BIOPESTICIDE CONTROL OF SOME IMPORTANT OKRA (*Abelmoschus esculentus* (L.) MOENCH) INSECT-PESTS AND VIRAL DISEASES IN THREE OKRA CULTIVARS

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DECLARATION

This thesis is the result of research conducted by CHRISTIAN AKAMA in the Department of Nuclear Agriculture and Radiation Processing of the School of Nuclear and Allied Sciences, University of Ghana, under the supervision of DR. SAMUEL AMITEYE and DR. ANDREW SARKODIE APPIAH.

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DEDICATION

This thesis is wholly dedicated to the Almighty God for bestowing on me His sufficient grace and mercies throughout my studies.

Also to my lovely wife, and siblings for their prayers and inspiration to me during difficult times and the sacrifices they have made towards this feat.

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LIST OF ABREVIATIONS

ACRONYM	MEANING
ACMV	African Cassava Mosaic Virus
BNARI	Biotechnology and Nuclear Agriculture Research Institute
ELISA	Enzyme-Linked Immunosorbent Assay
FOASTAT	Food and Agriculture Organization's Statistical Database
ISEM	Immunosorbent Electron Microscopy
IEM	Immune Electron Microscopy
Mabs	Monoclonal Antibody
MPs	Movement Proteins
BSc.	Bachelor of Science
OkYCV	Okra Yellow Crinkle Virus
OLCVD	Okra Leaf Curl Virus Diseases
OMV	Okra Mosaic Virus
OYVMV	Okra Yellow Vien Mosaic Virus
OYVMVD	Okra Yellow Vein Mosaic Virus Diseases
OYMV	Ononis Yellow Mosaic Virus
Pabs	Polyclonal Antibodies
RCBD	Randomised Complete Block Design
spp.	Species
SqLCV	Squash Leaf curl Virus
TGMV	Tomato Golden Msaic Virus
TLCV	Tomato Leaf Curl Virus
TAS-ELISA	Triple Antibody Sandwich
VITGS	Virus Induced Transcriptional Gene Silencing
AST	Asontem Okra Cultivar
CHE	Chemical Pesticide (Akape)

CON	Control Treatment
DAS-ELISA	Double Antibody Sandwich ELISA
e.g.	Example
F1K	F1 Kirene Okra Cultivar
F1S	F1 Sahari Okra Cultivar
FAO	Food and Agriculture Organization
GAEC	Ghana Atomic Energy Commission
i.e.	That is
IPM	Integrated Pest Management
JAT	Jathropha extract
kg	Kilogram
LEM	Lemon grass extract
MAb	Monoclonal Antibody
MoFA	Ministry of Food and Agriculture
NEM	Neem extract
NNRI	National Nuclear Research Institute
OMV-free	Healthy Okra Cultivars Free of OMV Disease
PBS	Phosphate Buffered Saline
pNPP	Para-Nitrophenyl Phosphate
RAM-AP	Rabbit Antimouse Alkaline Phosphate
SDW	Sterile Distilled Water
TAS-ELISA	Triple Antibody Sandwich ELISA
ul	Microlitre
WAE	Weeks After Emergence
WAT	West African Taxon

ABSTRACT

The production, processing and marketing of Okra (*Abelmoschus esculentus* L. Moench), a vegetable valued for its rich nutritional and medicinal benefits, serves as an important means of employment and income generation to many peasant farmers. The production of the crop is, however, constrained by the incidence of pests (whitefly and flea beetle) and okra yellow vein mosaic virus [OYMV] and okra mosaic virus [OMV]) diseases. To overcome the health and environmental risks associated with the excessive use of synthetic agrochemicals, the predominant means of control of these pests and viral diseases, the efficacy of crude leaf extracts as biopesticides from Neem, Jathropha and Lemon grass on the incidence and severity of OMV and OYVMV as well as crop damage due to whitefly and flea beetle, were evaluated in three okra cultivars [F1 Kirene (F1K), FI Sahari (F1S) and Asontem (AST)]. Among the three plant extracts tested, Neem extract treatment induced significantly ($p<0.05$) the lowest mean count of whitefly [ASTNEM (18.91), F1KNEM (22.17), F1SNEM (24.49)] compared to Jathropha extracts [ASTJAT (27.99), F1KJAT (28.73), F1SJAT (28.74)] and Lemon grass extract treatments [ASTLEM (34.22), F1KLEM (32.77), F1SLEM (32.67)]. Similar results were obtained for mean population of flea beetle. With respect to insect pests damage to the okra cultivars, Neem extract caused significantly ($p<0.05$) the lowest severity of damage [F1KNEM (1.53), F1SNEM (1.58), ASTNEM (1.63)] compared to Jathropha extract treatment [F1SJAT (2.74), ASTJAT (2.75), F1KJAT (2.77)] and Lemon grass extract [F1SLEM (2.97), ASTLEM (3.64), F1KLEM (3.73)]. Similarly, the application of Neem extract significantly ($p<0.05$) reduced the mean incidence (21.84%) of the viral diseases than Lemon grass extract (25.28%), Jathropha extract (25.44%) and the Control (28.89%). *In-vitro* confirmation test using Enzyme-linked immunosorbent assay (ELISA) revealed that majority (86.67%) of the treatment combinations showed single infection of OMV disease while in 13.33% of the treatment combinations mixed-infection of OMV and OYVMV diseases was observed. In terms of yield levels, chemical pesticide treatment produced significantly

($p<0.05$) highest yield (186.92 kg/ha) compared to Neem extract treatment (144.81 kg/ha), Jathropha extract (139.06 kg/ha), Lemon grass extract (115.75 kg/ha) and the Control (94.02 kg/ha). However, Neem extract performed best among the tested plant extracts. Therefore, in a second experimental trial to ascertain the best efficacious dose for Neem extract application, varying concentrations of 20 ml/L, 30 ml/L and 40 ml/L were used and compared with the chemical "Akape" in the three okra cultivars (F1K, F1S and AST) instead of the 50ml/L applied in the first experimental trial. Treatment with "Akape" recorded significantly ($p<0.05$) the lowest mean whitefly populations than treatment with plant extracts. However, 20 ml/L Neem extract treatment produced significantly ($p<0.05$) lowest mean count of whitefly [F1KN1 (17.88), ASTN1 (23.95), F1SN1 (29.01)] compared to 40 ml/L Neem extract [F1KN3 (37.90), ASTN3 (39.57), F1SN3 (38.22)]. Similarly, although the application of the synthetic chemical (insecticide) "Akape" resulted in the best performance in reducing flea beetle populations, it was observed among the Neem extract concentrations, 20 ml/L treatment produced significantly ($p < 0.05$) lowest flea beetle mean count [F1KN1 (24.04), ASTN1 (25.61), F1SN1 (29.41)] compared to 30 ml/L [F1KN2 (49.89), ASTN2 (48.73), F1SN2 (51.92)] and 40 ml/L [F1KN3 (72.68), ASTN3 (65.88), F1SN3 (80.88)] treated okra cultivars. Okra cultivars treated with 20 ml/L Neem extract had significantly ($p<0.05$) lowest severity of insect pest damage compared to treatment with 40 ml/L Neem extract. Of the three concentrations of Neem extract applied, 20 ml/L (N1) significantly ($p<0.05$) reduced severity of mixed-infection of OMV and OYVMV) in all the three okra cultivars [ASTN1 (0.64), F1KN1 (0.84), F1SN1 (1.23)]. Yield of okra cultivars treated with chemical pesticide was significantly ($p>0.05$) highest (234kg/ha). However, yield obtained with 20 ml/L Neem extract treatment (207kg/ha) was significantly ($p>0.05$) highest compared to the other Neem extract doses. It is noteworthy that cultivar F1 Kirene recorded significant ($p<0.05$) the highest yield (207kg/ha) followed by F1 Sahari (139kg/ha) and Asontem (127kg/ha). From the obtained results, the cultivation of the okra cultivar F1 Kirene could be combined with Neem

extract at a concentration of 20 ml/L to obtain effective disease and pests control for high yields.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Okra (*Abelmoschus esculentus* (L.) Monech), is a semi-fibrous annual dicotyledonous herb belonging to the order Malvales and family Malvaceae (Bennett-Lartey and Oteng-Yeboah, 2008). The crop is a fruit vegetable widely grown in many parts of the world, particularly the tropical and subtropical countries (Martin, 1982), for its immature fruits and fresh tender leaves, medicinal value and as raw material for industry (Saifullah and Rabanni, 2009). Okra in its fresh or dried form is used mainly for thickening of soup or stew due to its high mucilage content (Schippers, 2000). Okra fruit has high dietary nutritive value with up to 20% protein and therefore, very important in the diet of a large section of the populations where okra is widely cultivated (Obeng-Ofori and Sackey, 2003). Okra fruits and leaves are high in vitaminA and vitaminC and calcium (NARP, 1993). The seeds are also a good source of oil, minerals and medically important compounds (Kumar et al., 2010). The vegetable is laden with good balance of amino acids such as lysine and tryptophan in diets (Sanjeet et al 2010). Medicinally, the crop is made up of insoluble and soluble fibre essential for maintaining healthy intestinal tract, lowering serum cholesterol, reducing the risk of heart diseases and cancer (Schneeman, 1998; Brown et al., 1999). Okra, therefore, serves as a good source of food for medicinal and industrial purposes (Gemede et al., 2015).

Agronomically, okra survives and performs relatively quite well in harsh biotic or abiotic environments in which most other crops fail due to its hardy nature. The crop is easy to cultivate and suited to regions with moderate rainfall. Okra is a warm season crop, requiring ample

moisture for germination (Yamaguchi, 2012). These attributes as well as its rich nutritional and market value make okra very popular crop cultivated by small holder farmers across the tropical regions of Africa. The crop serves as a good means of rural employment and income generation for many resource-poor women. In West Africa, particularly in Ghana mostly women produce and market the crop (Norman et al., 2011). The contribution of okra to the economy of Ghana is, therefore, quite significant. For example, in 2011 alone, the total production of okra in Ghana was 80,000 metric tonnes, yielding the nation about 50,000 US Dollars (FAOSTAT, 2013). The world's leading producers of the crop are India, leading in production with 70%, Nigeria (15%), Pakistan (2%), Ghana (2%), Egypt (1.7%) and Iraq (1.7%) Benchasri et al., 2012). In Ghana, Brong-Ahafo, Northern, Volta, Greater Accra and Central regions are the leading producers (Torkpo et al., 2006).

Obviously, the crop has a huge potential to well transform rural economies through employment and income generation. However, that notwithstanding, the rate of commercialization of the crop far lags behind the rate of increasing demand because of the current low yield levels. The low yield records do not make the crop very attractive for rapid adoption by local farmers. In Ghana, the average yield of okra is as low as 1.5 to 4.5 tha^{-1} compared to an estimated 30 tha^{-1} obtained in agriculturally developed countries (SRID-MOFA, 2007). The major production constraints limiting significant yields are the incidence of viral diseases and insect pest attacks. These result in significant economic losses with respect to poor fruit quality and reduced market premium.

The crop is known to be susceptible to many viruses with *okra mosaic virus* (OkMV) and *okra yellow vein mosaic virus* (OYVMV), being the most prevalent and devastating (Brunt *et al*, 1996; Swanson and Harrison, 1993). O*kra mosaic virus* (OkMV), the most prevalent virus

disease of okra in Ghana is transmitted by flea beetles (*Podagrica spp*), followed by *okra yellow vein mosaic virus* (OYVMV) which is transmitted by the whitefly (*Bemisia tabaci*) (Brunt et al., 1996). These pests are reported to transmit these viruses through their feeding activities (Jose and usha, 2003). Reports reveal that the most prevalent and most devastating insect pests of okra in Ghana are flea beetle (*Podagrica spp*.) sub-species *Podagrica uniforma* and *Podagrica sjostedti* (Bi- Kusi, 2013 and Asare-Bediako et al., 2014). The flea beetle feed and damage leaves by creating a lot of irregular perforations and thus, reduce significantly the photosynthetic leaf surface area. The physiological implication is that photosynthetic activity is significantly reduced in affected plants and consequently leads to very low yields in the crop (Echezona and Offordile, 2011).

It has been observed that the most venerable and devastating period of okra production to insect pests and virus attack are the flowering and fruiting stages, though all stages of growth and development are affected (Sastry and Singh, 1974). Commonly, infected plants show symptoms of mosaic, leaf curling, chlorosis, vein banding, vein yellowing, leaf yellowing and smaller leaves (Asare Bediako et al., 2014). These symptoms result in crop stunting and fewer or smaller fruits culminating in significant yield reduction. Consequently, reports of 100% field loses due to disease incidence are recorded. Depending on the developmental stage at which infection occurs, yield losses could range between 50 to 94% (Sastry and Singh, 1974).

Effective monitoring of virus incidence in plant disease studies requires efficient and cost effective virus detection techniques. In this regard, enzyme linked immunosorbent assay (ELISA) is the serological technique of choice for studying different types of viruses and their host relationships in available germplasm (Narayanasamy 2001; Akad and Czosnek, 2002). El-

gaied et al. (2008) explained that serological techniques are the most effective and convenient for easy and rapid detection of viruses.

The general practice is that viral diseases and insect pest damage in okra fields are managed using chemicals applications. Chemical control methods are, however, proving increasingly ineffective. Furthermore, chemical applications continually pose damaging effects on the environment, human and animal health. Consequently, all over the world, excessive use of agrochemical for plant disease and insect pest control are currently becoming increasingly unattractive and agricultural practices are being fashioned to avoid chemical use. A paradigm shift in plant disease control requires the use of biopesticides in the form of plant extracts. The use of plant extracts from locally available plant materials such as neem; *Azadirachta indica* extract, physic nut; *Jatropha curcas* extract, lemon grass; *Citronella squinant* is environmentally friendly and a cheaper approach to the control of viral diseases and pests in crops (Sarabani et al., 2002).

1.2 Problem statement

The potential yield of okra is in the magnitude of 30 – 40 t/ha if growth factors are well controlled (Camciuc et al., 1998), but there is a wide gap between this potential yield and realized yield due to a number of constraints. Among the major problems that affect okra production in Ghana are pests and diseases of which the okra mosaic and okra yellow vein mosaic virus are the most important. These viruses cause serious economic loss by interfering in the physiology of the plant and fruits development resulting in malformed and reduced fruit sizes. The fruits are mostly yellow, small, tough and fibrous (Ndunguru and Rajabu, 2004).

The control of insect pests and viruses of okra, compel farmers to resort to the use of synthetic chemicals such as Attack, Akape, Golan, and many other pesticides. The over reliance on these synthetic chemicals is harmful to the environment and as chemical residues in the fruits may be risky to the health of consumers and can kill non-targeted animals. Indeed, the continual use of high doses of synthetic insecticideshas significantly reduced the population of indigenous natural enemies and other beneficial organisms that would otherwise normally keep pest and disease populations under control (Wiktelius et al., 1999). Evidence show that many insect pests over time, have developed resistance to these chemicals (Aktar et al., 2009). Besides, synthetic insecticides are also expensive and many resource-poor farmers are unable to afford to buy the recommended chemicals to protect their crops against pest infestation. There is, therefore, the need to develop alternative methods of control that are relatively cheaper and less destructive to the environment and safer for consumers.

1.3 Justification and relevance of the study

Okra is the fourth most important vegetable crop in Ghana after tomato, pepper and gardens eggs. The fruits are rich in vitamins, calcium, potassium and other minerals (Camciuc et al., 1998). The mature okra seed is a good source of oil and protein (Oyelade et al., 2003) and has been known to have superior nutritional quality. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid (Savello et al., 1980), which is essential for human nutrition. Its mature fruits and stems contain crude fibre, which is used in the paper industry. Many studies in Africa, however, showed that despite the useful attributes of the vegetable, okra yields are very low (Fajinmi and Fajinmi, 2010). Insect pests directly cause reduction in crop yield and quality through feeding and indirectly by acting as vectors of important okraviruses and diseases (Ndunguru and Rajabu, 2004). Plant diseases need to be controlled to maintain the quality and

abundance of food, feed, and fibre produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides.

According to many researchers, chemical insecticides are the most effective control measure against these pests (Jackai et al., 1985). However, chemical insecticides have over the years been found to be toxic to the health of consumers as well as pollute the environment. Besides, they are very expensive and their use results in increased cost of production (Pretty and Waibel, 2005). Consequently, some pest management researchers have focused their efforts on developing alternative measures to synthetic chemicals for controlling pests and diseases. Among these alternative control measures, biological control is gaining wide acceptance, notably the application of plant extracts as biopesticides. There is emerging considerable interest in plant extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens (Soliman and Badeaa, 2002; Valero and Salmeron, 2003). Crops treated with some plant extracts produce and accumulate elevated levels of specialized proteins and other compounds which triggered the defence system of the plant against destructive diseases (Marrone, 2009). The use of biopesticides is of relevant consideration in okra production in Ghana where the smallholder farmer is constrained by lack of resources and the means to purchase expensive synthetic insecticides to control pests and diseases. Also worrying is the inability of most farmers to use synthetic insecticides effectively and efficiently due to inadequate technical knowhow and extension services.

1. 4 Objectives of the study

The main objective of this study is to assess the effectiveness of selected bio-pesticides in controlling insect pests infestation in three okra cultivars.

The specific objectives were to:

i. Determine the effect of the application of three aqueous leaf extract on the incidence and severity of okra mosaic and okra yellow vein mosaic virus.
ii. Determine the effect of the plant extracts on the insect pest populations on the okra cultivars.
iii. Determine the total flavonoids and elemental composition of the three plant extracts
iv. Compare the effect of the applications of plant extracts to that of Akape (synthetic chemical) on the incidence and severity of the two viral diseases of okra.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy, Centre of Origin and Distribution

Okra (Abelmoschus spp. (L.) Moench) is a dicot, belonging to the order Malvales, family Malvaceae and genus Abelmoschus (Schippers, 2000). Okra was previously included in the genus Hibiscus. Later, it was designated to Abelmoschus, which is distinguished from the genus Hibiscus by the characteristics of the calyx; spatulate, with five short teeth, connate to the corolla and caduceus after flowering (Kundu and Biswas, 1973; Terrell and Winters 1974). The section Abelmoschus was subsequently proposed to be raised to the rank of distinct genus by Medikus (1987). The wider use of Abelmoschus was subsequently accepted in taxonomic and contemporary literature. Although about 50 species have been described, eight are most widely accepted (IBPGR, 1990). Okra, *Abelmoschus esculentus* is of African origin (Essilfie et al. 2010; Akhtar et al., 2014). It was discovered around Ethiopia during the 12th century B.C and was cultivated by the ancient Egyptians (Purseglove, 1987). Currently, the crop is grown as a popular vegetable throughout the tropical and sub-tropical regions of the world (Kumar et al., 2010). Okra is primarily distributed throughout the intermediate savannah zone between the rain forest and the arid Sahel. The species is less frequently found in the rain forest zone but fairly well represented in the Sahel zone. On the contrary, per the West African Taxon (WAT), *A. caillei* does not occur in the Sahelian zone since it has a long life cycle and usually requires abundant continuous rainfall. Hamon and van Sloten, (1989) stated that since a natural interspecific hybrid of the two cultivated species occurs in the central part of Sudan, *A. caillei* is possibly more widely distributed than currently known.

The crop is grown in many parts of the world, especially in tropical and sub-tropical countries (Saifullah and Rabbani 2009; Kumar et al., 2010). It is grown on a large scale in Africa, especially in Nigeria, Egypt, Ghana and Sudan, (Benchasri, 2012). It is also very important in other tropical areas including Asia, Central and South America (FAOSTAT, 2008). There are a number of species, both wild and cultivated including *A. esculentus, A. caillei, A. moschatus, A. manihot, A. ficulneus* and *A. tetraphyllus*. Two main species in the genus *Abelmoschus* are cultivated; *A. manihot* L. and *A. moschatus* L. (Siemonsma, 1991; Osawaru et al., 2013).

2.2 Botany, Cytology and Use

The okra plant is a semi-woody, fibrous herbaceous annual with an indeterminate growth habit (Nonnecke, 1989). The plant forms a deeply penetrating taproot with dense shallow feeder roots reaching out in all direction in the upper 45 cm of the soil. The stems reach heights from 3 m in dwarf varieties to 7 m to 8 m in others. Harvesting is recommended at least every other day for size and quality (Ramu, 1976). About 35-40 days are required from anthesis to seed maturity. If fruits are allowed to mature, plant growth declines and few flowers develop, but with continuous harvesting, the plant continues to set fruit (Norman, 1992). Mature fruits should be removed and discarded as they reduce the plant growth and decrease yield (Ramu, 1976). The rate of allogamy differs according to type of variety and ecological conditions (Hamon and Koechlin, 1991). When ripe, the fruit becomes fibrous and splits longitudinally in five parts, showing 5 rows of seeds, with 50 – 100 seeds per fruit (Norman, 1992). Okra has alternate, palmate broad leaves and the flowers have five large yellow petals with a large purple area covering the base. The fruits, which are harvested immature, are pale green, green, or purplish and in many cultivars are ridged (Hamon and Koechlin, 1991). When mature, they are dark brown dehiscent or indehiscent capsules. Fruit shapes range from round to ridged and

short to long (Siemonsma, 1991). The plant and fruits may have small spines on them that create allergies in some people (Ariyo, 1993; Düzyaman, 1997).

