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**STUDIES ON THE BIOLOGY AND ACTIVITY LEVELS OF
CARBOXYLESTERASE AS AN INDICATOR OF INSECTICIDE
RESISTANCE IN POPULATIONS OF DIAMONDBACK MOTH, *PLUTELLA
XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE) ON CABBAGE IN
GHANA.**

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(FACULTY OF SCIENCE) AND CROP SCIENCE (FACULTY OF AGRICULTURE).**

DECLARATION

I do hereby declare that except for references to works of other scholars, which have been duly acknowledged, this thesis is my own original research, which has neither been presented in whole or in part to any other university for the award of a degree.

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Dedication

This work is dedicated to my husband and my daughter Saffie, who went with me the extra mile.



ABSTRACT

Biology of Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) was studied in the laboratory under controlled conditions, i.e.

28 ± 1 °C, 65-70 % r.h and a photoperiod of 12L: 12D hours. The results indicated that the developmental time of *P. xylostella* from egg to adult emergence took on the average 17.5 days. About 59 % of the eggs were laid on the first night of oviposition. Additionally females were generally larger (5.0mm) than males (4.5 mm).

Effects of temperature, pH, and substrate concentration on the hydrolysis of α - naphthyl acetate by carboxylesterase from *P. xylostella* were evaluated. Optimum ranges of these parameters were, 40-45 °C for temperature, 6.9-7.3, for pH, and 35-40 mM for substrate concentration.

Carboxylesterase analyses of *P. xylostella* populations collected around Accra indicated the probable incidence of insecticide resistance in the insect population. Carboxylesterase activity levels ranged from 2.06 to 28.29 nmol / :10min/ μ g protein. While the highest activity was recorded by populations from Kumoji Road and classified as moderately resistant, G.B.C (Ring Road) and Legon populations were classified as highly susceptible. The finding has enormous implications for the vegetable growers.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

Cabbage and other brassicas are agriculturally a diverse group of crops of European origin (Purseglove, 1969). They are cultivated from the arctic to the sub-tropics, and at higher altitudes in the tropics. Certain species and varieties are more adapted to the tropics than others (Ooi, 1986).

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

In the tropics the two main types of pests causing damage to brassica crops are the cabbage aphids, *Brevicoryne brassica* (L.), (which transmit turnip mosaic virus and several other viruses specific to cruciferae), and leaf-eating caterpillars (Norman, 1992). Among the leaf-eating caterpillars implicated in the damage of cabbage in Ghana is the notorious and cosmopolitan pest, Diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Brempong-Yeboah, 1992).

It is considered the most destructive insect pest of cruciferous crops throughout the world (Kfir, 1997). The activities of the insect pests on cabbage lower the market value of the crop.

Modern agriculture heavily relies on chemical inputs such as pesticides and fertilizers. These chemicals are expensive and may cast a heavy economic burden on vegetable growers who are mostly poor subsistent farmers. In a desperate attempt to protect their crop and investment, they resort to the indiscriminate use of insecticides to control insect pests. It has been observed that cabbage growers are now using unnecessarily large quantities of insecticides (Brempong-Yeboah, 1992; Owusu, pers com) and in various concoctions against the diamondback moth. Not only are the dosages applied very high, growers also spray at very short intervals. Interviews conducted among the growers, revealed that chemical applications are done at 2-3 day intervals (Unpublished data).

Some of the insecticides recently documented to be used by growers in Ghana include Permethrin, Deltamethrin, Decis, Biobit (*Bacillus thuringiensis*), Dursban (Chlorpyrifos), Dipel (*B. thuringiensis*), Karate (Lambda-cyhalothrin), Perfeckthion (Dimethoate), Ripcord, Actellic (Pirimiphos-methyl), and Neem seed extract (Mawuenyegah, 1994).

The success of chemical control of insect is immediate and convincing; optimum crop yields are obtained and the quality of the farm produce is improved (GTZ, 1979). However, lack of adequate information on the safe

and efficient use of pesticides to the farmers has resulted in the repeated and indiscriminate use of these chemicals, which in turn has created the problem of human health hazards due to their toxic residues that persist in/on food after their application (ICAR Extension Folder: 59); and the contamination of the environment. These toxic effects are more apparent in vegetables since they are mostly consumed fresh. This repeated and uncontrolled application of pesticides has also resulted in pest populations containing high proportion of resistant individuals. Depending on the mechanism, highly resistant strains often stay resistant for generations even without further exposure to the pesticide.

Since insecticide resistance, decimation of natural enemies, and resurgence of insect pests act in concert, farmers increase the concentrations of insecticides against the target pest. This, however, leads to higher selection pressure to the extent that high concentrations of the pesticide are no longer effective against the pest. This phenomenon was observed by Brempong-Yeboah (1992) in the Accra plains where some of the cabbage growers were overusing pesticides against *P. xylostella*. The insect became resistant and could no longer be controlled by the chemicals. This caused so much financial losses and frustration, that some of the cabbage growers gave up cultivation.

Insecticide resistance in diamondback moth, *P. xylostella* (L. Plutellidae) has occurred in many parts of the world since Anklersmit (1953) first

reported its resistance to DDT in Indonesia. More recently, resistance has been reported in Hawaii (Tabashnik *et al.*, 1987), Japan and Australia (Kao *et al.*, 1989). However, the occurrence of insecticide resistance in this insect may not be limited to these areas (Kao *et al.*, 1989). 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 resistance spectrum of *P. xylostella* covers all major groups of insecticides, i.e., chlorinated hydrocarbons, organophosphorus compounds carbamates, pyrethroids (Sun *et al.*, 1986; Lin, 1988) and even to the bacterial pesticide, *Bacillus thuringiensis* (Kfir, 1997; Wright *et al.*, 1997).

Except for a few chlorinated hydrocarbons that are now banned, organophosphorus insecticides: have had the longest history of use among vegetable growers for the control of the diamondback moth (Kao *et al.*, 1989).

Resistance mechanisms in diamondback moth proposed for synthetic chemicals include decreased penetration (Noppun *et al.*, 1987) enhanced detoxication by esterases (Maa and Chuang, 1983) glutathione-S-transferases and reduced sensitivity of acetylcholinesterases (Wu, 1983; Hama, 1987).

Esterases in general have been noted to play significant roles in

resistance to insecticides, particularly organophosphorus insecticides (Owusu *et al.*, 1996). Despite the known involvement of esterases in insecticide resistance of *P. xylostella*, nothing has been done to ascertain their levels in most African countries, especially Ghana. Owusu (in press) has hinted on the increasing trend of insecticide resistance in insect pests of Ghanaian vegetables and has thus called for a national monitoring network. Such a network is expected to monitor insecticide use pattern and develop a well-meaning resistance management scheme. Without such a study, resistance may reach such an alarming level where governments will have to spend millions of dollars to address it (Owusu, in press). This study was therefore undertaken to investigate the resistance status of *P. xylostella* using carboxylesterase activity as indicator.

1.1 The specific objectives of the study were:

- 1 To study the biology of *P. xylostella* in the laboratory.**
- 2 To investigate carboxylesterase variation among populations of *P. xylostella* on cabbage grown in different localities.**
- 3 To look at the properties of carboxylesterase from *P. xylostella*, to enable an efficient bioassay method to be developed for future monitoring.**
- 4 To review the general trend of insecticide resistance in *P. xylostella* and institute appropriate advice for resistance management.**

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ORIGIN AND IMPORTANCE OF CABBAGE

Cabbage *Brassica oleracea* var. *capitata* L (Brassicaceae) is an important global vegetable crop (Rice *et al.*, 1993). The edible part of the cabbage plant, which is the head, is made up of a series of overlapping expanded leaves, which cover a small terminal bud (Purseglove, 1969, Rice *et al.*, 1993). The crop is of very ancient origin and has been cultivated in Europe since 2500 BC (Purseglove, 1969). It was first introduced into England by the Romans and is now grown throughout the world including lowland tropics for its foliage bud (Purseglove 1969)

Cabbage is a biennial herb with a short thickened stem surrounded by a series of fleshy overlapping expanded leaves, which form a compact head. The older leaves surround the younger, smaller, more tender ones and the miniature stem. The head shape may be pointed or round and leaf color and shape are variable. The cultivation of cabbage in the tropics has increased in the last decade. The booming tourism industry and foreign investments drive in the developing Countries, which are attracting many foreigners, have increased the demand for cabbage. The popularity of the crop may also be due to the nutritional value of the vegetable. It is a major source of vitamins and mineral salts, which are necessary in ensuring a balanced diet. It is used as cooked vegetables, salads and as a secondary source of energy. Cabbage is source of roughage, which by providing an indigestible matrix stimulates intestinal muscles and, prevents

constipation through its laxative effect (Norman, 1992). The fibre content adds to the bulk of the food, which prevents the feeling of hunger. The large drumhead cultivars are grown for feeding to stock. It could also be sliced and fermented in its own juice together with salt called sauerkraut, which is a form of human silage.

Table 2.1 Amounts of nutrients per 100 g of edible portion of cabbage

Nutrient	Value
Water	90 g
Calories	23 kJ
Fat	0.2 g
Protein	1.5 g
Carbohydrate	5.0 g
Fibre	1.0 g
Sodium	20 mg
Calcium	24 mg
Potassium	20 mg
Phosphorus	28 mg
Magnesium	22 mg
Iron	0.5 mg
B-Carotene	18 µg
Thiamine	0.1 mg
Riboflavin	0.7 mg
Niacin	0.7 mg
Ascorbic acid	40 mg
Moisture content	92%

(Source: Rice *et al.*, 1993)

2.2 INSECT PESTS OF CABBAGE

Although cabbage production has been on a steady increase in recent years, certain factors militate against obtaining optimal yield of the crop. These factors include water, marketing problems, storage and insect pest infestation (Miyata, 1986; Hill and Waller, 1994). Insect pest infestation has arisen from monoculture and intensive cabbage production and also from the misuse of pesticides.

The practice of monoculture has created conditions favourable for specialized insect species to flourish and become notorious pests (Kumar, 1986). This situation has been compounded by the cultivation of other related varieties of *Brassica oleracea* such as Chinese cabbage and cauliflower on adjacent plots with cabbage farms. The different varieties of *B. oleracea* tend to have a similar insect pest spectrum (Hill and Waller, 1994). According to Way, (1976), the cultivation of related crops together, bridges gaps in the host plants sequence of insect pests resulting in upsurges in insect pest populations. Forsyth (1966) recorded 12 insect pests associated with cabbage in Ghana (Table 2.2).

Table 2.2 Insect pests associated with cabbage in Ghana

<u>Name</u>	<u>Order and Family</u>
<i>Allogista serricorne</i> Kibe.	Coleoptera : Alleculidae
<i>Lagria villosa</i> F.	Coleoptera : Lagriidae
<i>Melanagromyza lambi</i> (Hend)	Diptera : Agromyzidae
<i>Diacrisia investigatorum</i> (Karcsh)	Lepidoptera : Arctiidae
<i>Plusia signata</i> (F)	Lepidoptera : Noctuidae
<i>Amauris psyttalea</i> Plotz	Lepidoptera Nymphalidae
<i>Spodoptera littoris</i> (F)	Lepidoptera : Noctuidae
<i>Appias epaphia</i> Cram.	Lepidoptera : Pieridae
<i>Crocidolomia binotalis</i> Zell.	Lepidoptera : Pyralidae
<i>Hellula undalis</i> (F)	Lepidoptera : Pyralidae
<i>Hymenia recurvalis</i> (F)	Lepidoptera : Pyralidae
<i>Gymnognylus lucen</i> (Wlk)	Orthoptera : Gryllidae

The major insect pests of cabbage according to Hill and Waller (1994) are cabbage aphids, *Brevicoryne brassicae* (Homoptera: Aphidae) and the leaf eating caterpillars, which include the diamondback moth, *Plutella xylosyella* L. (Lepidoptera: Plutellidae).

The cabbage aphid transmits turnip mosaic virus and several other viruses specific to cruciferous crops (Hill & Waller, 1994). The leaf-eating caterpillars also disfigure the cabbage plant and may completely defoliate it. Thus, these insects, acting in concert, can devastate many cabbage plants over a very short time if growers do not institute control measures. diamondback moth, this pest has become the key pest of cabbage in Ghana (Brempong-Yeboah,

1992). It is of European origin and considered to be, the most destructive insect pest of cruciferous crops throughout the world. In many countries, diamondback moth has become resistant to every synthetic pesticide used against it in the field and even to the bacterial pesticide *Bacillus thuringiensis* (Kfir, 1997).

The Diamondback moth feeds only on plants belonging to the family Brassicaceae and it probably evolved on plants from this family. The host plant preference of *P. xylostella* reported by Kfir (1997) include such cruciferous crops as broccoli, cauliflower, cabbage, Indian mustard, turnip and Chinese cabbage.

2.3 DAMAGE CAUSED BY *P. XYLOSTELLA*

P. xylostella destroys foliage of brassicas. These include cabbages, mustard turnip Swede watercress, and radish. In South East. Asia, it is considered to be the most important factor limiting the successful production of cruciferous vegetables (Mckinley, 1992). It is mainly associated with increased area under cultivation especially, cabbage which is grown all year round. This provides an easily accessible food source for DBM as well as for other pests, as a result of which these crops suffer depredation throughout the year (Sakai, 1981). Also due to its rapid generation turnover and short lifespan, DBM has become the chief insect pest of crucifers. During the dry season, heavy infestations of *P. xylostella*, when not controlled can cause over 30 %

crop damage (Suderwohadi and Eveleens, 1977). Unlike many Lepidopteran pests, caterpillars of *P. xylostella* attack and skeletonize plants at the seedling or newly transplanted stage. Destruction of the apical buds of the seedlings by feeding larvae, results in headless plants or with multiple undersized heads.

The 1st and 2nd instar caterpillars feed as leaf miners on the spongy mesophyll cells. As the caterpillars eat practically all of the leaf materials except the upper epidermis, leaves are often covered by numerous windows. In heavy infestations, feeding is more intensive and the leaves can be destroyed completely. Early crop damage may cause perforated wrapper leaves, down grade the quality and thus the value of the harvested crop.

2.4 CONTROL OF DIAMONDBACK MOTH

Cabbage growers in Ghana protect their crops from the damaging activities of the insect pests of cabbage by resorting to indiscriminate and multiple insecticide applications on the crop. It has been observed by Brempong-Yeboah (1992) that in 1986, cabbage growers in the Accra Plains were using large quantities of various concentrations of insecticides, applied at frequent but irregular intervals, often 2-3 day intervals. The insecticides used on cabbage in Ghana include cymbush 10 EC, cymbush 25EC, Ripcord WEC, Roxion (Dimethoate), Ambush (Permethrin) (BrempongYeboah, 1992),

Dursban (Chlorpyrifor), and Actellic (Pirimiphos-methyl) (Mawuenyegah, 1994).

