PESTICIDAL EFFECTS OF EXTRACTS FROM DIFFERENT PARTS OF NEEM PLANT *{Azadirachta indica)*

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DECLARATION

I hereby declare that, this thesis contains the report of my research work and has not been submitted for the award of a degree at any other University. Information and excerpts from published works of others have been gratefully acknowledged in the text.

Sikod Raymond Dinga

03/04/98
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CERTIFICATION

This thesis entitled Pesticidal Effects of Extracts from Different Parts of Neem Plant by Sikod, Raymond Dinga meets the regulations governing the award of the degree of Master of Science (M.Sc) Biochemistry of Ahmadu Bello University, and it is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my parents "the one single person" who more than any other human has not only made my success possible but has also brought honour and nobility to the whole process.

This thesis is dedicated to you

PAPA AND MAME

with all my love.

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ABSTRACT

Various water dispersible powder (WDP) 5% concentration and dust preparation (DP) 5% concentration both based on crushed neem kernel and leaf, and an emulsifiable concentrate (EC) based on neem kernel oil were prepared. Their azadirachtin content were determined. These formulations (WDP and EC) were evaluated on the field against the two major pest of cowpea i.e. the leaf beetle *Ootheca mutabilis* (Sahl) and the pod sucking bug *Clavigralla tomentosicollis* (Stal) and in the laboratory (DP) against *Callosobrachus maculatus* (Fab) an economically important pest of stored cowpea grains. The various formulations were compared with each other and with a commercially available insecticide.

In the field evaluation, the various formulations not only significantly ($P < 0.05$) reduced damage due to *Ootheca mutabilis* and increase grain yield in cowpea, but also gave a performance comparable to that of the synthetic insecticide (Cypermethrin). None of the formulations gave complete protection, but treatments with neem seed water extract (NSWE) and neem cake water extract (NCWE) resulted in less foliar damage and a lower incidence of *Clavigralla tomentosicollis* than the other formulations. In most instances, untreated plots were severely damaged whereas those protected with various neem extracts were not attacked or only slightly damaged. This was confirmed by

a strong and significant ($P < 0.05$) correlation between azadirachtin and pesticidal effects ($r = -0.96, .86, -.97, -0.6$ and -0.6) for leaf damage, yield, insect population, seed damage and weight loss respectively. However, there were significant ($P < 0.05$) variations among the treatments.

Laboratory trials for post-harvest storage indicated that, grains treated with various neem dust preparation (DP) and neem oil (4ml/kg) caused significant ($P < 0.05$) reduction in number of progeny and survival rate of *Callosobrachus maculatus*. There was no adult survivors or progeny production in grains treated with neem oil. Neem oil and neem cake protected the grains against feeding by *Callosobrachus maculatus* resulting in lower percentage weight loss and damage seeds compared to untreated grains and the standard actellic dust 16 weeks after infestation. The germinative percentage of seed treated with these materials indicated that, they do not impair viability and hence could be used in preserving grains as well as seeds for planting.

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"ATTITUDES RATHER THAN KNOWLEDGE ARE CONVEYED BY A DISTINGUISH TEACHER"

HANS A. KREBS

CHAPTER ONE

INTRODUCTION

1.0 **INTRODUCTION**

Approximately one-third of the global food production is estimated to be destroyed annually by over 20,000 species of insect, diseases, weeds, nematodes, rodents and other field and storage pests. Highest losses of up to 42% occur in developing countries (McEwan, 1978).

Synthetic pesticides have so far been the principal means of crop protection. However, the increasing serious problem of pest resistance to pesticides and of contamination of the biosphere associated with the large scale use of broad spectrum synthetic pesticides have dictated the need for an effective biodegradable pesticide with greater selectivity (Schmutterer, 1990). This awareness, has generated a worldwide interest in the re-evaluation and use of old traditional botanical pest control agents.

Among all the plants studied over the past 25 years, neem tree (*Azadirachta indica*) has fascinated scientists from various fields of research as its secondary metabolites find use in agriculture, medicine, cosmetics, etc (NRC, 1992).

The isolation of Azadirachtin from neem seeds has gained worldwide attention in view of the insect antifeedant and growth inhibiting properties of the compound (Jacobson,

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1986). This tetranortriterpenoid (Limonoid) constitutes 0.2 - 0.6% of neem seed (Govindarchari *et al*, 1992) and is highly potent at low concentration against over 200 agricultural pests (Mordue and Blackwell, 1993). In addition, it is ecofriendly (Stark, 1992). These as well as the selectivity (Stark, *et al*., 1992) made it a viable alternative to the toxic synthetic pesticides.

The purpose of this study therefore, is to evaluate the pesticidal properties of various constituents of neem leaves and seeds prepared using "low tech" method adaptable by local farmers and/or village level cottage industries. The objectives of the study are:

(1) to determine and compare the levels of Azadirachtin from various constituents of the seeds and leaf extracts.

(2) To make formulations with these extracts, and examine their pesticidal effects on two pests attacking cowpea in the field, - leaf beetle *Ootheca mutabilis* sahl (chrysomelidae) and the predominant pod sucking bug (PSB) species *Clavigralla tomentosicollis* stal (Hemiptera - coreidae).

(3) To examine the effectiveness of the various formulations in reducing post-harvest storage losses of cowpea grains caused by *Callosobrachus maculatus* Fab (Bruchidae).

In the study presented here, it is hypothesized that, there is little or no difference in pesticidal effects of various extracts of neem that contained Azadirachtin as the active ingredient and this hypothesis has been tested.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 PESTICIDES

2.1.1 Definition and Classification

Pesticides are chemicals designed to combat the attack of various pest on agricultural and horticultural crops. They fall into many classes of which are the insecticides, fungicides, and herbicides. Others are rodenticides, nematocides, malluscides and ascaricides (Cremyln, 1990).

Pesticides may also be divided into two main types, namely, contact (or non systemic) pesticides and systemic pesticides. Contact or surface pesticides do not appreciably penetrate plant tissue and are consequently not transported or translocated within the plant vascular system. The disadvantages in their uses are that, firstly, they are susceptible to the effects of weathering (wind, rain and sunlight) over long periods and secondly new plant growth are left unprotected and hence open to the attack by the pests.

In systemic pesticides however, the chemical comes into intimate contact with the tissues of the host plants. In the case of fungicide for example, the danger of the fungicide causing damage to the host is especially formidable since both the host and the pest are plants and for success, the chemical must show selective toxicity to the fungus (Cremyln, 1990).

2.1.2 Important Biochemical Reactions in Pesticides

The biological action of a given pesticide is intimately related to its metabolism in the target organism and, in the case of systemic compounds, will also be affected by metabolism in the crops.

I. Insecticides

Insecticides which are designed specifically to combat insect pest, exhibit their mode of action by interference with the transmission of nervous impulses.

In most mammals and insects, acetylcholinesterase catalyses the hydrolysis of acetylcholine to choline which does not act as a transmitter. The combination of acetylcholine with the receptor causes the post-synaptic cell to pass an impulse. Subsequently, acetylcholinesterase hydrolysis the acetylcholine so that stimulation of the receptor ceases, and the synapse is then available for release of a new transmitter.

The organophosphorus and carbamate insecticides owe their insecticidal properties to the phosphorylation or carbamoylation of the enzyme acetylcholinesterase. This poison the enzyme so that it cannot catalyse the hydrolysis of acetylcholine to choline, consequently, there is an accumulation of acetylcholine at the synapse which prevents

the continuous transmission of the nerve impulse and the effective nervous coordination break down, the insect or mammal suffers convalsions and finally death.

DDT and other organochlorines insecticide act by interference with axonal transmission by binding to the nerve membrance and upsetting the sodium-potassium ion balance. Pyrethroids affect both the peripheral and central nervous system of the insects and the convalsion of the insect appear to be initiated by the loss of potassium (Cremlyn, 1990).

II. **Herbicides**

Herbicides are weed (plants growing where man does not wish them to be) killers. They exhibit their mode of action in various ways particularly by interference with the light reaction of photosynthesis (Cremlyn, 1990).

No herbicide is known to interfere with dark reaction, but above 50% of current herbicide act on the light reaction of photosynthesis by blocking electron transport between photosystem II and the first ATP production site. On the other hand, bipyridimium herbicides function by utilizing photosynthesis for the production of stabilized free radicals which in the presence of oxygen give rise to toxic entities that kill the plant.

Glyphosphate herbicide inhibits the biosynthesis of the aromatic amino acids (phenylalanine, tyrosine and tryptophan). Specifically, it poisons the enzyme 5-enolphyruvyl shikimate-3-phosphate synthase (EPSP) which controls a vital step in the biosynthetic pathway. Plant treated with glyphosphate slowly die due to lack of proteins arising from the blockage in the amino acid synthesis.

Also, sulphonylurea and imidazoline herbicides block the biosynthesis of essential branch chain amino acid (valine, leucine and isoleucine). This herbicide poison the enzyme acetolactate synthase (ALS) which catalyse the first step in the biosynthetic pathway. The blockage result in the disruption of cell division in the plant. The development of this highly potent herbicides has demonstrated that, the biosynthesis of amino acids is a major target for herbicides (Cremlyn, 1990).

III. **Fungicides**

Many commercially available fungicides targetted for fungi are applied to plant foliage as dust or sprays. They are known as protectant or surface fungicides and more recently as plant chemotherapeutants, and are absorbed by the plants via the roots, leaves or seeds and are translocated within the plant.

The fungicides furalaxyl and metalaxyl apparently act by interference with RNA synthesis and the benzimidazoles systemic fungicides were originally considered to owe their activity to DNA synthesis. Later studies indicate that, benzimidazoles prevent the assembly of tubalin into microtubules. Several antifungal biotics are probably antifungicidal as a result of interference with protein synthesis.

Azo fungicides (e.g. tiazoles) act by specifically blocking the step involving 14α demethylation of 24 methylene dihydrolanostrol, while morpholine fungicides on the other hand act at later stages of ergosterol biosynthesis by inhibition of D^8-D^7 isomerase or D^{14} reductase enzymes. Other fungicides act by blocking melanin biosynthesis.

2.1.3 Naturally Occurring Insecticides

A large number of different plant species contain natural insecticidal materials which possess the advantage that their use does not appear to result in the emergence of resistant insect strains to the same degree as the application of synthetic insecticides. Examples in ascending order of importance are nicotine, derris (rotenone) and pyrethrum.

I. Nicotine

Nicotine occur in tobacco plant as a salt with citric and malic acid to the extend of 1-8% and may be extracted from the leaves and roots of the plant by treatment with alkali, followed by steam distillation.

Nicotine function as a non persistent contact insecticide particularly as a garden spray against aphids, capsids, leaf miner, colding moth and thrips on a wide variety of crops. However, its use is rapidly declining and is being replaced by synthetic insecticide because of its comparatively high mammalian toxicity ($^{x}LD_{50}$(oral) to rat ≈ 50mg/kg) and its lack of effectiveness in cold weather. The compound is readily absorbed by the skin and any splashes must be washed off immediately.

Nicotin kills vertebrates because it mimics acetylcholine by combining with acetylcholine receptor at neuromuscular junction causing twitching, convulsion and finally death (Cremlyn, 1990).

II. Rotenoids

Retenoids Occur in the roots of *Derris elliptica* and is manufactured by grinding up the roots and mixing the powder with a clay diluent. Alternatively, the rotenoids can be extracted from the powdered roots with organic solvents and

the resultant resin by crystallization from ether or carbon tetrachloride gives rotenone which is a white crystalline solid.

Rotenoids are toxic to fish and many insects, but are almost harmless to warm blooded animals. It is also used for control of ectoparasite in livestock beside its use in horticulture. It is an extremely safe garden spray because it is degraded by light and air and does not leave residue (LD_{50} oral to rat = 135mg/kg) (Cremlyn, 1990).

The biochemical mode of its insecticidal action involves the inhibition of mitochondria electron transport by the binding of the retenone to the component of the chain blocking electron transport from NADH to ubiquinone, but NADH, dehydrogenase is not inhibited. The symptoms of insect poisoned by rotenone differ from those produced by insecticides acting on the nervous system and are characterized by reduction in oxygen consumption, depressed respiration, heartbeat, and eventual paralysis.

III. Pyrethroids

Pyrethrum is a contact insecticide from the flower heads of *Chrysanthenum cinerariaefolinum*. It is extracted with kerosene or ethylene dichloride and the extract concentrated by vacuum distillation. It contains four main insecticidal components which are collectively termed pyrethrins.

Pyrethrum owes its importance to the outstanding rapid knock down action (a few seconds) on flying insects combined with a very low mammalian toxicity due to its ready metabolism to non toxic products. Therefore, unlike DDT, pyrethrum is not persistent and leave no toxic residues which may be why the insecticide does not tend to induce the development of resistant insect population. Pyrethrum is used to control pests in stored food against household and industrial pest. Pyrethrum aerosol sprays are excellent home insecticides because of their safety and rapid action (Cremlyn, 1990).

However, a major disadvantage of pyrethrum especially for use against agricultural pest lies in its lack of persistence due to its instability in the presence of air and light. Insects can often recover from exposure to sublethal doses of pyrethrum which means that the compound must be mixed with small amount of other insecticides to ensure that the treated insects do not recover.

The symptoms of insect poisoned by pyrethroids clearly shows that the chemical attacks the insect nervous system. Pyrethroids causes hyperexcitation followed by convulsion and death in arthropods. The majority of the evidence indicates that the primary target of pyrethroid insecticide is the sodium channel in neuronal membranes which modulates the gating characteristics of the channel, keeping it open for abnormally long period. The effect on the sodium

channel alters the action potential, so that depolarization after potential is increase and prolonged. This results in repetitive firing of neurones followed by convulsion and blockage of nerve conduction. The predominance of a particular symptoms can often be related to a specific class of pyrethroids (Cremlyn, 1990).

