EFFICACY OF AQUEOUS NEEM SEED EXTRACT IN THE CONTROL OF TWO MORPHS OF GREEN PEACH APHID (*MYZUS PERSICAE* SULZ.) AND AGRONOMIC PERFORMANCE OF PEPPER (*CAPSICUM ANNUM* L.)

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DEPARTMENT OF BIOLOGICAL SCIENCES,
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OCTOBER, 2015.

DECLARATION

I declare that the work in this thesis entitled **"**EFFICACY OF AQUEOUS NEEM SEED EXTRACT IN THE CONTROL OF TWO MORPHS OF GREEN PEACH APHID (*MYZUS PERSICAE* SULZ.) AND AGRONOMIC PERFORMANCE OF PEPPER (*CAPSICUM ANNUM* L.)" has been carried out by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Fatima LAWAL	________________	____________
Name of student	Signature	Date

CERTIFICATION

This thesis entitled "EFFICACY OF AQUEOUS NEEM SEED EXTRACT IN THE CONTROL OF TWO MORPHS OF GREEN PEACH APHID (*MYZUS PERSICAE* SULZ.) AND AGRONOMIC PERFORMANCE OF PEPPER (*CAPSICUM ANNUM* L.)" by Fatima LAWAL meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

A field cage experiment was conducted to determine the aphicidal efficacy of aqueous neem (*Azadirachta indica* Juss) seed extracts in comparison with Neemazal and Deltrin against lemon and dark green morphs of the Green peach aphid (*Myzus persicae* Sulz.) and its effect on agronomic performance of four selected pepper varieties. Field experiment was conducted using a Randomized complete block design with three replications. The pre-infested pepper varieties were sprayed with three concentrations of aqueous neem seed extract (ANSE) at 0.2g/ml (100%, 75% and 50%). Aphid morphs mortality was assessed at 24hr, 48hr, 120hr and 168hr after treatment application. The present study indicates a high percentage mortality of aphid morphs in the treated plots as compared to tap water treated plots. At 168hr after treatment application 100% ANSE (94.69%), 75% ANSE (96.52%) and 50% ANSE (96.34%) showed better aphicidal efficacy than Deltrin (85.90%) when used as aphicide on lemon green morphs (LG) infested pepper varieties. ANSE concentrations resulted in higher percentage mortality than Neemazal for both morph infestation. Pepper plants infested with dark green morphs (DG) of *M. persicae* had optimum growth and fruit production at 50% ANSE concentration followed by Neemazal, 75% ANSE, Deltrin, 100% ANSE and then tap water. 50% ANSE resulted in a 66.70% increase in fruit yield over tap water treated plots and 11.12% increase over deltrin, while for LG morph infestation, yield of pepper treated with 50% ANSE increased by 33.34% over tap water treated plots. The improved growth and yield achieved at 50% and 75% ANSE concentration as compared to 100% ANSE could be attributed with low toxicity of ANSE constituents at these concentrations.

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LIST OF ABBREVIATION

%:	Percentage
v/v:	Volume per volume
NSE:	Neem seed extract
ANSE:	Aqueous neem seed Extract
LG:	Lemon green morphs of *Myzus persicae*
DG:	Dark green morphs of *Myzus persicae*
NIHORT:	National Horticultural Research Institute

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Agricultural activities have been faced with the destructive activities of numerous pests, insects, fungi, and weeds which lead to radical decrease in yields (Wondafrash, 2007). This crisis was reduced to a great extent with the advent of chemical pesticides. Over dependence and uninhibited use of chemical protection has necessitated for alternatives due to environmental concerns (Phoofolo *et al.,* 2013). Efforts have been devoted to the discovery and development of plant extracts and phytochemicals as alternatives to synthetic insecticides for pest management. The use of more biodegradable pest control materials with greater selectivity might help to avoid the disadvantage caused by the use of synthetic pesticides (Wondafrash, 2007).

Pervin *et al.* (2012) stated that plants have the richest source of renewable natural pesticides and their extracts provide a safe and viable alternative to synthetic pesticides. Botanicals are also compatible with the use of beneficial organisms, pest-resistant plants, and to preserving a healthy environment in an effort to decrease reliance on synthetic pesticides. Among the wider range of plants, Neem derivatives have shown great potential in controlling insect pests (Mahmoud and Maha, 2008).

Neem, (*Azadirachta indica* A. Juss) (Family: Meliaceae) is native to the arid regions of the Indian sub continent, from where it spread out to many Asian and African countries as well as Australia and South America (Mahmoud and Maha, 2008). Various Neem products have been studied extensively for their phytochemistry and exploitation in pest control programs and a number of bioactive components have been isolated from various parts of the Neem tree. Azadirachtin is currently considered as Neem's main agent for controlling insects (Dubhashi and Pranay, 2013). Himesh *et al.* (2011) described azadirachtins as a complex tetranor-triterpenoid of the limonoid class which are a family of natural phago-repellents and anti-feedants found in the Neem tree: It can be isolated in small amounts from all parts of the Neem tree, but it is present at highest concentration in the mature seeds (Schaaf *et al.,* 2000). Azadirachtin Structurally resembles insect hormones called "ecdysones," which control the process of metamorphosis as the insects pass from larva through pupa to adult. They exert a strong negative influence on behavior, postembryonic development and fecundity of insects resulting in significant reduction of general fitness (Hummel *et al.*, 2012). The insecticidal activity of azadirachtin has been demonstrated against numerous insect pests and its various modes of activity include disruption of feeding, reproduction, or development (Weathersbee and McKenzie, 2005). More than 450 species of insects have been tested with Neem products in the world and 413 of these are susceptible to Neem products used at different concentrations (Wondafrash, 2007).

When insects ingest this compound, their growth and development are inhibited due to the blocking of the biosynthesis of insect hormones, such as ecdysteroids. In addition to blockage of hormone synthesis, the development of reproductive organs such as the ovary and testis, is significantly inhibited, and the fertility and fecundity of the adults are also reduced (Ohn Mar Lynn *et al.*, 2010).

Pepper (*Capsicum* sp.) belonging to the family Solanaceae is one of the most varied and widely used vegetable in the world. Nigeria is known to be one of the major producers of pepper in the world accounting for about 50% of the African production (Idowu *et al.*, 2010). Although, pepper is widely cultivated throughout Nigeria, yields obtained by peasant farmers are often very low (Adigun, 2001). Pepper production in Nigeria has been faced with many biotic and environmental constraints, prominent among such constraints are pests and diseases which reduce yields, quality of marketable fruits and these subsequently results in considerable economic losses and wastage. In the tropics, particularly in Nigeria, many insect pests are associated directly with vegetable damage and yield losses while some others are most important as vectors of diseases (Umeh *et al*., 2002).

A survey report by Dagnoko *et al*, (2013) showed that the most economically important pests to peppers in West Africa are: Thrips (*Frankliniella* sp), Aphids (*Myzus persicae* Sulzer, *Aphis* sp, and *Macrosiphum euphorbiae* Thomas), Whitefly (*Bemisia tabaci* Gennadius), Root knot nematodes (*Meloidogyne* sp), the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann), Red spider mites (*Tetranychus* sp Koch), and Fruit borers (*Lepidopterae* sp). In addition to damages caused to the plants by direct feeding, some pests such as whiteflies, aphids and thrips are vectors of viruses.

Myzus persicae known as the green peach aphid is an important pest of the Solanaceae family; it is a cosmopolitan and phytophagous species of aphid and has been reported to be highly efficient as a virus vector (Peri *et al*., 2009). Pepper belonging to the Solanaceae family is often attacked by the green peach aphid. It is a direct pest of plants and high populations of *Myzus* can result in chlorosis, necrosis, wilting, stunting, flower and fruit abortion, leaf distortion and defoliation in pepper plant (James *et al*., 2004).

La Rossa *et al*. (2013) reported that aphid populations can grow to extremely high levels under favorable environmental conditions in a short time, covering sprouts, leaves, flowers, and fruits. This insect damages plants by leaf sucking, molding which results from honey dew production, and plant virus transmission. Like the whiteflies the green peach aphids excrete sugary, sticky liquid called honeydew that accumulates on leaves and branches and enhances the growth of certain fungus on them which turns it black thereby affecting photosynthesis, plant growth and yield (Shannag *et al*., 2014). This pest have been reported to cause great damages to most horticultural crops, including tomato, cucumber, sweet melon, cabbage, paprika, and pepper crops. Aphids suck sap from plant (phloem) using mouthparts modified for piercing and sucking. Some aphids feed on foliage while others feed on twigs, limbs, branches, fruits, flowers or root of plants (Webb, 2010). Some species inject toxic salivary secretions into plants during feeding. If left unchecked aphids can stunt plant growth, deform and discolor leaves and fruits or cause gall formation on leaves, stems and roots. In case of heavy attack plants wither resulting in drastic loss in seed yield and oil contents (Akbar *et al.*, 2012).

1.2 Statement of Research Problem

Diseases and insect pests are the major limiting factors in the production of high quality agricultural products (Pervin *et al.*, 2012). Insect pest cause about 35-40% yield loss in vegetables and this may reach up to 60-70% in most favorable condition (Umeh *et al*., 2002). Vegetable farmers use a wide range of pesticide at different levels to reduce damages caused by pest and diseases, as a result of which vegetable cultivation has become more expensive due to the increasing use of purchased inputs such as pesticides and fertilizers to sustain production levels.

Myzus persicae is a polyphagus and economically important pest with a worldwide distribution. It is a serious pest of the solanaceae family and vector of multitude of plant viral diseases. Efficient control of *Myzus persicae* requires repeated pesticide applications which are often unaffordable to peasant farmers and their use is associated with many undesirable and sometimes lethal consequences. . In addition, chemical control of aphid has become less effective due to the evolution of insecticide resistance in natural populations (Michereff *et al*., 2011). The greatest concern with use of chemical insecticides in vegetable production is their potential poisonous effects on human health through dietary exposure (Phoofolo *et al*., 2103).

1.3 Justification

Myzus persicae is resistant to most insecticide classes, making chemical control of this species particularly problematic (Radcliffe and Ragsdale, 2002). To combat problem of pest resistance and resurgence, effective chemical control of sucking pests requires an increased number of applications and application doses. As such botanical pesticides are important alternatives to minimize or replace the use of synthetic pesticides (Anand *et al.*, 2008). Extracts from various part of Neem are among the most used botanical pesticide and serve as a viable alternative for the management of many sucking insects.

Azadirachta indica is known to be active on more than 200 economically important species of insects and the fact that azadirachtin is selective toward phytophagous insects with minimal toxicity to beneficial insects increases its potential value to pest management (Weathersbee and McKenzie, 2005). Furthermore, there is a dearth of documented information on the effect of aqueous Neem seed extract in control of colour morphs of *M. persica*e on commonly cultivated varieties of pepper in Nigeria.

Pepper, an important source of vitamin and minerals is one of the most important vegetable crop grown in Nigeria, however, it production is low when compared with those from Western Europe (Idowu *et al*., 2010). It is therefore pertinent to screen available alternatives to chemical pesticidal protection that can be affordable and accessible to small scale farmers while ensuring safety to man and his environment.

Comment [ARE1]: Input production yield

1.4 Aim and Objectives

The broad aim of this research is to develop a cheap, available and environmentally safe Biopesticide which would increase pepper production by controlling colour morphs of *Myzus persicae* associated with pepper.

The Objectives of the Study were to:

1. Determine the effect of Lemon and Dark green morphs of *Myzus persicae* on growth and yield parameters of pepper varieties

2. Determine the effect of aqueous Neem seed extract on growth and yield parameters of pepper varieties

3. Determine the aphicidal efficacy of aqueous Neem seed extract on the population of Dark green and Lemon green morphs of *Myzus persicae.*

1.5 Hypotheses

1. The growth and yield parameters of pepper are not significantly affected by the activities of dark and lemon green morphs of *M. persicae*.
2. Aqueous Neem seed extract does not have significant effect on growth and yield parameters of pepper varieties.
3. Aqueous Neem seed extract does not have significant effect on population of *M. persicae* morphs found growing on pepper.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pepper (*Capsicum annum*)

Pepper (*Capsicum annum*) are vegetable crops belonging to the genus *Capsicum* of the night shade family Solanaceae. Capsicum species are diploid, most having 12 pairs of chromosomes (2n=24). It is one of the first plants to be domesticated and cultivated in the western hemisphere (Wang and Bosland, 2006) and it is believed to have originated from central and South America (Dagnoko *et al.*, 2013). The genus contains about 25 wild and 5 domesticated species (Sanatombi and Sharma, 2007) for consumption, these includes *Capsicum annuum* L. (hot and sweet peppers), *Capsicum frutescens* L. (bird- pepper) and *Capsicum chinense* Jacq which are the most commonly cultivated species in tropical and temperate zones of the world. The other domesticated species are *Capsicum baccatum* L., and *Capsicum pubescens* Ruiz and Pavon (Dagnoko *et al.*, 2013). *Capsicum* species can be further divided in to several groups based on size, shape, color, flavor and degree of hotness. Despite their vast trait differences most cultivars of peppers commercially cultivated in the world belongs to the species *C. annum* which includes both mild and pungent fruit types (Seleshi, 2011).

Commercially peppers can be classified by the concentration of capsaicin ($C_{18}H_{27}NO_3$) which determines the “hotness” of a variety. Sweet pepper also called bell pepper covers a wide variety of mild pepper which has lower capsaicin content when compared to hot pepper. They have a mild sweet flavor and crisp juicy flesh (Sanatombi and Sharma, 2007).

2.1.1 Overview of Pepper production

Pepper is one of the most cultivated vegetable and spice worldwide and it plays an important role as a constituent in many of the world food industries (Wang and Bosland, 2006). Pepper production and consumption have steadily increased worldwide during the 20th century due to their roles as both vegetable and spices**.** Just like other Solanaceae plants for example tomatoes and potatoes, peppers have become important components of diverse cuisine around the world. Food and Agricultural Organization statistics estimates world production of capsicum peppers in 2012 at 1.65 million ha with more than 24 million metric tons harvest: China was rated the world's largest producer with 10 million tons and nearly 50% of the world production, followed by Mexico and turkey. Production in tropical Africa was estimated at 1 million tons with Niger and Nigeria as the largest producers.

Comment [ARE2]: In full

Pepper is the second most utilized vegetable in Nigeria where it is produced all year round; it is grown in all parts of the country but mainly cultivated in the savanna ecological zones during the rainy and dry season under irrigation farming. Adetula and Olakojo (2006) reported that Nigeria is the largest producer of pepper in Africa covering about 50% of total Africa production; they also reported that a total of about 100 ha-200,000 ha is being assigned to pepper production annually in Nigeria. Pepper is an indispensable part in the diet of millions of Nigerians and this can be attributed to it increase popularity, value and importance over a long period (Olaniyi *et al.,* 2010). Consumption of pepper in Nigeria accounts for about 40% of average daily in-take either in soup, or as condiments for flavouring and colouring of meats, fish and other food materials. In Nigeria, Pepper fruits are consumed fresh or dried either as whole fruit or ground alone or in combination with a large number of other flavoring agents (Adetula and Olakojo, 2006). It is also used both in the raw and processed form where it is grounded with other vegetables like tomatoes and onions in stew and soup preparation.

2.1.2 Challenges of pepper production

Pepper production is low when compared to yield obtained in Western Europe. Idowu *et al.* (2010) reported that pepper production in developing countries is about 10-30% of that obtained in developed countries. This wide difference has been attributed to a number of biotic and abiotic production constraints. Among such constraints are the advent of pest and diseases which affect the quality and quantity of marketable fruits, others include poor farming practices, poor weed management and low soil fertility (Jaliya and Sani, 2004). Kurungi *et al.* (2010) stated that yield of pepper like most vegetables are seriously affected by a complex of pests and diseases.

2.1.3 Insect pests of pepper

Survey report by Dagnoko *et al*. (2013) showed that the most economically important pests to peppers in West Africa are: thrips (*Frankliniella* sp) which are usually found feeding on the leaves, flowers or fruits; aphids (*Myzus persicae*, *Aphis* sp, and *Macrosiphum euphorbiae*) feeding on young leaves and shoots; whitefly (*Bemisia tabaci*) feeding on the leaves, the Mediterranean fruit fly (*Ceratitis capitata*) feeding on the fruit flesh, red spider mites (*Tetranychus* sp) feeding on the leaves, and fruit borers (*Lepidopterae* sp). Similar studies carried out in hot pepper producing area of Uganda showed that aphids, thrips, whiteflies, mites, fruitflies and bollworm were the major pests affecting pepper yield in the areas (Karungi *et al.*, 2010). In addition to damages caused to the plants by direct feeding, some pests such as nematodes, whiteflies, aphids and thrips are vectors of viruses.

