EVALUATION OF ADIPOKINETIC RESPONSE IN THE ADULT DESERT LOCUST, SCHISTOCERCA GREGARIA UNDER THE INFLUENCE OF AZADIRACHTIN AND MELIA VOLKENSII EXTRACT.

BY

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A thesis submitted to the University Of Nairobi in partial fulfillment of the requirements for the Degree of Master of Science in Zoology.

1997
DECLARATION

The work presented in this thesis is the result of my own investigations and has not been presented for a degree award in another University. All sources of information have been specifically acknowledged by means of references.

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Thou art worthy, O Lord to receive glory and honour and power; for thou hast created all things, and thy pleasure they are and were created.

Rev 4: 11.
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<tr>
<td>A</td>
<td>Single lipoprotein termed A yellow (or lipophorin).</td>
</tr>
<tr>
<td>A+</td>
<td>That high molecular weight lipoprotein which carries diglyceride.</td>
</tr>
<tr>
<td>AKH</td>
<td>Adipokinetic hormone.</td>
</tr>
<tr>
<td>Aza</td>
<td>Azadirachtin.</td>
</tr>
<tr>
<td>C</td>
<td>That low molecular weight protein associated reversibly with A yellow lipoprotein and DGL to produce A yellow lipoprotein.</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic 3’ - 5’ adenosine monophosphate.</td>
</tr>
<tr>
<td>CC</td>
<td>Corpora cardiaca (Corpus cardiacum)</td>
</tr>
<tr>
<td>DGL</td>
<td>Diglyceride ; Diacyl glycerol.</td>
</tr>
<tr>
<td>MGL</td>
<td>Monoglyceride ; Monoacyl glycerol.</td>
</tr>
<tr>
<td>PTTH</td>
<td>Prothoracicotrophic hormone.</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions per minute.</td>
</tr>
<tr>
<td>TGL</td>
<td>Triglyceride ; Triacyl glycerol.</td>
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ABSTRACT

In order to develop realistic options and control strategies which are environmentally acceptable, sustainable utilization of indigenous botanical resources ought to be established.

Flight performance was reduced in the desert locust, *S. gregaria* Forskal after injections of 0.01 gland pairs of corpora cardiaca as a standard dose and treatment with a definable triterpenoids (limonoid) fraction extracted from *A. indica* and *Melia volkensii*, family Meliaceae. The cc extract only produced a hyperlipaemic response which was approximately double the initial resting lipid levels. From the data obtained in this study a rise in the haemolymph lipid level from 11.98 ± 1.63 μg/μl at zero time to 28.76 ± 2.97μg/μl 60 minutes from commencement of flight was realised and was significant at P < 0.05. In contrast, the blood lipid levels in azadirachtin-treated and flown locusts were less than those in the controls, which received cc extract only. Doses ranged between 0.1μg to 2μg of azadirachtin per locust. Using 0.1 μg - 0.25 μg/locust of *M. volkensii* triterpenoid fraction, the difference in total lipid concentration between resting levels and 60 minutes after treatment ranged from 1.9 to 8.9 μg/μl. A higher *M. volkensii* dose (0.5-2μg/locust) was not effective in inducing hyperlipaemic response at 60 minutes.

Unflown locusts which were injected with cc extract and treated with azadirachtin or *M. volkensii* extract showed increased hyperlipaemic response with maximal blood lipid
level occurring between 60 and 90 minutes. The control group which received cc extract only responded maximally within 30 minutes of treatment.

The poor flight activity observed in treated locusts confirmed earlier reports on the physiological effects of triterpenoid compounds. *A. indica* extract showed the highest effect on the reduction of flight speed within the initial 30 minutes. The mean flight speed in control locusts which received cc extract only was 2292 revolutions, while locust treated with 0.1µg of azadirachtin attained a mean flight speed value of 1688 revolutions in the first 30 minutes (76rpm and 56rpm respectively), a difference which was significant at P < 0.05. *M. volkensii* treated locusts also had significantly reduced speed compared to the controls. The flight pattern of locusts treated with *M. volkensii* extract showed a continued drop throughout the experimental period. The control group showed a mean speed of 14rpm between 60-90 minutes. This was a significantly high value compared to 6rpm in the treated group for *M. volkensii* doses above 0.1µg.

A high dose of the *M. volkensii* extract (0. 5-2 µg/locust) caused hind leg paralysis two days after treatment in adult *S. gregaria*. 
INTRODUCTION

1.1. General introduction

There are at least five important locust species in Africa that can cause extensive damage to the crops and general vegetation, thereby degrading the entire environment. These are the African migratory locust (*Locusta migratoria migratorioides* Reiche & Fairmaire), the red locust (*N. septemfasciata* Serville), the brown locust (*Locusta pardalina* Walker), the Senegalese grasshopper (*Oedaleus senegalensis* Krauss) and the desert locust (*Schistocerca gregaria* Forskal). Both the nymph and adult of these locust species feed on wild grass and agricultural crops, defoliating the trees and vegetation.

According to Bellen (1966), the extent of locust damage is related to their breeding habits, their abundance and their swarming behaviour. The swarm, if unchecked by pesticides or other methods of control will fly to infest plantations and farms, thus destroying crops. In the absence of vegetative cover loses its top-soil due to surface run-off. The advancement of desertification causes catastrophic events such as:

(a) disrupting the community’s day to day activities and often causing human emigration from plagued areas;
(b) causing abandonment of development projects thereby increasing poverty and malnutrition; and,
(c) Where the degree of vulnerability is high there is a tendency to despair and resignation because the population gets overwhelmed by their helplessness.
Odhiambo (1991) in a review of managing drought and locust invasion in Africa note that, Sahel Zone which lies south of The Sahara desert across the continent of Africa has shifted substantially, resulting in its enlargement. Observations on the Kalahari and Namib deserts as well as Somalia and North-Eastern Kenya (Unep, 1985) show that rapid deforestation has led to critical phenomenon of desertification.

The desert locust, which is the most widespread of all locust species during the frequent dry periods, lives as a herbivore in the Savannah ecosystem. The nymphs march as marauding migrants. Fragmentary laboratory evidence suggest that a shortage of food plants and their low water content may contribute to the intensity and range of movements of swarming adult locusts in the field. Much of the earlier work was concerned with the extraction of the major energy storage site (fat body) in the insect, to demonstrate correlation between the fat content and the inclination to fly. As discussed by Scoggin and Tauber (1950), many factors influence the lipid content of insects including nutrition, environmental temperature, sex, age, starvation and finally taxonomic position. Walker et al. (1970) also found that flight muscles of desert locust *S. gregaria*, contained 4 to 4.5 mg lipid per 100 mg wet weight of tissue at all stages of adult development. The use of fat as a major metabolic fuel during sustained flight was estimated at a rate of 4.1 mg/hr with the hydrolysis of diglyceride (DGL) and monoglyceride (MGL) significantly higher than triglyceride (TGL) (Crabtree and Newsholme, 1972b). Beenakkers (1965) calculated the value of the main haemolymph metabolite for energy during
prolonged flight by the locusts. During such flights energy reserves stored as TGL in the fat body cells (Kilby, 1965) was converted into DGL which is then used by the muscles. This conversion requires adipokinetic hormone (AKH). AKH is synthesised and stored in a neuroendocrine gland (neurohaemal) known as the corpus cardiacum from where it is released in response to physiological signals. The electron microscope has provided useful information about the cellular organisation of the corpus cardiacum, its relationship with the brain and the way in which secretory products may be released from it prior to their distribution throughout the body by the haemolymph. These physiological signals include:

1. crowding of the adults (which occurs prior to migration);
2. increased levels of octopamine in the haemolymph;
3. reduced levels of DGL and levels of trehalose in the haemolymph;
4. an increase in sodium chloride concentration in the haemolymph; and,
5. increased osmolarity of the haemolymph.

The three last events may not be the primary signals and may also occur during starvation (Jutsum et al. 1975) or after ovariectomy (Lee and Goldsworthy, 1976). After commencement of flight the second event sets in as the level of flight metabolite rise concomitantly. The last two, also independent events occur concurrently with production of metabolic water and osmoregulatory control by diuretic hormone to maintain haemolymph volume and osmotic homeostatic control during prolonged flight.
Studies with the light microscope on sectioned material clearly indicate that the corpus cardiacum functions as a storage depot for neurosecretory substances, synthesised by neurone cell bodies lying within the brain, notably in the protocerebrum, and passed along the nerve axons. Scharrer (1959) in an electron microscopic study, recognised an important additional feature, that the corpus cardiacum was also equipped with its own secretory material contained in a distinct lobe, the glandular lobe. In the adult migratory locust (Locusta migratoria migratorioides), the octopamine secreting neurones mediate the synthesis and release of these hormones (Orchard and Langer, 1983a). As mentioned above, provision seems to be made for the release both of secretions synthesised within the organ and those that are conveyed from the brain.

It is also known that extracts from the neem tree, Azadirachta indica (A. Juss) and Melia volkensii (Gurke) reduce flight performance in Locusta migratoria and Schistocerca gregaria Forskal (Wilps and Nasseh, 1993). It is however not established whether this reduction in flight performance involves the effects of adipokinetic hormone at receptor level or its release. The primary objective of this study was therefore, to determine the extent to which azadirachtin and another limonoid compound source are antagonistic to AKH. Corpus cardiacum glandular lobe extract was used as a source of AKH.
1.2. Justification.

The studies reported in this thesis aimed at:

(a) identifying effects of meliaceae extracts on haemolymph lipid concentration of adult locusts during rest and during flight; and,

(b) evolving a laboratory method of evaluating the effects of meliaceae compound(s) on flight speed and flight pattern of desert locusts.

If natural products could, in small amounts, effectively interfere with populations of desert locust and reduce the availability of physiological substrates, then environmentally friendly biopesticides whose active compounds meet much of the "criteria for biopesticides" present an interesting area for future study.
1.3. Objective.

