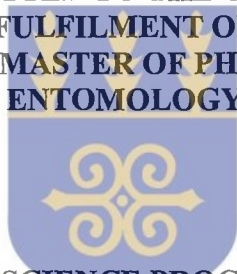


**PATTERNS OF PESTICIDE USE AND RESIDUE LEVELS IN  
EXPORTABLE PINEAPPLE (*Ananas comosus* L. Merr.)**

**BY**

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA,  
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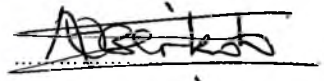


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## DECLARATION

I hereby declare that, with the exception of references to other people's work which have duly been acknowledged, this work is the result of my own tireless original research and that this thesis either in whole or in part has not been presented for any other degree elsewhere.



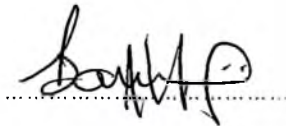
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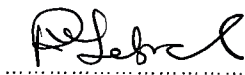
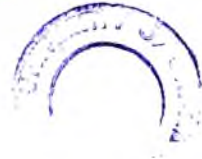
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## ABSTRACT

Pesticide use pattern on exportable pineapple (*Ananas comosus* L. Merr.) (Smooth Cayenne) was studied in the Akwapim South District of Ghana through administration of questionnaires. Among the several pesticides listed as being used, only nine, representing 43%, had been provisionally approved for use on exportable pineapples. Farmers handled pesticides carelessly without necessary caution though they had general awareness of hazards of pesticides.

Residue levels of chlorpyrifos and carbendazim in exportable pineapple (Smooth Cayenne) grown in the district were also determined using a Gas Chromatograph equipped with electron capture detector and Thin Layer Chromatography (TLC) by bioassay of *Aspergillus niger* Tiegh inhibition technique, respectively.

The detection limit of carbendazim in the pineapple peel using TLC is  $0.02\mu\text{g/g}$ . The carbendazim residue detected in the peel was in the range of  $0.04\pm 2\times 10^{-2}$  to  $0.05\pm 1\times 10^{-2}\mu\text{g/g}$ . Chlorpyrifos was detected both in the peel and the pulp.

The chlorpyrifos residue detected was within the range of  $0.005\pm 4.8\times 10^{-3}$  to  $0.02\pm 1\times 10^{-2}\mu\text{g/g}$ . These levels of residue were below pesticide permissible levels established by the European Union.

Regular monitoring of pesticide use and determination of residue levels are recommended to optimise the value of fresh pineapples and sustain the export potentials of the fruits.

## DEDICATION

This thesis is dedicated to my beloved children, Master Osei Aboagye Kwame and Miss Durowaa Aboagye Akosua.



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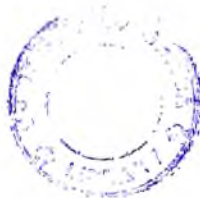
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## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background Information

Pineapple (*Ananas comosus* L. Merr.) is one of the most highly appreciated fruits in the world because of its delicious flavour, and the fact that it contains proteolytic enzymes that aid digestion (Bartolome *et al.*, 1995). Cabrera *et al.* (2000) reported that only orange and apple juices are consumed in greater amounts worldwide than pineapple juice. The annual consumption of the pineapple juice according to Cabrera *et al.* (2000) is estimated to be 200,000 metric tonnes.

In Ghana, the “Smooth Cayenne” cultivar (Plate 1) is the major variety cultivated for export, and production is centered in the Eastern region and parts of Central region due to the humidity that persists in those areas (Adjei-Boateng, 1998)

Ghana has experienced unprecedented growth in horticultural exports over the past five years. The value of horticultural exports is estimated above 40 million US dollars per annum and has increased steadily. Pineapple appears to be the leading horticultural export crop and its export grew in value by more than 70% between 1996 and 1999 (Kyofa-Boamah and Braun, 2002). The export of the product increased from 15,319 metric tonnes in 1994 to 25,124 metric tonnes in 1997, but suffered a reduction in 1998 due to unfavourable weather conditions (Ghana Matrix of Export Products & Markets, 2000). In the first quarter of 1999, Ghana exported 9,943 tonnes valued at US \$4.09 million (Ghana Export Promotion Council, 2000).



**Plate 1:** The fruit of Smooth Cayenne cultivar.

Ghana exported 28,511.600 metric tonnes of fresh pineapple valued at US\$11,853,127.83 in 2000 and in 2001, 34,933.400 metric tonnes valued at US\$13,225,375.24 was exported (Ghana Export Promotion Council, 2002).

Ghana's pineapple, known for its outstanding flavour and appearance, has found rapid acceptance in the European countries, particularly United Kingdom thus, opening tremendous opportunities for exports that fetch premium prices in the world market (Ghana Matrix of Export Product and Markets, 2000). European markets therefore account for nearly 60% of the total volume and continue to be a major importer of fresh pineapple from Ghana. Other developed countries and ECOWAS countries import 9.29% and 25.12%, respectively, of the total volume of these horticultural crops, while other African countries import 5.59% (Ghana Export Promotion Council, 2000)

Large-scale production of pineapple is done through monocropping. However, monocultures and continuous cropping coupled with required standards demanded in the European Union (EU) market have led to extensive use of pesticides on exportable fruits including pineapple. These practices of monocultures and continuous cropping in turn leads to a build up of unwanted pesticide residues.

Residue problems on pineapple do not only concern fungicides and ethephon, but also include fertilizers, herbicides, nematicides, and other plantation pesticide treatments (Teisson, 2000).

Of late, consumers have expressed deep concern about pesticide residues in fruits after pre- and post-harvest treatment. Children, in particular, may be more susceptible to

these risks owing to their higher overall consumption of fruits and vegetables (National Academy of Sciences, 1993).

In this regard the European Commission (EC), FAO/WHO and some nations have been evaluating the safety of residues in foods and establishing maximum residue limits (MRLs) to help ensure that pesticides are not overused and that any residue is safe for human consumption (PAN, 1998; Chan, 2000). Developing countries, including Ghana, as well as other countries exporting horticulture products to the EU must comply with pesticide residue levels established by the European Commission Legislation (FAO/WHO, 2000b).

Since 1993 the EU has been implementing a programme to establish harmonized MRLs that restrict levels of pesticide residues in foodstuffs sold in Europe. All products imported into EU must therefore comply with the new harmonized safety regulations (Chan, 2000).

## **1.2 Problem Statement**

Ghanaian exporters are experiencing problems in the European markets since there is no mechanism in place to regulate and control residue levels.

In view of strict enforcement of the MRLs harmonization programme in Europe over recent years, exporters of horticultural products in Ghana are liable to prosecution and severe penalties if their products do not comply with EU regulations. Pineapple export from Ghana to EU is therefore likely to face a bleak future due to changes in European market requirements and EU pesticide residues legislation, which will in turn, reduce

foreign earnings accruing from these exports to Ghana. This will threaten the livelihood of smallholder farmers by affecting their incomes thereby worsening unemployment and poverty in the areas where pineapples are grown.

### **1.3 Justification**

Chlorpyrifos and carbendazim are two of the approved pesticides for use on pineapples. Information on residue levels of these pesticides under contemporary agricultural practices in Ghana will be necessary to evaluate the need for any improvement on agricultural practices to ensure acceptance of Ghanaian pineapples on the International markets.

### **1.4 Objectives**

The objectives of the research were to:

- ascertain the pesticide use patterns for the production of the exportable pineapple (Smooth Cayenne) with the view to determine whether these pesticides are registered for use in Ghana, whether they are included in the EU approved pesticide list, whether contemporary agricultural practices in the pineapple growing areas will not lead to a build up of residues;
- provide baseline-monitoring data on the residual levels of chlorpyrifos and carbendazim in exportable pineapple (Smooth Cayenne) pulp and peel;
- determine and recommend practices that will ensure safe use of these pesticides and to ensure that residue levels fall within internationally acceptable limits.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

In Ghana residue levels have been detected in samples of cocoa beans and vegetables like cabbage and tomato (Mawuenyegah, 1994; Osafo and Frempong, 1998; Botchway, 2000). Unfortunately, samples of fresh pineapple fruits are not analysed to determine residue levels prior to export. The fresh pineapples are therefore analysed in the importing countries.

#### 2.1.0 PINEAPPLE (*Ananas comosus* L.Merr.)

##### 2.1.1 Botany

Pineapple is a native to Southern Brazil and Paraguay where wild relatives occur. It is strictly a tropical plant, and thrives best in areas where annual temperature is between 25 and 32 °C, rainfall is 100-150 cm and the humidity is high. The plant grows on a wide range of soils but well drained, slightly acidic, sandy loam is desirable (Kenneth *et al.*, 1987).

The plant is a terrestrial, herbaceous, perennial that may reach a height of 0.75 – 1.5m. with a spread of 0.9-1.2m. It is essentially a short, stout stem with a rosette of waxy, straplike leaves. The long-pointed leaves are 50-180cm, usually needle tipped and generally bearing sharp, upcurved spines on the margins. The leaves may be all green or variously striped with red, yellow or ivory down the middle or near the margins. As the stem continues to grow, the leaves become compact, stiff and short at the apex. Occasionally, a plant may bear 2 or 3 heads, or as many as 12 fused together instead of the normal one (Samson, 1986; CRFG, 1996; Morton, 1999).



At blooming time, the stem elongates and enlarges near the apex and puts forth an inflorescence of small purple or red flowers, each accompanied by a single red, yellow or green bract. The oval to cylindrical-shaped, compound fruit develops from many small fruits fused together. It is both juicy and fleshy with the stem serving as the fibrous but fairly succulent core. The tough, waxy rind made up of hexagonal units, may be dark green, yellow, orange-yellow or reddish when the fruit is ripe. The flesh ranges from nearly white to yellow depending on cultivars (CRFG, 1996; Morton, 1999).

Many cultivars are grown for local consumption; however, smooth cayenne types are the most important commercially due to their adaptations to canning and their spineless leaves. Red spanish, abacaxi, sugarloaf and queen are cultivars of importance for fresh local consumption with better flavour than cayenne types (Samson, 1986).

### **2.1.2 Importance of pineapple**

Ripe pineapple fruits are eaten fresh, and it is only necessary to remove the crown, rind, eyes and core. Pineapple is utilized in curries and various meat dishes. The fermented pulp is made into a popular sweetmeat in the Philippines (Morton, 1999).

Pineapple juice is prepared as syrup or is utilized in confectionery and beverages, or converted into powdered pineapple extract, which has various roles in the food industry. The juice of the peel can be made into vinegar or mixed with molasses for fermentation and distillation of alcohol (Morton, 1999).

Bromelain, or bromelin, a protein obtained from pineapple peel is used for tenderizing meat and chill proofing beer. Pineapple leaves yield a strong, white, silky fiber. Certain cultivars e.g. 'Perolera' are grown especially for fiber production and their young fruits are removed to give the plant maximum vitality.

Pineapple crowns and pineapple waste from the processing factories are used to feed cattle, pigs and chickens. Expendable plants from old fields can be processed as silage for maintaining cattle when other feed is scarce (Morton, 1999).

Pineapple juice is taken as a diuretic and to expedite labour, also as a gargle in cases of sore throat and as an antidote for seasickness (Morton, 1999).

The pineapple fruit with the crown intact is often used as a decoration and there are variegated forms of the plant universally grown for their showiness indoors or outdoors. Potted, ethylene treated pineapple plants with fruits have been used as indoor ornamentals (Morton, 1999).

### **2.1.3 Pests of pineapple**

#### **Pineapple mealybugs**

Wherever pineapple is grown in Ghana, mealybugs pose serious threat to fruit production. These mealybugs include pink pineapple mealybug, *Dysmicoccus brevipes* and the gray pineapple mealybug, *D. neobrevipes* (Cudjoe *et al.*, 2002). Toxin secreted by mealybugs feeding on pineapple plants cause mealybug wilt of pineapple (Carter, 1956). Field symptoms of mealybug wilt occur in four stages (Carter, 1956). The first stage starts with a reddening of the leaves and a slight inward reflexing of the leaf

margins. In stage two, definite colour changes from red to pink occur and in stage three the reflexing of the leaves becomes pronounced. Finally in stage four, the tips of affected leaves dry up and in severe cases, the plant becomes moribund.

Mealybug wilt syndrome, clearly included root collapse, and wilted plants invariably have poor roots (Carter, 1963). It was observed that the first symptoms are shown to be the cessation of elongation of roots, followed by collapse of the entire root systems.

Carter (1963) also indicated that the period for the development of symptoms varies with the age of the plant at time of infestation and indicated that symptom severity and relative recovery are affected by the frequency of infestation.

In addition to causing mealybug wilt, the toxic secretion of the species of mealybug (*Dysmicoccus brevipes* and *D. neobrevipes*), expresses itself in a number of ways including growth depression, accompanied by colour changes and this is especially noticeable in small seedlings (Carter, 1963).

The important nematode genera attacking pineapple on a worldwide basis are *Pratylenchus brahyurus*, *Meloidogyne javanica*, *Rotylenchulus reniformis* (Guerout, 1975). *Meloidogyne javanica* is more prevalent in Ghana (Cudjoe *et al.*, 2002). Raski and Krusbery (1984) reported that many other nematodes were found associated with pineapple roots, but either the degree of pathogenicity was not demonstrated or the amount of damage was not significant.

African mole cricket, *Gryllotalpa africana* and African giant cricket, *Brachytrypes membranaceus* and other insects may gnaw the bracts of the fruit before harvest and leave hole-shaped scars, which reduce the market value of the fruit (Adjei-Boateng,

1998). Termites, *Macrotermes spp.*, fruit boring caterpillars, and symphilids are serious pests in Ghana (Gerken *et al.*, 2001)

The pineapple mite, or so-called red spider (*Dolichote-tranychus* (or *Stigmacus*) *floridanus*) also attacks leaf bases and is troublesome during prolonged droughts, heavily infesting the slips. The palmetto beetle (*Rhynchophorus cruentatus*), which feeds on palm logs, enters the bud and lays eggs in young fruits and the fruit stalk. The sap beetle (*Carpophilus humeralis*) is one of the main insect pests of pineapple fruits in Puerto Rico, Hawaii and Malaysia and is especially attracted to fruits affected by gummosis. In Brazil, larvae of the large moth, *Castnia licus*, and of the butterfly, *Thecla basilides* damage the fruit. Cutworms eat holes in the base of the immature fruit (Morton, 1999). *Phytophthora cinnamoni* and *Pythium arhenomanes* cause rotting of the roots of pineapple. *Ceratocystis paradoxa* causes leaf spot, stalk rot, base rot, or water blisters and fruit rot. The fungus enters the fruit through wounds. *Phytophthora nicotianae* var *parasitica*, *Phytophthora cinnamoni*, and *Phytophthora palmivora* cause heart rot (Carter, 1963; Morton, 1999; Cudjoe *et al.*, 2002).

#### **2.1.4 Chemical control of pests and diseases**

Farmers around the world have used pesticides as an insurance policy against the possibility of a devastating crop loss from pests and diseases. They reason that as long as it is profitable, and no better alternatives are available, the spraying of pesticide is a good investment (Hardy, 1995). Chemical control method is very effective, rapid in curative action, adaptable to most situations, flexible in meeting changing agronomic and ecological conditions and economical. (Metcalf, 1975; Newsom *et al.*, 1976). There

are majority of pest outbreaks for which chemical control remains the only method of choice (Hill, 1993).

Pesticides that are used to control foliar insect pests of pineapple in Ghana include chlorpyrifos, diamethoate, diazinon, cymethoate and fenitrothion (Abutiate, 1991; Kyofa–Boamah and Blay, 2000). Soil inhabiting and root eating pests are controlled with chlorpyrifos, cadusofos, carbosulfan and ethoprophos (Kyofa –Boamah and Blay, 2000).

Heart (top) rot, root rot and basal rot are controlled by dipping the planting materials in fungicides including fosetyl-aluminium, metalaxyl, benomyl, carbendazim, triadimefon and triadimenol before planting (Abutiate, 1991; Kyofa- Boamah and Blay 2000; Cudjoe *et al.*, 2002).

Fungicides that are used for post–harvest treatment include maneb, carbendazim, imazalil, copper hydroxide (Abutiate, 1991; Kyofa-Boamah and Blay, 2000; Cudjoe *et al.*, 2002).

Herbicides applied on the fields with grasses as predominant weeds are glyphosate, fluazifop-butyl and ametryne. Fields with broadleaf weeds as predominant weeds are applied with either diuron or bromacil (Kyofa- Boamah and Blay, 2000).

The provisional pesticides approved for the production of exportable pineapples in Ghana are summarized in Table 1.

