A TOXICOLOGICAL STUDY OF *MELIA VOLKENSII* (GURKE) EXTRACTS ON *LOCUSTA MIGRATORIA MIGRATORIODES* (R & F)

By
JACQUES M. KABARU

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DECLARATION

This is my original work and has not been presented for a degree in any other university.

JACQUES M. KABARU

Signature....

Date. 4th March 1996

This thesis has been submitted for examination with our approval as University supervisors.

PROF. RICHARD W. MWANGI

Signature.....

Date. 4th March 1996

PROF. ELIUD N. WAINDI

Signature.....

Date....18/3/96...

DEDICATION

This Thesis is dedicated to my family. First to my late father Edward Muwangi who valued the search for knowledge above most things, my mother and best friend Nduta who did not see much of me during the study, my son Eddy Malcom for coping with the loneliness when I worked long hours in the laboratory, my sisters, brothers and grandmother Wambui for their love.

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LIST OF ABBREVIATIONS AND SYMBOLS

Anova	Analysis of variance
$CaCl_2$	Calcium chloride
cm	Centimetre
ED_{50}	Dose effective to 50% of the animals tested
d.f.	Degrees of freedom
g	Gram
i.e	Id est
KCl	Potassium chloride
kg	Kilogram
L	Litre
LC_{50}	Concentration lethal to 50% of the animals tested
LD_{50}	Dose lethal to 50% of the animals tested
Log	Logarithm
M	Metre
mg	Milligram
$MgCl_26H_2O$	Magnesium chloride
Min	Minute
ml	Millilitre
mm	Millimetre
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
$NaH_2PO_42H_2O$	Sodium dihydrogen phosphate
NB	Nota bene
nm	Nanometre
No	Number
ppm	Parts per million
R&F	Reiche and Fairmaire
®	Registered trade mark
Sq	Squared
TLC	Thin layer chromatography
µl	Microlitre
µg	Microgram
V	Volume
V/V	Volume to volume

°C	Degrees celsius
%	Percent
>	Greater than
<	Less than

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ABSTRACT

In a study designed to investigate the insecticidal potential of *Melia volkensii* (Gurke), various tests for biological activity in different alcoholic extracts were carried out on *Locusta migratoria migratoriodes*(R&F). Attempts were also made to improve extraction methods. Several extracts of *M. volkensii* were tested for antifeedant and toxic effects on *L. migratoria* nymphs and adults and their mechanism of action tentatively established. The presence of active substances was routinely monitored using the *Aedes aegypti* 2nd instar larvae bioassay.

Various concentrations of ethanol and methanol were tested for their efficiency in extraction of biologically active substances from *M. volkensii* fruit. The most efficient were found to be 60% ethanol and 80% methanol. A comparative study of fruits from Embu and Tsavo areas of Kenya revealed no difference in the content of insecticidal substances.

Various parts of *M. volkensii* were screened for biological activity. In the *A. aegypti* 2nd instar larvae bioassay, seed coat and root bark extracts had LC_{50}'s of 100 and 120 ppm respectively while stem wood had an LC_{50} of 1700 ppm in 48 hours. In locust antifeedant tests, root wood, root bark, endocarp, fruit coat and testa extracts showed high activities while stem wood, stem bark and leaves showed low activities. In fruits, more than 90% of the active material was recovered from the fruit coat and endocarp. Ripe and unripe fruits had equal quantities of active material.

In storage stability tests, the extracts and *M. volkensii* fruit powder were found to be highly stable, with extracts stored for two years at room temperature retaining insecticidal activity.

A procedure developed in this investigation demostrated that biologically active material was recoverable by precipitation from concentrated 80% methanol extract solutions by cooling to 0°C.

Antifeedant effects were induced in locusts through topical treatment, injection or food treatment. A drastic reduction in food intake resulted in locusts treated directly on the labial and maxillary palps. This effect was found irreversible with time. The rate of peristalsis of the alimentary canal in locusts was however, not affected by the extracts.

Acute toxicity tests on locusts revealed high contact poison activity in one *M. volkensii* fruit fraction named B. This activity was enhanced by addition of olive oil to the extract solution. All crude and purified fractions showed stomach

poison activity on locusts when administered as bait at concentrations above 0.1%. This was not enhanced by purification of the crude extracts. Locusts injected with doses above 100µg of extract per gram of body weight went into paralysis in a few hours. In this state, peristalsis of the alimentary canal, malpighian tubule activity and heart pulsations were not impaired. A state of stupor could also be induced in locusts in 24 hours through topical treatment with doses above 1000µg/g of body weight.

The onset of paralysis in locusts treated with *M. volkensii* fruit extracts was enhanced by high post-treatment temperatures. A post-treatment temperature rise from 15 to 40 °C increased the toxicity of the extracts on locusts by a factor of ten.

Compared to azadirachtin, *M. volkensii* fruit extracts revealed low growth inhibitory effects on *L. migratoria* 5th instar nymphs.

The combined insecticidal and physical properties of *M. volkensii* extracts, i.e. relative antifeedant, stomach poison, contact poison, good thermostability, long shelf-life and positive temperature coefficient of paralytic action would make the tree an attractive source of botanical locusticide for large-scale application especially in the tropics.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The environment in the tropics is conducive to proliferation of insects, many of which cause serious damage to crops in the field and in storage. In addition, others adversely affect the health of man and his livestock. Man in tropical environments has thus been confronted with the need to protect himself, his livestock, agricultural crops and stored produce from pests. Failure to do so has caused disease, food shortages and famines. To survive in such a challenging environment, control of pests particularly insects, has been a prime necessity.

Insect pests have mainly been controlled with synthetic insecticides in the last fifty years. The advent of dichlorodiphenyltrichloroethane (DDT) in the 1940's brought in a whole array of broad-spectrum synthetic insecticides in quick succession. Eradication of insect pests forever appeared a distinct possibility within a matter of time. However, the problems of pesticide resistance, pest resurgence and detrimental effects on non-target organisms including man soon became apparent (Rembold, 1984).

Most insecticidal compounds fall within four main classes, the organochlorines, organophosphates, the carbamates and pyrethroids. Out of these the major classes in use today are organophosphates and carbamates. These combine high insecticidal activity with low persitence when compared to organochlorines (Dorow, 1993). Further, restrictions on the organochlorine insecticides has resulted in a decline in their use in developed countries where the economic situation can accomodate the use of alternatives.

The problems associated with the use of environmentally hazardous insecticides are amplified when they have to be used to control pests like locusts. The areas to be sprayed are very large and the ecosystems fragile, and therefore any negative impact on the environment would be enormous. To date locust control methods mainly entail spraying with organochlorines like heptachlor, organophosphates like fenitrothion, chlorpyriphos, malathion and dimethoate, synthetic pyrethroids like deltamethrin and lambdacyhalothrin and carbamates like bendiocarb (Dorow, 1993). There has been a need to develop compounds with less bioaccumulation potential that are more degradable and applied at lower dose rates than the earlier generation of insecticides. This is exemplified by pyrethroids which have been shown to have limited impact on the aquatic and terrestrial environment, their ready degradation and to the use of very low dose rates (Yamamoto, 1970). One of the main areas from which there appears to

be potential of developing environmentally safe insecticides especially for use in locust control is botanicals.

The list of plants reported to possess some form of insecticidal activity is long. Most of the reports are purely speculative since no comprehensive data is available on such activities. It has however been proven that access to ethnobotanical information increases the chances of finding plants with biologically active substances (Balick, 1990). There would be no need to suppose that the pool of plants with potential as economic sources of insecticides has been exhausted considering that even the taxonomic identification of plants especially in the tropical forests is still incomplete. New species are still being described. Of the 250,000 or so species of higher plants known to exist on earth, only a relative handful have been thoroughly studied for all aspects of their biological activity, yet the plant kingdom has yielded 25% or more of the drugs used as medicine today (Farnsworth, 1984,1988). New sources of commercially valuable industrial and medicinal materials therefore remain to be discovered from the plant kingdom (Balandrin *et al*., 1985).

Despite the difficulties occurring in the development of botanical insecticides such as complex modes of action, doubtful availability of commercially viable bulk extraction materials and problems in registration of active crude mixtures, the lack of lead structures for synthetic pesticide research and the growing acceptance of "imperfect" pesticides as a result of the withdrawal of existing synthetic products in certain markets will no doubt generate more interest in insecticides from plants (Naumann, 1993). Biologically active compounds derived from selected plant species have been commonly used in the past to control pests in many tropical countries. The earliest insecticides i.e., nicotine, pyrethrum and rotenone were derived from plants. Indeed the chemical pesticide approach had its beginning in the use of botanical pest control materials (Saxena, 1987).

Insect damage to plants results from feeding or from the transmission of pathogens during feeding. Chemicals which reduce pest injury by rendering plants unattractive or unpalatable can be considered as potential insecticides. Due to their biodegradable nature and relative safety to non-target organisms, research on the biological activity and chemistry of antifeedants has been emphasized.

The potential of the *Meliaceae* trees as a source of natural insecticides has been studied for more than two decades. A wide range of biological activities of *Meliaceae* extracts on insects have been reported. These include, antifeedant, antiovipositant, repellent, growth-regulating activity (Schmutterer, 1995). The research efforts on the *Meliaceae* in the past have mainly concentrated on two species- *Azadirachta indica* and *Melia azedarach* that are native to Asia (Schmutterer, 1995). A plethora of

compounds have been isolated and characterised from these trees and their insecticidal activity on several insects investigated (Schmutterer, 1995). The main active compound appears to be azadirachtin (Butterworth and Morgan, 1971).

The development of commercially viable botanical insecticides from *A. indica* has progressed considerably in the recent years culminating in such products as "Margosan-o®" (Larson, 1987) and registration of azadirachtin-containing *A. indica* extracts in several countries (Naumann, 1993).

Recently, other *Meliaceae* trees have attracted interest as potential sources of botanical insecticides. These include the *Melia* genus whose history and taxonomy was first described by Mabberley in 1984. There are two species that are indigenous to Africa- *M. volkensii* and *M. bombolo* (Dale and Greenway, 1961). *Melia volkensii* grows mainly in Kenya, Somalia and Tanzania. In Kenya the tree grows in Tsavo, Kitui and Embu areas. During past locust invasions in Eastern Africa, local people observed that *M. volkensii* plants were not consumed by swarms. The insects also did not use the tree for perching. This led to the traditional practice of *M. volkensii* infusions being used to control ticks and fleas in households where human beings and animals shared shelter (Local ethnobotanical sources). This insect repellent or antifeedant activity was however not properly documented until Mwangi (1982) reported locust antifeedant and growth inhibiting activity in methanolic extracts of *M. volkensii* fruit.

The little data that is available on the insecticidal activity of *M. volkensii* fruit extracts is encouraging. Since Mwangi (1982) reported insect antifeedant and growth-inhibition in *M. volkensii* fruit extracts, more data especially on the mosquito larvicidal activities have emerged (Mwangi and Mukiama, 1988; Mwangi and Rembold, 1987,1988) and investigations on the locusticidal activity initiated (Wilps *et al.*,1993). Crude extracts of *M. volkensii* fruits have been reported to be fairly thermostable (Alsharook *et al.*, 1991) and the pure compounds isolated and characterised include salannin and volkensin (Rajab *et al.*, 1988; Rajab and Bentley, 1988). Both *A. indica* and *M. volkensii* have shown potential as sources of locusticides and field trials have been carried out against *S. gregaria* (Nasseh *et al.*,1993). However, comprehensive data on the mode of action of the active compound(s) in *M. volkensii* fruit extracts is unavailable.

1.2 LITERATURE REVIEW

There are about 500 species in the superfamily *Acridoidea* in Africa (Dirsh, 1965). Many of these insects belonging to the family *Acrididae* are potentially serious pests. They include the African migratory locust *Locusta migratoria migratoroides* (R & F) and the desert locust *Schistocerca gregaria* (Forskal) *Locusta migratoria migratoriodes* is distributed in Africa, Asia, Europe, Madagascar, New Zealand, Australia, New Guinea and the Pacific Islands (Steedman, 1990). In Africa, *L. migratoria* has breeding grounds in the middle Niger Delta in Mali and the Lake Chad Basin (Kumar, 1986; Lamb, 1974). A major outbreak occurred in 1925 causing serious destruction of crops, mainly cereals. Northern and East African countries were invaded before the outbreak finished in Southern Africa in 1934 (Kumar, 1986).

Schistocerca gregaria breeds in low rainfall areas with sufficient moisture for egg development and hatching e.g. Africa, Arabia, Iraq, Iran, Pakistan and India (de Pury, 1973). In 1958 Ethiopia lost 167,000 tons of grain to *S. gregaria*. In the same year *S. gregaria* swarms occupied 1000 square kilometers of Somalia consuming 80,000 tons of food a day (Kumar, 1986). These figures of losses are estimates. Exact and comprehensive data are not available. New methods of measuring crop losses that may lead to more accurate assessment of damage caused by locust swarms are however being developed (Krall, 1994). These few examples of locust plagues illustrate the locust menace in Africa, a continent which is not self-sufficient in food and therefore cannot afford to lose its fodder and food crops to pests.

Man has faced locust plagues for thousands of years with mostly futile attempts at controlling them. Until recently only mechanical methods were used, such as collecting, herding and then crushing or burning them (Bennet, 1993). Control of locusts in the last 50 years has been done by poisoning with insecticide laced baits or sprays (Dorow, 1993). The array of spray equipment include standard hand sprayers,vehicle mounted sprayers, aerial sprayers and ultra low volume sprayers (Dorow, 1993). The best strategy of control would be to destroy nymphs at breeding sites but this requires extensive organisation and ability to carry out regular field surveys for evidence of mass breeding, often in remote areas. Remote sensing techniques are now being used to identify potential outbreak areas (Voss and Dreiser, 1994).

For the last fifty years synthetic insecticides were deemed capable of eradicating insect pests. Subsequently the negative effects of the long lasting chlorinated hydrocarbons, mainly dieldrin on the environment were discovered. Safer insecticides were therefore sought. New insecticides with shorter periods of persistence appeared more suitable, but with these, blanket-cover application became necessary, therefore

again having a negative impact on the environment and increasing the costs enormously. In addition there remained the constant risk to humans and animals from the use of the pesticides. For example, safe and environmentally sound use of insecticides was not ensured during the 1986 to 1989 grasshopper and locust control programmes and human and environmental exposures were at times dangerously high (Franzen, 1993). There were serious leakages of malathion in Algeria, dimethoate in Khartoum, Sudan and of fenitrothion; dimethoate and heptachlor in North Yemen (Franzen, 1993). The synthetic pesticides used in the past have sometimes produced doubtful results. The problems encountered include high costs and in many cases only moderate success along with major ecological damage (U.S.Congress, 1990).

The use of conventional broad-spectrum insecticides causes environmental problems. In addition to pollution of the environment by residual insecticides e.g. dichlorodiphenyl trichloroethane (DDT), dieldrin, fenitrothion and azinphosmethyl, insects useful to farmers may be destroyed along with pests by broad-spectrum insecticides like demeton and omethoate (Hill and Waller, 1984). There exists also the problem of insecticide resistance in pests. It is estimated that 350 pest insect species are now resistant against one or more of about 50 commercial insecticides (Rembold, 1984). Against this background, research efforts were intensified worldwide with the aim of identifying new locust control methods. These efforts have focused on the use of new classes of substances like myco-insecticides (Bateman,1992) e.g. the spores of the pathogenic fungi *Metarhizium flavoviridae* and *Beauveria bassiana* (Wilps and Nasseh, 1994), *Metarhizium anisopliae* (Zimmerman *et al*, 1994), biocides based on active plant constituents (Mwangi, 1982; Mwangi and Rembold, 1987,1988; Mwangi and Mukiama 1988 ; Rembold, 1994) and on new application methods (Wilps and Nasseh, 1993). There is need to develop more selective and biodegradable insecticides. The search also continues for more insect-specific controls like application of pheromones (Ferenz *et al*, 1994) hormone mimics e.g. juvenile hormone analogues (Dorn *et al*, 1994) and chitin synthesis inhibitors like benzoylphenyl ureas (Hajjar, 1985; Wilps and Nasseh, 1994).

The pool of plants possessing insecticidal substances is enormous (Jacobson, 1975a). These have generated extraordinary interest in recent years as potential sources of natural insect control agents. Today over 2000 species of plants are known that possess some insecticidal activity (Crosby, 1966, Ahmed *et al.*, 1984, Jacobson, 1971). In many cases the plants have a history of use as folk remedies and are still in local use by different societies throughout the world to kill or repel insects (Secoy and Smith, 19-83).

The first insecticidal compounds to be used by man were from plants, the biological activities of which were known from the earliest recorded times. Dioscorides (AD 40-90) mentioned the usefulness of opium, colchicine and aconite (McIndoo, 1945). These were used as medicines and sometimes as insecticides. *Veratrum album* (White hellebore) and *V. nigrum* were well-known medicines and insecticides of the Romans. As early as 5 BC during the ancient Mediteranean period, seeds were treated with insecticidal plant extracts to protect them from insect pests of stored products so that they could remain viable. Houseleek and cuccumber extracts were used for seed steeps. Other insecticidal and insect-reppelent plants mentioned by classical writers include bitter lupin, absinthe, bay, cedar, garlic, fig, hellebore, oak, squill, asafetida, cassia and pomegranate (Smith and Secoy, 1975). In the middle of the 17th century sabadilla, nicotine, pyrethrum and rotenone were recognised as effective insect-control agents (Crosby, 1966). At around this period, nicotine was commercialised as an insecticide in America and pyrethrum and sabadilla were likewise commercialised in Europe (McIndoo, 1945). Large-scale investigations of insecticidal plants were first undertaken in the 1920's most notably in the United States of America and in England. These investigations resulted in the worldwide commercialisation of *Derris* and *Lonchocarpus* roots as insecticides (McIndoo, 1945; Ware, 1982).

The most economically important of the natural plant compounds used in commercial insect control are the pyrethrins from the flower heads of pyrethrum (*Chrysanthemum cinerariaefolium* Vis. and *C. coccineum* Willd.). Pyrethrum flower heads have been used as a natural contact insecticide since the early 1800's in Persia and Yugoslavia and by 1828, was being processed for commercial insect control (Tyler *et al*, 1976). The use of pyrethrins as sources of insect control agents was limited owing to inadequate supplies (Otieno, 1983). In addition the instability of these compounds to light and rapid metabolism limited their potency and application (Casida, 1983). These limitations gave impetus for the synthesis of active analogues, termed pyrethroids, modelled on the naturally occurring pyrethrins. The pyrethrins are particularly appropriate insecticide models because of their rapid paralytic action which is useful against flying insects. They act by stimulating insect nerve cells and fibres to discharge repetitively and later paralyse them (Matsumura, 1975). Against birds and mammals, these compounds are not highly toxic owing to rapid metabolic degradation by these organisms (Yamamoto, 1970). The pyrethrins are formed by esterification of two acid moities- chrysanthemic acid and pyrethric acid with three alcohol moities pyrethrolone, cinerone and jasmolone. The resultant esters are the

pyrethrins which include pyrethrins I and II cinerins I and II (LaForge and Barthel, 1944) and jasmolins I and II (Godin *et al*., 1966)

Pyrethroids are prepared by modifying the structure of pyrethrins by altering the alcohol or acid moities. Pyrethroids synthesized by alteration of the alcohol moiety include (s)-bioallethrin tetramethrin, resmethrin and phenothrin. Kadethrin is prepared by altering the acid moiety of resmethrin (Schechter, 1949). Permethrin the first photostable pyrethroid is prepared by replacing the isobutenyl side-chain of phenothrin with a dichlorovinyl group. Introduction of an alpha-cyano substituent to the photostabilized pyrethroids enhances insecticidal activity. Such reactions have resulted in the production of very potent insecticides e g decamethrin (Elliot and Janes, 1978).

Roots of certain tropical plants in the *Leguminosae* especially in the species of *Derris*, have a long history of use in the Far East as fish poisons, hunting aids and insecticides (Saxena , 1983). In other tropical areas, roots of other plants in the *Leguminosae* including *Lonchocarpus*, *Tephrosia*, *Mundulea* and *Millettia* species have been used for similar purposes. The biological activity of these roots has been attributed to a group of isoflavenoid compounds known as rotenoids. The first rotenoid isolated was rotenone, isolated in 1892 by Geoffroy from *Lonchocarpus nicou* (Windholz *et al*., 1983) and in 1912 by Nagai from *Derris chinensis*. Other sources of rotenone are *D. elliptica* , *D. malaccensis* , *L. utilis* and *L. urucu* (Saxena, 1983)

Rotenone is less acutely toxic against mammals than against fish and insects. Against insects, rotenone is active as a contact and stomach poison. It decreases oxygen uptake by specifically inhibiting the NADH-dependent dehydrogenase step of the mitochondrial respiratory chain (O'brien, 1966). These insecticides are especially valuable for the control of leaf-chewing beetles and caterpillars (Fukami and Nakajima, 1971). Ten compounds which are structurally related to rotenone have been isolated from various plants in the *Leguminosae* (Martin, 1942). There have been few attempts made to identify the toxic moieties of rotenone or to synthesize structurally simpler analogues (Bowers, 1983b).

The wood and bark of *Quassia amara* (*Simaroubaceae*) has been used as an insecticide for a long time in the tropics (Crosby, 1971). However interest in it's insecticidal properties rose in the west in the early 1990's (McIndoo, 1945). In the 1930's chips from the woody parts of *Q. amara* and *Picrasma excelsa* were used to control sawflies and aphids, both as contact and stomach poison (Crosby, 1971). The major insecticidal principle in the *Quassia* chips is the C20 triterpenoid (decanortriterpenoid) quassin (Valenta *et al*., 1961). Many related quassinoids have

been isolated from other plants in the *Simaroubaceae* (Polonsky, 1985). Several have antifeedant activity against *Locusta migratoria* (Odjo *et al*., 1981) antifeedant and growth inhibitor activity against the tobacco budworm *Heliothis virescen* and the black cutworm *Agrotis ipsilon* (Lidert *et al*., 1987). The narrow spectrum of activity of *Quassia* chips and high costs of chips production and transport has undermined the use of *Quassia* (Crosby, 1971). Interest in the quassinoids has increased in recent years because of their other biological activities especially antileukaemic activity (Polonsky, 1985).

Over 6000 phytoalkaloids are known (Harbone and Turner, 1984) and many of these exert actions upon animal nervous systems (Robinson, 1981). Important sources of these alkaloids are *Nicotiana rustica* (tobacco), *Veratrum sabadilla* (sabadilla), *Ryania* and *Physostigma venenosum*. Tobacco leaves have been used as an insecticide for about 3000 years (McIndoo, 1945). The leaves were either extracted, used as a dust or burned to protect crops from leaf eating insects. The major biologically active principle, nicotine was first isolated in 1828 (Schery, 1954). Related pyridine-based alkaloids from tobacco leaves, such as anabasine and nornicotine were also isolated and are also insecticides (Schmeltz, 1971).

Nicotine has been isolated from a number of species of *Nicotiana* as well as from unrelated species in the genera *Atropa*, *Equisetum* and *Lycopodium* (Schmeltz, 1971). *Nicotiana rustica* (*Solanaceae*) has the highest known content of nicotine, while *N. tabacum* is the most common commercial source (Morgan and Wilson, 1985). The use of nicotine in insect control has dropped steadily because of the high cost of production, disagreable odour, extreme mammalian toxicity, environmental lability and limited insecticidal activity (Schmeltz, 1971, Tyler *et al*., 1976). Attempts to use nicotine as a model of new insecticides have not been commercially successful. Nicotine which acts as an agonist on a specific type of acetylcholine receptor (nicotinic cholinergic receptor) (Murdock *et al*., 1985 has strict structural requirements.

Veratrum sabadilla and *V. album* (hellebore) have long been used as insecticides. The locusticidal properties of *Sabadilla* were recognised in the sixteenth century (McIndoo, 1945; Labreque, 1983) and was developed as a commercial insecticide in the 1940's (Roark, 1947). The toxic principles of *Sabadilla* and hellebore include over 30 alkaloids that are known collectively as veratine (Crosby, 1971). These alkaloids are strong irritants to mucous membranes and some are teratogenic (Crosby, 1971). They must be made less toxic to non-target organisms and more persistent in the field if they are to be successful commercial insecticides.

The wood of some species of *Ryania*, a genus of tropical American shrubs and trees belonging to the *Flacourtiaceae* has been used since the 1940's against the European corn borer, sugar cane borer and codling moth (Crosby,1971). The ground stem of *R. speciosa* is employed in a commercial insecticide formulation. Although *Ryania* is a minor insecticide, it has broader implications because of it's efficacy and type of action (Casida, 1987). It causes cessation of feeding and flaccid paralysis in insects owing to it's poisoning of muscle (Casida, 1987). The insecticidal principles of *Ryania* are ryanodine (Rogers *et al.*, 1948, Wiesner, 1972) and 9, 21-Didehydroryanodine (Waterhouse *et al.*, 1984). Although ryanodine is highly toxic to mammals, it's hydrolysis product ryanodol has low toxicity to mice and yet is a potent knockdown agent for insects (Casida, 1987).

Physostigma venenosum (*Leguminosae*) has long been used for the toxicity of it's seeds (Lewis and Elvin-Lewis, 1977). The toxic principle is predominantly the indole alkaloid physostigmine. Physostigmine reversibly inhibits acetylcholinesterase and can thereby prolong and exaggerate the effects of acetylcholine (Murdock *et al.*, 1985). Although physostigmine itself was never an important insecticide, it may have been the model for the synthesis of stable insecticidal N-methylcarbamates.

Despite the relative safety of well known botanical insecticides, most of these substances have their drawbacks hindering large scale application especially in the control of locusts. None of these insecticides have been used extensively as locusticides (Steedman, 1990, Peveling, 1994). For example, nicotine is highly toxic (Matsumura, 1975). Natural pyrethrins are highly unstable, while pyrethroids have low activity at high temperatures (Corbett *et al.*, 1984). Pyrethroids are also toxic to fish, aquatic insects and crustaceans (Casida, 1973). Further, several insects have exhibited resistance to pyrethroids (Corbett *et al.*, 1984). Rotenone is unstable and very toxic to fish (Matsumura, 1975). For these reasons, the search for new safer and more effective insecticides from plants is justified.

Another group of plant compounds with potential as insect control agents are behaviour modifiers (attractants and repellents), insect growth regulators (IGR's) and antifeedants. Plant attractants have been used in insect control both to lure insects to traps, to poison baits and to detect and monitor insect populations (Metcalf, 1975). Examples of practical application of plant compounds for trapping of insect pests include phenylpropanoids from *Umbelliferae* for the carrot fly *Psila rosae* and various monoterpenes from cotton seed oil for the boll weevil *Anthonomus grandis* (Stadler, 1983). Certain plants have been used since antiquity as insect repellents. These have been used mainly to repel blood sucking mosquitoes, flies, mites and

ticks. Examples are oils of citronella, turpentine,cedarwood and eucalyptus (Schery, 1954).

The insect growth regulators specifically affect growth and development of insects (Saxena, 1983). These compounds include analogues and antagonists of two groups of endogenous insect hormones, namely the juvenile hormones and the moulting hormones. The juvenile hormone analogues include juvabione from *Abies balsamea*, juvocimenes from *Ocimum bacilicum*, echinolone from *Echinacea angustifolia* and farnesol from many plant oils (Jacobson *et al.*, 1975b). Thousands of synthetic analogues have been prepared. Two of these, kinoprene and methoprene have been registered in the United States of America for the control of mosquitoes, manure-breeding flies and stored products pests (Staal, 1982). Antijuvenile hormone compounds have also been isolated from other plants. Examples are precocenes I and II isolated from *Ageratum houstonianum* (Bowers *et al.*, 1976). Disruption of the normal titres of the moulting hormones ecdysterone and ecdysone can result in abnormalities and death. An example of a moulting hormone analogue is ponasterone A. Larvae of the pink bollworm *Pectinophora gossiella* fed on ponasterone A were unable to moult (Klocke, 1989). Most insects are unaffected by ingested or topically applied ecdysteroids hence the low commercial use.

Antifeedants are substances which when tasted by insects, result either temporarily or permanently, depending on potency, in the cessation of feeding (Kubo and Nakanishi, 1977). The existence of and potential for antifeedant compounds, both natural and synthetic in practical insect control has been known for some time. For example the Bordeaux mixture (copper sulphate, hydrated lime and water), acts as a feeding deterrent to fleas, beetles, leaf hoppers and the potato psyllid *Paratrioza cockerelli* (Metcalf, 1975). Only more recently have the problems associated with total reliance on synthetic pesticides necessitated the re-evaluation of antifeedant compounds.

Several groups have in the recent years been examining plant extracts and pure compounds for insect-antifeedant activity (Kubo and Nakanishi, 1977,Munakata, 1977,Jacobson *et al.*, 1978;Mwangi, 1982;Rembold, 1989;Schmutterer, 1990). Although none of the plant compounds have thus far been developed into widely used commercial products, several of the more active ones have been synthesized in the hope of making these compounds and simpler structural analogues more widely available for testing. For example, several chemically rearranged azadirachtins have been shown to have antifeedant activity against the African leafworm *Spodoptera littoralis (Ley et al., 1991)*.

The antifeedant and other biological activities of extracts and pure compounds from some *Meliaceae* are well documented For more than 25 years the potential of neem tree as a pest management tool has been studied A wide range of biological activities of neem extracts and active compounds isolated from neem have been reported, antifeedant, antioviposition, repellent, growth-regulating and biocidal. The precise mechanisms of these effects have not been elucidated, although various hypotheses have been formulated

The neem tree, *Azadirachta indica* (A Juss), the China berry *Melia azedarach* (L), *Melia toosendan* and *Melia volkensii* (Gurke) belong to the family *Meliaceae*. Several compounds with biological activity have been isolated from *Meliaceae* trees-from fruits, bark, root bark, blossoms and leaves The structure of these compounds which are mainly tetranortriterpenoids suggest a biogenetic relationship to limonin the bitter principle of citrus trees (Arigoni *et al.*, 1960, Arnott *et al.*, 1960) and for this reason they are generally referred to as limonoid compounds The structures of these compounds have been studied extensively (Feuerhake and Schmutterer, 1982;Feurhake, 1984). The limonoid compounds identified in *M. azedarach* and *A. indica* are azadirachtin (Appendix 1) (Butterworth and Morgan, 1971), salannin (Appendix 2) (Henderson *et al.*, 1964), nimbin (Henderson *et al.*, 1963, azadiradione, azadirone and epoxyazadiradione (Lavie and Jain, 1967), meliantriol (Lavie *et al*, 1967a) melianone and melianol (Lavie *et al.*, 1967b), meldenin (Connolly *et al.*, 1968) and nimbolide (Ekong, 1967) Leaves and fruits of *A. indica* and *M. azederach* contain at least three limonoid compounds, azadirachtin, salannin, and meliantriol which have insect antifeedant activity. The most studied limonoid compound is azadirachtin whose structure has recently been revised (Broughton *et al.*, 1986). Most of the studies in this area have been carried out using either methanolic extracts of *A. indica* and *M. azedarach* or azadirachtin purified from these extracts by TLC and HPLC.

