EVALUATION OF NEEM (*AZADIRACHTA INDICA*) SEED EXTRACTS FOR THE CONTROL OF MEALY BUG (*DYSMICOCCUS* SPP.) AND MEALY BUG ASSOCIATED PINEAPPLE (*ANANAS COMOSUS*) WILT.

BY

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DECLARATION

I hereby declare that, except for references to work of other researches which have been duly cited, this thesis consist entirely of my original research work carried out at Greenspan Farms and University of Ghana, and that no part of it has been presented for another degree elsewhere.

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ABSTRACT

Mealybug associated pineapple wilt is a major constraint to pineapple production in Ghana. Aqueous and methanolic neem seed extracts and a standard insecticide, dimethoate were evaluated for their efficacy against the pineapple mealybug in laboratory, screen house and field studies. The pineapple cultivar used was Smooth Cayenne. In the screen house treated suckers were planted in plastic pots filled with soil. Destructive sampling techniques were applied to obtain the number of live mealybugs at two weekly intervals for 10 weeks. In the screen house experiment, methanolic neem seed extract at the highest dosage (6 g/l) was found to be most effective treatment against the pineapple mealybugs.

In the field, dimethoate and methanolic neem extract were equally effective in reducing the mealybug population than the control. The population of mealybugs was reduced to almost zero in dimethoate and methanolic neem extract treated plants. The extract also promoted healthy leaf development, reduced wilting and rotting of pineapple roots.

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CHAPTER ONE

1.0 INTRODUCTION

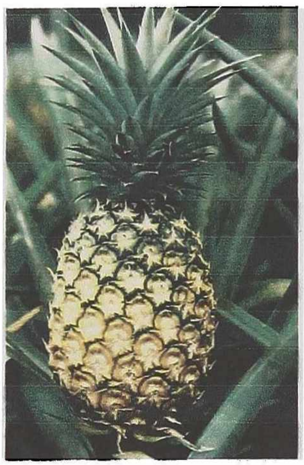
Pineapple (*Ananas comosus* (L.) Merr) is grown for fresh fruits. The plant was introduced to West Africa in 1602 (Vickery and Brian, 1979) and most of the commercial crops of pineapple are canned. Sugar-syrup, alcohol and citric acid may also be obtained from the juice. Besides canned slices, pieces and juices, pineapples are also made into jam, chutnuy, crystallized or glace fruits. Fresh pineapples contain about 14% sugar, 0.3% protein and vitamins A, B and C (Vickery and Brain, 1979). Fruit residue after the extraction of the juice may also be made into bran for use as cattle feed. The leaves yield about 2 - 3% white silk fibre, 38 - 90cm in length, which is used for cordage (Vickery Brain, 1979).

The plant is essentially a xerophytes and will extended period of drought. Uniform moisture is good for growth and economic production; however, excess moisture can result in total loss of the root system if pathogens are present. The crop is vegetatively propagated by crowns, butts, slips or suckers. The rosette long and narrow leaves channel any water falling on the plant to the roots.

Generally, the following cultivars are grown for commercial purposes: Queen Victoria, Smooth Cayenne, Sugar Loaf and other Hybrid Varieties (Plate I).
In Ghana, the cultivar mostly grown for export in "Smooth Cayenne". Despite high export earnings, production of pineapple drinks, cordage, fibre and fresh fruits, the cultivation of the crop faces several problems. Among the major constraints are disease and pest damage to both the plant and the fruit whilst mealybugs feed on the plant causing wilt (Plate IV), crickets and locust feed on the bracts of the fruit (Plate II).

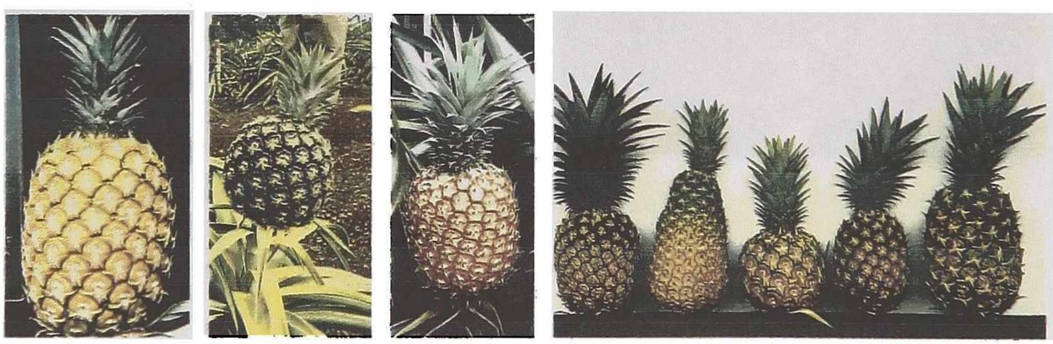
Mealybug wilt disease appears to have viral etiology (Sanford, 1988) and is transmitted by mealybugs. The mealybug species associated with the diseases are *Pseudococcus brevipes. Dysmicoccus brevipes* and *Dysmicoccus neobrevipes* (Plate III) (Ito, 1938; Carter, 1973).

Principal varieties commercialized



Smooth Cayenne.

Queen Victoria.



Hybrids under selection

Plate I : Some pineapple varieties

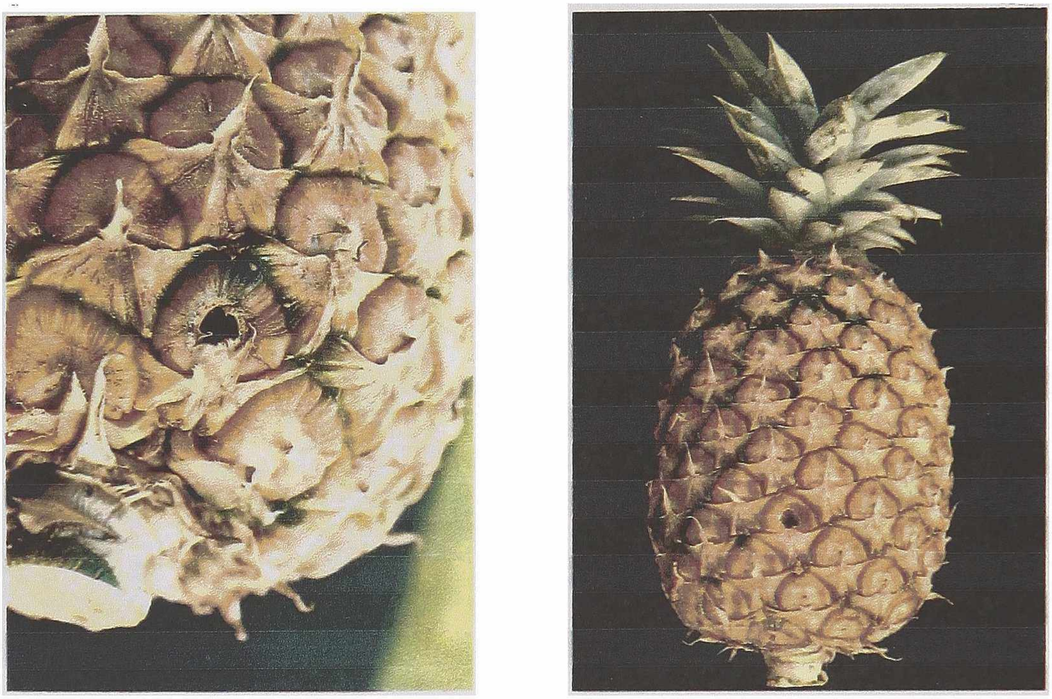


Plate II : Bract of fruits gnawed by Crickets

Mealy bug: *Dysmicoccus brevipes*

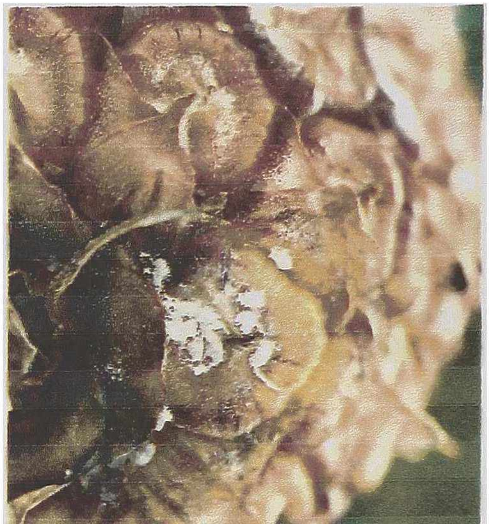


Plate III : Fruits infested by Dysmicoccus brevipes

Plate IV : Healthy and wilted plants with fruits

Plate V : A healthy smooth Cayenne sucker

Plate VI : A wilted smooth Cayenne sucker

The disease causes cessation of root growth followed by wilting of the leaves (Carter, 1935). It was first described in Hawaii in the early 1900's and is the damaging disease particularly to the predominant cultivar, "Smooth Cayenne" (Collins, 1960). Mealybug wilt severely limits fruit quality and yield of pineapple production. Currently in Ghana, the disease is gradually assuming alarming proportions with its incidence in virtually every commercial pineapple plantation. Estimates on incidences rates are not available but it is believed to vary between 5% to 80% (Owusu Bennoah, Personal communication, 1998.)

In order to control the activities of the mealybugs and the attendent ants, *Solonopsis germinata* Forel and *Pheidole megacephala* (Fabr), synthetic insecticides such as dimethoate, perfecthion, dursban, terminus, diazinon and pyrenex are applied at various stages of development of the plant and its fruits. The indiscriminate use of these synthetic insecticides has its own associated problems including the development of resistance, elimination of beneficial insects, resurgence of both major and potential pests, environmental contamination, high cost and human health hazards (Metcalf, 1980). It is, therefore, necessary to find alternative substances, which have insecticidal properties, but with comparatively fewer side effects and hence are socio-economically viable to replace the rather toxic synthetic insecticide (Jackai *et al.*, 1992).

Substances that have found promising for use as insect control agents on food crops and vegetables include extract from the neem plant, *Azadirachta indica* A Juss. (Schmutterer and Hellpap, 1989). The seed extract of neem has been found to have systemic activity and is active at low concentration with negligible mammalian toxicity (Lowery *et al.*, 1993). The extract demonstrates diverse behavioural and physiological effects on insects including repellency, interference with oviposition, feeding deterrence, growth disruption, interference with development and reproduction (Schmutterer, 1990). Neem extract has been tested on the mealybug *Planococcus citri* Risso (Planococcidae) on coffee in India (Kumar, 1985) and on the variegated grasshopper, *Zonocerus variagatus* L. (Pyrgomorphidae), the Legume pod borer, *Maruca testuraris* Geyer; and the cowpea coried bug, *Clavigralla tomentosicollis,*Stal (Olaifa *et al.*, 1991). Additionally, insecticidal activity of neem has been noted against aphids (Goyal *et al.*, 1971) and *Ephestia kuehniella* (Sharma *et al.*, 1980). Indeed neem affects over 200 species of insects worldwide (Schmutterer, 1990). Neem has also shown activity against nematodes

(Egunjobi and Afolami, 1976), fungi (Kher and Chaurasia, 1977) and viruses (Rai and Sethi, 1972).

The bioactive compounds of neem are terpenes, one of which is azadirachtin. This terpeniod was first isolated from neem seed (Butterworth and Morgan, 1971). It was found that even at concentrations as low as 4g/l, the compound prevented *Schistocerca gregaria* Forskal from feeding.

The main objective of the present study was to evaluate the potential of neem seed extracts (NSEs) for the control of mealybugs and mealybug associated wilt in pineapple.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of the crop

Pineapple is a strictly tropical plant, and thrives best in areas where annual temperature is between 25 and 32 °C, rainfall is 100 – 150 cm and the humidity is high. The plant grows on a wide range of soils but a well drained, slightly acidic, sandy loam is desirable.

The pineapple plant is a short-lived perennial with a short main axis, forming a rosette of leaves (Kenneth *et al.,* 1987). In the first year of growth the axis lengthens and thickens and bears numerous leaves in close-spirals. The leaves are dark green on the upper side and silvery-white on the lower side. They are fleshy, trough-like and grooved with sharp spines along the margins (Kenneth *et al.,* 1987). After 20 months the stem grows out into a spike-like inflorescence of 100 or more spirally arranged, trimerous flowers each subtended by a bract. 'Smooth Cayenne' has light purple flowers with red bracts, while other cultivars are lavender-coloured. The flowers consist of three sepals, three petals, six stamens, and three fused inferior ovaries with three branched stigmas (Kenneth *et al.,* 1987). The ovaries develop into fruitless (berries), parthenocarpically. These together with the fleshy axis of the inflorescence and the bracts, undergo coalescence, resulting in a compact, multiple, accessory fruit which is botanically called 'serosis' (Kenneth *et al.,* 1987).

The inflorescence grows to form the 'crown' found on top of the fruit. 'Slips' are side shoots that arise in the leaf axils beneath the fruit while 'suckers' develop from the axillary buds near the base of the stem.

