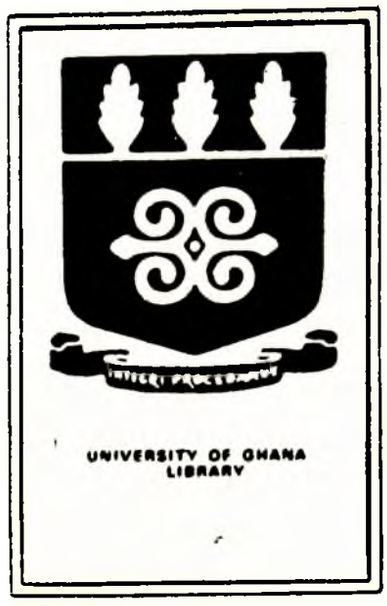

INSECTICIDE RESISTANCE IN DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* L. (LEPIDOPTERA: YPONOMEUTIDAE) FROM SELECTED CABBAGE FARMS ASSOCIATED WITH PYRETHROID AND ORGANOPHOSPHATE USE IN SOUTHERN GHANA

BY

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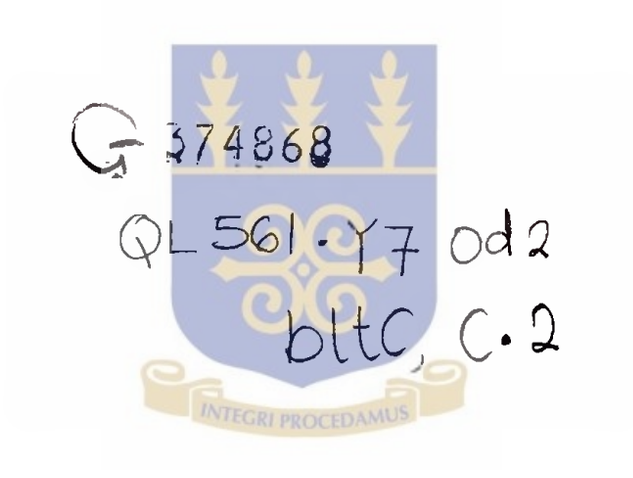

DECLARATION

I hereby declare that with the exception of references to other people's work which I have duly acknowledged all the experimental work described in this thesis was carried out by me and this thesis, either in whole or in part, has not been presented elsewhere for another degree.

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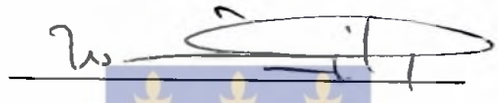

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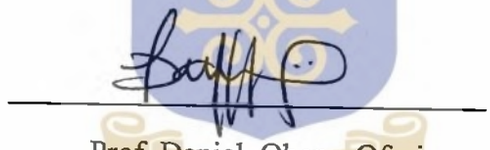

Prof. Daniel Obeng-Ofori

(Supervisor)

DEDICATION

To God, My Parents Mr. And Mrs. Charles Otieno, for offering me the best in life; my husband Jared, Daughter Alpha, for your unwavering thoughtfulness and sacrifice and the late Ben Akoth who never lived to see this work.

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ABSTRACT

Over dependence on insecticides for the control of diamondback moth (DBM), *Plutella xylostella* (L.) has resulted in development of resistant strains and health hazards due to toxic residues in cabbage. The present study was undertaken to evaluate resistance in DBM and to assess the contribution of residues of insecticides used for DBM control on resistance development. A preliminary survey conducted in Accra and Mampong-Akuapem, revealed inappropriate agronomic practices as the main cause of resistance development and health hazards due to insecticide residues on cabbage. Three pyrethroids viz lambda-cyhalothrin (pawa), cypermethrin (cypercal) and deltamethrin (deltaplan), an organophosphate– chlorpyrifos (dursban) and a biopesticide- *Bacillus thuringiensis* (*B.t.*) (dipel) were selected for the study. Wild DBM pupae were sampled from three sites in Accra (Dzorwulu, Airport and Madina) and a site in Mampong-Akuapem. These populations were used to establish a DBM colony, which was reared on potted insecticide-free cabbage in a screen house. Early 4^{th} instar larvae were used for leaf residue bioassays for *B.t.* and larvae immersion for organophosphate and pyrethroids. Compared to the recommended dosage the LC_{95} of dursban were 106, 74, 193, 114 fold in DBM populations from Airport, Madina, and Dzorwulu and Mampong respectively. Similarly, for Pawa the LC_{95} to the recommended dosage were 103, 77, 100, 58 fold for Airport, Madina, Dzorwulu and Mampong respectively. In contrast, only 3-fold tolerance to dipel was recorded in Airport and Madina, 2 fold in Dzorwulu and 4 fold in the Mampong DBM populations. The study revealed that most of the field-observed resistance among the conventional insecticides might be attributed to cross and multiple resistance. There was however lack of cues for cross-resistance between the conventional insecticides and *B.t.* Molecular studies of *B.t.* resistance using a PCR based method and further resolution using polyacrylamide gel electrophoresis (PAGE) showed the *B.t.* resistant gene to have multiple bands, including the diagnostic band,

as compared with bands from the susceptible strains. When the wild larvae were tested for carboxylesterase activities using the naphthyl acetate-diazo blue coupling reaction, significantly higher activities of α- and β- naphthyl esterases were recorded in the Mampong DBM population than the Accra samples. Compared to previous findings, the mean activity of α–naphthyl esterase had doubled, while an eight-fold increase was registered for β–naphthyl esterase. Although this result has enormous implications for cabbage farming, there was no relationship between resistance levels obtained by bioassay and activities of α- and/or β– esterases. The involvement of other metabolic detoxification enzymes or resistance mechanisms is suggested. Nonetheless, polyacrylamide gel electrophoretic zymogram study using α–naphthyl acetate revealed presence of bands, which are associated with higher resistance in DBM. The Mampong population showed higher frequencies of such bands than the Accra populations. Residues of chlopyrifos-methyl, pirimiphos-methyl and pyrethroids (Cypermethrin, lambda-cyhalothrin and deltamethrin) were estimated on cabbage samples using biotoxicity to brine shrimp nauplii after fractionation of the insecticide residues using solid phase extractor (SPE). The residue levels of chlopyrifos were found to be higher than the FAO/WHO recommended maximum residue level (MRL). However, residues for pyrethroids could not be detected for most of the study sites due to low brine shrimp mortalities, except for Dzorwulu and Airport B. Compared to earlier findings the residue levels recorded were not only above the WHO/FAO recommended MRL but a 12-18 fold increase was also recorded in residue levels of pyrethroids. Results from this study have shown that the insecticide residue problem for cabbage should be taken seriously in Ghana. Farmers need to be educated on proper use and handling of insecticides in order not to compromise human health. The study suggests that a more integrated approach using IPM principles, careful and selective use of conventional insecticides coupled with judicious use of *B.t.* could help reduce insect pests and the associated problems on cabbage.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Cabbage and other Brassicas are a diverse group of crops of European origin (Purseglove, 1969). They are cultivated from the arctic to sub – tropics, and at higher altitudes in the tropics as well as in the tropical lowlands on the West African coast (Hill, 1983) certain species or varieties are more adapted to the tropics than others (Ooi, 1986).

In Ghana, the cultivation and consumption of cabbage has been on the increase especially in the urban areas where there is high demand for the vegetable. This high demand, which has led to vegetable growers resorting to monoculture and intensive cultivation has in turn altered the natural ecosystem in such a way that an insect such as Diamondback moth (DBM), once part of the background fauna, has become a major pest (Kumar, 1986).

The diamondback moth (DBM) *Plutella xylostella (*Linnaeus) is an important pest of Brassicae plants worldwide. It is implicated as notorious and cosmopolitan pest (Brempong –Yeboah, 1992 and Talekar and Shelton, 1993). This insect is believed to be the most universally distributed of all Lepidopterans. Out of the 191 countries worldwide, DBM is listed to be present in 145 countries scattered over all possible climatic and ecogeographical zones, implying that it has a high propensity to adapt to variety of ecological habitats (Country Watch, 2002). It has been reported to attack both cultivated as well as many wild Brassica plants (Harcourt, 1986). The host range of DBM is limited to crucifers that contain mustard oils and their glucosides (Thorsteinson, 1953, 1955; Gupta & Thorsteinson 1960; Nayar & Thorsteinson, 1963; Hillyer & Thorsteinson, 1971).

In recent years, DBM has become the most destructive insect-pest of cruciferous plants throughout the world, and the annual cost of managing it is estimated to be $1 billion (Talekar, 1992). The pest is able to attack the crop from the nursery stage causing about 52 % loss in marketable yield in cabbage by rendering the vegetable unattractive, obnoxious, and therefore unmarketable.

There are various DBM control approaches such as cultural, biological, host-plant resistance, sex pheromone and chemical methods among others. Among all the control methods, chemical crop protection is the most effective and convincing, optimizing crop yields and improving quality of farm produce (GTZ, 1979).

In recent times, protected cabbage production, mainly by use of insecticides has been stepped up considerably worldwide. Brempong-Yeboah (1992) observed that cabbage growers in Accra plains used unnecessarily large quantities of insecticides, and in various concoctions against DBM. Some of the insecticides documented to have been used in Ghana include; Permethrin, Deltamethrin, Decis, Biobit & Dipel (*Bacillus thuringiensis*), and Dursban (chlorpyrifos). Others are Karate (Lamdacyhalothrin), Perfeckthion (Dimethoate), Ripcord and Actellic (Pirimiphos-methyl) [Mawuenyegah, 1994].

Large scale and indiscriminate use of insecticides for the control of this pest, necessitated by the ever increasing demand for quality food and better public health has resulted in a number of problems (Joia *et al.*, 2004) such as insecticide resistance and health hazards due to toxic residues that may persist in or on food after their application in amounts above prescribed maximum residue limit (MRL) [Oudejans, 1991]. The latter problem becomes more acute if the xenobiotics are used close to harvest as well as during transit and in vegetable yards (Kumari *et al.*, 2002). The residues also have hazardous effects on both the ecosystems and the environment in general including the atmosphere, soil and

water bodies (Carson, 1962). The toxic effects are more apparent in vegetables since they are mostly consumed fresh.

Insecticide resistance in DBM has occurred in many parts of the world since Ankersmit (1953) first reported its resistance to DDT in Indonesia. Resistance has been reported in Hawaii (Tabashnik *et al.*, 1987), Japan and Australia (Kao *et al.*, 1989). However insecticide resistance may not be limited to these areas only (Kao *et al.*, 1989). Diamondback moth is notorious for resistance to a wide range of insecticides (Syed *et al.*, 1989; Syed, 1992). These cover all major groups of insecticides, chlorinated hydrocarbons, organophosphorus insecticides, carbamates, synthetic pyrethroids (Sun *et al.*, 1986; Lin, 1988) and even some bacterial pesticide, that is, *Bacillus thuringiensis* (*B.t.*) based products (Tabashnik *et al.*, 1990; Hama, 1992; Kfir 1997; Wright *et al.* 1997), as well as insect growth regulators (Yuxian, 2001).

As hazards of conventional, broad-acting pesticides are documented, researchers look for pesticides that are toxic only to the target pest, have low toxicity to non-target organisms including beneficial insects (Edelson *et al.*, 1993; Liu, 1999; Liu and Sparks, 1999), and fewer environmental hazards. One of these pesticides is the soil bacterium *Bacillus thuringiensis* Berliner which has demonstrated its potential as effective and environmentally safe alternative to synthetic chemical insecticides for lepidopterous pests (Wilding, 1986) thus fulfilling the requisites of a microbiological control agent against agricultural pests and vectors of diseases (Aronson, 1994; Kumar *et al.*, 1996). However the use of genetically engineered crops to produce the *B.t.* toxin may greatly expand its use, and speed up the development of higher level of resistance.

Lepidopteran larvae being the world's most damaging crop pest are, the primary target of *B.t.* producing transgenic plants (McGaughey and Whalon, 1992). With many of such plants grown yearly, other insects are likely to develop resistance quickly unless effective countermeasures are designed and implemented (Mellon and Rissler, 1998). This calls for a better understanding of genetic basis of resistance to develop such countermeasures.

Resistance mechanisms in DBM proposed for synthetic chemicals include decreased penetration (Noppun *et al.*, 1987), enhanced detoxification by esterases (Maa and Chuang, 1983), Glutathione-S-transferases and reduced sensitivity of acetylcholinesterase (Wu, 1983; Hama, 1987). However, resistance to pyrethroids can be attributed to an inherited or induced mixed-function oxidase complex (Hama, 1987). Esterases in general have been noted to play significant roles in resistance to insecticides particularly organophosphates (Owusu *et al.*, 1996)

1.2 Justification

The repeated and indiscriminate use of pesticides in crop protection has created problems such as human health hazard due to toxic residues that persist in/on food after their application and environmental pollution among others. Although consumer awareness and legislation on pesticide residues in food are not that high in Ghana, pesticide management specialists are very concerned about the risks posed by pesticide residues in food to the Ghanaian public. This concern, specifically in respect to cabbage wholesomeness, is due to suspected indiscriminate use of insecticides on the crop.

Despite extensive use of pyrethroids in agriculture, comparable studies on DBM resistance to chemical pesticides are lacking (Beeman and Schmidt, 1982). This is because when new and more potent insecticides replace those that have become less

effective, laboratory tests to confirm the existence of resistance are not always performed. The increasing trend of insecticide resistance in insect pest of Ghanaian vegetables has led to scientists calling for the establishment of a national pesticide resistance-monitoring network (Owusu, 1997). Therefore, susceptibility study and identification of the biochemical and genetic mechanisms of resitance development by DBM and their correlation to insecticide residue levels in cabbage crop is a necessary first step to provide baseline data for the success of any insecticide resistance-monitoring programme. This information can be used for the establishment of a more effective resistance management scheme.

Apart from resistance development, the success of conventional pesticides is also threatened by increasing awareness of their toxicity to natural enemies (Johnson and Tabashnik, 1991) and their harmful effects to the environment. Microbial insecticides are a promising alternative. The most widely used microbial insecticide, *B.t.*, is highly toxic to certain pests, yet it has little or no adverse effect on most non-target organisms, including humans (Wilcox *et al.,* 1986). Resistance of DBM to conventional insecticides has been documented in Ghana (Brempong-Yeboah, 1992). Kaiwa (2000) also reported high activity of carboxylesterase as an indicator of insecticide resistance in populations of DBM in Ghana, yet *B.t.* is considered to be highly effective against DBM that are resistant to conventional insecticides (Sun *et al.,* 1986). At the same time, evidence of *B.t.* control failures has been documented in Florida (Shelton & Wyman 1992), Philippines (Kirsch & Schmutterer, 1988) and Malaysia (Syed *et al.,* 1990). Therefore there was the need to establish a baseline data for susceptibility status of DBM population in Accra suburbs (Ghana) to *B.t.* alongside the conventional insecticides should DBM show increased resistance to the conventional insecticides, since an

insecticide use pattern survey conducted in 1997 proved *B.t.* to be the most highly used insecticide by the cabbage growers in Accra suburbs (Ninsin, 1997).

1.3. OBJECTIVES

1.3.1 Main objective

The main objective of this study was to determine the level of DBM resistance to some commonly used insecticides, evaluate some of the resistance mechanisms and correlate these with insecticide residue levels in cabbage.

1.3.2 Specific objectives

To achieve the main objective the following specific objectives were considered.

i) To conduct a preliminary survey to determine the insecticide use patterns on cabbage in Accra-suburbs and Mampong-Akuapem.

ii) To determine and compare the level of insecticide resistance in *P. xylostella* to formulations of Lambda-cyhalothrin, Deltamethrin, Cypermethrin, Chlorpyrifos and *Bacillus thuringiensis* using bioassay.

iii) To determine the levels of non-specific carboxylesterases as a measure of resistance levels in DBM.

iv) To use polyacrylamide gel electrophoresis to establish qualitative differences in carboxylesterases isozymes in DBM.

v) To evaluate *B.t.* resistance mechanism of DBM using polymerase chain reaction (PCR) –based method.

vi) To determine levels and types of insecticide residues using Brine shrimp bioassay and compare the residue levels detected on cabbage to the FAO/WHO MRL.

vii) To correlate the level of resistance in DBM with residue levels in cabbage.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE CABBAGE PLANT

Cabbage, *Brassica oleracea* var. *capitata* L. (Brassiceae) is an important global vegetable (Rice *et al.*, 1993). It is a biennial herb with a short thickened stem surrounded by a series of fleshy overlapping expanded leaves, which form a compact head; the edible part of the plant (Purseglove, 1969). The older leaves surround the younger, smaller, tender ones and the miniature stem. The head shape may be pointed or round and leaf colour and shape are variable.

2.1.1 Origin, distribution and taxonomy

The crop is of very ancient cultivation and has been grown in Europe since 2500 BC. It was introduced into England by the Romans and is now grown throughout the world (Purseglove, 1969). However, CPC (2001a) asserted that the wild cabbage (*B. oleraceae* L. var. *oleraceae*) is indigenous to the Mediterranean region, South-West Europe and Southern England UK, where it grows on sea cliffs. It was brought into cultivation about 5000 years ago and gave rise to numerous cultivated forms, varying widely in vegetative morphology. Cabbages are cultivated from the arctic to the subtropics and at high altitudes in the tropics as well as in the tropical lowlands on the West African coast (Hill, 1983). Cabbage (*Brassica Oleracea* var. *Capitata*) belongs to the family Brassiceae (=Cruciferae) and the order Brassica (=capparidates). Members of the family cruciferae occur in temperate and tropical climates and represent a diverse, widespread and important plant group that include cabbage, broccoli, cauliflower, collards, rapeseed and Chinese cabbage, the most important vegetable crop grown in China (Li, 1981).

The Brassica is an agriculturally diverse group of high value crops (Tsunoda, 1980). Members of this plant group are cultivated for various edible parts, such as roots of

radishes and turnips, stems of kohlrabi, leaves of cabbage and other leafy brassicas. The seeds of mustard and rape are consumed as fresh, cooked, or processed vegetables. Crucifers are grown in tropical and temperate climates and in a variety of cropping systems from back yard gardens to larger-scale fully mechanized farms. The cultivated Brassica group is generally considered to belong to one species *Brassica oleraceae*, including white, red, and savoy cabbage, cauliflower, broccoli, Brussels sprout, kohlrabi and different kinds of kale (CPC, 2001a). Although information on distribution or introduction of the crop to Ghana is not well documented, it is believed that the British introduced it into this country and the crop has been grown on small scale since 1940 (Sinnadurai, 1992).

2.1.2 Agronomy

Cabbages like other brassicas are grown from the seed. Sowing is done in nurseries in the field. The land should be well drained and fertile. It should be well cultivated to achieve fine tilth for planting. Proper weed control is important for optimum yield. The crop responds well to organic manure and inorganic fertilizers, particularly nitrogen (CPC, 2001a).

Although cabbage production in Ghana has been on a steady increase over the last decade, there are certain constraints to attaining optimum yield and profit (Brempong-Yeboah, 1992). These constrains include shortage of land, clean water, marketing problems and insect pest infestation, which happens to be a global problem (Miyata *et al.*, 1986; Cartwright *et al.*, 1987; Hill and Waller, 1994). Insect pest infestation has arisen from monoculture and intensive cabbage production in peri-urban areas. Monoculture has created suitable conditions favourable for specialized insect species to flourish and become notorious pests (Kumar, 1986).

The insect pest problem has been augmented by the cultivation of other varieties of *Brassica oleracaea* such as Chinese cabbage and cauliflower on adjacent plots to cabbage farms, since as a group the varieties of *B. oleraceae* tend to have a similar insect pest spectrum (Hill and Waller, 1994). This practice of cultivating related crops together, according to Way (1976), bridges gaps in the host plant sequence of insect pests resulting in upsurges in pest infestation.

2.1.3 Pests

Like other brassicas, cabbage has a wide spectrum of pests, Hill (1983), listed 14 major pests of brassicas, of which *Plutella xylostella* is one, and a range of minor pests. The crop protection compendium (CPC, 2001a) listed 57 major pests and 28 minor ones including pathogens for brassicas and also listed DBM as one of the major pests of brassicas.

Hill and Waller (1994) mentioned the cabbage aphid, *Brevicoryne brassicae* (L.) (Homoptera; Aphididae) and leaf-eating caterpillars, which include the DBM, as the insect pests that cause the most damage to the *Brassica* spp. in the tropics. Forsyth (1966) recorded several insects on the cabbage plant (Table 1.) His findings have been confirmed by other workers (PPRSD, 2000).

The cabbage aphid transmits turnip mosaic virus and several other viruses specific to the cruciferous crops (Hill and Waller, 1994). The leaf-eating caterpillar also disfigures the cabbage plant and may completely defoliate it.

Table 1. Insect pests associated with cabbage in Ghana (Source: Forsyth, 1966)

Name	**Order and Family**
Allogista serricorne (Kibe)	Coleoptera: Alleculidae
Lagria villosa (F.)	Coleoptera: Lagriidae
Melanagromyza lambi (Hend)	Diptera: Agromyzidae
Diacrisia investigatorum (Karosh)	Lepidoptera: Arctiidae
Plusia signata (F.)	Lepidoptera: Noctuidae
Amauris psyttalea (Plotz.)	Lepidoptera: Nymphalidae
Spodoptera littoralis (F.)	Lepidoptera: Noctuidae
Appias epaphia (Cram)	Lepidoptera: Pieridae
Crocidolomia binotalis (Zeu)	Lepidoptera: Pyralidae
Hellula undalis (F.)	Lepidoptera: Pyralidae
Hymenia recuryalis (F.)	Lepidoptera: Pyralidae
Gymnognylus lucen (Wik.)	Orthoptera: Gryllidae

Thus, these insects acting in concert can devastate many cabbage plants over a very short time if growers do not institute control measures (Kaiwa, 2000). Diamondback moth has become the key pest of cabbage in Ghana (Brempong-Yeboah, 1992)

2.2 THE DIAMONDBACK MOTH

2.2.1 Description

The diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Yponomeutidae), is a small moth of about 1/3 inch (6mm) long, grey or brownish in colour, with pronounced antennae and a wingspan of about ¾ of an inch. It is recognized by the three pale or yellow triangular markings along the inner margin of each of the forewings (Sorensen, 1996). This is more pronounced in the males. When at rest, the closed /folded wings present an image of light coloured diamond shapes along the mid-dorsal line of the wings where they meet, which gives the moth its common name (Fig.1). When viewed from the side, the tips of the wings can be seen to turn upward slightly (Talekar and Shelton, 1993; Sorensen, 1996; Hutchison *et al.* 2004). The eggs are small measuring about 0.44 x 0.26 mm and are spherical, yellowish to white (Sorensen, 1996). The larvae vary in coloration from light brown at hatching through pale to dark green when fully grown. The larval body is wider in the middle than both ends and slightly tapering at both ends with prolegs on the last segment forming a distinct V-shape at the rear end (Suterra, 2004). The body is covered with tiny, erect black hairs. The larva wiggles rapidly when disturbed, and spins down from the plant on a strand of silk and drop over the edge. It climbs back on the leaf once the danger is gone (Talekar and Shelton, 1993). This behaviour is believed to be a parasitoid evasion response but can result in contamination of leaf surfaces with pathogens if they exist in the soil reservoir (Capinera, 2001) this behaviour distinguish them from other cabbage-infesting species.

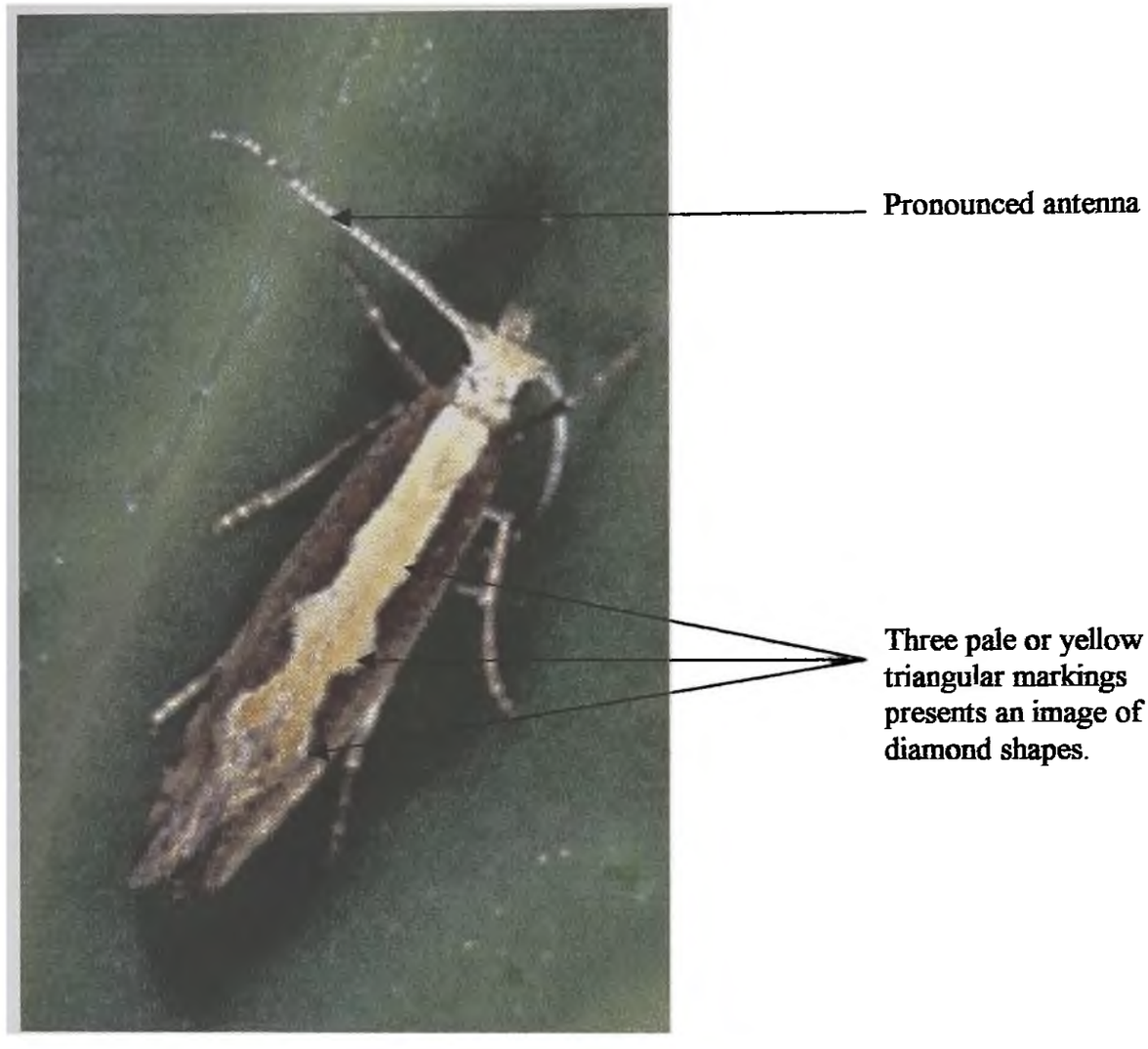


Fig 1. Adult diamondback moth (Credit: James Castner, University of Florida)

Pupation takes place in a net-like silken cocoon about 9-12 mm long. The new pupae appear greenish and are normally found on the underside of the leaves along the ridges of the leaf veins (Hill, 1983) or in soil debris (Hutchison *et al.*, 2004).

2.2.2. Origin and distribution of DBM

The diamondback moth is probably of Mediterranean region (European) origin, but is now found throughout the North Americas, southern portion of South America and in Europe, India, Southeast Asia, Australia, and New Zealand (Hardy, 1938). It is reported to be highly dispersive (Capinera, 2001). Although DBM had been considered to have an European origin, (Hill, 1983; Talekar *et al.*, 1990; Talekar and Shelton, 1993), on the basis of the large complex and sexual forms of parasitoids and host plants of DBM found in South Africa, a recent report by Kfir (1998) suggested that DBM may have originated from South Africa and dispersed to Europe (Palaearctic Region) and not vice versa. However, other reports indicate that the parasitoid complex of DBM is even more diverse in Eastern Europe and so that may be the origin (Talekar and Shelton, 1993). Furthermore, DBM feeds exclusively on Brassicas, which are of Mediterranean origin; there should be no doubt that its origin is Europe and not South Africa. Li (1981) asserted that, DBM has a very high propensity to adapt to a wide variety of ecological habitats.

2.2.3 Biology and Ecology

2.2.3.1 Life cycle

The DBM goes through four separate and distinct stages of its life cycle; egg, larva, pupa, and adult (Fig. 2).

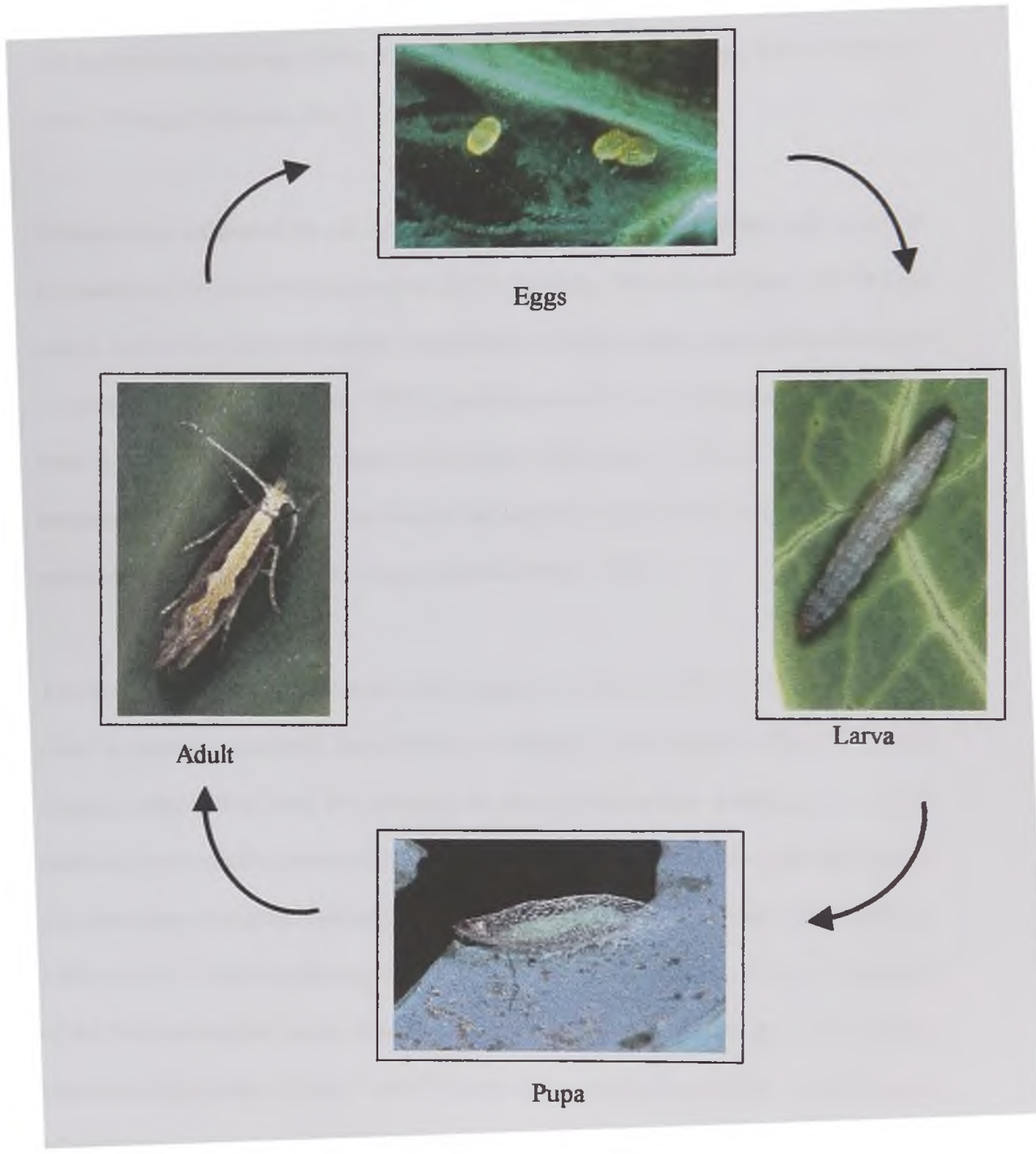


Fig. 2 Life cycle of diamondback moth showing the four stages (Credit: James Castner, University of Florida)

Eggs: After mating, the female deposits small (0.44x 0.26mm), eggs singly or in small groups of 2-8 eggs in a depression on the surface of foliage or occasionally on the stem and leaf-petiole (Capinera, 2001). Females lay between 250 -300 eggs, but an average is about 150 eggs (Capinera, 2001).

Oviposition is influenced by secondary chemicals, temperature, trichomes, and waxes on leaf surface (Uematsu and Sakanoshita, 1989; Pivnick, 1990). In addition, lack of light during normal daylight stimulates oviposition, but light during night hours does not completely inhibit it (Sorenson, 1996). Incubation lasts 5 to 6 days averagely ranging from 3 days at high temperatures (Ooi and Keldesman, 1979) to 8 days at low temperature (Harcourt, 1957). Just before hatching, they darken and young larvae can be seen coiled beneath the chorion or egg -shell (Harcourt, 1957).

