THE USE OF BIOPESTICIDES TO CONTROL INSECT PESTS OF PAWPAW (*CARICA PAPAYA*)

BY

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ABSTRACT

Laboratory and field experiments were carried out between June 1997 and January 1999 at Paradise farm near Nsawam in the Eastern Region of Ghana to determine the major insect pests of papaya, *Carica papaya* and to evaluate the effectiveness of *Bacillus thuringiensis* and neem seed extract for controlling the insect pests of papaya. Methanolic and aqueous neem seed extracts were tested at the following concentrations 0 , 50, 75 and 100 g/l. *Bacillus thuringiensis* was applied at the rate of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 g/l. Percentage mortality and leaf damage caused by insect pests were determined in the laboratory and in the field. The nature of the damage caused by the insect pests on the various growth stages of the plants was assessed by visual observation. The major insect pests recorded on papaya at Paradise farm were the variegated grasshopper, *Zonocerus variegatus*, mealy bugs, *Planococcus sp* and the Mexican bean beetle, *Epilachna sp*. It was observed in the field that the population of these insect pests were significantly ($p<0.05$) reduced by the higher doses of methanolic and aqueous neem extracts as well as the *Bacillus thuringiensis* treatments. The neem seed extracts and *Bacillus thuringiensis* preparations had significant ($p<0.05$) effect on the mortality of adult and nymphs of *Z. variegatus* in the laboratory and in the field compared to the control. Mortality increased with increasing dosage. Hundred grams per litre of methanolic and aqueous neem seed extract and 1 g of *B. thuringiensis* per litre of water were equally effective and induced the highest mortality of adults and nymphs of *Z. variegatus* compared to the other treatments. The least leaf damage was recorded on papaya plants treated with the highest dosage of neem extract and *B. thuringiensis* tested in the laboratory. Growth and yield parameters including plant girth, height, spread of leaf, number of leaves, number of flowers, fruit weight and the final yield per hectare were also determined in the field... Methanolic and aqueous neem seed extracts promoted vegetative growth of papaya plants by significantly ($p<0.05$) increasing plant height, girth, number of leaves, flowers and yield compared to the untreated plants. The results of the experiment indicate that 100 g/l of aqueous and methanolic seed extracts could be used to control pests of papaya in the field. However, aqueous extraction may be recommended because it is cheaper and the extraction is also easier.

DECLARATION

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

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DEDICATION

This work is affectionately dedication to my parents Mr. and Mrs. Akakpo and wife, Mrs. Gifty Akakpo in appreciation for their help, love and deep concern about my welfare, which enabled me to complete this work successfully.

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1.0 INTRODUCTION

Pawpaw, *Carica papaya*, belonging to the family Caricaceae, is one of the non-traditional export crops in Ghana. It is presently receiving much attention among commercial farmers and exporters. A total of 4700 tons of pawpaw was exported to Europe in 1998 (Ghana Export Promotion Council, 1998) . Pawpaw is a small tropical plant native to South America (Rice and Rice, 1986). It normally grows with a single unbranched trunk which may reach 10 m in height. The crown is covered by large palmate lobe leaves on long and hollow petioles up to 100 cm in length (Rice and Rice, 1986). The trees are primarily dioecious although hermaphrodite forms are common in some cultivars. Papaya is primarily propagated from seeds, which germinate in 2-4 weeks after planting. It does well on a wide range of soil types provided the drainage is good. Papayas are drought tolerant once established, but in areas with prolonged dry season, small fruits are produced. (Rice and Rice, 1986). The fresh fruits are exported while the latex in the plant contains a high percentage of papain, which is a proteolytic enzyme used in meat tendering, brewing, tanning and textile manufacturing. (Storey, 1958; Rice and Rice, 1986). Hawaii is the major producer of papaya for fresh fruit export while Sri Lanka and Tanzania are the primary producers of papain (Rice and Rice, 1986).

Like any other plant, papaya is attacked by several species of insect pests which are known to have significant effect on plant health, vigour and yield. The common insect pests known to attack pawpaw are the fruitflies, *Ceratitis capitata* (Wied.) and *Dacus dorsalis* (Hend.) which pierce and lay eggs into the fruits (Lloyd-Thomas, 1990).

Adult females of *D. dorsalis* respond to the odour of ripe fruits and will lay more eggs in them than matured green fruits (Jang and Light, 1991). The papaya fruitfly *Toxotrypana curvicauda* Gerst is a serious pest of pawpaw in south Florida. The female deposits her eggs into immature fruit by means of a long ovipositor, and the larvae feed in the central seed mass and later into the flesh, frequently rendering it unfit for human consumption (Wilson, 1927). The grasshopper, *Zonocerus variegatus* (L) is responsible for leaf defoliation in pawpaw (Harris *et al.*, 1991). Other insects reported on papaya in Hawaii are the red mite *Tetranychus sp*. and the larvae of the moth *Cryptoblades aliena* Swezey which feed under a web on the floral stems and beneath the flower clusters (Storey, 1958). Birds and snails have also been reported as serious pests of pawpaw (Lloyd-Thomas,1990).

To increase yield and reduce damage caused by insect pests, farmers use synthetic chemicals such as cypermethrin, diaxocarb, dimethoate, lindane, karate, cymethoate to protect the plants (Oudejans *et al.*, 1988). These synthetic insecticides though effective are often used at such high dosages and rates that they lead to the development of resistance in insect pests and pollution of water sources (Schmutterer and Hellpap,1988). There is also the emergence of secondary insect pests after killing the natural enemies. In addition, synthetic insecticides have toxic residues in the fresh fruits which may be hazardous to consumers and domestic animals (Oudejans *et al.*, 1988). To alleviate these problems associated with synthetic chemicals, insecticides of plant and microbial origin are currently receiving much attention (Steffens and Schmutterer, 1982). Pyrethrum, rotenone and nicotine, which are all plant derivatives, were among the first compounds used to control agricultural insect pests. (Morgan and Thornton, 1973).

Azadirachtin, a triterpenoid obtained from the neem tree, *Azadirachta indica* A Juss (Meliaceae), has been shown to possess some growth-regulating and anti-feeding properties against insects (Warthen, 1979; Schmutterer, 1990; Jackai, 1993;). Neem products are active against a wide range of insect pests of many crops world wide (Jacobson, 1981; Schmutterer,1985). Notable examples are its effectiveness against pest infestations on cassava (Olaifa and Adenuga, 1988), cowpea (Cobbina and Osei Owusu, 1988) and vegetables (Afreh-Nuamah *et al.*, 1998, Ankra, 1998). Neem, because of its effective insecticidal properties, has been crowned as the king of future pesticides (Nasampagi, 1993). All parts of the tree are biologically active (Singh, 1993). However, the fruits are the most important source of the active ingredients that affect insects in various ways. The active ingredients are readily biodegradable to harmless metabolites and therefore no residues are left in food.

Biological control by means of entomopathogens and other microbial organisms involves the application of microorganisms onto crops for ingestion by insects or directly onto the insects (Oudejans, 1991). The best known example is a bacterium, *Bacillus thuringiensis* which is active against lepidopteran larvae. Two varieties of *B. thuringiensis* (Kurtaski and Israelensis) are currently being widely produced and marketed under trade names such as Bactospeine, Bactimos, Dipel and Thuricide. These formulations when sprayed onto crops, kill leaf-eating caterpillars which ingest them (Oudejans, 1991). *B. thuringiensis.* was found to be pathogenic to lepidopteran pests, mosquitoes, beetles and dragonflies(de Barjac,1978; Dejoux, 1979;).

This study was therefore conducted to evaluate the efficacy of neem seed extracts and *B. thuringiensis* against insect pests of papaya.

The specific objectives were;

(i) To collect and identify the insect pests of papaya.

(ii) To evaluate the effectiveness of *B. thuringiensis* , methanolic and aqueous neem seed extracts to control insect pests of papaya.