In Ghana, the "Smooth Cayenne" cultivar is the major variety cultivated for export. Pineapple production in Ghana is centred in the Eastern region and parts of Central region due to the high humidity that persists in those areas. The crop is grown as a monoculture and it is among the major foreign exchange earning crop. Despite its contributions to the economy, pests and diseases hinder production. Crickets, locusts and other insects may gnaw the bracts of the fruit before harvest and leave hole-shaped

scars, which reduce the market value of the fruit (Plate II). Plant-parasitic nematodes also constitute a major limitation to roots development because the roots do not re-generate when damaged. Wherever pineapple is grown, mealybug wilt can also be a major problem. The disease is associated with the gray pineapple mealybug. *D. neobrevipes* Beardsley and the pink mealybug, *D. brevipes* Cockerel (Carter, 1963). Other diseases of pineapple include heart rot of the fruit, which is caused by *phytophthora parasitica* Dastur, P. *cinnamomi* Rands or *P. palmivora* Butler, and root rot is generally caused by *P.cinnamomi* or *Pythium arrhenomanes* Drechs (Carter, 1963). Black and brownrots of the fruit have also been found to be a problem.

2.2 Mealybug wilt of pineapple

The disease is unique among the phytotoximias and is most complex because studies had led to a reappraisal of the diseases to include the concept of a latent factor which is transmissible by mealybugs but is separate and distinct from the actual wilt inducing secretion (Larson, 1960). Larson used the word "wilt" in a generic sense to include all wilting conditions brought about by death of roots.

The disease occurs practically in all tropical areas of the world where pineapples are grown commercially. Carter (1933, 1956) reported the incidence of the disease in Africa, Central and South America, the Caribbean areas, Ceylon, Malaysia, Australia and Fiji.

2.2.1 Symptoms and aetiology of mealybug wilt of pineapple

The consistent association of mealybugs with the diseases earned it the name Mealybug Wilt of Pineapple. It was postulated to be caused by a toxin secreted by mealybug feeding on pineapple plants Carter (1956). Field symptoms of mealybug wilt had been designed into four stages (Carter, 1956). The first stage starts with a reddening of the leaves and a slight inward reflexing of the leaf margins. In stage two, definite colour changes from red to pink occur and in stage three the reflexing of the leaves become pronounced. Finally in the stage 4 the tips of affected leaves dry up and in severe cases, the plant becomes moribund (Plate VI).

The mealybug wilt syndrome, however, clearly included root collapse, and wilted plants invariably have poor roots (Carter, 1948). It was observed that the first symptom is shown to be the cessation of elongation of roots, followed by collapse of the entire root system before leaf symptoms develop. Carter also indicated that the period for the development of symptoms varies with the age of the plant at time of infestation and indicated reported that symptom severity and relative recovery are affected by the frequency of infestation.

The expression of symptoms as described above, refers to symptoms in Smooth Cayenne, the commercial variety in Hawaii, Costa Rica, Ivory Coast and Ghana. However, symptom expression may vary with variety and hybrids (Carter and Collins, 1947). Singh (1984) described the characteristics of early symptoms as drying and wilting of mature leaves beginning at the leaf tips and the appearance of a reddish-yellow colour. Their work indicated that at later stages, the central whorl of affected plants losses turgor as the leaf tips become brown and curl back tightly. If the plants become affected early, no fruit is formed and root development is retarded. Carter (1960) reported that plants wilted a second time in a considerable number of cases following re-infestationby mealybugs after recovery had taken place. It was noted that control by application of insecticides reduced mealybug populations at low levels but wilt once more develops if these same low populations became attended by ants and increase in size.

Carter (1951) provided data that called for a modification of the 'toxin hypothesis'' that some field-grown pineapple plants are negative sources for mealybug wilt. That is, mealybugs fed on them do not wilt the next plants on which they feed, and that pineapple seedlings not previously infested with mealybugs are also negative sources.

Carter (1962) establishment the fact that a latent factor is transmitted through vegetative reproduction. Carter (Carter, 1962) considered possible relationship between latent factor and the inherent toxicity of the insect's secretions and accepted the hypothesis that this toxicity provides a stress factor which aids in the establishment, concentration, and distribution of the latent factor in the infected plant, but rejected the hypothesis that the latent virus is activated by the insects secretion to become the aetiological agent of mealybug wilt. Instead, evidence was presented supporting the hypothesis that the actual wilt-inducing secretion is a toxin, synthesized by the insect when feeding on a plant infested with the factor.

Carter (1962) reported that there was the existence of a transmissible latent factor essential to the positive source value. It was stated that the latent factor is separate and distinct from the actual wilting secreting since positive source value develops without occurring. Although, the aetiology of mealybug wilt of pineapple remains unknown, a biological agent transmissible by mealybugs, has been implicated as the cause of this devastating disease (Ito, 1962). A long, flexuous, rod-shaped virus has been isolated from pineapple (cultivar: Smooth Cayenne) with symptom of mealybug wilt (Gunasinghe and German, 1989). Based on virus morphology, molecular weight of the coat protein, (23,000 Da) and the approximate size of the genomic RNA, the pineapple virus was assigned tentatively to type II closterovirus group (Gunasigbe and German, 1989). This closterovirus particle was detected serologically in cultivated pineapple (Smooth Cayenne) throughout the Hawaiian Islands (Ullman *et al.*, 1989). It remains to be proven whether the detection of such closterovirus particle is of aetiological to be proven ~~whether the detection of such~~ closterovirus particale is of aetiological significant.

2.2.2 Mealybugs and ants associated with pineapple

Wherever pineapple is grown, mealybug wilt can be a problem. This disease is associated with the gray pineapple mealybug *D. brevipes* Beardsley (Coccidae) and the pink pineapple mealybug, *D. brevipes* Cockerel (Coccidae) Beardsley *et al.*, 1982; Carter, 1963). Bearsley *et al.* (1982) reported that mealybug wilt was a problem in Hawaii because of excellent control of the ants that attend the natural enemies of the mealybug. Three species of ants in the family, Formicidae are important in Hawaii the big-headed ant *Pheidole megacephala* Fabricius, the Argentine ant, *Irodomyrmex humilis* Mayr and the fire ant, *Solenopsis germinata* Fabricius (Beardsley *et al.*, 1982). Beardsley *et al.* (1982) suggested that in Hawaiian plantations, fields where mealybug wilt was essentially eradicated, control can be maintained by ant surveillance and application of control agents on field borders only, since ants are generally responsible for movement of mealybugs into the fields.

2.2.2.1 Relationship between the mealybugs and the ants

Phylips (1934), in a study of ants in pineapple fields traced the influx of ants into the fields, finding intense activity and nest building on the edge and where the mealybug populations were highest; a steady thinning-out of activity farther on, roughly coincided with size of mealybug colonies. It was reported that *D. neobrevipes* (Coccidae) appears to be completely dependent on the activity of ants for its vigorous growth and reproduction, but not all ant species fulfil the mealybug's needs in this respect equally. *S. germinata*, *P. megacephala* Fabricius and the Argentine ant, *I. humilis* Mayr can occasionally build huge nests around colonies of mealybugs on scattered pineapple plants, but these colonies are of *D. brevipes* (Coccidae) (Beardsley *et al.*, 1982).

2.2.3 Epidemiology of mealybug wilt

Corbett and Pagden (1941) compared the prevalence of wilt in pineapple grown in lateric mineral soils to peat soil and recommended that new planting be restricted to peat soils and this was confirmed by order in Council and has been the policy in Malaysia. These authors reported that the factors affecting field incidence of pineapple wilt include soil, agronomy and pineapple variety and therefore healthy plants in Malaysia were to be found principally in virgin, peat soil areas.

Carter (1942) noted that in pineapple growing on the fringe of the plant's geographic economic range, low temperature during winter reduced the size of mealybug colonies and heavy rains also reduced leaf populations of mealybugs and favoured the establishment of subsurface colonies. These factors minimized wilt incidence. In 1938, plantings in Kenya showed only small and scattered colonies of mealybugs on small plantings on virgin land but with the use of old sisal land for pineapple planting wilt increased considerably in 1956 (Carter, 1960). Furthermore, it was indicated that the incidence of scattered wilt in an otherwise well controlled field could well be due to pockets of fungus infection, which might attack roots, weakened by mealybug feeding.

In Hawaii, Carter (1932) observed that mealybug infestation appeared to start from the outer edges of pineapple fields and gradually work in towards the centre. It was shown that there were scattered infestations in-field block, but heavy infestations occurred at short distance from the edge of fields. It was further stated that infestation occurred much more rapidly and was much greater in a new field contiguous to an old infested one than when the edge of the field was bordered by wild grasses and shrubs.

2.2.4 Other toxic effects of mealybug feeding on pineapple

Apart from the specific feeding which leads to mealybug wilt, the toxic secretions of the species of mealybugs *(D. brevipes* and *D. neobrevipes),* expresses itself in a number of ways including growth depression, accompanied by colour changes as typical reaction to mealybug feeding, and this is especially noticeable in small seedlings (Carter, 1948). A long-term carry-over effect of mealybug feeding was reporting by Carter and Collins in 1947. Carter (1963) reported that mealybugs from negative source plants ensured freedom from any complications with the wilt secretion and resulted in premature development of fruits. Increased susceptibility to wilt by later infestation of mealybug from positive source lead to colour changes and reduced vigour due to feeding by virgin colonies and difference in vigour of growth of plants infested with mealybugs from positive sources. Although these plants did not wilt, they expressed genetic weaknesses.

2.2.5 Nematode and disease problems of pineapple

The pineapple plant has three botanical characteristics that may contribute to its disease and nematode problems (Collins, 1960). First, roots originate adventitiously and do not regenerate if killed to stem. As the stem grows, roots continue to originate in the leaf axils. Only roots that contact the soil at planning are functional to absorb soil nutrients, moisture, and serve to anchor the plants. Collins (1960) also reported that the fruit originates from 100 - 200 individual florets that develop acropetally, and lastly, that the plant is a xerophyte and will survive extended periods of drought. The important nematode genera attacking pineapple on a worldwide basis are *Pratylenchus brachyurus* (Godfrey) Filijer and Schuurmans Stekhoven, *Meloidogne javanica* (Treud) Chitwood, and *Rotylenchulus reniformis* Linford and Oliveria (Guerout, 1975).

Among these, *P. brachyurus* is the most important nematode in Brazil and La Cote d'Ivoire; *P. brachyurus* and *M. javanica* predominate in South Africa; *M. javanica* is the primary pineapple nematode in Australia (Raski and Krusbery, 1984) and *M. javanica* and *Rotylenchulus reniforms* prodominate in Hawaii.

Raski and Krusbery (1984) reported that many other nematodes were found associated with pineapple roots, but either the degree of pathogenicity were not demonstrated or the amount of damage has not been significant.

2.2.6 Some other diseases of pineapple

Pineapple heart rot like mealybug wilt occurs universally. It can be caused by *Phytophthora parasitica* Dastur, *Phytophthora cinnanomi* Rands, and *Phytophthora palmivora* (Butler) Butler. Root rotting in pineapple is generally caused by *Phytophthora cinnamoni* or various *Phythium* species, most commonly *Pythium arhenomanes* (Drechs) (Carter, 1963).

The soft rot of the lower stem tissues (Butt rot) is caused by *Ceratocystis paradoxa* (de Seynes) Moreau, while yellow spot is caused by a strain of tomato spotted wilt virus transmitted from host weeds such as *Emilia fosberyii* Nicholson by the onion thrip, *Thrips tabaci* Lind (Rohrbach and Apt, 1986).

2.3 Botanical characteristics of the neem tree

Azadirachta indica A Juss (Maleaceae) is a fast growing plant that can reach a height of 15 to 20m, and under favourable conditions up to 35 - 40m. It occurs in southern and southeastern Asia as well as in tropical areas of Africa, America and Australia (Schmutterer, 1987). The plant is evergreen but sheds most of its leaves under extended dry periods. The trunk is relatively short, straight with the sapwood greyish-white and heartwood reddish when first exposed to air but changes to reddish-brown after exposure. The root system consists of a strong taproot and well developed lateral roots reaching over 18m (Benge, 1989). Vesicular-arbuscular mycorrhiza (VAM) is associated with the rootlets. Habte *et al.* (1993) categorized neem as VAM dependent species. The flowers are white, fragrant and arranged in axillary manner; normally more-or-less drooping panicles are about 25 cm long. The inflorescence branches up to 250 flowers (Gruber, 1991).

The fruits are globrous olive-like drupes varying in shape from elongate oval to nearly roundish and when ripe are 1.4 – 2.8 x 1.0 – 1.5 cm. They exocarp is thin and the bitter-sweet pulp (endocarp) is yellowish-white and fibrous. The endocarp of the seed encloses one, rarely two and very rarely three elongated seed kernels with brown testa (Gruber, 1991).

2.3.1 Ecology of the neem tree

The neem tree thrives in areas with sub-arid to sub-humid conditions with an annual rainfall between 400 – 1,200 mm. Neem grows in many different types of soil, but it develops best on well drained, deep sandy soils. In some African countries (Benin) neem trees are found growing in lateritic soils and in the Carribbean (Haiti) on calcareous soils. *A. indica* exists on stony shallow soils, on soil with a waterless subsoil, and also on soils with a hard calcareous or clay pan near the surface (Radwanski and Wickens, 1981). The plant also grows on alkaline or saline soil.

Rice (1993) reported that in Australia neem thrives on soil with pH value, which is slightly acid. Neem leaf litter can change the pH value of the top layer of the soil under neem trees from 5.4 to 6.8 (Radwanski and Wickens, 1981). Radwanski and Wickens (1981) again reported that neem exists at annual mean temperature of between 21 – 32 °C and altitudes up to 700 – 800 m and occasionally 1,000 m above sea level.

