UNIVERSITY OF CAPE COAST

STUDY OF OKRA LEAF CURL DISEASE AND ITS WHITEFLY VECTOR (*Bemisia tabaci*) VECTOR IN THREE AGRO-ECOLOGICAL ZONES IN THE VOLTA AND OTI REGIONS OF GHANA

CHARLES AMANKWA ADZIM

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BY

CHARLES AMANKWA ADZIM

Thesis submitted to the Department of Crop Science, School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of Doctor of Philosophy in Crop Science

JULY 2023

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature……Date……...

Name: ……

Supervisor's Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisors Signature ……Date ……

Name: ……

Co-supervisor's Signature ……Date…….

Name……

ABSTRACT

Research was carried out on okra leaf curl disease (OLCD) and its whitefly vector in the three agro-ecological zones of the Volta and Oti regions. The study evaluated the prevalence and severity of the disease, the level of the whitefly population and the impact of weather variables on their abundance. In order to evaluate the prevalence of the disease, farmers' knowledge of leaf curl disease, agronomic methods, and use of pesticides to manage pest and disease outbreaks, questionnaires were administered. The study also assessed quantitatively the intensity (severity and incidence) of the disease. The study then examined the resistance levels of 21 okra varieties to the disease and its vector during the major and minor okra seasons and further examined the molecular diversity of the Begomoviruses responsible for okra leaf curl disease. The majority of farmers observed whitefly and Okra leaf curl disease on their farms during both minor and major seasons. The mean incidence of the disease ranged from 69.69% to 80.62%, with a mean severity index of 1.18 to 1.44. The mean disease incidence was higher in coastal with 77.42%, than in transition (72.56%) and forest (71.85%) zones in both year one and two. Generally, the minor season had a much higher level of significance of disease incidence than the major season. A total 38,504 whiteflies were estimated where 25,978 for minor season and 12,526 in the major season. Varieties Kobinami, 1097, and 2033 were the most susceptible varieties to okra leaf curl disease. PCR using degenerate primers detected the presence of Begomoviruses. Sequence analysis of the PCR products revealed the presence of Okra yellow crinkle virus (OYCrV), which is responsible for okra leaf curl disease in the Volta and Oti regions. This is the first report of Okra yellow crinkle virus (OYCrV) in Ghana.

KEY WORDS

Coat protein

Incidence

Insecticide

Okra leaf curl disease

Okra yellow crinkle virus

Pesticide

Polymerase Chain Reaction (PCR)

Sequencing

Severity

Whitefly

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DEDICATION

My late father, Mr. Martin Kwame Yeboah, my beloved wife and kids, and my entire family are all honored in this work.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Vegetables are a great source of nutrients for maintaining good health, and preventing disease in the human diet (Ntow *et al.*, 2006). They added that vegetables play a significant socioeconomic role in improving nutrition through diet diversification and creating jobs for people in both rural and urban areas. African countries benefit greatly from the cultivation of vegetables such as tomatoes, okra, chilies, cucumber, and garden eggs in the form of foreign exchange to European countries such as Germany, Belgium, the United Kingdom, Italy, and Switzerland (Armah, 2010). After tomato, pepper, and garden egg, okra is Ghana's fourth most popular vegetable (Oppong-Sekyere *et al.*, 2012).

Okra (*Abelmoschus esculentus* L.) is grown as an important vegetable crop throughout the world's tropical and subtropical regions. Its white fibrous fruits or round pods with white seeds, as well as its young, immature fruits and fresh leaves, are used in salads, soups, and stews (Oppong-Sekyere *et al.*, 2012). Every part of the plant, particularly the leaf, flowers, and fruit, is consumed fresh or dry. The young, immature pods are consumed in a variety of ways, including boiling, frying, cooking, and drying for future use (Ndunguru & Rajabu, 2004; Agbo *et al.*, 2008).

Okra cultivation is concentrated exclusively in the developing countries of Asia and Africa with poor productivity, especially in African countries (2.25t/ha) compared with India, which ranks first in the world with a productivity of 11.44 t/ha. The world okra production was estimated at 4.8

million tons, with India leading the production by 70%, followed by Nigeria (15%), Pakistan (2%), Ghana (2%), Egypt (1.7%) and Iraq (1.7%) (Raemaekers, 2001; Gulsen *et al.,* 2007; FAOSTAT, 2008). The yield of okra in Ghana is estimated to be around 2000-3000kg/ha, depending on the cultivar, harvesting frequency and harvesting period (Cudjoe *et al.*, 2005 and MoFA, 2007). Sugri *et al.* (2015) estimated yields ranging from 1.27 to 9.5 tons per acre after evaluating different okra genotypes. According to other surveys, national production is around 120,000 Mt on 19,500 ha of arable land with a yield potential of 5.5 Mt/ha (Oppong-Sekyere *et al.,* 2012). Okra provides employment and income for rural people, particularly during the lean season, which is the dry season, when those with irrigation facilities from rivers, lakes, wells and dams harvest to make a lot of money (Sugri *et al.,* (2015). Oppong-Sekyere *et al.* (2012) stated that okra production has a high commercial value, particularly for rural women farmers in northern Ghana, where both fresh and dehydrated products are sold to supplement household income.

1.2 Statement of the problem

Despite the importance of okra, farmers face extremely low yields in Ghana. For example, the average productivity of okra in West Africa (2.5 t ha^{-1}) is very low when compared to East Africa (6.2 t ha^{-1}) and North Africa (8.2 t ha^{-1}) (Cudjoe *et al.,* 2005; FAOSTAT, 2008). The decline in okra production in Ghana appears to be the result of several factors, of which pests and diseases are the most significant constraints (Asare-Bediako *et al.,* 2014). Many pests have been reported to attack the crop at different stages of growth (Dabire-Binso *et al.,* 2009; Echezona *et al.,* 2010). Among the insect pests,

whiteflies (Bemisia tabaci) are an economical pest of many field and horticultural crops worldwide, including okra (Oliveira *et al.,* 2001). They can cause direct damage to plants by feeding on them, as well as indirect damage by transmitting viral diseases. Whitefly has been linked to the transmission of over 111 plant-pathogenic virus species as well as the induction of plant physiological disorders (Jones, 2003). It is difficult to manage *B. tabaci* populations and, in particular, the viral plant diseases that it transmits. This is due to the pest's rapid population growth, rapid evolution of insecticide resistance, and the relatively safe location of the individuals on the underside of the leaves (Kumar *et al.,* 2017). Viral plant diseases transmitted by *B. tabaci* are incurable; hence, the primary management strategies focus on vector control through the prevention of transmission and/or utilization of host-plant resistance (Hilje *et al.,* 2001; Antignus, 2007; Kumar *et al.,* 2017)

1.3 Justification

Okra leaf curl disease (OLCD) and okra mosaic disease (OkMD) are the two most common plant viral diseases identified in Ghana, according to Asare-Bediako *et al.* (2014), who also noted that the okra plant is susceptible to at least 19 plant viruses. Okra leaf curl disease is the most serious disease of okra and is transmitted by white flies (*Bemisia tabaci* Gen.) (Ali *et al.,* 2012; De Lucia *et al.,* 2017). Okra leaf curl disease (OLCD) is the most common, well-studied and major disease of okra reported in Ghana (Asare-Bediako *et al.,* 2014). The leaf curl disease of okra was reported in the Central region of Ghana as having disease incidence ranging from 36.4 percent to 80 percent, reaching epidemic proportions and posing a serious threat to okra cultivation (Asare-Bediako *et al.,* 2014; 2018). Okra leaf curl disease can result in crop

losses ranging from 30% to 100% (Basu, 1995; De Lucia *et al.,* 2017), depending on the cultivar, location, and planting date (Venkataravanappa *et al.,* 2015). Affected plants exhibit wrinkled leaves, upward or downward curling of apical leaves, vein distortion and thickening, leaf yellowing, stunted growth, and in severe cases, no fruits (Tiendrebeogo *et al.,* 2010; Askira, 2012). Okra leaf curl diseases transmitted by *B. tabaci* are incurable (Antignus, 2007), causing average economic losses to have been estimated to range between $1,950 and $11,100 USD per hectare of crop, depending on the okra variety globally (Tiendrebeogo *et al.,* 2010).

An in-depth study of whitefly species responsible for the type of begomoviruses transmitted is necessary in Ghana okra production systems. Plant-to-plant transmission is the mode of action for begomoviruses (family Geminiviridae), which are transmitted by the whitefly *Bemisia tabaci* Genn (Brown, 2007). Jones (2003) reported that whiteflies have been connected to approximately 111 plant-pathogenic virus types that upset the physiological balance of plants. For instance, Cotton leaf curl Gezira virus (CLCuGV), Okra yellow crinkle virus (OYCrV), and Hollyhock leaf crumple virus (HoLCrV) have all been connected to the known begomoviruses in Africa that cause Okra leaf curl disease (Tiendrebeogo *et al.,* 2010). Begomoviruses that cause the leaf curl disease have been extensively studied in certain African countries; nevertheless, it is unknown which virus, spread by whiteflies, causes the disease in Ghana's okra. Hence, an accurate diagnosis is essential for managing plant diseases in crop management systems and anticipating crop loss from plant pathogen infection (Van der Want and Dijkstra, 2006; Aboul-Ata *et al.,* 2011).

In contrast to earlier decades, which relied on biological techniques that were too slow and unsuitable for large-scale application, the fight against viral disease has shifted in the last three decades to rely on advances in molecular biology and biotechnology to develop quick, precise, and sensitive techniques for the detection of plant viruses. As a result, microscopical observations, serological approaches, and molecular techniques have been established to identify plant virus infections (Makkouk and Kumari, 2006). Polymerase chain reaction, a molecular technique now widely employed in virology and other scientific fields, has emerged as the new gold standard for identifying a wide range of templates. According to Kralik and Ricchi (2017), PCR is currently employed in laboratories for the identification of plant viruses and is based on the specificity of primers. For instance, Cotton leaf curl Gezira virus (CLCuGV) was reported by Tiendrebeogo *et al.* (2010) to be the virus causing/associated with okra leaf curl disease in Burkina Faso using PCR.

Additionally, the discovery of the okra yellow crinkle virus and the okra leaf curl disease diseases in the Ivory Coast, Mali, and Cameroon was through the use of molecular work (Askira, 2012). In Ghana, OLCD has been extensively diagnosed through the use of symptoms rather than PCR to identify the viral strain causing the disease. Understanding the begomoviruses that cause okra leaf curl disease can aid in the quick, accurate, and effective identification of a newly emerging viral strain in the Volta and Oti regions, as well as other areas. The difficulty in identifying the best ways to manage the disease and vector has been a great concern to all stakeholders; hence it is envisaged that the results from the study will improve the income of farmers

and encourage them to increase okra production when quick diagnosis is made.

The current study's goal was to discover the begomoviruses that cause the disease known as okra leaf curl disease and are transmitted by whiteflies. The main focus will be on the dynamics of the whitefly population, their influence on the prevalence and severity of OLCD, and the testing of specific varieties of okra for vector and disease resistance.

1.4 Main Objective

The current study aimed to assess the prevalence and incidence of okra leaf curl disease and its whitefly vector, as well as the genetic diversity of the associated begomoviruses.

1.4 Specific Objectives of the study are to:

1. Assess farmers' perceptions of the occurrence/prevalence and management of both okra leaf curl disease and its vector, the whitefly, respectively
2. Assess the incidence and severity of okra leaf curl disease and whitefly population in the Volta and Oti regions over two growing seasons.
3. Examine various okra accessions for resistance against the whitefly vector and okra leaf curl disease in a field setting.
4. Identify and ascertain the genetic diversity/molecular variability of the begomoviruses responsible for the okra leaf curl disease.

1.6 Hypothesis

- ✓ Ho: There is no significant difference in the incidence of okra leaf curl disease in the three agro-ecological zones in the Volta and Oti regions.

  H_A: There is a significant difference in the incidence of okra leaf curl disease in the three agro-ecological zones in the Volta and Oti regions
- ✓ Ho: There is no molecular variation among begomoviruses infecting okra in the Volta region and other parts of the world.

