Evaluation of neem (*Azadirachta indica A.* Juss) seed extracts for the management of some cocoa mirid species.

By

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DECLARATION

I hereby declare that, except for references to other peoples' work which have been duly cited, this work is the result of my original research and that this thesis has neither in whole nor in part been presented for a degree elsewhere.

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ABSTRACT

Laboratory and field studies were conducted to investigate the toxicity, antifeedant activity, moulting inhibition property and persistence of two neem seed extracts against the cocoa mirids *Distantiella theobroma* (Dist.) and *Sahlbergella singularis* Hagl. The study also investigated the effects of neem extracts on nontarget arthropods. Propoxur (Unden 200 EC) was used for baseline comparison. In the laboratory bioassays, the neem was applied at the concentrations of 5%, 10%, 15%, 20%, 25%, and 30% weight/volume (w/v) (water extract) and 0.77%, 1.54%, 3.08%, and 4.62% (neem oil).

The neem extracts acted mainly as antifeedants and stomach poisons against the mirids. The 20% neem extract provided the greatest antifeedant effect (84.3%) and gave the highest nymphal mortality (93.3%) 30 hours after treatment. Moulting was significantly inhibited (>50% inhibition) by the neem oil. LC_{50} values estimated from regression equations were 8.17% and 2.67% for the water extract and neem oil, respectively. LT_{50} values for the 20% neem seed water extract and 4.62% neem oil were 16 and 24 hours respectively. LC_{50} for propoxur is 2.73×10^{-3} % (Marchart, 1971).

In subsequent experiments, the 20% neem seed extract and 0.3% and 3.0% neem oil were tested in the field. Two application methods, spraying a tree from one side of the trunk into the canopy (T1 method), or spraying from both sides of the tree (T2 method), were compared using the "Urgent" GmbH motorised knapsack mistblower at the 2nd and 3rd nozzle restrictor positions.

When the mistblower was operating at the 2nd restrictor position, the resulting application rates were 75 and 480 litres/ha for the T1 and T2 methods, respectively. Corresponding application rates at the 3rd restrictor position for T1 and T2 methods

were 100 and 640 litres/ha. Results from the field study confirmed the short residual activity of neem observed in the laboratory. Reduction in mirid numbers after 48 hours of treatment at the 2nd restrictor position were 80.3%, 51.7% and 80.4% for the 20% neem seed water extract and 0.3% and 3.0% oil treatments, respectively using the restrictor 2. Restrictor 3 gave 88.9% reduction in mirid numbers after 48 hours of treatment. No significant difference in reduction in mirid numbers was found between the two different restrictor settings ($P = 0.05$) within 48 hours.

When trees were sprayed from two sides, a relatively better result was obtained than when they were sprayed from one side only. The addition of 4.5 ml propoxur per litre of neem extract increased efficacy significantly. The T2 method gave a more prolonged control of the mirids as the population did not recover even after two weeks of treatment. The more enhanced spray coverage achieved using the T2 method was therefore promising. Current trend of pesticide application technology however, is towards low volumes of application. For this reason, application of neem and propoxur mixture, using the T1 method (75 liters per ha.) may offer a more practical approach of mirid pest management especially in small scale farming systems. Neem was observed to be toxic to some nontarget insects, particularly ants, and spiders. Further investigation into neem/propoxur combinations and hazardous effects on nontarget species is suggested.

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To my grandmother Felicia Akosua Dansoah

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CHAPTER 1

1.0 GENERAL INTRODUCTION

The cocoa mirids, *Distantiella theobroma* (Dist.) and *Sahlbergella singularis* Hagl. are the most important pests of cocoa in West Africa (Entwistle, 1972). Annual crop loss attributed to mirids is estimated between at 25 and 30% of total yield (Stapley and Hammond, 1959.

The main method of mirid control in cocoa is by the application of chemical insecticides. Insecticides are largely nonspecific and may be harmful to many beneficial insects and the environment. Development of resistance in insect pests to insecticides, for example, resistance of mirids to lindane in Ghana in the early 1960s (Dunn, 1963), spray drift onto adjacent agricultural lands and contamination of water bodies are also hazards of indiscriminate use of chemicals.

Compounds from plants that have proven to be effective in insect pest control include the precocenes from *Ageratum houstomianum* Mill., ryania from *Ryania speciosa* Vahl., caffeine, and nicotine from tobacco among others (Coats, 1994). Antifeedants also offer a potential approach for pest management by rendering treated plants unattractive or unacceptable to insect pests (Saxena and Khan, 1987).

Azadirachtin, $C_{37}H_{48}O_{13}$ (Reed *et al.*, 1982; Green *et al.*, 1987) is one of the main triterpenoids from the neem tree (*Azadirachta indica* A. Juss) whose antifeedant properties has been well-documented (Butterworth and Morgan 1971; Ruscoe, 1972; Rembold *et al.*, 1980; Redfern *et al.*, 1981; Simmonds *et al.*, 1995). Water extracts of leaves and seeds of the neem tree are used in many developing countries as protectants against many economically important crop pests (Heyde *et al.*, 1984) and it was supposed that neem seed extracts could as well be effective against cocoa mirids. The fact that no previous work has been done to verify the efficacy of neem against tree crop pests

therefore provided a valuable opportunity for testing biopesticidal activity of neem against cocoa mirids. This research was undertaken with the following objectives:

1. to evaluate the potency of neem (a water extract and a formulated product, "bioprodotto") against cocoa mirids,
2. to evaluate the biological efficacy of different spray nozzles and application methods using neem extracts.
3. to assess any adverse effects on nontarget arthropod species.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The economic importance of cocoa:

Cocoa (*Theobroma cacao* L.: Sterculiaceae) originated from the Andes in the upper Amazon basin (Mossu, 1992). The crop was introduced into Ghana by Tetteh Quarshie from Fernando Po in 1879 (Hammond, 1962) and it has since become the main foreign exchange earner for the country. It is also an important crop in other West African countries, as well as in South America and the Far East. Indeed, Ghana was the world's leading cocoa producer, and maintained her world leadership from 1910 to 1977, when she was overtaken by La Cote d'Ivoire and Brazil (Gill & Duffus, 1989). In 1965/66, production reached an all-time peak of 580,000 tonnes. However, in the late 1970s and early 1980s, production fell to a very low level - 159,000 tonnes in 1983 (Gill & Duffus, 1989). The major causes of this downward trend were: decline in producer price, high incidence of pests and diseases, unavailability of such production inputs as insecticides, fungicides, fertilizer, pesticide sprayers and labour (The Cocoa Sector, 1983).

The Cocoa industry provides employment opportunities for a large number of Ghanaians and generates large amounts of foreign exchange which is used to finance various development programmes. In the 1992/93 crop year, the total cocoa output was 312,123 metric tonnes out of which total export was 273,249 tonnes. Total FOB (free on board) value for the period was equivalent to C165,357,425,217.91 (COCOBOD *Ann. Rep.*, 1993).

Cocoa is used for the manufacture of beverages, industrial alcohol, soft drinks, marmalade, animal feed (from cocoa pod husk), toilet soap, and cosmetics (from cocoa butter).

2.2 The cocoa plant:

Cocoa is a tree in the "C" storey of the evergreen tropical rain-forest. Its status as an under-storey tree perhaps led to the belief that cocoa must be cultivated under shade (Purseglove, 1984). Cocoa grows mainly at low elevations below 300 meters at optimum temperatures of 21-32°C and annual rainfall of 1,300-3,000 mm. In addition, cocoa requires a deep, well-drained, well-aerated soil with good crumb structure and adequate supplies of water and nutrients (Purseglove, 1984). Recent cultivation of cocoa under limited or no shade has resulted in the build-up of pests and diseases (Owusu-Manu, Personal communication). Over 1500 insect species are known to feed on cocoa but only a few are of economic importance (Entwistle, 1972). These fall into three main categories: (1) the mirids which are world- wide; (2) insects which attack the young tree which after maturity is no longer susceptible e.g. the cocoa "bollworm" (E*arias biplaga* Wlk.) and (3) insects which are important mainly as vectors of diseases e.g. the mealybugs (*Planococcus citri* (Risso), *Planococcoides njalensis* (Laing) and *Ferrisia virgata* (Ckll) which transmit the cocoa swollen shoot virus (CSSV) (Entwistle, 1972).

2.3. The cocoa mirid:

Mirids are perhaps the most destructive insect pests of cocoa in West Africa and though the mealybug vectors of swollen shoot disease have been considered important in this respect, their devastations have not been as widespread as those caused by the mirids (Johnson, 1962). Four mirid species commonly feed on cocoa in W. Africa to the extent of being considered pests. These are: *Distantiella theobroma* (Distant) (Fig. 1 a), *Sahlbergella singularis* Hagl. (Fig. 1 b), *Bryocoropsis laticollis* Schum and *Helopeltis*

Spp. *D. theobroma* and *S. singularis* are the widely distributed and most damaging pests; *S. singularis* seems to occupy the whole of the W. African cocoa growing regions (from Sierra Leone to Cameroon) while *D. theobroma* occurs in the central part of the region - from Eastern Cote d'Ivoire to Western Nigeria (Entwistle and Youdeowei, 1964).

Mirids have been known as serious pests of cocoa in Ghana since 1908 (Dungeon, 1910) and because of their devastating effects on the plant, the local farmers called them "Sankonuabe" which literally means "go back to planting oil palms" (Johnson, 1962).

2.3.1 **Temporal distribution of cocoa mirids:-** Studies have shown that on the average, the population of mirids is low from February to July and high from August to January (Williams, 1953; Johnson, 1962) with a peak in November. A second peak usually occurs in January and / or February. The earlier peak is on pods and after harvesting *S. singularis* becomes widely dispersed while *D. theobroma* moves to shoot tips in the area (Johnson, 1962). The population of both species decline in late February due to low humidity. It is also possible that combined effect of predators and parasitoids may be significant (Collingwood, 1968). Control measures based on this cycle on Amelonado Cocoa has worked very well until quite recently when it has been fund necessary to review mirid temporal distribution on hybrid cocoa. Hybrid cocoa bears throughout the year and there is some indication that this has affected temporal distribution of mirids Mirids, in the field, are highly aggregated thus, few trees within an area may have mirids on them.

2.3.2 **Parasitoids and predators of mirids:-** Cocoa mirids are subject to attack by many insects and spiders. The principal predators are the reduviids, mantids and salticid spiders (Squire, 1947). Predatory ants are mainly *Oecophylla* spp., *Crematogaster* spp., *Macromischoides* sp., *Pheidole* sp., *Camponotus* sp. and *Platythyrea* sp. (Leston, 1970). *Crematogaster* spp. and *Pheidole* sp have, however, been observed to be associated with cocoa swollen shoot disease by attending the mealy bug vectors. Parasitism of *S. singularis* by the braconid wasp *(Euphorus salbergellae* Wlk), is of minor importance. Though over 20 per cent parasitism has been recorded, the average is about 0.2% (King, 1971; Kumah, 1975). *D. theobroma* appears to have no record of consistent parasitism. A hymenopteran, *Encyrtus cotterelli* Wtstn. (Encyrtidae), was bred from a third instar nymph of *D. theobroma* by Waterston (1922) at Mampong in Ashanti, and has not been recorded since.

2.3.3 **Alternative host plants:-** Alternative host plants of mirids have been listed by Entwistle and Youdeowei, 1964; Padi, *et al.*, 1996, Appendix 1). *S. singularis* has 17 alternative hosts including several *Cola* species whilst *D. theobroma* is found on citrus, the silk cotton tree *Ceiba pentandra* L. Family : Bombacaceae and the baobab *Adansonia digitata* L., (Bombacaceae).

2.3.4 **Life cycle and damage:-** The adult female lays her eggs beneath the bark of an unhardened stem or in the cortex of the pod with only two fine filaments showing from the surface. The incubation period of the egg varies from 13 - 18 days (Johnson, 1962). There are five nymphal instars, each lasting about 3-5 days. Under laboratory conditions, a female begins to lay eggs 7 days after emergence and may lay up to 30-40 eggs. Adult longevity in the insectary is about three weeks (Raw, 1959).

