and 13.1% against *S. frugiperda, T. castaneum* and *C. puncticollis* respectively as presented in figures 4.15. Meliavolkenin was previously isolated from the root bark of *M. volkensii* by bioactivity-directed fractionation using the BST and was found to be moderately cytotoxic against three human solid tumor lines (human lung carcinoma, human breast carcinoma, and human colon adenocarcinoma) [47,76,82,295,296]. Its antifeedant activity against insect pests has, however, not been reported in literature.

Salanninolide had antifeedant activity of 83.5%, 69.3% and 23.2% against *S. frugiperda, T. castaneum* and *C. puncticollis* respectively. This is the first report of salanninolide being isolated directly from *M. volkensii*. Salanninolide has previously been isolated from the seeds of *Melia dubia* and *A. indica* [297–299]. Salanninolide has also been processed through photo-oxidation of salannin in presence of UV light and oxygen [300,301]. Bioconversion of salannin using fungal strain *Cunninghamella echinulate* has also produced salanninolide [302].

Salanninolide has also been reported to have higher potency than azadirachtin in a choice test against *Spodoptera littoralis, S. gregaria* and *L. migratoria* with IA_{50} (concentration required to elicit antifeedant index of 50%) of > 1 x 10^{-2} M, 2.1 x 10^{-3} M and > 1 x 10^{-2} M respectively [297,302]. Isosalanninolide, an isomer of salanninolide, has also shown high antifeedant and insecticidal activity almost comparable to that of azadirachtin against these insect pests [303]. Salanninolide is also known to have medicinal properties [304]. In other

related studies, pure isolated compound luteolin-7-0-glucopyranoside, isolated from *Vernonia cinerea* showed 98.6% feeding-deterrence against *Spodoptera litura* at 3000 ppm concentration [253].

Even though toosendanin and salanninolide had strong antifeedant activity against *S. frugiperda* and *T. castaneum*, they however showed low activity against *C. puncticollis*. This could point out to possibility of synergistic effect of other antifeedant components of crude extracts or semi-refined extracts in *M. volkensii* against *C. puncticollis* hence low activity with a single pure compound. It has been reported that complex metabolite mixtures could act synergistically against their targets [228,258,304–308].

4.2.6 DETERMINATION OF EC_{50} VALUES OF ISOLATED PURE COMPOUNDS

EC_{50} is the effective concentration causing 50% antifeeding effect compared with the control. The EC_{50} values were done for the pure bioactive compounds, toosendanin and salanninolide, isolated from *M. volkensii*. The EC_{50} values are graphically presented in figure 4.16 (a) and (b) following linear regression analysis of the pure compounds toosendanin and salanninolide

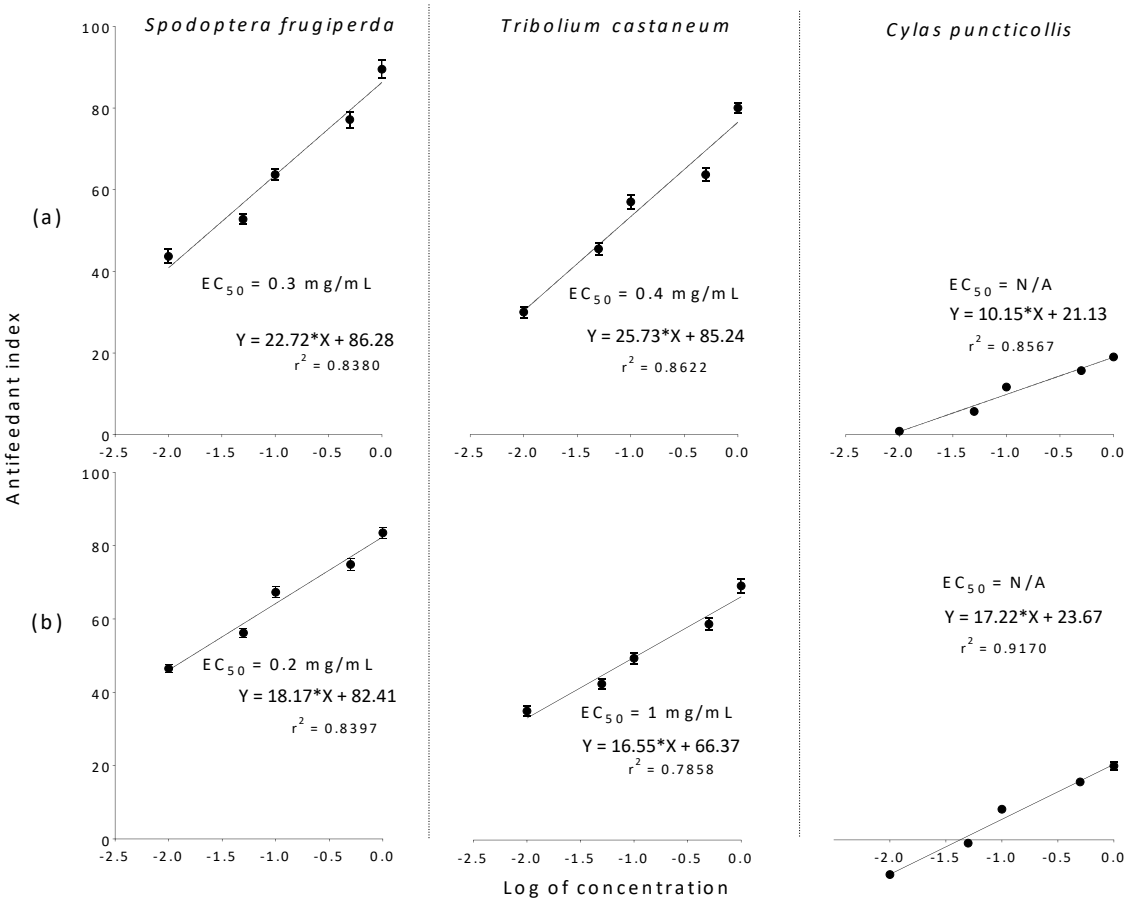


Figure 4.16: Regression analysis of toosendanin (a) and salanninolide (b) at different concentrations to determine the EC_{50} against various insect pests. The points are mean ± SEM of antifeedant index, based on 5 technical repeats and 3 independent biological repeats.

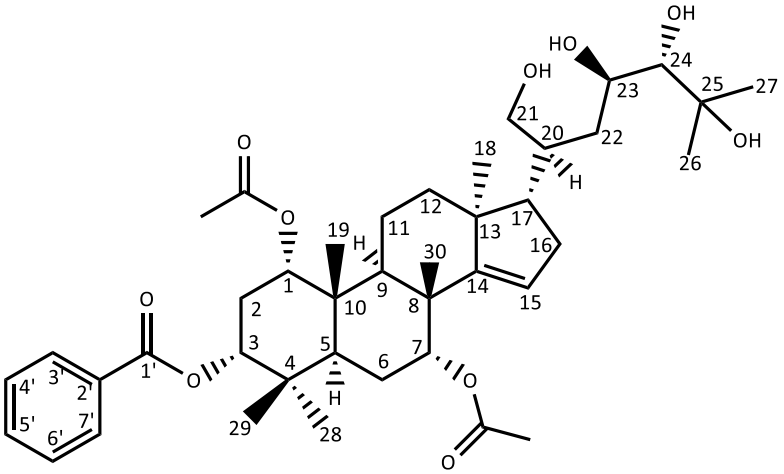
In this study, toosendanin had strong antifeedant activity against *T. castaneum* and *S. frugiperda* with EC_{50} of 0.4 mg/mL and 0.3 mg/mL respectively. Toosendanin, however, had no antifeedant activity against *C. puncticollis*. Salanninolide also recorded high antifeedant activity against *T. castaneum* and *S. frugiperda* with EC_{50} of 1mg/mL and 0.2 mg/mL. Against *C. puncticollis*, salanninolide had no antifeedant activity in this study. The lack of activity of

the pure compounds against *C. puncticollis* could be because other components of crude extracts or semi-refined extracts in *M. volkensii* act synergistically against *C. puncticollis* and this activity is lost when purification and isolation are done.

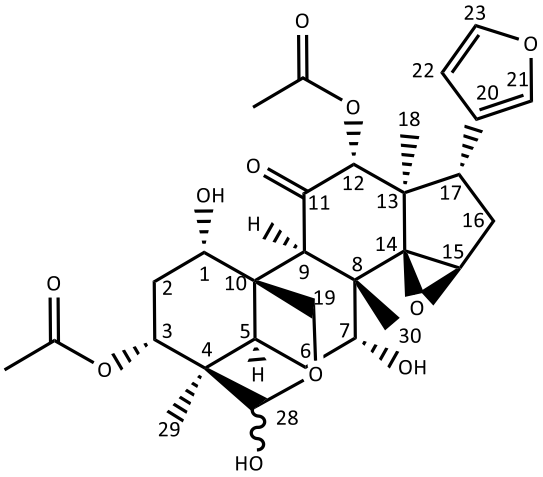
Previous studies have reported that salannin and volkensin isolated from *M. volkensii* have both shown antifeedant activity *S. frugiperda* with ED_{50} of 13 µg /cm^2 and 3.5 µg/cm^2 of leaf surface respectively [53]. Also, EC_{50} values of 3.6 x 10^{-7}, 4.7 x 10^{-4}, >1 x 10^{-3}, 1.4 x 10^{-4}, 6.3 x 10^{-4}, 4.3 x 10^{-4} and 2.6 x 10^{-4} µg/g were recorded for azadirachtin, salannin, salanninolide, isosalanninolide, nimbin, nimbinolide and isonimbolide were respectively against *S. frugiperda* [309]. Pure compounds 3-O-acetylsalannol, salannol and salannin have recorded EC_{50} values of 65.6 ppm, 77.4 ppm and 87.7 ppm respectively against *S. litura* larvae [310].

4.2.7 STRUCTURAL ELUCIDATION OF ISOLATED COMPOUNDS

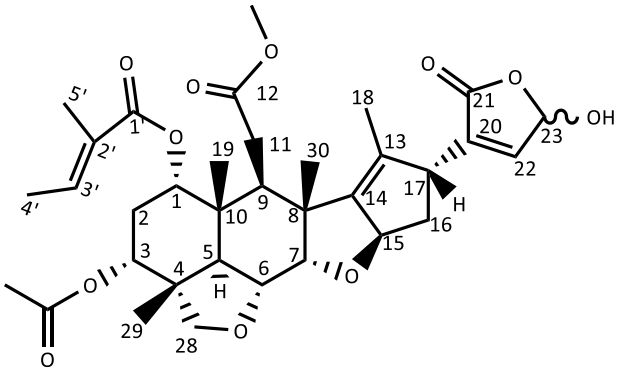
The present study resulted in the isolation of three pure compounds from *M. volkensii*. Compound **1** (reduced meliavolkenin) and compound **2** (toosendanin) were isolated from the nuts. Compound **3** (salanninolide) was isolated from the pulp in a bioactivity-directed isolation process. The chemical structures of these compounds are presented in figure 4.17.