This heavy reliance on insecticides in the control of insect pests of cabbage has been compounded by the availability of all year round suitable environmental conditions for pest survival, development and outbreak of the pests (Afun *et al.*, 1992), and a very large number of pesticides in the Ghanaian market. With exception of Mali and Guinea Bissau, Ghana was the biggest importer of pesticides in West Africa in the early 1980's (Bull, 1982).

The absence of appropriate legislation on the importation of pesticides, lack of education on their proper use coupled with low literacy background among the farmers (Youm *et al.*, 1990), and inadequate extension service have contributed to the widespread misuse of pesticides (Bull, 1982). All these factors have contributed to the use of pesticides on crops for which they are not recommended. For example chemicals meant for cocoa which are highly toxic, are used on vegetables and cocktail mixtures prepared by farmers are now in rampant use (NARP, 1993). Also the high cost of Agro-chemicals and generally low income among the growers act as a major constraint in the purchase of the recommended pesticides. This has resulted in most farmers using any pesticide they lay their hands on, which may be the wrong chemical, or chemical that is wrongly applied or sometimes the wrong equipment and formulation are used (NARP, 1993).

2.5 EFFECTS OF CHEMICAL CONTROL

The misuse of insecticides has hazardous effects on both the ecosystems and the environment in general. Carson (1962) highlighted the hazards and environmental consequences associated with the use of pesticides in her book- "The Silent Spring". Many researches and publications have since been carried out that substantiate the concerns raised in this book (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.

2.5.1 EFFECT ON HUMAN HEALTH

Insecticides are defined as chemicals used to kill or destroy insects.

Notwithstanding this definition 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). These insecticides are therefore not only toxic to insects but also to humans (Oudejans, 1991). Insecticide toxicity to humans could either be acute or chronic.

Acute toxicity is the immediate poisonous effect of a single dose of a toxicant (Hassall, 1990). Acute poisoning occurs when people come in direct contact with insecticides. The phenomenon in Africa has become 'the new developing world disease' (Anon.,1989). Most of the banned or heavily

restricted pesticides produced in the developed world are sent to developing countries where the illiterate farmers hardly understand the hazards associated with the use of such chemicals (Atteh, 1987). From the United States of America (U.S.A) 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. These chemicals however, find their way heavily into the markets of developing countries, which may have dire Consequences on the users (Weir and Schapiro, 1981). Although only 20% of the total world pesticide consumption is in the developing countries where majority of users are illiterates, about half of the poisoning cases and nearly three-quarters of the deaths are estimated to occur in the developing countries. (Oudejans, 1991).

Table 2.3 Fatal pesticide poisoning in Ghana (1986 – 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.

Source: FAO, 1989

These accidents come about from the fact that the users have inadequate knowledge on the proper handling and use of pesticides and improper disposal of containers after use, or sometimes may be 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 residue of such chemicals.

A lot of deaths have occurred due to poisoning from pesticides. The International Organization of Consumer Unions 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).

It is however impossible to obtain reliable statistics on the true extent of human morbidity and mortality due to insecticide use as most of these poisoning cases are not reported and are seldom subjected to laboratory verification (Davies, 1977).

Chronic toxicity is the exposure to repeated non-lethal doses of a potentially harmful chemical. Known responses include silicoses, lung cancer, brain damage and necrosis of the liver or kidneys. Other effects are damage to chromosomes (mutagenic effects) damage to foetus during pregnancy (teratogenic effect) and immunosuppressive effects. (Hassall, 1990; Matthews, 1992).

2.5.2 PESTICIDE RESIDUES IN FOODS

Pesticide residues in food are remnants of a pesticide or its metabolites that can be found in/on a crop after it has been used for pest control purposes (ICAR Extension Folder 59). The small but constant concentrations 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 the chronic toxicity and hazard of pesticides to humans, an array of long term toxicity studies and hazard evaluations are carried out on other smaller mammals like rats, rabbits, or dogs.

The following are certain chronic toxicity properties of some insecticides compiled by Extension Toxicology Network (EXTOXNET) (1993, 1995).

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

Deltamethrin:- Suspected chronic exposure effects in humans include choreoathretosis, hypotension, abortion and shock.

Cypermethrin:- This is a possible human carcinogen. Long term exposure to cypermethrin may also cause liver damage. Pathological changes in the cortex of the thymus, liver, adrenal glands, lungs and skin were observed in rabbits repeatedly fed with cypermethrin.

2.5.3 EFFECTS ON AGRICULTURAL SYSTEMS

Insecticides often disrupt the natural equilibrium between insects, pests and their parasites and predators. The natural enemies are thought 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 the natural enemies. For example, when the cabbage aphid, *Brevicoryne brassicae* was first sprayed commercially, though the initial kill was very high, the destruction of natural enemies resulted in the resurgence of *B. brassicae* outbreak ever seen in England (Ripper, 1956).

2.5.4 DEVELOPMENT OF RESISTANCE

Even before environmental concerns over the widespread use of the 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 completely to control the insect pests. It rapidly became apparent that insects had developed resistance to the organochlorine insecticides (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.

Resistance is exhibited in the field by a progressive inability of a given treatment to achieve control (O'Brien, 1967). When this happens, the insecticide is applied more frequently and in large quantities in an attempt to compensate for its decline in effectiveness. In consequence, further resistance is developed within the insect population until the usefulness of the insecticide is greatly diminished or lost entirely. Insect resistance to insecticides was first reported in the San Jose Scale, *Aspidiotus perniciosus* to lime sulphur (Melander, 1914, Quayle, 1922), and red scale, *Chrysomphalus aurantii* on apples and citrus respectively in U.S.A (Metcalf, 1983).

Insect resistance is a preadaptive phenomenon, i.e. 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 genotypes following the application of an insecticide (Crow 1957; O'Brien, 1967; Sawicki, 1979). The development of resistance proceeds most rapidly under circumstances in which the selection pressure exerted by an insecticide is widespread and continuous (Brown, 1964). Some pesticides lead to the development of resistance more quickly than others, particularly the organochlorine insecticides. The more persistent the

poisons and the more rapid the life cycle of the insect or acarine, the greater the risk that the development of resistance will be rapid (Hassall, 1990).

A survey of cases of resistance through 1980 showed that 428 species of arthropods have developed strains that are resistant to one or more insecticides that were once effective against them (Forgash, 1984) and by 1989 the number has risen to 504 species (Georghiou and Lagunes-Tejeda, 1991) (Table 2.3). About 60% of these are agricultural pests, and many of the others affect human health. The above statistics must be viewed only as indicators of broad trends and probably understate the extent of resistance since many cases have probably either not been investigated or remain unreported (Georghiou and Mellon 1983; Goerghoui and Lagunes-Tejeda, 1991).

Table: 2.4 Comparison of Records of Resistance to Pesticides in Arthropods reported through 1970, 1980, 1989.

	1970	1980	1989	Percentage Increases	
				1971-'80	1981-'89
Species with reported resistance	224	428	504	91.1	17.8
Cases of resistance by pesticide group					
DDT	98	229	263	133.7	14.8
Cyclodienes	140	269	291	92.1	8.2
Organophosphorus	54	200	260	270.4	30.0
Carbamates	3	51	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 pesticide groups	313	829	999	164.9	20.5

Source: Georghiou and Mellon (1983); Georghiou and Lagunes-Tejeda, (1991).

2.6 MECHANISMS OF INSECTICIDE RESISTANCE

Insecticide resistance is a dynamic, multidimensional phenomenon, which is dependent on biochemical, physiological, genetic, and ecological nature of the insect (Brattsten *et al.*, 1985). Considerable information on the biochemistry and genetics of resistance had been summarized by Busvine (1971), Matsumara (1985), Oppenoorth (1985) and Georghiou (1986). They identified resistance mechanisms to include reduced cuticular penetration of toxicants, enhanced metabolism by cytochrome P₄₅₀-dependent monooxygenases, hydrolases, or glutathione-S-transferases, and reduced sensitivity of mutant acetylcholinesterases to organophosphorus insecticides and carbamates and of other neuronal targets to pyrethroids, DDT and its analogues and chlorinated cyclodienes (Oppenoorth, 1985).

2.6.1. *Metabolic detoxification:* This is the most studied mechanism and refers to the process by which compounds are converted to harmless readily excretable metabolites thereby greatly decreasing their biochemical activity or toxicity (Wilkinson, 1983). Biochemical reactions leading to detoxification include oxidation, hydrolysis, dealkylation and dehydrochlorination brought about by several enzyme systems including mixed-function oxidases (MFO), esterases, glutathione S-transferases and DDT-dehydrochlorinase.

Mixed-Function Oxygenases (MFO). These are located in the microsomal portions of several tissues particularly the liver and shows substrate non-specificity. Microsomal enzymes have evolved as protective mechanism against naturally occurring toxicants such as nicotine, rotenone, and natural pyrethrins (Yamamoto *et al.*, 1969; Elliot *et al.*, 1972). Esterases are particularly important in degrading organophosphates. The larva of mosquito, *Culex tarsalis* was found to have higher carboxylesterase activity than susceptible counterparts (Matsumara and Brown, 1961).

Glutathione S-transferase plays a primary role in the resistance of organophosphate compounds (Tanaka *et al.*, 1981). Several workers have demonstrated glutathione dependent degradation of parathion and diazinon in resistant houseflies (Lewis 1969; 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 (Brattsten *et al.*, 1985).

Sequestration of synthetic insecticides can be more important in resistant development than appreciated (Brattsten *et al.*, 1985). The esterase responsible for resistance development in the green peach aphid, *Myzus persicae* (has high binding affinity but low catalytic reactivity and therefore functions as a storage protein for carbamates, organophosphates and pyrethroids. Toxic plant allelochemicals are frequently sequestered.

2.6.2 Target site insensitivity : Compared with a number of cases in metabolic resistance mechanisms, relatively few types of resistance are caused by a modified target site. Some arthropods develop resistance by altering the properties of the target site of action of a given insecticide. Target site insensitivity as a mechanism has been demonstrated in spotted spider mite, *Tetranychus urticae* (Smitsaert, 1964), leaf hopper, *Nephotettix cincticeps* (Iwata and Hama, 1972), and in numerous resistant insect species with cross-resistance to cyclodienes (Hama, 1983). The best example of an altered target as a cause of resistance to insecticides is that of acetyl cholinesterases with reduced sensitivity to organophosphorus insecticides and carbamates. A single gene has been shown to be responsible for the difference in acetyl cholinesterase sensitivity and the resistance caused by it in many arthropods (Oppenoorth, 1985). A major factor, *kdr* (knockdown resistance) located on chromosome 111 in houseflies which confers resistance to DDT and pyrethroids through target insensitivity mechanism has been demonstrated (Sawicki, 1973).

2.6.3. Alternative biochemical pathway. Another type of resistance mechanism is one that provides an alternative biochemical pathway to the one blocked by an insecticide. The blocked site is bypassed by the use of alternative pathway. Thus the California red scale, *Aonidiella aurantii*, became resistant due to alternative pathway for the terminal stages of cellular respiration and were no longer dependent upon enzyme systems disabled by cyanide.

2.6.4. Changes in behavior as a resistance mechanism. The mechanism of resistance may be strictly behavioral. For example, in the Mazoe valley of southern Zimbabwe, Murhead-Thomson (1960) found that *Anopheles* mosquitoes originally were abundant both outdoors and in human dwellings. The indoor mosquitoes virtually disappear from both treated and untreated dwellings after eight years of BHC treatments in dwellings and this condition persisted even after insecticidal treatments were discontinued. During the period of insecticide treatment, a strain evolved that lived and fed mainly out of doors.

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 by Gerold and Laarman (1964, 1967) for mosquitoes.

2.6.5. Economic implications of insecticide resistance. Insecticide resistance has given rise to a series of complex and interrelated economic and sociological problems that offer great challenges to mankind. The low cost of the old arsenicals and organochlorines made it possible for growers to apply these insecticides as inexpensive forms of crop insurance which created the pervasive philosophy of multiple applications that led to the 'pesticide treadmill' of cotton growers with many applications of 50-60 times in a single growing season (Flint *et al.*, 1981). Presently, however, it has become increasingly difficult and costly to discover and develop new insecticides, the cost rising from less than \$10 million in 1970 to over

\$20 million in 1980 (Braunholtz, 1981). The number of chemical compounds screened to obtain one commercial success has also risen. The accumulated impacts of these costs coupled with global inflation have resulted in exponential increases in the developmental cost of insecticides. The cost of resistance is exhibited at the farm level in terms of more frequent applications, higher dosages and changes to new and more expensive compounds. There is high risk of applicator and agricultural worker exposure from high rates of pesticide application, disruption of ecologically sound pest control strategies, increased incidence in human, animal, and plant diseases in whose transmission depends on insect vectors. And in the most extreme cases, the complete destruction of agricultural production systems on a local or regional basis can occur.

2.7. Resistance in Diamondback moth (DBM), P. xylostella

Insecticide resistance in diamondback moth (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 (Tabashnik *et al.*, 1987), Japan and Australia (Kao *et al.*, 1989). 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 *P.xylostella* larvae covers all major groups of insecticides i.e. chlorinated hydrocarbons, organophosphorus insecticides, carbamates, pyrethroids (Sun *et al.*, 1986; Lin, 1988) and even *Bacillus thuringiensis* (Kao *et al.*, 1989).

Resistance mechanisms in Diamondback moth proposed for synthetic chemicals include decreased penetration (Noppun *et al.*, 1987), enhanced detoxication by esterases (Maa and Chuang 1983) and glutathion S-transferase and reduced sensitivity of acetylcholinesterases (Wu, 1983). Resistance to some insecticides is known to be associated with high levels of carboxylesterase (Doichuangam and Thornhill, 1989; Maa *et al.*, 1983). The assumption in such cases has usually been that the insecticide is inactivated by hydrolysis. (Doichuangam and Thornhill, 1989).

2.8.MANAGEMENT OF RESISTANCE

It has now become increasingly difficult to develop new insecticides due to rising costs, (Miyata *et al.*, 1985). The number of chemical compounds screened to obtain one commercial success has also risen tremendously over the years (Braunholtz, 1981). The accumulated impacts of these costs coupled with global inflation have resulted in exponential increases in the developmental cost of insecticides.

The development of integrated pest management is the best way of circumventing the development of resistance (Philip *et al.*, 1980; Menn and Hollingworth, 1985). The evolution of resistance according to Georghiou (1983) is determined by genetic, biological and operational factors.

