SUSCEPTIBILITY OF TWO SPOTTED SPIDER MITE *Tetranychus urticae* KOCH (ACARI; TETRANYCHIDAE) TO SOME SELECTED INSECTICIDE IN THE GREATER ACCRA REGION OF GHANA

BY

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DECLARATION

I hereby do declare that with the exception of references to other scholars work which have been duly acknowledge, this thesis consist of my original work and has neither in whole nor part been presented to any other institution for the award of any degree.

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DEDICATION

I dedicate this research work to the memory of my late father Mr Bala Buba and to my mum, Mrs Barmini Bala Buba whose love, enthusiasm and inspirations have brought me this far.

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LIST OF ABBREVIATIONS

AChE Acetylcholine esterase

EC Emulsifiable concentrates

GABA γ - Aminobutyric acid

GST Glutathione S-transferase

IPM Integrated Pest Management

IRAC Insecticide Resistance Action Committee

LC50 Median lethal concentration of a pesticide expected to kill 50% of a test organism

LD50 Median lethal dose of a pesticide expected to kill 50% of a test organism

ABSTRACT

The two-spotted spider mite *Tetranychus urticae* is a pest of several plants including vegetables, fruits, ornamentals and field crops. Damage done to the host plant can cause leaf and fruit loss, complete defoliation and death of the host plant. They are mostly managed by the use of acaricides. Acaricides resistance has been reported in several countries, this is due to the fact that *T. urticae* fully becomes resistant to an insecticide after two to four years of use. The present study was initiated to monitor resistance by *T. urticae* to some selected insecticide in Accra. A survey carried out in the Accra suburbs revealed pattern and type of insecticide use for the control of pest, diseases and weeds. The survey also revealed that farmers have little knowledge about *T. urticae* and so do not specifically targets *T. urticae* in terms of control but that the insecticide used address the complex of pest affecting their crops. These insecticides are used in high dosages and at short intervals, this serves as a main cause of resistance development in mite pest. Susceptibility studies were carried out using five miticides- lambda *cyhalothrin*, Emamectin benzoate, prosular oxymatrine, imidacloprid and sulphur. Adult *T. urticae* were collected from four sites in Accra (Opeibea, Ashaiman, University of Ghana farm and Department of Crop Science Sinna's garden). These populations were used to raise colonies of spider mite in the screen house of the Department of Crop Science. Spray application was used to determine the resistance in *T. urticae* by application of different rates of acaricides against the different strains of the spider mite. Mortality was recorded 24 h after treatment. The LD_{50} values and slopes were estimated by probit analysis and resistance factor were calculated by dividing LD_{50} of field population by LD_{50} of susceptible strain. All field populations were quite resistant to Karate (up to 21.6-fold), the LD_{50} values for the susceptible strain was 0.007 ml/L and varied significantly from those of the field population which ranged from 0.084-0.151 ml/L. All field populations were

susceptible to Levo®, Protect® and Sulphur®. The University of Ghana farms, Sinna's garden and Ashaiman populations were susceptible to Imidacloprid except the Opeibea population which showed a low level of resistance (9-fold) to Imidacloprid. Optimal (non-lethal) concentration of the synergists (PBO and DEM) were also determined using the karate resistant population and a time series experiment was carried out to determine the time lapse between synergist and insecticide application. The optimal (non-lethal) PBO and DEM concentrations were 0.4 and 1.0 µl/ml respectively. Time series experiment showed significant difference between mortalities in mites exposed to karate immediately after pre-treatment with synergists and those with the miticide 1 h later. 1 h after pre-treatment with PBO gave a 94.8 and 94.7 % mortality in Opeibea and Ashaiman resistant population respectively. The 1 h pre-treatment with DEM also gave 88.2 and 85.05 % mortalities in Opeibea and Ashaiman resistant populations respectively. The synergist ratios obtained for Opeibea population was 4.3 and 2.9-fold for PBO and DEM where as that of Ashaiman is 2.2 and 2.6 for PBO and DEM respectively. The result of the synergist experiment suggests the involvement of glutathione-S-tranferases (GSTs) and cytochrome P450 monoxygenases as the possible mechanisms of resistance.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Mites are the most diverse representatives of the phylum Arthropoda. They belong to the subphylum Chelicerata and subclass Acari. Among the arachnids, Acari are the only group that feeds on plants. Around 7,000 species of Phytophagous mites are known worldwide which occur in five families namely Tetranychidae, Tenuipalpidae, Eriophyidae, Tarsonemidae and Tuckerillidae (Chillar *et al*., 2007).

In agriculture, the two main mite families of concern are the Tetranychidae and the Eriophyidae (Hoy, 2011). Tetranychidae, also known as spider mites, is a large family including about 1,200 species belonging to over 70 genera of worldwide distribution (Bolland *et al*., 1998). Of the more than 1,200 species of spider mites described the two-spotted spider mites *Tetranychus urticae* (Bolland *et al.,* 1998; Milegeon *et al.,* 2010) is the most injurious to Agriculture.

The two–spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), is the most economically important plant-feeding mite pest in the world (Van Leeuwen *et al.,* 2012). It is a cosmopolitan and highly polyphagous pest of many fruits, vegetables, ornamentals, and field crops. *Tetranychus urticae* is a generalist feeder and is among the most polyphagous arthropod herbivores (Agrawal, 2000), feeding on more than 1,100 plant species belonging to more than 140 different plant families, including those that are known to produce toxic compounds (Grbic 2011; Van Leeuwen *et al.,* 2012). *Tetranychus urticae* threatens greenhouse production and field, vine, and orchard crops, destroying economically important annual and perennial crops worldwide such as tomatoes, peppers, cucumbers, strawberries,

corn, apples, grapes, eggplant, almonds, peppermint, pawpaw and citrus (Jeppson *et al.*, 1975). Spider mites damage their host plants while feeding, using specialized piercing-sucking, stylet-like mouthparts to penetrate through the outer epidermal cells and into parenchyma cells (Park and Lee, 2002), and thus removing chlorophyll and other cell contents (Tomczyk and Kropczyńska, 1985). The loss of chlorophyll results in a visibly patchy discoloration of leaf tissue, as well as a reduced photosynthetic rate and production of nutrients (Park and Lee 2002). Economic injury occurs as high populations accumulate and feeding increases, leading to sufficient damage over a period of days. Extreme levels of damage can eventually cause leaf and fruit loss, complete defoliation, and death of the host plant (Van Leeuwen *et al.*, 2012).

Acaricide resistance in *T. urticae* has been reported from over 60 countries (DARP, 2013), easily earning it the title of the world's top resistant animal pest (Van Leeuwan *et al*., 2010). Tolerance documented to acaricides can result after a few applications (Sato, 2005). Furthermore, *T. urticae* can become fully resistant to new acaricides within two to four years, meaning that control of multi-acaricide resistant *T. urticae* has become increasingly difficult (Grbic, 2011).

The development of resistance in *T. urticae* has been reported for a number of compounds, including organophosphates, clofentezine, hexythiazox bifenthrin, fenpropathrin, bifenazate, fenpyroximate, pyridaben, tebufenpyrab, chlorfenapyr, etoxazole, spirodiclofen, abamectin, and milbemectin (Osakabe *et al*., 2009; Van Leeuwen *et al*., 2010). *Tetranychus urticae* has rapidly developed resistance to almost all types of acaricides due to its short life cycle, high biotic potential and parthenogenesis reproduction (Croft and Van De Baan, 1988).

According to Insecticide Resistance Action Committee (IRAC), 424 cases of *T. urticae* resistance have been reported to 94 active ingredients of acaricides, emphasizing the urgent necessity of efficient resistance management (http://www.pesticideresistance.com/search.php, accessed 15 February 2015).

1.1 Justification

Occurrences of pests and diseases have led to increased indiscriminate use of pesticide and other agrochemicals. Not surprisingly, pesticide use has increased over time in Ghana and is particularly elevated in the production of high-value cash crops and vegetables (Gerken et al. 2001). Dinham, (2003) estimated that 87% of Ghanaian farmers in rely heavily on the use of pesticides and these chemical pesticides are used improperly or in dangerous combinations (Obeng-Ofori *et al*., 2002). The problem is serious in areas where irrigated farming is practiced because of multiple cropping. The misuse of chemical pesticides is of so much concern that promotion of safe use of pesticides on vegetables has been placed on the agenda of Ghana's Food and Agriculture Sector Development Policy (Ministry of Food and Agriculture 2002). Efforts to manage *T. urticae* populations in field crops rely on the use of acaricides. Although acaricides have proven to be highly effective in protecting crops under extreme pressure from pest (Cooper and Dobson, 2007), this over reliance on synthetic chemicals in crop cultivation has generally caused mite resistance development and public concerns on their high residues in crop, soils and water (Owusu and Yeboah, 2007) as well as adverse effects on non-targets beneficial organism, and therefore there is a need for monitoring of *T. urticae* population.

No studies have been done on the susceptibility of Ghanaian populations of *T. urticae*, it is therefore necessary to conduct susceptibility test to know the resistance level of these pest to

commonly used insecticides so that potential solutions can be developed before the onset of severe economic losses to growers. The detection of resistance in its early stages aids in better resistance management and in reducing resistance related cost to farmers.

1.2 Main objective

The present study sought to investigate the susceptibility of *T. urticae* populations to commonly used insecticides in some parts of greater Accra region of Ghana.

Specific objectives

The specific objectives of this work were thus;

i. To determine the pattern and types of commonly used insecticides in these areas
ii. To assess the susceptibility of field populations of *T. urticea* to some selected insecticides
iii. To determine the possible roles of detoxifying enzymes using synergism assays.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and distribution of the two-spotted spider mite

The two-spotted spider mite *Tetranychus urticae* was described from European specimens and is considered to be a tropical species but it is also found in the subtropical regions (Fasulo and Denmark, 2000). *Tetranychus urticae* occurs in most parts of the world and has been recorded from most countries in Europe, Asia, Africa, Australia, the Pacific and Caribbean islands, North, Central and South America (Turtle and Baker, 1968; Bolland *et al.*, 1998).

2.2 Description and Biology of the two-spotted spider mite

The two-spotted spider mite is oval in shape, about 0.6 mm long (Tuttle and Baker, 1968). Colour varies from brown to orange-red and green (Metcalf and Metcalf, 1993). The female body is about 0.4 mm in length bearing 12 pairs of dorsal setae. The colour of the legs varies from pale to yellowish (Magdalena and Meyer, 1996).

The large dark spots are accumulation of body wastes, often visible through the transparent body wall but lacking in newly moulted mites (Tuttle and Baker 1968). The male is elliptical with a slender caudal end, longer legs, more mobile and smaller than the female (Coates, 1974). The axis of knob of aedeagus is parallel or forming a small angle with axis of shaft (Boudreaux, 1956). Both male and female undergo the same number of developmental stages but in the males time spent on each stage is shorter. The colour of the exoskeleton may vary according to their diet and environment. In autumn due to short days and poor host quality the diapause females become orange to brick red and creep into protected places such as on

the underside of leaves or on soil surfaces to hibernate, at this stage the mite becomes non-feeding and non-reproductive (Annecke and Moran, 1982; Davidson and Raupp, 1988).

Plate 1: Adult two spotted spider mite

The life cycle of *T. urticae* progresses through a series of five stages, egg, larva, protonymph, deutonymph, then finally moulting into an adult male or female (Crooker, 1985).

Adult females can lay an average of 5 or 6 eggs daily and 100-150 in a lifetime, over a period of 3-4 weeks (Sabelis, 1985). Spider mite females of the genus *Tetranychus* lay their eggs within or under webbing. Webbing may be used to protect against climatic factors such as wind and rain, and also protects the mites from natural enemies and exposure to chemicals (Gerson, 1985). Eggs are deposited singly on the underside of leaves. The eggs are translucent pearl-like spheres when initially laid but gradually becomes reddish as they develop (Van Leeuwen, 2012). The developmental period of the eggs varies from 3 days at 24ºC to 21 days at 11ºC (Cagle, 1949) until hatching into an orange six-legged larva which

turns green as they feed on chlorophyll. The larvae feed for a few days then search for protected areas to rest and moult into the protonymph.

The larvae, along with the next two eight legged nymphal stages (protonymph and deutonymph) are all active immature stages that feed on the host plant, that are followed by a period of quiescence. During the period of quiescence a mite attaches itself to the leaf substrate (Crooker, 1985). Time spent developing in each stage depends on temperature and humidity during the specific life stage (Cagle, 1949; Herbert, 1981). Spider mite can develop from egg to adult in 7-8 days at maximum temperatures of about 30-32ºC (Zhang, 2003).

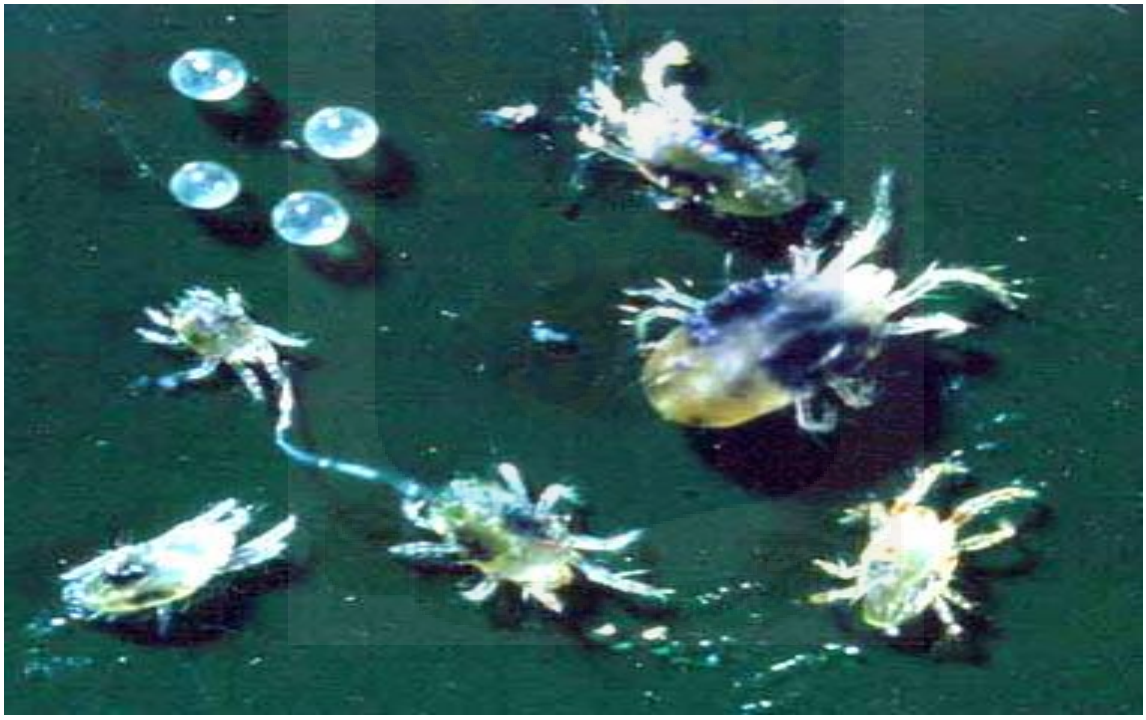

Plate 2: Life cycle of two spotted spider mite

(http://www.agf.gov.bc.ca/cropprot/tfipm/mites.htm)

Males reach maturity first, then search and wait besides female deutonymph in the resting state (Penman and Cone, 1972). Copulation occurs almost immediately after an adult female emerges (Crooker, 1985).