2.3.2 Uses

In Burma and Thailand leaves and inflorescences are edible and consumed as vegetable (Schmutterer, 1987). Neem twigs are used as toothbrushes in parts of Asia and Africa. Extracts from neem bark are components of toothpastes in Europe and India, and neem oil is used in preparing neem soap in India. (Schmutterer, 1987).

Extracts of neem have traditionally been used by farmers in Asia and Africa to ward off insect pests of household, agricultural, and medicinal importance. Neem derivatives comprise a complex array of novel compounds, which have diverse behavioural and physiological effects on insects. Repellency, feeding and oviposition deterrence, growth and reproduction inhibition and other effects have been attributed to the neem compounds – azadirachtin, salanin, meliantriol, 14-epoxyazaradion, salannolacetate, 3-deacetylsalannin, gedunin, nimbinen, and deacetylnimbinen (Jones *et al.,* 1989).

Most of these compounds showed antifeedant activity in the *Epilachna verivestis* (Coccinelidae) Schwinger *et al.,* 1984). Insect growth regulatory effects were seen in most other ingredient of neem seed kernels, namely 22, 23-dihydro-23-methoxy azadirachtin, 3-trigloyl azadirachtol, and 1-trigloyl-3-acetyl-11- methoxy azadirachtin (Kraus *et al.,* 1987). Some vilasinin derivatives with strong antifeedant activities were isolated from neem seed oil (Kraus *et al.,* 1987).

Neem derivatives affect a wide range of insect pests. Jacobson (1986) listed 123 insect species belonging to the order Coleoptera, Diptera, Heteroptera, Hymenoptera, Lepidoptera and Orthoptera and Homoptera in addition to three mite and five nematode species. Since 1986, neem derivatives have been found to affect 75 additional insect species belonging to different orders (Jacobson, 1986).

2.4 Aqueous extracts

Aqueous neem seed extracts (ANSE) are suitable for controlling a broad range of pest species, especially free-feeding coleopterans and lepidopterous larvae, as well as controlling leaf miners, piercing and sucking insects and grasshoppers (Jayaraj, 1993). There are different techniques for preparing aqueous extracts of neem. The Central Tobacco Research Institute in India recommended in 1979 to put 1 – 2 kg kernel powder in 100 litres of water. This was allowed to stand for 24 hours and sieved.

2.5 Alcohol extracts

Polar alcohol like ethanol and methanol are suitable solvents for extraction and this was first employed by Butterworth and Morgan (1968) to isolate the main active substance, azadirachtin, from neem seed kernels. For practical pest control, alcoholic macerates prepared from different parts of the neem tree proved to be effective against a wide range of pests (Butterworth and Morgan, 1968). Ascher (1981) studied the solubility of dried methanolic extracts prepared from neem seed kernel. In 1995 the first commercial neem insecticide –'Margosan – O' which is based on ethanolic crude extract from neem seeds was registered in the United States for control of various pests on non-food crops (Larson, 1985)

2.6 Protectant potentials of the neem tree

During the past two decades the biological activity of neem extracts has been investigated intensively, and a number if international neem conferences have been held (Schmutterer *et al.,* 1981; Schmutterer and Ascher, 1987), covering mainly the application of crude extracts in laboratory and field tests. In general, extracts of neem fruits, seeds, seed kernels, twigs, stem bark, and root bark have shown to posses insect antifeedant, insecticidal, insect growth disrupting, nematicidal, fungicidal (Jacobson, 1989; Randhawa and Parmar, 1993; Schmutterer and Ascher, 1987), bactericidal (Ara *et al.,* 1989) anti-inflammatory (Dhawan and Patnaik, 1993; Fujiwara *et al.,* 1984), antitumor (Fujiwara *et al.,* 1984) immunostimulating (Van Der Nat *et al.,* 1987; 1991), and other activities (Randhawa and Parmar, 1993).

Since the early investigations by Siddiqui (1942) more than 100 compounds have been isolated from various parts of the neem tree and several reviews on constituents of neem have been published to date (Champaigne *et al.,* 1992; Lee *et al.,* 1991). However, only relatively few pure constituents had been tested for biological activity and also little work has been done regarding structure-activity relationships. Most of the active principles belong to the group of tetranortriterpenoids, but biologically active diterpenoids, triterpenoids, pentanortriterpenoids and a small number of nonterpenoidal ingredients have been isolated (Kraus, 1986).

2.7 Effect of neem derivatives on insects

Pradhan *et al.* (1962) first demonstrated that a 0.001% aqueous suspension of crushed neem kernnal sprayed on cabbage plant totally stopped feeding by the desert locust *Schistocerca gregaria* Forsk (Acrididae) on treated foliage. Neem treatment also deterred the migratory locaust *Locusta migratoria* (L.) at a concentration of 0.001% suspension. Later studies on other pest species confirmed the feeding inhibition caused by neem derivatives (Jacobson, 1986). The repellent and antifeedant effects of neem derivatives on insect pests of rice have been studied in depth. Rice plants sprayed with neem oil using an ultra-low volume (ULV) spray applicator were unattractive to the brown planthopper, *Nilaparvata lugens* (Stal.), the Whitebacked planthopper, *Sogatella furcifera* (Horvath), the leaffolder, *Cnaphalocrocis medinalis* (Guenee), the ear-cutting caterpillar *Mythimna separata* (WLK.) and the rice armyworm, *Spodoptera mauritia acronynctoidea* (Bois) (Heyde *et al.,* 1984; Saxena *et al.,* 1987).

Gill and Lewis (1971) demonstrated neem's systemic antifeedant action. They observed that young bean plants systemically treated with an aqueous neem kernel suspension, ethanolic extract or pure azadirachtin at 1000, 100 and 10 ppm, respectively were hardly damaged by adults of the desert locust *Schistocerca gregaria*.

2.7.1 Effects of neem extracts on growth inhibition of insect

Schoonhoven (1982) reported that contact with azadirachtin disrupts food intake and increases the locomotory activity of insects. The growth inhibitory effects of neem derivatives are much more profound than its repellency or phagodeterrent effect. Leuschner (1972) reported that topical application of crude ethanolic extract of neem at 50µg to 4th or 5th instar nymphs of the East Africa coffee bug, *Antestiopsis orbitalis bochuana* (Kirk) caused morphogenetic defects similar to those caused by natural or synthetic hormonally active substances. Treatment of 4th instar nymphs caused abnormal wing cases, scutelli and hemielytra in the succeeding instar.

Crude methanolic extract of neem leaves or seed, neem oil and purified fractions of seed kernel inhibited the growth of the Mexican been beetle, *Epilachna verivestis* Mulsant (Coccinellidae), the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Chrysomelidae), and the diamondback moth, *Plutella xylostella* (L) (Plutelliadae) (Ruscoe, 1972). It was reported that growth of *Plutella xylostella,* the cabbage butterfly, *Pieris brassicae* (L), *Helicoverpa virescens* and the cotton stainer *Dysdercus fasciatus* Sign (Pyrrhocoridae) was affected if larvae or nymphs contacted azadirachtin-treated substrates or fed on treated food (Ruscoe, 1972).

The nymphs of rice leafhoppers, planthoppers and the rice bug caged on neem oil-or neem extract-treated rice plants and lepidopterous larvae fed on treated leaves suffered from ecdysial failure and other developmental defects (Ruscoe, 1972). Zebitz (1986) reported growth inhibitory effects of neem seed extracts and azadiractin on three species of mosquitoes. He observed that continuous exposure of first instar larvae to neem-treated water delayed their development.

Neem derivatives do not kill directly, but the insect eventualy succumb to behavioural and physiological stresses and starvation on treated plants (Mariappan and Saxena, 1983). Females of *Epilachna verivestis* (Coccinellidae) and *L. decemlineata*

(Chrysomellidae) were sterilized when exposed to neem components (Schmutterer, 1986). Schulz (1980) reported that the structure of ovaries of *E. verivestis* females were altered when they fed on bean plants sprayed with 1% methanolic neem kernel extract, and the egg-laying capacity of the queen of *Formica polyctena* Foerster ants (Formicidae) plunged when fed on a neem oil-treated diet (Schmit and Pesel, 1987). Saxena and Barrion (1987) reported that the frequencies of meiotic cells were significantly less in male progenies of *N. lugens* and *N. virescens* collected from rice plants sprayed with neem seed kernel extract.

Heyde *et al.,* (1984) reported that there was growth and development retardation of the first instars of *N. lugens; S. furcifera* and *N. virescens* on rice plants sprayed with enriched formulated neem seed kernel extracts (NSKEs). Saxena *et al.* (1987) reported that at higher concentrations nearly all nymphs of rice planthopper and leafhopper died before reaching the adult stage.

Moulting failures were observed in pea aphids, *A. pisum,* exposed to bean plants sprayed with NSKEs (Schauer, 1984). Bhathal and Singh (1993) reported that the 3rd and 4th instars of mustard aphids, Lipaphis erysimi with an azadirachtin-rich extract caused developmental abnormalities, such as incompletely developed reduced and crumbled wings. The treatments also delayed the emergence of greenhouse whiteflies, and adults were generally smaller and developed abnormally. Thus chronic effect of neem may be important in the long-term control of homopteran pests.

2.7.2 Effect of neem extracts on reproduction of insect

The fecundity of homopterain insects is strongly influenced by treatment with neem extracts (Schmutterer, 1990). Remhold (1989) has reported that neem exracts caused the inhibition of reproduction both in females and in males. This was thought to be due to disturbance of homeostasis of ecdysteroid titers and interference with the endocrine control of reproduction. Saxena and Barrion (1987) reported that the reduction in the frequency in the meiotic cells of progenies of *N. virescens* and *N. lugens* collected from rice plants treated with neem seed 'bitters' or kernel extract was attributed to cellular and chromosomal dysfunction during spermatogenesis, leading to nonviability and senescene of sperm cells. Schauer (1984) observed that the first instars of adult *A. pisum* on broad bean leaves treated with 0.002% methanolic NSKE produced 2 offspring per female per day in the control. Neem treatment is known to cause cytopathological alterations and resorption of developing oocytes in insects (Schulz and Schluter, 1984).

2.7.3 Effect of neem extract on oviposition and egg-hatchability in insect

Neem oil deterred egg laying by homopterans such as *N. lugens* (Saxena *et al.,* 1981), *Amrasca devastans* and *Bemisia tabacci* (Saxena and Basit, 1982). Saxena *et al.,* (1981) showed that females of the rice leaffolder, *Cnaphalocrosis medinalis,* laid about one third the number of eggs on neem oil-treated rice plants compared to the control plants. Fagoonee (1981) also observed that gravid females of the cabbage webworm, *Crocidolomia pavonoma* (or *Crocidoloma binotalis)* was deterred by ethanolic and methanolic neem leaf extracts sprayed on cabbage leaves. Saxena and Rembold (1984) tested volatiles from neem seed kernal against the cotton bullworm *Helicoverpa armigera* and concluded that neem seed kernal volatiles acted on repellents and not as contract ovipositional deterrent and contact with neem seed oil inhibited oviposition of the bullworm. Saxena *et al.,* (1984) stated that the application of neem cake to rice plants did not affect oviposition or egg hatching of L. *lugens. Heyde et al.* (1984) found in assays using three species of planthoppers and leafhopper on rice that settling by the brown plathopper, *Niilaparvata lugens,*and the white backed planthopper, *S. furcifera* was progressively reduced by increasing concentrations of neem oil (1 – 50 %) on plants.

Singh (1984) reported the antiovipositional effect of aqueous neem seed kernel extracts on the desert locust, *Suhistocerca gregaria.* Sexena *et al.* (1987) reported that the oviposition of *Nilaparvata lugens* females was reduced on rice plants sprayed with 7,500 ppm of neem seed extract but hatchability of eggs was not affected. Neem seed extracts, however, had no effect on the oviposition of *Sogatella furcifera* or *Nilaparvata virescens.*

Oviposition and hatchability of eggs by *Nilaparvata virescens* decreased on rice seedlings, which were systemically treated with 2,500, or 500ppm of neem seed extracts (Kareem *et al.,* 1988). Nicol (1993) reported the antiovipositional effect of neem seed kernel powder and aqueous neem seed kernel extract (20 g/1) on egg-laying females of desert locust, *Schistocerca gregaria.*

2.74 Antifeedant and repellent effect of neem extracts on insect

Antifeedants are compounds which, when perceived, reduce or prevent insect feeding (Saxena *et al.*, 1981). Heyde *et al.*, (1984) reported that rice plants treated with an ultra-low volume (ULV) spray of 3 % neem oil were found to be less attractive to *N. lugens* and the whitebacked planthopper, *Sogatella furcifera.* It was stated that rice plants treated with an ULV spray of neem oil repelled *N. lugens* and *S. furcifera* even without contact, indicating olfactory repellence. Neem oil at 0.52% deterred 50% of *N. lugens* population of 2^{nd} and 3^{rd} instar nymphs from alighting on treating rice plants (Chiu, 1984).

In China, application of 1.4 % emulsified neem oil was found to be effective repellent and feeding deterrent against the Asiatic citrus psyllid, *Diaphorina citri* (Chiu, 1984). The repellent action of neem is reported to result from the presence of volatile sulfur-containing compounds (Balandrin *et al.,* 1988). Neem oil and extracts have been reported to deter many homopteran insects. Food intake by planthoppers was deterred on rice plants sprayed with neem oil (Saxena *et al.*, 1981). Schoonhoven (1987) reported that the antifeedant may either stimulate the insects' specialized deterrent receptors or interact with receptors sensitive to phagostimulants, and this probably inhibits food intake. Jacobson *et al.* (1986) reported that a choice test, using 0.01 % hexane extract of neem seed deterred the California red scale, *Aonidiella auantii,* while 0.1% and 1% required to deter the yellow scale *A. citrina,* and the citrus mealybug, *Planococus citri* respectively.