(iii) To determine the optimum application rates of *B. thuringiensis*, methanolic and aqueous neem seed extracts against insect pests of papaya.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The origin and distribution of papaya

Papaya or pawpaw, *Carica papaya*, (Caricaceae) is a small tropical plant native to South America (Rice and Rice, 1986). Papayas are locally important throughout the tropics where they thrive in frost-free areas below 1500 m in elevation. Hawaii is the major producer of papaya for export of fresh fruits while Sri Lanka and Tanzania are the primary producers of papain, a proteolytic enzyme used in meat tendering, brewing, tanning and textile manufacture (Becker, 1958). Papaya production in Africa is mostly confined to plantings of few trees around private homes or in villages. In Tanzania large scale production is carried out for the production of papain (Rice and Rice,1986). In Ghana, pawpaw is grown in backyard gardens and marketed locally until the 1960s when exotic varieties including 'Solo' were introduced. Large scale commercial production started when the varieties 'Solo', 'Hortus Gold' and 'Bluestem' were re-introduced in the 90s. Even though exports are in the infancy, the prospects for increased export in the future appear to be optimistic (Abutiate, 1999).

2.1.1 Uses of papaya

The ripe fresh fruits are eaten throughout the tropics. They are used for making soft drinks, jams, and ice cream flavouring. Young leaves are sometimes eaten as spinach. The seeds are used in some countries as a vermifuge, counter-irritant and abortifacient (Becker, 1958; Storey, 1958). The papain, a proteolytic enzyme is used in meat tendering, brewing, tanning and textile manufacture (Becker, 1958).

2.1.2 Culture

Propagation is primarily from seed which germinates in 2-4 weeks after planting. To obtain plants of the best quality, seeds should be obtained from superior plants. In the cultivar 'Solo', which is the most important commercial cultivar, seeds should be obtained only from hermaphrodite plants which have been self-pollinated or crossed with another hermaphrodite offspring. Propagation by cuttings is possible but only the entire branches including the basal swelling should be used. Due to the non-branching growth habit of the papaya, trees produce few cuttings and propagation by cuttings is therefore impractical in commercial operations (Rice and Rice, 1986). Grafting of scions from desirable plants onto seedlings is also possible although this is not practised on a commercial scale. (Rice and Rice, 1986; Storey, 1958).

2.1.3 Cultivars

There are a number of cultivars of pawpaw cultivated world wide. Common examples are the 'Bluestem' (hermaphrodite) ; 'Graham' (dioecious); 'Fair child' (dioecious); 'Hortus Gold' (dioecious) and 'Solo' (hermaphrodite) (Rice and Rice, 1986).

2.2 Insect pests of papaya

Several species of insect and mite pests cause economic damage to papaya in the field. *Tetranychus sp* a mite, attacks the underside of leaves near the main veins, resulting in yellowing and falling of leaves (Wilson, 1927). The mites, *Hemitarsonemus latus* (Bank) and *Tenuipalpusn bioculatus* , feed on the underside of leaves of papaya (Wilson, 1927). The larvae of the moth *Cryptoblades aliena*, feeds under a web on the floral stems and beneath the flower clusters of papaya (Wilson, 1927). The Mediterranean fruit fly *Ceratitis capitata* and the fruit fly *Dacus dorsalis* deposit their eggs in ripen fruits whiles the fruit fly *Toxotrypana curvicanda* deposits her eggs into the immature papaya and the larvae which develop feed on the flesh of the fruits (Becker, 1958 and Wilson, 1927). The scale insect *Pseudoparlatoria ostriata* entrusts fruits and reduces the market value of the papaya fruits (Wilson, 1927). Other pests which feed on ripen fruits of papaya include birds, bats and snails (Becker, 1958)

2.2.1 Major insect pests of papaya in Ghana

Although there has not been any report of work on insect pests of papaya in Ghana, Abutiate (1999) listed the following insects which attacks the crop in Ghana. *Ceratitis capitata*, deposits her eggs in ripen papaya fruits in the field. Viral diseases such as papaya mosaic and papaya ring spot are transmitted by the aphids *Aphis gossypii* (Glov.). The red spider mites feed on the underside of leaves of papaya, resulting in defoliation whiles the grasshopper *Zonocerus variegatus* feeds on leaves of papaya in the field and the nursery.

2.3 The neem tree and its characteristics

2.3.1 Origin and botanical characteristics

The origin of *Azdirachta indica* is most probably Myanmar (formerly called Burma) in S.E Asia and parts of India such as Karnataka (Troup, 1921; Schumutterer, 1995). It was seen in Uganda in 1874. It was believed that the neem seed was first introduced into Ghana from India between 1919 and 1927 by the Colonial Governor, the late Brigadier General Sir Frederick Gordon Guggisberg (Aaba and Kwesi, 1934). It was first introduced to the northern part of the then Gold Coast, now Ghana, which spread to other West African countries like Nigeria and the Sahel regions. It was also taken by the Spaniards to Manila in the mid 16th century and reached Malacca shortly afterwards (Storey, 1958). It is distributed in Asia, Africa, and the Americas and Australia (Vartak and Ghate, 1990). *Azadirachta indica* is a fast growing plant that usually reaches a height of 15-20 m, and under very favourable conditions, up to approximately 35-40 m. (Benge, 1989). The leaves contain 12.40-18.27% crude protein, 11.40-23.08% crude fibre, 34.32-66.60% N-free extract, 2.27-6.24% ether extract, 7.73-18.37% total ash, 0.89-3.96 % calcium and 0.10-0.30 % phosphorus (Pennington and Styles, 1975). The fruits are green when young and yellowish-green to yellow when mature.

2.3.2 Chemical constituents and mode of action of neem products

The major constituent in neem is azadirachtin (a complex limonoid) which is one of the strongest insect antifeedant and ecdysis inhibitory compounds known from botanical source (Isao *et al.*, 1986; Warthen, 1989). It was observed that this component inhibits feeding in 20 species of Coleoptera, 25 species of Lepidoptera, 14 species of Hemiptera and 5 species of Orthoptera (Anon, 1992). Other constituents are azadiron, azadiradion, nimbin, meliantriol, nimbidin, vilasinin, gedunin, salannin, azadiracbtin, epoxyazadiradion nimbiem (Anon, 1992). Quercetrin-3- rhamnoside (quercitin), and quercetin-3 rutinoside (rutin) are reported to have medicinal and other uses (Kraus, 1984). Azadirachtin, salannin, meliantriol, nimbin and nimbidin also act as growth regulators and feeding deterrents in several insects including Mexican bean beetle, *Epilachna varivestis* (Muls). *Z. variegatus*, and *Schistocerca gregaria* (Forskål) (Steets and Schmutterer, 1975). Azadirachtin controls metamorphosis in insects from larva to adult (Steets and Schmutterer, 1975). Meliantriol causes insects to stop eating. Salannin is an antifeedant, inhibiting feeding in most insects, such as locusts, cucumber beetles, houseflies, the Japanese beetle and other insects (Anon, 1992). Neem has been observed to deter feeding in a number of insects notably lepidoptera larvae such as diamond back moth, *Plutella xylostella* (L), the large cabbage white, *Pieris brassicae* (L), the tobacco budworm, *Heliothis virescence* (F) and the fall army worm, *Spodoptera frugiperda* (Smith) (Nakanishi, 1975). Schmutterer *et al.*, (1993) obtained an anti- feedant response to aqueous extracts of neem seed in *Nomadocris septemfasciata* (Serv.) (Acrididae) on sorghum. Nymphs were more sensitive in their reaction than adults. Narayanan, *et. al.*, (1978) observed strong anti-feedant properties of 1 % solution of an acetone extract of neem leaves and alcoholic extracts of neem oil. Anti-feedant effects were also obtained in *Z. variegatus*. Olaifa and Akingbohungbe (1987), and Gomez (1990) recorded that aqueous extract of neem seed (60 g/l water) showed an anti-feedant effect against *Diabrotica balteata* (Lec.) and *Cerotoma ruficornis* (Olr) on cowpea.The repellency effect of neem has also been extensively studied. Aqueous neem seed extract showed a strong repellent effect on nymphs and adults of *S. gregaria* (Sarup and Srivastava, 1971). Neem products caused strong repellency against *N. septemfasciata* (Acrididae) (Schmutterer, *et. al.*, 1993; Langewald, 1994). Koul and Isman (1991) demonstrated that neem products depress feeding in lepidopteran pests following topical administration. Azadirachtin also disrupted the metamorphosis of treated larval instars and prepupal and early pupal stages of the Japanese beetle, *Papillio japonica* (Newm.) (Anon, 1992).

Steets and Schmutterer (1975) recorded the sterilizing effect of azadirachtin from neem on adults of *Epilachna varivestis* in the laboratory on bean leaves.