  H_A: There is molecular variation among begomoviruses infecting okra in the Volta region and other parts of the world
- ✓ Ho: There is no resistant okra variety among the existing varieties in Ghana.
- ✓ H_A: There is a resistant okra variety among the existing varieties in Ghana.

1.7 Significance of the study

The study aims to identify the begomoviruses responsible for the okra leaf curl disease and improve okra production by providing information for the development of effective management strategies to reduce the incidence and severity of okra leaf curl disease and the whitefly (*Bemisia tabaci*) population in Volta and Oti regions of Ghana. The successful outcome of this study will lead to an increase in okra yield where resistant varieties of okra will be developed across Volta and Oti as well as other regions, allowing for more effective income generation and poverty reduction.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 Okra's origin and distribution

The okra plant, *Abelmoschus esculentus,* also known as Lady's fingers, belongs to the genus Hibiscus and the section *Abelmoschus*, family *Malvaceae*. Later, in the year 1924, it was elevated to the genus status. The use of *Abelmoschus* in a broader sense was later accepted in the taxonomic literature (Hochreutimer, 1924). The calyx of the genus Hibiscus is spathulate, with five short teeth, connate to the corolla, and caducous after flowering (Kundu and Biawas, 1973). Most taxonomists revised the genus Abelmoschus, but the most comprehensively recorded investigations of the genus Abelmoschus were the results of the taxonomic review carried out by Borssum and Van (1966) and later by Bates (Bates, 1968).

There is some debate about the origin of okra, but it is thought to have originated somewhere in Ethiopia and was cultivated by the ancient Egyptians by the 12th century B.C. Its cultivation spread throughout the Middle East and North Africa (Tindall, 1983; Lamont, 1999). Some people think that India, West Africa, or Tropical Asia are the origins of *Abelmoschus esculentus* (Mardock, 1959). There is evidence of its presence in the wild along the White Nile in Sudan in the late nineteenth century (Singh and Bhantnagar, 1975). Okra arrived in the New World via Brazil and Dutch Guinea, where it was transported by African slaves via New Orleans to North America (Bish *et al.*, 1995).

Okra is a popular vegetable crop grown throughout the world's tropical and subtropical regions and can be found in any African market (Schippers,

2000). Okra is traditionally grown in African countries such as Cote d'Ivoire, Ghana, Nigeria, and Egypt, among others; however, Ghana, Burkina Faso, and Nigeria are the most important production countries (Raemaekers, 2001). Ghana's major okra-producing regions are Brong Ahafo, Ashanti, Northern, Volta, Greater Accra, and Central (NARP, 1993).

2.2 Propagation, Development, and Growth

Okra is an herbaceous annual plant that grows primarily from seeds. Before sowing, the seed is frequently soaked in water or chemicals to soften the hard seed coat. Typically, the seed is dibbled directly in the field, 2-3 seeds per hole, to a depth of 1-2 cm, with a gap of 25–45 cm (10–18 in) between rows (Olasotan, 2001). Seeds are planted 0.65–1.0 m (26–40 in) apart in commercial okra production. Six days for soaked seeds and three weeks for unsoaked seeds are the range of times that germination can take (Abidi *et al.,* 2014). The optimal germination percentage and emergence rate are found at 30-35°C (Akande *et al.,* 2003). Mulching, watering before the hottest part of the day, and sowing on ridge sides least exposed to direct sunlight all improve germination and early growth of okra (Doijode, 2001). From anthesis to seed maturity, it takes about 35-40 days (Norman, 1992), and it lasts 90-100 days (Nath, 1976).

Okra can grow to be 1.2–1.8 m tall and only survives one growing season as an annual plant. The leaves are alternate and palmately five-lobed, while the flower is axillary and solitary. Indeterminate growth is a characteristic of okra plants (Hamon *et al.,* 1990). Flowering occurs on a continuous basis but is highly dependent on biotic and abiotic stress. Temperature causes flower initiation and blossoming to be delayed because it

has a positive association with the number of vegetative nodes (Abd El-Kader *et al.*, 2010). One to two months after sowing, the plant will usually bear its first flower. After flowering, the fruit is a capsule that grows quickly. The greatest increase in fruit length, height, and diameter occurs between the fourth- and sixth-day following pollination. Fruit is most commonly plucked at this stage. When the fruit is ripe, it becomes fibrous and splits longitudinally into five parts, revealing five rows of seeds with 50 – 100 seeds per fruit (Norman, 1992). Some people are allergic to the plant and its fruits because they contain 13 small spines (Duzyaman, 1995).

2.3 Climate and Soil Requirements

Okra requires a long, warm, and humid growing season. It is sensitive to frost and extremely low temperatures, and develops poorly below 15ºC. An average temperature of 20°C to 30°C is thought to be ideal for growing, flowering, and fruiting (Akinyele and Temikotan, 2007). A temperature of 24°C to 28°C is ideal for normal growth and development. The first flower bud may show up in the third leaf axil at 24°C and in the sixth leaf axil at 28°C. Plants grow more quickly at warmer temperatures and reach the higher position earlier, so there may not always be a temporal delay associated with this higher position. Higher temperatures help with faster plant growth, but they delay fruiting. However, at temperatures above 40–42°C, flowers may desiccate and drop, resulting in yield losses. Okra tolerates poor soils but prefers well-drained sandy loams with a pH of 6-7 and high organic matter content (Adilakshmi *et al.*, 2008).

2.4 Okra Varieties

Okra is grown in many different types all over the world. However, biotic and abiotic stressors combined with a dearth of better varieties have resulted in low crop yields. For okra to be produced sustainably over time, new cultivars with higher yield potential and adaptability must be developed (Asare-Bediako *et al.*, 2016). According to Kumar *et al.*, (2017), before any variety or accession of okra was developed, it is believed that *Abelmoschus esculentus* (usually 2n = 130) is probably an amphidiploid (allotetraploid), derived from *Abelmoschus tuberculatus* P (2n = 58), a wild species from India, and a species with 2n = 72 chromosomes (poss *Abelmoschus caillei* (A. Chev.) Stevels is another edible okra species found in humid parts of West and Central Africa. There is strong evidence that *Abelmoschus caillei* is also an amphidiploid, with *Abelmoschus esculentus* as one of the parental species. Morphologically, *Abelmoschus caillei* differs from *Abelmoschus esculentus* in several ways, but the epicalyx is the most distinguishing feature: the width of the epicalyx segments in *Abelmoschus esculentus* is 0.5–3 mm and 4–13 mm in *Abelmoschus caillei*. On the basis of fruit form, the two okra species can be reliably (but not absolutely) distinguished.

The genus Abelmoschus is also thought to have four domesticated species. *A. esculentus* (common okra) is the most widely grown in South and East Asia, Africa, and the southern United States. The West African okra *A. caillei*, which has a longer production cycle, is also grown in the humid zone of West and Central Africa (Siemonsma, 1982). The plant height, degree of branching, coloration of different sections, maturation duration, and size and

form of the pods are all different amongst okra cultivars (AdeOluwa and Kehinde, 2011).

Several collections of okra species have been made around the world; for example, from 1982 to 1986, Biodiversity International, in collaboration with the Institut de Recherché pour le Development (IRD, formerly ORSTOM), conducted okra germplasm exploration in several West Central African countries. In addition to Asian and African collections, a core collection was formed at ORSTOM in Montpellier, France. However, breeding access to active collections from this core is no longer possible (Kumar *et al.*, 2010). The National Plant Germplasm System (NPGS) of the United States maintains and distributes over 3000 collections, as well as collections from Asia. Nonetheless, collections from countries such as Niger and Chad are underrepresented in the West African accessions. AVRDC – The World Vegetable Center has initiated countrywide explorations in collaboration with its partners and would like to continue exploring unexplored regions. For instance, between 2008 and 2009, 102 new accessions were gathered and regenerated for public use from Mali, Senegal, Niger, and Guinea. According to Jarvis *et al.* (2008), landraces—traditional varieties—retain significant genetic diversity in the form of on-farm richness and community evenness. This was discovered through the collection and analysis of varietal data on landraces and improved cultivars used by farmers in Burkina Faso.

Several scientists collected various okra varieties/germplasm and screened them for all types of diseases and pests. For example, 560 germplasm samples were collected from across India for research, and 20 genotypes were

infected with diseases. 10/OKYVRES-5, 10/OKYVRES-10, 10/OKYVRES-11, 10/OKYVRES-6, IC1124449, IC105675, IC411692, IC417885, IC433445, IC433532, IC411880, and BC0-1 were among the germplasm (Ayam *et al.*, 2018).

In Ghana, Asare-Bediako *et al.* (2016) identified 21 different accessions for various parameters, including Odumase, Antado, Asontem, Manshior, Fetri (Ewe), Krotetenye, Nkruma, Pbrnkruma, Bropo Asontem, Tuagya, Ogye abatan, UCC Campus, and Avalavi. Owusu-Sekyere *et al.* (2012) also identified some varieties, including GH 4487 Muomi, GH 3801 Pora, DA/08/001Wun mana, and Gbodro-wild. Sasilon, TZ-SMN-86, NB-55-Srivan, FV-Kpazeya, FV-Kpora napong, FV-Shie manna, NOKH 1004, FV-Unn, and other varieties are popular in Ghana's northern region (Sugri *et al.*, 2015).

2.5 The Economic Significance of Okra

Okra is an important part of the human diet because it contains protein, carbohydrates, vitamins, calcium, potassium, enzymes, and total minerals, all of which are often lacking in the diet of developing countries (Abidi *et al.*, 2014). It is grown specifically for its leaves and young pods, which are frequently consumed as a green vegetable. The consumption of young, immature okra pods as fresh fruits is important, and it can be consumed in various forms, such as boiled, fried, or cooked (Ndunguru and Rajabu, 2004). Okra has high nutritional values such as protein, carbohydrates, and vitamin C and plays an important role in the human diet. The fruit is very helpful in treating chronic dysentery, spermatorrhoea, and genito-urinary problems (Gopalan *et al.*, 2007; Dilruba *et al.*, 2009; Pal *et al.*, 2013). Additionally,

reports indicate its therapeutic usefulness in the treatment of ulcers and hemorrhoids (Abidi *et al.*, 2014)

2.6 Production, Yield, and International Trade of Okra

Okra pods are typically harvested every other day from the time the first pod forms. For okra to be marketable, the fruits must be picked between 5 and 10 days after flowering (Adetuyi *et al.*, 2008). Harvesting is usually done in the early morning, after which it is sold in the market (Moekchantuk and Kumar, 2004). It is critical to harvest this plant frequently in order to maximize yield. Over a harvest period of 30-40 days, yields of up to 2-3 t/ha of green pods, with approximately 4-6 fruits per plant, are possible (Tindall, 1986). The majority of farmers collect seed from their local cultivar or rather a diverse landrace. Leaving the seed in the pods is the simplest way to keep it (Moekchantuk and Kumar, 2004)

The fibrous fruits or pods of *Abelmoschus esculentus*, which contain spherical, white seeds, are what make the plant popular worldwide (Abidi *et al.*, 2014). Around the planet, from the Mediterranean to the equatorial zones, *A. esculentus* is found. While yields exceeding 40 t/ha are feasible under perfect circumstances, a vegetable yield of 10 t/ha is regarded as an excellent crop. Yields of roughly 10–15 t/ha are also achievable with proper management (NARP, 1993). According to Ahmad *et al.* (2015), the average yield of okra is 6.5-7.5 t/ha of green fruits during the dry season and 11.5-12.5 t/ha during the rainy season. Benchasri (2012) also reported an optimum yield of 6.6 t ha^{-1} for okra. The global okra production is estimated to be 6 million tons per year (Sathish-Kumar *et al.*, 2013). In 2010, the total global cultivation area was 0.43 million hectares, with a production of 4.54 million tons (Abidi

et al., 2014). Gulsen *et al.,* (2007) also estimated global okra production at 4.8 million tons, indicating a yield decrease. Okra is widely cultivated in developing Asian and African countries, but productivity is low, particularly in African countries, with a low yield of 2.25 t/ha compared to other countries (NARP, 1993). Although West and Central Africa produce more than 75 percent of Africa's okra, average productivity in the region is very low (2.5 t/ha) when compared to East (6.2 t/ha) and North Africa (8.8 t/ha). Nigeria is the largest producer in West Africa (1,039,000 t), followed by Cote d'Ivoire, Ghana, and others (FAOSTAT, 2008). India is the world's largest producer (67.1 percent), this is followed by Nigeria (15.4%) and Sudan (9.3%) (Kumar, 2006; Varmudy, 2011).