Compound **1**, Reduced meliavolkenin



Compound **2,** Toosendanin



Compound **3**, Salanninolide

Figure 4.17: Chemical structures of isolated compounds

a. Compound 1

Compound **1** was isolated as a white powder. The ESI mass spectrum of compound **1** showed an ion peak in negative mode at *m/z* 711 corresponding to the fragment $[M - H]^-$ and in positive mode at *m/z* 735 corresponding to $[M + Na]^+$ as presented by the LC-MS profile in figure 4.23. This suggested a molecular weight of 712 for compound **1**. The ^1H NMR and ^{13}C NMR spectra of compound **1** are presented in figures 4.19 and 4.20 respectively. The structure was confirmed by COSY and HMBC correlations presented in figure 4.18; and the COSY and HMBC spectra are shown in figures 4.21 and 4.22 respectively. The above spectral data revealed the structure of compound **1** as reduced meliavolkenin and the spectral data of the compound isolated matched well with those reported earlier [82]. The characteristic ^1H NMR and ^{13}C NMR data of compound **1** is listed in Table 4.13.

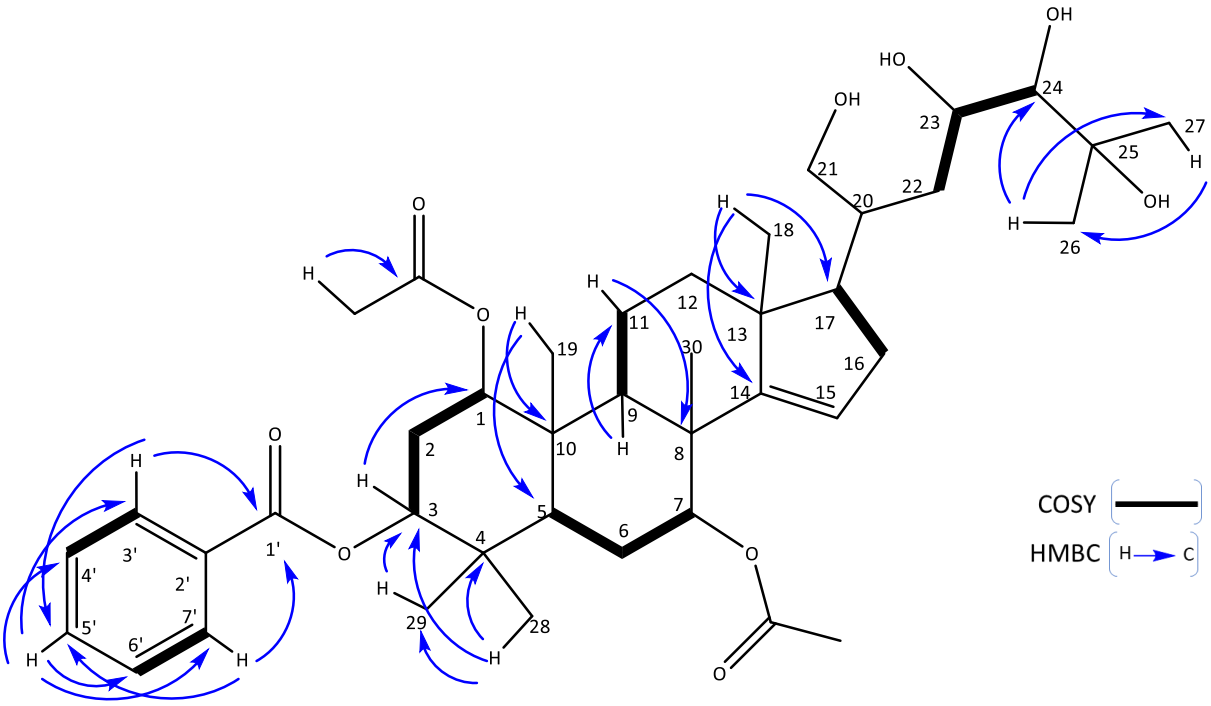


Figure 4.18*:* COSY and HMBC correlation of compound **1**

Table 4.13: Characteristic ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data of compound **1**, reduced meliavolkenin

Position	$\delta_{C,}$ type	δ_H (J in Hz)
1	72.7, CH	4.71, t (2.6)
2	25.5, CH_2	2.15-2.33, m
3	77.1, CH	4.89, t (2.5)
4	36.6, C	-
5	37.4, CH	2.54, t (8.0)
6	22.9, CH_2	1.82-1.87, m
7	75.6, CH	5.18, t (2.5)
8	42.2, C	-
9	35.5, CH	2.64, dd (11.3, 6.2)
10	40.3, C	-
11	16.2, CH_2	1.24-1.28, m and 1.46-1.51, m
12	35.0, CH_2	1.46-1.54, m and 1.70-1.77, m
13	46.5, C	-
14	159.3, C	-
15	119.4, CH	5.35, br d, (2.1)
16	35.0, CH_2	1.93-2.03, m and 2.22-2.30, m
17	56.1, CH	1.60-1.67, m
18	16.2, CH_3	1.00, s
19	20.0, CH_3	1.08, s
20	40.8, CH	1.80-1.86, m
21	65.3, CH_2	3.36-3.42, m and 3.90, dd (10.2, 2.8)
22	37.5, CH_2	1.64-1.78, m
23	77.9, CH	3.09, br s
24	71.6, CH	4.00, br d (7.9)
25	74.0, C	-
26	27.2, CH_3	1.29, s
27	26.4, CH_3	1.31, s
28	28.1, CH_3	0.91, s
29	21.5, CH_3	1.00, s
30	26.8, CH_3	1.15, s
1-AcO	21.1	2.07, s
7-AcO	21.0	1.65, s
1’	165.3, C	-
2’	130.8, C	-
3’	129.5, C	8.09, dd (8.3, 1.1)
4’	128.3, C	7.43, t (7.7)
5’	133.0, C	7.57, t (7.4)
6’	128.3, C	7.43, t (7.7)
7’	129.5, C	8.09, dd (8.3, 1.1)
AcO	169.7	-
	170.1	-