Offspring of both sexes is produced by a fertilized adult female with a sex ratio of 3:1 female: male (Overmeer and Harrison, 1969). Arrhenotokous parthenogenesis occurs in unfertilized eggs, resulting in the production of haploid males (Helle, 1985). The haplodiploidy genetic system enables a single female to initiate a new colony and cause a potential outbreak.

2.3 Economic importance of two-spotted spider mite

Modern agricultural system provides an ideal environment with high quality host crops for spider mite population growth. Large scale monoculture, constant irrigation, fertilization and continued application of increasing doses of pesticides also increases the food quality of crops for the pest and has reduced the natural enemies of pest (Bhana, 2014). The importance of mite pest worldwide has become prominent as a result of its rapid reproduction rate and development of resistance to acaricides.

Tetranychus urticae have a needle like piercing and sucking mouthpart which is used to penetrate plant tissues and feed on cell chloroplasts on the underside of the leaf. As feeding continues the upper surface of the leaf develops whitish or yellowish stippling characteristic, which may join and become brownish (Park and Lee, 2002).

Plate 3: Cluster of spider mites hanging before dispersal from the tip of an eggplant.

Depending on developmental stage, the stylet length of *T. urticae* is 132±27 µm but can vary from 103 µm (larvae) to 157 µm (adult females) (Avery and Briggs, 1968; Sances *et al*., 1979). The degree of leaf damage is a function of its stylet length and leaf thickness (Park and Lee, 2002). Damage done to leaves caused by *T. urticae* may result in a reduction in overall plant health or death (Park and Lee, 2002). Chhillar *et al*. (1998) estimated that an adult spider mite consumes about 50 % of its body mass per hour. Hundred photosynthetically active leaf cells are punctured and emptied per mite. Indirect effects of feeding thus include decrease in photosynthesis and transpiration (Park and Lee, 2002) leading to reduction in the amount of harvestable material (Hussey and Parr, 1963).

2.4 Effect of weather on two-spotted spider mite

The biology, distribution and abundance of mites are affected by various abiotic factors. Temperature is one of the most important factors responsible for build-up of *T.urticae* population. Several authors have reported a positive relationship between mite population and high temperature (Singh and Singh, 1993; Dhar *et al*., 2000; Putatunda and Tagore, 2003; Gulati, 2004; Haque *et al*., 2011). However Sunita, (1996) reported positive relationship between mite population and low temperature. Spider mites are reported to be most abundant during warm and dry weather which favours its multiplication and spread (Jeppson *et al.,* 1975).

Relative humidity is another abiotic factor found to affect mite population build-up. Pande and Yadav, (1976) reported a positive relationship between relative humidity and mite population build-up, non-significant negative relationship was reported by other authors (Singh and Singh, 1993; Dhar *et al*., 2000).

Heavy rains have also been reported to be a major cause of mortality for this pest as it is easily dislodged from plants by rain (Singh and Singh 1993; Gulati, 2004). Qui and Li, (1988) reported that low wind velocity favours the population build-up of tetranychid mites.

2.5 Management of two-spotted spider mite

2.5.1 Integrated pest management (IPM)

Integrated Pest Management focuses on environmentally friendly pest management options (Lacey and Brooks, 1997), which has been developed into an alternative approach to the conventional chemical based pest control system, currently used by the agricultural industry. The primary objectives of IPM are to maintain pest damage below economic injury levels and so as to reduce the adverse impact of chemicals in the environment and to maintain long-term

sustainable food production system (Thacker, 2002). IPM involves use of selective pesticide and other sustainable practices such as cultural control, physical control, host plant resistance, botanicals etc. Some of these methods are discussed briefly below for use against *T. urticae*.

2.5.1.1 Cultural control

Cultural control is a form of manipulating the environment for pest management (Hajek, 2004). Cultural control can be achieved by regular scouting of crops to identify *T. urticae* outbreaks to know the level of infestation. Intercropping or establishing barriers with non-host crops, burning of infested plants in the hot dry months help to reduce mite densities. Weeds are common mite hosts and are eliminated to prevent the spread of the two-spotted spider mite. Also, separation of infested and newly planted crops reduces the spread of mites (Magdalena and Meyer, 1996).

Irrigation and fertilization increases plant vigor and reduces crop stress. It has been documented that this reduces the susceptibility of plants to *T.urticae* infestation (Walsch *et al.,* 1998). Other cultural practices such as planting of cover crops, mulching and crop rotation also help to reduce pest establishment (Arancon *et al.,* 2005). Mites are easily dislodged from plants when a steady stream of water is sprayed on the plant (Lester *et al.,* 1997).

2.5.1.2 Host-plant Resistance

Breeding and selection of *T. urticae* resistant plant has been carried out on a several crops, such as *Impatiens* (Al-Abbasi and Weigle, 1982), soyabean (Mohammad and Rodriguez, 1985), *Pelargonium* (Chang *et al*., 1972), cucumber (de Ponti, 1980), *Vigna angularis* (Aguilar *et al*., 1996), strawberry (Shanks and Moore, 1995; Easterbrook and Simpson, 1998; Olbricht *et al*., 2014), watermelon (Lopez *et al*., 2005; El-Saiedy *et al*., 2011), maize (Mead

et al., 2010), tomato (e.g. Saeidi and Mallik, 2012) and citrus (Agut *et al*., 2014). However, the resistance may be polygenic in most cases (Easterbrook and Simpson, 1998), and so is difficult to exploit by plant breeders.

Agut *et al*., (2014), attributed the mechanisms of host-plant resistance of *T. urticae* to flavonoid pathways in citrus. Olbricht *et al*., (2014), reported that leaf trichomes on *Fragaria* entraps mites on tomato (Saeidi and Mallik, 2012), increased peroxidase and polyphenol oxidase activity in melon (Shoorooei *et al*., 2013), antibiosis and antixenosis in bean (Kamelmanesh *et al*., 2010), phytochemical compounds in watermelon, where El-Saiedy *et al*. (2011) reported a negative relationship between mite infestation and tannins, and nitrogen and protein content in maize leaves (Mead *et al*., 2010).

2.5.1.3 Biological control

Biological control is defined as the use of a living organism to suppress a specific pest organism, making it either less abundant or less damaging than it would be (Hajek, 2004). In biological control, human intervention by enhancing the numbers of or introducing new natural enemies of target organisms so as to restore the natural balance result in a safer and environmentally friendly alternative of pest control (Burges and Hussey, 1971; Gullan and Cranston, 1994). Bacteria, fungi, nematodes, protozoa and viruses are entomopathogens regarded as key bio control components of IPM (Bidochka *et al*., 2002; Thacker, 2002) in pest control programmes.

Tetranychus urticae has been the subject of some of the most successful examples of biological control by use of the phytoseiid mite *Phytoseiulus persimilis*. Hussey *et al*. (1965) were among the first researchers to have used *Phytoseiulus persimilis* in glasshouses on various crops and since then, it has been successfully used on different crops in a range of protected and unprotected environments.

However, *P. persimilis* is active only under a limited range of conditions (Gorski and Eajfer, 2003), and so other species of phytoseiid mites have also been used against *T. urticae*. For example, *Amblyseius idaeus* and *Phytoseiulus macropilis* have been used on strawberry and cucumber in Brazil (Watanabe *et al*., 1994). Metwally *et al*. (2005) investigated life table and prey consumption of the predatory mite *Neoseiulus cynodactylon*, and concluded that *T. urticae* was a profitable prey species of this phytoseiid as a facultative predator.

Neoseiulus californicus has shown promise as an agent in conservation biological control of *T. urticae*; the natural control of the mite in strawberries was used as the basis for developing an integrated management plan, using acaricide only when necessary (Greco *et al*., 2011).

Complete eradication of the pest is not the aim since it will disturb the balance between the pest and the biological control agents (Schuster and Murphy, 1991). Thus, the success of biological control depends on the activities of these natural enemies in order to facilitate the reduction of density and distribution of the pest and eventually the potential agricultural damage (Gullan and Cranston, 1994).

2.5.1.4 Botanical pesticides

Botanical pesticides such as garlic, chilli, soap extracts have been tested against spider mite in Namibia, Neem and Tephrosia in Zimbabwe, Kenya and Malawi (Keizer and Zuurbier, 1998) and insecticidal soaps containing potassium salts and fatty acids (Recep *et al*., 2005). Essential oils have also been used in pest control. Besides exerting acute toxicity to insects and mites, essential oils show sub-lethal effect as repellents, anti-feedants and reproduction inhibitors. Essential oils extracted from caraway seeds, eucalyptus, mint, rosemary, basil, oregano, thyme, and other plants have shown a significant acaricidal activity (Aslan *et al*., 2004; Choi *et al*., 2004; Miresmailli *et al*., 2006). These oils could be useful as fumigants in the control of phytophagous mites in greenhouses. Plant oils such as cottonseed oil (Rock &

Crabtree, 1987) soybean oil (Lancaster *et al*., 2002; Moran *et al*., 2003) and rapeseed oil (Kiss *et al*., 1996; Marčić *et al*., 2009) have also proven to be effective in control of mite pest well.

2.5.1.5 Entomopathogenic fungi

Studies have shown that entomopathogenic fungi, especially ascomycetes plays a vital role in regulating populations of harmful arthropods if used in biological control (Hajek & Delalibera, 2010), or applied as mycoinsecticides and/or mycoacaricides (Maniania *et al*., 2008; Jackson *et al*., 2010). The conidia and blastospores of *Beauveria basssiana, Hirsutella thompsonii*, *Lecanicillium* sp., *Metharizium anisopliae, Isaria fumosorosea*, *Neozygites floridana* (Chandler *et al*., 2000; Maniania *et al*., 2008), are used for formulation of fungal-based biopesticides. The main advantage of fungi as control agents is their ability to infect insects by penetrating the cuticle at any developmental stage. This means that insects of all ages and feeding habits, even sap-suckers, are susceptible to fungal disease (Gullan and Cranston, 1994). At the beginning of the 1980s, only one mycoacaricide was available (Mycar), formulated from conidia of *H. thompsoni* and intended for supression of citrus rust mite. Quarter of century later, there are some 30 commercial products acting against tetranychid, eriophyoid, and tarsonemid mites, mostly formulated as wettable powder or oil dispersions, and one third of which is made from conidia of *B. bassiana* (Faria and Wraight, 2007).

2.5.2 Chemical control

Despite the hazards of conventional pesticides, some use is unavoidable (Gullan and Cranston, 1994), chemical pesticides are predominantly used in the control of *T. urticae* because they are readily available, easy to apply, effective, cheap and as a result of the lack of reliable alternative control measures (Sanderson and Zang, 1995). Some examples of chemical control which has been used for the control of *T. urticae* include, abamectin,

bifenazate, bifenthrin, clofentizine, dicofol and methyl bromate (Motoba *et al.*, 2000; Rauch and Nauen, 2003; Simpson *et al.*, 2004; Recep *et al.*, 2005; Fahnbulleh, 2007; Piraneo, 2013). *Tetranychus urticae* develops resistance to chemical groups after a few years of use which makes its control very difficult (Cranham and Helle, 1985). It has also developed cross-resistance to other chemical groups (Thwaite, 1991; Al-Jboory *et al*., 2004). Although chemical control play a significant role in the control of the two-spotted spider mite, mites still continue to cause a notable yield loss in field and greenhouse crops (Kim and Seo, 2001).

2.5.2.1 Pesticide usage pattern

In order to produce healthy-looking, damage free fruit and vegetable to meet the demand of the growing population, fertilizers and insecticides are used to increase productivity and to control pest. Chemical pesticide use is a common practice in controlling pest and diseases in vegetable cultivation in Ghana (Ntow, 2007) since farmers' reason that as long as there is no better alternative to pest control, the spraying of pesticides is good investment (Hardy, 1995). Although the use of chemicals gives rapid curative action, effective and economically adaptable in all situations in meeting changing agronomic and ecological conditions (Metcalf, 1975). Not surprisingly pesticide use has increased overtime in Ghana and is particularly elevated in the production of vegetable (Gerken *et al*., 2001). Chemical pesticides are used improperly and in dangerous combinations (Obeng-Ofori *et al*., 2002). Mixing of pesticides was encouraged by the farmers' desire to have rapid knockdown of pests. Studies conducted by other authors (Wintuma, 2009; Kingsley, 2010) revealed similar findings among farmers in Accra. Though farmers claimed the combination was effective in controlling pest, however, combination of two insecticides with the same or different mode of action or mixing of two unrelated insecticides are dangerous (Obeng-Ofori *et al*., 2002) and results to cross or multiple resistance (IRAC, 2011). Medina, (1987), stated that the idea of mixing chemicals by farmers is questionable because the combinations used are indiscriminate. The

practice of using indiscriminate combinations of pesticides, particularly of insecticides, may have contributed to the increase in incidences of insect pest infestation of tomato in Ghana (Biney, 2001) thereby defying some of the basic principles of insecticide management. For instance, Metcalf (1980), in his recommendation of strategies for pesticide management, states that the use of mixtures of insecticides must be avoided, since mixtures of insecticides generally result in the simultaneous development of resistance.

Historically mite pests have been controlled by the introduction of acaricidal compound with a novel mode of action. *Tetranychus urticae* resistance to acaricides is a well-documented event (Grbic *et al*., 2011). Resistance to acaricides have been reported in over 60 countries to 95 acaricidal/insecticidal active ingredients (Van Leeuwen *et al*., 2010; DARP, 2013). Acaricides resistance have been reported from Norway (Fahnbulleh, 2007), North pacific (Piraneo, 2013), Turkey (Sokeli *et al*., 2007) and Australia (Nauen *et al*., 2001). Factors contributing to the development of resistance in *T. urticae* include high fecundity, short generation time and changes in agro-ecosystems (Cranham and Helle, 1985).

2.5.2.2 Effects of chemical control

Indiscriminate use of insecticides has hazardous effect on both the human health and the environment. Hazards caused by the prolonged use of pesticides may affect human health directly or indirectly through residues in food and other biotic systems (Metcalf, 1980).

2.5.2.2.1 Human health

Insecticides are not only toxic to insects but to humans as well. The World Health Organization (WHO) reported that 20% of pesticides use in the world is concentrated in developing countries posing danger to human health as well as environment (Hurtig *et al*., 2003). McCauley *et al*., (2001) noted that families residing in agricultural areas had noticeable levels of pesticide in their body systems. In Benin, endosulfan the most commonly

used insecticide in cotton during 1999/2000 growing season contributed to food poisoning deaths of approximately 70 Benin citizens (Vodouhe, 2001). In Ghana, high levels of pesticide residues in foodstuff has led to an outcry over the inappropriate use of pesticides on vegetables cultivated in urban and peri-urban areas (Koteh *et al*., 2008). In July 2004, several people were admitted to hospital at Tarkwa in the Central Region of Ghana after eating cabbage sprayed with excessive amounts of organophosphate (Ghana News Agency, 2004).