Melia azedarach extracts have antifeedant effects on *Mythimna separata* (Chiu, 1987) and azadirachtin purified from *M. azedarach* has antifeedant effects on *S. gregaria* (Butterworth and Morgan, 1968) The same compound isolated from *A. indica* has antifeedant effects on the larvae of the blowfly *Phormia terrae novae* (Wilps, 1987) and *S. gregaria* (Butterworth and Morgan, 1971, Zanno *et al*, 1975) *Meliaceae* seed extracts have been reported to cause antiovipositant effects in various insects. *Melia azedarach* extracts deter oviposition in the rice gall midge *Orseolia oryzae* (Chiu, 1987) and *A. indica* seed oil deters oviposition in *Heliothis armigera* (Saxena and Rembold. 1984) while azadirachtin purified from *A. indica* inhibits oviposition in *L. migratoria* (Rembold and Sieber, 1981) A reduction in fecundity has

been reported in insects treated with either purified azadirachtin or *A. indica* seed extracts. Purified azadirachtin has been reported to cause reduced hatchability of eggs and failure of phallus erection in the milkweed bug *Oncopeltus fasciatus* (Dorn *et al*, 1987), poor oocyte growth in *L. migratoria* (Rembold and Sieber, 1981) and inhibition of egg development in *L. migratoria* (Rembold *et al*., 1984) Extracts of *A. indica* seeds have been reported to cause a reduction of egg production in the Colorado potato beetle *Leptinotarsa decemlineata* (Schmutterer, 1987), in *Formica polyctena* (Schmidt, 1987) and *Mythima separata* (Sharma *et al*, 1984) The Mexican bean bettle, *Epilachna varivestis* when treated with *A. indica* extracts lay deformed, moist, discoloured eggs often infected with fungi (Schulz and Schluter, 1984). Growth-regulating effects of azadirachtin and *A. indica* extracts have been reported in several insects. These effects are expressed usually as interference with moulting of larvae or pupae and extension of periods between instars. Purified azadirachtin from *A. indica* causes formation of long-living permanent nymphs in *Oncopeltus fasciatus* (Dorn *et al*., 1987), failure of pupa maturation in *Phormia terrae* novae (Wilps, 1987) and delayed larval development in *Ostrinia furnacalis* (Chiu, 1984). In *L. migratoria*, periods between instars are extended by azadirachtin treatment (Rembold *et al*., 1984, Mordue *et al*., 1985). When *Epilachna varivestis* is treated with azadirachtin after a moult or as mid-instars, they do not moult to a further instar (Schulz and Schluter, 1984) Treatment with *A. indica* extracts causes formation of larval-pupal intermediates in *Callosobruchus analis* (Naqvi, 1987), formation of larval-pupal intermediates and supernumerary larvae in the tobacco hornworm *Manduca sexta* (Haasler, 1984) and prolonged larval period in *Mythima separata* (Sharma *et al*., 1984) and *Aedes aegypti* (Zebitz, 1984). Fifth instar *L. migratoria* treated with azadirachtin results in long-living nymphs and vitellogenin which is normally synthesized in adult females is detected in such nymphs (Rembold *et al*, 1984) Further, Shalon and Pener, (1984) have reported that long-living nymphs resulting from treatment of fifth instar *L. migratoria* with azadirachtin exhibit adult sexual behaviour. Treated fifth instar males make attempts to mate with normal adult females.

Azadirachtin and *A. indica* extracts have been reported to cause changes in the gross morphology and histology of insects. Extracts of *A. indica* seeds cause development of *Callosobruchus analis* adults with reduced wings and swollen abdomen and in mosquitoes, wings, legs and mouthparts are deformed (Navqi, 1987). Fifth instar *L. migratoria* treated with azadirachtin produce adults with curled wing tips (Mordue *et al*., 1985) Mid-instars of *Epilachna varivestis* treated with *A. indica* extracts are unable to build a continuous epidermal epithelium The

corpora cardiaca disintegrate and in males, lysis of testis occurs (Schulz and Schluter, 1984). Azadrachtin-treated larvae of the Asiatic corn borer *Ostrinia furnacalis* show atrophied brain and hypertrophied corpora cardiaca, corpora allata and prothoracic glands (Chiu *et al.*, 1985). High concentrations of azadirachtin or *A. indica* extracts may cause death of larvae, nymphs or pupae. High doses of azadirachtin have been reported to cause death of *Phormia terrae novae* (Wilps, 1987) and of fifth instar *L. migratoria* nymphs (Mordue *et al.*, 1985) and high doses of *A. indica* extracts have been reported to be lethal to cause death of *A. aegypti* larvae (Zebitz, 1984).

Changes in haemolymph constitution have been reported in insects treated with either azadirachtin or *A. indica* extracts. Sieber and Rembold (1983) reported lowered ecdysteriod levels in the haemolymph of fifth instar *L. migratoria* treated with *A. indica* seed extract. In the same insect, Rao and Subrahmanyam, (1986) found lowered haemolymph protein and amino acid levels. Polyacrylamide gel electrophoresis of haemolymph proteins revealed two additional protein bands in treated females. Chiu *et al.*, (1985) have reported lowered ecdysteroid levels in the haemolymph of *Ostrinia furnacalis* larvae treated with azadirachtin.

The effects of the biologically active limonoid compounds on insect enzymes has not been thoroughly studied, however, Navqi (1987) reported inhibition of housefly cholinesterase, acid phosphatase and alkaline phosphatase by *A. indica* extracts. Azadirachtin has been suggested to act by mimicking ecdysone (Appendix 1) or interfering with processes controlled by ecdysone (Leuschner, 1972) and juvenile hormones (Abraham and Ambika, 1979). However, radioimmunoassays have demonstrated that no cross reactivity occurs between ecdysone and azadirachtin (Koul *et al.*, 1987; Sieber and Rembold, 1983) therefore the possibility of azadirachtin acting as a ecdysone mimic is unlikely but inactivation of enzymes that control ecdysteroid metabolism cannot be ruled out. It is also not clear whether azadirachtin may be acting directly on ecdysteroid receptors (Schluter *et al.*, 1985). More clues on the mode of action of azadirachtin have recently emerged. Rembold *et al.* (1989a) have reported accumulation of radio-labled anti-azadirachtin antibody in the corpora cardiaca of locusts treated with azadirachtin and accumulation of 22,23 dihydroazadirachtin in malpighian tubules of the same insect (Rembold *et al.*, 1989b). The precise mode of action of azadirachtin is however still not clear.

Ecdysone may act in concert with bursicon, the hormone which controls cuticular tanning in insects. Extracts of *A. indica* inhibit cuticular tanning in *A. aegypti* pupae (Zebitz, 1984). Bursicon is produced by the cerebral neurosecretory cells although it is primarily released from the ganglia of the ventral nerve cord (Cottrel, 1962a, 1962b; Fraenkel and Hsiao, 1962, 1963; Mills *et al.*, 1965, Vincent, 1971). The

hormone increases the permeability of blood cells to tyrosine (Mills and Whitehead, 1970;Post, 1972) and is involved in the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA) (Seligman *et al.*, 1969) Dihydroxyphenylalanine decarboxylase, the enzyme which decarboxylates DOPA to dopamine in the pathway leading to the formation of the tanning quinone is induced *de novo* by ecdysone Further, ecdysone influences the formation of a protein which reacts with the tanning quinone to produce sclerotin in the cuticle Therefore interference with ecdysone levels may also affect the cuticular tanning process.

Recently, extracts from other trees belonging to the *Meliaceae* family have been reported to possess antifeedant and growth-regulating effects on insects. Extracts of *Melia toosendan* bark have been found to contain a compound, toosendanin with antifeedant effects on *Ostrinia furnacalis* larvae (Chiu, 1984) and the cabbage worm, *Pieris rapae* (Chiu, 1987). The findings suggest that there may be many more potential insecticidal compounds in *Meliaceae* trees than previously thought This suggestion is supported by the findings of Schmutterer and Zebitz, (1984) and Ermel *et al.*, (1984) working on the growth regulating effects of *A. indica* seeds obtained from Africa and Asia on larvae of *A. aegypti* and *Epilachna varivestis*. Growth-regulating efficacy was not correlated with the azadirachtin content of the extracts in some cases. These results suggest the presence of other compounds in *A. indica* extracts besides azadirachtin which may exert synergistic or antagonistic effects.

Work done by Mwangi (1982) with *M. volkensii* extracts suggest that the fruits of the trees may contain a compound other than azadirachtin with antifeedant and growth-regulating effects on insects. In *S. gregaria* adults and nymphs, the extracts had antifeedant effects and intermoult periods were prolonged. In addition, insects treated with extracts showed lowered haemolymph carbohydrate content and elevated lipid content Two of the growth-regulating compounds found in *M. volkensii* are volkensin (Appendix 2) and Salannin (Rajab *et al*,1988) Other compounds from *M. volkensii* fruit whose structures have been elucidated include 1-cinnamoyltrichilinin, 1-tigloyltrichilinin, 1-acetyltrichilinin, ohchinin-3-acetate, (Appendix 2) (Rajab and Bentley, 1988,) Balan (1993) has also isolated meliacin (Appendix 2), a limonoid compound with demonstrable larvicidal and growth regulating activity against *A. aegypti* and *Epilachna varivestis* Four new compounds with activity in the *Artemia salina* bioassay and showing cytotoxicity against human tumour cell lines have recently been isolated from *M. volkensii* root bark These compounds are the apotirucallane triterpenes, meliavolkensins A and B (Appendix 3) (Zeng *et al.*, 1995a), meliavolin, an apotirucallane triterpene and meliavolkin a tetranortriterpene (Appendix 3) (Zeng *et al*, 1995b) However, it is still not clear

whether these are the most potent insecticidal compounds found in *M. volkensii*. Further work by Mwangi and Rembold (1988) has shown that the *M. volkensii* extracts prolong larval instars and may cause death during moulting in *A. aegypti* pupae which develop from treated larvae.

Recently, more clues on the mechanism of action of these extracts have been emerging. Mwangi and Mukiama (1988) have reported peculiar coiling suggestive of neural or muscular disturbance in *Anopheles arabiensis* larvae treated with TLC fractions of *M. volkensii* fruit extracts. Recently, *M. volkensii* extracts have been shown to be ovicidal, pupicidal and antiovipositant against *A. aegypti* (Awala, 1994). In field trials *M. volkensii* extracts have been reported to reduce fitness and cause mortality in *S. gregaria*. Insects treated through injection showed reduced hyperlipemic response to injected adipokinetic hormone (Wilps *et al*., 1993). Initial reports indicate that *M. volkensii* extracts may be capable of halting and even reversing gregarisation in *S. gregaria* (Nasseh *et al*., 1993). The extracts have been reported to cause mild retardation of larval development in at least one non-target insect, the predatory *Reduviid* bug *Coranus arenaceus* (Peveling *et al*.,1994).

There have been few attempts to unravel the nature of the mechanism of the antifeedant action of *M. volkensii* extracts on locusts. Significant work has been done in this area on *A. indica*. Contact chemoreceptors located on the segments and dome of the maxillary and labial palps of *L. migratoria* respond to feeding deterrent extracts from *A. indica* (Haskell and Mordue, 1969,Haskell and Schoonhoven, 1969,Blaney, 1974,Blaney and Duckett, 1975). Basic data on the effects of *M. volkensii* extracts on locust chemoreceptors is not available. Mordue *et al* (1985) has shown that azadirachtin has a direct effect on *L. migratoria* gut muscles, reducing contraction and therefore inhibiting peristalsis with concommitant antifeedant action. Similar investigations on the antifeedant effects of *M. volkensii* have not been done.

The action of several insecticides is known to be influenced by environmental factors. In tropical areas, temperature is a critical factor. The insecticidal activity of DDT, pyrethrins and pyrethroids increases with decreasing temperature (Narahashi, 1989,Harris and Kinoshita, 1977). Temperatures in locust prone areas are high. Wilps *et al*., (1993) has reported night time temperatures of 20 ±5^{o}C and day time shade temperatures of 45 ±5^{o}C in Anou Mekkerene, Niger. Although *M. volkensii* extracts have been reported to be thermostable, (Alsharook *et al*., 1991) no literature on the effect of ambient temperature on their toxicity on insects is available. While *M. volkensii* fruit from Embu and Tsavo have been reported to contain biologically active substances by several workers (Mwangi, 1982,Mwangi and Rembold, 1988,Mwangi and Mukiama, 1988,Rajab *et al*., 1988), no comparative study on the

content of the active principles in fruits emanating from the two areas has been documented. Ermei *et al.*, (1984) has reported that kernels from Indonesian *A. indica* contained more azadirachtin than those from Niger There has been speculation that the bark of *M. volkensii* contains biologically active substances (Kokwaro, 1976,Rajab et al., 1988) although these have not been bioassayed Comprehensive data is only available for whole fruits. Singh, (1987) has reported differences in the distribution of biological activity in various parts of the neem tree

Data available on solvents used in the extraction of biologically active substances in *M. volkensii* includes 95% methanol (Mwangi,1982) and 80% methanol (Mwangi and Rembold, 1988;Mwangi and Mukiama, 1988). Mansour *et al.*, (1987) has reported that the toxicity of *A. indica* seed kernel extracts to *Tetranychus cinnabarium* increased with decreasing polarity of the extracting solvent.

From the foregoing it is evident that several aspects of the biological activity of *M. volkensii* extracts are unknown and need to be clarified. It is not known whether the toxicological effects of *M. volkensii* extracts on insects are similar to those caused by azadirachtin or extracts from other *Meliaceae* trees

1.3 OBJECTIVES OF THE STUDY

This study was undertaken with a view of generating basic information on the insecticidal potential of *M. volkensii* with *L. migratoria* being the test insect. The study was multi-faceted. One investigation focussed on technical aspects regarding extraction, purification and stability of *M. volkensii* extracts in storage. The second series of investigations carried out on locusts were preceeded by activity-monitoring bioassays done on the 2nd instar larvae of *A. aegypti*.

The specific objectives of the study were:

1. To determine the best commonly available solvent system(s) capable of extracting biologically active substances from *M. volkensii* fruit.

2. To determine the content of biologically active substances in *M. volkensii* fruit from Tsavo and Embu areas.

3. To determine the richest source of biologically active substances within the *M. volkensii* tree.

4. To attempt simplifying the procedure for the extraction of biologically active substances from *M. volkensii* without loss of activity.

5. To determine the stability of *M. volkensii* extracts in storage.

6. To determine the chronic effects of *M. volkensii* extracts on *L. migratoria* nymphs with regard to moulting and growth rate.

7. To investigate the general mode of action of *M. volkensii* extracts on *L. migratoria*.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Experimental insects

Locusta migratoria migratoriodes (Reiche and Fairmaire) used in this study were reared in the Department of Zoology, University of Nairobi as described by Hunter-Jones, (1966). The insects were fed on wheat bran and wheat seedlings. Larvae for larvicidal bioassays were obtained from an *Aedes aegypti* mosquito colony maintained in the Department of Zoology, University of Nairobi since 1980. The mosquito larvae were fed on ground dog biscuit, while adults were reared on sugar solution and allowed to take blood meals from the blood vessels of rabbit ears.

2.2 Experimental reagents

All reagents used in this study except solvents used in bulk extraction of crude extracts from *M. volkensii* and plant oils used in locusticidal formulations were of analytical grade. Azadirachtin A was a donation from Prof. H. Rembold of Max-Planck Institute for Biochemistry, Munich, Germany.

2.3 Plant material

Melia volkensii (Gurke) fruits (Plate 1), leaves, stem bark, stem wood, root bark and root wood used in this study were obtained from from Tsavo, 250Km East of Nairobi and Embu, 150Km North of Nairobi, Kenya (Figure 1). The altitude, temperature, rainfall and humidity data of these areas is presented in Appendix 4. The plant materials were dried under shade at temperatures below $30^{o}C$ and pulverised in a hammer mill fitted with a sieve of 1mm diameter pore size.

2.4 *Aedes aegypti* 2nd instar larvae bioassay

All extracts and fractions of *M. volkensii* were routinely tested for the presence of activity by the *A. aegypti* 2nd instar larvae bioassay (Mwangi and Rembold, 1988). Tests were performed in 40ml of 0.08% NaCl solution (Zebitz, 1984) contained in 250ml glass jars. The test material was dissolved in 60% ethanol so that the final volume did not exceed 50µl. Larval food consisted of 0.1g finely ground dog biscuit per jar per day. The number of larvae in each jar was 20. Controls in all cases received 50µl of 60% ethanol. After 48 hours dead larvae were removed and counted. The larvicidal activity of *M. volkensii* extracts was tested at a minimum of six different concentrations. Each bioassay was done in triplicate, the results pooled and subjected to probit analysis (Finney, 1952).

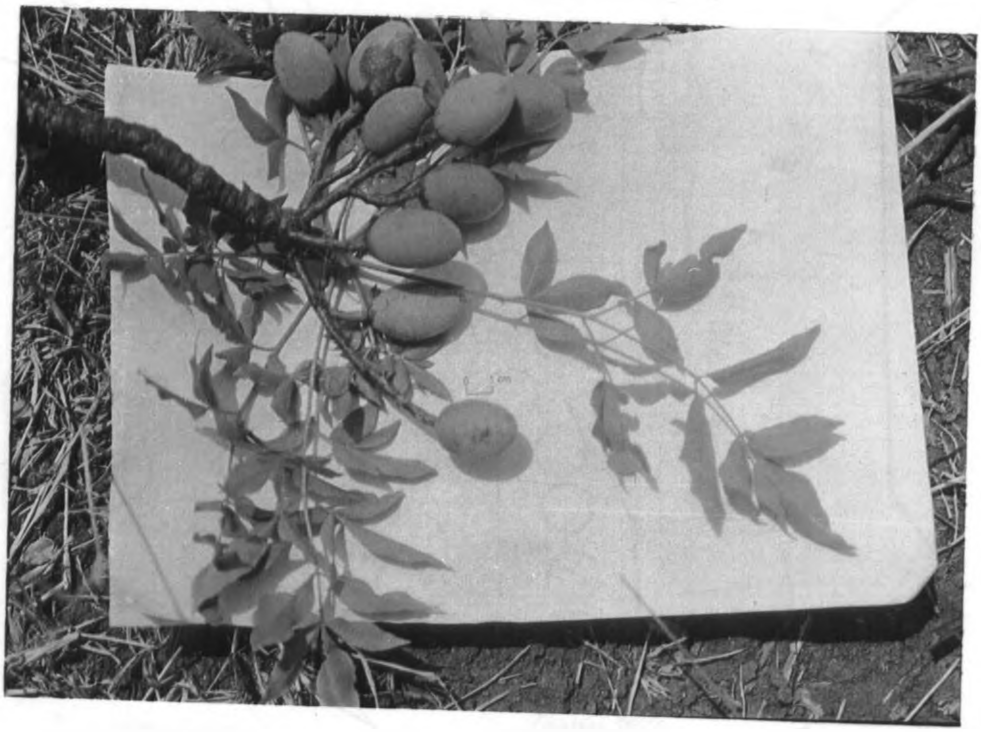


Plate 1: **Ripe *Melia volkensii* fruits from Embu**

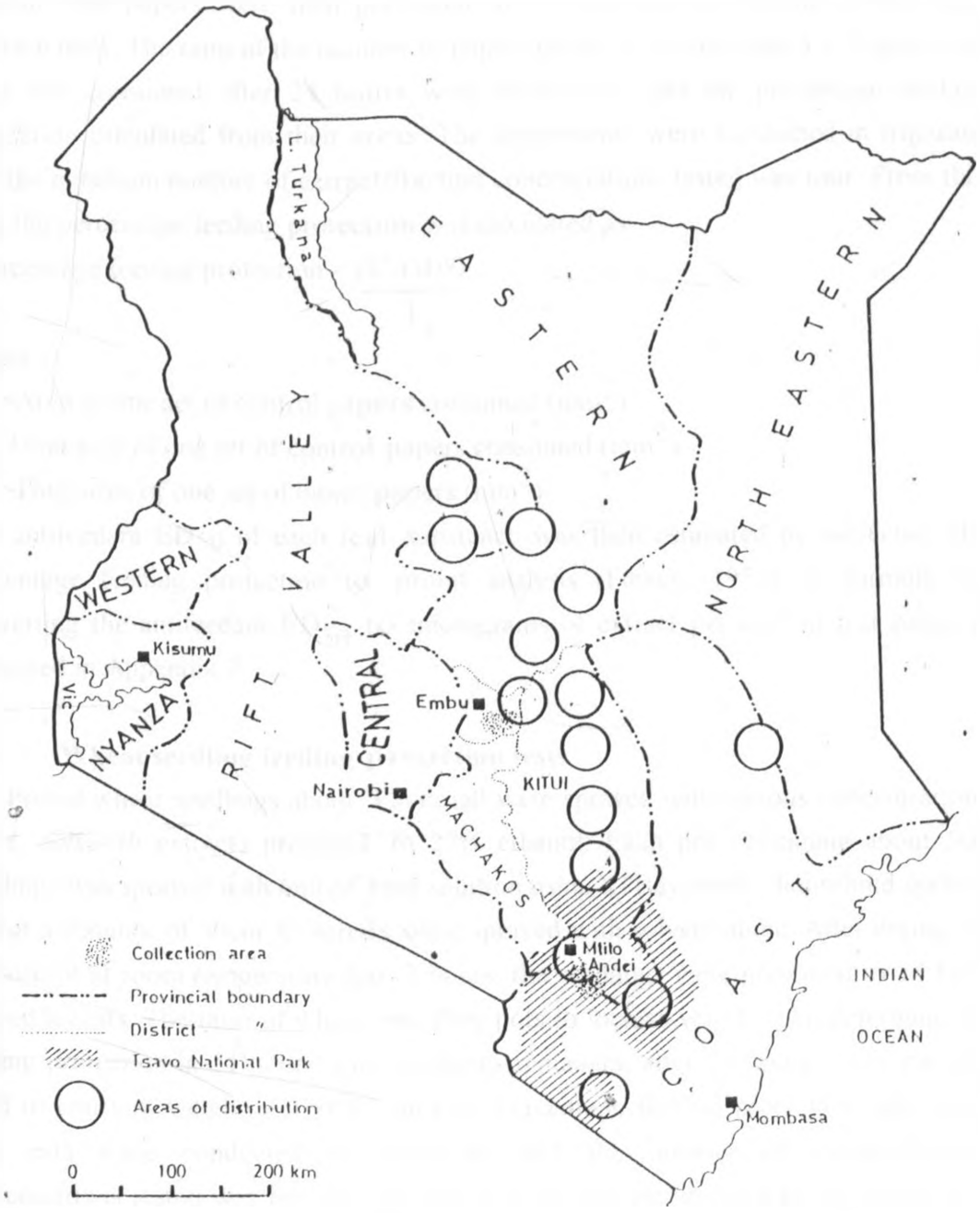


Figure 1: Map of Kenya showing the geographical distribution of *Melia volkensii* (Beentje, 1994) and the areas where fruits used in the study were collected

2.5 Paper feeding protection tests

Fifty microlitres of test solution in ethanol were applied to Whatman® number 1 qualitative grade filter paper squares measuring 2x2cm impregnated with 0.25M sucrose and dried at 40°C for 30 minutes. Control papers were treated with 50µl of ethanol. The papers were then presented to 24 hour starved locusts in one cage (Choice test). The ratio of the number of paper squares to locusts was 3:1. Papers that were not consumed after 24 hours were recovered and the percentage feeding protection calculated from their areas. The experiments were conducted in triplicate and the minimum number of extract/fraction concentrations tested was four. From this data the percentage feeding protection was calculated as:

$$\text{Percentage feeding protection} = \frac{(C-t)100}{T}$$

Where:

C=Area of one set of control papers consumed (mm^2)

t=Total area of one set of control papers consumed (mm^2)

T=Total area of one set of intact papers (mm^2)

The antifeedant ED_{50} of each test substance was then estimated by subjecting the percentage feeding protection to probit analysis (Finney, 1952). A formula for converting the antifeedant ED_{50} to micrograms of extract per cm^2 of test paper is presented in Appendix 7.

2.6 Wheat seedling feeding protection tests

Potted wheat seedlings about 15cm tall were sprayed with various concentrations of *M. volkensii* extracts prepared in 25% ethanol. Each pot containing about 500 seedlings was sprayed with 6ml of test solution using a "Baygon® " household sprayer held at a distance of 30cm. Controls were sprayed with solvent alone. After drying off the solvent at room temperature for 2 hours, the seedlings were presented to 24 hour starved locusts. The ratio of wheat seedling pots to locusts was 1:3. To determine the feeding protection in 24 hours, the seedlings remaining after 24 hours were cut and dried to constant weight at 100°C and the percentage feeding protection calculated. The tests were conducted in triplicate and the number of extract/fraction concentrations tested was five. From this data the percentage feeding protection was calculated as:

$$\text{Percentage feeding protection} = \frac{(C-t)100}{T}$$

Where:

C = Dry weight of control seedlings consumed (g)

t = Dry weight of treated seedlings consumed (g)

T = Dry weight of 500 intact seedlings (g)

The antifeedant ED_{50} of each test substance was then estimated by subjecting the percentage feeding protection to probit analysis (Finney, 1952)

2.7 Test for the suitabilty of water, methanol and ethanol in the extraction of biologically active substances from *M. volkensii* fruit

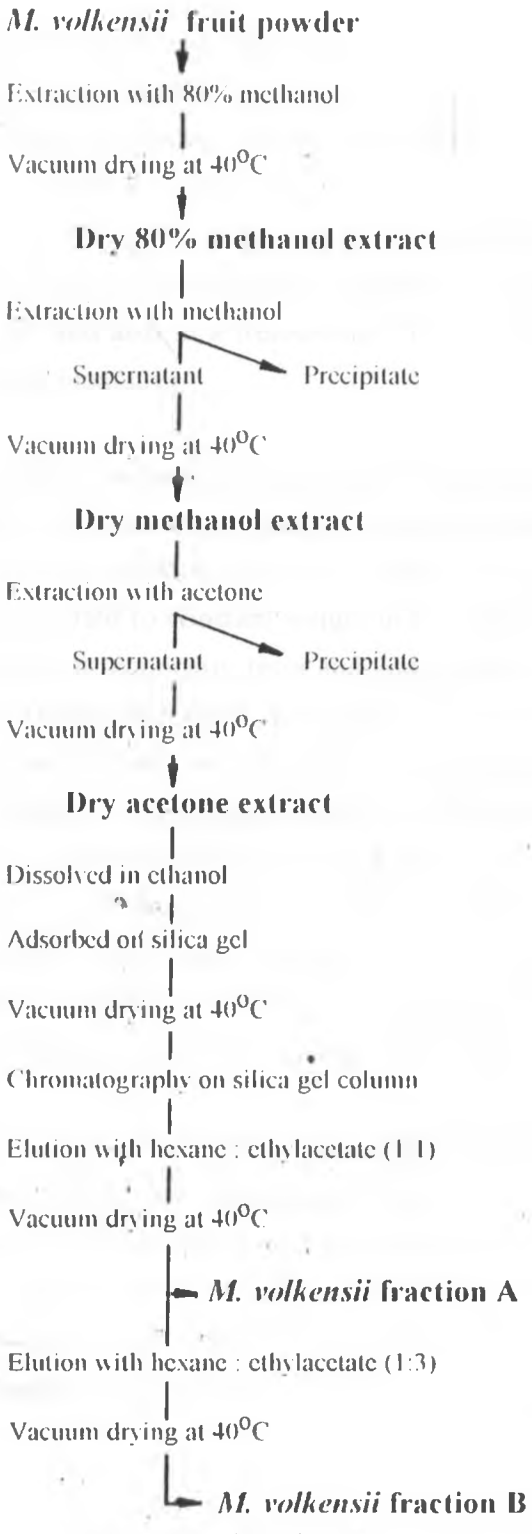
Water, and a range of methanol and ethanol solutions in water were tested for their efficiency to extract biologically active substances from *M. volkensii* fruit powder. The concentrations of ethanol tested were 20%, 40%, 50%, 60%, 70%, 80%, 95% and 99.7%. In the case of methanol, the concentrations tested were 20%, 40%, 50%, 60%, 70%, 80%, 95%, and 99.6%. One hundred grams of *M. volkensii* fruit powder was extracted with 300ml of each solvent system with regular stirring for 24 hours in airtight glass containers. The decanted extracts were filtered, concentrated in a rotary evaporator at 40^{O}C and dried in a freeze-drier. The extracts were kept dessicated at 4^{O}C. These extractions were conducted in triplicate. The yield of dry extract was determined followed by larvicidal and antifeedant tests. The yields of dry extracts were subjected to analysis of variance. The 48 hour LD_{50}'s of the extracts in the *A. aegypti* 2nd instar larvae bioassay were determined by probit analysis (Finney, 1952). The same statistical method was used to determine the ED_{50}'s of the extracts in the locust antifeedant tests.

2.8 Preparation of crude *M. volkensii* fruit extract

Melia volkensii fruit powder was extracted by adding 80% methanol at a ratio of 3:2 (Litres 80% methanol:Kilograms powder). During routine extractions the slurry of powder and solvent was left to stand for 7 days after which the supernatant was decanted, filtered, reduced in volume in a rotary evaporator at 40^{O}C, and dried in a freeze-drier. The extraction procedure was repeated three times. The dry crude extract was kept dessicated at 4^{O}C.

2.9 Preparation of *M. volkensii* fruit fractions A and B

Two purer extracts termed *M. volkensii* fractions A and B were routinely prepared from the 80% methanol crude extracts as described by Mwangi and Rembold, (1988) (Figure 2). In this procedure, cold acetone soluble material from 80% methanol *M. volkensii* fruit extract was dissolved in ethanol to a concentration of 1g/ml. This mixture was then added to silica gel at a ratio of 1g gel to 1.5ml solution. This solution was then dried in a rotatory evaporator under vacuum at 40^{O}C and in a



(Mwangi and Rembold, 1988)

Figure 2: A flow chart showing the procedure for the preparation of *M. volkensii* fractions A and B.

freeze-drier. Portions of 20g of the silica gel with the adsorbed extract were then applied on top of a glass column of 5cm internal diameter and 40cm height, packed with 100g of silica gel saturated with ethylacetate hexane (1:1). *Melia volkensii* fraction A was recovered by eluting the column with 2L of ethylacetate hexane (1:1), while *M. volkensii* fraction B was recovered by eluting the same column with 2L of ethylacetate hexane (3:1). The fractions were then dried under vacuum in a rotary evaporator at 40°C and also in a freeze-drier. The fractions were kept dessicated at 4°C until needed for bioassays.

2.10 Screening for biological activity in *M. volkensii*

All parts of *M. volkensii* were tested for content of biologically active substances. These plant materials which included fruits, leaves, stem bark, stem wood, root bark and root wood were dried to constant weight in the shade below 30°C. From some of the dry fruits, fruit coat, endocarp, testa and endosperm were extracted manually. All the plant material except testa and endosperm were pulverised to a fine powder with a hammer mill fitted with a 1mm mesh screen. The testa and endosperm were pulverised in a laboratory blender. This procedure was necessitated by the small quantities of these two materials recovered from the fruits. Three hundred grams of each powder was then extracted three times with 1L of 80% methanol with daily stirring for 7 days in airtight containers. The extracts were then decanted, filtered and the volume reduced in a rotary evaporator at 40°C, followed by drying in a freeze-drier. The extracts were kept dessicated at 4°C until needed for bioassays.

2.11 Comparison of activity and yield of biologically active material in *M. volkensii* fruit from Embu and Tsavo

One kilogram batches of fruit powder prepared from fruits collected from Tsavo and Embu areas were extracted 3 times with 1.5L of 80% methanol for 7 days. The extracts were decanted and purified following the standard procedure (Figure 2). The yields of dry fractions were determined followed by *A. aegypti* larvicidal and locust antifeedant tests.

2.12 Test for content of biologically active substances in ripe and unripe *M. volkensii* fruits

Ripe and mature unripe fruits of *M. volkensii* were collected from Tsavo and dried in the shade at 30°C and powder prepared by the standard protocol. Three hundred grams of each fruit powder was extracted three times with 1L of 80% methanol with daily stirring for 7 days in airtight plastic containers. The extract was decanted,

filtered, reduced in volume in a rotary evaporator under vacuum at 40°C. and dried in a freeze-drier. After the yields were determined, the biological activity in the extracts was determined by the *A. aegypti* larvae bioassay and the locust antifeedant test.

2.13 . Recovery of biologically active substances from 80% methanol extract of *M. volkensii* fruit by precipitation at low temperatures

In this procedure outlined in Figure 3, 1L of 80% methanol extract of *M. volkensii* fruit was concentrated in a rotary evaporator at 40°C and left to stand at 0 to 4°C for 12 hours. The resultant precipitate and supernatant were dried in a freeze-drier and tested for both larvicidal activity by the *A. aegypti* 2nd instar larvae bioassay and locust antifeedant activity. The activities and yields of these extracts were compared with that of acetone supernatant and acetone precipitate obtained by purifying 1L of an identical sample of 80% methanol *M. volkensii* fruit extract through the standard procedure up to the "acetone step".