**Table 1:** Provisional list of pesticides approved for the production of exportable pineapple.

Trade Name	Active ingredient	Type
Furadan	Carbofuran	Nematicide
Marshall	Carbosulfan	„
Diuron	Diuron	Herbicide
Fusilade	Fluazifop-butyl	„
Roundup	Glyphosate	„
Dursban	Chlorpyrifos	Insecticide
Cypermethrin	Cypermethrin	„
Perfekthion	Dimethoate	„
Ridomil	Metalaxyl	Fungicide
Kocide	Copper Hydroxide	„
Goldazim	Carbendazim	„
Thiabendazole	Thiabendazole	„
Imazalil	Imazalil	„
Ethrel	Ethephon	Growth regulator
Calcium carbide	Calcium carbide	„

**Source:** Suglo *et al.* (2001)

## **2.2.0 CONSEQUENCES OF THE USE OF PESTICIDES**

### **2.2.1 Pests resurgence and resistance**

The over reliance on pesticides has resulted in the resurgence of pests which occur as the target species is initially suppressed by the insecticide treatment, but later shows a rapid population recovery after the decline of the treatment effect. Additionally, pesticide control results in the resurgence of the target species due to either development of a resistant biotype and/or destruction of natural enemies (Hill, 1993). Critchley (1995) cited by Gerken *et al.*, (2001) reports that in Ghana the development of resistance is suspected for white fly, *Bemisia tabaci* and diamond back moth, *Plutella xylostella*). McKinlay (1992) reported that over 400 species of insects have developed resistance in areas where pesticides are applied intensively. At least 520 species of insects and mites, 50 plant diseases and 113 weeds have become resistant to pesticides meant to control them (PAN, 2000). Pesticide application also results in outbreak of a secondary pest or pests, due to the alteration of the agro-ecosystem, usually by the destruction of natural enemies (Hill, 1993). Though there are no reported cases of pests on pineapple resistant to pesticides in Ghana, it is worth investigating.

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### **2.2.2 Contamination of the air**

Pimentel (1985) cited by Altenburger (1995) reports that when pesticides are applied, only a small fraction of the amount sprayed actually reaches the target such as crop pests and pathogens. More than half of the amount of pesticides applied may go directly into the atmosphere during spraying (Ramade, 1986 cited by Altenburger, 1995).

Pesticides eventually contaminate the atmosphere, passing into the air through wind-drift, evaporation of active ingredient from the surface of the soil and from plant

surface, and through wind erosion of contaminated soil particles. Pesticides move freely in the air or are bound to the aerosol and return as either dry or wet deposits to the surface of the earth. In various studies, the largest amount of pesticides was found in rainwater during those months when pesticide spraying in agriculture is heaviest (Altenburger, 1995).

### **2.2.3 Contamination of soil**

Pesticides are sprayed from the air indiscriminately over canals, leftover spray is poured away without thought, and empty containers are rinsed out near streams or rivers. Sudden rain showers wash pesticides off the leaves, through surface runoff, soil erosion and leaching that finally arrive in ground water (Altenburger, 1995).

Many pesticides are adsorbed rapidly and non-specifically in the humus layer (Ottow, 1985 cited by Altenburger, 1995). Most pesticides become fixed by components of the soil (clay minerals, organic matter), which delay their leaching down into the groundwater. The presence of pesticides directly or indirectly influences the microfauna in the soil and can alter breakdown of organic matter and the circulation of nutrients (Pimentel, 1985 cited by Altenburger, 1995). Pesticide residues in the soil can be directly toxic to soil organisms or can exercise a subtle effect, e.g. influence activities, behaviour, reproduction and metabolism of soil organisms (Altenburger, 1995). This permanently impairs and alters the closely interactive ecosystem.

Herbicides such as dinitro-ortho-cresol (DNOC), diguat, 2,4 – dichlophenoxyacetic acid and 2,4,5 – trichlophenoxyacetic acid have more or less striking toxic effect on

epigeal predatory arthropods (beetles and spiders) living on the soil surface (Altenburger, 1995). The vast majority of non-persistent pesticides do indeed detoxify as they decompose. Often disregarded, however, is the fact that pesticides not only destroy pests, but that other sensitive soil life is also disrupted. This degrades soil fertility, ultimately producing an essentially biologically sterile soil (Altenburger, 1995).

In Ghana pesticides like aldrin, parathion and dieldrin have been banned because of their high toxicity and persistence in the environment that can produce residue problems in subsequent crops (Gerken *et al.*, 2001). Residues of lindane and endosulfan disappeared in incubated Wacri and Adenta soils within six weeks (Antwi, 1996). Soil samples from coastal savanna forest zones in Ghana when analyzed revealed the absence of residues of the following herbicides: atrazine, simazine, ametryne, diuron and metobromuron (Afful, 2002). The residual levels of these pesticides have not been studied in areas where pineapples are extensively grown in Ghana.

#### **2.2.4 Water contamination**

Run-offs from heavily sprayed crops near rivers or wrong disposal of pesticide waste can harm inland water bodies. This has negative consequences on animal and plant life within the water (EPA, 1997). Seepage of contaminated rainwater that has washed off pesticides from crops or irrigation water also contaminates underground water. The aforementioned menace has negative effects on drinking water network making it necessary to filter and purify water before use. Contamination of rivers, inland water bodies, and rainwater can also lead to the contamination of soil (Gerken *et al.*, 2001).

According to investigations carried out to assess chemical management in Ghana, the level of concern for water pollution and soil contamination is considered to be high (EPA, 1997). Kyei-Baffour and Mensah (1993) did a specific case in two tomato production areas to assess the water pollution potential from agrochemicals. The results received mainly from interviews with farmers and qualitative observation show that intensive tomato production is mainly done at riverbanks for purposes of easy irrigation. This creates the possibility of run-off fertilizer and pesticides right into the river. Many farmers used the water from the river to clean the equipment after applying fertilizer and pesticides. Some even clean the equipment right in the river.

#### **2.2.5. Pesticide poisoning**

The overuse, abuse or over-use of pesticides can have serious consequences in food. Large scale poisoning by pesticide-contaminated food is unusual but not unknown. In late October 1998 children aged between 3-4 years were served with milk contaminated with the organophosphate insecticide, parathion in Peru. They begun vomiting and convulsing within half an hour of eating their contaminated breakfast. Twenty-four children died and another twenty-one were treated at hospital and discharged (Korsah-Brown, 2000). In January 1999, four people in the capital city of Chad died after eating a pesticide-contaminated meal. Six others who shared the meal with the victims showed symptoms of poisoning (diarrhoea and vomiting) but survived under hospital treatment (Tagoe, 2000).

In March 1999, three children in Ghana died after consuming fruits containing high residue of carbamates (Gerken *et al.*, 2001). PAN (2000) reported that every year three

million people suffer from severe pesticide poisoning and 20,000 out of this number even die. It is believed that up to 80% of these deaths occur in developing countries where pesticide use regulations are poorly enforced (Tagoe, 2000). This problem is compounded by a general lack of training and equipment to handle these chemicals. This estimate of poisoning only reflects acute poisoning cases. The long-term damage is not even registered. The prevalence of toxic products applied in many developing countries gives rise to concern for consumer safety in those countries and in produce for export (PAN, 1998).

The farmers' field survey carried out by Gerken *et al.*, (2001) in Ghana identified headache, general weakness and dizziness, body pains, nausea and vomiting, stomachache and diarrhoea as acute poisoning symptoms through pesticide application. Clarke *et al.* (1997) undertook a field study to examine the extent of pesticide-associated symptoms in farmers involved in irrigation projects in Ghana. Blood tests for cholinesterase as an indication of residues of organophosphates showed a lower activity band in the farmers compared to the teachers. This was the indication of higher residues in the blood of the farmers (Clarke *et al.*, 1997). The longer the period of handling of pesticides the lower the activity of the enzyme.

Adetola *et al.* (1999) cited by Gerken *et al.* (2001) analyzed organs of the body, body fluids, foods and drinks submitted by various hospitals and other state institutions in the country to the Forensic Science Laboratory of the Ghana Standards Board. About 1,215 toxicological cases were examined between 1989 and 1997. Out of this, 963 cases tested positively for chemical poisoning; 30% of chemical poisoning was directly

related to the misuse of pesticides. The main causes for deaths were carbamates (126 cases), organophosphates (66 cases) and organochlorine (74 cases)

#### **2.2.6.0 Pesticide Residues in food**

Pesticide residue means any specified substances in food, agricultural commodities, or animal feed resulting from the use of pesticides. The term includes any derivative of pesticides such as conversion products, metabolites, reaction products, and impurities considered to be toxicologically significant (FAO/WHO, 1993). According to PAN (1998) residues can arise from:

- \* The use on a crop of legally allowed pesticides according to good agricultural practice.
- \* Over-use of a pesticide, or use too close to harvest, of legally permitted pesticide.
- \* Illegal use of a pesticide that is not approved for that crop.
- \* Incorrect use of pesticides for post-harvest treatment.

The use of pesticides in food production leaves residues and strict standards need to be maintained to guarantee food safety. Food safety depends on strict standards to prevent undesirable residues and provide consumers with sound and wholesome products.

The public concern about food safety in the developed world has led to the establishment of maximum residue levels (MRLs), which restrict levels of pesticide residues in foodstuffs. According to Chan (2000), MRLs for foodstuffs are established both nationally and internationally with the key objectives of:

- \* Controlling the correct use of pesticides in terms of the registered use.
- \* Permitting the free circulation of food commodities that have been treated with

approved pesticides and comply with the established MRLs

- \* Minimizing the exposure of consumers to harmful or unnecessary intake of pesticides.

MRL levels are established, taking into account the persistence of the particular pesticide in a given crop, the toxicity of the chemical and how much of the final product is typically eaten by the consumer (Chan, 2000). It is important to note that these MRLs are not maximum toxicological limits. They represent the maximum amount of residue that might be expected in/on a commodity if good agricultural practice was adhered to during the use of a pesticide. Nonetheless, when MRLs are set, care is taken to ensure that maximum levels do not give rise to toxicological concerns (FAO/WHO, 1993).

#### **2.2.6.1 Good Agricultural Practices**

The MRL and acceptable daily intake (ADI) set by the FAO/WHO, European Union and the specific residue legislation of any individual country are based on Good Agricultural Practice (GAP) data.

According to Oudejans (1991) and FAO/WHO (1998) GAP in the use of pesticides includes the nationally authorized safe use of pesticides under practical conditions at any stage of production, up to the highest authorized use applied in such a manner as to leave residue which is the smallest amount practicable and which is toxicologically acceptable.

Good agricultural practice may vary considerably from region to region owing to differences in local pest requirements. Consequently, residues in food, particularly at a point close to harvest may also vary. In establishing Codex MRLs these variations due to differences in good agricultural practice are taken into consideration as far as

possible on the basis of available data. Food derived from commodity that complied with MRL is intended to be toxicologically acceptable (FAO/WHO, 1998).

Oudejans (1991) notes that, practices that will minimize the harmful effects of pesticide in humans, animals and the environment include the following:

- (a) Choosing the least toxic and least persistent pesticide that will effectively control pests in the field and in storage;
- (b) Choosing a formulation that combines maximum efficiency of the selected pesticide with minimum risk;
- (c) Applying on the target area only with the minimum amount of pesticide required while determining the number of treatments on a need basis in relation to actual pest infestation;
- (d) Selecting the method of application that offers optimum control with minimum contamination of crops and the environment;
- (e) Timing of the treatment in relation to vulnerable stages of a pests development;
- (f) The pre-harvest interval should be long enough to reduce pesticide residues.

#### **2.2.6.2. The menace of pesticide residues in the European Union**

Latest pesticide residue results from the European Commission (EC) suggest that residue safety breaches are increasing. The EC has analyzed the tests carried out by 17 national monitoring programmes in the European Union countries as well as Norway and Iceland of melons, peppers, cauliflower and wheat. The analyses indicated that 64% of the samples contained no detectable residues; nearly one-third of the food was contaminated. Of this, 4.3% exceeded the approved MRL. The levels of particular

concern were the levels of residues of endosulfan and methamidophos in peppers and melons (European Commission, 2001).

The EU report highlights the low level of residue testing for fruits and vegetables in the United Kingdom (UK). The UK samples in the monitoring programme showed 2.9% of the residues analyzed as above the MRL, whereas 30% of samples in Netherlands were above the MRL, 24% in Finland and 10% in Spain (European Commission, 2001).

The organophosphate, chlorpyrifos, was detected frequently in sample matrix in 12 countries including the UK. Another frequently detected pesticide in the UK was DDT, despite having been banned for use since 1984. There is increasing concern about multiple residues in food. The results showed that 14% of the samples contained residues of more than one pesticide, and in 2.2%, residues of four or more pesticides were detected. In Finland 29% of the samples contained multiple residues, and in France one sample contained eight or more pesticide residues (European Commission, 2001).



#### **2.2.6.3. Pesticide residue levels in Ghana**

Mawuenyegah (1994) carried out studies on residue levels of chlorpyrifos sprayed on cabbage cultivated on two separate fields at Legon. Brine shrimp (*Artemia salina* Leach.) lethality test was used to estimate the residue levels after observing a four-week post-harvest interval. The residue detected was 0.8mg/kg, which was lower than 1.0mg/kg recommended by Codex Alimentarius Commission.

Ninsin (1997) estimated Delthaphos 262 EC residue levels within two cabbage samples from Accra-Tema Metropolitan area using brine shrimp (*A. salina* Leach.) lethality test. The Deltaphos 262 EC consists of 250 g Triaphos and 12g Deltamethrin. This implies

that over 95% of the biological activity of Delthaphos 262 EC residue could be attributed to Triaphos. The residue levels of 0.2122 mg/kg and 0.6433 mg/kg obtained could be attributed to the presence of Triaphos. This suggested that the residue levels exceeded the FAO/WHO recommended MRL of 0.1 mg/kg). Ninsin, (1997) further analyzed residues of Lambdacyhalothrin within the same cabbage samples. The residues detected did not exceed the FAO/WHO MRL of 0.2 mg/kg in cabbage leaves.

Osafo and Frempong (1998) analyzed water and fish samples from three rivers that flow through areas of intensive vegetable farming from January 1993 to October 1995. While the analysis of water in 1993 showed low levels of lindane and no residues of endosulfan, a similar analysis done in 1995, however, showed significant residue levels for both pesticides. The highest quantities were found in the River Oda. The results obtained from the residue analysis in fish in 1995 were higher than for 1993. In general, the residues of both lindane and endosulfan found in fish were higher than those in water. This might have been caused by the accumulation of pesticides in fish. The residues in fish were under the lethal dose.

Ntow cited by Gerken *et al.* (2001) did more research on possible residues of lindane and other organochlorine pesticides. A total of 208 samples of water, sediment, tomato, other crops, farmers' blood and milk of women in the Akumadan area in the Ashanti Region of Ghana were analysed. The results of the water and sediment analyses showed significant levels of lindane and endosulfan. These levels, were however, lower than the levels reported by Osafo and Frempong (1998) for samples collected from the same area. The residues of lindane and other organochlorine pesticides tested in crops were,

however, lower the levels recorded in water and sediment. Most of blood and milk samples analyzed were positive for the presence of lindane and organochlorine pesticides. The detected levels were higher than those in the water, which is again an indication that there might be an accumulation of residues in animals and human beings. Ntow confirmed that residues of some pesticides are present in areas of highly intensive agricultural production, although there were some cases of low levels. As a result of their high level of persistence, there are indications that accumulation of these pesticides might cause serious health problems.

An analysis of samples of street-vended food in Accra, Ghana, carried out in 1999-2000 revealed disturbing levels of contamination by heavy metals, pesticides, microorganisms and mycotoxins. The 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). Pesticide residues were not found in any samples of bread and salad. Local analytical facilities and methods were not able to quantify the levels of chlorpyrifos present to see if they exceeded Codex MRLs of 0.2mg/kg (NRI, 2001).

Botchway (2000) analyzed pesticide residues in exportable quality cocoa beans collected from selected cocoa growing districts in the middle belt of Ghana and the two shipping ports at Tema and Takoradi. Analysis of the extract by gas liquid chromatography showed detectable amount of lindane residue but the level was about 10% of maximum residue level of 1.0 µg/g permitted by Codex Alimentarius Commission. The results of the research indicate that Ghana's exportable cocoa beans

are therefore of no immediate danger of being rejected by any importing country due to presence of lindane residues.

#### **2.2.6.4. Residues in pineapple**

Marchal *et al.* (1999) cited by Pinon (2000) analyzed ethephon residue levels in pineapple on arrival in France from West and Central Africa. Ethephon residues were analyzed on a blind basis by independent laboratories.