Larvae: After emergence, neonate larvae crawl to the underside of the leaf and then mine the spongy mesophyll tissue thereby forming a gallery (Hill, 1983; Ooi, 1986) whereas older larvae feed by chewing the lower leaf surface resulting in irregular patches. Larvae usually consume all tissue except the wax layer on the upper surface and the veins thus creating the characteristic "windowing", (Bhalla and Dubey, 1986; Salinas, 1986; Lu *et al.*, 1988; Sarnthoy *et al.,* 1989). The DBM has four larval instars. The heads of the first and second larval stages are black in colour and distinct from the green to brown coloured heads of the 3rd and 4th instars (Mau and Kessing, 2004). Overall length of each instar rarely exceeds 1.7, 3.5, 7.0 and 11.2 mm respectively (Capinera, 2001). Average duration of the larval instars is about 4.5 (3-7), 4 (2-8), and 5 (2-10) days respectively (Capinera, 2001). The fully grown fourth instar larvae pupate within a woven silk cocoon fastened to the veins on the under surfaces of leaves (Hill, 1983)

Pupae: The fully-grown larva constructs an open network cocoon where it feeds and spends a two-day period of quiescence marking the pre-pupal stage. The pre-pupa sheds its larval skin, which remains attached to the caudal end of the green pupa, which is encased in a delicate, netlike cocoon on the under surfaces of leaves or in other protected areas on the plant or in soil debris (Hutchison *et al.,* 2004; Mau and kessing, 2004). The pupal period varies from 4 to 15 days, (averaging at about 8.5 days) depending on temperature (Chelliah and Srinivasan, 1986; Hoy, 1988; Capinera, 2001), with optimum development temperature being at 27.5°C and the threshold temperature at 9.8°C (Yamada and Kawasaki, 1983). The yellowish pupa is 7 to 9 mm in length (Capinera, 2001). Adult moths emerge primarily between 1:00 and 4:00 pm with a peak at 2:00 pm (Sakanoishita and Yanagita, 1972; Pivnick *et al.*, 1990)

Adult: Most adults emerge during the first 8 hours of photo phase (Pivnick *et al*, 1990). They feed on water droplets / dew or nectar from wild flowers and have a 2-3 weeks life span. Adult males live from 3-58 days averaging 12.1 days and females live from 7-47 days, an average of 16.2 days (Capinera, 2001; Mau and kessing (2004). The moth is nocturnal, active at dusk and continues so into the night (Harcourt, 1954). Mating occurs at dusk of the same day the adults emerge. Female moths start laying eggs soon after mating and the oviposition period lasts between 4-10 days. The majority of the eggs are laid before midnight with peak oviposition occurring between 7- 8 pm. of the first night of oviposition (AVRDC, 1987 and Pivnick *et al.*, 1990). Photoperiod, temperature, age, larval foods significantly affect adult survival, oviposition rates and lifetime fecundity (Harcourt, 1957; Sivapragasam and Heong, 1984). The moths are weak fliers, usually flying within 2m off the ground, and not flying long distances (Capinera, 2001).

2.2.3.2 Host range and host specificity

The diamondback moth feeds only on members of the family Cruciferea. Virtually all Cruferous vegetable crops are eaten, including cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*), radish (*Raphanus sativus*), turnip (*B. rapa pekinesis*), brussels sprouts (*B. oleracea* var. *gemmifera*) chinese cabbage (*B. rapa cv. gr. pekinensis*), kohlrabi (*B. oleracea* var. *gongylodes*), mustard (*B. juncea*), rapeseed (*B. napus*), collard (*B. oleracea* var. *acephala*) kale (*B. oleracea*) and watercress (*Nasturtium officinale*). Not all are equally preferred, however, and ovipositing moths relative to cabbage will usually choose collard (Talekar and Shelton, 1993). In addition, DBM feeds on numerous cruciferous plants that are considered to be weeds. It maintains itself in the absence of more favoured cultivated hosts. The following crucifers have been reported to sustain feeding and reproduction of DBM: *Arabis glabra*, *Armoracia lapathifolia*, *Barbarea stricta*, *Barbarea vulgaris* and *Basela alba*. Others include *Beta vulgaris*, *Brassica caullorapha*, *Galinsoga ciliate*, *G. parviflora*, *Sinapis alba*, *Brassica napobnrassica* etc. Apart from both cultivated and wild plants of family Cruciferae, several ornamentals, such as wallflower, candytuff, stocks, and alyssum are host plants of DBM (Mau and Kessing, 2004). Alternate weed hosts are especially important to maintain DBM population in temperature countries in spring (early in the season) before cruciferous crops are planted (Louda, 1986). The host range of DBM is limited to crucifers that contain mustard oils and their glucosides (Nayar and Thorsteinson, 1963; Hillyer & Thorsteinson, 1971), recently a group of ICIPE scientists have reported growth, development and survival of DBM on snow peas (Loehr *et al.*, 2002).

2.2.3.3 Effects of environmental factors on DBM

Various environmental factors influence the biology and therefore abundance and occurrence of the DBM. For example rainfall is specifically important in the tropics. Although the pest breeds all year round in the tropics, it is reported to be most abundant during cool or warm and dry seasons (Talekar and Lee, 1985; Talekar and Shelton, 1993). The weather during the egg laying period influences DBM abundance; cool, cloudy weather reduces moth flight activity. Heavy rain can disrupt mating and egg-laying. If inclement weather persists, many female moths die before egg- laying is completed (Moller, 1988). Heavy rains appear to be detrimental to infestation (Talekar and Lee, 1985) and are reported to be a major mortality factor for this pest since young larvae are easily dislodged from plants by rain and can drown on the soil surface or in water trapped on the plants, or are washed off the leaves (Harcourt, 1963; Capinera, 2001). During rainy weather and high humidity more than half of the first three larval stages may perish by drowning (Waterhouse, 1987). Harcourt (1957) reported an average mortality of 56%. Hence it is not surprising that cruciferous crops with overhead sprinkle irrigation tend to have fewer DBM larvae than drip or furrow- irrigated crops. Best results were obtained with daily evening application of sprinkle irrigation (Capinera, 2001).

2.2.3.4 Pest damage and economic importance

Plutella xylostella causes considerable damage to leaves, stems, growing point's inflorescence and fruits/pods (CPC, 2001b). Larval feeding mainly causes the damage. Initial damage results in small incomplete holes caused by young larvae and larger complete holes caused by mature larvae (Hurchison *et al.*, 2004). The entire plant may become riddled with holes under moderate to heavy populations. It attacks the growing tips of young plants and is particularly damaging to seedlings, since it can arrest

development and disrupt head formation causing headless plants or multiple undersized heads (Suterra, 2004). Sometimes, it causes formation of deformed heads and also encourages soft rots in cabbage, broccoli, and cauliflower. In case of severe infestation the entire plant could be lost (Capinera, 2001). Injury to leaves is not usually serious, except when the wrapper or cap leaves of cabbage are perforated /injured, down grading the quality and thus the value of the harvested crop (Suterra, 2004).

The pest causes serious economic losses. Even though proper economic impact of the pest is difficult to asses especially in Africa, since it occurs in diverse areas in large and small scale farms, and there seems to be less reliable data on the value of crucifers and losses incurred due to DBM. Besides, accurate data on the cost of control is rare due to the large volumes of insecticides used to control it, their variable costs, variable number of applications and efficacy (Capinera, 2001). DBM has developed resistance to most insecticides (Talekar and Shelton, 1993). The high pest status of DBM can be attributed to several factors mainly: high fecundity and reproductive potential; this results in rapid turnover of generation (Talekar and Shelton, 1993). This is further enhanced by the pest's ability to migrate over long distances, absence of effective natural enemy, and its ability to establish faster in newly planted crucifers than its natural enemy complex (Lim, 1986). Effective control by natural enemy complex is further weakened by over dependence on chemical pesticides and high ability of DBM to develop resistance to all forms of pesticides used against it (Talekar *et al.*1990).

2.3 MANAGEMENT OF CABBAGE

Cabbage is grown both on large scale and small-scale farming and in both cases DBM has been the key pest and the main constraint in cabbage production.

2.3.1 Small-scale farming

In developing countries of the tropics and sub-tropics, production of crucifers is characterised by small-scale farms and intensive use of land, labour and pesticides. For fresh vegetable production, for the large cities residents, farms are located on the outskirts of city centres or cleared areas in the highlands accessible from the cities. Production of healthy looking, damage free vegetables for the wealthy city dwellers is important consideration in cultivation, especially plant protection. The mainstay of control is frequent use of insecticides (Talekar and Shelton, 1993). This sole reliance on insecticide for control facilitates the rapid build up of resistance. Nevertheless the first report on DBM resistance to an insecticide in 1953 came from one intensive production area in the tropics, Indonesia (Ankersmit, 1953; Johnson, 1953) decades before resistance in warm areas of United States (Magaro and Eldelson, 1990; Shelton and Wynam, 1992). To overcome resistance, farmers often increase doses of insecticides, sometimes every two days. These high levels of use have caused the DBM to become resistant to practically all insecticides in many areas. Additionally, high insecticide use, has led to excessive residue on produce. Moreover because pesticide residue monitoring is absent or not enforced, insecticide contaminated crucifers often pass easily through marketing channels.

2.3.2 Large Scale Farming

In developed countries, crucifers' production is characterized by large-scale farming, which includes the reduction of labour, the increase of management and capital, and the consolidation of land into larger holdings. Large-scale farming is common in North America and Europe, and is becoming increasingly common in Mexico and Central America. However crop protection decisions tend to be similar over relatively large

areas. The primary control method of DBM here involves insecticides applied by air or ground rigs (Hoy *et al.*, 1983, Cartwright *et al.*, 1987, Beck, 1992).

2.4 CONTROL

Several approaches have been used to control DBM infestation in brassicas. The most popular control approaches include:

2.4.1 Cultural control

Prior to introduction of synthetic insecticides in the late 1940s, DBM were not reported as major pests on crucifers because they were relatively well managed by the natural enemies and cultural methods. Due to the failure of insecticides to control the DBM, interest is growing in the use of cultural practices in commercial crucifer production. Some of the classical control measures that have been tried with some success are intercropping, use of sprinkler irrigation trap cropping, crop rotation and clean cultivation (Talekar and Shelton, 1993).

2.4.2 Biological Control

The use of living organisms, such as parasitoids has also been exploited in controlling DBM. Numerous parasitoids attack all stages of DBM. Although over 90 parasitoids species attack DBM, only about 60 of them appear to be important (Godwin, 1979). Among these, 6 species attack the eggs, 38 attack larvae and 13 attack pupae (Lim, 1986). Egg parasitoids belonging to genera *Trichogramma* and *Trichogrammatoidea* require mass release to be effective. The most predominant and effective larval parastioids include *Diadegma insulare* (Cresson) Hymenoptera: Ichneumonidae (Suterra, 2004) and *Diadromus subtilicornis* (Gravenhorst) (Hymenoptera: Ichneumonidae, and *Cotesia* (*Apanteles) plutellae*, which attacks the three larval stages (Mau and kessing,

2004). This approach has considerable promise, although widespread, indiscriminate use of insecticides has frustrated recent efforts, and delayed the establishment of parasitoids, and their beneficial effect (Talekar & Shelton, 1993). Along side parasitoids, fungi and granulosis virus sometimes occur in high-density in DBM larval populations (Capinera, 2001).

2.4.3 Plant resistance

Several studies have surveyed existing germplasm for resistance to Lepidoptera, including DBM, in crucifers. Crucifers differ in their susceptibility to attack by DBM. Mustard, turnip, and Kohlrabi are among the more resistant crucifers (Eigenbrode *et al.*, 1990). The most notable resistance came from germplasm in United States North Eastern Plant Introduction Station (Eigenbrode *et al.*, 1990)).

2.4.4 Sex pheromone

The use of DBM sex pheromone has also been exploited, particularly when used in combination with the augmentation or conservation of natural enemies (CPC, 2001b).

2.4.5 Insect-growth regulators

Insect-growth regulators and pathogens offer a promising control measure as alternatives to broad-spectrum insecticides, which often disrupt the control exerted by natural enemies (Kobayashi *et al.*, 1992).

2.4.6 *Bacillus thuringiensis*

Biologicals like *B. thuringiensis* have since their introduction provided fairly good control. The use of *B.t.* is considered important since it favours survival of parasitoids. *B. thuringiensis* contains crystal proteins which are toxic only to certain insects, but are harmless to non-target organisms including people, wildlife, and most beneficial insects (Schnepf *et al.,* 1998). Genes encoding *B.t.* toxins have been incorporated into and expressed by crop plants, thus providing environmentally benign control of insect pests (Schnepf et *al.,* 1998).

2.4.6.1 Mode of Action

When conditions are unfavourable for bacterial growth Bt. forms a spore (the dormant stage of the bacterial life cycle) and it also creates the toxic protein crystal. After the insect ingests *B.t.* during feeding, the crystal is dissolved in the alkaline gut of the insect. The insect's digestive enzymes then break down the crystal structure and activate the insecticidal component, called delta-endotoxin, which binds to the cells lining the midgut membrane and create pores in the membrane, upsetting the ionic balance in the gut. The insect soon stops feeding and starves to death (Swadener, 2004).

2.4.6.2 Resistance to *Bacillus thuringiensis*

Bacillus thuringiensis offered tremendous hope for the control of DBM in the first two decades of its introduction. Due to its complex mode of action, and lack of documented cases of resistance from field population (Devriendt and Martouret 1976; Krieg and Langebruch, 1981), scientists presumed that DBM was unlikely to develop resistance to *B.t.* under field conditions (Talekar and Griggs, 1986). However, eight insect species including the Indian meal moth which was the first insect to develop resistance to *B.t.* in the laboratory ((McGaughey, 1990; McGaughey and Whalon, 1992) and others include

gypsy moth (Rossiter *et al.*, 1990). Moreover DBM and tobacco budworm, have exhibited multiple resistance to *B.t.* strains (Ellis, 1991; Gould, 1992). Evolution of resistance has therefore become the most serious threat to the continued efficacy of *B.t.* toxins. Although DBM is the only insect with resistance to *B.t.* in open-field populations (Tabashnik, 1994), however the wide spread use of *B.t.* toxin-producing transgenic plants, may hasten evolution of resistance by other pests (McGaughey and Whalon, 1992; Mellon and Rissler 1998). Since genetic analysis indicated that resistance to *B.t.* was autosomal, recessive and controlled primarily by one or few loci, a better understanding of the genetic basis of resistance is essential for developing good countermeasures to reduce further resistance to this important biopesticide.

2.4.6.3 Molecular studies of *B.t.* resistant gene

Extended Polymerase Chain Reaction (PCR) methodology has recently been exploited to rapidly identify the *B.t.* resistant gene. It requires minute amounts of DNA and allows quick, simultaneous screening of many samples for faster identification and classification (Kumar *et al.*, 1996).

Principles of Polymerases Chain Reaction (PCR)

The PCR is an *in vitro* method used for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers which hybridize to opposite strands and flank the region of interest in the DNA (Saiki *et al.*, 1985). The reaction uses the DNA polymerase enzyme and its many repetitive series of cycles results in accumulation of large amounts of the target DNA. The utilization of a heat stable polymerase isolated from the thermophilic bacteria *Thermus aquaticus* commonly denoted as *Taq* polymerase has transformed PCR into a simple, robust reaction, which is now automated using a programmable thermal cycling device (Saiki *et al.* 1985). The reaction mixture contains

the DNA template, oligonucleotide primers, *Taq* polymerase, deoxynucleotides, and reaction buffer and the amplification reaction is done by cycling the temperature within the reaction tube (Saiki *et al.,* 1988). Precisely, the PCR reaction involves three main steps of repeated cycles: heat denaturation to separate the two strands of DNA, primer annealing to their complementary sequence at lower temperature and extension of the new strands determined from the 5′ – 3′ end by DNA polymerases, with the base sequence of the new strands determined by the DNA template. The reaction mixture is repeatedly heated and cooled until the desired amount of DNA template is amplified.

2.4.7 Chemical control of DBM

The use of chemicals has become the mainstay of DBM control.

2.4.7.1 Chemical insecticide use patterns

Because DBM larvae feed on cruciferous vegetables, which are highly valued, effective crop protection is necessary. This is because the production of healthy-looking damage-free vegetable for the relatively wealthy city dwellers is an important consideration for better economic gain from crucifer production (Talekar and Shelton, 1993). Historically, the mainstay of control has been the use of synthetic insecticides. Since farmers reason that so long as it is profitable, and no better alternative is available, the spraying of pesticides is a good investment (Hardy, 1995). Chemical control is effective, rapid in curative action, adaptable to most situations, flexible in meeting changing agronomic and ecological conditions and economical (Metcalf, 1975). There are majority of pest outbreaks for which chemical control remains the only method of choice (Hill, 1983). Protection of crucifer crops from damage often requires application of insecticide to plant foliage, sometimes as frequently as twice per week (Capinera, 2001). Farmers tend to increase their use of pesticides, despite their rising costs, in Senegal; farmers increased

application frequency from 15 days to every 3 days in cabbage and this can be attributed to increased pest infestations levels; repeated ineffective pesticide treatments. This made farmers to suspect fraudulent use of poor quality insecticides and as a consequence anxiety to minimize damage and increase yields increased (Williamson *et al.,* 2003).

The general use patterns of insecticides vary widely over geographic locations and decades. The driving forces behind these changing patterns are the development of new, more effective insecticides and the lost usefulness of older insecticides because of resistance. Factors that influence the development of resistance in DBM include high fecundity and reproduction potential, rapid generation turnover, long growing season, extensive acreage of crucifers, and frequent insecticide application (Yamada and Koshihara, 1978; Magaro and Edelson, 1990). DBM has a long history of becoming resistant to every insecticide used extensively against them. Ankersmit (1953) noted development of resistance to DDT in Lembang, Indonesia. In the Philippines, Barroga (1974) reported development of resistance by DBM, by confirming failures with EPN and Mevinphos.

2.4.7.2 Effects of chemical control

Indiscriminate or misuse of insecticides has hazardous effects on both the ecosystem and the environment in general. Carson (1962) highlighted the hazards and environmental consequences associated with the use of pesticides. Many researches and publications have since been carried out to support the concerns raised by this publication (Metcalf, 1980). Hazards caused by the prolonged use of pesticides may affect human health, directly and indirectly through residues in food and other biotic systems.

2.4.7.2.1 Effects on human health

Insecticides are chemicals designed to kill insects but most of the widely used insecticides are nerve poisons and general biocides with acute toxicity on a weight basis approaching equivalence between mammals and insects (Metcalf, 1980). Therefore, the insecticides are not only toxic to insects but also to humans (Oudejans, 1991).

Insecticide toxicity to humans can either be acute or chronic. Acute toxicity is an immediate poisonous effect of a single dose of a toxicant involving few target organs or systems (Hassal, 1990). Acute poisoning occurs when people come in direct contact with insecticides. This phenomenon in Africa is known as "the new developing world disease" (Anonymous, 1989). Most of the banned or heavily restricted pesticides produced in the developed world are sent to developing countries where the illiterate farmers hardly have knowledge of the associated hazards with the use of such chemicals (Atteh, 1987). From United States of America alone, out of 210,000 metric tons of pesticides produced, 53,000 are either banned or not recommended for use in the U.S.A. However, these chemicals find their way into markets of developing countries, which may have dire consequences on the users (Weir and Schapiro, 1981). Even though only 20% of the total world pesticide consumption is in the developing countries, where majority of users are illiterate, about half of the poisoning cases and nearly three-quarters of the deaths are estimated to occur in the developing countries (Oudejans, 1991). FAO compiled a list on pesticide poisoning cases in Ghana as shown it Table 2.

Table 2. Fatal Pesticide poisoning in Ghana (Souce, FAO, 1989)

Year	**No. of reported cases**	**Remarks**
1986	4	All staff of plant protection and Regulatory services
1987	9	All volunteers, one died at Navrongo in the Upper East Region (Armyworm control)
1988	6	All farmers one died in the Volta Region (Armyworm control)
1989	4	Two staff of Plant protection and Regulatory services the others were farmers

These accidents happened because the users had inadequate knowledge on the proper handling and use of pesticides and improper disposal of containers after use, or because the containers were used to store water or food. Acute poisoning may also result from the infection of food exposed to high concentrations of highly toxic chemicals or containing residues of such chemicals. Many deaths have occurred due to pesticides poisoning. The International Organization of Consumer Union puts the figure for 1986 at 375,000 human poisoning cases in developing countries of which 10,000 died (Oudejans, 1991). At Kadjebi in the Volta Region of Ghana, five members of a household died after eating okra that was sprayed with an insecticide (Atsu, 1996), and as recent as July 2004, several people were admitted to hospital at Tarkwa after eating cabbage sprayed with excessive amounts of organophosphates (GNA, 2004). However reliable statistics on the true extent of human morbidity and mortality due to insecticide use is difficult to obtain, since most of these poisoning cases are not reported and are rarely subjected to laboratory verification (Davies, 1977).

Chronic toxicity on the other hand is associated with repeated and prolonged exposure to non-lethal doses of potentially harmful chemical (Yang, 1987). Known responses include lung cancer, brain damage and necrosis of the liver or kidneys. Others may cause chromosomal mutation or damage the foetus during pregnancy (tetratogenic effect) and immuno suppressive effects (Hassal, 1990; Mathews, 1994).

2.4.8 Pesticide Residues

Pesticide residues in food are remnants of a pesticide or its metabolites that can be found in or on a crop after it has been used for pest control purposes. The small concentration of these toxic residues that persist after application may have serious biological consequences (Kumar, 1986). They may cause damage to the liver, heart and kidneys

(Jackai, 1995). They may also be neurotoxic, teratogenic, mutagenic or carcinogenic. To evaluate chronic toxicity and hazard of pesticides to humans, an array of long term toxicity studies and hazard evaluation are carried out on smaller mammals like rats, rabbits or dogs. Extension Toxicology Network (EXTONET) [1993, 1995] compiled properties of chronic toxicity of some insecticides as follows:

Dimethoate: - it is a possible human tetratogen. It is also a mutagen and carcinogen. It causes organ toxicity. The testicles of male rats exposed to dimethoate decreased in size. The rats also developed chronic kidney problems.

Deltamethrin: suspected chronic exposure effects on man include choreogthretosis, hypertension, abortion and shock.

Cypermethrin: is a possible human carcinogen. Long-term exposure that may cause pathological changes in the cortex of the thymus, liver, adrenal glands, lungs and skin observed in rabbits repeatedly fed on cypermethrin.

Bacillus thuringiensis: Suspected exposure to *B.t.* has caused respiratory, eye, and skin irritation, and one corneal ulcer after direct contact with its formulation. Also allergies to the "inert" (secret) ingredients. People with compromised immune systems may be particularly susceptible.

2.4.8.1 Effects of Insecticide Residues on Agriculture Systems

Insecticides disrupt the natural equilibrium between insects-pests, their parasites and predators. Natural enemies are said to be more susceptible to insecticides than the insect pests (Jackai, 1995). The resultant effect is the resurgence of the pest or new pest species whose populations were regulated by natural enemies. For example DBM was long considered a relatively insignificant pest before 1940s, but with the introduction of broad-spectrum synthetic insecticides, which destroyed the natural enemies, by 1970s it

was reported as a major pest and by 1980s become a more serious pest of crucifers due to insecticide resistance and presently the most troublesome since it has resisted virtually all insecticides available in the modern markets (Talekar and Shelton, 1993; Capinera, 2001).

Statistics of harm from pesticides show that each year 25 million people, from the Southern hemisphere, are poisoned through occupational exposure to pesticides; out of which 220, 000 die. Pesticides have also been linked to cancer: like higher concentrations of DDT have been found in the breast milk of mothers in Central America (Instant Essay, 2004).

2.4.8.2 Pesticide Residue Legislation

Consumer awareness and concern about perceived risks that potential residues of pesticides may pose on human health is challenging the agro-industry worldwide especially the fruit and vegetable industry, to minimize pesticide residues in food. In order to protect the health of consumers, many countries have set maximum permissible levels for residues of particular pesticides in food. For purposes of trading foodstuffs on the world market, most developing countries rely on international standards, established jointly by (FAO) and (WHO) through Codex Alimentarius Commission, which have since 1961 set maximum residue limits (MRL) for pesticides in foods (Gbewonyo, 2004). They have also been concerned about the "maximum acceptable daily intake" (ADI) value, which is expressed in milligrams per kilogram body weight, and is defined as "that daily intake which during an entire life-time appear to be without appreciable risk on the basis of all known facts at the time" (FAO/WHO, 1993). In most developed countries, and some developing countries, strict legal controls have been enacted which lay down reasonable safe levels of pesticide residues in food. For instance, in 1979, the Federal

Republic of Germany pesticide residues legislation covered more than 200 different ingredients (GTZ, 1979).

While in Denmark, the Danish National Food Institute continuously controls pesticide residues in Food by continuously analysing various food samples on the market (Hewleg, 1991). The Indian Institute of Horticultural Research also published recommended pesticides, concentrations, terminal residues, and waiting periods for specific crops. While in USA, the first country to enact pesticide residue laws (GTZ, 1979) to ensure the safety of food supply by regulating pesticide residues through the cooperative effort of the United States Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) (Gal and Mathews, 1992).

Maximum Residue Limits (MRL) and ADI set by FAO/WHO and specific pesticide residue legislation of any country are based on Good Agricultural Practice (GAP). GAP emphasizes the official recommended usage of pesticides under practical conditions at any stage of production, storage, transportation, distribution, and processing of food, agricultural commodities and animal feed. It accounts for variations in different regions concerning minimum quantities necessary to achieve adequate control, yet leaving smallest residue, which is toxicologically acceptable (Oudejans, 1991). Therefore official recommended usage of pesticides need to comply with the procedures, including formulation, dosage rates, frequency of application and pre-harvest intervals (PHI) approved by the national authorities (Oudejans, 1991).

2.4.8.3 Violation of pesticide residue legislation

Cases of pesticide residues violation have been reported in both developing and developed countries alike. Elderkin *et al.* (1995) reported that; there are 2.5 billion

pounds of pesticides being applied to agricultural products each year in the United States. This is ten times more than was applied 40 years ago. Moreover each year there are 10,000 pesticide related poisonings. For example, on July 4th 1985, over 300 Californians became sick after eating watermelons treated with the pesticide TENIK. Besides 44% of the foods (carrots, tomatoes and lettuce) tested in supermarkets were found to have some traces of pesticide residue on them (Elderkin *et al.*, 1995).

The developing countries are the worst hit by this catastrophe. In Benin; cotton production is the largest consumer of insecticides, utilizing about 80 % of imported chemicals followed by cowpea and garden vegetable production. Among the most commonly used insecticides is endosulfan, which during 1999/2000 growing season, contributed to the food poisoning deaths of approximately 70 Benin citizens. Consequently there was increased demand for chemical free vegetables, which were grown mainly by using neem, and papaya leaf extracts (Vodouhe, 2001).

In India, Kumari *et* al. (2002) concluded that 100% of the vegetables tested were contaminated. Out of 60 samples, 92% were contaminated with organochlorines, 80% with organophosphates, 41% with pyrethroids and 30% with carbamates. About 23% showed residues of organophosphate insecticides above the respective MRL values. In Mauritius, Choy and Seeneevassen (2004) sampled 115 vegetables and fruits and extracted pyrethroids residues. They recorded cypermethrin in 73% of tomatoes, 37% of the watercress and 31% of the beans, whereas deltamethrin was found in 53% of tomatoes and 19% of watercress samples analyzed.

2.4.8.4 Pesticide residues levels in Ghana

Mawunyega (1994) carried out a study on residue levels of chlorpyrifos sprayed on cabbage fields in Legon. Brine Shrimp lethality test was used after observing four weeks of post harvest interval. The residue detected was 0.8mg/kg, which was lower than 1.0mg/kg recommended by Codex Alimentarius Commission.

Osafo and Frempong (1998) analyzed water and fish samples from three rivers that flow through areas of intensive vegetable farming from 1993 to 1995. In 1993, only low level of Lindane was recorded, however in 1995 significant levels of Lindane and endusulfan residues were recorded. Higher levels of residues were recorded in fish than in water, indicating accumulation of pesticides in fish. The highest quantities were found in River Oda.

Analysis of street vended food in Accra, Ghana, carried out in 1999-2000 revealed some levels of contamination by heavy metals, pesticides, micro-organisms and mycotoxins. Organophosphate chlorpyrifos was detected in six out of eight samples of *waakye* (rice and beans) and one out of eight samples of *fufu* (cassava and plantain dough).

Botchway (2000) analysed pesticide residues in exportable cocoa beans from selected growing districts and two shipping ports. Analysis by Gas liquid chromatography showed detectable levels of lindane residues but the levels were about 10% of the MRL of 1.0 μg/g permitted by Codex Alimentarius Commission. Aboagye (2002) used Gas Chromatography to analyse residues of chlorpyrifos, and fungal inhibition to determine levels of carbendazim in exportable pineapples in Ghana. The range of chlorpyrifos detected was $0.005 \pm 4.8x\ 10^{-3}$ to $0.02 \pm 1x10^{-2}$ μg/g. This was below European Union MRL and therefore was no threat to the pineapple industry in Ghana.

Gbewonyo (2004) reported studies on residue levels in a number of agricultural products. It was found that residues detected on cabbages sprayed with Lambda-cyhalothrin (karate) did not exceed FAO/WHO levels, the levels of Deltaphos 262 EC (250g/l triazophos and 12g/l deltamethrin) used then (1996) by farmers, were above recommended MRL. It was concluded that 33% of cabbage samples assayed within Accra-Tema Metropolitan area of Ghana showed residue levels, which were likely to be 2-3 fold higher than the FAO/WHO MRL levels. It is evident from these examples that the extent of pesticide residue violation even in countries with residue legislations is high. This reaffirms the need to minimize pesticide residues in agricultural produce, especially vegetables. To minimize the pesticide residues in vegetables, pest control must balance economic pesticide management (Sances *et al.,* 1993) considering the requirement for multiple insecticide application in vegetable production. This level of decision making certainly requires greater information not only from the field with respect to pest density, location and potential to increase (Sances *et al.*, 1993), but also on insecticide use patterns (FAO/WHO, 1994) and chemical behaviour of insecticide once they are applied in the environment. Other variables such as surfactant, formulation type, micro and macro environmental factors and individual chemical properties of the pesticide in question, may also have profound effect on degradation and hence the amount of residues in the market place (Sances *et al.*, 1993).

2.4.8.5 Residue level estimation

The following method has been adopted in residue estimation.

2.4.8.5.1 Methods for residue analysis

Residues are present in very small quantities in heterogeneous compounds including biological materials. The process of residue analysis consists of the following steps:

i) Sampling: This procedure aims at obtaining a final sample representative of the lot in order to determine is average pesticide residue content. It is based on the nature of the crop and the history of the crop (NRI, 1994).

ii) Extraction: This is done in a solvent to remove the residues from other components of the sample matrix. Insecticides are soluble in both polar and non polar solvents e.g. methanol is used for extraction of fatty substances, while polar water miscible solvents like acetronitrile, methanol or acetone are recommended for non-fatty samples and those with high to medium moisture contents (Yeboah, 2001). Hexane has been exclusively utilised for extraction of chlorinated hydrocarbon and organophosphorus insecticides (Matsumura, 1985).

iii) Clean up: Clean up removes extraneous materials that are co-extracted from the analytical sample. Methods involved in general clean up include liquid-liquid partitioning, adsorbent column chromatography, gel permeation chromatography and solid phase extraction (SPE) (Olson, 1988: Hetzel, 2000; Yeboah, 2001). The basic principle of SPE (accumulator or concentrator) is based on other clean up techniques such as adsorbent column chromatography, which is based on the interaction between a chemical dissolved in a solvent and an adsorptive surface. Where the clean up is either achieved by the extractives or the pesticides being adsorbed onto the absorbent i.e. the solvent passes through the column, dissolving and removing residues (eluting) leaving extractives attached to the absorbent or the co-extractives pass through the column and pesticides are eluted with appropriate solvent systems. Using the latter principle therefore, the SPE packing materials or cartridges retain the pesticides when the extract is passed through without retaining co-extractives and then eluted with appropriate solvent system. Conversely SPE can retain the co-extractives and allow the pesticides to pass

through. The column materials commonly used are florisil, alumina, silica gel, magnesium oxide and carbon (Yeboah, 2001).

iv) Sample concentration: This is aimed at reducing the volume of the solvent carrying the insecticide residues without losing residues, thus reducing the residues to detectable level (Hetzel, 2000). This is done by using either nitrogen gas evaporator or rotary evaporator.

v) Identification and quantification

After providing the residue containing extract with or without cleanup, the steps for resolution, detection, measurement, quantification, and confirmation are performed sometimes, after derivatization. Advances in the technologies available in these areas have been responsible for the improvements in both selectivity and detection limits over the past half century.

vi) Evolution of detection methods

In 1940s and 1950s, gravimetric and bioassay were the mainstay in trace analysis with detection level of about 1 ppm (Seiber, 1982). By 1950s to 1960s colometric and spectrophotomeric methods responded to whole classes of compounds rather than individual chemical species but with improved detection limits in magnitude, e.g. the Schechter Haller method for DDT. In 1950s paper and thin layer chromatography added the analysts' ability to resolve individual chemicals in a fairly complex mixture. These qualitative techniques were best suited for screening for presence of specific pesticides. Quantitatively they could also compare spot intensities visually. Since 1960s to the present, gas and high-performance liquid chromatography (HPLC) has nearly eliminated PC and TLC (Beckman, *et al.*, 1963).

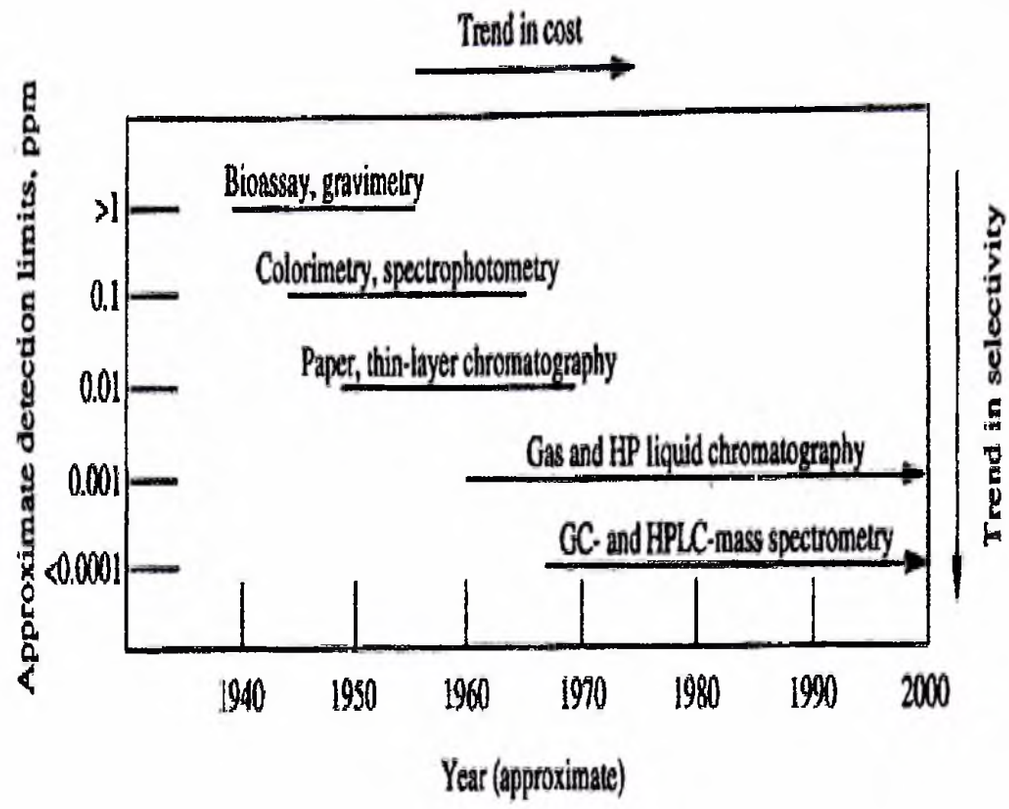


Fig. 3 The evolution of analytical methodology for organic toxicants in environmental samples (Seiber, 1982).