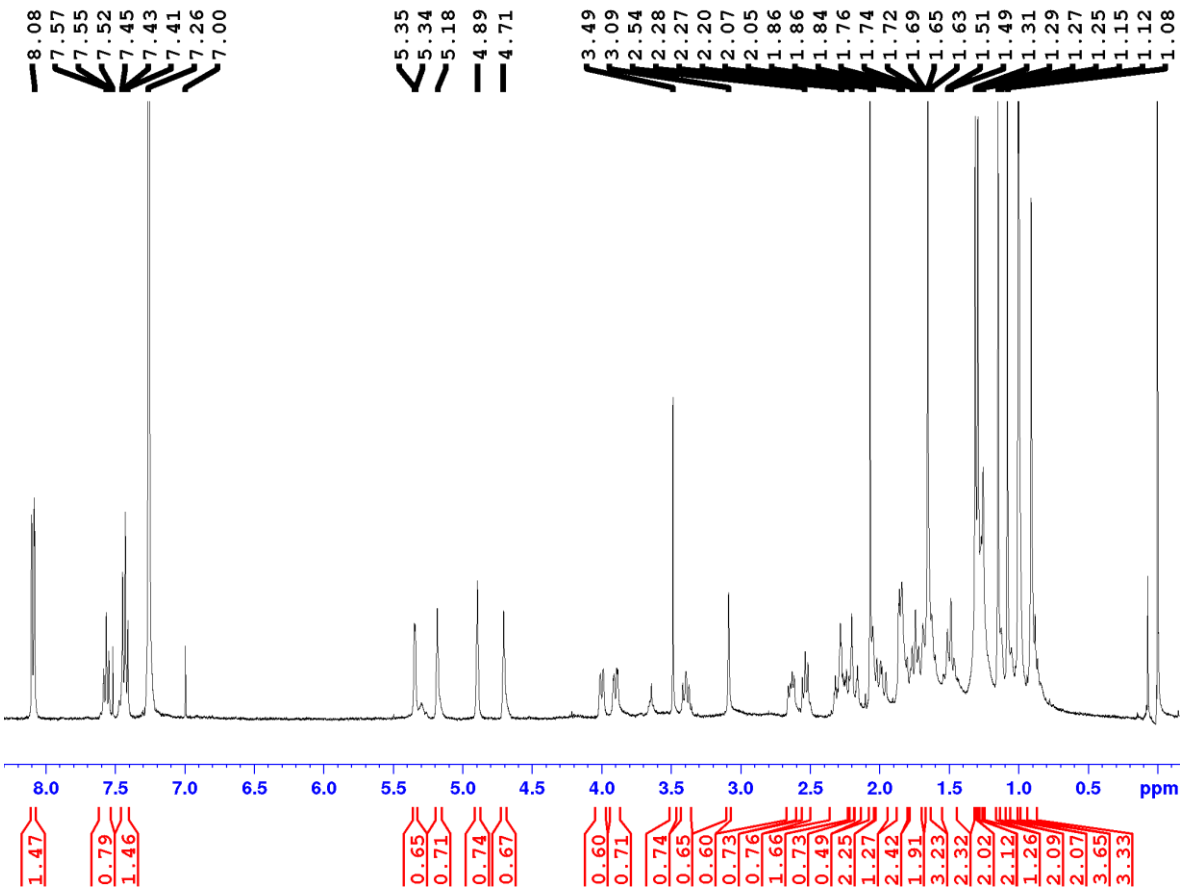


Figure 4.19*:* ^{1}H NMR spectrum of compound **1,** reduced meliavolkenin

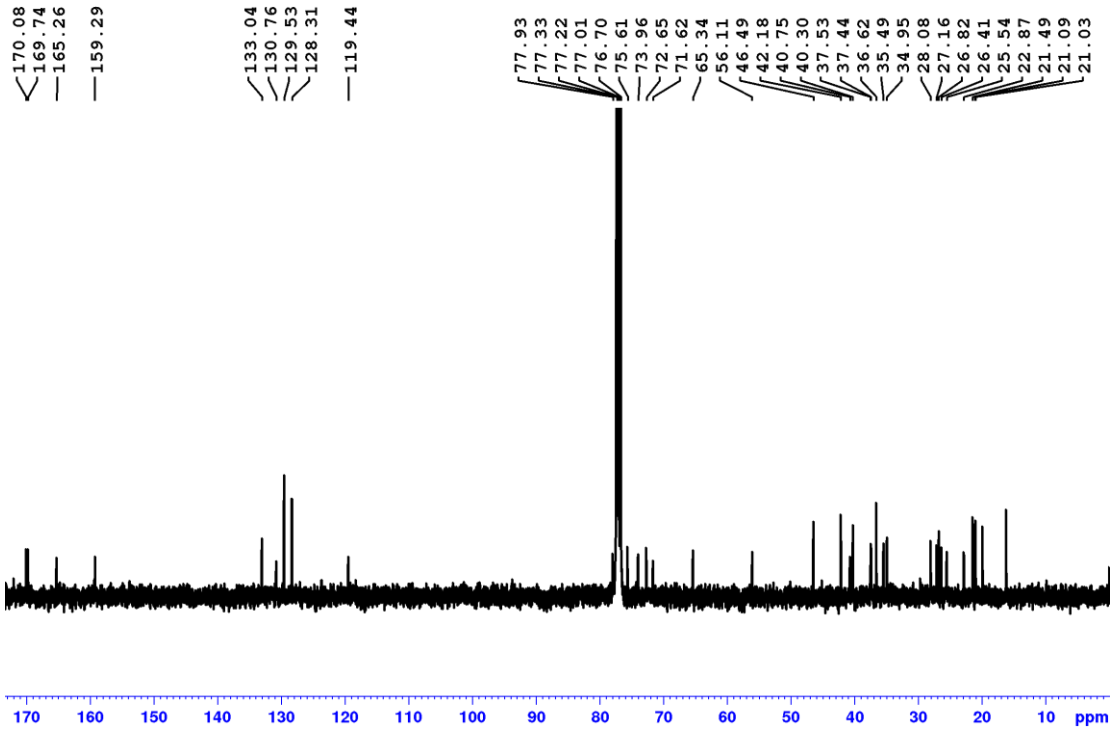


Figure 4.20*:* ^{13}C NMR spectrum of compound **1,** reduced meliavolkenin

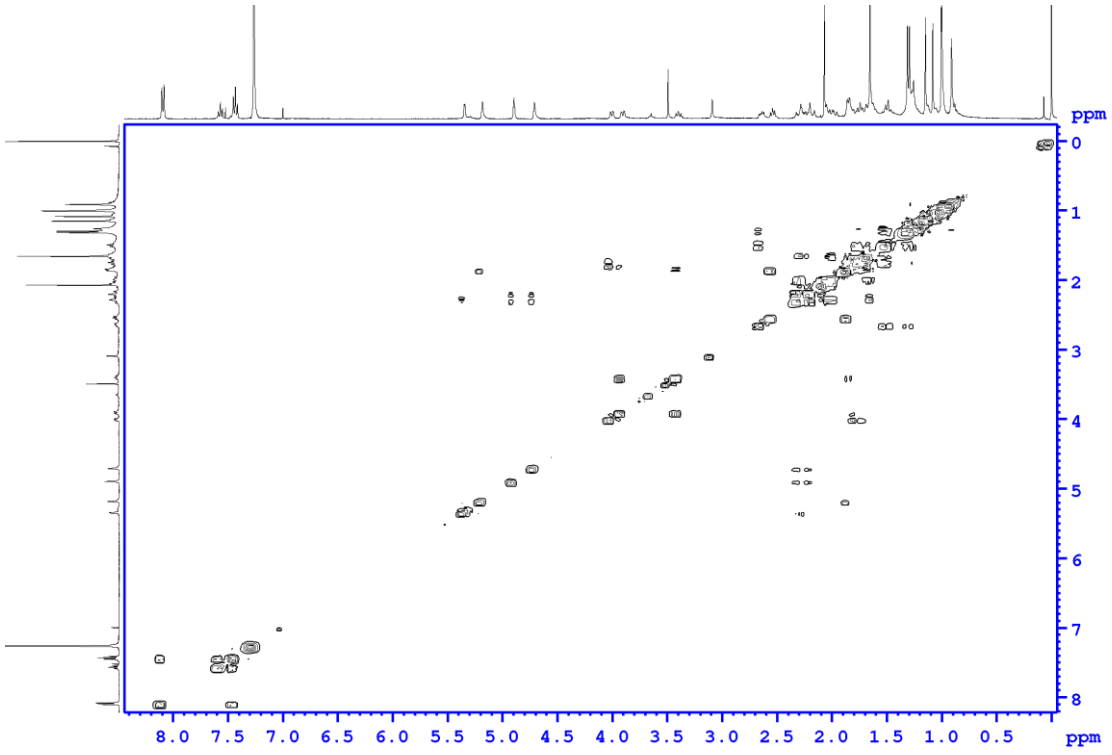


Figure 4.21*:* COSY spectrum of compound **1,** reduced meliavolkenin

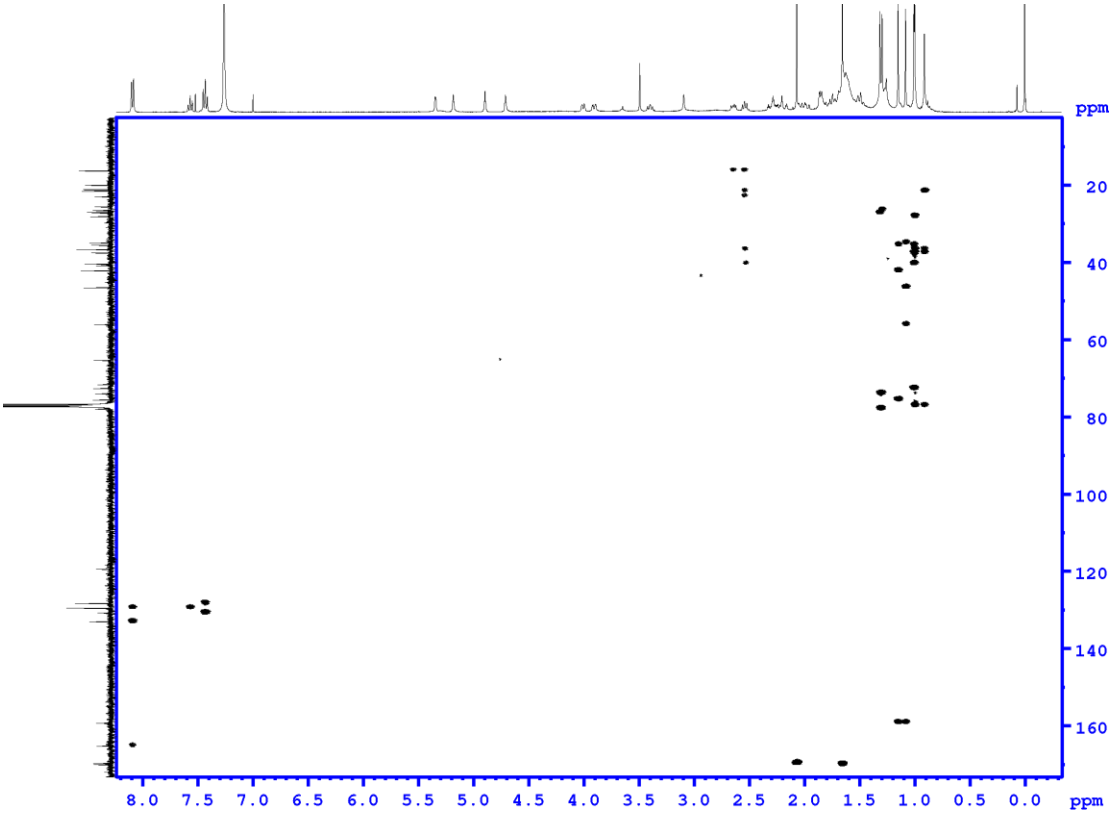


Figure 4.22*:* HMBC spectrum of compound 1, reduced meliavolkenin

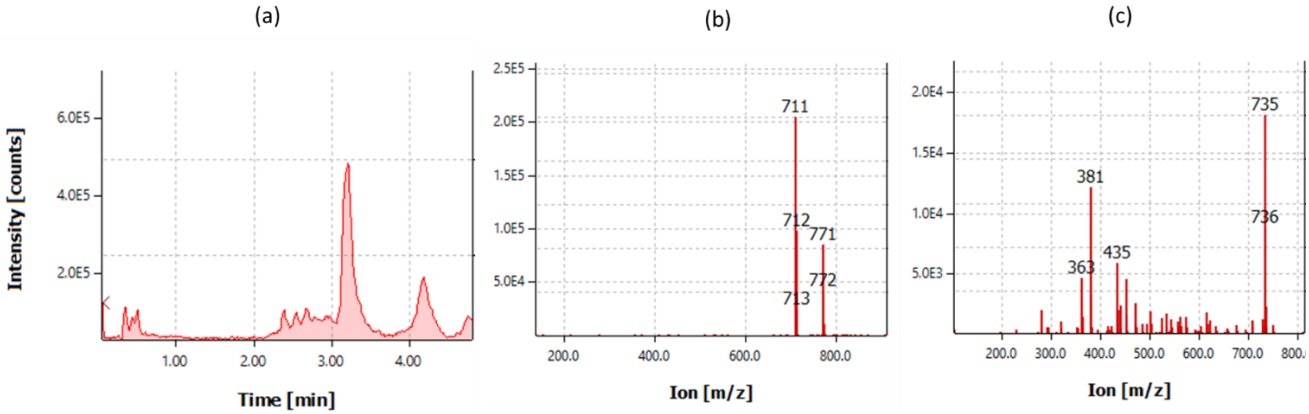


Figure 4.23*:* LC-MS profile of compound **1**, reduced meliavolkenin; (a) chromatogram (b) ESI- MS spectrum (c) ESI+ MS spectrum

b. Compound 2

Compound **2** was isolated as a white powder. The ESI mass spectrum showed an ion peak at *m/z* 573 in negative mode corresponding to $[M - H]^-$. In positive mode, *m/z* 557, *m/z* 497 *m/z* 437 in corresponding to $[M + H - H_2O]^+$, $[M + H - H_2O - CH_3COOH]^+$ and $[M + H - H_2O - 2CH_3COOH]^+$, respectively as presented by the LC-MS profile in figure 4.29. This suggested a molecular weight of 574 for compound **2.** Through comparison of experimental NMR spectral data and those previously reported in literature [311], compound **2** was identified as toosendanin present as a mixture of two epimers. The ^1H NMR and ^{13}C NMR spectra of compound **2** are presented in figures 4.25 and 4.26 respectively. The structure was confirmed by COSY and HMBC correlations presented in figure 4.24; and the COSY and HMBC spectra are shown in figures 4.27 and 4.28 respectively. The characteristic ^1H NMR and ^{13}C NMR data of compound **2** is listed in Table 4.14.

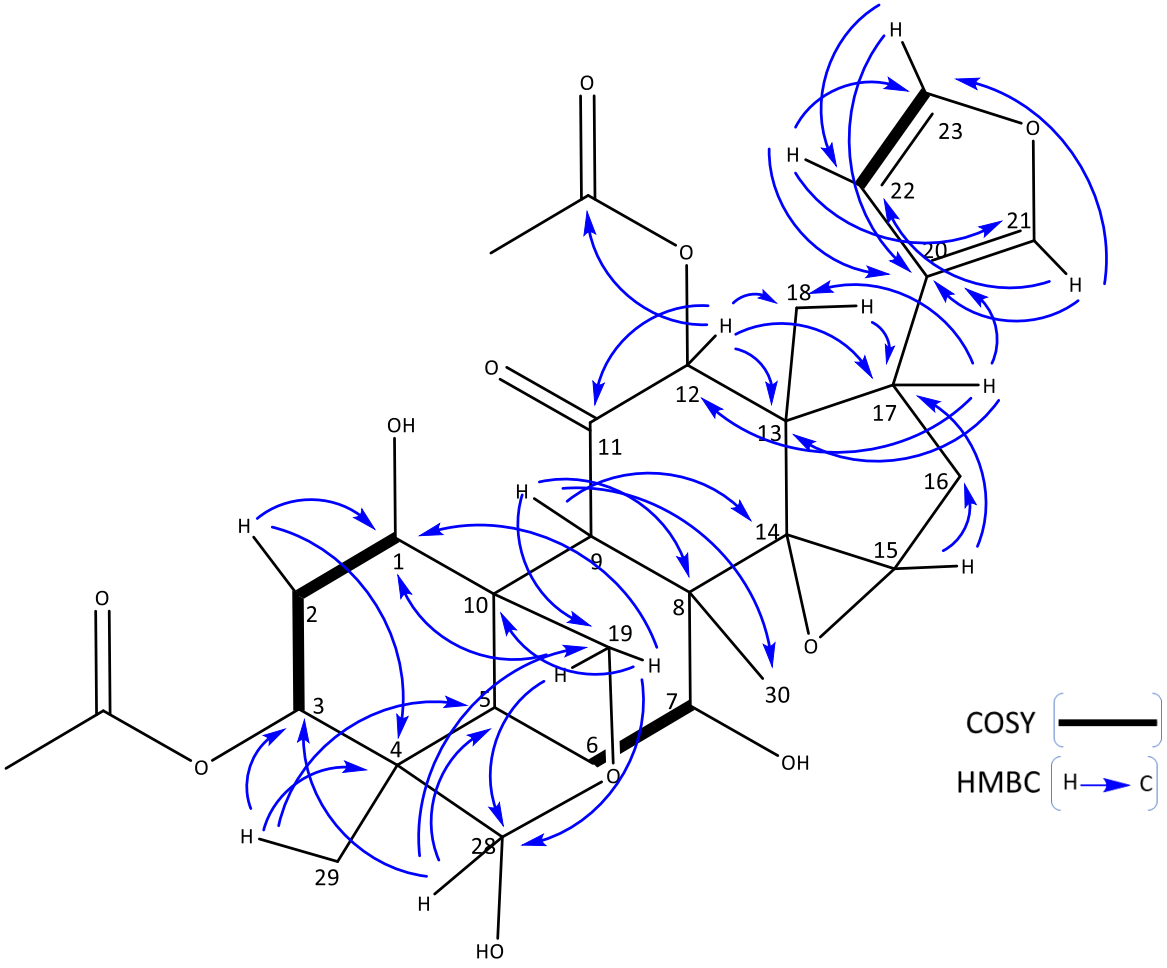


Figure 4.24*:* COSY and HMBC correlations of compound **2,** toosendanin

Table 4.14: Characteristic ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data of compound **2**, toosendanin.