The principle objective of the study was to assess the effects of compound(s) from *A. indica* and *M. volkensii* on the neuroendocrine control of lipid mobilization and flight performance in adult male locusts injected with corpora cardiaca tissue extract. Specific objectives were:

1. To determine the dose-response relationship of each compound in relation to haemolymph lipid concentrations during rest and during flight;
2. To determine the effect of each plant extract on flight performance; and
3. To assess *M. volkensii* extracts as potential locust control agents.
CHAPTER TWO

REVIEW OF LITERATURE.

2.1. Possible Endocrine control of phase forms in locusts.

A hormone can exert widespread effects by interacting with different effector tissues. For instance, adipokinetic hormone acts on the brain, suboesophageal connectives, fat body cells, lipoprotein profile, lipid oxidation in flight muscle and malpighian tubules of locusts (Beenakkers, 1969; Mayer and Candy, 1969; Goldsworthy, 1977a; Mwangi and Goldsworthy, 1977; Goldsworthy, 1983; O'Shea et al., 1984). The characteristics of the receptors in each tissue may differ just as the response may vary. Under crowding or during gregarisation in locusts the programming of related endocrine events result in swarming and enhanced flight performance (Pener, 1983). Several researchers who have worked on locust polymorphism remain uncertain about the endocrine control of locust phases. For instance, Albrecht and Lange (1978; 1979) reported that high temperature, increased daylength and high humidity induce a solitarizing effect on L. migratoria but could not explain the hormonal mechanisms involved. The existence of an endocrine factor which promoted melanization as a possible marker of gregarization in locusts was first claimed by Nickerson in 1954 and 1956. Nickerson injected the haemolymph of crowded S. gregaria hoppers into the haemolymph of isolated ones and obtained increased gregarious black patterns. Staal (1961) implanted extra corpora cardiaca into hoppers of Locusta and observed increased black patterns which
he claimed were caused by a factor from the corpora cardiaca.

However, Fuzeau-Braech (1985) outlined that the exact relationship between pigments and effects of endocrine factors on actual locust colouration was unknown, leaving the question of the endocrine control of pigmentation open to speculation. Whereas possible endocrine influences on aggregation behaviour of adult locusts was also unknown, it was true that active aggregation behaviour was a major factor in keeping the swarm coherent (Uvarov, 1977). The aggregative behaviour of marching hoppers studied in the laboratory by Ellis (1950, 1951) suggested that isolated hoppers spent less time on marching and marched more slowly than crowded hoppers. It was from this point of view that Uvarov (1966) proposed the term ‘gregarious’ and ‘solitarious’. In 1988, a desert plague of gregarious adults crossed the Sahel to reach the Atlantic Coast off West Africa. They made about 4,500 Km. of uninterrupted flight (Rainey, 1989). The solitary adult desert locusts were also capable of flight but the performance were much more limited. The longest established flight distance for solitary S. gregaria was about 300 Km (Uvarov, 1977) with no evidence of uninterrupted flight. These results were a demonstration of more intense responses of the crowded (gregarious) locusts with regard to a more intense flight behaviour.

2.2. AKH source, structure and role in sustaining flight.

Spencer and Candy (1976) provided evidence showing that partially purified extracts of the corpora cardiaca mobilized diglycerides from the locust fat body. This effect is
dependent on extracellular calcium. Goldsworthy et al. (1972a) found that the extract from the glandular lobes of the corpora cardiaca was 5-10 times more potent in mobilising stored lipids compared to the extract from the storage lobes. Stone and Mordue (1980), Beenakkers et al. (1985a) and Orchard (1987) reviewed the physiology of the corpora cardiaca as a source of AKH. The putative hormone termed 'adipokinetic hormone' by Mayer and Candy (1969a) was thought to have 'physiological effects' and was associated with the neurosecretory cells of the glandular lobe, where they were synthesised (Schooneveld et al. 1983). Presently, three AKHs (I, II-S, & II-L) and their chemical structures are known. AKHI from the glandular lobes of Locusta migratoria and Schistocerca gregaria was first isolated by Stone et al. (1976). AKH II from the locust glandular lobes was later isolated by Carlsen et al. (1979) and sequenced by Siegert et al. (1985).

The primary structure of the AKHs are shown below:

<table>
<thead>
<tr>
<th>Locusta migratoria</th>
<th>AKHI</th>
<th>pGlu-Leu-Asn-Thr-Pro-Asn-Trp-Gly-Thr-NH₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schistocerca gregaria</td>
<td>AKHI</td>
<td>pGlu-Leu-Asn-Thr-Pro-Asn-Trp-Gly-Thr-NH₂</td>
</tr>
<tr>
<td>Locusta migratoria</td>
<td>AKHII - L</td>
<td>pGlu-Leu-Asn-Ser-Ala-Gly-Trp-NH₂</td>
</tr>
<tr>
<td>Schistocerca gregaria</td>
<td>AKHII - S</td>
<td>pGlu-Leu-Asn-Ser-Thr-Gly-Trp-NH₂</td>
</tr>
</tbody>
</table>

These structures were elucidated by Stone et al. (1976); Carlsen (1979); and Siegert et al. (1985).

Bioassays of especially AKHI revealed that the hormone promoted formation and release of diglyceride (Spencer and Candy, 1976) from the fat body cells; induced marked hyper-
lipemia during flight (Tietz, 1967; Mayer and Candy, 1969a; Beenakkers, 1973; Spencer and Candy, 1974) and changed the haemolymph lipoprotein profile of the adult locust resulting in much improved lipid transport and reduced free "heparin soluble" A' lipoprotein during flight (Mwangi and Goldsworthy, 1977b). It also stimulated diglyceride oxidation in flight muscles (Robinson and Goldsworthy, 1977a; Goldsworthy, 1983) among other physiological actions. In addition, AKHI or the crude extract of the corpora cardiaca were shown to increase the levels of cyclic AMP in the fat body in vivo and in vitro (Orchard et al. 1982; Goldsworthy et al. 1986). Whilst AKHII elevated fat body cyclic AMP in vivo (Goldsworthy et al. 1986), flight as well induced elevations of fat body cyclic AMP (Orchard and Langer, 1984). Those elevations were coupled to the timing of octopamine release and adipokinetic hormones I and II release (Orchard and Lange, 1984). The involvement of cyclic AMP suggested the action of the hormone on fat body, leading to the action of lipase which hydrolyses the triglycerides to form diglycerides which are transported to the flight muscles where they are used for the generation of energy during flight (Goldsworthy, 1983).

The locust with a lifestyle requiring both short-term flight bursts and long-term migratory flights exhibit three separate time periods during flights in energy utilization. According to Rowan and Newsholme (1979), the initial or take-off period (0-10 min) is powered by the oxidation of muscle glycogen and proline. The glycogen quickly gets exhausted. Beenakkers et al. (1981b) observed that during the next 20 minutes the fuel for flight was largely through the oxidation of
haemolymph trehalose. After the 20 - 30 minutes period the locust entered the third phase of flight which was under the control of AKH and where lipids took over as the major fuel for any flight exceeding 30 minutes (Goldsworthy et al. 1973). Mwangi and Goldsworthy (1981) showed that there was a rapid disappearance of accumulated free "heparin-soluble" lipoprotein A as it binds to diglyceride from the fat body during flight. After two hours of flight (on a flight mill) a steady-state concentration of total diglyceride (12 - 15 µg/µl) and heparin soluble diglyceride (6 - 7 µg/µl) were maintained in both cc extract injected flown and non-injected flown locusts. This clearly indicated that a homeostatic control (Fig 2.1) existed and was usually matched to the demand for lipid oxidation in sustained flight.

The physiological significance of these adipokinetic peptides within the central nervous system cannot be ruled out. It is worth noting the work done on neurone immunoreactivity to (Tyr 1)-adipokinetic hormone I antibodies described in the brain and suboesophageal connectives of locusts (Schooneveld et al. 1983). The study indicated that the three neurones were AKH-sensitive although no direct evidence exists yet which associates AKH-like peptides with skeletal neuromuscular transmission (O'Shea et al. 1984).
Fig. 2.1  A diagrammatic representation of the diacylglycerol mobilization and utilization in Schistocerca (Mwangi, 1977; Orchard, 1987).
Azadirachtin and limonoid compounds—source, structure and roles as biopesticides.

The neem-tree *Azadirachta indica* (A. Juss) has been known for a long time for its medicinal and plant protection properties (Warthen, 1979; 1989). However, it is only during the last three decades that active compounds were isolated (Butterworthy and Morgan, 1968).

![Chemical structure of Azadirachtin (1)](image1)

Azadirachtin (1) by Ley et al. (1991) and Zanno et al., (1975).

![Chemical structure of Salannin (2a)](image2)


![Chemical structure of Volkensin (2b)](image3)


Fig. 2.2 The chemical structures of Azadirachtin (1) and *Melia volkensii* limonoids (2).
A. indica thrives mainly in the arid zones of tropical countries (Benge, 1989). Neem exists along the East African coast in Somalia (Mogadishu), Kenya (Mombasa) and Tanzania (from Tanga to Moshi; Zanzibar to Pemba) and continue to spread due to neem projects (SAARC, UNEP, ICIPE) involving the member-countries (Ahmed, 1995). In areas with sufficient rainfall (ca 800-1200mm per year) neem can regenerate naturally from seeds. Neem can also regenerate by coppice and root suckers. According to Radwanski (1977), 66% of the total growth takes place during the first 3 years. The fruit yield vary considerably and according to Radwanski (1977) an average single tree produces about 20.5kg in a season. While, Hedge (1993) recorded a yield of 25kg of seeds per year from mature tree.

The most active compound from the neem tree is azadirachtin (Butterworthy and Morgan, 1968). A maximum concentration of azadirachtin can be obtained by drying the seeds for 6-12h in the sun and then for several days in the shade. the outer 'shell' (endocarp) and kernel are cracked by gently threshing in a hand operated decorticator. The shell particles are then removed by winnowing. The extraction process takes several hours, as the active ingredients are not fully extracted in short time spans. Alcoholic extracts are suitable for controlling a wide range of pest species, especially free-feeding coleopterous and lepidopterous larvae as well as leafminers and hoppers (Pradhan et al., 1962; Warthan, 1979, 1989; Sieber and Rembold, 1981b; Barnby and Klock, 1987; Lee et al., 1991).