2.1.4 **Evolution of Botanical Pesticides**

Before the use of pesticides, man was often totally at the mercy of insects, fungi, rodent, etc. that attacked his crops. For centuries, various kinds of plant materials have been used to combat insects, rats and other pests in different parts of the world. At present, over a thousand plants are known to have insecticidal properties, yet only a few are of commercial value. Pyrethrum, nicotin, derris and cube are the best known and most commonly used insecticide of vegetable origin (Kochhar, 1986). Even these known insecticides got overshadowed by the development of DDT, chlordane, aldrin, melathion and others. The discovery of DDT and its subsequent introduction in controlling pests came as a great boom to mankind.

DDT and other synthetic pesticides dominate the scene of crop protection due to their efficacy and other advantages such as quick knock-down, long shelf life, and ease of storage and shipment. However, indiscriminate and

continuous use has led to problems such as toxicity to non target organisms, development of resistance in different pests, environmental pollution and the health hazards associated with pesticides residue (Ahmed *et al.*, 1984).

Thus, development of alternative plant protection technologies become imperative and, as a result, botanical pest control materials have been under investigation. Many plant species possess selective action against a number of pests through biological activities such as antifeedants, toxic and insect growth regulatory effects. The neem based insecticides (NBIs) seem to have advantage in that they:

(1) are unlikely to loose their potency due to the build up of genetic resistance in the pest;

(2) attack many harmful insects pests but leave mammals, birds and beneficial insects like bees unharmed;

(3) act as systemic agents in certain plant species. As they are absorbed and transported throughout the plant when aqueous neem extract is applied to soil the crop becomes protected from within (Ahmed *et al.*, 1984);

(4) are biodegradable.

2.1.5 Neem Based Insecticides

Environmentally benign pesticides which are selectively toxic, do not bioaccumulate and exhibit relatively short persistence in the environment. These are needed for modern intergrated pest management programs. Neem insecticides appear to fit this definition because they have been shown to be selective (Stark *et al.*, 1992), to have short persistence and pose less negative impacts to ecosystem than conventional insecticides.

Interest in neem insecticides has grown over the past 10 years as more insecticides are lost due to environmental and food safety problems (Koul *et al.*, 1990; Schmutterer, 1990). Two neem insecticides are now commercially available in the United States of America (Margosan-O, W.R. Grace and Co., Columbia, MD, and Azatin, Agridyne Technologies, Inc., Salt Lake City, U.T) and have recently received an exemption from residue tolerance on food crops by the United States Environmental Protection Agency. Several other products may soon be available.

Differences exist in the two neem insecticide formulation available in the U.S. Margosan-O (Mo) is composed of 0.25% Azadirachtin and 3-5% neem oil while Azatin has a 3% Azadirachtin content with no neem oil. Both Mo and Azatin are emulsifiable concentrate formulations. RH9999 another neem insecticide in the experimental stage, produced by

Rohm and Haas Co (Philadelphia PA) is a wettable powder that contains chemically modified (hydroxylated) azadirachtin (20% AI) and no neem oil. Neem guard (W.R. Grace and Co., Columbia MD) is a formulated neem oil product from neem seed kernels (90% AI) which has insecticidal activity for some plant species (Stark and Wlaters, 1995).

2.2.0 NEEM TREE

2.2.1 The Tree, Origin and Distribution

Azadirachta indica or *Anthelaea azadirachta* called the neem tree is a member of the mahogany family Maliacea. It is known as "Dogon Yaro" in Nigeria (Hausa), in other English speaking countries as "Neem or Indian Lilac". In France as "Azadira d' inde or margousier"; in Spain as "Margosa or Nim"; in Germany as Niembaum; in India as "Neem or Nimb" and in Burma as "Tamar or tamar kua" (NRC, 1992).

Neem is a very fascinating tree. It is believed to be one of the most promising plants and may eventually benefit every person on earth as it provides solutions to many of man's problems particularly in combatting pests and diseases.

The neem tree is believed to have originated in Assam and Burma. Some believe it is a native of the whole Indian

subcontinent while others believe it came from dry forest areas of southeast Asia including Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia (NRC, 1992).

Neem trees are widely distributed in tropical Asia, India, Adamans, Japan and the tropical regions of Australia. It was introduced to Africa ealier this century from India and has been established in thirty countries including Kenya, Cameroon, Nigeria and Ghana (NRC, 1992).

2.2.2 Physiology and Morphology

Neem trees are attractive, broad leaved, ever-green and can grow up to 30m tall and 2.5m in girth. Their spreading branches form rounded crown as much as 10m across. They retain their leaves, except during extreme drought when their leaves may fall off (NRC, 1992).

Neem has small white bisexual flowers which are borne in auxillary clusters. They have a honey-like scent that attracts bees. The bees produce neem honey which is popular and reportedly contains no trace of azadirachtin (NRC, 1992).

The fruit is a smooth elipsoidal drupe up to almost 2cm long. When it is ripe, it is yellow or greenish yellow with a sweet pulp enclosing the seed. The seed is composed of a shell and a kernel (sometimes two or three kernels)

each about half the seed's weight. It is the kernels that are mostly used in pest control as they contain more pesticidal ingredients than the leaves which are also used.

A neem tree usually begins fruiting after 3-5 years and becomes fully productive in ten years and from then can keep producing up to 50kg of fruits annually. Neem trees can live for more than two centuries (NRC, 1992).

2.2.3 Propagation and Growth

The neem tree is easily propagated both sexually and asexually. It can be planted using seeds, seedlings, root suckers or tissue culture (Bankshi, 1976). However, it is normally grown as seed either planted directly on the site or transplanted as seedlings from the nursery.

The neem tree grows almost anywhere in lowland tropics. It thrives in areas with annual rainfall of 400-1200mm and hottest condition up to over 50°C but will not withstand freezing or extended cold. Neem is therefore found mostly in the tropics. It does well at elevations from sea level to perhaps 1000m near the equator (Bankshi, 1976).

Neem is renowned for good growth on dry infertile sites. It performs better than most tress on sterile, stony, shallow and acidic soil. It however, cannot withstand wet feet and quickly dies if the site becomes water logged

(Bankshi, 1976). This is why neem is not found in swamps and riverine areas.

Neem often grows rapidly, is seldom affected by weeds and can dominate almost all competitors (except the very young plants). Neem trees spread very widely under favourable conditions since their seeds are distributed by birds, bats and baboons. Also, regeneration under old trees is oven abundant where the ripe fruits drop (NRC, 1992).

2.2.4 Pests and Diseases of the Neem Tree

Most neem tree are reported to be remarkably pest free, this may be as a result of the pesticidal properties of the neem tree. However, in Nigeria, fourteeen (14) insect species and one parasitic plant have been recorded as pest of the neem tree (Schmutterer, 1990). In recent years a very serious trend has emerged. In some parts of Africa (mainly Lake Chad basin and Cameroon), a scale insect *Aonidella Orientallis* has become a serious pest, it feeds on the tree sap and though the pest may not harm mature trees seriously, it kills the young ones (CAB-IIBC, 1987).

Despite the fact that the leaves contain fungicidal and antibacterial ingredients (Zech, 1984) certain microbes attack different parts of the tree causing diseases like root rot, blight on the stem, and twigs, leaf spot, powdery mildew and bacterial blight (Desai, 1966; Bankshi, 1976).

Also a cancker disease that discolours the wood, which seems to coincide with a sudden absorption of water after long droughts, has been observed (NRC, 1992).

Deficiency of zinc or potassium drastically reduces growth and causes chlorosis of the leaf tips and margin, their shoot exude much resin while the older leaves fall off (NRC, 1992). Young neem trees may be prevented from growing to maturity by fire which kills them outrightly. Sudden and intense sunlight as well as rodents which gnaw away the tree bark at the base cause much physical damage. Mature trees are seldom adversely affected by fire as they almost regrow especially if the dead parts are quickly cut away (NRC, 1992).

2.2.5 **Uses of the Neem Tree**

The neem tree is full of potentials. It appears to be God-sent solution to many of man's problems. No wonder it has been described as "A tree for solving global problems" (NRC, 1992). Its uses are numerous. Neem products include medicinals, soft pesticides (especially for plant and crop protection), industrial raw materials and contraceptives (Saxena, 1987).

2.2.5.1 **Insecticidal Uses**

By 1990, researchers had shown that neem extracts could

affect almost 200 insect species. These include many that are resistant or almost impossible to control with conventional pesticides. These include potatoe white fly, green peach aphid, western floral trips, diamond black moth and several leaf miners (NRC, 1992).

Generally, neem products are medium to broad spectrum pesticides of plant eating insects. They affect members of most if not all orders of insects. Some of the insects affected and their effects are shown below:

INSECTS	EFFECTS
Housefly	Inhibit feeding, disrupt molting and repels.
Yellow fever mosquitoe	Kills larvae, disrupts molting.
Head lice	Kills, very sensitive to the oil.
Cockroach	Reduce fecundity and molts, reduce number of fertile eggs.
Bean aphids	Reduce fecundity and disrupts molting.
Cowpea weavil	Inhibit feeding.
Rice weavil	Inhibit feeding and disrupt growth.
Migratory locust	Stops feeding, convert gregarious nymphs into solitary forms, reduce fitness, adults cannot fly (Wartem, 1989).

Neem seems remarkably mild to spiders, butterflies and insects such as bees that pollinate crops and trees. It is also mild to Lady bugs that consume aphids and wasps that act as parasites on various insects (Khan and Wassile, 1987). This is mainly due to the fact that, neem products have to be ingested to be effective. Thus, insects that do not feed on plant tissue are spared while those that do are affected.

2.2.5.2 **Medicinal Uses of Neem**

One of the most celebrated use of neem especially around this part of the world, is its use an antimalarial. In Nigeria, brewed leaves are used as cure for malaria. The tree has relieved so many of different pains, fever, infections and other complaints that it has been called the village pharmacy (NRC, 1992). It is a ready source of low cost analgestic (pain relieving) and antipyretic fever reducing (Badam, 1987).

Neems preparations are reportedly efficacious against a variety of skin diseases, septic sores and infected burns (Radwanski and Wickens, 1981). The leaves are applied in form of poultices or decoctions and are recommended for boils, ulcers and eczema (NRC, 1992). The oil is used for skin diseases such as srofula, indolent sores and ringworms.

In addition, neem has been reported to be effective against certain pathogenic fungi that affect the human body (Radwanski and Wickens, 1981). Some of them are listed below:

Trichophyton	an athlete foot fungus that affects the hair, skin and nails.
Epidermophyton	ringworm that invades hair, toe nails and skin.
Trichosporon	a fungus of the intestinal tract.
Microsporon	a ringworm that invades hair, skin and rarely nails.
Geotrichum	a yeast-like fungus that causes infection of the bronchi, lungs and mucous membrane.
Candida	a yeast-like fungus that is part of the normal floral but leads to lessions in the mouth, vagina, hands and lungs when out of control (Patel and Trivedi 1962).

Neem oil has also suppressed pathogenic bacteria including *Staphylococcus aureus* and *Salmonella typhosa* (Radwanski and Wickens, 1981; Schneider, 1986).

2.2.5.3 **Industrial Uses of Neem**

Beyond all the possible pesticides and pharmaceutics, neem provides many useful and common place materials. For

instance, oil extracted from the seeds goes into soaps, waxes and lubricants as well as fuels for lighting and heating (Steets and Schmutterer, 1986).

Neem leaves and cakes have demonstrated great potential as fertilizers. Other products obtainable from neem are, neem glue from resins tapped from its bark (Anderson and Hendrie, 1971), tannin and fibre for rope production, timber, neem honey, methane gas, and a carbohydrate base for other industrial purposes (Deuvkumar, 1985).

2.2.5.4 **Contraceptives and Birth Control Pills**

Materials from neem seeds have been shown to have contraceptive properties. The oil is claimed to be a strong spermicide and when used intravaginally, have proven effective in reducing birth rate in laboratory animals (Saxena, 1987). A recent test involving the wives of more than 20 Indian army personnel has further demonstrated its effectiveness (Saxena, 1987).

Other neem compounds show early promise as a long sought oral birth control pills for men. In preliminary trials, they reduce fertility in male monkeys and a variety of other mammals (Saxena, 1987) without inhibiting sperm production. In addition, the effect seems to be temporary which could be a big selling point that could help its rapid and widespread adoption.

2.3.0 ACTIVE INGREDIENTS IN NEEM TREE

2.3.1 Major Active Ingredients in Neem

The neem kernel contains about four main ingredients and twenty minor ingredients. In the main, these compounds belong to a general class of compounds called triterpenes or specifically limonoids. At least nine of the limonoids have demonstrated ability to block insect growth, affecting species that include the deadliest of agricultural and human pests. New limonoids are still being discovered in neem but Azadirachtin, Salannin, Malantriol and Nimbin are the best known and for now the most significant (NRC, 1992).

Azadirachtin is found to be the most abundant of these limonoids and may therefore serve as a substance representative of neem's biological activity (Ermel *et al.*, 1984; Warthen *et al.*, 1984).