Position	$\delta_{C,}$ type	δ_H (J in Hz)
1	70.34, 70.56 CH	4.27 br s, 4.33 br s
2	35.4, 36.2 CH_2	1.86-1.88 m and 2.85 dt (16.3, 4.7)
		1.81-1.82 m and 2.85 dt (16.3, 4.7)
3	73.5, 76.3 CH	5.36 br d (3.4), 4.88-4.90 m
4	40.0, 40.1 C	-
5	25.5, 27.9 CH	2.50-2.56 m, 2.70 br s
6	25.6, 27.4 CH_2	1.69-1.75 m and 1.91-1.96 m
		1.70-1.77 m and 2.63 dd (13.8, 4.1)
7	70.4, 70.63 CH	3.64 br s, 3.61 br s
8	42.47, 42.52 C	-
9	48.4, 48.7 CH	4.59 s
10	41.78, 41.80 C	-
11	206.8 C	-
12	78.5, 78.7 CH	5.33 s, 5.32 s
13	45.7, 45.8 C	-
14	72.1, 72.3 C	-
15	58.67 CH	3.76, s
16	33.7 CH_2	1.91-1.94 m and
		2.20-2.25 m
17	38.2, 38.3 CH	2.97 br dd (11.0, 6.3)
18	15.7 CH_3	1.32 s, 1.33 s
19	58.71, 63.9 CH_2	4.18 d (12.3) and 4.50 d (12.5)
		4.22-4.33 m
20	122.5, 122.6 C	-
21	140.7, CH	7.13 s
22	111.90, 111.93 CH	6.14 br s
23	142.41, 142.44 CH	7.33 br s
3-AcO	169.9, 170.0 C	-
	21.4, 21.5 CH_3	2.09 s, 2.10 s
12-AcO	170.49, 170.51 C	-
	20.77, 20.79 CH_3	1.99 s
28	96.1, 96.4 CH	4.79 s, 4.88 s
29	18.5 CH_3	0.89 s
30	22.5, 22.8 CH_3	1.15 s, 1.18 s