2.5.2.2.2 Agricultural systems

Modern agricultural system and over reliance on the use of chemicals disrupts the natural balance between insect pest and their natural enemies. These therefore lead to pest resurgence and introduction of new pest species whose populations were once regulated by natural enemies. The concept of secondary pest outbreak was introduced on spider mite as a paradigm. Advances in agricultural production after the 2nd world war based on the extensive use of insecticide, fertilizer, irrigation and other cultural practices induced increase in spider mite population far above economic threshold (Hufkar *et al*., 1970; McMurtry *et al*., 1970; Jeppson *et al*., 1975; Metcalf, 1980). The use of acaricides has increased substantially over the half past 20th century since the first serious and widespread outbreak of spider mites population during the 1950's earning it the title of world most serious mite pest.

2.6 Resistance Development

Resistance to an insecticide may be defined as an inherent ability of an insect population to survive doses of toxicant which would prove harmful to majority of the individuals in a normal population of the same species (Brown and Pal, 1971).

According to Sawicki (1987), resistance represents a genetic change in response to assortment by toxicant that may impede control in the field. Generally, pests are known to

develop resistance to chemicals due to frequent uncontrolled use and weak effect from common pesticides which are not actually potent against them (Fahnbulleh, 2007); and this resistance, in part, comes from their genetic makeup (Zhang, 2003).

According to the Insecticide Resistance Action Committee (IRAC) resistance is the selection of a heritable characteristic in an insect population that results in the repeated failure of an insecticide product to provide the intended level of control when used as recommended, while Gunning, (1991) defined resistance as failure of an insecticide to control a population of insects due to a genetically transmitted capacity to tolerate more insecticide than usual. Resistance can also be considered as a form of self-defense, because pest subjection to the many environmental stresses or dangers such as: humidity, temperature, radiation, predation/parasitism, diseases, and pollutants in the form of pesticides or plants allelochemicals inhibit their activities for survival. It is the apparent expression of pests' natural response to the above stresses through resistance mechanisms that is regarded as self-defence (Koehn and Bayne, 1989; Scott, 1995). As a result, the chemical is applied more frequently and in large quantities to make up for the decline in effectiveness. Resistant genes are normally present in a population but at a low frequency. They are derived from the initial population by the selective mortality of the more susceptible genotype growing during the application of an insecticide (Crow, 1957; O'Brien, 1967; Sawick, 1979). Development of resistance is rapid when selection pressure exerted by insecticide is widespread and continuous and occurs after the insect has been exposed to chemicals for several generations.

Kumar (1984) reported that *Heliothis virescens* developed resistance to DDT after 15 years of use. Resistance to organochlorine compounds by the boll weevil *Anthonomus grandis* took 25 generations (Graves *et al*., 1967). The more rapid the lifecycle of the insect and the more persistent the chemical is in the environment the rapid the development of resistance (Hassal, 1990).

2.6.1 Mechanisms of insecticide resistance

According to Brattsten *et al*., (1985) insecticide resistance is a multi-dimensional phenomenon affected by biochemical, physiological, genetic and ecological nature of the insect. They are metabolic detoxification, target site insensitivity and behavioural resistance.

2.6.1.1 *Metabolic Detoxification*

Metabolic resistance is based on the normal biochemical reactions that occur in insect which enables them to detoxify or prevent the activation of toxic materials. The biochemical reaction responsible for detoxification of xenobiotics in insect includes esterases, monooxygenases and glutathione-S-tranferases (GSTs). These enzyme systems are often enhanced in resistant insect strain enabling them to metabolise or degrade insecticide before they are able to exert a toxic effect (IRAC, 2011)

Cytochrome P450 – dependent monooxygenases

The cytochrome P450 monooxygenases belong to a very large family of water-repelling, hemecontaining enzymes involved in the detoxification of many identical internally originated elements such as juvenile hormones, ecdysteroids and pheromones, and externally originated substances such as plant allelochemicals, insecticides and promutagenes (Eldefrawi *et al*., 1960; Nordhus, 2005). As for its association with acaricide resistance, the process of fluvalinate resistance in the *Varroa* mite is a good example (Hillesheim *et al*., 1996). Cytochrome P450 oxidizes biochemical change in insecticides through O-, S-, and N-alkyl hydroxylation, aliphatic hydroxylation and pixilation, aromatic hydroxylation, ester oxidation and nitrogen and thioester oxidation (Brogdon and McAllister, 1998).

Glutathione- S - transferases (GSTs)

Glutathione –S – transferase is another enzymes class that has been found to be involved in metabolic resistance (Scott, 1995). It plays a vital role in detoxification of Organophosphate insecticides (Salinas and Wong, 1999; Nordhus, 2005) thereby accelerating the breakdown of pesticides or their metabolism (Stumpf, 2001) so as to increase water solubility and later the elimination of lipophilic substances (Motoyama and Deuterman, 1975; Stumpf, 2001). They can convert the poisonous substances of pyrethroid, organophosphate (OP), DDT, cyclodiene and carbamate insecticides into harmless substances. An increased GST production has been associated with resistance to all major classes of insecticides (Prapanthadara *et al*., 1995; Vontas *et al*., 2001; Hemingway *et al*., 2004; Nordhus, 2005), but the process involved in this elevated enzyme production is not well known (Enayati *et al*., 2005; Nordhus, 2005). Enzymatic detoxification has the potential to confer cross resistance to toxins independent of their target site (Stumpf, 2001). Despite the fact that much is known about insect GSTs and their role in the biochemical changes (metabolism) of insecticides, little information is available concerning the enzyme in spider mite (Stumpf, 2001) therefore, resistance mechanisms linked to GST in mites are difficult to elucidate.

Nonspecific Esterases

One of the most common metabolic resistance mechanisms is that of elevated levels, or activity, of esterases enzymes, which hydrolyse ester bonds or sequester insecticides. These esterases comprise six families of proteins belonging to α/ß hydrolase fold superfamily. Nearly all of the strains of *Culex quinquefasciatus* which resist a broad range of organophosphate (OP) insecticides have been found to possess multiple copies of a gene for esterases, enabling them to overproduce this type of enzyme. In contrast, strains of malathion resistant *Anopheles* have been found with non-elevated levels of an altered form of esterase

that specifically metabolises the OP malathion at a much faster rate than that in susceptible individuals.

In Diptera, they occur as a gene cluster on the same chromosome. Individual members of the gene cluster may be modified in instances of insecticide resistance, for example, by changing a single amino acid that converts the specificity of an esterase to an insecticide hydrolase or by existing as multiple-gene copies that are amplified in resistant insects (Brogdon and McAllister, 1998).

2.6.1.2 *Target site insensitivity*

Target site insensitivity is the second most common resistance mechanism encountered in insects. The site of action has been altered to decrease sensitivity to toxic attack. Alterations of amino acids responsible for insecticide binding at its site of action cause the insecticide to be less effective or even ineffective. Three different target site resistance mechanisms have been found: γ - aminobutyric acid (GABA), Acetylcholine esterase (AchE) and Sodium channel (knockdown resistance, kdr). All of these are well known targets of insecticides, and resistance alleles of each have been found (Scott, 1995). Insecticides act at this site within the insect, typically within the nervous system. The site of action can be modified in resistant strains of insects such that the insecticide no longer binds effectively. This result in the insects being unaffected or less affected, by the insecticide than susceptible insects. For example, the target site for Organophosphorus and carbamate insecticides is acetylcholinesterase (AChE) in the nerve cell synapses and the target of organochlorines (DDT) and synthetic pyrethroids are the sodium channels of the nerve sheath. DDT-pyrethroid cross-resistance may be produced by single amino acid changes (one or both of two known sites) in the axonal sodium channel insecticide-binding site. This cross-resistance

appears to produce a shift in the sodium current activation curve and cause low sensitivity to pyrethroids.

An unresponsive AChE resulting in OP resistance is found in many different insect species and has also been discovered in *T. urticae* strains from Germany (Voss and Matsumura, 1964; Smissaert *et al*., 1970), New Zealand (Ballantyne and Harrison, 1967), and in a few other tetranychid pest species, including the carmine spider mite (*Tetranychus cinnabarinus*) from Israel (Zahavi and Tahori, 1970), the kanzawa spider mite, *Tetranychus kanzawa*, from Japan (Kuwahara, 1982) and *Caloglyphus berlesei* (Blank, 1979). A point mutation in the γ – aminobutyric acid (GABA) receptor can confer an increased resistance to cyclodiene insecticides. This has been found in a range of different insect species (Bloomquist, 1994).

2.6.1.3 *Behavioural resistance*

Behavioural resistance describes any modification in insect behaviour that helps to avoid the lethal effects of insecticides that will otherwise prove lethal. Insecticide resistance in mosquitoes is not always based on biochemical mechanisms such as metabolic detoxification or target site mutations, but may also be conferred by behavioural changes in response to prolonged exposure to an insecticide. For example, a species of *Anopheles* in Africa was reported to avoid insecticide residues inside houses by remaining outdoors (Brown, 1958). Behavioural resistance is not as important as physiological resistance but might be considered to be a contributing factor, leading to the avoidance of lethal doses of an insecticide (IRAC, 2011).

2.6.2 Forms of resistance

2.6.2.1 *Cross resistance*

Cross resistance occurs when a resistance mechanism, that allows insects to resist one insecticide, also confers resistance to compounds within the same class, and may occur between chemical classes, depending on mechanism. The phenomenon of cross resistance is a relatively frequent one in vector populations (IRAC, 2011). For example, DDT and pyrethroid insecticides are chemically unrelated but both act on the same target site, the voltage gated sodium channel. Earlier use of DDT has resulted in several insect species developing resistance to DDT due to the *kdr* mutation at the target site. Where these mutations have been retained in the population, the insects have some resistance to all pyrethroids in addition to DDT. Cross resistance can also occur between OP and carbamate insecticides when resistance results from altered ACHe

2.6.2.2 *Multiple resistance*

Multiple resistances are a common phenomenon and occur when several different resistance mechanisms are present simultaneously in resistant insects. The different resistance mechanisms may combine to provide resistance to multiple classes of products. It is also quite common for the contribution of different mechanisms to change over time as selection processes evolve (IRAC, 2011).

2.6.3 Resistance in two-spotted spider mite

Resistance in mites to acaricides was first observed by Compton and Kearns in two-spotted spider mite, *T. urticae* Koch against ammonium potassium selenosulfide (Selecide) in 1937. It has been found that mites are capable of speedily developing extensive resistance to many acaricides (Cranham and Helle, 1985; Knowles, 1997; Stumpf and Nauen, 2001) after one to

four years of use and often induces a high degree of cross-resistance. Factors that contributes to resistance in two-spotted spider mites include great egg laying potential, polyphagous feeding habit, short life-cycle, cross fertilization and high mutation rates coupled with their extremely dispersal behaviour all help to boost resistance development in the species (Croft and Van de Bann, 1988). The continuous exposure of *T. urticae* to different pesticides in order to contain them below economic threshold has resulted in resistant populations found in more than 40 countries in both greenhouses and field conditions (Georghiou and Lagunes-Tejeda, 1991), and resistance to at least 85 different compounds has been published (http://www.pesticideresistance.org accessed 21 May 2015).

2.7 The role of synergists

Synergists are compounds that enhance the toxicity of some insecticides, although they usually have limited toxicity themselves. At non-toxic concentrations, insecticide synergists act by inhibiting certain enzymes naturally present in insects that would otherwise breakdown and detoxify insecticide molecules. The use of synergists has a valuable place in increasing the activity of certain insecticides on insects with specific resistance mechanisms and prolonging the useful life of those insecticides where resistance is developing.

Synergists, including piperonyl butoxide (PBO), *S,S,S*-tributyl phosphoro-trithioate (DEF), and *N*-Octyl bicycloheptene dicarboximide (MGK-264) and Diethyl maleate (DEM), enhance the effect of several classes of insecticide, including the pyrethroids, organophosphates and carbamates. Synergists act by blocking metabolic pathways that would otherwise break down pesticides, thus restoring susceptibility to the agent. Piperonyl Butoxide (PBO) is co-formulated with insecticides such as carbaryl, methomyl, fenvalerate, permethrin, parathion, malathion and dimethoate; S,S,S-tributyl phosphorotrithioate (DEF) is co-formulated with

malathion and permethrin; and Diethyl maleate (DEM) is co-formulated with parathion, malathion and dimethoate (Bernard and Philogene, 1993).

Insecticide synergists have also been used to investigate resistance mechanisms and to identify the specific metabolic pathways targeted. Tolerance of honey bees, *Apis mellifera*, to pyrethroids is largely reversed by PBO and at low levels by DEF, suggesting a significant role of cytochrome P450s and a lesser role of esterases in detoxification of the chemical. However, the metabolic routes blocked by synergists are not yet fully understood and maybe dependent on the species of arthropods (Johnson *et al*., 2006). For example, although initial evidence in the 1970's suggested that PBO acts as a specific inhibitor of monooxygenases (P450s), recently, esterases of pyrethroid resistant strains of the cotton bollworm, (*Helicoverpa armigera*) were shown to be inhibited by PBO (Young and Gunning, 2005). In a study conducted with insecticide resistant strain of the German cockroach, *Blatella germanica*, PBO and DEF were tested as synergists to propoxur (a carbamate insecticide). Both were shown to inhibit cytochrome P450 monooxygenases at different levels (Sanches-Arroyo *et al*., 2001), thus, suggesting that this enzyme family is primarily responsible for the metabolism of the pesticide.

2.8 Resistance management

Effective resistance management depends on early detection of the problem and rapid assimilation of information on the resistant insect population so that rational pesticide choices can be made (WHO, 2006). The ability to use other pesticides in order to avoid or delay the development of resistance in pest populations hinges on the availability of an adequate supply of pesticides with differing modes of action. This method is perhaps not the best solution, but it allows a pest to be controlled until other management strategies can be developed and brought to bear against the pest. These strategies often include the use of pesticides, but used

less often and sometimes at reduced application rates (Karaagac, 2010). The goal of resistance management is to delay evolution of resistance in pests. The best way to achieve this is to minimize insecticide use. Thus, resistance management is a component of integrated pest management, which combines chemical and non-chemical controls to seek safe, economical, and sustainable suppression of pest populations. Alternatives to insecticides include biological control by predators, parasitoids, and pathogens. Also valuable are cultural controls (crop rotation, manipulation of planting dates to limit exposure to pests, and use of cultivars that tolerate pest damage) and mechanical controls (exclusion by barriers and trapping). Because large-scale resistance experiments are expensive, time consuming, and might worsen resistance problems, modelling has played a prominent role in devising tactics for resistance management. Although models have identified various strategies with the potential to delay resistance, practical successes in resistance management have relied primarily on reducing the number of insecticide treatments and diversifying the types of insecticide used. For example, programs in Australia, Israel, and the United States have limited the number of times and periods during which any particular insecticide is used against cotton pests. Resistance management requires more effective techniques for detecting resistance in its early stages of development.

Pest resistance to a pesticide can be managed by reducing selection pressure by this pesticide on the pest population. In other words, the situation when all the pests except the most resistant ones are killed by a given chemical should be avoided. This can be achieved by avoiding unnecessary pesticide applications, using non-chemical control techniques, and leaving untreated refuges where susceptible pests can survive. Adopting the integrated pest management (IPM) approach usually helps with resistance management. When pesticides are the sole or predominant method of pest control, resistance is commonly managed through

pesticide rotation. This involves alternating among pesticide classes with different modes of action to delay the onset of or mitigate existing pest resistance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in four localities within Accra metropolis (Ashaiman, Opeibea, Sinna's garden and University of Ghana farm (Legon). These areas were chosen because of the differences in farming practices, rainfall and pesticide usage pattern. The Accra metropolitan area is located within the greater Accra region in Southern Ghana. It is a coastal savannah ecological zone characterized by dry climatic conditions with two peak rainy seasons from April to June and September to October. The annual rainfall ranges between 740 and 890 mm per annum and a temperature of 26 C and 30 C the relative humidity is 65-75 % (Dickson and Benneh, 1988).