2.3.1.1 Chemistry and Synthesis of Azadirachtin

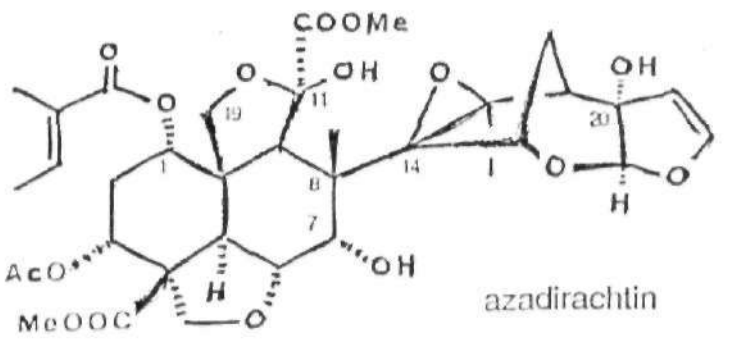
Azadirachtin exists in various forms. These are azadirachtin A-K among which azadirachtin A is the most abundant. Azadirachtin A was first extracted by Butterworth and morgan in 1968. They established the molecular formular as C_{35} H_{44} O_{16} and delineated important structural features. Azadirachtin has a molecular mass of

720 and exist as a micro-crystalline solid. It has a melting point of between 155-158°C and is unstable in aqueous solution (Govidachari *et al.*, 1992).

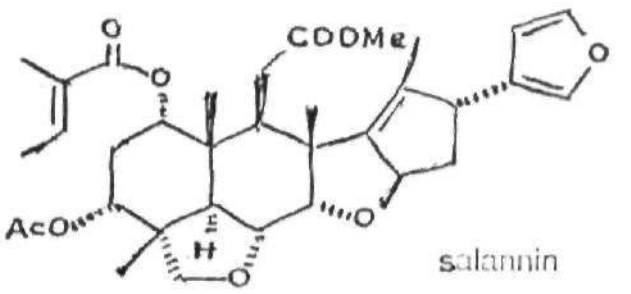
Structurally, azadirachtin belong to a new type of highly oxygenated triterpenes. Plants synthesize such compounds by a well-known route from acetate through mevalonate and squalene to one of several cyclized triterpenes in this case, the parent terpene is aportirucallol or apoeuphol.

The structure of several derivatives of aportirucallol in neem seeds were already known but azadirachtin evidently was not closely related to any of them. It was very different from the parent structure and contained a surprsing 16 oxygen atoms (Broughton *et al.*, 1986).

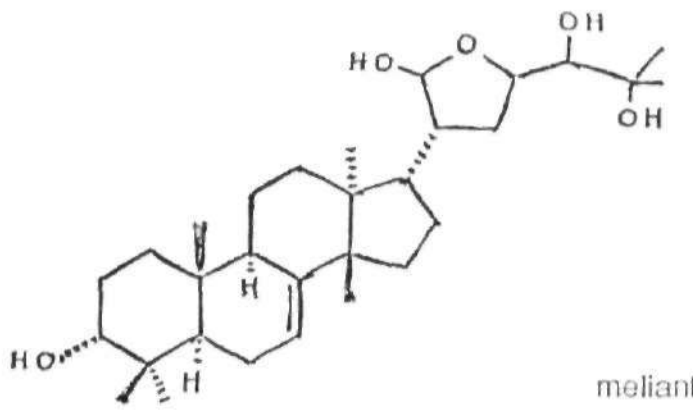
STRUCTURES OF COMPOUNDS MENTIONED



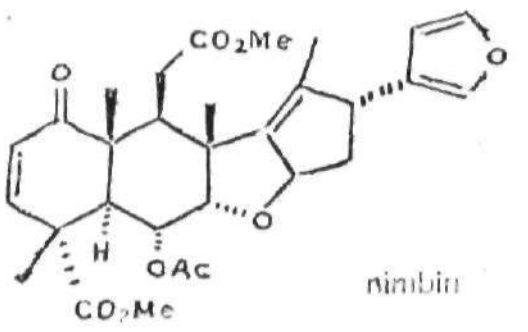
azadirachtin



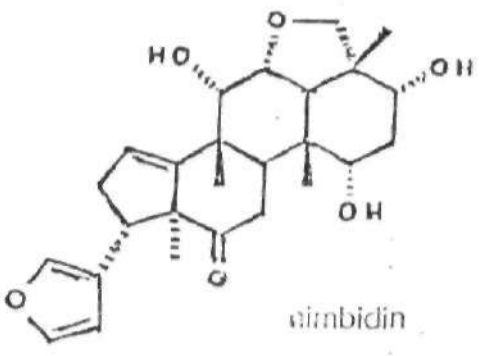
salannin



meliantriol



nimbin



nimbidin

2.3.1.2 **Mode of tion of Azadirachtin**

Azadirachtin exhibits its mode of action through antifeedant and growth inhibitory effects. Antifeedants are substances that are not necessarily food repellants but cancel out the signal to the appropriate organ in the insects to initiate feeding on the host. In the presence of antifeedant, the insect may starve to death while remaining on the host plant, possibly because the antifeedant makes the host plant distasteful to insect so feeding is inhibited. The majority of antifeedant do not directly kill the insect (Cremyln, 1990). Azadirachtin in particular interferes with the power of taste of the insects. It is frequently so repugnant to insects that scores of different leaf chewing species, even ones that normally strip everything living off plant, will starve to death rather than touch plants that carry traces of azadirachtin (NRC, 1992).

In addition to the antifeedant property, azadirachtin inhibits growth. This is because, this hormone-like compound "mimics" and blocks the part of the brain of the insect that produces the hormone necessary for growth and development. In many cases for instance, the insect may be ready to change from one developmental stage to another e.g. larvae to pupae while the molting hormone is not there, thus dying without producing a new generation. These deep seated hormonal effects are reasons for neem's

2.4.0 EXTRACTION OF ACTIVE INGREDIENTS

Azadirachtin and other active ingredients can be extracted using either water or alcohol. The simplest technique is to grind the kernel and extract them with water. This may be done by steeping them overnight in a cloth bag suspended in a barrel of water.

Alcohol extraction is the most direct process for producing neem-based insecticides (NBIs) in concentrated form since limonoids are highly soluble in alcoholic solvents. The grated kernels are usally steeped in ethanol or methanol giving a yield of active ingredients varying from 0.2-6.2% (NRC, 1992). In comparing extraction methods, it was found that the azeotropic mixture of methanol and methyl tertiary-butyl ether was most efficient and simple. It gave a yield of 4-5% (Kraus *et al.*, 1985).

2.5.0 PRODUCTS FROM NEEM SEED PROCESSING

2.5.1.1 Neem Oil

The neem oil is the most important of all the neem products. In composition, it is very much like other vegetable oils. It contains mainly triglycerides of oleic acid 42%, stearic acid 20%, linolenic acid 20%, palmitic acid 18% and linoleic acid 1% (NRC, 1992). The oil is generally dark, greeninsh yellow, bitter and smelly due to

its sulphur content. Six limonoids; nimbandiol, deacetylminbin, 6 acetylnimbandiol, deacetylsalannin, nimbin and salannin as well as two yet unidentified compounds, believed to be limonoids have been identified in neem oil (Stark and Walters, 1995).

To obtain the oil industrially, the kernels are pressed in an oil expeller, the oil yield in this case is frequently as high as 50% of the kernel weight. In the laboratory however, the oil is extracted using hexane or petroleum spirit. This gives yield of 45% (Devukummar, 1985).

The oil is used as fuel in lamps, in soap making and in other non-edible products. Its potential use as a spermicide has been reported (Saxena, 1987).

2.5.1.2 **Neem Cake**

The seed residue left after the lipid has been extracted is referred to as the cake. Its compositions are as follows:

Crude protein	13 - 25%
Carbohydrate	26 - 50%
Crude fibre	8 - 26%
Fat	2 - 3%
Ash	5 - 18%
Acid soluble ash	1 - 17%

Though too bitter to use as animal feeds, the cake has

unique promise as a fertilizer. It contains more nitrogen, phosphorus, potassium, calcium and magnessium than farm yard manure or sewage sludge (MacMillan, 1962).

It has been widely used to fertilize cash crops particularly, sugar cane and vegetables. In addition, it has been found to protect plant roots from nematodes and white ants probably due to its residual content of limonoids (Ketkar, 1976).

Studies have shown that the cake actually makes the soil more fertile probably due to the presence of an ingredient that blocks soil bacteria from converting nitrogen compounds into nitrogen gas which is useless to plants (Ketkar, 1976).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 REAGENTS, APPARATUS AND ANALYTICAL INSTRUMENTS

3.1.1 Reagents and Chemicals

All the reagents used were of analytical grade and were obtained from British Drug House (BDH) Poole, England. Synthetic cypermethrin (cymbush) and pirimiphos methyl (actellic dust) were obtained from the Department of Crop Protection in the Institute for Agricultural Research (IAR) Zaria, Nigeria.

3.1.2 Equipments and Analytical Instruments

Spectronic D21, EEI type flame photometer, Pye Unicam. Spectrophotometer alongside a rotary vacuum evaporator and oven (drying) were used.

3.2 Plant Materials and Sampling

Ripe neem fruits were harvested in the first week of July from five randomly selected trees in Samaru (11°11′ and 07°28′E668m). Some of the fruits and kernels were picked from the ground under the trees while others were harvested from the trees. The fruits were stored at room temperature in the laboratory in polythene bags before preparations. All the leaves used were freshly harvested ones.

3.3 Preparation of Extracts and Concentrates

Five different extracts were prepared from the seed, seed cake and leaves of neem. These include neem seed water extract (NSWE), neem leaf water extract (NLWE), neem cake water extract (NCWE), neem cake methanol extract (NCME) and neem oil emulsified concentrate (NOEC).

3.3.1 Neem Seed Water Extract (NSWE)

Neem seed kernels were pulversized using a grinding machine. Five percent weight per volume (5% w/v) of the powder was dispersed in water and left overnight. This was then filtered using a cheese cloth to obtain the filtrate referred to as neem seed water extract (Jackai, 1993).

3.3.2 Neem Leaf Water Extract (NLWE)

Fresh neem leaves were crushed with a mortar and pestle. Five percent weight per volume of the crushed leaves (5% w/v) was dispersed in water for two hours. Filtration was carried out as above (3.3.1) and the filtrate is referred to as neem leaf water extract (Grant *et al.*, 1984).

3.3.3 Neem Oil Emulsifiable Concentrate (NOEC)

Neem seed kernels were ground in a grinding machine and soaked with continuous stirring in hexane for two hours

(25% w/v). After decantation, the supernatant was distilled using a rotary vacuum evaporator and the solvent recovered. One hundred grams of the seed kernel yielded 35g of an oily yellowish green residue. The oil was stored in the refrigerator. For field application, the oil were emulsified with sodium dodecyl sulphate (SDS), by mixing 5ml of neem oil with approximately 5g of sodium dodecyl sulphate (emulsifier). This was further diluted in water to give a concentration of 5% v/v of the emulsifier in water to assess the impact of using water to provide the total amount of liquid to spray the plot.

3.3.4 Neem Cake Water Extract (NCWE)

Neem cake is the residue left after the lipid has been extracted from the seed kernel. Five percent weight per volume of the neem cake powder (5% w/v) was dispersed in distilled water and the suspension left overnight. The filtrate obtained after filtration was used as neem cake water extract.

3.3.5 Neem Cake Methanol Extract (NCME)

Cake powder (25g) was soaked in 100cm^3 of methanol with continuous stirring for two hours. The supernatant was distilled using a rotary vacuum evaporator. The solvent was recovered from the supernatant and a yellowish paste obtained as neem cake methanol extract. This was further

diluted in water to give a concentration of 5% v/v of the neem cake in water.

3.4 Determination of Azadirachtin

3.4.1 Concentration of Samples (Neem Extracts)

Twenty cubic centimeter of each of the samples to be analysed were put in rotory vacuum evaporator and the volume reduced to approximately $3cm^3$. They were then collected in sample bottles for T.L.C. analysis. They were however dissolved in $10cm^3$ of methanol before spotting on T.L.C. plates.

3.4.2 Preparation of Chromatographic Plates

Five 20 x 20cm plates were soaked in saturated sodium carbonate solution overnight. They were rinsed several times with distilled water before drying in an oven. With careful handling, the plates were made grease-free by wiping them with tissue paper soaked in petroleum spirit. 200g of silica gel was homogenously suspended in a 2 litre round bottom flask with approximately 480ml of distilled water by swirling the flask. The flask was subsequently allowed to stand for 1 hour and the suspension was rapidly spread with a spreader set at layer thickness of 1.5mm into a previously cleaned grease free plates. The plates obtained were kept for two days to dry at room temperature

and were subsequently activated for 2 hours at 120°c in a dry oven before use.

3.4.3 **Spotting Technique**

Aliquots containing about 5 - 10μl of the sample (neem extract) or the standard Azadirachtin dissolved in methanol were applied to the chromatographic plates with a glass capillray micropipette in holder. Applications were made in small increments under a stream of warm air from a heat gun to facilitate rapid drying and to minimize spot diffusion.

3.4.4 **Solvent Systems**

The spotted plates were placed in closed glass tanks lined with Whatman paper no. 4 saturated with the developing solvent to a distance of 10cm from the origin. First development made at room temperature took about 55 minnutes, subsequent runs took about 45 minutes. After a run, the plates were dried with the aid of a heat gun.

A wide variety of solvent systems were surveyed including a number that have previously been utilized for both paper chromatography and T.L.C., most of these generally gave unsatisfactory results under our condition. The system that produce distinctly superior separation was methanol and was employed alone (100%) for this studies.