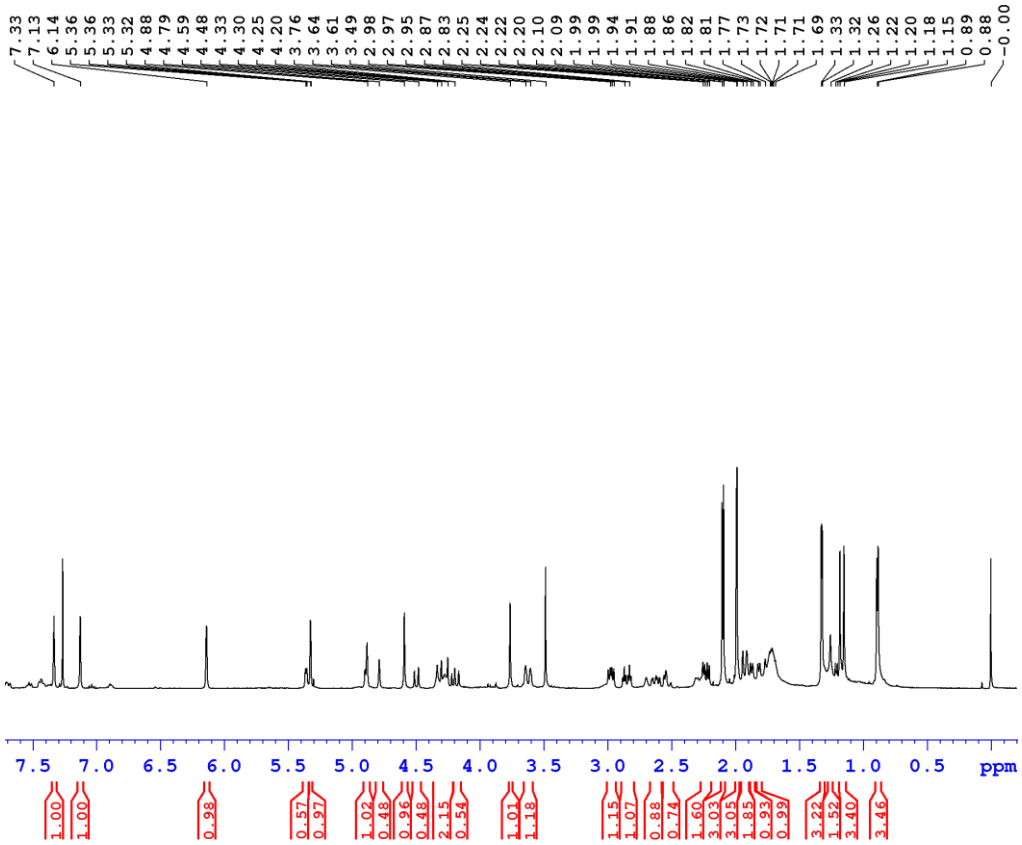


Figure 4.25*:* ^{1}H NMR spectrum of compound **2,** toosendanin

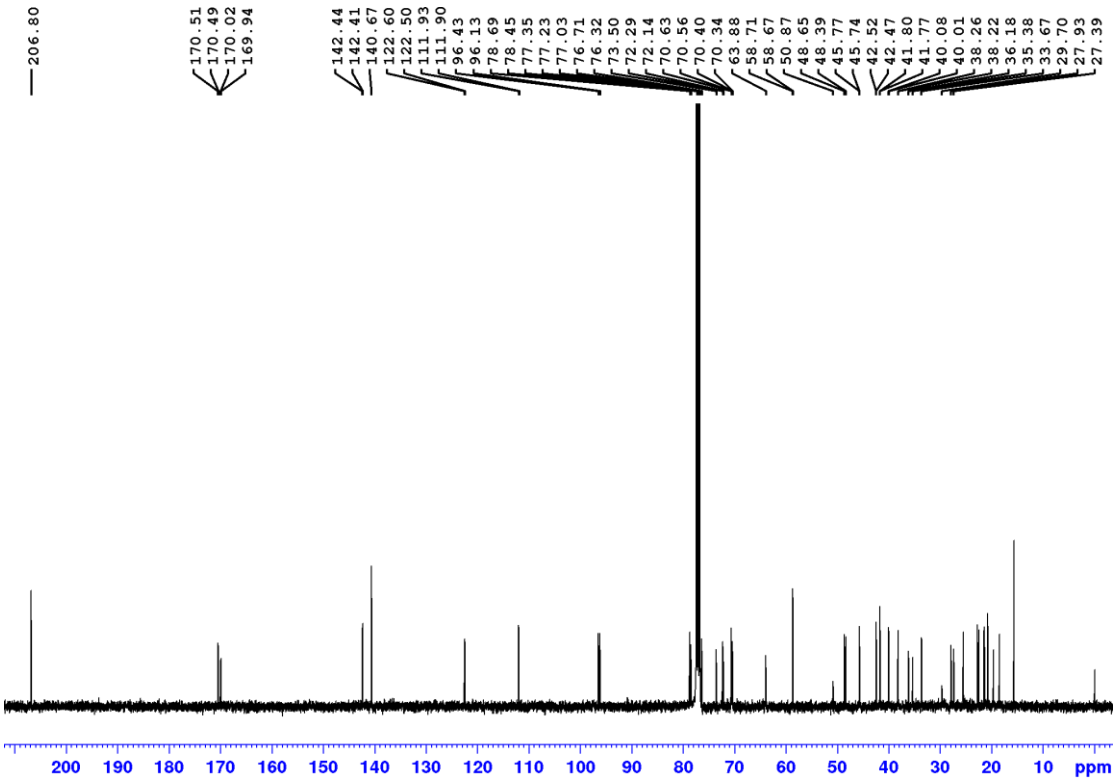


Figure 4.26*:* ^{13}C NMR spectrum of compound **2,** toosendanin

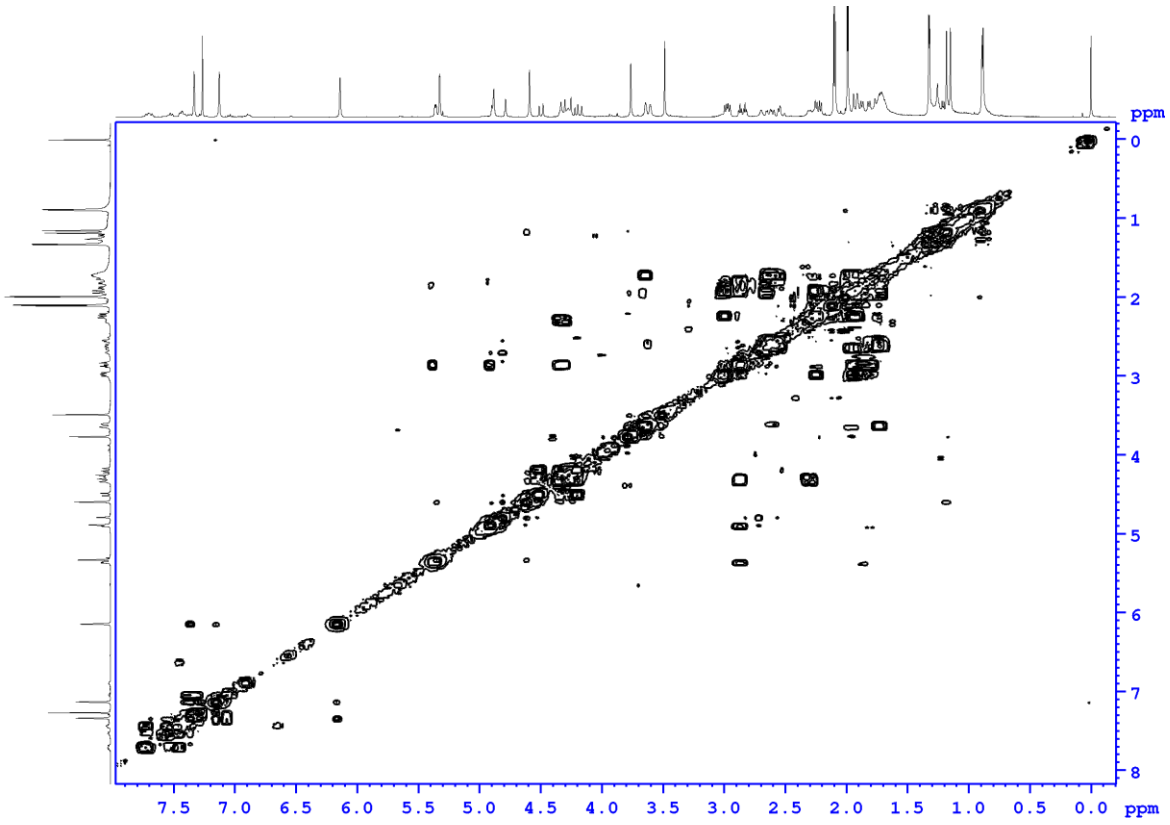


Figure 4.27*:* COSY spectrum of compound **2,** toosendanin

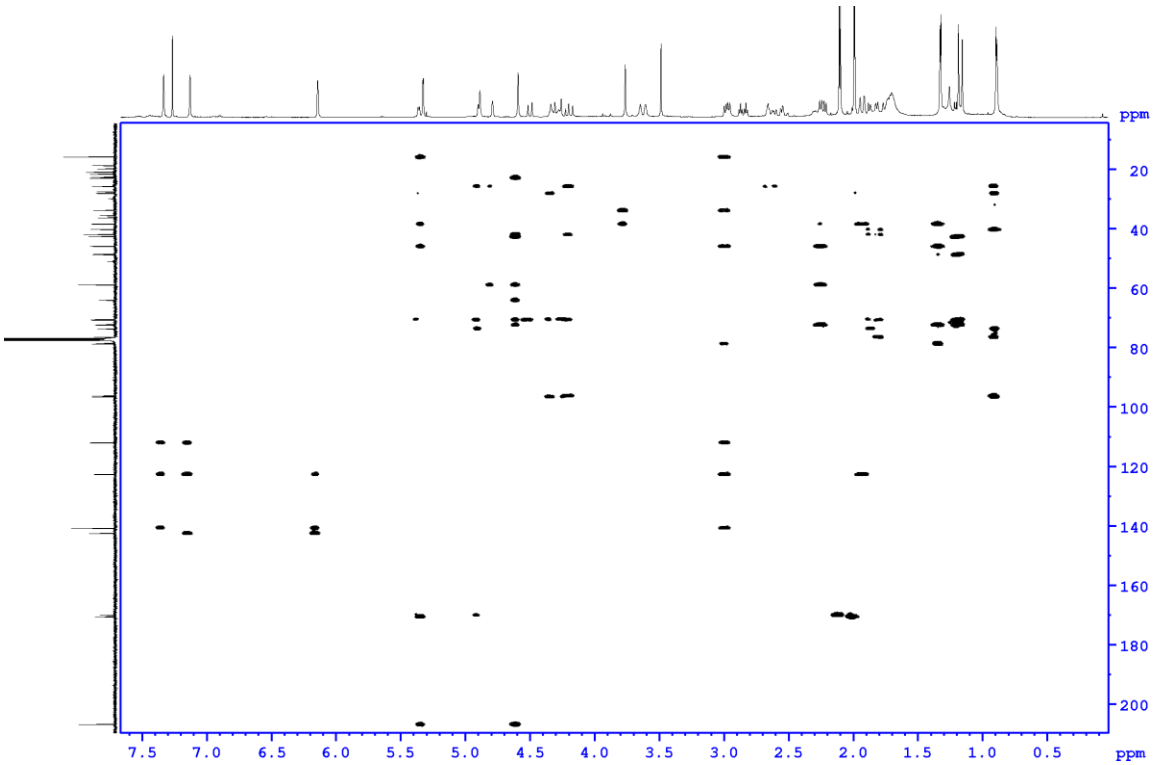


Figure 4.28*:* HMBC spectrum of compound **2,** toosendanin

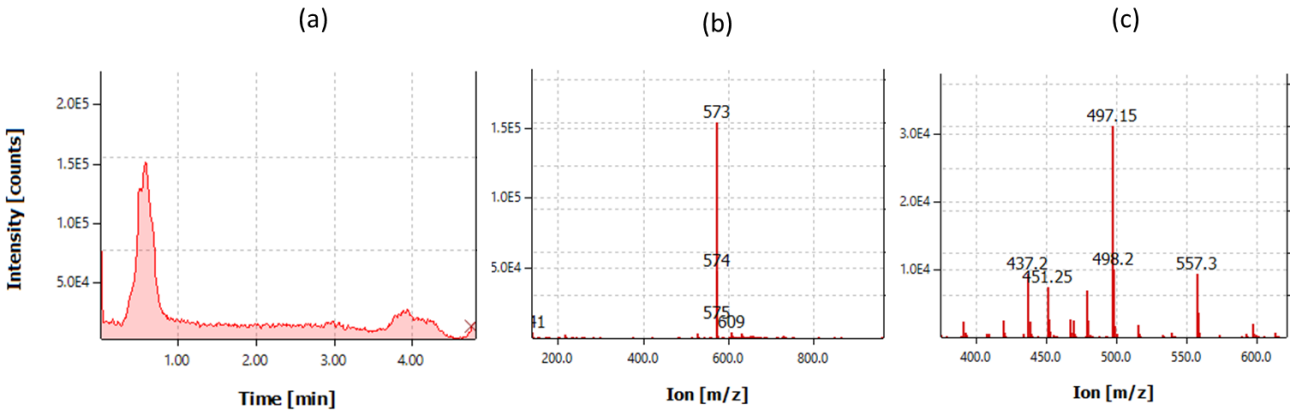


Figure 4.29*:* LC-MS profile of compound **2**, toosendanin: (a) chromatogram (b) ESI- MS spectrum (c) ESI+ MS spectrum

c. Compound 3

Compound **3** was isolated as a white powder. The ESI mass spectrum of compound **3** showed an ion peak at *m/z* 627 corresponding to $[M - H]^-$, *m/z* 629 corresponding to $[M + H]^+$, *m/z* 651 corresponding to $[M + Na]^+$ and *m/z* 646 corresponding to the fragment $[M + NH_4]^+$ as presented by the LC-MS profile in figure 4.35. This suggested a molecular weight of 628 for compound **3**. The NMR spectral data of the compound isolated in the present study match well with those reported earlier [302], and compound **3** was elucidated as salanninolide, as a mixture of two epimers at C-23 (88:12 from the ^{1}H NMR spectrum). The ^{1}H NMR and ^{13}C NMR spectra of compound **3** are shown in figures 4.31 and 4.32 respectively. The chemical structure was also confirmed by COSY and HMBC correlations shown in figure 4.30; the COSY and HMBC spectra are presented in figures 4.33 and 4.34 respectively. The characteristic ^{1}H NMR and ^{13}C NMR data of the major epimer of compound **3** are listed in Table 4.15.

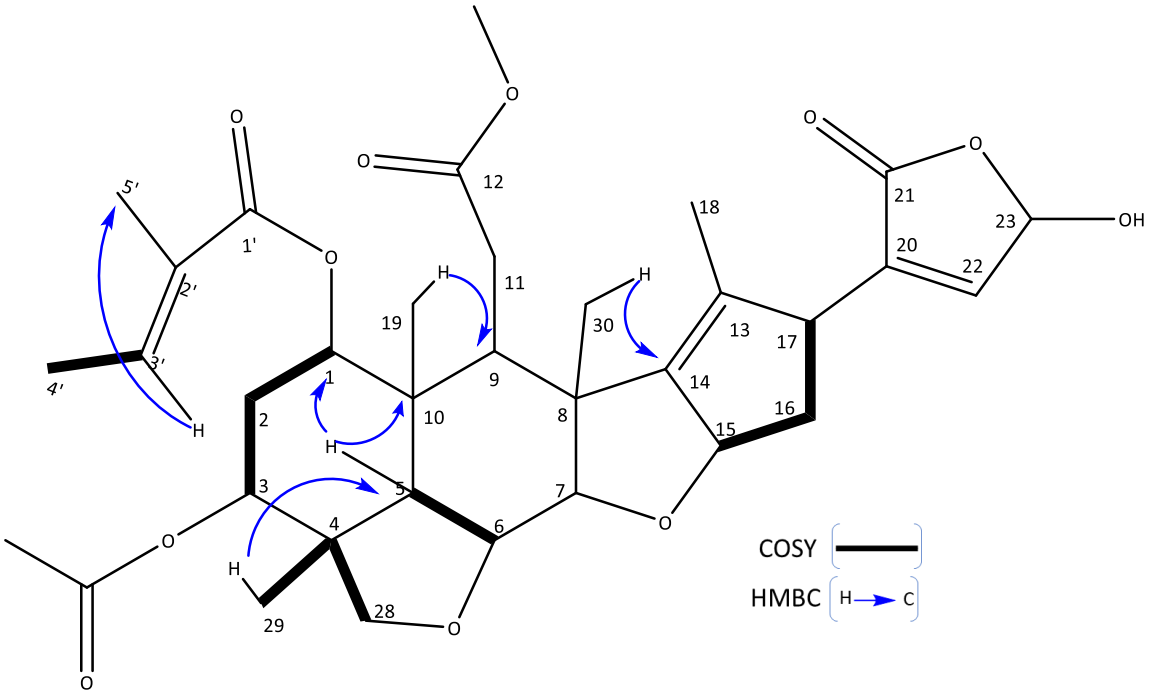


Figure 4.30*:* COSY and HMBC correlations compound **3**, salanninolide

Table 4.15: Characteristic ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data of compound **3**, salanninolide (400/100 MHz, $CDCl_3$).