Adults and nymphs of *D. theobroma* and *S. singularis* feed on unhardened tissue such as developing pods and chupons. The insect inserts its stylets into the plant tissue and saliva is injected into the plant. Dissolved cell contents are sucked back by the insect, leaving a small sunken water-soaked area known as a feeding lesion. It is usual, however,

for stem lesions to become infected with parasitic fungi notably *Calonectria rigidiuscula* Berk and Br. (Sacc.) (Crowdy, 1947; Goodchild, 1952) causing die-back disease.

Mirid damage (Fig. 2) starts when there is a break in the canopy which occurs from falling trees, cutting of swollen shoot diseased trees or from poor canopy development (Williams, 1953). Canopy breaks attract mirids and may become foci for local population build-up. Pockets so initiated increase light penetration and other conditions that lower humidity especially in the dry season. Regenerative shoot growth during the rainy season renders the pockets more attractive so that the same groups of trees become heavily attacked year after year while the area of the degraded pocket increases. Although the population of mirids is generally low the damage done is often very severe (Entwistle, 1965). This is because the damage is most obvious after peak numbers. It has been estimated that 6 mirids per 10 trees must be considered the economic threshold (Owusu-Manu, 1997). Where damage covers a large area of exposed cocoa in the form of fan shoot death, the condition is referred to as "blast". If the more persistently attacked trees have their crown extensively reduced by die-back, the condition is described as "stagheaded" (Williams, 1953). *B. laticollis* and *Helopeltis* spp feed mainly on pods but their feeding punches are superficial and are therefore considered as minor pests (Raw, 1959).

Annual crop loss through mirid damage has been estimated at 25 per cent for

Ghana (Stapley and Hammond, 1959) at the seedling stage. The effect of damage results entirely in reduction of potential pod production. Affected seedlings may fail to become established and where seedlings are not killed outright, mirid damage may delay bearing by several years (Johnson, 1962).

2.4. Chemical control of mirids in Ghana :

The need for routine chemical control was first recognized by Dungeon in 1909 (Dungeon, 1910) when he used kerosene/soap emulsion as stem paint. Cotterell (1943) tested nicotine sulphate, lime sulphur and found nicotine sulphate effective at 0.1 per cent while lime sulphur caused leaf scorch even at low concentrations. However, the use of nicotine sulphate was discontinued because it was found to be expensive and toxic to mammals. When dichlorodiphenyl trichloroethane (DDT) and lindane (Gammalin) were tested, the latter gave a better control of mirids than DDT and was subsequently recommended in 1957 (Hammond, 1957).

The large scale use of lindane had its adverse effect when in 1962, some populations of *D. theobroma* were found to be resistant to it in the Pankese area (Dunn, 1963). The search for substitutes to lindane was, therefore, directed towards the carbamate and organophosphorus insecticides (Collingwood and Marchart, 1971) and later the pyrethroids (Owusu-Manu, 1985). Comparative tests proved the carbamates to be more suitable for mirid control than the organophosphates as many combine high miridicidal activity with low mammalian toxicity (Collingwood and Marchart, 1971).

Pyrethroids, on the other hand were found to be expensive to use on cocoa (Owusu-Manu, 1985). Currently, spray treatments with lindane at 280 g a.i./ha, promecarb (Carbamult) at 280 g a.i./ha and propoxur (Unden) at 210 g a.i./ha are the recommended insecticides in Ghana (Owusu-Manu, 1995). These insecticides are

alternated every two years so as to reduce the possibility of the mirids developing early resistance to any of them.

2.4.1 **Application equipment and method:-** The choice of a suitable application machinery and method is very important in chemical pest control programmes. The efficacy of any pesticide partly depends on the pesticide being effectively distributed on the target. The application equipment should be able to deliver the toxicant to achieve a very good coverage on the cocoa tree (Omole and Ojo, 1982).

The standard method of pesticide application on mature cocoa in Ghana is by motorized knapsack mistblowers. In neighbouring countries of Nigeria, La Cote d'Ivoire and Cameroon, the hydraulic compression sprayer and other smaller hand-operated types are used (Omole and Ojo, 1982). In Cameroon, hand-held fogging device (the swing fog) is used as the standard method and good mirid control is claimed (Bruneau de Mire, 1966). This method of application has the advantage of covering large areas quickly but all treatments should be applied in the early morning when the smoke is carried up evenly and held in the canopy for a sufficient length of time to give an effective kill. Generally three methods of application have been developed in Ghana; these are: (1) covering a tree thoroughly on one side from the trunk into the canopy (T1 method); (2) covering both sides of the tree with the insecticide (T2 method) and (3) directing the nozzle upwards behind the operator (the Gammalin method) (Peterson *et al.* 1966; Marchart, 1971).

2.4.2 **Spray timing:-** Early workers recommended two sprays in June and July followed by two additional sprays in November and December (Hammond, 1957). Later trials demonstrated that plots having this recommended schedule had higher mirid population build-up between August and November than the series of plots where four sprays were

applied between the period August and December (Collingwood and Marchart, 1971). Currently, four applications are done from August to December at 28 day intervals leaving out November for pod harvesting. This recommendation is to ensure that treatment coincides with the main period of mirid population increase (Collingwood, 1969; Owusu-Manu, 1973). Present studies in Ghana have demonstrated that two treatments a

year, in September and October, could give effective control of mirids for the rest of the year (Owusu-Manu, 1997).

2.5 Side effects of toxic chemicals in pest management:

Concern is being expressed worldwide about the widespread use of chemicals for pest and disease control. Some problems which have become apparent with total reliance on broad spectrum insecticides include:

i. Elimination of beneficial natural enemies: the contribution of these natural enemies in pest management is often eliminated by insecticides. For instance the use of dieldrin on cocoa for the control of the mealybug, *P. njalensis* was followed by excessive damage by the pod borer *Marmara* spp. and the stem borer *Eulophonotus myrmeleon* Fldr. (Entwistle et al., 1959). The pod feeders *Pseudotheraptus devastans* (Dist.) (Lodos, 1967) and *Bathycoelia thalassina* (H-S) (Owusu-Manu, 1975) rose to major pest status where carbaryl and Gammalin, were used respectively, to control mirids. Major imbalances may be caused in the ecosystem by the use of some persistent contact insecticides.

ii Resistance to pesticides: the widespread and indiscriminate use of insecticide may lead to development of resistance in insect populations (e.g. mirid resistance to lindane - Dunn, 1963). This results in increased dosages being used at greater expense and with severer effect on beneficial natural enemies.

iii. Hazardous nature of pesticides: poor formulation, improper storage and packing or mis-use of pesticides may pose danger of toxicity especially amongst illiterate farmers who form the bulk of the farming community in Ghana. There is also the danger of unacceptably high pesticide residues in food and feed.

2.6 Development of IPM system for cocoa mirids:

Attention should be given to the development of environmentally safe and sustainable strategies for use at the farmer level. For example, the selection of *T. cacao* varieties resistant or tolerant to mirids, black pod, and CSSVD would be advantageous. Recently, methods of reducing the number of spray frequency, and the development of safer and biodegradable alternatives such as semiochemicals are being studied at C.R.I.G. (Padi and Hall, 1994; Owusu-Manu, 1995). Numerous plant species possess compounds with insecticidal activity some of which are readily extracted, synthesised and formulated for field application (Coats, 1994). Nicotine from tobacco for example is toxic to insects as well as other organisms including man. The pyrethrins originally extracted from chrysanthemum flower (e.g. pyrethrum) and later synthesized from petroleum derivatives (e.g. Allethrin) are widely used as household insecticides because of their relatively low mammalian toxicity combined with a rapid "knockdown" effect.

The leaves, fruits and the seeds of the neem tree, *Azadirachta indica* A. Juss (Meliaceae) contain active triterpenoids including azadirachtin, salannin (Reed *et al.*, 1982) (Fig. 3), nimbin, deacetylnimbin and thionemone (Simmonds *et al.*, 1992). Home-made formulations of neem extracts have been used for field pest control by farmers in India and some third world countries. Recent studies have demonstrated that neem extracts and neem-derived compounds were effective against as many as 198 different insect species (Saxena, 1988). Margosan-O, Azatin, RH-999 (PT), Neem PT1-EC4

(Sundaram and Sloane, 1995), Neemark (Bhathal and Singh, 1994), RD 9-Repelin (Mansour *et al.*, (1993), Neem Azal-F (Dimetry and El-Hawany, 1995) and Bioprodotto are examples of recently developed industrial formulations of neem. Warthen *et al.*, (1978) reported that azadirachtin isolated from ethanolic extracts of neem seeds inhibited the feeding of the fall armyworm, *Spodoptera frugiperda* (Smith). A similar effect has been reported on the

diamond-back moth *Plutella xylostella (L.)* (Tan and Sudderuddin (1978). Kareem *et al.*, 1989) observed that when fed on rice seedlings raised from seeds treated with 2.5% neem kernel extract or with 2% neem cake, fewer *Nephotettix virescens* (Distant) and *Nilaparvata lugens* (Stal) nymphs reached the adult stage.

In a study to test various plant extracts against stored grain insects, Jilani and Malik (1973) found the neem to possess the maximum repellency effect. Flour beetles (*Tribolium* spp) fed on neem-impregnated flour failed to reproduce and their feeding activity was greatly reduced. Neem Azal-F, a commercial product of neem seed kernel extract with 5% azadirachtin content was reported to deter feeding of adult and first instar nymphs of the cowpea aphid, *Aphis craccivora* Koch (Dimetry and El-Hawany, 1995). The extract also had aphicidal and growth inhibiting effects. Another commercial products of neem, Neemark, was found to deter feeding in the hairy caterpillar *Spilosoma obliqua* Walker. Antifeedant activity relative to the control ranged between 6.7% (at the lowest concentration of 0.313%) and 86% at the highest concentration of 5% (Bhathal and Singh, 1994).

In Togo, a methanol suspension of neem leaves (2-4%) was found to be as active as the synthetic insecticides Mevinphos (0.05%) and Deltamethrin (0.02%) against the diamond- back moth, *P. xylostella* (Dreyer, 1987). Field trials in Thailand have shown that piperonyl butoxide added to neem extract increased the efficacy of the neem and the

combination was as active as cypermethrin (0.025%) against *P. xylostella* and *Spodoptera litura* F. (Sombatsiri and Tigvattanont, 1987).

A comparative toxicity study by Kirsch (1987) in the Philippines using neem and BAY SIR 14591, an insect growth regulator, showed that the hormone was marginally better than the neem in the control of *Heliothis* spp on tobacco and *P. xylostella* on cabbage. Margosan-O has been reported to inhibit larval feeding in the cabbage white butterfly, *Pieris brassicae* (Linn) (Luo *et al.*, 1995). Simmonds *et al.*, (1995) used behavioral and electrophoretical bioassays to evaluate antifeedant activity of azadirachtin and 56 azadirachtin analogues against larvae of the armyworm *S. littoralis*. They found that none of the analogues was as active as azadirachtin although many showed significant activity at higher concentrations.

Under laboratory and field conditions, neem seed oil and neem seed extract have been found to be as effective as the pyrethrum for the control of aphids on pepper and strawberry (Lowery *et al*, 1993). Jacobson *et al.*, 1978) reported that water extracts of neem seeds were promising as antifeedants against the cucumber beetle *Acalymma vittatum* (F.). Similar results have been reported for the desert locust, *Schistocerca gregaria* (Forksål) (Gill and Lewis, 1971) and the variegated grasshopper, *Zonocerus variegatus* L. (Olaifa and Akingbohungbe, 1987).

Fecundity inhibitory effect of azadirachtin against the confused flour beetle, *Tribolium confusum* J. du Val was reported by Hu and Chiu (1993) and against the pea aphid *Acyrthosiphon pisum* (Harris) by Stark and Rangus (1994). Insecticidal activity of extracts prepared from neem leaves or seeds has been reported against larvae of *Maruca testulalis* (Geyer), the legume pod borer, the cowpea coreid bug *Clavigralla tomentosicollis* (Stål.) (Jackai *et. al.*, 1992) and *Z. variegatus* (Olaifa and Adenuga, 1988). In the savanna zone of Nigeria, neem plantations cropped to pearl millet, and

sorghum had lower grasshopper populations than either grazing land or plantations of *Acacia arabica* L. (Amatobi *et al.*, 1988). In Ghana, neem extracts were found to effectively reduce damage by insect pests of the egg plant, okra and cowpea (Cobbina and Osei Owusu, 1988; Tanzubil, 1992; Afreh-Nuamah, 1995).