3.4.5 Detection of Spots

Of a number of spraying agents compared, iodine produced the best results. About 10g of iodine chips were thrown inside a glass tank containing the plates. After about 30 minutes, Azadirachtin appeared as a dark grey spot in each of the samples and the standard. The R_f values were calculated as follows:

$$R_f = \frac{\text{distance moved by spot (cm)}}{\text{distance moved by solvent (cm)}}$$

3.4.6 Spectrophotometric Determination of Relative Concentration of Azadirachtin in Samples

The absorbance of the pure azadirachtin was obtained by dissolving 4.21mg of the pure azadirachtin in $100cm^3$ of methanol to give a concentration of $4.21 \times 10^{-3}g/100cm^3$. The absorbance was measured at 214nm.

Spots corresponding to azadirachtin (similar R_f values) for each of the sample were scrapped off from the plate. They were eluted in $10cm^3$ of methanol and the silica gel removed by flitration. The absorbance of each of the sample was taken at 214nm.

The relative concentration of azadirachtin in the samples were determined as follows:

$$A = \Sigma cL$$

$$Conc = 4.21 \times 10^{-3} g/100cm^3$$

$$\Sigma = \frac{A}{CL}$$

$$= \frac{0.68}{4.21 \times 10^{-3}}$$

$$= 161.52$$

.. $\Sigma^{1\%}_{1cm}$ of sample in methanol at 214nm = 161.52

e.g. concentration of azadirachtin in neem water extract with absorbance of 0.42

$$C = \frac{A}{\Sigma}$$

$$= \frac{0.42}{161.52}$$

$$= 0.0026g/100ml$$

$$= 2.6mg/100ml$$

$$= 0.26mg/10ml$$

$$= 0.026mg/ml$$

3.5 Field Experiments

3.5.1 Experimental Plot Design

Field trials were carried out in 1996 at the National Research Institute for Chemical Technology (NARICT), Basawa, Zaria in Kaduna State of Nigeria. Cowpea variety sampea 7 (IAR48) was used. The seeds were sown on 7th August, 1996.

The plots were randomized using complete block design with seven treatments including two controls in three replicates. Each plot measured 3.0 x 0.5m^2 and contained three rows. Intracrop and intrarow spacing were 25cm and 75cm respectively. Two seedlings were maintained perhill in all plots and there were 12 plants per plot. Plots were fertilized with NPK (15-15-15) applied 20 days after sowing.

3.5.2 Field Application

Field experiment had the following seven treatments:

(1) Neem seed water extract (NSWE)

(2) Neem leaf water extract (NLWE)

(3) Neem cake water extract (NCWE)

(4) Neem cake methanol extract (NCME)

(5) Neem oil emulsifiable concentrate (NOEC)

(6) Synthetic insecticide (cypermethrin)

(7) Water (control)

Weekly application was done with a hand sprayer and the leaves were sprayed to the point of run off. Locally available brown sugar (10g or 20g of the neem material and 7g of brown sugar) were added to the water extracts for better adherence (Jackai, 1993). Treatment commences 3 weeks after planting and continued for 6 weeks. At pod formation (about 10 weeks after planting), the plots were sprayed twice with the various formulations. Spraying was done between 8:00 and 10:00h on each spraying day.

3.5.3 Treatment Monitoring and Assessment

The pesticidal effects of the treatments were assessed by counting the number of insects (*Clavigralla tomentosicollis*) and estimating foliar damage by *Ootheca mutabilis*. The population of the insects were recorded at 8, 9, 10, 11 and 12 weeks after planting (WAP)from five plants obtained at random per plot. The foliar damage was however assessed at intervals of 3, 4, 5, 6, 7 and 8 weeks after planting on a visual scale of 0 - 7 which is a modification of Karel, (1989): 1=no damage, 2 = 1-5% damage, 3 = 6-10% damage, 4 = 11-20% damage, 5 = 21 - 30% damage, 6 = 31-50% damage and 7 > 50% damage.
Harvesting of the dry pods was done weekly from the 15th week to the 18th week and yield was assessed.

3.5.4 Data Analysis

Data collected from the field trials were subjected to analysis of variance and means were separated by Duncan's multiple range test.

3.6 Post-harvest Storage Experiments

3.6.1 Culturing of Beetles

Laboratory trials were carried out in the Storage Entomology Laboratory of the Department of Crop Protection. *Callosobrachus maculatus* were cultured in the laboratory at 27 ± 2°C and 70 - 75% relative humidity. The food media used were cowpea grains. Ten pairs of *Callosobrachus maculatus* were placed inside a glass jar containing 350g of cowpea grains. The jars were covered with a nylon mesh held with a rubber band. They were maintained for 7 weeks.

3.6.2 Grains Used

Grains used for the study were cowpea sampea 7 (IAR48) obtained from the Institute for Agricultural Research (IAR) Samaru, Zaria. The grains were fumigated with phostoxin (aluminium phosphide) for 48hrs to ensure that it was free from insect and allowed to "vent" for 24 hours before being used.

3.6.3 **Neem Materials and Dosage**

Neem seed, neem cake, neem leaf, neem seed residue and neem oil were used as pesticides for treating the cowpea before storage.

Neem seed powder was obtained by air drying the neem seeds (7 days), milled and sieved to get a finner powder. Five percent weight per weight (5% w/w) of the seed powder was used for treatment.

Neem seed cake powder was obtained by sun-drying the residue obtained from the oil extraction (3.3.3) milled and sieved. Five percent weight per weight (5% w/w) of the cake was used for treatment.

Neem leaves were dried for 7 days and then milled and sieved. Five percent weight per weight (5% w/w) of the leaves were used for treatment.

Neem seed residue refers to the residue left after filtration of the seed water extract. This was air-dried, milled and sieved. Five percent weight per weight (5% w/w) of the seed residue was used for treatment.

Neem oil used was the one prepared in section 3.3.3. The oil was applied at a rate of $4cm^3/kg$.

3.6.4 Infestation

Each of the five neem formulations were mixed separately with 250g of cowpea grains in glass jars at the dosages indicated in section 3.6.3 above. The jars were then shaken thoroughly to facilitate proper admixture. Control jars contained 250g of cowpea only or with actellic dust at 15ppm. The jars and their contents were allowed to settle for 10mins after which the test insects were sexed and five pairs of the non-active stage of *Callosobrachus maculatus* obtained from the laboratory culture were introduced into each jar. The glass jars were covered with nylon mesh held in place with rubber bands. The experiments, consisting of seven treatments plus two controls and replicated 3 times were arranged in a completely randomized design on a laboratory table where the laboratory temperature fluctuated between 27-29°C, and 70-75% RH. The first sample was drawn from each jar 30 days after infestation and percent seed damage and weight loss determined. Subsequent sampling were done monthly, and at the end of the 4th month, percent seed viability and number of beetles were determined.

3.6.5 Damage Assessment

Samples of 100 grains were taken from each jar and the number of damaged grains (grains with characteristic emergent hole) and undamaged grains were counted and

weighed. Percentage weight loss was calculated following method of FAO (1985) as follows:

$$\% \text{ weight loss} = \frac{UaN - (U + D)}{UaN} \times 100$$

where:

Ua = average weight of one undamaged grain

N = total number of grains in the sample

U = weight of undamaged fraction in the sample

D = weight of damaged fraction in the sample

Similarly, percentage seed damage is given as follows:

$$\% \text{ seed damage} = \frac{N_D}{N_T} \times 100$$

where:

N_D = number of grains with characteristic emergent hole

N_T = Total number of grains

Each treatment was repeated 3 times.

3.6.6 Inventory of Insect Fauna

The number of insects (*Callosobrachus maculatus*) in each jar was estimated using the "coning and quartering" machine. Insect fauna were determined after sieving sample with a 2.00 mm mesh 18 weeks after storage (WAS). The number of dead insects in each jar were counted.

3.6.7 Determination of Seed Viability

Twenty grains of cowpea were immersed in 5% sodium hypochloride (NaOCl) solution for 5 mins and rinsed several times with distilled water. The seeds were then placed in 9cm petri-dishes lined with filter paper moistened with distilled water. The petri-dishes were wrapped in aluminium foil and then incubated at 28°C for 48hrs to germinate. The percent seed viability were calculated as follows:

$$\% \text{ seed viability} = \frac{N_G}{N_T} \times 100$$

where:

N_G = number of seeds that germinated

N_T = total number of seeds

The determination was carried out in triplicate on petri-dishes.

3.6.8 Data Analysis

Data collected were subjected to analysis of variance and means were separated by the least significance difference test. Data were transformed were necessary.

CHAPTER FOUR

RESULTS

4.1 FIELD EXPERIMENTS

4.1.1 Damage by the Foliar Feeding Beetle (*Ootheca matabilis*

Ootheca matabilis (sahlberg) (Coleoptera: chrysomelidae) is the commonest foliar beetle. It is shiny, light brown or orange and damage is done by the adults feeding between the veins of the leaves. Table 4.1 shows the leaf damage rating for six weeks (3 - 8 weeks) after planting (WAP). All the neem treatments and the synthetic insecticide treatment (T_7) show significantly ($P < 0.05$) lower leaf damage ratings than the untreated control (T_6). Differences in the effectiveness of the various treatments were however obvious. At most evaluation dates, leaf damage was rated significantly ($P < 0.05$) lower on the plants treated with neem formulations than the synthetic insecticide treatment (T_7). Though none of the neem formulations protected the cowpea plants completely, there was a general reduction in the incidence of foliar damage caused by *Ootheca mutabilis*. In particular, treatments with neem seed water extract (NSWE) and neem cake water extract (T_5) appear most effective.

TABLE 4.1: Effect of Various Neem Formulations and Conventional Insecticide (cypermethrin) on Damage to Cowpea Leaf Caused by the Leaf Beetle (*Ootheca mutabilis*)

		LEAF DAMAGE RATING[Z]					
		WEEKS AFTER PLANTING (WAP)					
TREATMENTS		*3*	*4*	*5*	*6*	*7*	*8*
T_1	Neem seed water extract	3.1±0.2c[Y]	3.1±0.2e	1.5±2.6d	1.9±0.9e	1.2±2.1cd	1.7±1.5cde
T_2	Neem leaf water extract	4.1±1.2b	3.8±0.2bcd	2.0±1.4cd	2.0±1.7cde	1.4±2.4bcd	1.6±1.4de
T_3	Neem oil emulsified concentrate	3.9±0.2bc	3.3±0.5de	2.1±1.8bcd	2.2±2.3cde	1.6±1.4bc	1.8±2.4cde
T_4	Neem cake methanol extract	4.2±0.4b	4.1±0.2b	2.6±2.4b	2.7±1.5bc	1.8±1.6b	2.1±1.8bcd
T_5	Neem cake water extract	3.6±1.0bc	3.1±0.2de	1.6±1.4d	1.7±1.5e	1.1±1.9d	1.2±2.1e
T_6	Untreated control	6.3±0.6a	5.9±1.2a	5.6±1.0a	5.1±0.2a	5.1±0.2a	5.7±1.5a
T_7	Synthetic insecticide (cymbush)	3.5±0.5bc	3.4±0.5cde	2.4±2.1bc	2.3±2.0bcde	1.8±1.6b	2.0±0.0cd

Average of 3 replications

[Y]within each column, means followed by a common letter do not significantly differ at 5% level (Duncan's multiple range test).

[Z]damage rating 1 = no damage, 2 = 1 - 5% damage, 3 = 6 - 10% damage, 4 = 11 - 20% damage, 5 = 21 - 30% damage, 6 = 31 - 50% and 7 > 50% damage.

4.1.2 Insect Population (*Clavigralla tomentosicollis*)

Clavigralla tomentosicollis (stal) (Hemiptera-coreidae) is a major pest of cowpea in Africa. It is medium sized, hairy and grey. The nymphs form large colonies on cowpea pods and both nymph and adult do serious damage to the pods. Fig. 4.1 shows the effect of various neem extracts on the incidence of *Clavigralla tomentosicollis* on cowpea plant from the 8th to 12th weeks after planting (WAP). The population of the pod *suckers was significantly* ($P < 0.05$) higher in untreated control plot (T_6) than those with various neem formulations. The population of the pod sucking bugs in the untreated plot (T_7) increased from the 8th to the 9th WAP followed by a decrease in the 10th week. Treatments T_5 and T_6 receiving neem cake water extract and the synthetic insecticide respectively had the least infestation by the suckers. There was no significant ($P < 0.05$) difference in insect population in treatments T_1, T_5 and T_7.