3.2 Survey

A survey was conducted on some selected farmers in the vegetable growing areas of Ashaiman, Opeibea, University of Ghana farm (Legon) and Sinna's garden (Department of Crop Science) within the Accra metropolis so as to obtain information which will guide in the selection of insecticide to use for the study. A semi structured questionnaire was administered to farmers to obtain information on the practise, types, frequency and use of insecticides. Ten farmers were randomly selected at each of the afore-mentioned areas. Questions were structured to suit farmers understanding. An administrator was present to help translate the questions in the local dialect.

3.3 Miticides

Commercial formulations of miticide tested were Karate® (*lamda*-cyhalothrin 5% EC Syngenta Crop Protection Pty Ltd, Pendle Hill, NSW)), Protect® (Emamectin Benzoate 1.9 % EC Syngenta Crop Protection Pty Ltd, Pendle Hill, NSW), Levo 2.4 SL™ Sineria Industries Ltd. Neosytou Georgiou, The Netherlands), Anty ataa® (Imidacloprid Bayer Australia Ltd, Pymble, NSW) and the synergists PBO (97.5% ultra PBO, Pu-SF-02-008, Endura® Fine Chemicals, Italy) and DEM 97% (Aldrich Chemical Company, Inc., Milwuakee, USA).

3.4 Field Sampling of spider mites

Adult *T. urticae* were collected between November and April 2015, from commercial Eggplants, Okra, Paw paw and Cassava farms established at Ashaiman and Opeibea. Collection of mites was also made at University of Ghana farm and Department of crop science Sinna's garden. Infested leaves were excised from the plants and carefully placed in a Paper bag and transported to the laboratory for bioassay. The *T. urticae* for the susceptible population were originally collected from cassava plant grown in the green house of the Department of Crop Science University of Ghana where miticides had not been used for well over three years and reared on eggplant in the greenhouse and kept breeding for about five generations.

Plate 4: Sample collection site at Ashaiman.

3.5 Cultivation of miticide-free Egg plant

Miticide-free eggplant seeds were grown in the green house of the Department of Crop Science University of Ghana (Legon). The seedlings were used to culture the mites for the synergism assay. New uninfested eggplant seedlings were rotated into the mite colony every ten days.

3.6 Determination of Miticide Concentrations

A preliminary experiment was conducted to determine the optimal miticide concentration that will kill 90% of the mites after 24 h. 10 adult mites were exposed to each miticide (serially diluted with distilled water starting from twice the recommended rate for each miticide). The result of this preliminary experiment led to select a concentration of 0.7 ml/L and 1.5 ml/L, 4

ml/L, 10 ml/L, 0.66 ml/L and 5 g/L for Protect®, Imidacloprid®, Karate®, Levo® and Sulphur® miticide respectively for use in subsequent bioassays.

3.7 Bioassay

Adult *T. urticae* collected from the field were used upon arrival in the Laboratory. The leaf disc bioassays were used to estimate the LD_{50} and LD_{90}, (the lethal concentrations that kill 50% and 90% of the population, respectively) of a particular miticide. My bioassay method closely mimics methods described by Piraneo, (2013) to determine the direct efficacy of some selected miticide against *T. urticae.* Each bioassay consisted of five serial dilutions with three replicates per tested concentration. Each test included a control where the insects were treated with distilled water. Infested leaves were cut into small disc and placed in a 90 mm petri-dish lined with filter paper. The number of *T. urticae* on each leaf disc was then counted under a microscope and recorded. The leaves were then sprayed with 1 ml of miticide dilutions at the different concentrations with a hand sprayer. All treatments were maintained at room temperature (27.0 ±2.0ºC) with a photoperiod of 12h: 12h (L: D). Mortality was accessed 24 h after treatment by probing each mite with a fine brush under a microscope. Mites unable to move were classified as dead. Mites were also classified as dead if they were twitching or were unable to move a distance equivalent to their body length.

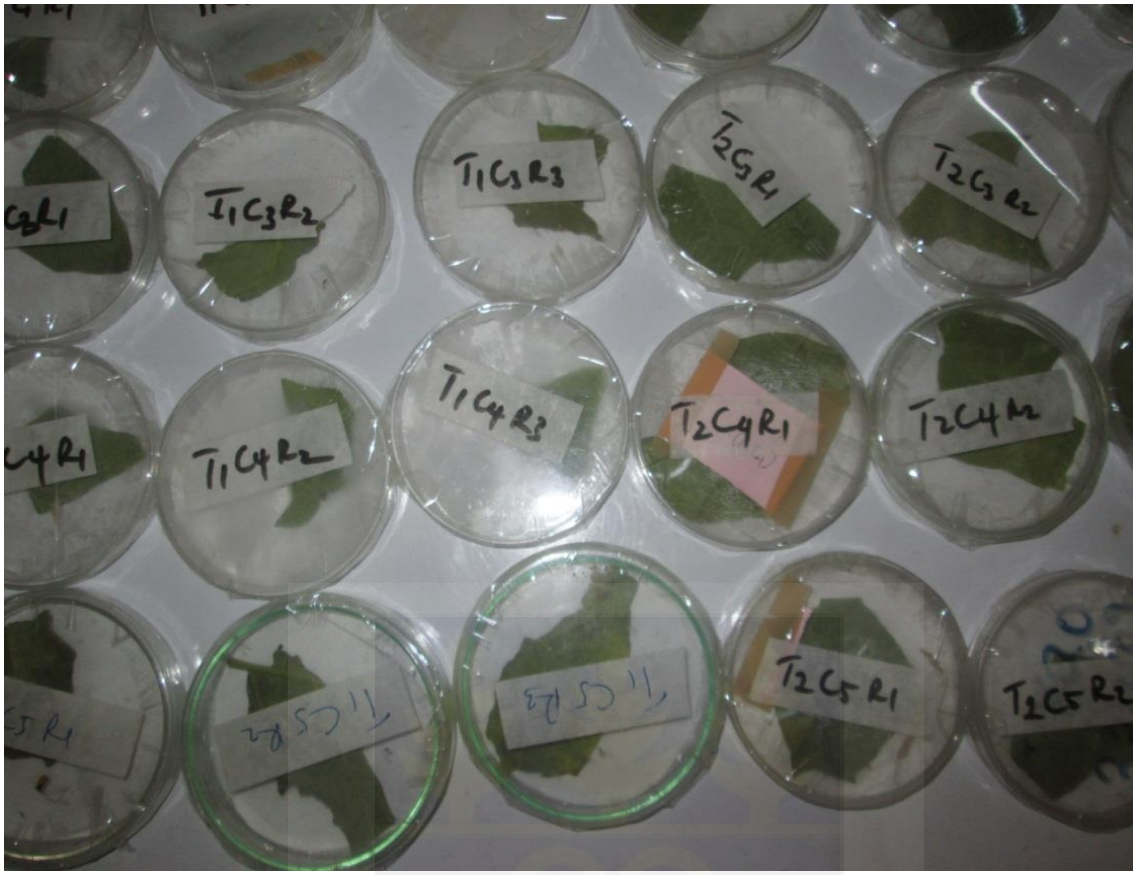

Plate 5: Experimental set up.

3.8 Determination of optimal (non-lethal) Piperonyl butoxide (PBO) and Diethyl Maleate (DEM) concentration

A preliminary experiment was conducted to determine the optimal (non-lethal) synergist concentration that the mites could tolerate and remain alive for 24 hours. Serial dilutions of the synergist were prepared in distilled water. Adult mites from Opeibea and Ashaiman population (Karate resistant population) were exposed to each synergist as previously described and maintained under same condition (3.6). The result of this preliminary experiment led to select a concentration (0.4 and 1µl/ml respectively) of each synergist for use in subsequent synergism assays.

3.9 Time series experiment with synergists

The optimal (non-lethal) PBO and DEM concentration determined from the above experiment were used for these assays. A time series experiment was carried out to determine the optimum time lapse between synergist and miticide application. The infested leaves were cut into leaf disc and the number of mites on each leaf disc were counted and placed in a 90 mm petri-dish lined with filter paper. The optimal PBO and DEM concentration determined from the above experiment was then applied. Karate miticide (0.151 ml/L and 0.140 ml/L) which produced 50 % mortality in the Opeibea and Ashaiman population respectively was then applied to the mites at 0, 1, 2 and 3 h after pre-treatment. All experiments were kept under lab conditions and mortality was recorded as stated earlier (3.6). 1 h pre-treatment period was used for both synergists.

3.10 Determination of the effect of PBO and DEM on Karate resistant populations

The optimal PBO and DEM concentrations determined from the above experiments were used for the assays. Infested leaves were cut into leaf disc and placed in a 90 mm petri dish lined with filter paper and pre-treated with 0.4 µl/ml and 1.0 µl/ml PBO and DEM respectively, karate miticide was serially diluted (at least five concentrations) and sprayed on each leaf disc at 1 h after treatment with synergist. For each treatment, there were three replicate Petri dishes each. The experimental conditions were the same as before and mortality was recorded as stated earlier.

3.11 Data analysis

Mortality data were subjected to probit analysis (Finney 1971) using a US Environmental Protection Agency probit program version 1.5 to determine the LD_{50}, slope and fiducial limits. Resistance factors (RFs) were determined by dividing the LD_{50} of the field populations by the LD_{50} of the susceptible population. Time-series experiments were analysed using analysis of variance (ANOVA) after the percentage mortalities were arcsine transformed. The means were separated using the least significant difference (LSD). The synergist ratio (SR) was determined by dividing the LD_{50} of the field population without synergists by the LD_{50} of the field population with synergists.

CHAPTER FOUR

4.0 RESULTS

4.1.1 Agronomic practices

The survey covered a total of forty (40) farmers in Accra suburbia. The survey showed that (10%) of the farmers had been involved in crop cultivation for a period of 5-10 years, (32.5%) for a period of 11-15 years and (27.5%) for a period of over 20 years (fig 1).

4.1.2 Pests and pest control practices

All the farmers indicated that the presence of pests were major reason for pesticide application. The farmers (50%) professed to take the necessary precautions before; during and after insecticide application and others (40%) relied on nose mask, gloves and long sleeves. A few (35%) sometimes took the necessary precautions, while the rest took absolutely no precaution though were aware of the associated health hazard of the pesticide.

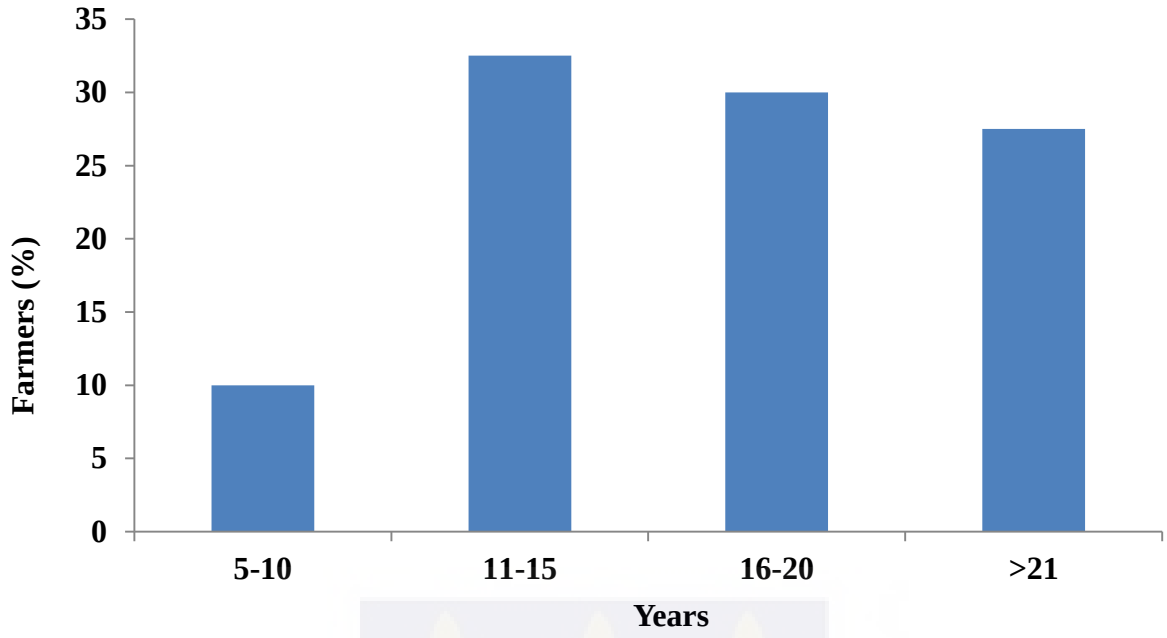

Fig. 1: Years spent in crop cultivation by farmers.

Several crops were cultivated with garden egg (80%), okra (35%), cowpea (72.5%), pepper (45%), Soya bean (55%) and pawpaw (35%) being host and most affected by spider mite (fig 2).

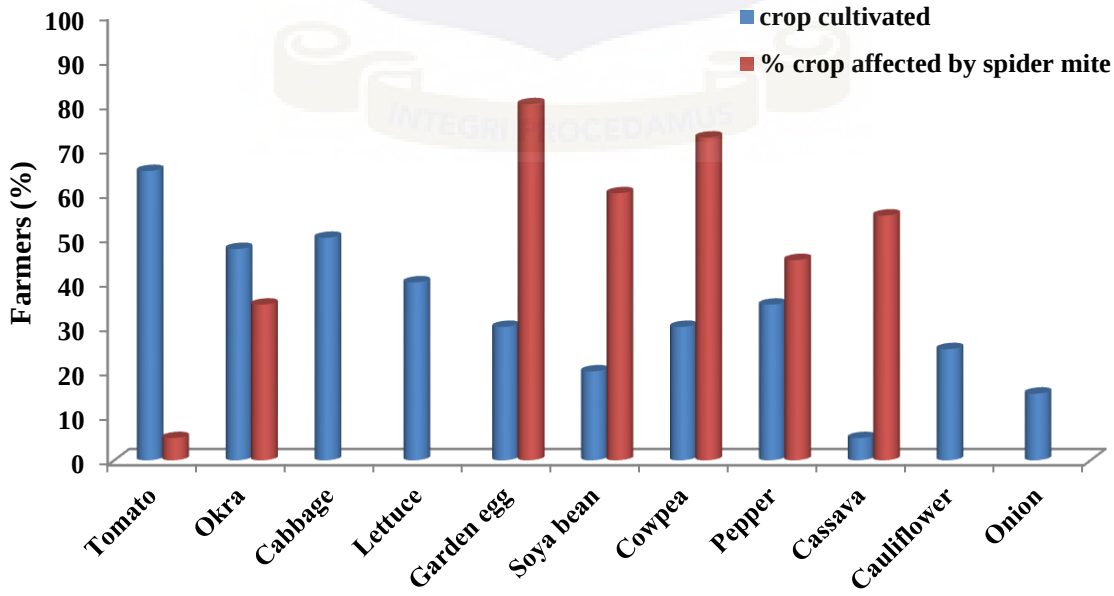

Fig. 2: Farmers perception of crops most affected by spider mites.