Micro-organisms have been isolated from neem leaves and from the endosperm of the seeds by Gupta (1992), who observed an antifeedant property when cabbage leaves treated with the micro-organisms were offered to the desert locust *S. gregaria.* An emulsified suspension of this micro-organisms was also reasonably effective against *Lipaphis erysimi* (Kalt.) infesting ornamental plants. It was, however, thought that the micro-organisms were photolytic as the toxic effect was only found in sunlight.

Stark and Rangus (1994) have observed that azadirachtin in the form of Margosan-0 reduced population of the pea aphid *A. pisum* in a concentration dependent manner. These authors noted that at a concentration equivalent to 100 mg/litre of azadirachtin, population increase was about 3.5 times less than in the control. In more detailed studies, azadirachtin significantly reduced the number of moults, longevity and fecundity of *A. pisum* reared on treated *Vicia faba* L. (faba bean) plants.

As with chemical insecticides, insects have developed resistance to azadirachtin (Feng and Isman, 1995). In their study to select for resistance to azadirachtin, the authors treated repeatedly two lines of the peach aphid, *Myzus persicae* (Sulzer) of the same origin with either pure azadirachtin or a neem seed extract at the equivalent concentration of the azadirachtin. After 40 generations, the azadirachtin selected line had developed 9-fold resistance to azadirachtin compared to a non-selected line, whereas the neem seed extract selected line did not. It was suggested that a blend of active constituents in a botanical insecticide such as neem might diffuse the selection process, mitigating the development of resistance. The above review underscores the need to explore the

potential in the neem tree for use in sound pest management strategies for the control of cocoa insect pests.

1 a. Adult female, *D. theobroma* 1 b. Adult female, *S. singularis*

Fig. 1: Two important cocoa mirid species from Ghana
(After Taylor, 1954).

Fig. 2: Mirid damage in a "capsid pocket".

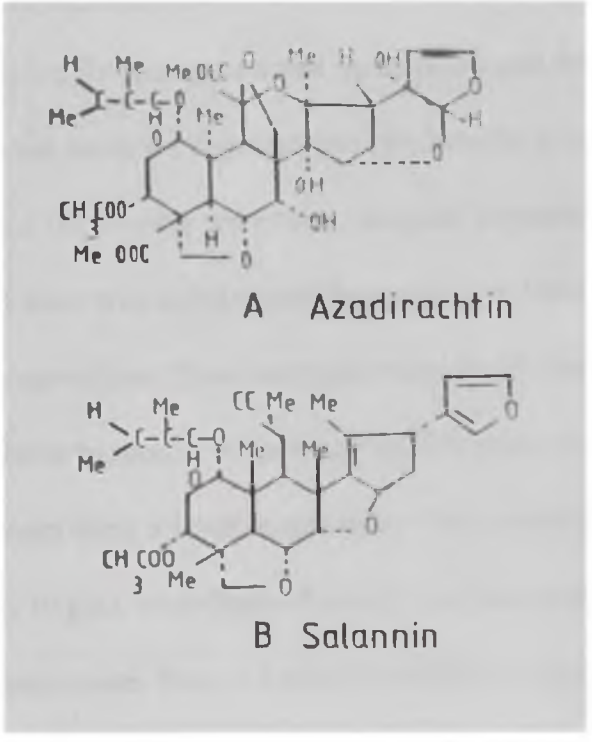

Fig. 3: Chemical structures of two triterpenoids, azadirachtin and salannin from neem. (After Reed *et al.* 1982)

CHAPTER 3

3.0 LABORATORY EVALUATION OF NEEM AGAINST *D. THEOBROMA*

3.1 Materials and methods:-

3.1.1 **Preparation of neem seed water extract and insecticide formulation:-** Neem seeds used in the present study were obtained from Accra and Kordiabe in the Greater Accra and Eastern Regions of Ghana respectively. The seeds were washed and dried in the sun for 10 days (Fig. 4) before packing into sacks or baskets. For each of the ten days, the seeds were exposed to the sun for 4 hours.The drying ensured that seeds were kept in good condition for storage to avoid germination and deterioration.

The dry neem seeds were ground into fine powder using an electric blender. 5, 10, 15 and 20 g of the powder were then weighed separately into 250 ml-capacity beakers. Distilled water was added to each beaker to give 100 ml of the estimated 5, 10, 15 and 20% w/v suspensions. These were left overnight for about 15-17 hours to ensure infusion of the active ingredient of the neem into the water medium. The column was filtered after 24 hours using a 1 mm mesh sieve. The control comprised distilled water only. Propoxur (210 g a.i. in 56 litres of water) was also prepared alongside the neem preparation for comparison. Thus, 1.8 ml of formulated propoxur was measured with a micropipette into a volumetric flask and topped with distilled water to 100 ml.

3.1.2 **Collection of test insects :-** Mirids were collected from Cocoa Research Institute experimental plot N17 at Tafo between August and September 1995. The samples were held at 29.0 ± 2.0°C in the insectary until use. During this time, they were fed on fresh chupons and young pods. Those insects that were injured during collection were either found dead or moribund after 24 hours and were discarded. At Tafo, *D. theobroma* was more common than *S. singularis* and for this reason, *D. theobroma* nymphs were used for all the laboratory studies.

Fig. 4: Drying of neem seeds.

3.1.3 **Toxicity test:-** Experimental cages were modified 450 ml capacity plastic containers (11.5 cm top diameter x 9 cm bottom diameter x 6 cm height) with 2 cm x 3 cm "windows" in the lids for ventilation (refer to diagram A of Fig. 5). "Windows" were covered with fine mesh screens. Each cage was lined with a 9 cm diameter Whatman filter paper. One or two cherelles supported by office pins were placed in each container as a source of food. A thin layer of silicone fluid was smeared around the rim of each container to prevent the insects from climbing out. Two experimental procedures were used to assess the toxicity of neem seed water extract on the mirids. 10 mixed population of 3rd, 4th and 5th instar nymphs of *D. theobroma* were either treated directly while on the pods or were placed on treated pods. Spraying was done using a hand-held mist applicator at 0.85ml of the solution per treatment. Each dosage level was replicated three times. Propoxur was included as a standard for comparison because it is one of the recommended insecticides for cocoa mirid control in Ghana. Control insects were treated with distilled water only. The set-up was held at 26.0 ± 1.0°C and mortality was recorded at hourly intervals for the first 12 hours and subsequently from the 24th, to the 30th hour. An insect was considered dead when it showed no sign of movement when lightly touched with a camel's hair brush or when it was found lying on its back without kicking.

Higher concentrations of neem extracts, 25 and 30% were also evaluated using mixed population of 3rd, 4th and 5th instars of *D. theobroma*. Mortalities and pod lesions were recorded after 30 hours. The experiment was repeated using "Bioprotto", a new oil formulation of neem (4% active ingredient) developed in Germany. The oil was tested at 0.77%, 1.54%, 3.08% and 4.62% using "Teepol" (liquid soap) as spreader. The capacity for spreading in water was demonstrated by a preliminary study which indicated good spreading capacity when the oil and "Teepol" were mixed in ratios of 10:3 respectively. Each dosage was replicated three times.

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Fig. 5: Experimental cages.

3.1.4 **Effect of neem on moulting:-** The effects of different concentrations of neem oil (0.77%, 1.54%, 3.08% and 4.62%) on moulting of *D. theobroma* was investigated by feeding 20 mixed populations of 3rd, 4th and 5th instar nymphs of *D. theobroma* on treated pods. The degree of moulting was determined from the numbers of exuviae cast after 30 hours of exposure. There were 3 replications.

3.1.5 **Neem extract antifeedancy test:-** This test was conducted to investigate antifeedant effect of neem seed extract on cocoa mirids as has been reported for other insect species (Butterworth and Morgan, 1971; Schmutterer, 1990). A cocoa pod was arbitrarily divided into two regions, proximal and distal ends and one end was treated with the 20% neem seed water extract. The other end was left untreated. In order to obtain a fair comparison of feeding lesions in the treated and untreated portions of the pods, the ends that were treated were interchanged so as to eliminate or minimize bias. Youdeowei (1968) had observed that over 60% of mirid feeding occurs on the peduncular end of the cocoa pod. 10 insects were released per pod and feeding lesions were counted after 24 hours and percent inhibition of feeding* calculated. There were three replications.

* % feeding inhibition = 100 – (lesions in treatment x 100)/(lesions in control).

3.1.6 **Residual toxicity test:-** 20 young cocoa pods growing under natural conditions were sprayed to drip point in the field with 20% neem extract using a hand-operated sprayer to assess the residual toxicity of the neem extract. Sprayed pods were harvested at intervals of 24, 48, and 72 hours after application, and one pod put into each plastic container. 10 mixed populations of 3rd, 4th and 5th instar nymphs were placed on each treated pod, and mortality count made 72 hours after exposure. The experiment was replicated three times.

3.1.7 **Fumigant action of neem seed extract:-** This experiment was carried out to determine whether the vapour emanating from the neem extract was repellent and contributed the the death of the mirids. The test apparatus was made from two plastic containers (refer to containers labelled B in Fig. 5). Each of the lower containers was filled with 100 ml of 20% neem seed extract which was then covered with 1 mm mesh cloth, held in place by sellotape. 10 mirids were placed on the mesh screens of each container together with pieces of cocoa chupons before covering with another container. The number of mirids that moved into the inverted container was recorded at 30 minutes intervals for 12 hours. Mortality was recorded after 30 hours.

3.1.8 **Stability of neem extract in storage:-** The prepared neem seed water extract (20% w/v) was stored under the laboratory condition (26.1 ± 1.2)°C and its potency was assessed after one, two and three days. Mixed populations of 3rd, 4th and 5th instar *D. theobroma* were confined on treated pods for 30 hours after which mortalities were recorded as described in section 3.2.3.

3.2.0 **Experiment to determine the efficacy of neem seed extracts obtained from neem seeds of different moisture levels:-** Neem seeds dried to the following moisture cotents, 24.3%, 14.2%, 11.8% and 7.7%, were used in the preparation of water extracts (20% w/v) for testing against 3rd, 4th and 5th instars of *D. theobroma.* Insects were exposed for 30 hours. Moisture contents were estimated from four groups of 50-g samples of neem seeds taken from the same seed stock which were weighed before and after oven-drying at 95°C for 48 hours. The per cent moisture content (fresh weight basis) was calculated as:

$$\frac{\text{(Initial weight - Final weight) X 100}}{\text{(Initial weight)}}$$

3.3 **Statistical analysis**

Percent mortality in the controls were corrected for using Abbott's formula* (Abbott, 1925). Percentages were transformed to arcsine values to meet ANOVA assumptions of normality and homogeneity of variances. Statistical analysis of the data by the analysis of variance technique was carried out according to procedures outlined by Steel and Torrie (1980). Number of pod lesions in the antifeedancy study were analysed by Chi-square analysis (X^2). Concentrations and time required for 50% mortality (LC_{50} and LT_{50}, respectively) were determined by linear regression relationships (Lowery *et al.* 1993).

* Corrected mortality =

$$\frac{\text{Percent mortality in treatment} - \text{percent mortality in control} \times 100}{100 - \text{percent mortality in control}}$$

3.4 **Results**

3.4.1 **Toxicity of neem to *D. theobroma*:** Results of the pre-treatment test, when the insects were on pods before treatment, and the post-treatment test, when the insects were confined to treated pods were not significantly different at (P=0.05) and for this reason, data for the two experiments were pooled for the analyses; regression coefficients were 3.73 ann 4.27, respectively. The results indicate that both the crude extract and oil formulation of neem were moderately toxic to 3rd, 4th and 5th instar nymphs of *D. theobroma*. The 20% neem extract caused 93% mortality while the 4.62 % neem oil caused 80% mortality (Tables 1 and 2). The LC_{50} values were 8.17% and 2.67% for the neem extract and oil formulation respectively. LT_{50} values for the 20% neem extract and 4.62% oil were 16 and 24 hours, respectively (refer to Figs. 6-9 for Probit response / curves). Estimated LC_{50} for propoxur is 2.73 x 10^{-3}% (Marchart, 1971).