The residue levels recorded in a first series of observation of 99 batches were: 22 batches < 0.5mg/kg, 52 batches >0.5 mg/kg but < 2mg/kg, and 25 batches > 2 mg/kg. These results mean that with a limit of 0.5mg/kg that came into force on 1 July 2001, only about 22% of the batches could have entered European Union countries. Later observations of 20 batches revealed the following residue levels: 11 batches < 0.5 mg/kg, and 9 batches > 0.5mg/kg but <2 mg/kg. Although this appears lower, 45% of the batches would still have been rejected.

Marchal *et al.* (1999) cited by Pinon (2000) further analyzed imazalil and triadimefon residue levels in the same pineapple from West and Central Africa .The residue levels recorded for imazalil was higher than the detection threshold of 0.01 mg/kg. In the other cases, only 33% of the fruits had triadimefon levels lower than the detection threshold of 0.01mg/kg.

Kyofa-Boamah (2001) analyzed ethephon and triadimefon residue levels in exportable pineapples selected from farms in Ghana. The pineapples were sampled from different farmers' fields in which ethephon was sprayed with different spray concentrations of

200 ml, 90 ml, and 50 ml /15 litters of water. The post-harvest interval observed by these farmers was seven days. Seventy-two fruits sampled from twelfth boxes were sent to a laboratory in Germany by air for residue analysis. The residue levels recorded in fruits sprayed with ethephon concentrations of 200ml, 90ml and 50ml/15litters of water were 3.31mg/kg, 1.13mg/kg and 0.90 mg/kg, respectively. This situation is alarming and the results from farmers practice reveals very high doses applied to immature fruits. However, there was no detection of residue level of triadimefon (Bayleton 5 %) applied on the stem as post-harvest treatment.

### **2.3.0 ANALYTICAL METHODS FOR PESTICIDE RESIDUE ANALYSIS**

Residues are present in extremely small quantities in generally heterogeneous materials including biological materials. Essentially the process of residue analysis consists of the following steps:

- a. Sampling.
- b. Extraction
- c. Clean up of interfering materials
- d. Concentration of samples
- e. Identification and quantification of pesticide residues

#### **2.3.1 Sampling**

The objective of sampling procedure is to obtain a final sample representative of the lot in order to determine its average pesticide residue content. Sampling for residue analysis is done based on the nature of the crop and crop treatment history (NRI, 1994).

When sampling commodities/crops for analysis, the sample(s) taken should be representative of the larger bulk of produce. Uniform application of pesticides is difficult to achieve and a range of residues may result. It is therefore important for the sampling to cater for this variability. (NRI, 1994).

### 2.3.1.1 Sampling in packaged produce

For packaged produce the following sampling plan is recommended.

No. of cans , packages or containers in the lot	Minimum no. of primary samples to be taken
1 –25	1
26-100	5
101 – 250	10
> 250	15

**Source:** FAO/WHO (2000a)

### 2.3.1.2 Primary field sampling

Field sampling techniques can be complex. Essentially the frequency of sampling, i.e. the number of sample per farmer's plot depends upon the size of the plot.

The following sampling plan is recommended in primary field sampling.

Area of plot (ha)	No. of primary samples
Less than 0.5	4
0.5 to less than 2.5	4 to 8
2.5 to less than 25	8 to 20
25 to less than 250	20 to 70

**Source:** NRI, (1994).

### 2.3.1.3 Laboratory sample

The sample sent to the laboratory for analysis represents quantity of material removed from the field sample. The laboratory sample may be the whole or part of the bulk sample. In the case of large sized fresh fruits with weights greater than 250 g. the minimum size of each laboratory sample should be 2kg (at least 5 fruits). The minimum number of primary samples should therefore comply with the minimum number of units required for the laboratory sample. The portion of product to be analyzed may include parts that are not normally consumed. In pineapple for instance, the crown is removed and the whole fruit is analyzed (FAO/WHO, 2000a).

### 2.3.2 Extraction

Extraction is performed with a solvent to remove the pesticide residue of interest from other components of the sample matrix. Pesticides such as insecticides are soluble in both polar and nonpolar solvents and less soluble in aqueous solutions (Hetzel, 2000). The relatively polar solvents e.g. methanol is recommended for the extraction of fatty substances (cheese, peanuts, fatty tissue of animals). For nonfatty samples and those having high to medium moisture contents, polar water miscible solvent such as acetonitrile, methanol or acetone is used (Matsumura, 1985; Yeboah, 2001). Hexane has been exclusively utilized for extraction of chlorinated hydrocarbon and organophosphorus insecticides (Matsumura, 1985)

Sample extractions are effected by surface rinsing, maceration with solvent and soxhlet extraction (Yeboah, 2001). The surface rinse is acceptable for loosely held or easily dislodged residues. The extraction is not intense to remove residues from the sample matrix or residues that are bound or absorbed in the sample. Maceration with solvent can be used for a wide range of pesticides and substrates. In order to disrupt the gross structural components of the sample to facilitate the extraction of residues, the analytical sample needs to be disintegrated in a high-speed homogenizer or blender in the presence of appropriate solvent or mixture of solvent. Soxhlet extraction is for more vigorous form of extraction, uses less solvents, and more efficient for a wide range of pesticides.

### 2.3.3 Clean up

The extract is cleaned up to remove extraneous materials that were co-extracted from the analytical sample. Methods involved in the 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 partitioning allows for the use of two immiscible solvents being shaken together to obtain the separation of the required residues from the extraneous co-extractives or their transfer into a suitable solvent for subsequent analysis.

Adsorbent column technique is based on the interaction between a chemical dissolved in a solvent and an adsorptive surface. The clean up can be achieved with either the co-extractives or the pesticides adsorbed onto the adsorbent. In the former, solvent passes through the column, dissolving and removing (“eluting”) residues, while leaving co-extractives attached to the adsorbent. In the latter, the co-extractives pass through the column and the pesticides are eluted with appropriate solvent system. The most commonly used column materials are florisil, alumina, silica gel, magnesium oxide and carbon (Matsumura, 1985; Olson, 1988; Yeboah, 2001).

Gel permeation (or size exclusion) chromatography is a technique that separates compounds from one another on the basis of differences in molecular size. Preparative-size columns are used and samples are placed at the top of the column and eluted with an appropriate solvent. Many of the co-extractives e.g. fats and oils in samples have larger molecules and therefore eluted before the pesticides.

SPE (accumulator or concentrator) technique is based on other clean up techniques such as adsorbent column chromatography. The SPE packing materials or cartridges retain the pesticide 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 pesticide to pass through.

#### **2.3.4 Concentration of sample**

The eluate (cleaned up extract) is concentrated using either nitrogen gas evaporator or rotary evaporator. The idea of concentration is to reduce the volume of the solvent carrying the pesticide residue without losing residue, thereby concentrating the pesticide to a detectable level (Hetzel, 2000).

#### **2.3.5 Identification and quantification of residues**

##### **(a) Bioassay method**

Insect bioassay systems are very specific for separating insecticide chemical from any other group of toxins and contaminants. Test animals are selected on the basis of high pesticide sensitivities and by the ease with which large numbers of them can be reared (Matsumara, 1985). Among these test organisms, the instars of *Artemia salina* Leach are suitable for detecting insecticide residues. The adult *Artemia* is found to be sensitive to a broad range of compounds at concentrations of 0.01 ppm in about forty-five minutes to two hours (McLaughlin, 1991).

##### **(b) Chromatographic methods**

For the routine analysis of pesticide residues in food products, chromatographic methods are by far the most popular. The common chromatography equipment are gas chromatography (GC), high performance liquid chromatography (HPLC), and thin layer chromatography (TLC) (Hetzel, 2000).

The multiple residue methods are preferable in order to include as many pesticides as possible in a single run. This makes the analysis of a large number of pesticides, relatively cost-effective. GC methods have always been predominantly applied in the last decades, due to the unsurpassed separation power of the capillary columns and the choice of various selective and/or sensitivity detectors (De Kok, 2002). The detectors of GC that make the use of the equipment very convenient include electron capture, flame ionization, flame photometric, nitrogen-phosphorus, and thermal conductivity detectors (Hetzl, 2000).

In recent years, the introduction of the routinely used gas chromatograph-mass spectrophotometers (GC-MS) methods, allowing automated identification (based on full spectra comparisons) and quantification, has further improved the performance of GC-multiple residue methods, not only as to the scope of one single method but also the quality of the data produced (De Kok, 2002).

Liquid chromatographic (LC) methods have also gained popularity due to the introduction of more new, polar and/or thermally labile pesticides. Well-known multiple residue methods developed are those for N-methylcarbamates, phenylureas and benzoylureas, using UV, diode-array or fluorescence detection. Similar trend noticeable in the last decade(s) with GC-MS seems to emerge now with LC-MS. Many LC-MS methods, both single and multiple methods are being developed. LC-MS is nowadays the method of choice especially, for single-residue analysis of pesticides that are not amendable to GC because time for analyses is short since cleanup can be minimized or even omitted (De Kok, 2002).

Tin Layer Chromatography technique is based upon partitioning a pesticide between a solvent and a thin layer of adsorbent, which is usually silica or alumina oxide that has

been physically bonded to a glass or plastic plate. Samples are applied in a solvent as spots or bands at the edge of the plate and the plate is then placed in a tank containing a solvent. The solvent migrates up the plate by capillary action, taking the pesticide with it and depositing it at a given distance from the edge of the plate. Following complete development, the plate is removed from the tank and the spots or bands left by the migration of the solvent are detected using several techniques such as visualization under UV light. Other techniques use reagents to produce colours resulting from chemical reaction that is specific for pesticide/reagent combination. Amounts of pesticide can be determined semi-quantitatively by comparison with standards that are developed on the same plate as the unknowns (Olson, 1988).

#### **2.4.0 METHODS FOR RESIDUE ANALYSIS OF SPECIFIC PESTICIDES**

##### **2.4.1. Carbendazim**

Methods for determining residues of carbendazim and its by-products in plant involve isolation of the residue by extraction with an organic solvent and purification of the extract by a liquid-liquid partitioning procedure. Measurement of the residues may be determined by procedures using reversed phase high-performance chromatography, high-speed cation exchange liquid chromatography, immunoassay or gas-liquid chromatography after triflouracetyl derivatization.

In a method tested on apples, cucumbers, grapes, sugar beets, strawberries and cherries for the determination of carbendazim (Gorbach and Kuenzler, 1972) the sample is homogenized with ethyl acetate, HCl, NaHCO<sub>3</sub> and Na<sub>2</sub>SO<sub>4</sub>. The aqueous phase is adjusted to pH 4.5 with ethyl acetate and re-extracted with 1M HCl. The aqueous phase

is analyzed by UV spectrometry. The limit of detection was 0.05 mg/g and average recovery of  $90 \pm 5\%$ .

Kirkland *et al.* (1973) modified this method by using ethyl acetate extraction and liquid-liquid partitions for clean up, with the introduction of HPLC with UV detection. Approximately 20 crops were tested, and the recoveries of benomyl and carbendazim were within the range 0.05 mg/kg (the limit of determination) to 2 mg/kg. Recoveries were generally well above 70%. Miller *et al.* (1990) developed a version of the Kirkland *et al.* (1973) method using extraction with ethyl acetate, acidification with hydrochloric acid, liquid-liquid partitions and HPLC determination. The method is suitable for enforcement purposes, with a limit of determination of 0.05 mg/kg and recoveries ranging from  $77 \pm 6\%$  to  $93 \pm 11\%$ .

Prince (1984) developed another HPLC method for carbendazim residues in plant tissues. After samples are extracted several times with ethyl acetate, the combined extracts are acidified and the ethyl acetate evaporated. The aqueous phase is washed with hexane, adjusted to pH 10 and re-extracted with ethyl acetate. After evaporation of the organic phase, the residues are dissolved in 0.1  $\text{H}_3\text{PO}_4$  and determined with UV detection. The limit of determination was 0.05 mg/kg and recoveries ranged from 76 % from rice to 120 % from lettuce.

McNally (1990) developed an analytical method for the determination of benomyl and its derivatives (carbendazim) in pineapples involving extraction with acetone followed by acidification with hydrochloric acid and cation exchange chromatography. A 1.0 M potassium hydroxide eluate from the exchange column was extracted with ethyl acetate, transferred to methanol solution and analyzed by reversed -phase HPLC with UV

detection at 280 nm. Recoveries from the pulp and peel were 67-120 % for benomyl, and 68-114 % for carbendazim.

A method validated for application to peaches, cucumbers, and tomatoes with a limit of detection of 0.05 mg/kg differs from others in using no organic solvent for the extraction. Samples were extracted with hot hydrochloric acid, followed by filtration and partition into dichloromethane. Subsequent liquid-liquid partitions eventually led to reconstitution of the residue in orthophosphoric acid for determination by ion exchange HPLC with UV detection (Du Pont, 1996).

Residues of carbendazim can be extracted from tomato with ethyl acetate, partitioned into 0.2 M HCL and back into ethyl acetate after adjusting the pH to 12.0. If necessary, further clean up is effected through an amino-bonded solid-phase extraction cartridge, before HPLC with UV detection. The limit of determination was 0.05 mg/kg and mean recovery was 89 % (Taylor, 1996; Taylor and Ferreira, 1997). In another method, combined extraction and clean up was achieved with methanol and solid-phase extraction columns. Elution of the residue with acetone and methanol was followed by HPLC with UV detection. The limit of detection was 0.01 mg/kg and recoveries at 0.02 to 0.5 mg/kg ranged from 74 to 90 % (Melkebeke and Geuijen, 1996)

In a quantitative method, the fungicides are extracted from fruits and vegetables with a mixture of acetone/dichloromethane and petroleum ether. The extract is cleaned up by solid-phase extraction on diol-bonded silica cartridges and the fungicides determined by HPLC with UV and fluorescent detectors in tandem. The limit of determination was 0.05 mg/kg (as carbendazim) and recoveries ranged from 90 to 11%. A qualitative and

semi-quantitative determination for screening was done, where the fungicides are extracted with ethyl acetate and applied to a TLC plate. After development of the chromatogram in ethyl acetate, a suspension of fungal spores of *Penicillium cylopium* is applied to the plate. After incubation, white spots appear on a green background. The diameters of the spots are proportional to the logarithms of the amounts applied on the plate. The limit of determination ranges from 0.01 to 0.1 mg/kg and recovery is over 90 % (Netherlands, 1996).

In another development, Specht (1985) reports that plant material can be analyzed for carbendazim after the sample is homogenized with NaHCO<sub>3</sub>, ethyl acetate and celite for 3 minutes. The organic phase is extracted with sulfuric acid and the residues re-extracted with dichloromethane. The organic extract is evaporated to dryness, the residue dissolved in acetone and potassium carbonate solution and the compound derivatized with pentafluorobenzyl bromide. The solution containing the derivative is purified on mini gel column and the residues determined by GLC with an ECD. The limit of determination was 0.05 mg/kg and recovery was 65-90 % in cereals.

#### **2.4.2 Chlorpyrifos**

Wetters (1973a,b) described a method for determining chlorpyrifos residues in sugar beet and processed fractions. The sample is extracted with acetone (liquid processed fractions with methanol) and cleaned up by a hexane-acetonitrile solvent partition and a silica gel column. Chlorpyrifos is determined by GLC with flame-photometric detection of phosphorous. The limit of determination was 0.01 mg/kg and recoveries from sugar

beet leaves, roots, wet pulp, dry pulp and dry cake samples fortified at 0.01 to 1.0mg/kg were in the range 79-112 %.

Chlorpyrifos residues were extracted from orange pulp and peel with dichloromethane in the presence of anhydrous sodium sulphate (Hollick and Sandenskog, 1976). Clean up was effected by solvent partition and florisil column chromatography. Chlorpyrifos residues were measured by GLC with flame-photometric detector. Analytical recoveries from pulp and peel fortified at 0.01-1.0 mg/kg ranged from 81-112 %.

Wettters (1977) used Wettters (1973a,b) method to determine chlorpyrifos residues in oranges. Analytical recoveries from whole oranges and orange peel and pulp fortified at 0.01, 0.1, 1.0 and 2.0 mg/kg were in the range 79-104 %. Orange juice samples were extracted by blending with methanol, but the solvent partition and column chromatography clean up and GLC determination were as described by Wettters (1973a). Recoveries of chlorpyrifos from orange juice fortified at 0.01, 0.1 and 1.0 mg/kg were in the range 73-100 %.