Detection limits of low ppb and even ppt are attainable, especially with clean samples of water, air, and soil and biological matrices. The use of mass spectrophotometry in connection with GC and HPLC has increased their detectability and selectivity although the cost has limited their use. Lastly, immunoassay, which has the potential of cutting into chromatography-based bastion of pesticide residue methodology, is currently under development.

vii) Bioassay method

The methods of analysis employed include Biological (Bioassay) and Chemical (analytical). Test animals are selected on the basis of high pesticide sensitivities and the ease with which large numbers of them can be reared (Matsumura, 1985). Bioassay has been used to determine pesticide residue levels (Sarode and Rattan, 1981). Ramasubbaiah and Rattan (1978) indicated that there is a high degree of coefficient of correlation (agreement) between bioassay and calorimetric chemical assay. Brine Shrimp, *Artemia salina* leach, a test organism is commonly used in the bioassay to detect and estimate levels of the insecticide residues (Grosch, 1967). This is because it is found to be sensitive to a broad range of compounds at concentrations of 0.01 ppm in about 45 minutes to two hours (McLaughlin, 1991). Although bioassay is rapid, inexpensive, and convenient, it lacks the ability to distinguish metabolites, impurities and alteration products of pesticides from the parent pesticidal compound. Hence Gas chromatography (G C) has been offenly used to determine the quantity and confirm the identity of the residue detected. GC can be fitted with Electron capture detector (ECD), Flame Ionising Detector (FID) or nitrogen Phosphorus detector (NPD) depending on the type of compounds being analysed (GTZ, 1979). The methods used for analysis can be multiple residue method (MRM), which can determine various residues of different pesticide;

while selective (MRM) is used on small numbers of chemically related pesticides and Single residue method (SRM) determines one pesticide (FDA.1994).

2.4.9 Integrated pest management (IPM)

Sole reliance on insecticides is not an effective or sustainable practice for the management of DBM in the long run. DBM is often a secondary pest, becoming a problem when insecticide use for primary pests destroys its natural enemies and selects for resistance in the population (Endersby, 2004). The probable way forward in controlling DBM is therefore to develop IPM programmes given that even chemicals, which have been the mainstay of its control, can no longer hold its population. A number of successful IPM programmes have been developed for DBM in large-scale brassica cultivation areas; one of the most successful is in the Bajio region in Mexico where about 15,000 ha of brassicas are grown annually. This programme was initiated in 1987 after a control failure of DBM despite an average of nine applications of synthetic insecticides. The programme relies on scouting thresholds, crucifer free periods and the judicious use of *B. thuringiensis*, and has resulted in over 50% reduction in the use of insecticide sprays (Talekar and Shelton, 1993). Similar success has been achieved in Jamaica (Ivey and Johnson, 1998), Singapore (Ng *et al.*, 1997), and the Philippines (Rejesus *et al.*, 1996). At Weija-Accra, Ghana, farmers abandoned cabbage production due to crop loss due to DBM infestation. But using agronomic skill acquired through training in Framers field schools coupled with the use of Neem extracts, they resumed cabbage production on a highly profitable way and environmentally sound manner (Youdeowei, 1999).

2.5 RESISTANCE DEVELOPMENT

Diamondback moth has developed resistance to most of the commonly used insecticides. Long before environmental concern over negative effects of the use of organochlorine insecticides developed, entomologists were already encountering a major problem in insect pest control. The organochlorine insecticides, which initially had been so effective, were performing erratically or in some cases failing. It became apparent that these insects had developed resistance to the organochlorine (Soderlund and Bloomquist, 1990).

Sawicki (1987) defined resistance as a genetic change in response to selection pressure by toxicants that may impair control in the field, while Wegorek *et al.* (2002) defined resistance as the naturally-occurring, inheritable adjustment in ability of an individual in a population to survive a plant protection product treatment that would normally give effective control. However, different views exist based on individual's focus/interests on resistance, for example; a biologist would view resistance in terms of "X-fold increase in lethal doses or percent survival at discriminating dose", while a geneticist would view it as "a change in gene frequencies," and a biochemist would view it as "a modification at the pesticide's target site", while to an industrialist, it is when the target pest's response to a commercial product development to a level where the product no longer performs as intended (Thompson, 2004). This circle between resistance development and insecticides spraying is presented in figure 4. Resistance is therefore exhibited as progressive inability of a given treatment to control a pest population (O'Brien, 1967). As a result, the insecticide is applied more frequently and in large quantities to compensate for the decline in effectiveness. Consequently, further resistance is developed within the insect population until the usefulness of the insecticide is greatly reduced and may totally loose effectiveness in that particular population in the long run.

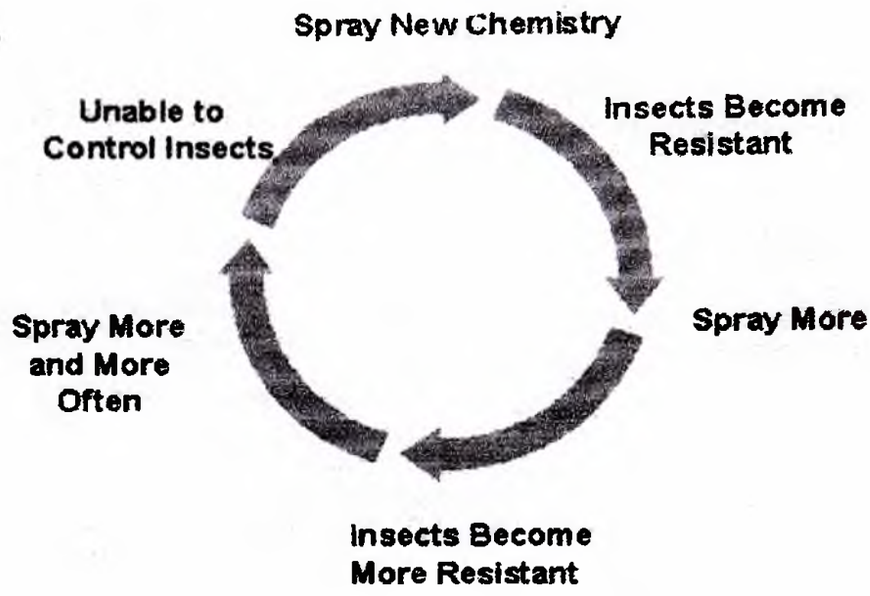


Fig. 4. Perceived pesticide treadmill (Thompson, 2004)

The resistant genes are usually present at very low frequencies in normal populations and resistant strains are derived from the initial population by the selective mortality of the more susceptible genotype growing during the application of an insecticide (Crow, 1957; O'Brien, 1967; Sawicki, 1979). Resistance development becomes rapid when selection pressure exerted by insecticide is widespread and continuous (Brown, 1964) and occurs after the insect has been exposed to chemicals for several generations. Graves *et al.* (1967) reported that it took 25 generations of intensive selection for the boll weevil *Anthonomus grandis* to develop resistance to organochlorine compounds. DDT was used for 15 years before *Heliothis virescens* developed resistance to it (Kumar, 1984). Brown (1977) has stated that development of resistance to the organochlorines (DDT and Cyclodienes) took about 10 years that for cyclodienes developed somewhat faster than the DDT group. It was further stated that development of resistance to organophosphates came to fruition 10 years after cyclodienes resistance. However, the resistance, to carbamates takes as much time as the organophosphate resistance but developed a little faster when it builds on a base of OP resistance (Kumar, 1984). The more persistent the poison is and the more rapid the life cycle of the insect or acarine and the greater the risk that the development of resistance will be rapid (Hassal, 1990). Table 3 is an indicator of broad trend or probably wider-estimate of the extent of resistance since many cases have either not been investigated or remain unreported (Georghiou and Mellon, 1983; Goerghiou and Lagunes-Tejeda, 1991).

Table 3. Comparison of Records of Resistance to Pesticides in Arthropods (Source, Georghiou and Legunes-Tejeda, 1991)

Pesticide group	**1970**	**1980**	**1989**	**Percentage 1971 – 80**	**Increases 1981 – 89**
No. of species with resistance cases	224	428	504	91.1	17.8
DDT	98	229	23	133.7	14.8
Cyclodienes	140	269	291	92.1	8.2
Organophosphorus	54	85	260	270.4	30.0
Carbamates	3	48	85	1600.0	66.7
Pyrethroids	3	22	48	633.3	118.2
Fumigants	3	17	12	466.7	
Other	12	41	40	241.7	-
Total for all pesticides groups	313	829	999	164.9	20.5

2.5.1 Mechanisms of insecticide resistance

Insecticide resistance is a dynamic multi-dimensional phenomenon, which is affected by biochemical, physiological, genetic and ecological nature of the insect (Brattsten et *al.*, 1985). Considerable information on the biochemistry and genetics of resistance is discussed by (Busvine, 1971; Matsumura, 1985; Oppenoorth, 1985; Georghiou, 1986). They identified resistance mechanism to include cuticular penetration of toxicants, enhanced metabolism by cytochrome P_{450} dependent monoxygenase, hydrolases or glutathione- S- transferases, and reduced sensitivity of mutant acetylcholinesterases to organophosphates and carbamates and of other neuronal targets to pyrethroids, DDT and its analogues and chlorinated cyclodienes (Oppenoorth, 1985). Resistance mechanisms in DBM proposed for synthetic chemicals include, decreased penetration (Noppun *et al.* 1987), enhanced detoxification by esterases (Maa and Chuang, 1983) and Glutathione-S-transferases, and reduced sensitivity of acetylcholinesterase (Wu, 1983; Hama, 1987). However, resistance to pyrethroids can be attributed to an inherited or induced mixed-function oxidase complex. Esterases in general have been noted to play significant roles in resistance to insecticides particularly organophosphates (Owusu *et al.*, 1996).

2.5.1.1 Metabolic detoxification: This refers to the process by which non specific enzymes break down toxic, lipophilic compounds into less toxic usually more soluble compounds for excretion thereby greatly decreasing their biochemical activity or toxicity (Wilkinson, 1983; Mallet, 1989). Biochemical reactions leading to detoxification include oxidation, hydrolysis, dealkylation and dehydrochlorination caused by several enzyme systems including mono-oxygenases (mixed function oxidases, microsomal oxidases and cytochrome P_{450} dependent oxidases), hydrolases (esterases) and transferases (glutathione-S-transferase) and DDT dehydrochlorinase.

Mixed-Function Oxygenases (MFO): Mixed function oxidases are located in the microsomal portions of several tissues particularly the Malpighian tubule. Microsomal enzymes have evolved as protective mechanisms against naturally occurring toxicants such as nicotine, rotenone and natural pyrethrins (Yamamoto *et al.*, 1969; Elliot *et al.*, 1972).

Glutathione–S-transferases: Play a key role in the resistance of organophosphate compounds (Tanaka *et al.*, 1981). Many researchers have demonstrated glutathione dependent degradation of parathion and diazinon in resistant houseflies (Lewis and Sawicki, 1971). Mechanisms of physiological resistance to toxic chemicals include reduced penetration, sequestration and excretion. Delayed penetration provides more time for detoxification of the incoming dose (Brattsen *et al.*, 1985).

Alternative biochemical pathway; This mechanism provides an alternative pathway to the one blocked by the insecticide. The blocked site is by-passed by the use of the alternative pathway. Altered acetylcholinesterase gives rise to resistance to certain organophosphates in cattle tick, in the mosquito *Aedes albimanus,* and the two-spotted spider mite. The Indian meal moth became resistant to *B.t.* by using altered binding sites on the gut wall, likewise, the California red scale, *Aonidiella aurantii*, became resistant due to alternative pathway to the terminal stage of cellular respiration and was no longer dependent upon enzyme systems disabled by cyanide.

2.5.1.2 Target site insensitivity

When compared with the case of metabolic resistance mechanisms, a modified target site causes relatively few types of resistance. Some arthropods develop resistance by altering the properties of the target site of action of a given insecticide. This mechanism has been

demonstrated in Spotted spider mite, *Tetranychus urticae* (Smissaert, 1964), leafhopper, *Nephotettix cincticeps* (Iwata and Hama, 1972), and in numerous resistant insect species with cross-resistance to cyclodienes (Hama, 1987). The best example of altered target as a cause of resistance to insecticides is that of acetylcholinesterase with reduced sensitivity to organophosphates and carbamates. A single gene has been shown to be responsible for the difference in acetylcholinesterase sensitivity and resistance caused by it in many arthropods (Oppenoorth, 1985). The esterase responsible for resistance development in the green peach aphid *Myzus persicae* has high binding affinity but low catalytic reactivity and hence functions as a storage protein for carbamates, organophosphates and pyrethroids. A major factor knockdown resistance (kdr) located on chromosome 111 in houseflies which confer resistance to DDT and pyrethroids through target insensitivity mechanism has been demonstrated (Sawicki, 1973).

2.5.1.3 Changes in behaviour as a resistance mechanism

Resistance mechanism can strictly be behavioural e.g. in Mazoa valley of southern Zimbabwe, Muirhead–Thomson (1960) found that *Anopheles* mosquitoes originally were abundant both outdoor and in human dwellings. The endophagic (indoor) mosquitoes eventually disappeared from both treated and untreated dwellings after eight years of BHC treatments in human dwellings and this condition persisted even after insecticidal treatment were discontinued. This suggests that during the period of insecticide treatment, an exophagic strain evolved that lived and fed outdoors.

In some cases the mechanism of resistance may represent an enhanced ability of the pest to detect a toxicant and initiate a response to avoid it. This phenomenon has been studied by Kirkpatrick and Schoof (1958) for houseflies and for mosquitoes (Gerold and Laarman, 1964).

2.5.1.4 Cross resistance and multiple resistance

An insect population is considered resistant if its response to an insecticide in detection tests drops significantly below its normal response (Georghiou & Mellon 1983). Resistance develops mostly due to a genetic mutation that enables the insect to counteract or circumvent the activity of an insecticide.

Cross-resistance denotes the resistance of a strain of insects to chemicals other than those they were selected against. This is possible if the compounds are closely related and have the same or a similar mode of action. As a result one compound causes selection for detoxification mechanism common to members of a group of chemical and the population becomes resistant to several closely related chemicals, in absence of selection pressure against each (French *et al.,* 1992).

Multiple-resistance on the other hand, is caused by the presence of separate detoxification mechanisms for unrelated insecticides, selected for independently (French *et al.,* 1992). Thus a single strain becomes resistant to several different compounds, using different mechanisms. This may be the result from intensive (indiscriminate) use of different chemical groups without following resistance management principles (Oppenoorth & Welling 1976). Multiple resistance often results from the simultaneous or consecutive use of several insecticides. However, it is sometimes difficult to discriminate between cross-resistance and multiple resistance, since genetic linkage may result in different cross resistances, particularly during selection in the laboratory (Oppenoorth & Welling 1976).

These two phenomena have been greatly exploited by many insect species. By 1984 there were 1797 cases of resistance in arthropods to insecticides, and by 1991, resistance to at

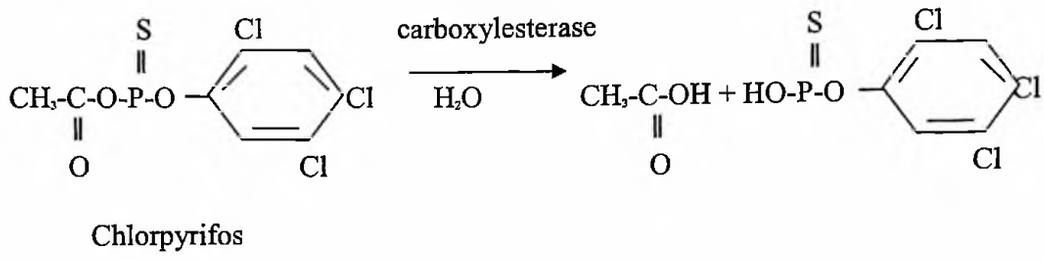
least one insecticide had been recorded for 504 species (Georghiou, 1986; Georghiou and Lagunes-Tejada, 1991). So far as at 1989, insect species resistant to the greatest number of insecticides is the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphidae), which has a documented resistance to 71 synthetic insecticides (Georghiou and Lagunes-Tejada, 1991), followed by DBM resistant to 51 compounds and Colorado potato beetle, *Leptinotarsa decem-lineata* (Say) (Coleoptera: Chrysomelidae), resistant to 37 compounds (Georghiou and Lagunes-Tejada, 1991). Other species that have developed resistance to most insecticides used against them include the cotton leaf worm, *Spodoptera littoralis*, in Egypt; the cattle tick, *Boophilus microplus*, in Australia; the housefly *Musca domestica*; and many species of *Anopheles* mosquitoes worldwide (Forgash 1984; Georghiou, 1986). Colorado potato beetle and DBM have developed resistance to all synthetic insecticides used against them including the biopesticide *B.t.* (Talekar and Shelton, 1993). While the biochemical resistance mechanism in *M. persicae* is based on increased levels of esterase-4 (Scott, 1990).

2.5.2 Resistance in Diamondback moth

Insecticide resistance in DBM has occurred in many parts of the world since Ankersmit (1953) first reported DDT resistance in Indonesia. More recently, resistance has been reported in Hawaii, Japan and Australia. However, the occurrence of insecticide resistance in this insect pest may not be limited to these areas (Kao *et al.*, 1989). The resistance spectrum of DBM covers all groups of insecticides; that is chlorinated hydrocarbons, organophosphorus insecticides, carbamates, pyrethroids (Sun *et al.*, 1986; Lin, 1988) and even *B.t.* (Kao *et al.*, 1989).

Resistance mechanisms in DBM proposed for synthetic chemicals include, decreased penetration Noppun *et al.* (1987), enhanced detoxification by esterases (Maa and

Chuang, 1983). Glutathione-S-transferases and reduced sensitivity of acetylcholinesterase (Wu, 1983; Hama, 1987). However, resistance to pyrethroids can be attributed to an inherited or induced mixed-function oxidase complex. Esterases in general have been noted to play significant roles in resistance to insecticides particularly organophosphates (Owusu *et al.*, 1996). Esterases are important in degrading organophosphates. The hydrolysis of organophosphates and pyrethroids by carboxylesterases, including site of action on some pyrethroids are shown in Fig 5 (A-D). The larvae of mosquito, *Culex tarsali* was found to have higher carboxyl esterase activity than susceptible strains (Matsumura and Brown, 1961). The DBM populations collected around Accra in Ghana, showed varying levels of Carboxylesterases activity, and hence insecticide resistance levels (Hama and Hosoda, 1988). The majority of individuals collected were classified as moderately resistance and may be low as compared with the populations in places like China, (Zhu, *et al.* 1996), New Zealand (Cameron *et al.*, 1997), Japan (Hama, 1990) or South Africa (Sereda *et al.*, 1997), however, there is evidence of probable increasing use of insecticides on cabbage indicating that insecticide resistance in DBM will become a limiting factor in the commercial cultivation of cabbage in the near future and therefore, there is need to urgently address the problem before it gets out of control (Kaiwa, 2000)



Chlorpyrifos

Fig. 5 (A) Hydrolysis of chlorpyrifos by carboxylesterase (ref)

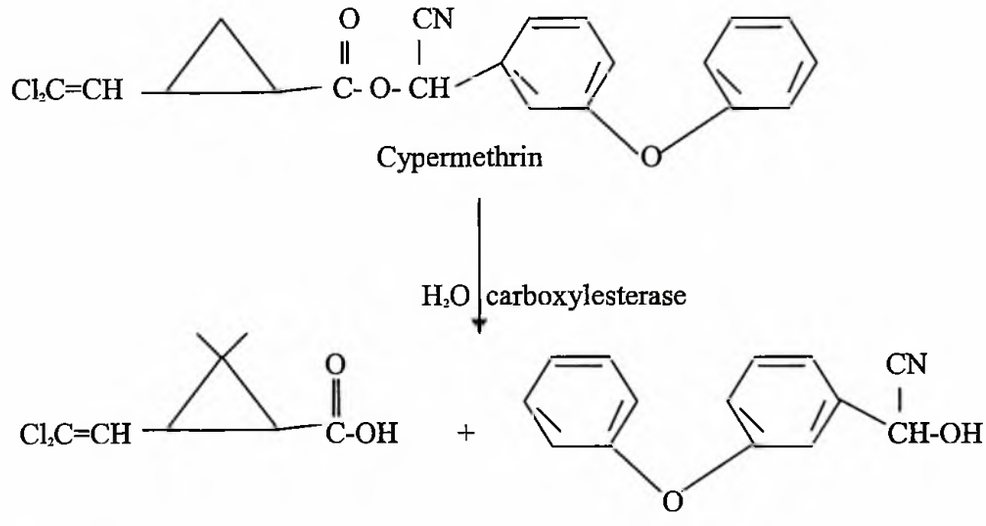


Fig. 5 (B) Hydrolysis of cypermethrin by carboxylesterase

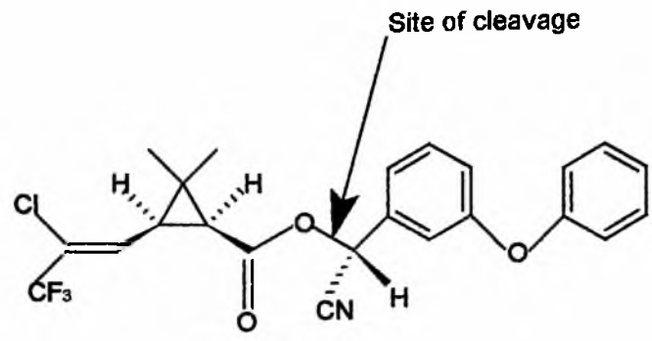


Fig. 5 (C) Hydrolysis of lambdacyhalothrin by carboxylesterase

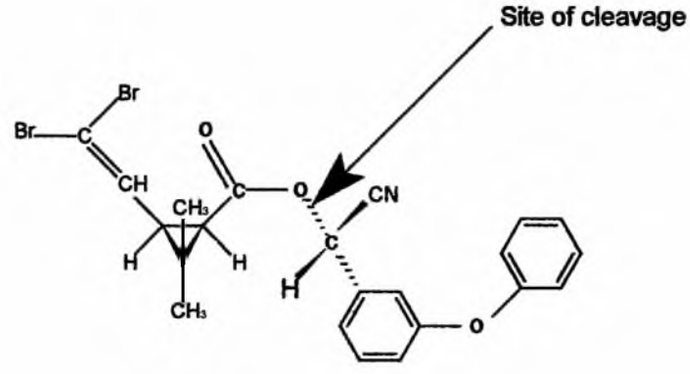


Fig. 5(D) Hydrolysis of deltamethrin by carboxylesterase

2.5.3 Management of resistance

The evolution of resistance according to Georghiou (1983) is determined by genetic, biological and operational factors. Some of the operational factors that promote resistance development include; prolonged exposure to insecticide, lack of refuges, large geographical area and selection before mating, while the biological factors comprises of little migration between population, monophagous species, short generation time, large numbers of offspring / generation, mobile species and increasing potential for exposure. Though control failure is attributed mainly to resistance, there are other factors that may lead to control failure. This includes poor chemical application methods and environmental condition, which may be due to old and obsolete application equipment, which provides poor coverage. Hot and dry conditions are known to be conducive to outbreaks of DBM and, this was probably the key reason for DBM outbreak in broccoli in California in 1997 (Shelton and Zhao, 2004).

For effective management of DBM, there is need to delay resistance development, this can be achieved by starting resistance management program before resistance is detected and strategically applying insecticides to vulnerable developmental stages and under environmental conditions that render the insects susceptible and the insecticide effective. Secondly, the choice of insecticides to be used may also decelerate resistance development; for example choosing insecticides with short persistence or residual effect while avoiding formulations that release their active ingredients slowly since these leads to prolonged exposure to insecticides which, is a prerequisites to resistance development. Consequently, insecticides should be used intermittently at the lowest possible effective rates/dosages and on the other hand; higher doses strategy is needed to kill the rare heterozygous population developing resistant. Besides, insecticides, which help conserve natural enemies, should be preferred like the *B.t.*'s, spinosad, emamectin benzoate and

indoxacard which will be friendly to natural enemies than pyrethroid, carbamate and organophosphate insecticides.

Since IPM is the most effective and promising method for controlling DBM, some of the IPM principles need to be incorporated to delay resistance development, this is based on using other control methods like cultural practices, host plant resistance etc. whenever possible, coupled with judicious use of insecticides like restricting insecticides to local instead of area-wide treatments to include spot treatment. Other cultural practices like, avoiding continuous cultivation of crucifers by having a host-free period and preservation of untreated refuges can help delay resistance. It is also important to monitor susceptibility to all insecticides on a regular basis in representative fields in each region. This is achievable through regular field scouting and treatment according to threshold levels.

Even with the judicious use of insecticides in IPM, care should be taken to avoid mixing insecticides for use against DBM. Since mixtures usually cannot delay the onset of resistance but often accelerate and complicate it. Mixtures are also undesirable due to cost, environmental risk, and residues or ineffective (differential persistence of components of the mixtures), despite their popularity with pesticide manufacturers and distributors, mixtures and high doses have many negatives to be practical in pest management (Mallet, 1989).

However attempts have been made to manage DBM by using multiple attacks that aims at achieving control through the action of independent acting forces by the use of insecticides in mixtures and using unrelated insecticides in rotations or in combination with other chemical and non-chemical measures and with synergists, which counteract

the insect defence mechanisms (Georghiou, 1983). This requires that the member chemicals are reciprocally unaffected by cross–resistance. Insecticides remain the most reliable and effective means of controlling pests, but should be used carefully, selectively and only when alternative methods do not exist or are uneconomical. So far, minimizing use is the only strategy really proven to work satisfactorily (Mallet, 1989).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals, reagents, equipment and software

The sources and or manufacturers of the chemicals and reagents, and equipment used are listed in Appendix I. The various buffers and solutions used were prepared as described in Appendix I.

3.2 Study area

The study was conducted in three localities within Accra suburbs and Mampong-Akuapem. The global positioning system (GPS) device was used to determine the specific geographical coordinates of the sampling sites. Dzorwulu (05° 37.05N, 00°11.70W), Airport (05° 35.72N, 00° 10.89W), Redco-Madina (05° 40. 36N, 00° 10.24W) and Mampong-Akuapem (05° 24.74N, 00°36.25W). These sites were chosen because of the differences in agronomic practices of the farmers, rainfall and insecticide use patterns. Accra suburb is located within the greater Accra Region in Southern Ghana. It is a coastal savannah ecological zone, characterised by dry climatic conditions with two peak rainy seasons from April to June and September to October. The annual rainfall ranges between 740 and 890 mm per annum and a temperature range of 26°C and 30°C. The relative humidity is 65-75 % (Dickson and Benneh, 1988). While Mampong-Akuapem records the highest mean monthly temperature of 30°C between March and April and the lowest of 26°C in August. Relative humidity is 65-75% throughout the year and the vegetation consists mainly of grass with isolated patches of shrub and sparse trees. The major rainfall season falls in June –July and followed by a long dry season (Yawson *et al.*, 2004). A sampling site in Accra suburbs is shown in plate 1

Plate 1. Sample collection site at Dzorwulu. A cabbage farm destroyed by Diamondback moth.

3.3 Questionnaire Survey

A preliminary survey to determine insecticide use patterns on cabbage was carried out at the beginning of the work. A questionnaire (Appendix II) was prepared to obtain information needed for quantification (Horton, 1995) on insecticide usage, type of insecticides used, frequency of use, mode of application, history of the farms and the prevailing agronomic practices was administered to the farmers. The questionnaire was structured to suit the farmers understanding, and they responded to the questions in the presence of an administrator who helped to translate the questions into the local language in some cases, or to further simplify the questions. The data obtained was analysed with a view to making a decision on the appropriateness of the site for sample collection and the most commonly used insecticides to be selected for the study. To guide on planning and conducting of the formal survey, a reconnaissance survey (Rhoades, 1995) was done to obtain basic information on the location of cabbage farms, insect pests of cabbage, and other agronomic practices that may affect insecticide residues and insecticide resistance.

Locating cabbage farms for the survey was difficult and growers in perennial cabbage growing farms were approached for information on where to find more growers. Besides, extension officers, pesticide sellers, grocers and friends were also approached for further information on the same.

The choice of farmers from a particular cabbage growing area depended on the number of growing areas identified from the informal survey and the number of growers in a particular area. In each growing area, a random representative number of growers (at least 3) were selected and spoken to (Table 4).

Table 4. Number of respondents interviewed in each cabbage growing area

GROWING AREA	NUMBER OF RESPONDENTS
Airport-West-Accra suburbs	4
Airport-(CSIR) -Accra suburbs	4
Dzorwulu-Ablemkpe- Accra suburbs	4
Adabraka –Accra suburbs	4
Atomic area- Madina Accra suburbs	3
Redco-Madina -Accra suburbs	6
Mampong-Akuapem	5

3.4 Insecticides

The insecticide formulations used were dependent on the results of the preliminary survey. The commercial formulations of test insecticides were purchased from the local chemical sale points namely Dizengoff, Agrimat House and Aglow-Accra. The products were diluted with water to obtain a range of test concentrations. The three most commonly used pyrethroids (Pawa 2.5 EC, Deltaplan 12.5 EC and Cypercal 250 EC), one organophosphate (Dursban 4E EC) and a *B.t.* formulation (Dipel 4E DF) were selected.

3.5 Culturing of Diamondback moth

For obtaining reliable and reproducible information from the physiological and toxicological experiments of any insect, it is desirable to have uninterrupted supply of physiologically homogeneous individuals. Therefore, development of laboratory rearing techniques is necessary (Sing, 1977). Mass rearing aims at producing the maximum number of insects within a short time using the most economical conditions such as minimum labor and space. Therefore the rearing materials and equipment should be kept

as simple and inexpensive as possible but they must be nutritionally and behaviorally optimal for the insect.

3.5.1 Field Sample collection

Wild fourth instar larvae and pupae were collected from infested plants in various cabbage growing areas of the selected sites from September to December 2004. A composite of pupae collected from 2-4 fields within a radius of 2-3 km served as the sample for each location as recommended by (Vastrad *et al.,* 2004). Samples were collected into plastic Petri dishes using fine camel hairbrush. The Petri dishes were lined with moistened tissue paper to avoid overheating during collection and transportation. The larvae were sent to Noguchi Memorial Institute of Medical Research (NMIMR) of University of Ghana and stored at a temperature of -80°C to prevent loss of the esterase activity (Zhu and Brindley, 1990) and later used for enzyme assay. The Pupae collected from the fields were used to establish a DBM colony at the Sinna's Garden of Crop Science department of University of Ghana in rearing cages (30 cm^3) as shown in Plate 2. The temperature during the period of study was 28+1°C with relative humidity of 65-70% and a photoperiod of 12h: 12h (L: D). The method of rearing was modified from Liu and Sun (1984) as adopted by Osae (2002), in which the adult moths were sustained on cotton wool impregnated with 50% (v/v) honey solution and potted-cabbage seedlings were provided as oviposition substrate. Hatched larvae were fed on pesticide-free potted cabbages grown in a screen house. Oviposition was synchronized in order to attain same age of insects at anytime. This was done by putting every potted cabbage into the oviposition cage for 24h to allow a maximum of 24h difference in age. Early fourth instar larvae of uniform size and weight from preferably F1 and F2 generations were tested for susceptibility to the range of insecticides used for the bioassays.

3.5.2 Reference (Susceptible) strain

Samples of the reference strain were provided by International Centre of Insect Ecology and Physiology (I.C.I.P.E.) Kenya. The samples were preserved in absolute ethanol. The origin of the colony is in Coast Province, Wundanyi District, Chawia (03° 28 39.6S and 038° 20 54.7E) and Werugah (03° 21 48S, 038° 19.43E).

3.5.3 Cultivation of insecticide free cabbage

Soil samples collected from a virgin land were sterilized using hot water treatment and left to cool in conjunction with the use of natural sun's heat on soil mass covered by black polythene sheet for 2 weeks. The samples were put into a tray and used for nursing the seedlings. Cabbage (var Oxylus) seeds were thinly sown in a nursery tray and shaded immediately after sowing. The shade was removed after one week when germination commenced and the seedlings were watered twice a day. After the seedlings were established, thinning was done to maintain one plant per spot. The sterilized soil was well mixed with poultry and farmyard manure and then put into 15cm diameter plastic pots. Four weeks after sowing, the seedlings were pricked out and transplanted into the pots. Agronomic practices like watering, weeding and mulching were carried out where necessary. Insect pests were controlled by non-chemical methods like handpicking and crushing. Six weeks after transplanting, the seedlings had 10-12 leaves and were used to rear DBM larvae. The cabbage was ready for harvesting 60 days after transplanting. Some heads were sampled and harvested at the root junction. The cabbage heads were sliced longitudinally and 50 grams of each sample were weighed into labeled beakers and then transferred into conical flasks containing 25ml hexane solution. The flasks were stored in a refrigerator at 4°C and the cabbage used as controls in the residue level estimations.

3.6 Determination of level of resistance

Various methodologies were employed to determine insecticide resistance of DBM to the selected insecticides as described below;

3.6.1 Dose-response bioassay

Resistance can be verified through a combination of laboratory bioassay and field performance. Laboratory bioassay is when a set of test insects are subjected to different doses of the insecticides, and then after a specific period of time the insects are judged to be dead / moribund or alive. This is underpinned by the principle of toxicology, which asserts that there is a relationship between a toxic reaction (the response) and the amount of poison received (the dose). It operates between two extremes; a low dose below, which no response can be measured and a maximum response which when reached, any further increases in the dose, will not result in any increased effect. From the assay, a dose-mortality line can be determined and the LC_{50} value calculated. The LC_{50} value is the dose required to kill 50% of individuals within a population. Bioassay has therefore become the principal method used by entomologists to determine and compare the toxicity of insecticides to insect pests. This is possible by using the dose mortality standard measures like 50% and 95% mortality points which can be used to compare different populations (Cochran, 2004).

3.6.1.1 Dose-response bioassay for Organophosphates and Pyrethroids

Since organophosphate and pyrethroids act by contact, the larvae immersion bioassay protocol was adopted from Zhao *et al.* (1994) with slight modification. Plastic cups chalices (about 2.74 cm depth) were improvised by having both ends open (see Plate 4). The wide bottom end (radius 3.42 cm) was fitted with a netting material and the top end (radius 2cm) left open. Different concentrations of the insecticides below and above the critical levels (chlorpyrifos: 3g/L, cypermethrin: 0.1g/L, deltamethrin: 0.0375g/L and

lambda-cyhalothrin: 0.083 g/L) were prepared in Petri dishes. Ten early 4th instar larvae were introduced into the container and immersed into serially diluted test solution in a Petri dish for 2 seconds. Excess solution was drained off with tissue paper. Cabbage leaves were provided for food and para film was used to seal the top open end. Mortality data was taken every 12h for three days. Any larvae that did not respond to pencil-tip prodding were considered dead.