Farmers reported pest such as Aphids, Whiteflies, Diamond back moth, Thrips, Spider mites, crickets, grasshoppers and tomato bollworm affecting their crops. Diamond Back Moth and tomato bollworm were reported as the most destructive (fig 3).

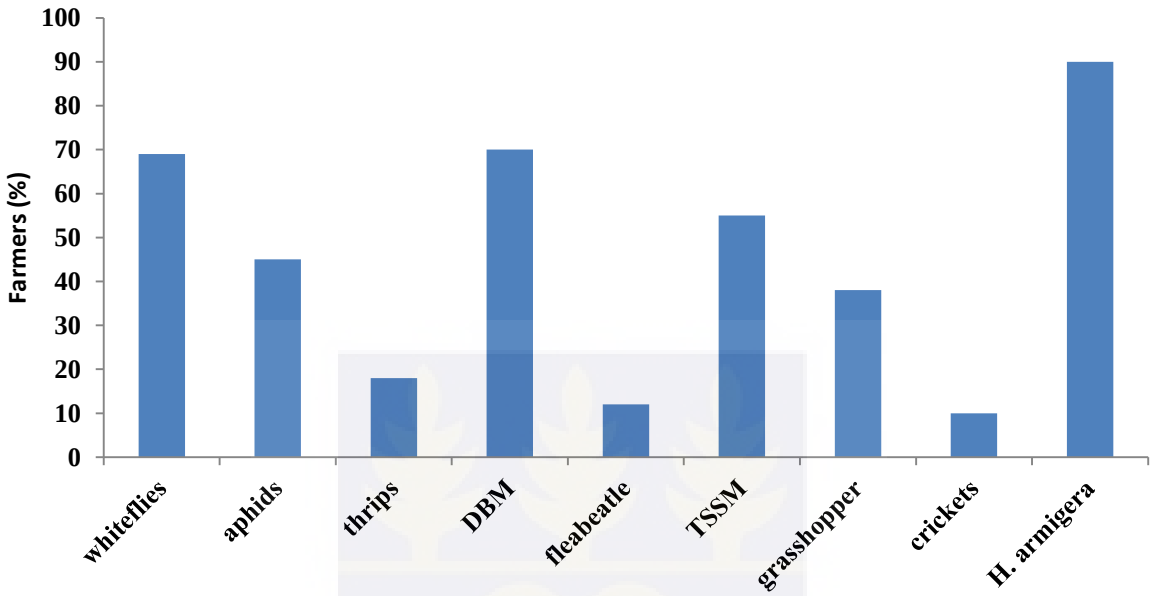

Fig.3: Insect pests of crops as reported by farmers.

4.1.3 Pesticide usage pattern

All the farmers interviewed indicated that they use agrochemicals on their crops. The main groups of agrochemicals used included insecticides, fungicides, herbicides and fertilizers (inorganic and organic manure). Of the agrochemical used; 11 types were insecticides, 3 fungicides, and 2 herbicides. The highest proportions and quantities of insecticides used were organophosphates, organochlorines and pyrethroids. These insecticides were applied as either single formulations or mixture (coctails). The neonicotinoid Imidacloprid which had a (30%) use among farmers with (22.5%) of the farmers using the product reported that it was effective. Also, (10%) of farmers surveyed sprayed cocktails of insecticide on their crops. Insecticides were classified as unlabelled if their labelling were either unclear to be read or there was none. Among the groups of fungicides; Mancozeb®, Maneb® and Cuprofix® were

the mostly used products against crop fungi. Glyphosate and Round up®) were the commonly used herbicides (fig 4).

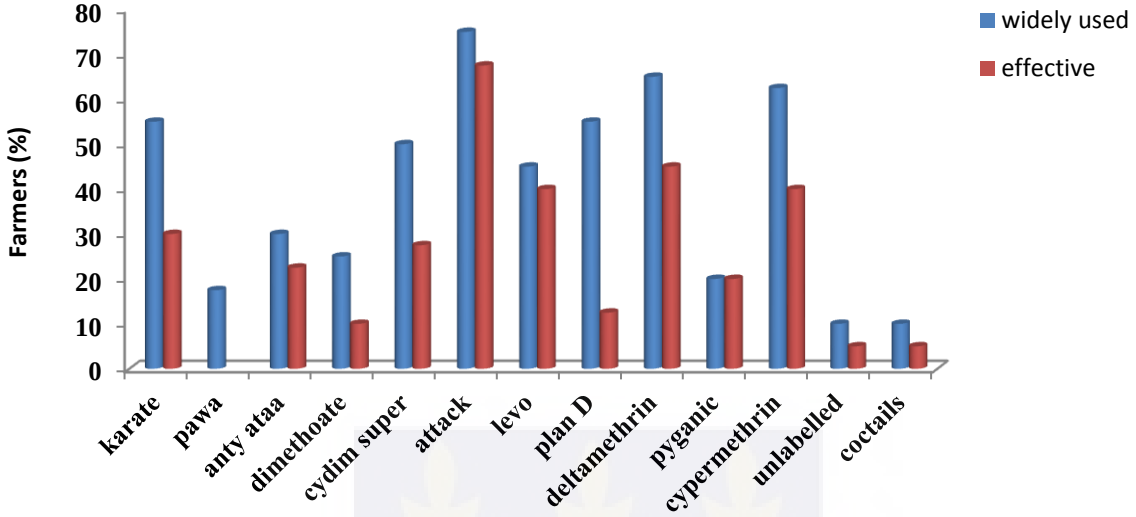

Fig.4: Commonly used insecticides and those perceived as effective by farmers.

4.1.4 Source of information

The study revealed that (65%) of farmers most commonly obtained general information of crop protection and agricultural practices from experience, (30%) claimed to have received advice from Extension officers and (50%) received advice from other farmers. The mass media was another means of educating and disseminating information on pest control strategies but was not fully exploited by farmers and was cited by (12.5%) of the farmers surveyed (fig 5).

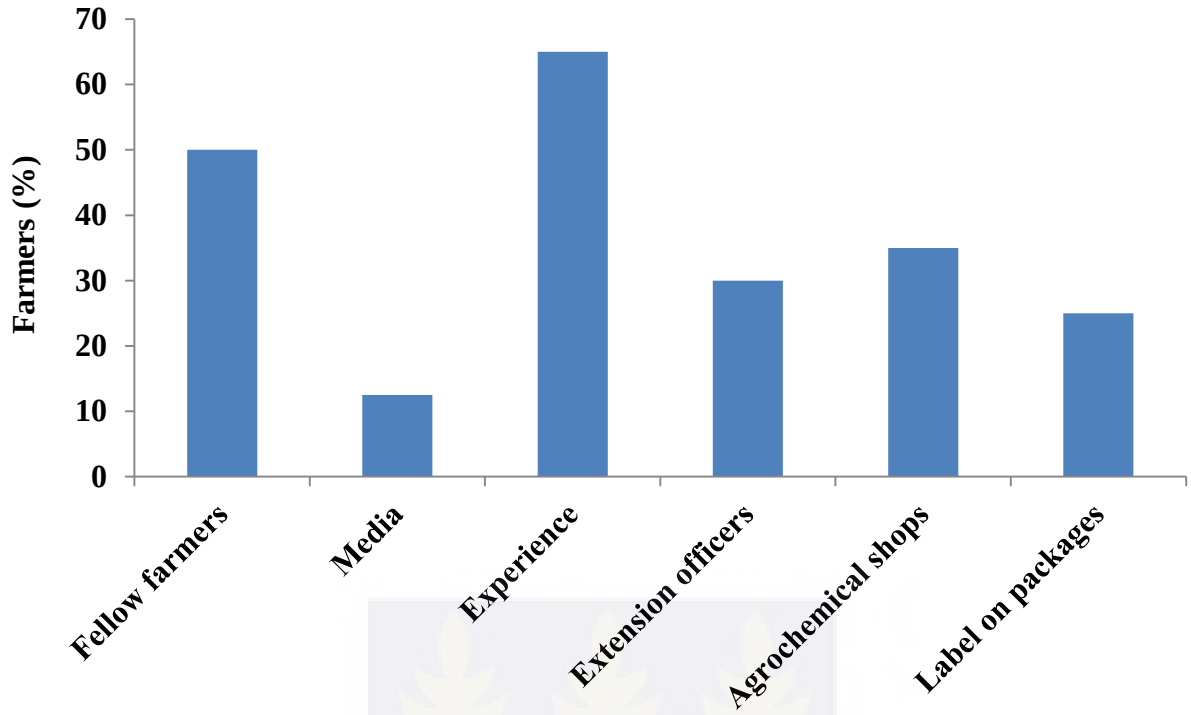

Fig.5: Source of farmers' knowledge about insect pest control.

4.1.5 Mode of insecticide application

Most of the farmers applied pesticides by use of Knapsack sprayers fitted with different nozzles tips. The farmers were able to give the various application rates of the insecticides used per sprayer. Majority used the lid of the insecticide container as standard measure of dosages. In addition to this, dosages in the farms were difficult to estimate because the application equipment was not calibrated before use. Remnants of pesticides were discarded in the farm by pouring them on the ground or burying.

4.1.6 Insecticide application dosage

The result also showed that most farmers (68%) applied the recommended dose whilst (32%) exceeded the recommended dose but none of the farmers surveyed applied insecticide below the recommended rate (fig 6).

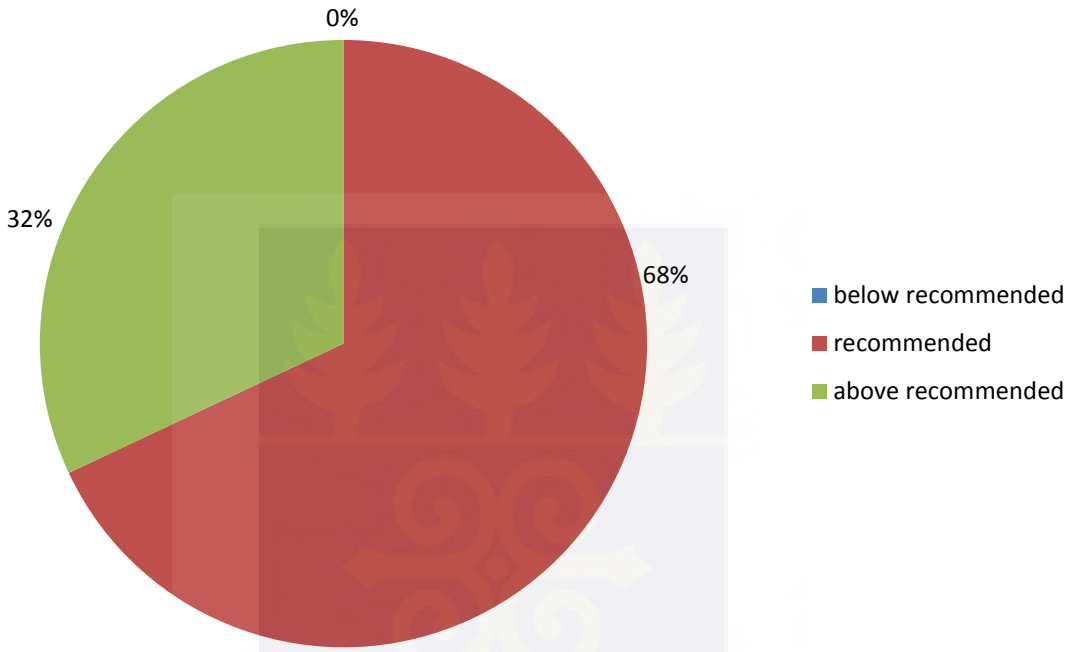

Fig. 6: Insecticide dosage application rate by farmers.

4.1.7 Timing of insecticide application

The timing of pesticides application mostly depended on presence of pests in different crops and their potential damages to the crop as well as farmers' perception regarding pest management practices. The first application of insecticides was generally carried out two weeks after transplanting of seedlings (fig 7).

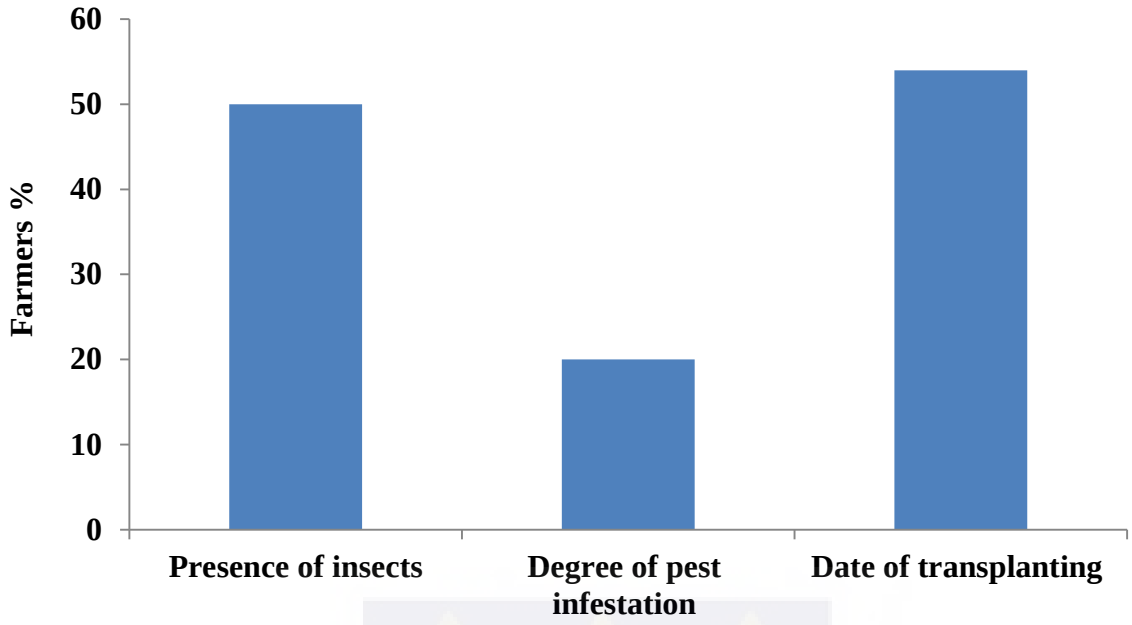

Fig.7: Timing of insecticide application by farmers.

4.1.8 Frequency of insecticide application

Based on the farmers' recollection on pesticide type and application frequency, weekly pesticide spraying was the most common, with (47.5%) of the farmers spraying insecticide. (25%) sprayed fortnightly, (12.5%) sprayed four times in a season; some (5%) of the farmers did not take note of the number of times they applied chemical on their crops (fig 8).