There is significant variation in chromosome numbers and ploidy levels in Abelmoschus (Bisht and Bhat, 2006). Different authors have variably reported the chromosome number 2n for *Abelmoschus esculentus* L. (Moench). The most frequently observed somatic chromosome number is 2n=130, although Datta and Naug (1968) suggested that the numbers 2n=72, 108, 120, 132 and 144 are in regular series of polyploids with n=12. This makes the existing taxonomical classifications at the species level in the genus *Abelmoschus* quite ambiguous. There was therefore a detailed cytogenetical study on Asian okra and related species, which have provided more evidence of the existence of amphidiploids in the genus (Siemonsma, 1982a).

Okra has several uses nutritionally, economically and industrially. The nutritive value of okra is due to the availability of the essential components carbohydrate, protein, fat, iron, calcium, fibre, thiamine, nicotinamide, riboflavin, and ascorbic acid in the various plant parts (Arapitsas, 2008; Fajinmi and Fajinmi, 2010; Keatinge *et al*., 2011). Mature seeds of 100 g okra contain 20% edible oil and 20.23% crude protein due to high lysine content and it is a good source of vitamin C (Siemonsma and Kouame, 2004). Okra seed oil is rich in unsaturated fatty acids such as linoleic acid which is essential for human nutrition. Okra seeds are used as a substitute or additive in feed compounds (Savello et al., 1980). Dried okra seeds, can be used to prepare vegetable curds, or roasted and ground and used as coffee additive or substitute (Moekchantuk and Kumar, 2004). Okra is a very important soup condiment that is consumed daily in almost all homes and restaurants. The tender pods of okra are used in stews or cut into slices, sun dried and then ground as a powder and used as a favourite Sudanese dish called “Weika”

(Abdelmageed, 2010). Similarly, the older immature pods and leaves that are yet to develop fiber are also cut into slices, sun dried and ground and used in soups in the dry season when fresh fruits are scarce (Siemonsma and Kouame, 2004; Oppong-Sekyere, 2011). Industrially, the mature fruit and stem of okra contain crude fibre which is used in the paper industry (Anderson and Pharis, 2003). Okra mucilage is usually used to glace certain papers and also useful in confectionery among other uses such as bioabsorbent (Adetuyi et al., 2008; Kumar et al., 2010).

2.3 Climate, Soil Requirements and Agronomic Practices

Okra is well adapted to a climate with a long, warm growing season. The crop is usually grown in many different types of soils but it thrives well in well–drained sandy and clay loam soils. It grows well at a maximum average temperature of 35ºC with a minimum average above 18ºC. It may be grown at elevations from sea level up to 30 meters above sea level. Okra is usually planted twice a year, from April to June and October to January in Ghana and also in other parts of Africa (MOFA, 2013).

High yields can be obtained if proper agronomic practices are followed. For instance, sowing high quality seeds, good land preparation, effective application of the recommended rates of fertilizer, irrigation and removal of weeds when necessary are recommended. Agronomic practices also include the cultural management practices from seed selection, land preparation to post-harvest handling practices to obtain maximum yield and best quality okra pods (MOFA, 2013).

The effect of NPK fertilizer rates and method of application on growth and yield of okra is documented by Omotoso and Shittu (2007). The band and ring methods of application are

commonly used in okra. The recommended fertilizer rate of 150kg ha^{-1} is most appropriate for okra production.

Weeds compete with okra for nutrients, space, sunshine and water as in many other crops. Weeds also serve as alternate host for pests and diseases in okra. Olabode et al., (2007) observed that integrated weed control method was more effective in controlling weeds than isolated applications. Ayvaz et al., (2010) recommended that combining chemical weed control method with cultural method of weed control is very effective in reducing weed populations and decreased dry matter. Some registered and commonly used herbicides for weed control in okra production in Ghana include Glyphosate, Gramoxone, Sarosate, Gramoquat (non–selective) and Chemostom, Amin salt, Propanil (selective) (Williamson et al., 2008). The indiscriminate application of these chemicals can result in several adverse effects on the environment. Fianko et al., (2011) identified the use of Glyphosate, fluazifop–butyl, ametryne, diuron or bromacil as the main herbicides normally employed in weed control on vegetables fields in Ghana.

Aside the use of herbicides to control weeds, Abla, (2015) studying the influence of mulching on the growth and yield of okra reported that mulching is very effective in controlling weeds on okra fields and contributed to increased growth and yields of okra. Ugese et al., (2016) also investigated the effects of organic and inorganic mulches on the growth and yield of okra and obtained similar results. These researchers further explained that weeds are controlled better in their early stages of growth. Hand hoeing is also used in the absence of mechanized system of farming.

2.4 Scale of Okra Production and Importance

China is the world leading producer of okra closely followed by India. These two countries produce over 100 million tons of okra from an area of around 15 million hectares (Benchasri, 2012). In Africa, Nigeria is the largest producer with 1,039,000 tons followed by Cote d'Ivoire and Ghana (FAOSTAT, 2008). Okra production in West Africa is very low at 2.5 t/ha compared to East and North Africa with production levels of 6.2 t/ha and 8.8 t/ha respectively (FAOSTAT, 2010). In Ghana, yields levels of okra are mainly dependent on cultivar, time and frequency of harvest. An average of 6-12 tons/ha can be obtained (MOFA, 2013).

Okra is the fourth most popular vegetable after tomato, pepper and garden egg in Ghana (Oppong-Sekyere et al., 2012). It is often the vegetable of choice among rural and urban consumers and even at food joints. National production hovers around 120,000 Mt produced on 19,500 ha of arable land with yield potential of 5.5 Mt\ha. Okra production serves an important source of income to farmers particularly in the dry season where commercial production is carried out using smallholder irrigation schemes such as dug-outs, small dams and along river banks. On arable lands, they may appear as a sole, inter or boarder crop. In most parts of Ghana, okra production has a strong commercial value particularly to rural women farmers, where both fresh and dehydrated products are sold to supplement household income (Opong-Sekyere et al., 2012).

The existing okra cultivars are land races which have been recycled for many decades. Some of these landraces are late maturing and photoperiod-sensitive with low marketing and export potential; due to fruit colour, shape and pubescence. However, some of the landraces have multi-purpose traits such as high yielding, good drying properties and

resistance to drought, diseases and pests. Most of the improved cultivars and hybrid seed introduced to many parts of Ghana are not widely adapted by farmers due to their relative susceptibility to some biotic and abiotic stresses. To surmount these constraints, it is necessary to employ interventions that will increase access to improved seed, develop efficient postharvest technologies to increase production, proper storage, utilization for increased economic returns to farmers (Bennet-Lartey and Oteng-Yeboah, 2008).

Okra is an annual crop, which requires warm conditions for growth and good yield. The crop is a significant part of the commerce of vegetables in almost every market all over Africa (Schippers, 2000). According to Oppong-Sekyere et al., (2011), in Ghana okra can be found in markets all year round in the fresh immature or dehydrated fruit form, particularly in Northern Ghana due to its strong commercial value for poor women farmers and its importance in the diets of the inhabitants. Okra is grown purposely for its leaves and young pods which are frequently eaten green as vegetable. Okra mucilage is suitable for medicinal and industrial applications. Industrially, okra mucilage is usually used to glace certain papers and also useful in confectionery (Farinde et al., 2007). Worldwide production of okra as fruit vegetable was estimated at 6,000,000 tons per year. In West Africa, it was estimated at 500,000 to 600,000 tons per year. Schippers (2000) observed wide genetic diversity in okra with the most important production regions localized in Ghana, Burkina Faso and Nigeria. Okra is among the three most important vegetables grown by 28% of the rural poor in Ghana.

2.5 Health Benefits and Nutritional Value of Okra

The okra fruit is a reservoir of important and valuable nutrients (Candlish et al., 1987), nearly half of which is soluble fibre in the form of gums and pectins. Soluble fibre helps to lower

serum cholesterol and reducing the risk of heart disease (Torkpo et al., 2006). The other half is insoluble fibre which helps to keep the intestinal tract healthy, decreasing the risk of some forms of cancer, especially colorectal cancer (Schneeman, 1998). Okra has several health benefits, as it is rich in vitamin A, thiamin, vitamin B6, vitamin C, folic acid, riboflavin, calcium, zinc and dietary fibre (Norman, 1992). In addition to its usefulness as a vegetable crop, okra fruit is useful medicinally, in curing ulcer and suppressing the pains and effects of haemorrhoid. The mucilage has been used as a plasma replacement or blood volume expander (Siemonsma and Kouame, 2004).

Okra is recommended for pregnant women, as it is rich in folic acid, which is essential in the neural tube formation of the foetus between the 4th and 12th weeks of pregnancy (Allen, 2007). It is rich in amino acids, with the likes of tryptophan, cystine and other sulphur amino acids. It is the ideal vegetable for weight loss and is a storehouse of health benefits, provided it is cooked over low flame to retain its properties (Düzyaman and Vural, 2001). Savello et al., (1980), reported that, a 100 g edible portion of okra fruit contains 90 g water, 2 g protein, 1 g fibre and 7 g carbohydrates. Its energy value is 145 kJ/100 g and it is a good source of vitamins and minerals. It is also very rich in calcium (70-90 mg/100 g). Therefore, the consumption of okra plays an important role in human nutrition.

2.6 Production Constraints of Okra

Okra is attacked by several species of insect pests and infected by a few diseases from seedling stage to harvesting. Economic losses depend on the degree of damage, pest density, environmental conditions, stage of growth and the plant part damaged by the pest (Siemonsma, 1991). Cercospora leaf spot and powdery mildew are two fungal diseases that attack okra at late vegetative to reproductive stage. Fungal infection can spread rapidly in the field due to

crowded and overlapping broad leaves of the plants. Besides wind spreading the fungal spores to plants, people harvesting daily and passing along the okra rows are also responsible for the widespread infection in the field (Siemonsma, 1991). Stem borers (*Earias spp*) damage shoots at early vegetative stage. The plants then develop branches to compensate for the damage by stem borers. Unlike other crops such as eggplant, development of more branches in okra is disadvantageous. Pods from branches are less and smaller. Fruit worm, stem and pod borer feed on flowers and bore inside the pods thereby damaging the flowers and no pods are developed. Cutworm (*Agrotis spp*) usually feeds on the leaves (Lamont, 1999). Damage by cutworm also greatly affects the photosynthetic ability of the plant and drastically limits effective partitioning of assimilates.

Damping off at seedling stage can cause tremendous losses unless most of the seeds sown are treated with fungicides. Leafhoppers, aphids and whiteflies attack at seedling to early vegetative stage and transmit the yellow vein mosaic virus. Infected plants produce poor quality pods. Rapid increase in leafhopper population during the dry season causes hopperburn in okra especially when there is no source of yellow vein mosaic virus in neighbouring fields. Leaves turn red and eventually dry up due to feeding by high density of sucking insects at the vegetative stage predisposing them to leaf curl and defoliation (Wammanda, 2010).

2.6.1 Viral Diseases of Okra

The main viral diseases caused by viruses; Okra yellow vein mosaic virus (OYVMV), Okra mosaic virus (OMV) and Okra leaf curl virus (OLCV). These viral diseases are responsible for yield loses in the global production of okra. Infected plants produce poor quality pods. (Kucharek, 2004). Okra yellow vein mosaic disease (OYVMD) is one of the devastating and

widely distributed disease in okra fields around the globe including Ghana. The disease is caused by Okra yellow vein mosaic virus (OYVMV) belong to Begomovirus, family Geminiviridae. It is transmitted mechanically by whitefly, leafhoppers and aphids in persistent circulative manner (Ghanem, 2003). Incidence of OYVMV was observed on plants with characteristic symptoms of mosaic pattern on leaves, vein clearing, formation of small fruit and stunting in severe case (Ali et al., 2000). Heavy yield losses (75%) were recorded worldwide along with the characteristic chlorosis yellowing of leaves, malformation and small size of fruits (Solankey, 2014).

2.7 Integrated Management Control of Pests and Diseases of Okra

Okra and many other vegetables are consumed fresh and, therefore, requires that in the production these vegetables, the control of pests and diseases should be strategically achieved with substances that pose no or minimal acceptable toxicity levels (Aziz et al., 2011). Frantic research efforts are, therefore, ongoing to explore alternatives that integrate various the common available pest control methods and techniques in order to reduce the over dependence of synthetic insecticides. To achieve this goal, integrated pest management (IPM) has proven to be the most appropriate approach to sustainable okra production. A common approach in IPM, is the use of preferred host plants as trap crops to attract and concentrate insect pests and their natural enemies instead of the cash crop. This IPM strategy enable effective suppression of pest populations by mechanical removal of trapped pests in small plots and reduce the damage in the cash crop. Trap crops have been tested and reported to be effective in the control of insect pests in soybeans and many other plant species (Javaid and Joshi, 1995).

Another important method used in IPM that is widely practised mainly in the humid tropics, mixed cropping, has been shown to be more efficient than sole or monocropping (Adelana, 1984) inreducing the incidence and significance of pests and diseases crop damage (Southwood and Way, 1970). The use of natural enemies against main pests is also recommended as an important component of IPM in okra pests and disease control. The lepidoptera *Earias spp.* has been reported to be effectively controlled by *Trichogramma spp.* known to be parasites of eggs of the lepidoptera (Anonymous, 2010). The use of biopesticides such as *Azadirachta indica* (Neem) as well as many other botanicals and their products have proven potent in many IPM control methods for controlling a wide variety of insect pests (Khuhro et al., 2014). Khuhro et al., 2014 reported that crude neem extracts prepared from neem seeds collected from field has significantly reduced the population percentage insects; similarly pod damage on treated okra plot was lower than untreated plots (Wubneh, 2016). Ultimately, the objective of the integrated control approaches is to obtain maximum return at minimum cost without disturbing the crop ecosystem (Adejonwo et al., 1991).

2.8 Control Strategies for Okra Viruses and Pests

Control of pests and diseases is vital in any agricultural crop production in order to maintain the quality and abundance of food, feed, and fiber produced by growers. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years (Wisniewski and Wilson, 1992). Plant disease control eliminates the pathogen and minimizes disease devastation and loss of crop yield. Various methods are used tocontrol the diseases and pests in plants. Chemical method of

disease control is the most widely used in crop cultivation (Jackai and Oyediran 1991). Other disease control measures includes, cultural control methods, breeding for disease resistance, and biological control methods and the use of plant extracts.

2.8.1 Cultural Control Methods

Acording to Howard, (1996) cultural methods of disease control encompass various combinations of agricultural cropping, harvesting and storage, tillage, crop rotation, soil management, growing of resistant varieties, and planning of land use. In addition, disease conditions are minimized by proper selection of the land or field, choice of time of sowing, selection of varieties, seed and plant stock and linking these with the integration of the appropriate cultural practices. These control strategy and measures enable the host plant to avoid contact with the pathogen or to ensure that the susceptible stages of the host and the favourable conditions for pathogen infection do not coincide. The location or geographical area for cropping is also an important consideration in order to avoid areas of very heavy loads. For instance, fungal and bacterial diseases are more severe in wet areas than in dry regions.

2.8.2 Chemical Control Methods

Agrochemicals are used in boosting yields and controlling insect pests, diseases and weeds in agriculture. These chemicals act on pests and diseases that destroy agricultural produce (Powers et al., 2009). The application of agrochemicals has, therefore, increased agricultural production and boost yields tremendously. Currently, synthetic insecticides are the main means of insect pests control both in the field and in storage (Jackai and Oyediran 1991; Jackai and Adalla, 1997). Agrochemicals have proven efficacy and potency against a wide range of pest species of agricultural crops. Chemical control is generally practiced by farmers for higher

gains, but its injudicious utilization pose environmental and health problems. Due to the activities of a wide spectrum of insect pests which ravage crops in the field at different growth stages, farmers spray their farms as many as eight to ten times during the growing season (Adipala et al., 2000). Over reliance on chemical control leads to problems of pest resistance, resurgence of pests, pesticide residues, destruction of beneficial non-target fauna and environmental pollution. Chemical residues in the environment has been reported to destroy natural enemies of pests, cause crop pollination problems due to decreased honey bee populations as well as domestic animal poisoning, contamination of livestock products, fish and wildlife losses. Contamination of underground water and rivers is also a major risk associated with excessive chemical use (Epidi et al., 2008). Most insecticidal compounds used to control pests on okra production fall within four main classes - organophosphates, organochlorines, carbamates and pyrethroids. As a result of the associated problems, organochlorine has been reportedly banned in developed countries as well as in developing countries like Ghana.

2.9 The use of Leaf Extracts in the Control of Okra Pests and Diseases

Botanical insecticides or biopesticides are naturally occurring chemicals extracted from plants which break down readily in the soil and are not stored in plant or animal tissue. The effect of biopesticides are not as persistent or long lasting as in the use of synthetic pesticides (Gillespie et al., 2011). Botanical insecticides are generally pest–specific and are relatively harmless to non-target organisms. These natural plant insecticides have been proven to be effective, biodegradable, low cost, low technological base, selective and environmentally friendly (Iram et al., 2009). It is also noteworthy that the possibility of insect developing resistance to botanical insecticide is less compared to the effect in chemical pesticides (Scott et al., 2005).

Furthermore, plant extracts act as mortality agents, repellents, anti-feedants, attractants, oviposition deterrents and sterility agents (Isman et al., 2010).

Research on the use of natural pesticides as an alternative to synthetic insecticides (Iloba and Ekrakene 2006; Dimetry, 2014) for both field and storage crop protection are increasing because of their low toxicity to human beings and minimal harm to the environment. Oparaeke et al., (2005) and Asawalam and Osondu (2013) reported that the effect of plant extracts on crops yield and yield component is dependent on the effectiveness of the individual plant extract. However, many require other plant spices with different mode of action, depending on the ratio and rate of application to increase their potency (Jeyaparvathi, 2013).

Over 2000 species of plants are known to possess insecticidal activities (Birch, 2011). Such plant materials include powders, water extracts, oil and wood ash from plants like Neem tree (*Azadirachta indica*), groundnuts (*Arachid hypogeal*), nutmeg (*Myristica fragrans*) and coconut. Others are leaf extracts from offish bean (*Toprasla vogelli*), ginger (*Zingiber offficinale*) garlic (*Allium sativum*), African Black Pepper (*Piper guineensis*) tobacco (*Nicotiana tabacum*), cashew (*Anacadium occidental*), (Oparaeke et al., 2000; 2006).

2.9.1 Neem Extracts

Neem (*Azadirachta indica*) is a tree in the Family Meliaceae. It is one of two species in the genus Azadirachta, and is native to India. It is a tropical evergreen tree and is also found in other southeastern countries example South East Asia, Australia, South and Central America. In India, neem is known as “the village pharmacy” because of its healing versatility, and it has been used in medicine for more than 4,000 years due to its medicinal and other healing properties. Neem extracts can affect nearly 200 species of insects some of which are pests

resistant to chemical pesticides or extremely difficult to control with them (Oladimeji and Kannike, 2010). Neem products do not necessarily kill insect pests – they are not always biocides or pesticides, but incapacitate them in several other ways, for example by interfering with development and growth of insects, act as anti-feedants on the host plant, or prevent them from depositing their eggs. Often, the precise effect is unknown (Bhattacharyya, 2007; Chaube et al. 2014). Neem extracts effectively reduced pests damage leading to increased yields (Jackai and Oyediran, 1991; Tanzubil, 2008). Neem products have shown efficacy against pod borer (Maruca vitata), pod sucking bugs complex (*Clavigralla tomentosicollis*) and other insect pests (Zongo et al., 1993). Schmutterer (1992), observed that neem products have shown activity on a wide range of insect pests. Islam et al., (2010), discovered that commercial neem, Neem Azal T/S significantly reduced the number of Aphis craccivora in cowpea.

2.9.2 Extracts from Physic Nut (Jatropha)

Jatropha curcas L. is a shrub of the Euphorbiaceae family, which originated from Central America. The Jatropha genus is widespread across tropical countries (Heller, 1996). The plant is succulent and highly resistant to drought. In many West and Central African countries, *J. curcas* is used as a means of delimiting fields for protection of cereal crops against strong winds and grazing by animals. (Henning, 2008). *J curcas* seeds are rich in an oil used as biofuel. Extensive research has, therefore, carried out to establish and develop the renewable energy potential of the plant. In addition to its use as a biofuel, Jatropha oil has also been used as a biopesticide (Solsoloy and Solsoloy, 1997).

Many reports exit that present the results of the test of oil emulsions against insects that attack stored maize grains and mung beans by *Sitophilus zeamais*, and *Callosobruchus chinensis*. The

efficacy of Jatropha oil on the control of *Amrarsca biguttula, Aphid gossypii* and *Helicoverpa armigera*, major insects that affect cotton plants have also been reported. It was observed that Jatropha oil ismore effective than Deltamethrine on *A. gossypii*, while the opposite effect could be observed on *A. biguttula.* For *H. armigera,* synthetic insecticides were more effective than Jatropha oil at the start of treatment, as the oil affects only insect growth and its effect is therefore slower (Solsoloy and Solsoloy, 2008).