The earliest study to demonstrate the antifeedant activity of the neem tree, Azadirachta indica (Pradhan et al., 1962)
showed that crops treated with an aqueous suspension of neem seemed protected from attack by locusts. A few years later, Butterworth and Morgan (1968) isolated azadirachtin, the active triterpenoid (limonoid) compound from neem seeds and demonstrated that this compound was a very potent antifeedant against the desert locust, *Schistocerca gregaria*. During the next decade azadirachtin was shown to have varied activity from species to species (Warthen, 1979; 1989). The insects' responses resulted from the compound affecting the centres that controlled feeding or hormones involved in food metabolism (Barnby and Klocke, 1987). Lee *et al.* (1991) demonstrated that azadirachtin disrupted normal growth and development in 1st instar larvae of *Spodoptera frugiperda*. The lepidopterous larvae were repelled by azadirachtin and consequently reduced their food intake (Barnby, 1987). The last nymphal instar of *L. migratoria* showed incomplete ecdysis (Rembold and Sieber, 1981b). Later, Rembold and Sieber (1983) demonstrated the connection between endocrine events and triterpenoid treatment, suggesting that azadirachtin in neem extracts led to inhibition of oogenesis and ovarian ecdysteroid synthesis.

All the tissues including brain, haemolymph, suboesophageal ganglion, malpighian tubules and corpora cardiaca studied showed traces of azadirachtin with significant accumulation in the malpighian tubules of *L. migratoria* (Rembold *et al*. 1988) and high accumulation in the brain fibres and storage lobes of the corpora cardiaca (Subrahmanyam and Rembold, 1989). The high azadirachtin levels in the neurosecretory system resulted in a poor rate of incorporation of radioactive cysteine into the
corpora cardiaca after administration of enriched azadirachtin neem extract (Subrahmanyan et al. 1989).

Another plant from Meliaceae family, *Melia volkensii* has also been reported to contain biologically active and insecticidal compounds. It is common in arid and semi-arid areas of Kenya, where it is planted by farmers in their homesteads for shade, timber and for livestock fodder. It grows as a tall, woody tree bearing olive-like fruits. It is predominantly an East African tree, primarily growing in Somalia, Ethiopia, Kenya and Tanzania between 350 and 1075 metres above sea level. Propagation is from seed kernels and vegetatively by means of suckers. Each tree can produce up to 100 Kg of dry fruits per season, three times a year. To prepare the active fraction, the seeds are dried in the shade, the hard shell is broken in a hammer mill and the sieved powder extracted with suitable organic solvents. The concentrated extract can be formulated for application.

The effects of *Melia volkensii* compounds on insects include morphological defects, growth inhibition, mortality, reduced physical fitness and reduced flight performance. Further studies (Mwangi and Mukiama, 1988; Mwangi and Rembold, 1986, 1989; Mwangi and Kabaru, 1991; Wilps and Nasseh, 1991; Nasseh et al. 1993; Wilps et al. 1993) have shown that the active compounds cause feeding inhibition and growth disruption depending on the dosage, the stage of the insect tested, mode and application. Attempts to obtain active fractions have failed to restore the original levels of biological activity implying that synergism cannot be ruled out. So far, the following compounds,
all of which are triterpenoids (limonoid) have been identified to somewhat contribute to the biological activity, albeit difficult to quantify:

Ohchinin-3-acetate, 1-Tigloyl-trichilinin, 1-cinnamoyl-trichilinin, meliacin, volkensin and salannin.

The early investigations by Balan, (1993) listed the above fractions obtained from various parts of *M. volkensii* tree.

Although the current knowledge indicates the existence of vulnerable target insect body systems to these meliaceae compounds, they cannot be adequately utilized without further research.
CHAPTER THREE

MATERIALS AND METHODS

3.1. Test Insects.

*S. gregaria* Forskal were reared in the Department of Zoology of the University Of Nairobi, under crowded conditions as described by Goldsworthy *et al.* (1972b). Sexes were reared together in crowded conditions at 30°C at a constant photoperiod. The insects were fed on wheat seedlings and bran. The experimental insects were at least 10 days old since their final moult into adulthood. It was assumed that under the rearing conditions, the insects were in a gregarious phase. Only males were used in the study. The eleventh tergite in males have articulated conspicuous cerci and the ninth sternite is very keeled or crested in shape. The females have simple structures.

3.2. Plant source and preparation of extracts.

3.2.1 *Azadirachta indica* extract

Enriched neem extract containing 20% azadirachtin was a preparation from Prof. Rembold of Max Plank Institute in Germany. Different dilutions of azadirachtin were prepared in 60% ethanol in concentrations of 1,000 ppm; 2,500 ppm; 5,000 ppm; 15,000 ppm and 20,000 ppm.

3.2.2 *Melia volkensii* seed kernel extract.

All extracts were prepared from dry whole fruits of *Melia volkensii*. Fruits were collected in Embu district, Kenya. The fresh fruits were dried under shade to a constant weight and crushed to a fine powder using a hammer mill. The powder was
soaked in 65% ethanol and left to stand for 5-7 days at room temperature forming a uniform paste to allow for extraction of active compounds with a vacuum pump. The material was extracted by using 120 liters of ethanol for every 100kg at room temperature. The crude alcohol extract was concentrated with a rotary evaporator. The alcohol was recovered at a temperature below 50°C to avoid destroying the active compounds. The concentrate was allowed to stand overnight, thus forming two layers of ‘gum’ which settled at the bottom and an oily layer at the top.

The oily layer when left overnight separated into the aqueous bottom layer and a small amount of top oily layer. The bottom aqueous layer was enriched by the use of ethyl acetate and fed into the rotary evaporator in order to recover the ethyl acetate, leaving behind a residue of the active triterpenoid fraction which was precipitated with hexane. After filtration, the yellow precipitate was crushed into a powder and concentrated in vacuo. The yield was 25% of the dry crude sample of Melia extract (Wilps and Nasseh, 1993). The powder was used throughout in the current study.

3.3. Preparation of CC extract (AKH source).

The head of a mature male or female desert locust was removed with a single stroke of the scalpel. It was bisected longitudinally along a line passing through the ridge to the left antenna. The locust head was divided into two unequal halves. The larger (right) was placed onto a waxed dish, with the cut surface uppermost. The corpora cardiaca were dissected out
under a double eye-piece microscope of medium magnification on power and freed from adipose tissue and air sacs. The lobes of the corpora cardiaca were disrupted in 100 µl of 50 percent methanol using a hand homogenizer. The homogenate was collected and the homogenizer rinsed with 50 µl of methanol in distilled water (50:50), centrifuged at 8800g for five minutes and the supernatant removed, dried down at 60°C in a gentle stream. The supernatant was then re-dissolved in 100 µl of simple saline (Table 3.1) to make 0.01 pairs of cc extract for 10 µl injection per locust (Mwangi, 1977).

3.4 Application of materials

All extracts in diluted forms were prepared to desired concentrations and injected in volumes of 10µl into the 3rd or 4th abdominal segments with a 100µl Hamilton syringe. To prevent oozing of haemolymph from the puncture a gentle backward push and a forward pull directed the fluid back into circulation.

The corpora cardiaca tissue extract was administered prior to treatment with various plant fractions at zero time. The same set of locusts were used for the control, which were injected with cc extract only and each treatment test of locusts, which received cc extract plus plant extract.

3.5 Measurement of total lipid levels in the haemolymph.

The analysis of haemolymph total lipid level (vanillin-positive material) was carried out according to the method of Jutsum and Goldsworthy (1974). Haemolymph was collected at 30 minutes interval using calibrated 5µl capillary tubes and pooled for experimental tests. The samples of haemolymph were taken from
a small puncture made in the base of one hind leg, while the abdomen was gently pressed to apply pressure to allow the haemolymph to ooze out. The blood was blown into 100 μl concentrated sulphuric acid. The mixture was then heated in a dry bath at 100°C for 20 minutes to charr. After cooling 2-3 μl of vanillin reagent (Table 3.2) was added to dissolve the dried precipitate and assayed for the total lipid level by the method of Jutsum and Goldsworthy (1974). The optical density of the solution was determined against a blank at 546 nm using a Unicam SP500 spectrophotometer. A series of standard lipid stock solutions (Table 3.3) containing 1 g/100 ml of stabilised ethanol (Boehringer Ltd) was similarly heated. A linear relationship exists between the optical density and the amount of lipid present at a range of between 0.11-1.00 O.D and 0-60 μg/μl respectively (Fig. 3.1). The value of lipid concentration was read from the standard curve after determining the O.D of collected sample in vanillin solution. The percentage change in the lipid concentration of the haemolymph was defined as:

\[
100 \times \frac{A - B}{B}
\]

whereby:

A = lipid conc. after foremost reading

B = lipid conc. foremost reading
APPENDICES (PREFERABLE FORMULATION).

3.1 COMPOSITION OF SIMPLE SALINE

NaCl - 7.00 g  
KCl - 0.375 g  

Dissolved in 1 litre of distilled water and stored at 4°C.

3.2 VANILLIN REAGENT FOR THE SPECTROPHOTOMETRIC MEASUREMENT OF LIPIDS.

1.98 g of vanillin were mixed with 668 ml of orthophosphoric acid and dissolved by heating in an oven at 60°C. After cooling, this was made up to 1 litre with distilled water and stored in the dark at room temperature.