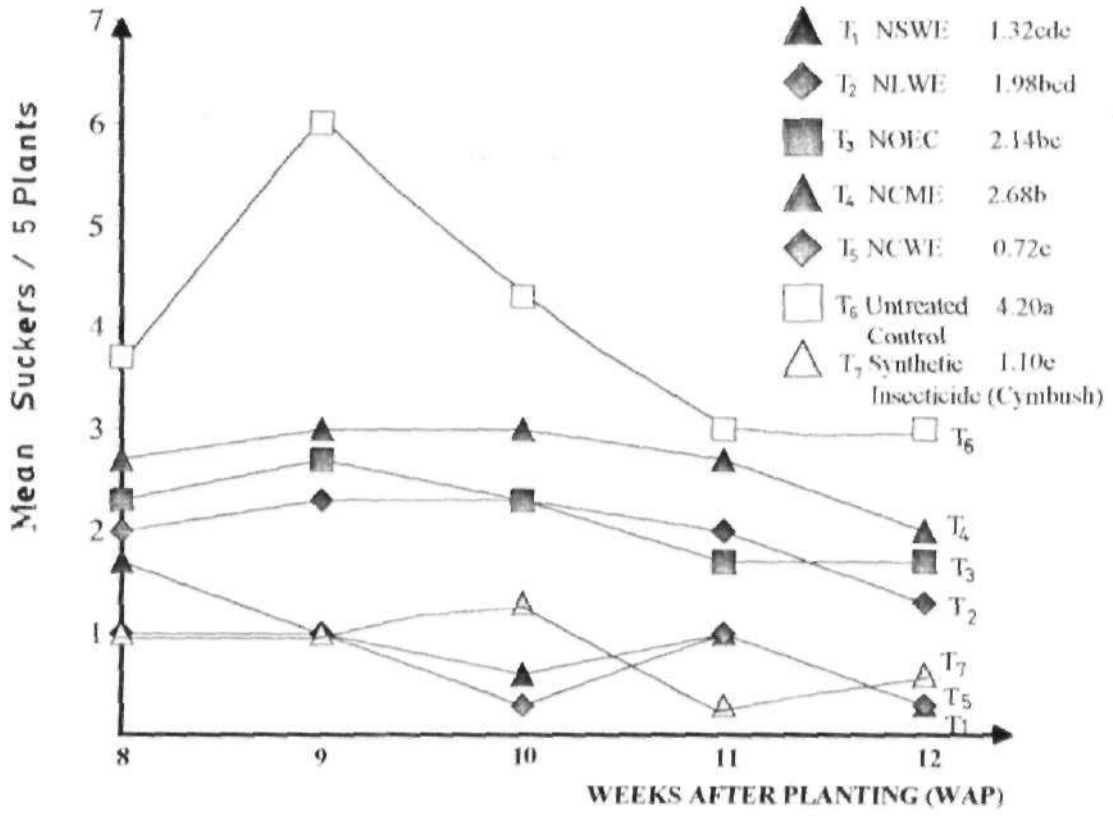


Fig. 4.1 Effect Of Various Neem Formulations On The Incidence Of Suckers (Pod Borers) On Cowpea Plant (Number Next To Curve And In Legend Refers To Treatment Numbers).

Mean Insect Population (Suckers) (*Clavigralla tomentosicollis*) With A Common Letter Do Not Differ Significantly At 5% Level (Duncan's Multiple Range Test).

4.1.3 **Yield**

The mean yield of cowpea per plot was observed to be higher in the various neem treatments and the synthetic insecticide treatment (T_7) than in the untreated control (T_6) (Fig. 4.2). Differences in the yield between neem extract and the synthetic insecticide treatment on the one hand and between the various neem treatments on the other hand were obvious with the neem seed water extract treatment (T_1) having the highest and the untreated control (T_6) the lowest.

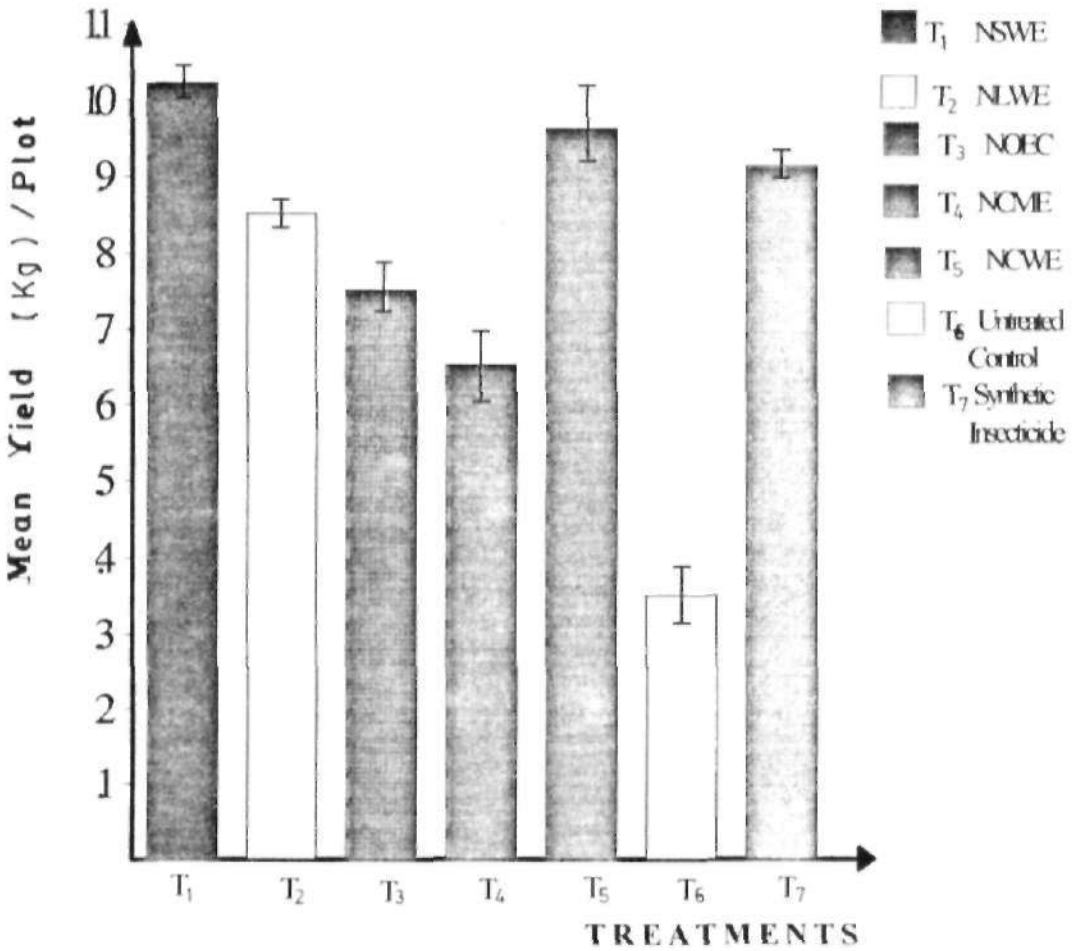


Fig. 4.2

-Mean Yield Of Cowpea Per Plot (3.0 X 0.5M) Treated With Various Neem Formulations

-3 Replications Per Treatment

-I Bars Indicate Standard Deviation.

4.1.4 Azadirachtin Levels and Linear Regression Parameters

Relative Azadirachtin levels in the various neem extracts are shown in Table 4.2. The results shows clearly that, the azadirachtin concentration is not the same in the various extracts. The neem cake water extract receiving treatment (T_5) and the neem water extract of the seed (T_1) have high values unlike the methanol extract of the cake (T_4). The differences in the levels of Azadirachtin is clearly reflected in their pesticidal effects where T_5 and T_1 were outstanding.

There was a significant ($P < 0.05$) correlation obtained ($r = -0.96$) between azadirachtin and leaf damage by *Ootheca mutabilis* for example and has the estimation equation as:

$$Y = 3.94 - 6.78x$$

meaning the intercept on the Y axis from Fig. 4.3 is 3.94 and the slope is -6.78. The estimate of the standard error is 0.13. The strong (negative) correlation coefficient of -0.96 means that $[(100)\ (-0.96)^2]$ 92.16% of the leaf damage is accounted for by the linear function of Azadirachtin in the treatment. The relatively high value of r obtained is also indicative of the closeness between the estimated regression line and the observed points (Fig. 4.3). The linear regression however becomes invalid if extrapolated outside the range of the minimum and maximum value of x corresponding to Y as determined from the estimation equation in drawing the regression line.

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TABLE 4.2: Relative Concentration of Azadirachtin (mg/ml) of Various Neem Extracts Used in Field Trials

Treatments	Absorbance max 214nm	Concentration of Azadirachtin mg/ml
T_1 Neem seed water extract (NSWE)	0.42	0.026
T_2 Neem leaf water extract (NLWE)	0.32	0.019
T_3 Neem oil Emulsified concentrate (NOEC)	0.36	0.022
T_4 Neem cake methanol extract (NCME)	0.27	0.017
T_5 Neem cake water extract (NCWE)	0.47	0.029

The concentration of the extracts used as treatments were five percent weight per volume (5% w/v) of the neem material in water.

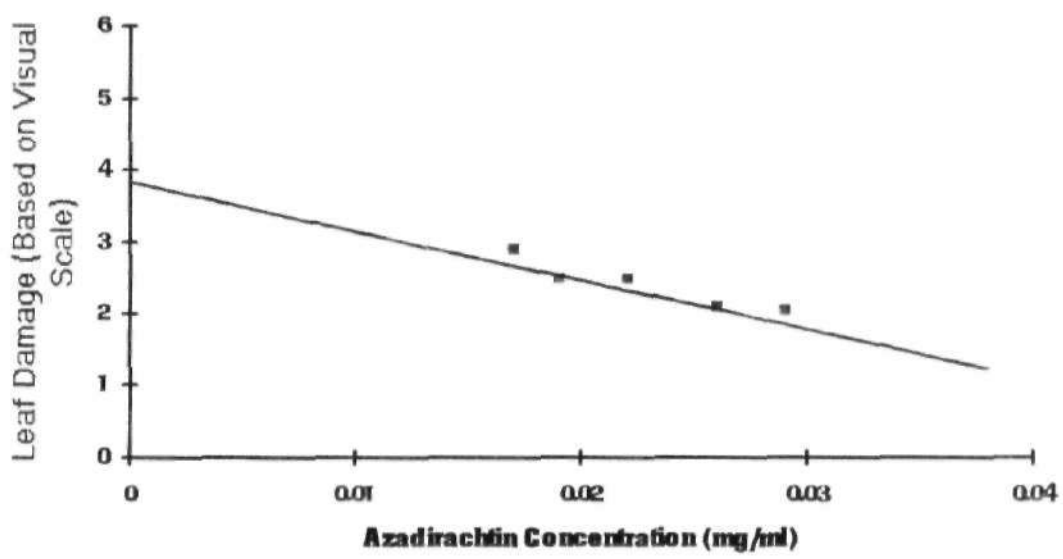


Fig. 4.3
Estimated Linear Regression Between Leaf Damage by Ootheca mutabilis And Azadirachtin Concentration

4.2 POST-HARVEST STORAGE EXPERIMENTS

4.2.1 Assessment of Percent Seed Damage and weight Loss due to *Callosobrachus maculatus* Infestation on Cowpea Seeds

Callosobrachus maculatus (Fabricius) [Coleoptera:bruchidae] is the most important storage pest of cowpea throughout the tropics. It is the larvae that penetrates the seed and do extensive damage by feeding from inside. Percent seed damage is based on exit holes. Table 4.3 and Fig. 4.4 give the percent seed damage and weight loss respectively.

Of all the neem materials tested, only the neem oil treatment (T_1) and the neem cake treatment (T_4) provided satisfactory protection to the stored grain against seed damage and weight loss due to the weavils. T_1 protected the grains against the weavils with no noticeable weight loss. All the cowpea seeds in the untreated control (T_7) suffered damage 16 weeks after infestation (WAI) whereas only 8% and 0% in the neem cake treatment (T_4) and the neem oil treatment (T_1) respectively were damaged. Damaged caused by the weavils was also significantly ($P < 0.01$) higher in the neem leaf treatment (T_2) and the standard actellic dust treatment (T_6). These treatments (T_2 and T_6) probably enhance the feeding activity of the weavils. The standard actellic dust treatment (T_6) however provided a reasonable level of protection up to 8 WAI only after which seed damage and weight lost become serious with no significant ($P < 0.01$) difference when compared with the untreated control (T_7) 16 WAI.

Table 4.3: Percent Seed Damage to Cowpea Treated with various Neem Materials and Stored for 16 Weeks Following Artificial Infestation with *Callosobrachus maculatus* (Original values in Parenthesis; Analysis done on Data after Arcsine Transformation (0.5 = arcsin $\sqrt{percentage}$

	Treatments	PERCENT SEED DAMAGE Weeks after Infestation (WAI) 8	12	16
T_1	Neem oil (4ml/kg)	(0.0 ± 0.0) 0.0	(0.0 ± 0.0) 0.0	(0.0 ± 0.0) 0.0
T_2	Neem leaf dust (5% w/w)	(23.4± 5.7) 27.3b[Y]	(78.0 ±2.0) 62.0b	(100.0 ± 0.0) 90.0a
T_3	Neem seed dust (5% w/w)	(10.7± 1.3) 18.0c	(31.0 ±2.6) 33.8d	(52.0 ± 5.2) 45.8b
T_4	Neem cake dust (5% w/w)	(0.0 ± 0.0) 0.0	(3.0 ±2.6) 10.0f	(8.0 ± 3.6) 16.4d
T_5	Neem seed residue dust (5% w/w)	(3.1 ± 3.0) 10.0d	(17.0±7.0) 24.4e	(34.0 ± 0.0) 35.7c
T_6	Actellic dust (standard) 15ppm	(9.0 ± 3.0) 17.8c	(49.0 ±2.0) 56.7c	(99.0 ± 1.0) 89.7a
T_7	Untreated control	(37.6 ± 6.6) 37.8a	(100.0 ±0.0) 90.0a	(100.0 ±0.0) 90.0a
	LSD	*0.8*	*1.7*	*1.3*

- Average of 3 replications
- Y within each column; means followed by a common letter do not differ significantly at 1% level.