2.14 Recovery of biologically active substances from *M. volkensii* fruit powder pre-washed with water

In this procedure, *M. volkensii* fruit powder was first washed with water and dried before being subjected to the standard extraction procedure. Three 500g batches of *M. volkensii* powder were washed vigorously three times with 3L of water. The powder was left to settle and the supernatant discarded. The powder was dried to constant weight at 40°C and then subjected to the standard extraction procedure with 80% methanol. Three 500g batches of unwashed powder were simultaneously extracted with 80% methanol. The resultant crude extracts were weighed and tested for biological activity in the *A. aegypti* larvicidal test and the locust antifeedant test.

2.15 Preparative thin layer chromatography of *M. volkensii* extracts

Kiesegel® 254, 60F was used as the adsorbent in thin layer chromatography of *M. volkensii* fruit fractions A and B. A slurry of the gel was made in water. The ratio of gel weight to volume of water was 110g of gel:220ml water. The slurry was then applied to glass plates of size 20x20cm to a thickness of 1mm using a "Shandon®" TLC plate coating equipment. The plates were heated at 60°C for 2 hours followed by further heating at 110°C for 12 hours. The plates were then left to cool and stored at room temperature ready for use. The test samples (fractions A and B) were dissolved in ethanol to a concentration of 400mg/ml. These solutions were applied to the plates as a streak. The loading per plate was 200mg. The dry samples were then chromatographed using chloroform and acetone at a ratio of 7:3. After running for one

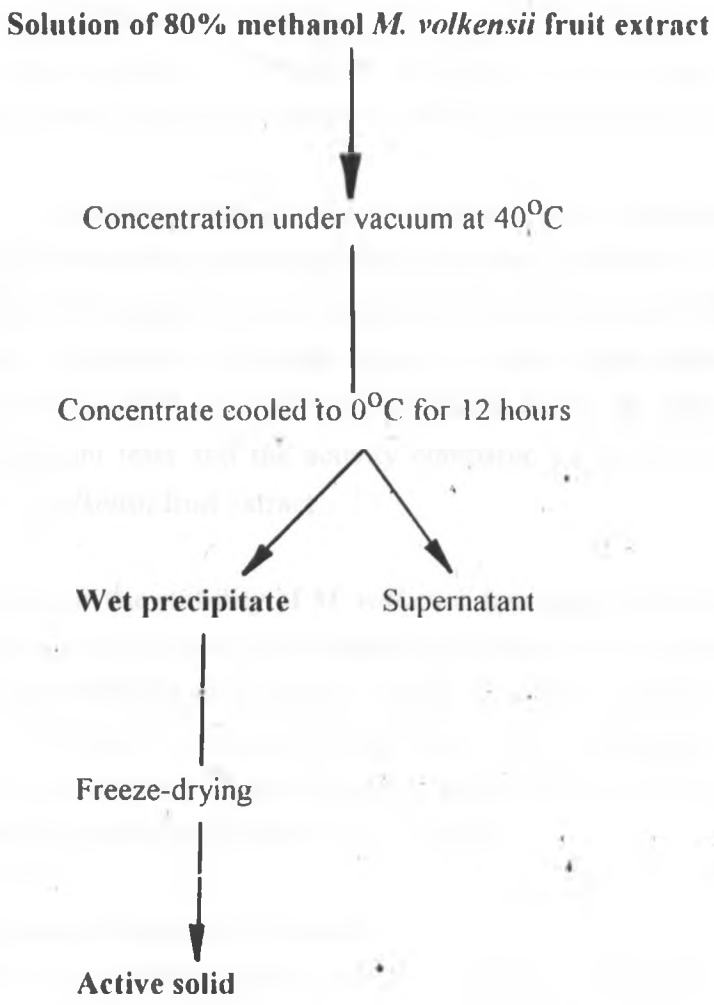


Figure 3: **A flow chart showing the procedure for the precipitation of an active fraction from 80% methanol extract of *M. volkensii* fruit**

hour, the plates were dried at 40^{O}C for one hour. Bands in the chromatograms were visualised under long wave ultraviolet light (340nm) and scraped off. The gel from each band was then eluted with three times equal volume of absolute ethanol for 12 hours at room temperature. The eluate was filtered, dried under vacuum in a freeze-drier, weighed and stored in a dessicator at 4^{O}C until needed for bioassays.

2.16 Test for the stability of *M. volkensii* fruit crude extract in storage

Melia volkensii fruit powder prepared from fruit collected in Embu area of Kenya and soaked in 80% methanol for 7 years was donated by Prof. R.W.Mwangi, Zoology Department, University of Nairobi, Kenya. Crude extract recovered from the wet powder by the standard protocol was bioassayed by the *A. aegypti* larvicidal and the locust antifeedant tests and the activity compared to that of freshly prepared 80% methanol *M. volkensii* fruit extract.

2.17 Test for the stability of *M. volkensii* fractions A and B in storage

Solutions of *M. volkensii* fruit fractions A and B at a concentration of 100mg/ml in ethanol were kept for two years in airtight glass vials. One set of the solutions was stored at 4^{O}C, the other at room temperature. These solutions were then bioassayed by both the *A. aegypti* 2nd instar bioassay and the paper feeding protection test and their activity compared with that of fresh samples.

2.18 Topical treatment of locusts

Locusts were either treated with *M. volkensii* fruit extracts on the labial and maxillary palps or on the neck membrane beneath the forward projection of the pronotum. In both cases the quantity of extract required was dissolved in 4µl of acetone. Controls received 4µl of acetone. In the case of palp treatment, 1µl of test solution was applied to the tip of each palp.

2.19 Injection of locusts with *M. volkensii* extracts

Solutions of *M. volkensii* extracts for injection were prepared in 60% ethanol. Locusts were injected with 5µl of the solutions in the intersegmental membrane between the 2nd and 3rd sternites with a microlitre syringe fitted with a gauge 26 hypodermic needle. Control locusts were injected with 5µl of 60% ethanol.

2.20 Composition of buffered saline used in locust dissections

Locusts were dissected in Maddrell and Klunsuwan (1973) Ringer,(NaCl,6.721;KCl,1.49;$CaCl_2 2H_2o$,0.29;$MgCl_2 6H_2o$,0.41;Glucose,1.98;$NaHCo_3$,1.85;$NaH_2Po_4 2H_2o$,0.94g/L).

2.21 Measurement of faecal output in locusts

Faecal output of locusts was measured after collecting the faeces produced in 48 hours by a known number of locusts kept in one cage. The faecal pellets were dried to constant weight at 80°C and the mean faecal output per insect determined. The initial number of insects per group in these experiments was 10 and the tests were conducted in triplicate. When faecal output was determined in nymphs for several days, moulting nymphs were removed from the test cages and discarded.

2.22 Test for effect of addition of plant oils on the contact poison activity of *M. volkensii* fraction B

Plant oils were added to *M. volkensii* fraction B solutions in acetone at a ratio of 2:3 (acetone : oil) and the mixture tested for contact poison activity on *L. migratoria* adults. Mortality was recorded after 48 hours. There were two sets of controls; one set was untreated while the other was treated with acetone/plant oil mixture. There were three groups of ten locusts in every test. The plant oils tested were; *M. volkensii* fruit oil, salad oil, coconut oil, castor oil and olive oil. *M. volkensii* oil was prepared by extracting 100g of ground endosperm for 6 hours 3 times with 500ml of hexane. The filtered hexane extract was then dried to constant weight at 40°C and the resulting oil stored at 4°C. The other plant oils were of technical grade. The mortality caused by the various formulations were compared by the t-test and by analysis of variance (Zar, 1984).

2.23 Tests for stomach poison activity in *M. volkensii* extracts

Potted wheat seedlings, 15cm tall, planted at a density of 500 per pot were sprayed with 6ml of *M. volkensii* fruit extract solutions in 25% ethanol. Control seedlings were sprayed with 6ml of 25% ethanol. The seedlings were dried at room temperature for two hours before being presented to 24 hour starved 5th instar *L. migratoria* nymphs or adults in rearing cages in a no choice test. After 24 hours the treated seedlings were removed and the insects fed on untreated seedlings for several days as the mortality rate was monitored daily. There were three groups of ten locusts. The LD_{50} of each extract was then estimated from the mortality data by probit analysis (Finney, 1952).

2.24 Measurement of abdominal movements in locusts

Five day old *L. migratoria* adults were mounted upside-down with adhesive tape on a stage fitted with a lever and pen system. Vertical movements were transmitted by a taut thin nylon thread anchored with a steel hook to the 3rd sternite to a kymograph recording device. After allowing the insects to acclimatize for 5 minutes, they were treated with *M. volkensii* extracts *in situ* by injection. The amplitude of abdominal movements was measured to the nearest 0.5mm while the frequency was measured to the nearest cycle.

2.25 Tests for effects of *M. volkensii* fruit extracts on the rate of peristalsis in locusts

Three day old 5th instar *L. migratoria* nymphs were used in these experiments. The insects were fed wheat bran and wheat seedlings followed by a meal on glass fibre discs (Whatman) of diameter 1cm impregnated with 0.25M sucrose. The insects were then injected with 50µg of either fraction A or B in 5µl of 60% ethanol on termination of the glass fibre disc meal. Controls were injected with 5µl of 60% ethanol. After three hours the insects were killed in Co_2, the gut dissected out and the position of the glass fibre pellet determined. There were 15 insects per group and the experiment was performed in triplicate. The pooled results were subjected to the χ^2 test (Zar, 1984) at probability 0.05.

2.26 Measurement of the rate of heartbeat in locusts

The pulsation of the heart was observed in adult locusts under a dissecting microscope through the semi-transparent dorsal areas of the 2nd and 3rd tergites. The number of pulsations in two minutes were recorded 4 times in 4 individual locusts and the mean beats per minute calculated. These determinations were done in adult *L. migratoria* paralysed within 48 hours through feeding on wheat seedlings sprayed with a 1% solution of *M. volkensii* fraction B, injection with 100µg fraction B/g of body weight and by topical treatment with fraction B dissolved in acetone:olive oil (2:3) at a dose of 1000µg/g of body weight. Control insects in every case received the solvent alone. The rate of heartbeat in control and treated insects was then compared by the t-test (Zar, 1984).

2.27 Test for chronic effects of *M. volkensii* fruit fractions in locusts.

Two to three day old *L. migratoria* 5th instar nymphs and three day old adults were used in the following experiments to test for the chronic effects of *M. volkensii* fruit extracts. Mortality was monitored in insects treated topically and by injection

with *M. volkensii* fractions A and B. Moulting was monitored in nymphs injected with fractions A, B and azadirachtin. In tests for the effect of topical treatment on moulting, only fractions A and B were used. Body weight gain and faecal output were determined in male and female nymphs injected with fractions A and B at doses of 25µg/g of body weight and azadirachtin at a dose of 2µg/g of body weight. The same parameters were determined in nymphs treated topically with fractions A and B at doses of 200µg/g of body weight. In all these experiments on chronic effects, the various determinations were done every other day. The data from tests on the effects of *M. volkensii* fractions A and B and azadirachtin on moulting in *L. migratoria* 5th instar nymphs was subjected to analysis of variance (Zar, 1984).

2.28 Measurement of oxygen consumption in locusts

Measurement of oxygen consumption in locusts was done in a simple respirometer. Ten adult *L. migratoria* males with clipped wings were placed in horizontally lying 300ml bottles containing cotton wool liberally soaked with a saturated solution of potassium hydroxide. The cotton wool was placed on a glass dish and covered with steel gauze to exclude the locusts. The bottles were then corked with a hollow rubber bung fitted with a 5ml graduated pipette. About 100ul of soapy water coloured with methylene blue was applied to the tip of the pipette so that a visible bubble was formed and was seen to migrate as the insects respired. Thus the volume of oxygen consumed could be estimated from the graduations of the pipette. The system was allowed to stabilize for 5 minutes after which volume readings were taken every 10 minutes for 30 minutes and the mean volume of oxygen consumed/min/g of body weight calculated.

2.29 Test for the effect of post-treatment temperature on the acute toxicity of *M. volkensii* fruit fractions A and B on locusts treated by injection

Five day old *L. migratoria* adults were injected with solutions of *M. volkensii* fractions A or B in 60% ethanol. Controls received the solvent alone. In one experiment, groups of 10 locusts were treated with 300µg of extract/g body weight and kept at temperatures ranging from 15 to 40^{o}C and the numbers of those paralysed in 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours at each temperature recorded. The data collected was then subjected to analysis of variance. In the second experiment, two groups of insects were treated with increasing of *M. volkensii* fraction B. One group was kept at 15^{o}C the other at 40^{o}C and the mortality in 48 hours determined. The insects were placed in well ventilated plastic cages of dimensions 15x15x15cm and fed wheat seedlings. The Relative Humidity was maintained at 55 to 60%. All the tests were

done in triplicate and there were 10 insects per replicate. The 48 hour LD_{50} of *M. volkensii* fraction B at 15°C and at 40°C was then estimated by probit analysis (Finney, 1952).

2.30 Test for the effect of post-treatment temperature on the acute toxicity of *M. volkensii* fruit fractions B in locusts treated topically.

Groups of 10 five day old *L. migratoria* adults were treated topically on the neck membrane with doses of 1000µg of *M. volkensii* fraction B per gram of body weight. Fraction B was dissolved in acetone:olive oil (2:3). Controls received solvent alone. The locusts were then kept at temperatures ranging from 15°C to 40°C and the numbers of those paralysed in 1, 1.5, 2, 3, 4, 5, 6, 12 and 24 hours at each temperature recorded. The insects were placed in well ventilated plastic cages of dimensions 15x15x15cm and fed on wheat seedlings. The Relative Humidity was maintained at 55 to 60%. All the tests were done in triplicate and there were 10 insects per replicate. The data collected was then subjected to analysis of variance (Zar, 1984).

CHAPTER THREE

RESULTS

3.1 The yield, larvicidal and antifeedant activity of extracts obtained from *M. volkensii* fruit powder with a range of ethanol and methanol concentrations.

3.1.1 The yield of dry material from *M. volkensii* fruit powder extracted with a range of ethanol and methanol concentrations

Tables 1 and 2 show the yield of dry material from 300g batches of *M. volkensii* fruit powder extracted with a range of concentrations of ethanol and methanol respectively. Water alone extracted significantly more material than 'pure' ethanol and methanol (Anova, $F_{2,6(1)}=24.794$, $P=0.0013$). The yield of material increased with rising water content in both solvents (Anova, $F_{16,34(1)}=4.180$, $P=0.0002$). In extractions with various concentrations of ethanol, the yields ranged from 0.57 ±0.05% recovered with 99.7% ethanol to 0.89 ±0.03% recovered with 20% ethanol. In the extraction with various concentrations of methanol the yields ranged from 0.61 ±0.02% recovered with 99.6% methanol to 0.91 ±0.02% recovered with 20% methanol. The yield of dry extract through water extraction was 0.96 ±0.03%.

3.1.2 The larvicidal activity of extracts obtained from *M. volkensii* fruit powder with a range of ethanol concentrations

The results of *A. aegypti* 2nd instar larvae 48 hour toxicity bioassay of material obtained by extraction of similar 300g batches of *M. volkensii* fruit powder with increasing concentrations of ethanol are presented on Table 3. The equations of the regression lines from probit mortality versus log dosage plots and the lower and upper confidence limits of the LD_{50} of each extract are shown. Concentrations of ethanol between 50% and 99.6% produced extracts with LC_{50}'s below 400ppm. Ethanol concentrations below 40% yielded extracts with low larvicidal activity with LC_{50},s above 500ppm. Highest activities were recovered by ethanol concentrations between 60% and 95% which recovered extracts with LC_{50}'s below 240ppm, while 99.7% ethanol recovered extracts with a higher LC_{50} of 268.9ppm.

3.1.3 The larvicidal activity of extracts obtained from *M. volkensii* fruit powder with a range of methanol concentrations

Table 4 shows the results of *A. aegypti* 2nd instar larvae bioassays of material obtained by extraction of similar 300g batches of *M. volkensii* fruit powder with

Table 1: **The yield of dry extract from 300g batches of *M. volkensii* fruit powder extracted with a range of ethanol concentrations.**

Concentration of ethanol (%)	Percentage yield of extract (Mean ±S.E.,n=3)
0	0.96 ± 0.036
20	0.89 ± 0.036
40	0.79 ± 0.033
50	0.80 ± 0.073
60	0.69 ± 0.076
70	0.66 ± 0.043
80	0.71 ± 0.106
95	0.56 ± 0.020
99.7	0.57 ± 0.056

Table 2: **The yield of dry extract from 300g batches of *M. volkensii* fruit powder extracted with a range of methanol concentrations.**

Concentration of methanol (%)	Percentage yield of extract (Mean ±S.E.,n=3)
0	0.96 ± 0.036
20	0.91 ± 0.023
40	0.84 ± 0.086
50	0 82 ± 0.046
60	0.76 ± 0.043
70	0.72 ± 0.033
80	0.70 ± 0.073
95	0.71 ± 0.030
99.6	0.61 ± 0.023

Table 3: **The toxicity of extracts obtained from *M. volkensii* fruit powder with a range of ethanol concentrations to *A. aegypti* 2nd instar larvae. (Number of larvae per bioassay = 20).**

Extract	Equation of regression line	R-Sq.(%)	48 hour LC_{50} (ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm)		t-Value	d.f.
				Lower	Upper		
0% Ethanol‡‡	---	---	---	---	---	---	---
20% Ethanol‡‡	---	---	---	---	---	---	---
40% Ethanol	Y=-9.76+5.34x	83.5	548.3	301.8	996.1	3.18	2
50% Ethanol	Y=-10.1+6.23x	74.1	276.9	157.1	488.1	2.93	3
60% Ethanol	Y=-8.95+5.99x	83.5	228.5	136.6	382.1	3.89*	3
70% Ethanol	Y=-11.7+7.31x	93.6	201.3	142.0	285.4	6.65*	3
80% Ethanol	Y=-7.53+5.53x	79.8	206.3	110.1	386.5	3.45*	3
95% Ethanol	Y=-2.36+3.21x	67.3	231.3	110.5	484.1	2.49	3
99.7% Ethanol	Y=-4.94+4.11x	85.5	268.9	175.1	412.9	4.20*	3

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}.

‡‡ Data insufficient for regression analysis.

Table 4: **The toxicity of extracts obtained from *M. volkensii* fruit powder with a range of methanol concentrations to *A. aegypti* 2nd instar larvae.(Number of larvae per bioassay = 20).**

Extract		Equation of regression line	R-Sq.(%)	48 hour LC_{50} (ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm)		t-Value	d.f.
					Lower	Upper		
0%	Methanol‡	---	---	---	---	---	---	---
20%	Methanol‡	---	---	---	---	---	---	---
40%	Methanol‡	---	---	---	---	---	---	---
50%	Methanol	Y=-13.2+6.89x	97.9	429.2	365.6	504.1	9.58*	2
60%	Methanol	Y=-10.1+6.30x	80.2	263.5	158.6	437.7	3.49*	3
70%	Methanol	Y=-10.2+6.40x	85.8	244.6	155.9	383.8	4.26*	3
80%	Methanol	Y=-8.36+5.73x	81.1	231.5	134.1	399.1	3.59*	3
95%	Methanol	Y=-9.48+6.14x	78.8	244.6	140.4	426.2	3.34*	3
99.6%	Methanol	Y=-11.5+6.84x	83.8	266.6	169.0	420.3	3.94*	3

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}.

‡ Data insufficient for regression analysis.

increasing concentrations of methanol. The equation of regression lines from probit mortality versus log dosage plots and the confidence limits of the LD_{50}'s are shown. Concentrations of methanol between 60% and 99.6% produced extracts with LC_{50}'s below 300ppm. The highest activities were recovered by methanol at concentrations of 70% and 95% which recovered extracts with LC_{50}'s below 250ppm. Methanol concentrations below 50% yielded extracts with low larvicidal activity, their LC_{50}'s being above 500ppm. As was the case with ethanol extractions, extraction of the fruit powder with 99.6% methanol resulted in extracts with a high LC_{50} of 266.6ppm.

3.1.4 The antifeedant activity of extracts obtained from *M. volkensii* fruit powder with a range of ethanol and methanol concentrations

The results of paper antifeedant tests on 24 hour starved *L. migratoria* 5th instar nymphs of extracts resulting from extraction of *M. volkensii* fruit powder with increasing concentrations of ethanol and methanol are presented on Tables 5 and 6 respectively. The extracts were tested at concentrations of 1000ppm, 500ppm, 100ppm and 50ppm. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50} of each extract are shown. Extraction of *M. volkensii* fruit powder with ethanol concentrations of 50% and above resulted in extracts giving 24 hour antifeedant LD_{50}'s below 240ppm. Extraction with methanol at concentrations of 60% and above yielded extracts giving 24 hour antifeedant LD_{50}'s below 260ppm. Water extracts tested at a similar concentrations had very low antifeedant activity giving a 24 hour feeding protection of less than 3% in 24 hours. The trend of antifeedant activity in the extracts was similar to that observed in the *A. aegypti* 2nd instar larvae bioassay. Material recovered from *M. volkensii* fruit powder with low concentrations of ethanol or methanol showed low antifeedant activity.

3.2 Distribution of biologically active substances in different parts of *Melia volkensii*

3.2.1 Weight distribution of various tissues of *M. volkensii* fruit

The weight of various dry tissues from 1000g of *M. volkensii* fruit is shown on Table 7. The weights of dry tissue recovered from the dissected fruit were significantly different ($F_{3,8(1)}=7.8326$, $P=0.000$). The weight of material recovered from endocarp and fruit coat was 588 ±15.5g and 354.3 ±10.7g respectively. Endosperm and testa produced small quantities of 41.3 ±7g and 19.6 ±2g respectively.

Table 5: **The locust antifeedant activity of extracts resulting from the extraction of *M. volkensii* fruit powder with a range of ethanol concentrations (Paper test).**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
0% Ethanol‡	---	---	---	---	---
20% Ethanol	Y=-0.20+1.93x	95.4	494.7	4.558	1
40% Ethanol	Y=-1.44+2.48x	98.5	395.2	8.062	1
50% Ethanol	Y=-0.99+2.52x	93.6	238.2	3.836	1
60% Ethanol	Y=-0.89+2.56x	98.9	199.9	13.247*	2
70% Ethanol	Y= 0.45+2.07x	97.0	157.8	8.000*	2
80% Ethanol	Y=-2.62+3.56x	91.9	138.2	4.777*	2
95% Ethanol	Y=-0.84+2.54x	96.8	199.2	5.465	1
99.7% Ethanol	Y=-0.52+2.43x	98.9	186.9	9.273	1

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}

‡ Data insufficient for regression analysis.

Table 6: **The locust antifeedant activity of extracts resulting from the extraction of *M. volkensii* fruit powder with a range of methanol concentrations (Paper test, n=3).**

Extract		Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
0%	Methanol‡	---	---	---	---	---
20%	Methanol‡	---	---	---	---	---
40%	Methanol‡	---	---	---	---	---
50%	Methanol‡	---	---	---	---	---
60%	Methanol	Y=-1.38+2.69x	98.2	235.4	7.370	1
70%	Methanol	Y=-2.06+3.05x	87.1	206.4	2.605	1
80%	Methanol	Y=-0.66+2.56x	96.5	162.5	7.386*	2
95%	Methanol	Y=-1.34+2.71x	97.3	218.5	8.447*	2
99.6%	Methanol	Y=-1.87+2.86x	98.4	252.4	7.902*	1

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}.

‡ Data insufficient for regression analysis.

Table 7: Weight distribution of various tissues of 1000g of *M. volkensii* fruit.

Part of fruit	Mean weight of fruit part (g ±S E.,n=3)
Fruit coat	354.3 ±10.7
Endocarp	585.0 ±15.5
Endosperm	41.3 ±7.0
Testa	19.6 ±2.0

3.2.2 The yield of 80% methanol soluble material from different parts of *M. volkensii*

The weight of 80% methanol soluble material from 300g batches of powder from different parts of *M. volkensii* is presented in Table 8 The weight of 80% methanol soluble material recovered from various tissues varied considerably ($F_{9,20(1)}=135.82$, $P=0.000$). Root bark, leaves and fruit coat had the highest yields of over 35g per 300g of plant powder while testa and endosperm had the lowest yields of 6.3 ±0.44g and 4.83 ±0.73g per 300g of plant powder respectively. All parts of *M. volkensii* therefore contained 80% methanol soluble substances.

3.2.3 The larvicidal activity of 80% methanol extracts of different parts of *M. volkensii*

The results of the *A.aegypti* 2nd instar larvae 48 hour mortality bioassays of 80% methanol extracts of various parts of *M. volkensii* are presented in Table 9. The equations of regression lines from probit mortality versus log dosage and the LC_{50}'s are presented. Extracts of testa and root bark had high larvicidal activity with LC_{50}'s of 95.4ppm and 120.3ppm respectively. Extracts of whole fruit, fruit coat, endocarp and root wood had larvicidal activity with LC_{50}'s ranging from 216.5ppm to 263.3ppm. Stem bark and stem wood had activities with LC_{50}'s of 455.7ppm and 502.3ppm, respectively while endosperm had a higher LC_{50} of 754.7ppm. Leaf extracts had the lowest larvicidal activity with an LC_{50} of 1490.1ppm .

3.2.4 Antifeedant activity of 80% methanol extracts of different parts of *M. volkensii*

Table 10 shows the results of paper antifeedant tests of 80% methanol extracts of various parts of *M. volkensii*. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50} of each extract are presented. Extracts from whole fruit, fruit coat, endocarp, testa and root bark had the highest antifeedant activity giving antifeedant ED_{50}'s below 120ppm. Stem wood, and root wood had moderate activity with ED_{50}'s of 328.9ppm and 211.1ppm, respectively. Extracts from leaves had low antifeedant activity with an ED_{50} of 1234.2ppm. No feeding protection was produced by endosperm extracts even at a concentration of 2000ppm.

Table 11 shows the results of antifeedant tests on *L. migratoria* 5th instar nymphs carried out with wheat seedlings sprayed with up to 3000ppm solutions of 80% methanol extracts of various parts of *M. volkensii*. As in the paper antifeedant tests, the equations of regression lines from probit feeding protection versus log concentration

Table 8: **The yield of 80% methanol extract from 300g batches of powder from various parts of *M. volkensii*.**

Plant part	Weight of extract in grams (Mean ±S.E ,n=3)
Whole fruit	25.5 ± 2.02
Fruit coat	37.5 ± 1.44
Endocarp	14.4 ± 1.16
Endosperm	4.8 ± 0.73
Testa	6.3 ± 0.44
Stem wood	16.3 ± 0.44
Stembark	15.9 ± 0.87
Leaves	45.0 ± 1.15
Root wood	13.2 ± 1.17
Root bark	35.7 ± 1.45

Table 9: **The toxicity of 80% methanol extracts of different parts of *M. volkensii to A. aegypti* 2nd instar larvae (Number of larvae per bioassay = 20).**

Part of plant	Equation of regression line	R-Sq.(%)	48 hour LC_{50}(ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm) Lower	Upper	t-Value	d.f.
Whole fruit	Y=-14.0+7.93x	94.1	253.9	223.7	288.1	9.81*	6
Fruit coat	Y=-8.88+5.68x	95.1	276.4	246.4	310.0	10.81*	6
Endocarp	Y=-7.42+5.33x	85.5	216.5	174.9	268.0	6.43*	7
Endosperm	Y=-1.75+2.27x	82.3	754.7	446.2	1276.7	5.29*	6
Testa	Y=-5.00+5.26x	83.3	95.4	62.30	146.0	5.90*	7
Stem wood	Y=-9.10+4.57x	24.8	502.3	168.8	1495.5	1.00	3
Stem bark	Y=-2.55+2.82x	89.0	455.7	359.4	577.8	6.36*	5
Leaves	Y=-3.32+2.50x	77.5	1490.7	176.9	12560.2	2.62	2
Root wood	Y=-3.03+3.32x	94.2	263.3	231.4	299.7	8.99*	5
Root bark	Y=-6.90+5.81x	90.1	120.3	92.4	155.6	7.98*	7

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}.

Table 10: **The locust antifeedant activity of 80% methanol extracts of various parts of *M. volkensii* (Filter paper test).**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
Whole fruit	Y=-0.03+2.59x	97.4	87.5	12.329*	4
Fruit coat	Y= 3.14+1.35x	41.1	21.9	1.669	4
Endocarp	Y=-1.00+3.02x	95.3	96.9	7.769*	3
Testa	Y= 0.42+2.61x	84.8	119.3	4.089*	3
Stem wood	Y=-1.62+2.63x	99.7	328.9	32.424*	3
Stem bark	Y= 0.66+2.35x	95.5	70.3	9.181*	4
Leaves	Y=-0.75+1.86x	94.6	1234.2	7.241*	3
Root wood	Y=-0.23+2.25x	93.0	211.1	6.331*	3
Root bark	Y= 0.35+2.38x	95.2	90.0	8.885*	4

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}.

Table 11: **The locust antifeedant activity of 80% methanol extracts of various parts of *M. volkensii* (Wheat seedling test).**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
Whole fruit	Y=-5.20+3.13x	99.1	1814.6	15.000*	2
Fruit coat	Y=-0.16+1.56x	95.0	2030.9	7.575*	3
Endocarp	Y= 0.09+1.63x	96.0	1028.7	8.459*	3
Testa	Y=-0.34+1.92x	92.7	604.3	6.165*	3
Stem wood	Y=-5.08+2.79x	99.8	$1.9X10^7$	22.147*	1
Stem bark	Y=-2.12+2.22x	99.9	1611.4	222.022*	2
Leaves	Y=-4.71+2.69x	99.6	4070.7	15.551*	1
Root wood	Y=-1.93+2.18x	99.9	1509.7	41.824*	2
Root bark	Y=-0.24+1.74x	97.5	1026.8	10.913*	3

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}

plots and the 24 hour ED_{50} of each extract are shown. The antifeedant activity pattern generally corresponded with the results obtained in the paper feeding protection tests. Extracts of whole fruit, fruit coat, endocarp, testa, stem bark, root wood and root bark had moderate antifeedant activity with ED_{50}'s below 2040ppm. Extracts of stem wood and leaves had low antifeedant activity with ED_{50}'s of $1.9X10^7$ppm and 4070.7ppm respectively. Extracts of endosperm did not show antifeedant activity even at the high concentration of 3000ppm.

3.3 The biological activity and yield of crude extracts and fractions of *M. volkensii* fruit extracts from Embu and Tsavo areas

3.3.1 The yield of crude extracts and fractions from *M. volkensii* fruit from Tsavo and Embu areas

Results presented on Table 12 show the total yields of 80% methanol extract, methanol fraction, acetone fraction, fraction A and fraction B from 1Kg batches of *M. volkensii* fruits from Tsavo and Embu areas. The yields of these extracts and fractions from fruits collected from both areas were not significantly different (t-test, $P > 0.05$).

3.3.2 Larvicidal and antifeedant activity of extracts and fractions from *M. volkensii* fruit from Tsavo and Embu

3.3.2.1 Larvicidal activity of extracts and fractions of *M. volkensii* fruit from Embu and Tsavo

In the *A. aegypti* 2nd instar larvae 48 hour mortality bioassay, the 80% methanol extracts, the resultant acetone fractions and fractions A and B prepared from *M. volkensii* fruits from Tsavo and Embu areas had comparable activities. The equations of regression lines from log dosage versus probit mortality plots and the confidence limits of the LC_{50}'s of these extracts and fractions are shown in Table 13. Extraction of fruit from both areas with 80% methanol produced material with LC_{50}'s below 250ppm while the acetone fractions had LC_{50}'s below 70ppm. Fractions A and B resulting from the fractionation of the 80% methanol extracts both had LC_{50}'s below 60ppm.

3.3.2.2 Antifeedant activity of extracts and fractions of *M. volkensii* fruit from Tsavo and Embu

Results of comparative paper antifeedant tests carried out on *L. migratoria* 5th instar nymphs with 80% methanol extracts of *M. volkensii* fruit powder from Tsavo and

Table 12: The yield of extracts and fractions from 1 kg of *M. volkensii* fruit from Tsavo and Embu.