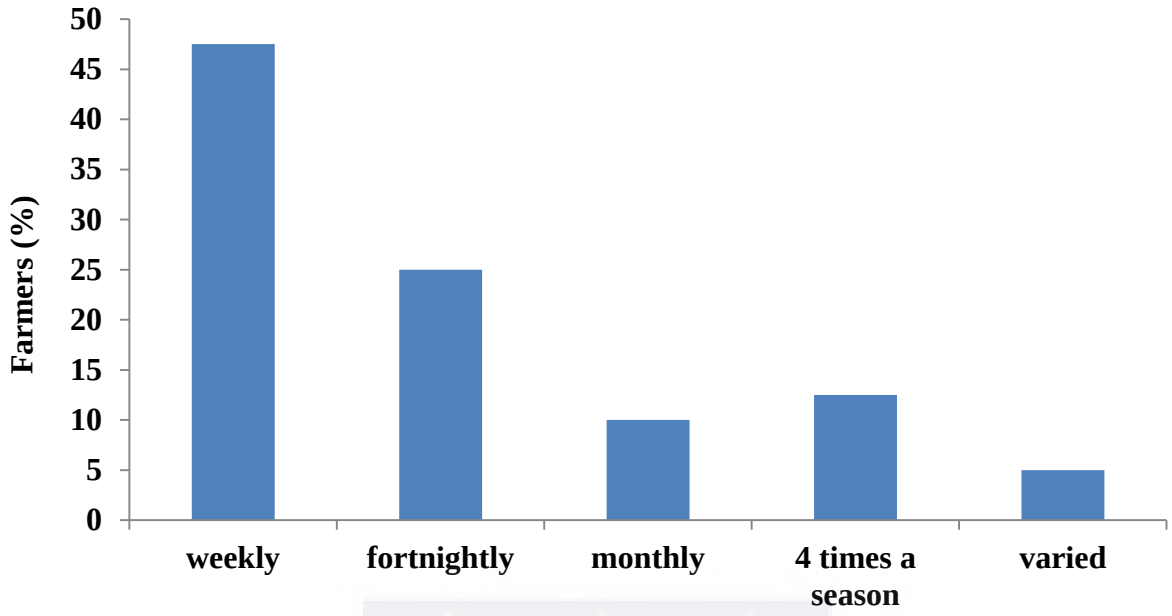

Fig.8: Frequency of insecticide application by farmers.

4.1.9 Reason for insecticide change by farmers

When farmers were asked their reason for changing the use of some insecticide, (70%) of the farmers attributed the decline in the usage of those insecticides to ineffectiveness against the target pest while (45%) of the respondents said the insecticides were out of stock compelling them to look for an alternative even though the previous ones had not failed. Of the farmers surveyed, (10%) cited cost as their sole reason for changing insecticide (fig 9).

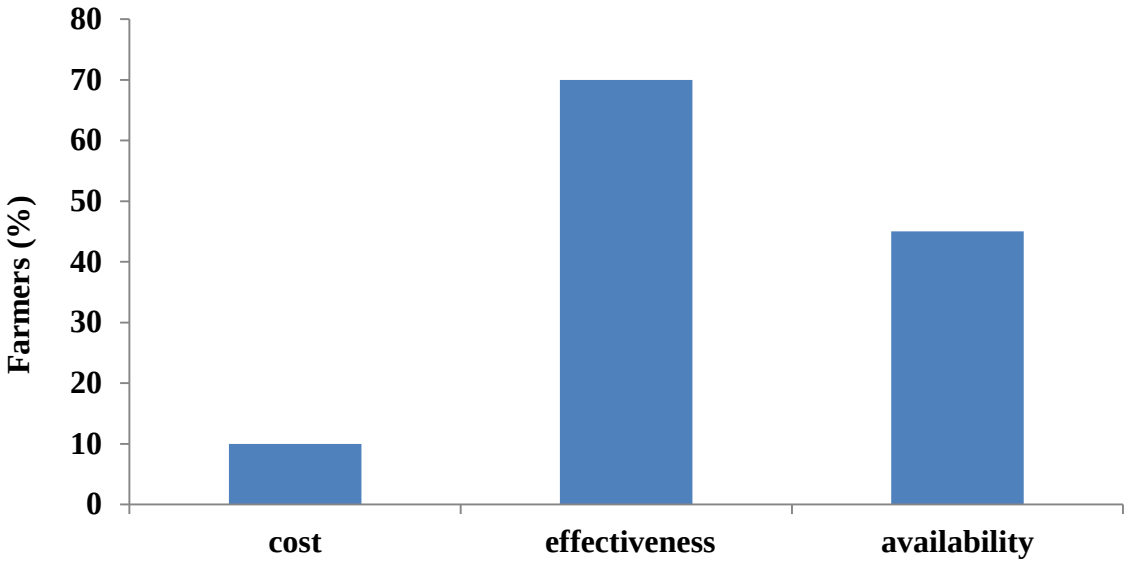

Fig.9: Reasons for insecticide change by farmers.

4.1.10 Method of disposal of empty insecticide containers

Disposal of the empty pesticide containers was mainly by throwing away in farms and waste (60%), others burnt or buried them in pits (35%), while the rest (12.5%) put them to other uses.

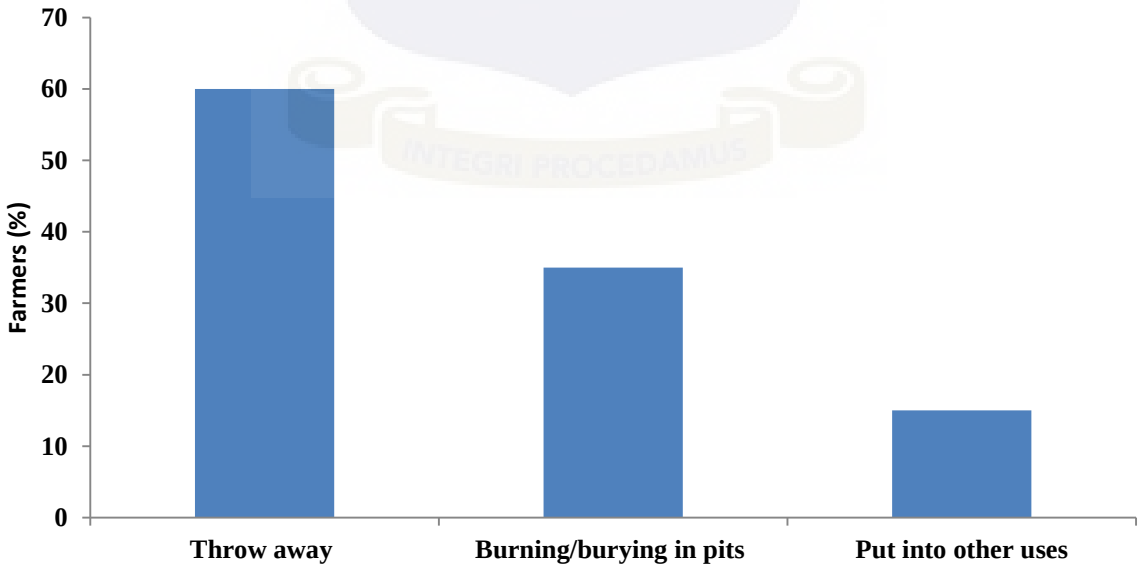

Fig.10: Methods of insecticide package disposal by farmers.

4.2 Susceptibility of two-spotted spider mite to miticides

The response pattern of four population strains of the two-spotted spider mite against five miticides was examined by the spray application method. Susceptibility of the four populations differed in all of the five miticides treatment compared to the susceptible population (table 1). The LD_{50} values of all field populations were significantly higher than those of the susceptible strain for the five miticides assayed. All field populations were resistant to Karate (ranging from 12.0-21.6-fold) differing significantly from the reference strain but did not differ amongst each other. The field populations showed differences in response with regards to Imidacloprid insecticide. The Opebia area populations recorded the highest LD_{50} value to Imidacloprid (0.072 ml/L) and differed significantly from Ashaiman and Sinna's garden population (0.014 and 0.022 ml/L respectively) but not the School farm, which recorded a significantly higher LD_{50} value for Imidacloprid (0.055) compared to Sinna's garden population. However, all the field populations were susceptible to Levo® and Sulphur® as there were no significant differences amongst the field population. The slope of regression values obtained from the Probit analysis for all the five miticides were low ranging from 0.567-1.115. This is indicative of the fact that the field populations' response to the toxicity test was quite heterogeneous. The resistance ratios for Protect ranged from 7-11, 1.8-9.0 for Imidacloprid, Levo, 2.0-4.0 and Sulphur, 4.3-11.0. Karate recorded the highest resistance ratio ranging from 12.0-21.6 (Table 1).

Table 1: Response of Four *T. urticae* Populations to Five Miticides by spray application.

Insecticide/ population	N	LD_{50}	95%CL	Slope±SE	RF
Protect					
Susceptible	150	0.001	0.000-0.004	0.749-0.177	-
Opeibea	207	0.007	0.002-0.017	0.859±0.159	7.0
Sinna's garden	258	0.007	0.002-0.018	0.772±0.123	7.0
Ashaiman	192	0.011	0.002-0.036	0.587-0.105	11.0
School farm	186	0.008	0.002-0.024	0.567-0.106	8.0
Imidacloprid					
Susceptible	150	0.008	0.002-0.023	0.766-0.153	-
Opeibea	261	0.072	0.011-0.219	0.700-0.146	9.0
Sinna's garden	309	0.022	0.006-0.056	0.660-0.096	2.8
Ashaiman	192	0.014	0.004-0.013	0.857-0.129	1.8
School farm	252	0.055	0.016-0.123	0.754-0.120	6.9
Karate					
Susceptible	150	0.007	0.001-0.023	0.646-0.143	-
Opeibea	273	0.151	0.038-0.375	0.793-0.137	21.6
Sinna's garden	222	0.094	0.025-0.242	0.680-0.118	13.4
Ashaiman	225	0.139	0.036-0.333	0.761-0.131	19.9
School farm	216	0.084	0.034-0.175	0.790-0.119	12.0
Levo					
Susceptible	150	0.003	0.001-0.009	0.748-0.147	-
Opeibea	150	0.012	0.003-0.028	1.115-0.262	4.0
Sinna's garden	183	0.007	0.002-0.021	0.648-0.127	2.3
Ashaiman	282	0.006	0.002-0.013	0.762-0.118	2.0
School farm	213	0.009	0.001-0.005	0.658-0.105	3.0
Sulphur					
Susceptible	150	0.003	0.000-0.011	0.633-0.141	-
Opeibea	150	0.033	0.799-0.123	0.009-0.176	11.0
Sinna's garden	228	0.017	0.002-0.020	0.584-0.097	5.7
Ashaiman	297	0.013	0.005-0.025	0.911-0.128	4.3
School farm	249	0.02	0.006-0.047	0.644-0.108	6.7

N, total number of mites tested; RF, resistance factor. LD_{50} field population/LD_{50} susceptible strain.

The optimal PBO and DEM concentrations were 0.4 and 1.0 µl/ml for the karate resistant population, respectively. Time series experiment showed significant differences (<0.005) between mortalities in mites exposed immediately to karate after pre-treatment with PBO and DEM and those with the insecticide 1 or 2 h later (Table 2 and 3).

Table 2: Optimal concentration of PBO and DEM on Karate resistant population.

Synergist concentration (µl/ml)	**Mean + SE %mortality**	
PBO	**Opeibea**	**Ashaiman**
0.4	0	0
2.0	29.44 ± 2.42	25.00 ± 4.81
10	91.16 ± 5.25	86.11 ± 7.35
DEM		
1.0	0	0
5.0	21.03 ± 2.41	22.09 ± 2.0
25	92.31 ± 7.69	70.79 ± 7.70

Table 3: Time-series experiments using karate resistant population

Population	Insecticide/synergist concentration	Time (h)	Mean ± SE % mortality
Opeibea area	0.151 ml/L + 0.4 µl/ml	0	48.9±3.22a[a]
		1	94.8±2.60[b]
		2	83.2±4.10[bc]
		3	79.5±3.61[c]
Opeibea area	0.151 ml/L + 1.0 µl/ml	0	48.7±1.28[a]
		1	88.2±6.05[b]
		2	83.5±3.40[bc]
		3	76.7±3.00[c]
Ashaiman	0.139 ml/L + 0.4 µl/ml	0	50.0±1.92[a]
		1	94.7±2.68[bc]
		2	81.4±9.44[c]
		3	68.5±5.69[ac]
Ashaiman	0.139 ml/L + 1.0 µl/ml	0	48.2±4.30[a]
		1	85.05±2.52[bc]
		2	63.34±3.49[a]
		3	62.74±2.01[a]

Means followed by the same letter do not differ significantly ($P<0.005$) from one another.

4.3 Determination of the effect of PBO and DEM on Karate resistant populations

The optimal PBO and DEM concentrations determined from the above experiment were 0.4 and 1.0 µl/ml respectively. Time lapse between pre-treatment with synergist and application of miticide was 1 h. Both PBO and DEM synergised effectively with karate insecticide. The synergist ratios for PBO and DEM were 4.3 and 2.9 respectively for the Opeibea population and 2.2 and 2.6 for Ashaiman population (Table 4).

Table 4: **Response of two *T. urticae* Karate resistant populations pre-treated with Synergist**

Population	N	LD_{50}	95% CL	Slope±SE	RF
Opeibea	273	0.151	0.038-0.375	0.793±0.137	21.6
Opeibea + PBO	150	0.035	0.007-0.105	0.748±0.127	4.3¶
Opeibea+ DEM	150	0.053	0.018-0.107	0.601±0.107	2.9¶
Ashaiman	225	0.139	0.036-0.333	0.761±0.131	19.9
Ashaiman+PBO	150	0.062	0.012-0.121	0.436±0.238	2.2¶
Ashaiman+DEM	150	0.053	0.007-0.209	0.704±0.089	2.6¶

N= total number of larvae tested, RF, resistance factor. LD_{50} field population/LD_{50} susceptible strain population. ¶Synergist ratio. LD_{50} of field population without synergist/LD_{50} of field population with synergist. PBO, piperonyl butoxide; DEM, diethyl maleate.

CHAPTER FIVE

5.0 DISCUSSION

Pest and disease are important constraints to crop production. The present study revealed that intensive small scale irrigated farming was common on small pieces of land with 2 to 6 cropping seasons per year. Pest and disease infestation varied with season. According to the survey, most farmers sprayed in the dry season than the rainy season because they claimed occurrence of pest and disease were more prevalent during the dry season than rainy season and most farmers grew vegetables during the dry season than the rainy season. For example mites' population were reported to be most abundant during warm and dry weather which favoured their multiplication and spread (Jeppson *et al*., 1975). The damage caused by this pest had led to increase in use of chemicals (Sanderson and Zang, 1995).

The practice of monoculture of different crops on small portions of the farmland was very common among the farms surveyed. Crops such as cowpea, soya bean, pepper, garden egg and okra were cultivated during a single cropping season. This practice create conducive environment by enhancing pest infestation and reducing the natural enemies of mites (Gulan and Cranston, 1994).

The present study revealed the use of chemicals such as herbicide, insecticide and fungicides. These chemicals were used extensively from inception of crop cultivation suggesting an erroneous symphony of crop cultivation and insecticides. Dinham, (2003) estimated that 87 % of Ghanaian farmers relied heavily on the use of pesticide to control pest and diseases. This practice is the same in many African countries such as Botswana where about 98% of vegetable farmers rely on the use of chemicals to control pest (Obopile *et al*., 2008).