3.6.1. 2. Dose-response bioassay to Dipel

A *Bacillus thuringiensis* var. *kurstaki*) insecticide formulation Dipel was used in the bioassay to establish the susceptibility status of DBM., using leaf residue bioassay shown in Plate 3 as described by Tabashnik *et al.,* (1987; 1990), Ninsin (2004) with slight modifications. One week after eggs were laid on cabbage plants, larvae were used for bioassays. Different concentrations of *Bacillus thuringiensis* var. *kusrtaki* formulation below and above the recommended rates (0.206 g/L) were serially diluted in distilled water assuming a spray volume of 200 litres/ha (Marina and Gatehouse, 2001), into 5-10 concentrations. Larvae of uniform age were selected from the reared DBM population and exposed to cabbage leaf discs (5 cm diameter) which were previously immersed in test solutions for 5 second and air-dried for one hour at room temperature (25°C) in standard size Petri dishes. Once the leaf discs had dried they were placed individually in clean Petri dishes (6 cm diameter), lined with a slightly moistened tissue paper and 10 early-fourth instars larvae were transferred to each dish.

For each concentration, four replicates of 40 larvae were tested, for each toxin and strain. Untreated cabbage leaves were dipped in distilled water as a control and 10 larvae introduced likewise. The Petri dishes were cleaned daily to avoid moisture build up and the larvae were provided with freshly treated leaf material for each bioassay every

second day until they pupated. After exposure, mortality was assessed after every 12 h at 25°C for 3 days. Larvae that did not show coordinated movement or did not respond to pencil-tip prodding were considered to be dead (Sun and Johnson, 1960). Whenever the control mortality exceeded 20 per cent the data was rejected d and fresh batch of larvae were used for the treatment. Resistant insect groups were preserved separately in 70% alcohol for further investigation to detect the presence of resistance genes as recommended by (Vastrad *et al.,* 2004). To help predict field performance using laboratory data, discriminating concentration method for resistance monitoring was used for comparison of concentration mortality tests (Zhao *et al.,* 1994). This enabled the population to be classified as either resistant/tolerant or susceptible to a single dose (Shelton and Zhao, 2004).

When a resistant strain was established, cross and multiple resistance was evaluated to determine which insecticides could be used to manage the resistant DBM populations. The protocol was modified from Liu *et al.* (2001) and Ninsin (2004). Larvae that survived during the bioassay were again reared to adults and their F1 generation used to test for cross-resistance with other insecticides, which were not used for resistance selection on the parents. The protocol described under section 3.6.1.1 was used to test the larvae using the critical dosages and mortality data was recorded every 12h for three days.

The data for each replicate were pooled and analyzed by probit analysis (Finney, 1971), using MINITAB software programme to determine lethal concentration (LC) values at 50% and 95% levels at 95% confidence intervals (CI). When LC values were compared, they were judged to be significantly different when the corresponding 95% CI values did not overlap. The slope of the regression line was also recorded.

Plate 2. A metal net cage (50cm3) with metal board floor. Inside is a potted cabbage plant about six weeks old used for oviposition and further feeding of the hatching larvae.

Plate 3. Potted cabbage plant showing diamondback moth 3rd to 4th instars larvae.

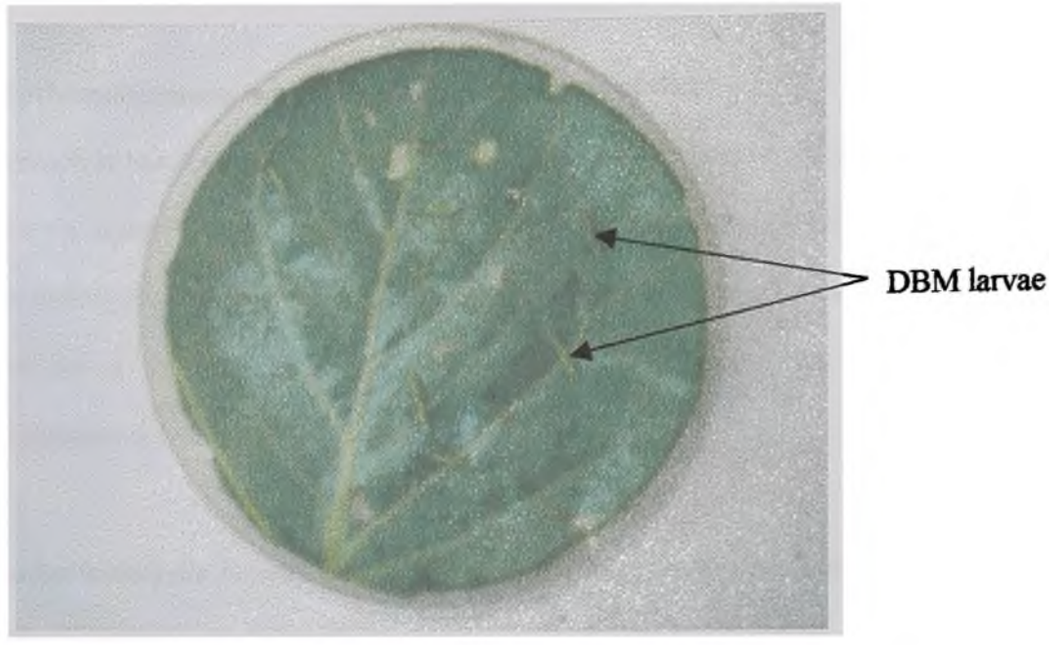


Plate 4. Leaf disk dip (leaf residue) bioassay set up. Plastic petri dish with insecticide treated cabbage leaf disk (about 5cm diameter) and ten early fourth instars DBM larvae introduced into the petri dish.

Plate 5. Larvae immersion bioassay set up. Plastic cup "holy communion cup" (about 2.74cm depth, bottom and top radii are 3.42cm and 2cm respectively). With net-like material at the bottom and an open top end.

3.6.2 Carboxylesterase activity in field population of DBM

The approach is based on the activity of carboxylesterase, which has been confirmed, to be positively linked to resistance in DBM (Doichuanngam and Thornhill, 1989) and involves hydrolysis of naphthyl acetate by carboxylesterase. Naphthyl acetate is a general substrate for a variety of hydrolases and is commonly used for determining carboxylesterase activity (Brown and Brogdon, 1987).

Quantitative hydrolytic activity of esterase enzymes was determined with two model substrates, α- and β- naphthyl acetate at predetermined optimum substrate concentration (30 mM), temperature (40°C) and pH value (7.2) (Kaiwa, 2000). The specific esterase activity was quantified by the amount of naphthol produced and special staining solution added to allow colour development to enable spectrophometric readings to be taken at specific wavelengths.

DBM specimens were assayed for carboxylesterase activity by naphthyl acetate- diazo blue coupling reaction (Owusu, 1992). The individual larvae were homogenised in a buffer and carboxylesterase assay was performed based on the original method developed for housefly esterase (Van Asperen, 1962) and adopted for cotton aphid carboxylesterase (Owusu *et al.,* 1996). A standard curve was plotted as reference.

3.6.2.1 Establishment of standard calibration curves

3.6.2.1.1 α-Naphthol

A 0.2M solution of α-Naphthol (MW 144.2) in 10ml of absolute ethanol was prepared. This solution was serially diluted 1 in 10 to give a concentration of 0.002M. This concentration was further serially diluted 1 in 2 to obtain the concentrations $10x10^{-4}$M, $5x10^{-4}$M, $2.5x10^{-4}$ M, $1.25x10^{-4}$M, $6.25x10^{-5}$M in test tubes. The different concentrations were then used to establish the calibration curve. Into each of the five test tubes

containing 2.8 ml of 0.07M phosphate buffer (pH 7.2), was added separately 0.1 ml of the prepared α-naphthol solution. A test tube containing ethanol (0.1ml) was also set up as a blank. Sodium dodecyl sulphate-fast blue B salt (SDS-FBS) solution (0.5ml) was added to each test tube and the mixture incubated for 15 minutes for colour development. The absorbances were measured using a Shimadzu Double –beam spectrophotometer at 600nm against the blank that had α-naphthol being replaced by absolute ethanol. The absorbance values were converted to micromoles of α- Naphthol produced by reference to the standard curve. The calibration curve was obtained from the results using linear regression in MINITAB statistical software programme (See Appendix IV).

3.6.2.1.2. β-Naphthol

A 0.5M solution of β-Naphthol (MW 144.2) in 10ml of absolute ethanol was prepared. This solution was serially diluted 1 in 10 to give a concentration of 0.005M. This concentration was further serially diluted 1 in 2 to obtain the concentrations $25x10^{-4}$, $12.5x10^{-4}$, $6.25x10^{-4}$, $3.125x10^{-4}$ in test tubes. The different concentrations were then used to establish the calibration curve. Into each of the five test tubes containing 2.8 ml of 0.07M-phosphate buffer (pH 7.2), was added separately 0.1 ml of the prepared β-naphthol solution. A test tube containing ethanol (0.1ml) was also set up as a blank. SDS-FBS solution (0.5ml) was added to each test tube and the mixture incubated for 15 minutes for colour development. The absorbances were measured at 450nm against the blank that had β-naphthol being replaced by absolute ethanol. The absorbances of the sample measurements were converted to micromoles of β-Naphthol produced by reference to the standard curve. The calibration curve was obtained using linear regression in MINITAB statistical software programme (See Appendix IV).

3.6.2.1.3 Calibration curve of bovine serum albumin (BSA)

Bovine serum albumin (0.1 g) was dissolved in 100 ml distilled water to obtain 0.1% stock solution. This was first diluted 1 in 10 after which 1 in 2 serial dilutions were made to obtain concentrations ranging between 10×10^{-2} g/L – 0.625×10^{-2} g/L which were used to establish the calibration curve. A volume of 0.1 ml BSA solution was transferred into test tubes and 2 ml of reagent B [50 ml of Reagent A (10 g of sodium carbonate and 2 g sodium hydroxide in 500 ml distilled water) to 0.5 ml each of 1 % $CuSO_4$ and 1 % sodium tartrate] was added and the mixture was allowed to stand for 20 minutes for colour development. Readings were taken on a spectrophotometer at 750 nm against a control that lacked BSA solution.

3.6.2.2 Enzyme preparation and assay

Individual larvae of DBM were separately homogenized using sterilized plastic pestle in a 1.5 Eppendorf tube-containing 0.3ml of potassium phosphate buffer (pH 7.2). The resultant homogenate was centrifuged at 4000 rpm in an Eppendorf centrifuge 5415C for 2 min at 1 min interval and was then used as enzyme source for carboxylesterase assay.

A reaction mixture consisted of incubating 0.1 ml of the homogenate with 0.1 ml of 30 mM α-naphthyl acetate, in absolute ethanol for 10 min. at 40 ^{0}C in 2.8 ml of phosphate buffer (pH 7.2) in a shaking water bath. After incubation, 0.5 ml of (SDS-FBS) was added for colour development. This mixture was incubated for 15 min and read at 600 nm with a spectrophotometer against a control that lacked enzyme. The homogenate (0.1ml) was also incubated for 10 min. at 40°C with β-naphthyl acetate in absolute ethanol as substrate. The mixture was incubated with SDS-FBS for 15 minutes to allow colour development after which it was read at 450 nm on the spectrophotometer against a control that lacked the enzyme. For DBM larvae with higher activity above the

measurable range, the homogenate was appropriately diluted 1 in 10 with phosphate buffer before assay.

3.6.2.3 Protein determination

Protein contents of all enzyme preparations used were determined by the method of Lowry *et al.* (1951) as adapted by Owusu *et al.* (1994) and Kaiwa (2000) with slight modifications. Aliquots (0.ml portions) of the homogenized enzyme solution were transferred into test tubes and 2 ml Reagent B (see 3.5.2.1.3) was added to it. This mixture was allowed to stand for about 30 minutes. The dilute phenol reagent (0.25 ml) was added and the reaction mix was incubated for further 20min to allow for a blue colour development. Readings were taken on a spectrophotometer at 750nm against a control that lacked an enzyme source.

3.6.3 Polyacylamide gel electrophoretic analysis of esterases

Resistance mechanism for organophosphate and pyrethroid insecticides in DBM was reported to involve the combined effect of increased oxidative and hydrolytic enzymes (Siegfried *et al.,* 1990; Siegfried and Scott, 1991). Even though the hydrolytic enzymes have been quantified and documented, polyacrylamide gel electrophoresis can be used to further characterize the esterase enzyme based on the band sizes, electrophoretic nobilities and staining intensity of different isozymes.

3.6.3.1 PAGE analysis of DBM esterase isozymes

Each insect was homogenized in 33ml of sample buffer using sterilized plastic pestle in a 1.5ml Eppendorf tube to break the cells and centrifuged at 4000 rpm in an Eppendorf centrifuge 5415C for 15-20 minutes and supernatant used for electrophoresis.

Non-denaturing discontinuous (2.5%; 7%) polyacrylamide gel electrophoresis (PAGE) was performed in tris–glycine buffer (pH 8.3) with slab plate ATTO CORPORATION, Japan. Fifteen millilitres of supernatant was loaded along with 50g sucrose in running buffer at pH 8.9, into wells of the polymerized stacking gel. The electrophoresis was conducted at a constant voltage of 150V supplied by an electrophoresis power supply pack ATTO AE-3121 (ATTO- Corporation, Japan) until the tracking dye had moved to the interface between the stacking gel and separating gel.

Staining of the gel for protein bands corresponding to esterase activity was done in a plastic tray containing staining solution [(100ml of 0.2 M phosphate buffer, pH 6) containing 2ml α-naphthyl acetate (30mM) and 0.2g fast blue BB salt], at room temperature in the dark for 45 minutes. After the protein bands appeared, the gel was transferred from the staining solution into the fixing solution (7% acetic acid) overnight in a slow mechanical shaker to destain the background. The destained gel was then visualized under an ordinary light illuminator and stored in distilled water to prevent it from drying up. While still soaked in distilled water, the destained gel was transferred onto chromatographic filter papers 3mm thick (Tokyo Roshi Kaishi, Ltd, Tokyo, Japan) and overlaid with a cellophane membrane previously softened by immersion in distilled water then left to dry over a flat hard board for 3 days.

3.6.4 PCR amplification of *B.t.* resistant gene

The DBM genomic DNA was extracted and Polymerase Chain Reaction (PCR) was used to detect *B.t.* resistant gene.

3.6.4. 1. DNA extraction

The PCR method was slightly modified from Heckel *et al.* (1999) for amplification of Bt resistance gene in DBM. The amplification process utilised two specific primers c39-451p1 (5′-CCG TGC TGA GCA TTG GAC AGT GAG-3′) and c39-451p2 (5′-TTA ACT ATA TTT GTT GGT GAC GAT AAG GTG-3′). The primers anneal on the rDNA of both susceptible and resistance strains of DBM, the amplified products are expected to be 325 bp.

DNA was extracted from the adult DBM using the Bender buffer method modified from Collins *et al.* (1987). Each adult was homogenized using sterilized plastic pestle in a 1.5ml eppendorf tube containing 200µl Bender buffer (preheated at 65°C) followed by incubation at 65°C for 30 minutes. Saturated phenol+chloroform buffer (125µl) was added to the homogenate, vortexed briefly and spun at 14000 rpm for 10 minutes. The supernatant was transferred into a fresh tube, and 250µl of chloroform was added. This mixture was vortexed briefly and spun at 14000 rpm in the Eppendorf centrifuge 5415C for 10 minutes. The supernatant was then transferred into a fresh tube and 250µl of pre-chilled absolute ethanol and 10µl of potassium acetate 8 M added, and mixed well by tube inversion followed by incubation at -40°C for 1 hour. This was followed by final centrifugation at 10,000 rpm for 10 minutes to pellet the DNA after which the supernatant was discarded. The pellet was rinsed with 200µl of 70% ethanol the tube swirled gently and the DNA re-pelleted by centrifugation at 10,000 rpm in the Eppendorf centrifuge 5415C for 5 minutes. The supernatant was discarded and the tube opened and inverted over a paper towel to evaporate to dryness before being re-dissolved in 25µl TE + RNAse (5µg/ml) or sddH_2O and incubated in ice for about an hour followed by storage at -20°C till needed for PCR.

3.6.4.4 Polyacrylamide gel electrophoresis of PCR products

Polyacrylamide gel electrophoresis was used to resolve the bands whose sizes were close and beyond the resolution of the agarose gel electrophoresis of the susceptible and the resistant DBM strains. The composition of the 7% polyacrylamide gel preparation used and solution preparation are outlined in Appendix I1. A vertical electrophoresis gel tank (BIORAD, USA) was used. Before loading the samples the wells were flushed with sddH_2O to remove excess urea. Fifteen microliter of the PCR products of each reaction was mixed with 10 µl of bromophenol blue dye and loaded into each well and the gel run at 13mA and 100 volts for 7 hours. Thereafter, the glass plates were separated and the gel carefully transferred into a plastic tray containing 5% ethidium bromide for 5-10 minutes to stain. The gel was then photographed as described under section 3.6.4.4

also subjected to solid phase extraction (SPE) in Alltech Prevail C_{18} solid phase extractor.

3.7.4. Solid phase extraction

One millilitre aliquot of cabbage extract was pipetted into a vial and the solvent completely dried (evaporated) under nitrogen gas and then redissolved in 0.5 ml of hexane. The SPE was equilibrated by allowing 2 ml methanol to run through the tube till the solvent front was about 1 mm above the column packing. After equilibration, the sample taken up in hexane (0.5 ml) was applied. Methanol, ethyl acetate, and hexane were used in that order to elute the insecticide residues in order of polarity. The eluent was collected in 2ml fractions for each solvent into pre-weighed vials. The eluted fractions were dried under nitrogen gas and the vials reweighed to obtain weights of extracted residues. The residues were re-dissolved in appropriate volumes of eluted solvents to give sample concentrations of 10mg/ml. Portions of the samples (250µl) were used for brine shrimp toxicity bioassay and mortality data was recorded after 24 hours.

3.7.5 Bioassay of cabbage extracts

A modified methodology outlined by Meyer *et al.* (1982) was adopted.

3.7.5.1 Hatching of Brine shrimps

The brine shrimps eggs used for the bioassay were obtained from Brine Shrimp Direct, California, USA. To hatch the eggs, a saline solution of concentration 25 g/L was prepared by dissolving sea salt in distilled water. This was filtered through Whatman number 1 filter paper and used to fill a small-perforated dividing tank (about ¾ full). A spatula was used to transfer the brine shrimp eggs to the covered half of the tank, the other half of the tank being open, allows shrimps to move towards light after hatching.

3.7 Residue Level Estimation

Residues are present in very small quantities as heterogeneous compounds, including biological materials like plants. For the successful analysis of residues in cabbage head samples, several steps were followed, these included sampling to get a representative of the whole lot of material for average quantification of residues and extraction, in a solvent to remove the residues from other components of the sample matrix. The extract was cleaned up to remove extraneous materials that were co-extracted from the analytical sample. The eluates were then concentrated to reduce the volume of the solvent containing the insecticide residues and the residues were finally quantified and identified using brine shrimp *Artemia salina* Leach, as a test organism (Bioassay).

3.7.1 Sampling

Insecticide residue level was determined using mature and marketable cabbage heads from the cabbage farms in the selected study sites as well as on the insecticide free cabbage heads grown in a screen house, which served as control.

3.7.2 Cabbage samples

Guided by the survey, particular farms were selected for collection of cabbage samples. The sampling was based on the following facts

i. The farms with the highest or lowest pest infestation, observed from the level of destruction on the leaves.

ii. The most commonly used organophosphate and synthetic pyrethroid insecticides.

iii. The farmers' agronomic practices like the mode of watering or concentration of the insecticides applied.

3.7.2.1 Sampling for cabbage

After selecting the farms, sampling was only done when the farmers were harvesting their cabbage for sale. This precaution was necessary to ensure that cabbage heads selected were ready for sale and for consumption. To randomize the cabbage head sampling, the field was mapped on a piece of paper, and numbers given to rows of plant and each plant in each row. The numbers of the rows were balloted for before picking ballots for plants in each row and the particular heads harvested were kept in polythene bags. The cabbage heads were sliced longitudinally into cone shapes and 50 grams of each sample was weighed into labelled beakers, and then transferred into a conical flask and 25ml hexane was added, and then was stored at 4°C for residue extraction.

3.7.3 Extraction, concentration and analysis of insecticide residues

Extracting mixture consisting of 55 ml hexane and 20 ml butanone (ethyl-methyl ketone) was used for extraction. Thirty millilitre of hexane was used for extraction after which the sample was blended for 4 minutes, at a minute interval, and the extract decanted into a 250ml flask. Further portions of the extracting solvent (35 ml and 40ml) were added and the homogenate blended, the extracts obtained were decanted into a flask. The homogenate was centrifuged in a bench top centrifuge at 3000 rpm (rotor radius, 11.5 cm) for 5 minutes at room temperature. The pooled extracts were concentrated using the Rotary Vacuum Evaporator to about 2 ml.

The concentrate was transferred into a 10 ml vial. The rotary vacuum flask was then rinsed twice, each time with 2 ml of hexane, and added to the concentrated extracts in the vial. The samples were dried under nitrogen gas and re-dissolved in 5 ml hexane. The vials were wrapped in aluminium foil and stored in refrigerator. The crude extract was

also subjected to solid phase extraction (SPE) in Alltech Prevail C_{18} solid phase extractor.

3.7.4. Solid phase extraction

One millilitre aliquot of cabbage extract was pipetted into a vial and the solvent completely dried (evaporated) under nitrogen gas and then redissolved in 0.5 ml of hexane. The SPE was equilibrated by allowing 2 ml methanol to run through the tube till the solvent front was about 1 mm above the column packing. After equilibration, the sample taken up in hexane (0.5 ml) was applied. Methanol, ethyl acetate, and hexane were used in that order to elute the insecticide residues in order of polarity. The eluent was collected in 2ml fractions for each solvent into pre-weighed vials. The eluted fractions were dried under nitrogen gas and the vials reweighed to obtain weights of extracted residues. The residues were re-dissolved in appropriate volumes of eluted solvents to give sample concentrations of 10mg/ml. Portions of the samples (250µl) were used for brine shrimp toxicity bioassay and mortality data was recorded after 24 hours.

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Hatching tank was left under fluorescent light at 22°C for 48 hrs before nauplii were used for bioassay.

3.7.5.2 Calibration curves for brine shrimp toxicity to the standard insecticide

The standard curves for the insecticide standards; chlorpyrifos-methyl, pirimiphos-methyl, deltamethrin, cypermethrin, lambda-cyhalothrin and permethrin were established to be used in estimation of the insecticide concentrations in the cabbage heads. A preliminary bioassay was done to determine the concentration that gives 10% and 90% mortality. Based on this, 100µl of each of the standard solutions of chlorpyrifos-methyl, deltamethrin, cypermethrin and permethrin were serially diluted (1 in ten dilution) to give two stocks of concentrations 10µg/ml and 1µg/ml using these solutions as stock, 0.5ng/ml, 5ng/ml, 50ng/ml, 500ng/ml concentrations of each of the standards were transferred into a 10ml vials, dried under nitrogen gas and used for the bioassay. However 200 µl of each of standard solutions of lambda-cyhalothrin and pirimiphos-methyl were used, to make a stock solution of 20µg/ml and (1 in 10 dilution) 2µg/ml. Using these two stock solutions, 1ng/ml, 10ng/ml, 100ng/ml and 1000ng/ml concentrations of these insecticides were used for the bioassay. The insecticide standards were dissolved in hexane. The dried sample in each vial was taken up in acetone (40µl) and sea-water (5ml) was added for the brine shrimp bioassay. Four replicates were bioassayed for each insecticide standard. Mortality data was recorded for each insecticide standard dilution and the control. The data was analysed using probit package in MINITAB 12 windows software to determine LC_{50} and LC_{95}, and the curves were obtained for all the standard insecticides.

3.7.5.3. Bioassay to determine appropriate solvent that elutes the insecticide standards

Preliminary bioassay was conducted using known volumes of the insecticide standards to determine the solvents, which eluted the insecticides from the C_{18} SPE tubes. Different solvents were selected in order of their polarity: methanol, ethyl acetate and hexane were used. The SPE tubes were preconditioned using 2ml of methanol, after which 100µl of each of the insecticide standards (pirimiphos-methyl, chlorpyriphos, deltamethrin, cypermethrin and lambda-cyhalothrin) was passed through the SPE. Two ml of each of the extracting solvents from methanol, ethyl acetate and hexane were added to the tube. The eluents were collected into 10ml vials and dried under nitrogen gas. Forty microlitres of acetone was added to re-dissolve the content in the vials. A volume of 2ml sea salt (25 g/L) was added to each vial after which 10 brine shrimps were added into each vial and made up to 5 ml mark with sea water. The set up was kept under fluorescent light and mortality count taken after 24 hr and another set up without the extract served as a control.

The Abbots formula (Abbot, 1925) was used to correct for deaths in the control samples.

$$CM = \frac{(\%T - \%C)}{(100 - \%C)} \times 100$$

Where, CM = corrected mortality

%T = percent test effect mortality

%C = percent control mortality

The corrected percentage mortalities were used to estimate the concentration of insecticide residues in cabbage, from the linear regression equation of dosage– mortality curves of the standard insecticides.

3.8 Data Analysis

SPSS version 10 (SPSS Inc. USA) analytical package was used for analyzing the survey data, all mortality data was corrected for natural mortality using Abbott's formula (Abbott, 1925) and Probit package in MINITAB 12 windows software was used to determine LC_{50} and LC_{95} values (Finney, 1971). Analysis of variance was used to test for significance in the activity of carboxylesterase enzymes and MRL values of the residues and Least significant Difference (LSD) used to separate means in Genstat 5 Release 3.2 Copyright 1995, Lawes Agricultural Trust (Rothamsted Experimental Station) statistical package.

CHAPTER FOUR

RESULTS

4.1 Insecticide use pattern survey

4.1.1 Agronomic practices

The survey covered a total of 30 cabbage farms in Accra suburbs and Mampong-Akuapem. It revealed that all the cabbage growers visited were small-scale farmers since 14 (47%) of the farms cultivated were less than ½ hectare (ha); 7 (23%) of the farms were about a hectare; 7 (23%) were 1-2 ha. and only 2 (7%) of the farms were more than 2 ha in size. Most of them were perennial cabbage farmers. Since 11 (36%) of the farmers had been involved in cabbage production for a minimum period of 5-10 years. Five (17%) of the farms have been cultivated continuously for over 20 years (Fig.6). The main equipment mostly used on the farms was watering can. This was fitted to the shower type nozzle by only 3 (9%) of the farmers, while the rest fitted flat metal plate. Farmers at Mampong were using bucket and cup and only 3 (10%) of the farmers used water hose (Fig.7).

4.1.2 Pests and pest control

The survey showed that 28 out of 30 of the farms surveyed had a problem with insect pest infestation. Twenty-two of the farmers reported that DBM was the most serious and destructive pest of cabbage in the farms (Fig. 8). With regard to period of prevalence, DBM was mostly found in dry season; 23 (77%) of the farmers confirmed this, while 6 (20%) said the pest was present all year round. Chemical control was the most predominant method used by 29 (97%) of farmers to control pests while a few practiced IPM, which include cultural approach like crop rotation and frequent watering.

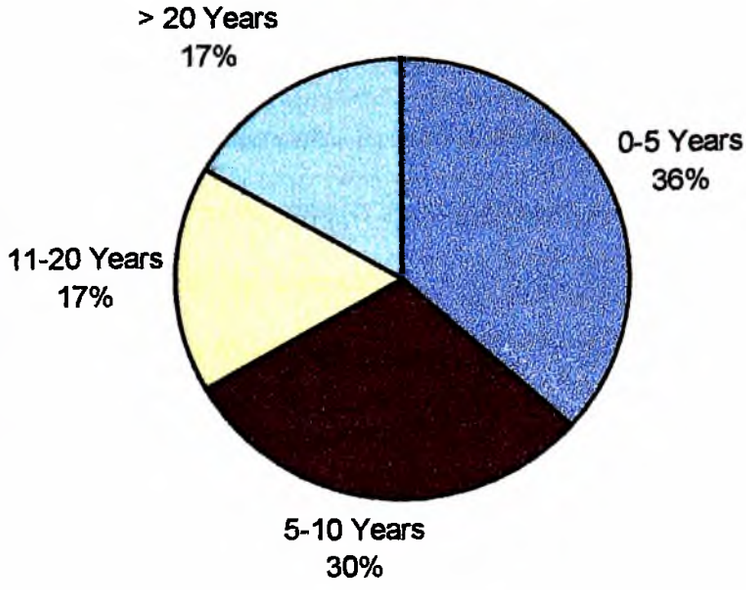

Fig. 6 Duration under which the farms have been used for Brassicas production

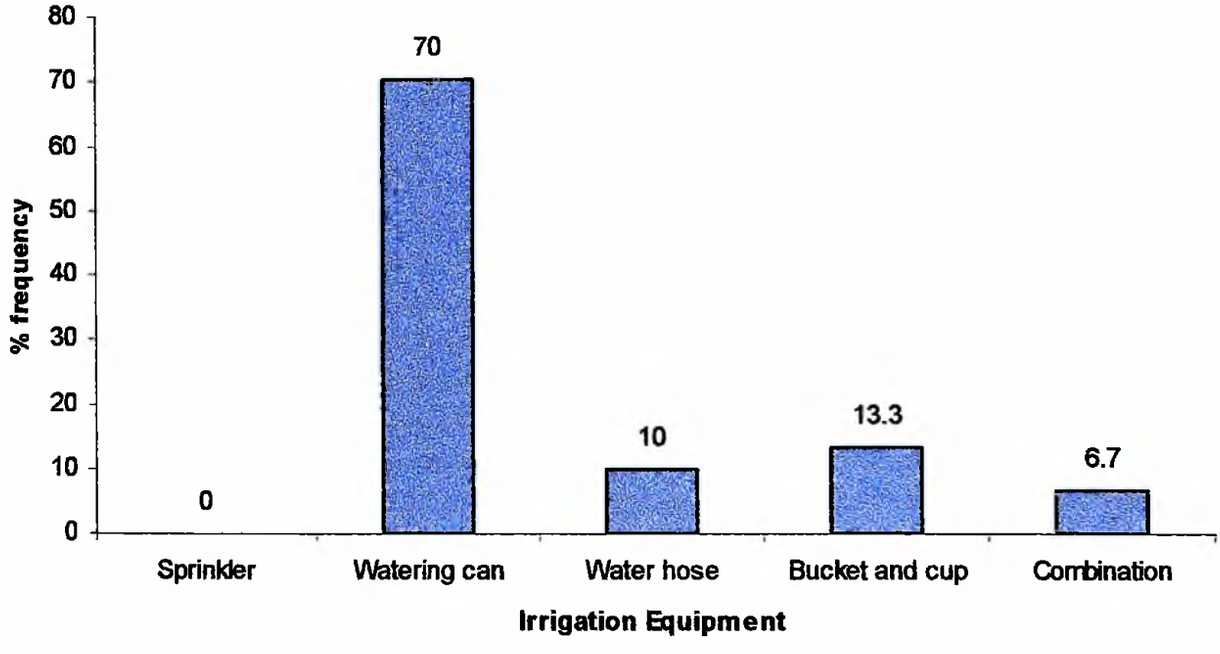

Fig. 7 Types of irrigation equipment used in cabbage farms

4.1.3 Insecticides and their use pattern

The survey sought information on current and previously used insecticides. Currently the most widely used insecticides to control cabbage pests in the study areas are as shown in Table 5 and the most popular insecticides on cabbage farms previously are shown in Table 6. When all the insecticides were grouped into their various classes, the results showed that apart from growth regulators, the use of organophosphates was on the rise (Fig.9). On the contrary, pyrethroids and the biopesticides: (*B.t.* formulations; Biobit and Dipel) usage has declined over the years. Twenty (66.67%) of the farmers attributed the decline in usage of these insecticides to ineffectiveness against the target pests especially Dipel, Biobit and cymbush while 8 (26.67%) respondents said the chemicals were out of stock, compelling them to look for alternatives even though the previous ones such as (karate and biobit) had not failed. Some of the farmers stopped because the chemicals were too expensive.

Table 5. Current insecticide use pattern in Accra suburbs and Mampong-Akuapem

Agrochemicals	**Class**	**Total (%) usage**
Rimon	Growth regulator	10 (32.39%)
Regent	Growth regulator	6 (19.72%)
Dursban	Organophosphate	3 (9.86%)
Dize DDVP	Organophosphate	2 (7.04%)
Cyperdim super	Pyrethroid+organophosphate	2 (5.63%)
Dipel	Biopesticide	1 (4.23%)
Pawa	Pyrethroid	1 (2.82%)
Amektin	Growth regulator	1 (2.82%)
Others (karate[1], Cypercal[2], Deltpaz[3], Deltaplan[4], Decis[5], Actelic[6], Cyperphos[7], D336[8] and Polytrine[9], Biobit[10]	1-5=Pyrethroids 6-9=Mixtures (Sps+Ops) 10=Biopesticide	4 [12.68%]

Table. 6. Previous insecticide use pattern in Accra suburbs and Mampong-Akuapem

Agrochemicals	**Class**	**Total (%) usage**
Dipel	Biopesticide	5 (18.18%),
Karate	Pyrethroid (SP)	5 (18.18%),
Biobit	Biopesticide	4 (12.73%),
Regent	Growth regulator (IGR)	3 (10.91%),
Cymbush	Pyrethroid (SP)	2 (7.27%),
Polytrine	Mixture (Op + SP)	2 (5.45%),
Cydimsuper	Mixture (Op + SP)	2 (5.45%),
Actelic	Mixture (Op + SP)	2 (5.45%),
Orthine	Pyrethroid (SP)	2 (5.45%),
Dursban	Organophosphate (OP)	1 (3.64%),
Others (Cyperphos[1], Rimon[2], Neem[3] and Thionex[4]	1=Mixture, 2=IGR 3=Biopesticide, 4= Op	2 [7.27%].