2.3.3 Work on neem in Ghana.

Neem products have been shown to be effective protectants of grains against infestation by grain weevils, grain beetles, grain borers, cowpea beetles and other storage moths (Cobbina and Appiah-Kwarteng, 1989; Owusu- Akyaw, 1991; Allotey and Dankwah, 1994; Obeng-Ofori, 1997). Neem products were also found to be effective against field pests of cocoa, cereals, legumes and vegetables as well as cocoa mirids, fruit borers, plant bugs and leaf rollers in Ghana (Cobbina and Osei-Owusu, 1988; Adu-Gyamfi, 1989; Wiafe, 1992; Ahadze, 1993, Adu-Acheampong, 1997; Owusu Ansah *et al.*, 1998; Ankra, 1998).

2.4 Extraction of neem products

2.4.1 Aqueous neem extract

One of the simplest extraction techniques is the aqueous extract suspension, which is prepared from ground neem seed or leaf (Schmutterer and Hellpap, 1988). Aqueous extracts are prepared by grinding neem seeds. The pulverized seeds are allowed to soak in water for a minimum of six hours. The suspension is then filtered and the filtrate is used to spray on crops (Przybyszewski, 1993).

2.4.2 Extraction using alcohol

Numerous studies on the use of neem products against insect pests have utilized a variety of extraction techniques and a wide range of concentrations of different parts of the neem tree. One of the most effective extraction techniques is the alcohol extract suspension. This is prepared from ground and dried neem seeds or dried neem leaves (Schmutterer and Hellpap 1988). The seeds are extracted fresh or dried in the shade at ambient temperature to avoid decomposition of the active ingredients (Schroeder and Nakanishi, 1987). Methanol, ethanol or acetone are used as solvents for extraction of neem seeds (Uebel *et al.*, 1979; Schroeder and Nakanishi, 1987). Ninety five percent ethanol was found to be one of the best solvents for extraction of neem seed compared to methanol (Butterworth and Morgan, 1968; Morgan and Thornton , 1973)

2.5 *Bacillus thuringiensis*

Bacillus thuringiensis, is a spore-forming bacterium that produces a crystal of toxic protein (delta-endotoxin) with each spore. When spores and crystals are eaten by a susceptible insect, the mouth parts and gut are paralysed and the gut epithelium is destroyed. The insect dies in a few hours to about three weeks, depending on the dose eaten and the type of pathogenic action induced (de Barjac, 1978). It persists on foliage for periods varying from three days to six weeks depending on the condition. This microbial insecticide is comparable to a stomach poison. It is effective only when ingested by the insect and has no contact action. Spores are killed by uv light (de Barjac, 1978).

2.5.1 Origin and biological characteristics of *B. thuringiensis*

Bacillus thuringiensis was successfully isolated by analysing both moist and dry soil samples from ponds or stream banks which were known to be mosquito breeding sites in the Negev, Israel, (Goldberg and Margalit, 1977)

Bacillus thuringiensis group is composed of aerobic, gram–positive endospore-forming rods. Two strains, belonging, respectively to the serotype 1 (*B. thuringiensis*) and the serotype 3a, 3b, (Kurstaki), had been identified showing a high pathogenicity against larvae of Lepidoptera and Culicidae (Hall *et al.*, 1977; Panbangred, *et al.*, 1979).

2.5.2 Effectiveness of *B. thuringiensis* against insects

Bacillus thuringiensis has been found to have larvicidal effect in the laboratory on larvae of *Culex pipiens* (Wied), *Aedes aegypti* (L) and *Anopheles sergentii* (Theo.) (Goldberg, *et al.*, 1979). The bacterium was incorporated into a buoyant formation in which cells were concentrated in the upper 2-3mm of water. The estimated dosages ED_{95} for controlling *C. pipiens* complex was $8x10^4$ cells /ml and for *A. sergentii* was $6x10^5$ cells/ml (Garcia and Goldberg, 1977; Goldberg and Margalit, 1977) . The mortality rate of the larvae of the blackfly (Simuliidae) reached 60 % in 12 hours under ambient temperature (Guillet and Escaffre, 1979). A field trial was carried out in a natural pond containing all stages of *A. aegypti* larvae. The water had a temperature of 11^0C, pH of 6.8 and a salinity of 2.5 % NaCl. The effective dosage was10 kg/ha for the standard powder and 2.8 kg for another primary powder produced at the industrial level (de Barjac, 1979). *Bacillus thuringiensis* was also evaluated in the field on insects such as the black fly, *Simulium damnosum* (Theobald) and *C. pipiens* mosquito. The minimal concentrations of the primary powder inducing a complete mortality was 0.1mg/l against *A. aegypti* and 0.4 mgl/l against *C. pipiens* (Sinégre, *et al*., 1979). Neither the nymphs of mayfly, dragonfly, damselfly nor the adults of Ranatra species and copepods were susceptible to the bacterium (Prasertphon, 1979). The bacterium was not pathogenic to the moth, *Anagasta kuehniella* (Zell), *Plutella maculipennis* (Curt.) and *Prodenia litura* (F) (de Bacjac, 1978) but showed low toxicity against *Euproctis chrysorrhoea* (L) caterpillars (40 % mortality in 7 days) (Garcia and Goldberg, 1977).

2.5.3 Work on *B. thuringiensis* in Ghana

The use of *B. thuringiensis* for the control of insects in Ghana is at its infant stages. However, Owusu-Ansah (1999) applied *B. thuringiensis* to control the insect pests of local garden egg, *Solanum integrifolium* in the field at the rate of 0.8 g/l. It was observed that the neem seed water extract and karate were more effective than *B. thuringiensis*. *B. thuringiensis* is active against lepidopterous pest of most crops. Notable examples are its effectveness against lepidopterous pests of egg plant (Braima and Timbilla, 1987) and cabbage (Braima and Timbilla, 1990; 1991).

2.5.4 Production of *B. thuringiensis*

Bacillus thuringiensis is produced by submerged liquid fermentation. It can be grown on nutrient agar media used for the commercial production of the other strains of the bacterium. (Goldberg and Margalit, 1977; Goldberg, *et al.*,1977). *Bacillus thuringiensis* used to prepare standard formulation "IPS-78" is cultivated on the following medium at 30°C for 35 to 40 hours: wheat flour (15 g), glucose (10 g) pepton (5 g), yeast extract (5 g) $KH_2PO_4.7H_2O$(0.1 g), $MgSO_4.7H_2O$ (0.5 g), Nacl (3 g), $FeSO_4$ (0.1 g) and water. The spore-crystal mixture is precipitated with acetone and collected by centrifugation (de Barjac, 1979).

2.5.5 Formulation and specifications of *B. thuringiensis*

The standard formulation of *B. thuringiensis* prepared was "1PS-78" (de Barjac, 1979; WHO, 1979), wettable and dispersible powders, liquid concentrates and granules are being developed in several countries.

Special formulations had been developed for blackfly control as it was found that the effectiveness of the bacterium was related to the size and consistency of the formulation particles (Guillet, *et al.*, 1979; Guillet and Escaffre, 1979).

2.5.6 **Stability of *B. thuringiensis***

The delta-endotoxin of *B. thuringiensis* can withstand 80 °C for eight hours (de Barjac, 1979) without losing activity but exposure to 120 °C for 15 minutes causes inactivation (de Barjac, 1978). No significant loss in larvicidal activity was observed following heat shock for 20 minutes at 60 °C.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Laboratory experiments

A preliminary experiment was conducted in the laboratory to determine the optimum application rates of *B. thuringiensis*, methanolic and aqueous neem seed extracts. The experimental design used was Completely Randomized Design (CRD)._Glass jars each measuring 15 cm in width and 45 cm in height were used. Four jars each containing two papaya leaves treated with methanol or aqueous neem seed extract were evaluated at the following concentrations: 0, 50, 75 and 100 g/l. Ten adult *Z. variegatus* were introduced into each jar for 48 hours. Each treatment was replicated four times. The same procedure was repeated for the third nymphal stages. In a similar experiment, six jars each containing two papaya leaves treated with *B. thuringiensis* at the rates of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 g/l were tested against *Z. variegatus*. Ten adult *Z. variegatus* were introduced into each jar and left for 48 hours. Each treatment was replicated four times. In the two experiments described above dead insects were recorded after 48 hours and percent mortality was calculated. Percentage leaf damage caused by the insects in the various treatments was also determined. Since natural mortality was observed, the data was corrected using Abbots formula (Abbot, 1925) before transforming the data using arcsine transformation.