Fraction	Mean weight of extracts and fractions in grams ±S.E.(n=3)			
	Area of collection			
	Tsavo	Embu	t-Value	d.f.
80% Methanol	30.7 ± 0.66	29.7 ± 0.33	-1.34 Ns	4
Methanol	20.7 ± 1.20	23.0 ± 1.00	1.49 Ns	4
Acetone	11.8 ± 0.60	11.3 ± 0.66	-0.56 Ns	4
A	6.8 ± 0.15	6.9 ± 0.37	0.24 Ns	4
B	1.4 ± 0.05	1.5 ± 0.05	1.22 Ns	4

Ns = Not significantly different, $P < 0.05$.

Table 13: **The toxicity of extracts and fractions of *M. volkensii* fruit from Embu and Tsavo to *A. aegypti* 2nd instar larvae (Number of larvae per bioassay = 20).**

Source of extract/fraction	Equation of regression line	R-Sq.(%)	48 hour LC_{50}(ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm) Lower	Upper	t-Value	d.f.
Tsavo A	Y=-1.830+4.03x	86.7	53.1	35.9	78.4	5.71*	5
Embu A	Y=-0.824+3.33x	92.8	56.6	50.6	63.3	20.22*	5
Tsavo B	Y=-1.370+4.12x	98.8	39.3	24.6	62.8	5.76*	5
Embu B	Y=-1.890+4.42x	86.9	66.1	55.7	77.9	8.03*	5
Tsavo Acetone	Y=-0.612+3.09x	96.8	66.1	55.7	77.9	12.26*	5
Embu Acetone	Y=-3.080+4.47x	85.3	66.8	46.3	96.5	5.39*	5
Tsavo Crude	Y=-12.80+7.43x	93.5	246.9	215.8	282.5	9.32*	6
Embu Crude	Y=-14.50+8.25x	90.1	236.1	199.2	279.9	7.39*	6

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}.

Embu and the subsequent fractions A and B are presented in Table 14. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50} of each extract are shown. In the tests, 80% methanol extracts of fruits from Tsavo had an ED_{50} of 285.5ppm. A similar extract of fruit from Embu had an ED_{50} of 276.8ppm. Fractions A and B derived from fruits from both areas showed greater antifeedant activity with ED_{50}'s below 120ppm.

3.3.2.3 The toxicity of injected *M. volkensii* fruit extracts and fractions on *L. migratoria* 5th instar nymphs

The 48 hour mortality in *L. migratoria* 5th instar nymphs injected with *M. volkensii* fruit 80% methanol extract, acetone fraction and fractions A and B is shown in Table 15. The regression equations of probit mortality versus log dosage plots and the confidence limits of the LD_{50}'s are presented. The 80% methanol extracts had low activity with an LD_{50} of 209.1µg/g of body weight while the acetone extract had moderate activity with an LD_{50} of 88.4µg/g of body weight. Fractions A and B exhibited high toxicity with LD_{50}'s of 51.7 and 33.6µg/g of body weight respectively. The pattern of the toxicity of injected *M. volkensii* extracts and fractions on locusts was apparently similar to that observed in the *A. aegypti* 2nd instar larvae bioassay.

3.3.2.4 The toxicity of topically applied *M. volkensii* fruit fractions A and B on *L. migratoria* 5th instar nymphs

The equations of regression lines of probit mortality versus log dosage plots and the confidence limits of the LD_{50}'s of fractions A and B applied topically on *L. migratoria* 5th instar nymphs are shown in Table 16. Fraction B showed considerable toxicity with an LD_{50} of 2091.7µg/g of body weight in 48 hours. Fraction A caused very low mortality even at the highest dose of 2000µg/g of body weight tested. This difference in the activity of *M. volkensii* fruit fractions A and B when applied topically on locusts was not apparent when the same fractions were administered through injection.

3.4 The biological activity and yield of 80% methanol soluble material from ripe and unripe *M. volkensii* fruit

3.4.1 The yield of 80% methanol soluble material from ripe and unripe *M. volkensii* fruit

Both ripe and mature unripe *M. volkensii* fruit produced similar amounts of dry 80% methanol extract. Beginning with 300g of each fruit powder ripe fruit produced

Table 14: **The locust antifeedant activity of 80% methanol crude extracts and fractions A and B derived from *M. volkensii* fruit from Tsavo and Embu areas (Filter paper test).**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
Embu crude	Y=-4.89+4.05x	98.8	276.8	12.763*	2
Tsavo crude	Y=-5.51+4.28x	98.9	285.5	13.511*	2
Embu A	Y=-1.57+3.17x	94.6	118.2	7.283*	3
Tsavo A	Y=-2.03+3.27x	97.2	141.2	10.287*	3
Embu B	Y=-0.96+2.88x	97.5	117.3	10.787*	3
Tsavo B	Y=-0.66+2.87x	92.0	93.8	5.904*	3

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}.

Table 15: **The toxicity of injected *M. volkensii* fruit extracts and fractions on *L. migratoria* 5th instar nymphs.(Number of nymphs per bioassay =10).**

Extract/fraction	Equation of regression line	R-Sq.(%)	48 hour LC_{50}(ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm) Lower	Upper	t-Value	d.f.
A	Y=-3.57+5.00x	95.2	51.7	41.1	65	7.71*	3
B	Y=-0.64+3.43x	94.4	43.8	33.6	57.2	8.20*	4
Acetone	Y=-4.58+4.92x	98.1	88.4	76.9	101.5	12.43*	3
Crude	Y=-5.63+4.57x	90.2	209.1	144.9	301.6	5.25*	3

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}.

Table 16: **The toxicity of topically applied *M. volkensii* fruit fractions A and B on *L. migratoria* 5th instar nymphs.(Number of nymphs per bioassay =10).**

Fraction	Equation of regression line	R-Sq.(%)	48 hour LD_{50}(ug/g)•	95% Confidence limit for 48 hour LD_{50} (ug/g) Lower	Upper	t-Value	d.f.
A	---	---	>2000‡	---	---	---	---
B	Y=-1.99+2.09x	94.4	2091.7	1028	4255	5.80*	2

* Significant at $P < 0.05$

• Calculated Log LD_{50} transformed to LD_{50}.

‡ Insufficient data for regression analysis.

6.93 ±0.06g of extract while unripe mature fruit produced 6.83 ±0.2g of extract. These yields were not significantly different, ($t=0.47$;d.f.=4, $P>0.05$).

3.4.2 The larvicidal activity in ripe and unripe *M. volkensii* fruit

The results of *A. aegypti* 2nd instar larvae bioassay of 80% methanol extracts of ripe and unripe *M. volkensii* fruit are shown in Table 17. The equations of regression lines from probit mortality versus log dosage plots are presented. The activities of the two extracts were not significantly different. Extracts from ripe fruits had an LC_{50} of 270.2ppm while that from unripe fruits had an LC_{50} of 282.7ppm in 48 hours.

3.4.3 The antifeedant activity in ripe and unripe *M. volkensii* fruit

Results of paper antifeedant tests on 24 hour starved *L. migratoria* 5th instar nymphs of 80% methanol extracts of ripe and unripe *M. volkensii* fruit are presented in Table 18. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50} of both extracts are shown. Extracts from both types of fruits showed feeding protection of 65% at a concentration of 500ppm. The 80% methanol extracts of ripe fruits had an ED_{50} of 323.9ppm, while extracts of unripe fruits had an ED_{50} of 308.4ppm.

3.5 Improved procedures for the recovery of biologically active substances from *M. volkensii* fruit

3.5.1 Improved recovery of biologically active substances from 80% methanol *M. volkensii* fruit extract by precipitation at low temperatures

The yields of "acetone step" fractions of *M. volkensii* fruit obtained through the standard purification procedure (Figure 2) and through the cold precipitation procedure (Figure 3) are shown in Table 19. Beginning with 1L batches of standard 80% methanol extract solutions, the yield of "acetone step" fractions through the standard procedure was 2.66 ±0.04g while through the cold precipitation procedure the yield of a similar fraction was 3.33 ±0.09g. The cold precipitation procedure yielded significantly more active fraction than the standard procedure ($t=-6.83$,d.f=4 $P<0.05$). The yield of the less active acetone precipitate and water supernatant was 3.73 ±0.12g and 3.82 ±0.15g, respectively. These yields were not significantly different, ($t=-0.45$;d.f=4,$P>0.05$).

The results of *A. aegypti* bioassays of "acetone step" fractions obtained from 80% methanol extracts of *M. volkensii* fruit through the standard procedure and those obtained by the cold precipitation procedure are presented in Table 20. The

Table 17: **The toxicity of 80% methanol extracts of ripe and unripe *M. volkensii* fruits to *A. aegypti* 2nd instar larvae (Number of larvae per bioassay = 20).**

Condition of fruit	Equation of regression line	R-Sq.(%)	48 hour LC_{50}(ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm) Lower	Upper	t-Value	d.f
Ripe	Y=-11.6+6.83x	98.2	270.2	248.2	294.1	12.88*	3
Unripe	Y=-11.3+6.67x	99.4	282.7	269.3	296.7	21.81*	3

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}.

Table 18: **The locust antifeedant activity of 80% methanol extracts of ripe and unripe *M. volkensii* fruit (Filter paper test).**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
Ripe fruit	Y=-5.87+4.33x	98.8	323.9	12.801*	2
Unripe fruit	Y=-5.23x+4.11x	97.8	308.4	9.423*	2

* Significant at $P < 0.05$.
• Calculated Log ED_{50} transformed to ED_{50}.

Table 19: **The yield of "acetone step" *M. volkensii* fruit fractions through the standard extraction procedure and the cold precipitation procedure.(n=3).**

Yield of fractions from 1L of standard 80% methanol extract (g ±S.E)			
Fractions of standard procedure		Fractions from cold precipitation	
Acetone supernatant	Acetone precipitate	Water Precipitate	Water supernatant
2.66 ± 0.04	3.73 ± 0.12	3.33 ± 0.09	3.82 ± 0.15

Table 20: **The toxicity of "acetone step" material recovered from 80% methanol *M. volkensii* fruit extract through the standard procedure and by the cold precipitation procedure to *A. aegypti* 2nd instar larvae (Number of larvae per bioassay = 20).**

Fraction	Equation of regression line	R-Sq.(%)	48 hour LC_{50}(ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm) Lower	Upper	t-Value	d.f.
Acetone supernatant	Y=-2.91+4.37x	95.9	67.3	47.9	94.5	10.82*	5
Water precipitate	Y=-2.31+3.87x	96	79.7	58.4	108.7	10.89*	5
Acetone precipitate	Y=-25.1+10.4x	76.5	684.9	289.3	1620.7	2.55	2
Water supernatant	Y=-14.4+7.18x	88.6	495.3	364.7	672.7	3.95	2

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}.

larvicidal activities of the highly active end products from both procedures were quite similar. The regression equations from probit mortality versus log dosage plots and the LD_{50}'s are shown. In these bioassays the LC_{50}'s of the acetone precipitate and the water supernatant were 684.9ppm and 495.3ppm respectively, while that of the acetone supernatant and the water precipitate was 67.3ppm and 79.7ppm, respectively.

Results of antifeedant tests of the 'acetone step' products and of the standard extraction procedure and the cold precipitation products are presented in Table 21. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50}'s of the acetone supernatant, water precipitate, acetone precipitate and water supernatant fractions are shown. The acetone supernatant and the water precipitate both showed high antifeedant activity with ED_{50}'s of 155.2ppm and 169.2ppm respectively. The acetone precipitate and water supernatant exhibited low antifeedant activity giving 24 hour ED_{50}'s of 1477.1ppm and 1052.5ppm, respectively.

3.5.2 Improved recovery of biologically active material from *M. volkensii* fruit powder by pre-washing with water

In this procedure, extraction of water pre-washed *M. volkensii* fruit powder with 80% methanol produced a fast drying active extract. Through this novel procedure, 4.66 ±0.33g of solid was recovered from 500g of fruit powder, a yield of about 0.94% while the same weight of powder extracted by the standard procedure produced 12.50 ±0.68g, a yield of 2.5%. These yields were significantly different ($t=-10.31$;d.f.=4,$P<0.05$). The equation of regression lines from probit mortality versus log dosage plots and the LD_{50}'s of these extracts in the *A. aegypti* 2nd instar larvae bioassay are presented in Table 22. In this bioassay, the 80% methanol of *M. volkensii* fruit powder pre-washed with water had an LD_{50} of 71ppm . This was much lower than the LD_{50} of standard 80% methanol extract of *M. volkensii* fruit which had an LD_{50} of 183.2ppm. The activity of this material recovered in the water pre-washing procedure was comparable to that of the acetone extract in the standard extraction procedure which had an LC_{50} of 67ppm in the *A. aegypti* bioassay. The results on the antifeedant activity of the same two extracts are shown in Table 23. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50} of both extracts are shown. The extract derived from *M. volkensii* powder pre-washed with water had an antifeedant ED_{50} of 139.5ppm, while the standard 80% methanol extract had an ED_{50} of 249.1ppm. Pre-washing *M. volkensii* fruit powder with water therefore increased the larvicidal and antifeedant activity of the resultant 80% methanol extract.

Table 21: **The locust antifeedant activity of "acetone step" material recovered from 80% methanol *M. volkensii* fruit extract through the standard extraction procedure and the cold precipitation procedure(Filter paper test).**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
Acetone supernatant	Y=-2.69+3.51x	93.9	155.2	6.810*	3
Water precipitate	Y=-2.51+3.37x	96.0	169.2	8.443*	3
Acetone precipitate	Y=-0 80+1.83x	99.1	1477.1	14.933*	2
Water supernatant	Y=-4.52+3.15x	99.1	1052.5	10.789	1

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}

Table 22: **The toxicity of 80% methanol extracts of standard *M. volkensii* fruit powder and that of powder pre-washed with water to *A. aegypti* 2nd instar larvae (Number of larvae per bioassay = 20).**

Fraction	Equation of regression line	R-Sq.(%)	48 hour LC_{50}(ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm) Lower	Upper	t-Value	d f
Standard procedure	Y=-0 806−2 56x	97 7	183.2	139 8	240.2	11 18*	3
Water pre-wash procedure	Y=-2 86−4 25x	95 6	71	52 9	95 3	9 37*	4

* Significant at P < 0 05

• Calculated Log LC_{50} transformed to LC_{50}

Table 23: **The locust antifeedant activity of standard 80% methanol extracts of *M. volkensii* fruit powder and that of powder pre-washed with water (Filter paper test).**

Extract	Equation of regression line	R-Sq (%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
Standard procedure	Y=-4.25+3.86x	95.6	249.1	6.577*	2
Pre-wash procedure	Y=-2.27+3.39x	94.6	139.5	7.247*	3

* Significant at $P < 0.05$

• Calculated Log ED_{50} transformed to ED_{50}

3.6 Effect of prolonged storage on biological activity of *M. volkensii* fruit extracts

3.6.1 The larvicidal and antifeedant activity of methanolic solutions of *M. volkensii* fractions A and B stored for two years at $4^{O}C$ and at room temperature

In the present study, *M. volkensii* fractions A and B were tested for both larvicidal and antifeedant activity after storage for 2 years as methanolic solutions at a concentration of 100µg/ul. One set of solutions was stored at $4^{O}C$, the other at room temperature. The regresssion equations from probit mortality versus log dosage plots and the LC_{50}'s of the stored and freshly prepared fractions A and B tested in the *A. aegypti* 2^{nd} instar larvae bioassay are presented in Table 24. The LC_{50} of fractions stored at both room temperature and at $4^{O}C$ was similar to that of a freshly prepared extract. Both extracts had LC_{50}'s below 56ppm. Likewise, the LC_{50} of fraction B stored at both room temperature and at $4^{O}C$ was very close to that of a freshly prepared extract. Both had LC_{50}'s below 40ppm.

Results of paper antifeedant tests of all samples are presented in Table 25. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50}'s of fresh and stored fractions A and B are shown. All samples showed high antifeedant activity with ED_{50}'s below 160ppm.

3.6.2 Larvicidal and antifeedant activity of 80% methanol extract of *M. volkensii* fruit stored at room temperature for seven years

The equations of regression lines from probit mortality versus dosage plots and LC_{50}'s in the *A. aegypti* 2^{nd} instar larvae bioassay of 80% methanol extracts of fresh *M. volkensii* powder and that of powder stored soaked in 80% methanol for 7 years are presented in Table 26. The 7 year old extract had an LC_{50} of 223.6ppm which was comparable to an LC_{50} of 247.9ppm in fresh 80% methanol extracts.

Results of paper antifeedant tests of the 7 year old extract compared to those of fresh extracts are shown in Table 27. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50}'s of both extracts are shown. The antifeedant activity of the two extracts did not differ substantially. Freshly prepared 80% methanol extract had an ED_{50} of 197.2ppm, while the seven year old extract had a similar ED_{50} of 213.1ppm.

Table 24: **The toxicity of methanolic solutions of *M. volkensii* fruit fractions A and B stored at 4°C and at room temperature for 2 years to *A. aegypti* 2nd instar larvae (Number of larvae per bioassay = 20).**

Fraction	Equation of regression line	R-Sq (%)	48 hour LC_{50}(ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm) Lower	Upper	t-Value	d.f.
A at RT	Y=-0.382+3.10x	98.8	54.8	47.8	62.8	15.84*	3
A at 4°C	Y=-0.891+3.45x	98.8	52.3	45.4	60.2	15.51*	3
B at RT	Y=-1.100+3.93x	98.6	35.9	29.9	43.1	14.45*	3
B at 4°c	Y=-3.120+5.24x	93.5	36.6	24.8	54.1	6.54*	3
Fresh A	Y=-1.510+3.74x	96	55.1	42.8	70.9	8.44*	3
Fresh B	Y=-2.780+4.95x	86	39.8	23.1	68.7	4.28*	3

* Significant at $P < 0.05$. • Calculated Log LC_{50} transformed to LC_{50}

Key: A at RT = Fraction A stored at room temperature - A at 4°C= Fraction A stored at 4°C
B at RT = Fraction B stored at room temperature B at 4°C= Fraction B stored at 4°C
Fresh A = Freshly prepared fraction A Fresh B= Freshly prepared fraction B

Table 25: **The locust antifeedant activity of freshly prepared *M. volkensii* fruit fractions A and B and that of A and B stored for two years as methanolic solutions at room temperature and at 4°C (Filter paper test).**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
A at 4°C	Y=-1.90+3.30x	94.9	123.3	7.471*	3
A at RT	Y=-1.66+3.21x	94.6	118.8	7.267*	3
Fresh A	Y=-2.81+3.58x	94.9	151.9	7.501*	3
B at 4°C	Y=-1.57+3.17x	93.6	118.2	6.631*	3
B at RT	Y=-0.68+2.81x	99.6	105.0	9.236*	3
Fresh B	Y=-1.42+3.13x	94.7	112.5	7.293*	3

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}.

Key: A at RT = Fraction A stored at room temperature. A at 4°C= Fraction A stored at 4°C
B at RT = Fraction B stored at room temperature B at 4°C= Fraction B stored at 4°C.
Fresh A = Freshly prepared fraction A. Fresh B= Freshly prepared fraction B

Table 26: **The toxicity of 80% methanol extracts of *M. volkensii* fruit stored at room temperature for 7 years to *A. aegypti* 2nd instar larvae (Number of larvae per bioassay = 20).**

Extract	Equation of regression line	R-Sq.(%)	48 hour LC_{50}(ppm)•	95% Confidence limit for 48 hour LC_{50} (ppm)		t-Value	d f.
				Lower	Upper		
Fresh	Y=-9.71+6.14x	95.1	247.9	212.9	288.9	8.85*	4
Stored	Y=-13.4+7.86x	90.1	223.6	179.4	278.7	6.05*	4

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}.

Table 27: **The locust antifeedant activity of freshly prepared 80% methanol extract of *M. volkensii* fruit and that of a similar extract stored at room temperature for seven years (Filter paper test).**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
Fresh extract	Y=-2.55+3.29x	96.5	197.2	9.086*	3
Seven year old	Y=-3.08+3.47x	97.0	213.1	8.083*	2

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}

3.7 Yields and antifeedant activity of thin layer chromatography fractions of *M. volkensii* fruit

3.7.1 Yield and RF values of TLC fractions of *M. volkensii* fractions A and B

Thin layer chromatography of *M. volkensii* fractions A and B using chloroform:acetone (7.3) resulted in 7 bands in both cases which were visualised with long wave ultraviolet light (340nm). The RF values and weight of dry material recovered through elution of bands resulting from thin layer chromatography of *M. volkensii* fraction A are presented in Table 28. Most of the material was recovered in the more lipophilic bands A1 and A2 (Anova, $F_{6,14(1)}=309.79$, $P=0.000$). Similar results on the chromatography of *M. volkensii* fraction B are presented in Table 29. Unlike the results on the chromatography of fraction A, the recovery of material from fraction B was not restricted to few bands. It was instead spread out over bands B1, B2, B3, B4 and B5 (Anova, $F_{6,14(1)}=59.26$, $P=0.000$).

3.7.2 Antifeedant activity of TLC fractions of *M. volkensii* fruit

Data on the antifeedant activity of fractions A and B TLC fractions is presented in Tables 30 and 31 alongside that of fractions A and B. The equations of regression lines from probit feeding protection versus log concentration plots and the 24 hour ED_{50}'s of the fractions are shown. The results show significant enhancement of antifeedant activity through TLC purification. Thin layer chromatography fractions A3, A4 and A5 had higher activity than unfractionated fraction A. These TLC fractions of fraction A had ED_{50}'s below 112ppm, while fraction A had lower activity with an ED_{50} of 128.5ppm. Similarly, TLC fractions B2, B3 and B4 all showed higher antifeedant activity than unfractionated fraction B. These TLC fractions of fraction B had ED_{50}'s below 100ppm, while fraction B had a lower activity with an ED_{50} of 169.5ppm. There was a difference in the activities of fractions eluted from the origin of migration in the TLC of *M. volkensii* fractions A and B. The fraction eluted from the origin in the chromatography of A had no measurable antifeedant activity while the corresponding fraction from B had considerable antifeedant activity with an ED_{50} of 340.8ppm.

3.8 The chronic effects of *M. volkensii* fractions A and B on *L. migratoria*

In the following experiments the effects of topically applied and injected *M. volkensii* fractions A and B on moulting, growth rate and mortality in locusts was

Table 28: **The RF value and yield of TLC fractions of *M. volkensii* fraction A.(The solvent system was chloroform:acetone (7:3)(V/V).)**

Fraction/Band	RF value	Mean weight (mg±S.E.)of TLC fractions recovered from 1200mg of fraction A
A1	0.93	102.3 ± 4.74
A2	0.80	93.3 ± 3.52
A3	0.66	30.4 ± 0.43
A4	0.56	13.5 ± 1.29
A5	0.34	14.6 ± 2.10
A6	0.18	2.3 ± 0.16
A7	0	2.2 ± 0.20
Total	---	258.7 ± 5.67

Table 29: **The RF value and yield of TLC fractions of *M. volkensii* fraction B.(The solvent system was chloroform:acetone (7:3)(V/V).)**

Fraction/Band	RF value	Mean weight(mg±S.E)of TLC fractions recovered from 1200mg of fraction B
B1	0.93	45.0 ± 4.33
B2	0.84	56.7 ± 3.33
B3	0.75	64.0 ± 1.06
B4	0.62	46.7 ± 3.52
B5	0:35	39.0 ± 5.62
B6	0.11	1.3 ± 0.20
B7	0	1.3 ± 0.10
Total	---	253.9 ± 6.07

Table 30: **The locust antifeedant activity of TLC fractions of *M. volkensii* fraction A (Filter paper test)**

Extract	Equation of regression line	R-Sq (%)	24 hour ED_{50}•(ppm)	t-Value	d.f.
A1	Y=-2.93+3.27x	85.1	266.1	3.388	2
A2	Y=-3.09+3.43x	95.2	228.3	6.272*	2
A3	Y=-0.41+2.83x	95.4	81.6	7.929*	3
A4	Y=-0.19+2.92x	89.4	59.9	5.026*	3
A5	Y=-1.37+3.11x	94.0	111.7	6.850*	3
A6	Y=-15.29+7.21x	98.1	651.8	7.182	1
Fraction A	Y=-1.98+3.31x	94.3	128.5	7.028*	3

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}

Table 31: **The locust antifeedant activity of TLC fractions of *M. volkensii* fraction B (Filter paper test)**

Extract	Equation of regression line	R-Sq.(%)	24 hour ED_{50}•(ppm)	t-Value	d f.
B1	Y=-3.41+3.50x	93.5	252 8	5.362*	2
B2	Y= 0.35+2.49x	98.0	140.8	11.978*	3
B3	Y= 0.38+2.73x	89.7	93.5	5.118*	3
B4	Y=-0.76+2.95x	96.0	89.6	8.492*	3
B5	Y=-0.35+2.79x	99.7	82.7	32.836*	3
B6	Y=-2.39+3.44x	94.9	140.7	7.444*	3
B7	Y=-5.89+4.30x	98.0	340.8	9.733*	2
Fraction B	Y=-2.78+3.49x	98.6	169.5	14.762*	3

* Significant at $P < 0.05$.

• Calculated Log ED_{50} transformed to ED_{50}

investigated. The efficacy of injected sub-lethal doses of fractions A and B were also compared with a sub-lethal dose of azadirachtin A in causing delay in moulting.

3.8.1 The effects of topically applied *M. volkensii* fractions A and B on mortality rate in *L. migratoria* 5th instar nymphs and adults

The mortality over a period of 14 days amongst *L. migratoria* 5th instar nymphs and adults treated topically with fractions A and B was investigated. The regression equations and LD_{50}'s from probit mortality versus log dosage plots from these tests are presented in Table 32 for fractions A and B. There was a distinct difference in the contact activity in the two fractions. In tests with both nymphs and adults fraction A had LD_{50}'s above 7000µg/g body weight. Fraction B caused considerable mortality through contact. It had LD_{50}'s of 8µg/g and 10µg/g body weight in nymphs and adults, respectively.

3.8.2 Mortality rates in *L. migratoria* 5th instar nymphs and adults injected with *M. volkensii* fractions A and B

The equation of regression lines from probit mortality versus log dosage plots and the LD_{50}'s of fractions A and B injected into *L. migratoria* 5th instar nymphs and adults are presented in Table 33. The mortality was recorded over a period of 14 days. Both extracts caused significant mortality at doses of over 40µg/g of body weight. However as in the results of topical treatment tests on adults and nymphs with fractions A and B, fraction B was about 3 fold more active than A. In injection tests with nymphs, A had an LD_{50} of 29.4µg/g while B had an LD_{50} of 10.9µg/g. In tests on adults, a similar pattern of activity was observed where A had an LD_{50} of 60.9µg/g and B an LD_{50} of 18.2µg/g of body weight. In a few instances, nymphs died during the moult. Such nymphs showed signs of having been unable to shed the exuvium. They had a crack in the old cuticle at the pronotum and in some cases showed separation of the old cuticle from the last abdominal segment.

3.8.3 The effects of *M. volkensii* fractions A and B and azadirachtin on moulting in *L. migratoria* 5th instar nymphs.

The mean number of days ±S.E. to the final moult in *L. migratoria* 5th instar nymphs treated topically with fractions A and B are presented in Table 34. The nymphs were 2 to 3 days old at treatment. The two groups of controls moulted in 9.33 ±0.33 days and 8.67 ±0.3 days. Nymphs treated with fractions A and B at doses ranging from 40ppm to 200ppm did not show considerable delayed moulting as they all moulted before day 11. Analysis of variance did not reveal significant differences in the mean

Table 32: **Mortality in 14 days amongst *L. migratoria* 5th instar nymphs and adults treated topically with *M. volkensii* fractions A and B.(Number of insects per bioassay=20).**

Fraction	Equation of regression line	R-Sq.(%)	48 hour LD_{50}(µg/g)•	95% Confidence limit for 48 hour LD_{50} (µg/g) Lower	Upper	t-Value	d.f.
A (Nymph)	Y=-3.68+0.164x	35.1	7144.9	0.01	5×10^9	1.27	3
B (Nymph)	Y=-3.43+2.09x	81.2	8.0	1.10	59.3	3.60*	3
A (Adult)	Y=-3.27+0.256x	57.5	36307.0	0.53	2×10^9	2.02	3
B (Adult)	Y=-2.96+2.49x	75.5	10.0	1.20	87.7	3.04	3

Insect stage treated in parenthesis.

* Significant at $P < 0.05$

• Calculated Log LD_{50} transformed to LD_{50}.

Table 33: **Mortality in 14 days amongst *L. migratoria* 5th instar nymphs and adults injected with *M. volkensii* fractions A and B.(Number of insects per bioassay=20).**

Fraction	Equation of regression line	R-Sq.(%)	48 hour LD_{50}(µg g)•	95% Confidence limit for 48 hour LD_{50} (µg/g) Lower	Upper	t-Value	d.f.
A (Nymphs)	Y=-4.09+6.31x	91.7	29.4	20.2	42.9	6.67*	4
B (Nymphs)	Y=-2.55+2.80x	71.6	10.9	4.1	29.7	3.89*	6
A (Adult)	Y=-14.2+10.9x	76.8	60.9	41.2	90.3	3.15	3
B (Adult)	Y=-2.4+2.29x	60.7	18.2	6.7	49.5	3.04*	6

Insect stage treated in parenthesis.

* Significant at $P < 0.05$

• Calculated Log LD_{50} transformed to LD_{50}.

Table 34: **The mean duration of the imaginal moult of *L. migratoria* 5th instar nymphs treated topically at the age of 2 to 3 days with *M. volkensii* fractions A and B.**

Dose (µg/g of body weight)	Days (±S.E) to final moult	
	A treated*	B treated†
0	9.33 ± 0.33 (18)	8.67 ± 0.30 (18)
40	10.11 ± 0.41 (18)	7.67 ± 0.33 (3)
80	10.47 ± 0.47 (17)	----
100	10.00 ± 0.40 (15)	----
200	10.00 ± 0.38 (17)	----

* Anova, no significant delay in moulting, $F_{4,80(1)}=1.077$, $P=0.3735$

† Anova, no significant delay in moulting, $F_{1,18(1)}=1.455$, $P=0.2433$

Number of insects moulting in parenthesis.

duration of the imaginal moult in the controls and the treated nymphs. There was however severe mortality in the B treated insects. The mean number of days ±S.E. to the final moult in 2 to 3 days old *L. migratoria* 5th instar nymphs injected with *M. volkensii* fraction A and B and azadirachtin are presented in Table 35. Fractions A and B were injected at doses ranging from 10ppm to 40ppm while azadirachtin was injected at doses of 0.5, and 1ppm. The controls and all insects treated with fractions A and B moulted before day 11. Nymphs treated with azadirachtin at a dose of 1ppm showed greatly extended instar with some insects moulting at day 23. Analysis of variance revealed a significant delay in moulting among the insects treated with *M. volkensii* fraction B, ($F_{3,48}$ (1)=4.872, P=0.0049) and azadirachtin, ($F_{2,16}$(1)=32.080, P=0.0000). In a few instances, insects treated with azadirachtin at doses of 2µg/g and 4µg/g of body weight survived as nymphs up to 38 days when the experiment was terminated. Azadirachtin caused considerable mortality at doses above 1µg/g of body weight. Azadirachtin treated insects dying in the early days had a very soft cuticle while those surviving for many days as nymphs had a very hard cuticle.

3.8.4 The effect of injected *M. volkensii* fractions A and B and azadirachtin A on body weight gain and faecal output in *L. migratoria* 5th instar nymphs

The body weight gain over a period of 10 days in male *L. migratoria* 5th instar nymphs injected with 25µg/g of body weight with fractions A and B is shown in Figures 4 and 5. Results of a similar experiment with female nymphs are presented in Figures 6 and 7. All the insects reached a peak body weight in about 10 days. However the body weights of treated insects remained lower than those of the control group throughout the duration of the experiment.

Results of a similar experiment on male *L. migratoria* 5th instar nymphs with azadirachtin at a dose of 2µg/g of body weight are presented in Figure 8. Treated insects gained very little weight and even showed a decrease in body weight after 28 days. The production of faeces by the same group of insects is shown in Figure 9. Both control insects and those treated with fractions A and B had similar patterns of faecal output with peaks at day 10. Azadirachtin treated nymphs showed a dramatic decrease in faecal output which remained low over the duration of the experiment.