About 35 viruses are known to infect pepper worldwide (Green and Kim, 1994). Among these viruses, 11 were reported in Africa out of which Pepper Venial Mottle Virus (PVMV) and Tomato Yellow Leaf Curl Virus (TYLCV) are the most economically important and the most widespread in the Western Africa sub-region. Fajinmi *et al.* (2011a) stated that Pepper venial mottle virus is a major constrain to the cultivation of pepper in some parts of Nigeria. Pepper infected by PVMV shows varied symptom expressions and disease severity on the leaves, stem and flowers or fruits. The virus causes severe and devastating destruction of pepper plants; and it has been reported that they are efficiently transmitted in nature by aphids, which are often difficult to control. Similar studies by Fajinmi *et al.* (2011b) identified *Myzus persicae* and *Aphis gossypii* as the most probable significant PVMV vectors.

2.2 *Myzus persicae* as an Agricultural Pest

Aphids are soft bodied insects belonging family Aphididae and the order Homoptera. They are very small, rarely over 5 mm in length, with piercing-sucking mouthparts that enable them to feed on plant sap. There are thousands species of aphids within numerous genera. Because they feed on plants, many are important pests of garden, orchard and field crops (Marie, 2004). *Myzus persicae* (Green Peach Aphid) is a polyphagous and economically important pest with a worldwide distribution (Iguchi *et al*., 2012). It is one of the most significant pests on agricultural crops worldwide (Blackman and Eastop, 2000). The green peach aphid *M. persicae* has been a particularly challenging taxon for over 200 years and it has characteristics that makes it both an important agricultural pest and difficult to discriminate (Marie, 2004). They belong to the family Aphididae and order Homoptera, and are very small, egg shaped, rarely over 1.2-2.5mm in length (Marie, 2004). They possess piercing-sucking mouthparts that enable them to feed on the sap transported in the phloem of plants. The winged forms can have black or brown colored heads and black markings on the body.

Myzus persicae has an extremely wide host range of over 100 plants including a wide variety of vegetables and ornamental crops. It is an important insect pest of the Solanaceae family and can attain very high densities on young plant tissue, causing water stress, wilting, and reduced growth rate of the plant. La Rossa *et al*. (2013) reported that aphid populations can grow to extremely high levels under favorable environmental conditions in a short time, covering sprouts, leaves, flowers, and fruits. Prolonged aphid infestation can cause appreciable reduction in plant yield because they consume plant nutrients and their sucking behavior can cause chlorosis and distortion of the leaves, abscission of blooms, and plant stunting and wilting. Lo *et al.* (1999) reported that large populations of *Myzus* developing on leaves of peach and nectarine plants will stunt or kill young shoots if left unchecked. High densities of these aphids can also cause actively growing leaves to curl, thereby forming pockets and folds that provide shelter to the aphids and, consequently, protection against insecticide treatments (Iguchi *et al*., 2012).
Among piercing-sucking insects, aphids are especially important pests of agriculture; they cause plant damage both by the direct removal of nutrients and by transmitting the majority of insect-vectored plant viruses. It also secretes honeydew which attracts fungus causing the smutting of leaves and fruit, the growth of sooty molds also hampers

photosynthesis. *M. persicae* has a very high reproductive potential and can cause substantial injury to young plants thus causing eventual death (Rashid *et al*., 2013).

Aphids are economically important pests that are difficult to control because of their mobility, tremendous reproductive ability, and resistance to many synthetic pesticides (Lowery *et al*., 1993). Green peach aphids are distributed worldwide and several resistant strains have been identified using molecular techniques (Al-Antary and Khader, 2011). Over time, *M. persicae* populations have developed resistance against compounds of synthetic insecticides classes including organochlorines, organophosphates, carbamates, and synthetic pyrethroids (Ronald, 1991). Due to repeated usage of these insecticides *M. persicae* have evolved several insecticide resistance mechanisms, including the detoxification of insecticides by elevated esterases (Khan *et al*., 2011). Anstead *et al*. (2005) reported development of resistance in *M. persicae* to more insecticides than any other insect.

2.2.1 Biology and lifecycle of *Myzus persicae*.

Aphids are highly variable in both form and life cycle. Within the same species, there are winged and non-winged forms and there are often both asexual and sexual life cycles within which there are distinct phenotypes (Blackman and Eastop, 2000). Green peach aphids (*Myzus persicae*) also produce distinct color forms, green, yellow and red. Color variation among green peach aphids is common, and appears to be interclonal and sympatric in occurrence (Kerns *et al*., 1998). *Myzus persicae* spend most of the growing season as parthenogenetic viviparous females with a generation time of about one week. All parthenogenetic aphids are born as wingless nymphs, but can develop into either alate (winged) or apterous (wingless) morphs at maturity (Blackman and Eastop, 2000). Usually the formation of alate within a population is triggered by overcrowding or the presence of genetic plant resistance (Arogundade *et al*., 2012)

Aphids can reach pest proportions rapidly, It reproduces asexually all year round on a wide variety of secondary hosts including potatoes, tomatoes, brassicas, beets, cereals, pasture clovers, peas and peppers (Al-Antary and Khader, 2011). One aphid can bear 30 nymphs and during a favorable season, eight successive generations may follow. Thus, one aphid can theoretically give rise to 30^8 individually, that is, more than 6×10^{11} aphids. Fortunately, nature limits aphid populations through unfavorable climatic conditions, parasites, predators, and nutrient deficiencies in the host plant (Al-Antary and Khader, 2011).

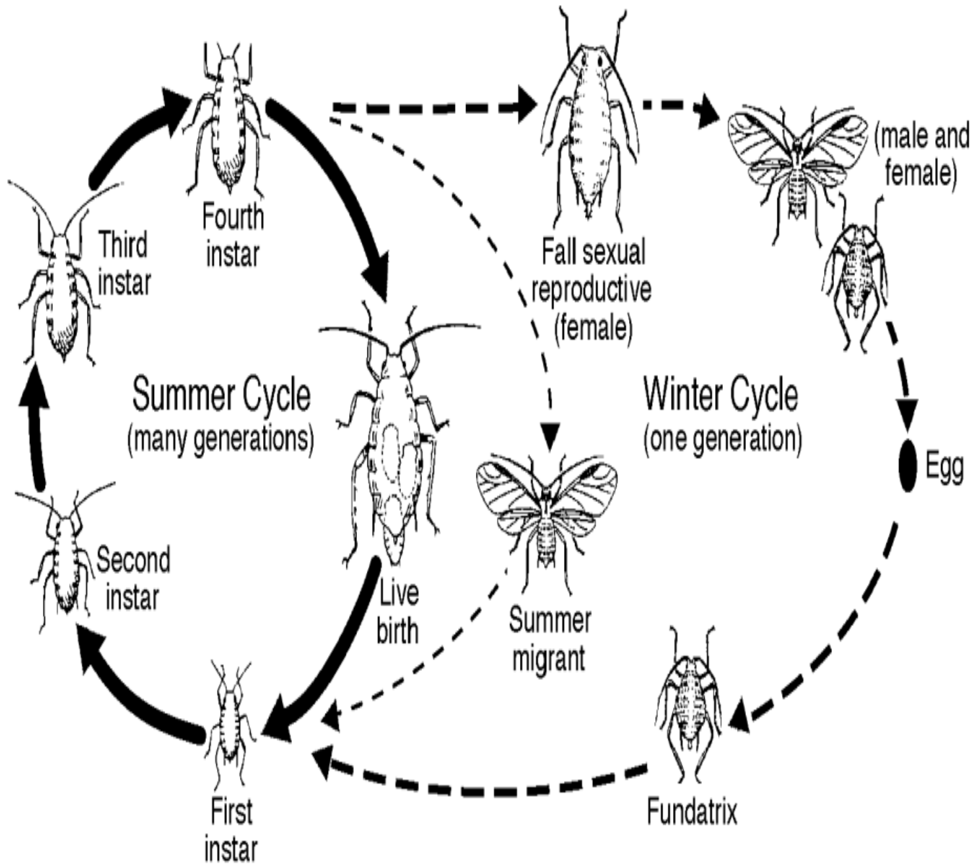

Figure 2.1: Life cycle of *Myzus persicae*

Source: http://asi.ucdavis.edu

2.3 Overview of Synthetic Pesticide Use in Nigeria

Over the years, the use of pesticides have substantially contributed in controlling agricultural pests, vector borne diseases and increasing crop yield in meeting food demand for the escalating population. However, despite the contribution of pesticide to the quality and quantity of agricultural yield, evidences in the last few decades have shown that they could pose potential hazards to human health and the environment when inappropriately handled (Adeola, 2012).

In parts of the developing world, pesticide poisoning causes more deaths than infectious diseases (Micheal *et al*., 2002).Human exposure to pesticides is an important health and social issue as it usually results in serious health problems such as epilepsy, stroke, respiratory disorders, stomach and intestinal upset, cancer, leukemia, brain tumors, liver tumors and convulsions (Tijani, 2006). Studies have shown that intense use of pesticides to kill resistant pests induces more resistance until further increases in pesticide use actually reduce agricultural yield (Micheal *et al*., 2002). Indiscriminate and uninhibited use of pesticide has also resulted in the disturbance of the ecosystem, principally in the form of pollution of natural water bodies, poisoning of aquatic organisms, groundwater contamination, reduction in wildlife populations and destruction of natural vegetation (Rehman *et al*., 2012).

In Nigeria, the use of pesticides is an indispensable tool in combating damage from pests and ensuring sustainable food production with improved yield and greater availability of food all year round (Oluwole and Cheke, 2009). In developing countries such as Nigeria where adequate workers education and effective regulatory measures are lacking, the use of agricultural pesticides have been accompanied by ignorance, over use and misuse/ abuse and this has subsequently resulted in an increase in the incidence of food poisoning across the nation. A survey conducted by Oluwole and Cheke (2009) on pesticide use by farmers in Ekiti state, Nigeria revealed that 83.6% of pesticide used in the surveyed areas were classified as highly hazardous by World Health Organization (WHO) in 2004 and the use of such pesticides were already restricted in many developed countries. In view of the several disadvantage of synthetic pesticide use, it is therefore incumbent to promote the use of botanicals which are safer, cheaper and environmentally friendly means of controlling agricultural pest and diseases.

Comment [ARE3]: Year?

2.4 Neem (*Azadirachta indica*) and potential use in pest control

With increasing concern and greater public awareness of long-term health and environmental effects of synthetic pesticides, the search for new compounds for pest control has increased since the early 1990s (Khater, 2012).The use of conventional insecticides has raised some concern about their threat to the environment and development of insecticide resistance in insects; therefore new trends in crop protection are encouraging the search for harmless pesticides or pesticides with very low toxicity and also for ways of reducing the use of pesticides (Anand *et al.*, 2008). To overcome the problems of synthetic chemical hazards, one of the best control measures is the use of plant origin products. Several botanicals have been screened for their phytochemistry and potential use in pest control programs. Currently, about 200 plants with insecticidal activities are known (Mondal and Mondal, 2012). Botanical insecticides are currently studied extensively because of the possibility of their use in plant protection as an alternative method to the broad use of conventional pesticides (Medhini *et al.*, 2012).

Comment [ARE4]: ?

Among the wider range of plants, Neem derivatives have shown great potential in controlling insect pests. One of the most studied botanical species with good pesticidal attributes is the Neem tree (*Azadirachta indica*), whose extracts have shown considerable activity and multiple modes of action against agricultural pests, forestry insects, and insects of public health concerns (Kelany, 1997). Rimpi-Das *et al.* (2010) reported that among the natural insecticides investigated in recent years, the constituents of Neem tree (*Azadirachta indica*) have shown great promise in controlling numerous (nearly over 200 species) insect pests of cultivated crop plants. Neem seed extracts are among the most used botanical pesticide and serve as a viable alternative for the management of many sucking insects.

2.4.1 Neem Tree (*Azadirachta indica*)

Neem (*Azadirachta indica)* belongs to the order Rutales and the family Meliaceae. It is 15-20 meters in height and grows well on a wide range of soil. It is drought hardy and produces ovoid-oblong fruits which are drupes and about 1-2 cm long, with one kernel in the seed. It has many common names which are region specific and have various meanings in respective regions. In Nigeria it is called Dogonyaro in Hausa, Aforo-oyingbo in Yoruba and Ogwu akuma in Igbo (Akeem, 2008). The seed kernels of Neem yield about 90% of a fixed oil comprised primarily of glycerides. The yellow, bitter oil has garlic- like odor and contains approximately 2% of bitter principles including nimbidin, nimbrin, nimbinin, nimbidol and other related minor limonoid triterpense (Ogemah, 2003).

Comment [ARE5]: Scientific name

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2.4.2 Geographic Distribution of Neem *(Azadirachta indica)*

Neem, (*Azadirachta indica* A. Juss) is native to the arid regions of the Indian sub-continent, from where it has spread out to many Asian and African countries as well as Australia and South America (Mahmoud and Maha, 2008). Although the exact origin is uncertain, it is thought to have originated from Myanmar (Burma) (Ketkar, 2000). The Neem tree is now widely distributed by introduction into tropical and subtropical zones of Asia, America, Australia, South Pacific Islands and it is found in not less than 78 countries worldwide and Global occurrence is estimated at somewhere between 64 to 91 million trees (Ketkar, 2003).

2.4.3 Ecology of Neem *(Azadirachta indica)*

Comment [ARE7]: Scientific name

Neem can grow in tropical and subtropical regions with semi-arid to humid climates. It can grow well in areas with annual rainfall of 400-1200mm and at elevations from sea level to1000m. At higher altitudes (1000-1500), growth may be slow. The best soils are deep, well drained and sandy (Schmutterer, 1995), although the range of soils on which it can grow is also wide. Soil water availability appears to be the most critical factor (Appiagyei, 2010). According to Ogemah (2003), Neem can grow on both alkaline and saline soils with a pH of 6.2 – 7.0 although pH of 5.9 and 10.0 may also be tolerated. Optimum temperatures range from 21^0c -32^0c. The tree can tolerate temperatures as high as 50^0c, but low temperatures below 40^0c are unfavorable. According to Childs *et al.* (2001), the Neem tree is drought tolerant and thrives in many of the drier areas of the world. Four to five years old trees can bear fruits on average giving 30-50 kg of fruit per tree (Wondafrash, 2007). All parts of the tree contain the effective ingredients, but are most highly concentrated in the seeds (Schaaf *et al*., 2000).

2.4.4 Chemical Composition of Neem *(Azadirachta indica)*

Comment [ARE8]: Scientific name

Various Neem products have been studied extensively for their phytochemistry and exploitation in pest control programs and a number of bioactive components have been isolated from various parts of the Neem tree. Although, new limonoids are still being discovered in Neem; Azadirachtin, salanin, meliantriol, and nimbin are the most known and significant components. Among the natural products obtained from Neem tree, one of the most promising natural compounds is Azadirachtin, an active compound extracted from the Neem tree whose antiviral, antifungal, antibacterial and insecticidal properties have been known for several years (Mondal and Mondal, 2012). It can be isolated in small amounts from all parts of the Neem tree, but it is present at highest concentration in the mature seeds (Schaaf *et al.*, 2000). About 413 different species/subspecies of insect pest listed by Schmutterer (1995) have been found to be susceptible to Neem products. Mondal and Mondal (2012) also reported that azadirachtin is active in nearly 550 insect species, mostly in orders Coleoptera (beetles and weevils); Dictyoptera (cockroaches and mantids); Diptera (flies); Heteroptera (true bugs); Homoptera (aphids, leaf hoppers, wasps, and ants); Isoptera (termites); Lepidoptera (moths and butterflies); Orthoptera (grasshoppers, katydids); Siphonaptera(fleas); and Thysanoptera (thrips).

2.4.5 Chemical structure and biosynthesis of Azadirachtin

The triterpenoid azadirachtin was first isolated from the seeds of the tropical Neem tree by Butterworth and Morgan in 1968 (Wondafrash, 2007). It has a definite structural formula (C_{35} H_{44} O_{16}), which resembles insect hormones called ecdysones, these hormones control the process of metamorphosis as the insects pass from larva through pupa to adult (Hummel *et al.*, 2012). Azadirachtin is a highly oxidized limonoid whose biosynthesis involves a series of oxidation and rearrangement reactions (Mordue and Blackwell, 1993). The fruit is the most important part of Neem that affects insects in various ways. Azadirachtin-A, the major bioactive secondary metabolite of *Azadirachta indica* and it is well known for its excellent insecticidal, antifungal and growth disruptive activity against a variety of insect pests. They exert a strong negative influence on behavior, postembryonic development and fecundity of insects resulting in significant reduction of general fitness of the pests.

2.5 Success of Neem *(Azadirachta indica)* in Pest Control

Comment [ARE9]: ?

The insect repellent and growth disrupting effects of Neem seed extracts, particularly the Neem seed chemical azadirachtin, are now well established and have been studied in many insect pest groups (Wondafrash, 2007).The products of *A. indica* are cheap, easy to prepare, eco-friendly and low-cost alternatives to agrochemicals. Extracts from various parts of *A. indica* have been compared with commercial pesticides on various crop pests where they have been found to be efficacious, and equally or more cost effective (Mondal and Mondal, 2012).