Mirids exposed to the neem treatments died rather slowly. Death rarely occurred before the fourth hour after treatment. In consequence, mortalities were generally low

(i.e. less than 40%) during the first 20 hours after treatment (Appendix 2). After the 20th and 24th hours, however, the hourly mortality rates rose sharply in the neem oil and neem seed water extract treatments, respectively. It was also observed that before death, mirids became less mobile and showed reduced activity for periods ranging between 1-5 hours. During this time, they did not feed but occasionally made sluggish movements. It was observed that when mirids were treated with the neem extracts, they dropped their antennae prior to death. Tremors of the legs were also observed. Increasing the neem concentration to 25% and 30% resulted in no significant increase in mortality indicating that the minimum effective dose of 20% was optimal for mirid control.

Table 1: Percent mortality of mixed population of 3rd, 4th and 5th instar *D.theobroma* after a 30 hour exposure to neem seed extract and propoxur.

Treatment	Percent mortality ± SEM
Control (water)	8.3 ± 3.1[a]
5% NSE	38.3 ± 4.8[b]
10% "	46.7 ± 12.0[b]
15% "	81.7 ± 5.5[c]
20% "	93.3 ± 2.1[d]
Propoxur (1.8%)	100 ± 0.0[d]

Mean of 6 replicates ± SEM; Means within a column followed by the same letter are not significantly different according to Tukey's HSD test (P =0.05).
NSE = neem seed extract. SEM = Standard error of mean.

3.4.2 **Effect of neem seed oil on moulting:** Moulting by *D. theobroma* nymphs was generally greater in the control than in the neem oil treatments. About 50% of the nymphs had cast their skin in the water treatment after 30 hours whereas the percentage was 25% or less in the neem oil treatments (Table 2).

Table 2: Effect of neem oil on moulting of *D. theobroma*

Treatment	No. of exuviae	% mortality ± SEM
Control	10	15 ± 5.0[a]
0.77% NSO	5	35 ± 2.9[b]
1.54% NSO	1	45 ± 4.4[bc]
3.08% NSO	2	50 [c]
4.62% NSO	1	80[d]

NSO = Neem Seed Oil

Mean of 3 replicates of 20 mirids each. Means in a column followed by the same letter(s) are not significantly different according to Tukey's HSD test (P = 0.05).

3.4.3 **Antifeedant effect of neem extract on *D. theobroma*:** *D. theobroma* nymphs were found to be deterred from feeding when put on neem treated pods (Table 3 and 4). Feeding inhibition tended to increase with increasing neem extract concentration. Similarly, *D. theobroma* nymphs, when offered a choice of neem-treated and untreated portions of cocoa pods, avoided the neem treated ends and concentrated on the untreated ends. Consequently, significantly lower numbers of lesions were recorded in the treated ends (Table 4).

Table 3: The deterrent effect (as % inhibition of feeding) of neem extract on *D. theobroma*.

Treatment	No. of lesions on pods	*Percent feeding inhibition
Control	176.17± 20.71	0.0[a]
5%	111.00 ± 19.76	35.9 ± 9.7[b]
10%	101.67 ± 12.27	48.9 ± 4.7[c]
15%	50.33 ± 14.74	73.5 ± 5.6[d]
20%	27.67 ± 4.48	82.1 ± 4.2[e]
Unden 200 EC	2.50 ± 1.52	98.7 ± 0.7[f]

*Compared with pod lesions in distilled water treatment.
Column means followed by the same letter are not significantly different according to Tukey's HSD test (P = 0.05).

Table 4: Chi-square analysis for number of pod lesions found on treated and untreated portions of pods in the mirid antifeedancy test.

Trial No.	Observed lesions		Total	Expected (1:1) lesions		X^2
	Treated	Untreated		Treated	Untreated	(df = 1)
1.	20	75	95	47.5	47.5	31.84**
2.	12	42	54	27	27	16.76**
3.	2	96	98	49	49	90.16**
Total	34	213	247	123.5	123.5	138.67**
				df=3		

** Significant at 1% level.

3.4.3 **Residual toxicity of neem to *D. theobroma*:-** The persistence of neem was short. Mean mortality of exposed mirids was less than 50% in the neem treatment after 72 hours while that in the control was 13.3% (Table 5).

Table 5: Residual toxicity (% mortalities) of neem to *D.theobroma* following 72 hours of exposure to treated pods harvested at various intervals.

Treatment	Hours after which sprayed pods were harvested from the field		
	24	48	72
20% NSE	43.3 ± 3.3[a]	40.0 ± 10.0[a]	13.3 ± 8.8
Control	13.3 ± 3.3[b]	13.3 ± 6.7[b]	10.0 ± 0.0
LSD (P = 0.05)	2.6	8.7	ns

Mean of 3 replicates. NSE = Neem seed extract.
Means in a row followed by the same letter are not significantly different, LSD (P = 0.05).

3.4.5 **Fumigant action of neem on *D. theobroma*:-** No mortality occurred within the 24hour study period. The odour that was produced by the extract did not repel the insects as they continued to feed on the chupons placed on the mesh.

The implication is that neem does not act as a fumigant.

3.4.6 **Stability of neem extract in storage:-** The efficacy of the neem extract decreased with time. The freshly prepared extract and the two and three day old extracts killed 96.3, 66.7 and 60% nymphs, respectively (Table 6).

Table 6: Percent mortality of mixed population of 3rd, 4th and 5th instar *D. theobroma* after a 30 hour exposure to crude neem extract stored for various periods.

Treatment	Storage Period (days)		
	1	2	3
20% NSE	96.3 ± 3.3[a]	66.7 ± 6.7[b]	60.0 ± 5.8[b]
Control (water)	0.0[b]	6.7 ± 6.7[b]	3.3 ± 3.3[b]

Mean of 3 replicates ± SEM. Means in a column or row followed by the same letter are not significantly different (P = 0.05).

3.4.7 **Efficacy of neem seeds at different moisture levels:-** Results of toxicity of neem extracts prepared from seeds with different moisture contents against mirids are summarised in Table 7. Seeds with the highest moisture content (24.3%) gave 90% mortality after 30 hours. The least (7.7% m.c.) gave 76% mortality.

Table 7: Percent mortality of *D. theobroma* exposed to neem seed extracts prepared from seeds of varying moisture content.

Treatment	Moisture content			
	24.3%	14.2%	11.8%	7.7%
20% NSE	90.0[a]	84.0 ± 2.4[b]	80.0 ± 3.2[c]	76.0 ± 5.1[d]
Control (water)	6.7 ± 3.3[b]	8.0 ± 3.7[b]	8 ± 3.7[b]	10.0 ± 3.2[b]

Mean of 5 replicates ± SEM . Means in a **row** followed by the same letter are not significantly different according to Tukey's HSD test (P = 0.05).

3.5 Discussion

Results of this study indicate the presence in neem seed extract of compounds capable of causing delayed toxicity effects when mirids fed on neem-treated cocoa pods. In the laboratory, mortality rate of mixed population of 3rd, 4th and 5th instar nymphs of *D. theobroma* was low i.e. less than 40% during the first 24 hours. Thereafter, mortality increased (Appendix 2). This response could be due to an initial feeding inhibition which led to starvation and death of the nymphs. The results from this work, where, the efficacy of the neem increased with increasing concentration is consistent with results of Lowery *et al.* (1993) who found that neem extracts controlled aphids on strawberry and pepper in a dose dependent manner. In the study, there was no evidence of toxicity or repellent effects of neem vapour on mirids though there was persistently strong odour. Neem extract had no fumigant activity. Consequently, the mirids that were exposed were found feeding equally on both water or neem treated chupons.

For the range of neem concentrations tested, mortality increased with concentrations. The 20% w/v neem extract was the most effective concentration causing 93.3% mortality (Table 1). Increasing the neem concentration to 25 and 30% resulted in no significant increase in mortality indicating that the minimum effective dose of 20% was optimal for mirid control. For the neem oil, 4.62% concentration caused 80% mortality while the lower concentrations were less toxic (Table 2).

The LC_{50} values were 8.17% and 2.67% for the water extract and neem oil respectively. Estimated LC50 for propoxur is $2.73x10^{-3}$% (Marchart, 1972). LT_{50} values for the 20% water extract and 4.62% oil were 16 and 24 hours, respectively meaning the 20% neem acted faster than the 4.62% oil. From the study, whether mirids were treated directly while on pods or were placed on treated pods did not seem to matter since the two methods gave comparable results. Based on 95% confidence limits, comparison of the regression coefficients (3.73 and 4.27, respectively) indicated no significant difference. It was for this reason that data from the two experimental procedures were pooled for analysis. A study conducted on aphids in Canada showed the same results (Lowery *et al.* 1993). This confirms the likelihood that contact toxicity of neem did not contribute significantly to the death of the insects; otherwise, mirids treated directly on pods should have had greater mortality values. This indicates the effect of neem on mirids is more of antifeedant and stomach poison than contact.

Efficacy of neem seeds appeared to have been affected by seed moisture since mortality of mirids tended to decrease with decreasing seed moisture content. Mirid mortality was greatest (93%) at the highest moisture content (24.3%) with diminishing effects as moisture content declined. It is possible that the amounts of azadirachtin and other compounds present in the neem seed are moisture dependent, and that deterioration

may have occurred with reduction in seed moisture. It is advisable therefore, on account of this, that seeds are used when freshly collected.

Moulting as measured by the number of exuviae cast was interrupted by the neem. At concentrations of between 0.77% and 4.62%, only 5 to 25% of the nymphs cast their skin compared with 50% for the water treatment. Isman *et al.* (1991) observed that 5th instar-nymphs of the migratory grasshopper, *Melanoplus sanguinipes* Fab. that consumed azadirachtin had great difficulty in moulting. Lowery and Isman (1994) have also observed that on canola plants treated with neem, pupation and adult emergence of *Coccinella undecipunctata* (L.) and *Eupodes fumipennis (*Thompson) were reduced by between 50 and 100%.