Insecticide management to delay resistance development can be achieved by:

- 1. Choosing insecticides with short persistence.**
- 2. Choosing insecticides to which the pest is not cross-resistant.**
- 3. Avoiding slow release formulations.**
- 4. Applying insecticides to a vulnerable developmental stage and under environmental conditions that render the insects susceptible and the insecticide effective.**
- 5. Using insecticides intermittently at the minimum dosage.**
- 6. Restricting insecticides to local use.**
- 7. Substituting broadcast for placement applications where feasible, and using unrelated insecticides in rotations or in combination with other chemical and non-chemical measures and with synergists, which counteract the insects' defense mechanisms.**

Georghiou (1983) grouped all strategies for resistance management into three categories, namely:

- 1. Management by moderation-** an approach recognizing that susceptible genes are a valuable resource that must be preserved in an attempt to reduce selection pressure.
- 2. Management by saturation-**, which implies the use of high dosages to annihilate the entire population in situations where high value crops or insect vectors of disease are involved. A high dosage of rapidly biodegradable compound such as fumigants, bacterial toxins

and juvenile hormone mimics can saturate the defense mechanism of the insect.

3. Management by multiple attack that aims at achieving control through the action of independent acting forces achieved through the use of insecticides in mixtures and in rotations. 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.

CHAPTER THREE

3.0. STUDIES ON THE BIOLOGY OF DIAMONDBACK MOTH

P. XYLOSTELLA (L.) (LEPIDOPTERA: PLUTELLIDAE)

3.1 INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.) is one of the many important lepidopterous insect pests of cultivated vegetables. This insect is widely distributed and is a serious pest of cruciferous crops in many parts of the world (Salinas, 1977). It has been recorded beyond latitude 60° N including the temperate zones and the tropics (Ooi, 1986). It therefore has the ability to survive in a wide range of temperatures.

The adaptability of the insect to different climatic conditions and its recognised status as a major pest in both temperate and tropical regions make the study of the biology of DBM important from the economic as well as the biological points of view.

DBM is an oligophagous insect and will feed only on plants with mustard glucosides (Thorsteinson, 1953 in Ooi, 1986). (Plate 1) An important economic group of plants with these glucosides are members of the family Cruciferae, which are essentially temperate climate crops. However, these crucifers, in particular the genus *Brassica*, have spread (from their original home) to other regions of the world. It is thus very likely that DBM spread along with the

spread of these crucifers (Ooi, 1986).

Although a lot of work has been done on the biology and other aspects of this insect in other countries, little documentation is available on the pest in Ghana. The focus of this work is to study the biology of the insect under laboratory conditions and report on its important parameters that are of great concern to pest managers.

3.2.0 MATERIALS AND METHODS

3.2.1 *LIFE HISTORY OF DBM*

Investigations on the biology of DBM were carried out in the Entomology/Food Security laboratory of the Department of Zoology, University of Ghana. The temperature during the period of study was 28 ± 1 °C with relative humidity of 65-70% and photoperiod of L: D 12:12. Initial culture of the moth was raised for the study from caterpillars collected from cabbage plants at the University of Ghana farm. Cabbage leaves were used for rearing the larvae. After pupal emergence, adults were transferred to plastic containers measuring 8.5 x 4.5 cm with perforated lids, which contained cabbage leaves. Because the adults don't feed on the cabbage leaves, they were provided with 10% sugar solution. Cabbage leaves were provided for egg laying and were replaced daily. Leaves with eggs were transferred to separate containers for studies on period of incubation. The number of eggs laid by females was recorded. Oviposition was observed for 4 days.

Newly hatched larvae were transferred to new containers with cabbage leaves. First and second instar larvae were observed for moulting. Matured larvae, i.e. 3rd and 4th instar larvae were kept in separate containers for pupation.

For duration of life cycle, ten newly hatched larvae were transferred into plastic containers provided with food. Three replications were kept. Fresh food (cabbage leaves) was provided daily. Data was recorded on larval and pupal development as well as adult emergence. Male and female longevity was also recorded. Ten percent (10%) glucose solution was provided as food to adult insects. For measurements of the length and wing expanse, five samples of each stage were taken and measurements made on a millimetre graph paper under the microscope.

3.2.2 RESULTS AND DISCUSSION

OVIPOSITION

Oviposition took place during the night. The eggs were generally laid singly or in groups of two (< 0.5mm in size) on the leaves along the midrib or the other major veins. However, because of confinement females did not follow any definite pattern of egg laying. Eggs were indiscriminately laid on the walls of the rearing container. Abro *et al.* (1992) also reported a similar behaviour of *P. xylostella* females under laboratory conditions. The average number of eggs laid by females during a four-day period was 145. It was observed that about 59% of the eggs were laid on the first night of oviposition, 29% on the second day, 9% the on third day and 3% on the fourth day. Working on *Plutella*

maculipennis (Curt.), Moller (1988) observed that the largest number of eggs was deposited on first night and the rate of oviposition declined steadily.

The pre-oviposition, oviposition and post-oviposition periods (which are the periods before the onset of egg laying, onset of egg laying and after egg laying, respectively) averaged 2.5, 3.5 and 6.5 days respectively (Table 3.1).

EGGS

The eggs were minute yellowish; oblong (oval) in shape with average length (estimated) of < 0.5 mm. Mean incubation period was 3.50 days, the range being 3-4 days. Before hatching, the colour of the egg changed from yellow to orange, or yellowish-brown.

LARVAE

The freshly hatched larvae were whitish to pale green with a light brown head. On average they measured about 1.4 mm. Under field conditions freshly hatched first instar larvae are found mining the leaf and feeding inside on the mesophyll tissues from the lower surface. After moulting to second instar larvae they become surface feeders and fed from the lower epidermis of the leaves and skeletonize it, leaving behind a transparent upper epidermis and veins. Similar observations made under laboratory conditions also confirmed this behaviour. The first instar larvae were found buried inside (mining) leaf tissues for the first 2-3 days and after that they moulted to second instar and came out of the leaf mines. Wu (1968) made similar observations on the feeding behaviour of *P. xylostella* in Taiwan.

Table 3.1. Life cycle of DBM in the laboratory.

Developmental stage	Duration (days)	
	Mean	Range
Pre-oviposition	2.5	2-3
Oviposition period	3.5	3-4
Incubation (eggs)	3.5	3-4
Larval periods		
1 st instar	2.5	2-3
2 nd instar	2.5	2-3
3 rd instar	2.5	2-3
4 th instar	1.5	1-2
Pupal period	5.0	4-6
Adult longevity	10.5	10-11
Life cycle Duration	17.5	14-21

Table 3.2. Body measurements of the different stages of DBM

Developmental stage	Length (mm)		Breadth/ wingspan (mm)	
	Range	Mean	Range	Mean
Egg	< 0.50		< 0.10	
Larval stage				
1 st instar	1.20-1.30	1.25	0.18-0.25	0.21
2 nd instar	4.5-6.4	5.40	0.18-0.25	0.24
3 rd instar	6.50-9.50	7.75	1.00-1.20	1.10
4 th instar	7-8.50	8.00	1.30-1.60	1.45
Pupal	4.5-5.0	4.75	2.50-3.20	3.85
Adult				
Male	4.5-5.0	4.75	12.00-13.00	12.50
Female	4.5-5.5	5.00	12.00-13.50	12.75

The larvae underwent three distinct moulting resulting in four instar stages (Table 3.2). A full-grown larva averaged 8.5 mm in length and was green (Plate 1), slightly stout and smooth with short scattered black hairs. These hairs appear as black spots on the segments of the 1st and 2nd instar larvae. The total average larval period was 7.5 days with range of 7-8 days. The average body length for 1st, 2nd, and 3rd instar larvae were 1.35, 3.15, and 5.50 mm respectively. (Table 3. 2).

PUPA

The mature caterpillar formed gauzy loosely spun cocoon. It afterwards shortened its body longitudinally but remained active. The newly pupated organism was yellowish green, but changed to brown after 2-3 days and gradually attained dark brown colour by the time of adult emergence. The mean length of the pupa was 4.75mm. The average pupal period lasted 5.0 days.

ADULTS

The male moths were slender and dark brown with whitish back, while the female moths were greyish brown back (Plate 1) . The male measured 4.8-5.0mm in length and 12.62mm in wing expanse and the female 4.5-5.5mm in length and 12.50-13mm in wing expanse (Table 3.2). The average longevity of the adult insect was 10.5 days. The pre-oviposition (period between emergence and egg laying), oviposition period and post-oviposition (end of egg laying and death) periods averaged 3.5 days (3-4 days), 5.7days (5-7 days) and 6.5days (5-8 days).

The total period for one generation, ₃₄ which is from egg to adults, was 14 –21

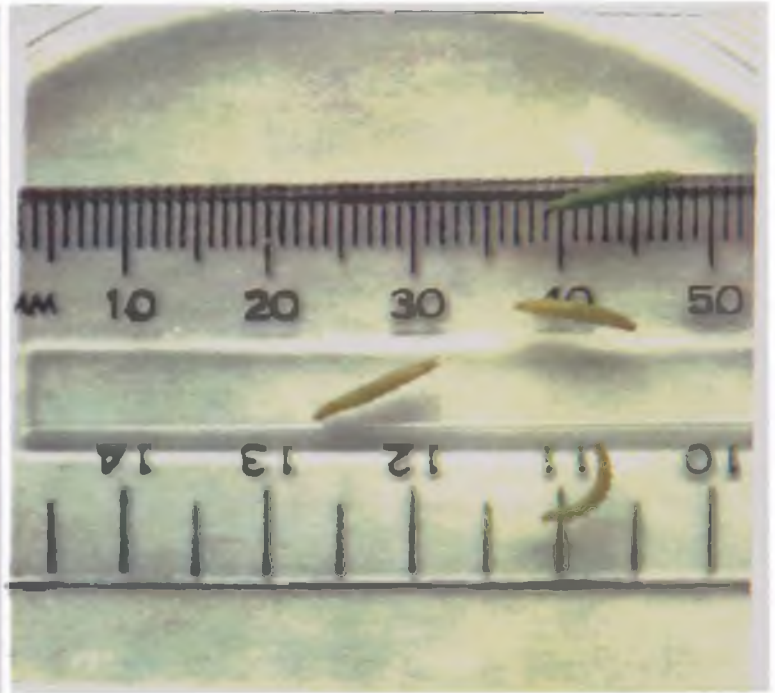
days.

The incubation period for the eggs lasted 3–4 days. The 1st and 2nd instar larvae took 2 days each to moult into the next developmental stage. The 3rd instar larvae also took 2 days to mature into the 4th instar larvae, which in turn took 1–2 days before moulting into pupae (Table 3.1).

Plate 1. The Larval, Pupal and Adult Stages of *P. xylostella*, and damage done to Cabbage.



1A. Damage done to Cabbage leaves by larvae *P. xylostella*



1B. Larvae of *P. xylostella*



1D. Adult of *P. xylostella*



1C. Pupae of *P. xylostella*

CHAPTER FOUR

4.0. INDIVIDUAL VARIATION OF CARBOXYLESTERASE ACTIVITY IN FIELDS POPULATIONS OF *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE)

4.1. INTRODUCTION

Cruciferous vegetables especially cabbages are economically very important to vegetable growers. These farmers use intensive agronomic practices because the sale of these vegetables provides an important source of ready income. However, cabbage production around Accra in recent years has been seriously affected by a steady increase in pest deprecations.

Cabbage also provides an easily accessible food source for DBM as well as for other pests, as a result of which these crops suffer deprecation throughout the year (Sakai, 1981). Also due to its rapid generation turnover and short lifespan DBM has become the chief insect pest of crucifers. During the dry season, *P. xylostella*, can cause over 90% yield loss while during the wet season yield loss can be over 30%. (Suderwohadi and Eveleens, 1977).

Since the demand for vegetables in terms of quality and quantity is ever increasing, farmers tend to take an 'insurance approach' (Ooi *et al.*, 1978) to reduce risk of crop loss due to pests. Thus, insecticides are considered the most effective means of protecting their crops against insect damage as they provide rapid control of whole pest complexes of major cruciferous pests.

Usually farmers use large quantities of chemicals, often spraying 'cocktail' of compounds. When 'cocktails' are used or tank combinations of two or more chemicals are used, these compounds are often used at the dosages recommended for each compound individually, so that the actual application rate is doubled or triple the recommended rate (Magallona, 1986). As a consequence of frequent insecticide use, DBM has developed resistance to practically all categories of insecticides (Sudarwohadi *et al.*, 1977). This is the bane of scientists, because farmers are faced with increased of pesticide use and subsequent input cost. The pest also exhibits cross-resistance to various conventional insecticides. (Teh *et al.*, 1978).

Although a more integrated approach towards the management of this pest has been recommended by several workers (Lim, 1974; Ooi and Sudderuddin, 1978; Beck and Cameron, 1990), the practicability and success of such an approach have not been convincingly demonstrated (Lim *et al.*, 1986). The first properly documented case of insecticide resistance in DBM to DDT was noted in Lembang, Java by Ankersmit (1953). Since then, conventional insecticides have become obsolete due to the development of resistance to these insecticides. DBM has developed high levels of resistance to various insecticides (Hama, 1987). These include organophosphorus insecticides (Noppun *et al.*, 1986), carbamates (Sun *et al.*, 1978), synthetic pyrethroids (Liu *et al.*, 1981; Hama, 1986; Horikiri and Mikano, 1987) and *Bacillus thuringiensis* Berliner preparations (Tanaka and Kimura, 1990). It has been demonstrated that resistance by *Myzus persicae* to organophosphorus and carbamate

insecticides are due mainly to an increase in the activity of carboxylesterases which hydrolyses and sequestrates these insecticides to avoid their interaction with the site of action, i.e. acetylcholinesterase receptors. Insect esterases perform both physiological and defensive functions and are found in both soluble and membrane-bound forms (Maa *et al.*, 1990). Among insect species, the carboxylesterase of green peach aphid (Sawicki, 1979) *Culex* mosquitoes (Georghiou and Pasteur, 1980), Housefly (Oppenoorth 1965) and leafhoppers (Ozaki and Kassai, 1970) have been studied extensively because of their involvement in resistance to insecticides (Maa *et al.*, 1990)

High activity levels of carboxylesterase have been confirmed to be positively linked to resistance in aphids (Devonshire, 1975; Devonshire & Moore, 1982; Hama and Hosoda, 1988; O'Brien *et al.*, 1992; Owusu *et al.*, 1995, Owusu *et al.*, 1996b), *Culex tarsalis* (Matsumura & Brown 1961, 1963), Houseflies (Niwa *et al.*, 1977) and *P. xylostella* (Doichuanngam & Thornhill, 1989).