A laboratory study by Rimpi-Das *et al*. (2010) to evaluate the effect of Neem kernel aqueous extract on larval weight, larval duration, mortality percent, adult emergence percent and antifeedant of red slug caterpillar (*Eterusia magnifica*) in tea plant revealed that the aqueous extract was effective in deterring the growth and development of the pest as the concentration of the extract increased. Boursier *et al*. (2011) reported that crude water extracts of Neem seeds have been tested in the field against several pests in tropical and subtropical countries and their efficacy has been found to be satisfactory to excellent. Lowery and Isman (1994) demonstrated that nine species of aphids were susceptible to the insect growth regulating (IGR) activities of Neem seed oil or AZA, and the mortality occurred mainly during failed attempts to molt. Similarly studies by Egho and Ilondu (2012) also showed that Neem seed extract significantly reduced aphid population (*A. craccivora*) when compared to the unprotected plots (control).

Laboratory and field trials with formulated Neem seed oil (NSO) and Neem seed extract (NSE) by Lowery (1992) demonstrated that these materials were potentially very effective as aphicides. In the same study laboratory trails showed a significant reduction in numbers of green peach aphids, *Myzus persicae* (Sulzer), on pepper; the study further revealed that mortality of aphids was between 94 -100% within nine days when Neem seed oil were applied to leaf disk at a concentration of 1.0%. Under field condition it was observed that NSO and NSE were effective or better than the commonly used botanical insecticide pyrethrum for the control of aphids on pepper, cabbage, and strawberry, but they were ineffective for the control of aphids on lettuce. Laboratory and greenhouse experiments by Nisbet *et al.* (1993) revealed that concentration as low as 25 ppm in AZA-treated diet to study its effect on the peach-potato aphid *Myzus persicae* resulted in a post-ingestive secondary antifeedant effects as well as drastic reduction in fecundity. No nymphs were produced by aphids previously fed on diet containing 25 ppm AZA and all nymphs produced by aphids that had ingested diet containing 10-20 ppm Aza were non- viable (El- Shafie and Basedow, 2003).

The efficacy of Neem seed products in reducing the transmission of potato virus Y to sweet pepper by the green peach aphid, *Myzus persicae* was studied by Lowery *et al.* (1997), their study revealed foliar applications of 1.0% or 2.0% Neem seed oil to infected source plants or to uninfected recipient plants inhibited *Myzus* from transmitting the virus to the plants. They also reported that the Neem seed oil interfered with virus acquisition and inoculation in a manner comparable to that of commercial horticultural oils, while an oil-free Neem seed extract did not reduce rates of transmission compared with controls. Apart from the seed of Neem other parts of the tree such as the leaves and bark have been exploited in pest control programs. Studies by Govindan and Nelson (2009), showed that leaf powder of *A. indica* caused 42.2% mortality of rice weevils and minimum adult emergence was observe (18 adults) in *A. indica* treated plots when compared to 98.00 adult weevils in untreated control.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted at the Botanical Garden of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Ahmadu Bello University is located on latitude 11˚ 13' North, Longitude 7˚ 12' East and on Altitude 630 meters above sea Level. The Departmental garden is located on latitude 11˚ 84.516'' North, Longitude 7˚ 39. 175'' East and on Altitude 2184 ft above sea level.

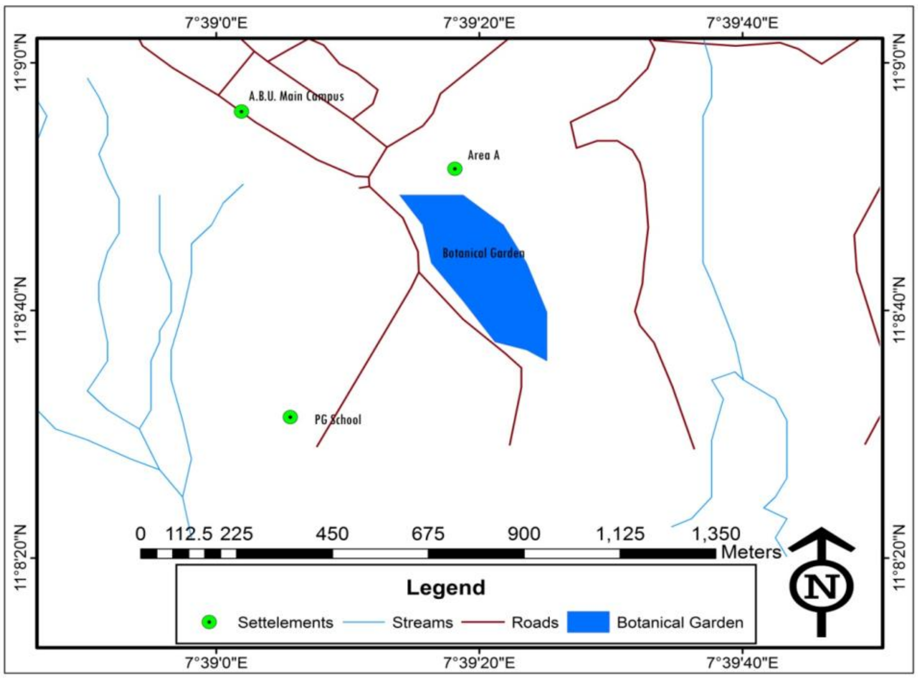

Figure 3.1 Map showing study area.

3.2 Rearing and Maintenance of Aphid Colonies

Nymphs of Lemon and dark green morphs of *M. persicae* used for raising the culture were collected from infected cabbage and pepper plantations around Bassawa, Zaria, Kaduna State, Nigeria (Plate 1 and 2). The insects were collected alongside with the plant parts where they were found. Identification of the collected insects as *Myzus persicae* was done at the Insect museum laboratory, Department of crop protection, A.B.U, Zaria, Nigeria using an aphid identification guide (Blackman and Eastop, 2000). Green peach aphids that were positively identified were used to infest several plants of a susceptible pepper cultivar (California wonder) and a local variety of chili pepper (Tsidifi). Stock cultures were maintained on pepper plants in aphid-proof cages. Each morph of *Myzus persicae* was cultured separately to avoid mix up of the aphids. The wooden culture cages (1.5m wide x1m long) were made from net of small mesh size and kept in a cool well shaded environment at a temperature of 25 ± 3°C.

New pepper plants were placed near the existing plants once it was noticed that the later have succumbed to aphid feeding pressure, this was done to facilitate movement of the aphids to the new plants and also ensured the continuity of the aphid colony throughout the period of the experiment (Plate 3).

Plate 1: Apterous *Myzus persicae* (Lemon green morph) as seen under the USB microscope

Plate 2: Dark green morphs of *Myzus persicae* (Apterous)

Plate 3: Potted pepper plants infested with *Myzus persicae.*

3.3 Source of Neem Seeds

Matured seeds of *Azadirachta indica* were obtained from Neem trees within the main campus of Ahmadu Bello University, Zaria. Yellowing of the fruits was taken as an indicator of their ripening. The seeds were collected by three methods

(a) Using straw broom to gather the already fallen seeds which were packed in plastic bags.
(b) Shaking the branches of the tree vigorously to detach the ripe fruits and to cause them to fall on the ground.
(c) Handpicking of ripe fruits on branches near the ground.

The seeds were depulped (removal of the outer layer) by soaking them in water and rubbing them between the palms to remove the seed coat. After depulping and cleaning, the seeds were shade dried under room temperature for three weeks. The dried seed was then crushed into fine powder with the aid of a mortar and pestle.

3.3.1 Preparation of Aqueous Neem Seed Extract

Aqueous neem seed extract was prepared according to methods described by Stefens and Schmutterer (1982) with modifications: 1kg of crushed Neem seed was weighed using a weighing balance and soaked in 5 liters of water for 24 hours, after which it was sieved through a fine muslin cloth. Tween 80 was added to the extract at the rate of 1ml per 1 liter of the extract, this served as an emulsifier to dissolve the active substances in the Neem powder and to make them stick to the plant surface, this mixture served as the stock solution from which serial dilutions were made. The aqueous neem seed extract stock solution was then serially diluted with distilled water to obtain the following concentrations: 50% (5:5 v: v), 75 % (7.5:2.5 v: v) and 100% (plant extract stock solution): 50% dilution was made by diluting 500mls of extract in 500mls of distilled water and 750mls of the extract was diluted in 250mls of distilled water. Each plant was sprayed from all side with 30mls of the treatments until runoff (Feng and Isman, 1995) using a handheld sprayer (2 liter capacity sprayer). Each dilution was prepared on the day of the experimental trial.

3.4 Source and Description of Pepper Varieties

In this study four varieties of sweet pepper (California wonder, Yolo wonder, Kano T and one local chili pepper variety locally called Danzagade) were used. California and Yolo wonder were obtained from Premier seed company, Zaria, while Kano T was obtained from National Horticultural Research institute (NIHORT), Bagauda, Kano state, Nigeria.

Calfornia wonder and Yolo wonder are exotic sweet pepper varieties, which reach maturity at 72-75days after sowing; they produce green fruit which are lobed. Kano T is an improved variety of local sweet pepper known as “Tatase”, it also produce lobed green fruits which turns red on maturity. Dazagade is a chili pepper variety characterized with production of moderately long fruits. Pepper varieties are usually planted in nurseries under irrigation and subsequently transplanted when the rains become established.

3.4.1 Raising of Pepper Nursery and Transplanting

The pepper seeds were sown on nursery beds which were covered with fresh straw to serve as mulch. Germination was noticed 7 days after sowing and the mulch was removed. Watering of the nursery was done daily until transplant. All agronomic practices of raising pepper seedling as described by Jaliya and Sani (2004) were followed. Weeds were removed regularly and transplanting was done at four weeks after sowing, at this stage the plants possessed about 4 true leaves. The transplanting activity was done early in the morning to reduce stress caused by transpiration. Seedlings were pulled out from the nursery bed with the aid of a hand trowel which was used to uproot the plants from the soil. The plants were uprooted with a ball of earth around the roots and care was taken to prevent the damage of plants root.

3.5 Preparation of Experimental Plots

Prior to transplanting of pepper seedlings the experimental site was weeded, the soil was pulverized to loosen it up and stones were removed, compound fertilizer NPK 15:15:15 was applied at a rate of 22g/m^2. Occasional top dressing with NPK was carried out when required; the application was done by ring placement into drills 5 cm deep and 7.5 cm away from the plant and covered with soil. Weeding was done weekly to prevent them from competing with the plants. The experimental plot used was covered completely with a wire net of 0.2cm x 0.2cm mesh size and polythene bags to act as barrier against predators and other insects.

3.6 Experimental Design

A separate experiment was set up for trials with Lemon and Dark green morphs of *M. persicae*. The experiment was laid down in a Randomized Complete block Design (RCBD). Each experimental site consists of 18 plots of 3m x 2.5m size; plots were separated by 50cm spacing. A table of random numbers was used to distribute the pepper varieties randomly in each plot**.**

A total of six (6) treatments were used, the treatments were replicated thrice and each replication consisted of four (4) pepper varieties with five replication per plant. The treatments consisted of: T1 - Neem Seed Extract (100%), T2 - Neem Seed Extract (75%), T3 - Neem Seed Extract (50%), T4 - NeemAzal (0.7%), T5 - Deltrin (0.75%), T6 -Tap water (neutral control)

NeemAzal applied at a recommended rate of 0.7% was formulated by diluting 7mls of the Biopesticide in 1000mls of water, this served as the positive Biopesticide check. NeemAzal-T/S 1% was kindly supplied by Dr H. Kleeberg from Trifolio-MGmbH Lahnau, Germany. This commercial Neem product consists of 4% NeemAzal-T/S i.e. 1% Azadirachtin A (10g/l) and 3% other Neem substances. It contains about 51% plant oil. The product is now registered in Germany as commercial insecticide for the control of greenhouse and some field pests (El Shafie, 2001).

A Cypermethrin derivative commercially known as Deltrin was applied at the recommended rate: 7.5mls of the pesticide was diluted in 1000mls of water.

3.7 Inoculation of Pest and Treatment Application

Inoculation of aphids was done at one week after transplanting, at this stage the plants had fully recovered from transplanting shock and have continued normal growing. Insects were inoculated according to method described by Ochieng and Nderitu, (2011); approximately 25 apterous *M. persicae* were introduced on young fully expanded leaves of each plant using a pencil brush. Initial infestation was done at one week after transplanting and subsequent re-infestation was done to maintain aphid population (Holmes and Hassan, 1997).

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Treatment was applied after second infestation; the aphids were allowed 24hours acclimatization period before treatment application. Four rounds of treatment application were done bi-weekly using a 2 liter capacity hand held sprayer (Saljoqi and Van Emdem, 2003). Each plant was sprayed from all sides with the treatment till point of runoff as described by Feng and Isman (1995).

3.8 Data Collection

The population change of *M. persicae* was determined by visual assessment of all parts of the pepper plants using a magnifying lens. Five plants per pepper variety were randomly selected per plots (El- Sayed, 2013). Pretreatment counts were taken before spray application while post treatment count of *M. persicae* population found on pepper plants was made 24, 72, 120 and 168hrs after treatment application to determine the population dynamics of the pest (Akbar *et al.*, 2008).

The efficacy of respective treatments in reducing population of *M. persicae* (Percentage reduction over control) was calculated by adopting methods used by Henderson and Tilton (1955).

Percent reduction in population= $1 - \left(\frac{T_a}{C_a} * \frac{C_b}{T_b}\right) * 100$

Where:

T_a = No. of insects after treatment

T_b = No. of insects before treatment

C_a = No. of insects in untreated check after treatment

C_b = No. of insects in untreated check before treatment

Weekly data was collected on plant vegetative parameters (plant height, number of leaves, leaf length, leaf diameter, leaf area, number of branches) and yield parameters (number of flower, fruit setting from flowers, number of fruits). Plants were also accessed for presence and number of curled leaves, chlorosis, stunting and disease incidence and severity using a scoring scale of 0-1.

Where:

0 = absence

1 = presence

Stunting of plant would be determined by measurement of plant height (cm) using a meter rule and comparing it with that of the treated control.

3.9 Statistical Analysis

Data on plant growth and yield parameters were subjected to Analysis of variance (ANOVA) using SAS analysis tool pack and where significant, separation of means was done by Duncan's multiple range tests. Pearson correlation test was also carried out to determine relationship between plant growth parameters and aphid number after treatment. Principal coordinate analysis was used to group pepper varieties on the bases of viral disease symptoms.

CHAPTER FOUR

4.0 RESULTS

4.1 Aphicidal Efficacy of Aqueous Neem Seed Extract on *Myzus persicae*

4.1.1 Effect of ANSE on Population of Dark green morphs of *M. persicae*

The mean mortality of Dark green morphs of *Myzus persicae* and reductions in aphid infestation was significantly ($p<0.05$) higher over control (tap water) in all concentrations of aqueous neem seed extract (ANSE). Mortality of *Myzus* reduced with decrease in the concentration of the aqueous neem seed extracts. At 24hours after spraying with 100% concentration of ANSE, a reduction (83.92%) in dark green morphs of *M. persicae* was obtained (Table 4. 1), this reduction was significantly lower to the reduction efficacy (98.25%) obtained for the synthetic pesticide (Deltrin). However, the percentage mortality increased to 97.11% at 168hr which was statistically not different from 96.5% obtained with Deltrin). At 24hr after spray treatment of the pepper varieties with 75% and 50% concentration of the extract resulted in reduction in the aphids by 73.08% and 69.58% respectively (Table 4.1).

At 168 hours after treatment application significant ($P < 0.05$) aphicidal efficacy was observed with the ANSE treatments (100%, 75% and 50%), the effect of the neem extracts was comparable to the synthetic pesticide used (Deltrin). A reduction in the percentage mortality was observed for Deltrin at 120hrs and 168 hrs after spray. None of the treatment resulted in 100% mean mortality. NeemAzal which is an azadirachtin based Biopesticide had the lowest percentage reduction of 63.29% at 24 hours after spray, however, the percentage reduction increased as the time of observation increased (Table 4.1).

Table 4.1: Effect of Aqueous Neem Seed Extract, NeemAzal and Deltrin on Percentage (%) reduction of Dark green morphs of *Myzus persicae*.

	Concentration (%)				
Time (HOURS)	100% ANSE	75% ANSE	50% ANSE	NEEMAZAL 0.7%	DELTRIN 0.75%
24	83.92^{b}	73.08^{c}	69.58^{d}	63.29^{d}	98.25^{a}
72	91.88^{b}	82.48^{c}	79.49^{c}	79.06^{c}	97.01^{a}
120	95.46^{a}	95.46^{a}	95.13^{a}	90.64^{b}	99.47^{a}
168	97.11^{a}	96.96^{a}	95.72^{a}	92.85^{b}	96.5^{a}

Means with different superscripts across row are significantly different ($P < 0.05$), Duncan's multiple range test (DMRT).