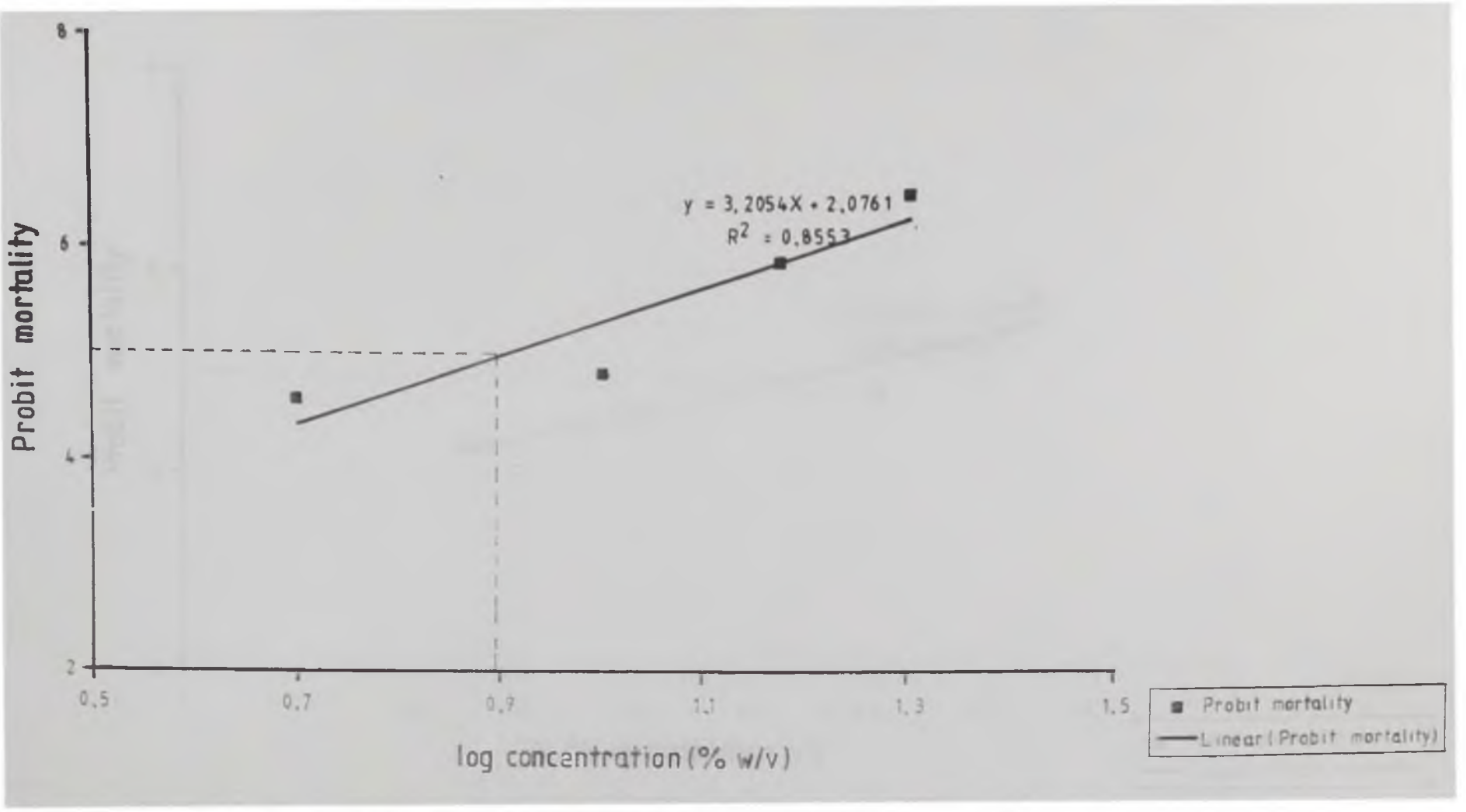

Fig. 6 Relationship between probit mortality and log concentration

(CRUDE NEEM EXTRACT)

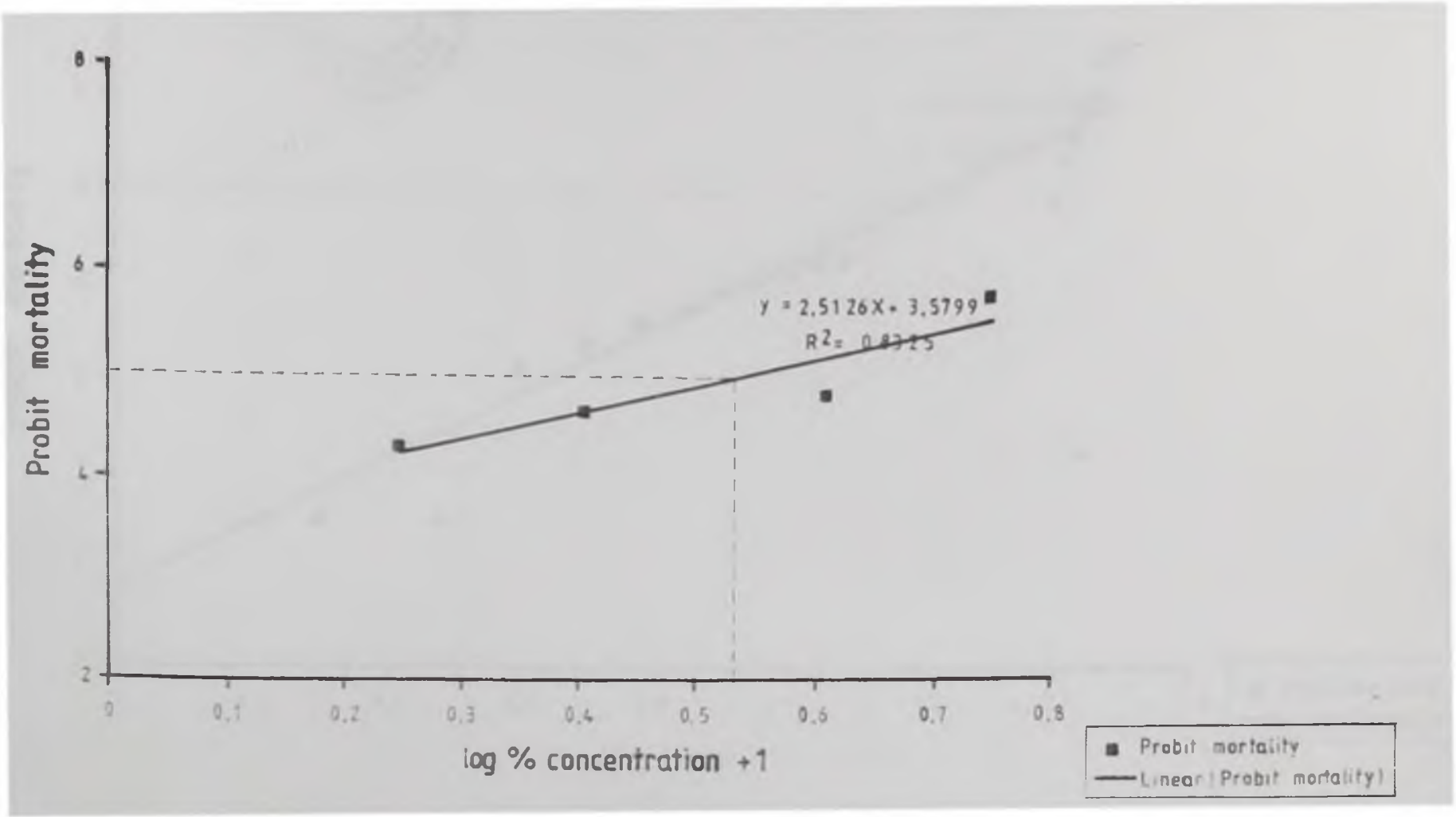

Fig. 7 Relationship between probit mortality and log concentration

(NEEM OIL)

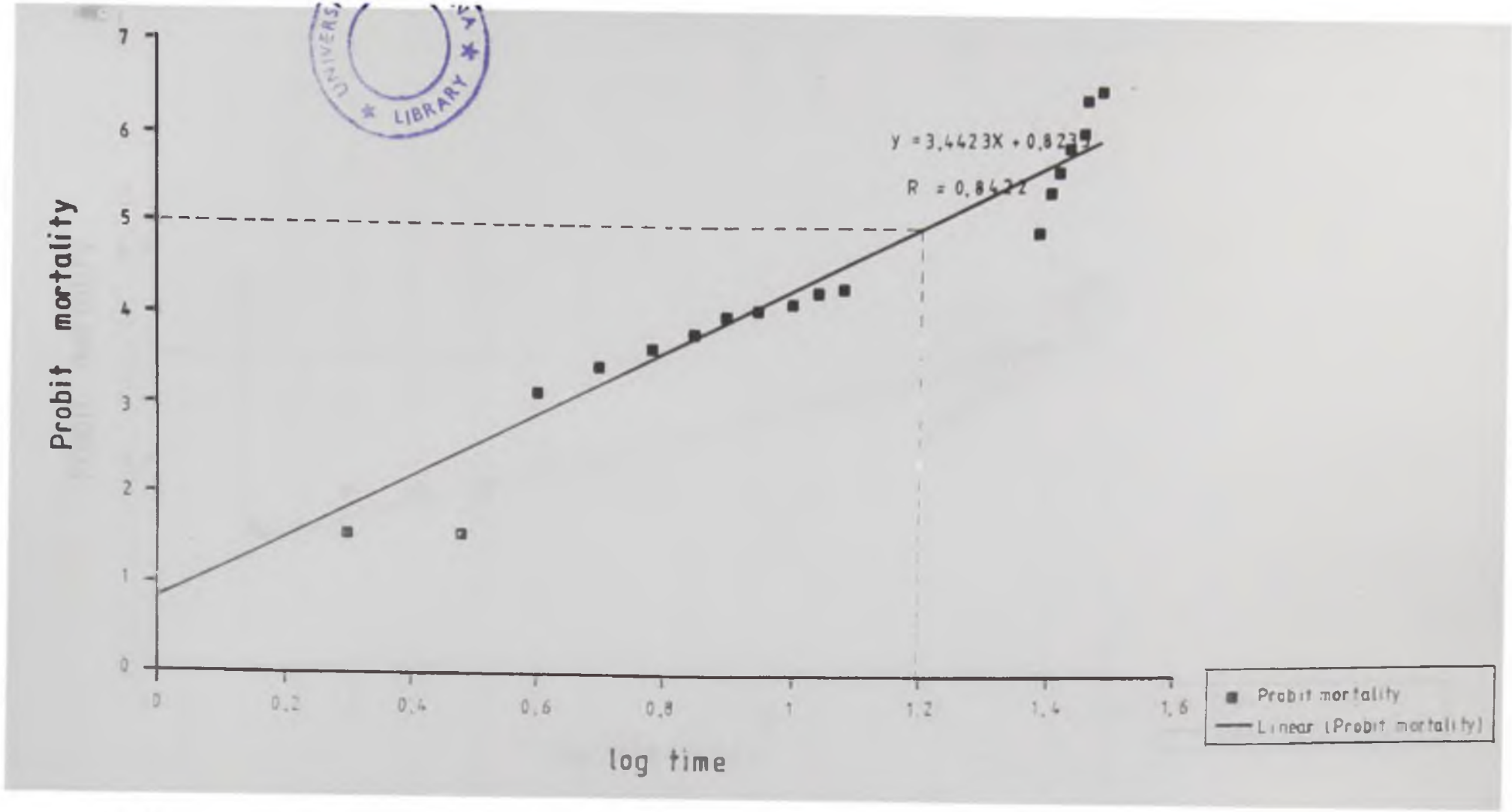

Fig. 8 Relationship between probit mortality and log time (hours)(neem oil)

(20% w/v CRUDE NEEM EXTRACT)

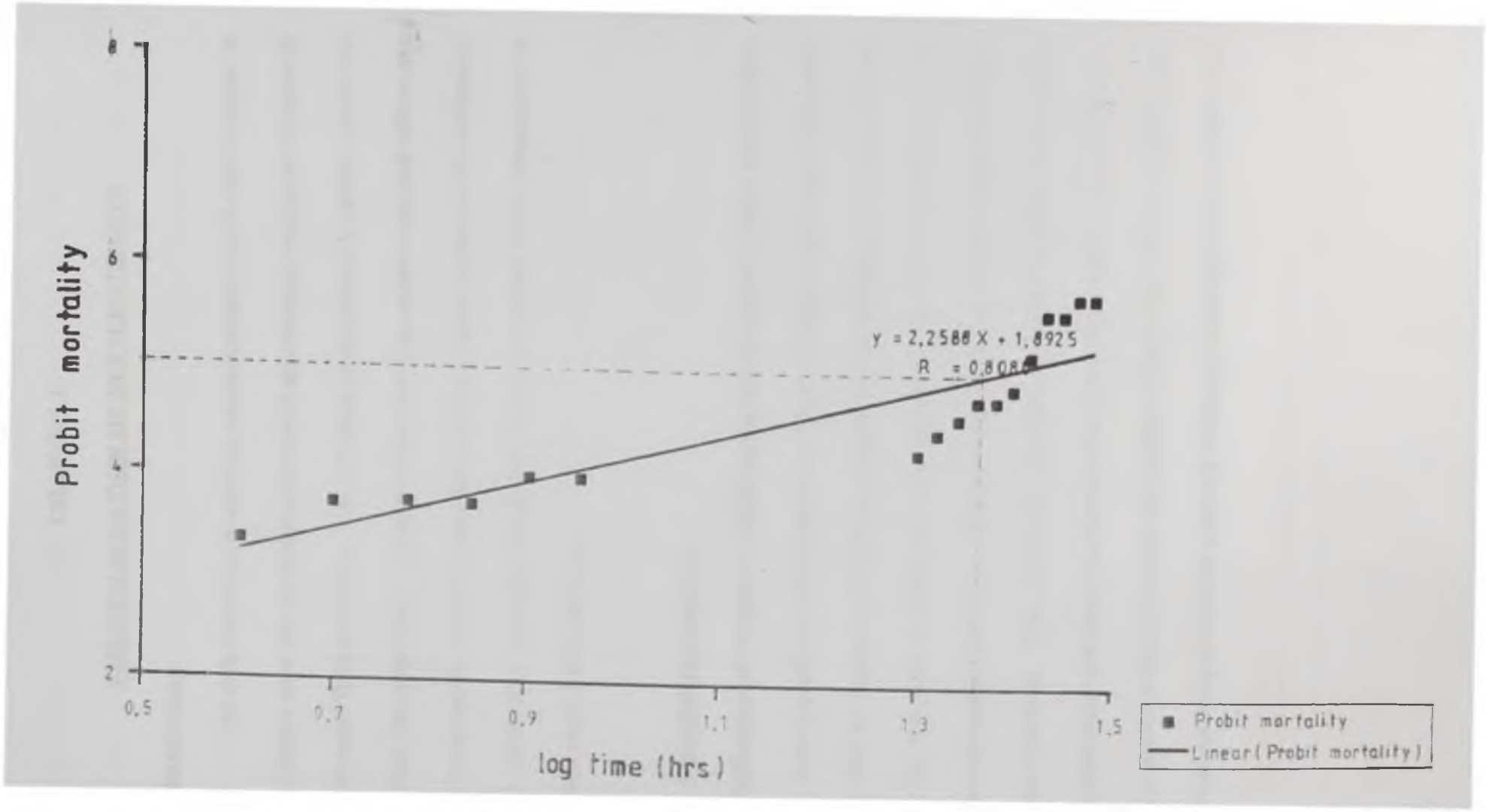

Fig. 9 Relationship between probit mortality and log time

(4·62% NEEM OIL)