2.9.3 Extracts from Lemon Grass

Lemon grass (*Cymbopogon Citratus*), a perennial plant from the grass family with long, thin leaves, is one of the largely cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, Africa and America (Sharaby, 1988). It contains 1-2% of essential oil on dry basis (Tzortzakis and Economakis, 2007). The chemical composition of lemon grass essential oil is varying widely upon genetic diversity, habitat and agronomic treatment of the culture (Carlson et al., 2001).

Lemon grass essential oil is characterized by a high content of citral (composed of neral and geranial isomers (c. 69%)), which is used as a raw material for the production of ionone, vitamin A and beta-carotene (Paviani and Dariva, 2006). Several studies reported antimicrobial activities, even for human pathogenic fungi, by lemon grass oil, (Skaria et al., 2006). Indeed, the lemon grass oil exhibited a broad spectrum of fungitoxicity by inhibiting completely growth of 35, 45, and 47 fungal species at 500, 1000, and 1500 ppm, respectively, and its fungitoxic potency remained unaltered for 210 days of storage, after which it started to decline, with considerable interests in the application of lemongrass oil for the preservation of stored food crops (Mishra and Dubey, 1994). Moreover, the essential oil of *C. citratus* was superior to

synthetic fungicides like Agrosan GN, Dithane M-43 and copper oxychloride (Mishra and Dubey, 1994; Adegoke and Odesola, 1996). Lemon grass as well as oregano and bay oil inhibited all microorganisms examined at ≤ 2% (v/v) (Adegoke and Odesola, 1996; Al Yousef, 2013). Moreover, lemon grass oil was non phytotoxic in nature, since it did not exhibit any adverse effects on germination and seedling growth of wheat and rice (Mishra and Dubey 1994).

2.10 Techniques for Virus Detection in Plants

An important prerequisite in developing control measures for plant viral diseases is the efficient detection of virus infections. In this regard, many viral coat protein and nucleic acid based techniques have been developed. One of the most widely used serological coat protein based methods is Enzyme- linked immunosorbent assay (ELISA). Serology based techniques have been effective in detection of plant viruses due to the high specificity of antigen and antibody reaction (Astier et al., 2007). ELISA technique therefore depends on these antigenic properties of the viral coat protein.

ELISA applied with either monoclonal antibody (MABS) or polyclonal antibodies (PABS) are the most extensively used for the detection of begomoviruses and tymoviruses. These viruses are characterized by the presence of coat proteins that have substantial amount of homology in their amino acid and therefore contribute to serological determination (Narayanasamy 2001; Akad and Czosnek, 2002). Serological techniques provided early evidence of leaf curl virus in diseased tissues of okra plant. ELISA has become extremely preferred for virus detection as a result of its sensitivity, accessibility of quantifiable data and its ability to handle a huge number of samples in a rapid way (Akad and Czosnek, 2002; Van Regenmortel and Dubs,

1993). ELISA is also efficient in detection of specific virus strains that exist in even low concentration in infected plant tissues (Wong, 2000). Many different types of the ELISA techniques are used (Van Regenmortel and Dubs, 1993). Some examples are direct antigen coating (DAC-ELISA), triple antibody sandwich (TAS-ELISA) and double antibody sandwich (DAS) ELISA (Narayasamy, 2001). The sensitivity of these variations in ELISA may vary depending on the tissues tested, host-virus combinations, or the level of susceptibility of the cultivar to the virus concerned.

Another advantage of ELISA is the versatility of the technique. For instance, polyclonal antiserum ELISA developed for the detection of tomato yellow leaf curl virus (TYLCV) was successfully used to detect okra leaf curl virus (Mansoor et al., 2003). Also, double monoclonal antibody ELISA using antibodies to African cassava mosaic virus was used to detect most geminiviruses including okra leaf curl virus (Givord et al., 1994).

The next set of techniques for viral detection involving viral nucleic acids tests apply mainly polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), and restriction fragment- length polymorphim (RFLP) (Shang et al., 2011). These viral detection techniques have been widely used successfully both in the laboratory and on the field (Shang et al., 2011). These methods are high throughput and can be used to run large plant samples.

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CHAPTER THREE

EFFECT OF THREE DIFFERENT PLANT LEAF EXTRACTS ON THE INCIDENCE AND SEVERITY OF OKRA MOSAIC DISEASE AND OKRA YELLOW VEIN MOSAICVIRUS DISEASE ON THREE OKRA CULTIVARS

3.1 INTRODUCTION

Plant viruses are of great concern to farmers, researches and policy makers because of the enormous losses they cause in different crops of food and economic importance. The cultivation, processing and marketing of the crop serves as a reliable source of employment and income generation for many women particularly in Ghana (Norman et al., 2011). The contribution of okra to the economy of Ghana is, therefore, quite significant. However, one of the major constraints to the production of okra in Ghana is low crop yield. Compared to agriculturally advanced countries where yields as high as 30 tha-1 is obtained, in Ghana, the average yield of okra is as low as 1.5 to 4.5 tha-1 (SRID-MOFA, 2007). The low yield levels of the vegetable discourage good adoption of it by local farmers for commercial production and use.

The major yield-limiting factor is the incidence of viral diseases and insect pest attacks. Among the diversity of plant viruses of concern, okra yellow vein mosaic virus (OYVMV), okra mosaic virus (OkMV) and okra leaf curl virus (OLCV) are the most important. These viral diseases are responsible for yield losses in the global production of okra. Infected plants produce fewer and smaller poor pods with respect to reduced fruit quality and marketable value which account for considerable economic losses (Anjorin et al., 2013). Commonly, yield losses

could range from 50 to 94% and in some instances complete crop failure depending on the stage of development at which infection occurs (Sastry and Singh, 1974). Okra mosaic disease (OkMD) is one of the most devastating and widely distributed viral diseases of okra in Ghana (Asare Bediako et al., 2014). The disease is caused by Okra mosaic virus (OkMV); a member of the Tymovirus group transmitted by the flea beetle *Podagrica* spp (Asare-Bediako et al., 2014)

Plant viral diseases are controlled largely by insecticides targeting the insect vectors. However, chemical control of plant virus diseases is increasingly becoming discouraged due to its phytotoxic effects. It is evident that society cannot continue to tolerate their harmful effects on the environment and non-target organisms. Nonetheless, synthetic pesticides application still remains the primary agricultural pest control strategy. One way to manage this menace is to develop pest management systems that are based on judicious application of synthetic insecticides. Thus there is the urgent need for the development of alternative control strategies (Mochia et al., 2011).

The alternative pests and disease control approach that is gaining wide use in crop protection against pests and diseases in recent years, is the applications of botanical extracts. Plant extracts/products have been found to be effective against a wide range of pathogens (Srivasata et al., 2010; Chakraborty and Chakraborty, 2013). Higher plants possess endogenous virus inhibitors, of which proteinaceous antiviral substances are of particular interest (Balasubramanyam et al., 2007). Therefore, plant extracts are important sources of a diverse range of antimicrobial compounds. According to Pretali et al., (2016), botanicals are one of the groups of safe insecticides which have a broad spectrum of anti-pest activity, relatively to specific mode of action, low mammalian toxicity and more tendency to disintegrate, in nature

or metabolic in a biological system. Moreover, the preparation and application at farm level are more convenient for the farmers and are quite incorporable into integrated pest management programmes.

It is envisaged that botanical insecticides could replace the expensive chemicals currently used in many developing countries. In addition, other factors such as availability, affordability and cost effectiveness have been cited as justifications for the use of bio-pesticides (Pavela, 2007). Moreover, the ease of preparation, application and incorporation into integrated pest management programmes at farm level makes the product convenient for farmers. In this study, the effect of three plant extracts, namely Neem; *Azadirachta indica* extract, Physic nut; *Jatropha curcas* extract, Lemon grass; *Citronella squinant* extract on the incidence of okra mosaic disease were investigated on three cultivated okra varieties under field conditions, and their effect compared to a known commonly used chemical insecticide Akape ®.

3.2 Objectives of study

The main objective of this study was to evaluate the effect of the application of three plant extracts on the incidence and severity of Okra mosaic disease.

The specific objectives were:

1. To determine the incidence and severity of OkMD and OYVMD following the application of the three leaf extracts.
2. To confirm the infection of the okra plants by OkMV and OYVMV using the enzyme-linked immunosorbent assay technique.
3. To determine the effect of the application of the plant extracts on fruit yield of the three okra cultivars.

3.3 MATERIALS AND METHODS

3.3.1 Study area and location

The field experiment for this study was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) between July 2017 and March 2018. The study area is located at Kwabenya, Accra on latitude 5º 40' N, longitude 0º 13' W with Ochrosol (Ferric Acrisol) soil type, derived from quartzite Schist. The site is well drained and has an elevation of 76 m above sea level within the coastal savannah agro- ecological zone. The maximum and minimum average temperatures for the period of study were 30.7 and 26.0 0C respectively with average annual rainfall of 220 mm. The highest and lowest relative humidity is between 75 and 60% (Akaho et al., 2003: Dickson and Benneh, 2004).

3.3.2 Land preparation, planting and cultural practices

The experimental field was lined and pegged and laid out into four blocks with five plots per block, each plot consisting of three sub plots. Seeds of three varieties of okra namely F1 Sahari (FIS), F1 Kirene (FIK), and Assountem (AST) were acquired and planted in a randomized complete block design with four replications. Four seeds were planted per stand and later thinned to one plant per stand two weeks after emergence leaving twenty plants per sub plot. There were twenty plants per sub plot and five plants out of the twenty were tagged as experimental plants on which data was taken. Routine cultural practices like watering, fertilizer application and weed control were carried out as and when necessary from germination of the seeds until maturity when the okra fruits were harvested. N.P.K (15-15-15) fertilizer was applied 21 days after seed emergence at the rate of 250 kg ha^{-1}.

3.3.3 Preparation and application of leaf extracts

Three biopesticides namely Neem (*Azadirachta indica)* extract, physic nut (*Jatropha curcas)* extract, Lemon grass (*Citronella squinant)* extract and a commercial pesticide (Akape®) were assessed for their ability to reduce insect pest infestation of field grown okra. Leaf extracts were prepared by grinding 50g neem leaf per litre of water (Biswas, 2013), 50g Jatropha leaves per 500ml of water (Jide-Ojo et al, 2013), and 50g Lemon grass leaves per litre of water (Ali Safdar, et al., 2005). The resulting solution was sieved with a fine mesh and 15ml each of neem, lemon grass and jatropha leaf extract concentrate was diluted in I litre of water. The three extracts alongside a commercial pesticide was sprayed on the okra plants with the help of a portable hand sprayer. Spraying was done on both the adaxial and abaxial sides of the okra leaves. The treatments were applied fortnightly starting one week after emergence.

3.3.4 Estimation of Disease incidence (DI) and Symptom Severity

Where initiation of symptom development was found, it was tracked and recorded for six consecutive weeks and the results was considered as disease incidence. Based on visual examination of symptoms, and following a procedure outlined by Sankara and Acharyya (2012), the disease incidence within the okra field was estimated as follows:

$$\text{Disease Incidence} = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants\ per\ plot} \text{X } 100\%,$$

Severity of symptoms was scored on a five point scale (0 – 4), modified after Ali et al., (2005), where; 0 No symptom; 1 Very mild symptoms, initial vein clearing, initial leaf yellowing, mild curling and blistering; 2 Leaf completely yellow and inter-veinal regions remain green or

yellow and blistering; 3 Severe curling, yellowing, stunting and blistering; 4 Severe yellowing, curling, blistering and deformed pods (All leaves of the plants get affected).

3.3.5 Confirmation of virus infection by ELISA test

The ELISA kits used for the detection of OkMV were purchased from DSMZ Plant Virus Collection, Braunschweig, Germany. The test format used for OkMV was the Double antibody sandwich ELISA (DAS-ELISA), following the protocol of the manufacturer except a slight modification of adding 2% skimmed milk to the wash in order to minimize non-specific binding. All tests were carried out at molecular-biology laboratory of the Biotechnology Centre of the BNARI, Ghana Atomic Energy Commission.

3.4 RESULTS AND DISCUSSION

3.4.1 Okra viral disease symptoms observed on the experimental field

Symptom expression started at approximately 2 weeks after plant emergence and persisted throughout the study. Diseased okra plants expressed various degrees of symptoms including leaf yellowing and distortions, mosaic, vein clearing, leaf curling and stunting (Figure 4.1). . The symptoms were indicative of viral infections and were more prominent in young actively growing leaves (Figure 3.1). These symptoms have been reported in virus-infected okra plants (Asare Bediako et al., 2014).

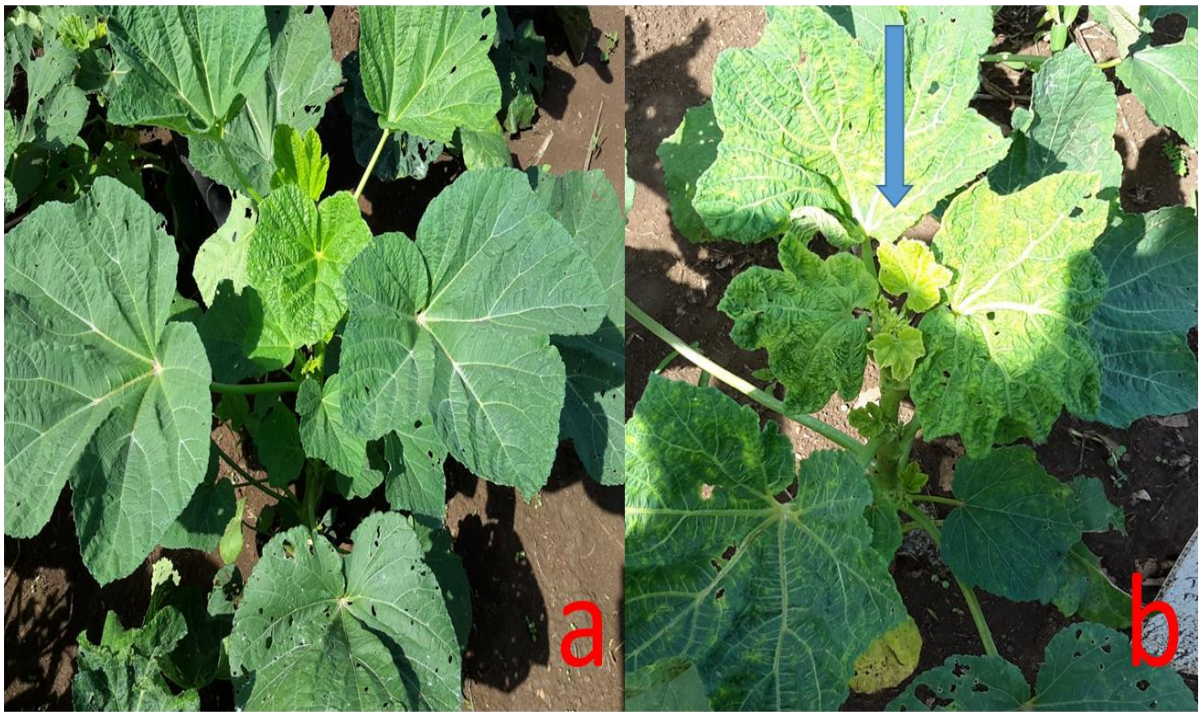

Figure. 3.1: Okra plants (a) Healthy okra plant (b) Okra mosaic virus-infected plant showing leaf yellowing, distortions and leaf mosaic symptoms

3.4.2 Effect of phytopesticide treatment on disease incidence of three okra varieties.

There were significant effects of phytopesticides on the incidence of Okra mosaic virus and Okra yellow mosaic virus as indicated by proportion of infected plant per cultivar. The effects of the leaf extracts on viral disease incidence on the three okra varieties are shown in Figure 3.2. Disease incidence (DI) was generally high and varied significantly among the treatments. However, okra plants treated with the chemical and neem extract had a much lower disease incidence compared to the Jatropha and Lemon grass extract and the control. Disease incidence was relatively low in F1 Kirene plants throughout the study irrespective of the treatment. This cultivar had previously been rated as highly susceptible (Boateng et al., 2019), thus the low disease incidence could be attributed to the biopesticides. The effect of plant extracts on Okra

mosaic virus incidence and yield related Parameters of Okra has been demonstrated (Bhyan et al., 2007). On the contrary, DI on cultivar F1 Sahari was generally high for all varieties irrespective of the treatment. This could possibly be due to the extreme susceptibility of the cultivar as reported by Boateng et al., 2019. Disease incidence was low in Asutem and F1 Sahari plants treated with Neem extract compared to those treated with Lemon and Jatropha extracts.

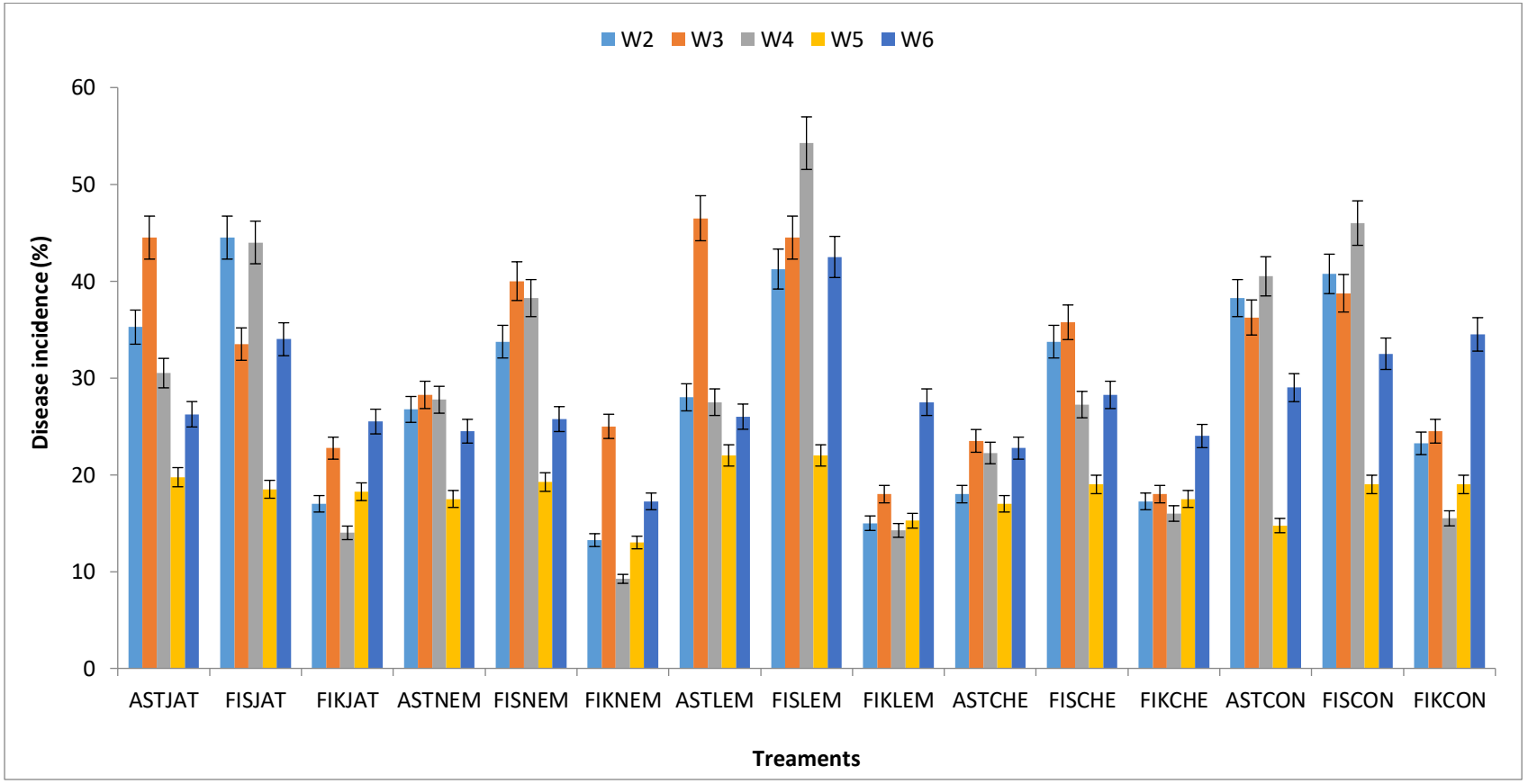
W2
W3
W4
W5
W6
60
50
40
30
20
10
0
Disease incidence (%)
ASTJAT
FISJAT
FIKJAT
ASTNEM
FISNEM
FIKNEM
ASTLEM
FISLEM
FIKLEM
ASTCHE
FISCHE
FIKCHE
ASTCON
FISCON
FIKCON
Treaments

Figure 3.2 Effect of different bio-pesticide treatments on mean viral disease incidence of three okra varieties

3.4.3 Effect of bio-pesticide treatment on mean symptom severity of three okra varieties.

The viral symptom severity scores for the three okra varieties are shown in Figure 3.3. Even though the differences in the mean symptom severity among the treatment were not significantly different from each other statistically, slight differences were recorded. Plants of cultivar F1 Sahari were most severely affected by the viral disease irrespective of the treatment throughout the study. On the contrary, F1 Kirene was the least affected in terms of severity of symptoms, showing mild symtoms throughout the study. The impact of viral infection is influenced by the virulence of the specific virus strain (or strains for mixed infections), the host genotype and environmental conditions (Paudel and Sanfaçon, 2018). Therefore, these observations could be as a result of the genetic makeup of the cultivars.