The selection of host plant by an insect is mainly governed by the responses of the insect's gustatory and olfactory sensilla. Butterworth and Morgan (1968) obtained 100 % antifeedant activity in hungry 5^{th} instar nymphs of *Sohistocerca gregaria* at a concentration of 70µg azadirachtin / litre offered on sucrose-impregnated filter papers. Using the same method Haskell and Mordue (1969) obtained almost total inhibition of feeding with a concentration as low as 1×10^{-6} % azadirachtin.

Schmutterer *et al,* (1993) reported that treatment of corn plants with azadirachtin-enriched neem oil water extracts showed that treated plants were significantly less infested than untreated control plants. They attributed this to the migration of the locust nymphs to untreated plants. Furthermore, adult *Chrotogonus homalodemus* (crididae)

were repelled from cabbage leaves, which had been dipped in aqueous neem seed kernal extract (Grahn, 1993). Nicol (1993) reported that an aqueous extract of seed shells (endocarp) of neem showed a strong repellent effect on nymphs and adults of *Schistocerca gregaria*.

Apart from its direct effect on test neurones, azadirachtin can modify an insect's response in less intermediate ways. The neural responsiveness of tasts sensilla to azadirachtin can be modulated by exposing the insects to diet containing low concentrations of azadirachtin (Simmonds and Blaney, 1985). These indirect effects (i.e. modification of an insects responses and the neural responsiveness of taste sensilla to azadirachtin) could result from the compound affecting the centres that control feeding, or the hormones that are involved in food metabolism (Barnby and Klock, 1987).

However, Rafa (1987) showed that the efficacy of azadirachtin can very depending on which crop it is sprayed on. Similarly, azadirachtin has been found to stimulate at least one "deterrent neurone" in the maxillary styloconic sensila of larvae of the Lepidopterans, *Pieris brassica* and *Lymantria dispar* (Schoonhoven, 1982), *Spodoptera exempta, Spodoptera littorades, Helionthis virescens*and *Helicoverpa armigera* (Blaney and Simmonds 1990). Dorn (1986) found that injection of azadirachtin into nymphs and adults of *Locusta migratoria* induced inhibition of midgut peristalsis, and concurrent deflection of serotoninergic cell bodies in the frontal ganglia. These authors suggested therefore that azadirachtin has its effect on the gut by interfering with the functioning of serotoninergic cells of the stomatogastric nervous systems and thereby blocking midgut peristalsis.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental sites

Laboratory, screen house and field experiments were conducted. The laboratory experiment was conducted at the Department of Biochemistry, University of Ghana, Legon while the screen house experiment was carried out in the screen house of the Zoology Department. The field experiment was conducted on Greenspan Farm at Agenkiti near Aburi in the Eastern Region of Ghana.

3.2 Preparation of neem seed extracts

Mature neem seeds were collected from Ashaley Botwe, Madina, near Accra. The seeds were kept in sealed polyethylene bags for four days to enable the pulp to soften. The pulp was then washed off in tap water and the seeds dried in a hot-air oven at 35°C for four days. The dried seeds were milled into powder suing a laboratory mill. One hundred and twenty gramme portions of the powder was extracted with 1.5 litres of water and methanol to give 8 % solutions on weight per volume basis over a period of 24 hours. The mixture was then filtered to obtain the filtrate. The filtrate from the methanolic extract was concentrated using rotary vacuum evaporator and stored at 10 °C till required. Samples of the concentrated solution were dried using a dessicator to determine the concentration of the extractive materials in both extracts used for the treatment of suckers for the screen house and field experiments.

3.3 Repellency bioassay

Adult mealybugs were selected from field populations for the bioassay. Field populations of mealybugs were preferred because they have a characteristic genetic make-up reflecting the prevailing agro-ecological conditions. Repellency test was conducted according to the method of Talukder and Howse (1993). Whatman filter paper (12.5 cm in diameter) was cut in two halves. Four ml of solutions of aqueous neem seed extract at a concentration of 6.0 g/1 of extractive material was applied to half filter papers as uniformly as possible using a pipette. The treated half circles were air dried to evaporate the solvent (water) completely. The other half-circles were also treated with 4 ml of water and then allowed to stay for 20 minutes to evaporate the water. Full-circles were remade by attaching treated halves to untreated halves of the same dimension using sellotape.

Precautions were taken so that attachment did not interfere with the free movement of mealybugs from one half to another, but the distance between the filter-paper segments was sufficient to prevent seepage of test samples from one half of the paper to another. The filter papers were placed into petri dishes with the seam oriented in four different directions to avoid any incident stimulus affecting distribution of mealybugs. Ten adult mealybugs were released at the centre of each filter paper circle and the petri dish was covered. Mealybugs present on each strip were counted every 10 minutes for one hour. This was repeated for the methanolic extract.

Percentage repulsion (PR) were calculated using the following formula: PR (%) = (Nc – 50) x 2 (Talukder and Howes, 1993) where Nc is the percentage of mealybugs present in the control half. Positive values (+) expressed repellency and negative values (-) attractancy. Data for PR (also known as Repellency Rate) were analysed using analysis of variance (ANOVA) after transforming into arcsin values.

The results were then categorised according to the following scale:

Class	Repellency Rate (%)
O	>0.01 to <0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

3.4 Screen house experiment

Nine batches with each batch made of 16 healthy-looking but mealybug infested suckers of "Smooth Cayenne" cultivar were selected from Greenspan Farm. Each group of selected suckers with not less than 20 mealybugs per sucker by visual inspection was exposed separately to one of the following treatments. Treatments were applied by dipping the sucker in the appropriate solutions.

1. Methanolic extract at 1.5 g/1 of extractive material;
2. Methanolic extract at 3.0 g/1 of extractive material;
3. Methanolic extract 6.0 g/1 of extractive material;
4. Aqueous extract at 1.5 g/1 of extractive material;
5. Aqeuous extract at 3.0 g/1 of extractive material;
6. Aqeuous extract at 6.0 g/1 of extractive materiral;
7. Dimethoate treatment at 0.53 g/water (Di)
8. Aqeuous treatment (Cw)
9. No treatment applied (Co)

The suckers were then allowed to dry for 24 hours before planting in plastic pots filled with soil collected from Greenspan Farm at Agenkiti. The pots were completely randomised to ensure equal green house lighting. Each batch of 16 suckers was sprayed at 2 weeks intervals with their respective treatments. Four suckers were randomly selected and uprooted at two weeks intervals and the number of live mealybugs counted.

3.5 Field experiment

3.5.1 Determination of levels of N, P and K

Before the start of the field experiment soil samples were taken from the plot to be used and their N.P.K level determined as follows:

3.5.1.1 Total nitrogen content of soil

Total nitrogen content of soil from each plot was determined by the micro Kjeldahl method (Black, 1965). Two grammes of air-dried soil sieved through 2 mm diameter mesh was transferred into a 950 ml Kjeldahl flask. Few drops of distilled water were added to moisten the soil. Five millilitre of concentrated H_2SO_4 was added and the contents digested to clear. The cooled digest was transferred with distilled water to 50 ml volumetric flask. Five millilitre of 40 % NaOH solution was added to 5 ml of the digest and distilled into 2 % boric acid. The distillate was titrated against 0.0012M HCI from green to reddish end point. The % N was calculated as follows:

$$\%N = \frac{\text{ml HCI used x Molarlity x 1400}}{\text{mg of sample}}$$

3.5.1.2 Determination of available phosphorus in soil

Available phosphorus in soil from each plot was determined by the Bray's I method (Bray and Kurtz, 1945). The reagents used were:

a. IM Ammonium fluoride (NH_4F) solution was prepared by dissolving 37 gm of NH_4F in distilled water and the solution diluted to 1 litre.

b. 0.5M hydrochloric acid was prepared by diluting 20.2ml conc. HC1 to a volume of 500 ml with distilled water.

c. Extracting solution was also prepared by adding 15 ml of 1M NH_4F and 25 ml of 0.5M HCI to 460 ml of distilled water. This gave a solution composition of 0.03M NH_4F in 0.025M HCI.

d. Dickman and Bray's reagent was prepared by dissolving 15g of ammonium molybdate (AR), $(NH4)_6MO_7O_{24}.4H_2O$, in 300ml of distilled water, warmed to about 60 °C and filtered after cooling. 342ml of conc. HCI was added to make up to 1 litre.

e. 40 % $SnCl_2$ stock solution was prepared by dissolving 10g of $SnCl_2$. $2H_2O$ (AR) crystal in 25 ml of conc. HCI. A working solution was made by diluting 0.5 ml of the stock solution to 66 ml with distilled water.

f. Standard P solution was prepared by dissolving 0.439g of KH_2PO_4, in 500 ml of distilled water, and 25 ml of 7M H_2SO_4 were added and the volume was made up to 1 litre with distilled water to give 100 ppm stock solution of phosphorus. From this, 2 ppm solution was prepared by 50 time dilution of the stock solution.

3.5.1.2.1 Extraction

Five grammes soil sample was weighed and transferred to a 100 ml conical flask and 50 ml of extractant solution was added. The contents of the flask were shaken for five minutes, and filtered through Whatman N$\underline{o}$ 42 filter paper to obtain a clear filtrate. A solution blank was prepared in which all the reagents were added, except the soil. The available P was calculated as follows:

Transmittance (%) of test solution	= T
Concentration of P as read from standard curve	= A ppm
Available P in soil	= A x 50 ppm

3.5.1.3 Determination of available potassium (K) in soil

Available potassium content of soil from each plot was determined by the ammonium acetate method (Mengel, 1968). The reagents used were:

a. Neutral molar NH_4OAc solution was prepared by dissolving 77 g of NH_4OAc in distilled water and the volume was made up to 1 litre. The solution was adjusted to a pH of 7.0, adding acetic acid solution.

b. Potassium chloride solution was prepared by dissolved 21g of AR grade KCI (dried at 60 °C for 1 hour) in distilled water, and made up the volume to 1 litre to obtain 1000 ppm K solution. This was treated as the stock solution of K.

c. A standard curve for K (working K standard) was obtained from the stock solution by taking measured aliquots and diluting with NH_4OAc solution to give 40 ppm of K. The K filter was inserted and the gas and air pressure regulated and the flame photometer reading was set at zero for the blank (NH_4OAc solution) and at 100 for 40 ppm of K. A calibration curve was drawn by plotting flame photometer readings against different concentrations (10, 15, 20, 25, 30, 35, 40 ppm) of phosphorus.

Five gramme of soil was weighed into a 150 ml of conical flask. Twenty-five millilitre of 1M NH_4OAc (pH 7) solution was added. The contents of the flask were shaken on an electric shaker for 5 minutes and filtered. The filterate was fed into the atomizer of the flame photometer, 100 of which was set with 40 ppm potassium solution and reading noted. The potassium concentration in the extract was then estimated the standard curve. From this concentration, the amount of potassium in the sample was calculated as follows:

Concentration (ppm) read from standard curve	= C
Dilution factor	= 5 times
Available potassium in the soil	= C x 5

3.6 Land preparation

A plot of 20.3 m x 19.2m was cleared and the thrash was burnt after one week of drying. The plot was chosen such that it was isolated from other plot cultivated on the farm.The land was then ploughed and harrowed.Planting was done on 21st January.

3.7 Treatment and Planting of Suckers

Suckers from "Smooth Cayenne" cultivar were obtained from Greenspan Farm. The suckers were selected from an old plot known to have incidence of mealybug infestation. Planting spacing of 30cm x 30cm x 90cm, double row was used. All treated suckers were further sprayed with their respective treatment at monthly intervals, two months after planting. Each experimental plot measured 6m x 6m and contained 100 plants. Each treatment was replicated four times. Plots were set out in Randomized Completed Block Design and the total plot size was 720m2.

Treatment applied were:

1. Methanolic neem seed extract at a concentration of 6.0 g/1itre of extractive materials;
2. Dimethoate (standard insecticide) at a concentration of 0.53 g/1 of water.
3. CW (suckers dipped and sprayed with water).

Liquid fertilizer comprising sulphate of potash and urea at a ratio of 2.5:1 was applied one month after planting. The first application was done four weeks after

planting and the secound application was done fifteen weeks after planting. The 3rd fertilizer application followed four weeks after the second application and the 4th application was done four weeks after the 4th application. After twenty-six weeks the 5th application was done and the last application was done when the plants were twenty-nine weeks old.

3.5.3 Data collection

Six plants per plot were selected at random from each experimental plot and uprooted and the following data were collected: Data was obtained as follows, every month for 6 months, beginning two months after planting:

a. **Number of healthy roots per sucker;**
b. **Number of rotten roots per sucker;**
c. **Number of mealybugs per sucker;**
d. **Number of wilted leaves per sucker**
e. **Number of healthy leaves per sucker;**

Wilted leaves were determined every month for 6 months by scrapping the upper surface of leaves and those with underlying tissues being white or yellowish were considered to be due to mealybug. Leaves with underlying tissues being green were attributed to water deficiency (Anon., 1954). Only leaves found to have wilt due to mealybugs were considered.