3.3 COMPOSITION OF LIPID STANDARD SOLUTION.

Cholesterol - 0.5 g  
Ethanol - 250 µl  

Dissolved and stored at room temperature.
Fig. 3.1 The standard curve for the spectrophotometric measurement of lipids. A relationship exists between optical density at 546 nm and lipid concentration in for the vanillin reaction. Mean value ± S.E. is shown for 7 samples at each point.
3.6 The flight roundabout

In order to assess the flight speed and flight behaviour in locusts it was necessary to construct a sensitive flight roundabout. The purpose of the flight roundabout was to contain locusts in a circular flight path and to count the number of revolutions at various times. An electronic counter was incorporated into the flight roundabout in order to record the number of revolutions. The flight roundabout consisted of a horizontal crosswire which rotated on a specific plane and onto which insects were attached; a detector or sensor which picked the interruptions from the plate was attached to the rotating crosswire frame and an electronic counter which converted the signal for display. The layout of the system presented in Plate 3.1 shows the three components (cross-wire, detector or sensor and an electronic counter). The interconnections are shown in Fig. 3.2. As the interrupting plate of the rotating cross-wire framework went past the slotted sensor or detector at every revolution, the recorder responded by one unit and a pulse was sent to the counter. To ensure minimum resistance to rotation, a low friction bearing pivoted the frame at the top of the pedestal. Fig. 3.3 shows the dimensions of the rotating frame, whose total mass in the absence of the insects was 58.2 g.

The sensor slotted up to the switch (R.S. Stk No. 304-560) comprised of a Callium-Arsenide infrared lights and an emitting diode, complete with an npn silicon photo transistor in a plastic package (see RS manual & data sheet no. 4276 of March 1987). The output in the form of pulses were counted as they arrive one after the other and were displayed onto the
counter. The counter is made of a fine digit cascade (five 7490 decade counter). The circuit details are given in Fig. 3.4.

3.7. Flight.

Adult male locusts were flown in groups of four. The locusts, which were at least 10 days old after the final moult were fastened to the flight roundabout arms with 4.92 inches radia, using a mixture of resin and beeswax in a ratio of 1:1. No attempt was made to select good fliers. They were suspended on the flight roundabout for a given period of time. Time intervals preferred were 30 min, 60 min, 90 min and 120 min. Locusts which stopped flying were stimulated by tapping the suspension arm and giving rudimentary tarsal touch. All experiments were carried out at a room temperature of 25 - 35°C.
Plate 3.1. Locusts attached to the flight roundabout.
Fig. 3.2 Elementary component and flow of signal from a simple flight roundabout

Fig. 3.3. Dimension of the rotating cross-wire frame (radius 2.145 inches)
Fig. 3.4. A detailed circuit diagram of counter-display for the flight round about
CHAPTER FOUR.

RESULTS.

4.1 TEST MATERIAL TREATMENT RELATED TO ADIPOKINETIC EFFECT IN
SCHISTOCERCA GREGARIA FORSKAL.

4.1.1 The adipokinetic response to corpora cardiaca extract.

Table 4.1 and Figure 4.1 shows that adult male *Schistocerca gregaria* injected with 0.01 pairs of corpora cardiaca and rested had the haemolymph lipid levels elevated from a mean of $8.9 \pm 1.13\mu g/\mu l$ to $13.6 \pm 1.26\mu g/\mu l$ at 30 minutes but later decreased significantly. The level stayed low to return to the initial resting of $7.5 \pm 1.57\mu g/\mu l$ which was observed at the end of the two hrs experiment. Adult male *Schistocerca gregaria* which were injected with 0.01 pairs of corpora cardiaca and flown had their haemolymph total lipid levels risen from $11.98 \pm 1.63\mu g/\mu l$ to maximum mean value of $28.76 \pm 2.97\mu g/\mu l$ at 60 minutes which was followed by a slight decline (Table 4.2).

From the data presented (Tables 4.1 and 4.2) unflown and flown locusts injected with cc extract exhibited an increase in haemolymph lipid levels which was significant ($P < 0.01$ level) at the 30 minute and 60 minute, respectively.
Fig. 4.1 Concentration of lipid (μg/ul) in locust haemolymph after injection of corpora cardiaca extract (0.01 pairs). Values are expressed as means of nine values ± standard errors of means.
4.1.2. The adipokinetic response to corpora cardiaca extract and enriched neem extract (azadirachtin).

a) Change in the haemolymph total lipid level in unflown adult male locust.

Adult male *S. gregaria* treated with 2 μg of azadirachtin per locust and rested showed significant (P < 0.05) increase in haemolymph total lipid from a resting level of 9.87 ± 0.58μg/μl to a maximum mean level of 18.40 ± 0.58μg/μl at 60 minutes (Table 4.1). Although those adult male locusts which were treated with cc extract only (on azadirachtin) also showed a similar change at 30 minutes, the peak response was somewhat delayed from 30 minutes to 60 minutes in all azadirachtin treated locusts (Table 4.1). Some treatment groups showed similar average changes in response. For instance injections of 0.01 pairs of corpora cardiaca extract and 0.5 μg of azadirachtin per locust caused 99.3% while 1.5 μg of azadirachtin yielded a 100.2% average change in total lipid at 30 minutes (Table 4.1 and Figure 4.2). Locusts treated with cc extract plus 0.1 μg and 2 μg of azadirachtin per locust showed a percentage change in total lipid concentration of 74.4% and 74.3%, respectively (Table 4.1 and Figure 4.2). These changes were significant at P < 0.01. By 120 minutes the control locusts which were injected with cc extract only, had their haemolymph lipid levels returning to normal (Figure 4.1). Those additionally treated with azadirachtin extract had not fully recovered their normal lipid levels (Figure 4.3 to 4.7).
Table 4.1: EFFECTS OF CC GLANDULAR LOBE (0.01 PAIRS) EXTRACT AND NEEM EXTRACT ON HAEMOLYPH LIPID IN RESTING UNFLOWN ADULT MALE SCHISTOCERIA GREGARIA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Observations</th>
<th>Change in Haemolymph total lipid level (µg/µl)</th>
<th>Period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc extract (0.01 Pairs)</td>
<td>9</td>
<td>8.9 ± 1.13</td>
<td>13.6 ± 1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.7 ± 1.16</td>
<td>8.5 ± 1.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.5 ± 1.57</td>
</tr>
<tr>
<td>cc + .1µg aza per animal</td>
<td>9</td>
<td>4.41 ± 0.19</td>
<td>7.69 ± 0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.41 ± 0.92</td>
<td>7.66 ± 0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.03 ± 0.68</td>
</tr>
<tr>
<td>cc + .25µg aza per animal</td>
<td>9</td>
<td>4.4 ± 0.23</td>
<td>5.9 ± 0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.41 ± 0.99</td>
<td>5.11 ± 0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.2 ± 0.37</td>
</tr>
<tr>
<td>cc + .5µg aza per animal</td>
<td>9</td>
<td>4.4 ± 0.13</td>
<td>8.77 ± 0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.35 ± 1.22</td>
<td>8.77 ± 1.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.35 ± 0.84</td>
</tr>
<tr>
<td>cc + 1µg aza per animal</td>
<td>9</td>
<td>5.27 ± 0.53</td>
<td>10.55 ± 1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.91 ± 1.83</td>
<td>9.97 ± 1.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.55 ± 1.81</td>
</tr>
<tr>
<td>cc + 2µg aza per animal</td>
<td>9</td>
<td>9.87 ± 0.58</td>
<td>17.2 ± 1.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.40 ± 1.75</td>
<td>14.5 ± 1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.72 ± 1.34</td>
</tr>
</tbody>
</table>

Treated animals were at least 10 days old after their last moult to adulthood

Percentage change calculated from proportional change = \(100 \times \frac{A-B}{B}\)

A: lipid conc. after foremost reading
B: lipid conc. foremost reading
EFFECT OF CC GLANDULAR LOBE (0.01 PAIRS) EXTRACT AND NEEM EXTRACT ON HAEMOLYMPH LIPID IN RESTING UNFLOWN ADULT MALE SCHISTOCERCA GREGARIA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% change in haemolymph total lipid level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period (min)</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>52.8</td>
</tr>
<tr>
<td>cc + 0.1μg aza per animal</td>
<td>74.4</td>
</tr>
<tr>
<td>cc + 0.25μg aza per animal</td>
<td>34.1</td>
</tr>
<tr>
<td>cc + 0.5μg aza per animal</td>
<td>99.3</td>
</tr>
<tr>
<td>cc + 1.5μg aza per animal</td>
<td>100.2</td>
</tr>
<tr>
<td>cc + 2μg aza per animal</td>
<td>74.3</td>
</tr>
</tbody>
</table>

33
Fig. 4.2. Effects of corpora cardiaca (0.01 pairs) and neem extracts on haemolymph total lipid levels in unflown adult male *Schistocerca gregaria*. Values represent mean of 9 observations.
Fig. 4.3. Effect of cc (0.01 pairs) extract and 0.1 μg/locust of neem extract on levels of haemolymph total lipid (μg/μg) in adult male Schistocerca gregaria. Mean ± S.E. of 9 observations are shown at each point.
Fig. 4.4. Effect of cc(0.01 pairs) extract and 0.25 ug/locust of neem extract on levels of haemolymph total lipid (ug/ul) in adult male Schistocerca gregaria. Mean ± S.E. of 9 observations are shown at each point.
Fig. 4.5. Change in haemolymph total lipid levels (µg/µl) with time after injections of 0.01 pairs of cc extract and 0.5µg/locust neem extract into adult male Schistocerca. Points represent mean ± S.E. for 9 locusts.
Fig. 4.6. Changes in haemolymph total lipid levels (µg/µl) with time after injection of 0.01 pairs of cc extract and 1.5µg/locust neem extract into adult male Schistocerca. Each point is the mean ± S.E. for 9 observations.
Fig. 4.7. Changes in haemolymph total lipid levels (µg/µl) with time after injections of 0.01 pairs of cc extract and 2µg/locust of neem extract into adult male Schistocerca. Points represent the mean ± S.E. for 9 observations.
b) Changes in the haemolymph total lipid level in tethered flight of desert locust.