Although a significant virus load is sustained resistant/tolerant cultivars, the plant growth is minimally affected and visible symptoms are either absent or mild (Paudel and Sanfaçon, 2018), resulting in less damage to the host.

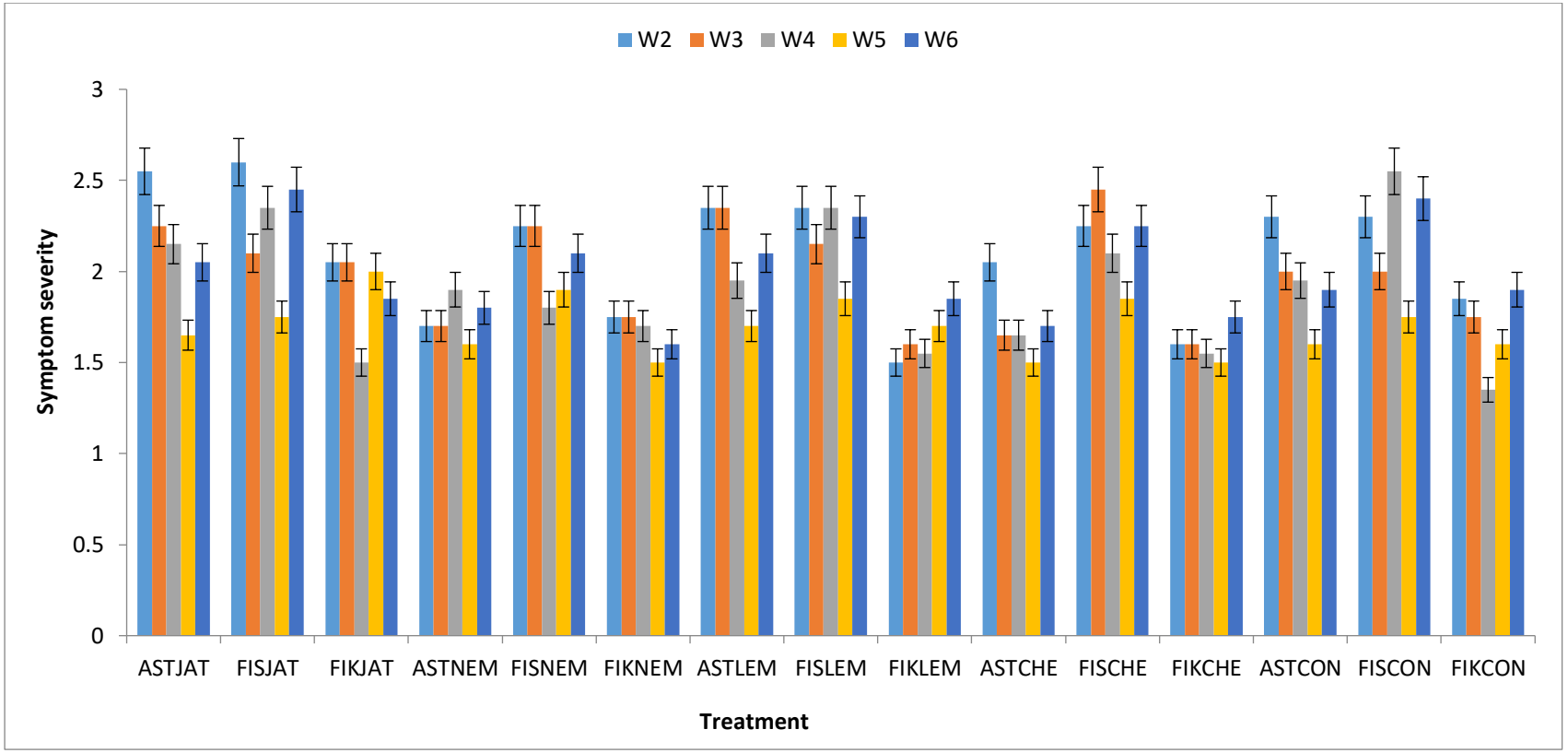

Figure 3.3 Severity of viral disease disease symptoms in three okra varieties treated with aqueous leaf extracts of Jatropha, Neem and Lemon grass

3.4.4 Confirmation of the presence of viruses by Enzyme-linked immunosorbent assay (ELISA).

Enzyme-linked immunosorbent assay (ELISA) is the most reliable serological detection method widely preferred for detecting plant viruses due its economy in the use of reagents, readiness of quantifiable data and ability to test a relatively large number of plant tissues same time within a short period of time (Narayasamy, 2001). Furthermore, the technique can be used to detect specific strains of viruses occurring even in low concentrations in infected plant parts (Wong, 2000). In this study, the technique successfully detected OkMV and OYVMV confirming that the visual symptoms observed on the okra plants on the field were due to the viral infection (Table 3.1). Okra mosaic virus was the most commonly detected by ELISA. The highest positive absorbance (0.252), indicating the highest virus titre was found in F1 Sahari plants treated with neem extract.

The incidence of Okra yellow vein mosaic virus infection was generally low among the varieties irrespective of the treatment applied,, with the highest positive absorbance (0.525) observed in in Asutem plants treated with neem extracts. The low incidence of OYVMV infection explains the why mosaic symptoms were most prevalent on the field. Furthermore, the degree of co-infection of the two viruses (6.67%) among the cultivars was low compared to a previous study (Appiah et al, 2020).

Table 3.1: Shows the results of ELISA confirming the presence or absence of the \Okra Mosaic Virus in the three okra varieties that were subjected to the pesticide treatments.

		OkMV			OYVMV		
Variety	Treatment	Absorbance@405nm	Elisa Reading (+/-)	% Infection	Absorbance@405nm	Elisa Reading (+/-)	% Infection
FIS	JAT	0.009	-		0.128	-	
	JAT	0.010	+	33	0.046	-	0
	JAT	0.001	-		0.108	-	
FIS	NEM	0.009	-		0.097	-	
	NEM	0.252	+	33	0.140	-	0
	NEM	0.003	-		0.099	-	
FIS	LEM	0.012	+		0.057	-	

	LEM	0.013	+	67	0.021	-	0
	LEM	0.007	-		0.076	-	
FIS	CHE	0.005	-		0.026	-	
	CHE	0.005	-	33	0.022	-	0
	CHE	0.234	+		0.078	-	
FIS	CON	0.245	+		0.292	+	
	CON	0.013	+	100	0.025	-	33
	CON	0.234	+		0.067	-	
FIK	JAT	0.007	-		0.057	-	
	JAT	0.011	+	67	0.025	-	0

	JAT	0.011	+		0.044	-	
FIK	NEM	0.009	-		0.059	-	
	NEM	0.010	+	33	0.042	-	0
	NEM	0.008	-		0.034	-	
FIK	LEM	0.008	-		0.031	-	
	LEM	0.007	-	0	0.028	-	0
	LEM	0.009	-		0.077	-	
FIK	CHE	0.008	-		0.017	-	
	CHE	0.005	-	0	0.025	-	0
	CHE	0007	-		0.024	-	

FIK	CON	0.004	-		0.152	+	
	CON	0.008	-	0	0.024	-	33
	CON	0.006	-		0.016	-	
AST	JAT	0.011	+		0.122	-	
	JAT	0.009	-	67	0.061	-	0
	JAT	0.011	+		0.050	-	
AST	NEM	0.005	-		0.351	+	
	NEM	0.007	-	0	0.525	+	67
	NEM	0.009	-		0.085	-	
AST	LEM	0.248	+		0.350	+	

	LEM	0.011	+	67	0.305	+	67
	LEM	0.226	+		0.094	-	
AST	CHE	0.011	+		0.012	-	
	CHE	0.235	+	33	0.031	-	0
	CHE	0.003	-		0.048	-	
AST	CON	0.006	-		0.059	-	
	CON	0.033	+	100	0.102	-	33
	CON	0.009	-		0.010	-	

Negative control=0.005. Values are means are four tests. All values equal or greater than the negative control × 2 are positive (0.005×2 = 0.01).

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3.4.5 Effect of biopesticide treatment on total yield of three okra varieties.

The effect of the biopesticides on the total yield of the okra cultivars is shown in Figure 3.4. The differences in the yield of the three okra cultivars were significant ($p<0.005$). The total yield for Asutem and F1S were generally low, but the lowest yield was recorded in the control plants of Asutem. Yield levels were significantly ($p<0.005$) highest in the chemically treated okra plants compared to all other treatments (Figure 3.4). In the control plants where no pesticides were applied, lowest yields were obtained. The yield of Jatropha extract treated FIS plants was observed to be significantly higher than the control. Similarly, Neem extract treated FIK and FIS plants produced significantly higher yield levels than the control. It was found that in the variety AST, comparing the control and biopesticide treatment, only Neem extract treatment was effective in producing a better yield. Similarly, in FIK, Jatropha and Lemon extract treatments were not effective except Neem. Lemon extract treatment was ineffective in enhancing yield in FIS. F1K performed best giving significantly ($p<0.005$) best yields irrespective of the treatment it received. F1 Kirene had been rated as highly susceptible in a previous study (Boateng, 2018), thus its high performance could be due to the effect of the pesticide treatment. The relatively low disease incidence and symptom severity throughout the study may have culminated in the high yield. The worst performing variety was F1S.

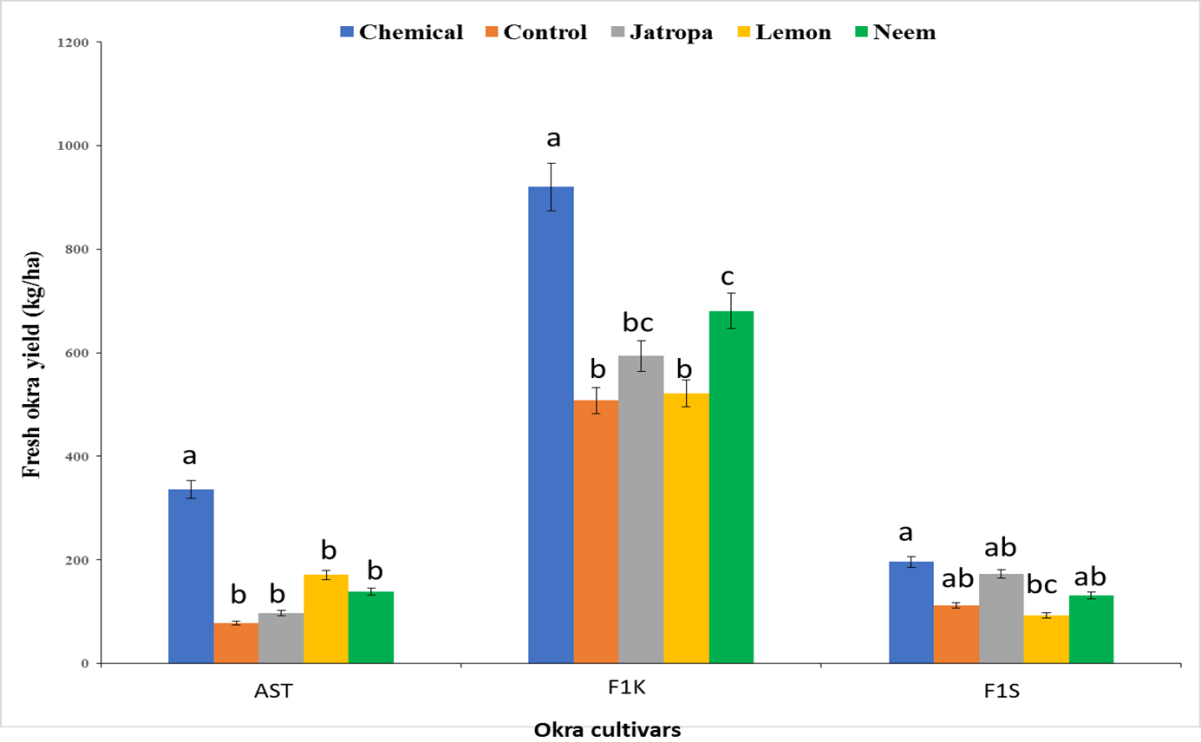

Figure. 3.4 Fig. 1.4: Effect of different pesticide treatments on the total yield of three okra varieties. AST = Asutem, F1K = F1 Kirene, F1S = F1 Sahari

3.5 Conclusion

The present study has demonstrated the effect of plant extracts in the control of viral diseases of okra. The positive control (chemical) gave the best results throughout the study, followed by the Neem plant extract. Even though the chemical treatment gave the best results, its negative effect on the environment and high cost necessitates the search for a more environmentally-friendly and cheaper approaches in dealing with plant viral diseases. Therefore, it is imperative to try varying concentrations of the Neem extract and at different seasons to determine its efficacy on the control of Okra mosaic and Okra yellow vein mosaic viruses.

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CHAPTER FOUR

EFFECT OF CRUDE LEAF EXTRACTS ON INSECT PEST ABUNDANCE ON OKRA

4.1 INTRODUCTION

Okra is one of the worlds' leading vegetables in terms of nutritional benefits and a host of industrial uses. The crop has become an important commodity of trade and export in Ghana. The total area under cultivation of okra in Ghana is about 19,500 ha of arable land with yield potential of 5.5 Mt\ha (Oppong-Sekyere et al., 2012).

The crop is attacked by a number of insect pests mainly white fly and flea beetle as well as viral diseases during different growth stages (Sardana et al., 2005; Gulati, 2004). Among viral diseases, okra mosaic virus (OMV) and okra yellow vein mosaic virus (OYVMV) are the most economic importance (Aziz et al., 2011). These viral diseases attack okra plants both at the vegetative and fruiting stages, resulting in a decline in quality and quantity of the produce. Due to high reproductive and damage potential of the feeding habits of the white fly and flea beetle vectors, their management on okra has become increasingly difficult. Farmers rely on the use of synthetic insecticides for the control of these insect pests. Since okra is sometimes consumed fresh, the use of toxic substances on the plant to control insect pests is not desirable (Kumar, 2015).

The yield of okra in Ghana continues to decline and is currently estimated at 2.5 t/ha (SRID-MOFA, 2007). Two flea beetle species, *Podagrica uniformis* and *Podagrica sjostedti* are responsible for leaf damage of okra (Obeng-Ofori and Sackey, 2003). Extensive leaf damage in the form of feeding creates holes on the leaves resulting in significant reduction of photosynthetic activities of the plant. The insect pests also feed on fruits, stems and flowers

which then culminate in poor crop performance and very low yields. The *Podagrica* species have also been implicated in the transmission of okra mosaic virus (Echezona and Offordile, 2011; Alegbejo, 2008).

The other insect pests of economic importance in okra production are the whiteflies (*Bemisia tabaci*) which feed on plant sap and transmit the *Okra yellow vein mosaic virus* (Echezona and Offordile, 2011; Ali et al., 2005).The species diversity of insects and their pest status varies from region to region with the variation in agro climatic conditions. Asare-Bediako et al., (2014) indicated that there is always a phenomenon of continual significant increase in insect populations globally. In Ghana, for instance, the rising insect pest populations has been attributed to poor agronomic practices such as the use of untreated seeds for cultivation and the continuous practice of mono-cropping by majority of local farmers in order to meet the increasing demand of the various staples in the country (Asare-Bediako et al., 2014).

It is the desire of farmers and agronomists to adopt safe and cost effective methods of farming, with the possibility of reducing the incidence of pest and diseases and the significance of their damage (Ntow et al., 2006). There is, therefore, the need to explore alternative control methods and techniques of pest control in order to reduce the sole dependence on chemical insecticides. Plant extract and exudes has been found over decades to poses active ingredients that has the potentials to repel some insect pests without any damage to non-targeted species and the environment (Dayan et al., 2009). *Azadirachta indica* (Neem), *Jatropha curcas* (physic nut), and *Citronella spp* (lemon grass) and a host of other plants are considered as effective botanical pesticides used to control a wide variety of insect pests (Patil, 2005 ; Khuhro et al. 2014). The increase in awareness regarding food safety has increased the demand for organically produced

food, necessitating the evaluation of biopesticides as safer alternatives to conventional insecticides (Akbar et al., 2010).

4.2 Objectives of the study

The principal objective of the study is to assess the efficacy of crude leaf extracts on the control of insect vectors of two viral diseases of okra Sin the coastal savannah agro-ecological zone of Ghana.

The specific objectives were:

1. To identify the elemental and phytoconstituents of the three plant aqueous extracts.

2. To assess the abundance of the insect species during the vegetative, flowering andfruiting stages of three selected okra cultivars.

3. To assess the extent of leaf damage on the okra cultivars during the vegetative, flowering and fruiting stages under field conditions.

4. To assess the efficacy of three different plant extracts on the control of insect vectors of OkMV and OYVMV.

4.3 MATERIALS AND METHODS

4.3.1 Soil and Rainfall Pattern of the Study Area

The research was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) research farms between July 2017 and March 2018. The study area is located at Kwabenya, Accra on latitude 5º 40' N, longitude 0º 13' W with Ochrosol (Ferric Acrisol) soil type, derived from quartzite Schist

(FAO/UNESCO, 1994).The maximum and minimum average temperatures for the period of study were 30.7 and 26.00C respectively with average annual rainfall of 220 mm. The highest and lowest relative humidity is between 75 and 60% (Akaho et al., 2003). The experimental site is well drained and has an elevation of 76 m above sea level within the coastal savannah agro-ecological zone.

4.3.2 Plant materials and field design

Plant materials used for the study consisted of three (3) okra cultivars namely F1 Kirene, F1 Sahare and Assontem. They were selected based on their susceptibility to okra mosaic virus (OMV) and okra yellow vein mosaic (OYVM) (Appiah et al, 2020).

Land preparation involved ploughing, harrowing, lining and pegging into 40 experimental plots using a Randomized Complete Block Design with four replications. Each replicate measured 35m x7m and separated by 2m from each other with 10 subplots within a block. Each subplot measured 3m x 3m and spaced from one another by 1m. Total size of the experimental area was 646m2. The okra seeds were manually sown to a depth of 2cm directly at a spacing of 0.50m x 0.60m between and within rows respectively. Four seeds per hill were sown and later thinned to one seedling per hill after emergence. Recommended agronomic practices including fertilizer application, weeding and watering were undertaken.

4.3.3 Selection plant extracts used

The plant species from which the biopesticides were prepared for this study are *Azadirachta indica* (Neem), *Jatropha curcas* (physic nut), and *Citronella spp* (lemon grass). The selection of these plants was based on their previously reported insecticidal properties and potency in

the control of many insect pests of vegetables (Ref…). Shown in Table 4.1 are the reported active ingredients and application concentrations of each plant extract.

Table 4.1: Plant extracts used in this study, their active ingredients and concentrations

Plant extract / Chemical	Active Ingredients		Concentration per acre
Neem	Azadirachtin		500 mL
Physic nut	Fatty acid		500 mL
Lemon grass	Citral		500 mL
Akape®	Imidacloprid	200g/L	75 mL

4.3.4 Preparation of aqueous extract

The leaves of the plant were harvested and washed to remove sand, dust and chemical contaminants. The fresh leaves were dried under shade, blended and prepared following the method of Rezaul Karim et al. (1992) and was filtered using muslin cloth. The final volume of each plant extract filtrate was made up by diluting 15 ml of extract with 1 litre of distilled water. The stickiness and adherence of each of the plant extract was enhanced by the addition of 100 ml of 5 % soap solution as surfactant.

4.3.5 Elemental and phytoconstituent analysis of aqueous extracts

4.3.5.1 Heavy metal analysis in the plant extracts

40 ml of plant extract was measured and transferred into a 100 ml borosilicate beaker containing acid wash. 25 ml of aqua regia was added to the sample in the ratio of 3:1 ml of 1 M HCl and 1 M HNO_3 in the fume chamber. The mixture was placed on a hot plate and heated at 45 °C for 3 hours. The mixture was allowed to cool to room temperature and transferred into a 50 ml volumetric flask and made up to the standard mark with deionized water after rinsing the reacting vessels, to recover any residual metal. The digested sample was then stored in pre-cleaned polyethylene storage bottles ready for analysis. The metal concentrations were determined using Fast Sequential Atomic Absorption Spectrophotometer (FSAAS 240) at the Nuclear Chemistry and Environmental Research Centre (NCERC), Ghana Atomic Energy Commission (GAEC), Kwabenya. The Spectrophotometer was earlier calibrated with analytical grade standard solutions of the five metals. A quality assurance programme was also put in place which involved analysis of blanks and duplicates and determination of % recovery of the metals. The working conditions of the FAAS 240 is summarised in Table 4.2.