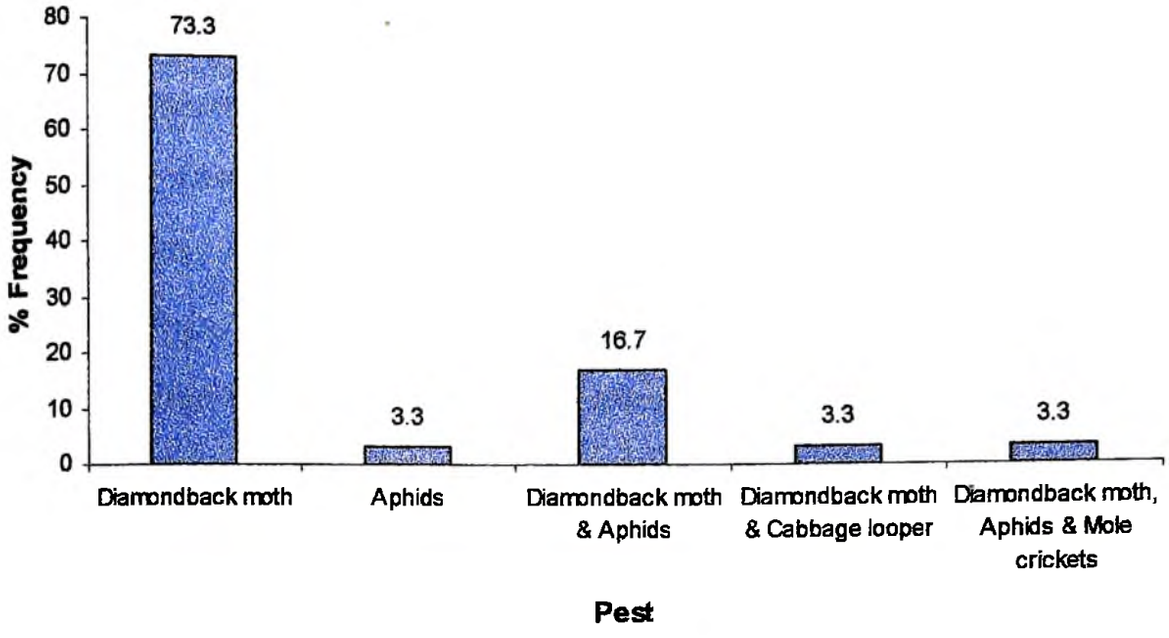

Fig. 8 Various pests of Brassicas in the cabbage farms

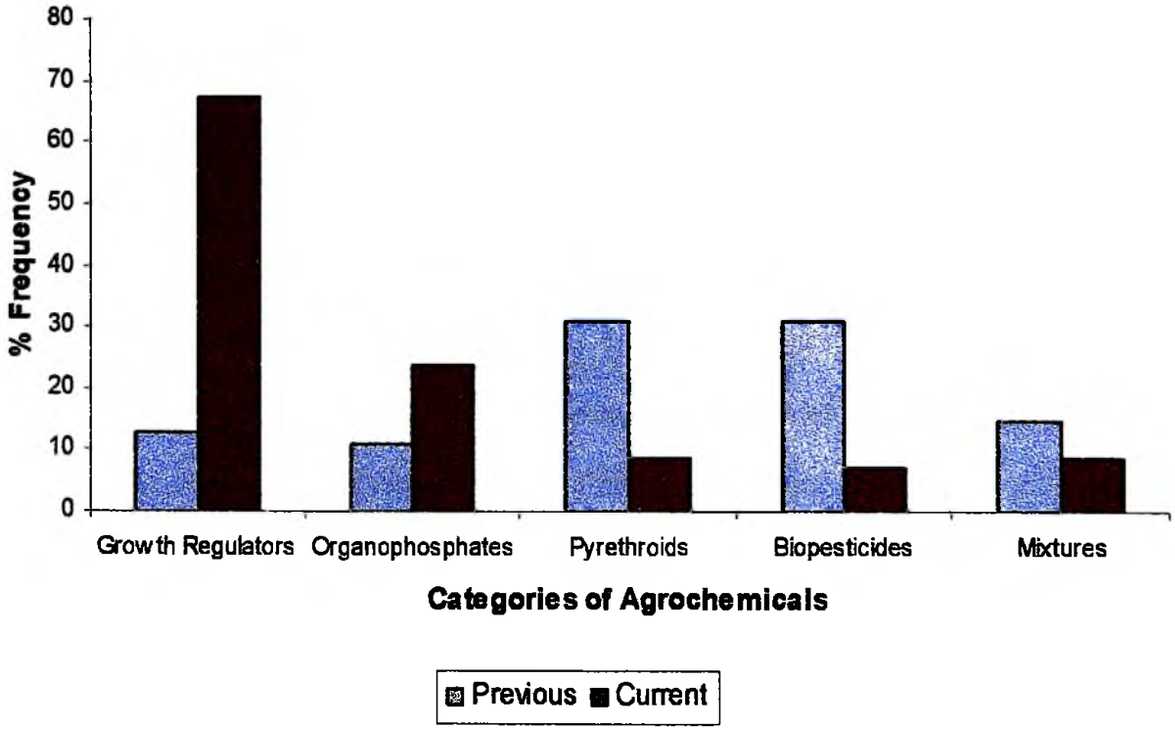

Fig. 9 Comparison between the previous and currently used agrochemicals in the cabbage farms

On insecticide use, spraying in 26 out of 30 farms started at seedling stage and these insecticides were either sprayed alternatively by 19 (63.33%) of the farmers or applied as cocktail mixtures by 11 (36.67%) of the farmers. The various combinations of insecticides used by growers were as follows:

i Regent and Rimon [**growth regulators**]

ii Dize DDV [**organophosphate**], Rimon [**growth regulators**] and Cydimsuper [**pyrethroid + organophosphate**]

iii Regent [**growth regulators**] and Dursban [**organophophate**]

iv Cydim super [**pyrethroid + organophosphate**] and Dursban [**organophosphate**],

v Rimon [**growth regulator**] and Deltapaz [**pyrethroid**]

vi Rimon, Regent [**growth regulators**] and Dize DDVP [**organophosphate**]

4.1. 4 Mode of insecticide application

The growers were able to give the various volumes of insecticides used per sprayer. Majority used the lid of chemical containers as standard measure of dosages and for Dipel, in Mampong, farmers used matchbox. In addition to this, dosages in the farms were difficult to estimate because the application equipment was not calibrated before use. The main mode of application was manually operated knapsack sprayer. This was attached to different nozzles. Fourteen (46.43%) used yellow polyjet, 12 (39.29%) blue/black, 3 (10.71%) green, and 1(3.57%) used red polyjet. It was also observed that 18 (60%) of the growers did not wear any protective clothes during spraying while 8 (26.67%) were partly protected by using gloves and facemasks and only 4 (13.33%) wore protective clothes.

4.1.5 Frequency of insecticide application

Apart from the use of various modes of application, the interval between insecticide spraying and irrigation varied greatly among the respondents. Ten (33%) of the respondents watered within the same day of insecticide spraying while majority, 16 out of 30 gave 1-3 days interval and the longest was by 1 (3%) farmer who used more than 5 days interval (Fig.10). The growers also sprayed the insecticides frequently and at short intervals, having 21 (70%) of them spraying at a frequency less than a week (Fig. 11).

This high frequency of spraying was reflected by 14 (46%) respondents who observed pre-harvesting interval (PHI) as short as less than a week (Fig.12). Although some growers used biopesticides like Biobit and Neem towards harvesting time with short PHI, others claimed they obeyed manufacturers' instructions concerning every group of chemicals used. Yet a few farmers during heavy pest infestation could spray the cabbages and sell them immediately.

The improper use of insecticides reflected above was because the common source of professional advice on use and handling of insecticides were fellow farmers and pesticide dealers, except for 11 (36%) who received advice from Agricultural extension agents of this number 3 (10%) were visited within 1-2 weeks, while 3 (10%) received their visits well above two years span and some could not tell the frequency of the visits due to their irregularity or they were not visited at all (Fig. 13).

With regards to insecticide residue awareness 19 (63.33%) had no idea about it (Table 7). Of those who were aware, the majority (82%) heard about the perceived risks caused by improper use of insecticides through the mass media (radio, news paper, television) as the main source of information (Fig. 14).

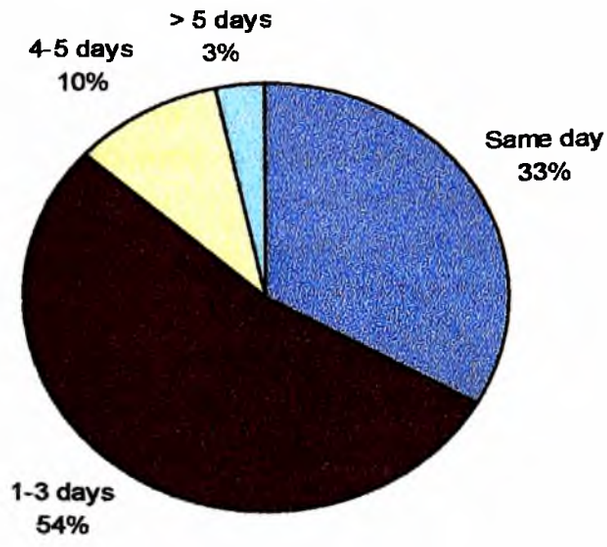

Fig. 10 Interval between insecticide spraying and subsequent irrigation

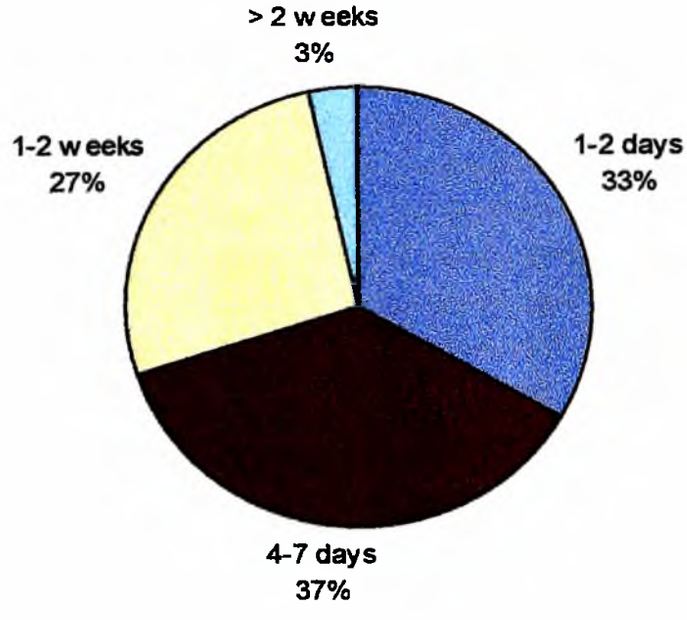

Fig. 11 Frequency of insecticide spraying

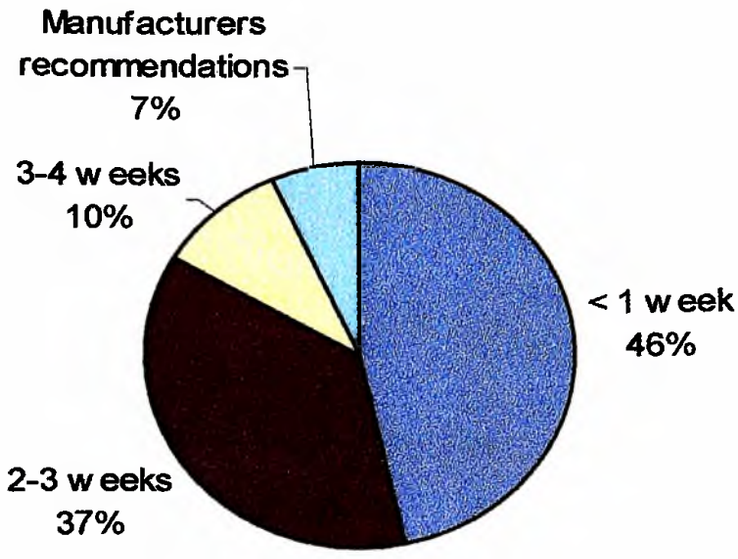

Fig. 12 Pre-harvest interval observed by cabbage farmers

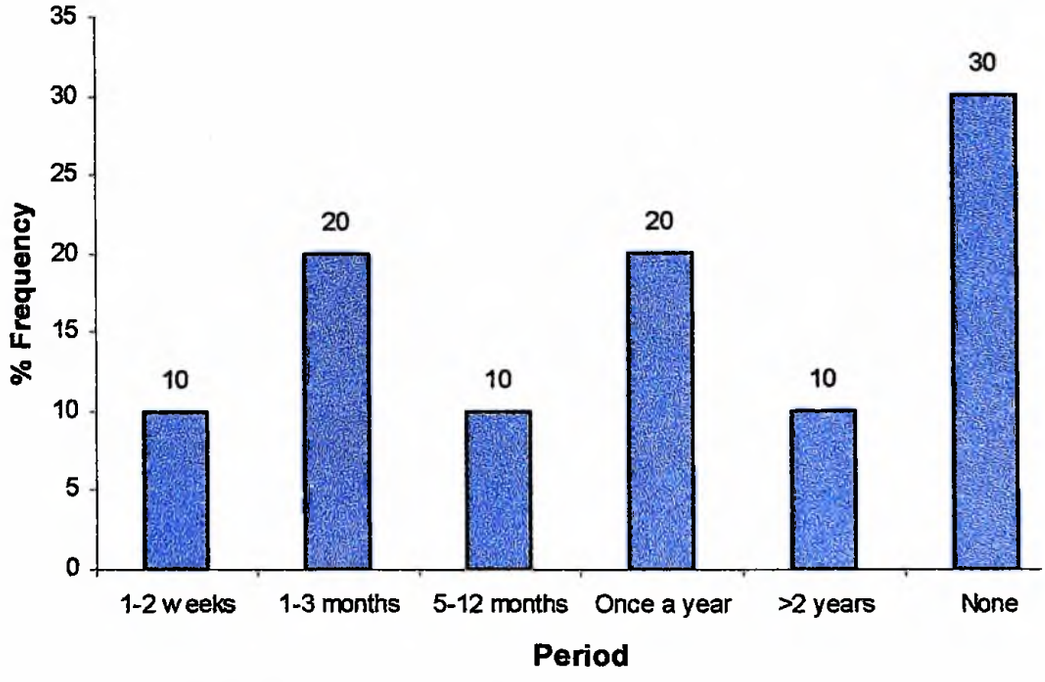

Fig. 13 Frequency of visits by the extension officers

Table 7. Farmers' Awareness about some agronomic practices and insecticide residues.

Practice/problems	Yes	No
Pest problem	93.3	6.7
Nursery treatment	86.7	13.3
Keeping of farm records	16.7	83.3
IPM training	16.7	83.3
IPM application	13.3	86.7
Residue awareness	36.7	63.3

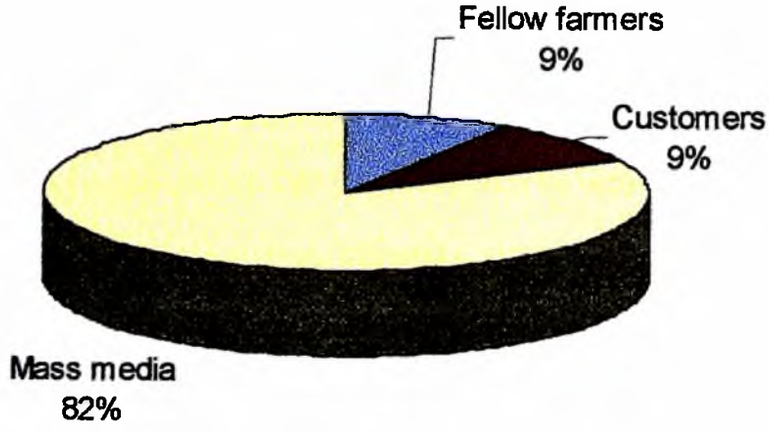

Fig. 14 Sources of information on insecticide residues awareness

4.2 Susceptibility of DBM to insecticides

Diamondback moth was found to be tolerant to all the insecticides assayed. Due to lack of reference strain, the recommended rate expected to give 95 % mortality was used as point of reference. This was compared to the LC_{95} to get the number of fold tolerance. Dose response mortality curves were used to determine the LC_{50} and LC_{95} values. The LC_{95} values were found to be much higher than the recommended dosage for all the insecticides assayed, which included Dursban (chlorpyrifos-methyl), Deltaplan (deltamethrin), Pawa (lambda-cyhalothrin), Cypercal (cypermethrin) and Dipel (*B.t.* var *kurstaki*). However, there were also wide variations in the response of the insect populations from different sites (Tables. 8).

4.2.1 Pawa (Lambda-cyhalothrin)

The LC_{95} estimated for Pawa for the Airport population was 8527.3mg/L which when compared with the recommended dosage of 83mg/L gave 102.7 fold tolerance. The Dzorwulu population of DBM followed closely with 100.3 fold tolerance and the least tolerant population was from Mampong which recorded 57.9 fold tolerance (Table 8). Pawa was the most highly tolerated pyrethroid in all the sites and overall it was second to dursban in all the study sites with respect to resistance.

4.2.2 Cypercal (cypermethrin)

Airport DBM population showed the highest level of tolerance to Cypercal, recording an LC_{95} of 8565.2 mg/L which when compared to the recommended dosage of 100mg/L gave 85.7 fold tolerance. Other Accra suburb sites recorded nearly similar values of fold tolerance. Mampong however recorded the lowest which was 54.6 fold tolerance.

4.2.3 Deltaplan (Deltamethrin)

This was shown to be the most effective of all the pyrethroids assayed. Among the conventional insecticides it had the lowest LC_{95} value for all the sites. However it was least effective in Dzorwulu where DBM population recorded an LC_{95} value of 2988.4mg/L against a critical dosage of 37.5mg/L giving 79.7 fold tolerance.

4.2. 4 Dursban (Chlopyrifos-methyl)

The bioassay revealed that Dursban was the least effective of all the insecticides assayed. It generally recorded the highest LC_{95} value in all the sites and except for Madina and also recorded the highest fold tolerance. Specifically, Dzorwulu population of DBM proved to be the most tolerant to Dursban with LC_{95} of 579438.1mg/L against recommended dosage of 3000mg/L giving a 193.2 fold tolerance. The Madina DBM population was comparatively less tolerant.

4.2.5 Dipel (*Bacillus thuringiensis* Var. *Kurstaki*)

In contrast to the conventional insecticides, DBM was found to show some significant level of susceptibility to Dipel in all the sites. The highest recorded LC_{95} for *B.t.* 892.7mg/L was recorded in Mampong as against a critical dosage of 206 mg/L, representing 4.3-fold tolerance. On the other hand, most of the Accra suburb populations were more susceptible giving about 2.8 fold in Airport with the Dzorwulu DBM population showing the least tolerance. In general, while Dzorwulu population showed the highest level of tolerance to the organophophates and pyrethroids tested, it showed the highest level of susceptibility to *B.t.*

Table 8. The response of *Plutella xylostella* (P1) generation of field-collected population to lethal concentrations of active ingredients of the selected insecticides.

	Insecticides	LC_{50} (mg litre^{-1}) (95% CI)*[a]	LC_{95} (mg litre^{-1}) (95% CI)	Slope	No. of fold tolerance*[b]
Airport	Pawa	2503.0 (2089.1 - 2941.2)	8527.3 (7581.8 - 9782.2)	3.0913	102.7
	Dursban	84034.8(64951.4- 106745.8)	318928.9 (269937.9-390425.8)	0.0781	106.3
	Deltaplan	572.5 (449.8-705.7)	2046.2 (1743.4 -2505.1)	12.872	54.6
	Cypercal	1819.0 (1269.5-2379.7)	8565.2 (7343.5-10319.7)	2.3567	85.7
	Dipel	166.5 (124.8-210.4)	577.8 (488.0- 717.4)	49.112	2.8
Madina	Pawa	2088.5 (1868.0-2335.7)	6355.0 (5764.2-7099. 7)	5.2946	76.6
	Dursban	74617.4(62046.7 -91825.9)	222920.4 (187335.3-276813.5)	0.0973	74.3
	Deltaplan	385.8 (345.0- 433.5)	1036.5 (916.2-1206.9)	34.841	27.6
	Cypercal	2475.0 (1856.1- 3106.0)	8056.0 (6947.7-9642.4)	3.3707	80.6
	Dipel	192.4(161.1-226.8)	587.0(518.8- 678.1)	37.779	2.9
Dzorwulu	Pawa	2923.5 (2585.5- 3289.5)	8328.4 (7586.5-9249.4)	3.5221	100.3
	Dursban	170573.3(141716.8- 206364.1)	579438.1 (495507.5-701492.1)	0.0552	193.2
	Deltaplan	801.7 (664.8 - 947.9)	2988.4 (2640.3- 3458.6)	8.4466	79.7
	Cypercal	2714.2 (2233.8-3242.9)	8188.1 (7142.9- 9650.9)	3.5684	81.9
	Dipel	126.9 (97.8-158.6)	466.0 (394.7- 575.0)	66.128	2.3
Mampong	Pawa	1759.1 (1544.5 - 2022.0)	4802.7 (4204.0 - 5648.9)	3.779	57.9
	Dursban	85496.6(71545.6- 101774.6)	342873.2 (297265.1- 407180.6)	0.0706	114.3
	Deltaplan	497.00 (431.0 -564.4)	1543.4 (1361.4 -1807.2)	22.492	41.2
	Cypercal	2274.9 (1905.3 -2726.3)	5462.9 (4685.6 -6616.3)	3.1493	54.6
	Dipel	247.6 (200.8- 307.5)	892.7 (741.3 -1137.3)	37.926	4.3

*[a] - 95% CI is 95% confidence interval in parenthesis

*[b]- Fold tolerance refers to LC_{95} as a proportion of recommended dosage (expected to give 95% mortality)

* Active ingredients for Pawa [Lambda-cyhalothrin], Cypercal [Cypermethrin], Deltaplan [Deltamethrin] Dursban [Chlorpyrifos-methyl] and Dipel [*Bacillus thuringiensis* var. *kurstaki*].

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*The insects were reared on insecticide free cabbage.

The slopes of the curves indicated the potency of the insecticides. Mortality curves with high slope values were more potent in controlling DBM, since low insecticide concentrations gave high larval mortality for example dipel (*B.t.*). The most potent of the pyrethroids was deltaplan and this was shown with relatively higher slope values compared to the other pyrethroids. While for dursban the least potent of the insecticides recorded the lowest slope values meaning higher concentrations only resulted in low larval mortality

4.3. Cues for Cross and multiple-resistance

The DBM larvae were shown to indicate development of cross-resistance to the commonly used pyrethroids and multiple resistance to the pyrethroids and organophosphates. These phenomena were tested across some selected sites. The DBM population that tolerated Pawa could be fairly controlled by dursban in Madina, where 42 (52%) mortality was recorded. Similar populations were tolerant to dursban in Mampong and Airport where only 21 (26%) and 24 (30%) out of 80 mortalities were recorded for both sites respectively. The DBM population tolerant to pawa when exposed to deltaplan at Airport recorded only 21 (30%) mortality. However, Deltaplan tolerant DBM population at the same site gave mortality of 8 (10%) for dursban. Conversely, when selection was done using Dursban and the population exposed to Pawa, mortalities ranging between 12 to32 (15-40%) were recorded in all the study sites. There was 90% mortality recorded in the DBM population selected for resistance by all the synthetic conventional insecticides bioassayed when exposed to *B.t.*

4.4 Carboxylesterase Activity in Field populations of Diamondback Moth

For each of the seven sub-study sites, 25 samples of DBM larvae were assayed for the two enzymes α- and β-carboxylesterases, giving a total of 350 larvae samples. The study sites were Mampong-Akuapem, Madina A (near Redco Junction), Madina B (Redco Area), Airport A (Airport West), Airport B (Opaibea Area near CSIR), Dzorwulu A (Around Power Station) and Dzorwulu B (Dzorwulu East near Kotobabi).

4.4.1 α-naphthyl esterase

The highest level of activity for α-naphthyl esterase was recorded in Mampong (Table 9). The Mampong DBM population was significantly different from all the other sites ($P < 05$). However the lowest activity was recorded in Madina B, which was significantly different from Madina A and Dzorwulu A. The enzyme activity in Madina A and Dzorwulu A were however not significantly different.

4.4.2 β-naphthyl esterase

B-naphthyl esterase activity for Mampong DBM population was also very high while very low activities were recorded for Accra sub-sites. Comparatively, higher activities were recorded in Madina and Airport A. However, statistical analysis proved that the activity of the enzyme was only significantly different between Mampong population and the rest of the sites, with the other six sites in Accra suburbs showing no significant difference in activity.

Analysis of variance showed that α- and β- naphthyl esterase activities were highly significant at both (0.05 and 0.01) probabilities (Appendix IV).

The frequency profiles of activity for the two enzymes for the various sites were shown to vary greatly (Appendix IV). In Mampong the modal frequency for α-naphthyl esterase activity was 10μmol/min/mg protein and β-naphthyl esterase 50mol/min/mg protein. While for the Accra surburb sites modal frequencies for both esterases varied greatly even within the same population. Specifically, for Dzorwulu DBM population α-naphthyl esterase activity was mostly recorded at 4μmol/min/mg in site A and 1 μmol/min/mg protein in site B. For β-naphthyl esterase the modal frequency was 5 and -2 μmol/min/mg protein in sites A and B respectively. While Airport populations had most frequencies of activity between 1 and 2 μmol/min/m activity for α- naphthyl esterase and β-naphthyl esterase had most frequencies at 15 and as low as –4 to 0μmol/min/mg protein activity in Airport A and B respectively. Likewise Madina sites had the most frequencies for α-naphthyl esterase at 4 and 1 μmol/min/mg protein activity recorded in site A and B respectively and for β-naphthyl esterase the modal frequencies were –5 to 0 and 5 μmol/min/mg activity for DBM populations in Madina A and B respectively (Appendix IV).

The study revealed that carboxylesterase had higher affinity for β-naphthyl acetate than for α-naphthyl acetate. This was indicated by the mean values of the two enzymes. In contrast, for DBM population in Accra surburbs, α-naphthyl acetate proved to be a better substrate for reaction with a mean of 1.77 μmol/min/mg protein and a range of 2.589-0.915 μmol/min/mg protein, while β-naphthyl acetate recorded a mean of 0.72 μmol/min/mg with arrange of 2.925 and -0.798 μmol/min/mg protein.

Mampong DBM population showed the widest disparity in activity for both enzymes but the Accra DBM populations for example recorded no significant difference for β-naphthyl esterase. This implied that though all the insects quantitatively showed some

carboxylesterase activity however there might be qualitative differences within insects even from the same population in one study site.

Concerning the insecticides used in the cabbage farms, lambda-cyhalothrin formulations (karate and pawa) were found in all the study sites except in Madina A and Mampong (Table 9). The bioassay showed DBM population in Mampong to be comparatively the most susceptible to Pawa (Table 8). *Bacillus thuringiensis* formulations (biobit and dipel) and dursban was used in all the farms except Madina B and Dzorwulu A. However, comparing with the susceptibility studies, Dzorwulu DBM samples were the least susceptible to dursban at the same time the most susceptible to dipel while the Madina populations were the most susceptible to dursban. Comparing these results to carboxylesterase activity, Madina B showed the least activity of α-naphthyl esterases followed by Dzorwulu A. However, the Madina populations comparatively exhibited relatively higher activity among the Accra surburb DBM samples for β–naphthyl esterases. While cypermethrin formulations were used in all the other sites except in Dzorwulu and Airport B. Contrastingly, DBM population in Airport was the most resistant to cypermethrin. In addition deltamethrin, which was used in most of the farms except Dzorwulu and Mampong, was least effective in DBM population in Dzorwulu.

Table 9. Carboxylesterase activity of DBM populations and insecticide used in cabbage farms in Mampong Akuapem and Accra suburbs

Population/ Site	**Insecticides used**	**Carboxylesterases activity Mean (± S.E) (µmol/min/mg protein**	
		α-NA	β-NA
Mampong-Akuapem	Dipel[1], dursban[2], cypermethrin[4], orthine[4],	9.55±1.25[a]	49.3±10.46 [a]
Madina A	Dize-DDVP[2], dursban[2], Decis[4], orthine[4], polytrine[5], biobit[1], actelic[2],	2.18±0.4[b]	1.14±1.03 [b]
Madina B	Dize-DDVP[2], decis[4], cydimsuper[5], karate[4], actelic[2], polytrine[5], thionex[2]	0.92±0.14[c]	2.92±0.95 [b]
Airport A	Amektin[3], dursban[2], dize-ddvp[2], deltapaz[4], actelic[5],biobit[1], dipel[1], karate[4],	1.05±0.25[bc]	1.26±0.8 [b]
Airport B	Dize-DDVP[2], pawa[4], polytrine[5], karate[4], dipel[1], deltaplan[4], cydimsuper[5], biobit, dursban[2]	1.55±0.39[bc]	0.25±0.65 [b]
Dzorwulu A	karate[4]	2.59±0.25[b]	0.45±0.8 [b]
Dzorwulu B	Amektin[3], karate[4], dursban[2], dipel[1], biobit[1], neem extract[1]	1.49±0.23[bc]	-0.8±0.51 [b]

*Regent[3] and rimon[3] were common to all the study sites

*The same letter within a column denotes no significance difference at 95% confidence level (0.05) probability level

*1 Biopesticides

*2 Organophosphates

*3 Growth regulators

*4 Pyrethroids

*5 Insecticide mixtures

4.5 Native Polyacrylamide Gel Electrophoresis (PAGE)

The results obtained from 120 DBM larvae collected from the four study sites revealed various distinct banding patterns in the field populations of DBM. The variants were classified based on the number of bands, staining intensity, and electrophoretic mobility. Between two to seven bands were detected in the zymograms. These bands were scattered throughout two zones of the gel: a fast moving upper zone and a slightly slower moving middle zone (Fig.15). All the isozymes migrated to the cathode end of the gel. The fast moving heavily stained zone, composed of one narrow and one wide band, was detected in all the zymograms examined. The middle zone-bands however varied in number, width, and staining intensity, with some faint bands in front of the main body of the middle zone, being present in most of the zymograms.

Diamondback moth esterases were shown to be highly heterogeneous in nature. This was shown through polymorphism of isozyme patterns and general variations. Thus more than one zymogram pattern was observed for DBM larvae even within the same population (Fig. 15). The electrophoregram revealed higher frequency of slow moving esterases, which confer more resistance in Mampong samples (Fig. 16) than for the Accra suburb sites which had majority of the zymograms with the fast moving esterases which are associated with less resistance. Besides, the zymogram patterns for the Accra populations were relatively similar, compared with the Mampong population, which reflected a wide range of zymogam patterns. Among the Accra populations Madina samples had relatively higher frequency of the slow moving esterases and the site with the least frequency of this type of esterase was Dzorwulu.

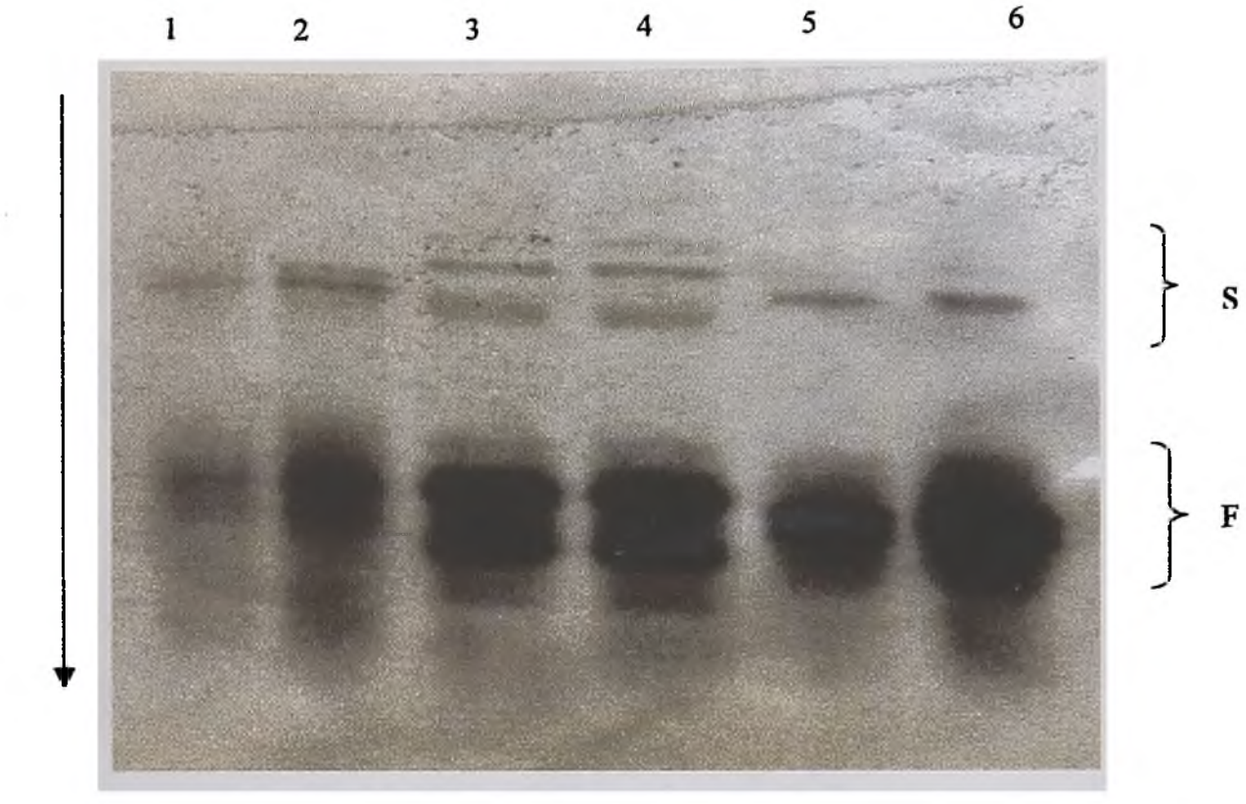

Fig. 15 Polyacrylamide gel electrophoresis of esterase isozymes of DBM larvae showing the difference between fast- moving (heavily stained) and slow moving (lightly stained) esterase isozymes. And the various zymogram patterns of the esterase isozymes within the same DBM population (Mampong).