Flown locusts treated with corpora cardiaca (0.01 pairs) and azadirachtin extracts elevated their haemolymph total lipids to maximum values in the first 30 minutes. This percent elevation of lipid levels was greater in treated locusts to controls at 30 minutes (Table 4.2). Later, the locusts treated with azadirachtin doses (0.1 μg to 2 μg) failed to assume a steady-state in their blood total lipid levels. Those locusts which were treated with cc extract only and flown, had significant increases in their blood lipid levels after 60 minutes. They also maintained steady-state concentrations (Figure 4.1, 4.4-4.8). At one hour of flight those locusts which had been injected with cc extract only had a rise in their haemolymph lipid level from the initial resting level of 11.98 ± 1.63 μg/μl to 28.76 ± 2.97 μg/μl, a significant (P < 0.01) increase of 140% (Table 4.2 and Figure 4.8). The highest dose of azadirachtin (2 μg/locust) also caused an elevation in lipid level, however, the change in haemolymph total lipid level was from 9.19 ± 1.92 μg/μl to 13.82 ± 2.09 μg/μl, an increase of only 50.41% (Table 4.2 and Figure 4.8). This change like in the other azadirachtin treated locusts, was still low. Figure 4.8 suggests that the gradual fall in the haemolymph lipid is evident in all azadirachtin treated locusts and not dependent on dose.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Observations</th>
<th>Change in haemolymph total lipid level (µg/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>9</td>
<td>11.98 ± 1.63</td>
</tr>
<tr>
<td>cc + .1µg aza per animal</td>
<td>9</td>
<td>5.08 ± 0.44</td>
</tr>
<tr>
<td>cc + .25µg aza per animal</td>
<td>9</td>
<td>7.22 ± 1.54</td>
</tr>
<tr>
<td>cc + .5µg aza per animal</td>
<td>9</td>
<td>10.13 ± 1.54</td>
</tr>
<tr>
<td>cc + 1.5µg aza per animal</td>
<td>9</td>
<td>5.63 ± 2.81</td>
</tr>
<tr>
<td>cc + 2µg aza per animal</td>
<td>9</td>
<td>9.19 ± 1.92</td>
</tr>
</tbody>
</table>

Treated animals were at least 10 days old after their last moult to adulthood

Percentage change calculated from proportional change - 100 x \( \frac{A-B}{B} \)

A: lipid conc. after foremost reading
B: lipid conc. foremost reading.
EFFECT OF CC GLANDULAR LOBE (0.01 PAIRS) EXTRACT AND NEEM EXTRACT ON HAEMOLYMPH LIPID IN FLOWN ADULT MALE SCHISTOCERCA GREGARIA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% change in haemolymph total lipid level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period of flight (min)</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>66</td>
</tr>
<tr>
<td>cc + 0.1μg aza per animal</td>
<td>150.2</td>
</tr>
<tr>
<td>cc + 0.25μg aza per animal</td>
<td>85.7</td>
</tr>
<tr>
<td>cc + 0.5μg aza per animal</td>
<td>50.7</td>
</tr>
<tr>
<td>cc + 1.5μg aza per animal</td>
<td>129.3</td>
</tr>
<tr>
<td>cc + 2μg aza per animal</td>
<td>43.2</td>
</tr>
</tbody>
</table>
Fig. 4.8. Effects of corpora cardica (0.01 pairs) and neem extracts on haemolymph total lipid levels in flown adult male *Schistocerca gregaria*. Values represent mean of 9 observations.
4.1.3. The adipokinetic response to corpora cardiaca extract and *M. volkensii* extract.

a) Changes in the haemolymph total lipid levels in unflown adult male desert locust.

Unflown locust injected with 0.01 pairs of cc and *M. volkensii* extracts exhibited higher lipid concentrations than those which did not receive *M. volkensii* treatment (Table 4.3). Haemolymph total lipid changes range from 6.17 ± 1.13 μg/μl to 9.48 ± 0.1 μg/μl for 1.5μg-2μg doses per locust, respectively, at the initial 30 minutes (Table 4.3). Those locusts treated with cc extract and low *M. volkensii* doses (0.1μg - 0.5μg/locust) showed a similar effect (Table 4.3). Lipid mobilization in unflown, *M. volkensii*-treated locusts were as high as 95.5% for 2 μg of per locust compared to the marginal (~52%) elevation for the control group at the initial 30 minutes (Table 4.3 and Figure 4.9). The peak time response for the control locusts injected with cc extract only was insignificant (P > 0.05). The peak time to response in locusts treated with low *Melia volkensii* doses (0.1μg - 0.5μg/locust) was delayed and shifted to between 60 - 90 minutes (Table 4.3 and Figure 4.9). Therefore, blood lipid elevations persisted longer in the locusts treated with *M. volkensii* doses compared to control locusts. Inspite of the marked haemolymph total lipid increase in the initial 30 minutes for locusts treated with 1.5μg-2μg of *M. volkensii* extract. The rested insects were unable to sustain these level, thereafter and showed a return to normal resting levels by 90 - 120 minutes of experiment (Figure 4.10-4.14).

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### Table 4.3: Effects of CC Glandular Lobe (0.01 pairs) Extract and Melia Extract on Haemolymph Lipid in Resting Unfown Adult Male Schistocerca Gregaria

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Observations</th>
<th>Change in haemolymph total lipid level (μg/μl)</th>
<th>Period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>9</td>
<td></td>
<td>8.9 ±1.14</td>
</tr>
<tr>
<td>cc+.1μg Melia/locust</td>
<td>6</td>
<td></td>
<td>14.75±1.26</td>
</tr>
<tr>
<td>cc+.25μg Melia/locust</td>
<td>6</td>
<td></td>
<td>12.46±1.65</td>
</tr>
<tr>
<td>cc+.5μg Melia/locust</td>
<td>6</td>
<td></td>
<td>7.4 ±1.00</td>
</tr>
<tr>
<td>cc+1.5μg Melia/locust</td>
<td>6</td>
<td></td>
<td>11.33±1.92</td>
</tr>
<tr>
<td>cc+ 2μg Melia/locust</td>
<td>6</td>
<td></td>
<td>9.88±1.51</td>
</tr>
</tbody>
</table>

Treated animals were at least 10 days old after their last moult to adulthood.

Percentage change calculated from proportional change \( \frac{100 \times A-B}{B} \)

A: lipid conc. after foremost reading

B: lipid conc. foremost reading
EFFECT OF CC GLANDULAR LOBE (0.01 PAIRS) EXTRACT AND MELIA VOLKENSII EXTRACT ON HAEMOLYMPH LIPID IN UNFLOWN ADULT MALE SCHISTOCERCA GREGARIA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% change in haemolymph total lipid level</th>
<th>Period of flight (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc extract (0.01 pairs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cc + 0.1µg Melia per locust</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cc + 0.25µg Melia per locust</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cc + 0.5µg Melia per locust</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cc + 1.5µg Melia per locust</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cc + 2µg Melia per locust</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>52.8</td>
<td>8.98</td>
<td>-4.49</td>
<td>-15.73</td>
</tr>
<tr>
<td>cc + 0.1µg Melia per locust</td>
<td>30.2</td>
<td>86</td>
<td>98.3</td>
<td>35.9</td>
</tr>
<tr>
<td>cc + 0.25µg Melia per locust</td>
<td>51.0</td>
<td>98.2</td>
<td>49.3</td>
<td>4.0</td>
</tr>
<tr>
<td>cc + 0.5µg Melia per locust</td>
<td>117.6</td>
<td>200</td>
<td>100</td>
<td>75.7</td>
</tr>
<tr>
<td>cc + 1.5µg Melia per locust</td>
<td>63.3</td>
<td>18.79</td>
<td>-2.6</td>
<td>-20.2</td>
</tr>
<tr>
<td>cc + 2µg Melia per locust</td>
<td>96</td>
<td>71.2</td>
<td>14.4</td>
<td>-16.5</td>
</tr>
</tbody>
</table>
Fig. 4.9. Effects of corpora cardiaca (0.01 pairs) and Melia volkensii extracts on haemolymph total lipid levels in unflown adult male *Schistocerca gregaria*. Values represent mean of 6 observations.
Fig. 4.10. The concentration of lipid (µg/µl) in unflown and flown locust haemolymph after injection with extracts of cc (0.01 pairs) and Melia (0.1µg/locust). Bars represent mean ± S.E. (n=6)
Fig. 4.11. The concentration of lipid (µg/µl) in unflown and flown locust haemolymph after injection with extracts of cc (0.01 pairs) and Melia (0.25µg/locust). Bars represent mean ± S.E. (n=6).
Fig. 4.12. The concentration of lipid (μg/μl) in unflighted and flighted locust haemolymph after injection with extracts of cc (0.01 pairs) and Melia (0.5μg/locust). Bars represent mean ± S.E. (n=9).
Fig. 4.13. Concentration of lipid (μg/μl) in unflown and flown locust haemolymph after injection with extracts of cc (0.01 pairs) and Melia (1.5μg/locust). Bars represent mean ± S.E., (n=7).
Fig. 4.14. Concentration of lipid (μg/μl) in unflown and flown locust haemolymph after injection extracts of cc (0.01 pairs) and Melia (2μg/locust). Bars represent mean ± S.E. (n=6).
b) Changes in haemolymph total lipid levels during tethered flight of desert locust.

The effect of *M. volkensii* extract on flown locust haemolymph lipid level was recorded in Table 4.4. 0.01 pairs of corpora cardiaca extract elevated haemolymph lipid level in flown locusts from a mean of 11.98 ± 1.63μg/μl to 28.96 ± 2.97μg/μl in 60 minutes (Table 4.4). Locusts treated with corpora cardiaca extract and low *M. volkensii* doses (0.1μg-0.25μg/locust) had had an increase of between 11.54 ± 0.04 μg/μl and 15.30 ± 1.17 μg/μl in 60 minutes after which blood lipid levels declined (Table 4.4). From the same data, high *Melia volkensii* extracts (0.5-2 μg/locust) exhibited reduced hyperlipaemic action after 30 minutes, whereas, cc extract injected locusts showed significant change at P > 0.05 (Table 4.4).