Table 4.2: The working conditions of the FAAS 240

ELEMENT	WAVELENGTH nm	LAMP CURRENT nA	SLIT WIDTH nm	FUEL	SUPPORT
Fe	248.3	5	0.2	ACETYLENE	AIR
Cd	228.8	4	0.5	ACETYLENE	AIR
As	193.7	10	0.5	ACETYLENE	AIR
Zn	213.9	5	1.0	ACETYLENE	AIR
Mn	279.5	5	0.2	ACETYLENE	AIR

4.3.5.2 Elemental Analysis using FAAS

90 g Fresh leaves of the three biopesticides (Neem, Lemon grass and Jatropha) were ground with 700 ml of distilled water. The resulting solution was sieved with a fine mesh and referred to as the extract. 100 ml of the extract of each biopesticide was collected and sent to the Chemistry Department of the National Nuclear Research Institute of the Ghana Atomic Energy Commission for elemental analysis. The extract was subjected to the following protocol.

Weigh 2 g of sample into a 100 ml class A beaker. Add 6 ml of 1 M HCl and 2 ml of 1 M H_2O_2. Cover the beaker with a cling film, place it on the hot plate and digest it for 3 hours at a

temperature of 45 °C. After the acid digestion, transfer the sample into a 50ml measuring cylinder and top it to the 20 ml mark with double distilled water. Transfer the whole content into a test tube for Atomic Absorption Spectoscopy analysis.

4.3.5.3 Phytoconstituent analysis

A substantial amount of the leaves of the three biopesticides (Neem, Lemon grass and Jatropha) were air dried, grounded and sieved to obtain a fine powder. 100 g powder of each biopesticide was packaged and sent to the Centre for Scientific Research into Plant Medicine, Mampong for analysis. The powder was subjected to analysis to determine the following phytoconstituents; saponins, polyuronides, reducing sugars, alkaloids, phenolic compound, cyanogenic glycosides, flavonoids, anthracenosides, triterpenes, and Phytosterols. To determine the phytoconstituents in the dried leaves samples, they were subjected to the following protocols.

Saponins: Shake 2 ml of diluted solution (1:1) in a test tube of 1.6 cm diameter for 15 minutes. The occurrence of a foam column of at least 1 cm in height persisting minimum 15 minutes, indicates the presence of saponins.

Reducing compounds: The alcohol extract (0.5-1ml) is diluted with water (1-2 ml) add Fehlings solution (A and B) (0.5 – 1 ml) solutions and heat them. A brick red precipitate denotes the presence of reducing compounds.

Polyuronides: 2 ml of the aqueous extract are added dropwise in a test tube where 10 ml of alcohol or acetone have already been placed. If a thick precipitate is formed it denotes presence of polyuronides.

Phenolic Compounds: 2-3 drops of 5 % Ferric Chloride is added to 3 ml of aqueous extract. A sudden change in colour to black, bluish-black or dark green shows presence of phenolic compounds.

Alkaloids: The alcoholic extract is evaporated to dryness and about 5 ml of 10 % HCl is added to the residue containing alkaloids as salts of some organic acids. The alkaloids now become salts of the mineral acid. From the aqueous solution, the alkaloids are precipitated as bases with the help of ammonia solution (Ph=8.9, 10%) and extracted with a non-polar solvent (ether, chloroform). The ether or chloroform solution is evaporated to dryness in an evaporating dish. The residue is dissolved in hydrochloric acid solution (1.5 ml, 2%). The acidic solution in which the alkaloids are under a salt form was divided into three test tubes: one is the reference and in the other two test tubes 2-3 drops of Mayer's reagent are added. The occurrence is an opalescence or yellowish-white precipitate with Mayer's reagent. To 25ml alcohol extract, hydrochloric acid solution (10%, 15ml) is added by refluxing and heated up for 30 minutes. During the hydrolysis the solution becomes opalescent due to the precipitating aglycones obtained by the division of the glycosides. After cooling, the solution is 3 times extracted in separating funnel, with ethyl ether (10-12 ml). The ether extracts are placed together (30-36ml) and dehydrated with anhydrous sodium sulphate, resulting in an ether and an aqueous solution.

The ether extract will serve to identify the anthracenosides, flavonoid, steroid glycosides and triterpenes by means of the following series of reactions characteristic of each group.

Identification of anthracenosides: The ether extract (4 ml) is concentrated to 2 ml, then ammonia solution (25%, 1-2 ml) is added by shaking. A cherish-red colour of the alkaline solution indicates the presence of aglycones of anthracenosides (Borntrager's reaction).

Identification of Phytosterols and Triterpenes: The ether extract (10ml) is evaporated to dryness. The residue is dissolved successively in acetic anhydride (0.5 ml) and chloroform (0.5 ml). The solutions are transferred to a dry test tube. Concentrated Sulphuric acid (1-2 ml) (Liebermann-Burchard's reaction) is added at the bottom. At the separating level of the two liquids, reddish-brown or violet-brown ring is formed, the superior layer being green for phytosterols and red or violet for triterpenes.

Identification of Flavonosides: The ether (5 ml) is evaporated to dryness. The residue is dissolved in methanol (50%, 1-2 ml) by heating, then metal magnesium and 5-6 drops of concentrated hydrochloric acid are added. The solution becomes red for flavonols and flavonones (Shibata's reaction).

Cyanogenic glycosides: A small volume (5 ml) of the extract was added to 1 ml of chloroform in a test tube and corked with a yellow picric paper. The mixture was heated on a water bath for few minutes. A colour change from picric yellow to brown or red was a positive test (Sofowora, 1993).

4.3.5.4 Total Flavonoid Analysis

To further quantify the amount of flavonoids in the biopesticides 100g of prepared powder from the leaves of each plant was analysed at the Noguchi Memorial Institute for Medical Research laboratory for quantification of the total flavonoids in eachsample. To determine the total flavonoids, the powder was subjected to the following protocol.

To 0.1 mg of Quercitin, 1 ml of methanol was added. A 2x serial dilution was done to obtain seven different concentrations of Quercitin. Two concentrations (10 mg/ml and 5 mg/ml) of each extract was prepared by dissolving the extracts in distilled water. To 100 µl of each test

substance, 100 µl of Aluminium chloride was added. The mixture was then incubated at room temperature for 20 minutes, after which the absorbance was checked at 415 nm. The data from Quercitin was used to plot a calibration curve from which the flavonoid content of the extracts was extrapolated. From the absorbance, the total flavonoid content of each extract was calculated and expressed as quercetin equivalents.

4.3.6 Application of plant extracts

Five treatments namely T1= Neem extract, T2 = Physic nut, T3 = Lemon grass, T4 = Akape® and T5 = Untreated control were applied. Akape® is chemical pesticide which is currently being used by farmers to control insect pests of okra. The extracts and the chemical used with their doses were based on Onunkun (2012). The crop was sprayed at15, 30, 45 and 60 days after sowing. Spraying of the plant extracts was done early in the morning before sunrise because of the photodegradable nature of the extracts.

4.3.7 Leaf damage assessment

The extent to which insects had caused physical damage to the leaves of the okra plants in field was assessed by using a rating scale developed by Boateng et al (2019) as follows: 1 = very mild damage (1 to 15 holes); 2 = mild damage (16 to 30 holes); 3 = moderately severe damage (31 to 45 holes); 4 = very severe damage (46 to 60 holes); 5 = extremely severe damage (more than 60 holes)

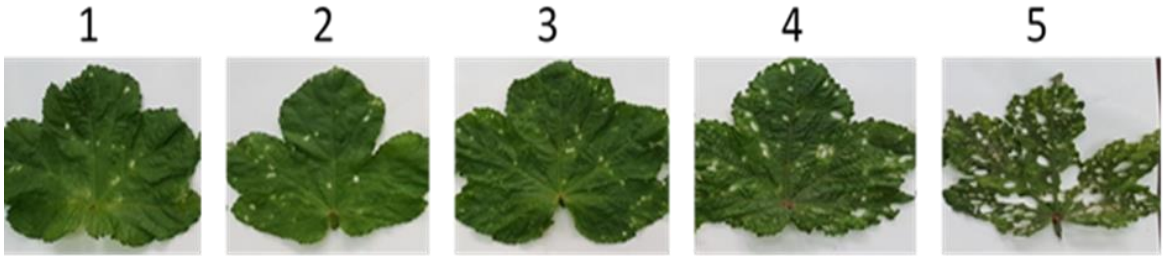

Figure 4.1 Insect damage rating scale (Boateng et al., 2019).

Leaves were visually assessed and scored for severity of damage using the damage rating scale described above (Figure 4.1). Leaf damage was determined by counting the total number of perforations created by the insects in all leaves found on the five randomly selected test plants. This was then divided by the total number of leaves on the five selected test plants to obtain the average number of perforations per leaf.

4.3.8 Flea beetle and whitefly monitoring

Data on insects were collected from five okra plants randomly selected from the middle rows. Five topmost fully expanded okra leaves were carefully examined by observing both the abaxial and adaxial surfaces. Insects found on the surfaces of the leaves were identified, counted manually and recorded. Data were taken weekly and the insects were counted between the hours of 6.00 am and 8.00 am when they are less active.

4.3.9 Data analyses

Quantitative data on insect abundance at various growth stages were subjected to one- way analysis of variance (ANOVA) while differences in treatment means were separated by the least significant different (LSD) test at 5% level of significance. All statistical analyses were done by GenStat statistical package (Version 12) and Microsoft Excel (2010 edition).

4.4 RESULTS AND DISCUSSION

4.4.1 Elemental Analysis

Atomic absorption spectroscopy (AAS**)** is a spectro-analytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. Atomic absorption spectroscopy is based on absorption of light by free metallic ions. AAS can be used to determine over 70 different elements in solution. Results from the elemental analysis are shown in Figure 4.2.

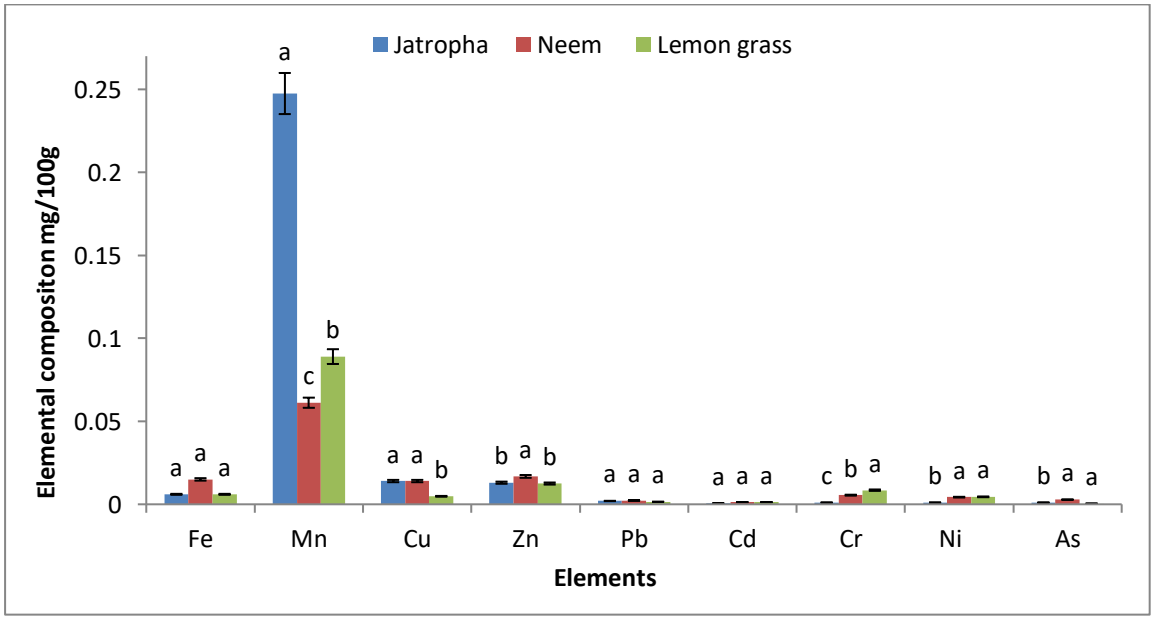

Figure 4.2: Elements identified in the biopesticides samples.

The elements found in the biopesticides samples were Iron, Manganese, Copper, Zinc, Lead, Chromium, Caesium, Nickel and Arsenic. All these elements are needed in various quantities

for various physiological roles in the plant. Almost all the elements identified are in the heavy metals group whose high abundance in the environment results in heavy metal toxicity. However, the quantities detected in the biopesticides were not high enough to cause harm to consumers who will consume okra treated with any of these biopesticides. The levels of these heavy metals were far below the WHO/FAO maximum permissible limits. For instance, manganese which recorded the highest concentration of 0.248mg/L was way below the WHO/FAO maximum permissible limit of 5.5 mg/L. WHO/FAO maximum permissible limit for iron is 4.5mg/L, lead is1.0 mg/L and Arsenic 0.26mg/L (Tiimub and Afua, 2013). However, the levels detected for iron, lead and arsenic in our study were all below 0.1 mg/L. The application of these plant extracts as biopesticides, therefore, poses no risks associated with heavy metal toxicity for both the environment and health of the consumer (Khan and Rahman, 2017). Figure 4.2 shows the most significantly abundant element to be manganese in all three plant extracts tested. Jatropha extracts contained significantly the highest quantity of manganese followed lemon grass and least in neem extracts. Lead, Caesium, Nickel and Arsenic were found lowest in quantity.

4.4.2 Phytoconstituents Analysis

Phytochemicals are naturally occurring in medicinal plants and offer protection from various diseases. Phytochemical properties are important attributesof many potent medicinal plants. Medicinal plants have so many phytochemicals each of which is possess particular important medicinal properties (Wadood et al 2013). The analysis was carried out to detect the presence of the following phytoconstituents; saponins, polyuronides, reducing sugars, alkaloids, phenolic compound, cyanogenic glycosides, flavonoids, Anthracenosides, Triterpenes, and Phytosterols. This analysis was only qualitative but not quantitative meaning this analysis only

shows the presence or absence of a particular phytoconstituent but could not detect the quantity of phytoconstituent in the sample. Some of the phytochemicals tested for in the three biopesticide extract were present and others were not detected. The most abundant phytochemical were saponins, reducing sugars polyuronides and flavonoids all of which play an important role in the potency of the plant extracts as biopesticides Table 4.3 shows all the detected phytoconstituents in the three biopesticides.

Table 4.3: Phytoconstituents in Jatropha, Neem and Lemon grass leaf extracts

Phytoconstituents	Jatropha	Neem	Lemon
Saponins	+	+	+
Polyuronides	+	+	+
Reducing sugars	+	+	+
Phenolic compounds	-	+	+
Cyanogenic glycosides	-	-	-
Alkaloids	-	-	-
Flavonoids	+	+	+
Anthracenosides	-	-	-
Triterpenes	-	-	+
Phytosterols	+	+	-

4.4.2.1 Total Flavanoid Analysis

Antioxidants or free radicals are generated in excess in a diverse array of microbial infections. Flavonoids are synthesized by plants in response to microbial infections. *In vitro* analysis of flavonoids are found to be antimicrobial substances against a wide array of microorganisms. Results from the analysis of total flavonoids ranks Neem to contain the highest (647.37mg/100gQE) followed by Lemon grass (624.78mg/100gQE) and Jatropha (333.78mg/100gQE). These high levels of flavonoids indicate that these plants are capaable of preventing pathogen attack and disease occurrence. This is consistent with work done by Alzohairy (2016), who reported that Neem and its constituents play a major role in the scavenging of free radical generation and prevention of disease pathogenesis. The total flavonoids analysis was undertaken at the Noguchi Memorial Institute for Medical Research (NMIMR). This Analysis was quantitative, so the quantity of flavonoid in each sample was detected. Figure 4.3 shows Neem sample recorded the highest flavonoid content followed by Lemon grass and Jatropha. . The quantity of flavonoids in the Neem was nearly twice as much as in Jatropha.

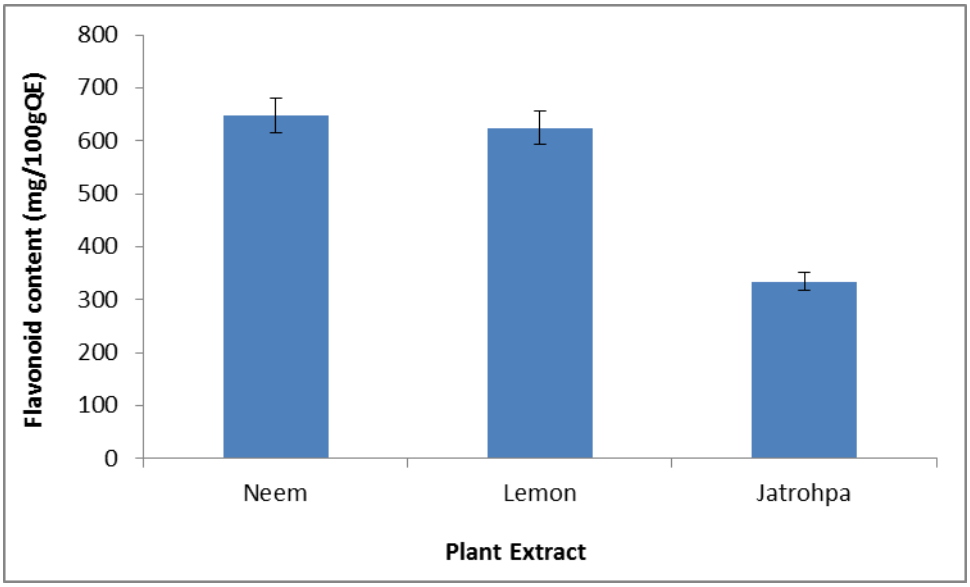

Figure 4.3: The quantity of flavonoids in the three biopesticides extracts

4.4.3 Chemical pesticide

The label on the chemical pesticide used suggests that Akape® insecticide is a broad spectrum systemic pesticide with an active ingredient of Imidicloprid which has a half-life of 1 – 3 years under anaerobic conditions and 39 days under aerobic conditions. It being a broad spectrum means that it can kill a wide range of pests including beneficial organisms in the environment. This means, when sprayed on okra, all the insects including beneficial ones will die. This attribute is not environmentally friendly (Abd-allah et al, 2009) and that it should not be the choice over environmentally friendly ones. It being systemic means that the active ingredient is transported through the vascular system to all parts of the plant. This also means that the active ingredient gets into the fruit as well hence dangerous to consumers. The half-life permits that the active ingredient is classified as persistent; meaning, its effect exists in the environment

even when the okra has long been harvested. This attributes renders the chemical harmful since it takes a longer period of time for traces of the chemical to leave the environment.

4.5 Effect of bio-pesticide treatment on mean insect abundance on three okra varieties.

Whitefly vector population assessment

The control of whitefly (*Bemisia tabaci*) on okra (*Abelmoschus esculentus* L.) consists primarily in the use of chemical insecticides which also affect other non-target insects. However, the emergence of bio-pesticides as safe option for insect pest control has created a renewed interest in their development and use in integrated pest management of crops. Thus, this study investigated the use of crude plant extracts as a means of environmentally-friendly approach to the control of the insect vectors of *Okra mosaic virus* (OkMV) and *Okra yellow vein mosaic virus* (OYVM). The effects of crude plant extract as bio-pesticides for insect vector control are shown in Figures 4.4, 4.5, and 4.6. Mean whitefly numbers found on the various treatments were significant for all cultivars from week one to week 8. Generally, the chemical treatment had low whitefly numbers with cultivar F1 Kirene. Chemical control of whiteflies especially with Imidacloprid has been found to be most effective control measure against whiteflies (Hemadri et al., 2018). Contrary, the control had high whitefly infestations for most treatments compared to the chemical treatment and the plant extracts. At week five, Asutem, F1 Sahari and F1 Kirene had average whitefly counts of 10.5, 7.5 and 8.1 respectively.

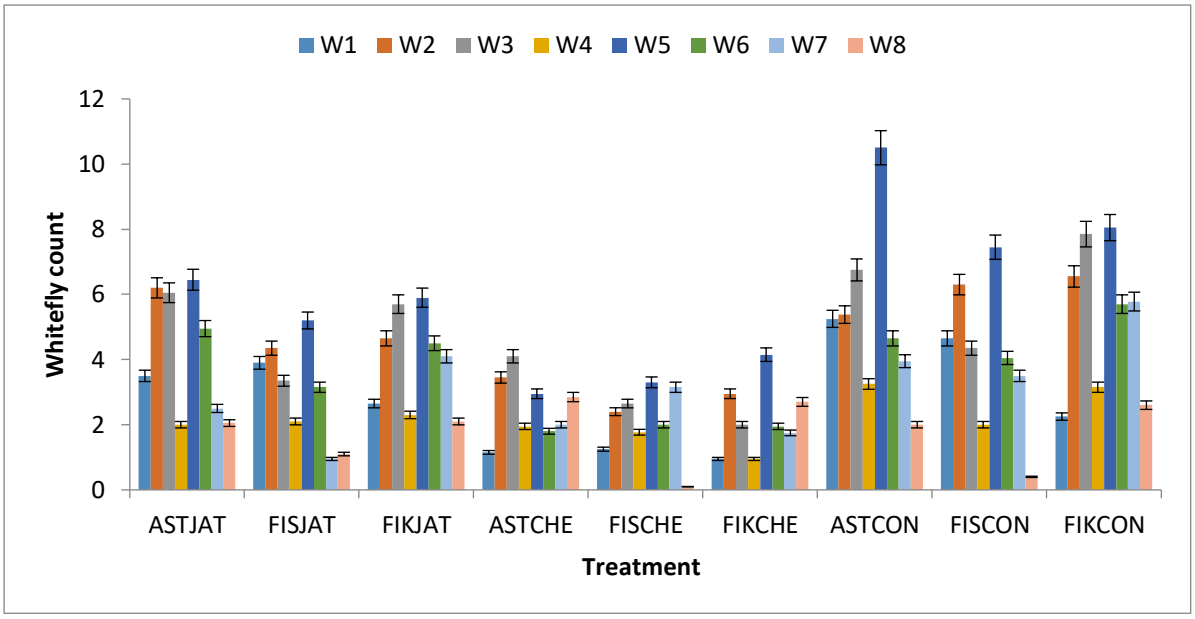

Figure 4.4: Variation in whitefly abundance among three cultivars of okra treated with aqueous Jatropha plant leaf extract.