Wettters (1985) extracted chlorpyrifos from oranges with methanol and cleaned up the extract on a C<sub>18</sub> Sep-Pak. After a solvent partition into hexane the chlorpyrifos was determined by GLC with a flame-photometric detector. The limit of detection was 0.01 mg/kg. Analytical recoveries of chlorpyrifos from samples fortified at 0.01 -2.0 mg/kg ranged from 71-103 %.

Robb (1991) used the Wettters (1985) method for chlorpyrifos on oranges. Analytical recoveries from samples fortified at 0.01-1.0 mg/kg ranged from 83-96%.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1.0 SURVEY ON PESTICIDE USE PATTERNS

##### 3.1.1 Development of questionnaires

In order to obtain an overview on pesticide use, a survey with questionnaires (Appendix 1) was conducted to obtain required data needed for quantification (Horton, 1995).

The data that were needed from the farmers include:

- \* Education and scale of production.
- \* Cropping period.
- \* Decision making in pesticides application.
- \* Acquisition of pesticides.
- \* Safe use of pesticides.
- \* Information on pesticides use.
- \* Problems associated with the use of pesticides.

##### 3.1.2 Selection of areas and respondents

The survey covered the major pineapple growing areas in the Akwapim South District of Ghana with Nsawam as the administrative district capital. The pineapple farmers in six (6) operational areas were randomly selected and interviewed (Table 2).

The pineapple farmers were categorized into small, medium, and large-scale producers depending on their current areas of cultivation. Farmers cultivating less than 2 hectares, 2-10 hectare and more than 10 ha were categorized as small, medium, and large-scale producers respectively (Gerken *et al.*, 2001). A total of 148 pineapple farmers were interviewed over a three-month period, from July -September, 2001.

**Table 2:** Number of Farmers interviewed in each Operational Area

<b>Operational Area</b>	<b>Number of Farmers</b>
Apentem	28
Awerase/Damang	12
Fotobi	28
Nsakyee	25
Oboadaka	27
Yaw Duodu	28
<b>Total</b>	<b>148</b>

## **3.2.0 ANALYSIS OF CHLORPYRIFOS AND CARBENDAZIM RESIDUES**

### **3.2.1 Reagents**

Hexane (analytical grade), acetone (pesticide grade), ethyl acetate (pesticide grade), methanol (pesticide grade) and anhydrous sodium sulphate (analytical grade) were obtained from BDH Laboratory Supplies, England. Chlorpyrifos standard (99.5% purity WP) and Carbendazim standard (98.5% purity WP) were obtained from Dr Ehrenstorfer GmbH (Germany).

### **3.2.2 Apparatus**

Encore Grinder (Speed-8000 RPM), centrifuge, Solid Phase Extraction (SPE) tubes, weighing balance, vials, Gas Chromatograph (GC), rotary vacuum evaporator, TLC tanks, TLC plates (10 cm x 20 cm silica gel 60), Eyla soft incubator SLI-600, 10  $\mu$ l calibrated micro syringe and spotting guide.

### **3.2.3 Preparation of standard solution**

#### **3.2.3.1 Chlorpyrifos**

Ten milligrams of chlorpyrifos standard was accurately weighed and transferred to a 2 ml vial, diluted to 1 ml with hexane and mixed as a stock solution. Further stock 100 $\mu$ g/ml was prepared and from which serial dilutions were made for the calibration curve.

### **3.2.3.2 Carbendazim**

Ten milligrams of carbendazim standard was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 100 ml methanol to give stock solution with a concentration of 100 $\mu$ g/ ml that was used for the calibration curve.

### **3.2.4 Sample collection**

The FAO/WHO (2000a) sampling procedure was used.

Pineapple fruits for chlorpyrifos residue analysis were sampled from five out growers of Farmapine Ghana Ltd.- Nsawam at five different locations in the Akwapim South District of Ghana. Five boxes of pineapple fruits for carbendazim residue analysis were also sampled from 100cartons at the pack house of Farmapine Ghana Ltd.- Nsawam prior to export. After sample collection in each case, a laboratory sample of about 2.4 kg was separated and sealed in polythene bag and sent to the Biochemistry Laboratory at the University of Ghana, Legon for the removal of the analytical sample for extraction. The analytical sample was sub-sample of either pulp or peel taken out for extraction.

### **3.2.5 Extraction**

The peel was separated from the pulp and extracted separately. The sample (either the pulp or peel) was chopped and mixed thoroughly and a sub-sample of 25g was taken out for blending.

#### ***Chlorpyrifos***

A sub-sample of 25g was blended with 50 ml methanol for 2-3 minutes. The homogenate was decanted into two centrifuge tubes and centrifuged at 5000 rpm for 5

minutes. The upper (organic extract) layers of the tubes were pooled together in a 100 ml conical flask. Twenty millilitres of the organic extract was diluted with 50 ml distilled water.

### ***Carbendazim***

A sub-sample of 25g was blended with 50 ml ethyl acetate for 2-3 minutes. The homogenate was decanted into two centrifuge tubes and centrifuged at 5000 rpm for 5 minutes. The upper (organic extract) layers of the tubes were pooled together in 100 ml conical flask. Thirty millilitres of the solvent phase was transferred into a 100ml conical flask containing 5g anhydrous sodium sulphate to remove moisture present.

The extraction procedure for Carbendazim and Chlorpyrifos residues was replicated for both the pulp and the peel and after each extraction; acetone was used to wash the blender and centrifuge tubes thoroughly.

### **3.2.6 Clean up**

The Solid Phase Extraction (SPE) C18 tubes with carbon as adsorbents were used to clean up the extracts for Chlorpyrifos residues. The SPE tubes were conditioned with methanol followed by distilled water. The organic extract was eluted into 100 ml beaker and the eluate was discarded. The SPE tube was rinsed with distilled water and dried by vacuum for 30 minutes. The tube was eluted with 3 x 0.5 ml hexane. The eluate was then evaporated to dryness under a stream of nitrogen gas and dissolved in 100  $\mu$ l of hexane and stored in a refrigerator.

### 3.2.7 Concentration of extracts

The moisture-free extract of carbendazim residues was concentrated using rotary vacuum evaporator. The concentrated extract was reconstituted into 1ml methanol and then evaporated under a stream of nitrogen gas to dryness. The dried extract was dissolved in 500 $\mu$ L of methanol and stored in a refrigerator to be used for spotting on a TLC plate.

### 3.2.8 Calibration Curve

#### 3.2.8.1 Chlorpyrifos

Hexane (680  $\mu$ l) was pipetted into a 2 ml vial and 320 $\mu$ l of chlorpyrifos standard solution (chlorpyrifos 32 $\mu$ g) was added. The concentration of 32 $\mu$ g/ml was serially diluted by factor 2 to concentrations of 16 $\mu$ g/ml, 8 $\mu$ g/ml, 4 $\mu$ g/ml, 2 $\mu$ g/ml, 1 $\mu$ g/ml, and 0.5 $\mu$ g/ml and 0.25 $\mu$ g/ml. A 5 $\mu$ l microsyringe was used to draw 1 $\mu$ l of each concentration and injected it into the injection pot of the gas chromatograph and the responses were recorded. A calibration curve was constructed by plotting the concentration against their respective peak areas.

#### GC Conditions

Shimadzu Gas Chromatograph GC-9A equipped with an electron capture detector (ECD); Column was SPB<sup>TM</sup> 608 Spelco<sup>TM</sup> Fused Silica Capillary Column (15 m x 0.53 mm x 0.5 $\mu$ m film thickness).

The column oven temperature was held at 190 °C. The temperatures of the injector and detector were 250 °C and 300 °C, respectively. The injection volume of the GC was 1 $\mu$ l. The carrier gas was helium set at a flow rate of 40 ml/min.

### **3.2.8.2 Carbendazim**

Three thousand two hundred microlitres (320 $\mu$ g carbendazim) of the stock solution was pipetted into a 2-ml vial and evaporated to dryness under a stream of nitrogen gas and then dissolved in 0.5 ml methanol. The concentration (320 $\mu$ g/500 $\mu$ l) was serially diluted by factor of two to concentrations of 160 $\mu$ g/500 $\mu$ L, 80 $\mu$ g/500 $\mu$ L, 40 $\mu$ g/500 $\mu$ L, 20 $\mu$ g /500 $\mu$ L and 10 $\mu$ g/500 $\mu$ L. These concentrations were used for the plotting of the calibration curve.

#### **(a) Preparation of Yeast extract-glucose-potassium nitrate (YGP) medium**

One and half grams each of glucose, agar-agar, yeast extract and 0.3 g of potassium nitrate were weighed into 100 ml de-ionised water in 250 ml conical flask. The mixture and 10 petri dishes were autoclaved at 121<sup>0</sup>C for 30 minutes. The YGP was allowed to cool to 45 <sup>0</sup>C in de-ionised water bath and 0.5 g oxytetracycline (antibiotic) was added and mixed thoroughly. The medium was poured into the 10 petri dishes and allowed to set (IAEA, 2000).

#### **(b) Culturing of *Aspergillus niger* Tiegh.**

The spores of *A. niger* were carefully removed from an old culture with a loop and streaked on the surface of the media in the petri dishes. The petri dishes were placed in an air-saturated incubator set at 25 <sup>0</sup>C. The new culture developed within 5 days (Plate 2) and after this period the petri dishes were covered with parafilm to arrest the growth of the fungus and kept in a refrigerator (IAEA, 2000).

**(c) Development of the TLC plates**

Ready-made, silica gel 60-coated TLC plates were activated in an oven at 105 °C for 30 minutes. The TLC tank was saturated with the vapour from 50 ml ethyl acetate used as the developing solvent. Lining the walls of the tanks with filter paper cut to the size of the tanks and allowing the vapour to soak the filter paper for 3 hours with the tank covered achieved the saturation. Each concentration (5 µl) was spotted on the activated TLC plates using 10 µl calibrated micro syringe. The TLC plates were developed by dipping them into the saturated TLC tanks where the developing solvent rises by capillary action. The solvent was allowed to move to more than two-thirds of the length of the TLC plates. The TLC plates were removed from the tanks after reaching quantify solvent front and allowed to dry.

**(d) Suspension of *A. niger* Tiegh spores.**

One and half grams each of agar-agar, glucose and yeast extract and 0.3g potassium nitrate were weighed into 70 ml distilled water in 250 ml conical flask. The mixture was autoclaved at 121 °C for 30 minutes. The agar-agar solution was removed from the autoclave and cooled to 50 °C in de-ionised water bath. *A. niger* spores were removed from the culture in a petri dish by adding 30 ml bi-distilled water and cautiously drawing with glass spatula until most of the spores were loosened. The suspension was added to the agar-agar solution and filtered through double layer gauze and then kept in 45 °C de-ionised water bath (IAEA, 2000).



**Plate 2:** Fresh culture of *A. niger* Tiegh in petri dishes.

**(e) *Spraying of the mixture of A. niger Tiegh spores and medium***

The spraying device was warmed up by immersing it into 40 °C de-ionised water bath to avoid the setting of the agar-agar. The mixture was transferred into the spraying gun and sprayed on four air-dried TLC plates until the adsorbent layers got thoroughly wet. The plates were then immediately transferred into a pre-saturated incubator held at 37 °C, and incubated for 48 hours. The incubator was pre-saturated by placing 3 petri dishes filled with de-ionised water on every shelf (IAEA, 2000).

**(f) *Detection and measurement of spots***

The location of a spot on a chromatogram is an index of the chemical nature of the compounds separated and hence can be used as a basis for identification of particular compounds. The relative rates of migration are usually expressed as  $R_f$  value (relative to solvent front) that is determined by the ratio of distances.

The  $R_f$  is expressed as:

$$\frac{\text{Distance of centre of the spot from starting point.}}{\text{Distance of solvent front from starting point}}$$

The average diameters of the spot given by each concentration, distance moved by the spot and the solvent front from the origin were measured. A calibration curve was plotted using the average diameters of spots against their corresponding concentrations.

### 3.3.0 Identification and quantification

#### *Chlorpyrifos*

A sub-sample of 25g was blended with 50 ml methanol for 2-3 minutes. The homogenate was centrifuged at 5000 rpm for 5 minutes. Twenty millilitres of the upper (organic extract) layer was diluted with 50 ml distilled water.

The Solid Phase Extraction (SPE) C18 tubes with carbon as adsorbents were used to clean up the extracts for chlorpyrifos residues as described under **section 3.2.6**. Thereafter 1 $\mu$ l of the extract was injected into the GC device. Residues of chlorpyrifos in the samples were identified on the basis of their respective retention times and quantified on the basis of their respective peak areas.

#### *Carbendazim*

A sub-sample of 25g was blended with 50 ml ethyl acetate for 2-3 minutes. The homogenate was centrifuged at 5000 rpm for 5 minutes. Thirty millilitres of the solvent phase was transferred into a 100ml conical flask containing 5g anhydrous sodium sulphate to remove moisture present.

The moisture-free extract of carbendazim residues was concentrated. The concentrated extract was reconstituted into 1ml methanol and then evaporated under a stream of nitrogen gas to dryness. The dried extract was dissolved in 500 $\mu$ L of methanol. Thereafter 5 $\mu$ l of the extract was spotted on each activated TLC plates and subjected to TLC methodology of analysis described under **section 3.2.8.2 (c-f)**. Residues of cabendazim in the samples were identified on the basis of their respective  $R_f$ s and quantified on the basis of their average diameters.

Carbendazim and Chlorpyrifos content in the samples were calculated using the following equation:

$$\text{Residue ((}\mu\text{g/g)} = \frac{C}{M} \times \text{dilution factor.}$$

Where C is the content of either carbendazim or Chlorpyrifos in the sample vial as estimated from the calibration curve ( $\mu\text{g}$ ). M is the weight of sample (g).

### 3.3.1 Recovery Test

A recovery test was carried out in triplicate and performed by fortified samples with  $2\mu\text{g}$  chlorpyrifos/g sample and  $4\mu\text{g}$  carbendazim/g sample. The fortified samples as well as blank were subjected to similar analytical procedures described under **sections 3.2.5, 3.2.6, 3.2.7 and 3.2.8.2 (c-f)**.

The concentration of the pesticides (Chlorpyrifos and Carbendazim) in the recovered extracts were calculated using the formula below:

$$\text{Recovery test (\%)} = \frac{\text{Pesticide (ppm) recovered from fortified sample}}{\text{Amount of pesticide (ppm) added.}} \times 100$$

### 3.3.2 Limit of detection (LOD) determination

#### *Chlorpyrifos*

The extracts of the fortified samples were serially diluted by a factor 2 to give different concentrations. One millilitre of each concentration was injected and the least concentration that gave response was noted.

LOD was calculated by the formula:

$$\text{LOD} = \frac{V_1 (\mu\text{L})}{V_2 (\mu\text{L})} \times \text{conc. fortified}$$

$V_1$  = volume injected.

$V_2$  = Final volume of fortified extract.

### ***Carbendazim***

Different volumes (4 $\mu$ l-0.5 $\mu$ l) of extract to which additional carbendazim was added was spotted on activated TLC plates and subjected to TLC methodology of analysis described under **section 3.2.8. 2 (c-f)**. The least volume of the fortified extract that gave a response was noted. LOD was calculated as:

$$\text{LOD} = \frac{V_1 (\mu\text{L})}{V_2 ((\mu\text{L}))} \times \text{concentration (ppm) added}$$

Where

$V_1$  = Least volume to give response.

$V_2$  = Volume of the fortified sample.

## CHAPTER FOUR

### 4.0 RESULTS AND DISCUSSION

#### 4.1 SURVEY ON PESTICIDE USE PATTERNS

##### 4.1.1 Education and scale of production

About 6.3%, 14.0%, and 10.5% of the small, medium, and large-scale producers, respectively, did not have any formal education (Fig. 1). Similarly, 22.8%, 10.5%, and 10.5% of the small, medium, and large-scale producers, respectively, had primary education. Additionally, 58.2% and 56.0 % of the small and large-scale pineapple producers, respectively, were Junior Secondary School/Middle School leavers. Secondary School (O/A Level, SSS) holders constituted 36.8% of the large-scale pineapple producers. The medium and large-scale producers had 8.0% and 10.5% made up of diploma and degree holders

Production of pineapple for export is a very lucrative venture. However, there is low level of involvement of graduates in pineapple farming which may be due to the perception that farming is meant for the less privileged in society. These results conformed to the survey conducted by Gerken *et al.* (2001), which indicated that only 11.8% of the farmers in the surveyed area had education higher than senior secondary school. This is the time to de-mystify the misconception about farming and entice graduates with background in agriculture to invest in pineapple production in order to reduce the perennial graduate unemployment in the country.