Lanes 1-6 = carboxylesterase isozymes, S = Slow moving esterases, F = Fast- moving esterases, Arrow = direction of gel movement

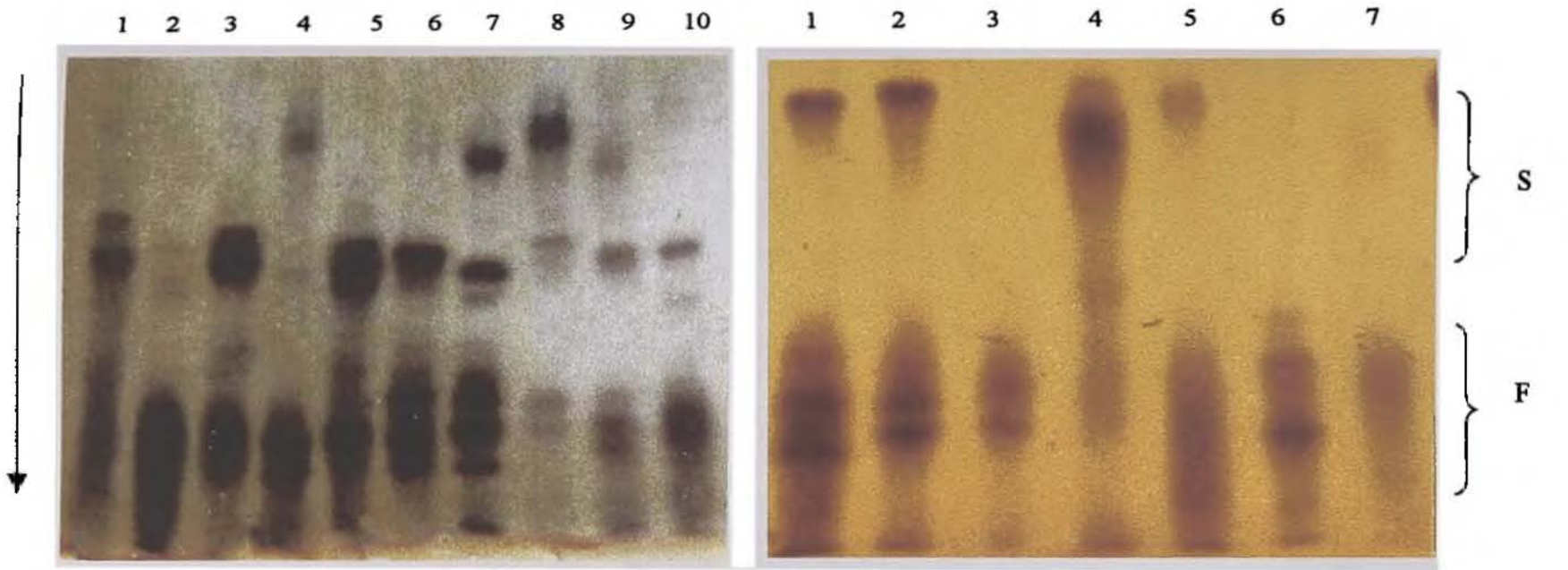

Fig. 16 Polyacrilamide gel electrophoresis of DBM larvae showing higher frequency of slow moving esterases in Mampong (gel A), than in a typical Accra-surbub site (Airport) (gel B).
Lanes 1-10 =carboxylesterase isozymes, S = Slow moving esterases, F = Fast- moving esterases, Arrow = direction of gel movement.

4.6 Molecular Identification of *B.t.* resistance gene

Bender buffer protocol was successfully used to extract DNA from a total of 140 samples of DBM. The genomic DNA was found to run together with 23.13kb in ethidium bromide stained 0.8% agarose gel electrophoresis (Fig.17). PCR amplification was successful for 65 out of the total 140 (46%) that were processed. The 65 consisted of 24 samples from Madina, 14 from Dzorwulu, 17 DBM samples from Mampong and 10 samples were from Airport. In addition to this, 14 laboratory reared reference/ susceptible DBM strains from ICIPE, Kenya were also analyzed for *B.t.* resistance gene.

Those that were PCR positive 41 out of 65 (61%) were characterized by the expected 325 bp amplified DNA product (Fig. 18). This 325bp band was shown by all the samples collected from Airport. In addition to this Mampong, Dzorwulu and Madina DBM populations had 26 out of 65 (40%) showing 760bp fragment (Fig. 19). Some samples from Madina and Mampong showed an extra 1100 bp band size. Most notably, were double bands of sizes 325 bp and 776 bp sizes amplified in four samples from Madina and a triple band of 325 bp, 776 bp and 1100 bp shown by two samples from Madina and Mampong. The susceptible strains also possessed a band size similar to the diagnostic 325bp DNA fragment. Since the size difference between the PCR products of the resistant and the susceptible gene differ by only 20bp, this cannot be easily resolved by agarose gel, but PAGE hence the latter was used.

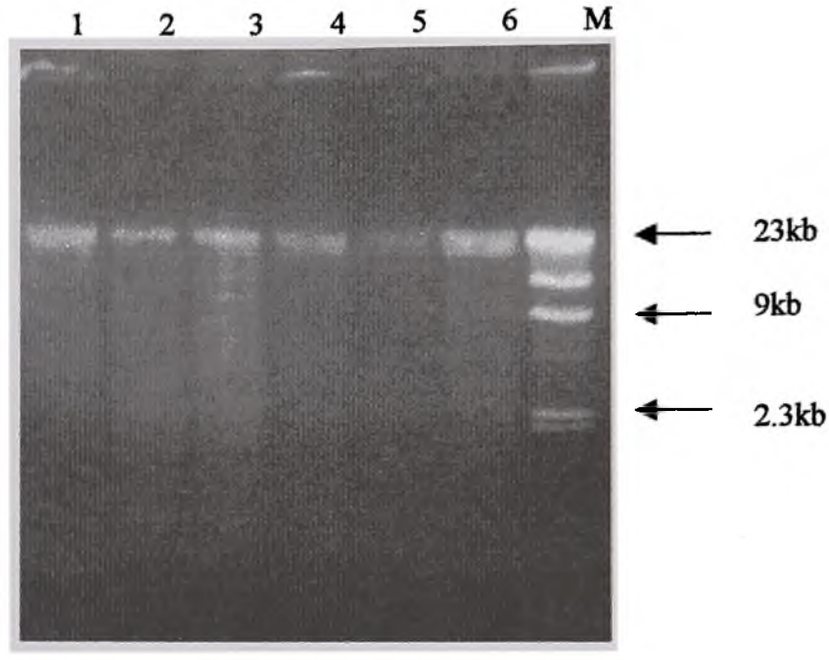

Fig. 17 Ethidium bromide stained 0.8% agrose gel electrophoresis of genomic DNA isolated from the DBM larvae Lanes 1-6 and molecular weight marker (Lane M). Population

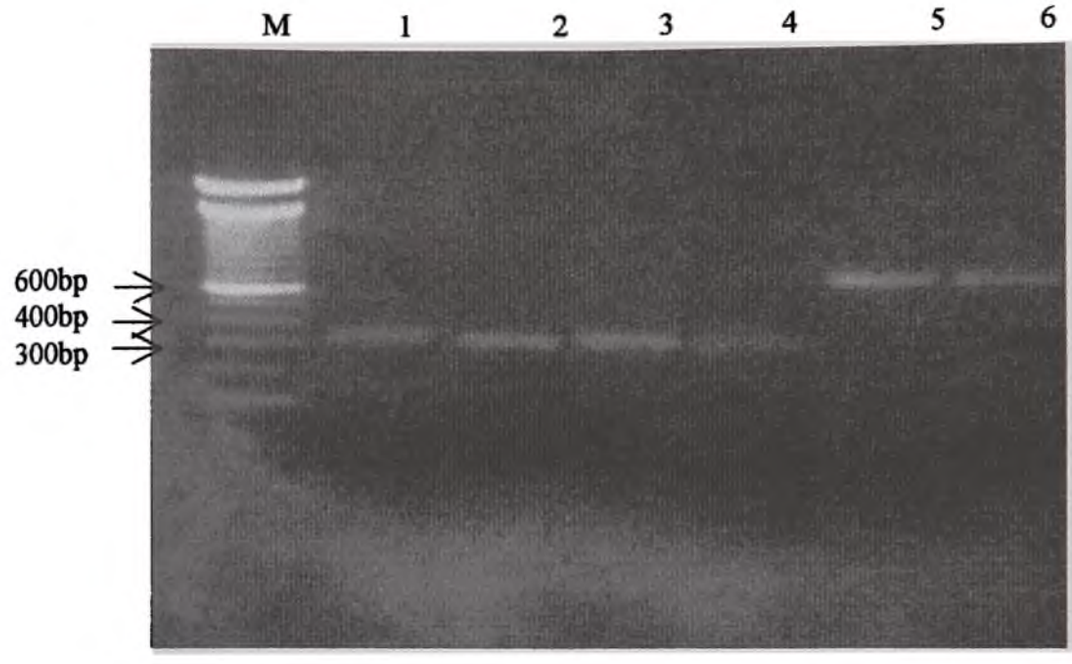

Fig. 18 Ethidium bromide stained 2% agarose gel elecrophoresis of PCR products obtained after amplification of DBM genomic DNA with specific primers (c39-451p1 and c39-451p2) for the *B.t.* resistant gene.

Lane M=molecular weight marker, Lane 1- 4 = 325 bp (expected gene band), Lanes 5 and 6 = 776 band size

4.7 Polyacrylamide gel electrophoresis of PCR Products

Polyacrylamide gel electrophoresis of PCR products of DBM larvae revealed presence of double and sometimes triple bands among the resistant populations, which were observed in all the study sites. The DNA fragments amplified were 325 bp, 776 bp and 1100 bp band sizes (Fig.19). Seven out 65 PCR products showed 325 bp and 1100 bp single bands from DBM samples from Airport and Mampong respectively. While 26 samples from Madina exhibited the 776 bp fragment. The double bands of sizes 325 bp and 776 bp were amplified in 13 DBM samples from Mampong, Madina and Dzorwulu. Nine out of 65 samples showed the triple bands of sizes 325 bp, 776 bp and 1100 bp. PAGE results also showed that all the susceptible strains of DBM produced single bands which were slightly above 20 bp the expected 325 bp bands size of the resistance strains, however the resistant strains had multiple bands (Fig. 20).

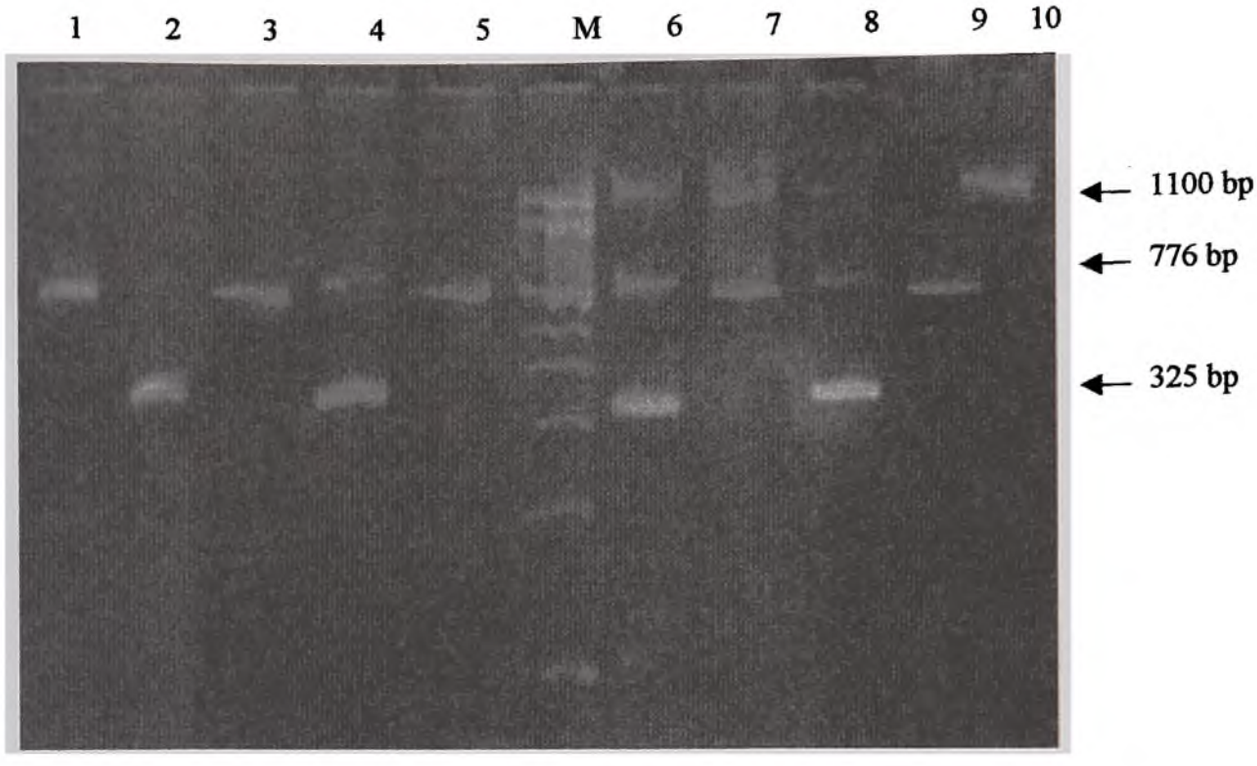

Fig. 19 Polyacrylamide gel electrophoresis of PCR products obtained from amplification of DBM DNA with specific primers (c39-451p1 and c39-451p2).

Lane 2 = single band 325 bp, Lanes 1, 3, 5 and 9 = Single band 776bp, Lane 10 = Single band 1100 bp, Lanes 4 and 7 = Double band sizes 325 bp and 776 bp), Lanes 6 and 8 = Triple band sizes 325 bp, 776 bp and 1100 bp, Lane M = 100 bp molecular weight marker.

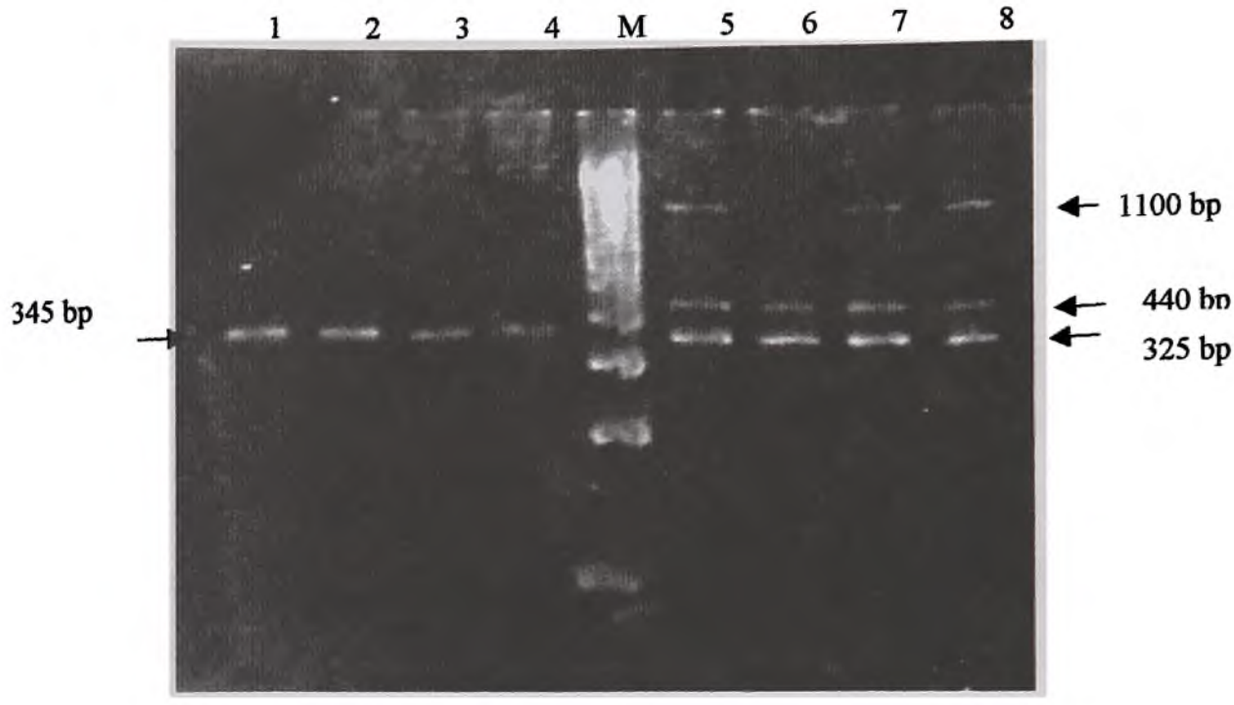

Fig. 20 Polyacrylamide gel electrophoresis of PCR products obtained from amplification of DBM DNA with specific primers (c39-451p1 and c39-451p2) showing the difference between *B.t.* susceptible and resistant DBM larvae.

Lanes 1-4 =susceptible strains, M = 100 bp ladder and Lanes 5-8 resistance strains of DBM.

4.8 Residue Level estimation

4.8.1 Brine shrimp bioassay for the insecticide SPE standards fractions

Mortality data recorded following bioassay of SPE fractions obtained by elution of the standard insecticides (permethrin, cypermethrin, deltamethrin, lambda-cyhalothrin, chlorpyrifos-methyl and pirimiphos-methyl) using methanol, ethyl acetate and hexane are shown in (Fig. 21). It was observed that pyrethroids were mostly eluted in hexane fractions which gave nearly 100% mortality, except for lambda-cyhalothrin which gave only 60% mortality. On the other hand, chlorpyrifos was eluted in methanol giving 100% brine shrimp mortality, while pirimiphos-methyl was eluted in ethyl- acetate which also gave 100% brine shrimp mortality. Thus, cypermethrin gave no mortality in ethyl acetate fraction, 10% and 100% of the brine shrimps died in methanol and hexane respectively. Furthermore, deltamethrin and permethrin gave about 40% mortality for ethyl acetate fractions but 100% mortality was recorded for hexane fractions. Chlorpyrifos only gave 100% mortality in methanol fraction while pirimiphos also gave 100% mortality in ethyl acetate, with no mortality being observed in the other fractions. Dose-response mortality curves of the insecticide standards were obtained from probit analysis and their LC_{50} and LC_{95} values and the slopes of the curves were recorded as shown in Table 10. The potency of pyrethroids based on LC_{50} was as follows: cypermethrin > permethrin>deltamethrin> lambda-cyhalothrin (Table 10). For organophosphates, chlorpyrifos was three times as potent as pirimiphos-methyl.

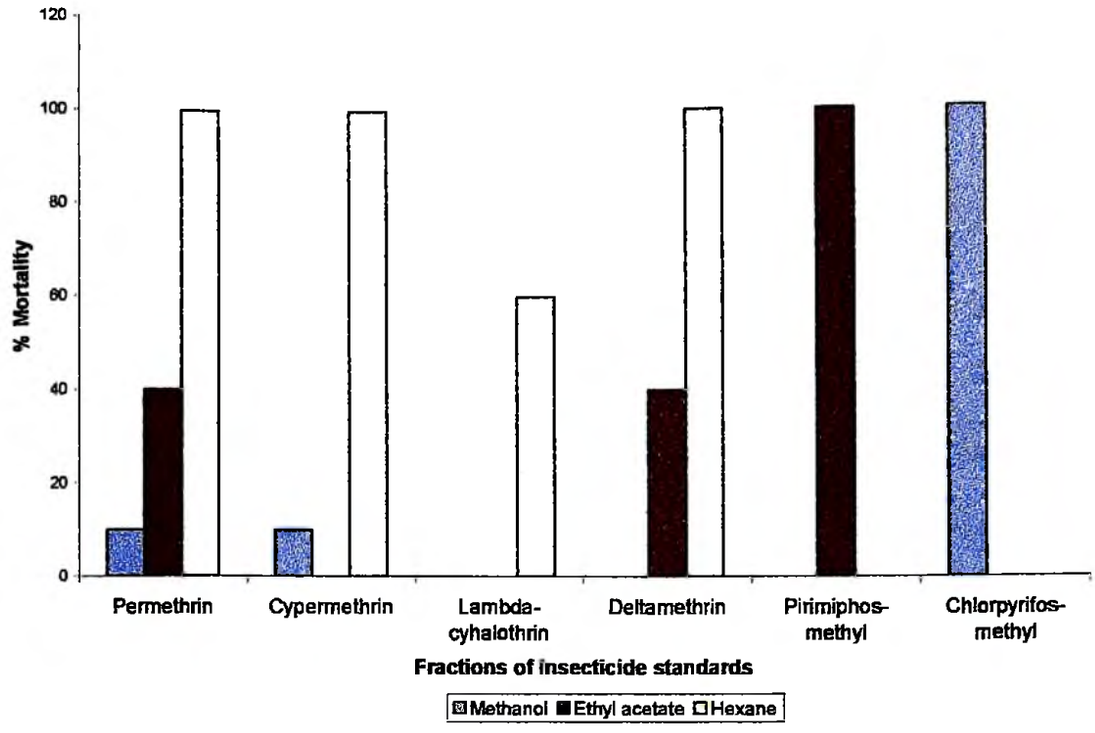

Fig. 21 Brine shrimp mortality profile of SPE fractions of insecticide standards

Table 10. Lethal concentrations of insecticide standards

Insecticides	LC_{50} (mg litre^{-1}) (95% CI)*[a]	LC_{95} (mg litre^{-1}) (95% CI)	Slope
Deltamethrin	374.0(286.9-524.7)	970.4(749.1-1411.9)	0.032
Cypermetherin	179.7(108.1-293.1)	634.0 (459.4-1053.5)	0.019
Pirimiphos-mehyl	684.1(483.9-1269.0)	1420.8(982.3- 2805.8)	0.015
Lambda-cyhalothrin	561.3(422.9- 64.5)	1413.6(116.8- 1960.3)	0.019
Chlorpyrifos-methyl	222.0(177.1- 284.7)	489.2(402.0-628.1)	0.067
Permethrin	234.7(171.3- 327.9)	766.3(596.9-1089.8)	0.037

*[a] CI- is the confidence interval

4.8.2 Estimation of residue levels in cabbage samples

The differential elution of the two organophosphate standards and pyrethroids from SPE (Fig. 21) with methanol, ethyl acetate and hexane were used as basis for fractionation of these insecticides from extracts of the residues obtained from cabbage. To estimate insecticide residue content in the different fractions, regression equations for standard insecticide concentration against brine shrimp probit mortality calibration curves were used (Appendix IV). Calibration curve of chlorpyrifos-methyl was used for methanol fractions, while pirimiphos-methyl and cypermethrin were used for ethyl acetate fraction and hexane fractions respectively.

4.8.3 Brine shrimp mortality profile for solid phase extracted (SPE) fractions from cabbage samples

A total of 60 brine shrimp nauplii were used for the bioassay of each of the fractions eluted from SPE. Fractions obtained for each of the 7 sub-sites were bioassayed for activity.

Fig. (22) Shows biological activity recorded for fractions obtained from all samples using the three selected solvents in the order: methanol, ethyl acetate and hexane.

Much higher mortality was generally recorded for methanol fractions compared with the hexane and ethyl acetate fractions. Between 42-93% mortality was recorded for methanol fractions. The ethyl acetate and hexane fractions gave relatively higher mortality in the Dzorwulu samples than for Airport and Madina. Level of mortality in ethyl acetate and hexane were lower for Mampong samples. Mortalities in Airport A samples were relatively low.

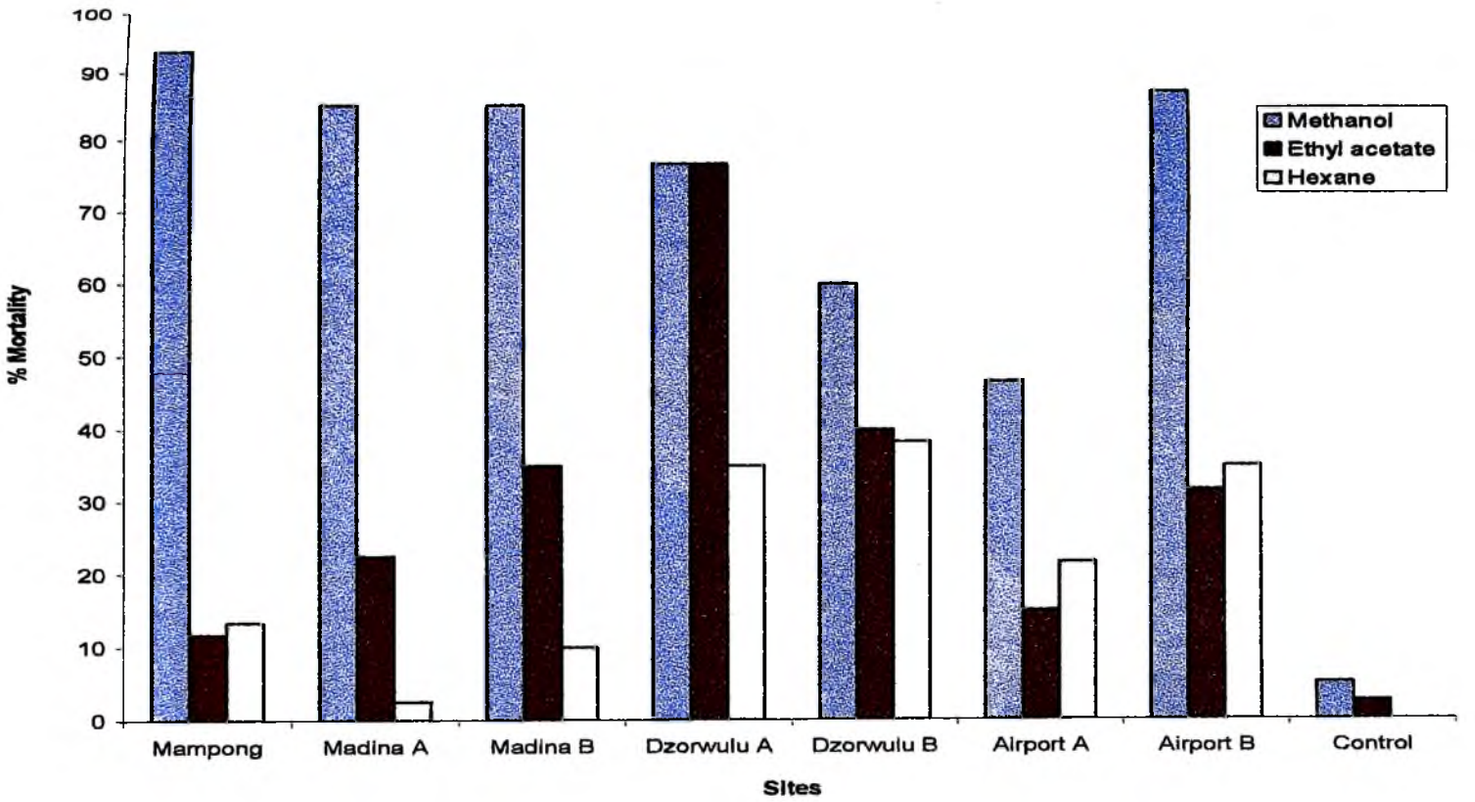

Fig 22. Brine shrimp mortality profile of solid phase extracted fractions from cabbage samples

* Control – insecticide free cabbage grown under screen house environment.

N/B A total of 60 Brine shrimp nauplii were used for each fraction of the sample extract while a total of 40 brine shrimps were used for each of the control fractions

4.8.4 Estimation of Maximum Residue Levels (MRL) of insecticides in the cleaned cabbage extracts.

The results of the MRL values of the cleaned cabbage extracts are shown in Table 11. Chlorpyrifos was eluted in methanol fraction, pirimiphos-methyl and pyrethroids in ethyl acetate and hexane respectively. The results showed very high values for chlorpyrifos-methyl in all the study sites. The highest value however was recorded in Mampong (124.1 mg/kg), likewise Airport B and the Madina samples recorded 114 mg/kg and 111.5 mg/kg respectively with the least MRL value being given by Airport A (55mg/kg). However, Least Significant Difference (LSD) at 95% confidence level showed no significant difference in MRL means for the study sites (Appendix VI).

For pirimiphos-methyl, cabbage samples from Dzorwulu sites though not significantly different recorded the highest values (14.6 mg/kg and 10.9 mg/kg). But MRL value for Dzorwulu A was significantly different from all the sites. Moderate MRL values were recorded for the Madina and Airport cabbage samples and the least value was recorded in Mampong.

Estimation of brine shrimp lethal activity of hexane fraction eluents was difficult due to the low mortalities recorded hence the pyrethroid concentration in Mampong, Madina A and B, Airport A and the control cabbage samples could not be determined from the calibration curve. The concentrations of pyrethroids determined in the three sites Dzorwulu B and A, Airport B were between 1.2 and 1.8 mg/kg respectively. The three were however not significantly different at 0.05 probability.

Cabbage samples from Dzrowulu were found to have high concentrations of both pyrethroids and pirimiphos-methyl, while Airport samples had comparatively high residue of all the three insecticides. Mampong cabbage samples recorded the highest concentration of chlorpyrifos but the least in pirimiphos-methyl and the pyrethroids levels in the samples were too low to be detected therefore could not be determined.

Table 11. Fractionated Insecticides from cabbage samples and estimated insecticide residue content

Site/Sample	Insecticide fractions	Total cleaned residue (g)	% Mortality	Total Active residues (mg) × 10^{-3}	% Active residues	Residual activity (kg)	MRL (mg/kg) of cabbage
Mampong	Chlorpyrifos	31.8	93.3	488.5	0.020	124144.1	124.1
	Pirimiphos	2.7	11.7	95.7	0.004	2086.3	2.1
	Pyrethroids	1.7	13.3	ND*	ND*	ND*	ND*
Madina A	Chlorpyrifos	31.6	85	441	0.018	111524.5	111.5
	Pirimiphos	4.4	22.5	180.3	0.007	6400.7	6.4
	Pyrethroids	2.7	2.5	ND*	ND*	ND*	ND*
Madina B	Chlorpyrifos	31.6	85	441	0.018	111524.5	111.5
	Pirimiphos	2.6	35	277.9	0.011	5835.9	5.8
	Pyrethroids	5.8	10	ND*	ND*	ND*	ND*
Dzrowulu A	Chlorpyrifos	31.5	76.7	393.5	0.016	99022.7	99.0
	Pirimiphos	3.0	76.7	603.3	0.024	14640.1	14.6
	Pyrethroids	2.1	35	74.1	0.003	1266.1	1.3
Dzrowulu B	Chlorpyrifos	31.1	60	298.5	0.012	74373.7	74.4
	Pirimiphos	4.3	40	316.9	0.013	10922.5	10.9
	Pyrethroids	2.2	38.3	101.4	0.004	1804.9	1.8
Airport A	Chlorpyrifos	30.9	46.7	222.5	0.009	54994.9	55.0
	Pirimiphos	3.4	15	121.7	0.005	3334.6	3.3
	Pyrethroids	1.4	21.7	ND*	ND*	ND*	ND*
Airport B	Chlorpyrifos	31.6	86.7	450.5	0.018	114038.7	114.0
	Pirimiphos	3.4	31.7	251.9	0.010	6818.1	6.8
	Pyrethroids	2.1	35	74.1	0.003	1235	1.2
Control*[c]	Chlorpyrifos	30.0	5	ND*	ND*	ND*	ND*
	Pirimiphos	1.6	2.5	ND*	ND*	ND*	ND*
	Pyrethroids	1.2	0	ND*	ND*	ND*	ND*

*c Control- Insecticide free cabbage samples grown in the screen house.
*a Active residue- the estimated concentration (µg/ml) of toxic residues per solvent using the calibration curves of the insecticide standards
*b % active residues- This is the fraction of the active residue in relation to the total residues extracted from the cabbage heads.
N/B Active residues for mortalities below 26% of pyrethroid fractions could not be estimated from the calibration curves of the insecticide standards
ND* (Not detected) Are fractions that could not be estimated (detected) from the calibration curve

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	Pyrethroids	2.7	2.5	ND*	ND*	ND*	ND*
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	Pyrethroids	1.2	0	ND*	ND*	ND*	ND*

*c Control- Insecticide free cabbage samples grown in the screen house.
*a Active residue- the estimated concentration (µg/ml) of toxic residues per solvent using the calibration curves of the insecticide standards
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	Pyrethroids	1.4	21.7	ND*	ND*	ND*	ND*
Airport B	Chlorpyrifos	31.6	86.7	450.5	0.018	114038.7	114.0
	Pirimiphos	3.4	31.7	251.9	0.010	6818.1	6.8
	Pyrethroids	2.1	35	74.1	0.003	1235	1.2
Control*[c]	Chlorpyrifos	30.0	5	ND*	ND*	ND*	ND*
	Pirimiphos	1.6	2.5	ND*	ND*	ND*	ND*
	Pyrethroids	1.2	0	ND*	ND*	ND*	ND*

*c Control- Insecticide free cabbage samples grown in the screen house.
*a Active residue- the estimated concentration (µg/ml) of toxic residues per solvent using the calibration curves of the insecticide standards
*b % active residues- This is the fraction of the active residue in relation to the total residues extracted from the cabbage heads.
N/B Active residues for mortalities below 26% of pyrethroid fractions could not be estimated from the calibration curves of the insecticide standards
ND* (Not detected) Are fractions that could not be estimated (detected) from the calibration curve

Table 11. Fractionated Insecticides from cabbage samples and estimated insecticide residue content

Site/Sample	Insecticide fractions	Total cleaned residue (g)	% Mortality	Total Active residues (mg) × 10^{-3}	% Active residues	Residual activity (kg)	MRL (mg/kg) of cabbage
Mampong	Chlorpyrifos	31.8	93.3	488.5	0.020	124144.1	124.1
	Pirimiphos	2.7	11.7	95.7	0.004	2086.3	2.1
	Pyrethroids	1.7	13.3	ND*	ND*	ND*	ND*
Madina A	Chlorpyrifos	31.6	85	441	0.018	111524.5	111.5
	Pirimiphos	4.4	22.5	180.3	0.007	6400.7	6.4
	Pyrethroids	2.7	2.5	ND*	ND*	ND*	ND*
Madina B	Chlorpyrifos	31.6	85	441	0.018	111524.5	111.5
	Pirimiphos	2.6	35	277.9	0.011	5835.9	5.8
	Pyrethroids	5.8	10	ND*	ND*	ND*	ND*
Dzrowulu A	Chlorpyrifos	31.5	76.7	393.5	0.016	99022.7	99.0
	Pirimiphos	3.0	76.7	603.3	0.024	14640.1	14.6
	Pyrethroids	2.1	35	74.1	0.003	1266.1	1.3
Dzrowulu B	Chlorpyrifos	31.1	60	298.5	0.012	74373.7	74.4
	Pirimiphos	4.3	40	316.9	0.013	10922.5	10.9
	Pyrethroids	2.2	38.3	101.4	0.004	1804.9	1.8
Airport A	Chlorpyrifos	30.9	46.7	222.5	0.009	54994.9	55.0
	Pirimiphos	3.4	15	121.7	0.005	3334.6	3.3
	Pyrethroids	1.4	21.7	ND*	ND*	ND*	ND*
Airport B	Chlorpyrifos	31.6	86.7	450.5	0.018	114038.7	114.0
	Pirimiphos	3.4	31.7	251.9	0.010	6818.1	6.8
	Pyrethroids	2.1	35	74.1	0.003	1235	1.2
Control*[c]	Chlorpyrifos	30.0	5	ND*	ND*	ND*	ND*
	Pirimiphos	1.6	2.5	ND*	ND*	ND*	ND*
	Pyrethroids	1.2	0	ND*	ND*	ND*	ND*

*c Control- Insecticide free cabbage samples grown in the screen house.
*a Active residue- the estimated concentration (µg/ml) of toxic residues per solvent using the calibration curves of the insecticide standards
*b % active residues- This is the fraction of the active residue in relation to the total residues extracted from the cabbage heads.
N/B Active residues for mortalities below 26% of pyrethroid fractions could not be estimated from the calibration curves of the insecticide standards
ND* (Not detected) Are fractions that could not be estimated (detected) from the calibration curve

CHAPTER FIVE

DISCUSSION

The questionnaire survey showed that insect pests were a major constraint to cabbage production in the selected sites. DBM was considered a key pest of cabbage in Ghana causing damage in 73% of the farms surveyed. DBM has been reported as a key pest of cabbage and other crucifers, in many other parts of the world (Miyata *et al.,* 1986; Cartwright *et al.,* 1987; Hill and Waller, 1994). The increasing pest infestation could be attributed to improper agronomic practices in the farms as was revealed by the present study. Most farmers in Accra suburbs applied water to the entire crop during irrigation, this washed off the insecticides applied especially those with contact action such as the pyrethroids and organophosphates, which are the most commonly, used insecticides against DBM. The duration between insecticide spraying and irrigation therefore was vital depending on the method used. Most farmers in Mampong justified watering the same day with spraying with insecticides since their bucket and cup method of watering was done underneath, hence did not wash off the chemicals to the extent done by the method used by their counterparts in Accra-suburbs.