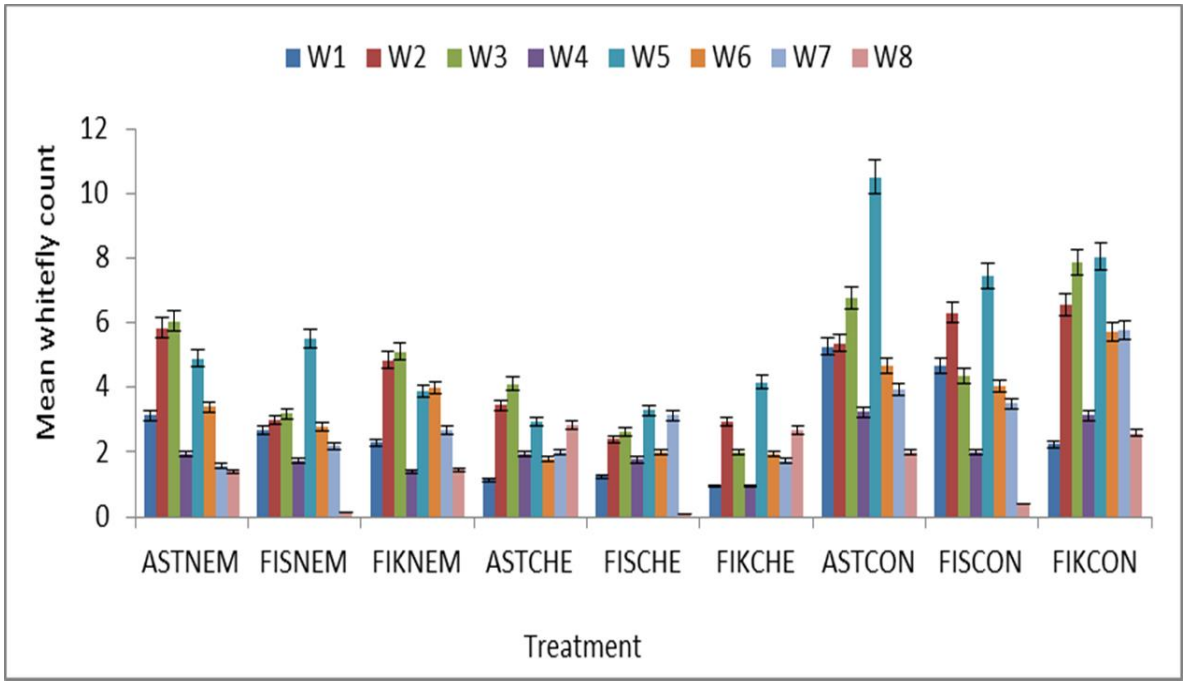

Figure 4.5: Variation in whitefly abundance among three cultivars of okra treated with aqueous Neem plant extract

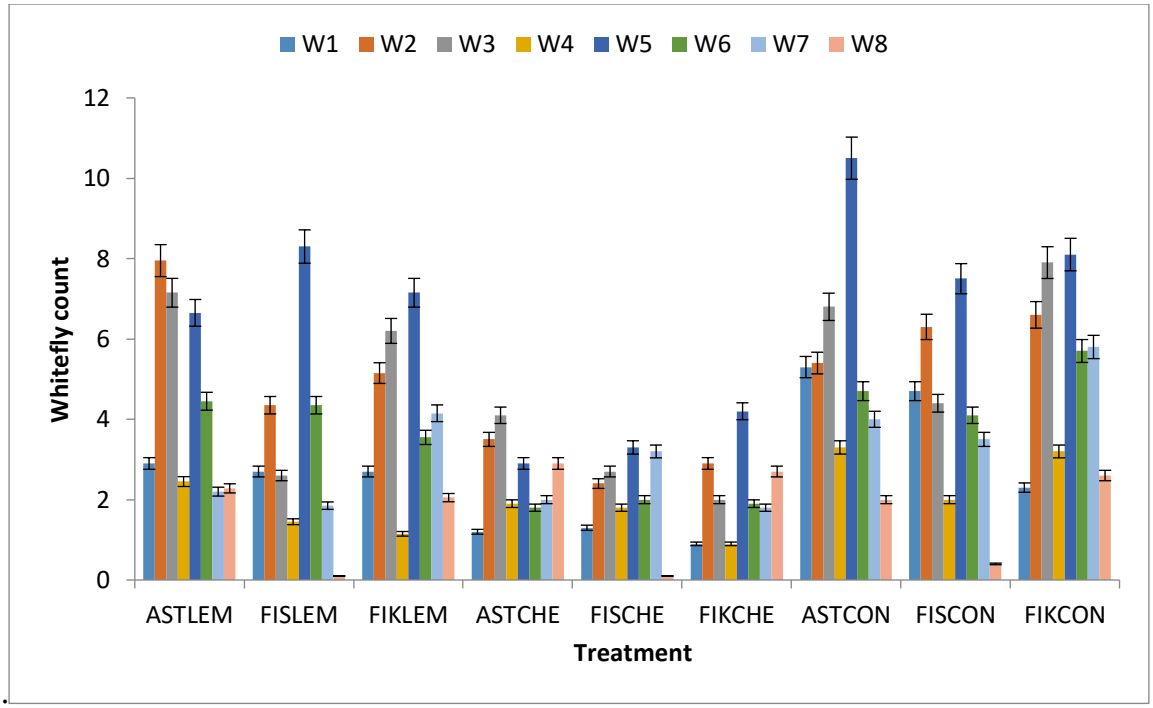

Figure 4.6: Variation in whitefly abundance among three cultivars of okra treated with aqueous Lemon grass leaf extract.

Among the plant extracts, neem performed better in terms of reduction in whitefly infestation (Figure 4.5). The highest average whitefly count for neem extract was 6.1 on Asutem plants, followed by F1 Kirene with mean insect count of 5.1 at 3 weeks after planting (WAP). The lowest infestation (0.2) was found in F1 Sahari at 8 WAP. Control of whiteflies using neem leaf extract has been demonstrated (Nzanza and Mashela, 2012). Extracts from neem has been identified as a strong anti-feedant and repellent, reducing growth and development and potentially delaying oviposition and preventing moulting. It has also been found to cause high mortality in more than 200 insect species, including whiteflies and aphids (Coudriet et al., 1985; Prabhaker et al., 1989; Liu and Stansly, 1995; Mitchell et al., 2004; Kumar et al., 2005; Kumar et al., 2006).

Even though Jatropha and Lemon grass extract performed better than the no treatment control, the effect was not as significant as observed for neem extract. Nevertheless, the efficacy of Lemon grass as a biopesticide for the control of cowpea weevil (*Callosobruchus maculatus*) (Uwamose and Okolugbo, 2016) and flea beetle (*Podagrica* spp.) (Ackah, 2013) have been reported. Extracts from *Jatropha curcas* has also been used in the control of insect pests of stored grains. (Silva et al., 2012)

The average whitefly count found on the control plants for the entire study period were 5.2, 4.2 and 5.3 for Asutem, F1 Sahari and F1 Kirene respectively. This is far lower than the numbers observed on okra by Khan et al., 2019 and Navneet and Tayde (2018). The three okra cultivars used in this study have medium to high density trichomes (Figure 4.7) and this may have resulted in the low whitefly numbers. According to Nausherwan et al. (2014) trichomes are known to confer significant resistance against some of the major insect pests including the sucking and chewing insect complex. The effect of trichomes on whitefly abundance in okra has been reported (Chu et al., 2000). However, the general declining trend recorded at week 6, 7 and 8 could be as result of plant senescence which has been found to reduce whitefly population on okra (Abd-Allah et al., 2009).

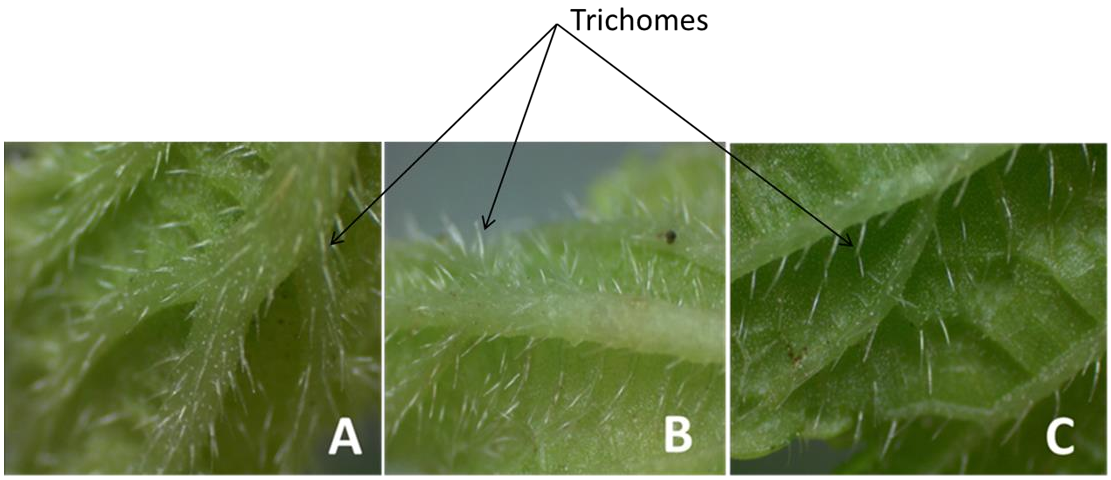

Figure 4.7: Trichomes found on A: F1 Sahari, B: Asutem and C: F1 Kirene under the light microscope.

4.6 Flea beetle vector population assessment

The flea beetle population observed on the three varieties of okra treated with the different plant extracts are shown in Figures 4.8, 4.9 and 4.10. The results did not show any consistency in vector decline over weeks of application. The commonest pest control method is usually by the application of synthetic insecticides but these are unfortunately dangerous to the ecosystem. This calls for a search for an alternative, more environmentally-friendly approach to insect pest control. The three plant extracts, even though were significant in reducing flea beetle population, were not as effective as the synthetic insecticide. Again, the neem treatment performed relatively better than the Jatropha and Lemon grass extracts, which were also better than the no extract control which had significant number of flea beetle infestations. Since the breakthrough in the insecticidal application of neem was attained by Pradhan et al. (1962), extracts from different parts of the plant have been extensively used in crop protection against different insect species. The potency of neem extract as a bio-pesticide has been demonstrated by several researchers. Kwaifa et al. (2015) reported that neem kernel extracts significantly

reduced infestation and population of flea beetles on field-grown okra. Additionally, aqueous neem leaf extracts significantly reduced flea beetle infestation of okra (Ackah, 2013). The effectiveness of neem extract in reducing flea beetle numbers in this study could be due to its anti-feedant repellent properties as reported by Echereobia et al. (2010). It is possible that their active ingredients may have affected the physiological activities of the insects as observed by Mordue (Luntz) and Blackwell (1993).

Although the Jatropha and Lemon grass were not as effective in reducing the populations of flea beetle as the neem, it did better than the no extract control. Leaf extracts from Jatropha and Lemon grass have been used in the control of flea beetle on okra (Onunkun 2012) and cowpea grain weevil (Uwamose and Okolugbo, 2015) respectively.

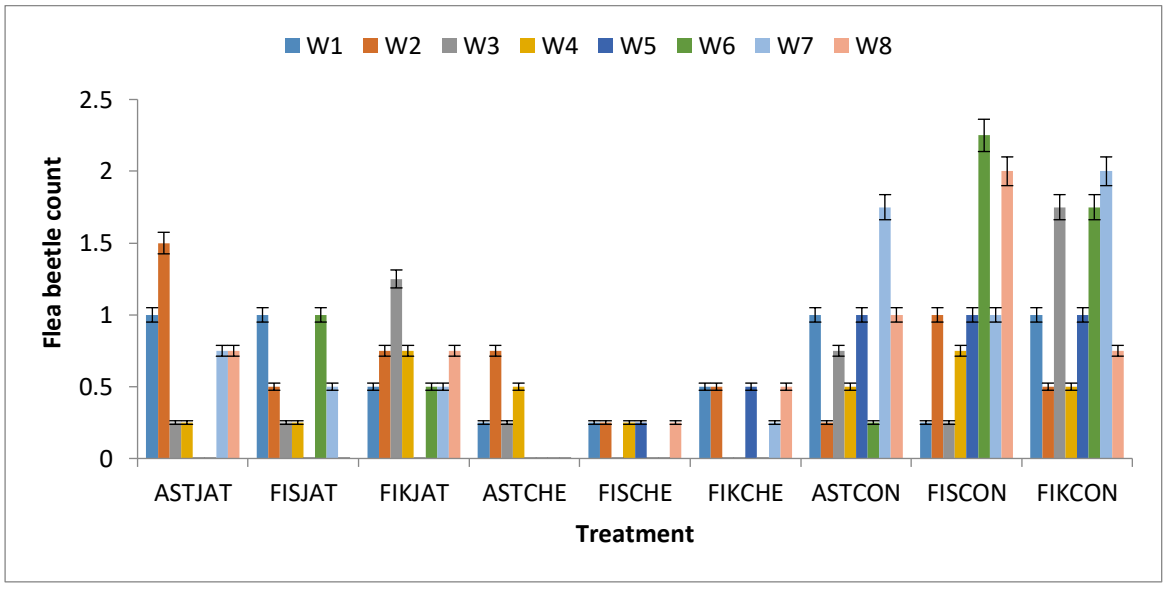

Figure 4.8: Flea beetle populations observed on three okra cultivars treated with Jatropha leaf extract.

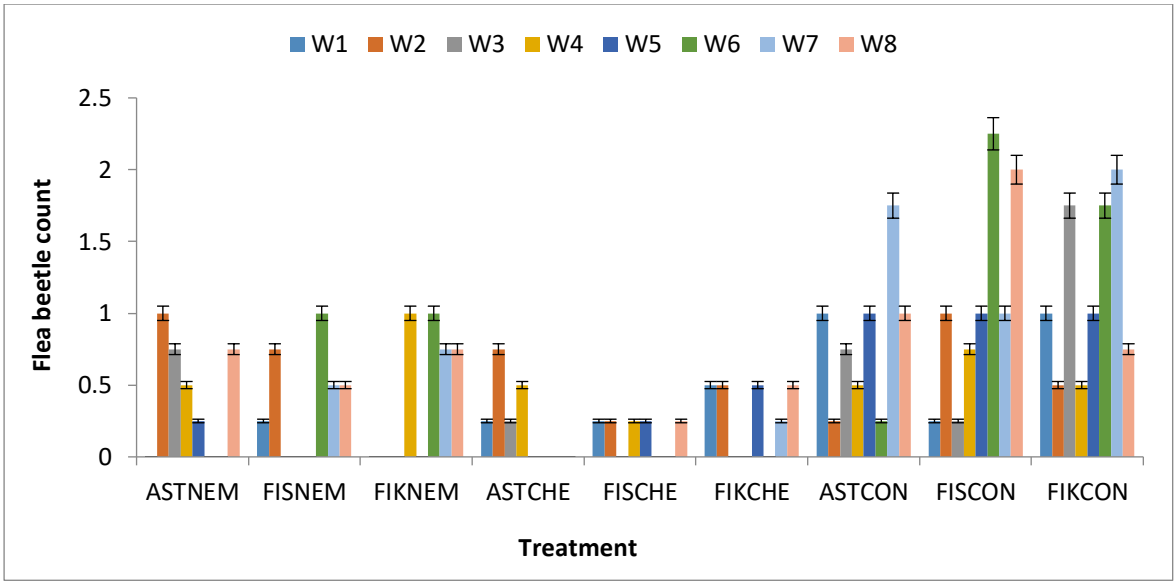

Figure 4.9: Flea beetle populations observed on three okra cultivars treated with Neem leaf extract.

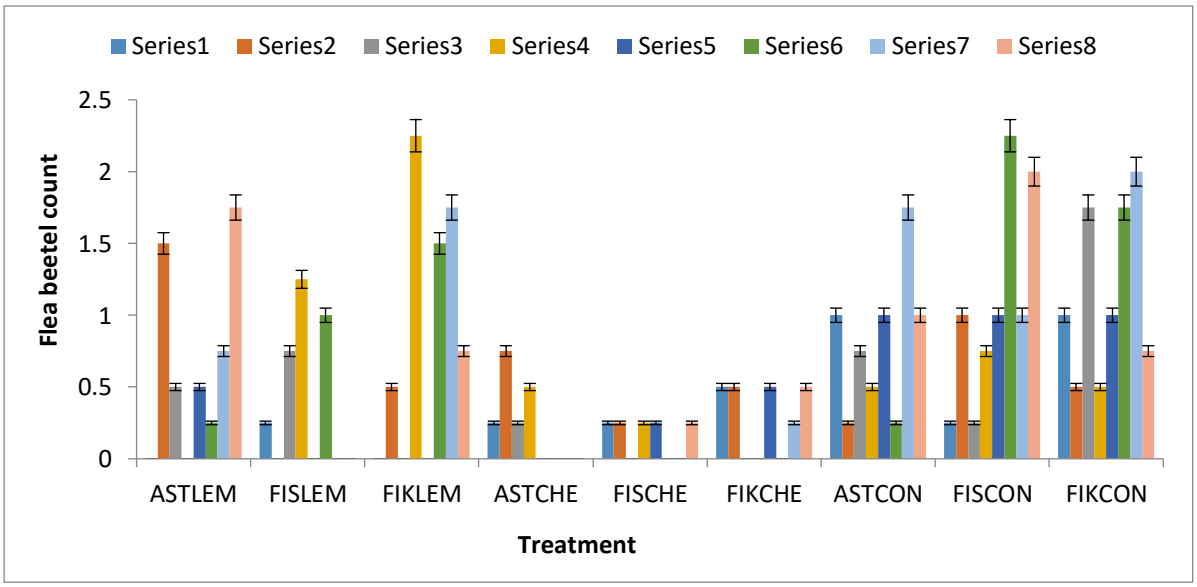

Figure 4.10: Flea beetle populations observed on three okra cultivars treated with Lemon grass extract.

4.7 Effect of plant leaf extracts on mean leaf damage of three okra cultivars.

Okra plants are attacked by various insect pests especially *Podagria* species at the various developmental stages of the crop. These insects have largely been controlled by synthetic insecticides which also affect non-target insects. Thus, the emergence of bio-pesticides (botanicals) as a safe option has created a renewed interest in their development and use in plant virus vector control as reported in several studies (Mobolade et al., 2014, Onunkun, 2012, Emeasor et al., 2017). The flea beetle (*Podagrica* spp.) has been identified as okra's most important insect pest in Africa, accounting for about 90% of the total insect population that attack the plant at the vegetative growth stage (Odebiyi, 1980) and limiting crop production. Among the flea beetle, *P. uniforma* and *P. sjostedti* are the common species that infest okra fields (Lana and Taylor, 1976; Vanlommel et al., 1996) resulting in significant yield losses. In the this study, leaf damage caused by Podagrica spp was significant and ranged from mild to moderately severe (Table 4.4).