Based on the above information, the present work was to test for resistance development in Ghanaian populations of *P. xylostella* by measuring the carboxylesterase activity of the insect, and to relate this information to the resistance of *P. xylostella* to insecticides.

4.2. MATERIALS AND METHOD

4.2.1 CHEMICALS: All the chemicals used were of the highest grade commercially available. Sodium carbonate anhydrous, disodium hydrogen phosphate, sodium hydroxide sodium tartrate, potassium phosphate, α -Naphthol, α -Naphthol acetate, β -Naphthol acetate, Sodium dodecylsulphate

(SDS), Folin-Ciocalteu reagent (Phenol reagent), Azoic diazoblu e B salt (Fast blue B salt/FBS), copper sulphate were purchased from SIGMA chemical industries Ltd, Japan.

4.2.2 SOLUTION PREPARATION

4.2.2.1 *ENZYME ASSAY*

- a. 0.558 g α -naphthyl acetate (MW 186.21) 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) was dissolved in 100 ml of absolute ethanol to make a solution with a concentration of 30mM as above.
- c. 5 g Sodium dodecyl sulphate (SDS) (MW 288.38) was dissolved in 100 ml distilled water in a beaker to give 5 % solution. 0.1g of fast blue B salt (MW 636.930) was dissolved in 10 ml of distilled water to make 1 % fast blue B salt solution. These two solutions were mixed in the ratio of 5:2 (a mixture of 2 parts of a 1 % solution of diazo blue B and 5 parts of a 5 % solution of sodium dodecylsulphate) by volume and stored in the dark one hour before the assay.
- d. 10 g sodium carbonate (MW 105.99) and 2g sodium hydroxide (MW 40) were dissolved in 500 ml distilled water. This gave 2 % sodium carbonate in 0.1M NaOH, which was labelled Reagent A.
- e. 11.933 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (MW 358.14) and 4.533 g KH_2PO_4 . (MW 136.09) were each dissolved in 500ml distilled water. These were mixed in a ratio of 6:4 (Na_2HPO_4 : KH_2PO_4) by volume. This gave 0.07M-phosphate buffer (pH 7.2).

4.2.2.2. PROTEIN ASSAY

Reagent A: This was prepared by dissolving 10 g of sodium carbonate and 2 g sodium hydroxide in 500 ml distilled water. This was replaced at the end of every month.

4.2.2.3. OTHER STOCK SOLUTIONS

a. 5 g CuSO_4 (MW 223.13) were dissolved in 500 ml distilled water in a beaker to obtain 1 % CuSO_4 solution

b. 5.925 g of sodium tartrate were dissolved in 500 ml of distilled water in a beaker to obtain 1 % sodium tartrate solution.

Reagent B: This was prepared by adding 50 ml of Reagent A (as described in preceding section) to 0.5 ml each of 1 % CuSO_4 and 1 % sodium tartrate, to give a ratio of 50:1. This mixing was done just before the start of assay.

Phenol Reagent : water (1:1) by volume was prepared by mixing equal volumes of phenol and distilled water. This was done just before the assay started because of the instability of the mixture. With exception of SDS and Phenol reagent, all stock solutions were stored at 5 °C in a refrigerator.

4.2.3 . INSECTS

Fourth instar larvae of diamondback moth, *Plutella xylostella* were collected from various cabbage fields around the Greater Accra area. Samples were collected into plastic containers using fine camel hairbrush and sent to the Zoology Department laboratory, University of Ghana. Larvae were stored at – 10 °C until ready for use.

Samples from the various populations were assayed for carboxylesterase activity by enzyme-naphthyl acetate-diazo blue coupling reaction (Owusu, 1992). Storage for several days was possible without a significant loss of the esterase activity (Zhu and Brindley, 1990).

4.2.4. ENZYME PREPARATION AND ASSAY

Individual larvae of *P. xylostella* were homogenized in 0.3 ml of potassium phosphate buffer (pH 7.2) using a glass well. The resultant solution was then used as enzyme source for carboxylesterase assay.

The carboxylesterase assay was based on the original method developed for Housefly esterase (van Asperen, 1962) as adopted for cotton aphid carboxylesterase (Owusu et al., 1993). A typical reaction mixture consisted of incubating 0.1 ml of enzyme with 0.1 ml of 30 mM α -naphthyl acetate in absolute ethanol for 10 min. at 40 °C in 2.8 ml of phosphate buffer (pH 7.2) in a shaking water bath. After incubation 0.5 ml solution mixture of sodium dodecyl sulphate-Fast blue B salt (SDS-FBS) was added for colour development and read at 600 nm on a spectrophotometer against a control that lacked enzyme. Enzyme solution was also incubated for 10 min. at 40 °C with β -naphthyl acetate in absolute ethanol as substrate. Colour development after incubation was as above with SDS-FBS, and read at 450 nm on the spectrophotometer against a control that lacked enzyme. For individuals with higher activity above the measurable range, the homogenate was appropriately diluted with phosphate buffer before measurement.

4.2.5. ESTABLISHMENT OF STANDARD CURVES

0.186g α -Naphthol (MW 144) was dissolved in 100 ml absolute ethanol to obtain a final concentration of 10 nmol/l for α -naphthol. Each test tube containing 2.8 ml of 0.07M phosphate buffer pH 7.2, received 0.1 ml each of the prepared α -naphthol solutions. A half ml of diazoblue-SDS solution (a mixture of parts of a 1% solution of diazoblue B and 5 parts of a 5% solution of SDS) was added to each test tube for colour development. The absorbances were measured on the Milton Roy spectrophotometer at 600 nm against a blank consisting of 2.8 ml phosphate buffer, 0.1 ml ethanol and 0.5 ml diazoblue-SDS solution. The absorbance of the sample measurements was then finally converted to nano-moles of α -Naphthol produced by reference to the standard curve.

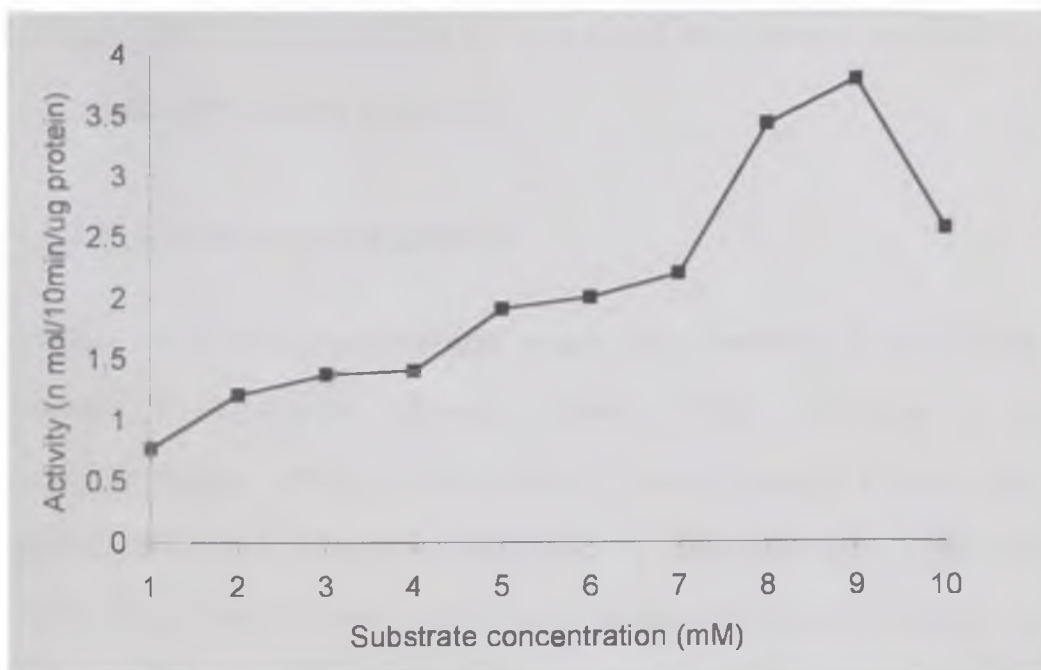


Fig.4.9 Standard curve for the hydrolysis of 1-Naphthol by carboxylesterase

4.2.6. PROTEIN DETERMINATION

Protein contents of all enzyme preparations used were determined by the method of Lowry *et al.* (1951) with slight modifications, as adopted by Owusu (1994).

0.1 ml portion of the homogenized enzyme solution was put into a test tube and 2 ml of Reagent B added to it. This mixture was allowed to stand for 20-30 minutes. 0.25 ml of the diluted phenol reagent was added and the reaction mixture allowed to stand for further 20 min. This ensures a blue colour development. Readings were taken on a spectrophotometer at 750 nm (single beam) against a control that lacked enzyme.

Enzyme activity was expressed as nano-moles of α -naphthol produced per microgram protein during 10 minutes.

4.3.0. RESULTS AND DISCUSSION

Varying levels of carboxylesterase activity were observed in the different populations of *Plutella xylostella*. Studied. These variations in the carboxylesterase activity in Diamondback moth populations may reflect insecticide resistance levels as suggested by (Doichuangam & Thornhill, 1989). The results may also reflect the trends and intensity of insecticide use patterns on vegetables (crucifers) farms.

The highest levels of carboxylesterase activity were observed in the populations at Dzorwulu, Tuba and Mallam Atta (Fig. 4.6, 4.2. and 4.3, respectively) and the lowest were observed in the samples collected from Crop Science, G.B.C. and Weija (Table 4.2)

The populations from Dzorwulu and Tuba showed an activity ranging from 13.43 to 44.34 n mol/ min / μ g protein and 9.38 to 73.55 n mol/ min/ μ g protein, respectively. The population from Kumoji Road (cantonments) showed the largest range from 2.88 to 98.46 n mnol/ min/ μ g protein. Despite this large range, the population can be described as moderately resistant with respect to carboxylesterase activity. It had an average activity <50 n mol/ min/ μ g protein, except for one individual insect that showed an abnormal activity more than

Table 4.1 Key to resistance classification (Owusu in press)

Colour intensity (Filter paper- Visual)	Absorbance (nm)	Carboxylesterase(κ) nmol/min/ μ g protein	Resistance Level
---	0 \approx 0.09	$\kappa < 5.63$	Highly susceptible
+	0.06 --- 0.18	$5.63 \leq \kappa < 12.00$	Susceptible
++	0.18 — 0.50	$12.00 \leq \kappa < 32.00$	Moderately resistant
+++	0.30 — 0.95	$32.00 \leq \kappa < 60.00$	Resistant
++++	0.65 \geq 1.50	$\kappa \geq 60.00$	Highly resistant

--- = no activity + = low activity ++ = medium activity
 +++ = highly activity ++++ = very high activity

Table 4.2 Average α -Naphthyl acetate and β -Naphthyl acetate linked carboxylesterase activity of *P. xylostella* at eight locations around Accra.

Population/ Location	Insecticide history	Carboxylesterase activity(\pm S.E) (nmol/min/ μ g protein)		Resistance classification ¹
		α -Na	β -Na	
Crop Science (Legon)	Fenvalerate, Perfekthion, Karate	4.11 \pm 2.03	1.73 \pm 0.66	Highly susceptible
Kumoji Road (Cantonments)	Karate, Dursban, Biobit, Dipel	28.29 \pm 26.61	31.91 \pm 24.49	Moderately resistance
G.B.C. (Ring Road)	DDT, Decis, Biobit	2.06 \pm 0.53	3.88 \pm 1.35	Highly susceptible
Dzorwulu	Biobit, Neem seed extract, Decis, Karate	22.21 \pm 10.71	7.96 \pm 2.92	Moderately Resistance
Weija	Perfekthion, Karate, neem seed extract	8.97 \pm 6.33	3.14 \pm 1.22	Susceptible
Tuba	Dursban, Dipel, Karate	27.85 \pm 19.21	36.09 \pm 19.23	Moderately susceptible
Tutu	Dursban, Dipel, Karate	10.19 \pm 3.08	3.92 \pm 0.97	Susceptible
Mallam Atta	Decis, Perfekthion	17.23 \pm 6.76	6.07 \pm 1.69	Moderately susceptible

¹ Refer to Table 4.1

α -Na= α -Naphthyl acetate

β -Na= β -Naphthyl acetate

the rest of the other individuals in the sample, with an activity of 98.46 n mol/min/ μ g protein and a frequency of >30 %. The reason for this could be due to migration of other individuals from other cruciferous vegetables grown within the same farm, which might have become highly resistant due to the frequent exposure to insecticides on these crops.

Contrarily, samples from the other populations collected from Dzorwulu and Crop Science Department (University of Ghana) showed occasionally individuals with high frequency of carboxylesterase activity. Although some individuals were sometimes detected with high carboxylesterase activity from these other locations, these occurrences were at very low frequency.

The high carboxylesterase activity levels observed at Dzorwulu and Tuba may be due to the fact that the populations have been subjected to high and intensive insecticide pressure at irregular but frequent intervals. The major factors accounting for the pattern of insecticide use are likely to be the ever-increasing high demand for vegetables in terms of quantity and quality. The farmers thus tend to take an 'insurance approach' to reduce risk of crop loss due to pest by using chemicals at very short and irregular intervals. In addition, there may be long history of insecticide use in these areas consequently exposing the pests to chemicals especially organophosphorus insecticides and carbamates, over a long period of time- a pre-requisite for development of insecticide resistance.

Another factor may be the type of chemicals used mostly DDT, organophosphorus insecticides and carbamate insecticides to which *P. xylostella* has developed resistance.