Position	$\delta_{C,}$ type	δ_H (J in Hz)
1	70.6, CH	4.89, t (2.8)
2	28.2, CH_2	2.1-2.3, m
3	71.2, CH	4.98, t (2.9)
4	42.7, C	-
5	40.3, CH	2.72, d (12.5)
6	72.5, CH	3.97, dd (12.5, 3.4)
7	86.0, CH	4.25, d (3.3)
8	48.3, C	-
9	39.2, CH	2.52, br d (9.7)
10	40.6, C	-
11	30.0, CH_2	2.1-2.4, m
12	174.6, C	-
13	132.8, C	-
14	147.8, C	-
15	87.4, CH	5.40-5.45, m
16	40.3, CH_2	2.1-2.4, m
17	48.8, CH	3.49, br d (6.5)
18	13.3, CH_3	1.83, d (1.6)
19	15.4, CH_3	0.93, s
20	137.4, C	-
21	171.4, C	-
22	141.7, CH	6.75, s
23	96.9, CH	5.96, d (12.5)
23-OH	-	5.26, d (12.6)
28	77.7, CH_2	3.72, d (7.4)
		3.61, d (7.6)
29	19.5, CH_3	1.21, s
30	16.4, CH_3	1.30, s
12-OMe	52.6, CH_3	3.44, s
3-OAc	21.0, CH_3	2.01, s
	170.4, C	
1’	166.5, C	-
2’	129.0, C	-
3’	137.3, CH	6.92-7.00, m
4’	14.4, CH_3	1.84-1.87, m
5’	11.9, CH_3	1.90, s