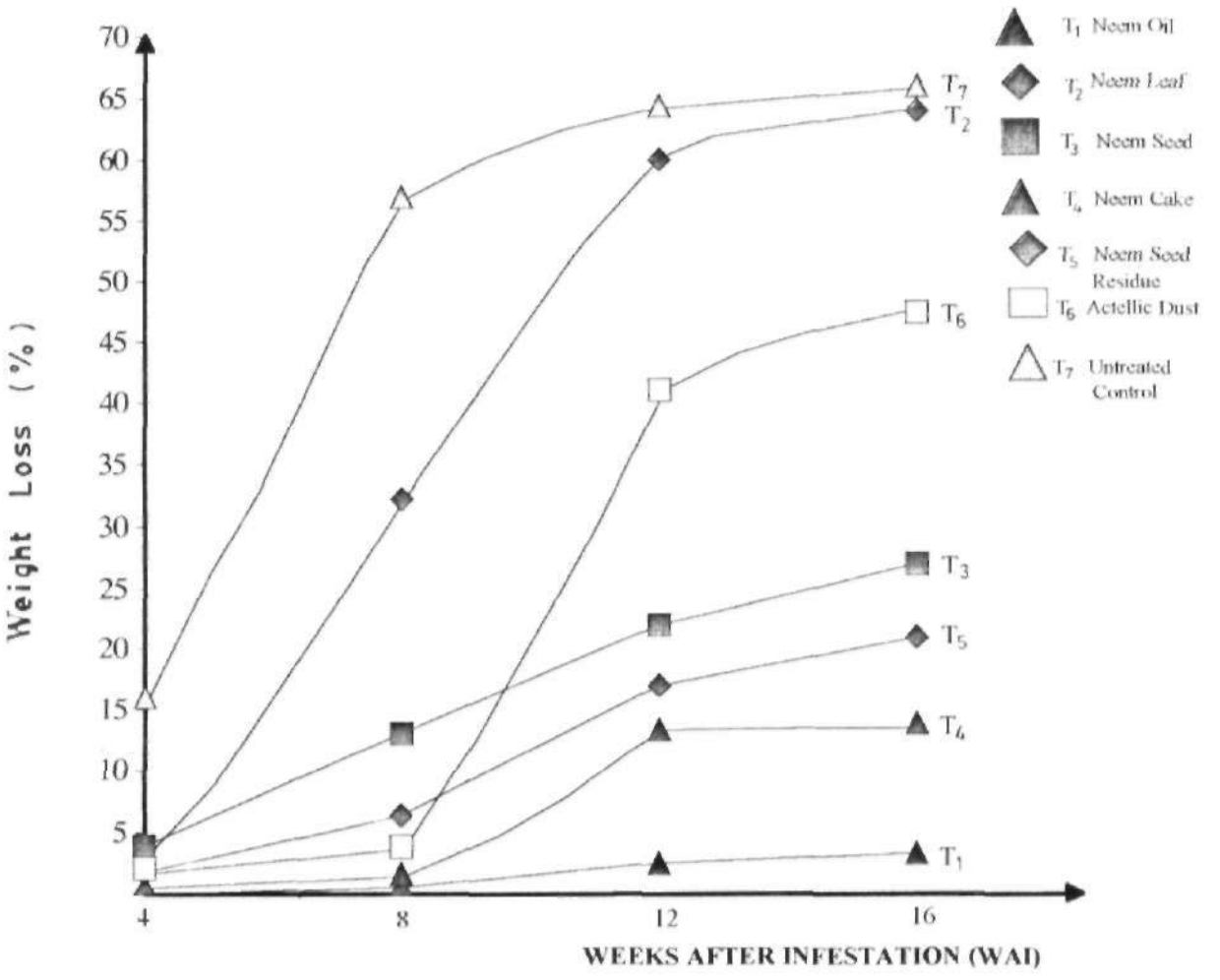


Fig. 4.4 Effect Of Various Neem Formulations On The Percentage Weight Loss Of Cowpea Grains (Number Next To Curve And In Legend Refers To Treatment).

4.2.2 Effect of various Neem treatments on Insect Population and Seed Viability

Table 4.4 shows the population of *Callosobrachus maculatus* and percent seed viability 16 weeks after infestation (WAI).

The population of the weavils were significantly ($P < 0.01$) lower in the oil treatment (T_1) and higher in the untreated control (T_7) and the standard actellic dust treatment (T_6).

The results also indicate that, there was no progeny production or adult survival in grains receiving the oil treatment (T_1) and significantly lower ($P < 0.01$) number of progeny production and survival rate of grains treated with neem cake (T_4) neem seed (T_3) and neem seed residue (T_5) were observed, when compared with the untreated control (T_7) and the standard actellic dust treatment (T_6).
Neem was considered larvicidal to *Callosobrachus maculatus* since upon dissection of the water soften cowpea seeds carrying eggs from which adult did not emerge, dead larvae were found.

The germinative percentage of the seeds showed that, besides the neem oil (95% seed viability) the neem cake (T_4), neem seed residue and neem seed treatments gave a reasonable percentage seed viability (85%, 85% and 65% respectively). Thus, the use of seed treated with these materials does not impair viability.

TABLE 4.4: Effect of Various Neem Formulations on Percentage Seed Viability and Insect Population (*Callosobrachus maculatus*) 16 Weeks after Infestation

	Formulation	Rate (a.i)	Population of (*Callosobrachus maculatus*)	% Seed Viability
T_1	Neem oil	4ml/kg	3(±0)d	95(±0)
T_2	Neem leaf dust	5%w/w	440(±20)b	25(±5)
T_3	Neem seed dust	5%w/w	49(±3)c	65(±5)
T_4	Neem cake dust	5%w/w	36(±4)c	85(±5)
T_5	Neem seed residue dust	5%w/w	38(±3)c	85(±0)
T_6	Actellic dust (standard)	15ppm	565(±25)a	0
T_7	Untreated control		544(±28)a	0
	LSD		24	

Average of 3 replications. In each column means followed by A common letter do not differ significantly at 1% level.

4.2.3 Azadirachtin Levels and Linear Regression Parameters

Table 4.5 shows the level of azadirachtin in the various neem treatments used as protectant of cowpea grains. There are obvious differences in the content of azadirachtin with the neem cake treatment (T_4) having the highest level.

There was no significant ($P < 0.05$ and $P < 0.01$) linear correlation observed between azadirachtin and percent seed damage or weight loss. This could however, be attributed to the presence of neem oil treatment in the data that has a very low azadirachtin content but yet proved best in controlling the weavils. From the estimation equation of azadirachtin and seed damage,

$$Y = 134.50 - 455.50x$$

having 134.50 as the intercept on the Y axis and 455.50 as gradient, the standard error estimate is 24.34. The correlation coefficient (negative) -0.60 means that about $[(100)(-0.60)^2]$ 40% of the seed damage is accounted for by the linear function of azadirachtin in the treatment. The relatively high value of r obtained is also indicative of the closeness between the estimated regression line and the observed points (Fig. 4.5). The linear regression becomes invalid if extrapolated outside the range of the minimum and maximum value of x corresponding to Y as determined from the estimation equation in drawing the regression line.

TABLE 4.5: Relative Concentration of Azadirachtin (mg/ml) of Various Neem Extracts Used in Laboratory Trials (Postharvest Storage Treatments)

	Treatments	Absorbance max 214nm	Concentration of Azadirachtin mg/ml
T_1	Neem oil	0.36	0.022
T_2	Neem leaf dust	0.32	0.019
T_3	Neem seed dust	0.42	0.026
T_4	Neem cake dust	0.47	0.029
T_5	Neem seed resude dust	0.43	0.026

The concentration of the extracts used as treatments were five percent weight per weight (5% w/w) of the neem material.

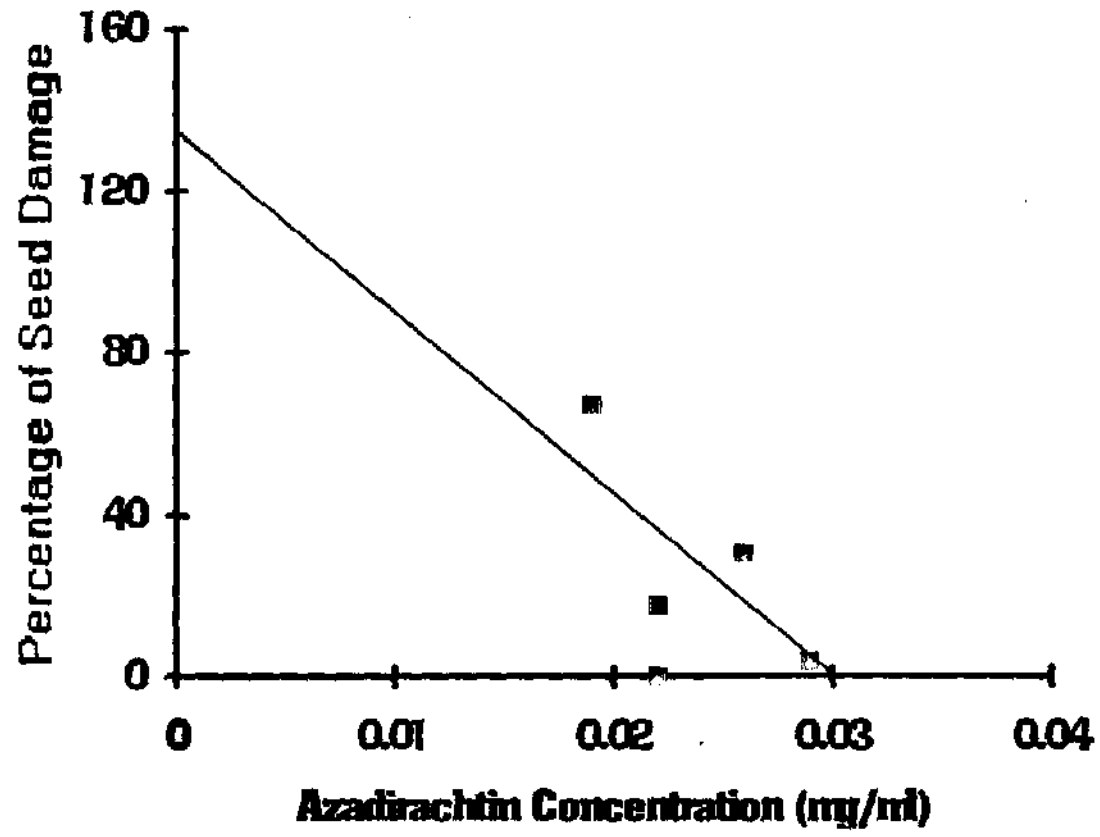


Fig. 4.5
Estimated Linear Regression Between Percentage Seed Damage And Azadirachtin Concentration

CHAPTER FIVE

DISCUSSIONS AND CONCLUSION

5.1 DISCUSSIONS

5.1.1 Antifeedant and the Biochemical Basis of Neem Action

The results from field trials showed that, neem extracts containing azadirachtin controlled the leaf beetle (*Ootheca mutabilis*) and the pod suckers (*Clavigralla tomentosicollis*) quite appreciably. Yield produced from these plots (sprayed with neem extracts) were quite good. This satisfying results of the fields trials gave the neem seed water extract and the neem cake water extract a good chance of being used chance to control the leaf beetle and pod suckers especially when considering the severe problem of resistance with insect pest.

The reduction in foliar damage of the plants treated with neem formulations in spite of the large populations of *Ootheca mutabilis* in these plots could be attributed to the antifeedant effect of the neem formulations, which may be as a result of phagodeterrance or the presence of antinutritional/toxic factors, most probably the two terpenoids: Azadirachtin and salannin. Earlier workers like Schmutterer (1990), and Jackai, (1993) reported similar antifeedant activity on various insects. Also, the suckers displayed initial negative feeding response on the pods, thus manifesting the well known antifeedant properties of neem. Although relatively high population of

suckers were recorded, these formulations gave considerable protection to the pods. Based on our observations and those of previous workers, (Saxena, 1989 and Schmutterer, 1990). The antifeedant and repellant properties of neem most probably prevented the suckers from feeding on the pods by rendering them unattractive to the insects.

Although the results do not attempt to explain the mode of action per se, and in some cases uses chemically obtained extracts (NCME) which are inappropriate for use by small scale farmers, the observed effects probably reflect manifestation of phagodeterrance due to antifeedants or mortality resulting from ingestion of the extracts, or both. In a recent report, Timmins and Reynolds (1992) suggested that azadirachtin inhibits the secretion of trypsin in the midgut of *Manduca sexta*. This may happen in other insects as well.

Antifeedants are generally highly complex molecule. Azadirachtin for example, is structurally similar to the insect hormone called "ecdysone" which controls the process of metamorphosis as the insect pass from the larva, to pupa and then the adult. Similar terpenoids which are antifeedant isolated from various plants have been shown to affect the insect metamorphosis by the compound interfering with the transport of this moulting hormone (Cremyln, 1990). The biochemical basis of the action of this terpenoid on insects is considered to be the disruption of

the moulting process by inhibition of the normal insect cuticle formation. The symptoms of poisoning by this compounds are death of treated larva due to their inability to shed the old cuticle at moulting time, and the distortions of the newly formed larve suggested that the compound impairs the mechanical properties of the new cuticle. Electron micrographs of the newly formed cuticle of the cabbage white caterpillars which have been treated with these compound show the cuticle lacks the lamellar structure of the untreated insect cuticle, and the compound may interefere with the final stage of chitin synthesis namely the polymerization of N-acetylglucosamine but the primary target is not definitely known (Cremyln, 1990). This probably could be the biochemical basis of the action of azadirachtin on insects since they all have the same terpenoid structure.