Table 35: **The mean duration of the imaginal moult of *L. migratoria* 5th instar nymphs injected at the age of 2 to 3 days with *M. volkensii* fractions A and B and azadirachtin.**

Dose (µg/g of body weight)	Days (±S.E.) to final moult		
	A Treated*	B Treated†	Azadirachtin• treated
0	9.15 ± 0.18 (20)	9.77 ± 0.15 (18)	10.80 ± 0.58 (9)
0.5	----	----	13.70 ± 1.10 (7)
1	----	----	23.30 ± 1.30 (3)
10	9.30 ± 0.21 (20)	9.87 ± 0.22 (16)	----
20	9.73 ± 0.34 (15)	10.75 ± 0.16 (8)	----
40	9.75 ± 0.25 (4)	9.60 ± 0.16 (10)	----

* Anova, no significant delay in moulting, F3,55(1)=1.170, P=0.3297.

† Anova, significant delay in moulting, F3,48(1)=4.872, P=0.0049.

• Anova, significant delay in moulting, F2,16(1)=32.080, P=0.0000.

Number of moulting nymphs in parenthesis

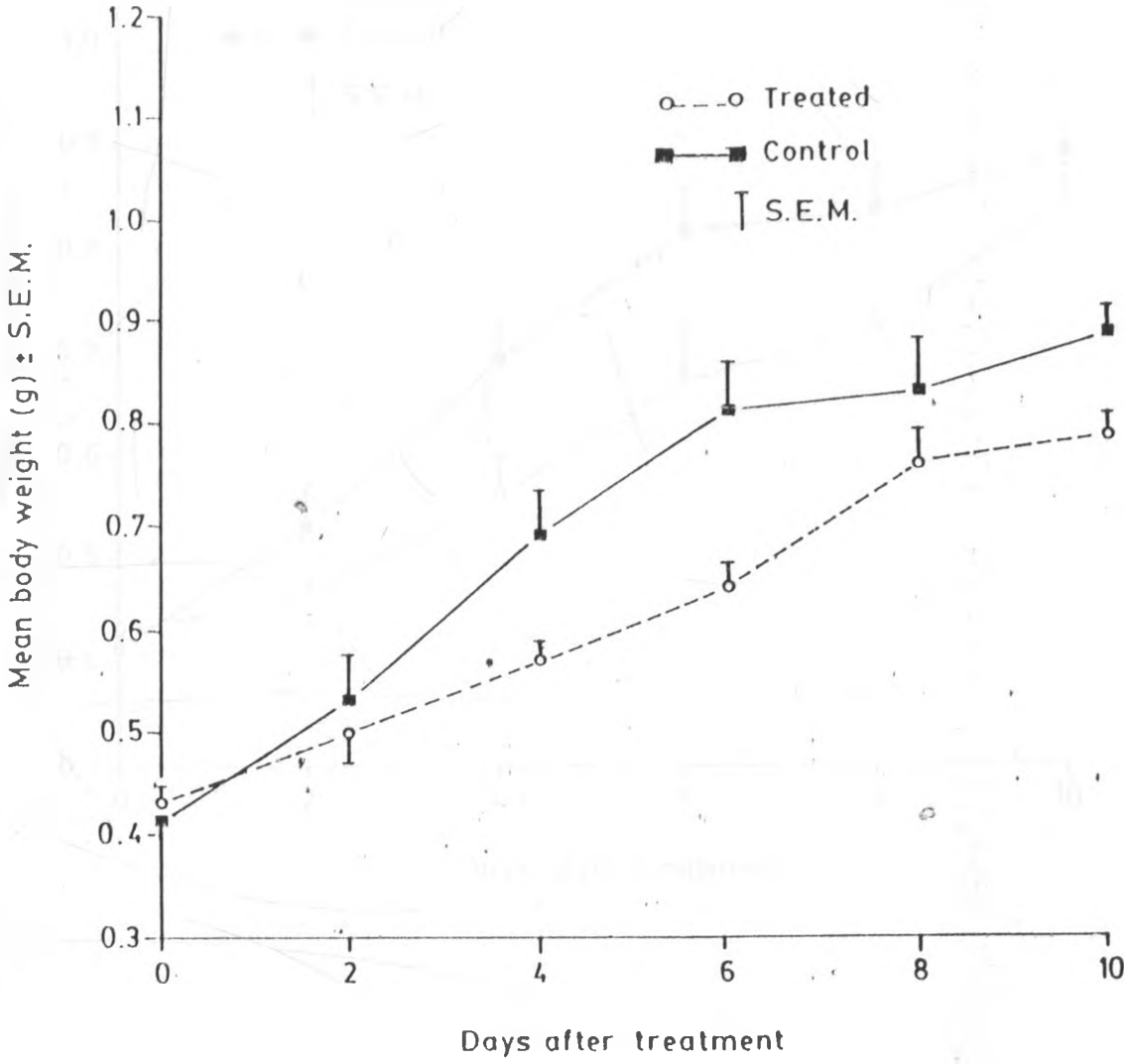


Figure 4: **Body weight gain over a 10 day period in male *L. migratoria* 5th instar nymphs injected with *M. volkensii* fraction A at a dose of 25µg/g of body weight at the age of 2 to 3 days.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate).

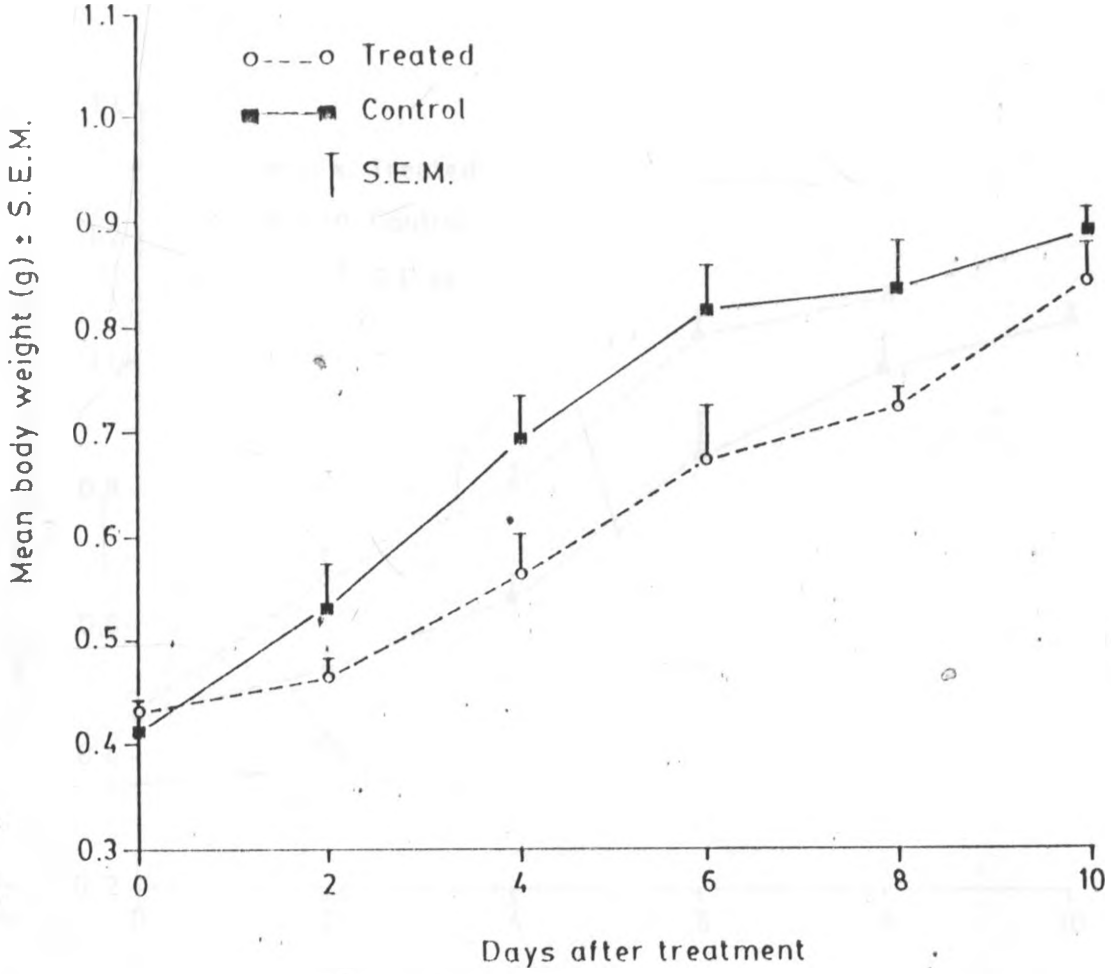


Figure 5: **Body weight gain over a 10 day period in male *L. migratoria* 5th instar nymphs injected with *M. volkensii* fraction B at a dose of 25µg/g of body weight at the age of 2 to 3 days.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate).

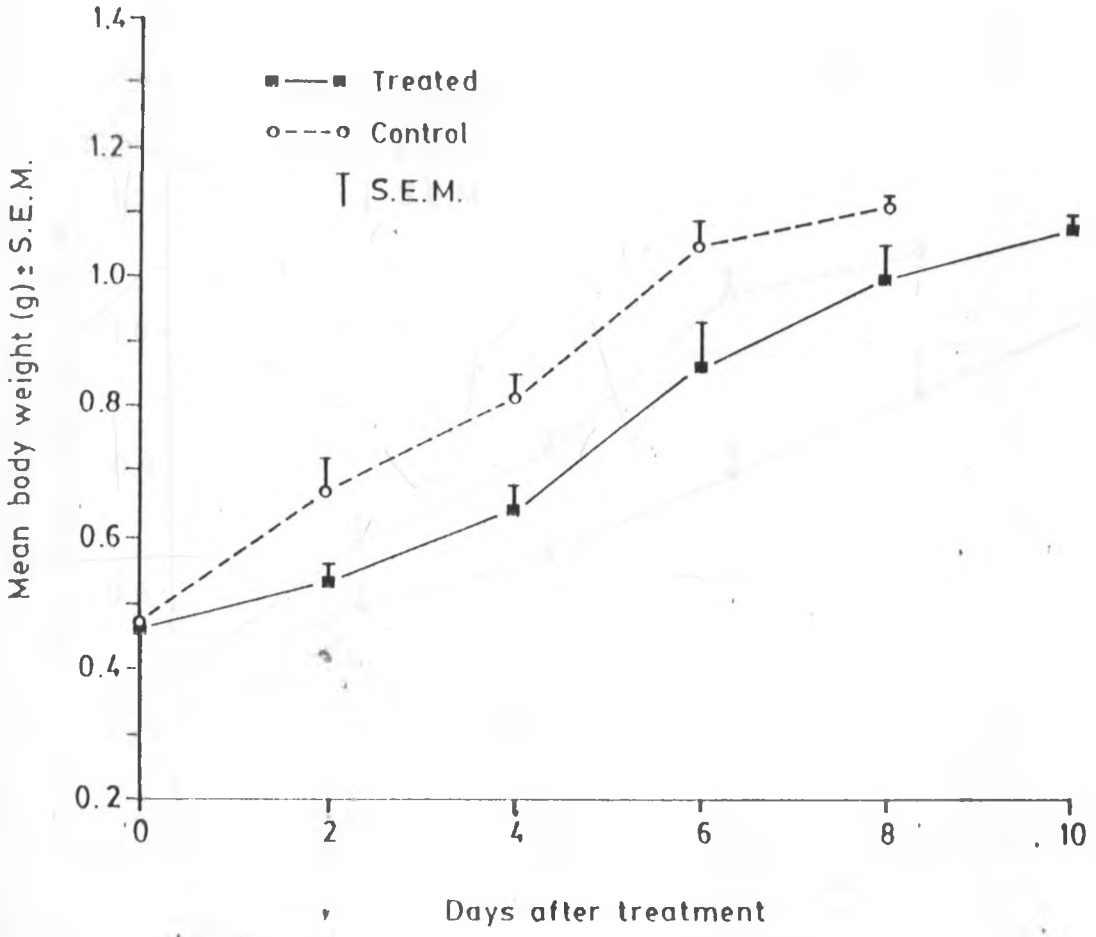


Figure 6: **Body weight gain in female *L. migratoria* 5th instar nymphs injected with *M. volkensii* fraction A at a dose of 25µg/g of body weight at the age of 2 to 3 days.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate. Body weight determinations were terminated when all nymphs in at least one group moulted to adults).

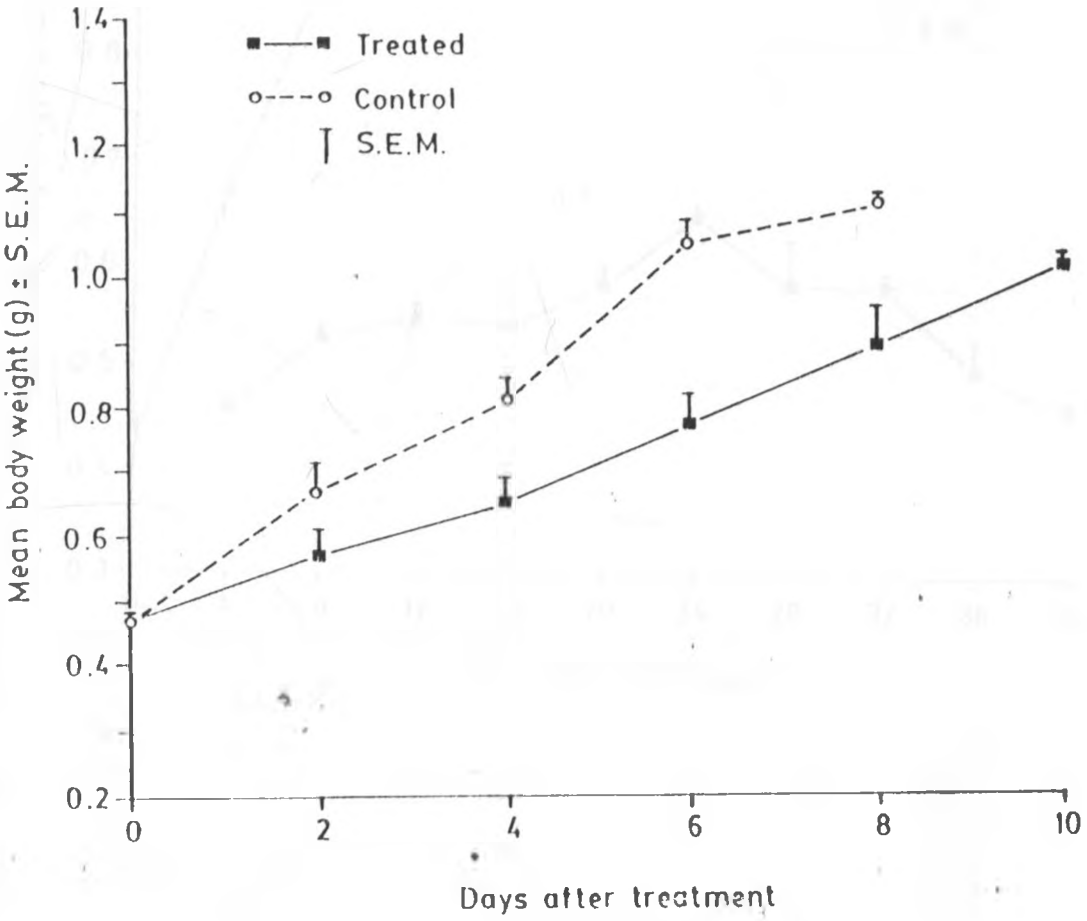


Figure 7: **Body weight gain in female *L. migratoria* 5th instar nymphs injected with *M. volkensii* fraction B at a dose of 25µg/g of body weight at the age of 2 to 3 days.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate. Body weight determinations were terminated when all nymphs in at least one group moulted to adults).

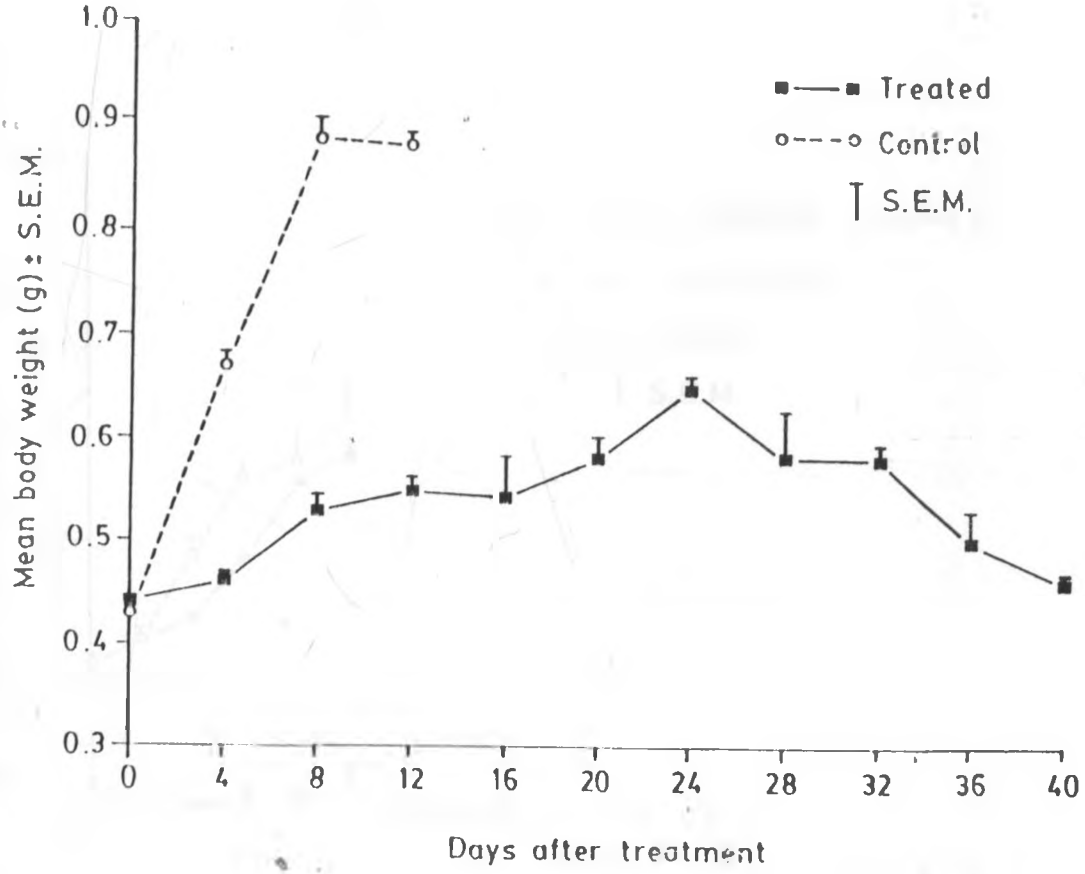


Figure 8: **The effect of azadirachtin injected at a dose of 2μg/g of body weight on body weight gain in male *L. migratoria* 5th instar nymphs.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate. Body weight determinations were terminated when all nymphs in at least one group moulted to adults).

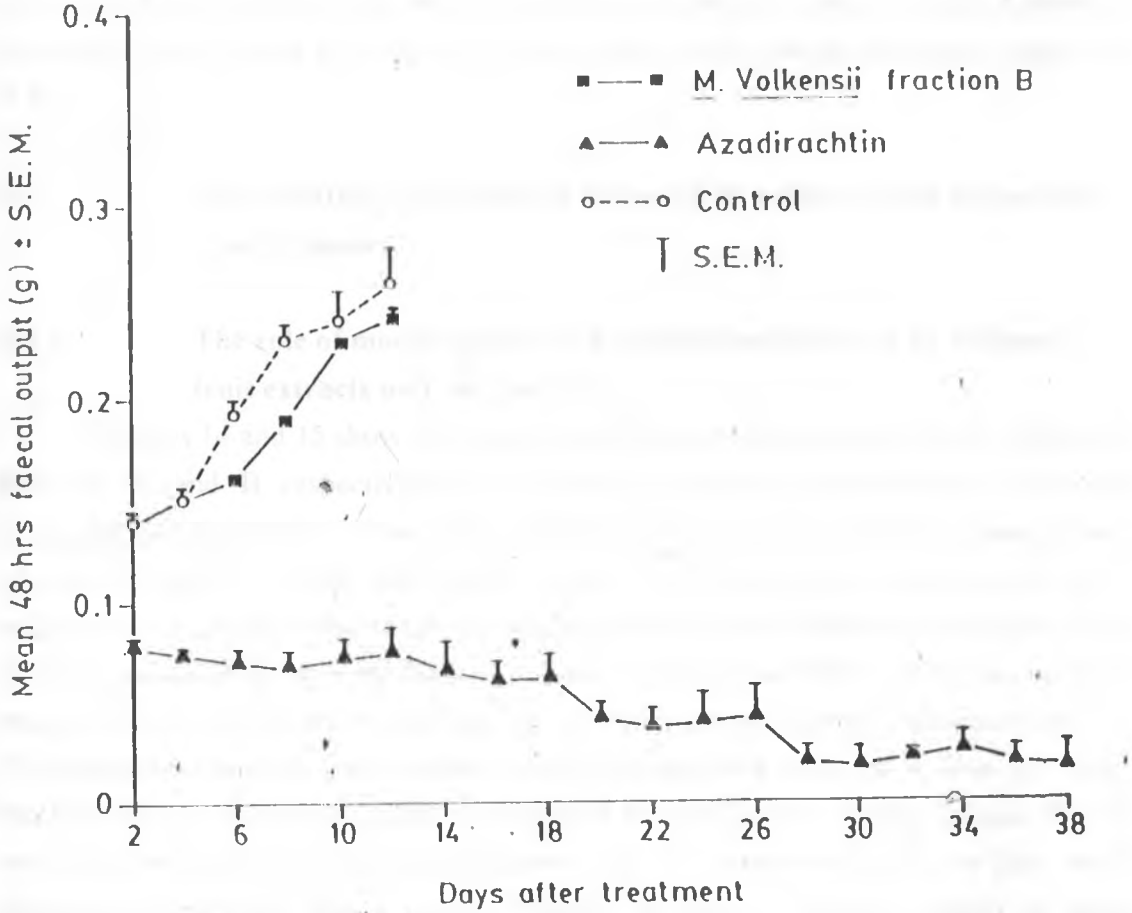


Figure 9: **Faecal output in male 5th instar *L. migratoria* nymphs injected with *M. volkensii* fraction B and azadirachtin at doses of 25ug and 2µg/g of body weight respectively.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate. Faecal output determinations were terminated when all nymphs in at least one group moulted to adults).

3.8.5 The effect of topical treatment with *M. volkensii* fruit fractions A and B on body weight gain in *L. migratoria* male and female 5th instar nymphs

The effect of topical treatment with *M. volkensii* fractions A and B at a sub-lethal dose of 200µg/g of body weight on body weight gain in male *L. migratoria* 5th instar nymphs is shown in Figures 10 and 11. Results of a similar experiment with female nymphs are presented in Figures 12 and 13. Treated insects never attained the maximum body weight reached by the control group. While the males reached a mean peak body weight of 0.85g in 10 days, treated ones had body weights of about 0.75g. The same trend was observed amongst the female nymphs. Control females reached a mean peak body weight of 1.08g in 10 days while treated nymphs had body weights of 0.8g.

3.9 The tentative mechanism of action of *M. volkensii* fruit extracts on *L. migratoria*

3.9.1 The role of mouth parts in the antifeedant effects of *M. volkensii* fruit extracts on *L. migratoria*

Figures 14 and 15 show the effects of palp and neck treatment with *M. volkensii* fractions A and B respectively on the 48 hour faecal output of two day old *L. migratoria* adult males. There was a reduction in faecal output with increasing doses of material applied. In the neck-treated insects, the faecal output was lowered to a minimum of 0.13g/insect/48 hours and 0.15g/insect/48 hours following treatment with fractions A and B respectively both at a dose of 400µg/g of body weight. In the palp treated insects, faecal output was lowered at relatively low doses of fractions A and B. The minimum faecal output attained was 0.11g/insect/48 hours at a dose of 30µg fraction A/g of body weight and 0.13g/insect/48 hours at a dose of 20µg fraction B/g of body weight. Palp treatment was therefore over 10 times more effective than neck treatment in reducing faecal output. Figures 16 and 17 show the effect of palp treatment with fractions A and B on body weight of *L. migratoria* adults over a period of 24 days. Controls attained maximum body weight in about 6 days while treated insects took 15 days. The maximum body weight attained by the treated insects remained below that of controls up to day 22. Figures 18 and 19 show the effect of palp treatment with fractions A and B on faecal output over a period of 24 days. In both cases control insects had a high faecal output in the first 8 days. After the 8th day, the faecal output declined and remained constant at about 0.15g/insect/48 hours for 24 days.

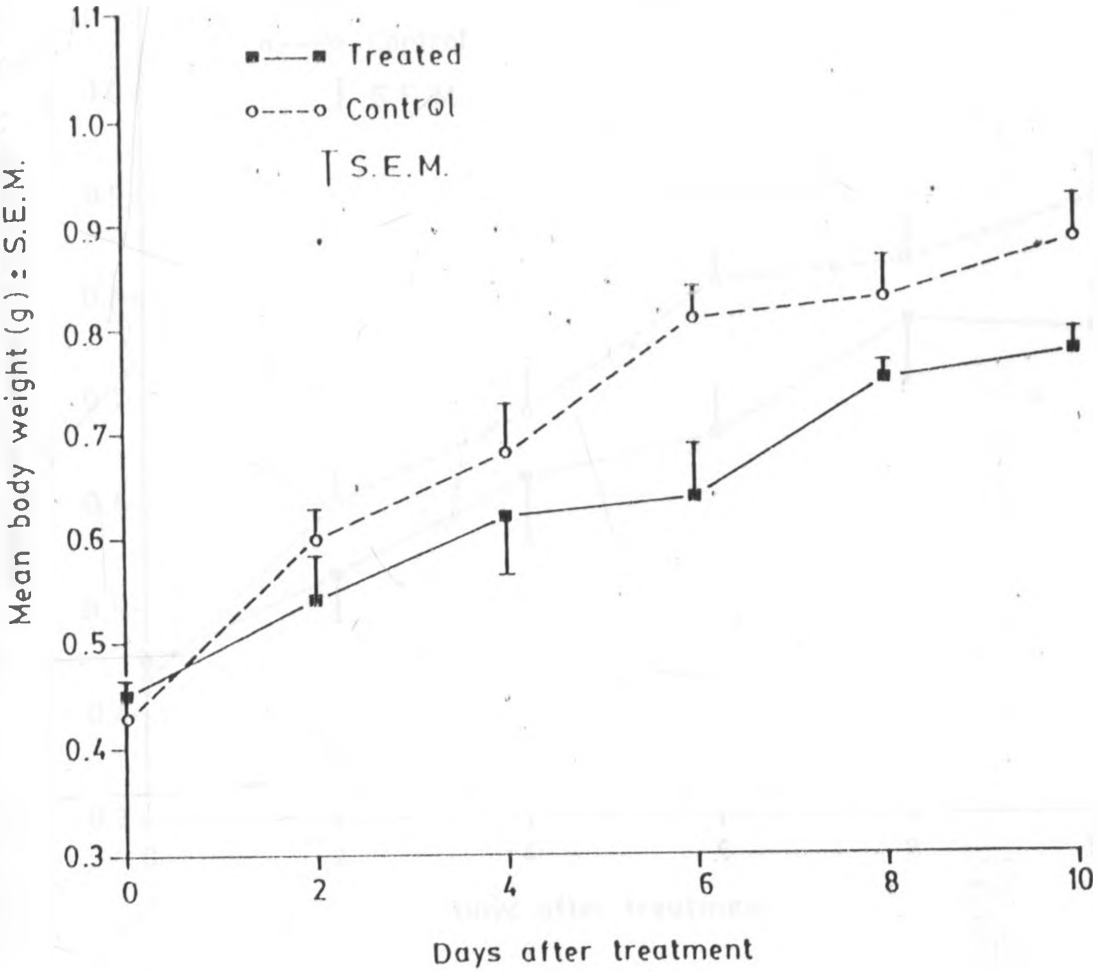


Figure 10: **Body weight gain over a period of 10 days in male *L. migratoria* 5th instar nymphs treated topically with *M. volkensii* fraction A at a dose of 200µg/g of body weight at the age of 2 to 3 days.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate. Body weight determinations were terminated when all nymphs in at least one group moulted to adults)

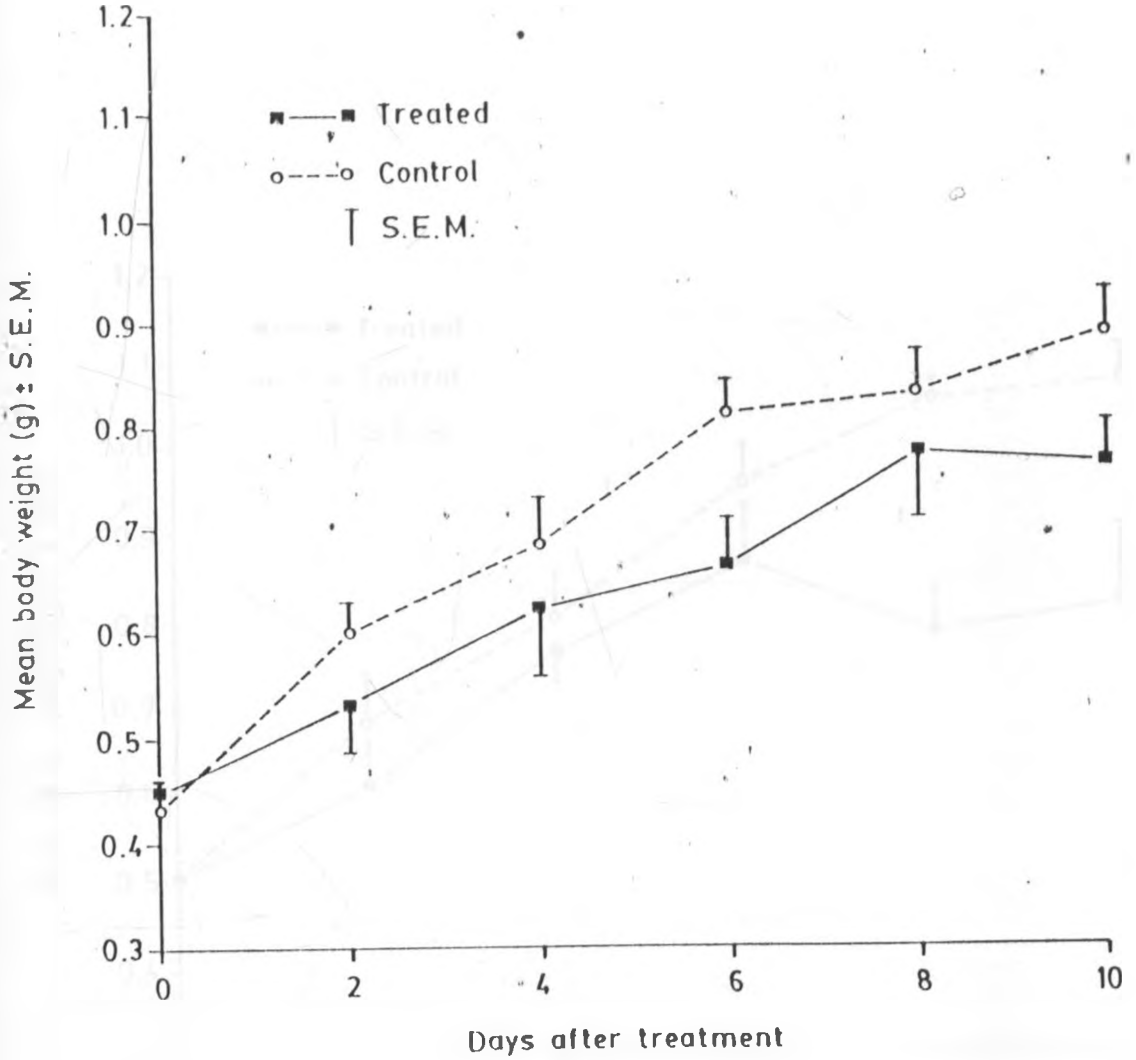


Figure 11: **Body weight gain over a period of 10 days in male *L. migratoria* 5th instar nymphs treated topically with *M. volkensii* fraction B at a dose of 200µg/g of body weight at the age of 2 to 3 days.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate. Body weight determinations were terminated when all nymphs in at least one group moulted to adults).