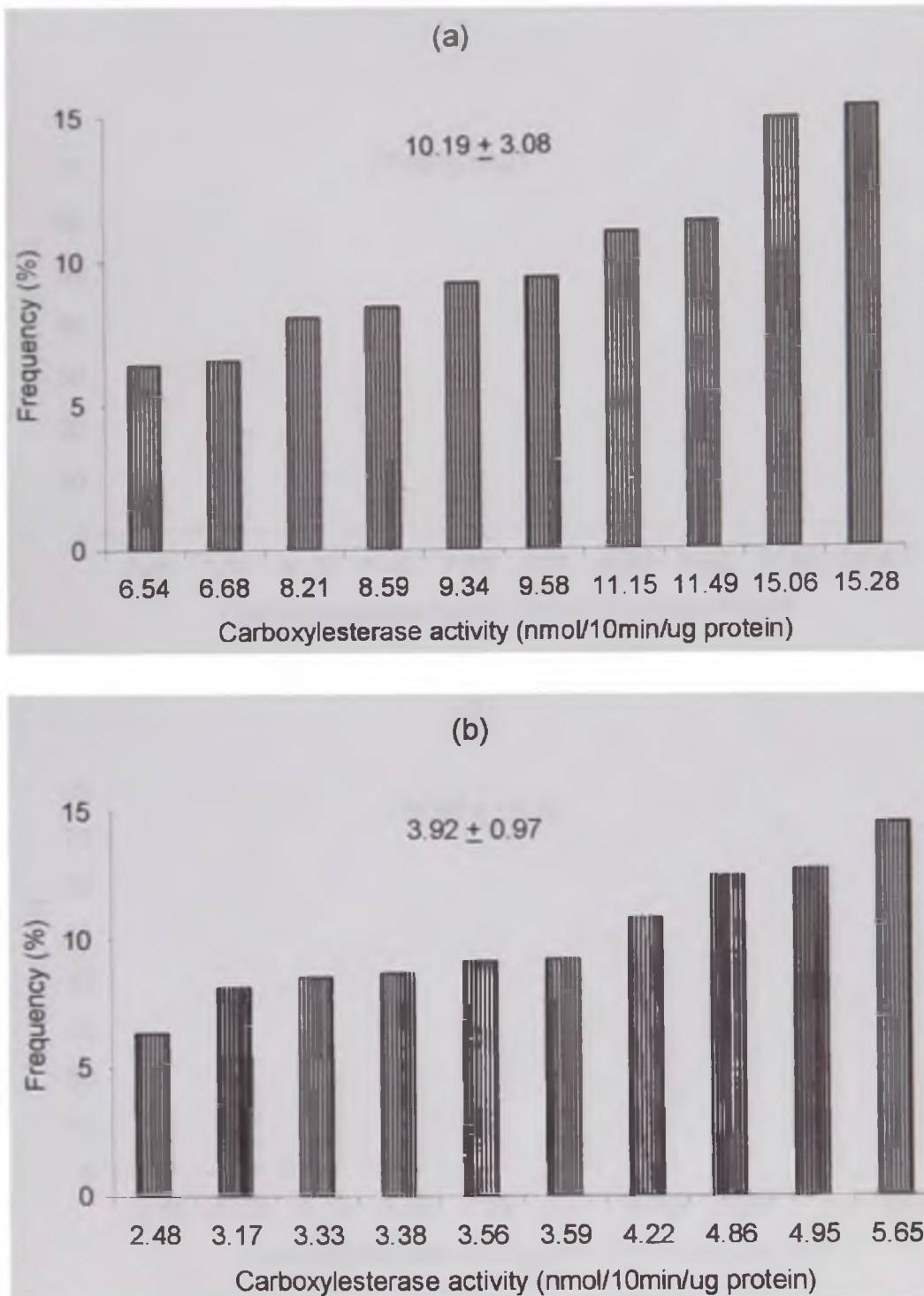


Fig 4.1 Frequency distribution of individual carboxylesterase activity in *P. xylostella* larvae collected from cabbage plants at Tutu. Values shown are averages with standard deviation of the activity. (a) 1 - Naphthyl acetate as substrate. (b) 2 - Naphthyl acetate as substrate

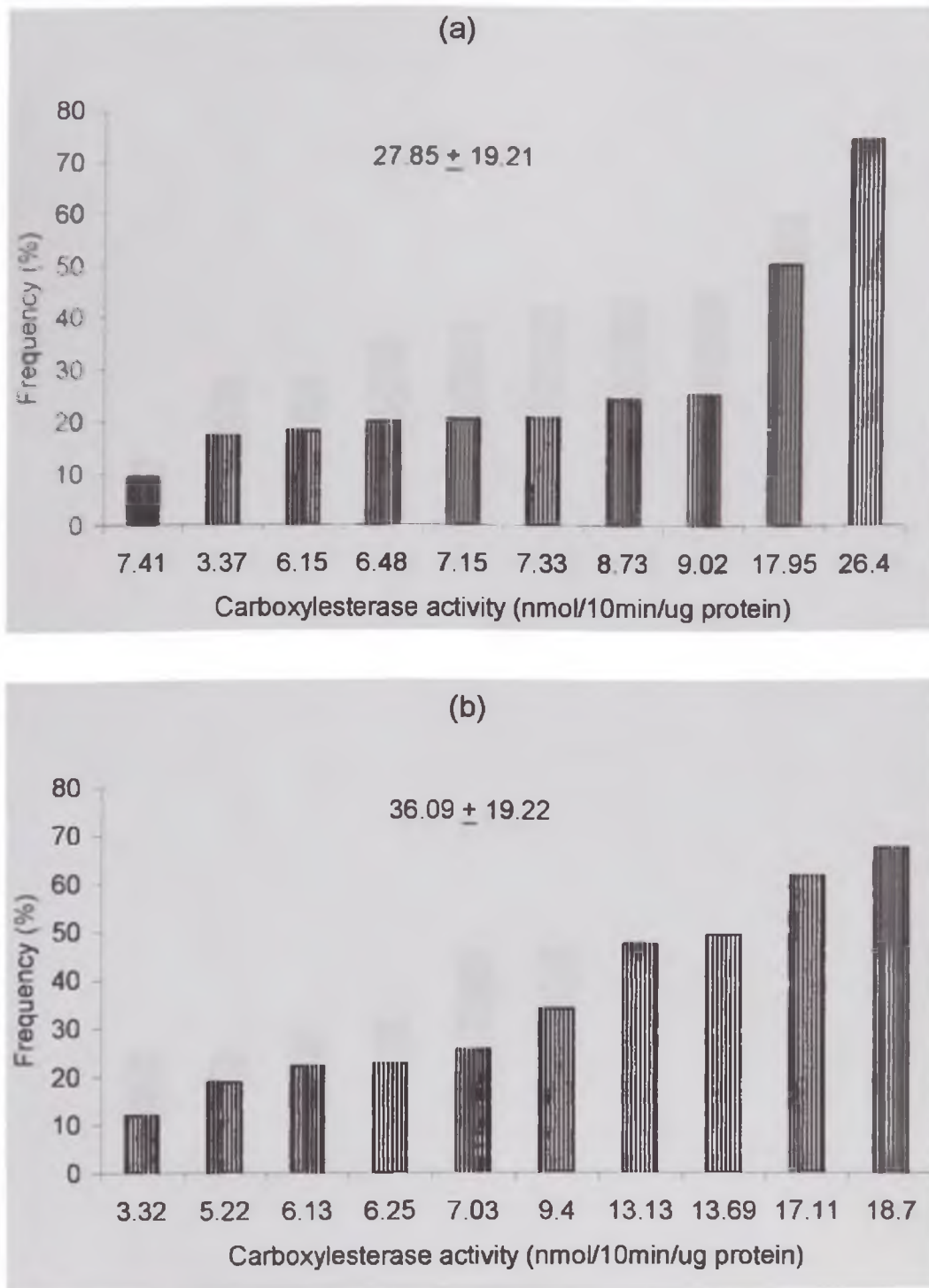


Fig 4.2 Frequency distribution of individual carboxylesterase activity in *P. xylostella* larvae collected from cabbage plants at Tuba. Values shown are averages with standard deviation of the activity.

(a) 1 - Naphthyl acetate as substrate. (b) 2 - Naphthyl acetate as substrate

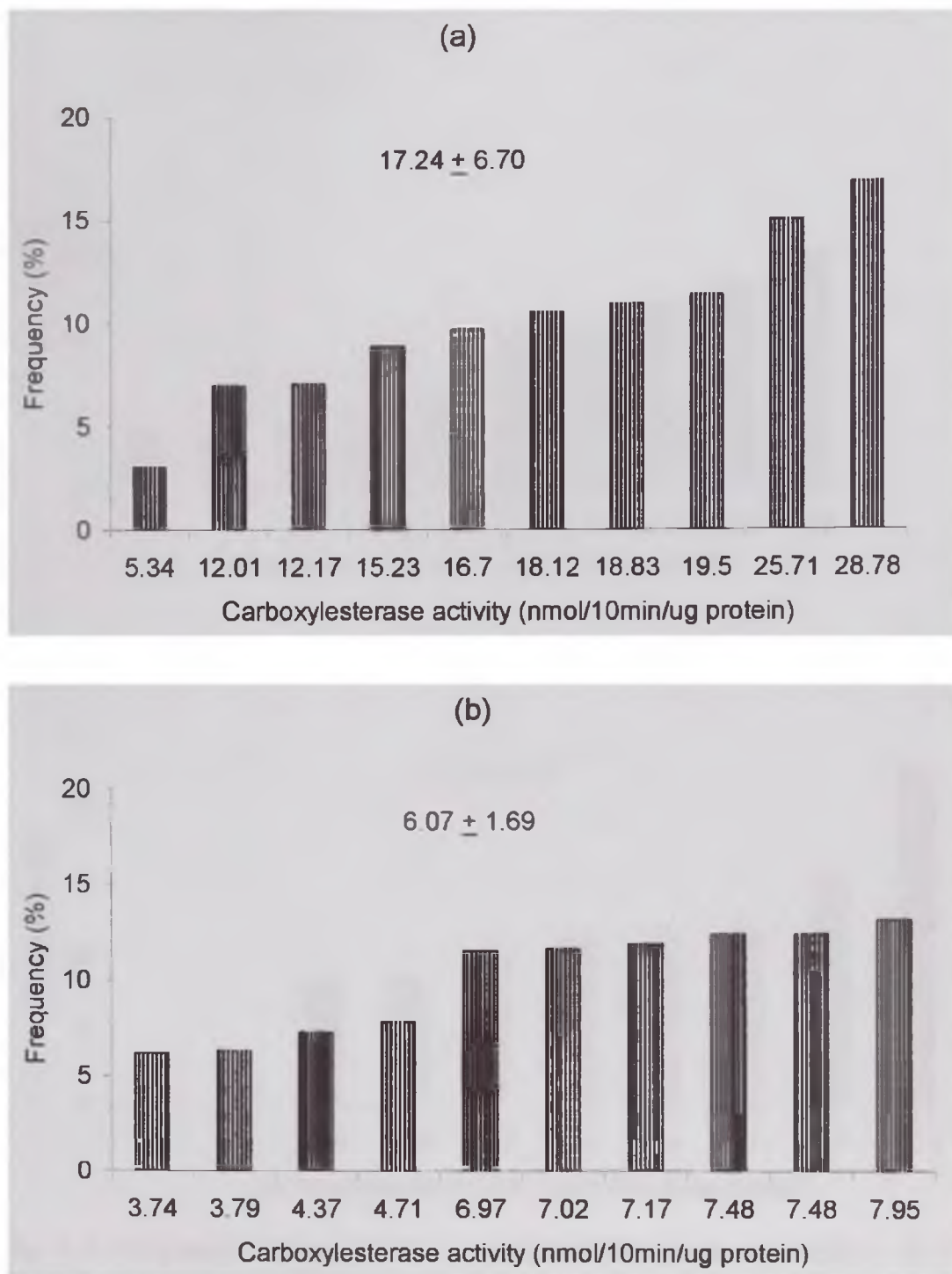


Fig 4.3 Frequency distribution of individual carboxylesterase activity in *P. xylostella* larvae collected from cabbage plants at Mallam Atta. Values shown are averages with standard deviation of the activity. (a) 1 - Naphthyl acetate as substrate. (b) 2 - Naphthyl acetate as substrate

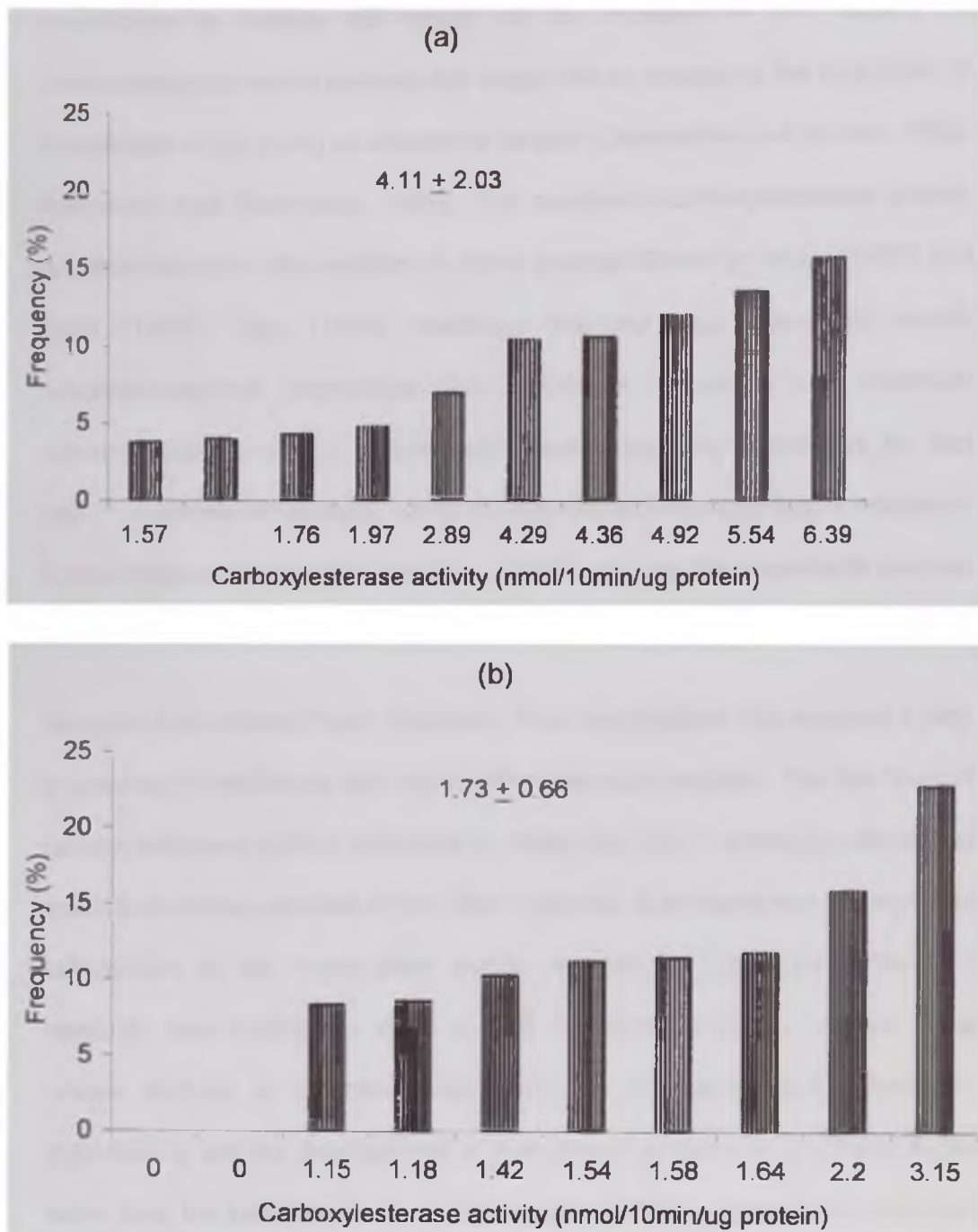


Fig 4.4 Frequency distribution of individual carboxylesterase activity in *P. xylostella* larvae collected from cabbage plants at Crop Science. Values shown are averages with standard deviation of the activity.