Table 4.4: Leaf damage assessment in three okra cultivars

Variety	Treatment	Damage Score/Average number of perforations per leaf* Weeks after planting			
		Week 2	Week 4	Week 6	Week 8
Asutem	Jatropha	**(1)** 4.60	**(1)** 9.50	**(1)** 3.00	**(1)** 5.70
F1 Sahari	Jatropha	**(2**) 30.40	**(2)** 24.9	**(2)** 19.35	**(2)** 21.1
F1 Kirene	Jatropha	**(2)** 19.75	**(2)** 24.15	**(1)** 11.4	**(2)** 25.95
Asutem	Neem	**(2)** 18.05	**(2)** 26.60	**(2)** 16.00	**(2)** 29.00
F1 Sahari	Neem	**(2)** 24.65	**(2)** 29.25	**(2**) 18.7	**(2)** 26.25
F1 Kirene	Neem	**(2)** 17.45	**(2)** 27.3	**(1)** 12.25	**(2)** 20.7
Asutem	Lemon grass	**(2)** 26.5	**(2)** 21.25	**(2)** 21.75	**(2)** 27.30
F1 Sahari	Lemon grass	**(2)** 22.65	**(2)** 25.825	**(2)** 22.15	**(3)** 32.95
F1 Kirene	Lemon grass	**(2)** 16.80	**(2)** 24.45	**(2)** 21.45	**(2)** 22.85
Asutem	Chemical	**(1)** 1.98	**(1)** 12.43	**(1)** 4.53	**(1)** 5.85
F1 Sahari	Chemical	**(1)** 2.70	**(1)** 14.60	**(1)** 9.85	**(1)** 8.60
F1 Kirene	Chemical	**(1)** 10.20	**(1)** 14.40	**(1)** 4.40	**(1)** 8.450
Asutem	Control	**(2)** 21.55	**(2)** 25.55	**(2)** 21.40	**(3)** 31.70

F1 Sahari	Control	**(3)** 34.4	**(2)** 28.3	**(2)** 26.95	**(3)** 38.73
F1 Kirene	Control	**(2)** 16.10	**(2)** 24.35	**(2)** 17.30	**(2)** 23.15

* Values in brackets indicate damage level on a five point scale whereas corresponding value represents the number of leaf perforations as follows: 1 very mild damage (1 to 15 perforations); 2 mild damage (16 to 30 perforations); 3 moderately severe damage (31 to 45 perforations); 4 very severe damage (46 to 60 perforations); 5 extremely severe damage (more than 60 perforations).

The damage done to the leaves were largely perforations in the leaf blade and leaf distortions resulting from the insects' feeding (Figure 4.11). Plants of the three cultivars treated with the chemical had mild leaf damage compared to those treated with the leaf extracts. However, plants of cultivar Asutem treated with Jatropha had mild leaf damage compared to those treated with neem and lemon grass extracts. In a related study, extracts of *Jatropha curcas* significantly reduced the population of flea beetles in okra (Onunkun, 2012, Emeasor et al., 2017).

Figure 4.11: Okra leaf damage resulting from the insect feeding. Leaf damage assessment was done under field conditions.

4.8 Conclusion

The current research has demonstrated the effect of aqueous plant leaf extract on the abundance of whitefly and flea beetle populations on three okra cultivars. Although the plant extracts were not as effective as the chemical insecticide in reducing the insect vector populations, they offered a minimal level of protection as seen in the case of neem**.** Further research is warranted to determine the optimal doses and the frequency of application especially for neem leaf extract that is required for effective management of the insect vectors.

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CHAPTER FIVE

ASSESSMENT OF DIFFERENT CONCENTRATIONS OF NEEM EXTRACT ON INSECT VECTOR INFESTATION AND INCIDENCE OF OkMV AND OYVMV

5.0 INTRODUCTION

As a result of the recent global concerns on consumption of safe and healthy foods (Henning, 2008), many commercial and peasant okra farmers have shown much concern in the production of safe and quality fruits. Notwithstanding, many okra farmers still rely heavily on the use of synthetic chemicals in controlling or managing pests and diseases. This practice, many at times compromise the quality of the okra fruits especially in situations where excess application of chemicals occur (Epidi et al., 2008). Hence, the exploration and introduction of biopesticides as safe and environmentally-friendly means of pests and disease control (Singh and Jackai 1985).

Dayan *et al.,* (2009) argued that, plant extracts and exudes possess active ingredients which has the potential to repel some insect pests without any negative effect on non-targeted species and the environment at large. Similarly, Patil (2005) and Khuhro et al., (2014) made independent claims that Neem (*Azadirachta indica*), Physic nut (*Jathropha curcas*), Lemon grass (*Citronella* spp.) and many other plants are considered as effective botanical pesticides in the control of a wide variety of insect pest. Furthermore, Iram et al. (2009) indicated that natural insecticides especially those of plant origin have proved to be effective, biodegradable, low cost, low technological base, selective and environmentally friendly. More importantly, Scott et al. (2005) stressed that the possibility of insect pests developing resistance to botanical insecticides are often low. Nevertheless, the applications of these biopesticides are more often

not regulated by farmers in respect to the right minimal concentration needed to control insect pests/diseases and the rate of application required (Dimetry, 2014). For instance, in my previous experiment, Neem extract proved to be more effective in reducing insect vector populations on the okra cultivars, severity of their damag as well as incidence and severity of *Okra mosaic virus* (OkMV) and *Okra yellow vein mosaic virus* (OYVMV) diseases. However, these results were achieved without taking into consideration the minimal concentration of the extract required to cause such activities. There was the need, therefore, to further ascertain the minimal concentration of the Neem extract required to substantially reduce the population of whitefly and flea beetle, severity of their damages as well as incidence and severity of viral diseases they transmit.

5.1 Objective of the study

The main objective of this study was to determine the most effective concentration of the Neem extract responsible for reducing whitefly and flea beetle populations as well as incidence and severity of OkMV and OYVMV diseases on the three okra cultivars.

5.1.1 Specific objectives

The specific objectives of this study were to:

1. Examine the effect of varying concentrations of Neem extract on abundance of whiteflies and flea beetles on the three okra cultivars.
2. Assess the influence of different concentrations of Neem extract on the severity of damage caused by whiteflies and flea beetles on the three okra cultivars.

3. Evaluate the effect of varying concentrations of Neem extract on the incidence and severity of *Okra mosaic virus* and *Okra yellow vein mosaic virus.*
4. Assess the impact of Neem extract concentrations on the yield of three okra cultivars.

5.2 MATERIALS AND METHODS

5.2.1 Soil and Rainfall Pattern of the Study Area

The research was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) farms between July 2017 and March 2018. The study area was located at Kwabenya, Accra on latitude 5º 40' N, longitude 0º 13' W with Ochrosol (Ferric Acrisol) soil type, derived from quartzite Schist (FAO/UNESCO, 1994).The maximum and minimum average temperatures for the period of study were 30.7 and 26.0 ºC respectively with average annual rainfall of 220mm. The highest and lowest relative humidity is between 75 and 60% (Akaho et al., 2003). The experimental site is well drained and has an elevation of 76 m above sea level within the coastal savannah agro-ecological zone.

5.2.2 Plant Materials and Field Design

Plant materials used for the study consisted of three okra cultivars (F1 Sahari [F1S], F1 Kirene [F1K] and Asontem [AST]) obtained from the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Kwabenya – Greater Accra region.

The research field was prepared by ploughing and harrowing after which the land was lined and pegged into four main blocks. The design used was randomized complete block design (RCBD) with 4 replications with each block having ten subplots. Each replicate measured 35 m x 7 m and separated by 2 m from each other while each subplot measured 3 m x 3 m and spaced 1 m from each other. The okra seeds were manually sown to a depth of 2 cm directly at a spacing of 0.50 m x 0.60 m between and within rows respectively. Four seeds per hill were sown and thinned to one seedling per hill after emergence. Recommended agronomic practices observed included fertilizer application and watering.

5.2.3 Selection and Collection of Plant Species for Extract

The selection of the plants (Neem, Physic nut and Lemon grass) used in the study was based on their potential insecticidal properties against many insect pests of vegetables (Onunkun et al., 2012). All the selected plants were collected from BNARI, GAEC, Kwabenya – Greater Accra Region. Akakpe®; a chemical insecticide was used as a check.

5.2.4 Preparation of the Plant Extracts

Preparation of the plant (Neem) extract was done with reference to the first (main) experiment using the following working concentrations (20 ml/L, 30 ml/L and 40 ml/L). The five treatments were N1= Neem extract (20 ml/L), N2 = Neem extract (30 ml/L); N3 = Neem extract (40 ml/L); CHEM = Akape® and CON = control (water). The crop was sprayed at 15 days after emergence and continued weekly until the 6th week.

5.2.5 Data Collections and Analyses

Data Analysis

Quantitative data on insect abundance were subjected to one-way analysis of variance (ANOVA) while differences in treatment means were separated using the least significant different (LSD) test at 5% level of significance. All statistical analyses were done using GenStat statistical package (Version 12) and Microsoft Excel (2010 edition).

5.3 RESULTS AND DISCUSSIONS

5.3.1 Abundance of Insect Pests on the Three Selected Okra Cultivars

Figure 5.1 shows the mean whitefly population observed on treated okra cultivars grown on a field over a period of 9 weeks. Generally, mean whitefly population recorded ranged between 7.96 (cultivar Asontem treated with synthetic chemical [ASTCHE]) and 39.57 (Asontem okra cultivar treated with 40 ml/L concentration of Neem extract [ASTN3]). Chemical treated okra cultivars [ASTCHE (7.96), F1SCHE (12.96) and F1KCHE (10.94)] recorded significantly ($p<0.05$) lower mean whitefly populations than the Neem extract treated-okra cultivars [F1KN1 (17.88), ASTN1 (23.95), F1SN1 (29.01); F1KN2 (22.79), ASTN2 (28.96) and F1SN2 (25.04), and; F1KN3 (37.90), ASTN3 (39.57) and F1SN3 (38.22)] and the controls [ASTCON (37.17), F1SCON (38.07) and F1KCON (37.52]. The synthetic chemical 'Akape' significantly decreasing the population of whitefly on the okra cultivars is consistent with the finding in the first experiment and corroborates the reports of Jackai and Oyediran (1991) and Jackai and Adalla (1997) who reported that, synthetic insecticides are the chief means of insect pests control – both in the field and in storage. The high level of efficacy of 'Akape' against the

whiteflies may partly be due to the high potentials of agrochemicals in reducing the growth and development of crop pests and/or diseases (Tanzubil 1991).

Among the three concentrations of Neem extract applied, 20 ml/L Neem extract treated okra cultivars [F1KN1 (17.88), ASTN1 (23.95) and F1SN1 (29.01)] had significantly ($p<0.05$) lower mean population of whitefly compared to 40 ml/L Neem extract treated-okra cultivars [F1KN3 (37.90), ASTN3 (39.57) and F1SN3 (38.22)]. Lower concentration of castor plant extracts (20 ml/L) has been found to be effective in reducing the population of insect pests on chili pepper (Islam *et al*., 2010). The poor performance of the 40 ml/L Neem extract in reducing population of whitefly suggests that high rate of pesticide application beyond the optimum concentration may result in a decreased rate of lethality of pests and disease-causing organisms (Khuhro *et al.*, 2014). It is, therefore, suggested that farmers apply the required concentration of Neem extracts on their crops in order to ensure high efficacy against insect pests and diseases.

There was no significant ($p>0.05$) difference between the mean whitefly population recorded in 40 ml/L Neem extract-treated okra cultivars [F1KN3 (37.90), ASTN3 (39.57) and F1SN3 (38.22)] and that of the control [ASTCON (37.17), F1SCON (38.07) and F1KCON (37.52)] (Figure 5.1). Among the treatments, F1KN1 (17.88) resulted in the least mean whitefly population followed by F1KN2 (22.79) while the highest mean whitefly population was recorded in ASTN3 (39.57). This finding contradicts the report of the first experiment where ASTNEM (18.91) had the lowest mean whitefly population followed by F1KNEM (22.17) and F1SNEM (24.49). However, the overall reduction in whitefly population observed on plants treated with 20 ml/L extract of Neem in this study could be attributed to the right concentration of the extract as suggested by Saikia et al., 2006).

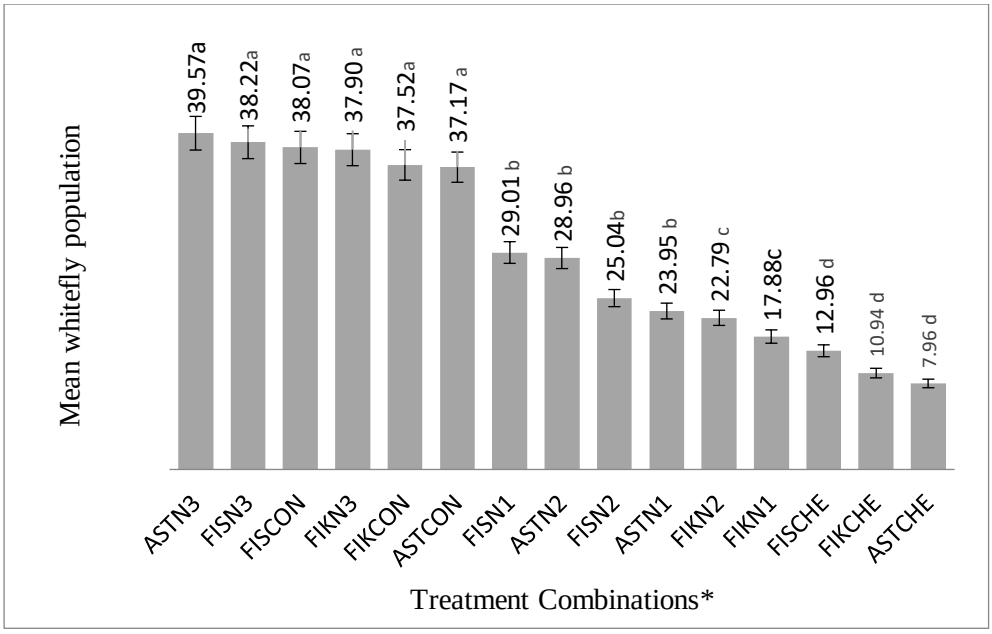

Figure 5.1: Mean whitefly population observed on neem and chemical pesticide-treated okra cultivars grown on the field.

*Treatment combinations: ASTN3 = Asontem okra cultivar treated with 40 ml/L concentration of Neem extract; F1SN3 = F1 Sahari okra cultivar treated with 40 ml/L concentration of Neem extract; F1SCON = F1 Sahari okra cultivar without any treatment; F1KN3 = F1 Kirene okra cultivar treated with 40 ml/L concentration of Neem extract; F1KCON = F1 Kirene okra cultivar without any treatment; ASTCON = Asontem okra cultivar without any treatment; F1SN1 = F1 Sahari okra cultivar treated with 20 ml/L concentration of Neem extract; ASTN2 = Asontem okra cultivar treated with 30 ml/L concentration of Neem extract; F1SN2 = F1 Sahari okra cultivar treated with 30 ml/L concentration of Neem extract; ASTN1 = Asontem okra cultivar treated with 20 ml/L concentration of Neem extract; F1KN2 = F1 Kirene okra cultivar treated with 30 ml/L concentration of Neem extract; F1KN1 = F1 Kirene okra cultivar treated with 20 ml/L concentration of Neem extract; F1SCHE = F1 Sahari okra cultivar treated

with chemical; F1KCHE = F1 Kirene okra cultivar treated with chemical, and; ASTCHE = Asontem okra cultivar treated with chemical.

Figure 5.2 indicates the mean flea beetle population observed on treated okra cultivars. Generally, the mean flea beetle population recorded ranged between 5.03 (F1KCHE) and 84.71 (ASTCON). Chemical treated okra cultivars [F1KCHE (5.03), ASTCHE (6.22) and F1SCHE (8.57)] had significantly ($p<0.05$) lower mean population of flea beetle compared to those treated with 40 ml/L of neem extract [ASTN3 (65.88), F1KN3 (72.68) and F1SN3 (80.88] and the controls [F1KCON (77.08), F1SCON (84.59) and ASTCON (84.71)]. Similar to findings of experiment one (this study) and that of Islam et al. (2010), there was a significant reduction in mean population of flea beetle in the synthetic pesticide-treated okra cultivars. Despite their negative effect on non-target organisms and the environment, synthetic pesticides, when applied in recommended doses, have the potential of reducing pest infestations.

Of the three concentrations of neem extract applied, okra cultivars treated with 20 ml/L had significantly ($p < 0.05$) lower flea beetle populations [F1KN1 (24.04%), ASTN1 (25.61%) and F1SN1 (29.41%)] compared to the 30 ml/L [F1KN2 (49.89%), ASTN2 (48.73%) and F1SN2 (51.92%)] and the 40 ml/L [F1KN3 (72.68%), ASTN3 (65.88%) and F1SN3 (80.88%)] (Fig. 6.2). This finding contradicts the results of Santos *et al.* (2004) and Mondédji and Nyamadorin (2019) in which higher concentrations of aqueous neem extract resulted in effective reduction in aphid and Lepidopteran spp. populations respectively.

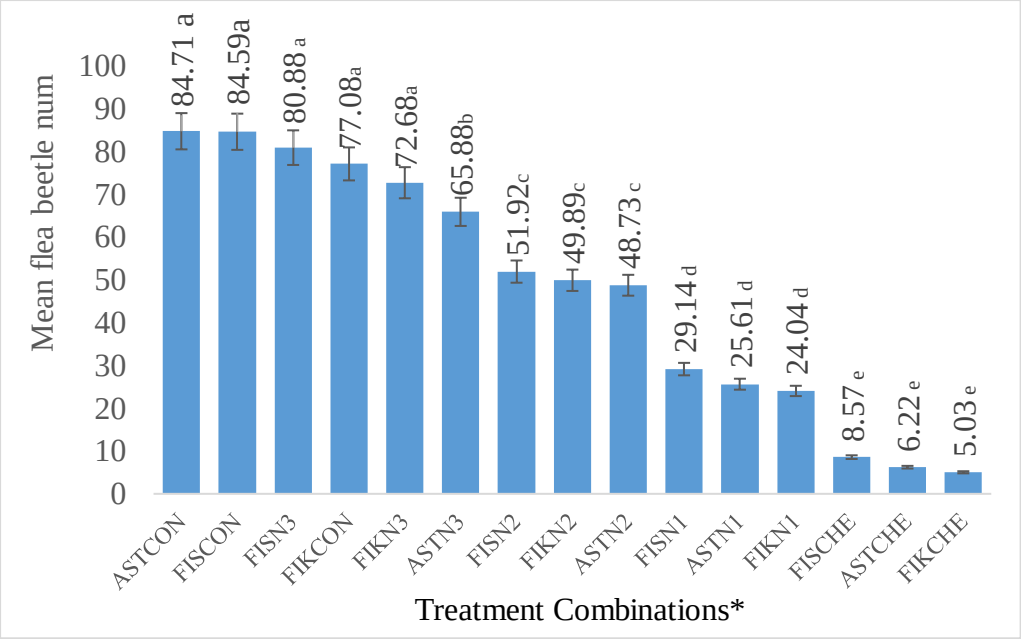

Figure 5.2: Mean flea beetle population observed on treated okra cultivars grown on a field.

*Treatment combinations: ASTCON = Asontem okra cultivar without any treatment; F1SCON = F1 Sahari okra cultivar without any treatment; F1KCON = F1 Kirene okra cultivar without any treatment; F1KN3 = F1 Kirene okra cultivar treated with 40 ml/L concentration of Neem extract; F1SN3 = F1 Sahari okra cultivar treated with 40 ml/L concentration of Neem extract; ASTN3 = Asontem okra cultivar treated with 40 ml/L concentration of Neem extract; F1SN2 = F1 Sahari okra cultivar treated with 30 ml/L concentration of Neem extract; F1KN2 = F1 Kirene okra cultivar treated with 30 ml/L concentration of Neem extract; ASTN2 = Asontem okra cultivar treated with 30 ml/L concentration of Neem extract; F1KN1 = F1 Kirene okra cultivar treated with 20 ml/L concentration of Neem extract; F1SN1 = F1 Sahari okra cultivar treated with 20 ml/L concentration of Neem extract; ASTN1 = Asontem okra cultivar treated with 20 ml/L concentration of Neem extract; ASTCHE = Asontem okra cultivar treated with

chemical; F1SCHE = F1 Sahari okra cultivar treated with chemical, and; F1KCHE = F1 Kirene okra cultivar treated with chemical.

5.3.2 Severity of Insect Pest Damage on the Okra Cultivars

Mean severity of insect pest damages on the three okra cultivars is shown in Fig. 5.3. The severity of insect pest damage progressively increased from 0.65 (very mild damage) in F1KCHE to 4.91 (very severe damage) in ASTCON. Okra cultivars treated with 20 ml/L neem extract [F1SN1 (1.38), ASTN1 (1.53) and F1KN1 (1.63)] had significantly ($p<0.05$) lower insect pest damage compared to those treated with 40 ml/L neem extract [F1SN3 (3.64), ASTN3 (2.97) and F1KN3 (3.53) – mild to moderately severe damages] and the control [F1SCON (4.72), ASTCON (4.91) and F1KCON (4.46) – very severe damages]. The mild damage recorded in okra cultivars treated with 20 ml/L Neem extract could possibly be ascribed to the ability of the extract to significantly induce mortality of both whitefly and flea beetle vectors compared to the 30 ml/L and the 40 ml/L neem extract. Nevertheless, all the Neem treated okra cultivars recorded lower pest damages than the controls which confirm its ability to effectively reduce pest damages leading to increased yields (Jackai and Oyediran, 1991; Tanzubil, 1991).

Furthermore, damage done to plants of cultivar Asontem treated with synthetic chemical [ASTCHE (1.16) – very mild damage] was not significantly ($p>0.05$) different from okra cultivars treated with 20 ml/L neem extract [F1SN1 (1.38), ASTN1 (1.53) and F1KN1 (1.63) – very mild pest damages] (Figure 6.3). The similarity in performance of the 20 ml/L neem extract and the synthetic chemical in reducing the severity of pest damages to the okra cultivars

confirms the efficacy of neem extract against insect pest damages (Tanzubil, 1991) especially, when applied in the right proportion.