CHAPTER FOUR

4.0 RESULTS

4.1 Yield of extract

The percentage yield of crude extractive materials obtained from the methanol extract was nearly three times higher than the amount from the water extract (Table 1).

Table 1: Yield from extraction with methanol and water

Solvent Used	Weight of powdered material (g)	Weight of extractive materials (g)	Yield (%)
Methanol	4275	1064	24.9
Water	1100	94	8.5

4.2 N P K levels in selected field plot field experiment

Table 2 shows the levels of nitrogen, phosphorus and potassium levels in the plots used for the field experiment. The mean nitrogen and phosphorus levels were 8.7 x 10 $^{-2}$ % and 7.87 ppm, respectively. Using the NH_4Ac the mean potassium level was 2.2 x 10 Cml/kg. No significant difference (P = 0.05) was obtained for the three nutrients in the different plots used for the field experiments.

4.3 Effect of neem seed extracts on population of mealybug in the screen house

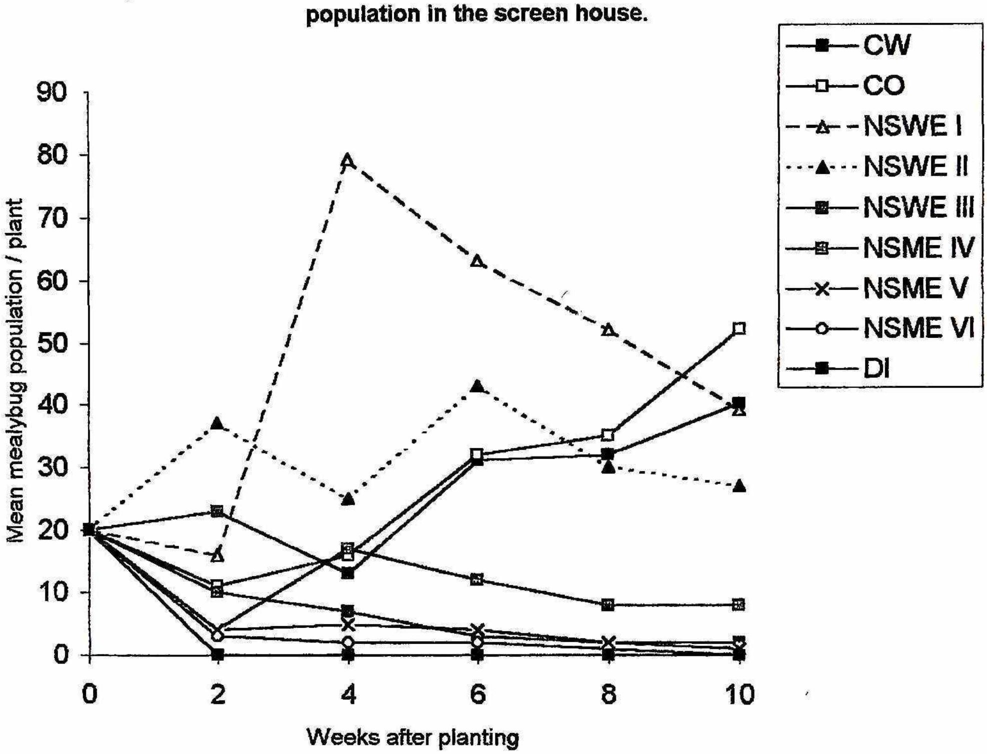
Figure 1 shows the effect of the different treatments on the population of mealybugs on pineapple suckers after planting in the screen house over a 10 week period. The population of mealybugs in the control (Co and Cw) plants were not significantly different from each other. No mealybug was found on pineapple plants treated with dimethoate. Of the neem-treated plants, the methanolic extract at 6.0 g/1 crude extractive materials was most effective in causing reduction in the population of mealybugs. The water extract at a concentration of 1.5 g/1 crude extractive materials was least effective (Fig 1). The water extract, which caused significant reduction in mealybug population, was the water extract at 6.0 g/1. In general methanolic extract

Table 2: Level of N. P. K. obtained

Plot No.

	1	2	3	4	5	6	7	8	9	10	11	12
N x 10^{-2}(%)	8.7	8.6	8.7	8.7	8.7	8.5	8.6	8.6	8.8	8.6	8.7	8.7
P (ppm)	7.86	7.87	7.87	7.88	7.87	7.87	7.87	7.89	7.87	7.87	8.85	7.86
K(Cmol/kg)	0.22	0.20	0.22	0.21	0.20	0.23	0.21	0.22	0.21	0.22	0.20	0.20

Fig.1 Effect of neem extracts and dimethoate on pineapple mealybug population in the screen house.



Cw and Co represent control: with and without water respectively.
I,II,III represent neem extract at concentrations of 1.5, 3.0, 6.0g/l respectively.
IV, V, VI are neem methanolic extract at concentrations of 1.5, 3.0, and 6.0g/l respectively. DI is dimethoate.
NSME and NSWE neem seed methanol and water extracts respectively
F < 0.001

above 3.0 g/1 crude extract reduced the mealybug population the 2 week after application.

4.4 Repellency of neem seed extract to pineapple mealybug

The neem seed extract (6.0 g/1 of crude extractive materials) had low repellent effect on the mealybugs on treated filter paper (Table 3). Of the two extracts tested the methanol extract was more repellent (26 %, class II) than the aqueous extract (13 %, class I) at the same concentration used.

Table 3: Percentage repellency of neem seed extract to pineapple mealybug

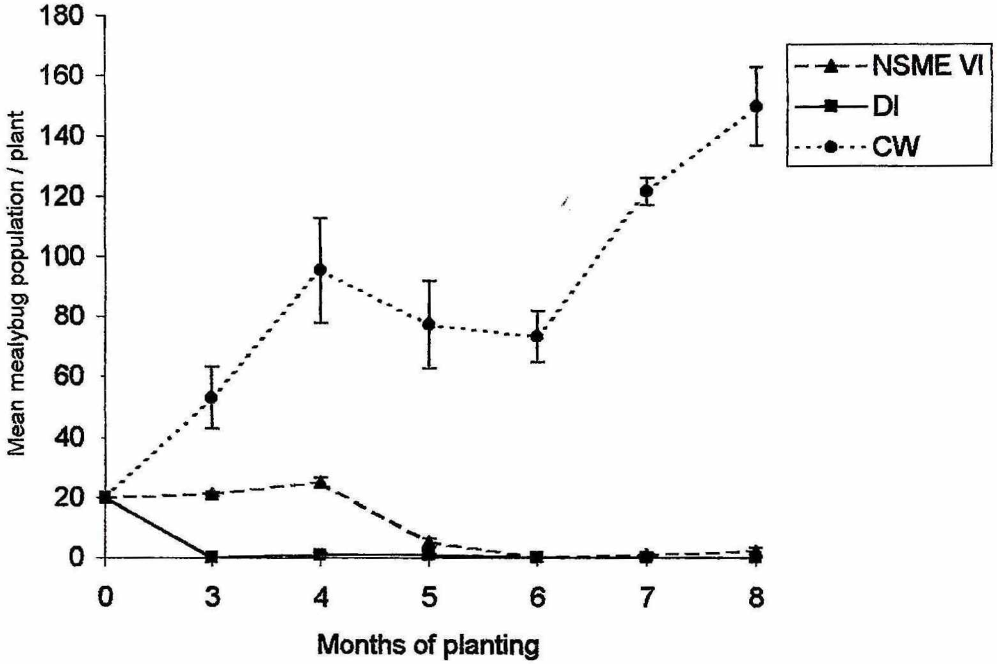
Extract	Dosage (g/l)	% repellency (min.)				
		10	30	60	class	repellency (%)
NSME	6.0	51	27	27	II	26.10
NSWE	6.0	0.0	27	27	I	13.28

Data obtained were transformed by arcsin % transformation and analysed by ANOVA
NSWE and NSME are neem seed methanol and water extracts respectively.

4.5 Effect of neem extract and dimethoate on mealybugs population on the field

Figure 2 shows the effect of methanolic neem seed extract and dimethoate on the population of mealybugs in the field over the eight-month period. Like the screen house, the control treatment (water only) was least effective in reducing mealybug population. In general, mealybug population increased over the eight-month period. The methanolic neem extract and dimenthoate caused a highly significant reduction ($P < 0.001$) in mealybug population in the field. Dimethoate completely eliminated mealybugs within three months, but the methonalic neem extract at 6.0 g/1 crude extractive materials, caused significant reduction only after four months, inducing over 95 % reduction in mealybug population (fig. 2). From the fifth month following the initial application the methanolic neem extract and dimethoate were equal in activity when few or no mealybug was found on treated plants.

Fig. 2. Effect of neem extract and dimethoate on pineapple mealybug population in the field.



VI and DI are methanolic neem extract at 6.0g/l and dimethoate respectively.
CW is control (water treated).
NSME is neem seed water extract
F < 0.001

4.6 Effect of neem seed extract on leaf development

Figure 3 shows the effect of methanolic extract and dimethoate on the number of leaves produced by pineapple grown in the field. Apart from the 8^th^ month, there was a general increase in the number of healthy leaves produced by the plants. Methanolic neem extract and dimethoate had no significant (P=0.37) effect on leaf development. However, the methanolic neem extract produced the highest number of healthy leaves.

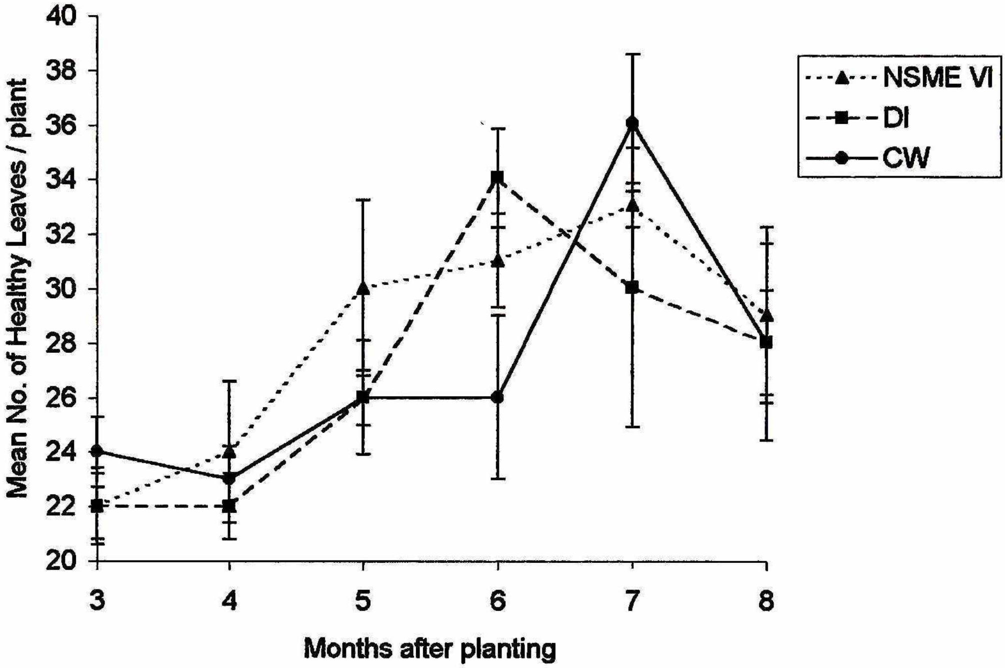
4.7 Effect of neem seed extract and dimethoate on wilting

The effect of methanolic extract and dimethoate on wilting is shown in figure 4. Apart from the 8^{th} month, there was an increase in wilting over the first seven months in the control. Fewer wilted leaves up to the 6^{th} (up to one per plant) were observed for the neem and the dimethoate-treated plants increased rapidly in the 7^{th} and 8^{th} months. These were, however, a significant difference (P = 0.001) between the effectiveness of these two treatments and the control in controlling wilting resulting from mealybug infection. But the difference between the methanolic neem extract and the dimethoate was not statistically (P=0.001).

4.8 Effect of neem seed extract and dimethoate on healthy root production

Figure 5 shows the effect of methanolic neem extract and dimethoate on root production. In general, the production of healthy roots was improved in all the treatments during the experimental period. The methanol extract initiated and promoted root growth better than dimethoate and the control after the 6^{th} month. There was no significant difference (P=0.001) in the development of healthy root. The methanolic neem seed exract and dimethoate reduced root rot after the sixth month of its application (Table 4). There was a significant difference in the abilities of the methanolic extract and dimethoate to reduce root rot when compared to the control (P=0.001). However, the difference in root rot between the methanolic extract and dimethoate was not significant.