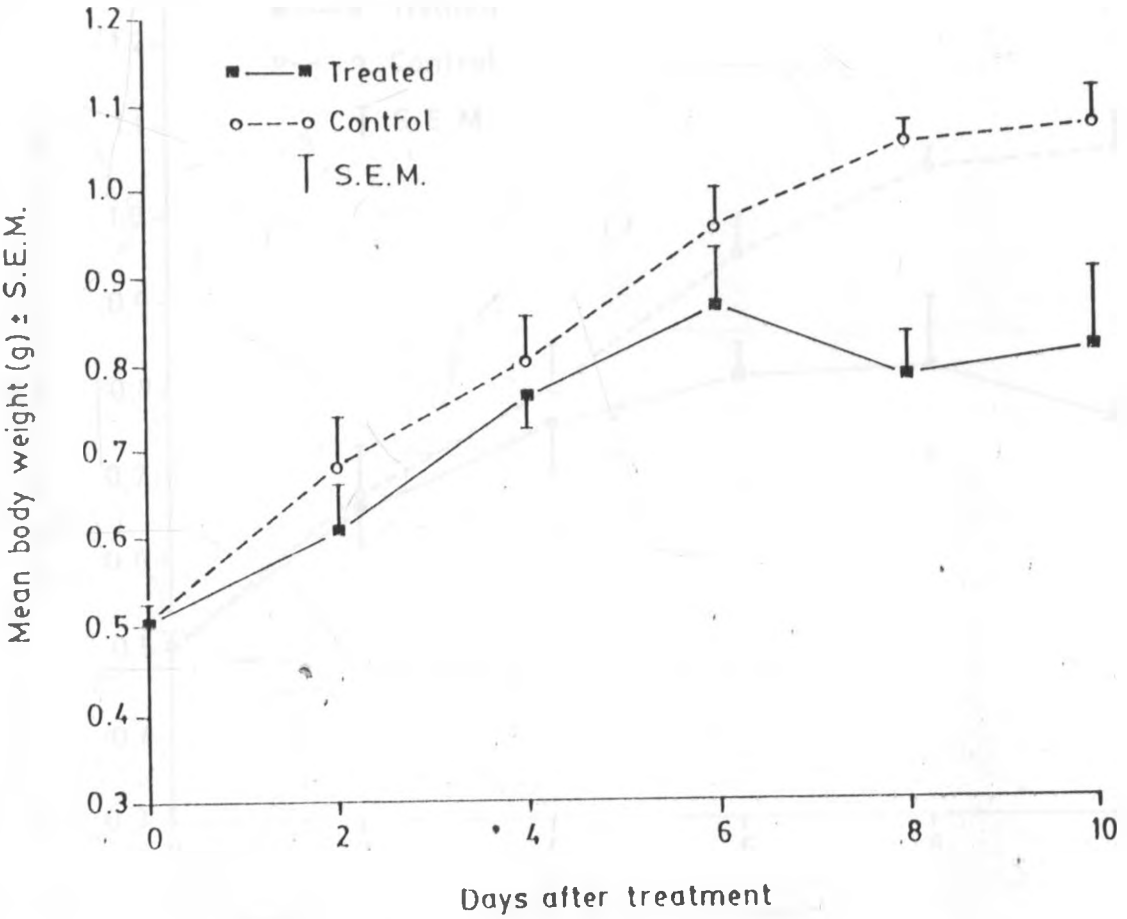


Figure 12: **Body weight gain over a period of 10 days in female *L. migratoria* 5th instar nymphs treated topically with *M. volkensii* fraction A at a dose of 200µg/g of body weight at the age of 2 to 3 days.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate. Body weight determinations were terminated when all nymphs in at least one group moulted to adults)

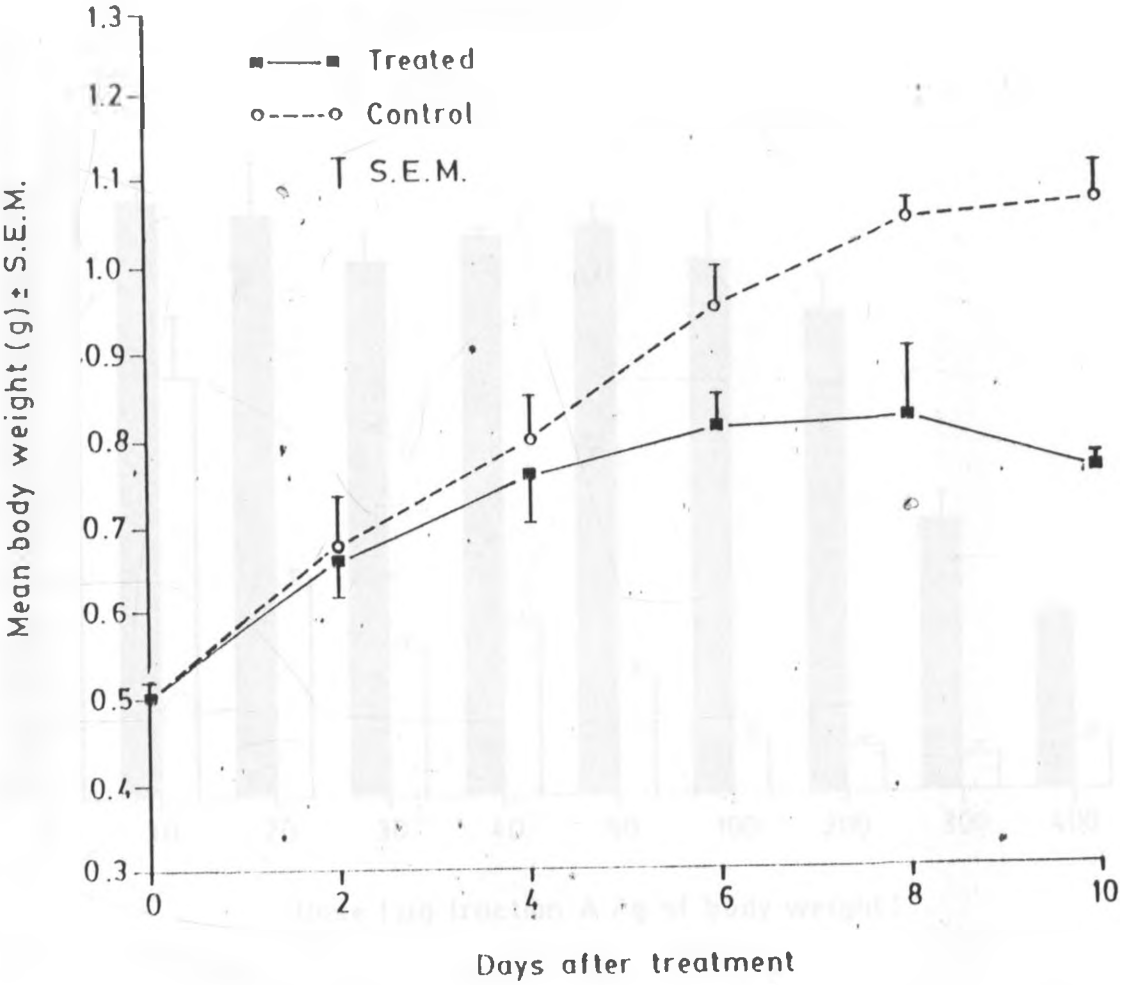


Figure 13: **Body weight gain over a period of 10 days in female *L. migratoria* 5th instar nymphs treated topically with *M. volkensii* fraction B at a dose of 200µg/g of body weight at the age of 2 to 3 days.** (The initial number of nymphs per group was 10 and the test was conducted in triplicate. Body weight determinations were terminated when all nymphs in at least one group moulted to adults).

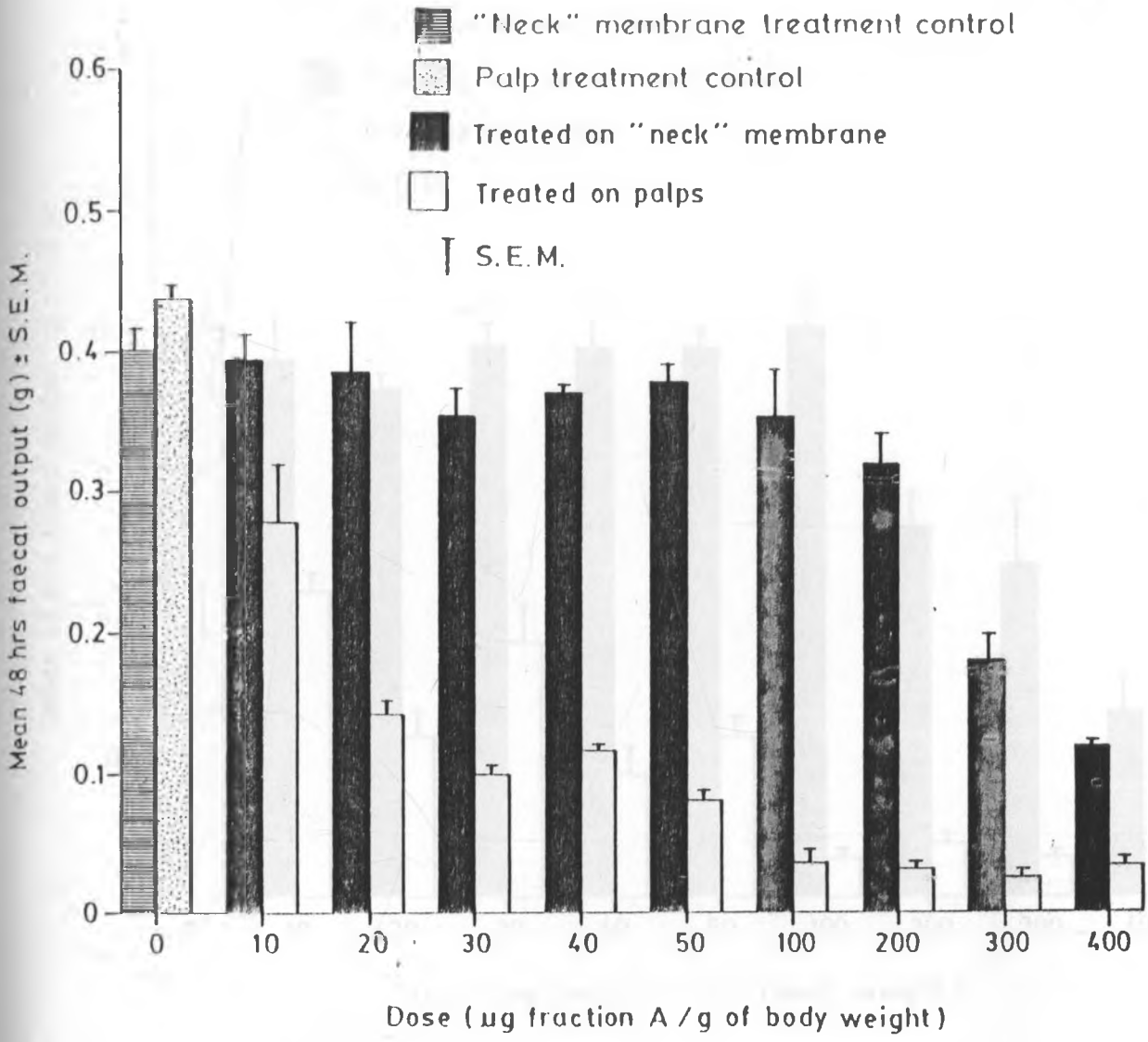


Figure 14: **The effect of topical application of *M. volkensii* fraction A on the palps and "neck" membrane of *L. migratoria* male adults on the faecal output in 48 hours.** (Faecal output was determined in 3 groups of 10 insects at every dose used).

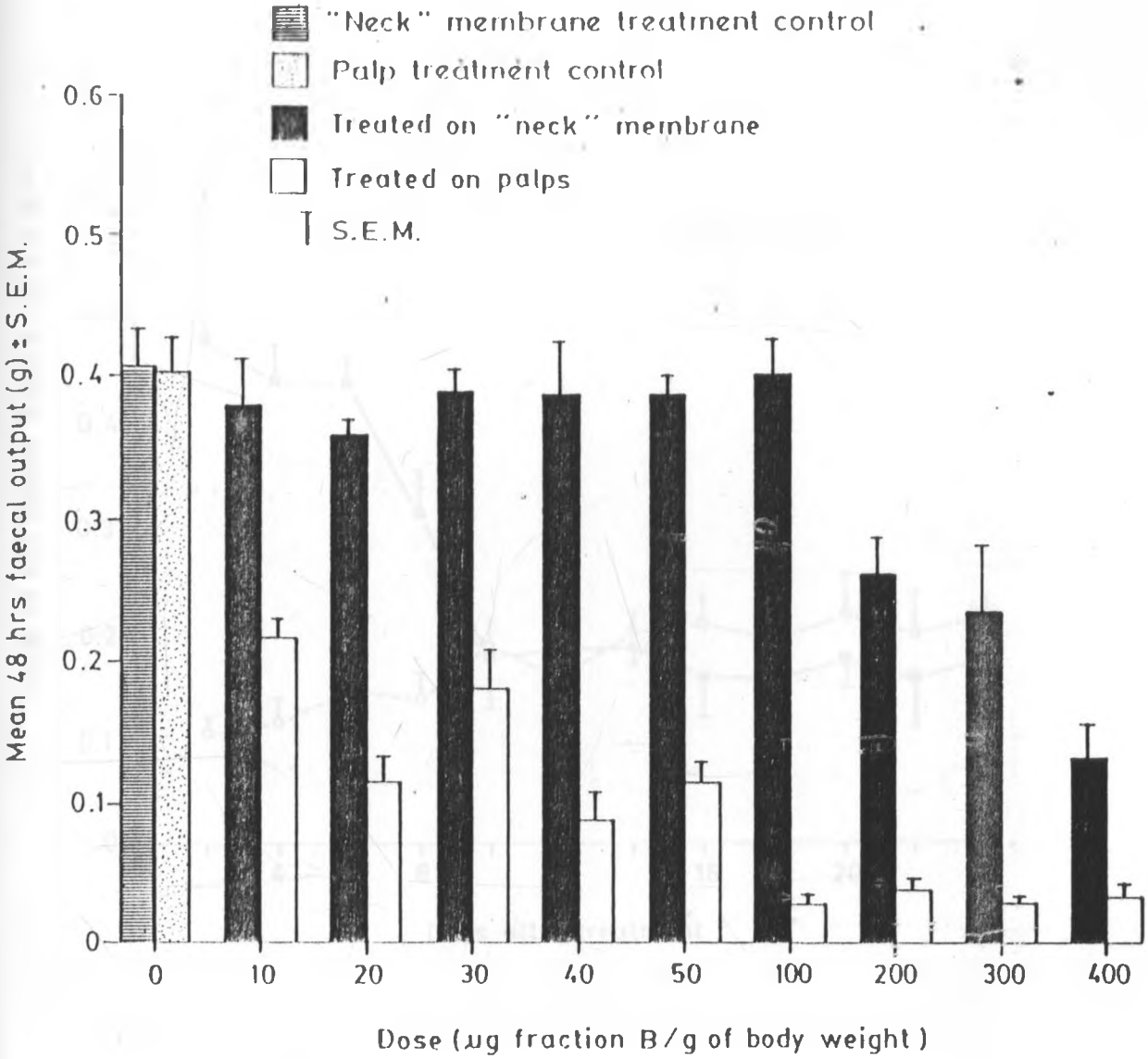


Figure 15: **The effect of topical application of *M. volkensii* fraction B on the palps and "neck" membrane of *L. migratoria* male adults on the faecal output in 48 hours.** (Faecal output was determined in 3 groups of 10 insects at every dose used)

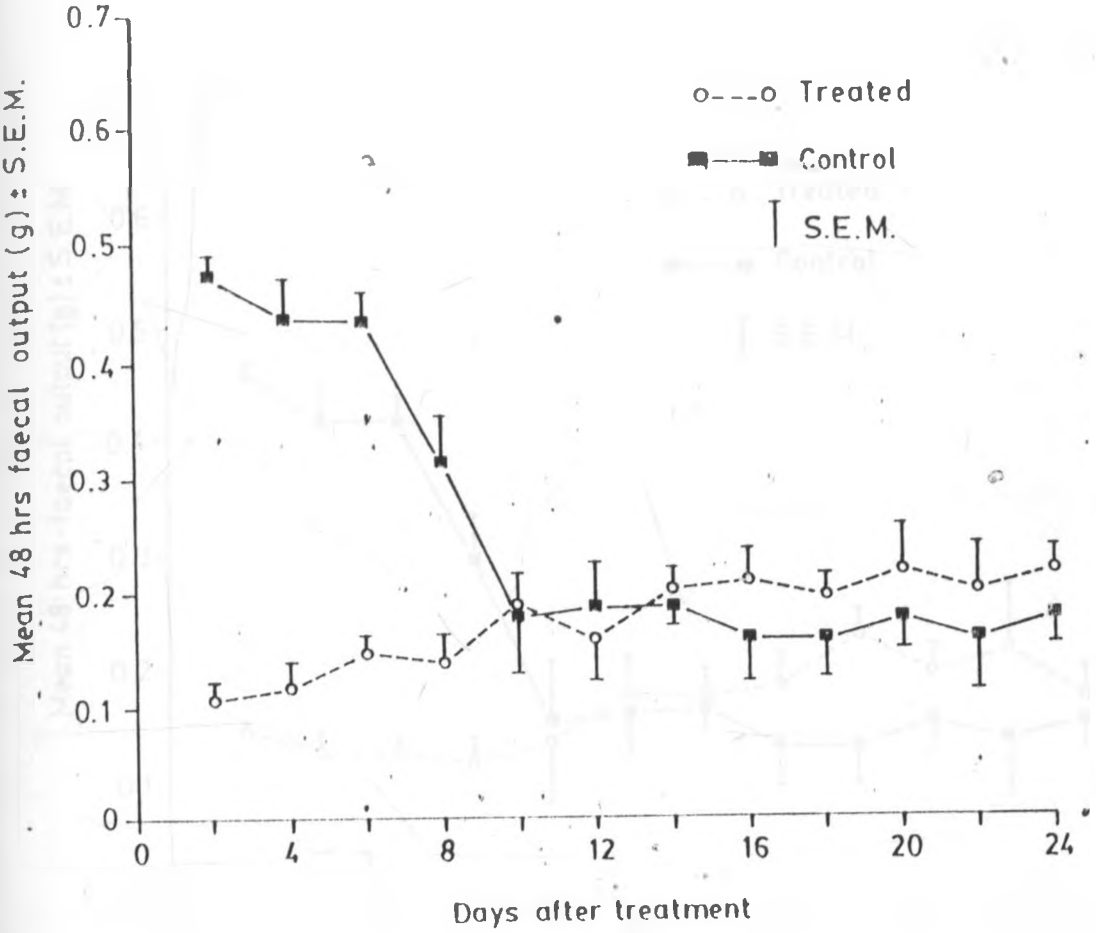


Figure 16: **The effect of topical application of *M. volkensii* fraction A on the palps of *L. migratoria* male adults at a dose of 40μg/g of body weight on faecal output over a period of 24 days.** (The initial number of insects per group was 10 and the determinations were conducted in triplicate).

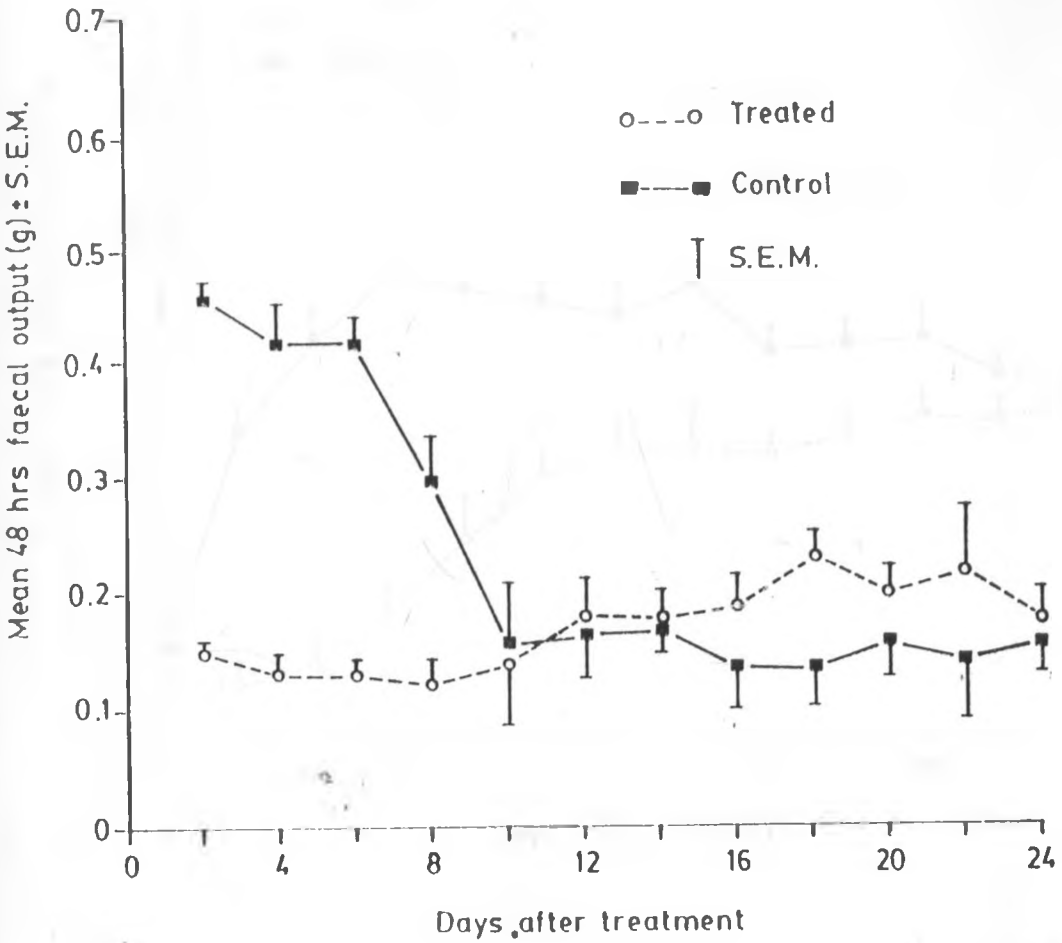


Figure 17: **The effect of topical application of *M. volkensii* fraction B on the palps of *L. migratoria* male adults at a dose of 40µg/g of body weight on faecal output over a period of 24 days.** (The initial number of insects per group was 10 and the determinations were conducted in triplicate).

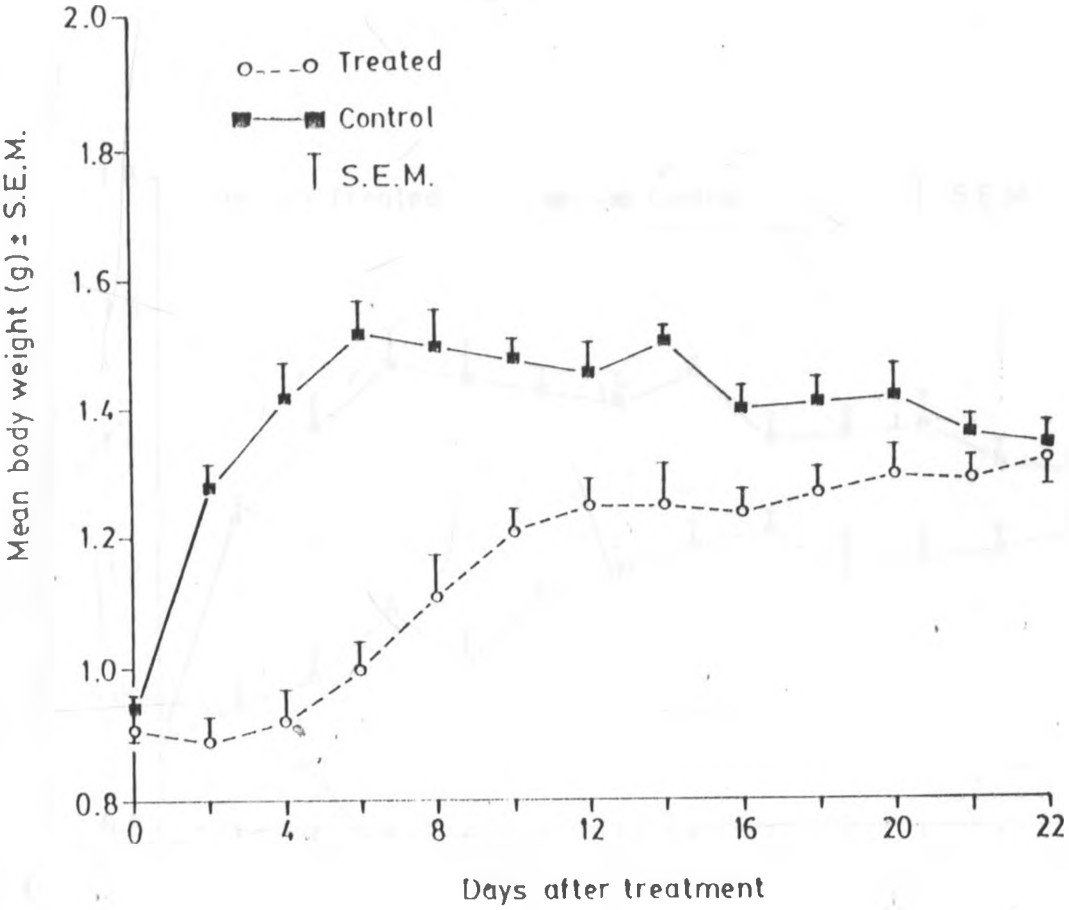


Figure 18: **Body weight gain over a period of 24 days in adult *L. migratoria* males treated topically on the palps with *M. volkensii* fraction A at a dose of 40µg/g of body weight.** (The initial number of insects per group was 10 and the determinations were conducted in triplicate).

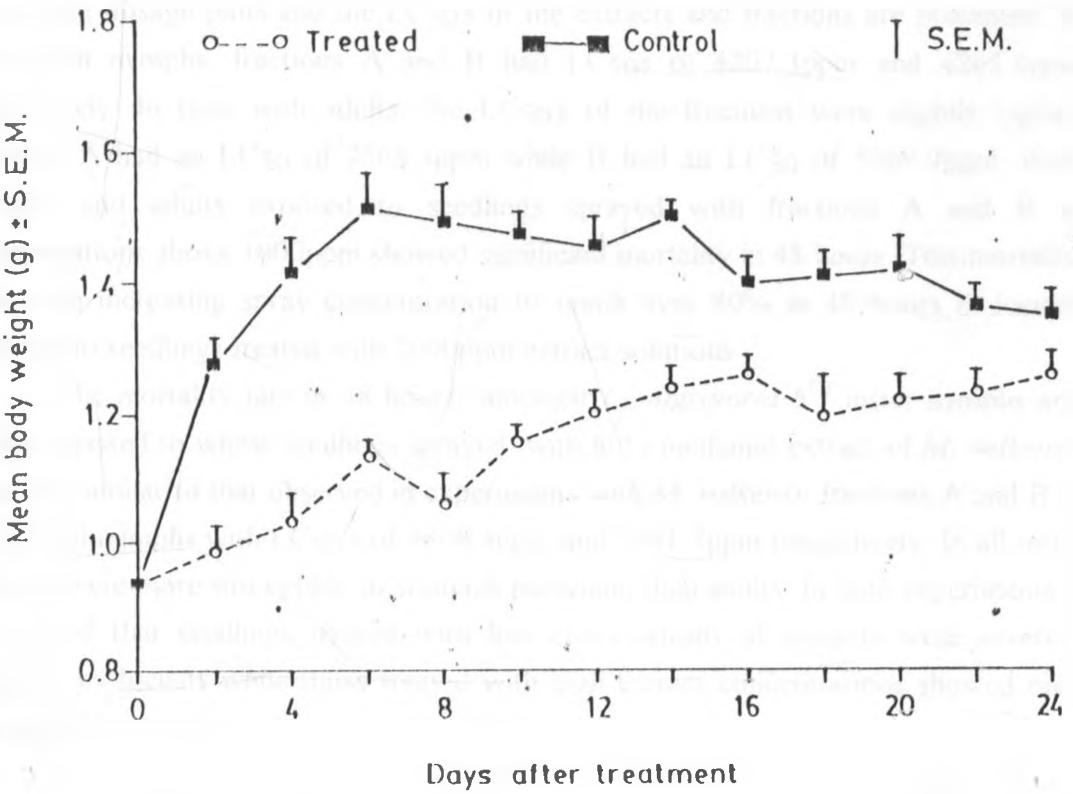


Figure 19: **Body weight gain over a period of 24 days in adult *L. migratoria* males treated topically on the palps with *M. volkensii* fraction B at a dose of 40µg/g of body weight.** (The initial number of insects per group was 10 and the determinations were conducted in triplicate).

3.9.2 Stomach poison activity of *M. volkensii* fruit extracts

During the present study it was observed in preliminary studies on antifeedant activity in *M. volkensii* fruit fractions by the wheat seedling feeding protection test that there was considerable mortality amongst locusts exposed to seedlings treated with high concentrations of *M. volkensii* fractions A and B and the 80% methanol crude extract. It was suspected that the deaths were due to poisoning through ingestion.

Table 36 shows the mortality in 48 hours in *L. migratoria* 5th instar nymphs and adults exposed to wheat seedlings sprayed with 80% methanol *M. volkensii* extract and fractions A and B. The equations of regression lines from probit 48 hour mortality versus log dosage plots and the LC_{50}'s of the extracts and fractions are presented. In tests with nymphs, fractions A and B had LC_{50}s of 4207.3ppm and 4265.8ppm respectively. In tests with adults, the LC_{50}'s of the fractions were slightly higher. Fraction A had an LC_{50} of 7568.3ppm while B had an LC_{50} of 5069.9ppm. Both nymphs and adults exposed to seedlings sprayed with fractions A and B at concentrations above 1000ppm showed significant mortality in 48 hours. This mortality rose with increasing spray concentration to reach over 80% in 48 hours in locusts exposed to seedlings treated with 2000ppm extract solutions.

The mortality rate in 48 hours amongst *L. migratoria* 5th instar nymphs and adults exposed to wheat seedlings sprayed with 80% methanol extract of *M. volkensii* fruit was similar to that observed in experiments with *M. volkensii* fractions A and B in adults and nymphs with LC_{50}'s of 4808.4ppm and 6441.7ppm respectively. In all tests, nymphs were more susceptible to stomach poisoning than adults. In both experiments it was noted that seedlings treated with low concetrations of extracts were severely attacked by locusts while those treated with high extract concentrations showed only very small bite marks.

3.9.3 The effect of addition of plant oils on the contact poison activity of *M. volkensii* fraction B

Results on the contact poison activity of *M. volkensii* fraction B on *L. migratoria* adults when applied topically as solutions in acetone and acetone/plant oil are presented on Table 37. There was very low mortality in control groups of insects receiving acetone/plant oil alone (Anova, $F_{5,12(1)}=1.64$, $P=0.223$). Considerable activity was however observed in insects treated with fraction B solutions in acetone and acetone/plant oil (Anova, $F_{11,24(1)}=19.558$, $P=0.000$). Acetone/olive oil solution of fraction B caused the highest mortality resulting in over 50% mortality in 48 hours (Anova, $F_{5,12(1)}=11.044$, $P=0.0004$).