The ability of *M. volkensii*-treated *S. gregaria* to mobilize lipid during flight was affected after the first 30 minutes (Figure 4.10-4.14). The peak response time for locusts treated with high *M. volkensii* extract doses was at 30 minutes. Locust injected with cc extract only, had a maximal response at 1 hr of 140%, while 2 μg of *M. volkensii* per locust showed an increase of -28.5% in (Table 4.4 and Figure 4.15). The change in haemolymph lipid level appeared to be reduced with dose increase (Figure 4.16).
Table 4.4: EFFECTS OF CC GLANDULAR LOBE (0.01 PAIRS) EXTRACT AND MELIA EXTRACT ON FLOWN ADULT MALE SCHISTOCERCA GREGARIA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Observations</th>
<th>Period of flight (min)</th>
<th>Change in haemolymph total lipid level (µg/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>9</td>
<td>0</td>
<td>11.98 ± 1.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>28.96 ± 2.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>21.28 ± 2.63</td>
</tr>
<tr>
<td>cc + .1µg Melia/locust</td>
<td>6</td>
<td>0</td>
<td>13.16 ± 0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>24.70 ± 0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>15.54 ± 1.43</td>
</tr>
<tr>
<td>cc + .25µg Melia/locust</td>
<td>6</td>
<td>0</td>
<td>8.75 ± 1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>24.00 ± 3.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>13.37 ± 1.58</td>
</tr>
<tr>
<td>cc + .5µg Melia/locust</td>
<td>6</td>
<td>0</td>
<td>8.7 ± 1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>13.77 ± 2.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>7.30 ± 1.97</td>
</tr>
<tr>
<td>cc + 1.5µg Melia/locust</td>
<td>6</td>
<td>0</td>
<td>9.25 ± 1.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>12.75 ± 1.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>7.89 ± 1.20</td>
</tr>
<tr>
<td>cc + 2µg Melia/locust</td>
<td>6</td>
<td>0</td>
<td>7.62 ± 0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>9.79 ± 1.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>4.91 ± 0.30</td>
</tr>
</tbody>
</table>

Treated animals were at least 10 days old after their last moult into adulthood.

Percentage change calculated from proportional change:

\[ \text{Percentage change} = 100 \times \frac{A - B}{B} \]

A: lipid conc. after foremost reading
B: lipid conc. foremost reading
EFFECT OF CC GLANDULAR LOBE (0.01 PAIRS) EXTRACT AND MELIA VOLKENSII EXTRACT ON HAEMOLYMPH LIPID IN FLOWN ADULT MALE SCHISTOCERCA GREGARIA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>66</td>
</tr>
<tr>
<td>cc + 0.1μg Melia per locust</td>
<td>73.2</td>
</tr>
<tr>
<td>cc + 0.25μg Melia per locust</td>
<td>72.8</td>
</tr>
<tr>
<td>cc + 0.5μg Melia per locust</td>
<td>61.2</td>
</tr>
<tr>
<td>cc + 1.5μg Melia per locust</td>
<td>94.6</td>
</tr>
<tr>
<td>cc + 2μg Melia per locust</td>
<td>80.5</td>
</tr>
</tbody>
</table>
Fig. 4.15. Effect of corpora cardiaca (0.01 pairs) and Melia volkensii extracts treatment on haemolymph total lipid levels in flown adult male Schistocerca gregaria. Values represent mean of 6 observations.
Fig. 4.16. Dose-response curve at 60, 90 and 120 min time intervals. (A) is a linear and (B) a logarithm Plot of Melia dose against change in haemolymph total lipid levels on flight (Mean ± S.E., n=5).
4.2. TEST MATERIAL TREATMENT RELATED TO FLIGHT SPEED AND FLIGHT PATTERN IN SCHISTOCERCA GREGARIA FORSKAL.

When locusts were tethered on to the roundabout their behaviour was erratic. Some locusts then flew consistently for the whole period of the experiment while the others flew well for only a short period yet others flew rather reluctantly. The majority of the treated locusts stopped flying frequently and needed stimulation to maintain flight. Control adult male locusts which were injected with 0.01 pairs of corpora cardiaca once flight had begun, in many instances did not require as much stimulation as treated locusts to keep them flying. The roundabout used was not friction-free and the opaque plate passing the sensor produced further resistance to rotation. These variabilities resulted in measurements of absolute flight speed being unprecise. Therefore to account for varying proportion of slow or non-fliers and the mechanical properties of flight roundabout, flight speed was statistically expressed as a percentage of the average flight speed during 30 minute intervals. The percentage change in flight speed against time was termed the "flight pattern". The data with absolute values of average flight speed (revolutions/min) for the control group compared with A. indica and M. volkensii treated animals are presented in Tables 4.5 and 4.6. The same set of locusts were used for each treatment test.
4.2.1. The effect of injection of corpora cardiaca extract on flight performance.

Injection of 0.01 pairs of corpora cardiaca extract only initially caused a significant average speed of locusts and a general reduction in the required stimulation. A maximum mean value of $2292 \pm 51$ revolutions was recorded in the initial 30 minutes but declined to $1592 \pm 24$ revolutions at 1 hr and to $681 \pm 66$ revolutions in 120 minutes (Table 4.5). The "flight pattern" in control locusts, which were injected with cc extract only resembled those of the treatment groups but flight speed in treated locusts remained comparatively low (Table 4.5 or 4.6).

4.2.2. The effect of injections of corpora cardiaca extract and neem extract on flight speed and flight pattern of desert locust.

Injections of corpora cardiaca (0.01 pairs) and neem extracts ($0.1 \mu g - 2 \mu g$) caused an immediate impairment of flight performance. Adult male $S. gregaria$ treated with 0.1 of azadirachtin per locust flew in short bursts (of about 5 minutes) and the average speed was $1688 \pm 98$ revolutions at 30 minutes compared to $2292 \pm 52$ revolutions which was faster for control locusts, injected with cc extract only. This change in flight speed was significant ($P < 0.05$) in cc extract alone injected locusts. (Table 4.5). The highest dose of azadirachtin ($2 \mu g$/locust) reduced the
apparent flight speed to 1270 ± 72 revolutions at the first 30 minutes (Table 4.5). The final flight speed at the end of experiment was reduced in azadirachtin treated locusts to between 326 and 600 revolutions compared to about 700 revolutions in control locusts (Figure 4.17). The azadirachtin treated locusts were (~18% to 25%) slower compared to control locusts as early as in the first 60 minutes and showed the impairment of flight to the end of experiment (Figure 4.18).
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Observation</th>
<th>Flight Speed (rev/min)</th>
<th>Period of light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 - 30</td>
</tr>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>5</td>
<td>2292 ± 51</td>
<td>1595 ± 24</td>
</tr>
<tr>
<td>cc+0.1μg aza/locust</td>
<td>5</td>
<td>1688 ± 98</td>
<td>1128 ± 46</td>
</tr>
<tr>
<td>cc+0.25μg aza/locust</td>
<td>5</td>
<td>1494 ± 53</td>
<td>1032 ± 49</td>
</tr>
<tr>
<td>cc+0.5μg aza/locust</td>
<td>5</td>
<td>1291 ±111</td>
<td>912 ± 76</td>
</tr>
<tr>
<td>cc+1.5μg aza/locust</td>
<td>5</td>
<td>1144 ±116</td>
<td>794 ±170</td>
</tr>
<tr>
<td>cc+2μg aza/locust</td>
<td>5</td>
<td>1270 ± 72</td>
<td>873 ±108</td>
</tr>
</tbody>
</table>

Treated animals were at least 10-days old after their last moult adulthood.
Fig. 4.17. Effects of corpora cardiaca (0.01 pairs) and neem extracts on flight speed of adult male *Schistocerca gregaria*. Values represent mean of 5 observations.
Fig. 4.18. Effects of corpora cardiaca (0.01 pairs) and neem extracts on flight pattern of adult male Schistocerca gregaria.
4.2.3. The effect of injections of extracts of 0.01 pairs of corpora cardiaca and *Melia volkensii* on flight speed and flight pattern of desert locust.

Table 4.6 shows that the adult male *Schistocerca gregaria* injected with 0.01 pairs of corpora cardiaca tissue extract and *Melia volkensii* extract in definable doses had impaired flight performance as early as in the initial 30 minutes. In contrast to control locusts which flew $2292 \pm 51$ revolutions. The *Melia volkensii* extract treated locusts flew within a mean flight speed range of 1425 to 2094 revolutions in the first 30 minutes (Table 4.6). Higher doses of *Melia volkensii* ($1.5 \mu g - 2 \mu g$/locust) apparently reduced flight speed. Figure 4.19 shows that flight performance declined by a range of approximately 500-700 revolutions at 1hr. As much as one-third reduction was realised with significant ($P < 0.05$) flight observed in control locusts, injected with cc extract only (Table 4.6). Locusts treated with $0.5 \mu g$, $1.5 \mu g$, to $2 \mu g$ of *M. volkensii* extract per locust showed a decline in flight up to 50% at 1hr (Figure 4.20). Flight in locusts treated with high doses of *M. volkensii* remained distinctly less than of control locusts throughout the test period and flew considerably (~18% - 27%) slower by the end of the experiment (Figure 4.20).
Table 4.6: FLIGHT SPEED IN FLOWN ADULT MALE SCHISTOCERCA GREGARIA ON TREATMENT WITH MELIA VOLKENSII EXTRACT AND CORPORA CARDIACA EXTRACT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Observation</th>
<th>Flight Speed (rev/min)</th>
<th>Period of flight (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 - 30</td>
<td>30 - 60</td>
</tr>
<tr>
<td>cc extract (0.01 pairs)</td>
<td>6</td>
<td>2292 ± 51</td>
<td>1595 ± 24</td>
</tr>
<tr>
<td>cc+0.1μg Melia/locust</td>
<td>6</td>
<td>1926 ±157</td>
<td>1506 ±137</td>
</tr>
<tr>
<td>cc+0.25μg Melia/locust</td>
<td>6</td>
<td>2094 ±208</td>
<td>1436 ± 22</td>
</tr>
<tr>
<td>cc+0.5μg Melia/locust</td>
<td>6</td>
<td>2069 ±153</td>
<td>1173 ± 72</td>
</tr>
<tr>
<td>cc+1.5μg Melia/locust</td>
<td>6</td>
<td>1425 ±246</td>
<td>989 ±173</td>
</tr>
<tr>
<td>cc+2μg Melia/locust</td>
<td>6</td>
<td>1586 ±176</td>
<td>827 ±208</td>
</tr>
</tbody>
</table>

Treated animals were at least 10-days old after their last moult into adulthood.
Fig. 4.19. Effects of corpora cardiaca (0.01 pairs) and Melia volkensii extracts on the flight speed of adult male Schistocerca gregaria. Vertical bars represent the mean values or 5 observations.
Fig. 4.20. Effects of corpora cardiaca (0.01 pairs) and Melia volkensii extracts on the flight pattern of adult male Schistocerca gregaria.
4.3. TEST MATERIAL RELATED TO HINDLEG OF DESERT LOCUST (*SCHISTOCERCA GREGARIA*).