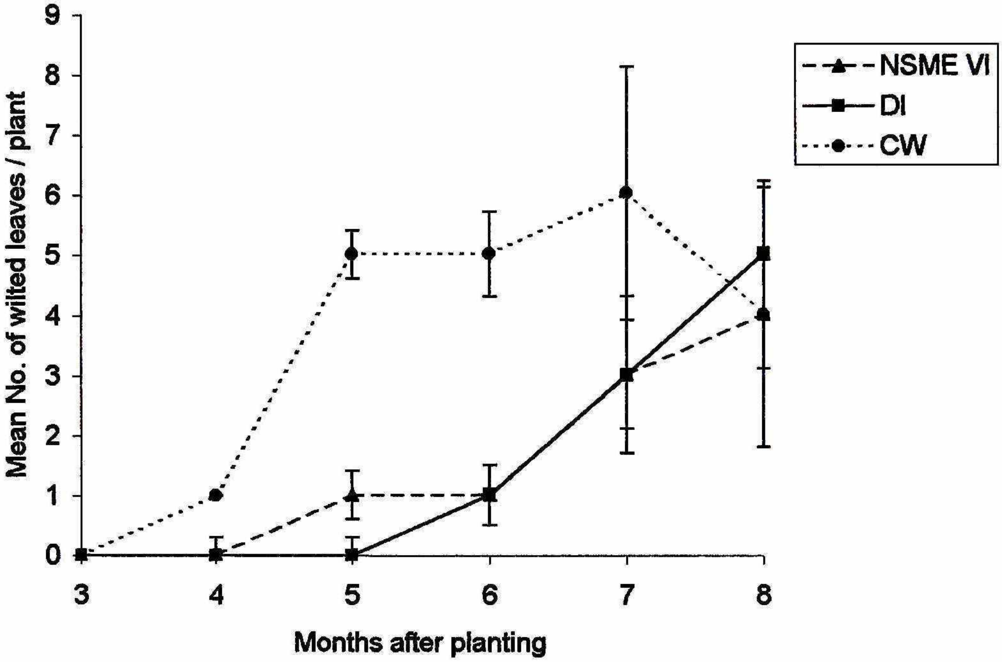
Fig. 3. Effect of neem extracts and dimethoate on pineapple leaf development in the field.



VI and DI are methanolic neem extract at 6.0g/l and dimethoate respectively.
CW is control (water treated).

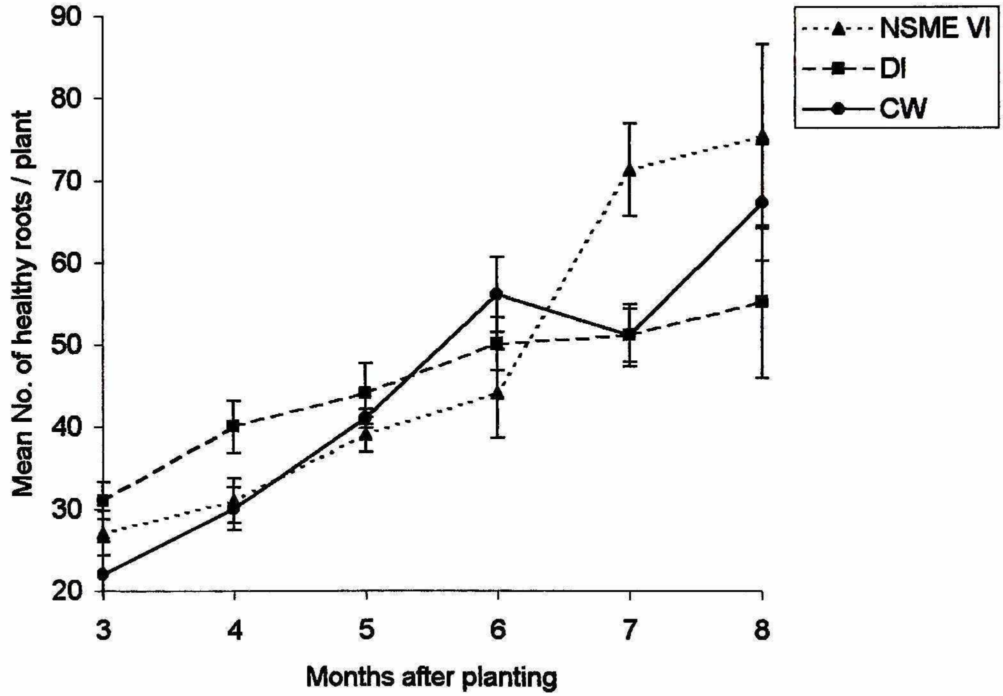
P = 0.001

Fig. 4 Effect of neem extract and dimethoate on pineapple leaf wilting in the field.



VI and DI are methanolic neem extract at 6.0g/l and dimethoate respectively.
CW is control (water treated).
P = 0.001

Fig. 5. Effect of neem extract and dimethoate on development of healthy pineapple root development in the field.



VI and DI are methanolic neem extract at 6.0g/l and dimethoate respectively.
CW is control (water treated).
P = 0.001

Table 4

Number of rotten roots observed per plant

Month

	March	April	May	June	July	August
TREATMENT						
NSME	0	1	1	0	1	2
DI	0	0	0	0	1	4
Cw	0	1	1	3	6	8

CHAPTER FIVE

5.0 DISCUSSION

The results obtained showed that methanol extracted more materials compared to water. This might be because the extracted materials are more soluble in methanol.

Copious excretion of sugary honeydew as a result of the feeding of the insects encourages the build of ant colonies and growth of sooty mould on plants, which make the fruits unsightly, thus reducing their market value. Because of this there was the need to find out the repellent effect of neem seed extract on *Dysmicocus* spp. From the results it was observed that the mealybug activity was higher on the filter paper half treated with methanolic extract compared to that with the water extract and the control. Thus more mealybugs moved away from the methanolic extract treated half to the control (water only). The difference between the two treatments was significant (P=0.001). Heyde *et al.* (1984) also reported that only 36% of *N. lugens* females alighted on plants sprayed with 3% neem oil and increased concentration decreased insect arrival. The oil repelled *N. lugens* and *S. furcufera* adults even without contact. The moderate repellent effect of methanol neem seed extract appears to confirm the findings of Balandrin *et al.,* (1988) who reported that the repellent action of neem extract may result from the presence of sulphur containing compounds and neem 'bitters'. Saxena (1987) has pointed out that the repellent action of neem extracts may be due to the strong odour of the presence of sulphur moieties, or other volatiles that may be present in neem oil from methanolic extract as indicated by Saxena and Rembold (1984).

The effectiveness of the methanol extract might be due to the methanol being able to extract more active ingredients than water. The methanol yielded more extractive materials compared to the water. Methanolic extract at 6.0 g/1 was as effective as dimethoate in controlling mealybug population in the screen house and therefore was chosen for the field.

Neem extract contains compounds with growth inhibition and chemosterilants action, making it biologically active against mealybug as reported by Svoboda and Robins (1971). The extract might have also acted as antifeedant causing the death of the mealybugs through starvation (Mordue and Evams, 1987). It could also be possible that the extract interacted with some receptors sensitive to egg laying and hatching. Schoonhoven (1987) reported that neem extract interacts with rceptors sensitive to phagostimulants in leafhopper on rice plants treated with 25 % neem oil. Kumar *et al.* (1989) reported the effectiveness of neem seed extract on *Planococus citri* on coffee. Jacobson *et al.* (1986) found that 1 % hexane extract of neem seed reduced *P.* citri population by 50 %. Reese and Beck (1976) showed that food treated with 1.5 nmol/g azadirachtin decreased relative growth rate of *P. saucia* by 50 % and attributed this to the decrease in the efficiency with which ingested and digested food was converted to insect biomass.

Healthy root development appeared to be promoted by the neem extract and this effect was different from the dimethaote and control after the sixth month. This effect may not be due to the reduction in mealybug population since dimethoate had 100 % reduction in mealybug population without significant effect on root development.

Root rot was reduced by about 75 % by the neem extract at 6.0 g/1 crude extractive materials whilst dimethoate registered 50 %. The reduction in root rot by the neem extract may be due to its initiation and promotion of healthy root development as well as the reduction in mealybug population.

Leaf development was improved by the neem extract. The improvement in leaf growth and development by the neem extract might be due to the high percentage nitrogen level in the extract (Grubber, 1991). This alao confirms the findings of Parmer and Ketkar (1993) that neem extracts act as a slow fertilizer release Kareem *et al.* (1989) also indicated that seedings grown from neem-treated seed developed vigorously and more robustly than those germinated from untreated seed.

Wilting was reduced by the methanolic neem seed extract after the seventh month compared to the dimethoate-treated and the control plants. The control however, had the highest number of wilted leaves after the forth month. Reduction in wilting by the neem extract might be due to the initiation and promotion of healthy roots and development of healthy leaves due to the high nitrogen level (Grubber 1991) and reduction in mealybug population.

The under utilized neem lots in Ghana could be tapped for the control of mealybug wilt of pineapple since it can reduce the mealybug population and the incidence of the disease. Such use of neem will open new opportunities for employment and income generation. The extracts could then be produced on a cottage industry level of by small-scale extraction plants at the village level.

CONCLUSIONS AND RECOMMENDATIONS

The effect of methanolic neem seed extract in controlling pineapple mealybugs, *Dysmicocus* spp population was studied in the laboratory, screen house and the field. Even though the use of synthetic inesticides is still the main method of controlling mealybugs on pineapple farms, the above study has amply shown that neem materials such as the seed extract, which is environmentally friendly can be used at high concentrations to achieve acceptable levels of mealybug control in pineapple farms in Ghana and other developing countries where neem is common. This approach should be practical and preferable over the used of synthetic insecticides which affect beneficial organisms such as natural enemies of pests, parasitoids, numerous predators and causes of off-farm effects (e.g. pollution of water bodies). From the study, the following results can be summarized as:

1. The neem seed extract caused low incidence of mealybug population, showing significant difference from the control.
2. The methanolic neem seed extract promoted leaf development. However, it was not significantly different from the standard insecticide and the control.
3. On wilting, the effect of the neem seed extract was not different from the standard insecticide. However, it performed better than the control.
4. The methanolic neem seed extract promoted root development and its effect was not different statistically from the standard insecticide or the control.
5. Root rotting was reduced significantly by the methanolic neem seed extract.

From the results presented above, it could be seen that neem seed extract is effective reducing the population build-up of mealybugs on pineapple and subsequently promoting leaf development. Owing the mode of action. Neem seed extracts should be considered suitable for integrated pest management programme on small-scale as well

as the commercial farmers for controlling mealybug wilt in Ghana and other developing countries.

Aqueous extract at higher concentration should be tested on the field.
Also hydrodistillation of neem seed to obtain volatiles should be tested in the screen house.

An attempt on studies on the effect of neem seed extract on the eggs of the mealybug was not successful due to the ovoviviparity nature of the insect. However, further studies should be conducted to determine the effect of neem seed extract on morphology and on the nymphs of pineapple mealybugs.

LITERATURE CITED.

Anon. (1954). "Pest of Pineapple," Rept. 6th Comm. Entomol. Conf., Dept. Agr. Malaysia, p. 270 - 271.

Ara, I., Siddique, B.S., Faizi, S. and Siddique, S. (1989). Diterpenoids from the stem bark of *Azadirachta indica*. Z. Naturforsch. 44: 1279 - 1282.

Ascher, K.R.S. (1981). Some physical (solubility) properties and biological (sterilant for *E. varivestris* females) effect of dried methanolic neem (A. indica) seed kernel extract. Proc. 1st Int. Neem Conf. (Rottach-Egern, Germany, 1980), pp. 63 - 74.

Balandrin, M.F.; Lee, S.M.; Klocke, J.E. (1988). Biologically active volatile organosulphur compounds from the seed of neem tree, *A. indica*. J. Agric. Food Chem. 36: 1048 - 1054.

Barnby, M.A. and Klock, J.A. (1987). Effects of azadirachtin on the nutrition and development of the tobacco budworm, Helionthes virescens (Fabr.) J. Insect Physiol. 33: 69 - 75.

Beardsley, J.W.Jr., Su, T.H., McEwen, F.L. and Gepling, D. (1982). Field investigations and the interrelationships of the big-headed ant, the grey pineapple mealybug and pineapple mealybug wilt disease in Hawaii. Proc. Hawaiin Entomol. Soc. 24: 51 - 67.

Beardsley, J.W. (1960). On the Taxonomy of pineapple mealybug in Hawaii, with a description of previously unnamed species (Homoptera: Pseucoccidae). Proc. Hawaiian Entomol. Soc., 17: 29 - 37.

Benge, M.D. (1989). Cultivation and propagation of the neem tree. In Jacobson, M. (Ed.) Focus on Phytochemical Pesticides. Vol. 1, The Neem Tree. p. 1 - 18 C.R.C Press, Boca Raton, Fl.

Bentley, M.D.; Hassanali, A..; Lwande, W.; Njoroge, P.E.W.; Ole Sitayo, E.N.; Yatagai, M., (1987). Insect Science Application 8: 85.

Bhathal, S.S. and Singh, D. (1993). Effects of AZT-VR-K and some commercial neem products on development, reproduction and mortality of mustard aphid, L. erysimi (Kaltenbach). World Neem Conf. (Bangalore, India), Abstr. p. 48.

Black, C.A.. (1965). Methods of Soil Analysis. Part I and II. American Society of Agronomy, Inc., Publishers, Madison, Wisconsin, U.S.A..

Blaney, M.D., Simmonds, M.J.S.,Ley,S.V., Anderson,J.C., Toogood, P.L.(1990). Antifeedant effects of azadiractin and structurally related compounds on lepidopterous larvae. Entomol. Exp.Appl. 55: 149 - 160.

Bray, R.H. and Kurtz, L.T. (1945). Determination of total, organic and available forms of phosphorus in soils. Soil Science 59: 39 - 45.

Butterworth, J.H. and Morgan, E.D. (1968). Isolation of a substance that suppresses feeding in locust. J. Chem. Soc. Commun., p. 23 - 24.

Butterworth, J.H. and Morgan, E.D. (1971). Investigation of the locust feeding inhibition of the seeds of the neem tree, *A. indica*. J. Insect Physiol. 17: 969 - 977.