(a) 1 - Naphthyl acetate as substrate. (b) 2 - Naphthyl acetate as substrate

Similar work done on an aphid species *Myzus persicae*, (Sulzer) has demonstrated that resistance to organophosphorus and carbamate insecticides is mainly the result of an increase in the activity of carboxylesterase which protects the target site by catalyzing the hydrolysis of insecticides or by acting as alternative targets (Devonshire and Moores, 1982; Soderlund and Bloomquist, 1990). The resistance-carboxylesterase activity relationships were also reported in *Aphis gossypii* Glover by Inoue (1987) and Saito (1989). Saito (1989) observed that the LC₅₀ values of certain organophosphorus insecticides had a positive correlation with individual carboxylesterase activity. These findings go a long way to buttress the fact that *P. xylostella* on cabbage farms in Ghana may have developed resistance to the different insecticides used in its control and has the potential to develop resistance to other chemicals that have been recently introduced.

Samples from Kumoji Road, Dzorwulu, Tuba and Mallam Atta included a high frequency of individuals with high carboxylesterase activity. The low level of carboxylesterase activity observed at Weija and G.B.C. although with similar insecticide history as most of the other locations, is probably due to the recent introduction of the neem seed extract insecticide formulation which is a relatively new insecticide used against *P. xylostella* at this location. One unique feature of insecticide resistance in the diamondback moth (*P. xylostella*) is that the development of resistance can take place quickly. At the same time, the insect can lose resistance quickly if the population is freed from insecticidal pressure (Motoyama *et al.*, 1990). In addition, organophosphorus resistance has been found to be unstable in diamondback moth compared

with pyrethroid resistance, which lingers on for a long period of time (Sun *et al.*, 1990).

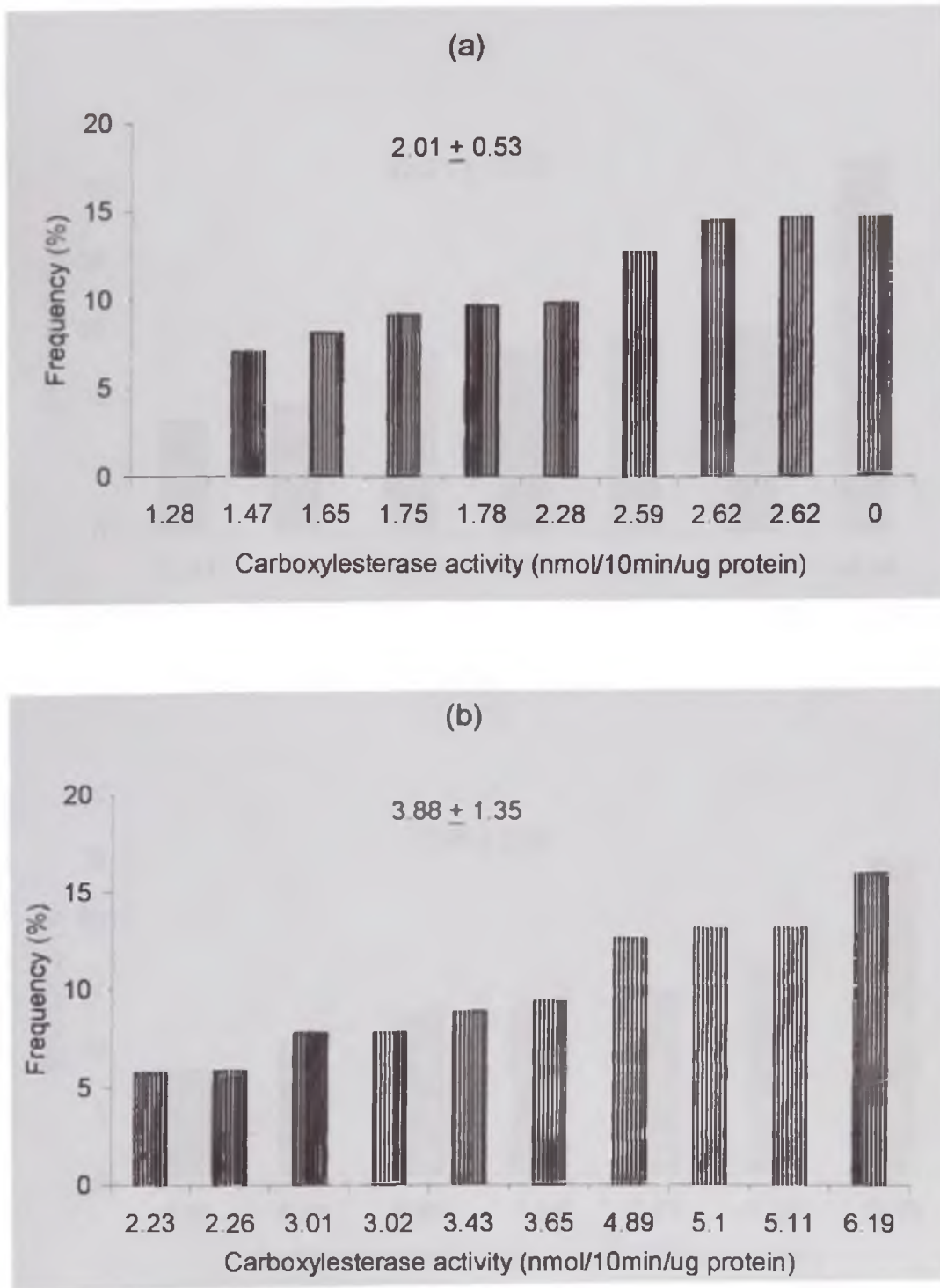


Fig. 4.5. Frequency distribution of individual carboxylesterase activity in *P. xylostella* larvae collected from cabbage plants at GBC (Ring Road). Values shown are averages with standard deviation of the activity. (a) 1 Naphthyl acetate as substrate. (b) 2 - Naphthyl acetate as substrate

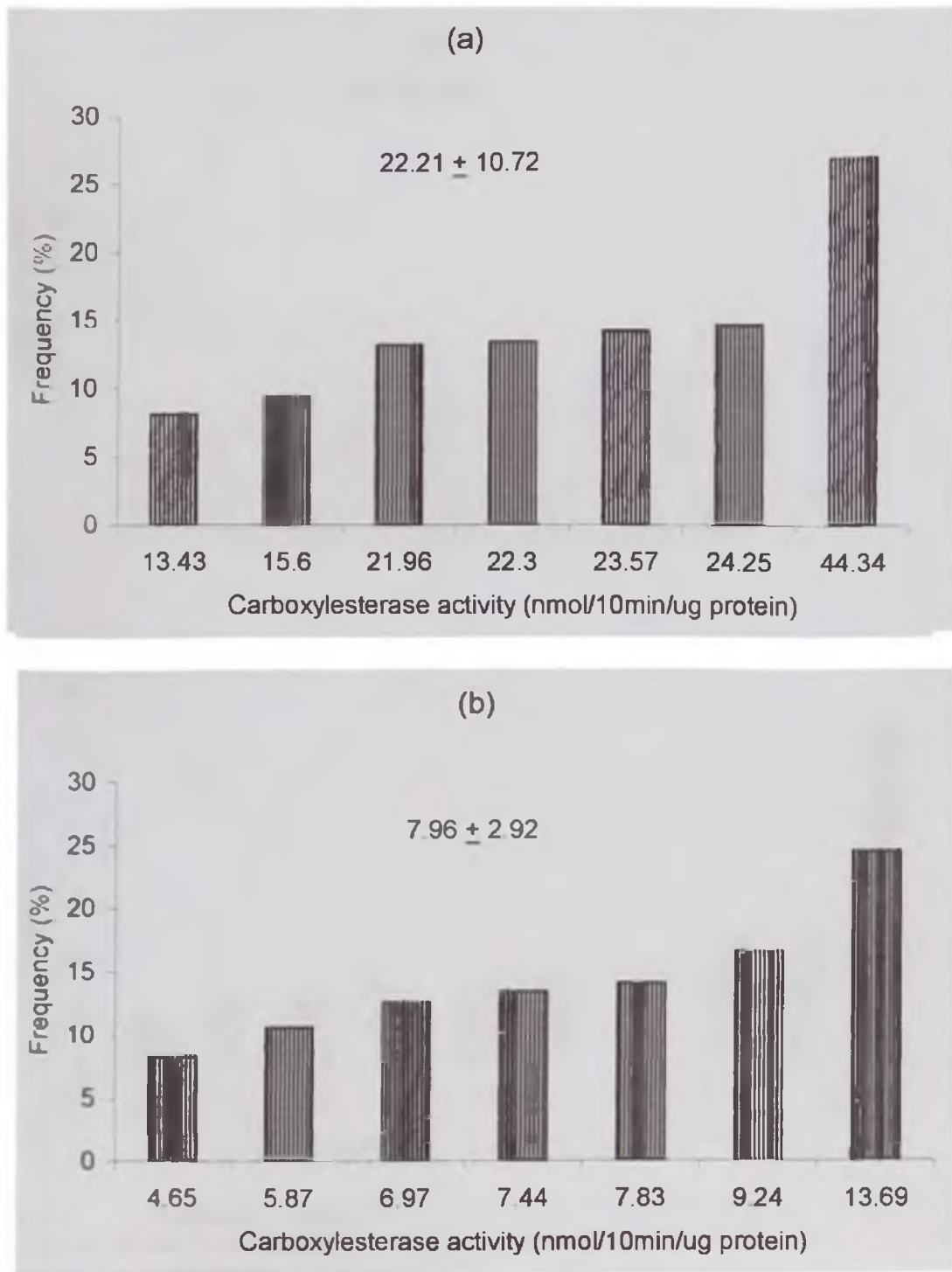


Fig. 4.6 Frequency distribution of individual carboxylesterase activity in *P. xylostella* larvae collected from cabbage plants at Dzorwulu. Values shown are averages with standard deviation of the activity. (a) 1 - Naphthyl acetate as substrate. (b) 2 - Naphthyl acetate as substrate

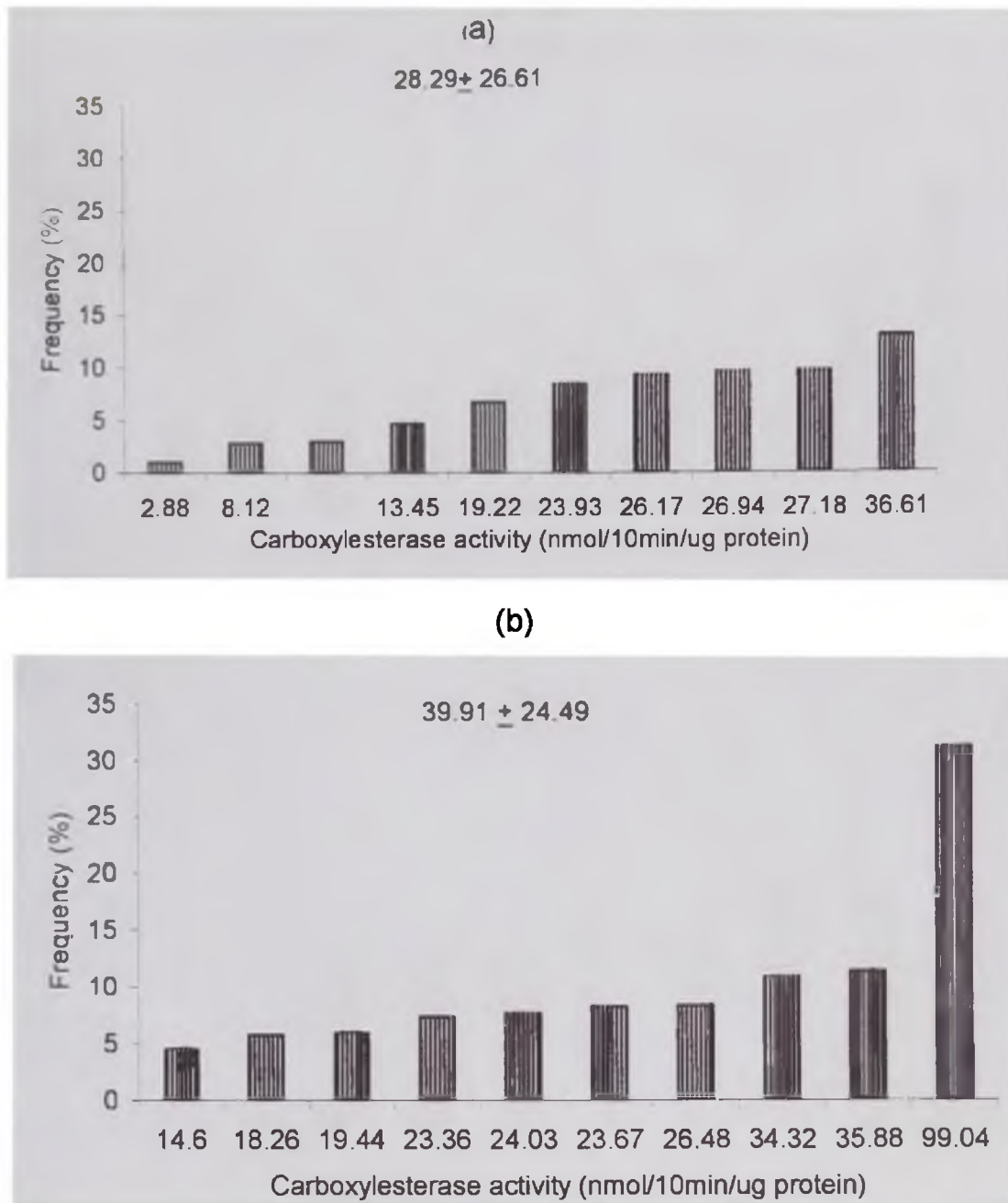


Fig. 4.7 Frequency distribution of individual carboxylesterase activity in *P. xylosteffa* larvae collected from cabbage plants at Kumoji Road. Values shown are averages with standard deviation of the activity.