4.1.2 Effect of ANSE on Percentage reduction of lemon green morphs of *M. persicae*.

Aqueous neem seed extract resulted in significant ($P < 0.05$) reduction in population of Lemon green morphs of *Myzus persicae* on pepper plants. At 24 hours after treatment, an increase in the mortality of aphid was observed with increasing concentration of the aqueous neem seed extract. The extracts acted in a concentration dependent manner. For all ANSE concentrations used, the aphicidal efficacy of the extract increased with an increase in time of exposure (Table 4.2) except at 120 hrs and 168 hrs where the percentage reduction for 100% ANSE (94.14% and 94.69% respectively) were slightly lower than that obtained when plant infested with Lemon green morphs of *Myzus* were treated with 75% and 50% concentration of ANSE. NeemAzal also showed an increase in aphicidal efficacy over time, it was equally as efficacious as the aqueous neem seed extract treatments at 168hours after spray.

A decrease in percentage reduction of aphids from 87.32% to 85.90% was observed from 120 hrs to 168 hrs when Deltrin was applied (Table 4.2), this was significantly lower ($P < 0.05$) than the percentage reduction obtained with ANSE at 120 hrs and 168 hrs after spray.

Table 4.2: Effect of Aqueous Neem Seed Extract, NeemAzal and Deltrin on Percentage (%) reduction of Lemon green morphs of *Myzus persicae.*

	Concentrations (%)				
Time (Hours)	100% ANSE	75% ANSE	50% ANSE	NEEMAZAL 0.7%	DELTRIN 0.75%
24	86.78^{a}	79.34^{b}	76.45^{b}	65.70^{c}	85.95^{a}
72	91.73^{a}	88.72^{ab}	89.10^{a}	87.97^{b}	90.6^{a}
120	94.14^{a}	95.58^{a}	97.05^{a}	89.97^{b}	87.32^{b}
168	94.69^{a}	96.52^{a}	96.34^{a}	90.45^{b}	85.90^{c}

Means with different superscripts across rows are significantly different ($P < 0.05$), Duncan's multiple range test (DMRT).

4.1.3 Effect of aqueous neem seed extracts on the number and fecundity of *Myzus persicae*

At 24 hours after treatment, mean number of Lemon green aphids per plant was highest in plants treated with 50% concentration of ANSE; followed by treatment with NeemAzal and then 75% ANSE (Figure 4.1). Mean number of lemon green aphids per plant was observed to be lowest in plants treated with 100% ANSE concentration and synthetic pesticide (Deltrin).

For all treatments except the control (tap water), there was a reduction in the mean number of aphid per plant 72 hours after application. At 120 hours after spray an increase in the mean number of aphids per plant was observed in plants treated with chemical pesticide and NeemAzal. However the mean number of aphids per plant for plants treated with synthetic pesticide further increased at 168 hours after spray (Figure 4.1) as new instars were observed on the plants. Fewer numbers of new instars were observed on plants treated with ANSE as well as NeemAzal hence the differences in the mean number of aphids obtained for these treatments at 168 hours after treatment application. At all time intervals there was a continuous development of aphids on plants treated with tap water (Figure 4.1 and Figure 4.2). For plants infested with dark green morphs of *Myzus persicae* similar trend in mean number of aphids was observed (Figure 4.1), however, the mean number of aphids per plant was lower when compared with plants infested with lemon green morphs (Figure 4.1 and Figure 4.2).

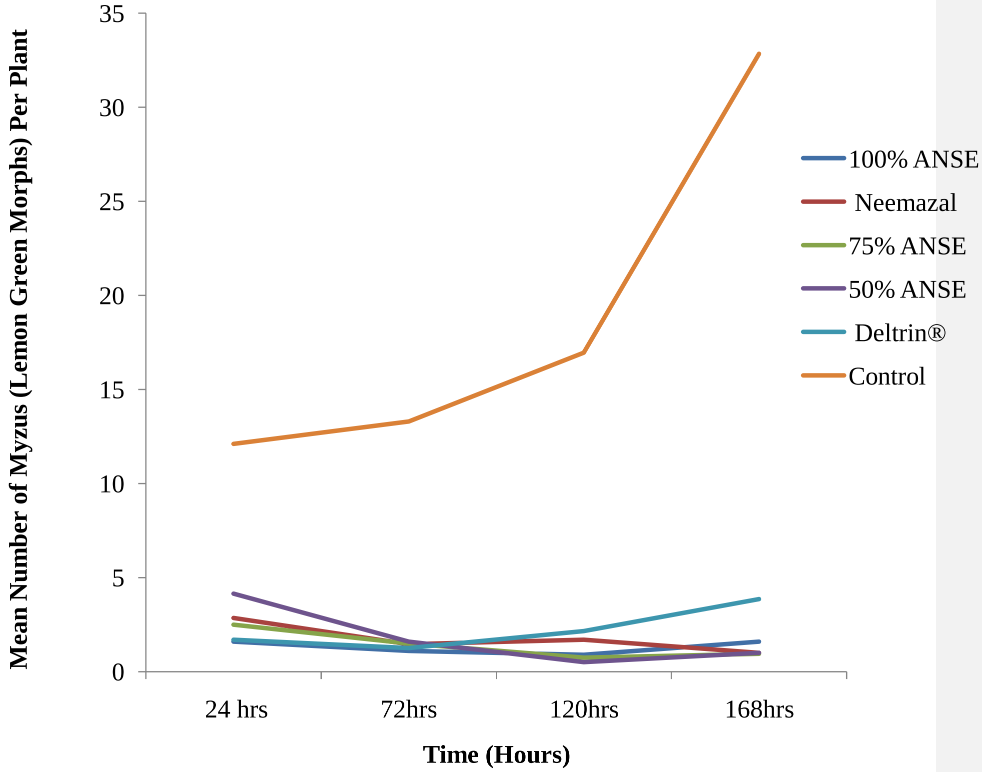

Figure 4.1: Effect of Aqueous Neem Seed Extract, NeemAzal and Deltrin on number of *Myzus persicae (*Lemon green morphs) per plant.
ANSE = aqueous neem seed extract.

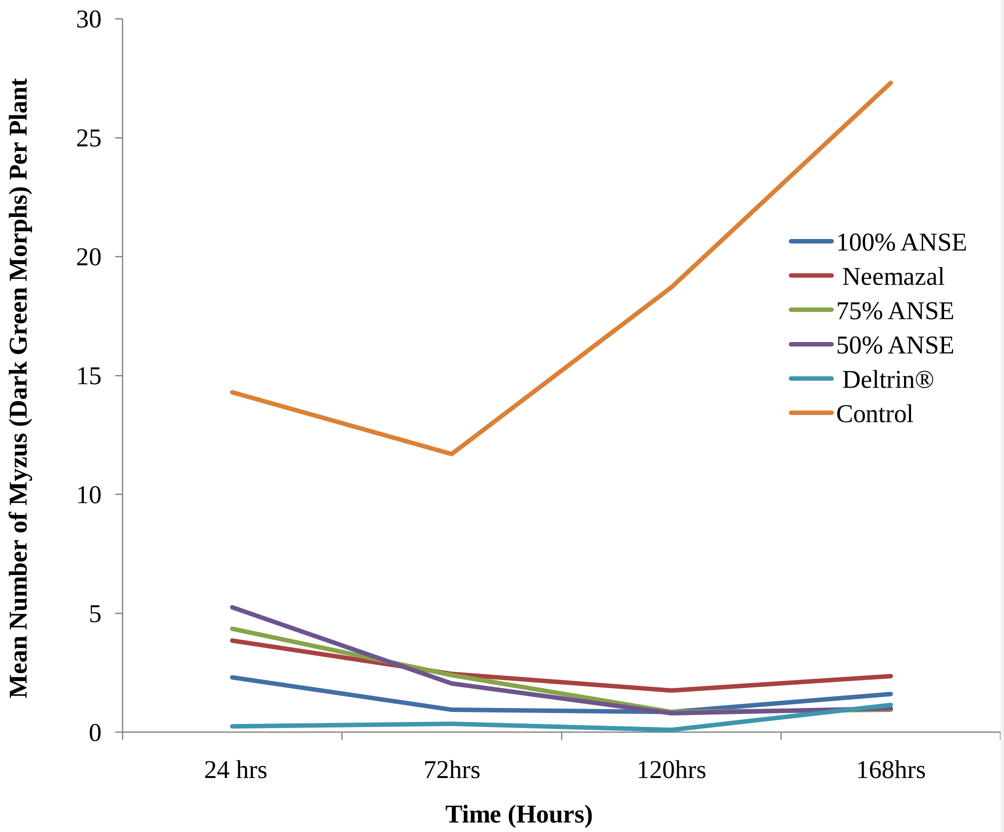

Figure 4.2: Effect of Aqueous Neem Seed Extract, NeemAzal and Deltrin on number of *Myzus persicae (*Dark green morphs) per plant.

ANSE = aqueous neem seed extract.

4.1.4 Infestation of *Myzus persicae* on pepper varieties

As the time interval increased the number of *Myzus persicae* on Danzagade variety of pepper increased (Table 4.3), although a drop in the number of aphids per plant was noticed across all the pepper varieties at 72 hrs after aphid infestation. Highest number of aphids for both morphs of *Myzu*s studied where found growing on Danzagade variety of pepper.

Analysis of variance for Lemon and dark green morphs of *Myzus persicae* showed a significant ($P < 0.05$) difference in the number of aphids amongst pepper varieties.

For lemon green morphs, number of aphids was highest in Danzagade, followed by Kano-T and then California wonder and lowest on Yolo wonder variety of pepper at 168 hours. Similar trend was observed in pepper plants infested with Dark green morphs of *Myzus*, however, in this infestation, California wonder least supported increase in the number of the aphids at 168 hour period of observation (Table 4.3).

Table 4.3: Infestation of Lemon and Dark Green morphs of *Myzus persicae* on four varieties of pepper.

Time (Hours)	Danzagade	Kano T	California Wonder	Yolo Wonder
24 Hrs I	7.90[b]	8.15[b]	8.70[a]	8.60[a]
II	9.20[a]	7.83[b]	7.03[c]	7.86[b]
72 HRS I	5.95[a]	4.48[c]	4.60[c]	5.25[b]
II	4.30[b]	3.55[c]	4.70[a]	4.90[a]
120HRS I	6.45[a]	5.20[b]	6.10[a]	4.55[c]
II	7.83[a]	3.99[c]	3.95[c]	5.36[b]
168 HRS I	11.05[a]	9.55[b]	8.30[c]	7.70[d]
II	11.20[a]	7.85[b]	4.40[d]	6.56[c]

Means with different superscripts across rows are significantly different ($p<0.05$), Duncan's multiple range test (DMRT). "I"- represents Lemon green Morphs of *Myzus persicae* while "II" represents Dark green Morphs.

4.2 Effect of Aqueous Neem seed extract on Plant Growth and Yield Parameters

4.2.1 Effect of aqueous neem seed extract on plant height

Treatment of pepper infested with both lemon and dark green morphs of *Myzus persicae* with aqueous neem seed extract did not significantly ($P < 0.05$) increase plant height over Deltrin and Neemazal (Figure 4.3). Amongst the ANSE treatments, highest mean plant height (15.80 cm) was observed in plants treated with 75% concentration of ANSE followed by 50% ANSE concentration (15.74 cm) which were not significantly different from that obtained with Neemazal (15.82cm). Plant height was highest in plots treated with Deltrin and lowest in tap water treated plots.

Similar trends were obtained in lemon green morphs infestation with the exception of plant height observed in 100% ANSE treated plots been significantly ($P < 0.05$) higher than that obtained with NeemAzal, 75% ANSE and 50% ANSE concentrations (Figure 4.3). In addition, plant height was significantly ($P < 0.05$) lower in lemon green morphs than dark green morphs of *Myzus persicae*.

Among the pepper varieties, plant height of California wonder and Yolo wonder infested with LG and DG morphs were not significantly influenced by all the treatment used. However, treatment of DG morphs infested Danzagade with 75% ANSE (18.94cm) and 50%ANSE (18.85cm) resulted in plant height which were comparable to Deltrin (17.50cm) and significantly higher than the control (14.95cm). For Kano T optimum plant height was obtained when plants were treated with Deltrin which were 18.23cm for DG and 14.24cm for LG morphs respectively (Table 4.4).

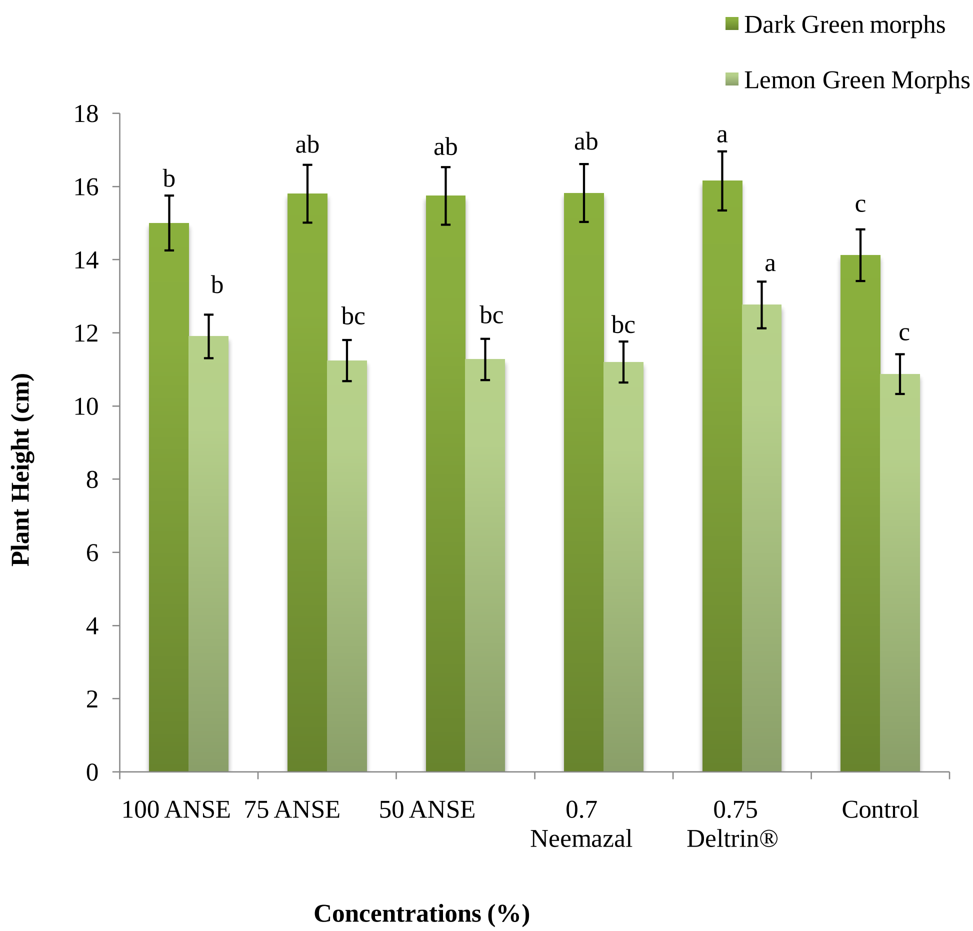

Figure 4.3**:** Effect of different concentrations of ANSE, NeemAzal and Deltrin on mean plant heights of pepper varieties infested with Lemon and Dark green morphs of *M. persicae.*

Means with different Duncan ratings (alphabets) are significantly different within morphs ($p < 0.05$). ANSE = aqueous neem seed extract.

Table 4.4: Effect of different concentrations of ANSE, NeemAzal and Deltrin on plant height (cm) of pepper varieties infested with Lemon green (LG) and Dark Green (DG) morphs of *Myzus persicae*.

		Plant Height (cm)			
Concentrations (%)	*Myzus* Morphs	Danzagade	Kano T	California wonder	Yolo Wonder
100 ANSE	LG	12.93 ± 0.95^{a}	11.71 ± 0.41^{b}	10.66 ± 0.62^{a}	12.31 ± 0.84^{a}
	DG	15.52 ± 1.23^{c}	16.70 ± 1.53^{a}	14.10 ± 1.63^{a}	13.33 ± 1.05^{a}
75 ANSE	LG	12.14 ± 0.76^{a}	11.63 ± 0.81^{b}	10.60 ± 0.48^{a}	10.59 ± 0.62^{a}
	DG	18.85 ± 2.40^{b}	15.33 ± 1.59^{b}	14.32 ± 0.75^{a}	14.52 ± 0.71^{a}
50 ANSE	LG	11.44 ± 0.67^{a}	12.06 ± 0.62^{ab}	10.14 ± 0.64^{a}	11.44 ± 0.59^{a}
	DG	18.94 ± 1.04^{b}	16.33 ± 1.67^{ab}	14.76 ± 1.01^{a}	12.91 ± 1.26^{a}
0.7 NeemAzal	LG	12.73 ± 0.59^{a}	11.91 ± 0.85^{b}	9.42 ± 0.71^{a}	10.72 ± 0.65^{a}
	DG	23.10 ± 1.33^{a}	15.80 ± 1.29^{b}	11.94 ± 1.30^{b}	12.43 ± 1.30^{a}
0.75 Deltrin	LG	13.79 ± 0.93^{a}	14.24 ± 1.33^{a}	10.96 ± 0.72^{a}	11.98 ± 0.73^{a}
	DG	17.50 ± 1.79^{b}	18.23 ± 1.61^{a}	14.02 ± 1.47^{a}	14.85 ± 1.85^{a}
Control	LG	11.54 ± 0.62^{a}	11.03 ± 0.61^{b}	9.40 ± 0.68^{a}	11.70 ± 1.36^{a}
	DG	14.95 ± 1.49^{c}	15.58 ± 1.06^{b}	12.96 ± 1.76^{ab}	13.00 ± 1.36^{a}

Means with different superscripts within a column are significantly different from each other ($p<0.05$), Duncan's multiple range test (DMRT). ANSE = aqueous neem seed extract.