Carter, W. (1973). Insects in Relation to Plant Diseases, 2nd Edition. John Wiley & Sons, New York.

Carter, W. (1963). Mealybug wilt of pineapple: A reappraisal. Ann. N.Y. Acad. Sci. 105: 741 - 764.

Carter, W. (1960). A study of mealybug populations (D. brevipes (Ckl.)) in an ant-free field. J. Econ. Entomol. 53: 296 - 299.

Carter, W. (1956). Notes on some mealybugs (Coccidae) of economic importance in Ceylon, Food and Agric Organization, U.N., Plant Protection Bull. 4: 49 - 52.

Carter, W. (1954). Etiological aspects of mealybug wilt, Phytopathology 35: 305 - 315.

Carter, W. (1951). The feeding sequence of Pseudococcus brevipes (CKL.) in relation to mealybug wilt of pineapple in Hawaii. Phytopathology, 41: 769 - 780.

Carter, W. (1949). Insect notes from South America with special reference to *Pseudococcus brevipes* and mealybug wilt, Econ. Entomol. 42: 761 - 766.

Carter, W. (1948).The effects of mealybugs feedings on pineapple plants grown in finely atomized nutrient solutions. Phytopayhology 38: 645 - 657.

Carter, W. and Collins, J L. (1947). Resistance to mealybug wilt of pineapple with special reference to a Cayenne-Queen hybrid. Phytopathology 37: 332 - 348.

Carter, W.(1942). Geographical distribution of mealybug wilt with notes on some other pest of pineapple, J. Econ. Entomol. 35: 10 - 15.

Carter, W. (1934). Mealybug wilt and green spot in Jamaica and Central America, Phytopathology 24: 207 -242.

Carter, W. (1933). The pineapple mealybug, *Pseudococcus brevipes* (Ckl.) and wilt of pineapples, Phytopathology 24: 207 -242.

Carter, W. (1932). Studies of populations of *Pseudococcus brevipes* (Ckl.) occurring on pineapple plants. Ecology 13: 296 - 304.

Casida, J.E. (1987). In Pesticide Science and Biotechnology; Greenhalg, R.; Roberts, T.R., Eds. Blackwell Scientific London p.75 - 80.

Champagne, D.E., Koulo, O., Isman, M.B., Scudder, G.G.E. and Towers, G.H.N. (1992). Biological activity of limonoids from the Rutales. Phytochemistry, 31: 377 - 394.

Chiu, S.F. (1984). The active principles and insecticidal properties of some Chinese plants, with special reference to Maliaceae. Proc. second Int. Neem Conf. (Rauischholzausen, Germany, 1983), pp. 255 - 261.

Collins, J. L. (1960).The Pineapple - Botany, Cultivation and Utilization. Leonard Hill, London p. 248.

Corbertt, G.H. and Pagden, H.T. (1941). A review of some recent entomological investigations and observations, Malaysia Agric. J. 29: 347 - 375.

Dethier, V.G. (1956). Repellents. Ann. Rev. Entomol. 1, 181 - 202.

Dhawan, B.N. and Patnaik, G.K. (1993). Pharmacological studies for therapeutic potential.In Randhawa, N.S., Parmar, B.S. (Eds). Neem research and development. Society of pesticide science, New Delhi, India, p. 242 - 249.

Dorn, A. (1986). Effects of azadirachtin on reproduction and egg development of the heteropteran *Oncopeltus fasciatus* Dallas. Z. Angew. Entomol. 102: 313 - 319.

Dover, M.J. and Croft, A.B. (1984). Getting Tough: Policy and Management of Pesticide Resistance (Washington, D.C.: World Resources Institute). 195th National Meeting of the American Chemical Society, Toronto, Ontario, Canada. June 5 - 11, 1988.

Dyck, V.A.; Thomas, B. (1979). The brown planthopper problem. In Brown Planthopper: Threat to Rice Production in Asia. Int. Rice Res. Inst., Los Banos, Philippines. pp. 3 - 17.

Egunjobi, O.A .and Afolami, S.A.. (1976). Effects of neem (*A. indica*) leaf extracts on populations of *Pratylenchus bruchurus* and on the growth and yield of maize. Nematologica, 22: 125 - 132.

Ermel, K., Pahlich, E., Schmutterer, H. (1987). Azadirachtin content of neem kernels from different geographical locations, and its dependence on temperature, relative humidity, and light. Proc. third Int. Neem Conf. (Nairobi, Kenya, 1986), p. 171 - 184.

Fagoonne, I. (1981). Behavioural response of *Crocidolomia binotalis* to neem. Proc. First Int. Neem Conf. (Rottach-Egern, Germany,1980) p.109-120.

Fujiwara, T., Sugishita, E.Y., Takeda, T., Ogihara, Y., Shimizu, M., Nomura, T.

and Tomita, Y. (1984). Further studies on the structure of polysaccharides from the bark of *Melia azadirachta*. Chem. Pharm. Bull. 32: 1385 - 1391.

Gill, J.S. and Lewis, C.T. (1971). Systemic action of an insect feeding deterrent. Nature (London) 232: 402 - 403.

Goyal, R.S., Gulati, K.C., Sarup, M.A., Kidwai and Singh, D.S. (1971). Biological activity of various alcohol extracts and isolates of neem seed cake against Rhopalosiphum nympheae (L.) and *S. gregaria* Forsk. Indian J. Entomol., 33: 67 - 71.

Gruber, A.K. (1991). Wachstum, Fruchtértrag und Azadirachtingehalt der Samen von Azadirachta indica auf vershiedenen standorten in Nicaraguua. Doctor, thesis,Techn. Univ. of Berlin, Germany.

Guerout, R. (1975). Nematodes of Pineapple: A review. PANS 21: 123 - 140.

Gunasinghe, U.B. and German, T.L. (1989). Purification and partial characterization of a virus from pineapple. Phytopathology 79: 1337 - 1341.

Habte, M., Muraleedhara, B.N., Ikawa, H. (1993). Response of neem to soil P concentration and mycorrhizal colonization. Neem Newsl. 3: July - Sept. , 1- 4.

Haskel, P.T. and Mordue, J.A. (1969). The role of mouthpart receptors in the feeding behaviour of *S. gregaria*. Entomol. Exp. Appl. 12: 591 - 610.

Heyde, J.V.D., Saxena, R.C. and Schmutterer, H. (1984). Neem oil and extracts as potential insecticides for control of homopterous rice pests. Proc. Second Int. Neem Conf. (Rauischholzausen, Germany, 1983), p.377 - 390.

Heyde, J.V.O., Saxena, R.C. and Schumutterer, H. (1980). Proc. 2nd Int. Neem Conf., Rauischholzlhausen, pp. 377 - 390.

Ito, K. (1962). Additional immunological evidence supporting the virus nature of mealybug wilt. Pineapple Res. Inst. News, 10: 158 - 162.

Ito, K. (1938). Studies on the Life History of the Pineapple mealybugs *Pseudococcus brevipes*. J. Econ. Entomol. 31: 291 - 298.

Jackai, L.E.N., Inang, E. E. and Nwobi, P. (1992). The potential for controlling post-flowering pests of cowpea, *V. unguiculata* (L) (Walp.) using neem *A. indica* A. Juss. Tropical Pest Management, 38 (1) 56 - 60.

Jacobson, M. (1989). Focus on phytochemical pesticides. Vol. I, The Neem Tree. C.R.C. Press, Boca Raton, FL.

Jacobson, M. (1986). Roles of allelochemicals. In: Natural resistance of plants to pests. Eds. Green, M.B.; Hedin, P.A.; ACS Symposium Series No. 296. American Chem. Soc. Washington, DC. p.220 - 232.

Jacobson, N. (1981). Isolation and identification of insects antifeedants and growth inhibitors from plants; an overview. Proceedings of the first Int. Neem Conf. (Rottach-Egern, 1980). pp 13 - 24.

Jayaraj, S. (1993). Neem in pest control: Progress and Perspectives. World Neem Conf. (Bangalore, India), Proc., pp. 37 - 43.

Jones, P.S.; Ley, S.V., Morgan, E.D.and Santafianos, D.(1989). The chemistry of the neem tree. In Jacobson, M. (Ed). Focus on phytochemical pesticides vil. 1, The Neem Tree. CRC Press, Boca Raton, Fl. p. 19 - 45.

Kareem, A.A., Saxena, R.C., Boncodin, M.E.N., Krishnasamy, V. and Seshu, D.V. (1989). Neem as seed treatment for rice before sowing: effects on two homopterous insects and seedling vigor. J. Econ. Entomol. 82: 1219 - 1223.

Kenneth, G., Rohrbach and Walter, J.A. (1987). Nematode and disease problems of pineapple. Plant Disease Reporter, 70: 196 - 199.

Kher, A.. and Chaurasia, S.C. (1977). Antifungal activity of essential oils of three medicinal plants. Indian Drugs, 15: 41 - 42.

Kraus, W., Bawmann, S., Bokal, M., Keller, U., Klenk, A., Klingle, M., Pohnl, H., and Schwinger, M. (1987). Control of insect feeding and development by constituents of *M. azadarach* and *A. indica.*

Kraus, W. (1986). Constituents of neem and related species. A revised structure of azadrachtin. Stud. Org. Chem., 26, 237 - 256.

Kumar, M.G., Bhat, P.K. and Ramaiah, P.K. (1989). Potential role of kerosene and neem derivatives in integrated management of mealybug on coffee.

Larsen, L.D. (1988). The commercialization of neem. In: The Neem Tree, (Jacobson, M. , Ed.). Boca Raton, FL: CRC Press. p. 155 - 170

Larson, R.O. (1985). Stable anti-pest neem seed extract, U.S.A. patent No. 4,556,562. Dec. 3, 1985. Official Gazette of U.S. Patents and Trademarks 1: 265.

Larson, L. D. (1960). Diseases of Pineapple. Hawaiian Sugar Planters Assoc. Pathol. Physiol. Ser. Bull., No.10.

Lee, S.M., Klocke, J.A.., Barnby, M.A.., Yamasaki, R.B. and Balandrin, M.F. (1991). Insecticidal constituents of *A. indica* and *M. azadirach.* In Hedin, P.A.. (Ed.). Naturally Occurring Pest Bioregulators. ACS Symposium Series No. 449, American Chemical Society, Washington, D.C., pp 293 - 304.

Leuschner, K. (1972). Effect of an unknown plant substance on shield bug

Naturwissenscaften 59: 217 - 218.

Lowery, D.T.; Isman, M.B. and Brard, N.L. (1993). Laboratory and field evaluation of neem for the control of Aphids (Homoptera: Aphididae). J. Econ. Entomol. 86 (3) 864 - 870.

Maramorosch, K., Guan, T. and Ghosh, B.K. (1984). Virus-like particles associated with pineapple wilt disease. (Abstr.). Proc. Int. Congr. Virol. 6th Ed.

Mariappan, V. and Saxena, R.C. (1983). Effect of mixtures of custard-apple oil and neem oil on survival of Nephtetix virescens (Homoptera: Cicadellidae)and on rice tungro virus transmission. J. Econ. Entomol. 76: 573 - 576.

Mengel, K. (1968). Exchangeable cations of plant roots and potassium absorption by plant. In "The role of potassium in agriculture". American Soc. Agron., Madison, U.S.A., p. 311 - 318.

Metcalf, R. (1980). Changing role of insecticides in crop protection. Ann. Rev. Entomol. 25: 219 - 256.

Mital, H.C. (1971). Protection of cowpea from insect infestation with the aid o fixed oils. J. W. Afr. Serv. Assoc. 16: 45 - 48.

Mitchell, W.; Brandwijk, M.G. (1962). The medicinal and poisonous plants of

Southern and Eastern Africa. Livingstone. London, p. 1457.

Mittler, T.E. (1967). Water tension in plants: an entomological approach. Ann. Entomol. Soc. Am. 60, 1074 - 1076.

Mordue, A.J. and Evans, K.A. (1987). Thephysiological effects of azadirachtin in the locust. In Labeyrie, V., Fabres, G., Lachaise, D.(Eds.) Insect-Plants, p. 43 - 48.

Nicol, C. M.Y.(1993). Influence of neem oil on the fecundity of the desert locust, *Schistocerca gregaria* Mitt. Dtsch. Ges. Allg. Angew Entomol., XXX.

Norton, W. G. and Alwang, J. (1993). Introduction to economics of Agricultural Development. McGraw-Hill series in Agricultural Economics. p. 213.

Olaifa, J. I.; Adedokun, T. A. and Adenuga, A. O. (1991). Antifeedant and growth-regulating effects of neem products on the variegated grasshopper, *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae). J. of African Zoology 105 (2) 157 162.

Parmer, B.S. and Ketker, C.M. (1993). Commercialization. In Randhawa, N.S. and Parmer, B.S. (Eds) Neem Research and Devellopment. Soc. of Pesticide Science, New Delhi, India. p. 270 - 283.

Periera, J. (1983). The effectiveness of six vegetable oils as protectants of cowpea and bambara groundnut against infestation by *C. maculatus*. J. Stored Prid. Res.

19: 52 - 62.

Phylips, J.S. (1934). The biology and distribution of ants in Hawaiian pineapple fields. Expt. Sta. Pineapple Producers Coop. Assoc. Bull., 15: 1 - 57.

Pradaham, S., Jotwani, M.G. and Rai, B.K. (1962). The neem deterrent to locusts. Indian Farming, 12: 7 - 11.