Monoculture enhanced pest infestation on cabbage by lepidopterous insects and aphids since it creates conducive environment for particular insect pest to flourish (Kumar, 1986). Creating a host free period was difficult due to lack of coordination of activities amongst the growers with the result that growers cultivate cabbage at different times of the year. Consequently, growers were unable to synchronise time for insecticide spraying schedules, resulting in unsprayed cabbage plants serving as refuge at a time when some farmers would have sprayed their crops.

Since Brassicas (cabbage, cauliflower and chinese cabbage) have similar pest spectrum (Hill and Waller, 1994), all year round cultivation of these varieties of Brassica in

adjacent plots to cabbage bridged the gap in host plant sequence of DBM (Miyata *et al.*, 1986) resulting in upsurges in its infestation (Way, 1976). In addition, DBM can survive on host residues throughout the off seasons. This was a major source of re-infestation in the farms since most farmers either left the residue on the farms or threw them by the side of the farms. These practices made food available for the pest, and created a favourable condition for their survival, development and multiplication even during off seasons, leading to increased pest infestation and damage.

On pest control, growers relied exclusively on the use of insecticides since they believed they gave faster results once applied and most of them used the insecticides as prophylactic measure. The high rate and dosages of application lead to development of resistance in the pest. Consequently, the survey showed a decline in the use of pyrethroids and the biopesticide *B. thuringiensis* (Fig.9), which were the most widely used against pests of cabbage (Ninsin, 1997). This was attributed to their ineffectiveness against DBM. Thompson (2004) has proposed a perceived treadmill that clearly illustrates the circle between resistance development and insecticide spraying. The result of the present study further supports Thompson's assertion. This is because the use of organophosphates had increased, with a worse consequence due to their persistence in the environment, and this could enhance development of resistance due to prolonged exposure to the pest and also cause health hazards due to insecticide residue accumulation in edible materials.

Cabbage farmers were using pesticides labelled in French (cyperphos, Polytrine, D336 EC and Thionex). These could be a major source of hazards to consumers since the relevant information on the products label concerning the safe and effective use of these insecticides were written in French. Furthermore, these products were not registered

contrary to the pesticide Control and Management Act, 528 of Ghana. Davis (1997) reported that about one third of chemical products on the shelves in a shop in Kumasi were labelled in French. In addition, products that were recommended for use in cotton, coffee and cocoa were sold for use on all crops.

Farmers also reported the use of insecticide mixtures. This practice defied the principles of insecticide management. Metcalf (1980) recommended that the use of such mixtures should be avoided since it results in the simultaneous development of pest resistance because each compound seems to develop the residual inheritance of the supporting genome for resistance in the other (Metcalf, 1980. This gives rise to control failure for such a notorious pest as DBM, which is capable of quickly developing resistance to all insecticides, exposed to it (Miyata *et al.*, 1986). Growers were observed to use sub-lethal-doses of the insecticides and this also increases the development of resistance in the pest.

With regard to personal protection against contamination, it was found out that the application of insecticides by the cabbage growers was not carried out properly. Only four (13.33%) wore protective clothes during spraying, claiming that the equipment were expensive and uncomfortable. Thus these growers were predisposed to the risk of insecticide poisoning, "the New Developing World Disease" (Anonymous, 1989). Most of their knapsack sprayers often leaked and it was common for the sprayers to have skin rashes, headaches, and dizziness for a few days after insecticide application (Davis, 1997).

Farmers observed short Pre-harvest intervals. Indeed during heavy infestation, farmers sprayed and sold their produces the same day (Fig. 12). This exposed consumers to great

health risk since the cabbages were sold directly to consumers without passing through a marketing quality control board. Farmers also turned to pesticide sellers and fellow farmers for professional advice concerning identification of new pests and their control. The former recommended their new products that needed field trials for the new pest while the latter recommended the insecticides they had tried on their farms and were successful, although their practices in terms of rate of application and safety measures were not up to standard.

Although the increase in the use of OPs and the high residue levels of chlorpyrifos was a great health hazard since farmers were revealed to observe Pre-harvest interval as short as one day, yet OPs, require 21 days (PHI). The use of biopesticide should therefore be encouraged towards maturation of cabbage since its PHI is zero days (Shelton and Zhao, 2004) and it has less health hazards. More extension should be encouraged in conjunction with the Farmers Field Schools, which were reported to have reduced indiscriminate use of insecticides (Kaiwa, 2000).

Concerning pesticide residue awareness 19 out of 30 (63%) of the respondents were ignorant about perceived risks caused by improper use of insecticides. They attributed pesticide-poisoning incidences e.g. (Tarkwa and Sunyani) to lack of proper training on insecticide use since the farmers were reported to have used insecticides meant for cocoa on cabbage farms.

In order to ascertain the effect of the indiscriminate use of insecticide on DBM, a susceptibility study was carried out. The study showed that DBM was highly resistant to all the synthetic insecticides assayed. However, resistance to *B.t.* was very low (Table 8). Shelton and Zhao (2004) reported that resistance to Cry1A toxins of *B.t.* in DBM tends to be unstable, and one generation without selection can make the population susceptible

but resistance can quickly return when populations are challenged. Tabashnik *et al.* (1991) also reported that *B.t.* resistance in DBM declines in absence of selection. Hama (1992) also documented that DBM resistance to *B.t.* decreased within few generations. Conversely, McGaughey & Beeman (1988) reported that *Plodia interpunctella* resistance to *B.t.* did not decline even after two generations without selection. This apparent unstable resistance was demonstrated by the survey findings, which indicated that most farmers in Accra suburbs had reduced their use of *B.t.* due to low efficacy in controlling DBM. As a result the DBM populations in Accra were almost susceptible to *B.t.* whereas the Mampong population showed some level of resistance since *B.t.* is presently widely used by farmers in the area.

On the other hand the synthetic insecticides assayed (pyrethroids and organophosphate) have been reported to have more stable resistance (Shelton and Zhao, 2004). This was substantiated from the study, which recorded very high resistance to insecticides assayed even in areas where those particular formulations were not currently in use. For example, Dursban recorded very high level of tolerance (193 fold) in Dzorwulu, although only a few farmers were currently using it. Similarly, DBM population in Airport showed the highest tolerance to cypermethrin although currently cydimsuper, a mixture of cypermethrin (36g/L) and dimethoate (400g/L) was in common use in the area and not cypermethrin alone. This suggested not only the stability of resistance to conventional insecticides but also that multiple resistance and cross-resistance might be the major contributors to low efficacy of most conventional insecticides to DBM.

The study suggested the presence of cross-resistance among the pyrethroids assayed and multiple-resistance between the pyrethroids and organophosphates. In contrast, all the DBM resistant strains to the conventional insecticides were susceptible to *B.t.* This

suggests that *B.t.* resistance results from selection by *B.t.*, but not cross-resistance to other insecticides. This is not surprising because the mode of action for *B.t.* differs from that of the conventional insecticides. The *B.*t.'s δ endotoxin lyses cells in the midgut membrane while the pyrethroids and organophosphates, targets cholinesterases the high voltage sodium ion channel (Horway *et al.*, 1986). DBM populations showed cues of multiple resistance to both Pawa (lambda-cyhalothrin a pyrethroid) and Dursban (chlorpyrifos an organophosphate) in Mampong and Dzorwulu and therefore the two should not be used in rotation. The low tolerance recorded for Deltaplan (deltamethrin a pyrethroid) in Madina suggest that it may be judiciously used to control DBM population that were tolerant Pawa however this was not the case for Airport area where Deltaplan only recorded 30% mortality on a similar DBM population. This observation suggests similar mechanisms underlying DBM resistance to the assayed groups of insecticides. This confirmed the findings by other workers that DBM resistance covers all major groups of insecticides (Sun *et al.*, 1986 and Lin, 1988). As a result insects resistant to pyrethroids may develop resistance to organophosphates even without exposure to the latter group of insecticides. It was therefore not advisable to use pyrethroids with dursban in the management of DBM. The use of pyrethroids and organophosphates in rotation with dipel could be a better option, as this will reduce the selection pressure of the insecticides and preserve susceptible genes in DBM population. These results confirm the findings of Sun *et al.* (1986) that Bt is highly effective against DBM populations that were resistant to conventional insecticides.

The different levels of non-specific carboxylesterase activities observed in the DBM (Table 9) from different study sites indicate some level of resistance (Doichuanngam and Thornhill, 1989). The high carboxylesterase activity observed in DBM was a consequence of a long period of exposure to organophosphates and pyrethroids, which

was prerequisite for resistance development. Mampong recorded very high activity of the two enzymes followed by Madina and Dzorwulu population. The grand mean recorded in this study for α and β naphthyl esterases were 3.05μmol/min/mg and 9.1μmol/min/mg respectively. Comparing these findings with the work of Kaiwa (2000), which recorded means of 1.5μmol/min/mg and 1.2μmol/min/mg for α β–naphthyl esterase activities respectively; it was observed that the mean activity of α–naphthyl esterase had doubled while an eight-fold increase was registered for β–naphthyl esterase. However, in general, higher α–naphthyl esterase activity was recorded for Accra suburb sites. This confirmed the findings of Kaiwa (2000) on carboxylesterase activity in DBM in Accra suburbs. Similar findings were also reported by Villania *et al.* (1983) on *Culex pipiens* mosquitoes in selected locations around Accra by Poku (1999).

The results of the carboxyleterase activity did not reflect the findings of the insecticide use pattern survey, which showed high use of both groups (OP and SPs) of insecticides in the Accra suburbs than in Mampong (Table 9). Likewise this was contrary to the bioassay results, which recorded high resistance to all the conventional insecticides assayed (Table 8). This may suggest that apart from carboxylesterases, other enzymes such as oxidases and Glutathion-S-transferases may be involved in DBM resistance as reported elsewhere (Maa *et al.,* 1997). Carboxylesterase enzymes were also shown to have wide variations in activity even from same site especially in Mampong. This may suggest development of resistance at different times under selection by different insecticides.

An attempt was made to characterise the esterases to identify the isozymes present. Based on electrophoretic mobility and staining intensity, two main groups of esterase

bands were identified. These bands were scattered throughout two zones of the gel (Fig.15) a fast moving-heavily stained upper zone and a slow moving-light stained lower zone. Based on the preliminary characterization of esterases of DBM (Appendix IV) reported by Maa *et al.* (1990); Maa and Liao (2000) high level of resistance is more likely to be associated with slow moving esterases, which are more important than fast moving esterases in resistance monitoring. However, without genetic data it was difficult to attribute each isozyme to a given locus. The esterases isozymes combined in different patterns and intensities to give varying levels of resistance, although negative correlation between the banding patterns and resistance had been reported (Maa *et al.,* 1997). These workers argued that DBM had developed multiple resistance mechanisms, and its resistance to OP and SPs did not only depend on hydrolysis. In addition, enzyme activities of soluble esterases of different cultures were found not to correlate with frequency of slow moving esterases. However, the work of Owusu *et al.* (1995), on aphids emphasized that, band intensities rather than numbers were likely to be the principal factors determining the quantity of resistant-linked enzymes produced by an individual insect.

DBM population in Mampong-Akuapem showed higher frequency of slow moving esterases than the Accra surburb sites (Fig.16), which had majority of the zymograms with the fast moving esterases, associated with low resistance. This confirms the findings of the enzyme assays, for which the Mampong population had higher α– and β–carboxylesterase activity than the Accra sites. Besides the zymogram patterns for the Accra populations were nearly similar, while the Mampong population reflected a wide range of zymogam patterns (Fig.15). This pattern was also shown by the results for enzyme assay, which depicted wide deviations in activity, indicative of wide qualitative differences within the same population. Comparatively, Madina DBM population had

higher frequency of slow moving esterases while Dzorwulu was shown to record the least frequency. This confirmed the results of the carboxylesterases assay, which showed Dzorwulu population to be nearly susceptible for both enzyme substrates. The exhibition of different forms of electrophoretic banding patterns by resistant DBM strains and the presence of esterase heterogeneity suggest that resistance might have developed over different periods of time under varying degrees of pesticide selection. This supports the view that resistance development has become rapid when selection pressure exerted by insecticide is widespread and continuous (Brown, 1964). The heterogeinity shown in the characterization of carboxylesterase as well as the variation in gradient of the dose-mortality curves suggests the possibility of having different DBM species particularly in Mampong and some taxonomic work need to be done to elucidate this fact in Ghana

Polimerase chain reaction (PCR) was used to detect *B.t.* resistant gene. This molecular identification of *B.t.* resistant gene of DBM showed the expected 325 bp PCR fragment in both resistant and susceptible strains, implying that *B.t.* resistance was not as a result of deletion of the entire genome but of change in the genome (Heckel *et al.*, 1999). This finding supports the leading hypothesis that *B.t.* resistance was conferred by altered *B.t.* toxin binding sites in the larval midgut epithelium (Ferre *et al.*, 1991). The amplification of the additional 776 bp fragment could be because the primers may not be as specific as they were reported to be. Moreover the primers could be amplifying the section that represents the sequence-tagged site (STS) for the chromosome containing *B.t.* R-1, which corresponds to the *B.t.* resistance gene in DBM where the primers were cloned (Heckel *et al.*, 1999). The extra 1110 bp fragment however could also be a product of further mutation of *B.t.* resistant gene in DBM or a change in the target site in DBM, therefore sequencing the new areas amplified would have shown whether or not these mutations are relevant to *B.t.* resistance.

The first insect reported to have developed resistance to *B.t.* in open field was DBM (Mcgaughey and Whalon, 1992) and Indian meal moth in laboratory experiments (Mcgaughey, 1990). Others that have shown resistance in laboratory set up are tobacco budworm and Colorado beetle. DBM and tobacco budworm have even shown multiple resistance to other *B.t.* strains (Gould, 1992).

The varying levels of resistance recorded for DBM from the various sites may have arisen from continued exposure of these DBM populations to conventional insecticides. The present work showed stability of resistance to organophosphates and pyrethroids, which these farmers have been using. Brine shrimp toxicity bioassay directed SPE fractionation of extracted residues from cabbage was used to establish the identity and levels of organophosphates and pyrethroids the extracts. The organophosphates were eluted in the polar solvents, while the pyrethroids were generally eluted in hexane, anon polar solvent Yeboah (2001) has found that methanol extracted a lot fatty substances and extraneous materials, which may explain the high extractable levels of total residue in methanol fraction (Table 11). While Matsumura (1985) exclusively used hexane for extraction of organochlorines and organophosphate insecticides, this study proved otherwise. On the bother hand, the low residue levels obtained for hexane fractions may be due to hexane/ petroleum ether selective extraction of insecticides from plant materials found by the work of Gbewonyo (1991). Ethyl acetate with medium polarity may also demonstrate some levels of selective extraction, hence the lower level of ethyl acetate extracted residues.

In the Mampong cabbage samples, methanol fraction gave significantly high chlorpyrifos residues and consequently MRL, but the lowest levels of pirimiphos-methyl ethyl acetate fraction and pyrethroid hexane fraction residues. The survey showed that

the farmers were currently using chlorpyrifos (dursban) (Table 9) and DBM also in the bioassay recorded the highest (106 fold) resistance for chlopyrifos (dursban) at the same site (Table 8). In addition to these carboxylesterase activity for both enzymes was highest in Mampong. Additionally, the esterase isozymes recorded the highest frequencies of slow-moving esterases associated with high resistance for Mampong samples (Fig.16) justifying the high chlopyrifos residues detected.

Unlike Mampong, the Accra suburb sites gave much higher yield for all the three insecticides. This was substantiated by the insecticide use pattern survey, which indicated the use of most of the insecticides chosen for the residue estimation. Notably Dzorwulu A had significantly higher amounts of pirimiphos-methyl and low to undetectable levels of pyrethroid residues, in some sites due to low brine shrimp mortalities. The susceptibility studies showed that DBM population in these sites was highly resistant to all the conventional insecticides assayed (Table 8). Similarly, Airport samples generally showed all round presence of the three insecticides including high levels of pyrethroids. This concurred with the bioassay results, which showed Airport DBM population to record the highest resistance to cypermethrin and lambda cyhalothrin. This may be an indication of long-term use of these insecticides in the sites, and therefore prolonged exposure of the insect to them, which is prerequisite for resistance development (Brown, 1964). However, these high pyrethroids residues and resistance was not reflected in carboxylesterase activity and esterase zymogram pattern, studies suggesting that carboxylesterase was not solely responsible for the resistance observed in these DBM populations.

The generally low residual activity and MRL for pyrethroids could partly be because pyrethroids are photosensitive and are therefore less persistent in the environment as

compared to the OPs. However comparing these findings with the work of Ninsin (1997) on lambda-cyhalothrin there was 18 fold increase in MRL values at Dzorwulu B and 13 and 12 fold increase in Dzorwulu A and Airport B respectively over results obtained by Ninsin (1997). This was 6 to 9 fold above the FAO/ WHO MRL of 0.2mg/kg. The residue levels of pirimiphos-methyl ranged between 2.1mg/kg and 14.6mg/kg, which gave 2- 15, fold, increase above the recommended MRL 1mg/kg. The insecticide use pattern survey also showed an increase in the use of organophosphates and a reduction in the use of pyrethroids (Fig. 9). This was confirmed by the highest resistance recorded for dursban (chlorpyrifos-methyl) when bioassayed, and an equally high chlorpyrifos level detected in all the sites. The highest chlorpyrifos residue was recorded in Mampong 124.1mg/kg and the lowest 55mg/kg in Airport A samples (Table 11). This was 55 to 124 fold above the recommended MRL of 1mg/kg recommended by Codex Alimentarius Commission.

Works reported by many scientists in Ghana have shown concern for the use of chlorpyrifos. Mawunyega (1994) and Aboagye (2002) analysed residues on cabbage and on exportable pineapples respectively. Chlorpyrifos was also detected in 6 out of 8 samples of street vended *waakye* (rice and beans) and 1 out of 8 samples of *fufu* (cassava and plantain dough that were analysed in 1999-2000. However, residue levels recorded were lower than the level permitted by Codex Alimentarius Commission.

CONCLUSIONS AND RECOMMMENDATION

Poor agronomic and insecticides application practices by cabbage farmers have resulted in high increase in DBM resistance to all the conventional insecticides. As a consequence of the indiscriminate use of these conventional insecticides to control DBM, increased residue levels on cabbage heads has become a great source of health hazards to cabbage consumers. However, low resistance was recorded for *B.t.* leaving it as the only possible alternative for controlling DBM in the study sites.

A substantial increase in carboxylesterase activity compared with earlier studies together with esterase zymogram pattern studies further confirmed that DBM population have developed resistance to these pyrethroids and organophosphates assayed. The low carboxyleterase activity recorded suggests that apart from carboxylesterase, other enzymes could be involved in DBM resistance. Given that DBM has developed resistance to different classes of insecticides and most of the field observed resistance are due to multiple resistance activities of other enzymes such as oxidases and glutathione transferase should be determined since they have also been reported to be involved in DBM resistance. Use of enzyme inhibitors in the electrophoretic studies of the esterases, could help identify the particular esterase isozyme (acetylcholinesterase or carboxylesterase) that confers resistance to this group of insecticides.

The expression of *B.t.* resistance genes and the possibility of further mutations should serve as appoint of caution to scientists who are using transgenic plant campaign as the ultimate solution to insect pest problems. Rather, more integrated approach using IPM principles, including the use of parasitoids coupled with judicious use of the environmentally friendly biopesticides could be more effective in the management of DBM and other insect pests. This should be strengthened by using the Farmers Field

schools which was shown to be successful in controlling DBM at Weija cabbage farms in Accra especially when Neem extracts were incorporated in insect pest control.

Furthermore the use of spreader stickers could help increase efficacy of the insecticides with contact action this is because of the wax on cabbage leaves. The same has led to low efficacy of *B.t.* in controlling DBM, which stays on the underside of the cabbage leaves. Care should therefore be taken to ensure proper coverage during spraying and proper timing of application since *B.t.* has a field life of 2-3 days and would be most effective if applied soon after eggs of DBM emerge for larvae to feed when it is active (Mau and Kessing, 2004).

As a consequence of DBM resistance to conventional insecticides and further increase in their rate and frequency of application very high levels of insecticide residue were shown in Brine shrimp toxicity test. Chlopyrifos levels were far higher than the recommended FAO/WHO established MRL levels likewise pyrethroids levels compared to the works of earlier scientists and recommended MRL were also higher. The results of the present study have shown that the insecticide residue problem for cabbage should be taken seriously in Ghana. Urgent steps must be taken to help farmers control insect pests of cabbage without compromising human health. To achieve this, insecticide companies and researchers should educate farmers on proper use and handling of insecticides. In addition, the Environmental Protection agency of Ghana should prevent the importation of insecticides not registered for use in Ghana and the National Agricultural Extension Service should be strengthened since the extension service's education of cabbage farmers was found to be seriously inadequate.

For effective management of DBM, there is need to delay resistance development. This can be achieved by starting resistance management before resistance is detected and strategically applying insecticides at the most vulnerable developmental stages of the insect. The use of insecticides with short persistence is preferred to avoid prolonged exposure to insecticides, which is a prerequisite to resistance development. These insecticides should also be used intermittently at the lowest possible effective dosages/ rates. Alongside, IPM principles like cultural practices, host plant resistance should also be incorporated to delay resistance development. It is also important to monitor susceptibility of DBM and other major pests to all the insecticides on a regular basis in representative fields in each region. Finally, insecticides remain the most reliable and effective means of controlling pests but should be used carefully and selectively and only when alternative methods do not exist, or are not economical, so far, minimizing their use is the only strategy really proven to work satisfactorily.

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APPENDICES

APPENDIX 1: STANDARD SOLUTIONS

Preparation of standard solutions and buffers

All chemicals used were of the highest grade commercially available.

a) Solutions for Enzyme assays

a. 0.558 g α naphthyl acetate (MW 186.21 g/mol) was dissolved in 100 ml absolute ethanol to make a solution with a concentration of 30 mM.
b. 0.558 g β naphthyl acetate (MW 186.21 g/mol) was dissolved in 100 ml absolute ethanol to make a solution with a concentration of 30 mM. As above.
c. 4.5135 g Sodium dodecyl sulphate (SDS) (MW 260.32 g/mol) was dissolved in 100ml-distilled water to give 5% solution. Another 0.0498g (0.05) of fast blue B salt (MW 317.2 g/mol) was dissolved in 10ml of distilled water to make 1% fast blue B salt solution. These two solutions were mixed in the ratio of 5:2 (mixture of 2 parts of a 1% solution of azoic diazo blue B salt and 5 parts of a 5% solution of SDS by volume and stored in the dark one hour before the assay.
d. 11.933 g Na2 HPO_4. $12H_2O$ (MW 358.14 g/mol) and 4.533 g KH_2PO_4. (MW 136.09 g/mol) were each dissolved in 500ml-distilled water. These were mixed in a ratio of 6:4 (Na_2HPO_4: KH_2PO_4) by volume to make (0.07 M) phosphate buffer (pH 7.2).

Protein assay

a. Reagent A was prepared by dissolving 10 g of sodium carbonate (MW 105.99 g/mol) and 2 g sodium hydroxide (MW 40.0 g/mol) in 500 ml distilled water to give 2% sodium carbonate. This was replaced at the end of every month.
b. 5.595 g $CuSO_4$ (MW 249.68) were dissolved in 500 ml distilled water in a beaker to obtain 1 % $CuSO_4$ solution.
c. 5.925 g of sodium tartrate (MW 230.08 g/mol) were dissolved in 500ml of distilled water in a beaker to obtain 1 % sodium tartrate solution.
d. Reagent B was prepared by adding 50ml of Reagent A to 0.5ml each of 1% $CuSO_4$ and 1% sodium tartrate, to give a ratio of 50:1. Mixing was done just before the start of assay.
e. Phenol (folin-Ciocalteau) Reagent: water (1:1) by volume was prepared just before the assay started because of the instability of the mixture.

NB/ All stocks solutions were stored at 5°C in a refrigerator with exception of SDS and Phenol reagent,

b) Standard Solutions Used In Molecular Studies

Solution for DNA extraction

Bender buffer (pH 8.0)

0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCL pH 7.5, 0.05 M EDTA pH 9.1, 0.5% SDS stored at 4°C

0.5 M EDTA (pH 8.0)

186.1 g/l in 800ml of sddw, pH adjusted with NaHO pellets and stored at room temperature.

Ethidium bromide, EtB(10 mg/ml)

1g of EtBr was completely dissolved in 100ml ddw and stored in the dark at room temperature.

KAc(5M K 8M Acetate)

60 ml of 5M KAc and 11.5 ml glacial acetate acid in 28.5 ml distilled water autoclaved and stored at 4°C

RNase - 10mg/ml in water. Sterilised by filtration and stored at -20° C.

TE (pH 8.0) - 10mM Tris-HCL, 1 mM EDTA (pH 8.0),stored at room temperature.

TE+RNase

(5µg/ml) 5 µl of RNase (10mg/ml) solution, 995 µl of TE (pH 8.0). Stored at -20° C

Solution for Electrophoresis

Agarose Gels

10x TAE buffer, 242 g Tris-base, 57.1 ml glacial acetic acid, 100 ml 0.5 M EDTA. pH adjusted to 7.7 (with glacial acetic acid) and the volume made to 1 litre with sddw

Urea Polyacrylamide Gels

10 X TBE electrophoresis buffer

108g/L tris base, 55g/L Boric acid, 9.3g/L Na_2 EDTA and distilled water added to make up the volume to 1 litre and store at room temperature on the bench. It was Dilute to 1X working solution for electrophoresis.

40 % Acrylamide

38g acrylamide (MW 71.08), 2g bis-acrylamide (MW 154.2) in 100 sddH_2O. This was filtered and stored in the dark at 4 ^{0}C. The required constituents needed to prepare the 7% polyacrylamide; plug and running (separating) is given in the following table.

Ingredients	**5 % Polyacrylamide gel solution**
Water (ml)	30.00
Urea (g)	8.25
40 % acrylamide (ml)	11.66
10 X TBE buffer (ml)	5.50

10 µl ammonium persulphate (APS) and 5 µl of TEMED were added to 2.4 ml of the gel to plug the plates to avoid leakage. 60 µl of APS and 30 µl of TEMED were added to the rest of the solution to make the separating gel.

The following table gives the required quantities of ingredients needed to prepare stacking gel for the above running gel and plug.

Ingredients	**4.5 % Polyacrylamide gel solution**
Water (ml)	31.00
Urea (g)	8.25
30 % acrylamide / 0.8 Bis-acrylamide(ml)	7.50
10 X TBE buffer (ml)	5.50

3.5 ml of this solution were taken per gel, 30 µl of APS and 10 µl TEMED were added and used as stacking gel. The gel was stained in EtBr.

ii. Gel loading buffers

5x Orange G

20% (w/v) Ficoll, 25 Mm EDTA, 2.5mM EDTA. 2.5% (w/v) orange G. Stored at 4°C

6 X Bromophenol blue

0.25 % bromophenol blue was added to 40 % sucrose in water and stored at 4°C. Bromophenol blue xylene cyanol; 1 volume of bromophenol blue xylene cyanol and 4 volumes of cyanide.

DNA molecular weight size markers

The following DNA molecular weight markers were used at various stages of the study: those that were obtained from the manufacturers like 100bp ladder marker was diluted according to their recommendations.

i. hind III molecular weight maker

Digestion of y/DNA with Hind III generates 8 fragments of the following lengths: 23130 bp, 9416 bp, 6682 bp, 4361bp, 2322 bp, 2027 bp, 564 bp, 125 bp.

ii. 100 bp ladder molecular weight marker:

The first band size is 100 bp, the subsequent ones measure 200, 300…..1000 bp.

A reaction mixture of 4 samples was set

SDH_2O	64.8
Buffer ($MgCl_2$) $(NH_4)_2SO_4$ Tris-HC)	8
dNTP mix	1.6
Cp1	16
CP2	16
Taq	0.5
DNA template	1µl
	20µl

c) Protein (Esterases) Analysis By Polyacrylamide Gel Electrophoresis (PAGE)

The electrophoresis and PCR standard solutions were prepared using sterilised double distilled water (sddw). Where applicable, the solutions were autoclaved at 1211b/sq in. for 15 minute in an Eyela Autoclave (Rikikkaki, Tokyo).

0.558 g α naphthyl acetate was dissolved in 100ml absolute ethanol to make a solution with a concentration of 30mM.

Tris-HCL buffer (pH 8.9)

54.513g of Tris (121.14 MW) was dissolved in 300ml of distilled water to make 1.5M and pH adjusted using HCl.

Tris-HCl buffer (pH 6.7)

16.67ml of the above solution was made up to 50ml with distilled water to make a concentration of 0.05M.

0.1% Bromophenol Blue

0.1g of 1% aq bromophenol was dissolved in 10mls of distilled water and 1/10 dilution taken.

Homogenising Buffer

5ml of Tris-HCL buffer (pH 8.9) was added to 50g sucrose and 0.4 ml of 1% aq bromophenol blue, this was made up to 100ml solution.

Electrophoresis Buffer of (pH 8.3)

3.0285g of Tris (121.14 MW) was used to make 0.05M and 14.2633g of Glycine(75.07 MW) was used to make 0.38M , the two were dissolved in 500ml of distilled water.

10% Ammonium persulfate (APS)

0.1g was dissolved in 1ml of distilled water

7% Separating Gel

40% Acrylamide/Bisacrylamide (19:1)	3.5ml
1.5M Tris-HCL pH 8.9 (4x)	5.0ml
10% APS in dH_2O	0.5ml
TEMED	0.01ml
Distilled water	10.9ml
	20ml

2.5% Stacking Gel

40% Acrylamide/Bisacrylamide (19:1)	0.625ml
1.5M Tris-HCL pH 8.9 (4x)	2.5ml
10% APS in dH_2O	0.1ml
TEMED	0.005ml
Distilled water	6.8ml
	10 ml

Phosphate Buffer (pH 6)

13.8g of Sodium phosphate (138 MW) was dissolved in 5000ml of distilled water to make a solution of concentration 0.2M.

Staining Solution

0.1g of fast blue salt was added to 50ml of 0.2M phosphate buffer (pH6)/gel. And 1ml, of 30mM α naphthyl acetate also added and kept in the dark.

APPENDIX II: SURVEY

Preliminary survey to determine insecticide use patterns in the major cabbage growing areas in Southern Ghana

Questionnaire for Cabbage Producers

1. Survey Agent..... 2. Date.....

3. Farmer Name..... 4. Site.....

5. What is the size of your farm per season?

1 = <1/2 ha

2 = 1 ha

3 = 1-2 ha

4 = >2 ha

6. How long has your field been under brassica cultivation.....

7. What irrigation equipment do you use?

1 = sprinkler

2 = watering can

3 = water hose

8. If watering can, what type of nozzle is attached.....

9. Are insect pests a problem on your farm Yes [ ]No [ ]

10. Which is the most serious pest on your farm?

1 = diamondback moth

2 = cabbage looper

3 = aphids

4 = cabbage worm

5 = all the above

6 = other (specify)

11. Which season is it most serious

1 = dry season

2 = wet season

12. How do you control these pests?

1 = spraying insecticides

2 = cultural methods

3 = botanicals

4 = biological control

5 = IPM

6 = Other (specify)

13. If by use of chemicals, which type of insecticide do you use currently?.....

14. Which others have you ever used.....

15. Why did you stop using them?.....

16. Do you treat your cabbage nursery before transplanting Yes [ ] No [ ]

17. How do you use the various insecticides?

1. Alternatively (one after the other)

2. as mixture

18. State the precise dosage applied by you of the insecticides you use.....

19. How many cabbage plants do you spray with your stated concentration of insecticides?.....

20. What insecticide application equipment do you use?

1=Knapsack sprayer (hand operated)

2= other (specify).....