Gulsen *et al.* (2007), stated that India leads production by 70%, followed by Nigeria (15%), Pakistan (2%), Ghana (2%), Egypt (1.7%), and Iraq (1.7 percent). Yields are typically low (2–4 t/ha) due to non-intensive growing methods. Seed yields are in the 500–1000 kg/ha range (Sathish-Kumar *et al.*, 2013). In Ghana, yield potential for okra has been reported to be between 2000 and 3000 kg $ha^{-1,}$ depending on cultivar, harvesting frequency, and harvesting period (Cudjoe *et al.*, 2005; MoFA, 2007). Actual okra yields in Ghana are often low and have declined over time despite the crop's economic significance and health advantages (Asare-Bediako *et al.*, 2014).

2.7 Pests and diseases of okra

The development of virus and vector-resistant strains has been regarded as the most effective, cost-effective, and dependable method of controlling viral diseases and pest infestation. The first step in any virus resistance program was to identify germplasm that was immune or resistant to

viruses and pests. One of the most important factors in okra production is the prevalence of insect pests (Radake and Undirwade, 1981). According to Dhamhere *et al*. (1984), approximately 13 major insects and non-insect pest species attack okra at various stages of development. There are 30-40 insect pests that have been identified as attacking the okra crop and causing immeasurable damage (Ahmad *et al.,* 2015). Flea beetles (*Podagrica sp*.), cotton stainers (*Dysdercus superstitus*), white flies (*Bemisia tabaci*), and green stink bugs (*Nezera viridula*), among others, are reported as major insect pests on okra in Ghana (Obeng-Ofori and Sackey, 2003 and Senjobi *et al.,* 2013). Cotton jassids, *A. biguttula* (Ishida), spotted bollworm, *Earias insulana* (Fabr.), pink bollworm, *Pectinophora gossypiella* (Saunders), and cotton whitefly, *Bemisia tabaci*, were also identified as major insect pests of okra in Eritrea by Ahmad *et al.,* (2015).

Okra may be resistant or susceptible to pests and disease attacks, which may decrease or increase yield, respectively. The incidences of okra mosaic disease and okra leaf curl disease in Ghana were reported by Asare-Bediako *et al*. (2014) to be 78–83 percent and 63–70 percent, respectively. According to Ayam *et al. (2018), several okra varieties have been identified in India as having different levels of resistance to viral diseases*. The varieties included 10/OKYVRES-5 and 10/OKYVRES -10 (highly tolerant), 10/OKYVRES-11 and IC1124449 (moderately tolerant), IC105675, IC411692, IC417885, IC433445, IC433532, and BC0-1 (moderately susceptible), and IC41. According to Navneet and Tayde (2018), the number of whiteflies on selected okra cultivars in India ranged between 1.98 and 7.52, while OYVMV resistance ranged from 0.3% (highly resistant) in cultivar IIVR-10 to 20%

(moderately resistant) in cultivar HRB-55. Munthali and Tshegofatso (2013), in a study on major insect pests attacking okra, in Sebele, Botswana estimated yield losses caused by pod-damaging species on three cultivars: Clemson spineless, Asontem, and Legon 6. *Earias biplaga, Pachnoda rubrocincta, Mylabris* sp., *Dysdercus* sp., and *Aphis gossypii*. Pod damage caused by *P. rubrocincta* was similar on all three cultivars, averaging about 23% per plant. On Legon 6, Asontem, and Clemson spineless, *E. biplaga* caused 0.0, 2.0, and 16.7 percent damage, respectively. In Nigeria, Udengwu and Dibua (2014) tested 23 okra genotypes for resistance to okra leaf curl and okra mosaic virus disease, and yield declines of less than 10% were observed in the varieties Ebi Ogwu, Ojo Ogwu, Tongolo, VLO, Oru Ufie, and Ogolo, indicating that they are less resistant to the diseases.

Asare-Bediako *et al. (*2016) conducted a study on the phenotypic and serological screening of 21 okra genotypes against Okra mosaic virus infection under field conditions and observed mild symptoms on genotypes GH3760, GH2052, GH5332, UCC6, GH5302, GH5793, and GH2063 during both rainy and dry seasons. The fruit yield of 11.88 t ha^{-1} was highest in genotype GH5332 with mild symptoms. Genotype GH6105 had a high fruit yield (9.34 t ha-^{1}) but was susceptible to OkMV infection. In all okra genotypes, disease severity and yield were greater in the minor season than in the major season. According to the findings, genotype GH5332 exhibited partial resistance to OkMV, whereas genotype GH6102 was tolerant. Furthermore, Oppong-Sekyere *et al.* (2012) identified high resistance to the mosaic and Leaf curl diseases in three okra varieties, namely Atuogya, Wun mana, and Sheo.

2.7.1 Pests of Okra

Heavy infestations caused by several insect pests are one of the major constraints for okra production, causing not only quantitative but also qualitative loss to the crop (Pal *et al.*, 2013). Many insect pests infest okra from seedling to harvest (Al-Hamadany and Al-Karboli, 2017). The occurrence and severity of insect pest damage varies with crop growth stage, region, and season. On okra, two different insect species have been identified based on severity (Srinivasa and Rajendran, 2003). Insect pests that infest okra include those that attack the foliage, shoots, flowers, and pods (Hill, 1987; Munthali and Tshegofatso, 2013). *Aphis gossypii* (Glov.), *Empoasca* spp., *Ferrisia virgata* (Ckll.), *Dysdercus* spp., *Oxycarenus hyalipennis (Costa), Earias vittella (Stoll.), Earias biplaga (Wlk.), Earias insulana (Boisd.),* and *Helicoverpa* sp. Piercing and sucking insects of sap from leaves, shoots, and pods such as *Acrosternum hilare*, *Euschistus servus*, and *Leptoglossuis phyllopus*, as well as other hemipteran insects such as *Bemisia tabaci*, *Amrasca biguttula biguttula*, and *Dysdercus* superstitiosus, are important crop pests in Africa and other regions (Dhandapam *et al.*, 2003; Obeng-Ofori and Sackey, 2003). Some insects of the genus *Mylabris* also cause significant damage to okra flowers (Singh and Joshi, 2003). Flea beetles, *Podagrica uniforma* (Jac.), *Bemisia tabaci*, and *Nisotra sjostedt* (Jac.) was discovered to be the most damaging to crops in West Africa (Dabire-Binso *et al.*, 2009; Echezona *et al.*, 2010; Asare-Bediako *et al.*, 2018). Adetuyi *et al.* (2008) also reported jassid (Ishida), aphid (Glover), *Amrasca biguttula biguttula Aphis gossypii* whitefly (Genn), mite (spp.) and shoot and fruit borer as important pests affecting okra yield (*Bemisia tabaci, Tetranychus Earias* vittella. Fab).

Obeng-Ofori and Sackey (2003), for example, collected the following insect pests of okra: *Podagrica uniformis* Jac, *Aphis gossypii* Glov, *Sylepta derogata* (F.), *Spodoptera litoralis* Boisd, *Prodenia litura* (F.), *Dysdercus superstitious* (F.), *Epilachna similis* (F.), *Bemisia tabaci* (F.). *Lagria villosa* (F.), L. *cuprina* Thoms, *Mylabris temporalis* Wellni, *M. trifasciata* (Thumb.), *Lapidognatha* sp, and *Empoasca devastans* were among the minor pests identified (D.)

2.7.1.1 The whitefly (*Bemisia tabaci)*

Whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous pest that feeds on a wide range of crops, such as vegetables and ornamentals (Oliveira *et al.*, 2001). Most whitefly species are thought to be oligophagous, but most whitefly pest species are polyphagous. However, some oligophagous whitefly pest species, such as *Aleurocybotus* spp. and *Aleurolobus* spp., affect plants in the *Gramineae* family and *Asterochiton* spp. affect plants in the *Acer* spp. (Byrne *et al.*, 1990).

2.7.1.2 The Origins of the whitefly

Bemisia tabaci, also known as *Aleyrodes tabaci*, was discovered in a tobacco farm by Gennadius in Greece in 1889. Whitefly belong to the family Aleyrodidae in the suborder *Sternorrhycha* and the entire superfamily Aleyrodoidea, which is related to the superfamily Psylloidea and finally to the order homoptera (Richards and Davis, 1977). Quintance collected whitefly samples of *Physalis alkenkengi* L. in Southern USA in 1900 and described it as *Aleyrodes inconpicua* Quintance (Quintanee, 1900). Quintance and Baker transferred the species to the genus *Bemisia* in 1964, naming it *Bemisia inconpicua* (Quintance and Baker, 1914). After several years of identifying the first and second species, additional whitefly species from 14 different

countries were identified on a variety of host plants (Perring, 2001). Takhashy (1936) placed *tabaci* in the genus *Bemisia* and gave it the current name, *Bemisia tabaci* Gennadius. Currently, there are at least 30 morphologically indistinguishable species of whitefly, 11 of which have been placed in a high well-defined group based on biochemical and molecular markers (Dinsdale *et al.*, 2010; Hu *et al.*, 2011; Alemandri *et al.*, 2012). Insect systematists' difficulty in making good adult slide mounting has resulted in few distinguishing characteristics (Martins, 1987). However, the fourth nymphal stage is the best for identification because it allows for effective diagnosis (Gill, 1990). Crenulations on the margin, the structure and size of the thoracic tracheal folds, the shape of the lingua and opercula in the vasiform orifice, various pores and porettes on the nymph's dorsum, and the shape, size, and presence of dorsal setae all overlap significantly in taxonomic identification (Mound, 1963 and Gill, 1990)

2.7.1.3 Geographical distribution of whitefly (*Bemisia tabaci*)

Bemisia tabaci was described more than a century ago and has since become one of the most important pests in subtropical and tropical agriculture, as well as greenhouse production systems worldwide (Oliveira *et al.*, 2001). *Bemisia*, a member of the Aleyrodidae family, is thought to have originated in Africa and spread to other parts of the world, including the Neotropics and southern North America, according to Campbell *et al.* (1990). Others believe that the Indian subcontinent is the suspected origin of *B. tabaci* due to the presence of a large number of its natural hosts there. It has been proposed that the spread of the species occurred most likely through the movement of plant material by man from the Indian subcontinent to Africa,

Europe, and the Americas (Cock, 1986; Brown *et al.*, 1995). It easily adapts to new host plants and geographical regions, and it has now been reported from all of the world's continents except Antarctica. International transports of plant material and people have contributed to geographical spread in the last decade (Oliveira *et al.*, 2001). *B. tabaci* population outbreaks and *B. tabaci*-transmitted viruses have been a limiting factor in the production of food and fiber crops in many parts of the world since the 1980s (Brown, 1994). *B. tabaci* has now spread to Japan, Canada, and the Netherlands, where it is wreaking havoc on greenhouse crops (Oliveira *et al.*, 2001). It was discovered on *Euphorbia hirtella* in Brazil in 1928 and collected in Taiwan in 1933 (Mound and Halsey, 1978). *B. tabaci* was first reported causing damage to greenhouse ornamentals in the United States of America by Price *et al.* (1986) and has rapidly spread across the country, making it the most difficult pest to manage on many greenhouse crops. Whitefly has since been reported on various crops all over the world. Severe cotton infestations were recorded in Sudan and Iran (1950), El Salvador (1961), Mexico (1962), Brazil (1968), Turkey (1974), Israel (1976), Thailand (1978), Arizona and California, USA (1981), Ethiopia (1984) and Burkina Faso (1985) (Otoidobiga *et al.*, 2003). *B. tabaci* has been identified as a major pest in Algeria, Bahrain, Cyprus, Egypt, the Islamic Republic of Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Malta, Morocco, Pakistan, Saudi Arabia, Somalia, Sudan, Tunisia, Turkey, the United Arab Emirates, and Yemen (Traboulsi, 1994).