5.1.2 Rationale for using Neem Oil for Post-harvest Storage and its Relation to Azadirachtin

In the post-harvest storage experiment, excellent effectiveness was obtained with the neem oil and neem cake extracts, against *Callosobrachus maculatus* in preserving the grains. With these findings, the use of neem oil and neem cake extracts for grain preservation seems very promising. Studies from other parts of the world, showed that the range of insect pest that can be controlled by the neem is quite wide (Schmutterer, 1985; Saxena, 1989).

Neem fruit contains 4 main active ingredients (*Azadirachtin, Meliantrol, Nimbin and Salannin*) which bestow on the treated materials repallancy and antifeedant effects against the target insects. The poor performances of the neem leaf treatment (T_2) may thus be attributed to the lower content of the active ingredients and possibly also to the absence of fatty acid since the oil treatment (T_1) effectively controlled the weavils. Also peanut oil has been shown to be capable of penetrating weavil egg and curtailing their development (Singh *et al.*, 1976). It is thus possible that, neem oil exhibits similar properties, hence it's excellent control of the weavils. It is generally believed that oils increase insecticidal activity, this increase in efficacy caused by oils is mediated by increase in penetration into crops by insects and by increasing coverage and persistence (Anderson *et al.*, 1986).

The low value obtained for azadirachtin in the neem oil and its excellent pesticidal effects, could be attributed to its high lipid content and content of the other limonoids. Stark and Walters (1995) showed that neem oil and other oil increases the efficacy of neem insecticides, but a polar component of neem oil also contributed to the increased biological activity of neem insecticides. In studying the azadirachtin content of 12 neem oil samples, Isman *et al.*, (1990) reported a variation of azadirachtin from 50 - 4000ppm with a subsequent variation in bioactivity from 72

- 90%. Therefore, variation in content of azadirachtin observed considering the process of collection, storage and method of preparing the sample for analysis was not unexpected. This is however, reflected in the pesticidal effect, with the cake treatment (T_4) performing better than the others after the oil treatment (T_1).

5.1.3 The Use of Neem in African Agriculture

Neem grows widely in the Africa Savanna and elsewhere in the topics. Presently, widespread use is made of neem as antimalaria drug, so farmers and other likely users are already quite conversant with the plant as a source of medication. Additionally, some farmers already use it to control grasshopper damage on cowpea during dry season in a number of Northern States in Nigeria. Cowpea and many other legumes grown in many developing countries involve application of huge quantities of synthetic insecticides to secure a satisfactory crop yield. The reduction of synthetic insecticide input is very desirable not only for economic but also for ecological reasons. Successfully tested insecticide of plant origin such as neem extracts could help subsistence farmers to improve crop yield with a minimum cash input in the first place. Substitution of synthetic insecticide with neem extracts might however be feasible to a certain degree.

Neem-derived non synthetic insecticide are essentially free

of charge and there is no clear evidence of environmental hazards or health risks associated with the use of these derivatives. There is also much evidence that neem will control storage pest in Africa, and elsewhere despite the fact that its use against storage pest of cowpea is not widespread. This could be because of the lingering "bitter" taste and the rather strong garlic odour (particularly from the crude oil) that results from its use. There is good reasons to believe that, the use of neem in any form to protect cowpea against field pest would be more readily accepted since taste impairment is not going to pose a problem in this case.

So far, there is no recorded incidence of death or abnormal growth that is clearly attributed to the use of neem. Toxicological test carried out for the registration of marganson-O in the U.S. showed among other things that, neem based insecticides (NBIs) are essentially non mutagenic and non toxic to warm blooded animals. Other test with aqueous neem extracts also proved non toxic (Schmutterer, 1990). Since the use of neem in this project is on field crop and also for storage, it is unlikely that there will be any direct oral contamination, even in the unlikely such occurence, there is no known risk to the users.

The physiological effects on insects treated with neem extracts are not likely to be expressed in humans. The relatively weak contact activity and peculiar mode of

action makes NBIs very compartible with the environment. Development of resistance to NBIs have been extremely slow and it has been suggested (Schmutterer, 1990) that this can be further maintained by applying NBIs in combination with other bio-pesticides such as *B. thuringiensis*.

Finally, the procedure described here for the extraction is simple and inexpensive and requires no sophisticated equipments or skills. Every farmer can be taught to do it. Application can be done with a local broom or similar implements for farmers who cannot afford the cost of sprayers. With time, the income level of the farmers will be raised to the point of being able to buy a knapsack sprayer or other type of sprayers and their skills become progressively mechanized at individual or community level as evidence by small scale industries that operate in India and elsewhere in Asia (Saxena, 1989).

5.1.4 The Relevance of Neem in Other Control Strategy

Neem derived formulations obviously cannot be a panacea for the pest problems of tropical crops nor are they intended to be that, even though there are cases where neem based insecticides were as effective or more effective than synthetic insecticide (Schmutterer, 1990). Tropical crops will benefit from any control tactics that can be used and can certainly benefit from the use of insecticides (Ezueh, 1990).

Rather than use highly toxic synthetic insecticide, existing biointensive control measures (plant resistance, crop mixtures and beneficial organisms) can be complemented with what ever benefits can be derived from using neem products. Only low to moderate levels of host plant resistance are presently available in most field pest of cowpea. These levels can be better exploited if combined with a safe and inexpensive application of a natural product such as neem based insecticide (NBIs) in the appropriate crop association and plant configuration. This model will continue to be valid even if higher levels of host plant resistance would become available in the near future. In addition, it would also be possible and in fact desirable to combine the application of NBIs with microbial insecticides and other plant products. Such a mixture will not only widen the scope of activity, and increase the degree of efficacy, but also slow down the development of resistance by pest as they would have to deal with an array of compounds.

In some countries like India, NBIs have been produced and are available in open market (e.g. Repelin(R), Wellgro(R), Nimbosol(R) and Margosan-O(R) in the USA) (Saxena, 1989; Schmutterer, 1990). Though concern are being raised that it might pose risk as synthetic insecticides, the synthesis of the active principles of neem appears to be very difficult, without question, the use of this NBIs would be much more expensive than direct preparations by the

farmers. The tree grows everywhere, and no ownership problem exist at present, but this could change very quickly, if the use of neem become very popular and widespread. Presently, there are enough trees to go around, most of them grossely underutilized. Therefore, rather than encourage the synthesis of NBIs (despite the relevance in other agricultural system) the production of NBIs and their use in tropical pest management can be maintained at the level of village and community preparation.

5.2 CONCLUSION AND RECOMMENDATIONS

The results from this studies show clearly that, there exist a relationship between azadirachtin and pesticidal effects. Also, differences exist in pesticidal effects of neem extracts that contain azadirachtin, these extracts, compare favourably with their synthetic counterparts in both field and laboratory trials.

In view of the potentially vast amounts of cake that can be generated from the oil extraction of the seed and the secondary uses to which it can be applied (insecticide, nematicide, fertilizer and soil drench, etc) the use of neem ought to be given greater prominence than has hitherto been the case in tropical pest management.

As the use of neem is becoming widespread, it is necessary to conduct detailed studies to find out what component(s) of neem oil is (are) responsible for the excellent activity observed in the laboratory trials. If it is azadirachtin as we hypothesize, does the neem oil actually increase the penetration of azadirachtin?, or are some other mechanisms involved?.

It is also necessary to conduct detailed studies, on the possible long term effect of exposure to neem products, though the likely end users are also the same persons who use neem to treat malaria.

Finally, neem research has reached a stage of practical feasibility, further practice oriented trials should be carried out to support research findings and enable the rapid introduction of neem insecticides to farmers level, since the use of its extracts are economical, readily available, safe to use, and non-toxic to natural enemies of the pest.

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APPENDIX 1A: Data from Field Trials

Population of (*Clavigralla tomentosicollis*) Receiving Various Neem Formulations

Weeks after planting (WAP)

	8		9		10		11		12		XX
		X		X		X		X		X	
T_1	0, 2, 2	1.7	1, 0, 2	1	0, 0, 2	0.6	1, 2, 0	1	1, 0, 6	0.3	1.32
T_2	2, 2, 2	2	2, 2, 2	2.3	3, 2, 2	2.3	2, 2, 2	2	1, 1, 2	1.3	1.98
T_3	3, 2, 2	2.3	2, 3, 3	2.7	2, 3, 2	2.3	1, 2, 2	1.7	1, 2, 2	1.7	2.14
T_4	2, 3, 3	2.7	3, 3, 3	3	3, 3, 3	3	3, 3, 2	2.7	2, 2, 2	2	2.68
T_5	3, 0, 0	1	1, 2, 0	1	0, 0, 1	0.3	1, 1, 1	1	0, 0, 1	0.3	0.72
T_6	5, 3, 3	3.7	8, 6, 4	6	6, 3, 4	4.3	3, 3, 3	3	3, 3, 3	3	4.2
T_7	1, 2, 0	1	3, 0, 0	1	4, 0, 0	1.3	0, 1, 0	0.3	0, 0, 2	0.6	1.1

X = mean

XX = Grand mean

APPENDIX 1B: Leaf Damage Rating Caused by (*Ootheca mutabilis*) Receiving Various Neem Formulations

Weeks after planting (WAP)

	3		4		5		6		7		8		XX
		X		X		X		X		X		X	
T_1	3, 3, 3. 3	**3.1**	3, 3, 3. 3	**3.1**	0, 4.5, 0	**1.5**	2, 2.7	**1.9**	3.6, 0, 0	**1.2**	3, 2.1, 0	**1.7**	**2.1**
T_2	3, 4, 5.3	**4.1**	4, 3.7, 4	**3.8**	0, 3, 3.3	**2.0**	0, 3, 3	**2.0**	4.2, 0, 0	**1.4**	2.2, 2.6,0	**1.6**	**2.48**
T_3	4, 3.7, 4	**3.9**	3, 3, 3.9	**3.3**	2, 2.8, 0	**2.1**	0, 4.6, 2	**2.2**	2.2, 2.6,0	**1.6**	4, 4.4, 0	**2.8**	**2.48**
T_4	4, 4, 4.6	**4.2**	4, 4, 4.3	**4.1**	4.8, 3, 0	**2.6**	4.1, 4, 0	**2.7**	2.4, 3, 0	**1.8**	0, 3, 3.3	**2.1**	**2.9**
T_5	3, 3, 4.8	**3.6**	3, 3, 3.3	**3.1**	2, 2.6, 0	**1.6**	3, 2.1, 0	**1.7**	3.3, 0, 0	**1.1**	3.6, 0, 0	**1.2**	**2.05**
T_6	7, 6, 5.9	**6.3**	7, 6, 4.9	**5.9**	5, 5, 6.8	**5.6**	5, 5, 5.3	**5.1**	5, 5, 5.3	**5.1**	6, 7, 4.1	**5.7**	**5.6**
T_7	3, 3.5,4	**3.5**	4, 3, 3.2	**3.4**	0, 4, 3.2	**2.4**	3, 3.9,0	**2.3**	2.4, 3, 0	**1.8**	2, 2, 2	**2.0**	**2.5**

X = mean

XX = Grand mean

APPENDIX 2A: Results from Post-harvest Storage Experiments

Data for Percent Weight Loss

Weeks after infestation (WAI)

	4				8				12				16				
	R_1	R_2	R_3	X	R_1	R_2	R_3	X	R_1	R_2	R_3	X	R_1	R_2	R_3	X	XX
T_1	0	0	0	**0**	0,	0,	1.8	**0.6**	2,	2,	3.8	**2.6**	3,	3,	4.2	**3.4**	**1.65**
T_2	3,	3,	3	**3.0**	35,	34,	30.9	**32.2**	65,	60,	55	**60**	65,	70,	57	**64**	**40**
T_3	0,	5,	6.7	**3.9**	13,	13,	13	**13**	20,	24,	22	**22**	30,	27,	24	**27**	**16.5**
T_4	0,	0,	1.2	**0.4**	0,	0,	5.1	**1.7**	10,	10,	20.2	**13.4**	12,	12,	17.7	**13.9**	**7.35**
T_5	0,	0,	5.4	**1.8**	5,	4,	10.2	**6.4**	17,	19,	15	**17**	20,	20,	23	**21**	**11.5**
T_6	0,	0,	6.3	**2.1**	10,	5,	6.7	**3.9**	40,	40,	43	**41.0**	45,	45,	52.5	**47.4**	**26.4**
T_7	15,	14,	19	**16.0**	60,	60,	59	**56.9**	62,	64,	66	**64.4**	65,	66,	67	**66.0**	**56.8**

X = mean

XX = Grand mean

APPENDIX 2B: Data for Percent Seed Damage, Seed Viability and Insect Population

Weeks after infestation (WAI)

	8			12			16			Insect Population (*C. maculatus*)			% Seed Viability			
	R_1	R_2	R_3	X	R_1	R_2	R_3	X	R_1	R_2	R_3	X	XX	R_1	R_2	R_3	X	R_1	R_2	R_3	X
T_1	0,	0,	0	**0**	0,	0,	0	**0**	0,	0,	0	**0**	**0**	3,	3,	3	**3**	95,	95,	95	**95**
T_2	30,	20,	20.2	**23.4**	76,	78,	80	**78**	100,	100,	100	**100**	**67**	460,	440,	420	**440**	20,	30,	25	**25**
T_3	10,	10,	12.2	**10.7**	29,	30,	34	**31**	49,	49,	58	**52**	**31**	52,	50,	46	**49**	60,	65,	70	**65**
T_4	0,	0,	0	**0**	5,	0,	4	**3**	12,	5,	7	**8**	**3.6**	34,	33,	40	**36**	90,	85,	80	**85**
T_5	3.3,	0,	6	**3.1**	17,	10,	24	**17**	34,	34,	34	**34**	**18.0**	40,	35,	40	**38**	85,	85,	85	**85**
T_6	6,	12,	9	**9**	49,	51,	4.7	**49**	98,	99,	100	**99**	**50.3**	542,	592,	560	**544**	0,	0,	0	**0**
T_7	40.8,	30,	42	**37.6**	100,	100,	100	**100**	100,	100,	100	**100**	**79.2**	576,	532,	524	**544**	0,	0,	0	**0**