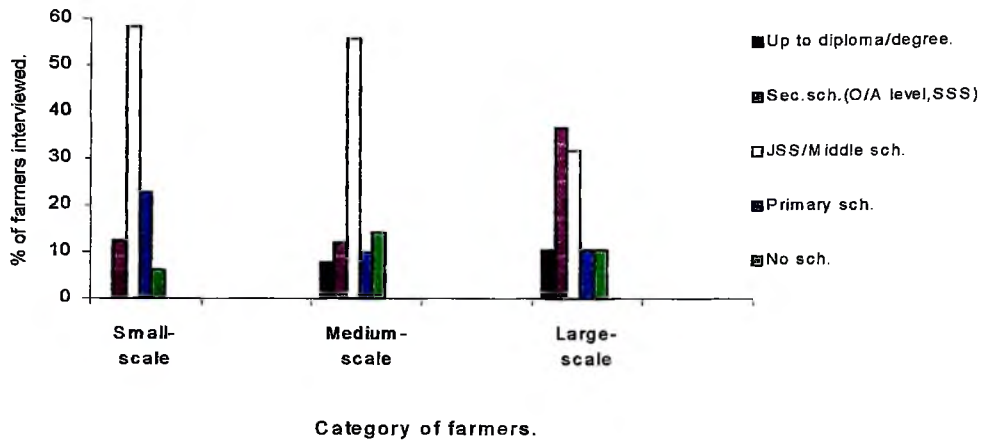


Fig. 1: Educational level of interviewed farmers.

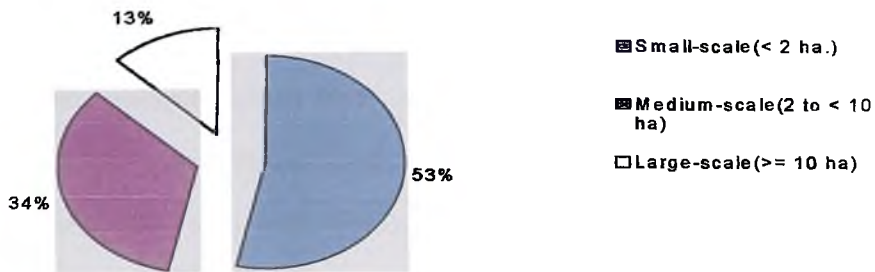


Fig. 2 : Scale of production.

The survey revealed that 53% of the 148 pineapple farmers interviewed were cultivating less than 2 hectares hence classified as small-scale producers (Fig.2). Medium and large-scale producers constituted 34 % and 13 %, respectively. The initial production of exportable pineapple involves the acquisition of land and planting materials, and other farm inputs that are not within the reach of many farmers.

#### **4.1.2 Cropping period**

The survey revealed that 42% of the pineapple farmers use the same piece of land for more than 3 years (Fig.3). Out of the 148 pineapple farmers interviewed, only 18 representing 12.2% had just started cultivating new fields.

The growing of pineapple over a large area for a long time provides an unlimited source of food and favourable microclimate for pest species and build up of pest population (Hill, 1993). Pineapple farmers essentially control weeds in their pineapple farms with herbicides without disturbing the soil (Abutiate, 1991). The soil inhabiting pests e.g. termites and symphilids, are therefore not killed by exposure to the sun and to desiccation, predators and parasites. Monoculture and continuous growing of crops including pineapple harbour fewer insect pests and eliminate the competing species. Many of the insects released from competitive pressure may increase in numbers and become serious pests (Hill, 1993). This practice of monoculture and continuous therefore necessitates intensive use of pesticides, with the subsequent high residue levels on crops.

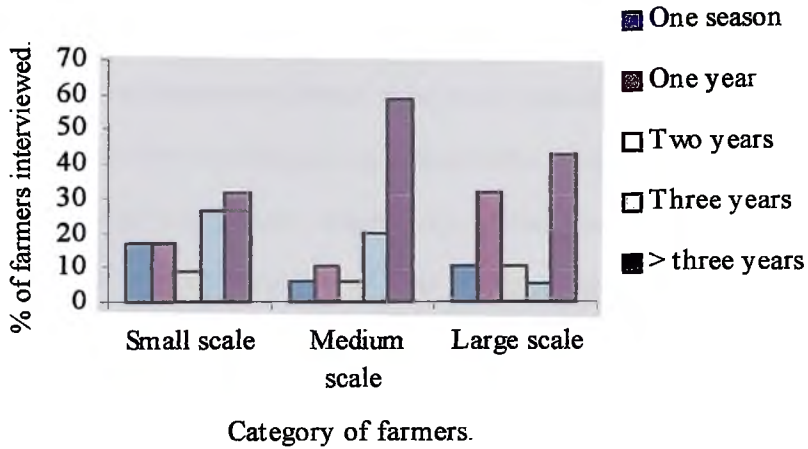


Fig. 3 Period of cultivation on the same field.

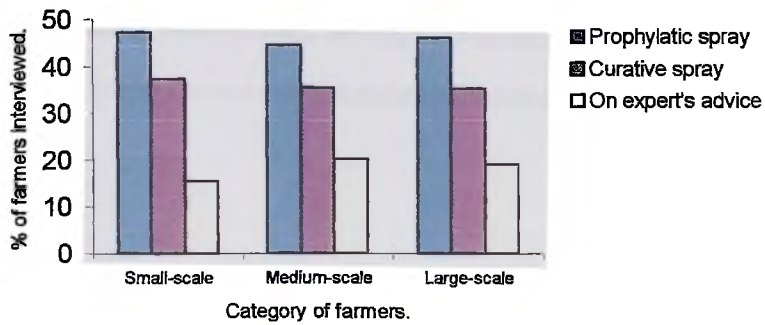


Fig. 4: Farmers decision on application of pesticide.

#### **4.1.3 Decision making in pesticides application**

Virtually, all the pineapple farmers use pesticide for field and post harvest treatment. In the survey, the pineapple farmers were given alternative questions on when and how they applied pesticides. The results in Fig. 4 show that 47.3%, 44.4% and 45% of the small, medium and large-scale farmers respectively applied pesticides on prophylactic basis, the synonym for calendar spraying. About 37.3%, 35.4%, and 35.2% of the small, medium, and large-scale farmers, respectively, applied pesticide based on individual decision (curative) on the incidence of pests at certain threshold level. Only 15.4%, 20.6%, and 18.9% of the small, medium, and large-scale farmers, respectively, sought expert advice before applying a pesticide. An expert in this context refers to Agricultural Extension Agents (AEAs) or staff from Produce Buying Companies or Co-operative Societies.

Most of the pineapple farmers claimed they had limited information on pest levels and nature of damage caused yet only few contacted experts before applying pesticide. With little knowledge in handling pests, farmers are expected to seek expert advice before applying pesticide to promote a sound pest management in pineapple field but this was not the case.

#### **4.1.4 Acquisition of pesticides**

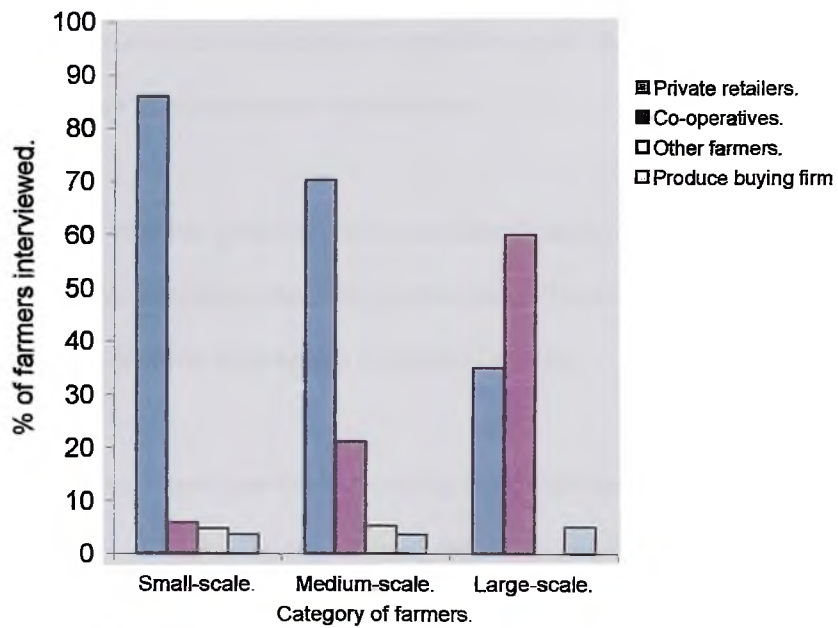
The survey revealed that 86% of the small-scale and 70.2% of the medium-scale farmers acquired their supplies of pesticides from private retailers, whereas 60% of the large-scale farmers obtained their pesticides from their Co-operative Societies (Fig.5).

It was observed that those who bought the pineapple offered little assistance to the

farmers in terms of pesticides supplies.

According to Gerken *et al.* (2001) the importation and distribution of pesticide in Ghana has been dominated by the private sector in recent years. It is therefore not surprising that most of the pineapple farmers acquire their supplies from private retailers.

In line with the frequent prophylactic strategies employed for pineapple protection, 69.1%, 68.5% and 95% of the small, medium and large-scale farmers, respectively acquired their pesticides in advance. About 30.9%, 31.5% and 5.0% of the small, medium and large-scale farmers, respectively, bought pesticides only after the appearance of the pests.



**Fig. 5: Sources of farmers' acquisition of pesticide.**

#### 4.1.5 The use of single pesticide

African mole cricket, *Gryllotalpa africana*, the African giant cricket, *Brachytrypes membranaceus*, termites, *Macrotermes spp.*, fruit boring caterpillars, symphilids (*Hanseniella spp.* and *Scutigera spp.*), mealybugs (*Dysmicoccus brevipes* and *D. neobrevipes*) and weeds were identified as a serious threat to pineapple fruit production in the survey area. In an attempt to control these pests the farmers used any available pesticide as an insurance against crop damage.

A total of twenty-one pesticides were enumerated during the survey (Table 3). Out of the twenty-one pesticides, only nine, representing 47%, were provisionally approved for use during production of pineapple in Ghana (Table 4).

The most widely used insecticide according to the field survey was chlorpyrifos, which was used by 70%, 81.3% and 63% of the small, medium and large-scale farmers respectively. Cypermethrin and dimethoate were the second rated insecticides used by these farmers. Very few (i.e. 4%) of the small-scale producers used other insecticides such as lambda-cyhalothrin and lindane.

The dominant herbicides used, according to the survey were diuron, bromacil, and fluazifop-butyl (Table 3). All other herbicides including ametryne, paraquat, and glyphosate were being used in isolated cases.

Among the fungicides used to control *Phytophthora*-causing diseases on the field, fosetyl-aluminium was predominant one used by 8.9%, 12%, and 21.1% of the small,

medium and large-scale farmers, respectively (Table 3). Only 1.3% of the small-scale and 5.3% of the large-scale farmers used thiophanate-methyl and metalaxyl respectively, to control of fungal diseases on the field. The farmers were skeptical about the survival of the wilted plants after treatment with fungicides on the field. This attests to the failure of 88.8%, 88.0% and 73.6% of the small, medium, and large-scale farmers respectively, to apply fungicides on the field and instead rogue the diseased plants (Table 3). Exporters used carbendazim and prochloraz to treat the fruits just before packing to control rots caused by *Ceratocystis paradoxa* that penetrates via wounds and cut stem.

The exporters and a few farmers sprayed the growth regulator, ethephon on the fruits in about 7 days before harvesting to activate and homogenize peel colour. Ethephon is an ethylene generator that causes the breakdown of the chlorophyll in the peel but without accelerating any other fruit maturation process. The uniform colouring of the fruits eliminates the need for multiple pickings and reduces harvesting cost. Additionally, the farmers/exporters apply ethephon to avoid the risk of the downgrading of fruits because naturally ripe fruits have black spots or serious blemishes even though they are sweeter (Teisson, 2000).

The use of provisionally unapproved pesticides for application on pineapple suggests that the farmers were unaware of the provisionally approved pesticides for the application on exportable pineapple in Ghana. This situation is worrying and poses a threat to the pineapple industry since the detection of residues of some of the 12 unapproved pesticides will have serious repercussion on pineapple export. The farmers should therefore be made aware of the provisionally approved pesticides and the consequences of the use of unapproved pesticides on pineapple industry.

**Table 3: Use of single pesticide**

<b>Pesticides</b>	<b>Percentage no. of farmers interviewed</b>		
	<b>Small-Scale</b>	<b>Medium-Scale</b>	<b>Large-Scale</b>
<i><b>Insecticides</b></i>			
Chlorpyrifos	70.0	81.3	63.0
Cypermethrin	6.0	6.8	25.9
Dimethoate	10.0	5.1	7.4
Diazinon	6.0	3.4	3.7
Endosulfan	4.0	3.4	0
Lambdacyhalothrin	2.0	0	0
Lindane	2.0	0	0
<i><b>Herbicides</b></i>			
Diuron	36.4	36.2	33.3
Bromacil	30.0	34.6	33.3
Fluazifop-butyl	29.4	24.4	27.8
Atrazine	1.6	4.0	3.7
Laso-atrazine	0.5	0	0
Glyphosate	1.6	0	1.9
Paraquat	0.5	0	0
Ametryne	0	0.8	0
<i><b>Fungicides</b></i>			
Fosetyl-aluminium	8.9	12.0	21.1
Metalaxyl	0	0	5.3
Thiophanate-methyl	1.3	0	0
Carbendazim	For post harvest treatment of fruits		
Prochloraz		”	
Non-application	89.8	88.0	73.6
<i><b>Growth regulator</b></i>			
Ethephon	12.7	16.0	21.1
Non-application	87.3	84.0	78.9

**Table 4:** Provisionally approved pesticides for application on pineapple in Ghana.

Trade Name	Active ingredient	Type
Diuron	Diuron	Herbicide
Fusilade	Fluazifop-butyl	„
Roundup	Glyphosate	„
Dursban	Chlorpyrifos	Insecticide
Cypermethrin	Cypermethrin	„
Perfekthion	Dimethoate	„
Ridomil	Metalaxyl	Fungicide
Goldazim	Carbendazim	„
Ethrel	Ethephon	Growth regulator

**Source:** Suglo *et al.* (2001)

#### 4.1.6 The use of combination of herbicides

It is interesting to note that all the small, medium, and large-scale farmers applied mixtures of two or three herbicides. About 36.3%, 54.9%, and 54.6% of small, medium, and large-scale farmers respectively applied mixtures of three herbicides (Table 5).

A higher percentage of small-scale farmers (about 67.7%) applied mixture of two herbicides. Small-scale farmers applied more mixtures than the other two groups.

The farmers claim that the use of a mixture of herbicides achieves effective control but surprisingly herbicides of the same mode of action are mixed, e.g. diuron and glyphosate that are broad-spectrum herbicides (Hassall, 1990) are mixed together and applied.

Fluazifop-butyl is a selective grass herbicide and should be used on the field with grass as predominant weed (Suglo *et al.*, 2001). The result of the survey revealed that fluazifop-butyl was mixed with diuron, a broad-spectrum herbicide. This combination is not necessary and can lead to waste of pesticides and economic loss, and increase the cost of production. The correct time of application (either as pre-emergence or post-emergence) of herbicides reduces the rate per hectare and achieves effective weed control.

**Table 5:** Combination of herbicides used by farmers

Combination of herbicides	Percentage no. of farmers interviewed		
	Small-Scale	Medium-Scale	Large-Scale
Diuron/Atrazine/Bromacil	2.6	5.8	0
Diuron/Bromacil/Fluazifop-butyl	29.8	45.1	54.6
Diuron/Glyphosate/Bromacil	1.3	0	0
Diuron/Fluazifop-butyl/Atrazine	1.3	0	0
Fluazifop-butyl/Paraquat/Atrazine	1.3	2.0	0
Diuron/Ametryne/Bromacil	0	2.0	0
Diuron/Atrazine	0	2.0	0
Diuron/Bromacil	24.7	27.5	27.3
Diuron/Fluazifop-butyl	27.3	9.8	9.1
Fluazifop-butyl/Bromacil	10.4	5.8	4.5
Fluazifop-butyl/Atrazine	0	0	4.5
Paraquat/Glyphosate	1.3	0	0

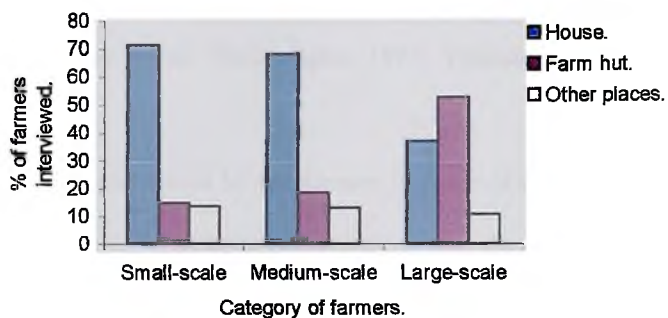
#### **4.1.7.0 The safe use of pesticide**

##### **4.1.7.1 Handling and application of pesticide**

About 71.6% of the small-scale and 68.5% of the medium-scale pineapple producers store their pesticides in the house. On the contrary 52.6% of the large-scale producers store their pesticides in the farm hut (Fig.6). Pesticides are hazardous chemical and therefore handling them can pose safety risk especially if the facilities and equipment are poorly designed or maintained. Pesticides and pesticide containers should be kept in a separate store/room or enclosure. This storage area must be exclusive for pesticides and empty containers. The store or room used for this purpose should be dry, well-ventilated, smooth cement floor, enough lighting and under lock and key (Yeboah, 1999; Suglo, 2000). However, these farmers store their pesticides in their houses where they and their families can be exposed leading to chronic and acute toxicity of these chemicals (Altenburger, 1995).