2.7.1.4 Biology and Morphology of Whitefly

Borror *et al.* (1989) classify the metamorphosis of whitefly as "Intermediate." The adult whitefly has five instars, the first of which is active, while the other four are inactive or sessile. According to Kedar *et al*. (2014), the whitefly has six life stages: egg, crawlers (1st nymphal instar), two sessile instars (2nd and 3rd instar nymphs), the 'pupa' (4th instar), and the adult or imago. Many whitefly pest species are multivoltine, meaning they have multiple generations per year (Byrne *et al.*, 1990). Freshly laid eggs are transparent, yellowish-white, and spindle-shaped. Every day, the egg color changes from pale brown to dark brown before hatching (100). *B. tabaci* eggs were ovoid or pyriform in shape (Gameel and Jewett, 1974). (Kedar *et al*. (2014) also reported that the incubation period on both cotton varieties and hybrids ranged from 3-5 days. Hussein and Trehan (1933) reported a similar incubation period ranging from 3-5 days from April to September. In contrast, Mohanty and Basu (1987) discovered that the whitefly incubation period ranged from 2 to 4 days from March to October. The crawlers (first instar nymphs) are oval in shape and whitish yellow (Bhardwaj and Kushwaha, 1984; Patel *et al.*, 1992; Kedar *et al.*, 2014). Crawlers crawl on the lower surface of the leaf for 1 - 2 hours after hatching, looking for a place to side and feed (Kedar *et al*., 2014). Crawlers are 0.3 mm long, transparent to opaque, and range in color from light green to yellow, light brown to dark brown, and black (Azab *et al.*, 1971). He stated that crawlers have four to five leg segments and two to three antennal segments.

Various researchers have reported on the duration of the first instar nymph. Kedar *et al*. (2014) observed that first instar nymphs live for 3-5 days.

Nymphal duration has been reported to be 2-5 days (Mohanty and Basu, 1987) and 3 to 9 days (Aneja, 2000). The second instar is about 0.4 m long, oval to elongated oval, while some are circular and nearly heart-shaped (Azab *et al.*, 1971). Kedar *et al*. (2014) observed that freshly moulted nymph was whitish yellow in colour, oval and flat. Petal *et al. (1992) also reported similar colours and shapes for* second instar nymphs. The mean duration of the second instar nymphal period was observed at 3.4 ± 0.6 days and 3.2 ± 0.8 on cotton (Aneja, 2000; Kedar *et al*., 2014).

Freshly moulted third instar nymphs are 0.5mm long and more oval and flattened. It started pale yellow and gradually darkened after feeding (Deotale *et al.*, 1992; Kedar *et al*., 2014). Petal *et al.* (1992), on the other hand, reported that the third instar nymphs were pale green in color and elliptical in shape. The average duration of third-instar nymphs was 3.3 days, 3.1 days, 2.5 - 5.0 days, and 2.0 - 4.0 days (Petal *et al.*, 1992; Kedar *et al*., 2014). The early fourth instar is 0.6 mm long, greenish-yellow, and elliptical, with small eyes like the previous instars (Azab *et al.*,1971). Other observations on the newly formed puparium include a yellowish-white color and an oval shape. Compound eyes that were well-developed reddish brown in color were clearly visible (Kedar *et al*., 2014). As reported by Kedar *et al*. (2014), this instar lasted for 4.0 and 4.3 days and 3.5 - 6.0 days on cotton variety and hybrid in 2012 and 2013, respectively. Wings are present in both sexes at the adult stage and measure about 2–3 mm in length. The wings are generally opaque, with a whitish powder or wax covering them. The abdomen lacks cornicles (tubular structures located dorsally near the abdomen's posterior end), and the hind wings are nearly as long as the

forewings (Borror *et al.,* 1989). Kedar *et al*. (2014) discovered that the fully developed adult emerged through an inverted 'T' shaped slit in the dorsal surface of the pupal case. A newly emerged adult is 0.8-0.9 mm long and whitish-yellow in color, but after a few hours, the color changes to pure white due to wax deposition on the wings and body (Azab *et al.,* 1971).

The majority of Aleyrodidae species have a wingspan of less than 3 mm and a body length of 1 mm to 2 mm. There are some "giant white fly" species, some of which can grow up to 5 mm in length. However, in some giant tropical species, males are significantly larger than females (Martin, 2007). The adult female has dark brown compound eyes, smoky white wings, and a pale-yellow broader abdomen with an ovipositor (Patel *et al.,* 1992; Kedar *et al*., 2014). On cotton, the female has a fecundity of 34 – 66.0 eggs. However, different researchers have reported varying fecundity ranges. Fecundity has been reported to be greater than 50 eggs (Basu, 1995), and Hussain and Trehan (1933) have 119 eggs. These variations in fecundity could be attributed to environmental factors such as temperature, relative humidity and rainfall, as well as different test varieties (Kedar *et al*., 2014). The adult male whitefly abdomen is narrow with a pair of claspers and smaller in size than the adult female, indicating sexual dimorphism between the male and female whitefly (Patel *et al.,* 1992). According to reports, the average adult male longevity ranges between 3.0 - 5.0 days and 3.0 - 6.0 days (Kedar *et al*., 2014). Azab *et al*. (1971) found that male adult longevity ranged from 2 to 17 days at different temperatures. Several researchers, however, have reported significant variations in the total life cycle of *B. tabaci* on cotton. *B. tabaci's* life cycle was completed in 14-107 days (Hussain and Trehan, 1933), 24 - 44

days (Aneja, 2000), 20 days (Palaniswami *et al.*, 2001), and 17.7 and 38.2 days at 25.5 and 20.0 °C, respectively (Powell and Bellows, 1992). Such differences could be attributed to differences in pest-rearing conditions and cultivars used in the study (Kedar *et al*., 2014)

2.7.1.5 Biotypes of *Bemisia tabaci*

B. tabaci is known for its genetic diversity, which manifests itself in a variety of biotypes. Biochemical or molecular polymorphism distinguishes the biotypes, which differ in characteristics such as host plant range, ability to cause plant disorders, attraction by natural enemies, expression of resistance, and plant virus-transmission capabilities (Brown *et al.*, 1995). Brown (1994) reported 1,100 species of whiteflies worldwide, of which only three are recognized as plant virus vectors. Wan *et al.* (2009) identified six biotypes in China: B, Q, ZHJ1, ZHJ2, ZHJ3, and FJ1. However, the B-biotype has been the most dominant of *B. tabaci*, appearing in China in the late 1990s and becoming the only biotype of *B. tabaci* prevalent in many regions of the country by 2005–2006. Costa and Brown (1991) stated that biotype B is most prevalent in the southwestern United States following an extensive outbreak of B. tabaci in the late 1980s, which has since spread worldwide.

2.7.1.6 Host range of *Bemisia tabaci*

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous pest that feeds on a wide range of crops, including vegetables and ornamentals (Ma *et al.*, 2004; Oliveira *et al.*, 2001; Jones, 2003). More than 600 different plant species have been found to contain *B. tabaci* (Mound and Halsey, 1978; Secker *et al.*, 1998). Mound and Halsey (1978) identified some plant species that are commonly attacked by whiteflies, with 50%

belonging to five families. Fabaceae, Asteraceae, Malvaceae, Solanaceae, and Euphorbiaceae are some of the families of plants. The number of host plants per plant family ranges from 99 species in the Fabaceae (Basu, 1995) to one species in each of the Begnoniaceae, Lythraceae, and Zygophyllaceae (Mound and Halsey, 1978). Greathead (1986) also reported a diverse host range of over 500 different plant species.

2.7.1.7 Damages caused by whitefly

B. tabaci is one of the world's most destructive pests of field and greenhouse crops. *B. tabaci* nymphs and adults both cause direct and indirect damage to their host plants. It pierces and sucks cell contents and excretes massive amounts of honeydew, which induces and increases sooty mould fungal development and reduces the plant's photosynthetic efficiency and, thus yield (Byrne *et al.,* 2003). It was discovered that *B. tabaci* infestation reduced chlorophyll pigments and moisture content in many plant leaves, including tomato leaves (Buntin *et al.,* 1993). They can cause direct damage to plants by feeding on them, as well as indirect damage by transmitting viral diseases. Whitefly has been linked to the transmission of over 111 plant-pathogenic virus species as well as the induction of plant physiological disorders (Jones, 2003).

2.7.1.8 Whitefly Vector Management

The white fly is the main agent responsible for transmitting the viruses that cause Okra leaf curl disease. Thus, vector control is key to the management of this disease (Kumar *et al.,* 2017). It is difficult to manage *B. tabaci* populations and, in particular, the viral plant diseases that it transmits. This is due to the pest's rapid population growth, rapid evolution of insecticide

resistance, and the relatively safe location of the individuals on the underside of the leaves. Because viral plant diseases transmitted by *B. tabaci* are incurable, the primary management strategies focus on the prevention of transmission and/or utilization of host-plant resistance (Antignus, 2007). Whitefly management entails four pillars of the integrated pest management approach: host plant resistance, biological control, chemical control, and cultural practices (Hilje *et al.*, 2001)

2.7.1.8.1 Chemical control of Bemisia tabaci

In most plants around the world, insecticides are commonly used to control *Bemisia tabaci*. For the control of okra pests, a wide variety of systemic and contact insecticides, as well as bio-pesticides, are currently recommended (Cahill, 1996). Short picking intervals of okra fruits, on the other hand, cause residue hazards to consumers when conventional pesticides are used repeatedly, in addition to killing natural enemies and eventually leading to resistance development. Several agricultural practices, such as monoculture and extensive pesticide use, have resulted in the development of several biotypes of *Bemisia tabaci* that differentially exhibit pesticide resistance and virulence, which has been observed to occur globally at the same time (Cock, 1986). Farmers use a variety of indiscriminate insecticides to control whiteflies, which has resulted in resistance, pest resurgence, and residual toxicity issues. To address these issues, the identification of safe molecules with improved insecticidal properties, lower mammalian toxicity, safety to natural enemies, and compatibility with the Integrated Pest Management concept is critical (Hemadri *et al.*, 2018). However, whitefly tends to develop resistance to a wide range of pesticides rather quickly (Bryne

et al., 1990). In many cropping systems, the primary strategy for controlling *Bemisia tabaci* is the use of insecticides. The main method used to manage B. tabaci conventionally in field and vegetable crops is foliar spraying active chemicals; the efficiency of this method depends on the coverage and deposition of the spray (Sharaf, 1986). Hemadri *et al.* (2018) investigated the best insecticide for the management of whiteflies in okra farms in India and discovered that foliar spray of Imidacloprid 17.8 SL @ 0.5 ml/l was most effective against whiteflies with a higher percent reduction of pest population (84.54%), followed by acetamiprid 20 SP @ 0.5 g/l, thiamethoxam 25 WG @ 0.3 g/l. Other researchers found imidacloprid 17.8 SL @ 25 g active ingredient/ha to be effective against whiteflies (Raghuraman and Gupta, 2005; Rajveer *et al.*, 2017; Preetha *et al.*, 2018). In Egypt, Kadil *et al.* (1991) reported that imidacloprid, diafenthiuron, and carbosulfan were effective against adults and immature *B. tabaci* cotton. Asare-Bediako *et al.* (2018) reported that farmers in Ghana use the following chemicals to control whitefly in okra farms: Acetamiprid, Lambda-cyhalothrin, Bifenthrin + Acetamiprid, Chlorpyrifos, Chlorpyrifos-ethyl, Imidacloprid, Bifenthrin, Pyrethrum, Pirimiphos-methyl, Sulphur.