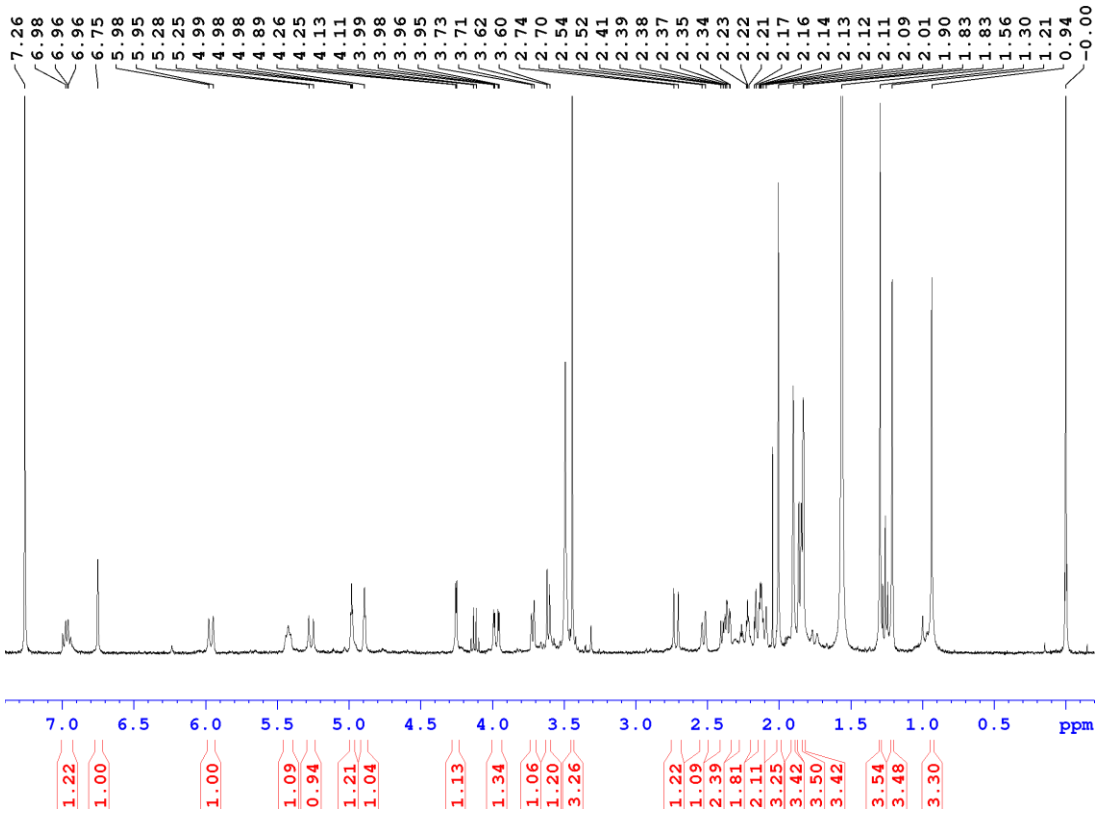


Figure 4.31*:* ^{1}H NMR spectrum of salanninolide

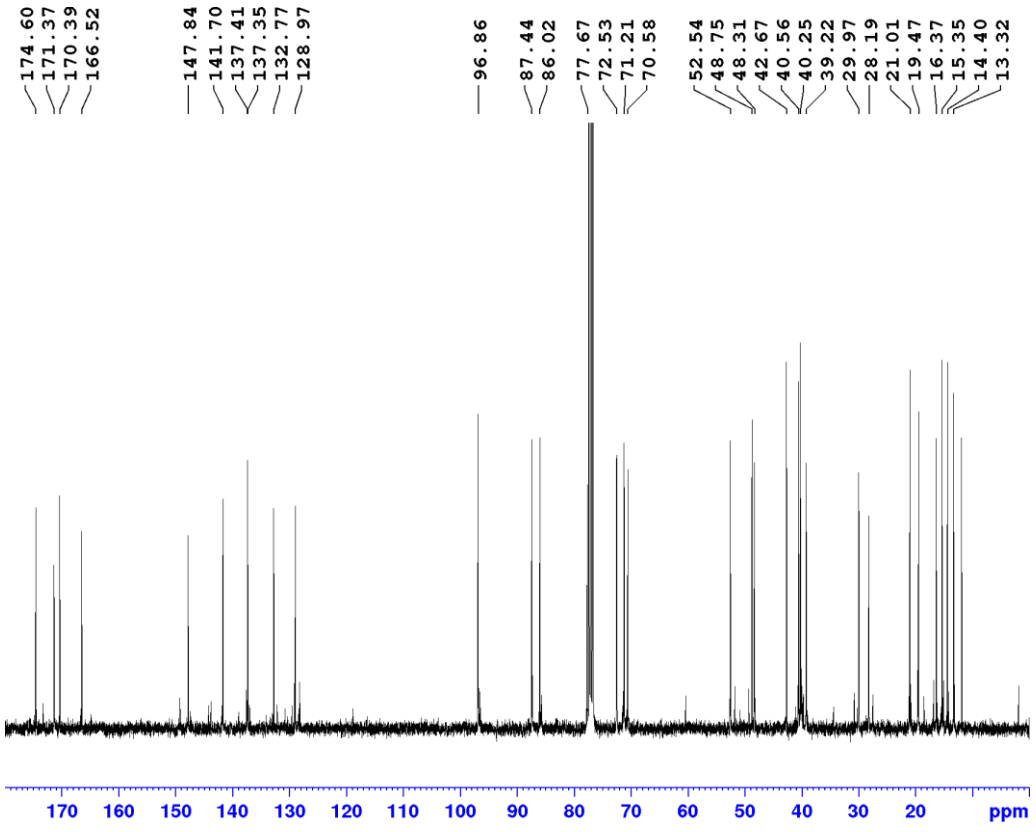


Figure 4.32*:* ^{13}C NMR spectrum of salanninolide

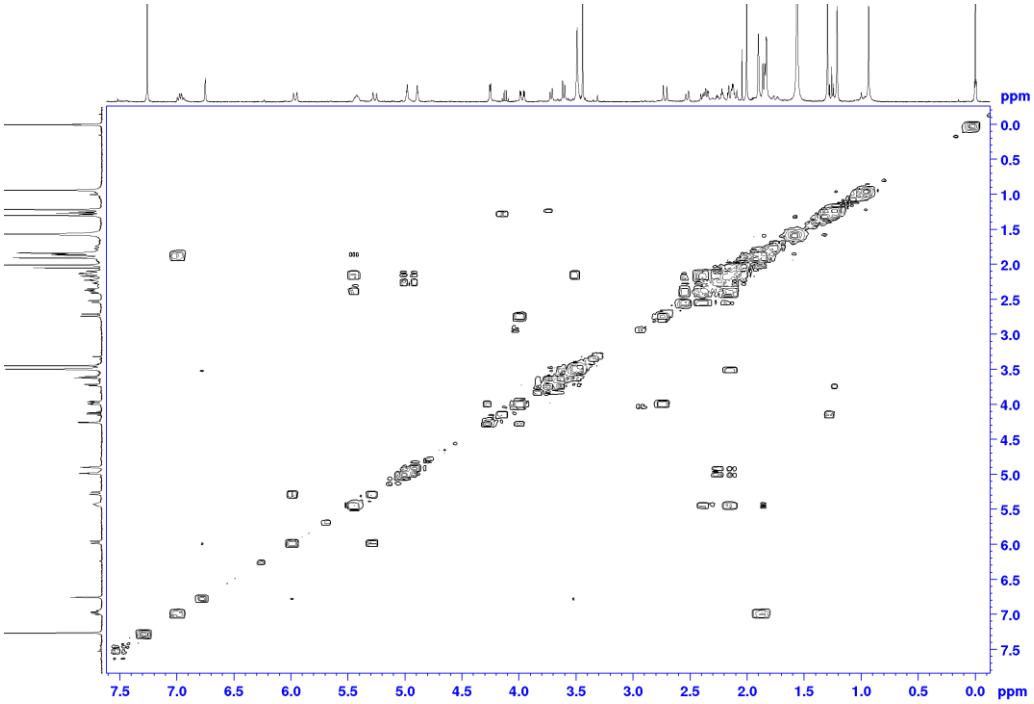


Figure 4.33*:* COSY spectrum of salanninolide

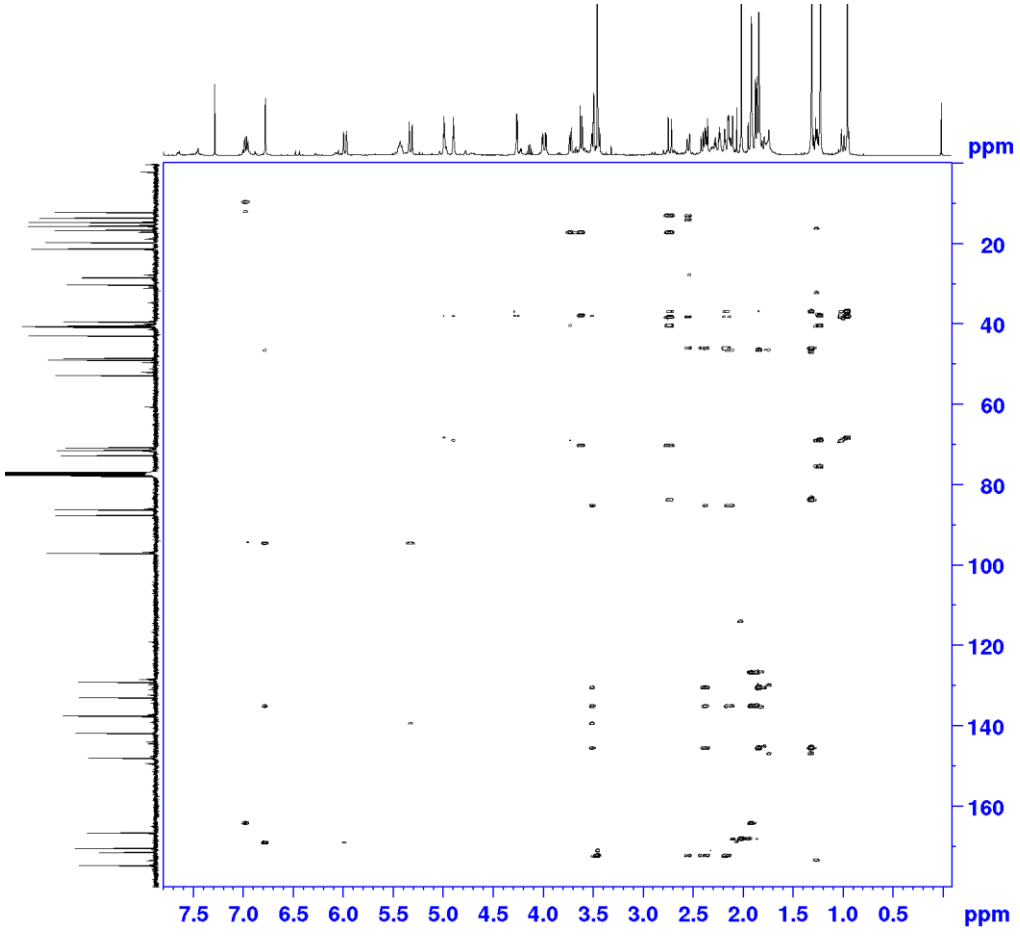


Figure 4.34*:* HMBC spectrum of salanninolide

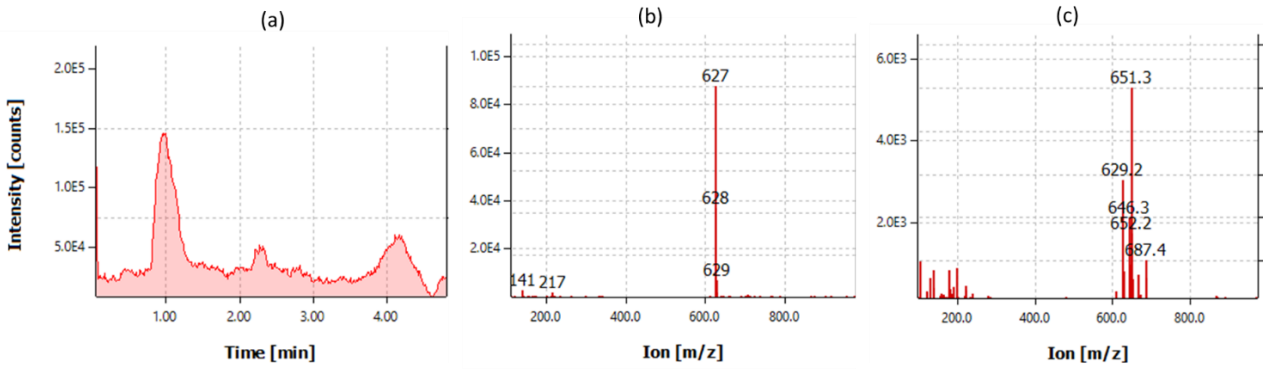


Figure 4.35*:* LC-MS profile of salanninolide: (a) chromatogram (b) ESI- MS spectrum (c) ESI+ MS spectrum

4.3 CONCLUSION

Plants are a rich source of a wide variety of chemical compounds and have been used as major source of indigenous pest control methods. Preliminary screening of *M. volkensii* nuts, bark, pulp and leaves showed varying antifeedant activity against *C. puncticollis, S. frugiperda* and *T. castaneum*. The present study resulted in isolation of three pure compounds, compound **1** characterized as reduced meliavolkenin was isolated from *M. volkensii* nut, compound **2** characterized as toosendanin was also isolated from the nuts while compound **3** characterized as salanninolide was isolated from the pulp in a bioactivity-directed isolation. Biotesting of the pure compounds **2** and **3** showed strong antifeedant activity against *S. frugiperda* and *T. castaneum* while low activity was observed against *C. puncticollis*. The fact that no adverse effects of these compounds on beneficial insects has been reported underscores their low toxicity against non-target organisms. However, the effects of salanninolide and toosendanin on beneficial vertebrates and insects should be studied. Altogether, salanninolide and toosendanin isolated from *M. volkensii* could form potential raw material for formulation of insect control products against *S. frugiperda* and *T. castaneum*.

CHAPTER 5

General discussion and future perspectives

5.0 General discussion

This study demonstrated the biocontrol potential of *M. volkensii* crude extracts and powders against *C. puncticollis, S. frugiperda* and *T. castaneum* in laboratory, greenhouse and simulated storage conditions. In-depth scientific research was conducted to evaluate efficacy of *M. volkensii* extracts leading towards the isolation of three pure compounds. Preliminary screening results showed that methanolic extracts from *M. volkensii* bark, leaf, nut and pulp all have certain degree of antifeedant effects. This effect could be attributed to the presence of phytochemicals in *M. volkensii* that possess antifeedant activity against insect pests. Such phytochemicals are produced by plants for self-defence against attacks by insects [24,312,313]. Previous studies have reported that *M. volkensii* contains insect antifeedant compounds such as salannin, volkensin, 1-acetyltrichilinin which are active against a range of insect pests [58,60,61,68]. Even though all the plant parts showed antifeedant potential, this study rated the nut and pulp extracts superior to the leaf and bark extracts in terms of antifeedant activity against the three insect pests evaluated. This could be an indication that stronger antifeedant phytochemicals are found in the fruit of *M. volkensii* which contains both the pulp and nut. In fact, most of the reported antifeedant compounds have been isolated from the fruit (which contains the nut and pulp). The *M. volkensii* nuts, pulp, bark and leaves used in this study are part of waste generated during seed extraction and timber harvesting of the tree and this makes *M. volkensii* a potential bioresource candidate for insect control with minimal interference of the tree.

Our greenhouse trials showed that *M. volkensii* nuts and pulp offered significant protection to maize and sweet potato against *S. frugiperda* and *C. puncticollis*, respectively. Moreover, the synthetic pesticides used in this study, Hable and Actellic dust, did not prove to be any more efficacious than *M. volkensii* nut extracts. The crude nut extracts offered significant reduction in sweet potato damage caused by *C. puncticollis* in greenhouse with tuber damage of 18% compared to negative control which showed 76% tuber damage. In fact, the efficacy of the nut extracts was higher than the commonly used synthetic pesticide, emamectin benzoate whose application resulted in 33% tuber damage. It is important to note that in

Kenya, where this study was conducted, there is no registered pesticide against *C. puncticollis* hence making this an interesting finding for sweet potato protection.

This study also observed temporary phytotoxicity in sweet potato caused by application of crude nut and pulp extracts. This was characterized by wrinkled leaves, scorching, general chlorosis, recurved leaf edges and twisted growing tips. Several factors could be attributed to the phytotoxicity observed in sweet potato treated with the nut and pulp extracts. The low levels of major plant nutrients (N, P and K) in sweet potato tissues (leaves and vines) could have been due to blockage of cambium, phloem or xylem in the sweet potato roots by the crude extracts thereby minimizing translocation of the nutrients from the soil. This could have led to systemic blockage of uptake of major plant nutrients by the crops thereby affecting their bioavailability in the plant tissues. Another reason for the phytotoxicity could have been due to the burning effect of crude extracts observed through leaf chlorosis and scorching. This could have led to breakdown in photosynthesis resulting in the nutrient deficiency recorded. Perhaps, in future work, application of lower concentrations of crude extracts could be explored to ascertain if the phytotoxicity effect observed was caused by high concentration of the crude extracts. Even though the pure isolated compounds, toosendanin and salanninolide, were not efficacious against *C. puncticollis*, no phytotoxic effects of these compounds on crops have been reported. The temporary phytotoxicity lasted for 7 days implying that affected crops could regenerate without impacting the overall development of cultivated sweet potato. Phytotoxicity caused by spraying plant extracts on crops has also been reported in previous studies [6,219,314].

In further greenhouse trials, *S. frugiperda* larvae damage on maize leaf and whorl was significantly reduced by application of *M. volkensii* nut and pulp crude extracts. This study showed that crude nut and pulp extracts can reduce maize whorl damage by up to 17% and 35%, respectively, while also minimizing leaf damage. Untreated maize, on the other hand, had 96% of their whorls damaged with extensive leaf damage also observed. Extensive leaf and whorl damage induced by *S. frugiperda* can lead to poor plant health, stunted growth or even death of the maize plant and ultimately reduced maize yield at harvest [166,315]. *M. volkensii* nut extracts could potentially offer maize protection by reducing leaf and whorl damage by *S. frugiperda*.

In simulated grain storage protection against *T. castaneum*, *M. volkensii* nut powder had comparable grain protection efficacy as the commonly used Actellic dust, a synthetic pesticide. *M. volkensii* nut powders, admixed with grains showed potential to slow down damage on grains by acting as antifeedant and growth inhibitor against *T. castaneum*. Application of *M. volkensii* nut powder in insect control could not only be cost effective to the farmers, but also a milestone in environmental protection. Other plant powders have also been explored for control of *T. castaneum* infestation in stored products [143,147,149,316,317].

The pulp powder, however, proved to be ineffective in protecting stored grains as it stimulated emergence of *T. castaneum* adults and larvae. Previous studies have reported that *T. castaneum* development is stimulated by protein-rich flour while carbohydrate-rich flour inhibits adult emergence [248–250]. The reason for the stimulating effect of *M. volkensii* pulp powder in this study is still unknown since protein and carbohydrate levels in *M. volkensii* nut and pulp powders were not different. Nevertheless, the findings in this study provide an innovative approach in use of plant-based pesticides for grain protection in Kenya. This study shows extension potential and can be easily adopted by local farmers or small-scale traders because of ease in application, practicability, low cost, availability of raw materials and sustainability.

In-depth studies to identify bioactive constituents of *M. volkensii* were done using bioactivity-guided isolation in tandem with analytical separation techniques including liquid-liquid extraction, column chromatography, preparative-HPLC and preparative-TLC. NMR and LC-MS techniques were used for structure elucidation of the isolated compounds. Fractionation of *M. volkensii* extracts showed that the antifeedant activity was mostly retained in the dichloromethane fraction. Subsequent separation using column chromatography revealed that bioactive fractions were eluted from the column with a hexane/ethyl acetate 50:50 and 25:75 solvent system. This observation, made for both *M. volkensii* nut and pulp, implied that the bioactive compounds from these plant parts could be of moderate polarity. Preparative-TLC and recrystallization of the bioactive fractions resulted in isolation of three pure compounds: compound **1**, characterized as reduced meliavolkenin, was isolated from *M.*

volkensii nut, compound **2**, characterized as toosendanin, was also isolated from the nuts while compound **3**, characterized as salanninolide, was isolated from the pulp.

This is the first report of isolation of reduced meliavolkenin **1** as a natural product directly from *M. volkensii*. Additionally, this is the first report of salanninolide **3** isolation from *M. volkensii*. Even though toosendanin **2** has previously been isolated from *M. volkensii* root bark, this is the first report of its isolation from *M. volkensii* nuts. Toosendanin **2** has previously been found in the bark of *M. toosendan*, *M. azedarach* and *M. volkensii* root bark [2,43–48,52], while salanninolide **3** has been isolated from the seeds of *Melia dubia* and *A. indica* [52–54]. Salanninolide **3** has also been obtained from salannin by photo-oxidation and through bioconversion using fungal strain *Cunninghamella echinulate* [52,55,56,302].

Reduced meliavolkenin **1** did not show activity against any of the insect pests. Toosendanin **2**, on the other hand, showed strong activity against *T. castaneum* and *S. frugiperda* with EC_{50} values of 0.4 mg/mL and 0.3 mg/mL, respectively. Salanninolide **3** also recorded high antifeedant activity against *T. castaneum* and *S. frugiperda* with EC_{50} of 1 mg/mL and 0.2 mg/mL, respectively. Both toosendanin **2** and salanninolide **3** had no activity against *C. puncticollis*. This could indicate the possibility of a synergistic effect of other antifeedant components of crude extracts or semi-refined extracts of *M. volkensii* against *C. puncticollis*, hence the low activity of a single pure compound. It has been reported that complex metabolite mixtures can act synergistically against their targets [58–61].

It has been reported that toosendanin **2** can cause 51.7% mortality against oriental armyworm, *Mythimna separata* Walker after 35 days when applied at 1 mg/ml [43,50]. Toosendanin **2** has also shown antifeedant and toxicity effects against *Pieris rapae* Linnaeus [269]. A 0.5% emulsifiable concentrate of toosendanin **2** has shown high control effect against pests in economically important crops [279]. Salanninolide **3** has been reported to have a higher efficacy than azadirachtin against *S. littoralis, S. gregaria* and *L. migratoria* [52,57]. Salanninolide **3** has shown stronger antifeedant activity than azadirachtin, salannin, isosalanninolide, nimbin, nimbinolide and isonimbolide against *S. frugiperda* [309].