CHAPTER 4

4.0 FIELD EVALUATION OF NEEM EXTRACTS

4.1 Introduction

As with synthetic insecticides, natural products which show promise in laboratory studies must also be subjected to careful and thorough evaluation of potential side effects under field conditions. Not only must a biopesticide be potent against the intended pest, but must also be applied in the field in an economical and efficacious manner. Consequently, following the effectiveness of neem extracts in the laboratory against *D. theobroma* nymphs, the present study was carried out to investigate its performance under field conditions.

4.2 Materials and methods

4.2.1 Calibration of "Urgent" GmbH knapsack mistblower using neem water extract:- Known quantities of 20% neem seed extract (2 litres) were placed in the spray tank and with the engine at full throttle (maximum revolutions), the time taken to discharge the two litres at restrictor or jet positions 2 and 3, were recorded. This procedure was repeated three times. The extract appeared more viscous than water due to suspended particles. Thus, during the calibration, particles blocked the fine-mesh filter provided at the tank outlet and resulted in erratic flow rates. Consequently, the sieves were removed but this resulted in unusually higher flow rates i.e. 0.6 litres per minute when operating at restrictor 2 and 0.8 litres per minute when set at restrictor No. 3.

4.2.2 **Site description and experimental design:-** Field evaluation studies involving a water extract of neem prepared from seeds, a formulated neem oil, and a mixture of one-third the field rate of propoxur and 20% neem extract were conducted during the 1996 main cocoa spraying season (August - December). The 2nd and 3rd restrictor positions of an "Urgent" GmbH knapsack mistblower were used. Spraying was done using two different application techniques i.e. (1) spraying one side of a tree from the trunk into the canopy (T1 method) or (2) from two sides to achieve adequate plant coverage.

4.2.2.1 **Efficacy of neem extract against mirids using the T1 application method:-** In late July 1996, three mirid infested cocoa farms as replications in a randomized complete block design (sizes ranging from 1 to 2 ha), were selected from three locations namely, Nsutam, Bosuso and Osiem about 20 km north of the Cocoa Research Institute, Tafo (Fig.10). Five plots each averaging 0.1ha were demarcated in each location with a strip of land (about 10 m) of untreated cocoa surrounding each plot to reduce effects of spray drift.

The treatments used were:

i. untreated control

ii. neem extract (20% w/v) sprayed at restrictor 2 (T1 method);

iii. neem extract (20% w/v) sprayed at restrictor 3 (T1 method);

iv. neem/propoxur (Unden 200 EC) mixture (4.5 ml propoxur/1000 ml of neem extract, 20% w/v restrictor 2;

v. Propoxur sprayed at the recommended field rate of 1050 ml (i.e. 210 g a.i.) in 56 litres/ha (using restrictor 2).

All treatments were applied in the morning before 9 am when the sun was low and when there was no wind. This was to prevent drying out and drifting of the spray into adjacent plots. Before the treatments were applied, trees were carefully examined for mirids and the numbers of *D. theobroma* and *S. singularis* were recorded. The effectiveness of each treatment was determined by counting again the numbers of live mirids 48 hours after the treatment. The trial was repeated after one month and the results

averaged. Application rates were 75 and 100 litres per hectare using the 2nd and 3rd restrictor positions, respectively.

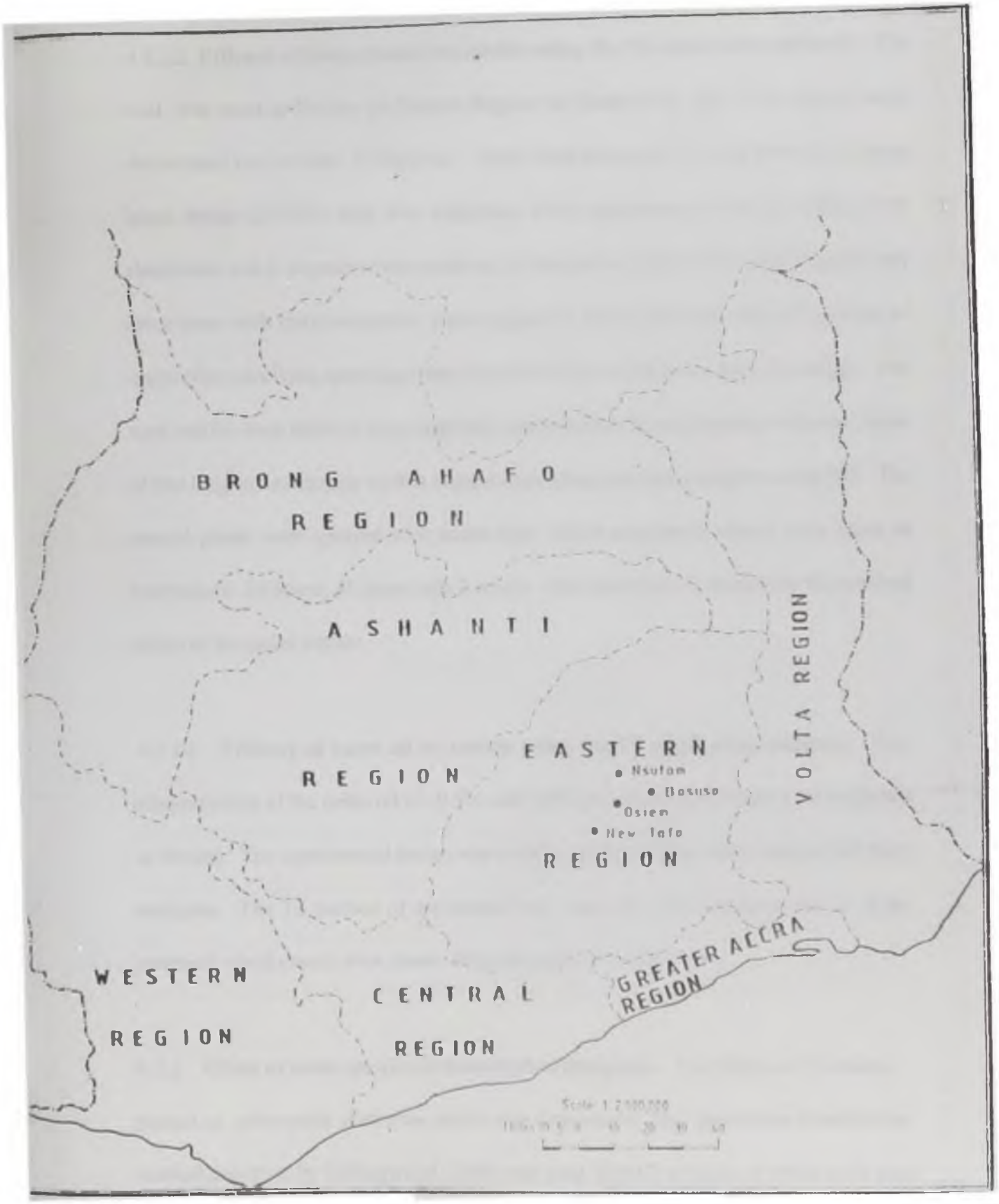

Fig. 10: Map showing locations of field trials in the Eastern Region of Ghana.

4.2.2.2 **Efficacy of neem extract on mirids using the T2 application method:-** The trial was sited at Bosuso in Eastern Region of Ghana (Fig. 10). Four blocks were demarcated into sixteen 0.1ha plots. These were arranged in a randomized complete block design (RCBD) with four replicates. Field assessment of the incidence of *D. theobroma* and *S. singularis* was made on all trees in each plot before spraying and only those trees with mirid incidence were tagged for the experiment. The T2 method of application involving spraying a tree from two sides of the trunk into the canopy, was used and the time taken to treat each tree was recorded. Measurements were also taken of tree heights and canopy widths using a measuring tape and a long wooden pole. The control plants were sprayed with water only. Mirid population counts were made at intervals of 24 hours, 48 hours and 2 weeks after treatment to determine the residual action of the neem extract.

4.2.2.3 **Efficacy of neem oil on mirids using the T2 application method:-** Two concentrations of the neem oil i.e. 0.3% and 3.0% and water treatments were evaluated at Bosuso. The experimental design was a randomized complete block design with three replicates. The T2 method of application was used using the restrictor No. 2. Post-treatment mirid counts were made 48 hours and two weeks later.

4.2.3 **Effect of neem sprays on nontarget arthropods:-** The effects of the neem extract on arthropods other than mirids was determined. The "pyrethrum knockdown" method described by Collingwood (1969) was used. Eight 3 m^2 sheet of white cloth were spread to cover the ground under trees selected to be sprayed with water only or 20%

neem extract (restrictor 2). The sheets were held close against the trunk and were intended to collect all arthropods that dropped from the tree. Twenty-four hours after the treatment, the sheets were collected and all arthrpods found dead were counted.

4.2.4 Statistical analysis

Percentage reduction in numbers in the controls were corrected using Abbott's formula (Abbott , 1925). Percentages* were transformed to arcsine values while actual numbers were transformed using logarithm transformation [$(Log_{10}(x +1)$] to meet ANOVA assumptions of normality and homogeneity of variances. Statistical analysis by the analysis of variance technique was carried out on the transformed data according to procedures outlined by Steel and Torrie (1980). Correlation analysis was used to compare relative sizes of trees and duration of spray.

* Percent population reduction =

$$\frac{\text{Pre-treatment count} - \text{Post-treatment count X 100}}{\text{Pre-treatment count}}$$

4.3 Results:-

4.3.1 **Efficacy of neem extracts on mirids using the T1 application method:-** The results of the field trials showed that all the treatments reduced the incidence of mirids except when neem extract and the 2nd restrictor was used (Table 8). There was, however, an increase in population in both the untreated and the treatment with the restrictor no. 2.

4.3.2 Table 8: Efficacies of neem and Propoxur treatments for controlling mirids on cocoa using the T1 application technique.

Treatment	48 hours Post-treatment % mirid reduction ± SEM
Unden (restrictor 2)	99.2 ± 0.8[a]
Neem + Unden (restrictor 2)	88.9 ± 0.6[ab]
Neem (restrictor 3)	51.7 ± 15.0[b]
Neem (restrictor 2)	* -35.8 ± 21.8[c]
Untreated control	* -98.6 ± 67.4[c]

Mean of 3 replicates. Means in a column followed by the same letter(s) are not significantly different according to Tukey's HSD test (P = 0.05). * Negative values were replaced by zeros before data transformation and analysis of variance.