Pineapple farmers use knapsack sprayers to apply pesticide on the field. However, planting materials are treated with insecticides by hand.

The survey revealed that most of the small-scale (about 56.9%) and medium scale (52.6%) farmers applied pesticides themselves (Table 6). Large-scale farmers employ labourers or caretakers who assist in the application of pesticides. Spouses, children, other farmers, and commercial sprayers also play a role in pesticide application.



**Fig. 6:** Storage of pesticides.

**Table 6:** Categories of person applying pesticides in the field.

Group of persons	Percentage of farmers interviewed		
	Small-scale	Medium-scale	Large-scale
Farmer himself	56.9	52.6	23.1
Spouse	2.6	0	0
Farmer's children	4.3	1.3	3.8
Labourers, caretakers	16.4	34.6	57.8
Other farmers	13.8	6.4	3.8
Commercial sprayers	6.0	5.1	11.5

Pesticides' protective clothings diminish but do not entirely remove the danger of pesticide absorption through the skin or respiratory tract. These include hat, overall/a long sleeved shirt and long pair of trousers, Wellington boots, apron, rubber gloves, goggles, and respirator (face mask) (Meinzingen, 1993; Yeboah, 1999; Suglo *et al.*, 2001).

The main protective items mentioned by the farmers in order of importance were long trousers & long sleeved shirts, Wellington boots, gloves, overalls, hats, respirators, and goggles (Table 7). None of the farmers used apron.

The general awareness of protective device seemed to be common. However, the transfer of knowledge into practice seemed to be weak. None of the farmers interviewed used the complete set of protective clothing that is technically desirable (Table 8). This suggests that the farmers neglect their personal protection while applying pesticide despite the known hazards of pesticide. It is therefore not surprising that some farmers are exposed to the danger of acute poisoning and general ill health (Gerken *et al.*, 2001). The common reasons assigned for not wearing protective clothing during pesticides application were unaffordable price, uncomfortable and unbearable in the hot weather, and unavailability.

**Table 7:** Use of protective clothing for pesticide application by farmers

Type of clothing	Percentages of farmers interviewed		
	Small-scale	Medium-scale	Large-scale
Hat	9.3	10.8	5.8
Long trousers & long sleeved shirts	27.9	27.8	25.7
Overall	11.4	9.2	10.0
Apron	0	0	0
Gloves	21.4	19.3	25.7
Wellington boots	24.3	26.1	25.7
Respirator	4.3	5.1	5.7
Goggles	1.4	1.7	1.4

**Table 8:** The combination of protective clothing used for pesticide application

Combination of protective clothing	% no. of farmers interviewed		
	Small-Scale	Medium-Scale	Large-Scale
Hat, long trousers & long sleeved shirts, gloves, Wellington boots & respirator.	1.3	4.0	0
Long trousers & long sleeved shirts, gloves & Wellington boots.	25.3	24.0	42.0
Hat, long trousers & long sleeved shirts & Wellington boots.	8.9	2.0	10.5
Long trousers & long sleeved shirts & Wellington boots.	5.0	12.0	0
Long trousers & long sleeved shirts, gloves, Wellington boots & respirator.	2.5	2.0	5.3
Hat ,long trousers & long sleeved shirts & gloves	1.3	2.0	0
Long trousers & long sleeved shirts, overall, gloves & Wellington boots.	13.9	20.0	15.7
Hat, overall, gloves & Wellington boots.	1.3	2.0	0
Hat, long trousers & long sleeved shirts, gloves & Wellington boots.	7.6	10.0	5.3
Hat, long trousers & long sleeved shirts, overall, gloves, Wellington boots & respirator.	1.3	2.0	5.3
Hat, long trousers & long sleeved shirts, overall gloves, Wellington boots, respirator & goggles	1.3	2.0	0
Long trousers & long sleeved shirts, overall & Wellington boots.	2.5	0	0
Long trousers & long sleeved shirts, overall, respirator & Wellington boots.	2.5	0	0
Hat, long trousers & long sleeved shirts, gloves respirator Wellington boots.	2.5	0	0

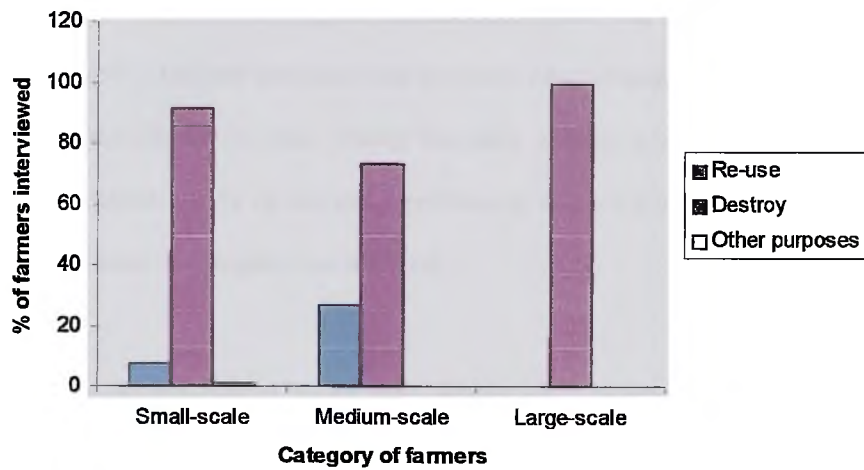
Table 8 Continue

Combination of protective clothing	% No.of farmers interviewed		
	Small-Scale	Medium-Scale	Large-Scale
Hat, long trousers & long sleeved shirts, respirator & Wellington boots.	1.3	0	0
Hat, long trousers & long sleeved shirts, overall, gloves, Wellington boots & goggles.	1.3	0	0
Long trousers & long sleeved shirts, overall, gloves, respirator & Wellington boot.	1.3	0	0
Long trousers & long sleeved shirts & gloves.	3.7	4.0	0
Long trousers & long sleeved shirts, overall & gloves.	7.6	0	5.3
Hat, long trousers & long sleeved shirts, overall, respirator, Wellington boots & goggles.	1.3	0	0
Long trousers & long sleeved shirts, overall, gloves. Wellington boots & goggles.	1.3	0	0
Overall, gloves, respirator, Wellington boots & goggles.	0	0	5.3
Hat, long trousers & long sleeved shirts, overall & gloves.	0	2.0	0
Long trousers & long sleeved shirts, respirator & Wellington boots	0	2.0	0
Long trousers & long Sleeved shirts, gloves, respirator, Wellington boots & goggles.	0	2.0	0
Hat, long trousers & long sleeved shirts, respirator, Wellington boots & goggles.	0	2.0	0
Hat, long trousers & long sleeved shirts,overall & Wellington boots.	2.5	0	0
Hat, long trousers & long sleeved shirts,overall Wellington boots & gloves	2.5	6.0	5.3

#### 4.1.7.2 Disposal of pesticide containers

About 73.3-100% of the farmers destroyed empty pesticide containers (Fig. 7). About 7.4% and 6.7% of the small- and medium-scale producers respectively re-used the empty containers for storage or for buying pesticides again. Few small-scale producers (about 1.2%) used empty containers for other purposes such as storage of kerosene.

The careful disposal of empty containers is an important part of safe pesticide use. Empty containers can be hazardous to children and domestic animals and therefore should never be converted for other uses (Yeboah, 1999; Suglo *et al.*, 2001). The empty containers are to be kept separate in pesticide store until final disposal in collaboration with Environmental Protection Agency (EPA) (Suglo *et al.*, 2001). The proper and safe disposal of empty pesticide containers avoids serious contamination and hazards to the environment and human life (Meinzingen, 1993).



**Fig. 7: Disposal of pesticide containers**

#### 4.1.7.3 Rates of pesticide application

The results of the survey revealed that most farmers applied higher rates of insecticides (Table 9). On the contrary most of the farmers applied the recommended rates of herbicides. Few farmers who applied metalaxyl to control diseases used the required application rate

One producer/exporter interviewed indicated that he had ceased post-harvest treatment of the fruits with fungicides for export. The rationale behind was to evade the possible high residual level. Only one indicated that the fruits were treated with carbendazim at the rate of 15ml/15litres of water during the rainy season, which of course is the required rate. About 59.1% of the exporters/farmers applied lower rate of ethephon whereas 36.4% used rates higher than required.

The lower rate of insecticides coupled with calendar spray enhanced insect's tolerance to these insecticides (Yeboah, 1999). It is therefore not surprising that some farmers sprayed their field about eight (8) times during the cropping period. The higher rate of insecticide spray eliminates most of the susceptible individuals leaving a high percentage of heterozygous resistant individuals (Matsumura, 1985) and with continuous breeding of these insects development of insecticide resistance can become far more rapid (Hill, 1993). The development of resistance could result in the use of higher rate of insecticides to control insect pests. This will result in economic loss and exposure of the applicators to chronic and acute toxicity of these pesticides (Matsumura, 1985). As a result of the use of mixture of herbicides, farmers end up

using lower rate of each herbicide. The use of herbicide mixtures for weed control often results in ineffective weed control and economic loss.

The research institutions in collaboration with Plant Protection and Regulatory Services Directorate of the Ministry of Food and Agriculture should therefore carry out supervised field trials and come out with recommended protocol for pesticides application.

The use of lower application rate of ethephon is preferable even though the ripening is delayed further than expected. Application of ethephon on immature fruits required very strong doses for effective results. However, this will result in the harvesting of immature fruits with low sugar content, excessive acidity, higher residues, internal browning and short shelf life (Pinon, 2000). The EU placed temporary ban on pineapple from Ghana in July 2001 due to higher levels of ethephon (growth regulator) detected after analyzing samples of the consignment (MTI, 2001; Kwawu, 2001). It is against this background that there is an indication that plans are in the offing to discontinue the use of the growth regulator and rely on natural ripening.

**Table 9:** Rates of pesticide application by farmers

<b>Pesticide</b>	<b>Percentage of farmers interviewed</b>		
	<b>Lower rate</b>	<b>Recommended rate</b>	<b>Higher rate</b>
<i>Insecticide</i>			
Chlorpyrifos	3.0 (< 0.5 lit)*	20.7 (0.5-1 lit)*	76.3 (> 1 lit)*
Cypermethrin	0 (< 0.6 lit)*	5.9 (0.6 lit)*	94.1 (> 0.6 lit)*
Dimethoate	13.3 (< 0.8 lit)*	13.3 (0.8 lit)*	73.4 (> 0.8 lit)*
<i>Herbicides</i>			
Diuron	23.7 (< 2 kg)*	71.0 (2-5 kg)*	5.3 (> 5 kg)*
Fluazifop-butyl	19.8 (< 1 lit)*	77.2 (1-5 lit)*	3.0 (> 5 lit)*
Glyphosate	25.0 (< 2 lit)*	50.0 (2-5 lit)*	25.0 (> 5 lit)*
<i>Fungicide</i>			
Metalaxyl	0 (< 1 kg)*	100 (1-3 kg)*	0 (> 3 kg)*
<i>Growth regulator</i>			
Ethephon	59.1 (< 3 lit)*	4.5 (3 lit)*	36.4 (> 3 lit)*

\*Rate of application/hectare

Source: Suglo *et al.* (2001)

#### **4.1.7.4 Pre-harvest Interval**

The results of the survey indicated that the fruits sprayed with ethephon for de-greening were harvested within 7 days. The pre-harvest interval observed for other pesticides ranged between 5-12 months. In pineapple cultivation no fertilizer or pesticide is applied later than 5 months before harvesting because applications of chemicals, apart from ethephon, can be stopped after flower induction (Pinon, 2000).

#### **4.1.7.5 Frequency of spraying**

The frequency of the spraying regime depended on the field situation. The survey revealed that 93.7% of all the pineapple farmers interviewed, sprayed insecticides and fungicides 1-4 times during the growing season. Few farmers (about 6.3%) sprayed 5-8 times during the period. The spraying intervals ranged between 2-3 months. The higher frequency of pesticide application might be due to the calendar spray practice by the farmers and also the lower rate of application.

#### **4.1.7.6 Awareness of existence of pesticide residues in fruits**

The results of field survey indicated that 57%, 54%, and 89.5% of the small-, medium-, and large-scale farmers, respectively, were aware that pesticide residues could be present in the pineapple fruits. Except for one producer/exporter whose samples were usually analyzed on arrival in Europe, the producers/exporters interviewed reported that samples of their fruits were normally not analyzed for residue levels of pesticides.

One producer/exporter reported the rejection of a consignment of the exported fruits due to internal browning caused by high levels of ethephon residue.

#### **4.1.8.0 Information on pesticide**

##### **4.1.8.1 Sources of information on pesticide use**

The survey revealed that 23.5 % of small-scale producers received information from other farmers. About 23.4% of the medium-scale farmers also received their information from experts that is Agricultural Extension Staff. The higher percentage of the large-scale farmers (about 25.0%) also claimed that their source of information on pesticides use was through their own experience (Table 10). All the farmers claimed they received little information on pesticides use from the dealers' materials.

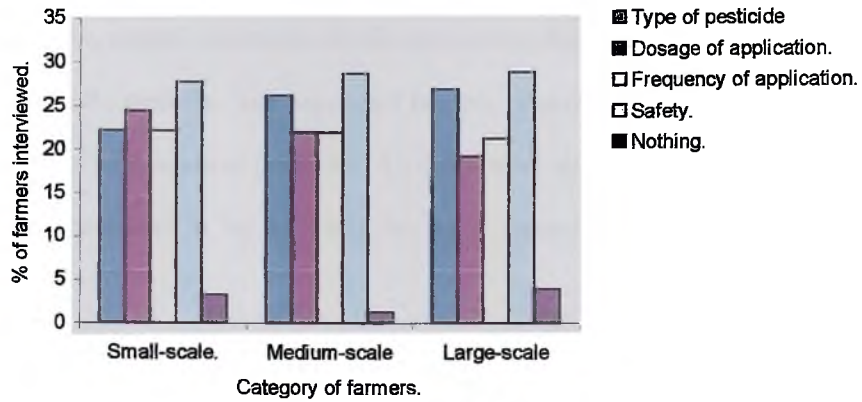


**Table 10:** Channels of information

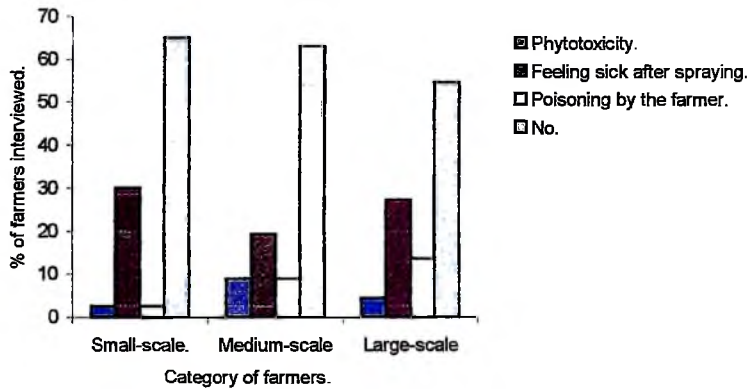
Source of information	Percentage of farmers interviewed		
	Small-scale	Medium-scale	Large-scale
Own experience	10.9	13.7	25.0
Label of the pesticide	17.7	18.3	12.5
Extension material	7.1	5.6	9.4
Dealer's material	5.1	3.0	3.1
News papers/journals	14.3	15.2	15.6
Other farmers	23.5	20.8	18.8
Advice by experts	21.4	23.4	15.6

#### **4.1.8.2 The need for more information on pesticide use**

Almost all the farmers interviewed indicated the inadequacy of their knowledge on the use of pesticides and therefore required more information. About 27.8% 28.7% and 28.8%, respectively, of the small-, medium-, and large-scale farmers wanted information on the safe use of pesticides (Fig.8). The next priority of the medium- and large-scale farmers was to have information on types of pesticides. About 3.3%, 1.3%, and 3.9% of the small-, medium- and large-scale farmers, respectively, indicated that they wanted no information on the use of pesticides. The farmers' realization of their need for more information on insecticide use is a healthy development towards the more rational use of pesticide, and that could lead to healthy environment and economic benefits.