3.2 Field experiment

3.2.1 Location of experimental site

The experiment was carried out at Paradise Farms, located at Obom, about 10 kilometers from Nsawam in the Eastern Region of Ghana from June 1997 to January 1999. The site had previously been cropped to cassava, maize and cowpea for the past six years. The land was ploughed, harrowed and levelled. Lining and pegging were subsequently carried out. The experimental design used was the randomized complete block design (RCBD). There were five treatments, which were replicated three times. A plot measured 16 m x 6 m and a block measured 80 m x 6 m. There were 3 blocks. The total land area for the experiment measured 80 m x 26 m. There were 15 plots. Each plot contained 24 plants. The planting distance was 2 m^2 and the distance between plots and blocks was 4 m.

Treatments used were:

T_1- Control (water only)

T_2- Methanol (90 %) only

T_3- Methanolic neem seed extract at 100 g/*l*

T_4- Aqueous neem seed extract at 100 g/*l*

T_5- *Bacillus thuringiensis* at 1 g/*l*.

3.2.2 Nursing of seeds, transplanting and fertilizer application

Seeds of papaya cv. 'Solo sunrise' imported from Hawaii University were soaked in a chemical called 'intercept' (It is a combination of a nematicide and fungicide) at 1% solution to control nematodes and soil fungi. The seeds were nursed on a seedbed measuring 2 m^2 and watered every other day. The seedlings were transplanted onto the field six weeks after nursing. Four weeks after transplanting sulphate of potash and rock phosphate fertilizers were applied at the rate of 150 g/plant and 450 g/plant, respectively. Also, a foliar fertilizer, 'siapton' (developed from compost manure) was applied at 1 %, six weeks after transplanting as a nitrogen source. Twenty kilograms of cowdung was also applied per plant to improve the organic matter content of the soil. The plants were irrigated two hours each day with drip irrigation at a rate of 2.3 litres per hour. Weeding of plots was done each month during the duration of the experiment.

3.2.3 Preparation of aqueous neem seed extract (ANSE)

Dropped neem fruits were collected from neem trees at 37 Military hospital in Accra, dried partly in the sun for about four hours. Drying was continued in the shade for 30 days. The dried neem seeds were milled in an electric miller into a coarse powder and mixed with water at the required concentrations.The mixtures were allowed to stand overnight according to Kumar and Sangappa (1984) and then strained using a muslin cloth before spraying.

3.2.4 Methanol neem seed extract.

The methanolic extract was prepared by grinding the neem seed and mixed with 90 % methanol in a glass jar. The mixture was stirred thoroughly and placed in a hot water bath at a temperature of 45 °C for six hours. The methanol was evaporated from the mixture. The required application rate was prepared by weighing the required weight of the evaporated extract and mixed with the right amount of water.

3.2.5 Formulation of *B. thuringiensis (Bt.)*

To obtain the following concentrations of *B. thuringiensis*. i.e. (0, 0.2, 0.4, 0.6, 0.8 and 1.0 g/*l*), the required weight of Bt. was weighed with a Metler balance and mixed with the required amount of water and sprayed.

3.2.6 Data collection and spraying schedule

Data collection and spraying started two months after transplanting when insects were first observed on the field and repeated each month till the end of the experiment. The experiment was conducted over a period of 17 months. Sampling of insects was done in the morning because most of the insects were less active during this time. Also during the morning wind velocity is low, which means the drift of the biopesticides was reduced. At each sampling, the middle eight plants were examined for insects out of 24 plants. Insects were collected using a sweep net or handpicked where appropriate. The variegated grasshoppers were collected using sweep net and hand picked where appropriate. While the mealy bugs and the Mexican bean beetle were hand picked. All insects collected were killed with 90% methanol and later identified using reference collections at Zoology and Crop Science Departments of the University of Ghana.

3.2.7 Assessment of leaf damage

Leaf damage was assessed by randomly selecting three leaves each from the middle eight plants and the amount of damage estimated by tracing the holes created as a result of the insect feeding on a graph paper. The total area damaged by insects was calculated (Coaker, 1957). The percentage leaf damage was calculated using the formula:

$$\% \text{ Leaf Damage} = \frac{\text{Area of damaged leaf}}{\text{Area of whole leaf}} \times 100\ \%$$

The figures obtained were transformed using the arcsine formula

$$X^1 = \text{sine}^{-1} \sqrt{x}/\ 10. \qquad (x = \%\ \text{leaf damage})$$

3.2.8 Yield data

Harvesting started 10 months after transplanting (Plate 3). The harvested fruits were separated into damaged and undamaged fruits i.e. those damaged by insects. They were counted, weighed and recorded. The yield per hectare (ha) of fruits obtained from each treatment was calculated based on plot size, plant spacing, mean fruit weight and number of fruits per plant. All data collected were analysed using analysis of variance and means were separated by Ducans Multiple Range Test.

Plate 1. Pre-pupa of Mexican bean beetle on papaya fruit

Plate 2. Damage on papaya leaves caused by *Z. variegatus*

Plate 3. Papaya plants after 10 months

CHAPTER 4

4.0 RESULTS

4.1 LABORATORY EXPERIMENT

4.1.2 Effect of methanol neem seed extract on *Z. variegatus*.

The mean percentage mortality of nymphs and adults of *Z. variegatus* fed on leaves treated with different dosages of methanol neem seed extracts are shown in Table 3. Significant differences ($p<0.05$) were observed among the treatments in terms of percentage mortality of nymphs and adults. The mean mortality of nymphs increased as the application rates increased (Table 3). However, there was no significant ($p>0.05$) difference between mean percentage mortality of nymphs on leaves treated with 50 g/l and 75 g/l of methanol neem seed extract. The highest mortality of nymphs was observed on leaves treated with 100 g/l of methanolic neem seed extract (Table 3). Similarly, the mean mortality of adult *Z. variegatus* increased with increasing concentrations of methanol neem seed extract on leaves (Table 3). It was observed that leaves treated with 75 g/l and 100 g/l showed about the same ($p>0.05$) percentage mortality of the adults. The highest mortality of adults was recorded on leaves treated with 100 g/l of methanolic neem seed extract (40%) (Table 3). In general mortality was dose-dependent.

4.1.3 Effect of methanol neem seed extract on leaf damage.

The percentage leaf damage caused by nymphs and adults *Z. variegatus* are shown in (Table 3) There were significant differences ($p<0.05$) in the leaf damage caused by adult *Z. variegatus* among the treatments. The leaf damage caused by adult *Z. variegatus* decreased as the application rates increased (Table 3). Leaves treated with 100 g/l of methanol neem seed extract recorded the least leaf damage (23.8%), while the control treatment had the highest leaf damage (69.7%) (Table 3). Similarly, leaf damage caused by nymphs decreased with an increase in dosage. The leaf damage caused by nymphs on leaves treated with 75 g/l and 100 g/l were similar ($p>0.05$). It was also observed that there was no significant difference ($p>0.05$) between leaves treated with 50 g/l and the control (Table 3). Leaves treated with 100 g/l had the least leaf damage by nymphs (11.2%) (Table 3).

Table. 3. The effect of different concentrations of methanol neem seed extract on insect mortality and percentage leaf damage caused by *Z. variegatus*.