Plate 4.1 shows an adult male *Schistocerca gregaria* whose right hindleg had characteristic hindleg paralysis as a result of injection of 0.5μg to 2μg of *Melia volkensii* extract. The leg in such cases remained stretched even after 2 days of the experiment. The visible image on examination (Plate 4.1) shows that the left hindleg of treated locust was also twisted sideways or backwards, leaving the claws out of place. Such animals were not able to stand and remained immobile.

Plate 4.1: Experimental locust with hindleg paralysis, evident two days after injection with corpora cardiaca (0.01 pairs) and *Melia volkensii* (0.5-2 μg) extracts.
a. General discussion.

Weiss-Fogh (1952) demonstrated that carbohydrate is the fuel used during the early stages of flight and lipid is used for prolonged flights. The concentration of lipids and carbohydrates in the haemolymph of the desert locust, *Schistocerca gregaria* is controlled and converted as energy reserves in a close inverse relationship (Weiss-Fogh (1952); Zebe (1959) and Beenakker (1969a) categorized three physiological types of flight muscles. The carbohydrate utilizers of dipterous insects (Chadwick, 1947); the lipid utilizers as in lepidopterous insects (Zebe, 1954) and combined carbohydrate-lipid utilizers as in Orthopterans (Krogh and Weiss-Fogh, 1951). These metabolic groupings result from the requirements of aerobic flight muscle metabolism and the need for instantaneous activation of metabolism to produce maximal energy from functioning pathways.

Among the important factors in activating and sustaining muscle metabolism during flight are individual hormones, regulating enzymes and co-enzymes tuned to the type of fuel being utilized. The substrate(s) from which flight energy is derived vary from species to species but the primary source of required metabolic energy amongst migratory species of insects and those which are non-feeding as adults is lipid. The haemolymph lipid content and
composition usually depend on age, sex, migratory status and the
taxonomic position of the organism under study (Gilbert, 1967;

b. Adipokinetic activity of the corpora cardiaca extract.

From the present study variable small amounts of lipid
content of 4.4 to 14.75 ± 1.26μg/μl were measured in the haemolymph
at resting level. Walker et al. (1970) suggested that the fat body
lipid contents varied by 4 to 4.5mg per 100mg wet weight of tissue
at all stages of adult development of male S. gregaria. It is
likely, that, these low lipid contents observed in the haemolymph
at zero time were related to large lipid build up needed as energy
reserves for migration (Goldsworthy et al., 1973). Normally the
locust take a considerable period of intense lipid accumulation in
the fat body (Hill et al. (1968) and could determine the cited low
or variable lipid levels measured in the haemolymph.

Besides the age and migratory status of the locust other
addition sources were involved in determining blood lipid levels.
Figure 4.1 shows that lipid content in the haemolymph is very
dependant upon the hormonal injection and the functional status of
S. gregaria 0.01 pairs of corpora cardiaca extract showed a
hyperlipaemic response, causing the blood lipid level in flown
locust to reach a peak value of about 28.76 ± 2.97 μg/μl from 11.98
± 1.63 μg/μl, an increase of 140% at 1hr (Table 4.2 and Fig 4.1).
The lipid concentration in the haemolymph was probably necessary to
supply adequate amount of fuel for flight. Several reports exist on the relationship between the corpora cardiaca and lipid mobilization in locusts (Spencer and Candy, 1974; Tietz, 1967; Mayer and Candy, 1969a; Beenakkers, 1973; Spencer and Candy, 1976). The action of AKH from the corpora cardiaca was reported to result in fat body cyclic AMP level increase (Stone and Mordue, 1980; Beenakkers et al. 1985a and Orchard, 1987) leading to activation of inactive lipase (Orchard et al. 1982; Goldsworthy et al. 1986). This action of the hormone was first observed in the hydrolysis of triglycerides to form diglycerides by Kilby (1965) and later by Spencer and Candy (1976). The protein acceptor (Mwangi and Goldsworthy, 1981) in the haemolymph of the corpora cardiaca-injected, flown locusts maintain an efficient mechanism to promote the association of the haemolymph lipoprotein to form high molecular weight A+ during flight. AKH also facilitates the increased take up of DGL for oxidation during flight (Goldsworthy, 1983; Beenakkers et al. 1984). The results in Table 4.2 and Fig. 4.1 show that the insect haemolymph lipid circulated in steady-state levels of $22.46 \pm 2.78 \mu g/\mu l$ and $21.28 \pm 2.63 \mu g/\mu l$ at 90 minutes and 120 minutes, respectively. The above results suggests that this peptide (pGlu - Leu -Asn - Phe ..... NH$_2$) which possess a pGlu residue at the amino terminus, belonging to the adipokinetic hormone (AKH) family has homeostatic hyperlipaemic effect.

The physiological details in the flown or unflown S. gregaria depend upon the precise functional requirements. Figure 4.1 and Table 4.1 show that, unflown adult male S. gregaria
responded to the injection of 0.01 pairs of corpora cardiaca extract with the peak response of 52% at 30 minutes. Mayer and Candy (1968) concluded that the poor AKH response observed in unflown adult Schistocerca was mediated in part by flight. Incidentally, the flow of impulse (Beenakker, 1969; Mayer and Candy, 1969b; Houben and Goldsworthy et al. 1974; Jutsum and Goldsworthy 1976; Cheeseman et al. 1976) during flight has a direct stimulation on neurosecretory structure to cause hormone release.

In this study, supporting laboratory evidence show, diminished hyperlipaemic response in A. indica and M. volkensii treated flown locusts. Azadirachtin and other biologically active compounds from meliaceae family showed a variety of physiological effects, ranging from poor flight performance to partial hindleg paralysis.

c. The effect of neem extract (Azadirachtin) on lipid levels.

The present study on S. gregaria brings out the possible influence of azadirachtin on the hormonal controlled adipokinetic effects. The adipokinetic effects include the combined activities of mobilization, transport and unloading of lipids, all of which are regulated by adipokinetic hormones (Spencer and Candy, 1976; Goldsworthy, 1983; Goldsworthy et al., 1986). In addition the adipokinetic family is also involved with flight muscle metabolism (Robinson and Goldsworthy, 1974, 1977a) and myotropic activity (O’Shea et al., 1984).
Unflown locusts injected with corpora cardiaca and azadirachtin extracts showed increased hyperlipemic response. High azadirachtin dose (1.5μg/locusts) caused an increase of 100.2% while control locusts, injected with cc extract only achieved as low as 52% increase in blood lipid level at the initial 30 minutes (calculated from Table 4.1). Incidentally, unflown treated locusts delayed the peak time response to 60-90 minutes. This, may have been due to direct interference with the corpora cardiaca glandular lobe, involved with secretory and release activity of hormone controlling adipokinetic effects (Spencer and Candy, 1976; Goldsworthy, 1983; Goldsworthy et al., 1986).

Flown locusts injected with cc extract only, increased their haemolymph lipid content by 140% at 60 minutes (Table 4.2 and Fig 4.1). While locusts treated with high azadirachtin dose (2μg/locust) increased their total lipid level by 50% at at 60 minutes (calculated from Table 4.2). There was a significant (P < 0.05) increase in blood lipid level of locusts injected with cc extract only. It therefore seems reasonable to assume that most of the blood total lipids mobilized in the azadirachtin treated locusts during flight were under the influence of impaired corpora cardiaca and the hormone titre were insufficient. The azadirachtin treated locusts showed upto 70% reduction in lipid mobilization at 60 minutes (see Table 4.2). Subrahmanyam and Rembold in 1989, using autoradiographic technique proposed that it was the endocrine events which failed
to mature in *L. migratoria* after azadirachtin treatment.

Subrahmanyan *et al.* 1984 injected radiolabelled cysteine in *L. migratoria*. The binding amino-acid was taken up by neurosecretory material of the corpora cardiaca in both controls and azadirachtin treated males. Their study support the suggestion that the potentially toxic compound affects the corpora cardiaca content release.

d. **Effects of *Melia volkensii* extract on lipid levels.**

The effect of crude *M. volkensii* extract to delay peak time response in unflown-treated locust and diminish increase in haemolymph lipid levels in flown-treated locust, suggest the presence of biologically active principles.

Unflown-treated locust shifted the peak response levels from 30 minutes to between 60 and 90 minutes (Table 4.3 and Figure 4.9). The rise was similar to values measured by Mwangi, (1982). The haemolymph total lipid level increased by 98.3% for 0.1µg *M. volkensii* per locust at 90 minutes, while cc extract injected locust had ~5% increase at the same time (calculated from Table 4.3). All adult male *Schistocerca* which were treated with *M. volkensii* extract and rested showed haemolymph lipid increase which was significant at P < 0.05 or 0.01 after 30 minutes (Table 4.3).

Due to diminished adipokinetic response in *M. volkensii* treated-flown *S. gregaria*, the haemolymph lipid levels decreased between 30% and 65% at one hour (calculated from Table 4.4).
This inability to mobilize lipid sufficiently is an indication that energy for long-distance flight would not be available after such treatment. These findings fit well with results obtained on *S. gregaria* by Wilps et al. (1993).