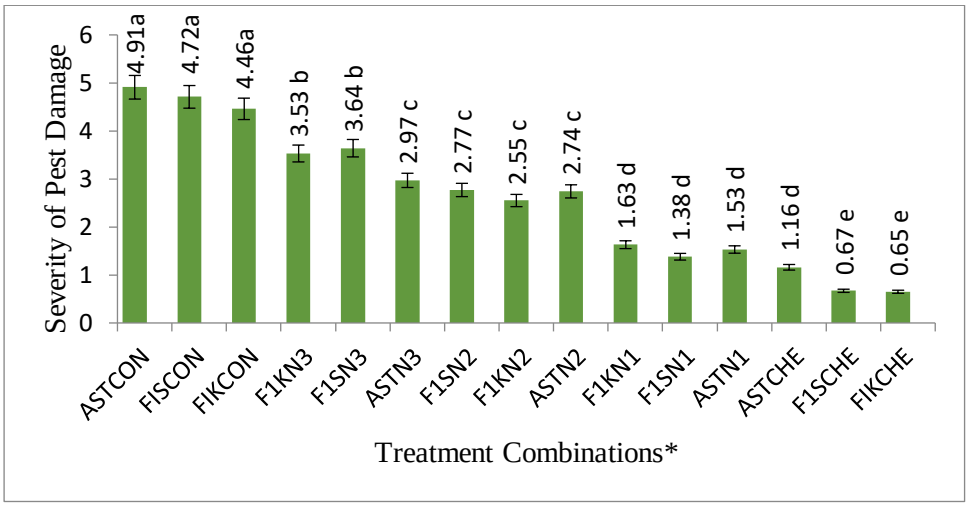

Figure 5.3: Mean severity of insect pest damages on pesticide-treated okra cultivars.

*Treatment combinations: ASTCON = Asontem okra cultivar without any treatment; F1SCON = F1 Sahari okra cultivar without any treatment; F1KCON = F1 Kirene okra cultivar without any treatment; F1KN3 = F1 Kirene okra cultivar treated with 40 ml/L concentration of Neem extract; F1SN3 = F1 Sahari okra cultivar treated with 40 ml/L concentration of Neem extract; ASTN3 = Asontem okra cultivar treated with 40 ml/L concentration of Neem extract; F1SN2 = F1 Sahari okra cultivar treated with 30 ml/L concentration of Neem extract; F1KN2 = F1 Kirene okra cultivar treated with 30 ml/L concentration of Neem extract; ASTN2 = Asontem okra cultivar treated with 30 ml/L concentration of Neem extract; F1KN1 = F1 Kirene okra cultivar treated with 20 ml/L concentration of Neem extract; F1SN1 = F1 Sahari okra cultivar treated with 20 ml/L concentration of Neem extract; ASTN1 = Asontem okra cultivar treated with 20 ml/L concentration of Neem extract; ASTCHE = Asontem okra cultivar treated with chemical; F1SCHE = F1 Sahari okra cultivar treated with chemical, and; F1KCHE = F1 Kirene okra cultivar treated with chemical.

5.3.3 Incidence of OkMV and OYVMV on the Okra Cultivars

Incidence (%) of OkMV and OYVMV diseases on the three okra cultivars treated with the three different concentrations of neem extract (20, 30 and 40 ml/L) is shown in Table 5.1. Nearly all plants of the different cultivars expressed symptoms of viral infections irrespective of treatment except the chemical treated-okra cultivars. Generally, mean incidence of the diseases was moderately high with a grand mean incidence of 42.02% compared to that of the first experiment (24.41%) although a few of the treatments had disease incidence below 30.0%. Majority of the okra cultivars exhibited symptoms of viral infections. Okra are generally attacked by a number of diseases during different growth stages with *Okra yellow vein mosaic virus* and *Okra mosaic virus* being the most important (Sardana et al. 2005, Aziz et al,. 2011). The relatively high incidence of viral diseases in this study may be due to the increased populations of flea beetle and whiteflies in the okra fields probably brought about by seasonal variation.

Among the okra cultivars, F1 Kirene had significantly ($p<0.05$) lower mean disease incidence (38.42%) compared to F1 Sahari (46.01%) and Asontem (41.64%) okra cultivars. These were however, not significantly different from each other. Furthermore, among the treatments, the chemical had the lowest mean disease incidence (23.50%) while 40 ml/L neem extract had the highest mean disease incidence (50.73%). Even though the 20 ml/L had a lower disease incidence (34.63%) than the 30 ml/L neem extract (41.80%) , there was no significant ($p>0.05$) difference between the means (Table 5.1). The low disease incidence for F1 Kirene in all treatments corroborates the results of the first experiment but contradicts the findings of Appiah *et al.*, 2020 which rated F1 Kirine as the most susceptible okra cultivar to OkMV and OYVMV.,

Table 5.1: Incidence (%) of OMV and OYVMV diseases on three okra cultivars treated with 20, 30 and 40 ml/L concentrations of Neem extract and Chemical pesticide over a period of 9 weeks on the field.

OKRA CULTIVAR	**DISEASE INCIDENCE (%) PESTICIDE TREATMENT**					
	Neem 1	**Neem 2**	**Neem 3**	**Chemical**	**Control**	**Means**
Asontem	34.6[b]	44.3[b]	48.90[b]	26.80[c]	53.60[a]	**41.64**[b]
F1 Kirene	33.5[b]	39.20[b]	43.60[b]	17.70[c]	58.10[b]	**38.42**[b]
F1 Sahari	35.8[b]	41.90[b]	59.70[a]	26.01[c]	66.62[a]	**46.01**[b]
Means	**34.63**[b]	**41.80**[b]	**50.73**[a]	**23.50**[c]	**59.44**[a]	**42.02**
STDEV	**1.15**	**2.55**	**8.21**	**5.04**	**6.61**	
CV (%)	**1.32**	**6.51**	**67.32**	**25.41**	**43.73**	

Lsd (P<0.05): Cultivar = 3.18; Pesticide = 2.60; Cultivar x Pesticide = 3.92; Neem 1 = 20 ml/L; Neem 2 = 30 ml/L, and; Neem 3 = 40 ml/L.

5.3.4 Severity of OMV and OYVMV on the Okra Cultivars

Figure 5.4 shows the mean severity of OkMV and OYVMV diseases on three okra cultivars treated with 20, 30 and 40 ml/L concentrations of neem extract. Generally, the mean severity of viral diseases was moderately low, ranging between 0.32 (no symptom: healthy plant) in ASTCHE and 2.35 (very mild symptoms: initial vein clearing, blistering, mild mosaic with curling) in ASTCON.

F1 Sahari okra cultivar recorded the highest disease severity [F1SCHE (1.04), F1SN1 (1.23), F1SN2 (2.12), F1SN3 (1.83) and F1SCON (2.35)] throughout the experiment whereas

Asontem okra cultivar recorded the lowest disease severity [ASTCHE (0.32), ASTN1 (0.64), ASTN2 (0.95), ASTN3 (0.71) and ASTCON (1.89)]. This finding partly contradicts the report of the first experiment where F1 Kirene okra cultivar recorded the least disease severity. Cultivar asontem had previously been rated as tolerant to OkMV and OYVMV (Appiah et al., (2020), Therefore, the significant reduction of the disease severity in this cultivar may probably be due to the ability of the cultivar to tolerate the presence of the disease on the field (Sergius et al., 2014).

Of the three concentrations of Neem extract applied, 20 ml/L (N1) significantly ($p<0.05$) reduced severity of the disease on all the three okra cultivars [ASTN1 (0.64), F1KN1 (0.84) and F1SN1 (1.23)] compared to the 40 ml/L neem extract [F1SN3 (1.83) and F1KN3 (1.25)] and 30 ml/L neem extract [F1SN2 (2.12), F1KN2 (1.18) and ASTN2 (0.95)]. This may suggest that, 20 ml/L concentration of neem extract is the permissible application concentration in managing OkMV and OYVMV diseases in okra.

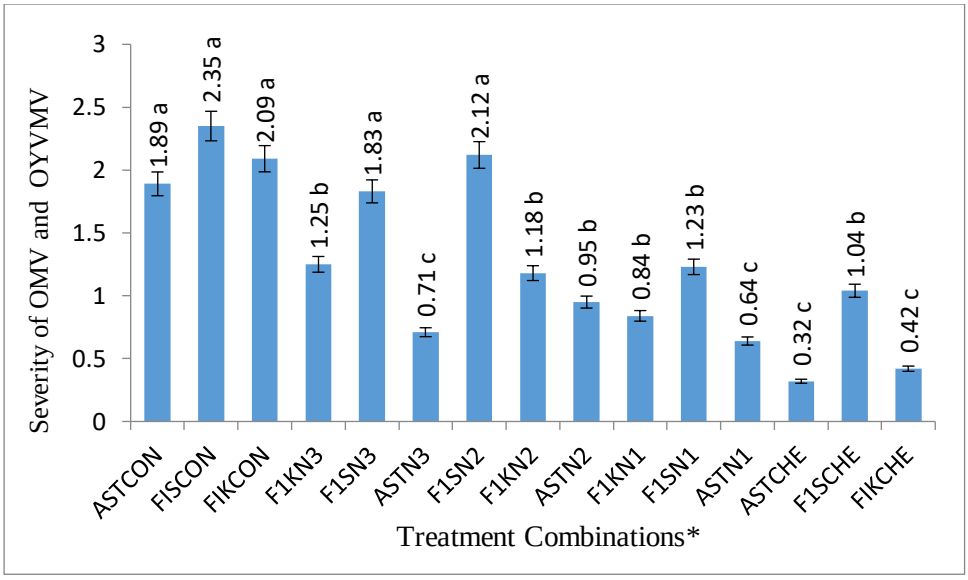

Figure 5.4: Severity of OkMV and OYVMV diseases on three okra cultivars treated with different concentrations of Neem extract,

*Treatment combinations: ASTCON = Asontem okra cultivar without any treatment; F1SCON = F1 Sahari okra cultivar without any treatment; F1KCON = F1 Kirene okra cultivar without any treatment; F1KN3 = F1 Kirene okra cultivar treated with 40 ml/L concentration of Neem extract; F1SN3 = F1 Sahari okra cultivar treated with 40 ml/L concentration of Neem extract; ASTN3 = Asontem okra cultivar treated with 40 ml/L concentration of Neem extract; F1SN2 = F1 Sahari okra cultivar treated with 30 ml/L concentration of Neem extract; F1KN2 = F1 Kirene okra cultivar treated with 30 ml/L concentration of Neem extract; ASTN2 = Asontem okra cultivar treated with 30 ml/L concentration of Neem extract; F1KN1 = F1 Kirene okra cultivar treated with 20 ml/L concentration of Neem extract; F1SN1 = F1 Sahari okra cultivar treated with 20 ml/L concentration of Neem extract; ASTN1 = Asontem okra cultivar treated with 20 ml/L concentration of Neem extract; ASTCHE = Asontem okra cultivar treated with chemical; F1SCHE = F1 Sahari okra cultivar treated with chemical, and; F1KCHE = F1 Kirene okra cultivar treated with chemical.

5.3.5 Impact of Different Concentrations of Neem Extract on Yield of the Three Okra Cultivars

Yield (kg/ha) of three okra cultivars treated with 20 ml/L, 30 ml/L and 40 ml/L concentrations of neem extract and a chemical pesticide on the field is shown in Table 5.2. Okra cultivars treated with chemical pesticide had significantly ($p<0.05$) higher yield (234.03 kg/ha) compared to the control (101.43 kg/ha) and the okra cultivars treated with 30 ml/L and 40 ml/L Neem extracts (138.87 kg/ha) and (125.90 kg/ha) respectively). However, there was no significant ($p>0.05$) differences between the yield of okra cultivars treated with chemical pesticide (234.03 kg/ha) and 20 ml/L Neem extract (191.0 kg/ha). The improved yield resulting from the application of synthetic chemical is consistent with the report of Epidi et al. (2008) and Powers et al. (2009). The parallel result recorded among the yield of okra cultivars treated with synthetic chemicals and 20 ml/L Neem extract is in agreement with the report of Khuhro et al., (2014) which indicates the high potential of correct application of Neem extract in improving the yield of okra.

In terms of cultivars, F1 Kirene recorded significant ($p<0.05$) higher yield (207.62 kg) followed by F1 Sahari (139.18 kg) and Asontem (127.94 kg) which was consistent with the yields recorded in the first experiment. This result partly contradicts the finding of Boateng (2018, unpublished) who noted in his research that F1 Sahari okra cultivar was able to give appreciable yield even in the presence of OkMV and OYVMV diseases, followed by F1 Kirene and Asontem okra cultivars. The disparity in the findings may possibly be ascribed to the application of pesticides treatments (which was absent in Boateng's experiment) to the okra

cultivars which might have conferred varying levels of protection to the cultivars in the fight against the viral diseases and subsequently translating into varying yields.

The mean grand yield (158.25 kg) recorded in this experiment is higher compared to that of the first experiment (136.11 kg). The high yields recorded in this trial may perhaps be explained by the low level of disease severity (ranging between 0.32 and 2.35) recorded during the production season compared to the previous findings. More often, crops give appreciable yields in the presence of diseases with lower severity levels (Aziz et al., 2011).

Table 5.2: Yield (kg) of three okra cultivars treated with extracts of various concentrations of Neem extract and Chemical pesticide on the field

TREATMENTS*	YIELD (kg)			
	OKRA CULTIVAR			Mean
	Asontem	F_1 Sahari	F_1 Karene	
Neem 1	155.70[a]	132.00[b]	285.30[a]	**191.00[a]**
Neem 2	122.00[b]	122.70[b]	171.90[a]	**138.87[b]**
Neem 3	109.80[b]	120.70[b]	147.20[b]	**125.90[b]**
Akape	174.30[a]	219.70[a]	308.10[a]	**234.03[a]**
Control	77.90[c]	100.80[c]	125.60[c]	**101.43[c]**
Mean	**127.94[b]**	**139.18[b]**	**207.62[a]**	**158.25**
STDEV	**38.04**	**46.43**	**83.29**	
CV(%)	**28.31**	**44.14**	**16.88**	

*Neem 1 = 20 ml/L; Neem 2 = 30 ml/L, and; Neem 3 = 40 ml/L; Akape = synthetic chemical.

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CHAPTER SIX

6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The synthetic chemical ('Akape') used in the study out performed all three plant extracts. Okra cultivars treated with chemical pesticide had significantly ($p<0.05$) higher yield (186.92 kg) compared to okra cultivars treated with Neem extract (144.81 kg), Jathropha extract (139.06 kg), Lemon grass extract (115.75 kg) and the Control (94.02 kg).

However, among the plant extracts, neem leaf extract exhited the best performance. Neem extract treated okra cultivars gave significantly ($p<0.05$) the lowest mean population of whitefly and flea beetle, disease incidence and severity as well as the best yields.

The *in-vitro* confirmation test (ELISA reactions) revealed that majority (86.67%) of the treatment combinations had single infection of OMV disease while a few (13.33%) of the treatment combinations had mixed-infection of OMV and OYVMV diseases. The cases of mixed-infections on the field recorded the least yields.

Among the three concentrations 20ml/L, 30ml/L and 40ml/L of Neem extract applied in the second trial, the 20 ml/L Neem extract treatment out performed the other two concentrations in all parameters measured. Neem extract at 20 ml/L gave the lowest flea beetle and whitefly populations, lowest severity of insect pest damages, significantly ($p<0.05$) lowest mean disease incidence. Neem extract applied at 20 ml/L also significantly ($p<0.05$) reduced disease severity due to mixed-infection of OMV and OYVMV.

There was significant ($p<0.05$) interactions between the okra cultivars and various pesticides applied. For instance, F1 Kirene treated with Neem extract recorded significantly ($p<0.05$) highest yield (157.92 kg) compared to Asontem cultivar without any pesticide treatment (74.38 kg). F1 Kirene recorded significant ($p<0.05$) highest yield (207.62 kg) followed by F1 Sahari (139.18 kg) and Asontem (127.94 kg).

6.2 Recommendations

On the bases of results obtained in this study, the following recommendations are made:

6.2.1 Okra cultivars, F1 Kirene and Asontem which exhibited a high level of performance in terms of yields and disease tolerance should be further studied and integrated into future breeding programmes.

6.2.2 Neem extract concentration (20 ml/L) which had parallel performances (pest and disease control) to the synthetic chemical 'Akape' needs to be further assessed (possibly, including lower concentrations) in multi-locational trials to affirm its potency and commercialization.

6.2.3 Farmers need to be extensively educated on the adoption of integrated pest management (IPM) system in the management or prevention of pests (flea beetle and whitefly) and diseases (OMV and OYVMV) in okra production.

7.0 APPENDICES

7.1 Appendix I: ANOVA for parameters recorded

7.1.1 Analysis of variance for elemental compositions

Variate: Elemental_compositions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Elements	8	10338.64	2584.66	105.97	<.001
Plant extracts	2	1204.97	602.49	24.70	<.001
Elements.Plant extracts	16	808.38	101.05	4.14	<.001
Residual	282	6878.00	24.39		
Total	299	19553.02			

7.1.2 Analysis of variance for flea beetle abundance

Variate: Flea_beatle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.2274	0.0758	0.75	
Block.*Units* stratum					
Treatment	4	2.9345	0.7336	7.28	<.001
Genotype	2	4.5890	2.2945	22.76	<.001
Treatment.Genotype	8	0.7161	0.0895	0.89	0.527
Residual	282	28.4274	0.1008		
Total	299	36.8943			

7.1.3 Analysis of variance for whitefly abundance

Variate: white_flies

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	21.563	7.188	5.92	
Block.*Units* stratum					
Treatment	4	154.849	38.712	31.87	<.001
Genotype	2	35.644	17.822	14.67	<.001
Treatment.Genotype	8	24.460	3.057	2.52	0.012
Residual	282	342.506	1.215		
Total	299	579.022			

7.1.4 Analysis of variance for severity of insect pest damages

Variate: Severity_insect pest damages

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	19.770	6.590	0.81	
Block.*Units* stratum					
Genotype	2	367.927	183.963	22.73	<.001
Treatment	4	237.647	59.412	7.34	<.001
Genotype.Treatment	8	56.373	7.047	0.87	0.542
Residual	282	2282.480	8.094		
Total	299	2964.197			

7.1.5 Analysis of variance disease incidence

Variate: Disease_incidence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.2773	0.0924	0.73	
Block.*Units* stratum					
Treatment	4	1.5769	0.3942	3.12	0.015
Genotype	2	12.3756	6.1878	49.01	<.001
Treatment.Genotype	8	1.1198	0.1400	1.11	0.357
Residual	282	35.6019	0.1262		
Total	299	50.9514			

7.1.6 Analysis of variance for disease severity

Variate: Disease_severity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	108.42	36.14	1.43	
Block.*Units* stratum					
Genotype	2	1842.39	921.19	36.49	<.001
Treatment	4	544.93	136.23	5.40	0.001
Genotype.Treatment	8	195.79	24.47	0.97	0.473
Residual	42	1060.23	25.24		
Total	59	3751.75			

7.1.7 Analysis of variance for okra yield

Variate: Okra_yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	27197.	9066.	1.63	
Block.*Units* stratum					
Genotype	2	1088036.	544018.	97.91	<.001
Treatment	4	152017.	38004.	6.84	<.001
Genotype.Treatment	8	71935.	8992.	1.62	0.149
Residual	42	233354.	5556.		
Total	59	1572539.			

7.2 Appendix II: Buffers used for the ELISA Test

7.2.1 Coating buffer

1.59g sodium carbonate (Na_2CO_3)

2.93g sodium bicarbonate ($NaHCO_3$)

0.20g sodium azide (NaN_3)

Make up to 1 litre with distilled water. The PH of the buffer is 9.6 and does not need to be adjusted.

7.2.2 PBS phosphate buffered saline (PBS)

8.0g sodium chloride (NaCl)

0.2g monobasic potassium phosphate (KH_2PO_4)

1.15g dibasic sodium phosphate (Na_2HPO_4)

0.2g potassium chloride (KCl)

0.2g sodium azide (NaN3)

Make up to 1 litre with distilled water. The PH of the buffer is 7.4 and does not need to be adjusted.

7.2.3 PBS –Tween (PBST)

PBS + 0.5ml Tween 20 per litre

7.2.4 Sample extraction buffer (PH 8.5)

0.05 M Tris containing 0.06 M Sodium sulphite

7.4.5 Conjugate buffer

PBST + 2% PVP + 0.2% egg albumen (e.g. Sigma A-5253)

7.2.6 Substrate buffer

97 ml diethanolamine

600 ml H2O

0.2 g sodium azide (NaN3)

Make up to 1 litre with distilled water. The PH of the buffer is 9.8 and does not need to be adjusted.

7.2.7 General extraction buffer

Tris	24.00g
Sodium chloride	8.00g
Polyvinylpyrrolidine (PVP)	20.0g
Tween 20	0.50g
Potassium chloride (KCl)	0.20g

Sodium azide (NaN_3)	0.20g

7.2.8 Inoculation buffer

KH_2PO_4	0.65g
Na_2H	4.05g
PVP	2%