Dosage (g/l)	Mean % mortality of nymphs x±SD	Mean % mortality of adults x±SD	Mean % leaf damage caused by nymphs x±SD	Mean % leaf damage caused by Adults x±SD
0	18± 1.5[a]	10± 5.6[a]	16.9± 2.5[a]	69.7± 7.6[a]
50	33± 5.2[b]	20± 6.6[b]	15.7± 2.5[a]	49.2± 5.1[b]
75	33± 1.5[b]	33± 4.4[c]	11.2± 2.0[b]	38.2± 3.7[c]
100	60± 6.8[c]	40± 5.5[c]	10.7± 2.1[b]	23.8± 3.6[d]

* Column means followed by the same letters are not significantly different at p> 0.05 Duncan's Multiple Range Test

4.1.4 Effect of aqueous neem seed extract on insect mortality

Mortality of *Z. variegatus* fed on leaves treated with different dosages of aqueous neem seed extract is shown in Table 4. Mortality of nymphs on leaves treated with 100 g/l of aqueous neem seed extract was higher than the other treatments (43%). The mean mortality of nymphs increased with increasing dosage. However, there were no significant differences ($p>0.05$) in the mortality of nymphs on leaves treated with 75 g/l and 100 g/l of aqueous neem seed extract and also between leaves treated with 50 g/l and the control treatment (Table 4). Similarly, the mortality of adult *Z. variegatus* increased as the dosage of aqueous neem seed extract increased (Table 4). The highest mortality of adult *Z. variegatus* was recorded on leaves treated with 100 g/l of aqueous neem seed extract (45%)and the lowest by the control treatment (18%). There were no significant differences ($p>0.05$)between leaves treated with 75 g/l and 100 g/l of aqueous neem seed extract and also between leaves treated with 50 g/l and he control treatment (Table 4).

4.1.5 Effect of aqueous neem seed extract on mean leaf damage

The percentage leaf damage caused by both adults and nymphs reduced considerably in aqueous neem seed extract (Plate 2) (Table 4). The least leaf damage caused by nymphs was recorded on leaves treated with 100 g/l of aqueous neem seed extract(7.7%) and the highest on the control treatment (Table 4). However, there were no significant differences ($p>0.05$) in the mean % leaf damage between leaves treated with 75 g/l and 100 g/l. Leaves treated with 50 g/l of aqueous neem seed extract were similar ($p>0.05$) in leaf damage to the untreated leaves (control) caused by nymphs (Table 4). The leaf damage caused by adult *Z. variegatus* showed significant difference ($p < 0.05$) among the treatments. The leaf damage caused by adults on leaves treated with 50 g/l was similar ($p>0.05$) to those treated with 75 g/l of aqueous neem seed extract. The least leaf damage caused by adult *Z. variegatus* was recorded on leaves treated with 100 g/l of aqueous neem seed extract (35%) (Table 4). The leaf damage caused by nymphs and adults of *Z. variegatus* decreased as the concentration of aqueous neem seed extract increased.

Table .4. The effect of aqueous neem seed extract on mean mortality and percentage leaf damage caused by *Z. variegatus*.

Application rate (g/l)	Mean % mortality of nymphs x±SD	Mean % mortality of Adults x±SD	Mean % leaf damage caused By nymphs x±SD	Mean % leaf Damage caused By adults x±SD
0	18± 6.0[a]	18± 2.5[a]	14.0± 1.5[a]	69.7± 7.6[a]
50	20± 8.7[a]	20± 6.7[a]	12.7± 1.3[a]	49.2± 5.1[b]
75	38± 11[b]	40± 9.0[b]	8.7± 1.2[b]	38.2± 3.7[c]
100	43± 5.6[b]	45± 6.5[b]	7.7± 1.0[b]	23.8± 3.6[d]

*Column means followed by the same letters are not signicantly different at p> 0.05 Duncan's Multiple Range Test

Effect of *B. thuringiensis* on mean mortality of *Z. variegatus*

The mean mortality of adults and nymphs of *Z. variegatus* on leaves treated with Bt. increased as the concentration increased (Table 5). Significant differences ($p < 0.05$) were observed among the treatments in terms of the mortality of the nymphs. Nymphs on leaves treated with 0.2, 0.4 and 0.6 g/l were not significantly different ($p > 0.05$) in percentage mortality (Table 5). The highest mortality of nymphs was recorded by leaves treated with 1 g/l of *B. thuringiensis* (Table 5). The mortality of adults recorded on leaves treated with 0.6 g /l of Bt did not show any significant difference ($p > 0.05$) from leaves treated with 0.8 g/l of Bt. Mortality induced by leaves treated with 0.2 g/l and 0.4 g/l Bt was also not Significant ($p > 0.05$). The highest mortality of adults was recorded on leaves treated with 1 g/l of Bt (83 %) and the lowest by the control treatment (5 %) (Table 5).

4.1.7 **Effect of *B. thuringiensis* on mean leaf damage caused by *Z. variegatus***

Significant differences ($p < 0.05$)were observed in leaf damage caused by adults and nymphs . The leaf damage decreased as the concentration of *B. thuringiensis* increased (Table 5). It was observed that there were no significant differences ($p > 0.05$) in leaf damage caused by nymphs on leaves treated with 0.2 g/l and 0.4 g/l B.t. Leaves treated with 1 g/l of Bt. had the least leaf damage caused by both nymphs (10%) and adults (22.5%) (Table 5). However, there were no significant differences ($p > 0.05$) in leaf damage caused by adults on leaves treated with 0.2, 0.4 g/l and the untreated leaves (control) (Table 5).

Table 5. Effect of *B. thuringiensis* on mean mortality and % leaf damage caused by *Z. variegatus*

Dosage (g/l)	Mean % mortality of nymphs x±SD	Mean % mortality of adults x±SD	% leaf damage caused by nymphs x±SD	% leaf damage cause by adults x±SD
0	8.0 ±3.1^{a}	5.0 ±2.5^{a}	72.0 ±5.8^{a}	71.0 ±8.0^{a}
0.2	20.0 ±6.2^{b}	18.0 ±7.6^{b}	54.0 ±10.0^{b}	65.3 ±6.3^{a}
0.4	33.0±6.5^{b}	15.0±2.6^{b}	42.0 ±6.6^{b}	63.7 ±4.2^{a}
0.6	40.0±9.9^{b}	55.0±3.4^{c}	28.0 ±4.7^{c}	49.5 ±7.7^{b}
0.8	65.0±6.4^{c}	63.0±7.6^{c}	17.0 ±5.1^{d}	35.4 ±11.2^{d}
1.0	83.0±6.5^{d}	85.0±12.7^{d}	10.0 ±2.5^{e}	22.5 ±7.1^{e}

Column means followed by the same letters are not significant at $p > 0.05$.
Duncan's Multiple Range Test

4.2 Insect fauna of pawpaw at Paradise farm

The major insect species encountered in the field were grasshoppers, *Zonocerus variegatus*, mealy bugs, *Planococcus* spp. and Mexican bean beetles, E*pilachna spp*. *Z. variegatus* were found defoliating leaves of pawpaw while the mealy bugs were found piercing and sucking cell sap of fruits. The *Epilachna spp.* were found pupating on the fruits which caused injury to the fruits when removed (Plate 1).Other pests encountered in small numbers on the fruits were the fruit fly, *Ceratitis capitata*, they deposit eggs into riped fruits; the birds, bats and the snails fed on riped fruits; the ants, *Oecophilla longinoda* and *Crematogaster* spp., were a nuissance to the pawpaw fruit harvesters.

4.2.1 Effect of neem extracts and B*t* on mean population of *Z. variegatus* in the field.

There were significant differences ($p<0.05$) in the mean population of *Z. variegatus* on leaves among the treatments in the field. The control treatment was significantly different ($p<0.05$) from the rest of the treatments. Plants treated with methanolic neem seed extract at 100 g/l recorded the least population of Z. *variegatus* (3.2) and the highest by the control treatment (16.37) (Table 6). However, there was no significant difference ($p>0.05$) in the mean populations of *Z. variegatus* found on plants sprayed with aqueous neem seed extract at 100 g/l and on plants treated with methanolic neem seed extract at 100 g/l. Also between mean populations of *Z. variegatus* on plants treated with *B. thuringiensis* at 1 g/l and 90% of methanol. (Table 6)

4.2.2 Effect of neem extracts and B*t* on mean leaf damage caused by *Z. variegatus* in the field

Significant differences ($p<0.05$) were observed among the treatments in terms of leaf damage caused by *Z. variegatus* in the field. It was observed that there were no significant differences ($p>0.05$) in the mean leaf damage caused by *Z. variegatus* on plants treated with 90% of methanol and water only (Table 6). Similarly, there were no significant differences ($p>0.05$) in leaf damage on plants treated with aqueous neem seed extract at 100 g/l, *B. thuringiensis* at 1 g/l and the methanol neem seed extract at 100 g/l. The least leaf damage caused by *Z. variegatus* was recorded on plants treated with methanolic neem seed extract at 100 g/l (2.82%) (Table 6).