21. Give reasons for your choice of equipment.....

22. If you use a knapsack sprayer (hand operated), what nozzle type do you use?

1. Polyjet red 2.Polyjet yellow 3.Polyjet green 4.Polyjet blue 5.Cone

23. Where do you buy your pesticides?.....

24. Do you wear protective clothing during insecticide application? Yes [ ] No [ ]

25. What is the interval (hours/days/weeks) between insecticide application and watering?.....

26. How many times do you treat your cabbage field before harvest.....

27. How long do you wait after last insecticide application before you harvest.....

28. Where do you get professional advice on proper insecticide use and handling?

1 = From Agric Extension Officers

2 =Fellow farmers

3 = Pesticide sales points

4=other (specify)

29. How often do you receive such advice from extension officers?.....

30. Do you keep farm records on your insecticide use patterns? Yes [ ] No [ ]

31. Have you had any training in IPM Yes [ ] No [ ]

32. Do you apply the methods you learnt from the IPM training Yes [ ] No [ ]

33. What percentage of your income is derived from the sale of cabbage.....

34. Do you have any knowledge on the problems associated with insecticide residues in foods?
1. Yes [ ] 2. No [ ]

35. If yes, please elaborate.....

APPENDIX III: BIOASSAYS

Susceptibility studies of Diamondback moth to pyrethroids and organophosphate insecticides at selected sites in Accra suburbs and Mampong-AKuapem

i) Chlorpyrifos-methyl

Mampong			Dzorwulu			Airport			Madina		
Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality
1560	6	15	2520	2	5	2520	5	12.5	1560	7	17.5
3000	7	17.5	3000	6	15	3000	10	25	2040	6	15
4440	19	47.5	3480	13	32.5	3480	5	12.5	2520	3	7.5
5400	7	17.5	3960	10	25	3960	10	25	3000	11	27.5
6840	7	17.5	4440	5	12.5	4440	6	15	3480	22	55
7800	8	20	4920	13	32.5	6000	9	22.5	3960	5	12.5
8760	6	15	5400	15	37.5	6840	11	27.5	4440	17	42.5
9720	7	17.5	8760	10	25	8760	15	37.5	4920	5	12.5
10680	5	12.5	14520	1	2.5	12000	35	87.5	6000	12	30
12600	14	35	29880	6	15	240000	34	85	6840	8	20
14520	17	42.5	38520	22	55	360000	38	95	7800	4	10
16440	8	20	57840	19	47.5	384000	40	100	8760	6	15
18360	16	40	115680	26	65				9720	7	17.5
28920	14	35	231360	24	60				12000	12	30
38520	14	35	462720	31	77.5				48000	13	32.5
48000	17	42.5	480000	37	92.5				120000	27	67.5
57720	29	72.5							192000	37	92.5
96000	31	77.5							384000	40	100
11520	30	75									
144120	26	65									
172920	29	72.5									
192000	32	80									
384000	39	97.5									
462720	37	92.5									

ii) Lambda-cyhalothrin

Mampong			Dzorwulu			Airport			Madina		
Dosage mg/ L	Total mortality	% mortality	Dosage mg/ L	Total mortality	% mortality	Dosage mg/ L	Total mortality	% mortality	Dosage mg/ L	Total mortality	% mortality
16.6	0	0	83	0	0	83	4	10	3.32	5	12.5
83	5	12.5	99.6	0	0	99.6	7	17.5	13.28	8	20
166	9	22.5	132.8	0	0	132.8	4	10	16.6	3	7.5
232.4	11	27.5	166	1	2.5	166	10	25	33.2	6	15
332	5	12.5	199.2	7	17.5	199.2	16	40	66.4	11	27.5
398.4	10	25	232.4	7	17.5	265.6	14	35	83	6	15
531.2	7	17.5	265.6	11	27.5	298.8	16	40	99.6	14	35
597.6	9	22.5	298.8	18	45	332	13	32.5	132.8	10	25
664	9	22.5	332	14	35	1328	18	45	149.4	4	10
796.8	17	42.5	398.4	12	30	2656	24	60	166	9	22.5
929.6	21	52.5	464.8	9	22.5	3984	25	62.5	199.2	12	30
1062.4	14	35	664	9	22.5	5312	25	62.5	232.4	7	17.5
1195.2	18	45	996	13	32.5	7968	35	87.5	265.6	11	27.5
1328	19	47.5	1328	21	52.5	8632	40	100	298.8	7	17.5
2656	22	55	2656	25	62.5	9296	40	100	332	15	37.5
3320	37	92.5	5312	36	90	10624	40	100	398.4	6	15
5312	39	97.5	6640	32	80				498	8	20
7968	39	97.5	7968	36	90				597.6	19	47.5
10624	40	100	8300	36	90				664	10	25
			8632	39	97.5				796.8	23	57.5
			9296	39	97.5				863.2	15	37.5
			10624	39	97.5				929.6	8	20
									1328	19	47.5
									2656	30	75
									3984	19	47.5
									4648	35	87.5
									4980	34	85
									5146	34	85
									5229	35	87.5
									5312	40	100

iii) Deltamethrin

Mampong			Dzorwulu			Airport			Madina		
Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality
37.5	9	22.5	25	0	0	31.25	14	35	12.5	1	2.5
75	8	20	31.25	0	0	37.5	13	32.5	25	3	7.5
112.5	14	35	37.5	6	15	150	14	35	37.5	5	12.5
150	17	42.5	75	11	27.5	175	15	37.5	75	6	15
225	19	47.5	100	37	92.5	225	13	32.5	100	17	42.5
300	5	12.5	125	15	37.5	300	8	20	125	16	40
350	22	55	150	22	55	600	19	47.5	150	11	27.5
400	16	40	200	13	32.5	1200	31	77.5	175	13	32.5
450	18	45	225	17	42.5	1800	36	90	200	14	35
500	17	42.5	300	5	12.5	2400	40	100	225	18	45
600	17	42.5	500	13	32.5				250	11	27.5
700	21	52.5	750	17	42.5				300	19	47.5
900	35	87.5	1000	28	70				350	16	40
1000	35	87.5	2000	30	75				400	30	75
1100	28	70	2500	30	75				450	16	40
1200	38	95	2750	40	100				600	31	77.5
			3000	40	100				700	25	62.5
			4000	40	100				900	37	92.5

iv) Cypermethrin

Mampong			Dzorwulu			Airport			Madina		
Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality
12.5	0	0	6.25	0	0	3.13	4	10	12.5	2	5
25	4	10	12.5	12	30	6.25	8	20	100	8	20
100	7	17.5	25	15	37.5	12.5	10	25	400	12	30
400	5	12.5	100	9	22.5	25	12	30	1600	27	67.5
1600	20	50	400	11	27.5	100	14	35	6400	34	85
3200	32	80	1600	9	22.5	400	20	50	8000	36	90
6400	37	92.5	3200	25	62.5	1600	28	70	9600	40	100
9600	40	100	6400	32	80	3200	35	87.5			
12800	40	100	7000	37	92.5	6400	34	85			
			9600	40	100	9600	35	87.5			
						11200	40	100			
						12800	40	100			

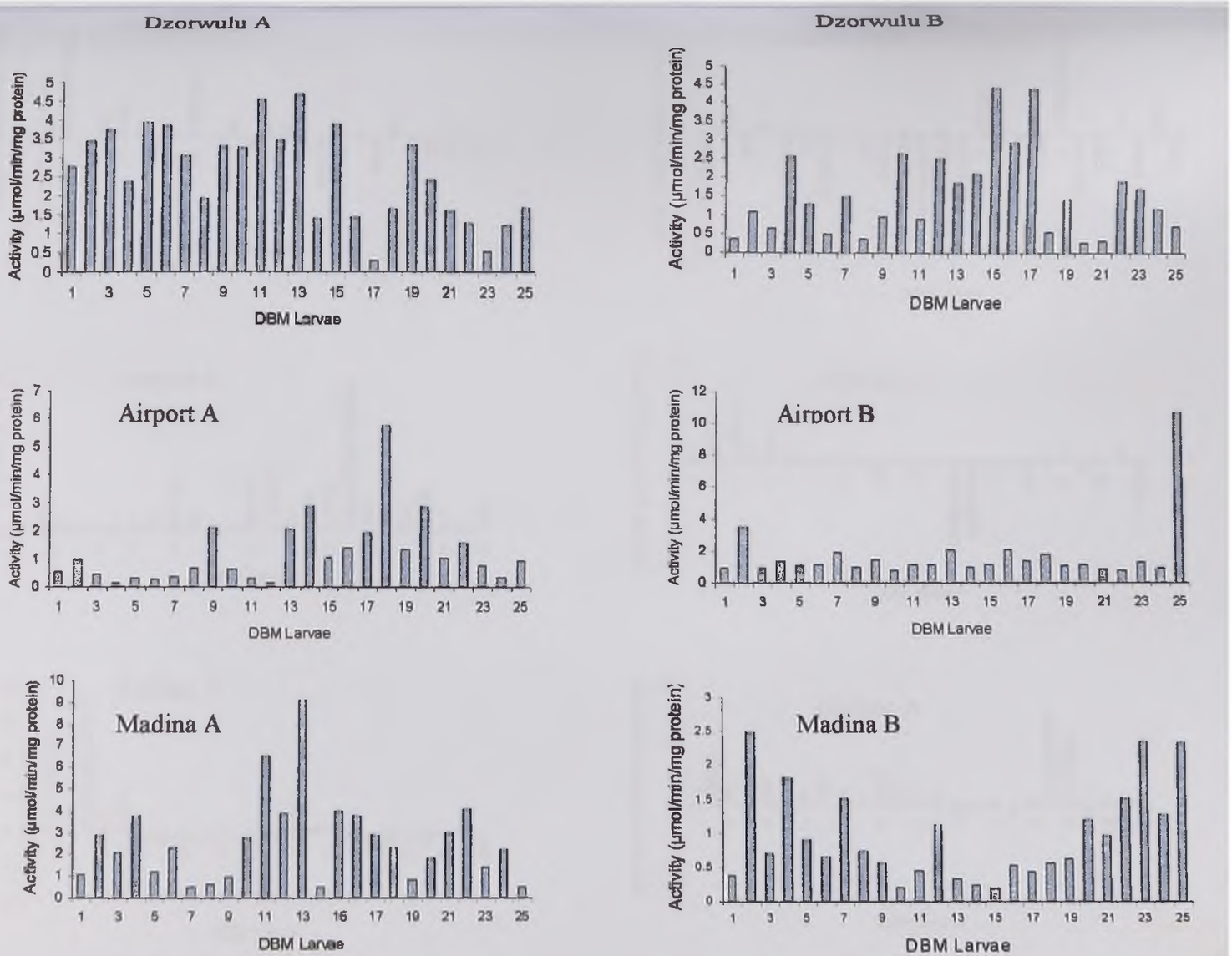
v) Bacillus thuringiensis var kurstaki

Mampong			Dzorwulu			Airport			Madina		
Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality	Dosage mg/L	Total mortality	% mortality
0.006592	7	17.5	0.1648	5	12.5	0.824	4	10	0.006592	0	0
0.03296	2	5	0.824	6	15	4.12	8	20	0.03296	2	5
0.1648	3	7.5	4.12	4	10	20.6	15	37.5	0.1648	4	10
0.824	10	25	10.3	19	47.5	103	21	52.5	0.824	8	20
4.12	11	27.5	20.6	20	50	206	23	57.5	4.12	5	12.5
20.6	5	12.5	103	21	52.5	412	35	87.5	10.3	15	37.5
41.2	18	45	206	30	75	618	37	92.5	20.6	15	37.5
82.4	18	45	309	28	70				103	25	62.5
103	23	57.5	412	37	92.5				206	24	60
206	31	77.5							412	38	95
309	26	65							515	35	87.5
412	26	65							618	36	90
618	26	65							824	39	97.5
									1030	40	100

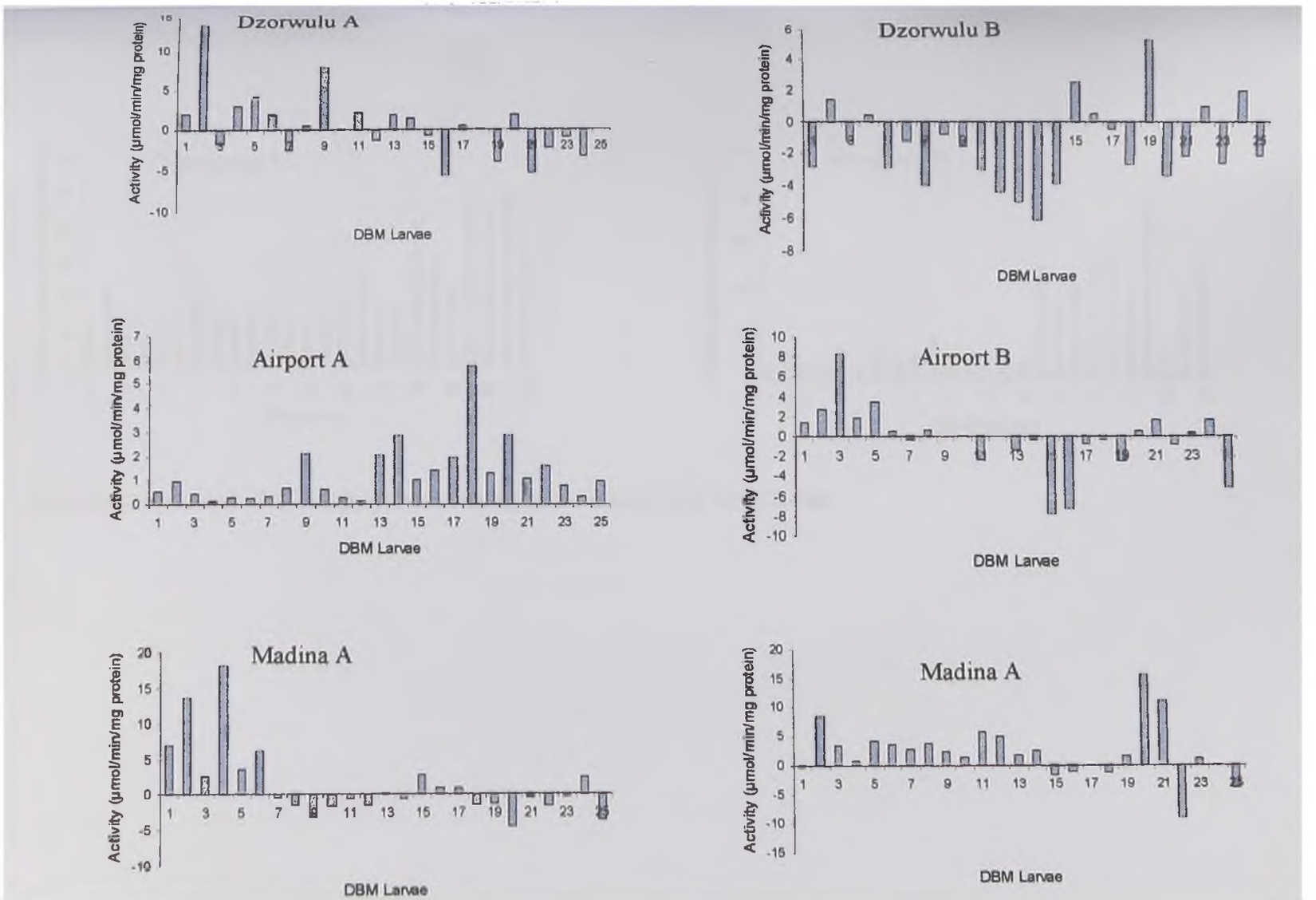
APPENDIX IV: ENZYME ASSAYS AND CARBOXYLESTERASE ISOZYME STUDIES

Activity for α-naphthyl acetate (α N A) and β-naphthyl acetate (β N A) (n mol/min/□g protein)

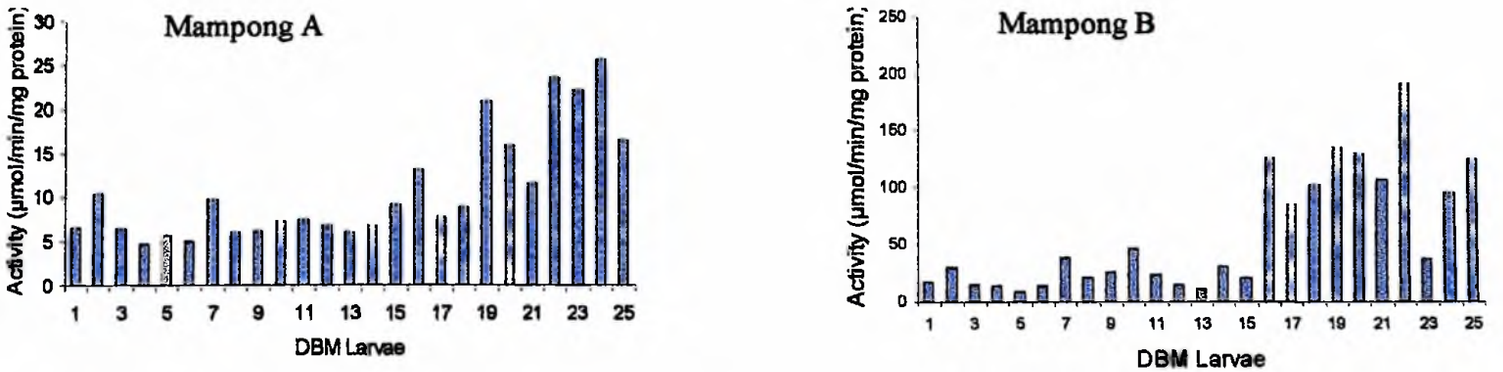
	Mampong		Dzorwulu A		Dzorwulu B		Airport A		Airport B		Madina A		Madina B	
Insect	αNA	βNA	αNA	βNA	αNA	βNA	αNA	βNA	αNA	βNA	αNA	βNA	αNA	βNA
1	6.643	17.877	2.747	2.045	0.361	-2.764	0.537	0.349	0.904	1.299	1.052	6.849	0.389	-0.437
2	10.373	29.73	3.438	13.647	1.07	1.414	0.965	-0.807	3.435	2.711	2.868	13.619	2.482	8.543
3	6.453	15.589	3.75	-1.632	0.641	-1.26	0.458	0.759	0.901	8.27	2.096	2.553	0.71	3.328
4	4.767	14.185	2.355	2.935	2.532	0.407	0.139	0.325	1.348	1.796	3.74	18.178	1.82	0.753
5	5.698	8.325	3.923	4.145	1.302	-2.83	0.305	0.466	1.109	3.473	1.162	3.602	0.905	4.253
6	4.975	14.103	3.85	1.853	0.495	-1.243	0.254	0.71	1.178	0.521	2.301	6.115	0.651	3.618
7	9.73	39.352	3.026	-2.45	1.482	-3.921	0.335	0.754	1.888	-0.392	0.518	-0.428	1.505	2.719
8	6.038	22.014	1.902	0.647	0.366	-0.79	0.655	1.814	0.981	0.645	0.594	-1.385	0.733	3.891
9	6.071	27.101	3.275	7.858	0.967	-1.488	2.095	5.334	1.484	0.061	0.938	-3.147	0.567	2.369
10	7.274	46.767	3.264	0.349	2.633	-2.914	0.615	1.381	0.804	0.102	2.735	-1.604	0.216	1.41
11	7.39	23.957	4.54	2.141	0.88	-4.386	0.311	-1.397	1.128	-2.296	6.507	-0.563	0.447	5.792
12	6.748	15.335	3.451	-1.211	2.491	-4.958	0.151	-3.515	1.141	-0.041	3.864	-1.386	1.131	4.982
13	5.911	11.057	4.684	1.925	1.857	-6.067	2.042	0.382	2.09	-1.394	9.118	0.237	0.339	1.711
14	6.8	31.541	1.389	1.547	2.103	-3.802	2.858	2.003	1.024	-0.419	0.521	-0.61	0.238	2.655
15	9.108	21.822	3.88	-0.645	4.386	2.418	1.004	-1.521	1.191	-7.811	3.963	2.708	0.196	-1.628
16	12.993	126.55	1.398	-5.541	2.931	0.465	1.386	2.172	2.074	-7.333	3.746	0.823	0.531	-0.964
17	7.733	86.148	0.279	0.636	4.365	-0.494	1.923	4.578	1.398	-0.758	2.863	0.888	0.428	0.083
18	8.851	102.181	1.636	0.178	0.567	-2.675	5.76	17.697	1.792	-0.412	2.299	-1.531	0.565	-1.193
19	20.762	135.304	3.32	-3.968	1.42	5.051	1.325	0.413	1.115	-2.511	0.821	-1.228	0.618	1.622
20	15.78	130.094	2.387	1.855	0.272	-3.324	2.869	7.943	1.17	0.476	1.805	-4.44	1.188	15.571
21	11.461	107.295	1.576	-5.227	0.305	-2.093	1.038	0.852	0.86	1.546	2.957	-0.489	0.955	11.007
22	23.364	191.381	1.27	-2.18	1.929	0.952	1.57	1.15	0.759	-0.865	4.023	-1.538	1.502	-8.898
23	22.04	37.736	0.52	-0.81	1.693	-2.538	0.768	1.542	1.272	0.397	1.432	-0.201	2.349	1.148
24	25.424	96.421	1.195	-3.284	1.18	1.91	0.351	0.657	0.894	1.566	2.164	2.409	1.265	0.082
25	16.29	125.091	1.662	0.101	0.7	-2.08	0.942	0.79	10.709	-5.201	0.488	-3.637	2.329	-3.954
Mean	9.546	49.31	2.589	0.451	1.491	-0.798	1.054	1.26	1.549	0.246	2.185	1.414	0.915	2.925



Distribution patterns of α-esterase activity in Diamond Back Moth larvae.



Distribution patterns of β-esterase activity in Diamond Back Moth larvae.



Distribution patterns of α- and β-esterase activity in Diamond Back Moth larvae.

Results of Determination of α- -Naphthol Calibration Curve

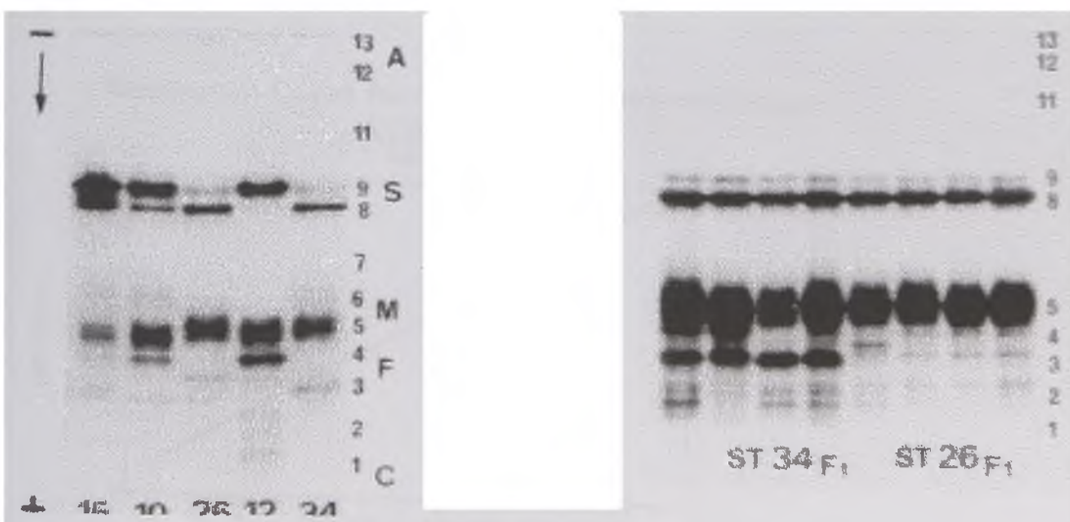
Concentration x 10^{-2} (M)	Absorbance (600nm)
0.20	1.433
0.10	0.673
0.05	0.331
0.025	0.146
0.0125	0.064
0.00625	0.027

Table 7: Results of Determination of β -Naphthol Calibration Curve

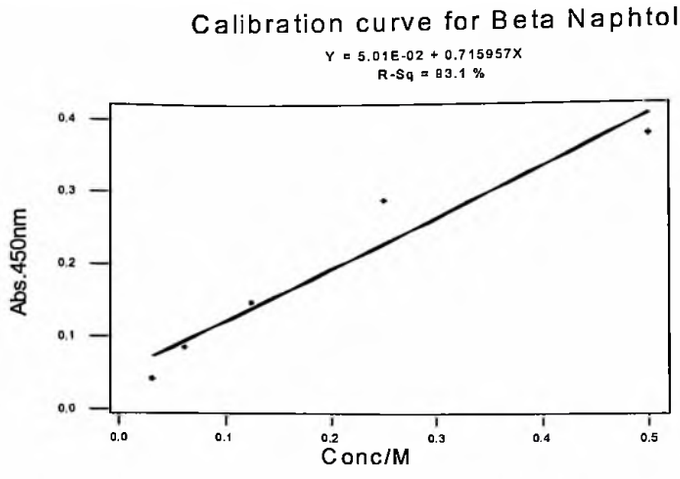
Concentration (M)	Absorbance (450nm)
0.5 x 10^{-2}	0.379
0.25 x 10^{-2}	0.289
0.125 x 10^{-2}	0.148
0.625 x 10^{-3}	0.086
0.3125 x 10^{-3}	0.042

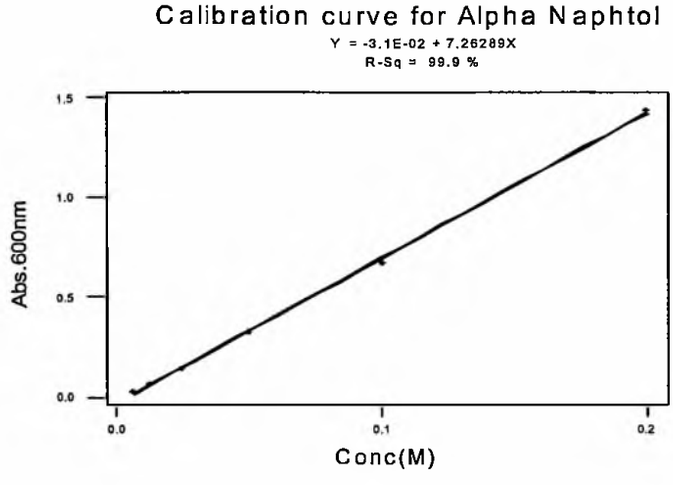
Table 8: Results of Determination of Calibration Curve For 0.1% BSA

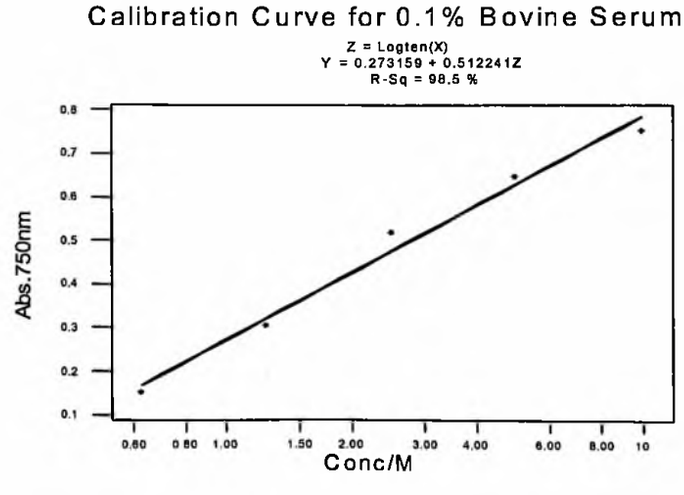
Concentration x 10^{-2} (mg/mL)	Absorbance (750nm)
10	0.612
5	0.594
2.5	0.493
1.25	0.315
0.625	0.187



Zymgram patterns of 5 strains of DBM populations and zymogram patterns showing the fast-and slow-moving esterase isozymes. (Maa and Liao, 2000)







APPENDIX V: RESIDUES ANALYSIS

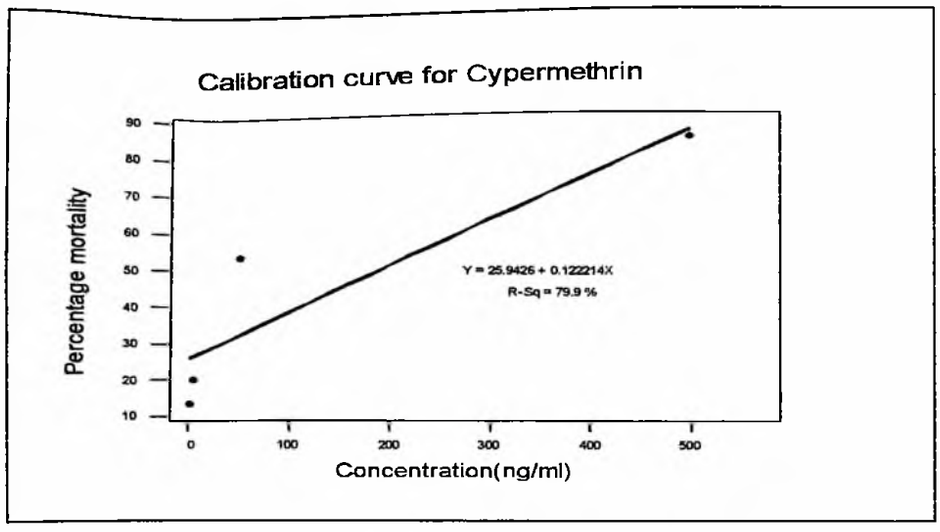
Site	Solvents	Residues wgt(□g/ml)	Total mort.	% mort.	Active*[a] resd, □g)	% Active*[b]. residues
Mampong	Methanol	127066.6667	56	93.3333	0.4885	0.0004
	Ethyl acetate	10900	7	11.6667	0.0957	0.0009
	Hexane	6733.33	8	13.3333	-0.1032	-0.0015
Madina A	Methanol	47150	51	85	0.4410	0.0009
	Ethyl acetate	17750	14	22.5	0.1803	0.0010
	Hexane	10950	2	2.5	-0.1918	-0.0018
Madina B	Methanol	58350	51	85	0.4410	0.0008
	Ethyl acetate	10500	21	35	0.2779	0.0026
	Hexane	3650	6	10	-0.1304	-0.0036
Dzorwulu A	Methanol	89666.66667	46	76.6667	0.3935	0.0004
	Ethyl acetate	12133.33333	46	76.6667	0.6033	0.0050
	Hexane	4800	21	35	0.0741	0.0015
Dzorwulu B	Methanol	405033.3333	36	60	0.2985	7E-05
	Ethyl acetate	17233.33333	24	40	0.3169	0.0018
	Hexane	8900	23	38.3333	0.1014	0.0011
Airport A	Methanol	54200	28	46.6667	0.2225	0.0004
	Ethyl acetate	13700	9	15	0.1217	0.0009
	Hexane	5433.333333	13	21.6667	-0.0350	-0.0006
Airport B	Methanol	108233.3333	52	86.6667	0.4505	0.0004
	Ethyl acetate	13533.33333	19	31.6667	0.2519	0.0019
	Hexane	8333.333333	21	35	0.0741	0.0009
Control*[c]	Methanol	120100	2	5	-0.0151	-1.3E-05
	Ethyl acetate	6400	1	2.5	0.0241	0.0004
	Hexane	4800	0	0	-0.2123	-0.0044

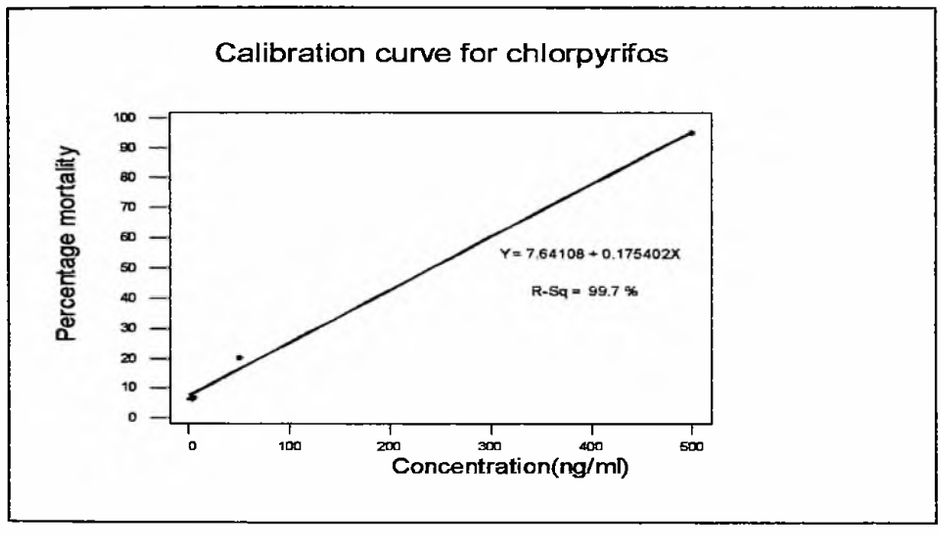
*c Control- Insecticide free cabbage samples grown in the screen house.

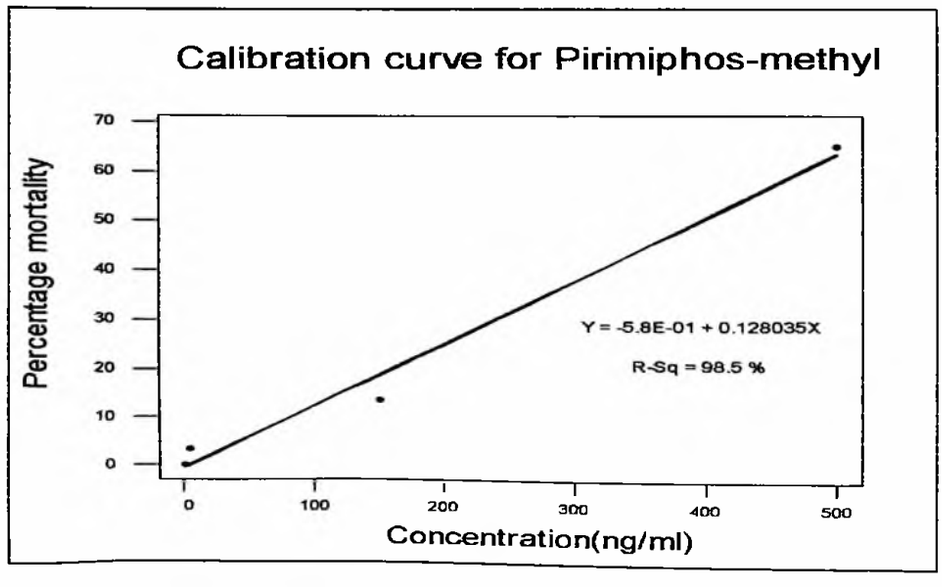
N/B Active residues for mortalities below 26% could not be estimated from the calibration curves of the insecticide standards

N/B A total of 60 Brine shrimp nauplii were used for each fractions of the sample extract while a total of 40 brine shrimps wee used for each of the control fractions

N/B A volume of 250μl of the cabbage extracts was used.







APPENDIX VI: STATISTICAL ANALYSIS

Analysis of variance
Variate: Alpha-naphthy esterase

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Location	6	1784.856	297.476	40.33	<.001
Residual	168	1239.049	7.375		
Total	174	3023.905			

F tab at 5 (2.16) 0.01 (2.92)

Analysis of variance
Variate:Beta naphthyl esterase

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Location	6	73191.1	12198.5	30.14	<.001
Residual	168	68002.9	404.8		
Total	174	141194.0			

F tab at 5 (2.16) 0.01 (2.92)

Analysis of variance
Variate: Chlorpyrifos-methyl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Location	7	42623.	6089.	0.82	0.586
Residual	13	96212.	7401.		
Total	20	138835.			

Analysis of variance
Variate: Pirimifos-methyl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Location	7	350.82	50.12	0.91	0.529
Residual	13	716.44	55.11		
Total	20	1067.26			

Analysis of variance
Variate: Pyrethroids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Location	7	112.302	16.043	2.01	0.131
Residual	13	103.701	7.977		
Total	20	216.003			