4.2.2 Effect of aqueous neem seed extract on leaves and leaf number.

Comment [ARE11]: what effect?

The mean number of leaves gradually reduced ($P < 0.05$) with increasing concentration of ANSE when infested with lemon green morphs of *Myzus* (Figure 4.4). Plants treated with 50% ANSE concentration had highest mean number of leaves followed by 75% ANSE concentration. At highest concentration (100% ANSE) reduction in mean number of leaves was observed for the morphs infestation. Additionally, 100% ANSE resulted in visible burns on the leaves of pepper plants infested with both morphs of *Myzus*, this burns were not pronounced with other ANSE concentrations.

Varieties infested with Dark green morphs of *Myzus persicae* showed highest mean number of leaves (21) at 75% concentration of ANSE, this was not significantly ($P<0.05$) different from treatment with 50% ANSE, NeemAzal (21 leaves) and Deltrin (19 leaves). Lowest number of leaves was observed in plants treated with tap water (Figure 4.4). For LG morph infestations, number of leaves in 100% and 75% ANSE treated plots were not significantly ($P > 0.05$) higher than that produced by pepper plants in tap water treated plots. The morphs population showed selective preferences of attachments to the leaves of the varieties irrespective of the treatment.

A highly significant ($P < 0.05$) interaction was observed between the number of leaves produced by the pepper varieties and treatment (Table 4.5). Treatment of Danzagade with ANSE improved leaf production over tap water treated plots. Leaf production was highest when treated with Neemazal for DG morphs infestation (36 leaves) and Deltrin for LG morphs infestation (28 leaves). 75% ANSE (30 leaves) and 50% (31 leaves) ANSE concentration had improved leave production as compared to deltrin (27 leaves) in the DG morphs infestations. These were significantly ($P < 0.05$) higher than the number of leaves produced when the plants were treated with Deltrin.

The number of leaves produced by Kano T was not significantly ($P > 0.05$) affected upon treatment with 75% ANSE, 50% ANSE, NeemAzal and Deltrin. Lowest mean number of leaves was obtained at 100% ANSE. As the concentration of ANSE increased, the mean number of leaves per plant increased in California wonder and Yolo Wonder. Highest mean number of leaves was obtained when the plants were treated with NeemAzal.

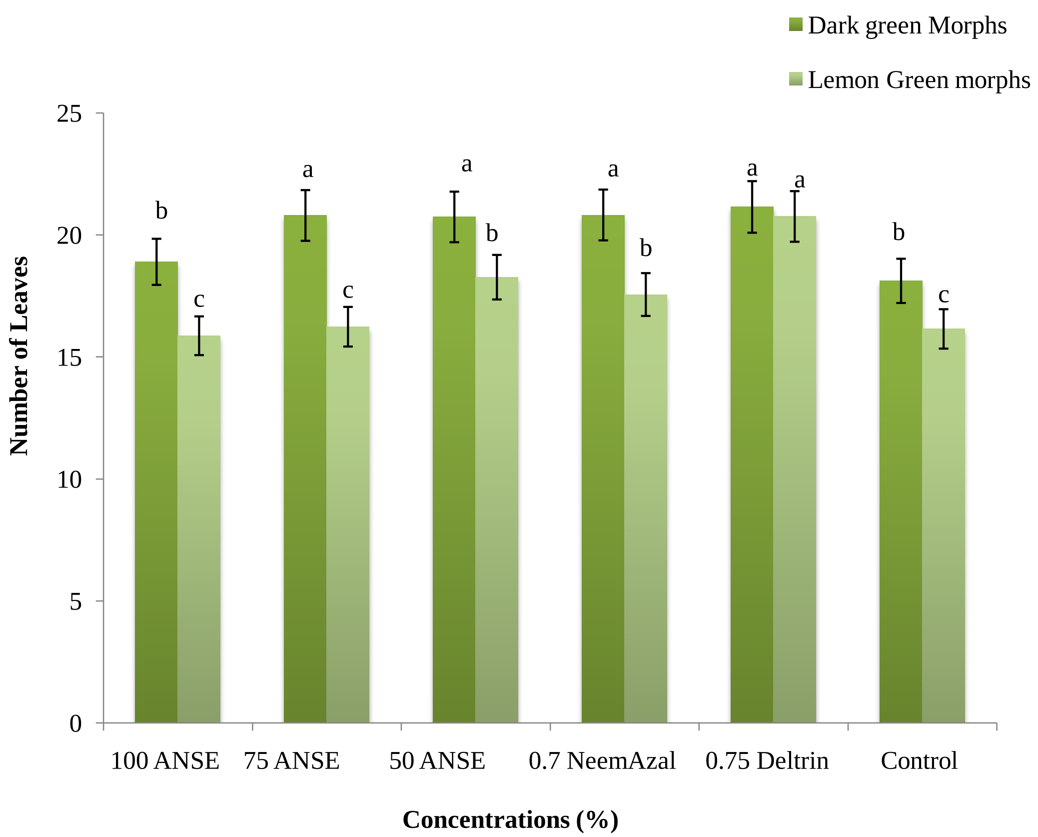

Figure 4.4: Effect of different concentrations of ANSE, NeemAzal and Deltrin on mean number of leaves on plants infested with Lemon and Dark green morphs of *M. persicae.*

Means with different Duncan ratings (alphabets) are significantly different within morphs (P<0.05). ANSE = aqueous neem seed extract.

Table 4.5: Effect of different concentrations of ANSE, NeemAzal and Deltrin on Number of Leaves of pepper varieties infested with Lemon green (LG) and Dark Green (DG) morphs of *Myzus persicae*.

		Number of Leaves			
Concentrations (%)	Myzus Morphs	Danzagade	Kano T	California wonder	Yolo Wonder
100 ANSE	LG	16.00 ± 1.52^{d}	17.00 ± 0.98^{b}	13.00 ± 1.10^{b}	18.00 ± 1.50^{b}
	DG	25.00± 4.98^{c}	22.00 ± 2.62^{b}	18.00 ± 1.30^{b}	17.00 ± 1.31^{a}
75 ANSE	LG	18.00 ± 1.40^{c}	16.00 ± 1.14^{c}	16.00 ± 1.13^{a}	17.00 ± 1.12 ^{b}
	DG	30.00 ± 3.14^{b}	24.00 ± 2.15^{a}	18.00 ± 1.35^{b}	18.00 ± 1.35^{a}
50 ANSE	LG	22.00 ± 1.12^{b}	17.00 ± 2.63^{b}	15.00 ± 1.07^{ab}	15.00 ±1.11^{bc}
	DG	31.00 ± 3.88^{b}	23.00 ± 2.34^{a}	16.00 ± 1.22^{bc}	16.00 ±1.31^{b}
0.7 NeemAzal	LG	23.00 ± 2.38^{b}	20.00 ± 1.72^{b}	16.00 ± 0.86^{a}	16.00 ± 0.97^{b}
	DG	36.00 ± 4.43^{a}	23.00 ± 2.91^{a}	21.00 ± 0.63^{a}	17.00 ± 0.88^{ab}
0.75 Deltrin	LG	28.00 ± 4.69^{a}	26.00 ± 2.18^{a}	16.00 ± 1.04^{a}	20.00 ± 1.12^{a}
	DG	27.00 ± 1.15^{b}	24.00 ± 1.76^{a}	16.00 ±0.94^{b}	16.00 ± 1.80^{b}
Control	LG	15.00 ± 2.01^{c}	18.00 ± 2.44^{b}	13.00 ± 1.43^{b}	15.00 ±0.89^{c}
	DG	21.00 ±3.47^{d}	23.00 ±2.85^{ab}	15.00 ±1.16^{c}	16.00 ±0.97^{b}

Means with different superscripts within a column are significantly different (P < 0.05), Duncan's multiple range test (DMRT). ANSE = aqueous neem seed extract.

4.2.3: Effect of aqueous neem seed extract on leaf diameter, leaf length and leaf area.

Plants infested with dark and lemon green morphs of *M. persicae* which were treated with synthetic pesticide had a higher mean leaf diameter followed by NeemAzal (Figure 4.5). However, for plant infested with the Dark green morphs only, the mean leaf diameter (2.56cm) upon treatment with 50% ANSE concentration were not significantly ($P > 0.05$) lower than the leaf diameter obtained upon treatment with Deltrin (2.50cm). However at highest concentration, the aqueous neem seed extract resulted in decreased leaf diameter for varieties infested with lemon and dark green morphs of *M. persicae* (Table 4.6).

Treatments amongst plants infested with Dark green morphs of *Myzus persicae* did not show significant ($P > 0.05$) increase in the mean leaf length of plants over Deltrin and Neemazal (Figure 4.6). In plants infested with lemon green morphs of *Myzus persicae*, the mean leaf lengths of pepper varieties treated with aqueous neem seed extracts were not significantly different from one another, however, these leaf length values were significantly ($P < 0.05$) lower than that obtained when treated with NeemAzal (4.51cm) and Deltrin, highest mean leaf length (5.54cm) was observed in plants treated with Deltrin (Figure 4.6).

Pepper varieties infested with lemon green morphs had lower leaf area (LA) when compared to those infested with dark green morph of *Myzus.* Among the ANSE treatments, 50% ANSE concentration resulted in highest leaf area of 7.52 cm^2, this was not significantly ($P > 0.0.5$) different from Neemazal (7.88 cm^2) but was significantly lower than that obtained with Deltrin (12.03cm^2). There was no significant difference between the leaf area values obtained for 100% ANSE and 75% ANSE concentrations in pepper varieties infested with lemon and dark green morphs respectively. In both infestations, greatest leaf area (12.03cm^2 for LG morphs and 10.68 cm^2 for DG morphs respectively) was obtained when plants were treated with Deltrin. Furthermore, treatment with 100% ANSE resulted in lowest mean leaf area (Figure 4.7) which was not significantly ($P > 0.0.5$) different from the leaf area of varieties treated with 75% ANSE.

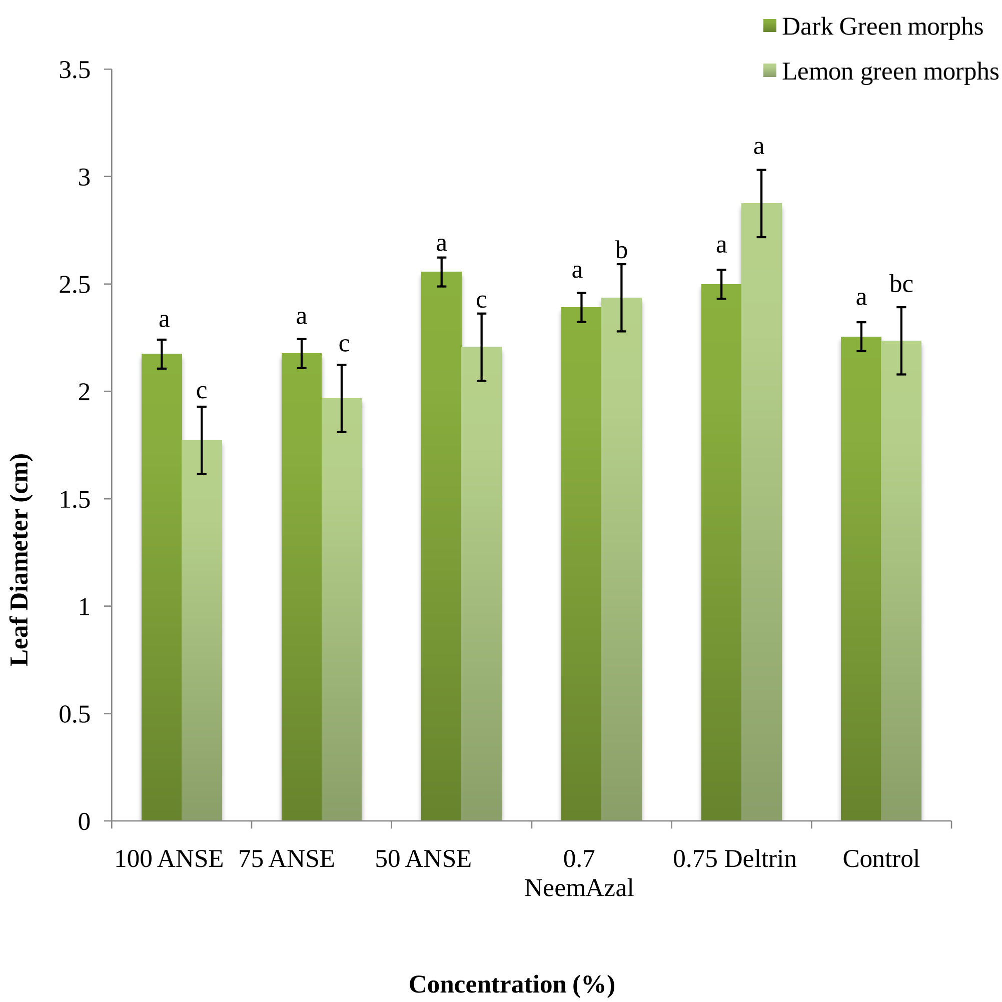

Figure 4.5: Effect of different concentrations of ANSE, NeemAzal and Deltrin on means of leaf diameter of plants infested with Lemon and Dark green morphs of *M. persicae.*

Means with different Duncan ratings (alphabets) are significantly different within morphs ($P < 0.05$). ANSE = aqueous neem seed extract.

Table 4.6: Effect of different concentrations of ANSE, NeemAzal and Deltrin on Leaf diameter (cm) of pepper varieties infested with Lemon green (LG) and Dark Green (DG) morphs of *Myzus persicae.*

		Leaf Diameter (cm)			
Concentrations (%)	Myzus Morphs	Danzagade	Kano T	California Wonder	Yolo Wonder
100 ANSE	LG	1.74 ± 0.31[c]	1.96±0.26[b]	1.42±0.24[c]	1.80±0.50[b]
	DG	2.15±0.26[a]	2.97±0.51[a]	2.69±0.39[a]	2.42±0.46[a]
75 ANSE	LG	1.81±0.35[c]	2.14 ±0.43[b]	2.12±0.22[b]	1.99± 0.23[b]
	DG	1.93±0.29[b]	2.27±0.28[bc]	2.60±0.46[a]	1.91±0.27[ab]
50 ANSE	LG	2.34±0.23[b]	2.20±0.25[ab]	1.99±0.14[b]	2.30±0.21[ab]
	DG	2.32±0.30[a]	2.43±0.43[b]	1.94±0.34[b]	2.01±1.98[b]
0.7 NeemAzal	LG	2.49±0.36[b]	2.13±0.31[b]	2.56±0.24[a]	2.57±0.24[a]
	DG	2.41±0.29[a]	2.35±0.25[b]	2.63±0.15[a]	2.19± 0.26[a]
0.75 Deltrin	LG	3.09±0.34[a]	2.88±0.40[a]	2.61±0.39[a]	2.94±0.52[a]
	DG	2.02±0.32[a]	3.22±0.79[a]	2.42±0.36[a]	2.35±0.28[a]
Control	LG	2.16±0.27[b]	2.52±0.29[a]	2.05±0.13[ab]	2.22±0.22[b]
	DG	2.36±0.35[a]	2.51±0.37[a]	2.00±0.21[b]	2.16±0.33[a]

Means with different superscripts within a column are significantly different ($P < 0.05$), Duncan's multiple range test (DMRT). ANSE = aqueous neem seed extract.