Table 6. The effect of neem extracts and B*t* on mean population of Z. *variegatus* and mean % leaf damage

Treatments	Mean number of *Z. variegatus* x±SD	Mean % leaf damage x±SD
Aqueous neem seed /100 g/l	4.33 ± 0.2 [a]	3.36 ± 0.2 [a]
B. thuringiensis 1g/l	10.73 ± 0.7 [b]	4.13 ± 0.2 [a]
Methanol neem seed extract /100 g/l	3.2 ± 0.4 [a]	2.82 ± 0.01 [a]
90% Methanol only	13.0 ± 0.9 [b]	6.98 ± 0.6 [b]
Control (water only)	16.37 ± 0.4 [c]	7.87 ± 0.3 [b]

Column means followed by the same letters are not significantly different at ($p > 0.05$) Duncan's Multiple Range Test

4.2.3 Effect of neem extracts and B*t* on mean number of mealy bugs on papaya fruits.

Number of mealy bugs recorded on fruits were significantly different ($p<0.05$) among the treatments (Table 7). It was observed that the number of mealy bugs on plants sprayed with 90% methanol was not significantly different from the plants treated with water only (control). The number of mealy bugs on plants treated with aqueous neem seed extract, methanolic neem seed extract and *B. thuringiensis* was similar ($p>0.05$).The control treatment recorded the highest population of mealy bugs (10.56) while the methanolic neem seed extract at 100 g/l recorded the least number of mealy bugs (3.56) (Table 7).

4.2.4 Effect of neem extracts and B*t* on mean number of pre-pupa of *Epilachna spp*. on papaya fruits

Significant differences ($p<0.05$) were observed among the treatments with regards to the mean population of pre-pupa of *Epilachna spp*. It was observed that the number of pupa of the beetle on plants treated with aqueous neem seed extract was not significantly different ($p>0.05$)from plants treated with methanolic neem seed extract. However, both treatments were significantly different from the rest of the treatments (Table 7). The least population of *Epilachna spp*. was recorded on plants treated with methanolic neem seed extract (2.78) and the highest on the untreated plants (control) (10.40).

Table 7. Effect of neem extracts and B*t* on mean populations of mealy bugs and pupa of epilachna beetle on pawpaw fruits

Treatments	Mean pop. of mealybugs x±SD	Mean pop of pupa epilachna beetle x±SD
Aqueous neem seed extract 100 g/l	3.90±1.0 [a]	4.56±0.5 [a]
B. thuringiensis 1g/l	5.65±0.4 [a]	8.10 ±1.2 [b]
Methanol neem seed extract 100 g/l	3.56 ±0.7 [a]	2.78 ±0.8 [a]
90% methanol	9.56±1.7 [b]	6.00±1.1 [b]
Control (water only)	10.56±1.1 [b]	10.40±0.8 [c]

Columns means followed by the same letters are not significantly different at $p > 0.05$
Duncan's Multiple Range Test

4.2.5 Effect of neem extract and B*t* on height, girth and spread of leaves of papaya plants

Significant differences ($p<0.05$) were observed among treatments in terms of plant height. The plants sprayed with aqueous neem seed extract were significantly ($p<0.05$) higher than those sprayed with 90% methanol (Table 8). There were no significant differences ($p>0.05$) in the height of plants treated with 90% methanol and water only. Also, plants treated with aqueous neem seed extract and methanolic neem seed extract showed no significant differences ($p>0.05$) in plant height (Table 8). However, plants treated with methanolic neem seed extract recorded the highest plant height (163.9) (Table 8). In general the height of plants increased with the application of biopesticides. There were significant differences ($p<0.05$) among the treatments in terms of plant girth. Plants treated with aqueous neem seed extract did not show any significant difference ($p>0.05$) in plant girth with those treated with methanolic neem seed extract. However, both treatments were significantly different ($p<0.05$) from the rest of the treatments (Table 8). It was also observed that plants treated with *B. thuringiensis*, 90 % methanol and water only were similar ($p>0.05$) in girth. Plants treated with methanolic neem seed extract recorded the highest plant girth (28.9 cm) (Table 8). Significant differences were ($p<0.05$) observed among the treatments in terms of spread of leaves. The spread of leaves among treatments increased considerably in relation to the application of biopesticides. Plants treated with aqueous neem seed extract were similar in leaf spread with those treated with methanolic neem seed extract. However, plants treated with methanolic neem seed extract recorded the highest leaves spread (209.8 cm). There were no significant differences ($p>0.05$) in the spread of leaves on plants sprayed with 90 % methanol and water only.

Table 8. Effect of neem extracts and Bt on height, girth and spread of leaves of papaya plants.

Treatments	Mean height/cm x±SD	Mean girth/cm x±SD	Mean spread/cm x±SD
Aqueous neem seed exract (100 g/l)	144.9 ± 5.8 [a]	25.1 ± 1.0 [a]	194.7 ± 2.4 [a]
B. thuringiensis (1 g/l)	123.0 ± 4.8 [b]	16.9 ± 1.6 [b]	140.2 ± 3.3 [b]
Methanol neem seed extract (100 g/l)	163.9 ± 3.3 [a]	28.9 ± 1.3 [a]	209.8 ± 3.6 [a]
90% methanol	103.4 ± 2.6 [c]	13.8 ± 0.2 [b]	123.6 ±2.6 [c]
Control (Water only)	97.1 ± 3 .2 [c]	14.2 ± 1.3 [b]	117.2 ± 2.7 [c]

Column means followed by the same letters are not significantly different at $p > 0.05$

Duncan's Multiple Range Test

4.2.6 Effect of neem extract and Bt on mean number of flowers and leaves

Significant differences ($p<0.05$) were observed among treatments in terms of number of flowers and number of leaves produced. No significant differences ($p>0.05$) were observed on the number of flowers produced by plants sprayed with aqueous neem seed extract and those sprayed with methanolic neem seed extract. Both treatments produced significantly ($p<0.05$) higher number of flowers than the other treatments (Table 9). Plants treated with 90% methanol recorded the least number of flowers (23.5) while those treated with methanolic neem seed extract recorded the highest mean number of flowers (60.79) (Table 9). Plants treated with *B. thuringiensis* , 90% methanol and the water only recorded similar ($p>0.05$) number of leaves (Table 9). There were no significant differences in the number of leaves on plants sprayed with aqueous neem seed extract and those with methanolic neem seed extract. The plants sprayed with methanolic neem seed extract recorded the highest number of leaves (21.20) (Table 9).

Table 9. Effect of neem seed extract and Bt on mean number of flowers and leaves

Treatment	No. of flowers	No. leaves
Aqueous neem seed / 100 g/l	59.7 ± 4.7^{a}	20.9 ± 7.0^{a}
B. thuringiensis 1 g/l	38.7 ± 1.6^{b}	18.1 ± 9.1^{b}
Methanolic neem seed extract at / 100 g/l	60.8 ± 3.3^{a}	21.2 ± 5.9^{a}
90% of methanol	23.5 ± 1.0^{e}	18.7 ± 5.6^{b}
Control (water only)	26.9 ± 1.8^{c}	18.4 ± 1.2^{b}

Column means followed by the same letters are not significantly different at $p > 0.05$ Duncan's Multiple Range Test

4.2.7 Effect of neem extracts and Bt on mean fruit yield

The yield of papaya fruits among all the treatments showed significant Differences ($p<0.05$). Plants sprayed with aqueous neem seed extract recorded the highest yield of undamaged fruits (161.6 kg/ha) (Table 10). Similarly, the highest number and weight of undamaged fruits were recorded by plants treated with aqueous neem seed extract (59.3 and 17.2 kg), respectively (Table 10).

Table 10. Effect of neem extracts and B. thuringiensis on fruit yield

			damaged fruits kg/ha
Aqueous neem Seed extract (100 g/l)	59.3±7.6a	17.2±3.2a	161.6±5.83 a
B. thuringiensis (1 g/l)	33.3±11.0b	9.7±1.7b	51.2±1.65 b
methanolic neem seed extract 100 g/l	58.0±9.2a	16.8±2.5 a	154.3±2.2 a
90% methanol	23.3±5.8c	6.8±1.7 b	25.1±0.15 c
control (water only)	18.3±3.2c	5.3±1.8 b	15.4±0.49 d

Duncan's Multiple Range Test

4.2.8 Effect of neem extract and Bt on mean fruit damage

Significant differences ($p<0.05$) were observed among treatments in terms of fruit damage caused by insect pests. Plants sprayed with aqueous and methanolic neem seed extract produced the least number and weight of damaged fruits (Table 11). The highest number of damaged fruits were recorded by the control treatment (23.1 kg/ha) while the least yield of damage fruits was recorded on plants sprayed with aqueous neem seed extract (4.2 kg/ha). Plants sprayed with aqueous neem seed also recorded the least number and weight of damaged fruits (12.0 and 2.2 kg), respectively (Table 11).