X = mean

XX = Grand mean

APPENDIX 3

To illustrate the procedure for analysis of variance (ANOVA) and least significant differences (LSD) test for data from a randomized complete block using insect population (*C. maculatus*)

	R_1	R_2	R_3	Σx	X
T_1	3	3	3	12	3
T_2	460	440	420	1220	440
T_3	52	50	46	148	49
T_4	34	33	40	107	36
T_5	40	35	40	115	38
T_6	542	592	560	1694	565
T_7	516	592	560	1632	544
Σx	1707	1685	1633	5024	

$\Rightarrow$ Correction factor (C.F) $= \frac{G^2}{rt}$ r = replication

t = treatment

$= \frac{(5025)^2}{21}$

$= \underline{1202410.7}$

$\Rightarrow$ Total SS $= \sum_{i=1} \sum_{j=1} X - C.F$

$= [3^2 + 460^2 + \ldots 560^2] - 1202410.7$

$= \underline{1242010}$

$\Rightarrow$ Replication SS $= \sum_{j=} R^2j - C.F$

$= \frac{1707^2 + \ldots + 1633^2}{7} - 1202410.7$

$= \underline{412.5}$

$\Rightarrow$ Treatment SS $= \sum_{i=} T^2_i - CF$

$= \frac{(12^2 + \ldots + 1632^2)}{3} - 1202410.7$

$= \underline{1238616}$

⇒ ERROR SS = Total SS - Rep SS - Tret SS
= 1242010 - 412.5 - 1238136
= 3286

⇒ Replication MS = $\frac{Replication\ SS}{r-1} = \frac{4125}{2} = 206$

⇒ Treatment MS = $\frac{Treatment\ SS}{t-1} = \frac{1238316}{6}$ 206386

⇒ ERROR MS = $\frac{ERROR\ SS}{(r-1)(t-1)} = \frac{3282}{12} = 274$

⇒ Computed F value for testing treatment = $\frac{Treatment\ MS}{ERROR\ MS} = \frac{206386}{274} = 735$

Construction of Table

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F	Table F	
					5%	1%
Replication	2	412	206	0.75ns	3.82	6.93
Treatment	6	1238316	206386	735**	3.00	4.82
ERROR	12	3282	274			
Total	20	1242010	62100			

ns = not significant, ** highly significant

⇒ LSD = tα Sd = $\sqrt{\frac{2 \times EMS}{Rep}}$

1.78

1.782 x $\sqrt{\frac{2 \times 274}{3}}$

LSD = 24

⇒

Arrange means in decreasing order

564 544 440 49 38 36 3

⇒ Compare each with LSD and take decision

$\underset{a}{\underline{564 \quad 544}}$ $\underset{b}{\underline{440}}$ $\underset{c}{\underline{49 \quad 38 \quad 36}}$ $\underset{d}{\underline{3}}$

⇒ Means with similar letter do not differ significantly at 1% level.

APPENDIX 4A: Yield from Cowpea Plots after Application of various Neem Extracts

Yield in kg/plot

	R_1	R_2	R_3	Σx	X	$\pm$ SE
T_1	1.1	0.98	1.0	3.08	**1.02**	0.064
T_2	0.85	0.87	0.84	2.56	**0.85**	0.015
T_3	0.88	0.70	0.68	2.26	**0.75**	0.11
T_4	0.68	0.70	0.58	1.96	**0.65**	0.06
T_5	1.18	0.93	0.78	2.9	**0.96**	0.2
T_6	0.37	0.35	0.40	1.12	**0.35**	0.2
T_7	0.90	0.85	0.99	2.74	**0.91**	0.1

APPENDIX 4B: Relative Concentration of Azadirachtin of various Neem Materials used in Field and Laboratory Trials

	Post-harvest Storage Experiment	Absorbance max at 214nm	Relative Conc. in mg/ml
T_1	Neem oil	0.42	0.026
T_2	Neem leaf	0.32	0.019
T_3	Neem seed	0.36	0.022
T_4	Neem cake	0.27	0.017
T_5	Neem seed residue	0.47	0.029

	Field Trials		
T_1	Neem seed water extract	0.36	0.022
T_2	Neem leaf water extract	0.32	0.019
T_3	Neem oil emulsified extract	0.42	0.026
T_4	Neem cake methanol extract	0.47	0.029
T_5	Neem cake water extract	0.43	0.020

APPENDIX 5: Linear Regression Parameters between Azadirachtin and Pest Effects Measured in Field Trials

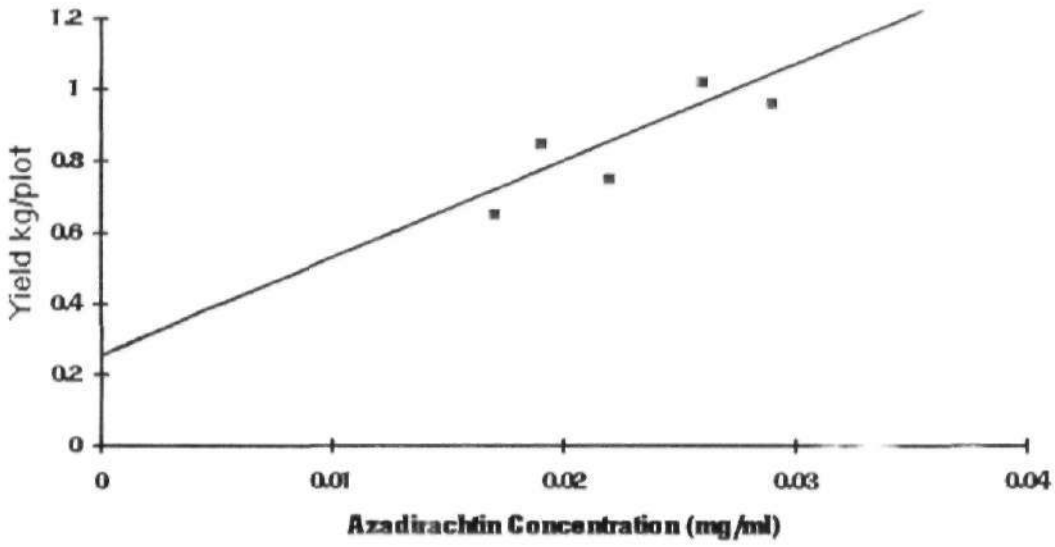
LINEAR REGRESSION DATA

	Parameters Measured	Intercept on Y-axis a	Slope b	Correlation coefficient r	Standard errod estimate S x Y	Estimation equation Y = a+bx
1	Azadirachtin and leaf damage	3.94	-6.78	-0.96*	0.13	Y=3.94 - 6.78x
2	Azadirachtin and insect population	5.20	-15.10	-0.97**	0.23	Y=5.20 - 15.10x
3	Azadirachtin and yield	0.23	-2.25	0.86*	0.10	Y=0.23 - 2.25x
4	Yield and leaf damage	4.24	-2.17	-0.95*	0.12	Y=4.24 - 2.17x
5	Yield and insect population	5.59	-4.52	-0.90*	0.39	Y=5.59 - 4.52x
6	Leaf damage and insect population	-3.31	2.11	0.96**	0.25	Y= -3.31 + 2.11x

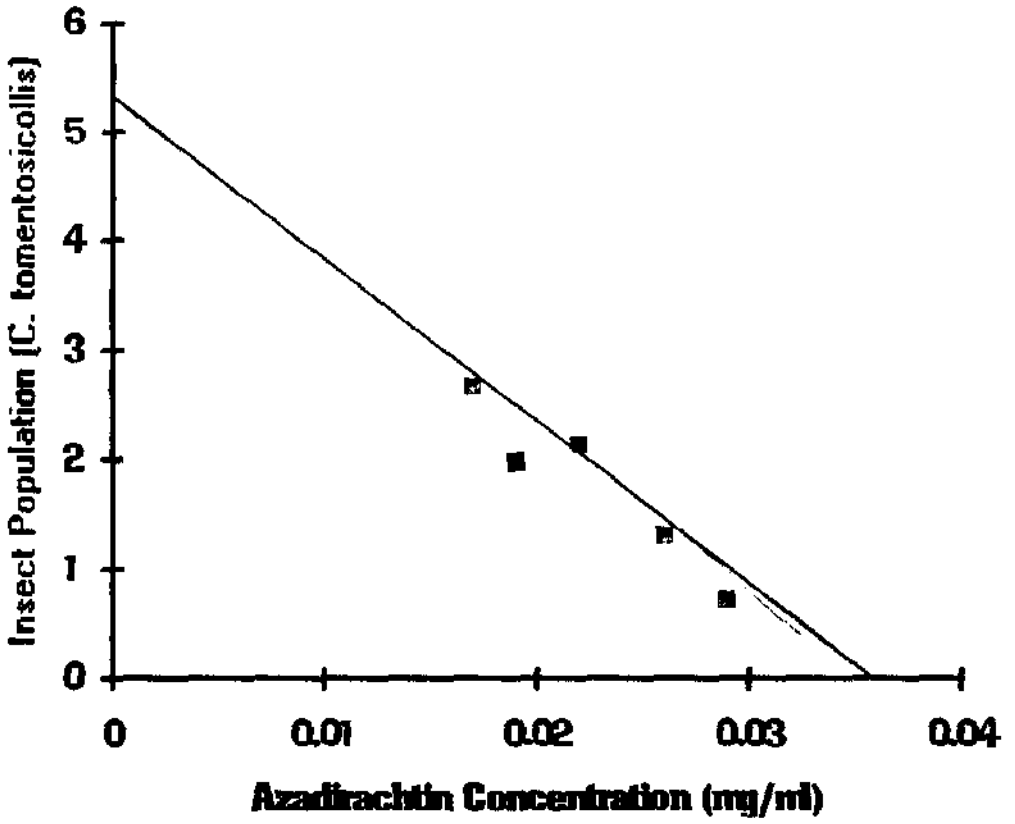
* = Significant at 5%

** = Significant at 1%

Insect population is suckers (*Clavigralla tomentosicollis*)



Estimated Linear Regression Between Yield And Azadirachtin Concentration

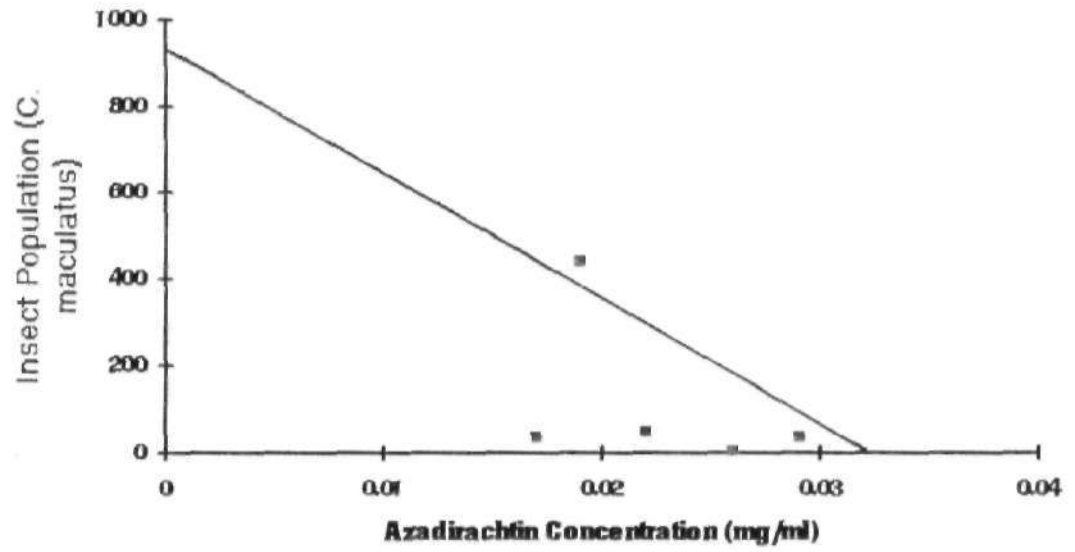


Estimated Linear Regression Between Insect Population (Pod Suckers) And Azadirachtin Concentration

APPENDIX 8: Linear Regression Parameters between Azadirachtin and Pest Effect Measured in Laboratory Trials for Postharvest Storage

LINEAR REGRESSION DATA

	Parameters Measured	Intercept on Y-axis a	Slope b	Correlation coefficient r	Standard error estimate S x Y	Estimation equation Y = a+bx
1	Azadirachtin and seed damage	134.50	-455.50	0.60^{ns}	24.34	Y=134.50-455.50x
2	Azadirachtin and weight loss	71.70	-229.25	0.60^{ns}	14.68	Y=71.70-229.20x
3	Azadirachtin and *insect population*	965.10	-3473.77	0.69^{ns}	154.20	Y=965.10-3473.77x
4	weight loss and insect population	-69.72	11.80	0.98^{**}	61.00	Y=69.72+11.80x
5	Seed damage and insect population	-36.18	6.23	0.92^{*}	83.32	Y=36.18+6.23x
6	Seed damage and weight loss	-38.90	-1.810	0.99^{**}	4.67	Y=-38.9-1.81x



Estimated Linear Regression Between Insect (C. maculatus) And Azadirachtin Concentration