(a) 1 - Naphthyl acetate as substrate. (b) 2 - Naphthyl acetate as substrate

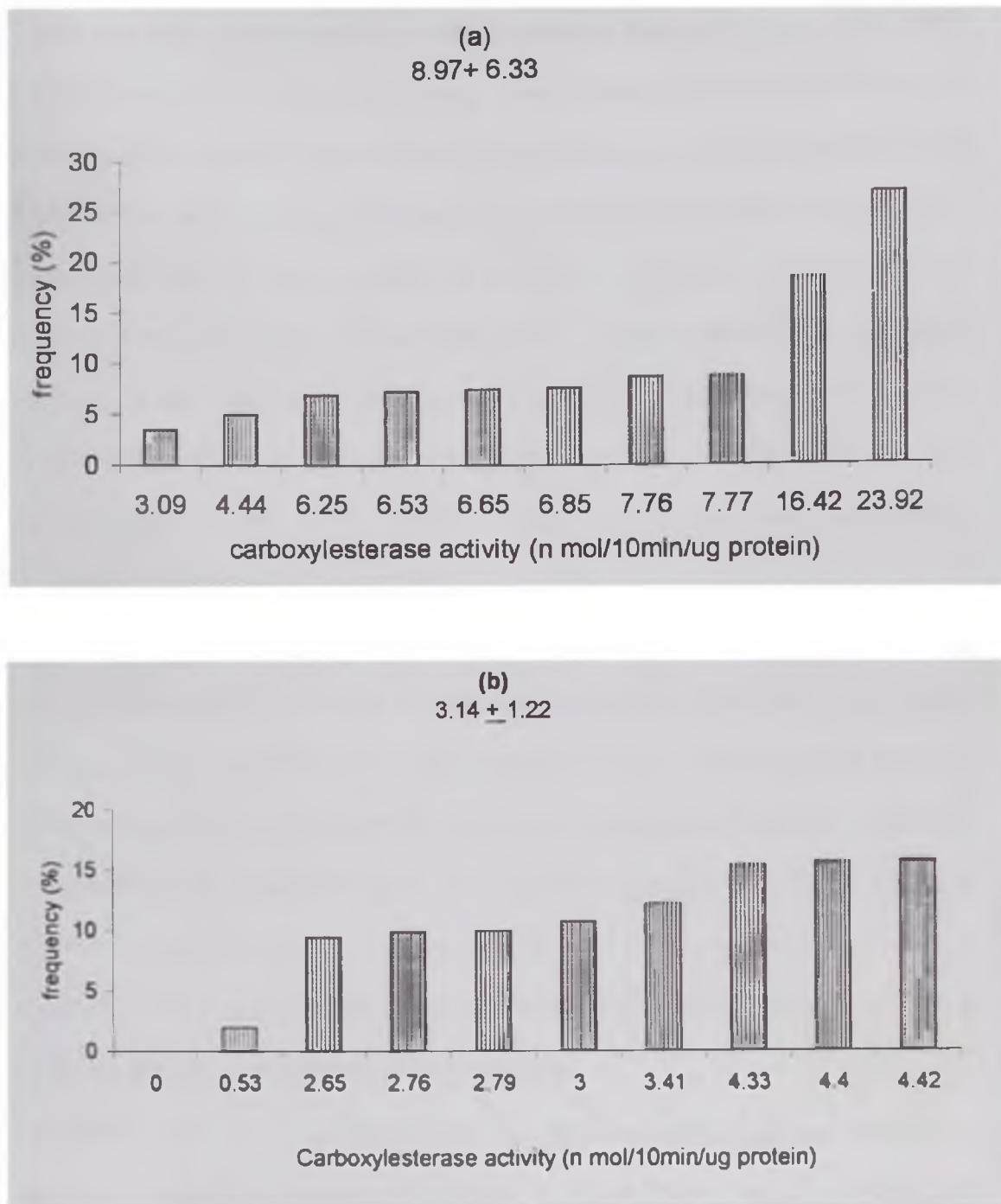


Fig. 4.8 Frequency distribution of individual carboxylesterase activity in *P. xylostella* larvae collected from cabbage plants at Weija. Values shown are averages with standard deviation of the activity.

(a) 1 - Naphthyl acetate as substrate. (b) 2 - Naphthyl acetate as substrate

These and other factors might have taken place at Weija and Kumoji road. With the withdrawal of the organophosphorus insecticides and carbamates, the insect could have considerably lost their resistance developed to those insecticides and thus the low activity observed. However at Tuba, (Fig.4.2) neem seed extract is sometimes used, yet high activity was recorded. The reason may be that, this product has been used at this location for a longer period than at Weija, considering the fact that *P. xylostella* has the ability to quickly develop resistance to all insecticides soon after introduction (Miyata *et al.*, 1986). Thus, the high activity level observed at this location suggesting that the insect has already developed resistance to the chemical.

Also, at Dzorwulu (Fig.4.5) the carboxylesterase levels could have been higher than what was observed but for the recently introduced farmers field schools. (IPM programme), an FAO- UNDP sponsored project geared towards alleviating pest problems on vegetable farms. This programme encourages farmers to look at other methods of controlling pests besides the use of chemicals. Since this is currently under trial at Dzorwulu, may probably be the reason responsible for the relatively low carboxylesterase activity observed.

Considering the two substrates used for the biochemical assay, that is α -Naphthyl acetate and β - naphthyl acetate, (Table 4.2). It was observed that on average, α - Naphthyl acetate was a better substrate for reaction than β - Naphthyl acetate, although the difference was not significant.

This had been reported also in both resistant and susceptible strains of *Culex pipiens* by Villania *et al.* (1983); and in mosquitoes in selected locations around Accra (Poku, 1999). The reason for this may probably be because the two substrates have very close stereo-chemical configuration and the relative low specificity of esterases.

CHAPTER FIVE

5.0 . SOME PROPERTIES OF CARBOXYLESTERASES FROM *PLUTELLA XYLOSTELLA* (L.)(LEPIDOPTERA:PLUTELLIDAE)

5.1. INTRODUCTION

Studies of Diamondback moth (DBM) resistance to insecticides have indicated the presence of three possible mechanisms. These are reduced chemical penetration, lower sensitivity of the target site, and enhanced activity of detoxification enzymes (Carboxylesterases) (Doichuanngam and Thornhill, 1989). Saito (1989) observed that the LC₅₀ values of certain organophosphorus insecticides had a positive correlation with individual carboxylesterase activity. Also from the study carried out on the individual carboxylesterase activity showed that resistance was related to carboxylesterase activity. With these unique properties of carboxylesterase being possibly responsible for insecticide resistance, the purpose of this work was to characterize activity of carboxylesterase in *P. xylostella* with regards to optimum temperature, pH and substrate concentration.

5.2. MATERIALS AND METHOD

5.2.1. OPTIMUM TEMPERATURE, PH AND SUBSTRATE CONCENTRATION

Homogenized enzyme solution (0.6 mls) was prepared by homogenizing two larvae of *P. xylostella* in a deep glass well with cold phosphate buffer as described in the preceding chapter. The enzyme solution was then filtered. Enzyme activity was obtained as described in the preceding chapter. Standard final concentration of α -naphthyl-acetate was 30 mM except for substrate

concentration study. The effect of temperature on the hydrolysis of 1-naphthyl acetate by esterases was studied at ten temperatures between 5 and 50 °C. . For determination of temperature below 20 °C, ice was added to tap water at 30 °C for incubations.

Effect of pH on esterase activity was studied at ten pH values ranging from 5.5 to 8.0 at 40 °C. 11.933 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (MW 358.14) and 4.533 g KH_2PO_4 (MW 136.09) were each dissolved in 500 ml distilled water to give 1/15 M. These solutions were then mixed in different proportions to give the desired pH. Except for this experiment, all other esterase determinations were done at pH 7.2.

The study on substrate concentration was done at ten different concentrations ranging between 5mM and 50mM. The standard concentration of α -naphthyl acetate was for all biochemical assays with the exception of substrate concentration determination, which was 30mM.

5.3. RESULTS AND DISCUSSION

Figure 5.1 shows a typical Temperature- Activity curve for the -. The effect of temperature on the hydrolysis of α -Naphthyl acetate by esterases was studied at ten temperatures between 5 and 50 °C. Enzyme activity increased gradually from the lowest at 5 °C to a maximum at 45 °C. Above 45 °C however, there was a significant reduction in activity. There was a gradual increase in activity from 5 to 25 °C, which was followed by a sharp increase to 30 °C. The increase became gradual again between 30 and 40 °C and a rise to 45 °C and

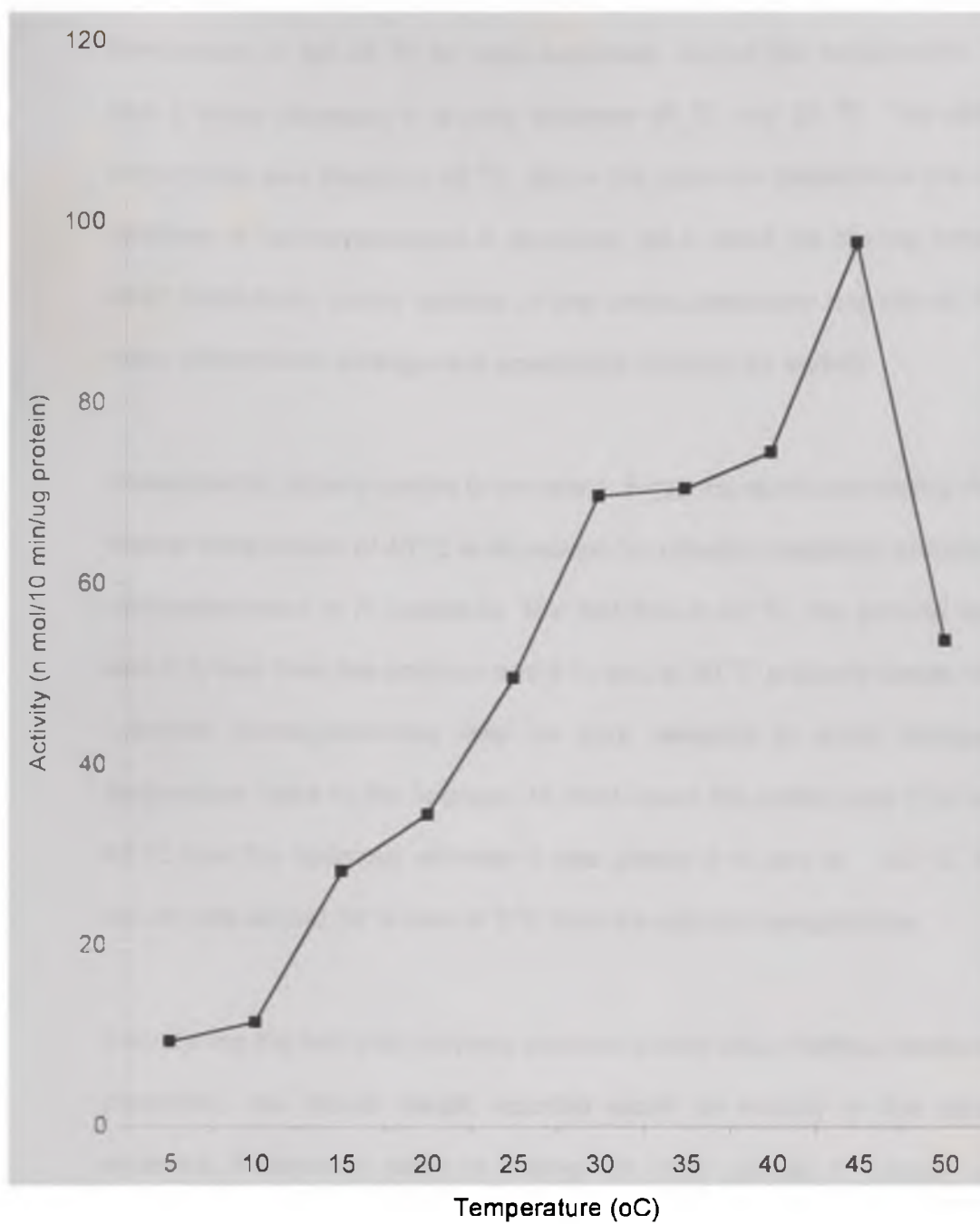


Fig. 5.1 The of temperature on the hydrolysis of 1-Naphthyl acetate. Incubation time was 10 min. Each point represents mean of three incubations.

at which point the optimum activity was attained. This lies within the optimum temperature of 40- 45 °C for most esterases. Above this temperature, there was a sharp decrease in activity between 45 °C and 50 °C. The optimum temperature was therefore 45 °C. Above the optimum temperature the native structure of carboxylesterase is disrupted. As a result the binding sites and other catalytically active centres of the carboxylesterase are altered in the three- dimensional arrangement specifically required for activity.

Consequently, activity begins to decrease. It may be worth speculating that an internal temperature of 45 °C is necessary for effective metabolic activity of carboxylesterase in *P. xylostella*. The fact that at 40 °C, the enzyme activity was 5 % less than the optimum and 8 % less at 50 °C probably shows that *P. xylostella* carboxylesterase may be very sensitive to small changes in temperature close to the optimum. In most cases the activity was 5 % less at 40 °C than the optimum, whereas it was almost 9 % less at 50 °C. Again activity was almost 18 % less at 5 °C than the optimum temperature.