2.7.1.8.2 Biological Control of *Bemisia tabaci*

Biological control is an effective method of combating this pest, providing environmentally friendly and cost-effective pest management. These attributes have prompted the development of alternative control methods, such as biological control of *B. tabaci* with the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae). This parasitoid is probably the most

widely used in greenhouses for the control of *Trialeuroides vaporariorum* (Westwood) and *Bemisia* spp. (Hoddle *et al.*, 1998).

2.7.1.8.3 Cultural control *Bemisia tabaci*

An intensive use of cultural practices is one of the most effective methods for controlling whitefly around the world. Thus, management through cultural practices entails manipulating existing or new components of the agroecosystem in order to reduce pest damage to non-economic levels (Hilje, 2002). Cultural practices can play an important role in integrated pest management (IPM) systems because of the preventative nature of targeting whiteflies. Cultural practices, however, have received disproportionately little attention from researchers, possibly due to the difficulty of testing using traditional methods. Crop-free periods, changing planting dates, crop rotation, and weed and crop residue disposal all perform well only on a regional scale, making them difficult to test or demonstrate experimentally (Hilje, 2002). The crop-free period is a cultural method used to synchronize cropping patterns over a large area in order to avoid the continuous presence of crops susceptible to whiteflies and/or whitefly transmitted viruses, which reduces the overall population levels of the vector and the amount of virus inoculum in the area. It may be impossible to eliminate all of the vector's plant hosts from a given area. This set of cultural practices aims to separate the crop from the sources of the insect and/or the viruses it vectors over time. Crop-free periods, on the other hand, can reduce the mass migration of insects from one crop to another (Hilje, 2002). For example, in the 1920s, cotton leaf curl disease was controlled in Sudan's Gezira region by a two-month "dead season," during which cotton was not planted and ratoon growth was removed (Bailey, 1930).

Several countries have tried crop-free periods and seen a significant reduction in the population of whiteflies. Crop residue must be removed in order to create crop-free periods. A relatively large proportion of infected plants left at the end of the crop season causes a high proportion of viruliferous whiteflies coming off virus-infected crop residues to be high. If infected plants are left on the field, the whitefly can still infect them (Hilje, 2002).

Another cultural practice worth pursuing is the timing of crop planting. Planting early or late affects the availability of pests. Planting okra early, depending on the season of insect abundance, will protect the okra from infestation. Planting late may also reduce the rate of infestation. In Mexico, for example, okra was planted early to reduce the population of whiteflies (Daz-Franco and Obregon, 1997). Weed removal from cropping areas can help reduce the availability of alternate hosts for the vector as well as potential sources of viral inoculum. The importance of weeds in *B tabaci* population dynamics and viral epidemics will vary depending on cropping season and plant-virus combination (Hilje, 2002).

Another cultural method for managing the pest is to exclude whiteflies from infesting okra. Growing the crop in a greenhouse or under insect-proof structures is one method of exclusion (Hilje, 2002). Horowitz *et al*. (1994) reported that in Israel, all tomatoes are grown inside enclosed structures made of solid plastic and/or fine screening to avoid TYLCV pressure. Another cultural method used to manage whiteflies on okra farms is high plant density on the field. If whitefly population control on okra is based on the concept of increasing crop plant density per unit area to decrease disease incidence, it is based on the principle that given a fixed number of vectors, the more crop

plants there are per unit area, the smaller the proportion of plants those insects can infect (Hilje, 2002). Powell *et al.* (1993) observed no significant difference in symptom rating of plants in a study on silver leaf symptom severity in Zucchini squash with row spacing ranging from 30 to 76 cm or 1 – 3 plants per hill. Another cultural practice used to manage whiteflies is behavioral manipulation, which involves disrupting whitefly host-searching behavior by interfering with visual or olfactory cues. It entails intercropping okra with two or more plant species in close proximity within a given plot. Crop associations can be formed to provide a haven for natural enemies and/or to manipulate the pest's host-seeking behavior in order to protect the main or most vulnerable crop. Planting a more preferred crop in close proximity to a less preferred whitefly-sensitive crop can be used to create a trap crop. Insects will infest the preferred trap crop first, reducing pest pressure on the primary crop. The preferred trap crop can be treated with an insecticide to either kill or prevent whiteflies from feeding on it (Hilje, 2002).

2.7.2. Okra Diseases

Numerous diseases afflict okra, diminishing the plant's capacity for output and limiting farmers' economic growth in Ghana and other regions globally. The following are some of the most common okra diseases in Ghana and around the world: YVMV (Yellow Vein Mosaic Virus), Cercospora Leaf Spot Fusarium wilt, Okra Leaf Curl Virus and Disease are just a few examples. (Moekchantuk and Kumar, 2004; Asare-Bediako *et al.*, 2014; Kumar *et al.*, 2017)

2.7.2.1 Okra Leaf Curl Disease

Okra Leaf Curl Disease is the most serious disease of okra and is transmitted by white flies (*Bemisia tabaci* Gen.) (Ali *et al.*, 2012). Okra leaf curl virus (OLCV) is the most common, well-studied and major disease of okra reported in Ghana (Asare-Bediako *et al.*, 2014). Yield losses from OLCD can reach 80% (Brown and Bird, 1992). Singh (1996) reported yield loss due to ELCV in okra ranging from 30% to 100.

2.7.2.2 Okra leaf disease symptoms and economic significance

OLCD symptoms include leaf curl, wrinkles, vein distortion, yellowing of the leaves, and reduced growth (Askira, 2012). Tiendrebeogo *et al. (2010)* also observed severely stunted plants with apical leaf curls (upward or downward), distortion, and thickening of the veins.

2.8 History of Family Geminiviridae

The *Geminiviridae* family is one of the largest plant virus families, with over 440 recognized members distributed globally across all land ecosystems with warm and temperate climates (Zerbini *et al.*, 2017). *Geminiviruses* are important plant pathogens that cause economically significant diseases in the majority of the world's tropical and subtropical regions. The maize steak and beet curly top disease's causative agent was unknown until virus particles with a unique twinned quasi-isomeric morphology associated with maize streak and beet curly top diseases were isolated (Bock *et al.*, 1974; Mumford, 1974). As a result, the name geminivirus, which means Gemini in the zodiac sign of twins, has remained a unifying feature of the virus family (Bock *et al.*, 1974). Zhang *et al.* (2001) stated that structural analyses revealed that the Maize Streak Virus was

associated with 22 x 38 particles composed of two incomplete T= 1 icosahedra, and 146 discovered a similar structure for the African cassava mosaic virus. Bottcher *et al*. (2004) reported that, during mechanical inoculation of some plant species with germinate particles associated with African Cassava Mosaic Virus, Maize Streak Virus and Bean golden mosaic virus (BGMV), which contained circular ssDNA and this genomic DNA were infectious when re-introduced to plants, setting geminiviruses apart from all plant viruses that had being characterized at that time. Some geminiviruses had divided genomes; this became evident in Beans golden mosaic virus (BGMV) and Tomato golden mosaic virus (TGMV).

Using the nucleotide sequence of a latent cassava virus, infectious clones were used to demonstrate a bipartite genomic structure, which was later renamed the ACMV (Stanley and Gay, 1983). Following that, the monopartite virus germiniviruses MSV, BCTV, and Tomato pseudo curly top virus were identified (Briddon *et al.,* 1996). Its members have a 2.7–5.2 kb circular, single-stranded DNA (ssDNA) genome encapsulated within twinned (geminate) icosahedral virions (Lazarowitz, 1992). There are now over 200 officially recognized geminivirus species (152). Geminiviruses are classified into seven genera based on their genome arrangement and biological properties: Mastrevirus, Curtovirus, Topovirus Becurtovirus, Eragnovirus, Turncurtovirus, and Begomovirus (Stanley *et al.,* 2005; Osei *et al.,* 2017). Since the nineteenth century, geminivirus infections have been observed in plants in tropical and subtropical regions of the world, exhibiting typical symptoms on crops such as cassava and croton in India, pepper, cotton, mungbean, okra, and chickpea in Pakistan, cassava and papaya in Uganda, and

okra in Cameroon (Wege *et al.*, 2000). The viruses in the Geminiviridae family replicate in the host cell nucleus, are transmitted by insect vectors in a non-propagative, persistent, and circular manner, and can infect phloem cells (Lazarowitz, 1992).

2.8.1 Taxonomy and Nomenclature of Geminiviruses

Viruses are classified into species, strains, and variants according to established rules (Van Regenmortel, 2006). Geminiviridae is the second largest family of plant viruses and geminiviruses. Taxonomy and nomenclature are becoming more complex as the number of viral genomic sequences deposited increases, so a rational and comprehensive approach to describing and classifying newly identified geminiviruses should be used (Brown *et al.*, 2012). Becurtovirus, Capulavirus, Curtovirus, Eragrovirus, Grablovirus, Mastrevirus, Topocuvirus, and Turncurtovirus all have a single genomic component, whereas Begomovirus has one or two. Phylogenetic analysis of complete genome sequences (DNA-A sequences in the case of bipartite begomoviruses) from representative species isolates reveals that geminiviruses are classified into nine genera (Brown *et al.*, 2012). Geminiviruses share similarities with other virus taxa. Geminiviridae and Nanoviridae, for example, have circular ssDNA genomes and replicate using a rolling circle mechanism. All of these viruses have highly conserved sequences; for example, geminiviruses have TARTATTAC, while nanoviruses have TAGTATTAC in the loop of a putative stem-loop structure within the IR, where a nick is introduced during replication initiation.

Interestingly, the majority of alpha satellites that are associated with begomoviruses have the nanovirus-like sequence TAGTATTAC, indicating

that they evolved from nanovirus components (Zerbini *et al.*, 2017). To ensure that taxonomic standards are clearer and nomenclature guidelines are more transparent, the International Committee on Taxonomy of Viruses proposed a recent set of demarcation criteria for the classification and naming of geminiviruses (Brown *et al.*, 2012). The proposed rule is that if the pairwise nucleotide sequence identity of a newly isolated geminivirus sequence to the previously reported geminiviral sequences is 89 percent (except for mastreviruses, which has a cut-off limit of 75 percent), it would be accepted as a new species, whereas a sequence identity greater than 89 percent would be classified as a member of the same species. When the nucleotide sequence of a geminivirus isolate is compared to all known strains and variants, if the pairwise comparison analysis is 93 percent, it is a member of a new strain of that species, and if it is less than 94 percent, it is a variant of that strain of the same species (Brown *et al.*, 2012). The following nomenclature structure is usually followed when naming a new virus: Name of the species, strain descriptor (symptoms, host, location, and/or a letter A, B, C, etc.) [Descriptor (country: location: [host]: year)] (Leke *et al.*, 2012). For example, each genus is named after the type species, such as Begomovirus-Bean golden mosaic virus (now known as Bean golden yellow mosaic virus) (Brown *et al.*, 2012). Zerbini *et al.* (2017) stated that the names of the various genera of geminiviruses were derived based on the host.

2.8.2 Genus Begomovirus

Begomoviruses are a newly emerging and economically significant group of plant viruses of the Geminiviruses (Fauquest *et al.,* 2008; Stanley *et al.,* 2005). Begomoviruses are the most numerous and economically destructive geminiviruses, transmitted by the ubiquitous whitefly *Bemisia tabaci*. The genus Begomovirus contains 196 species, making it the largest genus in the *Geminiviridae* family (Brown *et al.,* 2012). Morphologically, several researchers have identified various species of the Begomovirus, with 152 reporting over 200 species and the highest number of species being 322(Osei *et al.,* 2017). Brown *et al.* (2015) also reported 388 species that infect dicot plants and are transmitted by the *Bemisia tabaci* cryptic species complex. Begomoviruses are known to cause severe symptoms in their hosts, such as yellow mosaic, golden mosaic, and leaf curl. Pathogens that cause havoc include the African cassava mosaic virus, Bean golden mosaic virus, Cotton leaf curl Multan virus, and Ageratum leaf curl virus. Cabbage leaf curl virus, Allamanda leaf curl virus, Bean chlorosis virus, Bean dwarf mosaic , Chili leaf curl virus from India, Cotton leaf crumple virus, Cotton leaf curl Gezira virus, Okra enation leaf curl virus, Okra yellow mosaic virus from Mexico COVID-19 that causes papaya leaf curl African cassava mosaic Burkina Faso virus, Pepper golden mosaic virus, Abutilon golden mosaic virus, viruses that cause tomato yellow leaf curl include, among others, Tomato Yellow Leaf Curl Virus (Zerbini *et al.,* 2017). The classification of Begomovirus is based on the genome structure and phylogenetic relationship, host range, and insect vector type (Osei *et al.,* 2017). Begomovirus genomes are composed of either two bipartite genomic components, namely DNA-A

and DNA-B, of equal size of 2.8 kb, or a single component monopartite, homologous to the DNA-A component of bipartite viruses (Rojas *et al.*, 2005; Stanley *et al.*, 2005). There are three types of circular DNA satellites associated with begomoviruses: alpha satellites, beta satellites, and delta satellites (Zhou, 2013; Lazano *et al.*, 2016).