Table 11. Effect of neem extracts and *B. thuringiensis* on fruit damage

Treatment	Mean no. of damaged fruits	Mean weight of damaged fruit/kg	Mean yield of damaged fruits/kg/ha
Aqueous neem Seed extract 100 g/l	12.0±2.7[a]	2.2±1.0[a]	4.2±0.37[a]
B. thuringiensis (1 g/l)	22.3±5.9[b]	4.0±2.0[a]	14.0±0.48[b]
Methanol neem Seed extract (100 g/l)	14.0±2.1[a]	2.5±1.9[a]	5.6±0.05[a]
90% methanol	30.0±6.4[c]	5.4±1.0[a]	22.8±0.39[c]
Control (wateronly)	28.7±5.5[c]	5.1±2.1[a]	23.1±0.16[c]

Means in the colums followed by the same letters are not significantly different at $p > 0.05$.

Duncan's Multiple Range Test

CHAPTER 5

5.0 DISCUSSION

5.1 Insect mortality

It was established from the experiment that 100 g/l of aqueous and methanolic neem seed extracts caused significantly ($p<0.05$) higher mortality of nymphs and adults of *Z.variegatus* than the control. This may be due to the anti-feeding and repellent effect of the neem seed extracts which prevented the insects from feeding, hence their death through starvation (Jacobson, 1981; Rand-Hawa and Parmar, 1993). This confirms the work of Narayanan *et al.*, (1978), Sarup and Srivastava (1971) that aqueous and methanolic neem seed extract at 1 % was effective in the control of nymphs and adults of *Z. variegatus*. *B. thuringiensis* at the rate of 1 g/*l* also caused significant mortality ($p<0.05$) of *Z. variegatus* compared to the control treatment. This agrees with the work of Sinégre, *et al.*, (1979) that *B. thuringiensis* at a concentration of 1 g/l was able to control insects such as the black fly and mosquitoes in the field and in the laboratory.

5.2 Leaf damage

Leaf damage caused by adults and nymphs of *Z. variegatus* was very low on leaves treated with methanolic and aqueous neem seed extracts. This is an indication that the antifeedant and repellent activity of neem seed extracts prevented the insects from feeding on the plants (Schmutterer and

Ascher, 1984; Schmutterer, 1990). This confirms the work carried out in Madagascar in the maize farm where methanolic and aqueous neem seed extract significantly reduced the damage caused by nymphs and adults of *Z. variegatus* (Schmutterer *et al.*, 1993; Langewald, 1994). *Bacillus thuringiensis* at 1 g/l reduced leaf damage caused by nymphs and adults of *Z. variegatus* significantly than the control treatment. This was consistent with the results of the work conducted in Malaysia where Bt. reduced damage caused by *Z. variegatus* on rice plants (Oudejans, 1991).

Zonocerus variegatus was sensitive to methanolic and aqueous neem seed extracts in the field, therefore, resulting in the decrease in feeding activity and mobility. This resulted in low leaf damage caused by *Z. variegatus* in the field. This may be attributed to the repellent and antifeedant activity of neem (Schmutterer, 1990). Narayanan *et al.*, (1978) showed that neem extracts in methanol and water sprayed on plants reduced the feeding activities and decreased leaf damage caused by *Z. variegatus* and *S. gregaria* on cassava plants. From the studies, methanolic, aqueous neem seed extract and the Bt. sprayed on plants did not show any difference in the level of leaf damage since the three treatments gave comparable results.

5.3 Insects Population

The population of *Z. variegatus*, mealy bugs and pre-pupa of *Epilachna spp.* dropped when methanolic and aqueous neem seed extracts were sprayed on plants in the field compared to the control. This may be attributed to the insecticidal and antifeedant effect of the neem seed on the insects (Schmutterer and Ascher, 1984; Schmutterer, 1990). This confirms the work of Schmutterer and Ascher (1994) that methanolic and aqueous neem seed extracts were able to control insect pests like the grasshopper, *Z. variegatus* and the desert locust, *S. gregaria* on vegetables and tree crops. Similarly, plants sprayed with methanolic and aqueous neem seed extracts recorded low populations of pre-pupa of *Epilachna spp.* and mealy bugs than the control. This is consistent with the results of Schmutterer (1995) that neem products reduced the population of *Epilachna spp.*and mealy bugs on plants. From the study, there was no difference in insect pest numbers on plants sprayed with methanolic and aqueous neem seed extracts, however, plants sprayed with methanolic neem seed extracts recorded less number of insects. *Bacillus thuringiensis* at 1 g/l was effective in controlling *Z. variegatus* in the laboratory, but it could not control insects effectively in the field. This may be due to other environmental factors like tempereature and sunshine acting on *Bt.* on the field.The results obtained suggest good potential for the use of neem extracts to control insect pests of papaya in the field. Non-synthetic insecticides such as neem extracts represent an important component of integrated pest management of crop pests as they are broad spectrum in action, based on local materials and potentially less expensive. Many are also safe to the environment and harmless to man and other mammals.

5.4 **Plant growth**

In this study plants sprayed with 100 g/l of methanolic neem seed extract recorded the highest mean girth, height, number of flowers, number of leaves and spread of leaves of papaya plant in the field compared to the control. This shows that the neem products have growth promoting properties and thus confirms the work of Pennington and Styles (1975) that neem products contain high amount of nitrogen (43.32-66.60 %; 0.89-3.96 % calcium and 0.10-6.30 % phosphorus) which promotes growth in plants. Aqueous neem extract has also been shown to promote the vegetative growth of egg plant in Ghana (Owusu-Ansah, 1999).

5.2.4 **Yield**

The results showed that plants sprayed with methanolic and aqueous neem seed extract recorded the highest mean fruit yield of papaya, number and weight of fruits compared to the control. This shows that neem seed extracts were effective against the insects therefore preventing them from feeding on the plants, which resulted in the higher yield of papaya fruits. This is consistent with the work of Olaifa and Akingbohungbe (1987) that neem products are effective against several insect thereby promoting crop yield.

With the demand for organically grown products globally it will be ideal if the Ghanaian pawpaw farmer could adopt the use of neem extracts to control pests on his farms. They could take advantage of the large European market.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

The following conclusions and recommendations can be made from the study:

(1) The major insects pests encountered in the field were grasshoppers (*Z. variegatus*), mealy bugs (*Planococcus spp)*, and the *Epilachna spp*. Minor pests encountered were birds, bats, snails *Crematogaster spp* and *Oecophilla longinoda*.

(2) One hundred grams per litre of aqueous and methanolic neem seed extracts and 1 g/l of *B. thuringiensis* recorded the highest mortality of adult and nymph of *Z. variegatus* in the laboratory compared to the control treatment.

(3) 100 g/l of methanolic seed extract reduced the population of grasshopper, mealy bugs and Epilachna beetle in the field compared to the control.

(4) The leaf damage caused by *Z. variegatus* in the field was low on plants sprayed with 100 g/l of methanolic compared to the control.

(5) neem seed extracts promoted vegetative growth of papaya in the field.

(6) The highest fruit yield was recorded by plants sprayed with 100 g/l aqueous neem seed extract. From this experiment it may be recommended that 100 g/l of aqueous and methanolic neem seed extracts could be used to control pests

of papaya in the field. However, aqueous extraction may be recommended because it is cheaper and the extraction is also easier. It may be necessary to repeat this experiment to find the effect of neem seed extract and *B. thuringiensis* on growth and moulting of *Z. variegatus* in the laboratory. Also, to find the relative proportion of the growth promoting elements in neem products.