Although azadirachtin was earlier not mentioned as one of the biologically active compounds of crude *M. volkensii* extract (Balan, 1993) and neither are they related in their chemical structures (Figure 2.2). It is possible that that *M. volkensii* exerts a slow effect on increase in haemolymph lipid concentration in flown-treated locust through the same mode of action but this remains to be studied. Azadirachtin interferes with the function of the corpora cardiaca especially the release activity. Flown locusts treated with *Melia volkensii* showed insufficient lipid mobilization during prolonged flight. This would indicate that sustained fuel supplies were upset (Fig. 4.16)

e. The effect of injections with extracts of 0.01 pairs of corpora cardiaca and azadirachtin on flight speed and flight pattern of *Schistocerca gregaria*.

Injections of the corpora cardiaca extract in absence of azadirachtin resulted in high flight speed. The results in Table 4.5 show that flight speed after azadirachtin treatment was impaired as early as during the initial 30 minutes of flight. After treatment the locusts flew in short bursts. Earlier studies reveal that neem extracts affect insect response (Pradhan et al. 1962) and its fraction, azadirachtin exhibits potent effects (Butterworth and
Morgan, 1968). For example it affected centers that controlled hormones involved in food metabolism (Barnby and Klocke, 1987). The highest dose of azadirachtin (2μg/locust) impaired flight performance of the treated locusts in the initial 30 minutes as the number of revolutions declined (Table 4.5) compared to control locusts. Lee and Goldsworthy (1976) observed that the non-injected locusts exhibited high mean speed values of 3300 ± 113 revolutions in the first 30 minutes of flight. According to Rowan and Newsholme (1979), muscle glycogen and is often used for take-off or preflight warm up. While, short flight in locusts draw their energy from haemolymph trehalose until AKH level is able to mediate the sustained release of diglyceride from fat body to support long-term flight. The hyperglycaemic factor in the non-injected locusts ensure rapid and efficient supply of fuel during the early phase of flight. Following AKH injection, a reversed-phase intercepted high performance because of the less readily available energy from lipid. The approximate changes in flight speed for azadirachtin treated locusts were considerably (-18% - 25%) lower compared to controls after one hour (Figure 4.18). The efficiency of azadirachtin to lower flight performance persisted to the end of experiment with treated locusts flying between 325 - 600 revolutions compared to 700 revolutions for control locusts (Figure 4.17). Azadirachtin treatment may be interfering with either carbohydrate metabolism or lipid mobilization or both, since flight impairment occurs during both the trehalose phase of flight as well as the lipid phase. It would seem reasonable to suggest that
physiological damage of the neurohaemal organ subsequently reduced the availability of substrate for flight. Subrahmanyam and Rembold, (1989), in their study of target organs showed traces of azadirachtin with high accumulation in the brain fibre and storage lobe of the corpora cardiaca, thus implying affinity for azadirachtin receptors in such tissue. Not much is known about the direct mode of action, biodegradation and the nature of binding to the tissues (Rembold et al., 1983). Future studies may enlighten the organ specific concentration of this compound, azadirachtin.

f. Effect of injections of corpora cardiaca and Melia volkensii on flight speed and flight pattern in the desert locust.

From the results obtained in this study, Melia volkensii influenced the flight behaviour of adult male Schistocerca gregaria during flight. The maintained steady-state concentration of total lipid (~21 - 22μg/μl), in cc extract injected and flown locusts at 60-90 minutes, suggested that adipokinetic effects facilitated both the mobilization and the utilization available substrate for flight (Figure 2.1). It was obvious from the flight performance that injection with definable doses of M. volkensii possibly caused impairment of flight with transient decrease in flight speed (Table 4.6). The suppression of the development of flight performance in M. volkensii-treated locust was shown as early as at the initial 30 minutes (Table 4.6). It is significant that these flown locusts have trehalose utilization in the intial cruising take-off phase of
flight (Beenackers et al., 1981b). There are, however, indications from this study which suggest that *M. volkensii* treatment diminished the flight speed values in locusts. Robinson and Goldsworthy (1974) have shown that high concentrations of haemolymph lipid levels bring about competitive inhibition of trehalose utilization in the flight muscle. As was mentioned earlier, Lee and Goldsworthy (1976) observed that the non-injected locusts exhibited high mean speed values of 3300 ± 113 revolutions in the first 30 minutes of flight. These findings may explain the low flight speed in *M. volkensii* extract injected flown locust and even, yet lower flight speed in *M. volkensii*-treated locusts, which flew with reduced mean flight speed values of 1420 to 2069 revolutions in the initial 30 minutes. *M. volkensii* treatment lowered the flight speed of locust prematurely and the characteristic subsequent decline persisted throughout the entire experimental period (Figure 4.19). In the locusts, flight speed changes with time is thought to reflect change in substrate utilization (Weiss-Fogh, 1952) a process which is dependent upon the release of AKH from corpora cardiaca (Mayer and Candy, 1969b; Goldsworthy, Johnson, and Mordue, 1972a; Goldsworthy, Mordue, and Guthkelch, 1972b).

The flight pattern of *M. volkensii*-treated locusts also show a peculiar feature during 60-120 minutes of flight (Figure 4.20). The differences in capability between experimental locusts presumably reflect the effect of *M. volkensii* dose. The estimates of the quality of flight in locusts treated with high doses of *M. volkensii* (0.5µg - 2µg/locust), remained distinctly lower than
those of control locusts throughout the test period and flew considerably (~18% -27%) slower by the end of the experiment (Figure 4.20). The results agree basically with those of Wilps et al. (1993) and postulate damage at physiological level.

It is possible that the low flight intensity observed in *M. volkensii* (0.5μg - 2μg/locust) compared to those injected with cc extract only (Table 4.6 and Figure 4.19) was because of poor substrate availability for flight. If this interpretation is correct then it is possible to suggest an explanation for the effects of *Melia volkensii* extract on flight patterns of locusts. From the analysis of the flight pattern of locusts (Figure 4.20), it is clear that the *M. volkensii* extract influence not only the lipid phase but also the initial cruising take-off period before 30 minutes. Reports by Rembold (1989a), Subrahmanyam and Rembold (1989) and Subrahmanyam et al. (1989) showed that radiolabelled azadirachtin adhered onto the membranes of the corpora cardiaca and on the membranes of the brain. Furthermore, the afore-mentioned authors suggested that release activity in the corpora cardiaca was impaired. The decreased response following *M. volkensii* treatment could thus point to the same or identical interaction of the biologically active triterpenoids (Balan, 1993) interfering with the corpora cardiaca activity, eventually, blocking flight metabolism at one or more key steps.
g. The effect of *Melia volkensii* treatment on locust hindleg.

Figure 4.21 suggests that effect of *M. volkensii* on leg resulted from toxic doses of 0.5µg - 2µg per locust. Mwangi (1982) observed desensitization, a physiological effect that block the rising phase of the action potential. The partial desensitization was also observed in the present study on *S. gregaria* two days after *M. volkensii* treatment and the distortion of normal posture remained with paralysed hindlegs which hardly held the body weight.

Further studies with triterpenoid compounds of *M. volkensii* extract could possibly explain the tetanic spasms and loss of primary postural relations observed in the hindleg of the desert locust, *S. gregaria*.
SUMMARY AND CONCLUSION.

Flight performance was reduced in the desert locust after treatment with extracts of *A. indica* and *M. volkensii* containing triterpenoid compounds. Using 0.1µg to 0.25µg doses of *M. volkensii* per locust the haemolymph lipid levels increased between 1.91 ± 0.04µg/µl to 8.88 ± 1.40µg/µl. Higher *M. volkensii* doses (0.5µg to 2µg/locust) also showed diminished hyperlipaemia and was insignificant at 60 minutes of flight. The rise in haemolymph lipid level for cc extract only injected flown locust was from 11.98 ± 1.63µg/µl at zero time to 28.76 ± 2.97µg/µl, a significant (P < 0.05) increase of 140%, at 60 minutes.

Unflown locusts which were treated with azadirachtin or *M. volkensii* extract showed increased hyperlipaemic response with a shift in peak response time to between 60 and 90 minutes instead of 30 minutes observed in control locusts. There was an insignificant (P > 0.05) increase in blood lipid level.

The poor flight performance observed in azadirachtin treated locusts was considerably (18% - 25%) slower at one hour and remained lower to the end of the experiment. *M. volkensii* treated locusts also had insignificantly speed and flew considerably (18% - 27%) slower by the end of the experiment. The poor flight performance may have been due to decreased availability of substrate during flight in plant extract-treated flown locusts. A high dose of the *M. volkensii* extract (0.5µg to 2µg/locust) further led to paralysis in *S. gregaria* two days after treatment.
The present study shows that:

1). Extracts from members of meliaceae family *A. indica* and *M. volkensii* reduce the hyperlipaemic response during flight.

2). The effect by *M. volkensii* might be a widespread effect on different effector tissues especially those which are associated with the adipokinetic responses.

3). The biologically active compounds, azadirachtin and other triterpenoid compounds from *A. indica* and *M. volkensii*, respectively may have different targets in the locust. The effects of *Melia volkensii* may be more numerous and diffuse compared to those of azadirachtin. These maybe related to the poor hormonal and metabolic integration of carbohydrate utilization, thus causing a delay in peak performance during flight. It is also important to recognise that the locust haemolymph contains low levels of lipid during flight and regulatory system(s) which locusts may depend on to meet their changing demands initiated by hormones, photoperiod, temperature as well as other internal and external factors become desensitised. While azadirachtin may acton the corpora cardiaca in a generally similar way, there is no account of toxic action on skeletal muscles.


Goldsworthy, G.J.; Johnson, R.A. and Mordue, W. (1972a): *In vivo* studies on the release of hormones from the corpus cardiaca of


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Nickerson, B. (1956): Pigmentation of the desert locust,