Most farmers did not have specific insecticide for spider mite control; they expected the insecticides they used to address the pest complex affecting their crops. Insecticides such as lamda cyhalothrin, imidacloprid, ememactin benzoate were used to control pest on tomato, garden egg, pepper and cabbage while fungicides like maneb and mancozeb are used to control pest on cabbage. Farmers also used herbicides such as glyphosate to control growth of unwanted weeds in the farms. The choice of these pesticides was guided by effectiveness in controlling pest. The range of chemicals listed reflected the seriousness of pest problems and difficulties in control which prompted farmers to try different formulations; it could also be the indication that farmers were not using appropriate pesticides (Ntow, 2007).

None of the farmers applied pesticide below recommended rate, which was laudable as sub lethal dose have been found to cause pest resurgence and development of resistance. Application of insecticides commenced 2 weeks after transplanting of seedlings subsequently followed by weekly or fortnightly application. Weekly application of insecticide was a common practice amongst the farmers interviewed, the frequency of application coupled with insecticide dosage may exert selection pressure on the insect population thereby selecting for more resistant individuals. Decision to pesticide spray by farmers was guided by the presence of pest, this was borne out of farmers awareness of the threat that pest posed in limiting the yield of their crops as well as the farmers intolerance to the presence of pests and damage level rather than being informed on the basis of economic threshold.

General information on crop protection and agricultural practices mainly came from experience through years of cultivation, Agricultural Extension Officers and/or pesticide labels. To a more limited extent, information was also obtained from pesticide dealers or mass media.

Farmers interviewed used mixtures (cocktail) of two or more insecticides and this usually resulted in the efficacy of one insecticide masking the inefficacy of others in the mixture. Mixing of pesticides was encouraged by the farmers' desire to have rapid knockdown of pests. Studies conducted by other authors (Wintuma, 2009; Kingsley, 2010) revealed similar findings among farmers in Accra. Though farmers claim the combination was effective in controlling pest but combination of two insecticides with the same or different mode of action or mixing of two unrelated insecticides are dangerous (Obeng-Ofori *et al*., 2002) and results to cross or multiple resistance (IRAC, 2011). Medina, (1987), stated that the idea of mixing chemicals by farmers was questionable because the combinations used were indiscriminate. The practice of using indiscriminate combinations of pesticides, particularly of insecticides, may have contributed to the increase in incidences of insect pest infestation of tomato in Ghana (Biney, 2001) thereby defying some of the basic principles of insecticide management. For instance, Metcalf (1980), in his recommendation of strategies for pesticide management, stated that the use of mixtures of insecticides must be avoided, since mixtures of insecticides generally result in the simultaneous development of resistance.

Most farmers do not take necessary precaution before, during and after spraying. Rubber boots, long sleeves shirts, gloves and a piece of cloth to cover the mouth serves as protective cover for most of the farmers during spraying. Most of the farmers surveyed had become ill at one point from exposure to pesticide. The most frequent symptoms reported were weakness, headache and/or dizziness (Ntow *et al*., 2006).

With regard to pesticide application procedures, the knapsack was the most popular spraying equipment used. The commonest way of disposing pesticide remnants and sprayer wash water among the farmers interviewed was by throwing them on the field. Empty pesticide containers were thrown away, burnt or put to other use. Pesticide containers were seen lying

about at the edges of farms. Majority of respondents interviewed indicated they were unaware of the effects of pesticides to the environment. It was envisaged that poor disposal of pesticide remnants and containers together with indiscriminate use of pesticides in farms presented a potential pollution problem to the environment and public health. In addition, putting the pesticide empty containers into other uses as was reported by the respondents could also be dangerous to human health.

The level of resistance observed in all field populations to Karate showed the extent to which this product had been used to control pest in these areas. The commercial farms in this study (Opeibea area and Ashaiman) recorded the highest LD_{50} values of 0.151 and 0.139 ml/L corresponding to a 21.6-fold and 19.9-fold resistance, respectively. Yang *et al*. (2002) reported 9.5 and 51.8-fold resistance to bifenthrin and lambda cyhalothrin respectively in Kansas populations of *T.urticae*. The authors stated that there was a risk of cross resistance between bifenthrin and lambda cyhalothrin and also between bifenthrin and dimethoate as selection with dimethoate led to 15.9-fold cross resistance to bifenthrin. Selection with lambda cyhalothrin also led to a 2.8-fold cross resistance to bifenthrin. Wintuma (2009) and Kingsley (2010) reported an LD_{50} value of 0.323 ml/L and 1.56 µg ai/larva in Opeibea and Ashaiman population of whiteflies and DBM respectively. High LD_{50} values were also recorded in the University of Ghana farm and Sinna's garden population of spider mite. LD_{50} values were 0.094 and 0.084 ml/L, respectively. Though the susceptibility level of mite pest to this insecticide appeared to be low, some farmers claimed the product was effective in the control of pest. This was significant considering that these fields had continually been exposed to this product for the past 6 years. Therefore, there was a need to discourage the use of synthetic pyrethroids in pest control in these areas so as not to aggravate the resistance problem (Hama 1991). It should be noted that *T. urticae* develops resistance to chemical

groups after two to four years of use which makes its control very difficult (Cranham and Helle, 1985).

Presence of abamectin and emamectin benzoate reservoirs in parenchyma tissue accounts for their long residual activity on certain crops under field conditions, and their ability to control several Dipteran and Lepidopteran leafminers (Jansson & Dybas 1996). The result of this studies shows that all the population were susceptible to Emamectin benzoate. The LD_{50} values ranged from 0.007-0.011 ml/L and resistance ratios ranged from 7.0-11.0 fold. Dybas, (1989) reported about 15-fold resistance ratios to Emamectin benzoate in populations of the spider mites. Compos *et al*., (1995) first reported a decreased activity of abamectin in *T. urticae* while monitoring ornamental nurseries in California. Resistance ratios at the LC_{95} level ranged from 1-658 and it was documented that increased resistance ratios were correlated with the number of abamectin applications per year along with the total no of application of abamectin. Beers *et al*., (1998) reported a decrease *T. urticae* susceptibility to abamectin from population collected from pear orchards in Washington. Vassilious and Kitsin (2013) reported LC_{50} values to abamectin in greenhouse from Cyprus; their highest LC_{50} value record to abamectin indicated a 1356-fold increase over the susceptible population. *Tetranychus urticae* have been found to develop cross-resistance to other chemical groups (Thwaite, 1991; Al-Jboory *et al*., 2004). Sato *et al*., (2005) reported that *T. urticae* developed 342-fold resistance to abamectin after 5 selections and that in the resistant population the cross resistance was determined against milbemectin 16.3-fold and chlorfenapyr 2.3-fold. In the Pacific Northwest, Piraneo (2013) reported 86.29-fold resistance in 2012 and 107.64-fold resistance in 2013 after 12 selections with abamectin. However, Fahnbulleh (2007) reported toxicity to abamectin in Norwegian populations of *T. urticae*, resistance ratios ranged from 1.10 to 1.31- fold. Sokeli *et al*., (2007) reported high toxicity of abamectin to *T.urticae* in Isparta province resistance ratios ranged from <1 to 1.6-fold.

Except from Opeibea area, *Tetranychus urticae* populations from Ashaiman, School farm and Sinna's garden were susceptible to imidacloprid. The LD_{50} values were 0.014, 0.022 and 0.055 ml/L respectively whilst that of Opeibea was 0.072 ml/L. Owusu-Boateng and Amuzu (2013) reported that farmers in Opeibea area used mixtures of insecticide so that they could have a rapid knock down effect against pest. This practice could be the reason for insecticide resistance in this area.

Elbert *et al*., (1991) reported that at normal field rates Imidacloprid was not toxic to phytophagous mites. James and Price (2002) reported an increase in egg production of female *T.urticae* by 26% after 12 days of adult life by spray application with Imidacloprid.

Oxymatrine and oxymatrine based products have shown considerable toxic and anti-feedant activity against several pest species in laboratory bioassays (Mao and Henderson, 2007; Asghari-Tabali *et al*., 2009). Spider mite populations from all the four farms were highly susceptible to levo. The LD_{50} values ranged from 0.006 to 0.012. Wang *et al*., (2009) found the combination of oxymatrine/prosuler to have an LC_{50} of 150µg/L for carmine spider mites *T. cinnabarinus*. Marcic and Medo (2014) reported the LC_{50} value to be 14.8 µl/L after 96 h. The authors also stated that 24 h exposure to prosular oxymatrine at the concentration of 34.3 µl/L caused a corrected mortality of 60% and reduced net fertility of the surviving females by 90%. The use of this insecticide was not widespread thus the high susceptibility levels. Therefore there is a need to monitor its use to avoid resistance development in the future.

Sulphur is one of the oldest known insecticides used for the control or prevention of black spot, rust, leaf rust and powdery mildew on roses, other ornamentals, fruits and vegetables. It is also less frequently used as miticide. In the present study low LD_{50} values were recorded for all the populations this may probably be due to the fact that Sulphur® was less frequently

recommended for spider mite control. The LD_{50} values ranged from 0.013-0.02 ml/L and resistance ratios ranged from 4.3-11-fold. Kovach and Gorsuch (1986) recorded 60% mortality in South Carolina population of spider mites 48 h after treatment. The authors also reported low toxicity of sulphur to predatory mites since some phytoseid mites have developed resistance to sulphur (Jeppson *et al*., 1975), the use of sulphur in pest management system may help keep mites below economic injury level.

Synergism of karate by PBO and DEM resulted in increase in toxicity of karate in Opeibea and Ashaiman populations respectively and required 1 hour pre-treatment period. Resistance ratios fell from 21.6 to between 2.9 and 4.3 for DEM and PBO respectively in the Opeibea area population whilst that of Ashaiman fell from 19-fold to about 2-fold following the application of the synergist. Pasay *et al*., (2009) have shown in a laboratory experiment that application of PBO/permethrin drastically reduced the median survival time of permethrin resistant *Sarcoptes scabiei* variety *Canis* from 15 to 4 hours. Gunning *et al*., (1991) reported that PBO was likely to facilitate pyrethroid penetration through the cuticle. Eziah *et al*., (2008) also showed that a 30-fold and 1.9-fold synergist ratio using PBO and DEM in Esfenveralate-resistant populations of DBM with a 300 and 490-fold resistance respectively. These results were consistent with those obtained in the current study and suggest the involvement of glutathione-S- tranferases and cytochrome P450 monoxygenases in the observed resistance to Karate® in these locations.

The development of resistance is a major constraint to effective control of pest with pesticides. Early monitoring of resistance should be encouraged as it will provide pest control practitioners the option to develop a long term strategy to arrest further development and institute a sound integrated approach to solving pest problems (Obeng-Ofori *et al*., 2002).

CHAPTER SIX

6.0 Conclusion

The present study revealed that farmers in Accra relied heavily on the use of chemicals to control pest, disease and weeds on their farms. Farmers used different types of insecticide with different modes of actions. Use of insecticide mixtures, spraying on weekly basis were improper pest management practices and should be discouraged. There was also a need to sensitize farmers on the threats posed by spider mites since most farmers were unaware of them. The amount of pesticide used in crop cultivation could be reduced if the correct information about suitable insecticides and control method to use against crop pest was provided to the farmer. The results of this study revealed that all field populations of spider mites were susceptible to Levo® and Protect® miticide but resistant to karate. Resistance to karate had been reported in other insects at Opeibea area, Ashaiman and University of Ghana farm but not Sinna's garden (Wintuma, 2009; Kingsley, 2010). These provide the basis for resistance monitoring in spider mite in the future. The study also showed that both Piperonyl butoxide, (PBO) and Diethyl maleate DEM might be helpful in the management of resistance of spider mite to Karate® insecticide in Accra.

6.1 Recommendation

It is then recommended that further studies should focus on creating awareness to farmers through extension workers about the benefits of;

1. Use of synergists

2. Targeted application of pesticides and strategies for resistance management

3. The use of cocktails of pesticides

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APPENDICES

Appendix 1.0: EPA Probit Analysis Program used for calculating LC/EC values version 1.5

Appendix 1.1: susceptibility of *T.urticae* to Protect

Site: susceptible

Chi - Square for Heterogeneity (calculated) = 1.861

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	7.211835	0.512633	(6.207075, 8.216596)
Slope	0.749151	0.177089	(0.402057, 1.096245)
Spontaneous Response Rate	0.198904	0.063805	(0.073846, 0.323963)

Estimated LC/EC Values and Confidence Limits

Point	Exposure Conc.	95% Confidence Limits Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.000
LC/EC 15.00	0.000	0.000	0.000
LC/EC 50.00	0.001	0.000	0.004
LC/EC 85.00	0.027	0.008	0.220
LC/EC 90.00	0.057	0.016	0.779
LC/EC 95.00	0.175	0.039	5.480
LC/EC 99.00	1.422	0.186	239.481

Site: Opeibea

Chi - Square for Heterogeneity (calculated) = 6.039

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.841657	0.313075	(6.228030, 7.455285)
Slope	0.859309	0.159006	(0.547658, 1.170960)
Spontaneous	0.174952	0.058531	(0.060232, 0.289673)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.001
LC/EC 10.00	0.000	0.000	0.001
LC/EC 15.00	0.000	0.000	0.002
LC/EC 50.00	0.007	0.002	0.017
LC/EC 85.00	0.116	0.050	0.363
LC/EC 90.00	0.223	0.093	0.897
LC/EC 95.00	0.590	0.213	3.672
LC/EC 99.00	3.664	0.908	57.65

Site: Sinna's garden

Chi - Square for Heterogeneity (calculated) = 4.456

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.657091	0.242052	(6.182668, 7.131514)
Slope	0.772901	0.123393	(0.531050, 1.014752)
Spontaneous	0.159486	0.062087	(0.037796, 0.281176)

Response rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.001
LC/EC 15.00	0.000	0.000	0.001
LC/EC 50.00	0.007	0.002	0.018
LC/EC 85.00	0.157	0.070	0.435
LC/EC 90.00	0.327	0.140	1.101
LC/EC 95.00	0.964	0.361	4.692
LC/EC 99.00	7.342	1.908	80.021

Site: Ashaiman

Chi - Square for Heterogeneity (calculated) = 2.295

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.161765	0.208009	(5.754066, 6.569463)
Slope	0.587969	0.105086	(0.382001, 0.793937)
Spontaneous	0.148889	0.063017	(0.025375, 0.272403)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.001
LC/EC 15.00	0.000	0.000	0.001
LC/EC 50.00	0.011	0.002	0.036
LC/EC 85.00	0.612	0.192	3.078
LC/EC 90.00	1.599	0.462	11.408
LC/EC 95.00	6.633	1.547	87.293

LC/EC 99.00 95.633 12.932 4578.905

Site: school farm

Chi - Square for Heterogeneity (calculated) = 1.575

Parameter Estimate Std. Err. 95% Confidence Limits

Intercept 6.186069 0.234657 (5.726142, 6.645997)

Slope 0.567341 0.106441 (0.358717, 0.775964)

Spontaneous 0.134056 0.048543 (0.038912, 0.229200)