Considering the fact that enzymes possess a very usual thermal denaturation properties, the results herein reported would be exactly in line with the expected. References made to findings on other species of insects further strengthens this acclaim. Zhu and Brindley (1990) working on esterases of *Lygus Hesperus* Knight found an optimum range of enzyme activity to be 40 to 45°C. Also Owusu *et al.* (1993) determining the effect of temperature on hydrolysis of α -naphthyl acetate by carboxylesterases from *Aphis gossypii*

Glover and found the optimum temperature to range between 40 to 45 °C with the peak at 45 °C. The exception to any of the cases reported above was

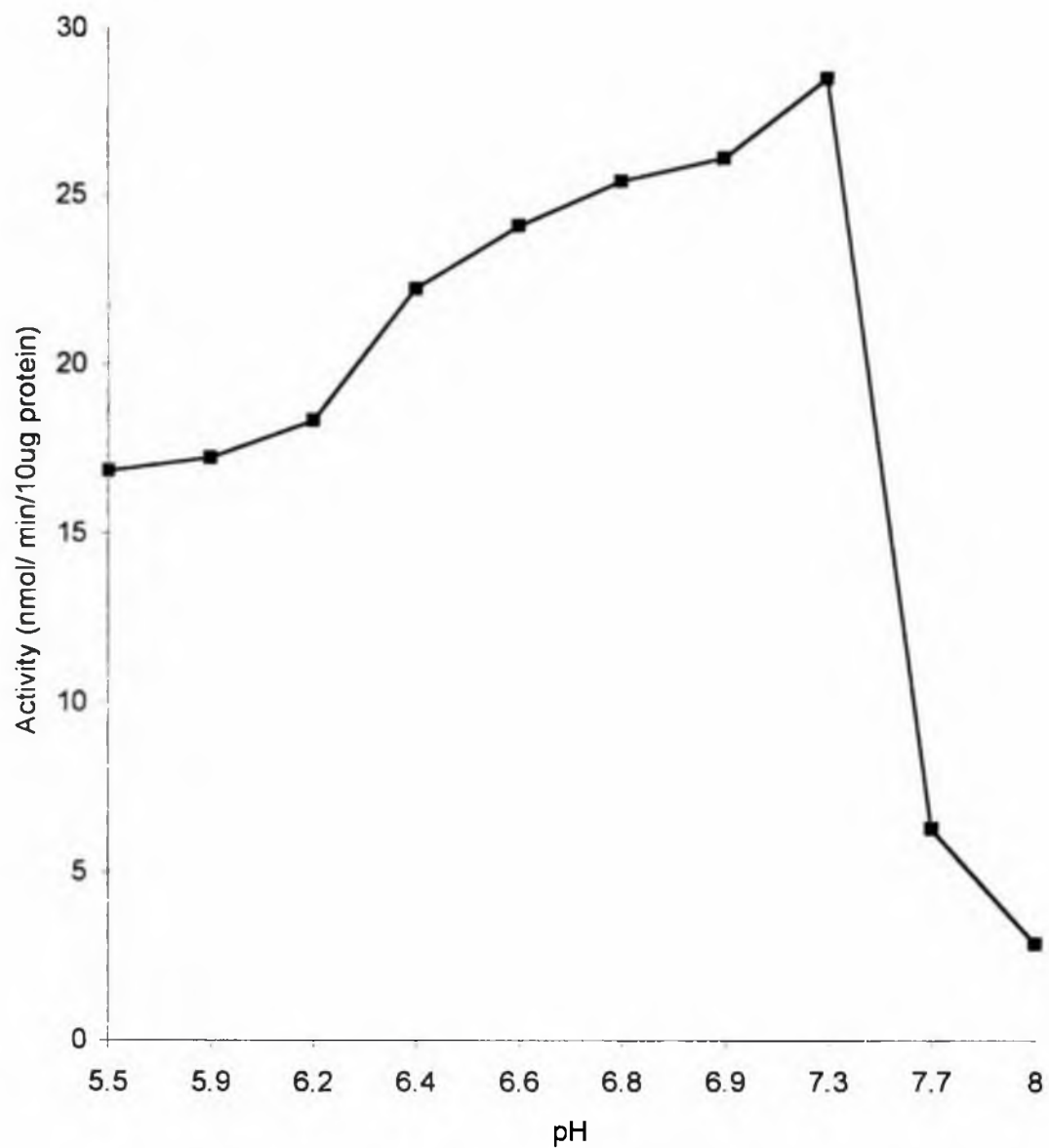


Fig.5.2 Effect of pH on the hydrolysis of 1-Naphthyl acetate. Time of incubation was 10 min. Each point represents the mean of three incubations.

found in *Triatoma infestans* (Klug) esterases by De Malkenson *et al.* (1984). In their study, temperature increases of up to 100 °C for 30 min without any loss in esterolytic activity being observed. This result still poses some problem considering the fact as stated earlier, that enzymes possess a very usual thermal denaturation properties.

The esterase activity towards α -Naphthyl acetate was studied at ten pH values ranging from from 5.5 to 8.0 with the reaction temperature being 40 °C (Figure 5.2). The enzyme activity increased between pH 6.4 and 6.6. This was again followed by a steady increase from 6.6 to 7.3, and decreased sharply when the pH was >7.3. The optimum pH range lasted from 6.9 to 7.3.

From the study, it can be asserted that a pH range from 6.9 to 7.3 is the most convenient for experimental purposes. This assertion accrues from the fact that under acidic conditions lower than pH 6.8, colour development with diazo-blue reagent is slow with occurrence of some precipitates, thus making photometric readings extremely difficult. (Owusu *et al.*, 1993). Their work on *Aphis gossypii* showed the optimum pH range from 6.8 to 7.2, a range that is very close to the range used in the present study.

The effect of substrate concentration on the esterase activity was studied at ten concentrations of α -naphthyl acetate between 5mM and 50mM at 40°C (Fig.5.3).

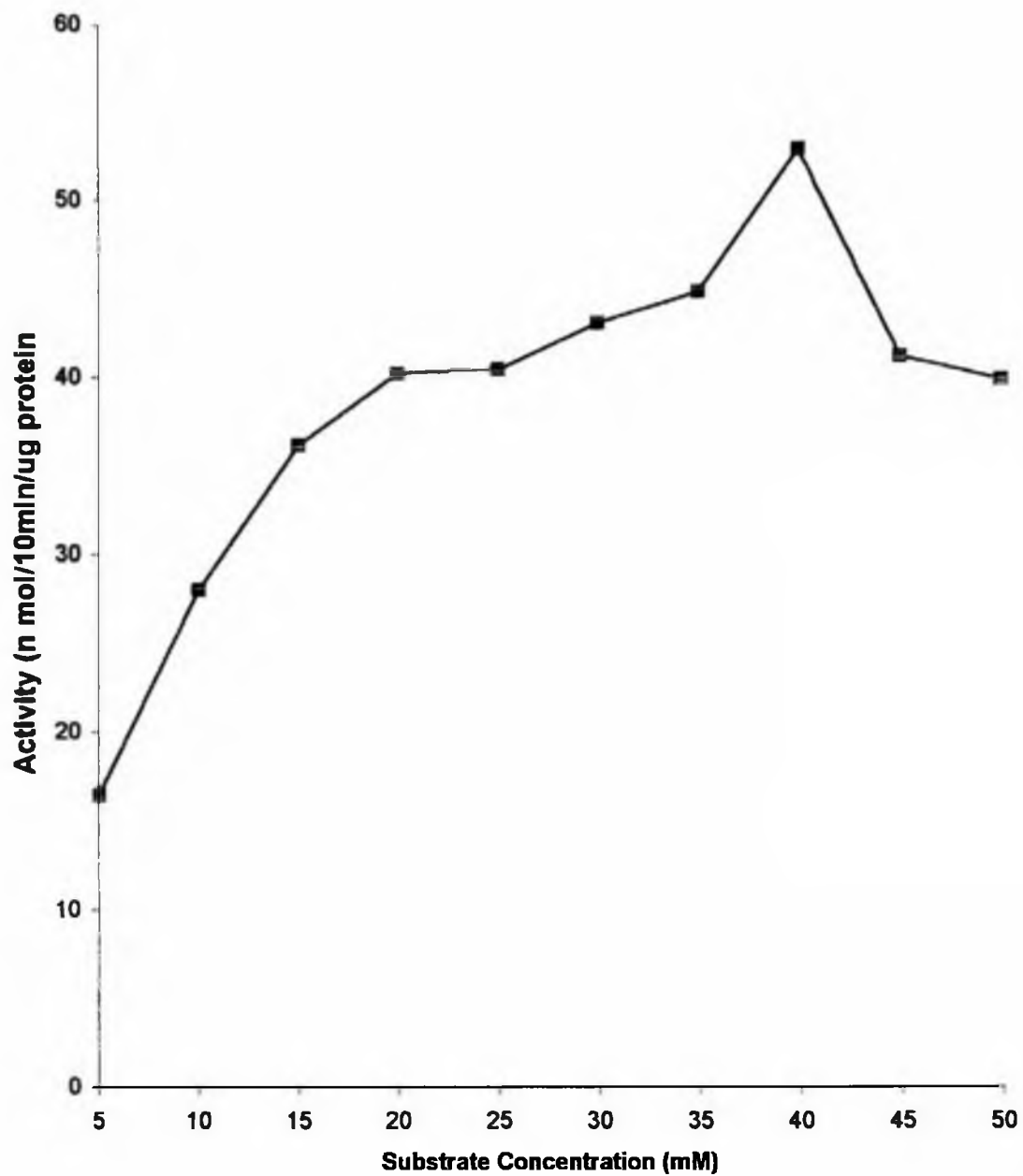


Fig.5.3 Effect of substrate concentration on the hydrolysis of 1-Naphthyl acetate. Time for incubation was 10 min. Each point represents the mean for three incubations.

decline in the esterase activity. The reaction of α -naphthyl acetate was further decreased with increase in substrate concentration indicating substrate inhibition.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

The life cycle of Diamondback moth varies considerably depending on the environment under which it develops (Ooi, 1986). Temperature plays an important role on the duration of the life cycle. Harcourt (1957) in Canada, Ho (1965) in Malaysia; Lee (1968) in Hong Kong; Bhalla and Dubey (1986) in India have reported that the life cycle of the Diamondback moth took 14 to 21, 10.8 to 27, 22 to 37, and 35.65 to 27.95 days, respectively. Even in the same region, as reported by Ko and Fang (1979) in Taiwan, a single generation took only 9 to 10 days under the most favorable conditions, while during winter one generation could take as long as 110 days.

From the study, it was observed that the developmental stages overlap. Moreover, the duration of the lifecycle shows that several generations can occur within a year thus enabling it to pass any modified or acquired factors (resistance genes) to several offspring within a very short time.

An in-depth information on *P. xylostella* has come mostly from Asia i.e. Japan, Malaysia, Phillipines, India, Taiwan, Pakistan etc. In all these countries, resistance to insecticides has been the major problem to its effective control. The populations collected around Accra, showed varying levels of carboxyesterases activity, and hence insecticide resistance levels (Hama and Hosoda, 1988). Majority of individuals collected were classified as moderately resistance. The

present results reflect the probable increasing use of insecticides on cabbage. The observed resistance levels though moderate, indicate that insecticide resistance in *P. xylostella* will become a limiting factor in the commercial cultivation of cabbage in the near future. This poses a serious national challenge to pest managers, especially the plant protectionists.

The relatively short life cycle and its high reproductive rate, raises serious concerns about the rapid spread of resistant genes under continued insecticide pressure. Since development of resistance by insects to pesticides is one of the most difficult steps towards effective pest control today, early detection of resistance should provide pest management experts the option to develop a long term strategy to arrest further development and institute a sound integrated approach to solving pest problems.

From these present results, it is very likely that the problem may not rest with Diamondback moth alone, but extended to other serious cabbage pests like the cabbage aphid, *Brevicoryne brassicae*, *Spodoptera littoralis*, *Hellula undalis*, etc. With the diamondback moth having the ability to quickly develop resistance to chemicals, the probability of losing most of the beneficial insects including pollinators and natural enemies is extremely high, due to increase in dosage and frequency of insecticide application.

Although resistance detected in *P. xylosteffa* populations collected around Accra, may be low as compared with the populations in places like China, (Zhu-Shu *et al.*, 1996), New Zealand (Cameron *et al.*, 1997), Japan (Hama, 1990) or South Africa (Sereda *et al.*, 1997), there is the need to urgently address the problem before it gets out of control.

The cause of resistance may be a complex of interplay of various factors of which biochemical mechanism of development by increased carboxylesterase levels from different areas under varied conditions may positively correlate with the magnitude of insecticide pressure. It can be highly speculated that insecticide use on vegetable farms would continue and be increased if one considers increasing brands of insecticides on the Ghanaian market. Although the major insecticides in use, in addition to the already organophosphates and carbamates, are biopesticides like *biobit* and *dipel*, and botanical products, the subsequent development of resistance to these newly introduced chemicals cannot be ruled out completely. In view of this, certain precautionary measures should be undertaken.

The use of insecticides to control Diamondback moth and most other pests is unavoidable to ensure high yields of quality cruciferous vegetables. In many cases, more frequent insecticide sprays are required to control this pest as opposed to other pests. There should however be a careful timing of application and rotation of chemicals. Judicious rotation of chemicals with different modes of

action will prolong the effectiveness of presently used insecticides substantially. Moreover, monitoring networks should be set up to monitor resistance in major insect pests in all national agricultural and medical based systems where insecticide application constitutes a major approach to pest control. However, the monitoring and research information alone is not sufficient in the implementation of a DBM management program. Bringing the information to the crucifer farmers is equally important. The farmer must understand and accept its concept. To achieve this studies should be carried out to understand the attitudes and behavior of the farmers (Ooi, 1986). This will facilitate the education of the farmers as well as the education of the scientists as to the need of the farmers. Cooperation between the farmers and the scientists will enhance a successful management program.

In conclusion, a thorough study and understanding of the problem of insecticide resistance as part of a comprehensive integrated package to control pests should be initiated by those involved in pest control, with the full support of both governmental and non-governmental organizations. This is because it is much more cost effective to prevent an emerging resistance to chemicals than to reverse it when fully developed.

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APPENDIX A

Effect of Temperature on carboxylesterase Activity

Temperature ($^{\circ}$ C)	Carboxylesterase Activity (n mol/ min / μ g protein)
5	9.36 \pm 0.13
10	11.51 \pm 0.45
15	28.20 \pm 0.24
20	34.54 \pm 0.11
25	49.71 \pm 0.33
30	69.91 \pm 0.07
35	70.73 \pm 0.72
40	74.64 \pm 1.07
45	97.64 \pm 0.27
50	53.73 \pm 0.72

Each Activity represents the mean of one determination with triplicate incubations. Substrate concentration and time of incubation was 30 mM and 10 min respectively.

APPENDIX B

Effect of substrate concentration on carboxylesterase Activity

Substrate concentration (mM)	Carboxylesterase Activity (n mol/ min/ μ g protein)
5	16.43 \pm 0.89
10	28.00 \pm 0.19
15	36.20 \pm 0.86
20	40.25 \pm 0.33
25	40.42 \pm 0.37
30	43.00 \pm 0.43
35	44.67 \pm 0.37
40	52.55 \pm 0.38
45	40.94 \pm 0.15
50	39.55 \pm 0.18

Each Activity represents the mean of triplicate incubations.
Temperature and time of incubation were 40 °C and 10 min respectively.

APPENDIX C

Effect of pH on Carboxylesterase Activity.

pH	Carboxylesterase Activity (n mol / min/ μ g protein)
5.5	16.85 \pm 0.56
5.9	17.25 \pm 0.16
6.2	18.35 \pm 1.32
6.4	22.25 \pm 0.44
6.6	24.10 \pm 0.23
6.8	25.40 \pm 0.38
6.9	26.05 \pm 0.18
7.3	28.35 \pm 0.44
7.7	6.25 \pm 0.10
8.0	2.85 \pm 0.30

Each Activity represents the mean of triplicate incubations. Temperature and substrate concentration were 40 °C and 30 mM respectively. Time of incubation was 10 min.