4.3.2 **Efficacy of neem extracts on mirids using the T2 application method:-** Mirids in the field were highly aggregated; thus out of about 140 trees in a plot, mirids were found on an average of about nine trees. Spraying time ranged from 16 to 70 seconds per tree, and tree heights, from about a metre to 5.2 metres (Appendix 3). Consequently, mean application rates were 480 litres per hectare (96 kg seed) and 640 litres per hectare (128 kg seed) for the restrictors 2 and 3 respectively. The neem treatments were effective in reducing mirid population on cocoa compared with the water treatment. The neem / propoxur mixture was significantly better than the neem alone (P= 0.05). In plots sprayed with the mixture, the population was effectively checked and could not recover one month after spraying (Figs. 11 and 12). There was no significant difference in population reduction between the restrictors (P=0.05) 48 hours after treatment. However, percentage reduction was less consistent 14 days after treatment (Table 9).

Table 9 Efficacies of neem and neem/propoxur mixture for controlling mirids on cocoa using the T2 application technique.

Treatment	Post-treatment % reduction in number of mirids ± SEM		
	24 hours	48 hours	14 days
Neem+Unden (restrictor 2)	100[a]	100[a]	100[a]
Neem (restrictor 3)	40.6±7.6[b]	88.9±2.1[b]	64.9±20.4[b]
Neem (restrictor2)	44.6±5.3[b]	80.3±6.0[b]	94.7±3.6[a]

Means in a **column** followed by the same letter (s) are not significantly different according to Tukey's HSD test (P= 0.05). (Corrected data, using Abbott's formula).

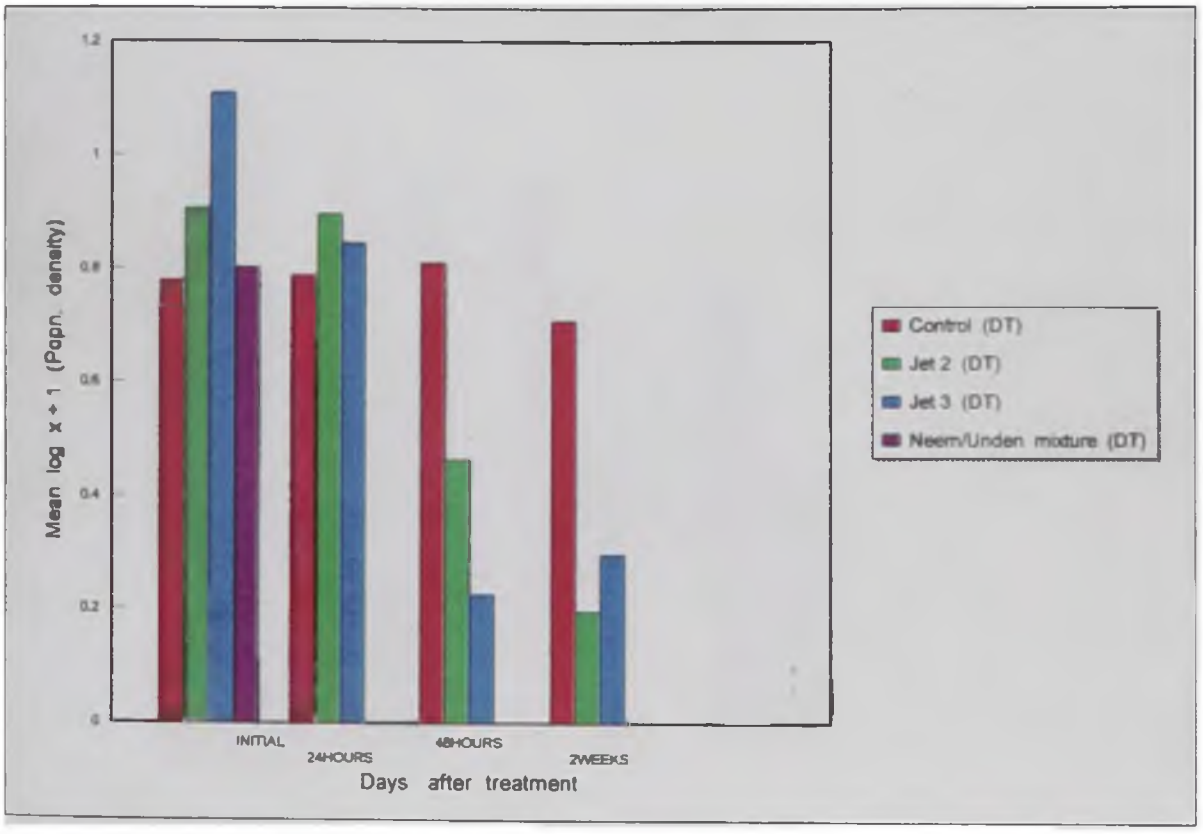

Fig. 11: Mean population densities of *D. theobroma* on 0.1ha plots before and after neem and neem plus propoxur application on cocoa.

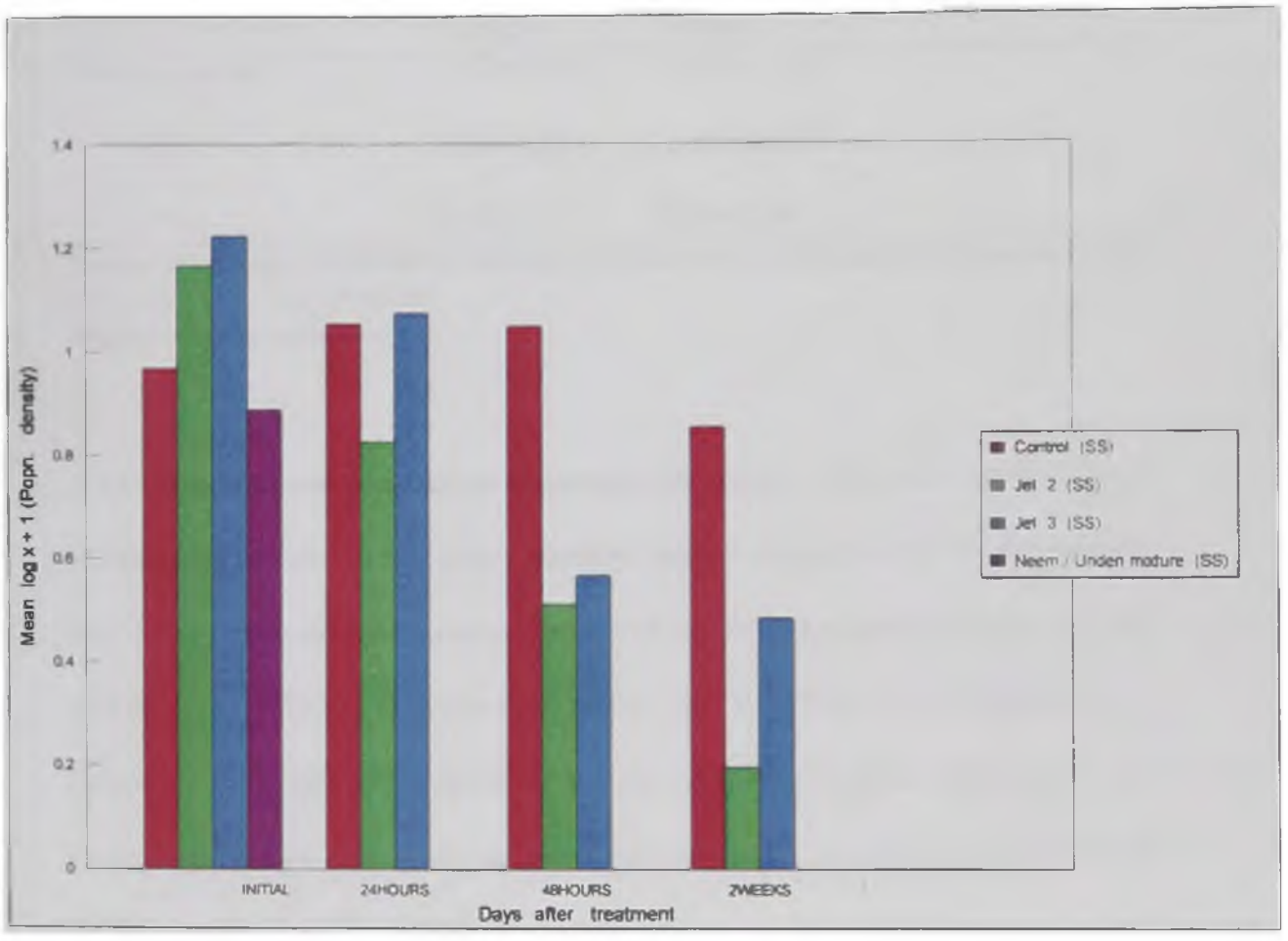

Fig. 12: Mean population densities of *S. singularis* on 0.1ha plots before and after neem and neem plus propoxur application on cocoa.

4.3.3 **Efficacy of neem oil on mirids using the T2 application method:-** The percent population reduction of mirids in plots treated with 3.0% neem oil was higher than plots treated with 0.3%. Both concentrations were significantly better than the water treated control (P = 0.05) (Table 10).

Table 10: Efficacy of neem oil treatments for controlling cocoa mirids.

Treatment	*Post-treatment % reduction in number of mirids ± SEM	
	48 hours	14 days
Water (control)	15.0±15.0[a]	12.25± 7.75[a]
0.3%	51.7± 12.1[b]	81.97± 0.73[b]
3.0%	80.4±2.7[c]	93.50±3.34[c]

Means in a **column** followed by the same letter are not significantly different according to Tukey's HSD test (P=0.05).
*Mean of three replicates.

4.3.4 **Effects of neem extract on nontarget arthropods:-** The results obtained from this study suggest that neem extracts could be harmful to a number of arthropods other than mirids when applied on cocoa. The list of insects and spiders that were killed is presented in Table 11. It appeared however, that the effect of the treatments on *Oecophylla longinoda* and *Camponotus* sp. was short-lived as greater numbers of these insects were present, actively foraging on the plants when inspection was made 48 hours and two weeks after the treatment.

Table 11: Effects of neem extract on nontarget arthropods

Species	Number(s) of insects recorded dead after 24 hours
Bathycoelia thalassina	2
Zonocerus variegatus	3
Unidentified orthopterans	2
Unidentified dictyopterans	2
Unidentified spiders	2
Oecophylla longinoda	>40
Unidentified weevils	1
Unidentified dipterans	2
Unidentified hymenopterans	8
Pheidole spp	5
Unidentified hairy caterpillars	2
Camponotus spp	4

4.4 Discussion

Results from the field study showed that neem seed water extract and neem oil were effective though less effective than propoxur, in reducing mirid numbers on cocoa. When the trees were sprayed from one side only (T1 method), all the treatments, except when the 2nd restrictor position was used, significantly reduced the incidence of mirids. Similarly, when the trees were sprayed from two sides, all the treatments compared with the water treatment, significantly reduced mirid population. The neem/propoxur mixture not only to give the best control, but also continued to effect some measure of control 14 days after treatment. At the end of the 14th day,

some level of recovery of *D. theobroma* was observed in some plots treated with neem at the restrictor no. 3 (Fig. 11 and 12). Consequently, percent reduction in mirid numbers using the T2 application method was less consistent after the 14th day of treatment (Table 9). For instance, there was 80% reduction in mirid numbers after the second day of treatment when spraying was done with the restrictor 2; this increased to 95% by the 14th day. In contrast, percent reduction in numbers decreased from 89% on day two to 65% on day 14 with the restrictor 3. Bernays (1985) also observed significant variation in the efficacy of neem in the control of the variegated grasshopper on cassava. Mirid numbers on the water-treated plants, however, continued to increase until after the 14th day when it dropped, presumably, by movement of adults into adjacent trees. This observation suggests that the persistence of the neem extract decreased with time. In effect, efficacy seemed to have been lost after 14 days (Figs. 11 and 12). This confirms the reports of earlier workers (Pradhan *et al.*, 1962; Ladd *et al.*, 1978; Meisner *et al.*,.1983; Bernays, 1985; Schmutterer, 1988; Barnby *et al.*, 1989; Afreh-Nuamah, 1995) that the residual activity of neem does not exceed 14 days. This could be due to degradation of the active constituents (e.g. azadirachtin) by ultraviolet light.