2.8.3 Genome Organization of the Begomovirus

2.8.3.1 Bipartite Begomovirus

A 200-nt segment, known as the common sequence, is the only sequence similarity between the DNA-A and DNA-B components (Lazarowitz, 1992). The replication origin (ori) of the CR consists of a stem-loop structure containing conserved iterated sequences (iterons) necessary for specific recognition and binding by Rep during replication, as well as an invariant nonanucleotide (TAATATTAC) sequence whose T7–A8 site is required for cleaving and joining viral DNA during replication (Arguello-Astorga *et al.*, 1994; Fontes *et al.*, 1994; Harrison and Robinson, 2002) AV1/V1 (coat protein, CP) and AV2/V2 (AV2/V2 protein) are two of DNA-A's six open reading frames (ORFs) on the virion-sense strand. On the complementary-sense strand, the ORFs are AC1/C1 (replication initiation protein, Rep), AC2/C2 (transcriptional activator, TrAP), AC3/C3 (replication enhancer, REn), and AC4/C4 (AC4/C4 protein). Two ORFs on the virus-sense strand, BC1 (movement protein, MP) on the complementary-sense strand and BV1 (nuclear shuttle protein, NSP) on the virus-sense strand, encode movement proteins (Rojas *et al.*, 2005; Seal *et al.*, 2006; Harrison and Robinson, 1999). The intergenic region (IR) that divides the opposing transcription units of begomovirus DNA-A and -B molecules often share the

common region (CR), a highly conserved area of roughly 200 nucleotides (nts) (Lazarowitz, 1992). Although beta satellites have been implicated in pathogenicity, alphasatellites have no known function and are almost certainly not involved in symptom induction (Mansoor *et al.*, 1999)

2.8.3.2 Monopartite Begomoviruses

The six open reading frames comprise the monopartite Begomovirus genome (ORFs). Coat protein genes CP or V1 and V2 are expressed from viral sense strands, while C1, REP C2, C3, and C4 are expressed from complementary strands (Navot *et al.*, 1991). ORFs are labeled as being encoded on the virion-sense (V) or complementary-sense (C) strand, followed by a component designation (A or B) if bipartite (bottom). Protein products that correspond to each other are labeled. ORF AV2/V2 is not found in New World begomoviruses. The "common regions" CRA and CRB (bottom) shared by the two genomic components of bipartite viruses are represented as grey boxes within the intergenic region (IR). The position of the stem-loop in the IR containing the conserved TAATATTAC sequence. CP stands for coat protein, and Rep stands for replication-associated protein. TRAP stands for transcriptional activator protein, REn stands for replication enhancer protein, MP stands for movement protein; and NSP stands for nuclear shuttle protein, as shown in figure 1 below.

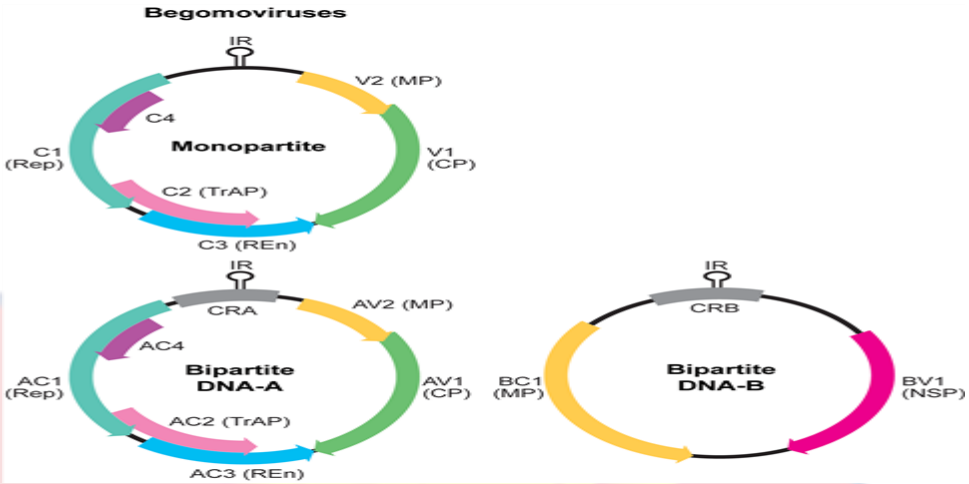


***Figure 1*: Begomovirus. Genomic organization of begomoviruses.**

2.8.3.3 Begomovirus-satellite complex

Satellites are viruses or nucleic acids that rely on helper viruses for replication but lack extensive nucleotide sequence identity to the helper virus and are unimportant in terms of proliferation (Mayo, 2005). The recently discovered DNA satellites (betasatellites) and nano virus-like DNA satellite molecules (alphasatellites) have been linked to the majority of monopartite begomoviruses (Briddon and Stanley, 2006). Only in plants infected with monopartite begomoviruses have alphasatellites been found in association with beta satellites (Mubin *et al.*, 2009). Satellite complexes are most common in the Old World, including Asia and Africa. However, alphasatellites have recently been linked to bipartite begomoviruses from the New World (Romay *et al.*, 2010). These 'satellite' molecules are roughly half the size of the 'helper' begomovirus molecules. DNA- molecules have one major ORF (C1) on their complementary strand, are replicated by a helper virus, and are responsible for symptom induction (Briddon and Stanley, 2006)

Saunder *et al.* (2000) experimented on *Ageratum conyzoides*, which failed to show signs of yellowing vein symptoms of the leaves after re-

inoculation with Ageratum yellow vein virus (AYVV), implying the presence of another factor responsible for symptom development. A number of recombinant molecules containing the begomovirus origin of replication (ori) were then isolated from the infected host plant *A. conyzoides*, and a novel ssDNA molecule, approximately half the size of helper begomovirus, was isolated from *A. conyzoides* plants that reproduce typical yellow vein symptoms in the host plants.

2.8.4 Vector Transmission, Begomovirus Infection and Replication Mechanism

The white fly is a vector of the Begomovirus, inoculating virus particles in phloem cells while feeding on its host, which is mostly dicot plants. When viral particles are uncoated and viral nuclei acids enter the nucleus, replication and transcription occur. The first stage of the infection cycle involves a whitefly vector injecting viral ssDNA into a plant cell. Geminiviruses replicate in the nucleus of infected cells via a double-stranded (ds) DNA intermediate. When a geminivirus first enters a host cell, the only viral protein present is the CP. Movement to the nucleus must thus be entirely dependent on the CP and the host transport mechanism (Figure 1). It is unclear whether the virus inoculated by the vector moves to the nucleus as an encapsulated virion or decapitates and moves as a nucleoprotein complex. The interaction with the host transport is the result of the CP's involvement. When the viral ssDNA enters the nucleus, it is converted into a transcriptionally active dsDNA intermediate that serves as a template for both transcription and replication (Gafni and Epel, 2002).

2.8.5 Identification of novel *begomoviruses*

Several begomoviruses have been identified all over the world. However, the begomovirus that infects okra in Ghana is yet to be identified. Following a complete genome sequence analysis, Ha *et al*. (2008) reported that nine of the viruses (six monopartite and three bipartite) belong to novel species, with five of them being identified for the first time in Vietnam. The viruses were named Corchorus golden mosaic virus (CoGMV), which infects jute mallow (*Corchorus capsularis*); kudzu mosaic virus (KuMV), which infects kudzu (*Pueraria montana*); Clerodendrum golden mosaic virus (ClGMV), which infects glory bower (*Clerodendrum philippinum*); Spilanthes yellow vein virus (SpYVV), which infects paracre. Ghanem (2003) identified a novel begomovirus in a research farm for the first time in Saudi Arabia, and the isolated virus was named okra leaf curl geminivirus-Saudi Arabian-isolate (OLCV-SA). In Africa, okra leaf curl diseases are caused by a complex of begomoviruses that include novel viral species such as Cotton leaf curl Gezira virus (CLCuGV), Okra yellow crinkle virus (OYCrV), and Hollyhock leaf crumple virus (HoLCrV), all of which are linked to Cotton leaf curl Gezira beta satellite (CLCuGB) and Cotton leaf curl Gezira alpha satellite (CLCuGA) (Jose and Usha, 2003). Tiendrebeogo *et al.* (2010), for example, collected 74 samples of okra plants infected with okra leaf curl disease across Burkina Faso, 48 of which were found to be infected with begomovirus using PCR amplifications with the universal primer pair VD360-CD1266, which recovered the conserved CP ORF. RCA was used to successfully obtain 23 begomovirus genome sequences ranging in length from 2761 to 2773 nucleotides (nt) from positive samples. As a result, based on the currently

applicable species demarcation threshold of 89 percent for begomoviruses, they concluded that all 23 begomovirus isolates isolated from okra in Burkina Faso belonged to the species Cotton leaf curl Gezira virus and the Niger strain. OELCuV, another novel species described by Venkataravanappa *et al.* (2011), was linked to a betasatellite. An alphasatellite DNA associated with Okra enation leaf curl virus (OELCuV), which causes enation and leaf curling in okra (*Abelmoschus esculentus*) plants, was identified as a novel species of begomovirus in Surat, India (Chandran *et al.*, 2013).

2.8.6 Diversification of begomoviruses in Africa

Geminivirus centers of diversification were identified from the phylogenetic tree built from all available sequences (Fauquet *et al.*, 2008) as the areas with the greatest number of geminiviruses. Australia, Japan, South China, the Indian subcontinent, Sub-Saharan Africa, the Mediterranean-European region, South America, and Central America are the eight distinct geographical centers of geminivirus diversification (Nawaz-ul-Rehman and Fauquet, 2009). Africa also has tropical or subtropical climatic conditions that are similar to those found in the Indian subcontinent, and begomovirus diversity is high. Begomoviruses of both types, bipartite and monopartite, have been reported from Africa. These begomoviruses infect cassava, tomato, tobacco, okra, and beans, among other plants (Leke *et al.*, 2012; Tiendrebeogo *et al.*, 2010).

2.9 Detection Methods of Plant Viruses

Plant viruses are much smaller than fungi and bacteria, making detection and management difficult for all stakeholders. When compared to fungi and bacteria, viruses are difficult to see with a light microscope. They can only be seen with a transmission electron microscope. They are composed of a coat protein and various types of nucleic acid, such as DNA or RNA, based on the nucleic acid core that contains genetic information (Strange, 2005). Over 1000 viruses have been discovered in plants around the world, excluding bacteria, fungi, and phytoplasma (King *et al.,* 2011). Aside from fungi, viruses are the world's second most economically important plant pathogen, causing farmers and other stakeholders billions of dollars in losses (Hull, 2002). Symptoms of viral diseases such as crinkling, browning of leaf tissues, mosaic, and necrosis may not be visible because plant virus infection causes no symptoms, and plants may exhibit these symptoms due to unfavorable weather, nutritional imbalances, infection by other types of pathogens, and even pests (Van der Want and Dijkstra, 2006). Accurate virus disease diagnosis is the first and most important step in any crop management system. Plant virus disease treatments after infection, on the other hand, do not always result in effective control; virus diseases are best managed if control measures are implemented before infection occurs. Farmers' use of healthy (virus-free) plant propagation material is one of the most effective approaches they can take (Makkouk and Kumari, 2006; Aboul-Ata *et al.,* 2011). In crop management systems, accurate diagnosis is the foundation for managing plant diseases and predicting crop loss due to plant pathogen infection (Van der Want and Dijkstra, 2006; Aboul-Ata *et al.,* 2011). In the last three decades, the

fight against the viral disease has shifted to advances in molecular biology and biotechnology to develop rapid, specific, and sensitive techniques for the detection of plant viruses, as opposed to previous decades that relied on biological techniques that were too slow and unsuitable for large-scale application. As a result, microscopical observations, serological techniques, and molecular methods, among others, have been developed to detect plant virus diseases (Makkouk and Kumari, 2006).