Table 36: **Mortality in *L. migratoria* 5th instar nymphs and adults 48 hours after feeding on wheat seedlings sprayed with *M. volkensii* fruit extracts and fractions.(Number of insects per bioassay=10).**

Fraction	Equation of regression line	R-Sq.(%)	48 hour LC_{50}(ppm)• (Concentration of spray)	95% Confidence limit for 48 hour LC_{50} (ppm) Lower	Upper	t-Value	d.f
A (Nymph)	Y=-3.13+2.25x	90.0	4207.3	1389.9	12705.7	4.25	2
B (Nymph)	Y=-1.54+1.79x	84.7	4265.8	1238.8	14655.5	4.07*	3
Crude (Nymph)	Y=-7.17+3.34x	65.0	4808.4	632.4	36559	1.93	2
A (Adult)	Y=-0.46+1.39x	75.6	7568.3	1315.2	43551.2	2.49	2
B (Adult)	Y=-0.66+1.52x	88.3	5069.9	1667.2	15417	4.76*	3
Crude (Adult)	Y=-3.20+2.14x	79.0	6441.7	1348.9	30831.9	2.74	2

Insect stage treated in parenthesis.

* Significant at $P < 0.05$

• Calculated Log LC_{50} transformed to LC_{50}

Table 37: **Mortality in *L. migratoria* adults 48 hours after topical treatment with *M. volkensii* fraction B dissolved in acetone and acetone: plant oil (2:3) at a dose of 1000µg/g of body weight.(Number of insects per bioassay= 10).**

Treatment	Mean number (±S.E)of locusts dead in 48 hours	t-Value (d.f.=4)
Control	0	----
Acetone + B	2.67 ± 0.33	----
Melia oil + acetone	0	
Melia oil + acetone + B	1.67 ± 0.33	----
Salad oil + acetone	0	
Salad oil + acetone + B	1.67 ± 0.33	----
Coconut oil + acetone	1.00 ± 0.57	
Coconut oil + acetone + B	2.67 + 0.67	-1.89*
Castor oil + acetone	0.3 ± 0.33	
Castor oil + acetone + B	2.33 ± 0.33	-4.24*
Olive oil + acetone	0.3 ± 0.33	
Olive oil + acetone + B	5.33 ± 0.33	-10.61*

* Significant enhancement of mortality at $P < 0.05$.

3.9.4 Effect of addition of olive oil on the contact activity of *M. volkensii* fruit extracts on *L. migratoria*

As reported earlier in the present study, *L. migratoria* 5th instar nymphs and adults treated topically on the neck with up to 200µg of *M. volkensii* fraction B per gram of body weight were poisoned and considerable mortality was observed in about 5 days. In these earlier experiments, the fraction was dissolved in acetone alone. Further tests demonstrated that plant oils increased the contact poison activity of *M. volkensii* fraction B with olive oil being the most effective plant oil for this purpose.

In the following experiments, the contact poison of fraction B dissolved in acetone olive oil (2:3) and acetone alone was investigated in more detail. Results presented on Table 38 show the equation of regression lines from probit mortality versus log dosage plots of *M. volkensii* fraction B applied topically on *L. migratoria* 5th instar nymphs and adults as a solution in acetone olive oil (2:3) and in acetone alone. High mortality was recorded in 24 hours amongst insects treated with over 500µg fraction B in acetone olive oil (2:3) per gram of body weight. The LD_{50} was below 900ppm in both nymphs and adults. These results contrasted sharply with those obtained through topical treatment with fraction B dissolved in acetone alone where very low mortality was observed in 24 hours. The LD_{50} of fraction B dissolved in acetone alone was above 4000ppm in both nymphs and adults. In both treatments, affected insects showed lack of coordination in about 12 hours before going into a stupor followed by death. Insects in stupor showed no visible response to tactile or acoustic stimulation.

3.9.5 The effect of *M. volkensii* fruit fraction B on the frequency and amplitude of abdominal ventilation movements in *L. migratoria* adults

The frequency and amplitude of abdominal ventilation movements in *L. migratoria* adults 1 hour after injection with *M. volkensii* fraction B is shown in Figure 20. Specimen kymograph records of these movements in individual locusts before and after treatment are presented in Plate 2. In individual locusts, the frequency of contractions remained fairly constant with values lying between 13.7 and 16.7 cycles per minute before and after treatment while the amplitude decreased considerably with increasing doses of fraction B. At a dose of 200µg/g body weight, the amplitude fell to 0.67 ±0.16mm while controls showed an amplitude of 2mm.

Table 38: **The effect of addition of olive oil on the contact poison activity of *M. volkensii* fraction B on *L. migratoria* 5th instar and adults.**

Fraction	Equation of regression line	R-Sq.(%)	48 hour LD_{50}(µg/g)•	95% Confidence limit for 48 hour LD_{50} (µg g) Lower	Upper	t-Value	d.f
Acetone:Olive oil (2:3) (Nymph)	Y=-10 9-5 68x	91.3	657.1	461 5	935 6	6.49*	4
Acetone (Nymph)	Y=-1.25+1 70x	91.9	4358 1	1448 1	13115 9	4.77*	2
Acetone:olive oil (2:3) (Adult)	Y=-15.4-7 04x	91.0	822.1	544.5	1241 1	5 51*	3
Acetone (Adult)	Y=-1.49+1.69x	82.9	5227.6	779.8	35042 9	3.12	2

Insect stage treated in parenthesis.

* Significant at $P < 0.05$

• Calculated Log LD_{50} transformed to LD_{50}.

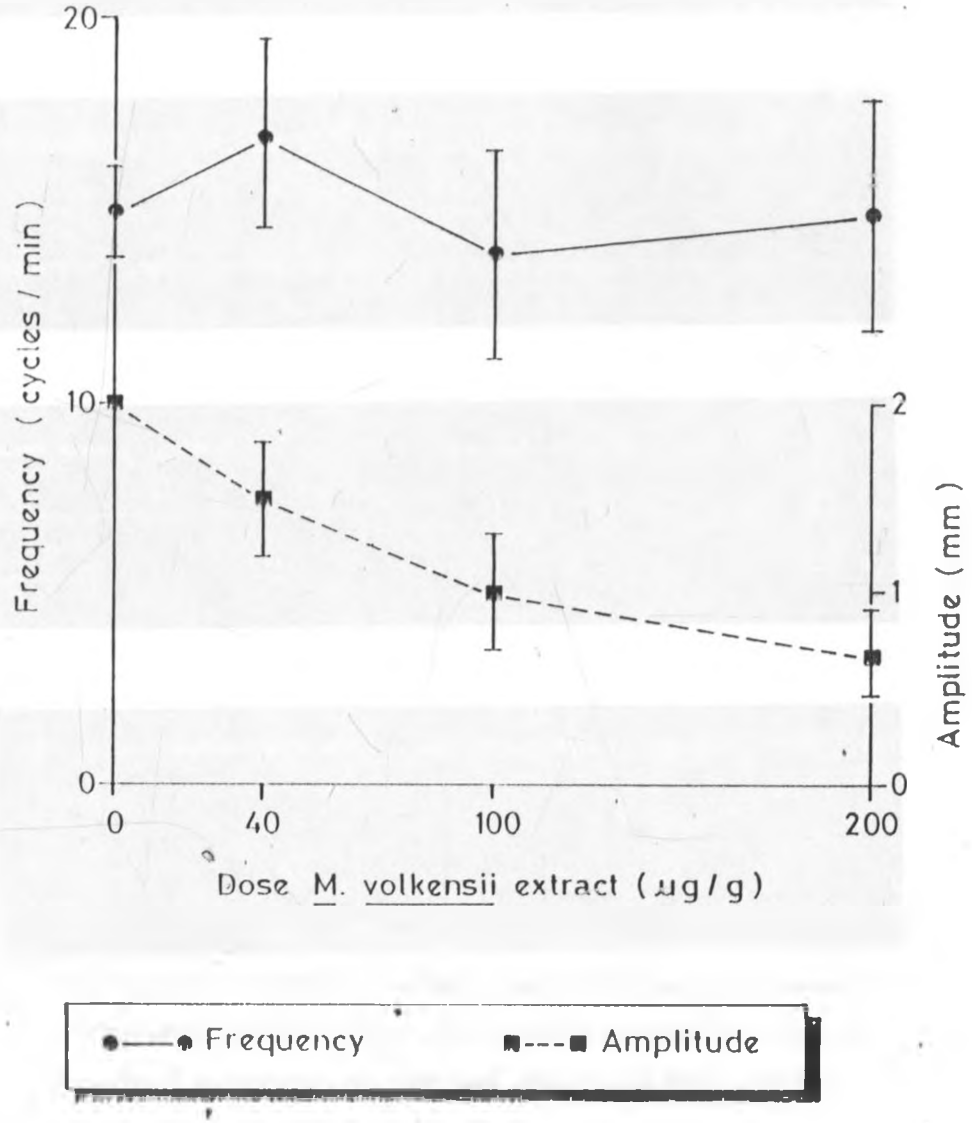


Figure 20: **The frequency and amplitude of abdominal ventilation movements in *L. migratoria* male adults 1 hour after injection with *M. volkensii* fraction B. (n=3).**

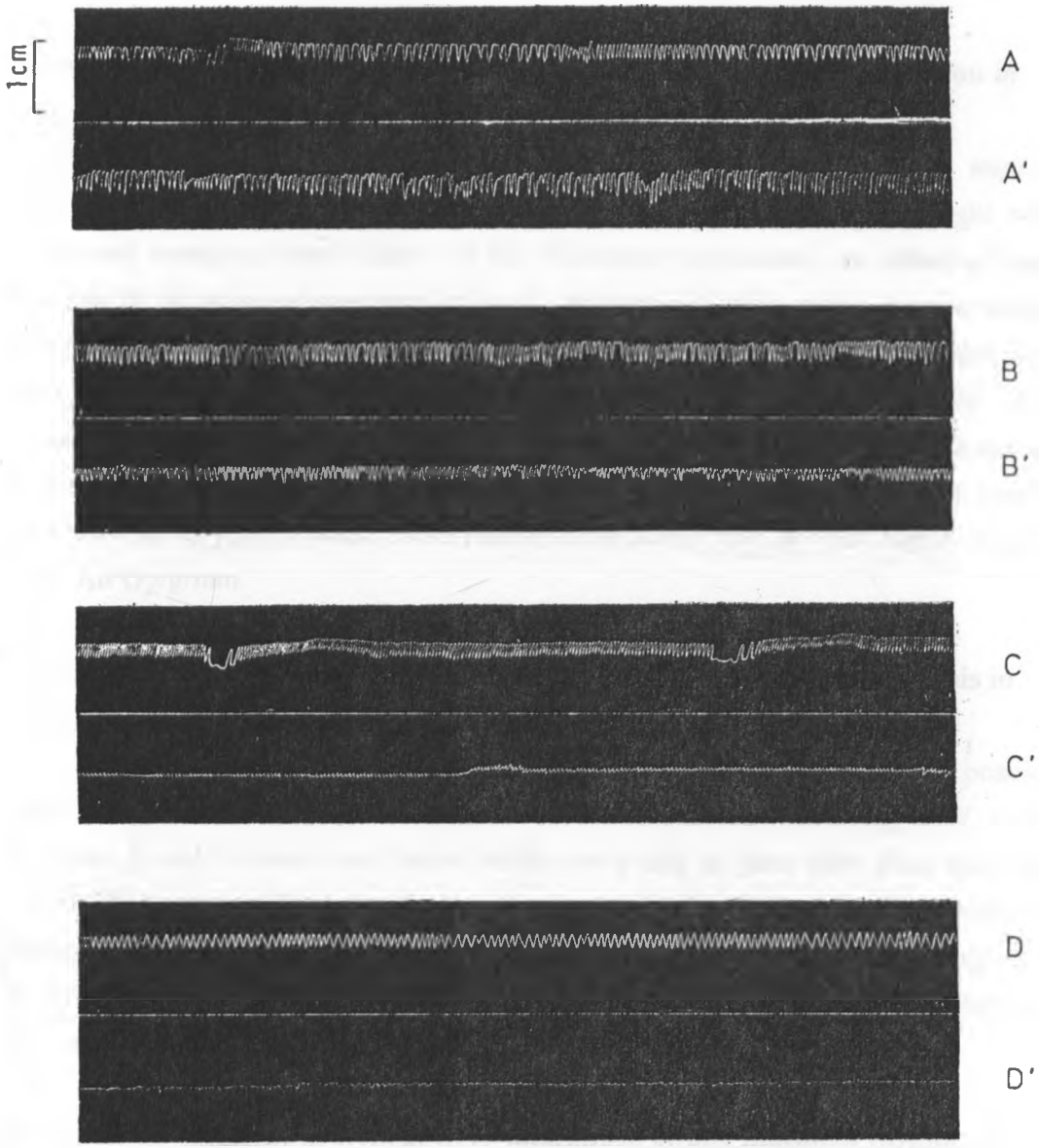


Plate 2: Kymograph recording of abdominal ventilation movements of *L. migratoria* adults 1 hour after treatment with *M. volkensii* fraction B at doses of 40, 100 and 200µg/g of body weight.

Key:

A-Recording from control locust before injection with 60% ethanol.
A'-Recording from control locust one hour after injection with 5µl of 60% ethanol.
B-Recording from experimental locust No.1 before injection.
B'-Recording from experimental locust No.1 one hour after injection with *M. volkensii* fraction B at a dose of 40µg/g of body weight.
C-Recording from experimental locust No.2 before injection.
C'-Recording from experimental locust No.2 one hour after injection with *M. volkensii* fraction B at a dose of 100µg/g of body weight.
D-Recording from experimental locust No.3 before injection.
D'-Recording from experimental locust No.3 one hour after injection with *M. volkensii* fraction B at a dose of 200µg/g of body weight.

3.9.6 Effect of *M. volkensii* fruit extracts on oxygen consumption in *L. migratoria*

In the present study, it has been demonstrated that injection of *L. migratoria* with *M. volkensii* fractions A and B at doses above 50µg/g body weight reduces abdominal pumping considerably. In the following experiment, the effect of injected fraction B on oxygen consumption in *L. migratoria* adult males was investigated. Figure 21 shows the rate of oxygen consumption in *L. migratoria* adult males injected with up to 400µg of *M. volkensii* fraction B per gram of body weight. Oxygen consumption in treated insects was reduced dramatically. The control insects consumed 21.9 ±0.69µl O_2/g/min. Locusts treated with 100µg B/gm of body weight consumed 14.1 ±0.85µl O_2/g/min while those treated with 200µg B/g of body weight consumed 3 ±0.3µl O_2/g/min

3.9.7 The effects of *M. volkensii* extracts on the rate of peristalsis in *L. migratoria*

Results presented in Tables 39 and 40 show the frequency of the position of glass fibre pellet in the gut of *L. migratoria* adults injected with 50µg of *M. volkensii* fractions A and B three hours after feeding on a diet of glass fibre discs impregnated with 0.25M sucrose. The glass fibre pellet was sought in the foregut, crop, midgut and hindgut. In the majority of cases, the pellet was located in the foregut and crop. The χ^2 test did not reveal a significant difference in the frequency of the location of the pellet in the treated and control insects.

3.9.8 Rate of heartbeat in *L. migratoria* adults poisoned through bait, injection and topical treatment with *M. volkensii* fraction B

Earlier findings in the present study indicated that locusts were paralysed when fed wheat seedlings sprayed with *M. volkensii* fractions A and B at concentrations above 0.5%. A similar state of stupor could be induced by injecting locusts with the fractions at doses above 80µg/g of body weight or by topical treatment at doses above 500µg/g body weight. In the continuing investigation on the mode of action of *M. volkensii* on insects, the following experiments were set up in an attempt to determine whether the stupor induced through ingestion was similar to that induced through injection and topical treatment. Table 41 shows the heartbeat rates of *L. migratoria* adults paralysed within 48 hours through feeding on wheat seedlings sprayed with 1% solution of fraction B in 25% ethanol, injection with 100µg B/g body weight and by topical treatment with fraction B dissolved in acetone:olive oil (2:3) at a dose of 1000µg/g of body weight. The rates were compared with those of control

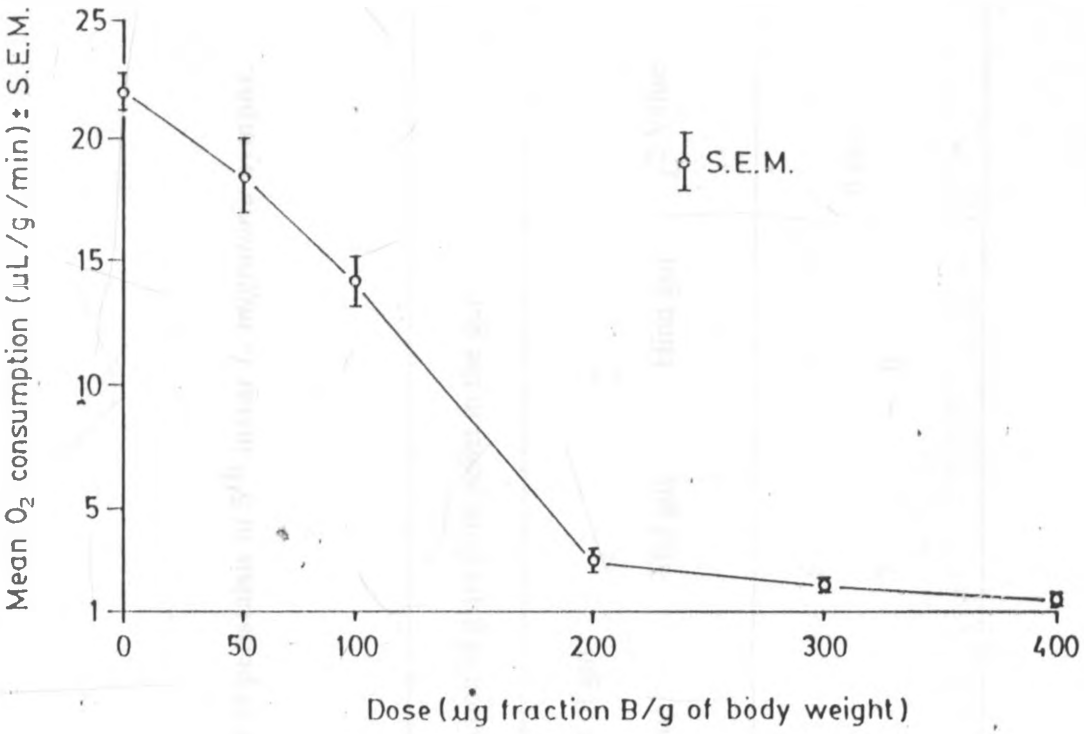


Figure 21: **The effect of injected *M. volkensii* fraction B on the rate of oxygen consumption in *L. migratoria* male adults. (n=4).** (Oxygen consumption was measured over a period of 40 minutes. Four groups of 10 insects were used at each dose tested).

Table 39: **The effect of *M. volkensii* fraction A on the rate of peristalsis in 5th instar *L. migratoria* nymphs.**

	Frequency of the position of glass fibre pellet in the gut				
	Part of gut				
	Fore gut	Crop	Mid gut	Hind gut	χ^2 Value
Control (n=45)	22	21	6	1	0.061
Treated (n=45)	22	20	7	0	

χ^2,0.05; df,3 = 7.815

Table 40: **The effect of *M. volkensii* fraction B on the rate of peristalsis in 5th instar *L. migratoria* nymphs.**

	Frequency of the position of glass fibre pellet in the gut				
	Part of gut				
	Fore gut	Crop	Mid gut	Hind gut	χ^2 Value
Control (n=45)	23	20	7	0	1.600
Treated (n=45)	17	15	12	4	

χ^2,0.05, df,3 = 7.815

Table 41: **Heartbeat rates of *L. migratoria* adults paralysed within 48 hours through feeding on wheat seedlings sprayed with 1% of *M. volkensii* fraction B (Bait), injection with 100µgB/g of body weight and by topical treatment with fraction B dissolved in acetone:olive oil (2:3) at a dose of 1000µg/g of body weight.**

	Heartbeat rate (Pulsations/min ±S.E.)		
Treatment	Control	Treated	t-Value (df=30)
Bait	70.0 ± 7.9	66.4 ± 5.5	0.3629 Ns
Injection	81.8 ± 2.3	78.0 ± 2.1	1.2310 Ns
Topical	86.8 ± 2.1	80.1 ±2.3	2.0337 Ns

Ns= No significant difference at $P < 0.05$

insects There was no significant difference in the heartbeat rates of controls and the three groups of treated locusts (t-test,df=30) Similarly, in all cases peristaltic movements of the gut and waving of the malpighian tubules was evident

3.10 The effect of post-treatment temperature on the toxicity of *M. volkensii* fruit extracts on *L. migratoria*

3.10.1 The effect of post-treatment temperature on the toxicity of injected *M. volkensii* fruit fraction B

Results presented in Table 42 show the effect of post-treatment temperature on the 48 hour LD_{50} of *M. volkensii* fraction B injected into *L. migratoria* adults. The equations of regression lines from probit mortality at posttreatment temperatures of 15°C and 40°C versus log dosage plots are presented. There was a sharp decrease in the LD_{50} with rising temperature. The extract had an LD_{50} of 15.4ppm and 159.6ppm at 40°C and 15°C, respectively.

3.10.2 The effect of post-treatment temperature on the onset of paralysis in *L. migratoria* adults treated topically and by injection with *M. volkensii* fruit fraction B

Results on the effect of post-treatment temperature on the onset of paralysis in *L. migratoria* adults injected with 300µg of fraction B/g of body weight are presented in Table 43 The percentage figures reported were subjected to arcsine transformation. There was a dramatic acceleration of the onset of paralysis with increasing temperature. While 90% of insects kept at 40°C were paralysed in 1 hour, only 6.2% of those kept at 15°C showed signs of paralysis. Results presented in Table 44 show the effect of post-treatment temperature on the onset of paralysis in *L. migratoria* adults treated topically with 1000µg *M. volkensii* fraction B in acetone:olive (2:3). The percentage figures reported were subjected to arcsine transformation. As in the experiment with injected extract, there was a dramatic acceleration of the onset of paralysis with rising temperature. While only 17.7% of insects kept at 15°C developed paralysis in 24 hours, 77.7% of those at 40°C developed paralysis in 2 hours. In these two experiments, none of the control groups of insects kept at 15°C and at 40°C were paralysed over the duration of the experiment Results of analysis of variance of the data presented in Tables 43 and 44 is also presented in Appendices 4 and 5, respectively.

Table 42: **The toxicity of injected *M. volkensii* fraction B to *L. migratoria* adults at post-treatment temperatures of 15°C and 40°C. (Number of insects per bioassay=10).**

Post-treatment temperature(°C)	Equation of regression line	R-Sq.(%)	48 hour LD_{50}(µg/g)•	95% Confidence limit for 48 hour LD_{50} (µg/g) Lower	Upper	t-Value	d.f.
15	Y=-18.3+10.6x	88.1	159.6	103.4	246.4	3.84	2
40	Y=-1.14+3.4x	91.2	15.4	9.4	25.1	8.52*	7

* Significant at $P < 0.05$

• Calculated Log LD_{50} transformed to LD_{50}.

Table 43: **The effect of post-treatment temperature on the time to the onset of paralysis in *L. migratoria* adults injected with *M. volkensii* fraction B at dose of 300µg/g of body weight.**

Post-treatment temperature (°C)	Percentage• (±S.E) of insects in paralysis								
	Time after treatment (Hours)								
	0.5	1	1.5	2	3	4	5	6	n
15	0	6.2 ±6.2	6.2 ±6.2	28.8 ±2.2	45.0 ±6.8	52.8 ±2.0	67 ±10.7	90	30
20	0	15.0 ±7.9	31.0 ±2.2	43.0 ±5.2	62.7 ±8.9	75.0 ±7.9	90	---	30
25	0	28.3 ±4.9	39.1 ±3.4	55.0 ±4.2	61.9 ±6.1	83.9 ±6.2	90	---	30
30	12.3 ±6.2	39.1 ±3.4	48.9 ±3.9	63.9 ±4.3	83.9 ±6.2	90	---	---	30
35	33.0 ±3.7	59.2 ±4.2	83.9 ±6.2	90	---	---	---	---	30
40	43.1 ±1.9	90	---	---	---	---	---	---	30

• Percentages converted to arcsine.

Table 44: The effect of post-treatment temperature on the time to the onset of paralysis in *L. migratoria* adults treated topically with *M. volkensii* fraction B in acetone:olive oil (2:3) at dose of 1000µg/g of body weight.

Post-treatment temperature (°C)	Percentage• (±S.E) of insects in paralysis									
	Time after treatment (Hours)									
	1	1.5	2	3	4	5	6	12	24	n
15	0	0	0	0	0	0	0	0	17.7 ±8.9	30
20	0	0	0	0	0	0	0	0	28.8 ±2.2	30
25	0	0	0	0	0	0	15.0 ±7.9	23.9 ±2.7	47.0 ±1.9	30
30	0	6.2 ±6.2	15.0 ±7.9	28.8 ±2.2	35.2 ±2.0	43.1 ±1.9	49.0 ±3.9	66.1 ±2.7	77.7 ±6.2	30
35	15.0 ±7.5	34.3 ±3.0	50.9 ±3.4	72.8 ±9.6	90	---	---	---	---	30
40	28.8 ±2.2	49.0 ±5.2	81.1 ±8.9	90	---	---	---	---	---	30

• Percentages converted to arcsine.

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

4.1 DISCUSSION

Biologically active compounds are extracted from plants using solvent systems whose choice is dictated by the nature of the compounds. The commonest solvent systems are water and organic solvents. Material with antifeedant effects on *S. gregaria has* been previously extracted from *M. volkensii* fruit with 95% methanol (Mwangi, 1982). From the same fruit, extracts with toxic effects on mosquito larvae have been extracted with 80% methanol (Mwangi and Mukiama 1988; Mwangi and Rembold, 1987,1988)

Despite the growing interest in the biologically active substances in *M. volkensii* fruit, no detailed study of the range of common solvent systems capable of recovering these compounds from the plant has been documented. The present study has revealed that methanol concentrations between 60% and 99.6% and ethanol concentrations between 50% and 99.7% effectively extracted biologically active compounds from *M. volkensii* fruit powder. All extracts thus recovered had significant toxic effects on *A. aegypti* larvae and antifeedant effects on *L. migratoria*. The effectiveness of the solvent systems to extract activity decreased with increasing polarity. Methanol which is more polar than ethanol extracted less active compounds when the two solvents were tested at similar concentrations below 50%. The observation that the activity of material extracted with increasing concentrations of solvent reached a peak is interesting. Low concentrations of organic solvent extract mainly inactive water soluble material. At very high organic solvent concentrations, the extracts consist of active material and inactive oil which lowers the specific activity of the extracts.

Phytochemical screening of medicinal plants has shown that various parts of plants differ in content of biologically active compounds. The plant parts commonly used for medicinal preparations are roots, root bark, stem, stem bark and leaves (Chhabra *et al.*, 1984). Harvesting of these plant parts especially on a large scale basis poses the danger of destroying precious rare plants that may not be regenerated easily. Little work has been done on the distribution of biologically active compounds in plant parts. Singh (1987) has tested various parts of the seed of *A. indica* for antifeedant activity on *S. gregaria* and found most activity in the seed kernel. Screening *of M. volkensii* for biologically active substances in various parts of the plant has not been reported although data is available for whole fruits (Mwangi, 1982; Mwangi and Rembold,1987,1988; Mwangi and Mukiama, 1988).

There has been speculation that the bark of *M. volkensii* contains biologically active substances (Kokwaro, 1976), although these have not been bioassayed. In the present study, all parts of *M. volkensii* have been shown to contain 80% methanol soluble material with larvicidal and antifeedant activity. The content of the biologically active substances however varies in different parts of the plant. The comfirmation of the presence of high activity in extracts from fruit parts is a finding with important conservational implications. Fruits are a convenient renewable resource from plants and therefore would be the material of choice for extraction of natural products. The indications are that despite the wide distribution of activity in all parts of the tree, it would not be justifiable to fell the tree for the purpose of extracting insecticidal compounds. The *M. volkensii* fruits are large and the tree fruits abundantly. Further, the fruit parts need not be separated before extraction.

The high larvicidal and antifeedant activity obtained from the testa does not justify the tedium of dissecting out this tissue as the yield of raw material is quite low. The testa constitutes only 2% of the dry weight of the fruit. The confirmation of activity in other parts of the plant other than the fruit is interesting. When *M. volkensii* has for good reasons to be cut down for example in furniture making, only the stem is used. The bark, roots and leaves would be available as a source of biologically active substances.

Plants from different ecotypes have been known to vary in the content of biologically active substances. An example of these is *A. indica*. Kernels from Indonesian *A. indica* have been shown to contain more azadirachtin than those from Niger (Ermel *et al.*, 1984). While *M. volkensii* fruit from Embu and Tsavo have been reported to possess biologically active compounds by several workers (Mwangi, 1982;Mwangi and Rembold, 1987;1988;Mwangi and Mukiama, 1988; Rajab *et al.*, 1988) no comparative study on the content of the active principles in fruits emanating from these two areas has been documented.

In attempts to develop and promote natural products for wide application, studies on the content of these substances in plants from different ecotypes are important. The aim of such studies is to identify and use the high yielding varieties. That *M. volkensii* fruit from Embu and Tsavo areas have been shown in the present study not to have differences in content and activity of biologically active substances is of immense importance. A large pool of trees is available for extraction of these substances for large scale application of the insecticide.

A large number of physiological and biochemical changes occur during the ripening of fruits. These include the degradation of starch or other storage carbohydrate, the production of sugars, the synthesis of pigments and volatile compounds and the

modification or partial modification of cell walls (Grierson *et al*., 1981). Drying of *M. volkensii* fruit is slow at 30^{O}C and takes up to four weeks. During this process, mature unripe fruits tend to ripen in the first few days. This phenomenon may result in these fruits acquiring chemical profiles similar to those of ripe fruits. The observation that both ripe and mature unripe fruits produce similar quantities of active material is of importance in the processing of large amounts of material. It would be instructive to harvest all mature fruits along with ripe ones without the need to sought them out.

In the present study it has been shown that it is possible to partially purify biologically active substances from *M. volkensii* fruit by cold precipitation from a concentrated 80% methanol extract without significant loss of yield and activity in the final product. This novel procedure in addition to evading the expensive use of acetone and pure methanol also skips the rotary evaporator step at which the 80% methanol extract is normally dried (Mwangi and Mukiama, 1988; Mwangi and Rembold, 1987;1988). Evaporation of the last quantities of water in the extract is extremely slow considering that this is done at 40^{O}C albeit in vacuum. This new method therefore saves time as well as resources.

Water soluble substances in *M. volkensii* fruit extracts are extremely difficult to dry in a rotary evaporator at 40^{O}C. Further, when dry, the extract is very hygroscopic making it difficult to handle. These water soluble materials which are co-extracted with more lipophilic substances by 80% methanol have very low biological activity. Removal of these water soluble substances would therefore solve the technical problems associated with their hygroscopic properties. In the present study, it has been demonstrated that pre-washing the *M. volkensii* fruit powder with water before extraction with 80% methanol produces a none hygroscopic extract with high activity without significant loss in yield. This novel procedure could thus be adopted as a first step in the extraction of biologically active substances from *M. volkensii* fruit.

Aedes aegypti 2nd instar larvae bioassays and antifeedant tests carried out on methanolic solutions of *M. volkensii* fractions A and B stored at room temperature for two years showed that the material lost very little activity in storage. Similar properties of stability were revealed by larvicidal and antifeedant tests carried out on solutions of 80% methanol extracts of *M. volkensii* fruit stored for seven years at room temperature. While it is known that freeze-dried *M. volkensii* fruit extract retains activity for long periods, the low loss of activity in methanolic solutions stored at room temperature even for as long as seven years is interesting. During the processing of this extract in the field especially in Africa where vacuum evaporators may not be available, it would be possible to perform the extraction and store the solutions at room temperature until needed for application without significant loss of activity.

Fractionation of *M. volkensii* fractions A and B by TLC enriches the antifeedant activity in several bands. The reasons for this apparently poor resolution can only be speculative. The phenomenon may be attributed to "tailing" of the compounds in the chromatogram or it could be that each band consists of certain active compounds distinct from those in other bands. The latter explanation seems more plausible because under UV light, the chromatograms showed distinct bands. There is no reason to predict the presence of only one compound with antifeedant activity in *M. volkensii* fruit. That most of the material recovered from fraction A by TLC appeared in the most lipophilic bands is not surprising. *M. volkensii* fraction A consists of compounds which are more lipophilic than those in B because in the silica gel chromatography protocol, A was eluted with a more lipophilic solvent system. The results contrast sharply with those of Mwangi and Mukiama, (1988) who found the most lipophilic TLC fractions of *M. volkensii* fruit to be most toxic to *A. gambiense* larvae. This pattern of toxic activity does not follow that of the antifeedant data presented in the present study although locust antifeedant activity does not necessarily follow mosquito larvicidal activity.