The results from this study also showed that when cocoa trees were sprayed from two sides (T2 method), significantly better spray coverage was achieved than when spraying was done from one side only (T1 method). Neem acts mainly as an antifeedant and a stomach poison (Isman *et al.*, 1991) and with the highly sedentary habit of mirid nymphs (Williams, 1953), optimizing spray coverage, is an important factor in mirid control with neem extracts.

It has generally been observed that pest behaviour greatly affects efficacy of an

application methodology. For instance, two species of aphids *M. persicae* and *Aphis gossypii* Glover, are equally susceptible to the entomopathogenic fungus *Verticillium Lecanii* (Zimm.) Viegas; however, control of *A. gossypii* was markedly less than that of *M. persicae* (Hall and Papierok, 1982). This effect was attributed to the higher mobility of *M. persicae* resulting in frequent contact with the fungal spores. The lack of adequate control of *A. gossypii* was overcome by the use of methods which optimized deposition of spores on the aphid (Sopp *et al.,* 1989).

Insufficient spray coverage coupled with lack of vapour activity of neem extract probably contributed to the poor performance of the T1 method of application. Population increase after treatment could have resulted from new nymphal instars hatching from eggs unaffected by the spray. Adding 4.5 ml of propoxur per litre of neem extract resulted in increased toxicity and duration of control (Figs. 11 and 12).

Application of the neem/propoxur mixture at 75 litres/ha i.e. about 340 ml (68 g a.i.) of propoxur/ha with the T1 method gave results comparable to that of propoxur alone when the latter was applied at 1050 ml (210 g ai.) /ha. There is however potential for increased spraying efficiency with the T1 method than the T2. Thus, there will be a considerable decrease in the total volume of application, the cost of carting water and the time required for application.

From the field study, it was obvious that neem could adversely affect a number of nontarget arthropod species some of which (*O. longinoda, Camponotus* sp. and salticid spiders) have been reported to be beneficial as mirid predators (Leston, 1970). Although the overall adverse effect of the neem treatment on the balance of arthropods was not investigated in this study, it appeared the effects on *O. longinoda* and *Camponotus* sp. was short-lived as greater numbers of these ants were seen foraging on the treated plants 48 hours and 14 days after treatment.

Few studies have been undertaken on the effects of neem on beneficial arthropod species (Spollen and Isman, 1996). Naumann *et al.*, (1994) found that sugar solutions containing 0.1% azadirachtin were avoided by honey bees. The number of bees, however, foraging in treated and untreated plots was not significantly different in field trials in which azadirachtin was applied at rates of up to 150 ppm (0.015%). In laboratory tests, Hoelmer *et al.*, (1990) found that leaves treated with 20 ppm (0.002%) azadirachtin repelled the coccinellid predator *Delphastus pusillus* LeConte and the whitefly parasitoid *Eretmocerus californicus* Howard, but repellency decreased over time. Joshi *et al.*, (1982) reported that neem did not deter parasitization of *Spodoptera litura* (F.) eggs by *Telenomus remus* Nixon. Stark *et al.* (1992) found that azadirachtin was effective against tephritid fruit flies but had little adverse effects on associated endoparasitoids. Spollen and Isman (1996) tested the effect of a neem insecticide on two predatory mite species *Phytoseiulus persimilis* Athias- Henriot, and *Amblyseius cucumeris (*Oudemans) of aphids. No significant differences in egg or adult mortality was found between treated and untreated mites for either species. In contrast, Klemn and Schmutterer (1993) reported reduced parasitism by *Trichogrmma* spp on diamond back moths, *P. xylostella* eggs treated with neem, and reduced adult emergence from treated parasitoid pupae. Further research may be necessary to determine the full impact of neem on beneficial arthropods.

CHAPTER 5

5.0 SUMMARY AND CONCLUSIONS

Laboratory and field tests indicated that the two neem extracts tested were toxic to cocoa mirids. Residual toxicity was short, and mortality fell below the acceptable 95% kill for field tested insecticides. However, considerable potential exists for the use of neem extracts for the control of cocoa mirids especially in small scale farming systems. This will not only be a success from a biological point of view but also will give the assurance of safeguarding the environment. Adding a little amount of propoxur (4.5 ml per litre of neem extract) significantly increased efficacy of neem. Current trend in pesticide application technology is towards reduction of amount of spray liquid to ensure application efficiency. Neem/propoxur mixture applied with the T1 methodology could offer a more practical approach to mirid control. The obvious advantages of low application rate are: less cost per hectare, reduction of residues in a final product and possibly, a less harmful effect on beneficial species.The study demonstrated that neem could be harmful to nontarget arthropod species. Further work therefore, is required to determine in detail the effects of neem on nontarget fauna and to accurately establish recommendation for the use of neem in integrated pest management (IPM) programmes.

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Appendix 1. Alternative host plants of *Sahlbergella singularis*.

recorded in West Africa

Host Plant	Order: Family
Berria amonilla	Tiliales: Tiliaceae
Ceiba pentandra Linn.	Tiliales: Bombacaceae
Bombax buonopozense	" "
Cola acuminata	Tiliales: Sterculiaceae
C. diversifolia	" "
C. gigantea v glabrescens	" "
C. lateritia v maclaudi	" "
C. millenii	" "
C. nitida	" "
Desplatsia dewevrei	Tiliales: Tiliaceae
Nesogordonia papavifera	Tiliales: Sterculiaceae
Sterculia foetida	" "
S. rhinopetala	" "
Theobroma bicolor	" "
T. microcarpum	" "
T. speciosum	" "
Gossypium sp.	Malvales: Malvaceae

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S. rhinopetala	" "
Theobroma bicolor	" "
T. microcarpum	" "
T. speciosum	" "
Gossypium sp.	Malvales: Malvaceae

Appendix 2: Cummulative percent mortalities of *D. theobroma* exposed to neem treated cocoa pods. (Corrected percent mortarities after Abbott 1925)

Neem extract					Neem Oil			
Time afte treatment (hours)	5%	10%	15%	20%	0.77%	1.54%	3.08%	4.62%
1	0	0	0	1.67	5.00	5.00	0	0
2	0	0	3.34	1.67	10.00	5.00	0	0
3	0	0	5.0	1.67	10.00	5.00	0	0
4	0	1.67	6.67	3.33	15.00	10.00	0	5.00
5	1.67	1.67	6.67	6.67	15.00	10.00	5.00	10.00
6	1.67	3.51	8.34	8.34	15.00	10.00	5.00	1.00
7	1.67	6.67	10.0	11.67	15.00	10.00	5.00	10.00
8	1.67	6.67	10.0	16.67	15.00	10.00	5.00	15.00
9	1.67	6.67	10.0	18.51	20.00	10.00	5.00	15.00
10	3.33	10.17	11.84	21.84	-	-	-	-
11	3.33	10.17	13.57	23.57	-	-	-	-
12	3.51	10.17	13.57	25.54	-	-	-	-
20	-	-	-	-	22.22	16.67	16.67	16.67
21	-	-	-	-	22.22	16.67	27.78	22.22
22	-	-	-	-	22.22	16.67	27.78	27.35
23	-	-	-	-	22.22	22.22	27.78	33.33
24	15.51	12.25	39.65	46.53	22.22	22.22	27.78	33.33
25	18.97	12.25	48.27	63.80	27.78	27.78	27.78	38.89
26	26.50	25.03	50.00	71.78	27.78	33.33	27.78	50.00
27	26.52	28.61	60.42	77.73	29.41	35.29	29.41	70.59
28	28.35	30.46	63.49	83.73	29.41	35.29	29.41	70.59
29	28.97	34.52	74.05	91.01	29.41	35.29	29.41	76.47
30	32.67	41.80	79.96	92.80	29.41	35.29	41.18	76.47

(-) No readings taken beyond 10.00p.m. until following morning.

Appendix 3(a):

Relationships between tree heights, canopy widths and the time taken to obtain complete spray coverage (restrictor 2).

Tree height (m)	Height of trunk up to first jorquette (m)	Width of tree canopy (m)	Spray duration (s)
1.30	-	0.80	22.7
1.44	-	1.24	21.0
1.60	1.0	2.25	30.8
1.65	-	2.05	26.4
1.75	1.2	1.09	30.0
1.85	1.0	0.88	30.5
2.01	-	1.25	40.2
2.03	1.0	1.41	49.4
2.06	1.54	0.70	23.0
2.06	1.49	1.45	33.9
2.12	-	2.37	39.2
2.14	1.0	1.60	32.5
2.20	-	1.60	28.6
2.30	1.0	1.70	49.3
2.35	1.30	1.56	48.6
2.41	-	2.92	40.2
2.47	1.20	2.50	65.0
2.49	1.74	0.46	28.2
2.58	1.44	1.80	31.3
2.60	1.46	1.75	52.4
2.60	1.0	1.09	25.0
2.65	1.58	1.45	44.8
2.70	1.33	1.60	31.3
2.73	1.37	1.25	48.6
2.73	1.25	2.25	33.1
2.85	1.26	1.73	46.2
2.95	1.40	2.55	40.0
2.96	1.12	1.25	66.0
3.0	1.0	3.35	62.0
3.45	1.10	4.0	56.9
3.69	1.0	1.70	50.4
3.72	1.37	3.38	50.0
3.76	1.38	2.35	59.3
4.0	1.83	3.20	51.0
4.0	1.57	4.05	40.2
4.36	1.66	3.55	60.0
4.40	1.84	3.75	60.0
4.41	1.44	4.37	66.0
5.45	1.53	4.49	60.2

Trees marked with dashes (-) have not formed jorquettes.
Coefficients of linear correlation: r_1 (Tree heights vs. spray duration) = 0.699**
r_2 (Canopy widths vs. spray duration) = 0.617**
** significant ($P \leq 0.01$)

Appendix 3(b): Relationships between tree heights, canopy widths and the time taken to obtain complete spray coverage (restrictor 3).

Tree height (m)	Height of trunk up to first jorquette (m)	Width of tree canopy (m)	Spray duration (s)
1.25	-	0.88	16.1
1.50	-	1.10	24.8
1.57	-	1.35	25.5
1.75	1.0	1.80	22.0
1.77	-	1.60	26.4
1.78	1.19	1.27	20.4
1.78	-	1.95	28.4
1.83 1.84	1.0	2.45	28.8
1.85	-	0.95	24.7
1.85	1.0	0.59	21.0
1.90	-	1.30	22.0
2.09	1.10	1.0	25.6
2.14	1.01	0.72	24.0
2.24	1.0	1.83	28.5
2.24	1.29	2.75	41.2
2.25	1.08	2.20	33.9
2.25	1.35	1.0	25.5
2.30	1.11	2.95	36.0
2.35	1.30	1.40	29.4
2.35	1.20	1.75	21.0
2.51	1.15	2.31	37.0
2.55	1.30	1.94	60.0
2.56	1.29	2.86	31.2
2.65	1.50	1.75	40.0
2.66	1.35	2.69	31.5
2.67	1.36	2.25	40.0
2.67	1.42	2.0	30.0
2.73	1.14	2.29	41.0
2.94	1.48	2.45	49.0
2.95	1.43	1.61	31.3
3.0	1.42	5.0	63.5
3.06	1.71	2.55	52.0
3.13	1.49	2.16	35.0
3.30	1.81	1.74	42.0
3.34	1.25	3.20	38.2
3.47	2.02	2.25	43.4
3.55	1.26	3.35	36.6
3.73	1.80	2.30	37.9
3.75	1.06	3.70	30.7
3.89	1.61	3.65	48.8
4.0	1.20	4.0	46.5
4.34	1.40	2.78	47.5
4.35	1.45	3.47	35.0
4.53	1.74	3.85	60.0
4.48	1.25	3.46	55.8
5.0	1.10	3.64	70.0
5.16	1.20	2.82	42.9
	2.08	3.05	61.0

Trees marked with dashes (-) have not formed jorquettes.
Coefficients of linear correlation: r_1 (Tree heights vs. spray duration) = 0.731**
r_2 (Canopy widths vs. spray duration) = 0.728** ** significant ($P \leq 0.01$)