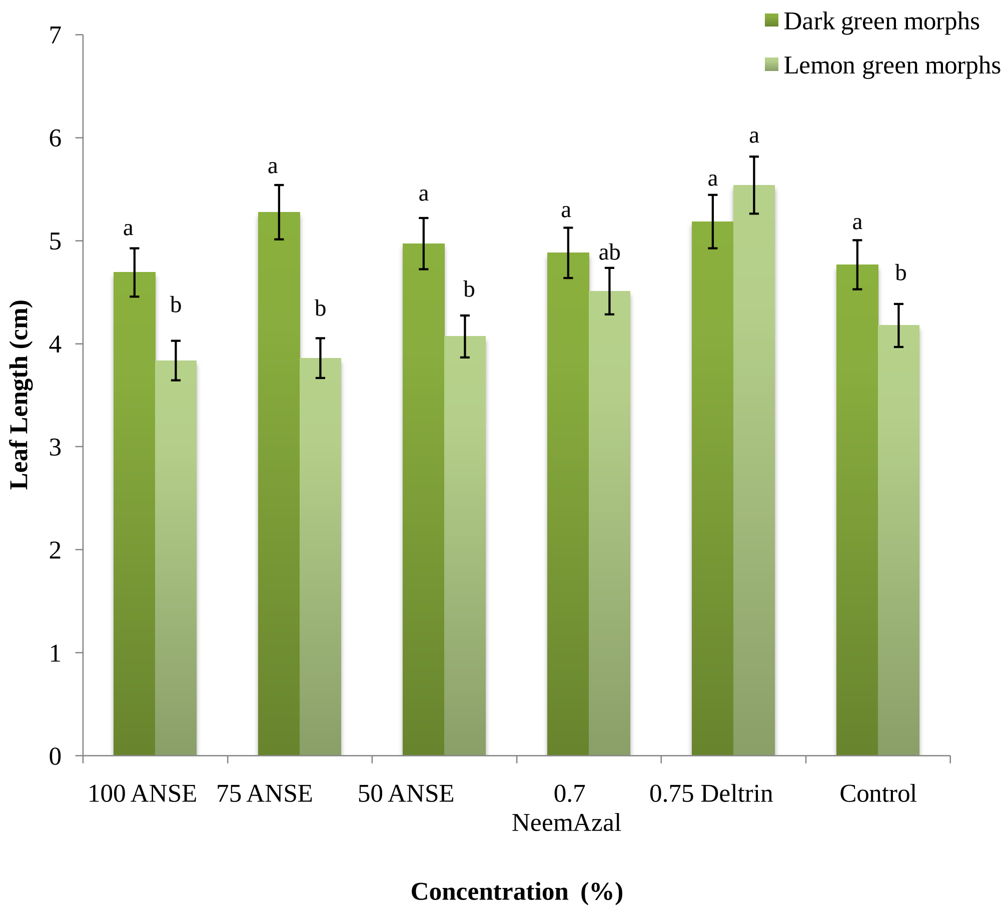

Figure 4.6: Effect of different concentrations of ANSE, Neemazal and Deltrin on means of leaf length of plants infested with Lemon and Dark green morphs of *M. persicae*.

Means with different Duncan ratings (alphabets) are significantly different within morphs (P< 0.05). ANSE = aqueous neem seed extract.

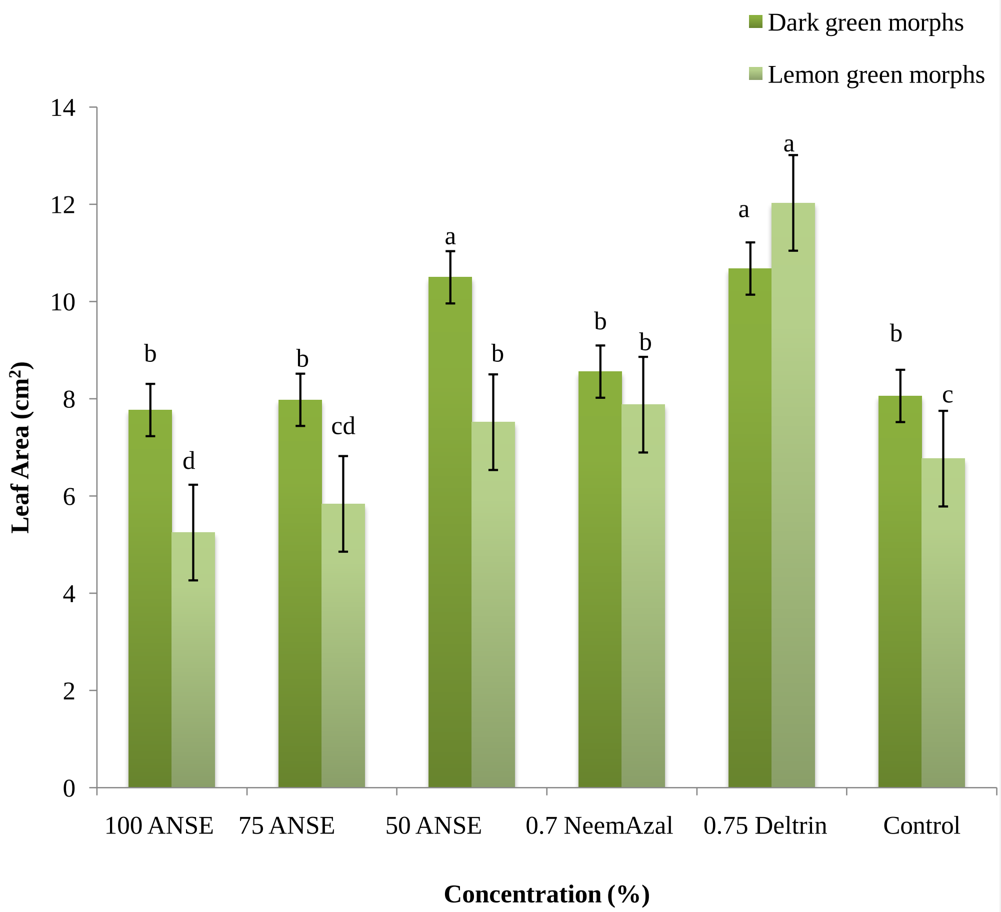

Figure 4.7: Effect of different concentrations of ANSE, NeemAzal and Deltrin on means of Leaf Area of plants infested with Lemon and Dark green morphs of *M. persicae.*

Means with different Duncan ratings (alphabets) are significantly different within morphs ($P < 0.05$). ANSE = aqueous neem seed extract.

4.2.4 Effect of aqueous neem seed extract on number of branches

The number of branches produced significantly ($P < 0.05$) differed amongst varieties and across treatments. Danzagade had the highest mean number of branches (Table 4.7) followed by Kano T, then California wonder and Yolo wonder. All varieties observed produced higher number of branches when treated with 50% ANSE except in California wonder infested with lemon green morphs of *M. Persicae*.

Lowest mean number of branches (1 branch per plant) was obtained for plants infested with lemon green morphs of *M. Persicae at* 100% ANSE concentration. 50% ANSE concentration presented the best values for branch numbers in both infestations (Figure 4.8).

Table 4.7: Effect of different concentrations of ANSE, NeemAzal and Deltrin on number of branches on pepper varieties infested with Lemon green (LG) and Dark Green (DG) morphs of *Myzus persicae*.

		Number of Branches			
Concentrations (%)	*Myzus* Morphs	Danzagade	Kano T	California wonder	Yolo Wonder
100 ANSE	LG	2.00 ± 0.28^{c}	1.00 ± 0.18^{d}	0.60 ± 0.15^{c}	0.40 ± 0.1^{b}
	DG	9.80 ± 0.59^{b}	10.40 ± 0.58^{a}	4.60 ± 0.41^{a}	3.40 ± 0.37^{b}
75 ANSE	LG	3.20 ± 0.27^{c}	5.60 ± 0.32^{b}	3.80 ± 0.23^{b}	3.60 ± 0.25^{a}
	DG	8.20 ± 0.45^{bc}	8.20 ± 0.39^{b}	2.40 ± 0.44^{b}	5.20 ± 0.29^{ab}
50 ANSE	LG	10.80 ± 0.25^{a}	10.80 ± 1.05^{a}	3.00 ± 0.57^{b}	2.80 ± 0.45^{a}
	DG	15.40 ± 0.52^{a}	8.00 ± 0.53^{b}	5.00 ± 0.32^{a}	7.40 ± 0.29^{a}
0.7NeemAzal	LG	11.80 ± 0.37^{a}	4.00 ± 0.33^{b}	0.40 ± 0.31^{c}	1.20 ± 0.33^{b}
	DG	7.60 ± 0.80^{c}	6.80 ± 0.32^{b}	4.40 ± 0.1^{a}	6.60 ± 0.3^{a}
0.75 Deltrin	LG	6.60 ± 0.28^{b}	3.00 ± 0.32^{c}	7.24 ± 0.24^{a}	1.20 ± 0.27^{b}
	DG	7.40 ± 0.64^{c}	9.60 ± 0.49^{a}	4.60 ± 0.27^{a}	3.20 ± 0.49^{b}
Control	LG	2.40 ± 0.41^{c}	4.00 ± 0.30^{b}	4.20 ± 0.39^{b}	2.20 ± 0.21^{a}
	DG	7.20 ± 0.60^{c}	6.00 ± 0.60^{bc}	5.00 ± 0.39^{a}	2.80 ± 0.31^{b}

Means with different superscripts within a column are significantly different ($P<0.05$), Duncan's multiple range test (DMRT). ANSE = aqueous neem seed extract.

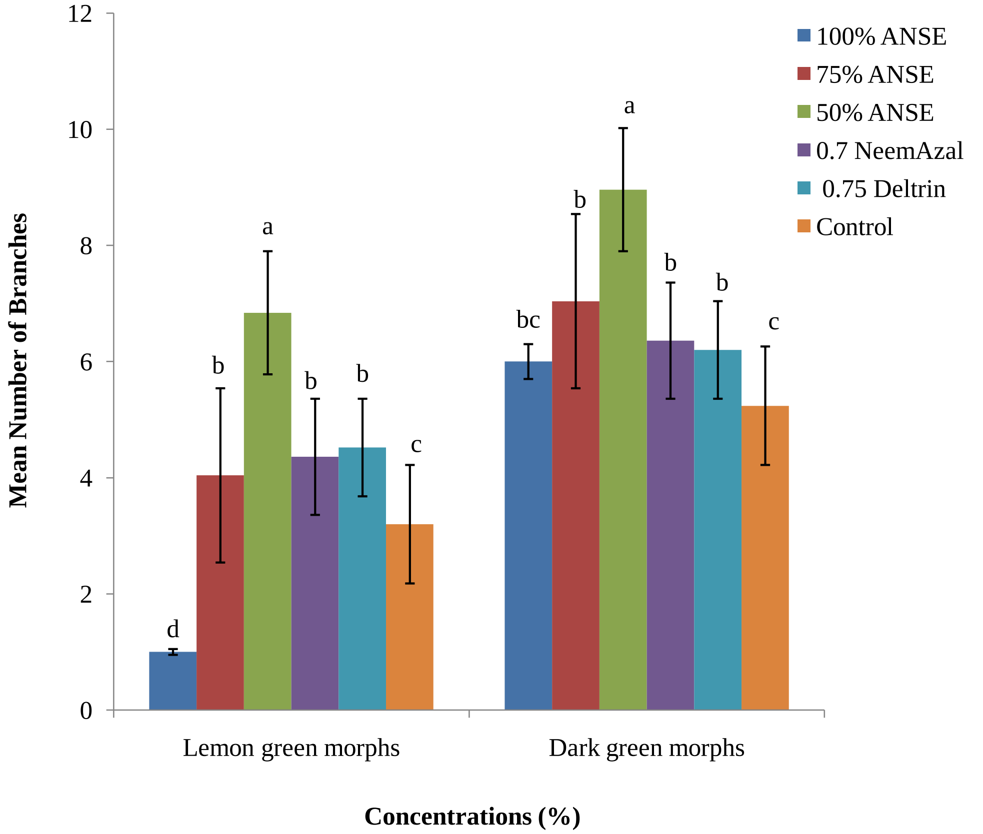

Figure 4.8: Effects of different concentrations of ANSE, NeemAzal and Deltrin on number of branches produced by plants infested with Lemon and Dark green morphs of *Myzus persicae.*

Means with different Duncan ratings (alphabets) are significantly different within morphs (P < 0.05). ANSE = aqueous neem seed extract.

4.2.5 Effect of aqueous neem seed extract on flowering of pepper plants.

Pepper varieties infested with dark green morphs of *Myzus persicae* produced significantly ($P < 0.05$) higher number of flowers when treated with 50% ANSE concentration (19 flowers per plant) and NeemAzal (16 flowers per plant), followed by Deltrin and 75% ANSE concentration (Figure 4.9). Least flower production was obtained when pepper plants were treated with 100% concentration of ANSE which was significantly different from that obtained with the control for Dark green morphs infested plants. In plants infested with lemon green morphs, treatment with Deltrin produced the highest (8 flowers) number of flowers per plant. There was no significant difference between the number of flowers produced in 75% ANSE, 50 % ANSE and Neemazal treated plots. In addition flower production in 100% ANSE treated plots was not significantly different from that obtained with the control (Figure 4.9).

Among the pepper variety Danzagade and Kano T responded better to the ANSE treatments, highest number of flower production was observed when Danzagade was treated with 75% concentration of ANSE and NeemAzal. Yolo wonder produced more flowers than California wonder but this was not significantly different (Table 4.8).

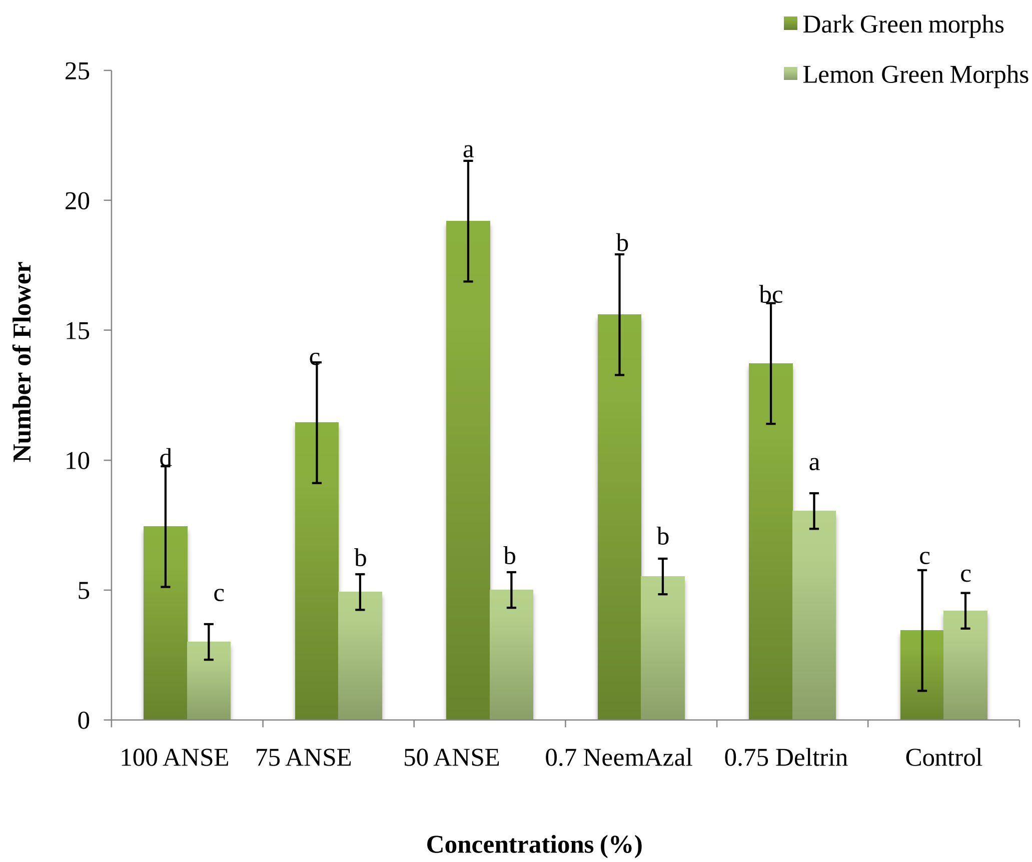

Figure 4.9: Effect of different concentrations of ANSE, NeemAzal and Deltrin on number of flower produced by plants infested with Lemon and Dark green morphs of *M. persicae.*

Means with different Duncan ratings (alphabets) are significantly different within morphs ($P < 0.05$). ANSE = aqueous neem seed extract.

Table 4.8: Effect of different concentrations of ANSE, NeemAzal and Deltrin on number of flowers produced by pepper varieties infested with Lemon green (LG) and Dark Green (DG) morphs of *Myzus persicae*.

		Number of Flower			
Treatment Concentrations (%)	Myzus Morphs	Danzagade	Kano T	California wonder	Yolo Wonder
100 ANSE	LG	4.20 ± 0.76^{c}	5.60 ± 0.52^{b}	2.00 ± 0.19^{bc}	2.00 ± 0.24^{bc}
	DG	8.20 ± 0.69^{d}	13.60 ± 0.92^{b}	5.40 ± 0.53^{bc}	5.00 ± 0.38^{c}
75 ANSE	LG	16.20 ± 1.36^{a}	0.60 ± 0.07^{c}	3.20 ± 1.04^{b}	11.40 ± 0.98^{a}
	DG	27.60 ± 0.64^{a}	11.20 ± 0.54^{c}	4.00 ± 0.18^{c}	19.40 ± 0.10^{a}
50 ANSE	LG	5.40 ± 0.98^{c}	4.80 ± 1.83^{b}	1.40 ± 0.25^{c}	0.40 ± 0.06^{d}
	DG	14.40 ± 0.79^{c}	8.60 ± 0.84^{c}	12.40 ± 0.05^{a}	10.40 ± 0.18^{b}
NeemAzal	LG	8.80 ± 1.88^{b}	9.40 ± 0.94^{ab}	0.20 ± 0.35^{d}	1.20 ± 0.48^{c}
	DG	26.40 ± 1.19^{a}	20.20 ± 0.15^{a}	10.60 ± 0.34^{ab}	19.40 ± 0.00^{a}
Deltrin	LG	7.20 ± 1.70^{b}	11.60 ± 1.16^{a}	7.00 ± 0.46^{a}	1.40 ± 0.07^{c}
	DG	17.80 ± 0.33^{b}	14.60 ± 0.93^{b}	3.60 ± 0.49^{c}	5.00 ± 0.21^{c}
Control	LG	2.00 ± 1.35^{d}	5.20 ± 0.92^{b}	0.80 ± 0.19^{d}	3.60 ± 1.47^{b}
	DG	14.40 ± 0.84^{c}	12.00 ± 0.47^{bc}	9.40 ± 0.12^{b}	8.00 ± 0.31^{bc}

Means with different superscripts within a column are significantly different ($P<0.05$), Duncan's multiple range test (DMRT). ANSE = aqueous neem seed extract.