**Fig. 8:** The need for more information on pesticide use.



**Fig.9:** Problems with pesticide use.

#### **4.1.9 Problems with the use of pesticides**

The serious problem confronting the farmers with regard to pesticides application was general ill health (Fig.9). Other problems were acute poisoning and phytotoxicity in the treated pineapple plants. The acute poisoning symptoms identified during the survey- included headache, general weakness and dizziness. Nevertheless, 65.0%, 63.1 %, and 54.6% of the small-, medium-, and large-scale farmers, respectively, did not experience problems with the application of pesticides. All the farmers ignored protective measures in handling the pesticides in the field and this might account for the acute poisoning recorded.

### **4.2 ANALYSIS OF CHLOPYRIFOS AND CARBENDAZIM RESIDUES**

#### **4.2.1 Calibration Curve and recovery test**

Calibration curves exhibited very good linearity for chlorpyrifos ( $R^2 = 0.9994$ ) (Fig 10) and carbendazim ( $R^2=0.9984$ ) (Fig 11). The good linearity of the curves indicates their reliability and thus very dependable (Ninsin, 1997). Fig.12 shows the gas chromatogram obtained after injection of 1 $\mu$ l of 2  $\mu$ g chlorpyrifos/g sample and blank.

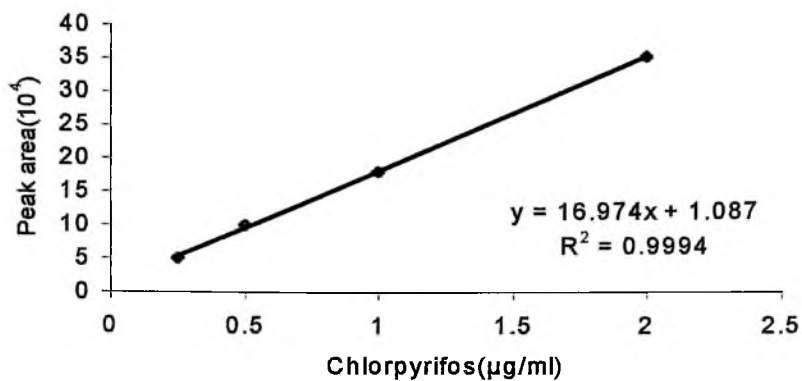


Fig.10:Calibration curve for chlorpyrifos.

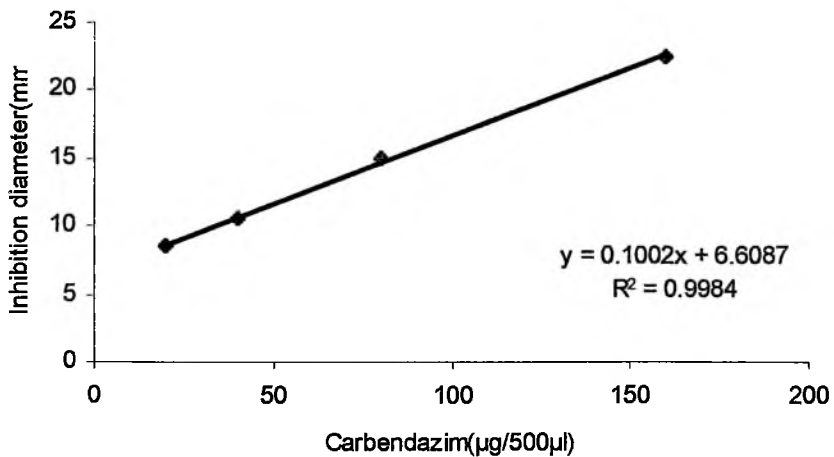
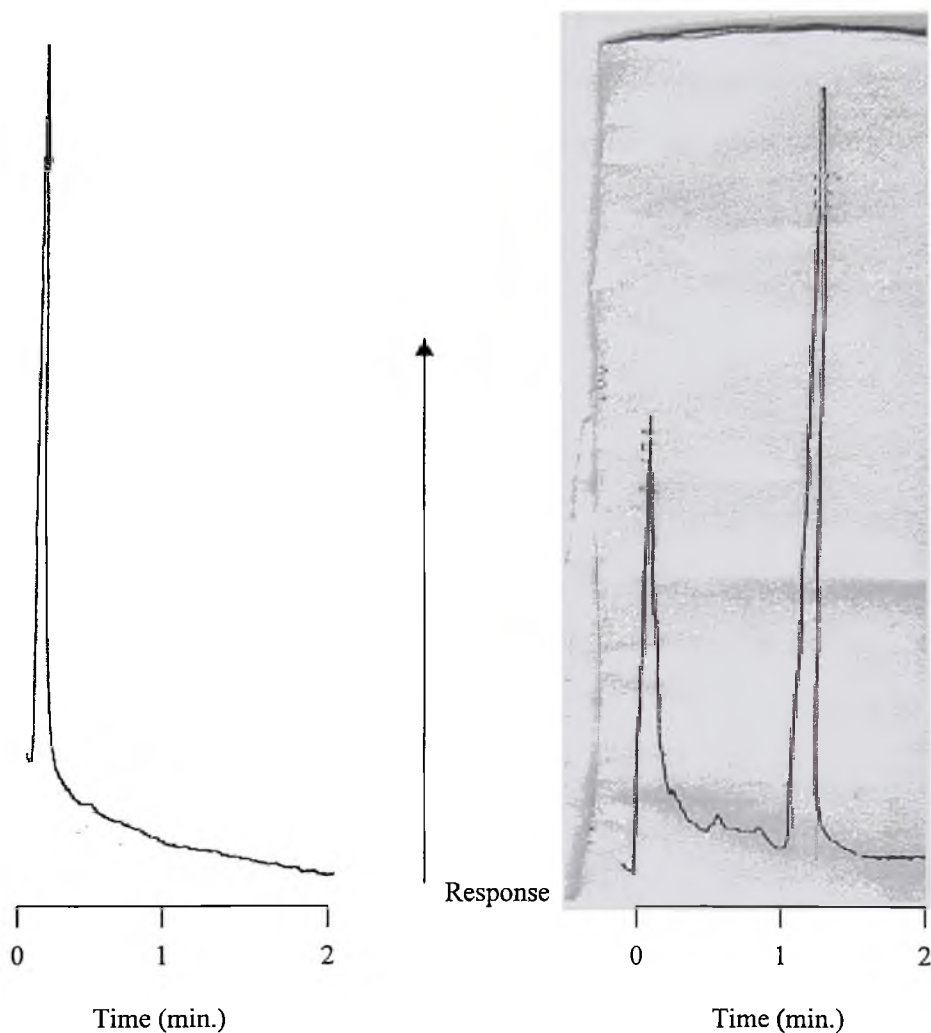


Fig.11.Calibration curve for carbendazim.

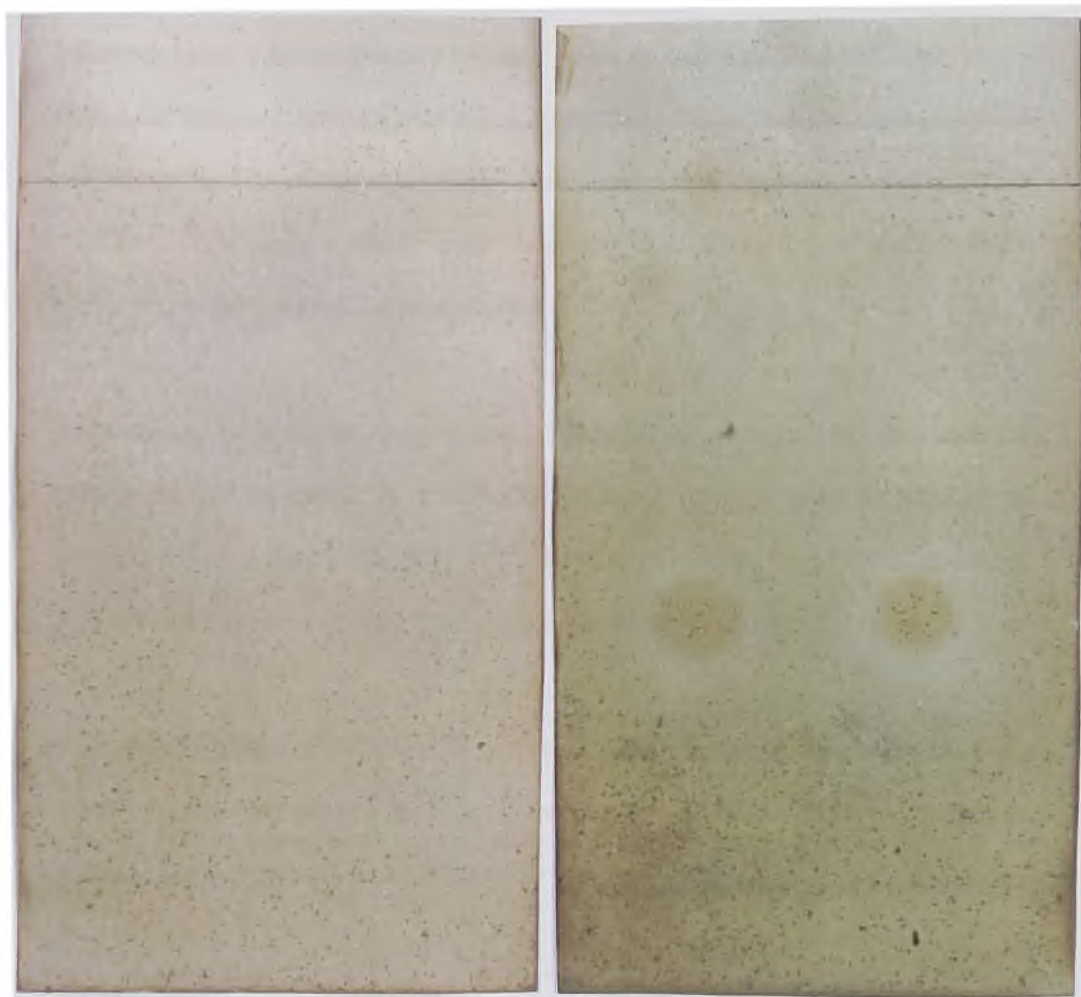
Recoveries from samples fortified with 4 $\mu$ g carbendazim/g sample and 2 $\mu$ g chlorpyrifos /g sample were 91.5 $\pm$ 2 % and 93.5 $\pm$ 0.9%, respectively. The analytical recovery of 91.5 $\pm$ 2% carbendazim from fortified pineapples agrees with the recovery of over 90 % obtained in similar technique employed in The Netherlands to detect carbendazim residue in fruits and vegetables (Netherlands, 1996). In that technique a suspension of *Penicillium cytopium* was applied to the TLC plates. The 93.5 $\pm$ 0.9% recovery for chlorpyrifos supports the 71-103% recovery obtained from orange by Wetters (1985). However, in that analysis, Gas Liquid Chromatograph with a flame-photometric detector was used.



**Fig.12:** Gas Chromatogram of (left) blank and (right) sample fortified with 2µg Chlorpyrifos/g sample (10x dilution).

*GC column: SPB<sup>TM</sup> 608 Spelco<sup>TM</sup> Fused Silica Capillary (15 m x 0.53 mm x 0.5µm film thickness); oven temperature: 190 °C; injector temperature: 250 °C; detector temperature: 300 °C; carrier gas: helium; flow rate: 40 ml/min.; retention time: 1.79 min; detector: ECD*

TLC plate spotted with blank sample had no visible inhibition spots (Plate 3a) whereas the plates spotted with 4 $\mu$ g carbendazim/g sample clearly showed inhibition spots (Plate 3b). This confirms significant inhibition of *Aspergillus spp.* when carbendazim is applied at recommended rate (FAO/WHO, 1995).



(a)

(b)

**Plate 3:**

(a) TLC plate spotted with blank.

(b) Inhibition spots on TLC plate spotted with 4 $\mu$ g carbendazim/g sample.

( $R_f$ -0.46, solvent system-ethyl acetate)

Inhibition spots were not detected on TLC plates spotted with 10µg/ml carbendazim. Gupta and Sharma (1989) reported that *A.niger* Tiegh, *Penicillium chrysogenum* Thom and *Mucor* sp. degraded carbendazim faster under high temperature and moisture conditions. This suggests that *A. niger* Tiegh may have rapidly degraded carbendazim (10 µg/ml) in the pre-saturated incubator set at 37<sup>0</sup> C.

Carbendazim inhibits the development of fungal germ tubes, the formation of appressoria and the growth of mycelia. Its fungitoxic action is based on blockage of nuclear division during mitosis and destabilizations of fungal cell structures (FAO/WHO, 1995).

With the 91.5±2% and 93.5±0.9 % recoveries obtained, the methods were therefore suitable for the analysis since the recovery ranging from 70 % to 140% is the acceptable analytical performance (Hill, 2000).

#### **4.2.2 Limit of detection (LOD)**

The LOD for carbendazim and chlorpyrifos were 0.02µg/g and 0.0001µg/g, respectively. These limits give an idea of the lowest practical concentration of carbendazim and chlorpyrifos residues that can be quantitatively measured and identified in pineapple using the GC and the TLC procedures.

#### 4.2.3 Residue levels ( $\mu\text{g/g}$ )

The pineapple samples contained residues of the pesticides analyzed but the residual levels obtained (Table 11) were below the permissible limits set by EU (COLEACP, 2000).

Fig.13 shows gas chromatogram of an extract of pineapple sample.

Plate 4 shows an inhibition spots of a peel extract from one of the samples.

The latest report released by European Commission indicates that carbendazim and chlorpyrifos residues have been persistently detected in fresh fruits and frozen vegetables sampled from EU countries from 1998-2000. The report revealed that less than 1 % of the samples had chlorpyrifos and carbendazim exceeding national and EU MRLs (European Commission, 2002). In another development 50 % of pineapple from non-EU countries sampled in the Netherlands in 2000 had carbendazim residue exceeding EU-MRLs (van der Schee, 2001). The results of the present study demonstrate that none of the pineapples contained carbendazim and chlorpyrifos residues exceeding EU-MRLs.

**Table 11:** Carbendazim and Chlorpyrifos residue levels ( $\mu\text{g/g} \pm \text{SE}^{++}$ ) obtained from pineapple peel and pulp.

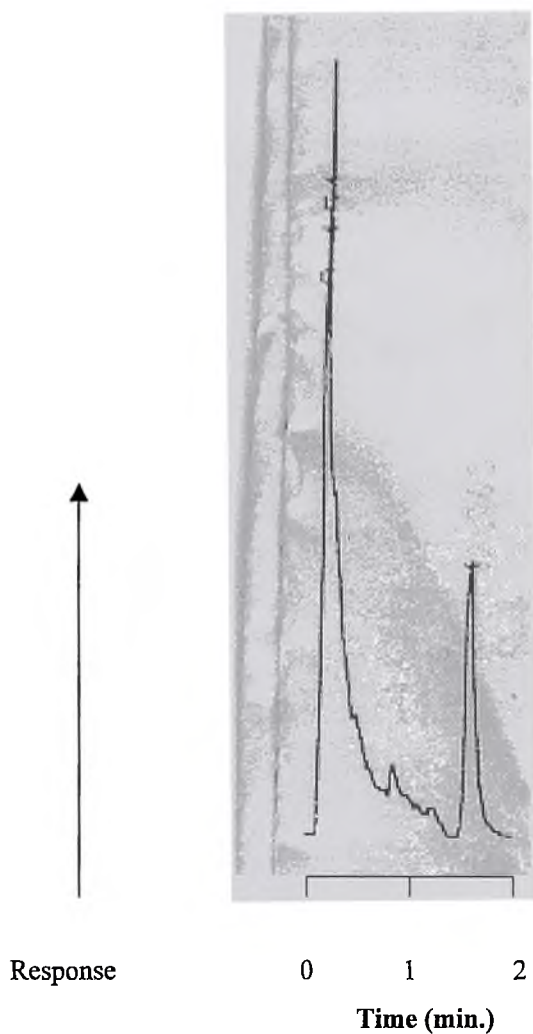
Pesticide	Sample site	Sample type		EU-MRL
		Pulp	Peel	
Chlorpyrifos	Field A <sup>xx</sup>	$0.02 \pm 3 \times 10^{-3}$	$0.02 \pm 6 \times 10^{-3}$	0.05 $\mu\text{g/g}$
	Field B	$0.01 \pm 6 \times 10^{-3}$	$0.005 \pm 4.8 \times 10^{-3}$	
	Field C	$0.01 \pm 4 \times 10^{-3}$	$0.008 \pm 3 \times 10^{-3}$	
	Field D	$0.02 \pm 6 \times 10^{-3}$	$0.008 \pm 3 \times 10^{-3}$	
	Field E	$0.02 \pm 1 \times 10^{-2}$	$0.007 \pm 1 \times 10^{-3}$	
Carbendazim				0.1 $\mu\text{g/g}$
	Box A <sup>@</sup>	<i>nd</i> *	$0.05 \pm 1 \times 10^{-2}$	
	Box B	<i>nd</i> *	$0.04 \pm 6 \times 10^{-3}$	
	Box C	<i>nd</i> *	$0.04 \pm 2 \times 10^{-2}$	
	Box D	<i>nd</i> *	$0.04 \pm 2 \times 10^{-2}$	
	Box E	<i>nd</i> *	$0.05 \pm 1 \times 10^{-2}$	

\*Not detected (*nd*) below the quantification limit of the method used (0.02  $\mu\text{g/g}$ ).