2.9.1 Serological Methods

Serological detection systems rely on antibodies developed in animals to respond to antigens (Torrence, 1998). For more than a half-century, serology has been used to detect plant viruses (Torrence and Jones, 1981). It entails the detection of viruses and the use of antigens to develop antibodies. Serological methods such as enzyme-linked immunosorbent assay (ELISA), tissue blot immunoassay (TBIA), and quartz crystal microbalance immunosensors (QCMI) have been reported (Jeong *et al.*, 2014)

2.9.2 Enzyme-Linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is a widely used method for virus detection that is highly sensitive, simple, fast, and, most importantly, can quantify virus content in plant tissue. The binding of the virus and specific antibody is visualized using an antibody that has been tagged with an enzyme that can react with a substrate to produce a colored, water-soluble product (Makkouk and Kumari, 2006). Clark and Adams (1977) described the first method, double antibody sandwich ELISA (DAS-ELISA), in which the antibody is bound to the solid phase (e.g., a polystyrene microtite plate), and the test samples, enzyme-labeled antibody, and substrate are added

sequentially, with unbound material removed by washing between steps. The color intensity, which is proportional to virus content, can be measured spectrophotometrically, with a positive test causing the substrate solution to turn colored and a negative test leaving the substrate solution colorless. Other ELISA variants, such as triple antibody sandwich ELISA (TAS-ELISA) and direct antigen coating ELISA, have been reported using different enzymes or universal conjugates (DAC-ELISA). Currently, most ELISAs are performed in polystyrene plates capable of binding antibodies or proteins with an enzyme-substrate reaction association.

Aside from the polystyrene plates, a variety of solid phase supports were found to be adequate. Immunoblots or dot-blots are assays in which antibodies or virus particles are bound to nitrocellulose membrane filters. Dot blot ELISA is often more sensitive than ELISA performed in a microtiter plate because it is faster, easier to perform, and uses fewer reagents (Banttari and Godwin, 1985). ELISA is a popular assay for detecting plant viruses in plant material, insect vectors, and seeds (Clark and Adams, 1977; Naidu and Hughes, 2001). It has been used to detect viruses such as CMV, Citrus tristeza virus (CTV), Potato leaf roll virus (PLRV), Potato virus X (PVX), Potato virus Y (PVY), okra leaf curl virus, Cotton leaf curl Gezira virus (CLCuGV), okra yellow crinkle virus (OYCrV), and Hollyhock leaf crumple virus (HoLCrV) (Sun *et al.,* 2001; Idris *et al.*, 2002; El-Araby *et al.,* 2009). When compared to molecular methods, ELISA requires a large sample to capture the antigen of interest from the sample, and it takes about 2 days to diagnose (Lievens *et al.,* 2005). Because ELISA is an antibody-antigen assay, the availability of antibodies capable of properly responding to the target agent is regarded as a

critical factor. ELISA frequently provides misdiagnosis due to false positives, which are primarily caused by non-specific reactions or cross-reactivity with certain factors in samples (Kirf and Genthe, 1993). Because of the lack of specificity, the antibody used in ELISA can respond to many strains with clearly different symptoms, and thus, strains of the virus that are very similar cannot be correctly differentiated by ELISA (Boonham *et al*., 2014). Although the sensitivity of ELISA was increased by including some additives in the extraction buffer, ELISA is generally less sensitive than molecular methods (Fegla and Kawanna, 2013)

2. 9.3 Polymerase Chain Reaction (PCR)

PCR has become the new gold standard for detecting a wide range of templates in a variety of scientific disciplines, including virology (Mullis and Faloona, 1987). PCR is a scientific technique for generating millions of identical copies of a specific DNA sequence within a tiny reaction tube (Saiki *et al.,* 1985). PCR allows for the creation of millions of copies of a specific nucleic acid sequence. This chemical reaction takes place due to the action of a DNA polymerase, which can copy a DNA strand. Target DNA, two oligonucleotide primers, a DNA polymerase, a mixture of deoxyribonucleotide triphosphates (dNTPs), $MgCl_2$, KCl, and Tris-HCl buffer are used in PCR. Before starting a new round of DNA amplification, the DNA is denatured, two sets of oligonucleotides are called forward and reverse primers anneal to the denatured complementary strand. Following that, primers direct DNA synthesis by the DNA polymerase. All reactions take place in a template-dependent order. The target sequences of interesting DNA are exponentially amplified as a result of this (Saiki *et al.,* 1988). PCR has been used in a variety

of applications, including cloning, gene manipulation, gene expression analysis, genotyping, sequencing, mutagenesis, and disease detection (Schaad and Fredrick, 2002; Makkouk and Kumari, 2006). PCR is currently used in the laboratory for the detection of plant viruses and is based on the specificity of primers. PCR is preceded by three steps: dsDNA separation at temperatures above 90^{o}C, primer annealing at temperatures between 50 and 75^{o}C, and optimal extension at 72-78^{o}C. A programmable thermal cycle controls the rate of temperature change, the length of incubation at each temperature, and the number of times each cycle is repeated.

The amplified DNA fragments are then separated using agarose gel electrophoresis, and the bands are visualized by staining the resulting bands with ethidium bromide and exposing them to ultraviolet light. The primer sets used determine the specificity of PCR testing. There are primers that are specific to a virus species and primers that are specific to a genus (Makkouk and Kumari, 2006). With reverse transcription and polymerase chain reaction, when compared to serological methods, the PCR (RT-PCR) technique is more sensitive, specific, inexpensive, and reliable. RT-PCR can detect plant viruses and their vectors (Peter *et al.*, 2009). PCR is effective against DNA viruses of the genera Geminivirus, Nanovirus, and Caulimovirus (Makkouk and Kumari, 2006). Viruses such as Cucumber vein yellowing virus (CVYV), Cucurbit yellow stunting disorder virus (CYSDV), Potato aucuba mosaic virus (PAMV), Potato yellow dwarf virus (PYDV), and Tomato chlorosis virus (ToCV) can be detected using this technique (Lee *et al.*, 2011).

Another type of PCR that outperforms ELISA is multiplex PCR, which detects two or more targets' DNA or RNA at the same time in a single

reaction. To detect more than two viruses or bacteria, the method necessitates the use of several specific primers (Chamberlain *et al*., 1988; Li *et al*., 2011). It is critical to quantitatively depict the progress of amplification (Lomeli *et al.*, 1989). With the passage of time, the PCR target amplification and detection steps occur concurrently. These methods necessitate the use of specialized thermal cyclers capable of monitoring the fluorescence emission from the sample. The thermal cycler's computer software monitors the data at each cycle and generates an amplification plot for each reaction. Fluorescent dyes that preferentially bind to double-stranded DNA are used to detect the PCR product. The use of fluorescent resonance energy transfer (FRET) probes in the reaction mixture can also improve the specificity of real-time PCR. The use of dual hybridization probes, which use two specially designed sequence-specific oligonucleotide probes, is another approach to real-time PCR. Finally, molecular beacons can be used to detect and quantify amplification products. Because there are no post-PCR processing steps, real-time PCR reduces the time required to perform nucleic acid assays. The main advantages of these methods include the reduction of contamination and the ability to perform quantitative applications (Cobo, 2012).

CHAPTER THREE

3.0 STUDY OF FARMER PERCEPTION OF OKRA LEAF CURL DISEASE AND WHITEFLY VECTOR IN THE VOLTA AND OTI REGIONS

3.1 Introduction

Vegetables are an excellent source of nutrients in the human diet for maintaining good health (Ntow *et al.*, 2006). After tomato, pepper, and garden egg, okra is the fourth most popular vegetable in Ghana (Oppong-Sekyere *et al.*, 2012). Under good management, okra yields of about 10-15 t/ha can be obtained (NARP, 1993). Despite the importance of okra, farmers in Ghana face extremely low yields. For example, okra productivity in West Africa is very low (2.5 t ha^{-1}) compared to East Africa (6.2 t ha^{-1}) and North Africa (8.2 t ha^{-1}) (Cudjoe *et al*., 2005), a situation that can be attributed to pests and diseases. Among the numerous pests and diseases identified in Ghanaian okra production, whitefly and okra leaf curl disease (OLCD) are regarded as the most economically significant (Asare-Bediako *et al.*, 2014). Controlling OCLD and its associated whitefly vectors is critical to increasing okra yield. Farmers have resorted to the wrongful use of pesticides in the management of the disease and its associated whitefly vector in order to increase okra yield. However, pesticide-based management of viral diseases and their vectors has been ineffective due to inappropriate application and use, which frequently results in the development of resistant pest strains.

Furthermore, synthetic pesticides are harmful to both human health and the environment (Asare-Bediako *et al.*, 2014). Okra leaf curl disease can cause significant losses in okra production if left unmanaged or managed incorrectly.

As a result, it is critical to understand what farmers know about the disease, their perceptions of crop yield damage, the control methods they employ, and the perceived effectiveness of these methods. Information on the farmers perception about the disease is essential for developing appropriate and effective management strategies. Despite the importance of this information, there have been few studies on the subject, particularly among smallholder farmers. As a result, this study assessed farmers' knowledge of okra leaf curl disease and its vector, the whitefly, in Volta and Oti region.

The specific goal of this aspect of the study are to:

1. Assess farmers' perceptions of okra leaf curls disease in the Volta and Oti regions
2. Determine the management practices used to control the OLCD and its associated whitefly vector in Volta and Oti regions.
3. Determine the effectiveness of management practices in disease control.

3.2 Materials and Methods

3.2.1 Study area

The research was carried out in three agro-ecological zones, namely coastal savanna, forest, and transition, which encompassed Ghana's Volta and Oti regions (Volta and Oti constituted, previously, the Volta region). The Volta and Oti region of Ghana is located between latitudes 5° 45'N and 8° 45'N and is bordered by the Volta Lake to the west, the Republic of Togo to the east, and the Atlantic Ocean to the south. The region is 20,570 square kilometers in size, accounting for 8.6 percent of Ghana's total land area. The vegetation is divided into five types: coastal strand mangrove swamps, woodland savannah, Savannah grassland, mangrove swamps, and deciduous

forest. The region has a population of 2,118,252, with over 291,224 people working in agriculture. Agriculture/Hunting/Forestry is the most important industry in the region, and indeed in all of the districts (GSS, 2013; GTA, 2020).

The region has a tropical climate with moderate temperatures ranging from 12 ^{o}C to 32 ^{o}C for the majority of the year. The rainfall pattern is bimodal, which means that it has two rainfall regimes throughout the year, the first from March to July and the second from mid-August to October. Rainfall figures vary greatly across the region, with the central highlands and forest zone receiving the most and the Sahel-savannah zone in the north receiving the least. The annual precipitation ranges between 513.9 mm and 1099.88 mm (GSS, 2013; GTA, 2020).