While this study did not investigate the possible mode of action of the isolated compounds in the insects, it is reported that most botanical pesticides significantly interfere with the digestive system causing insect malnutrition, growth inhibition and even death [292].

Toosendanin **2** is a digestive agent that adversely affects the digestive system targeting the microvilli of midgut cells causing destruction of midgut epithelial cells leading to regurgitation, paralysis and death of insects [292]. Steroid-containing compounds in plant extracts also inhibit proteins by blocking sterol carrier proteins, causing insects to grow abnormally [195].

Plant-based pesticides, which have traditionally been used in insect pest control, have emerged as safer alternative to synthetic pesticides because they are ecologically sound and have low mammalian toxicity. In fact, the first insecticides used by humans were derived from plants and their biological activities were known from the earliest recorded times [156]. Incorporating plant-based pesticides into integrated pest management programs reduces unnecessary usage of synthetic pesticides. Moreover, plant-based products also offer multiple modes of action including antifeedant activity, growth regulation, oviposition inhibition, repellency and toxicity thereby minimizing chances of pest resistance emergence. Additionally, in the context of agricultural pest management, botanical pesticides are best suited for organic food production, and consumers are willing to pay premium for organically cultivated food crops [7], [33]. There is, however, little awareness among smallholder farmers about the usefulness of botanicals in crop protection [1].

The fact that crude extracts from *M. volkensii* showed antifeedant activity indicates that they could be incorporated in an integrated pest management together with other control methods against *C. puncticollis*, *S. frugiperda* and *T. castaneum*. With more than 80% of Kenya being arid and semi-arid, *M. volkensii* could be a promising candidate not only to mitigate against desertification of arid and semi-arid lands but also could provide a valuable resource for insect control products. The tree could also be incorporated in semi-arid agroforestry systems as it is a browse for goats and cattle [54]. The application and use of *M. volkensii* is on the rise in Kenya and there is therefore need for more in-depth scientific studies on the plant to exhaust its potential value and further evaluate its insect control potential [55]. *M. volkensii* preparations could be utilized to avoid over-reliance on synthetic pesticides which have been reported to have detrimental consequences [13].

This study also provides new leads for development of insect control compounds and shows that *M. volkensii* extracts could be incorporated in integrated pest management. The pure bioactive compounds isolated in this study, salanninolide and toosendanin, could constitute

potential active ingredients for the formulation of insect control products against *S. frugiperda* and *T. castaneum.* During seed extraction, the fruits of *M. volkensii* are pulped and the nut that houses the seed is also removed [50]. This produces a large amount of waste whose economic value is unknown. These waste materials could be a useful feedstock for the formulation of botanical pesticides, as they have shown potential for insect control. Overall, *M. volkensii* presents a high potential pesticidal plant whose nuts and pulp could be incorporated in integrated pest management, especially considering the interest in botanical pesticides with non-toxic mechanisms against insect pests. The availability of renewable resources from the tree, such as fruits, stem bark, and leaves makes this plant a potential candidate for insect control with minimal interference on the plant. This fits into a circular economy where organic waste provides a useful product that could be combined with other pest control methods in an integrated pest management system. In this regard, *M. volkensii* could be further exploited as a source of natural insecticides.

5.1 Future perspectives

This study provides a basis for formulation of locally produced botanical insecticide using *M. volkensii* as feedstock. The various plant parts could be pounded or ground either when fresh or dry and crude formulations made could be sprayed on crops. Even though this study used methanol for extraction, aqueous extracts could be used by smallholder farmers. Addition of small quantities of vegetable oil or soap during aqueous extraction to improve extraction of less polar constituents and for better adherence to the plants foliage during application could be attempted [294]. This straightforward preparation process could be optimized to improve efficacy of the botanical pesticide prepared from *M. volkensii*. Even though pesticide registration does not normally allow registration of crude formulations, such preparations could be useful for non-commercial smallholder farming in Africa. While botanical pesticides could be relatively safer compared to synthetic pesticides, it is important for farmers to observe basic safety measures when preparing the formulations to avoid exposure during processing.

It is also important to note that even though *M. volkensii* powders directly admixed with the grains provided strong grain protection in this study, toxicity studies on the effect of the powders need to be undertaken to guarantee safety. Pesticide residue analysis of *M. volkensii*

extracts, salanninolide and toosendanin on sprayed crops could also be done in future to assure safety. Toxicological assessments would therefore be an interesting perspective for future research.

The environmental safety assessment of *M. volkensii* extracts and bioactive constituents has not been done in this study. It is however important to conduct further investigation on the effects of *M. volkensii* extracts, salanninolide and toosendanin on beneficial insects and non-target organisms, particularly pollinators, aquatic organisms, birds and other natural enemies. This is especially important because data on locally prepared botanical pesticides is largely unavailable in literature. From previous trials, commercial neem extract has been reported to be harmful to bees and wild pollinators while pure azadirachtin is classified as moderately toxic to bees and pesticides in this category is not recommended for application on blooming plants [22].

The reduced meliavolkenin isolated in this study did not show activity against *C. puncticollis*, *S. frugiperda* or *T. castaneum* and its possible bioactivity is still unknown. Cytotoxic activity of the reduced meliavolkenin could be investigated in future work since meliavolkenin is reported to have moderate cytotoxicity against human breast, colon and lung cancer cells [82].

Attempts to explore the biosynthesis pathways of the isolated compounds, toosendanin and salanninolide, from *M. volkensii* could an interesting perspective for further work. This would provide better understanding and help in attempts to metabolically engineer the bioactive compounds (toosendanin and salanninolide) more easily in economically important crops.

To ensure sustainability of *M. volkensii* as potential botanical pesticide, the tree must be grown to ensure adequate supply of leaves, nuts, bark and pulp. This would lead to afforestation of the arid and semi-arid lands where *M. volkensii* is a native plant, a much welcome boost to environmentalists. Investment in propagation materials to ensure planting of the tree in large quantities should be considered going forward.

While this study focused on *M. volkensii* nuts and pulp, the perspective of further research could focus on leaf and bark. These plant parts also showed certain extent of antifeedant

activity against *C. puncticollis, S. frugiperda* and *T. castaneum.* Further studies could be conducted to explore the bioactive principles in the leaves and the bark.

Cost-benefit analysis for locally prepared formulation could be performed to give an indication of economic viability taking into considerations the cost of production. The analysis should consider all the plant parts investigated in this study including leaves, bark, pulp and nut of *M. volkensii*. Previous economic analysis has reported that the cost of local formulations is lower than synthetic pesticides [29,323]. This makes local preparation an interesting resource for resource-limited growers in Africa.

A next step would also be to take this work out of the laboratory and greenhouse into the field for efficacy trials. The effect of topical application of *M. volkensii* extracts could also be exploited to ascertain their effects on insect pests. In addition, it would be important assess phytotoxicity of crude extracts on sweet potatoes at concentrations lower than 20 mg/mL. While this study reported temporary phytotoxicity of crude nut and pulp extracts in sweet potato, it would be necessary to assess the phytotoxic effect at lower concentrations. Investigating stimulative effects of pulp powders on *T. castaneum* would be an interesting area of further study. This study reported higher adult emergence of *T. castaneum*. The reason for this stimulative effect was not clear and this would form part of future perspectives. Finally, formulation of a botanical pesticide using the isolated compounds, toosendanin and salanninolide, could be prepared for further assessment and registration by pesticide regulatory authority in Kenya.

CHAPTER 6

References

6.0 REFERENCES

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Publications

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2. Jaoko Victor, Nji Tizi Clauvis Taning, Simon Backx, Pierfrancesco Motti, Jackson Mulatya, Jan Vandenabeele, Titus Magomere, Florence Olubayo, Sven Mangelinckx, Stefaan Werbrouck, and Guy Smagghe, "Laboratory and Greenhouse Evaluation of *Melia volkensii* Extracts for Potency against African Sweet Potato Weevil (*Cylas puncticollis*) and Fall armyworms (*Spodoptera frugiperda*)," *Agronomony*, vol. 11, no. 1994, pp. 1–9, 2021.

Participation in conferences and workshops

1. Jaoko Victor, Nji Tizi Clauvis Taning, Simon Backx, Jackson Mulatya, Jan Vandenabeele, Titus Magomere, Florence Olubayo, Sven Mangelinckx, Stefaan Werbrouck, and Guy Smagghe. "Laboratory and field evaluation of *Melia volkensii* crude extracts as biopesticide against African sweet potato weevils (*Cylas puncticollis*) and armyworms, *Spodoptera* spp.," in International Symposium on Crop Protection, 72nd, Abstracts, online (Ghent, Belgium), 18th May 2021.
2. Jaoko Victor, Nji Tizi Clauvis Taning, Simon Backx, Pierfrancesco Motti, Jackson Mulatya, Jan Vandenabeele, Titus Magomere, Florence Olubayo, Sven Mangelinckx, Stefaan Werbrouck, and Guy Smagghe. "Bioactivity-guided isolation of salanninolide and toosendanin from *Melia volkensii* and their antifeedant activity against *Spodoptera frugiperda* and *Tribolium castaneum*," in International Molecular Biology and Biotechnology Congress, 10th, Abstracts, online (Turkey), 4th – 8th October 2021.

3. Jaoko Victor, Nji Tizi Clauvis Taning, Simon Backx, Jackson Mulatya, Jan Vandenabeele, Titus Magomere, Florence Olubayo, Sven Mangelinckx, Stefaan Werbrouck, and Guy Smagghe. “Characterization of the bioactivity of pesticidal extracts of *Melia volkensii* against African crop insect pests,” in Chemical Research in Flanders, 2nd Symposium, Abstracts, Blankenberge, Belgium, 14th – 16th October 2019.

4. Jaoko Victor, Nji Tizi Clauvis Taning, Simon Backx, Jackson Mulatya, Jan Vandenabeele, Titus Magomere, Florence Olubayo, Sven Mangelinckx, Stefaan Werbrouck, and Guy Smagghe. “Evaluating the efficacy of *Melia volkensii* extracts as a potential botanical pesticide against African crop insect pests of economic importance” in 24th National symposium for Applied Biological Sciences, (Ghent, Belgium) 4th February 2019.

5. Jaoko Victor, Nji Tizi Clauvis Taning, Simon Backx, Jackson Mulatya, Jan Vandenabeele, Titus Magomere, Florence Olubayo, Sven Mangelinckx, Stefaan Werbrouck, and Guy Smagghe. “Evaluation of *Melia volkensii* as potential biopesticide against the African sweet potato weevil, *Cylas puncticollis*” in 14th IUPAC International Congress of Crop Protection Chemistry, (Ghent, Belgium), 19th – 24th May 2019.

6. Jaoko Victor, Nji Tizi Clauvis Taning, Simon Backx, Jackson Mulatya, Jan Vandenabeele, Titus Magomere, Florence Olubayo, Sven Mangelinckx, Stefaan Werbrouck, and Guy Smagghe. “Efficacy of *Melia volkensii* as a natural biopesticide against the African sweet potato weevils and armyworms” in AGRO 2019 Conference & Exhibition, (Nairobi, Kenya), 22nd – 24th October 2019.