Radwanski, S.A. and Wickens, G.E. (1981). Vegetative fallows and potential value of the neem tree in the tropics. Econ. Bot., 35: 398 - 414.

Rafa, A.F. (1987). Influence of host plant on deterrence by azadirachtin of feeding by fall armyorm larvae (Lepidoptera: Noctuidae). J. Econ. Entomol.80: 384 - 387.

Rai, A.. and Sethi, M.S. (1972). Screening of some plants for the activity against vaccinia and fowl-pox viruses. Indian J. Anim. Sci., 42: 1066 - 1070.

Randhawa, N.S.and Parmar, B.S. (1993). Neem research and development. Society of pesticide science, New Delhi, India.

Raski, D.J. and Krusberg, L.R. (1984). Nematode parasites of grapes and other small fruits, pp 457 - 506. In: Plant and Insect Nematodes. Nickle, W.R. (Ed). Marcel Derkker Inc., N.Y.

Reese, J.C. and Beck, S.D.(1976). Azadirachtin Toxicity to Peridroma saucia.

Ann. Ent. Soc. Amer. 69: 59 - 67.

Rembold, H. (1989). Azadirachtin, their structure and mode of action. In Arson, J.T., Philognene, B.J.R., Morand, P. (Ed.). Insecticides of plant origen. ACS Symp. Ser. 387, American Chem. Soc. , Washington, DC, p. 150 - 163.

Rembold, H., Sharma,G.K., Czoppelt, C. and Schumutterer, H.(1982). Azadirachtin: a potent insect growth regulato of plant origin. Z. angew. Ent. 93: 12 - 17.

Rembold, H., Sharma,G.K., Czoppelt, C. and Schumutterer, H.(1980). Evidence of growth disruption in insects without feeding inhibition by neem seed fractions. Z. Pflanzenkrankh. Pflanzenschutz 87: 290 - 297.

Rice, M. (1993). Development of neem research and industry in Australia. World Neem Conf. (Bangalore, India), Souvenir, p. 8 - 24.

Rohrbach, K.G. and Apt, W.J. (1986). Nematode and disease problems of pineapple. Plant Dis. Rep. 70: 81- 87.

Rohrbach, K.G., Beardsley, J.W., German, T.L., Reimer, N.J. and Sanford, W.G., (1988). Mealybug wilt, Mealybugs, and ants of pineapple. Plant Disease 72 (7) 558

Ruscoe, C.N.E (1972). Growth disruption effects of an insect antifeedant Nature New Biol. 236: 159 - 160.

Sanaa, M.M. (1992). Neem seed extract and powders as grain protectants to cowpea seeds against the cowpea weevil, *Callosobrochus maculatus* Fab. Egypt J. Agric. Res., 7 (2), 487 - 494.

Sanford, W.G. (1998). Mealybug wilt, mealybugs and ants on pineapple. Plant Disease 72:558 - 565.

Saxena, R.C. (1987). Neem oil - a potential antifeedant against insect pests of rice Pesticide Science and Biotechnology. Eds. Greenhalgh, R.; Roberts, J.R. (Eds) Blackwell Scientific, London, p. 139 - 144.

Saxena, K.N. and Rembold, M. (1984). Orientation and ovipositional responses of *Helionthes armigera* to certain neem constituents.

Saxena, R.C., Rueda, B.P., Justo, H.D. Jr., Boncodin, M.E.M. and Barrion, A..A .(1987). Evaluation and utilization of neem seed 'bitters' for management of planthopper and leafhopper pests of rice. Proc. Pest Control Council of the Philippines (Davao City, Philippines, 1987), p. 1 - 43.

Saxena, R.C. and Khan, Z.R. (1986). Safety of Neem to Beneficial Organisms and Man. Entomol. Exp Appl. 42: 279 - 284.

Saxena, R.C. and Basit, A. (1982). Inhibition of oviposition by volatiles of certain plants and chemicals in the leafhopper, Amrasca devastans (Distant). J. Chemical Ecol. 8: 328 - 338.

Saxena, R.C; Waldbauer, G.P.; Liquido, N.J.; Puma, B. C. (1981). Effects of neem seed oil on the rice leaf folder C. medinalis. In Schmutterer, H., Ascher, K.R.S., Rembold, H. (Eds). Natural Pesticides from the Neem Tree (A. indica) and other Tropical Plants, Proc. 2nd Int. Neem Conf. (Rauischholzhausen, Germany, 1983), p. 377 - 390).

Saxena, R.C. and Barrion, A. A. (1987) Cytogenetic effects of neem seed "bitters" (NSB) on green leafhopper males. Int. Rice Res. Newsl. 12: 24 - 25.

Sharma, G.K., Czopelt, H. and Rembold, H. (1980). Further evidence of insect growth disruption by neem seed fractions. Z. Angew. Entomol., 90: 439 - 444.

Schauer, M. (1984). Effects of variously formulated neem extracts on *Acyrthosiphon pisum* and Apis fabae. In Schmutterer, H. and Ascher, K.R.S (Eds). Natural Pesticide from the Neem Tree and Other Tropical Plants. Proc. second Int. Neem Conf. (Rauischholzausen, Germany, 1983), pp. 141 149.

Schmidt, G.H.and Pesel, E. (1987). Studies on the sterilizing effect of neem extract in ants. In Schmutterer, H. and Ascher, K.R.S. (Eds). Natural Pesticides from the

Neem Tree and other Tropical plants. Proc. 3rd Int. Neem Conf., Nairobi, p. 361 - 373.

Schmutterer, H. (1995). The Neem Tree, *A. indica* A. Juss, and other maliaceous plants.

Schmutterer, H. and Doll, M. (1993). The Marrango or Phillippine neem tree, *Azadiracta excelsa*: A new source of insecticdes with growth-regulating properties. Phytoparasitica 21: 79 - 86.

Shumutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, *A. indica*. Ann. Rev. Entomol. 35: 271 - 297.

Schmutterer, H. and Hellpap, C. (1989). Effects of neem on pests of vegetables and fruit trees. In Jacobson, M. (Ed.). Focus on phytochemical pesticides, vol. I, The Teem Tree pp. 69 - 96. C.R.C Press.

Schumuttrer, H.(1987). Insect growth-disrupting and fecundity-reducing ingredients from the neem tree and chinaberry trees. In: Handbook of Natural Pesticides, ed. E.D. Morgan, N.B. Mandava, III (Part B): 119 - 170. Boca Raton: C.R.C Press. 180 pp.

Schmutterer, H. and Ascher, K.R.S. (1987). Natural pesticides from the neem tree and Other Tropical Plants. Proc. 3rd Int. Neem Conf. (Nairobi, Kenya, 1986).

Schmutterer, H. (1986). Studies on the sterilizing effect of neem extracts in ants. I n Schmutterer, H. and Ascher, K.R.S. (Eds). Natural Pesticides from the Neem Tree and Other T ropical plants. Proc. 3rd Int. Neem Conf., Nairobi, p. 361 - 373.

Schmutterer, H., Ascher, K.R.S. and Rembold, H. (Eds) (1981). Natural pesticides from the neem tree. In Proc. first Int. Neem Conf. (Rottach-Egern, Germany, 1980) p. 286.

Schoonhoven, L.M. (1982). Biological aspects of antifeedants .Ent. Exp. Appl. 31: 57 - 69).

Schulż, W.D.; Schluter, U. (1984). Structural damages caused by neem in *E. varivestis*. A summary of historical and ultra structural data II. Tissue affected in adults. Proc. 2nd Int. Neem Conf. (Rauischholzausen, Germany, 1983), pp. 237 - 251.

Schulz, W.D. (1980). Natural pesticides from the Neem Tree. In Proc. 1st Int. Neem Conf. (Rottach-Egern, Germany), p. 351 - 360.

Schwinger, M.; Ehhmmer, B.; Kraus, W. (1984). Methodology of the *E. verivestris* bioassey of antifeedants demonstrated with some compounds from *A. indica* and *Melia azadirach*.

Siddique, S. (1942). A note on the isolation of three neem bitter principles from the neem oil. Curr. Sci. 11: 278 - 279.

Singh, R.P. (1984). Effect of water extract of deoiled neem kernel on 2nd instar larvae of Culex fatigans Wiedmann. Newsl. No.1 2: 16 - 21.

Simmonds, M.S.J. and Blaney, W.M. (1985). Some neurophysiological effects of azadirachtin on lepidopterous larvae and their feeding response. In Schumutterer, H. and Ascher, K.R.S. (Eds) Natural Pesticides from the Neem Tree and Other Tropical Plants, Proc. 2nd Int. Neem Conf. (Rauischholzhausen, Germany), p. 163 - 173.

Singh, B. (1985). Studies on the effect of neem kernel and certain less persistent insecticide the oviposition behavior and mortality of the desert locust, *S. gragaria* (Forsk). Ph. D. thesis, Agra. Univ., India Neem Newsletter 4 (1), 11 - 12 (abstr.).

Svoboda, J.A. and Robins, Z.R. (1971). Azadirachtin Effects on *Melanoplus sanguinipes*. Lipids 6:113 - 119.

Talukder, F.A. and Howse, P.E. (1993). Detrrent and insecticidal effects of extracts of Pithraj, *Aphanamixis porystachyta* (Maliaceae), against *Tribolium castaneum*. Journal of Chemical Ecology, 19: 2463 - 2471.

Ullman, D.E., German, T.L., Gunasinghe, U.B. and Ebesu, R.H. (1989). Serology of a closteroviruslike particle associated with mealybug wilt of pineapple. Phytopathology 79: 1341 - 1345.

Van Der Nat, J.M., Thart, L.A.., Van Der Sluis, W.G. and Labadie, R.P.(1987). Two functionally different immunomodulators from an aqueous bark extract of *A.. indica* A. Juss (Mäliaceae); Pharm. Weekb. Sec. Ed. 9; 224.

Van Der Nat, J.M., Thart, L.A.., Van Der Sluis, W.G., Labadie, R.P. and De Silva, K.T.D. (1991). Activity-guided isolation and identification of *A. indica* bark extract constituents which specifically inhibit chemiluminescence production by activated human polymorphonuclear leukocytes. Planta Med. 57: 65 - 68.

Varmo, R.C. and Pandey, G.P. (1980). Treatment of stored green gram seed with edible oils for pupation *C. maculatus*. Trop. Grain Legume Bull. 17: 8 - 11.

Vickery, M.L. and Brian, V. (1979). Plant Products of Tropical Africa. Macmillan Tropical Agriculture, Horticulture and Applied Ecology Series. p.37

Zebitz, C.P.W. (1986). Effect of three different neem seed kernel extracts and azadirachtin on larvae of five different mosquito species. J. Appl. Entomol. 102: 455- 463.

APPENDIX I

No. of mealybugs counted (screen house experiment).

Age of plants	2	4	6	8	10
Treatment					
Cw	23	13	31	32	40
Co	11	16	32	35	52
NSWE I	16	79	63	52	39
NSWE II	37	25	43	30	27
NSWE III	10	7	3	2	2
NSME IV	4	17	12	8	8
NSME V	4	5	4	2	1
NSME VI	3	2	2	1	0
DI	0	0	0	0	0

APPENDIX II

No. of mealybugs counted (field experiment).

Age of plant (months)	3	4	5	6	7	8
Treatment						
NSME VI	21	25	5	0	1	2
DI	0	1	1	0	0	0
Cw	53	95	77	73	121	149

APPENDIX III

No. of healthy leaves

Age of plant (months)	3	4	5	6	7	8
Treatment						
NSME VI	22	24	30	31	33	29
DI	22	22	26	34	30	28
Cw	24	23	26	26	36	28

APPENDIX IV

No. of wilted leaves

Age of plant (months)	3	4	5	6	7	8
Treatment						
NSME VI	0	0	1	1	3	4
DI	0	0	0	1	3	5
Cw	0	1	5	5	6	4

APPENDIX V

No. of healthy roots

Age of plant (months)	3	4	5	6	7	8
Treatment						
NSME VI	27	31	39	44	71	75
DI	31	40	44	50	51	55
Cw	22	30	41	56	51	67

APPENDIX VII

Analysis of variance for number of healthy roots produced (field experiment).

Source of variation	d.f	s.s	m.s	v.r	F. pr
Treatment	2	95.88	47.94	2.05	0.210
Residual	6	140.28	23.38		
Total	11	246.58			

APPENDIX VIII

Analysis of variance for number of healthy rotten roots produced (field experiment)

Source of variation	d.f	s.s	m.s	v.r	F. pr
Treatment	2	1.803	0.901	26.16	0.001
Residual	6	0.207	0.034		
Total	11	2.547			

APPENDIX IX

Analysis of variance for number of healthy leaves produced (field experiment).

Source of variation	d.f	s.s	m.s	v.r	F. pr
Treatment	2	6.112	3.056	1.18	0.370
Residual	6	15.56	2.592		
Total	11	45.187			

APPENDIX X

Analysis of variance for number of wilted leaves produced (field experiment).

Source of variation	d.f	s.s	m.s	v.r	F. pr
Treatment	2	0.7183	0.359	6.20	0.035
Residual	6	0.3478	0.058		
Total	11	1.4554			

APPENDIX XI

Analysis of variance for number of mealybugs counted (field experiment).

Source of variation	d.f	s.s	m.s	v.r	F. pr
Treatment	8	195.32	24.415	24.91	< 0.001
Residual	36	35.290	0.980		
Total	44	230.612			