<sup>xx</sup> Fields where samples were collected.

<sup>@</sup> Boxes sampled from the consignment to be exported.

<sup>++</sup> Standard error



**Fig 3:** Gas Chromatogram of pineapple extract of on sample with  
the solvent peak is on the left

*GC column: SPB<sup>TM</sup> 608 Spelco<sup>TM</sup> Fused Silica Capillary (15 m x 0.53 mm x 0.5 $\mu$ m film thickness); oven temperature: 190 °C; injector temperature: 250 °C; detector temperature: 300 °C; carrier gas: helium; flow rate: 40 ml/min.; retention time: 1.79min.*

*detector: ECD*



**Plate 4:** Inhibition spots on TLC plate spotted with extract from the peel.  
( $R_f$ -0.46, Solvent system-ethyl acetate).

Pineapple is one of the rare crops in which all pesticides, apart from ethephon, and fertilizer applications are halted after flower induction but the fruits are harvested five months later. The detection of chlorpyrifos residue in the pulp and peel therefore suggests that the plants may have absorbed the residue of the insecticide applied prior to flower induction or from cross contamination. Chlorpyrifos has pre-harvest interval of 2-6 weeks (Hill, 1993). Therefore the low residual level of chlorpyrifos can partly be attributed to the five months pre-harvest interval observed by these pineapple farmers, which might have led to the insecticide degradation due to climatic factors such as heat or sunlight and irrigation (Cabrera, *et al.*, 2000).

The slight differences in chlorpyrifos residue levels in samples from different locations may be mainly due to the use of different concentrations depending upon the pest density, number of applications and inappropriate methods of application (Hussain *et al.*, 2002).

The residual level of carbendazim in the pineapple peel was quantified but the pulp contained concentration not exceeding the detection limit of the method used (0.02 µg /g).

These results confirm the findings of Cabrera *et al.*, (2000) who did not detect carbendazim, applied in the field, in pineapple pulp within the detection limit of 0.1µg/g. Scalon *et al.*, (1996) cited by Cabrera *et al.*, (2000) also detected no traces of benomyl (as carbendazim) in mango pulp. However, in another study, benomyl (as carbendazim) was detected at levels of 0.08µg/g and 0.05µg/g in sub-products of pear and apples respectively that had been cultivated in the open field (Schnell *et al.*, 1997).

In the latter, a fluorescence detector, which is more sensitive, was used to quantify benomyl.

In post-harvest treatment, the base of the pineapple fruit is dipped into the fungicide solution before packing into cartons for export. The non-detection of carbendazim residue in the pulp can be attributed to the pineapple peel, acting as a protective shield, that prevented the diffusion of the fungicide (Cabrera *et al.*, 2000). It is important to emphasize that the degree of migration of residues from the peel to the pulp depends on the substrate and the chemical nature of the pesticide (Awasti, 1993). The detection of carbendazim in the peel can be attributed to the presence of epicuticular wax in pineapple peel that contains substances in which pesticides are soluble (Melnikov, 1971 cited by Cabrera *et al.*, 2000). According to Riederer and Schreiber (1995) after treatment of pesticides, the molecules penetrate into the epicuticular wax of the peel by diffusion, impeding their transport into the pulp.

The slight disparities in carbendazim residues in the pulp from samples in different boxes might be due to errors in measuring the fungicide for the post-harvest treatment.

Based on the results, carbendazim and chlorpyrifos residues detected do not pose a serious threat to public health. However, it is suggested that a continuous monitoring system for pesticides is needed for fruits and vegetable to encourage more rational use of pesticides.

## CHAPTER FIVE

### 5.1 CONCLUSION AND RECOMMENDATIONS

- There is low level of involvement of diploma/degree holders in pineapple farming. Only 4.1 % of the farmers had education higher than senior secondary school. Most of the farmers were cultivating less than 2 hectares and had cultivated on the same piece of land for more than 3 years.

- All the pineapple farmers used pesticide either for field application or post-harvest treatment of the fruits. However, the pesticides were selected without due cognizance of the provisionally approved pesticides for the production of exportable pineapple in Ghana. Out of twenty-one types of pesticides listed only nine were provisionally approved for production of exportable pineapple. These were:

Herbicides: Diuron, Fluazifop-butyl and Glyphosate

Insecticides: Chlorpyrifos, Cypermethrin and Dimethoate

Fungicides: Metalaxyl and Carbendazim

Growth regulator: Ethephon.

The farmers mixed herbicides irrespective of their modes of action, with the view to achieve effective weed control. The correct time of application (either as pre-emergence or post-emergence) of herbicides reduces the rate per hectare and achieves effective weed control.

- The farmers handled pesticides without caution despite the known hazards, thereby exposing themselves and their families to the toxic effects of these pesticides.
- Samples of pineapple fruits were not analyzed for pesticide residue levels prior to export since there was no commercial laboratory well equipped to carry out the analysis in Ghana.
- Carbendazim residue was analysed using Thin Layer Chromatography by bioassay of *Aspergillus niger* Tiegh inhibition technique. The recovery rate of the technique was  $91.5 \pm 2\%$ . The fungicide was detected in the peel and not in the pulp within the detection limit of  $0.02 \mu\text{g/g}$ . The Carbendazim residue detected in the peel was in the range of  $0.04 \pm 2 \times 10^{-2}$  to  $0.05 \pm 1 \times 10^{-2} \mu\text{g/g}$ . The residue of chlorpyrifos was also determined using Gas Chromatograph equipped with electron capture detector. The recovery rate of the technique was  $93.5 \pm 0.9\%$ . Chlorpyrifos was detected both in the peel and in the pulp. The range of chlorpyrifos residue detected was from  $0.005 \pm 4.8 \times 10^{-3}$  to  $0.02 \pm 1 \times 10^{-2} \mu\text{g/g}$ . These levels of residue were below European Union MRL and therefore pose no threat to pineapple industry in Ghana.

The pineapple farmers, even though they received information on the use of pesticides through various channels, there is the need to be regulated. It is therefore recommended that Plant Protection and Regulatory Services of the Ministry of Food and Agriculture (MOFA) should provide the farmers with the necessary training to optimize the value of their products.

The training programme should emphasize:

- Safe and effective use of pesticide.
- Pest management including pest recognition and biology.
- Pesticide resistant management.
- Integrated pest management principles.

MOFA in collaboration with various Institutions and other stakeholders should carry out supervised field trials to recommend to farmers the required rates of provisionally approved pesticides for pineapple production in Ghana.

A key limitation to the use of carbendazim and other benzimidazole is the development of resistance. It is therefore imperative for exporters to alternate or mix carbendazim with other non-benzimidazole fungicides.

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## APPENDIX I

**AFRICAN REGIONAL POST GRADUATE PROGRAMME IN INSECT SCIENCE  
(ARPPIS), UNIVERSITY OF GHANA, LEGON, GHANA**

**Questionnaire for the determination and assessment of farmers' pesticides use patterns  
on pineapple**

(Please either fill in the blank space provided, or tick where applicable)

1. District \_\_\_\_\_
  2. Village \_\_\_\_\_
  3. Operational Area \_\_\_\_\_
  4. Name of the farmer (first name) \_\_\_\_\_
- 
- |  |                       |                          |
|--|-----------------------|--------------------------|
|  | Small scale producer  | <input type="checkbox"/> |
|  | Medium scale producer | <input type="checkbox"/> |
|  | Large scale producer  | <input type="checkbox"/> |
- 
5. What is your educational level?
 

	Up to diploma/degree	<input type="checkbox"/>
	Sec. Sch. (O/A-level, SSS)	<input type="checkbox"/>
	JSS/middle school	<input type="checkbox"/>
	Primary school	<input type="checkbox"/>
	None	<input type="checkbox"/>
- 
6. How much land is available to you for farming?
 

	.....	Acres/ha
--	-------	----------
- 
7. How much land was under cultivation last year?
 

	.....	acres/ha
--	-------	----------

8. How long have you cultivated your last years land?
- One season
  - one year
  - three years
  - more than three years
9. Do you use pesticides?
- Yes,
  - No
10. How did you get to know about the Pesticides?
- Through a farmer
  - through a MOFA staff
  - through buyer of produce
  - through magazines journals etc
11. Where do you obtain your pesticides?
- Private retailers
  - Co-operative
  - Other farmers
  - Produce buying firm
  - Government institutions
  - NGOs
  - Others
12. When do you buy the pesticides?
- In advance
  - After appearance of pests of diseases

13. How do you apply pesticides to planting materials?
- By hand
- by machines
14. How do you apply pesticides in the fields?  
(*By hand includes broom, brush, home-made pump, cup, bottles*)
- By hand
- Hand pump (knapsack)
- Motorised sprayer
- Ultra-low volume sprayer
- Tractor with sprayer
15. How do you apply pesticides for post-harvest?
- By hand
- By machines
16. Who does the application?  
(*More than one answer is possible.*)
- By myself
- My wife
- My children
- Labourers, care takers
- Other farmers
- Commercial applicators
- Extension agents
17. What protective clothing do you use during application of pesticide  
(*More than one answer is possible.*)
- hat
- long trousers
- Overall
- Apron
- Gloves
- Respirator
- Wellington boot
- Goggles
- None

18. Where do you store your pesticides? In my house   
In a farm hat   
Other places
19. What do you do with empty pesticide containers? Re-use them   
Destroy them   
Use for other purposes
- (Other purposes: place specify) \_\_\_\_\_
20. Where do you get your information on pesticides from? own experience   
Label of the pesticides   
Extension material   
Dealer's material   
Newspapers   
Television   
Other farmers   
Personnel advice by experts   
 (More than one answer is possible.)
21. How often do you get technical advice Once per season   
Once per month   
Never
22. From which organization do you get professional advice. Extension service of MOFA   
Other producer organizations   
Retailer/trader of pesticides   
Buyer of the products   
NGOs   
Expert farmers   
 (More than one answer is possible.)



23. Which recommendations do you find useful?  
(*More than one answer is possible.*)
- |                          |                          |
|--------------------------|--------------------------|
| frequency of application | <input type="checkbox"/> |
| Dosage of application    | <input type="checkbox"/> |
| Type of pesticide        | <input type="checkbox"/> |
| Safety                   | <input type="checkbox"/> |
24. What would you like to have more information on?  
(*More than one answer is possible.*)
- |                          |                          |
|--------------------------|--------------------------|
| Type of pesticide        | <input type="checkbox"/> |
| Dosage                   | <input type="checkbox"/> |
| Frequency of application | <input type="checkbox"/> |
| Safety                   | <input type="checkbox"/> |
| Nothing                  | <input type="checkbox"/> |
25. Have you encountered any problem with pesticide use?  
(*More than one answer is possible.*)
- |               |                          |
|---------------|--------------------------|
| Phytotoxicity | <input type="checkbox"/> |
| Fell sick     | <input type="checkbox"/> |
| Poisoning     | <input type="checkbox"/> |
| None          | <input type="checkbox"/> |
26. Are you aware that pesticides residues could be present in fruits.
- |     |                          |
|-----|--------------------------|
| yes | <input type="checkbox"/> |
| no  | <input type="checkbox"/> |
27. What main export fruit crop do you grow?  
\_\_\_\_\_
28. How many acres do you cultivate?  
\_\_\_\_\_
29. What is the yield per season?  
\_\_\_\_\_
30. Do you use fertilizer?
- |                                  |                          |
|----------------------------------|--------------------------|
| (1) Yes, chemical fertilizer     | <input type="checkbox"/> |
| (2) Yes, manure (green, compost) | <input type="checkbox"/> |
| (3) Nothing                      | <input type="checkbox"/> |

31. Do you apply herbicides? Yes
- No

32. If yes, name the herbicides and rate of application

Herbicide	rate	Qty/acre/ha

33. What pesticides do you use to treat your planting material.

Pesticide	Rate

34. Do you use plant growth regulator(s) for flower induction? Yes
- No

35. If yes, name them and give rate of application

Name	rate	Qty/acre	Stage of growth

36. Do you use plant growth regulator(s) for fruit ripening? Yes
- No

37. If yes, name them and give rate of application.

Name	rate	Qty./acre/ha	Stage of growth
<hr/>			
<hr/>			

38. What pre-harvest interval do you observe after the application of growth regulator for fruit ripening

- One week
- Two weeks
- Other (specify) \_\_\_\_\_

39. How do you use your products?

- For home consumption
- For local markets
- For export

40. What kind of planting materials do you use?

- Certified planting materials
- Own selection

41. Which pest(s) needs to be control on this crop?

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42. Which type of pesticide(s), or mixture of pesticides do you use for pests control.

Name of pest	Pesticide	Formulation

43. What is the quantity of pesticide(s) used?

Pesticide	Frequency	Rate	Qty/acre

44. Approximately at what interval do you apply pesticides?

(Insecticides, nematicides etc)	Less than 1 wk	<input type="checkbox"/>
	One week	<input type="checkbox"/>
	Two weeks	<input type="checkbox"/>
	Other (specify) _____	

45. How do you take the decision?

Prophylactic use (calendar spray)	<input type="checkbox"/>
Depending on intensity (curative)	<input type="checkbox"/>
After advice from expert	<input type="checkbox"/>

46. What pre-harvest interval do you observe	One week.	<input type="checkbox"/>
after pesticide(s) (insecticides(s), nematicide(s)) application	Two weeks	<input type="checkbox"/>
	Other (specify) _____	

47. Which disease(s) need to be controlled on this crop? \_\_\_\_\_

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48. Which fungicide(s) or mixtures of fungicide do you use for disease control?

Disease	Chemical	Formulation

49. What is the amount of fungicide used?

Pesticide	frequency	rate	qty/acre/ha

50. Approximately at what intervals do you apply

fungicide(s)	Less than 1 week	<input type="checkbox"/>
	One week	<input type="checkbox"/>
	Two week	<input type="checkbox"/>
	Other (specify)	_____

51. How do you take the decision?

Prophylactic use (calendar spray)	<input type="checkbox"/>
Depending on intensity (curative)	<input type="checkbox"/>
After advice from expert	<input type="checkbox"/>

52. What pre-harvest interval do you observe after fungicide(s) application?
- |                        |                          |
|------------------------|--------------------------|
| One week.              | <input type="checkbox"/> |
| Two week.              | <input type="checkbox"/> |
| Others (specify) _____ |                          |

53. Which pesticide or mixture of pesticides do you use for post-harvest application?

Pesticides	rate	formulation
_____		
_____		
_____		

54. Do you keep farm records on your pesticide use patterns?
- |     |                          |
|-----|--------------------------|
| Yes | <input type="checkbox"/> |
| no  | <input type="checkbox"/> |

55. Give reasons for your answer.

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56. Do you use irrigation?

- |                            |                          |
|----------------------------|--------------------------|
| Manual                     | <input type="checkbox"/> |
| Manual mechanical (furrow) | <input type="checkbox"/> |
| Mechanical (pumps)         | <input type="checkbox"/> |
| No irrigation              | <input type="checkbox"/> |

57. Have you been transporting harvested

- |                                   |     |                          |
|-----------------------------------|-----|--------------------------|
| fruits with pesticide containers? | Yes | <input type="checkbox"/> |
|                                   | No  | <input type="checkbox"/> |

**Thank you**