Response Rate

Estimated LC/EC Values and Confidence Limits

Exposure 95% Confidence Limits

Point Conc. Lower Upper

LC/EC 1.00 0.000 0.000 0.000

LC/EC 5.00 0.000 0.000 0.000

LC/EC 10.00 0.000 0.000 0.000

LC/EC 15.00 0.000 0.000 0.001

LC/EC 50.00 0.008 0.002 0.024

LC/EC 85.00 0.545 0.155 4.809

LC/EC 90.00 1.474 0.352 21.100

LC/EC 95.00	6.438	1.131	198.678
LC/EC 99.00	102.278	9.351	14400.345

Appendix 1.2: susceptibility of *T.urticae* to imidacloprid

Site: Susceptible

Chi - Square for Heterogeneity (calculated) = 2.786

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.618414	0.320424	(5.990383, 7.246445)
Slope	0.776357	0.153197	(0.476091, 1.076622)
Spontaneous Response Rate	0.159430	0.052531	(0.056469, 0.262391)

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.001
LC/EC 15.00	0.000	0.000	0.002
LC/EC 50.00	0.008	0.002	0.023
LC/EC 85.00	0.178	0.063	0.849
LC/EC 90.00	0.368	0.122	2.443
LC/EC 95.00	1.082	0.300	12.532
LC/EC 99.00	8.162	1.453	299.797

Site: Opeibea

Chi - Square for Heterogeneity (calculated) = 3.902

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.801442	0.224949	(5.360541, 6.242342)
Slope	0.700189	0.146269	(0.413502, 0.986876)
Spontaneous	0.266044	0.069621	(0.129588, 0.402500)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.001
LC/EC 5.00	0.000	0.000	0.003
LC/EC 10.00	0.001	0.000	0.008
LC/EC 15.00	0.002	0.000	0.014
LC/EC 50.00	0.072	0.011	0.219
LC/EC 85.00	2.166	0.732	11.742
LC/EC 90.00	4.850	1.508	39.585
LC/EC 95.00	16.018	4.031	261.382
LC/EC 99.00	150.585	22.448	10229.071

Site: Sinna's garden

Chi - Square for Heterogeneity (calculated) = 3.244

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.090998	0.174063	(5.749834, 6.432161)
Slope	0.660362	0.096884	(0.470469, 0.850255)
Spontaneous	0.140044	0.060457	(0.021547, 0.258540)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.001
LC/EC 15.00	0.001	0.000	0.003
LC/EC 50.00	0.022	0.006	0.056
LC/EC 85.00	0.827	0.346	2.591
LC/EC 90.00	1.944	0.756	7.642
LC/EC 95.00	6.899	2.259	40.473
LC/EC 99.00	74.241	15.921	1020.510

Site: Ashaiman

Chi - Square for Heterogeneity (calculated) = 4.281

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.584781	0.227790	(6.138312, 7.031250)
Slope	0.857498	0.129803	(0.603083, 1.111912)
Spontaneous	0.168703	0.062725	(0.045763, 0.291644)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.001
LC/EC 10.00	0.000	0.000	0.002
LC/EC 15.00	0.001	0.000	0.003
LC/EC 50.00	0.014	0.004	0.033
LC/EC 85.00	0.229	0.107	0.568
LC/EC 90.00	0.443	0.202	1.272
LC/EC 95.00	1.175	0.486	4.491

LC/EC 99.00 7.324 2.254 53.549

Site: school farm

Chi - Square for Heterogeneity (calculated) = 1.402

Parameter Estimate Std. Err. 95% Confidence Limits

Intercept 5.952070 0.150260 (5.657560, 6.246579)

Slope 0.754126 0.120732 (0.517491, 0.990761)

Spontaneous 0.125421 0.044182 (0.038824, 0.212018)

Response Rate

Estimated LC/EC Values and Confidence Limits

Exposure 95% Confidence Limits

Point Conc. Lower Upper

LC/EC 1.00 0.000 0.000 0.000

LC/EC 5.00 0.000 0.000 0.002

LC/EC 10.00 0.001 0.000 0.005

LC/EC 15.00 0.002 0.000 0.009

LC/EC 50.00 0.055 0.016 0.123

LC/EC 85.00 1.294 0.607 3.652

LC/EC 90.00 2.735 1.194 9.783

LC/EC 95.00 8.293 3.054 44.797

LC/EC 99.00 66.417 16.256 850.250

Appendix 1.3: susceptibility of *T.urticae* to karate

Site: Susceptible

Chi - Square for Heterogeneity (calculated) = 1.976

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.404239	0.338725	(5.740338, 7.068141)
Slope	0.646367	0.143398	(0.365306, 0.927428)
Spontaneous	0.143990	0.059823	(0.026738, 0.261243)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.001
LC/EC 15.00	0.000	0.000	0.001
LC/EC 50.00	0.007	0.001	0.023
LC/EC 85.00	0.270	0.074	3.014
LC/EC 90.00	0.646	0.152	12.555
LC/EC 95.00	2.357	0.420	110.918
LC/EC 99.00	26.701	2.545	7277.590

Site: Opeibea

Chi - Square for Heterogeneity (calculated) = 6.074

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.652140	0.175470	(5.308219, 5.996061)
Slope	0.793648	0.137271	(0.524596, 1.062700)
Spontaneous	0.209912	0.061656	(0.089067, 0.330758)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.002
LC/EC 5.00	0.001	0.000	0.008
LC/EC 10.00	0.004	0.000	0.018
LC/EC 15.00	0.007	0.001	0.031
LC/EC 50.00	0.151	0.038	0.375
LC/EC 85.00	3.049	1.289	9.772
LC/EC 90.00	6.210	2.487	25.274
LC/EC 95.00	17.819	6.148	110.671
LC/EC 99.00	128.662	30.139	1966.573

Site: Sinna's garden

Chi - Square for Heterogeneity (calculated) = 1.944

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.698296	0.170873	(5.363385, 6.033206)
Slope	0.680458	0.118010	(0.449158, 0.911757)
Spontaneous	0.125569	0.056790	(0.014260, 0.236878)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.003
LC/EC 10.00	0.001	0.000	0.007
LC/EC 15.00	0.003	0.000	0.013
LC/EC 50.00	0.094	0.025	0.242
LC/EC 85.00	3.140	1.172	14.182
LC/EC 90.00	7.198	2.408	45.079
LC/EC 95.00	24.611	6.622	264.169
LC/EC 99.00	246.896	40.640	7913.326

Site: Ashaiman

Chi - Square for Heterogeneity (calculated) = 1.332

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.651617	0.150488	(5.356661, 5.946572)
Slope	0.761406	0.131055	(0.504538, 1.018275)
Spontaneous	0.125415	0.053377	(0.020797, 0.230033)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.001
LC/EC 5.00	0.001	0.000	0.006
LC/EC 10.00	0.003	0.000	0.014
LC/EC 15.00	0.006	0.000	0.026
LC/EC 50.00	0.139	0.036	0.333
LC/EC 85.00	3.202	1.403	10.205
LC/EC 90.00	6.720	2.741	27.845
LC/EC 95.00	20.162	6.920	131.631

LC/EC 99.00 158.293 35.647 2674.040

Site: school farm

Chi - Square for Heterogeneity (calculated) = 6.023

Parameter Estimate Std. Err. 95% Confidence Limits

Intercept 5.851677 0.176211 (5.506304, 6.197051)

Slope 0.790156 0.119765 (0.555416, 1.024896)

Spontaneous 0.122805 0.044258 (0.036061, 0.209550)

Response Rate

Estimated LC/EC Values and Confidence Limits

Exposure 95% Confidence Limits

Point Conc. Lower Upper

LC/EC 1.00 0.000 0.000 0.001

LC/EC 5.00 0.001 0.000 0.003

LC/EC 10.00 0.002 0.000 0.007

LC/EC 15.00 0.004 0.001 0.012

LC/EC 50.00 0.084 0.034 0.175

LC/EC 85.00 1.713 0.748 5.914

LC/EC 90.00 3.500 1.384 15.319

LC/EC 95.00 10.090 3.336 64.826

LC/EC 99.00 73.492 16.479 1022.642

Appendix 1.4: susceptibility of *T.urticae* to levo

Site: Susceptible

Chi - Square for Heterogeneity (calculated) = 5.992

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.867028	0.370392	(6.141059, 7.592998)
Slope	0.748621	0.147076	(0.460351, 1.036890)
Spontaneous Response Rate	0.125521	0.045703	(0.035943, 0.215099)

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.000
LC/EC 15.00	0.000	0.000	0.001
LC/EC 50.00	0.003	0.001	0.009
LC/EC 85.00	0.078	0.027	0.431
LC/EC 90.00	0.165	0.052	1.321
LC/EC 95.00	0.505	0.129	7.355
LC/EC 99.00	4.107	0.650	200.843

Site: Opeibea

Chi - Square for Heterogeneity (calculated) = 2.969

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	7.143714	0.474318	(6.214052, 8.073378)
Slope	1.115353	0.262105	(0.601626, 1.629079)
Spontaneous	0.211683	0.052309	(0.109157, 0.314209)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.001
LC/EC 5.00	0.000	0.000	0.002
LC/EC 10.00	0.001	0.000	0.003
LC/EC 15.00	0.001	0.000	0.005
LC/EC 50.00	0.012	0.003	0.028
LC/EC 85.00	0.102	0.043	0.424
LC/EC 90.00	0.169	0.068	0.969
LC/EC 95.00	0.357	0.127	3.499
LC/EC 99.00	1.458	0.368	42.779

Site: Sinna's garden

Chi - Square for Heterogeneity (calculated) = 2.373

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.380169	0.282147	(5.827161, 6.933177)
Slope	0.648092	0.127173	(0.398832, 0.897351)
Spontaneous	0.128032	0.057445	(0.015440, 0.240624)

Response Rate

Estimated LC/EC Values and Confidence Limits

Point	Exposure Conc.	95% Confidence Limits Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.001
LC/EC 15.00	0.000	0.000	0.001
LC/EC 50.00	0.007	0.002	0.021
LC/EC 85.00	0.295	0.096	1.957
LC/EC 90.00	0.705	0.200	7.233
LC/EC 95.00	2.561	0.563	53.286
LC/EC 99.00	28.832	3.568	2466.939

Site: Ashaiman

Chi - Square for Heterogeneity (calculated) = 3.578

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.714321	0.281170	(6.163228, 7.265414)
Slope	0.762458	0.118133	(0.530917, 0.993999)
Spontaneous	0.120696	0.051213	(0.020319, 0.221073)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.000
LC/EC 15.00	0.000	0.000	0.001
LC/EC 50.00	0.006	0.002	0.013
LC/EC 85.00	0.129	0.057	0.405
LC/EC 90.00	0.271	0.110	1.066
LC/EC 95.00	0.811	0.280	4.706

LC/EC 99.00 6.348 1.484 82.627

Site: school farm

Chi - Square for Heterogeneity (calculated) = 3.964

Parameter Estimate Std. Err. 95% Confidence Limits

Intercept 6.748228 0.264652 (6.229510, 7.266946)

Slope 0.658302 0.105055 (0.452394, 0.864210)

Spontaneous 0.117230 0.045666 (0.027725, 0.206736)

Response Rate

Estimated LC/EC Values and Confidence Limits

Exposure 95% Confidence Limits

Point Conc. Lower Upper

LC/EC 1.00 0.000 0.000 0.000

LC/EC 5.00 0.000 0.000 0.000

LC/EC 10.00 0.000 0.000 0.000

LC/EC 15.00 0.000 0.000 0.000

LC/EC 50.00 0.002 0.001 0.005

LC/EC 85.00 0.083 0.032 0.338

LC/EC 90.00 0.196 0.068 1.071

LC/EC 95.00 0.697 0.195 6.217

LC/EC 99.00 7.553 1.307 182.316

Appendix 1.5: susceptibility of *T.urticae* to sulphur

Site: Susceptible

Chi - Square for Heterogeneity (calculated) = 0.022

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.586844	0.369914	(5.861812, 7.311876)
Slope	0.633816	0.141558	(0.356363, 0.911269)
Spontaneous Response Rate	0.163533	0.063803	(0.038479, 0.288587)

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.000
LC/EC 15.00	0.000	0.000	0.000
LC/EC 50.00	0.003	0.000	0.011
LC/EC 85.00	0.135	0.036	1.509
LC/EC 90.00	0.330	0.076	6.444
LC/EC 95.00	1.235	0.216	59.546
LC/EC 99.00	14.678	1.366	4303.211

Site: Opeibea

Chi - Square for Heterogeneity (calculated) = 3.374

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.986101	0.259809	(5.476875, 6.495327)
Slope	0.799625	0.181846	(0.443208, 1.156043)
Spontaneous	0.199044	0.063504	(0.074577, 0.323511)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.001
LC/EC 5.00	0.001	0.000	0.004
LC/EC 10.00	0.001	0.000	0.009
LC/EC 15.00	0.003	0.000	0.016
LC/EC 50.00	0.058	0.009	0.176
LC/EC 85.00	1.156	0.386	7.103
LC/EC 90.00	2.342	0.726	21.988
LC/EC 95.00	6.666	1.709	127.103
LC/EC 99.00	47.428	7.551	3852.981

Site: Sinna's garden

Chi - Square for Heterogeneity (calculated) = 2.583

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.032766	0.184980	(5.670206, 6.395327)
Slope	0.584879	0.097980	(0.392839, 0.776919)
Spontaneous	0.128849	0.060056	(0.011139, 0.246559)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.000
LC/EC 10.00	0.000	0.000	0.001
LC/EC 15.00	0.000	0.000	0.002
LC/EC 50.00	0.017	0.004	0.050
LC/EC 85.00	1.014	0.342	5.101
LC/EC 90.00	2.663	0.796	19.076
LC/EC 95.00	11.133	2.607	143.737
LC/EC 99.00	162.790	21.872	7004.759

Site: Ashaiman

Chi - Square for Heterogeneity (calculated) = 3.174

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.729144	0.238588	(6.261510, 7.196777)
Slope	0.911365	0.128806	(0.658905, 1.163825)
Spontaneous	0.113277	0.052972	(0.009452, 0.217103)

Response Rate

Estimated LC/EC Values and Confidence Limits

	Exposure	95% Confidence Limits	
Point	Conc.	Lower	Upper
LC/EC 1.00	0.000	0.000	0.000
LC/EC 5.00	0.000	0.000	0.001
LC/EC 10.00	0.000	0.000	0.002
LC/EC 15.00	0.001	0.000	0.003
LC/EC 50.00	0.013	0.005	0.025
LC/EC 85.00	0.174	0.091	0.389
LC/EC 90.00	0.323	0.162	0.833
LC/EC 95.00	0.808	0.365	2.707

LC/EC 99.00 4.521 1.537 26.775

Site: school farm

Chi - Square for Heterogeneity (calculated) = 4.825

Parameter Estimate Std. Err. 95% Confidence Limits

Intercept 6.100797 0.184864 (5.738464, 6.463130)

Slope 0.644929 0.108194 (0.432869, 0.856989)

Spontaneous 0.121176 0.046339 (0.030351, 0.212001)

Response Rate

Estimated LC/EC Values and Confidence Limits

Exposure 95% Confidence Limits

Point Conc. Lower Upper

LC/EC 1.00 0.000 0.000 0.000

LC/EC 5.00 0.000 0.000 0.000

LC/EC 10.00 0.000 0.000 0.001

LC/EC 15.00 0.000 0.000 0.002

LC/EC 50.00 0.020 0.006 0.047

LC/EC 85.00 0.795 0.307 3.532

LC/EC 90.00 1.907 0.646 11.942

LC/EC 95.00 6.977 1.856 76.182

LC/EC 99.00 79.472 12.518 2643.617