Antifeedant and toxic activity of *M. volkensii* fruit extracts on *S. gregaria* has been reported (Mwangi, 1982). Locusts treated topically with a 95% methanol extract or fed on treated food showed poor growth rate and delayed moulting. Some treated nymphs were reported to be dying during ecdysis. Azadirachtin injected at doses below 10 µg/g in *L. migratoria* 5^{th} instar nymphs results in very prolonged stadia (Sieber and Rembold, 1983). Other effects include reduced faecal output and food intake. Data on effects of purified *M. volkensii* extracts on moulting in locusts is not available. In the current study, it has been demonstrated that the "purified" fractions A and B do not cause profound delay in moulting in *L. migratoria* 5^{th} instar nymphs which are treated either topically or by injection. Indeed significant delay in moulting was only observed in nymphs injected with *M. volkensii* fraction B only. In contrast, the moult inhibition activity of azadirachtin was dramatic. The results of the current study do not however rule out the presence of insect growth inhibitors in *M. volkensii* fruit extracts. It is speculated that the growth inhibiting effects of *M. volkensii* are masked by high toxic or antifeedant activity. It is also interesting to note that while most of the A and B treated nymphs which manage to moult survive as apparently healthy adults, azadirachtin treated nymphs that manage to moult develop into weak adults which die a few days later. The activity of *M. volkensii* on moulting in *L. migratoria* appears to be "all or nothing". The insects either die as nymphs or moult into normal adults.

Significant mortality has been reported over a 24 day period in *S. gregaria* feeding on food treated with methanolic extracts of *M. volkensii* fruit (Mwangi, 1982). It is

interesting to note that in the present study, although there were deaths in locusts presented with wheat seedlings sprayed with over 0.5% solutions of *M. volkensii* fruit extracts, the damage caused through feeding to these seedlings was very small. This observation suggests that the dying insects were poisoned by ingesting tiny quantities of the extracts. Poisoning of locusts through contact seemed unlikely. Results of earlier experiments in the current study have demonstrated that only *M. volkensii* fraction B possesses high contact poison activity on locusts and that this activity was only evident several days after treatment. *Melia volkensii* fraction A did not show high contact poison activity and yet in the current study it has been shown to be as potent as fraction B in poisoning locusts feeding on treated wheat seedlings. It is noteworthy that significant mortalities in this case are observed in as short a time as 48 hours. The observation that death occurs in 48 hours rules out the possibility of starvation being the cause of death as it has been demonstrated elsewhere in the current study and by other workers (Uvarov, 1966) that starved *L. migratoria* can survive much longer than 3 days.

Results of the study have demonstrated that *L. migratoria* 5^{th} instar nymphs and adults which are treated topically on the neck membrane with *M. volkensii* fractions A and B show reduced faecal output and poor body weight gain. The two fractions also had similar LC_{50}'s in the *A. aegypti* 2^{nd} instar larvae bioassay. High mortality was also observed amongst locusts treated with fraction B at doses of 200µg/g of body weight. When applied topically on locusts at high concentrations, the difference in the contact poison activity of fractions A and B was clear. It was surprising that fraction B had over three fold more activity than fraction A when applied on locusts topically at high doses. It had been proposed that fraction A which is more lipophilic than B would have greater cuticle penetrating properties. It is postulated that *M. volkensii* fruit has active compounds with different effects on locusts. There are antifeedants and repellants which occur in both fractions A and B and contact poisons which occur mainly in fraction B. The cuticle penetrating compound(s) in B deserve further investigation as their peculiar activity would be of enormous advantage in the development of locusticidal sprays.

Insecticides are formulated for maximum efficacy and economy. Insecticides that are not soluble in water may be presented for spray application as oil/water emulsions with emulsifiers or surfacants added. Further, poor cuticle penetrating properties of a contact insecticide may be improved by incorporating lipophilic solvents (Ware, 1982). *Melia volkensii* fraction B has earlier been shown in the current study to kill *L. migratoria* nymphs and adults when applied topically. Using acetone alone as solvent, significant mortalities were observed in about 5 days. By incorporating olive oil

in the treatment solution, significant mortality or stupor was induced in less than 24 hours. One possible explanation of this enhanced activity would be that olive oil which has a high boiling point remains on the cuticle for a long period therefore facilitating continuous penetration of the toxic substances so that a lethal concentration is reached in the insect faster.

The reason(s) why olive oil was more efficient than other plant oils as a solvent in topical application formulations can only be speculative at this stage. However synergistic effects cannot be ruled out. The results of this study are of immense importance in the future development of *M. volkensii* fruit extracts for field application. With better formulation it now appears possible to enhance the "knock down" activity in this extract. Such enhancement of activity was demonstrated on locusts by the acetone olive oil (2:3) formulation of *M. volkensii* fraction B applied topically at a dose of 1000ug/g of body weight, which caused a mortality of 28.8 ±2.2% in one hour at $40^{o}C$. This activity is attractive to the users of such products. It is also noteworthy that the findings reported in the current study open the way for further studies on the acute mode of action of the biologically active substances in *M. volkensii* on insects when applied through the topical route.

The mechanism through which *M. volkensii* fruit extracts produce antifeedant effects on locusts is not known. The results presented in the current study on the effect of treatment on the palps and treatment on the neck with *M. volkensii* fractions A and B on the faecal output of *L. migratoria* adult males show a marked difference in the response of these two regions to the extracts. The persistence of the effect of a single treatment with the extracts on the palps on body weight gain for up to 22 days after treatment is important because such a phenomenon would interfere with maturation of the insects leading to reduced fecundity. This poor weight gain is caused by reduced feeding as confirmed by results on the effect of treatment on the palps with fractions A and B on faecal output for 24 days. The control insects fed voraciously for the first 8 days during which their body weights increased considerably whereas the treated insects fed to a very small extent and lagged behind in weight gain. These results of the current investigation indicate that palps are involved in producing the antifeedant effects of *M. volkensii* fruit extracts on locusts. The findings also open the way for electrophysiological studies using purified compounds from *M. volkensii* fruit on palp chemoreceptors. Such studies would be of importance in the elucidation of the mode of action of these extracts on insects.

Feeding in locusts is not initiated until the foregut and hindgut are fairly empty (Bernays and Chapman, 1973; Simpson, 1983) and therefore inhibition of peristalsis would produce an antifeedant effect. Mordue *et al.*,(1985) have shown that

azadirachtin has a direct effect on locust gut muscles. It causes reduced contraction and therefore inhibits peristalsis. In the current study, there was insignificant inhibition of the rate of peristalsis in *L. migratoria* 5^{th} instar nymphs which were injected with 30µg of A or B. From the results of previous work in the current study, a dose of 30µg/g of body weight of either fraction A or B administered by injection in *L. migratoria* produced significant reduction in faecal output. It is therefore likely that the antifeedant effect is produced through other mechanisms rather than inhibition of peristalsis.

In the *A. aegypti* 2^{nd} instar larvae bioassay, the LC_{50}'s of A and B were considerably lower than that of the 80% methanol extract. However in wheat seedling antifeedant tests with locusts all these extracts showed similar activities. This observation indicates that in the standard purification procedure used (Mwangi and Mukiama, 1988;Mwangi and Rembold, 1987,1988) the mosquito larvicidal activity is enriched while the locust repellent activity is not.

The rate of oxygen consumption is an indicator of metabolic activity in an insect. In locusts, increased activity results in accelerated abdominal pumping. This is an indication of increased demand for oxygen. Oxygen consumption is therefore directly proportional to the rate of abdominal pumping. It is not surprising therefore that *M. volkensii* fraction B should inhibit both abdominal pumping and oxygen consumption in *L. migratoria*. It is noteworthy that there was significant oxygen consumption in insects treated with high doses of B. This could explain why the insects remain alive in a stupor for several hours after treatment.

In experiments done in the *S. gregaria* recession areas in the Southern Tamesna desert in Niger, Wilps *et al.*, (1993) reported a mortality rate of as high as 60% in 48 hours and of 100% amongst *S. gregaria* adults injected with ethanolic extracts of *M. volkensii* fruit at doses between 3.5 to 6.5µg/g body weight in experiments conducted at 20 to 40^0C. Such high mortalities have not been observed in laboratory experiments conducted in Nairobi, Kenya at 22^oC. At doses of 20ppm, *M. volkensii* extracts caused a mortality of less than 10% in *L. migratoria* adults in 48 hours. This large discrepancy in activity may partly be attributed to the effects of temperature on toxicity reported in the present study. The positive temperature coefficient of *M. volkensii* extract toxicity on locusts would be of great advantage as locust prone areas are generally hot. This discovery may also lead to further clues on the mode of action of these extracts. Increasing temperatures may lead to the acceleration of catabolic reactions resulting in accumulation of lethal amounts of end products like carbon dioxide and ammonia. It is speculated that at high temperatures, there occurs changes in the fluid structure of cell membranes which accentuate the toxic effects of the extracts. Such changes in membrane structure may allow better penetration of the extracts into the cells resulting

in accelerated disruption of certain metabolic pathways. Further, disruption of the integrity of cell membranes would result in cell death leading to decreased oxygen consumption. It would be interesting to compare the results of these laboratory experiments with field trials.

The results obtained in the present study reveal some aspects of the activity of *M. volkensii* which were previously not clear. For example, the antifeedant effect induced in topically treated insects is not absolute but relative since a large increase in the dosage did not result in a corresponding decrease in faecal output indicating that the insects continued to feed albeit at a reduced rate. These results are concordant with those obtained by baiting the insects with *M. volkensii* sprayed seedlings. *Melia volkensii* has been shown to produce a feeding protection of about 100% in *S. gregaria* (Mwangi, 1982). It is speculated that although the extracts produce a high repellency effect at high concentrations, it is not absolute. The insects feed to a very small extent and in the process ingest lethal quantities that act as stomach poison. It is unlikely that the mortalities caused through baiting were due to contact of the insects with *M. volkensii* treated plants since the extract forms a thin dry film on the leaves. Previous reports had shown that *M. volkensii* extracts could only cause significant mortality through topical application on locusts after several days (Mwangi, 1982). It is speculated that this low rate of mortality was due to the small amounts of active material penetrating the cuticle. At high doses it is possible to induce lethal effects in shorter periods of time.

Locusts paralysed by *M. volkensii* may remain in a stupor for over 24 hours. It was puzzling how the insects could remain alive for so long. The explanation that can be drawn from the results of the present study is that since the ventilation movements do not seem to cease immediately on treatment, but gradually, sufficient gaseous exchange occurs to maintain vital physiological functions, e.g. of the heart and gut. Such a situation is even more likely since the spiracles of paralysed insects remain partially open. These effects have been reproduced with more than one *M. volkensii* fraction indicating that the activity is most likely attributable to more than one compound.

Full scale application of *Melia volkensii* extracts by farmers appears feasible. One mature *M. volkensii* tree produces about 100kg of dry fruit per year from which 1kg of active ethanolic extract is obtainable. Ultra low volume application of a 5000ppm formulation of the extract in vegetable oil as done by Wilps *et. al.*, (1993) against *S. gregaria* in field trials at a rate of 5L/Hectare would cover 40 Hectares of farmland.

4.2 CONCLUSIONS

The following conclusions can be made from the results of the present study:

1. Biologically active substances are most effectively extracted from *M. volkensii* fruit with 60% to 80% ethanol or 70% to 80% methanol.

2. Mature and not necessarily ripe fruit are a rich source of the bioactive compounds in *M. volkensii*.

3. All parts of *M. volkensii* contain biologically active substances with the highest concentrations being localised in the fruit and root bark.

4. *Melia volkensii* fruit from Tsavo and Embu areas have equal content of biologically active substances.

5. In the production of *M. volkensii* fruit extracts, substantial economy in labour and solvents can be achieved by cold precipitation of the active fraction and also by pre-washing the fruit powder with water before extraction with the organic solvent.

6. *Melia volkensii* fruit extracts can be stored safely for several years even at room temperature without significant loss of larvicidal and antifeedant activity.

7. All *M. volkensii* fruit fractions have stomach poison activity against *L. migratoria*.

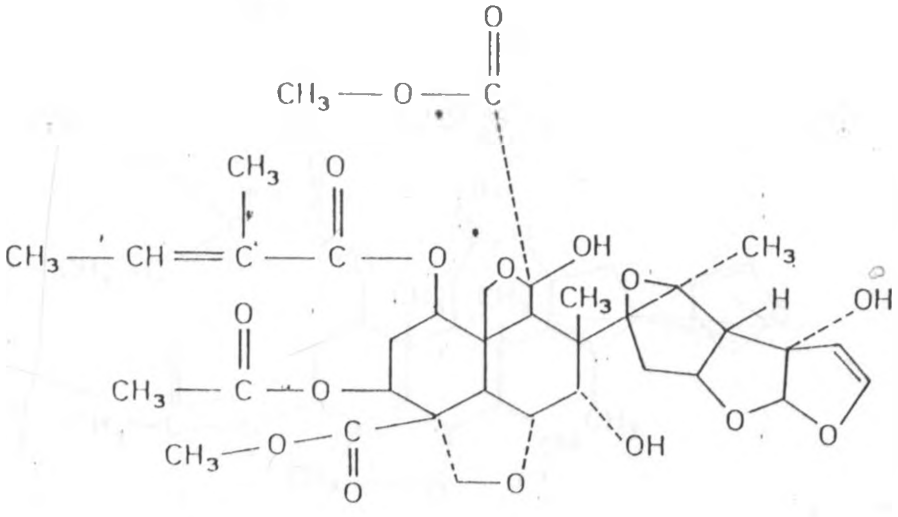
8. *Melia volkensii* fruit has compounds with contact poison activity against *L. migratoria* which occur mainly in fraction B. This activity is enhanced by the addition of olive oil or other plant oils.

9. It is tentatively concluded that the mode of action of *M. volkensii* fruit extracts on locusts is through antifeedant/repellent action elicited by low dose treatment topically and by injection and direct toxicity on either the skeletal muscles or associated nerves induced through high dose treatment by bait, topical and injection treatment.

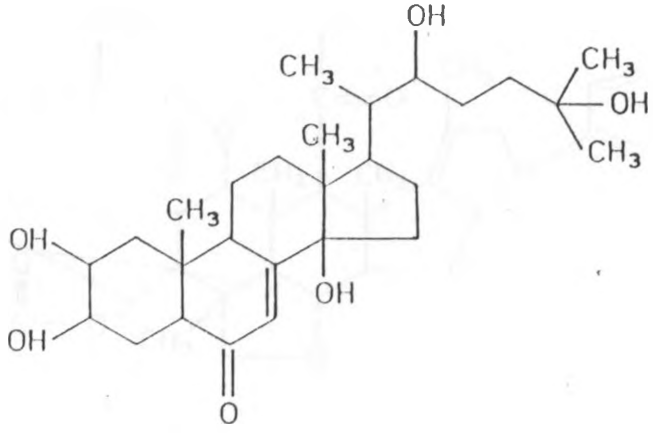
10. The acute toxicity of *M. volkensii* fruit extracts on *L. migratoria* has a positive temperature coefficient.

Appendix 1

Structural formulae of azadirachtin and α-ecdysone



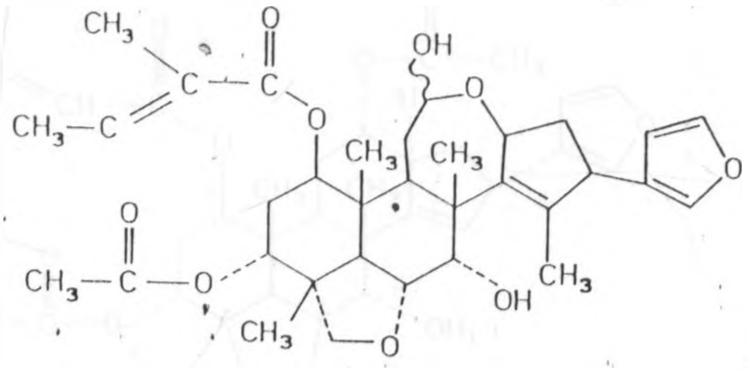
Azadirachtin (Zanno *et al.*, 1975)



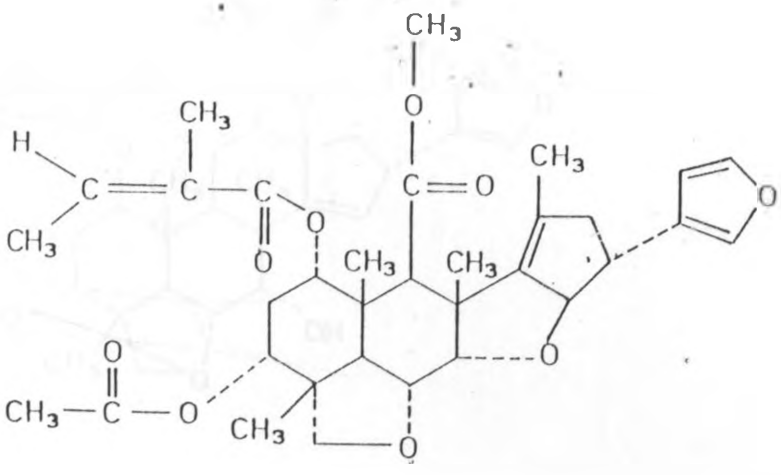
α-Ecdysone (Schneiderman, 1971)

Appendix 2

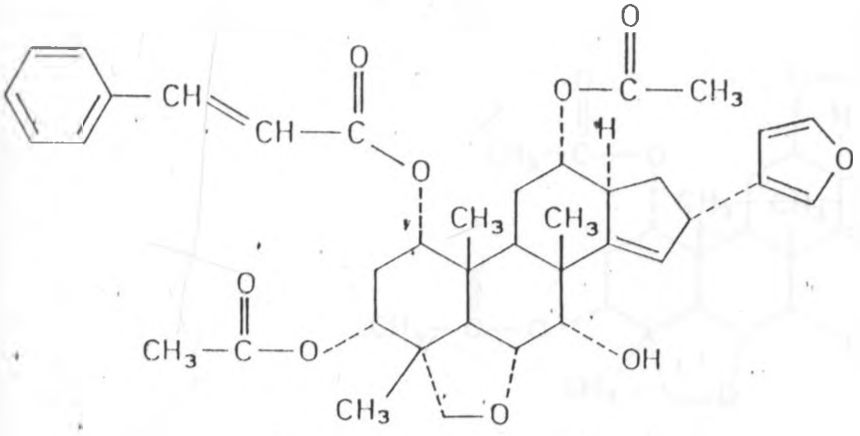
Structural formulae of limonoid compounds isolated from *Melia volkensii* fruit



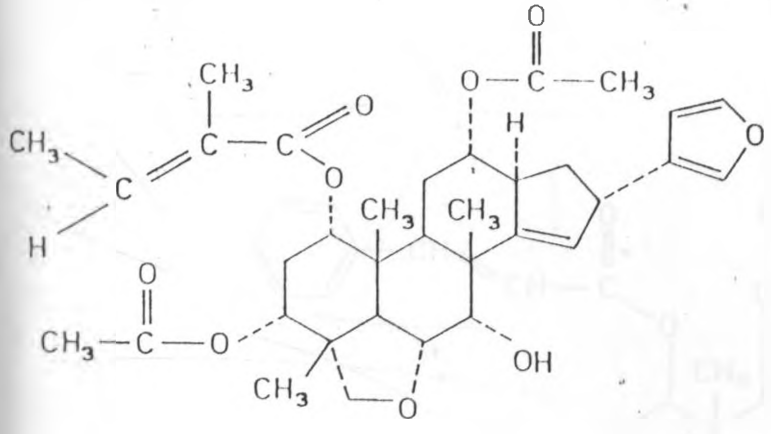
Volkensin (Rajab *et al.*, 1988)



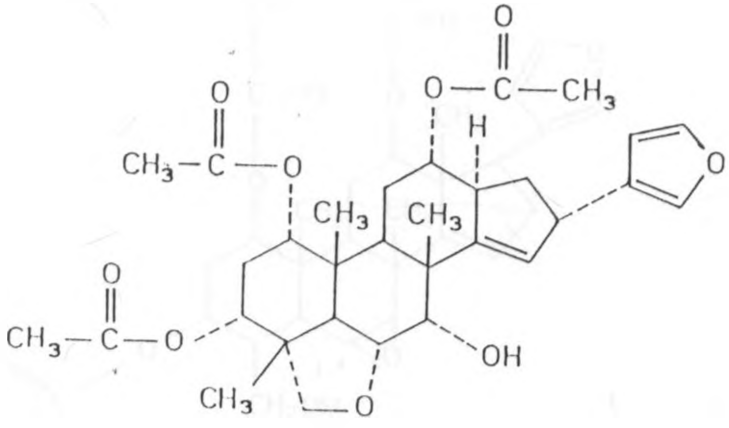
Salannin (Henderson *et al.*, 1964,Rajab *et al.*, 1988)



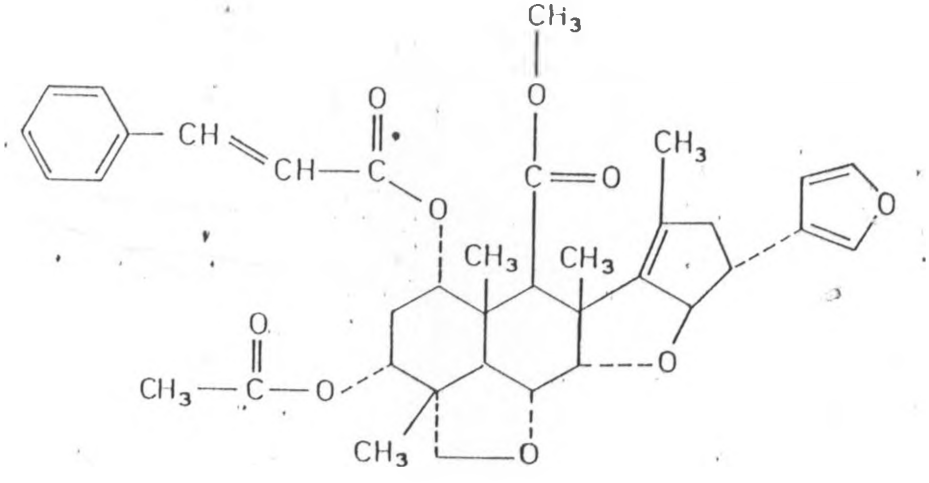
1-cinnamoyltrichilinin (Rajab and Bentley, 1988)



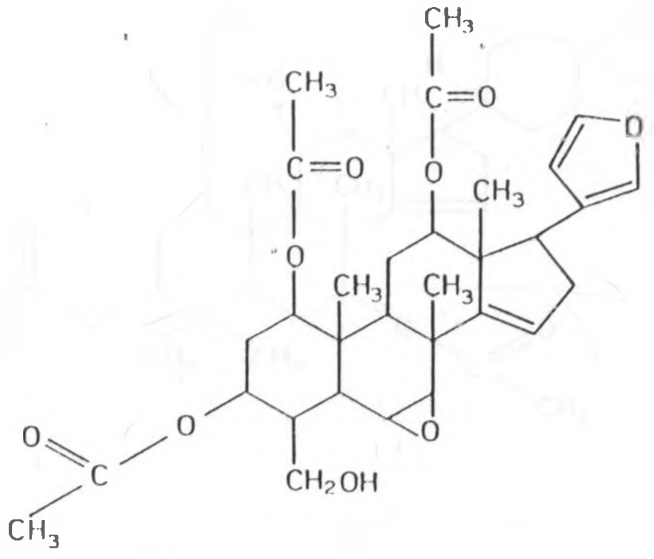
1-tigloyltrichilinin (Rajab and Bentley, 1988)



1-acetyltrichilinin (Rajab and Bentley, 1988)



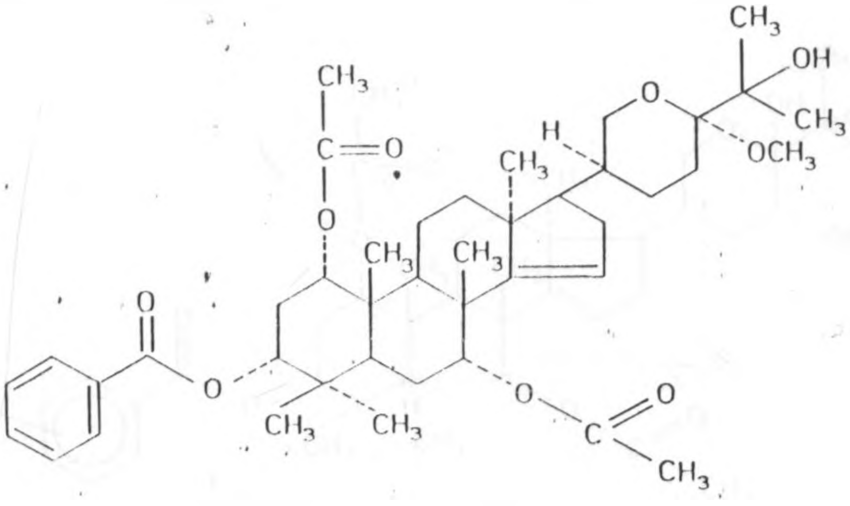
Ohchinin-3-acetate (Rajab and Bentley, 1988)



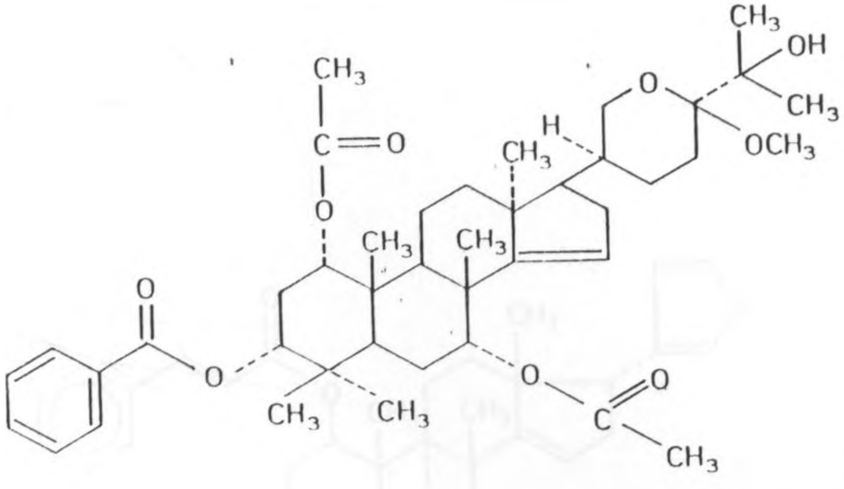
Meliacin (Balan, 1993)

Appendix 3

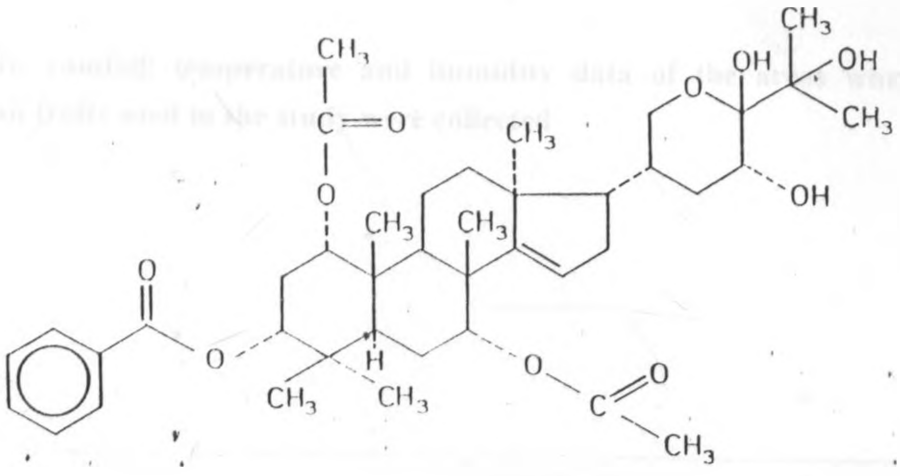
Structural formulae of limonoid compounds isolated from the root bark of *Melia volkensii*



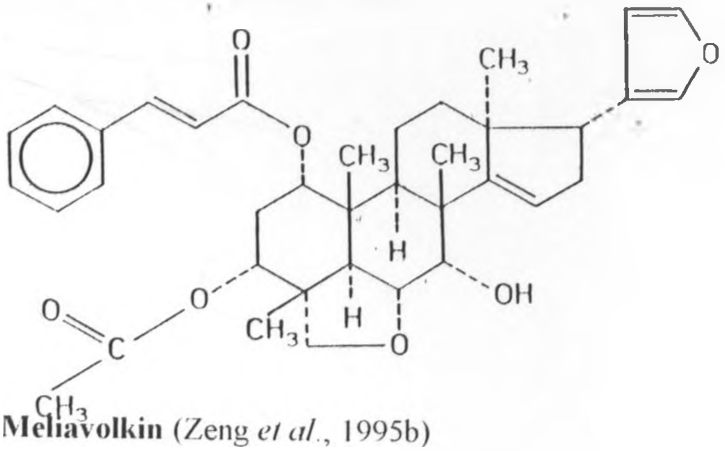
Meliavolkensins A (Zeng *et al.*, 1995a)



Meliavolkensins B (Zeng *et al.*, 1995a)



Meliavolin (Zeng *et al*., 1995b)



Meliavolkin (Zeng *et al*., 1995b)

Appendix 4

Altitude, rainfall, temperature and humidity data of the areas where *Melia volkensii* fruits used in the study were collected

Parameter	Collection area	
	Tsavo	Embu
Altitude (M)	500-1000	1000-1500
Mean annual rainfall (mm)	255-760	760-1015
Mean minimum annual temperature (°C)	18-22	10-14
Mean maximum annual temperature (°C)	30-34	26-30
Relative humidity (%)	60-70	60-70

Source: National Atlas of Kenya, 1970.

Appendix 5

Results of analysis of variance on the data on the effect of post treatment temperature on the onset of paralysis in *L. migratoria* adults injected with *M. volkensii* fraction B at a dose of 300µg/g of body weight.

	Time after treatment (Hours)							
	0.5	1	1.5	2	3	4	5	6†
F-Ratio	39.287	37.427	57.532	54.705	10.274	12.523	1.000	---
F-Probability	0.0000	0.0000	0.0000	0.0000	0.0005	0.0002	0.458	---
d.f. Between groups	5	5	5	5	5	5	5	---
d.f. Within groups	12	12	12	12	12	12	12	---

† No analysis carried out. All test locusts dead.

Appendix 6

Results of analysis of variance on the data on the effect of post treatment temperature on the onset of paralysis in *L. migratoria* adults treated topically with *M. volkensii* fraction B in acetone:olive oil at a dose of 1000µg/g of body weight.

	Time after treatment (Hours)								
	1	1.5	2	3	4	5	6	12	24
F-Ratio	13.210	34.434	45 803	100.145	2896.809	3166.642	138.346	732 996	48 415
F-Probability	0.0002	0.0000	0.0000	0 0000	0.0000	0.0000	0.0000	0 0000	0 0000
d.f. Between groups	5	5	5	5	5	5	5	5	5
d.f. Within groups	12	12	12	12	12	12	12	12	12

Appendix 7

Formula for conversion of ED_{50} to micrograms of *Melia volkensii* extract per cm^2 of filter paper used in paper antifeedant tests

Micrograms of *M. volkensii* extract per cm^2 of test paper=

$$\frac{(ED_{50} \times 50\mu l)/4cm^2}{1000\mu l}$$

Where: ED_{50}= Effective dose in ppm at which 50% of the test paper is protected.

50µl=Volume of extract solution applied to test paper of area $4cm^2$

1000µl=Volume of extract containing the weight of extract in micrograms numerically corresponding to the ED_{50} expressed in ppm.

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