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APPENDICES

Appendix 1

Analysis of variance on mortality caused by nymphs in methanol neem seed extract in the laboratory

Source of variation	df	ss	ms	Observed F	P
Total	15	3840.0			
Treatment	3	3726.0	1242	104.0*	< 0.05
Block	3	6.5			
Error	9	107.5	11.94		

Appendix 2

Analysis of variance on mortality caused by adult *Z. variegatus* in methanol neem seed extract in the laboratory

Source of variation	df	ss	ms	Observed F	p
Total	15	220.96			
Treatment	3	201.71	67.24	51.72*	< 0.05
Block	3	7.54			
Error	9	11.72	1.30		

Appendix 3

Analysis of variance on leaf damage caused by nymphs of *Z. variegatus* in methanol

Source of variation	df	ss	ms	Observed F	p
Total	15	151.6			
Treatment	3	124.52	41.51	14.36*	< 0.05
Block	3	2.07			
Error	9	26.02	2.89		

Appendix 4

Analysis of variance on leaf damage caused by adult *Z. variegatus* in methanol neem seed extract in the

laboratory

Source of variation	df	ss	ms	Observed F	p
Total	15	151.356			
Treatment	3	145.64	48.55	81.87*	< 0.05
Block	3	1.38	0.46		
Error	9	5.34	0.593		

Appendix 5

Analysis of variance on mortality caused by nymphs of *Z. variegatus* in aqueous neem seed extract

Source of variation	df	ss	ms	Observed F	p
Total	15	1582			
Treatment .	3	1441.5	480.5	41.57*	< 0.05
Block	3	36.5			
Error	9	104	11.56		

Appendix 6

Analysis of variance on mortality caused by adult *Z. variegatus* in aqueous neem seed extract in the laboratory

Source of variation	df	ss	ms	Observed F	p
Total	15	112.68			
Treatment	3	87.43	29.14	12.10*	< 0.05
Block	3	3.54	1.18		
Error	9	21.71	2.41		

Appendix 7

Analysis of variance on leaf damage caused by nymphs in aqueous neem seed extract in the laboratory

Source of variation	df	ss	ms	Observed F	p
Total	15	145.71			
Treatment	3	126.64	42.21	42.64*	< 0.05
Block	3	10.16			
Error	9	8.91	0.99		

Appendix 8

Analysis of variance on leaf damage caused by adult *Z. variegatus* in aqueous neem seed extract in the

laboratory

Source of variation	df	ss	ms	Observed F	p
Total	15	88.74			
Treatment	3	83.56	27.85	63.30*	< 0.05
Block	3	1.23	0.41		
Error	9	3.96	0.44		

Appendix 9

Analysis of variance on mortality caused by nymphs of *Z. variegatus* in *Bacillus thuringiensis* in the laboratory

Source of variation	df	ss	ms	Observed F	p
Total	17	10329.13			
Treatment	5	9825.71	1965.14	45.59*	< 0.05
Block	2	10329.13			
Error	10	430.98	43.10		

Appendix 10

Analysis of variance on mortality caused by adult *Z. variegatus* in *B. thuringiensis* in the laboratory

Source of variation	df	ss	ms	Observed F	p
Total	36	274			
Treatment	5	204.5	40.9	22.85*	< 0.05
Block	3	19.33			
Error	28	50.17	1.79		

Appendix 11

Analysis of variance on leaf damage caused by nymph of *Z. variegatus* in *Bacillus thuringiensis* in the laboratory

Source of variation	df	ss	ms	Observed F	p
Total	17	8786.94	516.88		
Treatment	5	8422.94	1684.59	49.96*	< 0.05
Block	2	26.77	13.39		
Error	10	337.23	33.72		

Appendix 12

Analysis of variance on leaf damage caused by adult *Z. variegatus* in *B. thuringiensis* in the laboratory

Source of variation	df	ss	ms	Observed F	p
Total	15	42.90			
Treatment	5	42.12	8.43	31.22*	< 0.05
Block	3	0.78	0.26		
Error	7	1.88	0.27		

Appendix 13

Analysis of variance on number of *Z. variegatus* in the field

Source of variation	df	ss	ms	Observed F	p
Total	14	136.71			
Treatment	4	73.24	18.31	2.76*	< 0.05
Block	2	10.33			
Error	8	53.00	6.63		

Appendix 14

Analysis of variance on leaf damage caused by *Z. variegatus* on the field

Source of variation	df	ss	ms	Observed F	p
Total	14	63.79			
Treatment	4	54.81	13.70	18.03*	< 0.05
Block	2	2.88			
Error	8	6.1	0.76		

Appendix 15

Analysis of variance on number of Mealy bugs

Source of variation	df	ss	ms	Observed F	p
Total	14	8877.9			
Treatment	4	4591.7	1147.93	4.08*	< 0.05
Block	2	2037.2			
Error	8	2249	281.13		

Appendix 16

Analysis of variance on neem seed extract and B*t* on number of pre-pupa of *Epilachna spp.* on fruits

Source of variation	df	ss	ms	Observed F	p
Total	14	10.13			
Treatment	4	5.94	1.484	3.72*	> 0.05
Block	2	1.0015	0.501		
Error	8	3.19	0.3988		

Appendix 17

Analysis of variance on height of plants

Source of variation	df	ss	ms	Observed F	p
Total	14	11448.03			
Treatment	4	11313.23	2828.31	183.06*	< 0.05
Block	2	11.24			
Error	8	123.56	15.45		

Appendix 18

Analysis of variance on effect of neem seed extract and B*t* on girth

Source of variation	df	ss	ms	Observed F	p
Total	14	30283.3	2163.1		
Treatment	4	5635.2	1408.8	469.6*	< 0.05
Block	2	27.1	13.6		
Error	8	24	3		

Appendix 19

Analysis of variance on effect of neem seed extract and *Bt* on spread of leaves

Source of variation	df	ss	ms	Observed F	p
Total	14	107797			
Treatment	4	61581	15395.25	3.57*	> 0.05
Block	2	11681			
Error	8	34535	4316.88		

Appendix 20

Analysis of variance on number of flowers

Source of variation	df	ss	ms	Observed F	p
Total	14	5904.42			
Treatment	4	3853.58	963.40	3.77*	> 0.05
Block	2	4.13			
Error	8	2046.71	255.84		

Appendix 21

Analysis of variance on effect of neem seed extract and B*t* on number of leaves

Source of variation	df	ss	ms	Observed F	p
Total	14	18.20			
Treatment	4	9.993	2.50	1.59	> 0.05
Block	2	4.54	2.27		
Error	8	12.533	1.57		

Appendix 22

Analysis of variance on number of undamaged fruits

Source of variation	df	ss	ms	Observed F	p
Total	14	4534.97	323.93		
Treatment	4	4126.98	1031.75	23.98*	< 0.05
Block	2	63.93	31.97		
Error	8	344.24	43.03		

Appendix 23

Analysis of variance on mean weight of undamaged fruits

Source of variation	df	ss	ms	Observed F	p
Total	14	6279.13	448.51		
Treatment	4	6113.54	1528.39	142.57*	< 0.05
Block	2	79.85	39.93		
Error	8	85.74	10.72		

Appendix 24

Analysis of variance on undamaged fruit Yield

Source of variation	df	ss	ms	Observed F	p
Total	14	62283.4			
Treatment	4	61811.5	1685.43	276.2*	< 0.05
Block	2	24.0			
Error	8	447.5	55.9		

Appendix 25

Analysis of variance on number of damaged fruits

Source of variation	df	ss	ms	Observed F	p
Total	14	939.64	67.12		
Treatment	4	827.48	206.87	25.57*	< 0.05
Block	2	47.42	23.71		
Error	8	64.74	8.09		

Appendix 26

Analysis of variance on weight of damage fruits

Source of variation	df	ss	ms	Observed F	p
Total	14	59.24	4.23		
Treatment	4	26.85	6.71	8.49*	< 0.05
Block	2	26.04	13.02		
Error	8	6.35	0.79		

Appendix 27

Analysis of variance on damaged fruits yield

Source of variation	df	ss	ms	Observed F	p
Total	14	1826.6	130.47		
Treatment	4	1515.53	378.88	21.26*	< 0.05
Block	2	168.5	84.25		
Error	8	142.57	17.82		

Appendix 28

Abbot's formula

$$P' = C + P(1-C)$$

$$P = \frac{(P'-C)}{(1-C)}$$

C- Proportion of insects dead in the control

P' = Total number of insects dead

P = Number of insects killed by neem extracts or Bt.