4.2.6 Effect of aqueous neem seed extract on fruiting of pepper plants

All plant infested with aphids showed reduced fruit set from flowers. However, highest fruiting was observed on plants infested with dark green morphs of *M. persicae* and treated with 50% ANSE. Poor fruiting was observed when varieties infested with lemon and dark green morphs of *Myzus persicae* were treated with tap water (control) (Figure 4.10). Similarly, very poor fruiting was observed when plants were infested with lemon green morphs of aphids, although, there was no significant difference in the number of fruit produced amongst the treatments this however was significantly higher than that obtained in tap water treated plots. The yield of pepper varieties infested with dark green morphs of *M. persicae* was significantly ($P < 0.05$) higher than that obtained from varieties infested with lemon green morphs of the aphids.

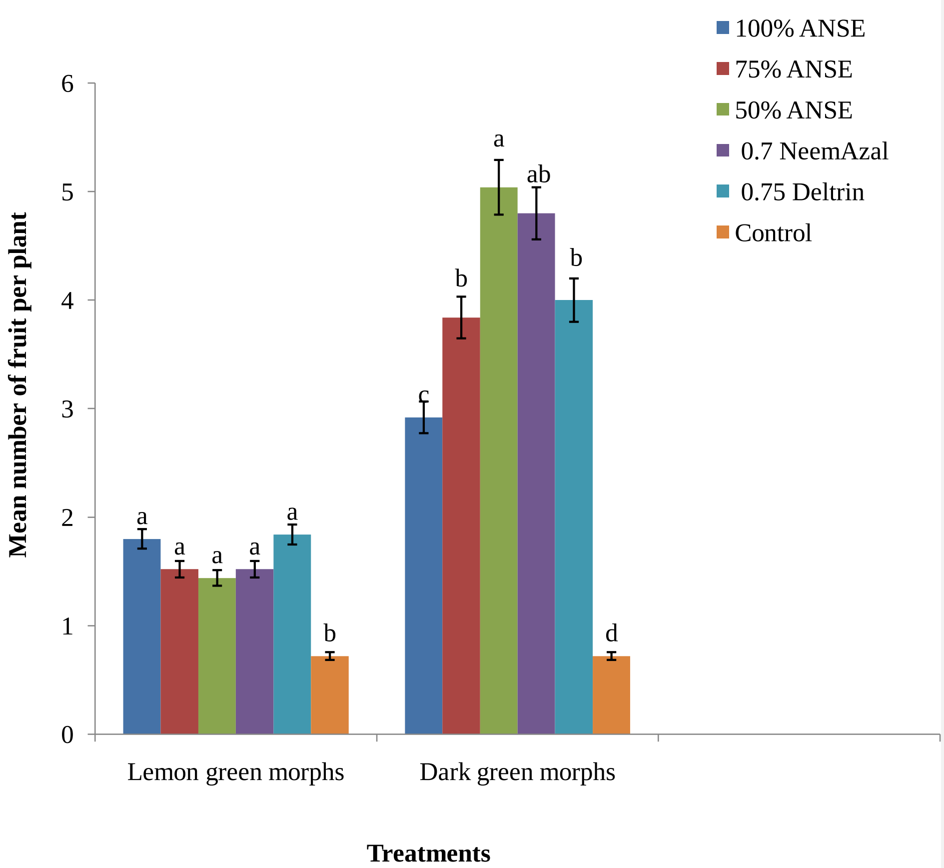

Figure 4.10: Effect of different concentrations of ANSE, NeemAzal and Deltrin on number of fruits produced by plants infested with Lemon and Dark green morphs of *M. persicae*.

Means with different Duncan ratings (alphabets) are significantly different within morphs ($P < 0.05$). ANSE = aqueous neem seed extract.

Table 4.9: Effect of different concentrations of ANSE, NeemAzal and Deltrin on mean number of fruits produced by pepper varieties infested with Lemon green (LG) and Dark Green (DG) morphs of *Myzus persicae*.

		Number of Fruits			
Concentrations (%)	Myzus Morph	Danzagade	Kano T	California wonder	Yolo Wonder
100ANSE	LG	0.60±0.00 [b]	0.80±0.00 [b]	0.80±0.00 [c]	1.00 ± 0.31[b]
	DG	0.00 ± 0.08[e]	1.60 ± 0.09 [c]	0.00 ±0.12[d]	2.80 ± 0.12[c]
75ANSE	LG	0.00 ±0.29 [b]	0.80 ± 0.34[b]	0.80 ± 0.37 [c]	0.40 ± 0.31[c]
	DG	2.20 ± 0.00[c]	3.80±0.09[b]	5.60 ± 0.12[a]	3.20± 0.10[c]
50ANSE	LG	0.60±0.31b	0.40 ±0.48 [b]	0.40±0.37[cd]	0.40±0.22 [c]
	DG	6.80 ± 0.11[a]	5.20± 0.07[a]	1.60 ± 0.07[c]	6.60 ± 0.10[a]
0.7 NeemAzal	LG	0.80 ± 0.39 [b]	0.80 ± 0.32[b]	0.20 ± 0.36 [d]	0.20 ± 0.36 [c]
	DG	5.40 ± 0.16[b]	4.00 ± 0.12[ab]	4.80 ± 0.05[a]	4.80 ± 0.05[b]
0.7Deltrin	LG	3.20 ± 0.53[a]	4.40 ± 0.35[a]	3.40 ± 0.37[a]	4.80 ± 0.20[a]
	DG	7.60 ± 0.07[a]	3.20 ± 0.15[b]	3.40 ± 0.05[b]	1.00 ± 0.07[d]
Control	LG	0.60 ± 0.32 [b]	0.00 ± 0.34[c]	1.00 ± 0.39[b]	0.40 ± 0.39[c]
	DG	0.40± 0.11 [d]	1.60 ± 0.15[c]	0.20 ± 0.12[d]	0.40 ± 0.07[e]

Means with different superscripts within a column are significantly different from each other ($P<0.05$), Duncan's multiple range test (DMRT). ANSE = aqueous neem seed extract.

4.3 Effect of Aqueous Neem Seed Extract on Leaf Curling and Crinkling

The leaves of plants curled inwards and showed marked crinkling which was usually associated with stunting, defoliation and chlorosis of the leaves. The curling symptoms appeared in all treatments before and after flowering. On dark green morph infested plants, severity of curled leaves was significantly ($P > 0.05$) lower amongst the treatments. Lowest number of curled leaves was observed in plants treated with the ANSE. Amongst the aqueous neem seed extracts higher number of curled leaves was observed in plants treated with 50% ANSE and 75% ANSE concentration (Figure 4.11). Highest incidence of curled leaves was observed in plants treated with tap water alone. Significant ($P < 0.05$) number of leaf crinkling was also observed in plants treated with NeemAzal and Deltrin. Amongst the pepper varieties leaf curling was more severe in California wonder and Yolo wonder, lowest incidence of leaf curling was observed in Danzagade, similar trend was observed in pepper varieties infested with lemon green morphs of *M. persicae* (Table 4.10).

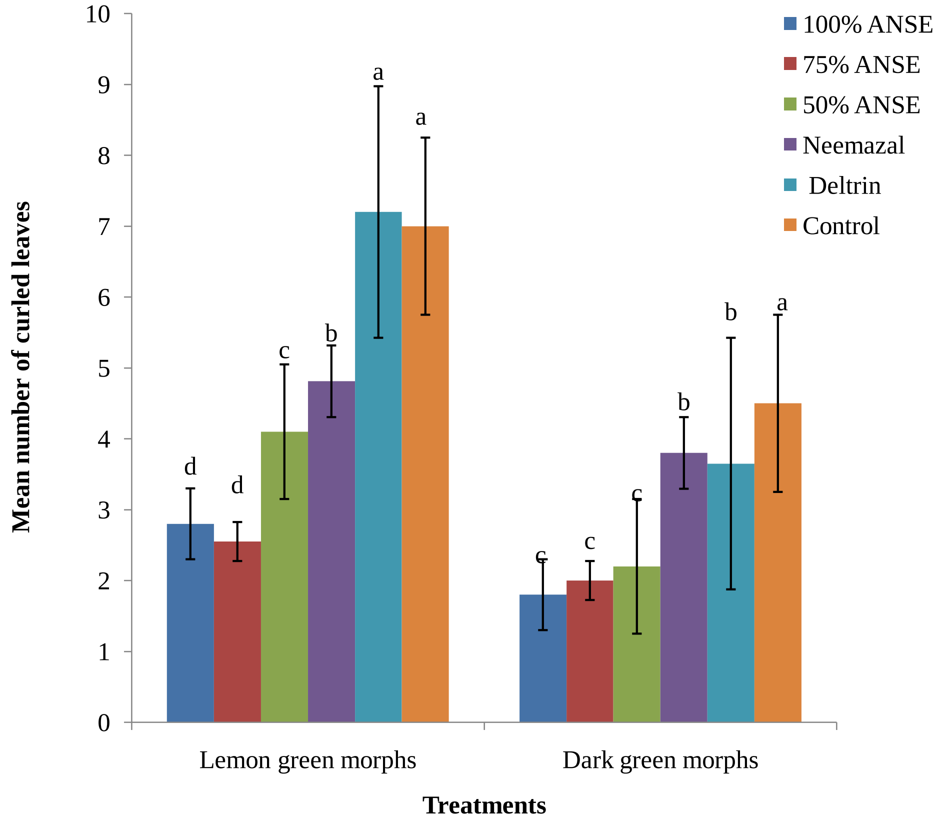

Figure 4.11: Effect of different concentrations of Aqueous NSE, NeemAzal and Deltrin number of curled leaves in plants infested with Lemon and Dark green morphs of *M. persicae*.

Means with different Duncan ratings (alphabets) are significantly different within morphs ($p < 0.05$). ANSE = aqueous neem seed extract.

Table 4.10: Effect of different concentrations of ANSE, NeemAzal and Deltrin on number of curled leaves on pepper varieties infested with Lemon green (LG) and Dark Green (DG) morphs of *Myzus persicae.*

		Number of Curled leaves			
Concentrations (%)	Myzus Morphs	Danzagade	Kano T	California wonder	Yolo Wonder
100 ANSE	LG	2.00 ±0.21 [bc]	1.00 ± 0.35[a]	2.40 ± 0.20[b]	4.80 ± 0.22[a]
	DG	1.00 ± 0.14[bc]	0.50 ± 0.02[b]	1.00 ± 0.14[c]	2.80 ± 0.14[bc]
75 NSE	LG	1.52 ± 0.30[c]	2.10 ± 0.17[a]	3.00 ± 0.20[b]	1.40 ± 0.18[c]
	DG	1.50 ± 0.33[b]	1.00 ± 0.05[ab]	2.80 ± 0.27[c]	4.20 ± 0.17[b]
50NSE	LG	3.00 ± 0.18[c]	1.70 ± 0.41[a]	5.80 ± 0.27[ab]	3.80 ± 0.23[b]
	DG	1.40 ± 0.80[b]	0.40 ± 0.31[b]	6.80 ± 0.12[b]	2.80 ± 0.17[bc]
0.7 NeemAzal	LG	3.50 ± 1.45[b]	1.60 ± 0.65[a]	7.00 ± 0.12[a]	4.20 ± 0.14[a]
	DG	2.80 ± 1.45[a]	1.60 ± 0.65[a]	6.80 ± 0.17[b]	3.80 ± 0.14[b]
0.75 Deltrin	LG	3.90 ± 0.23[b]	0.40 ± 0.28[b]	2.80 ± 0.22[b]	4.40 ± 0.27[a]
	DG	0.80 ± 0.15[c]	0.60 ±0.30[b]	0.60 ± 0.18[d]	2.00 ± 0.20[c]
Control	LG	4.80± 083[a]	2.40 ± 0.57[a]	9.00 ± 0.26[a]	5.00 ± 0.32[a]
	DG	1.00 ± 0.67[bc]	2.00 ± 0.28[a]	17.4 ± 0.31[a]	7.00 ± 0.34[a]

Means with different superscripts within a column are significantly different (P<0.05), Duncan's multiple range test (DMRT). ANSE = aqueous neem seed extract.

4.4 Response of Pepper Varieties to Curling, Stunting and Fruiting.

During the vegetative and reproductive development pepper varieties investigated showed different degree of severity to leaf curling and stunting which subsequently affected fruiting. The dendogram (Figure 4.12) revealed two (2) main groups and four (4) sub groups on bases of response of the pepper varieties to chlorosis, stunting and leaf curl incidence. The first main group consisted of all pepper varieties infested with dark green morphs of *Myzus persicae* while the second main group consisted of all pepper varieties infested with Lemon green morphs of *Myzus persicae*. California wonder (3) and Yolo wonder (4) infested with lemon green morphs showed similar response to chlorosis, stunting, curling and fruiting and were grouped together while the second sub group comprised of Dazangade (1) and Kano-T (2) infested with lemon green morphs. Similar grouping was observed in pepper varieties infested with dark green morphs of *Myzus persicae* with California wonder (7) and Yolo wonder (8) infested with dark green morphs grouped together and the last group consisting of Dazangade (5) and Kano-T (6) infested with dark green morphs (Figure 4.12).

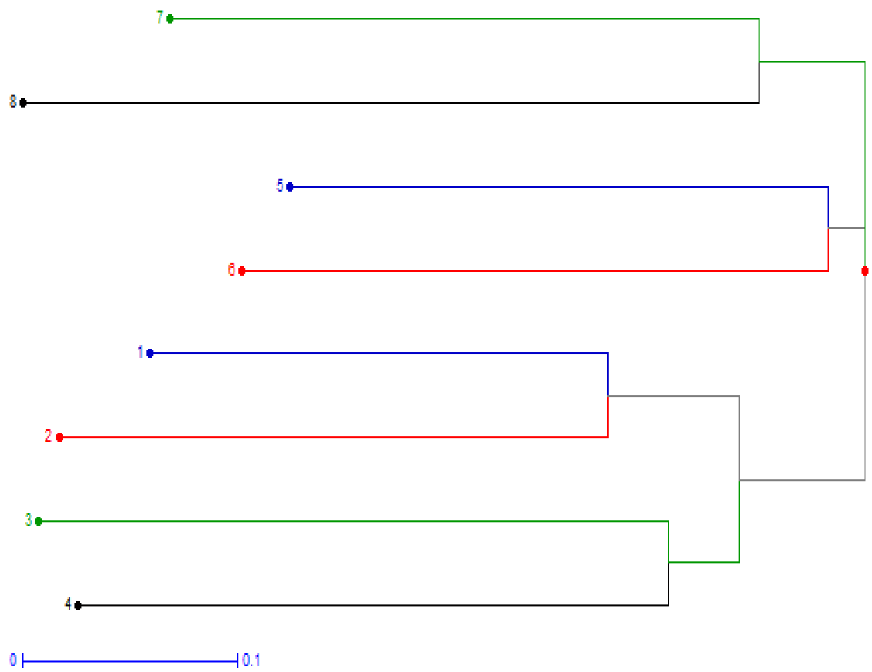

Figure 4.12: Response of pepper varieties infested with lemon and dark green morphs of *Myzus persicae* to disease symptoms.

Key: Green (3 and 7) = California wonder infested with L.G and D.G morphs respectively; Black (4 and 8) = Yolo wonder infested with L.G and D.G morphs respectively; Blue (1 and 5) = Danzagade infested with L.G and D.G morphs respectively; Red (2 and 6) = Kano T infested with L.G and D.G morphs respectively.

4.5 Correlation Analysis

Pearson correlation coefficient test to assess the relationship between plant growth parameters and duration of exposure to treatment (Table 4.11) revealed a strong positive correlation amongst the plant growth parameters. Leaf length positively correlated (r = 0.62) with leaf diameter, leaf area also correlated positively with leaf diameter (r = 0.09) and leaf length (r = 0.84). Number of Fruits (FF) showed a positive correlation (r = 0.64) with the number of flowers (NF). The duration of exposure were weakly and progressively correlated (r = 0.13, r = 0.18, r = 0.21 and r = 0.22) with the number of curled leaves (NC) at 24hrs, 48hrs 72hrs and 168hrs respectively. The number of fruits was also weakly associated (r = 0.11, r = 0.15 and r = 0.15) with the duration of exposure at 24hrs, 72hrs, 120hrs and 168 hours after treatment application respectively.

Table 4.11: Correlation coefficient among plant growth parameters and number of *M. persicae* with duration of exposure to treatment

	PH	LD	LL	LA	NL	NB	NC	NF	FF	24HR	72HR	120HR	168HR
PH	1.00												
LD	0.13*	1.00											
LL	0.27*	0.62*	1.00										
LA	0.23*	0.90*	0.84*	1.00									
NL	0.55*	0.20*	0.25*	0.23	1.00								
NB	0.49*	0.09	0.14	0.12	0.57*	1.00							
NC	0.35*	0.09*	0.10*	0.09*	0.39*	0.39*	1.00						
NF	0.15*	0.30*	0.16*	0.24*	0.40*	0.34*	0.21*	1.00					
FF	0.12	0.19*	0.07	0.12	0.23*	0.29*	0.16*	0.64*	1.00				
24HR	0.05*	-0.07	-0.06	-0.09	0.11	0.1	0.13*	0.06	-0.01	1.00			
72HR	0.11	-0.04	-0.06	-0.06	0.12	0.14*	0.18*	0.08	0.11*	0.54*	1.00		
120HR	0.17*	0.02	0.01	0.01	0.18*	0.18*	0.21*	0.12	0.15*	0.49*	0.69*	1.00	
168HR	0.19*	0.1	-0.01	-0.04	0.19*	0.20*	0.22*	0.09	0.15*	0.49*	0.65*	0.81*	1.00

PH- plant height, LD- leaf diameter, LL- Leaf Length, LA- Leaf area, NL- Number of leaves, NB- Number of Branches, NC- number of curled leaves, NF- Number of flower, FF- fruit from Flower

CHAPTER FIVE

5.0 DISCUSSION

Aqueous neem seed extract had a high aphicidal efficacy in the control of both lemon and dark green morphs of *Myzus persicae*. It can be assumed that the high mortality of aphids was due to the various active components contained in the extract, the doses and processing time of the aphids. An increase in percentage reduction of the aphid morphs with increasing time of exposure to ANSE could be attributed to its diverse effects which were initially via contact action and might have latter acted in a systemic manner. Lowery and Isman (1993) reported that crude formulations of neem seed extracts contain limonoids that contribute to it insecticidal properties.

The differential effects of ANSE concentrations against lemon and dark green morphs of *Myzus persicae* could also be attributed to the differences in the concentration of active ingredient in the aqueous extract. An increase in the concentration of the aqueous neem seed extract resulted in an apparent decrease in aphid density and in turn, high reduction percentages. Hellpap and Dreyer (1995) reported that crude extract if neem seeds these have been tested in the fields against several pests in tropical and subtropical countries and their efficacy has been found to be satisfactory to excellent. Lowery *et al.* (1993) proved the effectiveness of neem seed oil against aphids and found up to 50% reduction with 0.4% neem oil in the field conditions, also, Dimetry *et al.* (1996) reported that the percentage reduction in population of whitefly larvae reached maximum level one hour after treatment with different formulation of neem seed extract. Wondafrash *et al.* (2012)

reported that water extracts of neem seed and leaf have significant weight reduction effect on the larvae and pupae of *Helicoverpa armigera*.

The better aphicidal efficacy showed by ANSE as compared to Neemazal could be as a result of the high concentration of the extract used (0.2g/ml). In addition to the concentration levels, products containing azadirachtin in combination with other ingredient (for example Nimbin and Salanin) which have been reported to be present in water extract of neem exhibit better aphicidal activities than either ingredients alone (Stark and Walter, 1995). Boursier *et al.* (2011) reported that the presence of other terpenoids in crude neem seed extract could act as insecticides and or improve the effectiveness of azadirachtin through a synergistic effect of co-extracted compounds. The observation in our study was contrary to the findings of Kelany *et al.* (1997) who reported that NeemAzal formulation was more effective than neem kernel powder and aqueous neem kernel powder extract when used as surface protectants against potato tuber moth, the contrary observation in their study might have been due to a lower concentration of stock solution of the aqueous extract.

The lower aphid kill recorded in Neemazal as compared to ANSE for both lemon and dark green morphs of *M. persicae* could also be attributed to the high temperature prevailing in the study area, which might have enhanced the rapid degradation of azadirachtin which is the active ingredient in Neemazal. Azadirachtin, the major active ingredient in neem is photo and thermo labile and it easily degrades under light and high temperature condition (Wondafrash, 2007). El-Wakiel and Saleh (2009) reported that NeemAzal-T/S increased the mortality of *M. persicae* populations compared with the control and with aphid population before treatment, their results indicate that 88.8%

initial kill of aphids was achieved by NeemAzal-T/S 24hrs after the first spray. This observation was in line with the study of Metspalu *et al*. (2000) who reported that aphids are very susceptible to the contact action of NeemAzal T/S seemingly due to their thin cuticle and that the mode of action was via toxicity, repellant and deterrent mode of action. It was evident that the aqueous neem seed extract initially acted as a contact pesticide and later as a systemic pesticide which subsequently led to the death of aphids, thus a decrease in aphid population over time. The diverse effects of aqueous neem seed extract on insect pests include feeding deterrence, reproduction disturbance and insect growth regulation among others (Mordue and Blackwell 1993).

The Lemon and dark green morphs of *Myzus* responded in similar pattern to the ANSE. The similarity in the response of the aphids might have been due to their common ancestry, although no molecular studies were carried out to determine the differences in their genome.

The lower number of Lemon and Dark green morphs recorded on ANSE treated plots as the time of observation increased might have been due to the systemic actions of ANSE concentrations one of which could have been developmental inhibition of the nymphs which may have been caused by the ingestion of azadirachtin through the leaves. Azadirachtin inhibits the activity of ecdysone-20-monooxygenase, which converts ecdysone into 20-hydroxyecdysone, the active form of insect-molting hormone in the hymolymph (Ohn Mar Lynn *et al*., 2010). Ingestion of azadirachtin by adult females can reduce the molting hormonal level, which results in a delay or blockage of the growth and development (Morgan, 2009). This could also explain the reduced number of new instars in ANSE treated plots.

An increase in the number of aphids on plant treated with Deltrin at 168hours after treatment can be attributed to the development of resistance amongst the Lemon green morphs and or the failure of the insecticide to act systemically or as a reproduction deterrent. Foster *et al.* (2003) reported that some clones of *Myzus persicae* (926B) used in their study were still alive and reproducing 7 days after treatment with the full recommended dose of imidacloprid (2mg per plant) this demonstrates a potential for aphids with this level of tolerance to withstand field applications. Kerns *et al.* (1997) reported that red and green morphs of green peach aphids show only slight differences in their susceptibility to foliar and systemic insecticides; they also reported that Red-colored aphids were consistently more resistant to dimethoate and lambda-cyhalothrin, and are usually more resistant to endosulfan than green-colored aphids collected from the same field. This was partially in line with the finding of this study where Lemon green morphs appeared to be more tolerant to Deltrin than the Dark green morphs. Morphs of different colours can be found in a number of aphid species (Weisser and Braendle, 2001) but in contrast to wing polyphenism, little is known about the adaptive significance of aphid body colour (Kerns *et al.,* 1998). Weisser and Braendle (2001) reported that body colour was not significant in fecundity and production of winged form among pea aphids (*Acyrthosiphon pisum*).

The low proliferation rate of *Myzus persicae* observed in all the treatment during the first three sprays could have been as a result of the consistent heavy rainfall in the surrounding environs which might have been unfavourable to reproduction. Lawrence (1977) reported that low mean population of aphids recorded during the wet month as compared to that during the dry month indicates that heavy rainfall is an important factor in regulating the field population of *M. persicae* and was observed to have marked adverse effect on the aphid population. This was not in total agreement with the findings of Fajinmi *et al*. (2011b) who reported that rainfall may not be the reason for a sudden decline in aphid populations per leaf in agro- ecological zones around Nigeria, but little cover, high temperature, low humidity and the long dry season might have caused aphids to die as a result of non-availability of food.

A differential preference of *capsicum* cultivars by *Myzus persicae* could be attributed to the genetic makeup and differences in the growth pattern which might have resulted in the differential mechanisms of resistance either by antibiosis, antixenosis or tolerance. Danzagade best supported the growth of both morphs of *Myzus persicae*, this could be as a result of its branching pattern and more number of leaves which serves as a canopy and hide out for the aphids against pesticides and predators, this is evident from the strong positive correlation obtained between the number of leaves and the number of insect found on the pepper plant, a positive correlation was also observed between number of branches and number of aphids on the plant. This was in agreement with the findings of Fajinmi *et al.* (2011a) who reported that several studies shows that sparse vegetation cover as well as overcrowding has been reported to have impact on aphid population. Hegab *et al.* (2014) reported that pepper cultivars have pronounced effect on the number of *M. persicae*; they also reported in their study that yellow California and red California variety of sweet pepper had the lowest number of aphids. Lower number of aphid observed in California wonder and Yolo wonder variety of sweet pepper could be as a result of the less branching pattern observed in the cultivars in this study.

Reductions in growth parameters observed in these two cultivars might have been due to the fact that they are readily susceptible to a wide range of viral diseases in which *Myzus Persicae* serves as an important vector. This might have reflected in the high number of curled leaves, plant stunting and chlorosis observed in them. Donaldson and Gratton (2007) reported that aphid density and performance on uninfected soybean plants was nearly double that in virus infected plants.

Highest concentration of ANSE resulted in visible leaf burns of pepper varieties thereby causing premature wilting; this was accompanied by defoliation of the affected leaves. This could possibly explain the lower mean number of leaves observed in plant treated with 100% ANSE. This observation was in line with the findings of Olaifa and Adenuga (1988) who reported that neem products caused yellowing and subsequent shedding of leaves of cassava plants. Appiagyei (2010) also observed that when aqueous neem kernel extract was applied as foliar spray, the leaves of some tomato plant showed symptoms of burning and this caused some of the leaves to wilt prematurely four days after application. The phytotoxic effect observed in the current study might have been due to the high concentration of the neem seed extract. Abassi *et al.* (2003) also reported phytotoxic effect of neem oil in green house grown pepper plants.

Improved growth performance observed with 50% ANSE followed by 75% ANSE concentrations could be attributed with low toxicity of ANSE constituents at those concentrations. Rouf and Sardar (2011) reported a 68. 49% increase in pod yield of beans plants when treated with neem seed extract applied 150g/l at 7 days intervals. Gupta *et al.* (2005) mentioned that the mean grain yield of mustard was higher in the treatment of neem compared to control. Similar results obtained by Alabi and Olorunju (2004), stated that groundnut plants sprayed with neem seed extract gave higher yields than the plants that received other treatments (black soap and cow dung), apart from the un-treated plants.

The poor growth of pepper cultivar infested with Lemon green morphs of *Myzus*, might have been due to their acquisition of viral disease at an early stage, although symptoms of viral infection was observed in plant infested with Dark green morphs of *M. persicae* but this appeared only at a later stage when plants where almost at reproductive stage. The lemon green morphs of the aphids could be said to be deleterious to the growth and development of pepper than the dark green morphs, this was reflected in the higher incidence of aphids, stunted growth, poor fruiting and lastly curling of leaves. These was in contrast to the finding of Gillespie *et al.* (2008) who reported that the darker green morphs of *Myzus persicae* were more associated with outbreaks and are capable of causing death of growing plant tips in green house pepper than the lighter green and red morphs investigated.

Among the pepper varieties highest yield was obtained in Danzagade. It was characterized by production of high number of leaves, flowers and branches. The greater the numbers of branches that bear more number of leaves, the higher the photosynthetic capacity and hence assimilate production. These are consequently translocated to sink and hence higher yield (Yahaya and Gaya, 2012).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

A field cage experiment was conducted to determine the aphicidal efficacy of aqueous neem seed extract in the control of two colour morphs of *Myzus persicae* and its effect on agronomic performance of four selected pepper varieties. Separate experimental plots were set up for the aphid morphs. Lemon and Dark green morphs of *Myzus persicae* individually cultured and maintained on susceptible pepper cultivars were used to infest the test pepper varieties after transplant.

The experiment was laid out in a randomized complete block design (RCBD) consisting of six treatments, three replications and four varieties of pepper. The pre-infested pepper varieties were sprayed with three concentrations of ANSE at 0.2g/ml (100%, 75% and 50%) and their efficacy on aphid morphs mortality was assessed at 24hr, 48hr, 120hr and 168hr after treatment application. Plant mophometrics in ANSE treated plots were compared to Neemazal and deltrin treated plots which severed as positive Biopesticide check and synthetic pesticide check respectively. Plots treated with tap water alone served as the control.

The present study indicates a high percentage mortality of aphid morphs in the treated plots as compared to tap water treated plots. At 168hr after treatment application 100% ANSE (94.69%), 75% ANSE (96.52%) and 50% ANSE (96.34%) showed better aphicidal efficacy than Deltrin (85.90%) when used as aphicide on lemon green morphs infested pepper varieties. There was no significant ($p > 0.05$) difference in the percentage mortality of aphid morphs on pepper plants treated with 100%, 75% and 50% ANSE concentrations, however, the ANSE concentrations resulted in higher percentage mortality than Neemazal for both morph infestation. No case of pest resurgence was observed with ANSE treatments as compared with Deltrin.

Comparison of plant growth and yield mophometrics between lemon green and dark green morphs infested pepper varieties showed that the lemon green morphs of *Myzus persicae* was most deleterious to the pepper cultivars than the dark green morphs, this was reflected in poor fruiting, higher incidence of curled leaves and stunted growth of the studied pepper varieties. Crinkling and inward curling of pepper leaves which is associated with presence of viral disease symptoms on plants were found to be within the range of an average of 2-4 curled leaves in ANSE and Neemazal treated plots as compared to Deltrin and tap water treated plots.

Pepper fruiting was observed to be low when plants were infested with lemon green morphs of aphids, although, there was no significant difference in the number of fruit produced amongst the treatments (two (2) pepper fruits per plant) this however was significantly higher than that obtained in tap water treated plots (one fruit per plant).

An increase in the concentration of aqueous neem seed extract increase to 100% resulted in lower number of leaves, reduced leaf area, reduced number of flowers and fruits in both lemon green and dark green morph infested plants. The reduced yield obtained with 100% ANSE treated plots was associated with the phytotoxic effect on the leaves of plants which resulted in premature wilting and senescence and hence reduced the growth and yield of the pepper varieties.

For plants infested with dark green morphs of *M. persicae*, optimum growth and fruit production of about five (5) fruits per plant was obtained with 50% ANSE concentration followed by Neemazal, 75% ANSE, Deltrin, 100% ANSE and lastly tap water. 50% ANSE resulted in a 66.70% increase in fruit yield over tap water treated plots and 11.12% increase over deltrin, while for LG morph infestation, yield of pepper treated with 50% ANSE increased by 33.34% over tap water treated plots. There was no significant difference in the number of fruits produced in 75% ANSE treated plots and Deltrin treated plots. The improved growth and yield achieved with 50% and 75% ANSE concentration as compared to 100% ANSE could be attributed with low toxicity of ANSE constituents at these concentrations.

The efficacy of aqueous neem seed extracts in reducing population of *Myzus persicae* increased with increasing concentration and time of exposure. However, the growth and yield parameters were best with 50% ANSE.

6.2 CONCLUSIONS

1. Pepper varieties infested with Dark green morphs of *Myzus persicae* showed better growth and yield mophometric. Lemon green morphs of the *Myzus persicae* were more detrimental than the dark green morphs and posses economic threat to yield loss in the cultivation.

2. Optimum growth and yield of the pepper varieties was obtained in plants treated with 50% ANSE concentration. For DG morph infestations 50% ANSE resulted in a 66.70% increase in fruit yield over tap water treated plots and 11.12% increase over deltrin, while for LG morph infestation, yield of pepper increased by 33.34% over tap water treated plots.100% ANSE concentration corresponded to a decrease in yield of the pepper varieties.

3. At highest concentration ANSE resulted in 97.11% reduction for DG morphs and 94.69% for LG morphs of *M. persicae.* All ANSE concentrations used were found to be more effective aphicides than Neemazal, the extract acted in a concentration and duration dependent manner.

6.3 RECOMMENDATIONS

1. It is recommended that 50% concentrations of ANSE (100g/l) could be subjected to further studies as a potential substitute to synthetic aphicides used in control of *M. persicae* on pepper plants since it was able to significantly reduce *M. persicae* population as well as improved the yield of the plants over the control.

2. The significant reduction in the number of *Myzus persicae* on pepper plants treated with aqueous neem seed extract is an indication that they could be used as potential alternatives to chemical insecticides and can play a more prominent role in integrated pest management programs in the future.

3. This study serve as a baseline for further studies on the active ingredients/ metabolites in ANSE which could be separated and individually tested on pepper varieties to decipher efficacy and toxicity levels of each constituent.

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