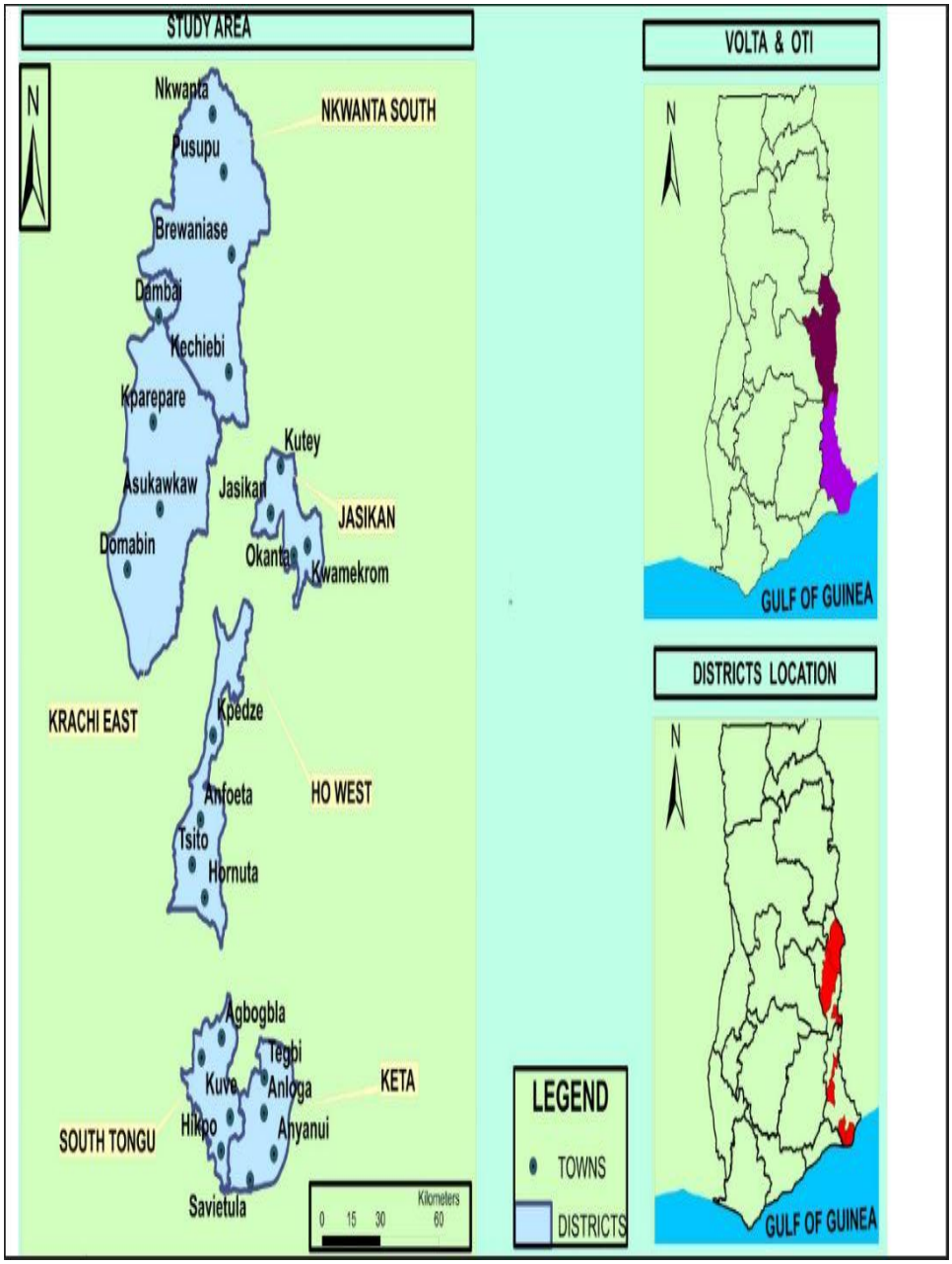


***Figure 2*: A Map of the Volta and Oti regions with surveyed districts and communities (marked Black)**

3.2.2 Experimental Design

In this study, a convergent parallel design and descriptive survey were used, in which quantitative and qualitative elements of the research were conducted independently over the same period, and the results were then integrated into the overall interpretation to answer the stated research questions and hypothesis.

The mixed method approach aided in the collection and analysis of quantitative data such as farmer background information, farm characteristics, farmers' awareness of Okra leaf curl disease (OLCD), farmers' knowledge of whitefly infestation, and okra farmers' pesticide use practices.

3.2.3 Population of the Study Area

The study population included all okra farmers from the three agro-ecological zones and the six districts that have okra farms with one year or more of farming experience. The region is home to approximately 15000 okra farmers (personal communication, MOFA)

3.2.4 Sampling Procedure

In the study, okra farmers were chosen from different communities in different districts from the region's three agro-ecological zones using a multi-stage sampling technique. Interactions with personnel from the Ministry of Food and Agriculture's (MoFA) regional and district directorates were used to gather information on the most important okra-growing areas in the region's various ecological zones. The multi-stage sampling technique entailed the following steps:

Stage 1: Selection of Clusters: The Volta and Oti region's okra-growing districts were divided into six clusters: Keta, South Tongu, Ho East, Jasikan, Nkwanta South, and Krachi East.

Stage 2: Determination of Sampling Frame: The region's okra farmer population is estimated to be 15, 000 people (Personal communication, MoFA). As a result, the study's sample frame includes 8,793 farmers with at least one year of okra farming experience. Determination of the sample size as follows;

Sample Size (n) = $N/(1+N(\alpha)^2)$ ..... (1)

Where N denotes the sample frame, n denotes the sample size and = the margin of error at the 95 percent (0.05) confidence level.

n = 8793/ (1+8793(0.05)2)

n = 8793/ (1 + 8793(0.0025))

8793/22.9 = n

384 people

384 farmers are involved

Stage 3: Cluster Sample Size Determination

A proportionate stratified random sample was estimated to generate the number of okra farmers from the Volta region's six okra districts to be included in the study. There were 384 farmers selected across all of the districts (Keta, South Tongu, Ho West, Jasikan, Nkwanta South, and Krachi East) with 16 from each community.

Stage 4: Sample Selection Technique

A simple random sampling technique was used to select the assigned number of okra farmers in various communities in each of the Volta and Oti region's six okra districts.

3.2.5 Non-Probability Sampling

The samples for the key informant interviews and observations were chosen using a non-probability sampling technique.

3.2.6 Sampling for farm observation

In the Volta and Oti regions, 96 farms (sixteen (16) farms from each district) were purposefully chosen as cases for direct observation of farm characteristics, agronomic practices, disease and pest status, pesticide use, and management practices.

3.2.7 Data Collection Instruments

As data collection tools, a semi-structured questionnaire and observation guides were used. Farmers should respond to semi-structured questions for both closed and open-ended questions. The observation guides aided in concentrating on what was expected to be observed during the observation process. Microsoft Office Word was used to create the instruments. The questions on the instruments were derived from the literature, scoping studies, and the researchers' perspectives on the research questions guiding the study. The semi-structured questionnaire includes a confidentiality statement, a questionnaire number, and open information about the interviewee's location. The questionnaire was divided into the following sections:

Section 1: Background information on farmers

Section 2: Farm characteristics

Section 3: Farmers' awareness of Okra leaf curl disease (OLCD)

Section 4: Farmers' knowledge of whitefly infestation

Section 5: Pesticides use practice by the okra farmers

3.2.7 Data Collection Procedures

Due to the significance of okra cultivation in the South Tongu district, a pre-tested structured interview schedule comprising both closed- and open-ended questions was created. The farmers were tracked down to their farms, homes, and marketplaces to administer the questionnaires. The one-on-one questions were asked in both the local dialect (Ewe and Twi) and English. After obtaining permission from the farmers, photographs were taken with them. Using an observation guide, key areas were observed on the selected okra farmers in each of the selected districts, as well as four farms in each community in the region. Farmers' fields were visited early in the morning to observe farm hygiene and cleanliness, agronomic practices such as land preparation methods used, weed control, fertilizer application, source of water, and pesticide use protocols, and to see if farmers are performing the activities if available and providing reasons in some cases regarding the activities they undertake. The presence of disease and pests, as well as the management practices used, were also observed. Photographs of the activities observed were taken with the farmers' permission.

3.3 Data Processing and Analysis

Following data collection, the questionnaire was cleaned by correcting spelling errors and the numbering of the questionnaire was double-checked. In the SPSS software, a template was created, and the data were entered as string and numeric values. The farmers' perception data (chi-square test) was analyzed using both inferential statistics and descriptive statistics (means, frequency distributions, and percentages). The elicited data was processed using IBM 'Statistical Package for the Service Solutions (SPSS) Software Package Version 20. Tables, figures, images, themes, and narratives were used to present the quantitative and qualitative data that had been analyzed.

3.4 Results

3.4.1 Background information on Farmers (respondents)

3.4.2 Age Distribution

Figure 3 shows the age distribution of the respondents. Farmers aged 20 to 29 formed the majority (42.4%), followed by those aged 40 to 49 (20.6%). Farmers aged 30 to 39 made up 20.1% of the population, while those over 50 made up 13.8 %. Respondents under the age of 18 made up only 3.1% of the total number of respondents.

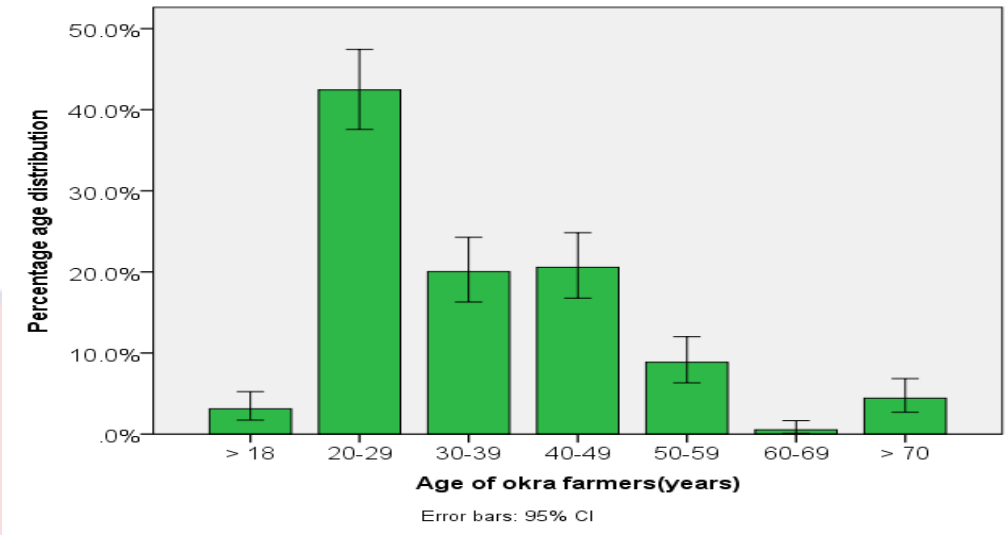
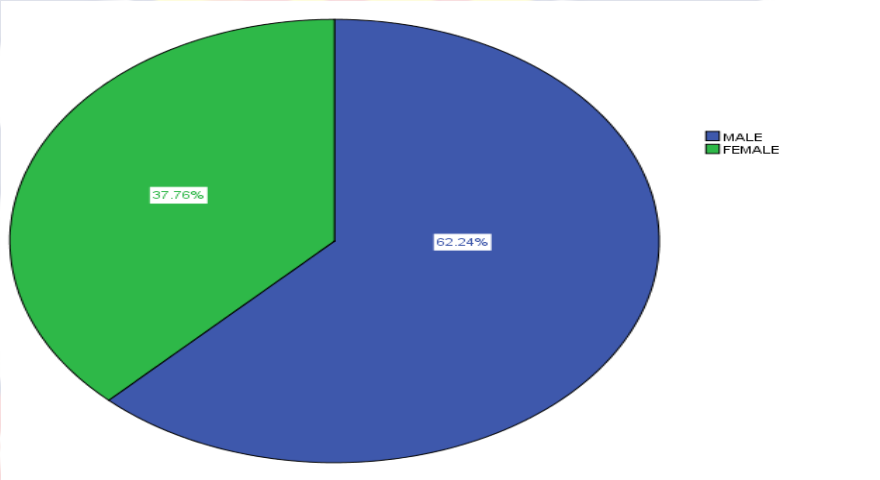


***Figure 3*: Percentage age distribution of respondents in the study area. N=384**

3.4.3 Gender Distribution

Figure 4 shows the gender distribution of respondents. The majority (62%) of respondents were male, while 38% were female.

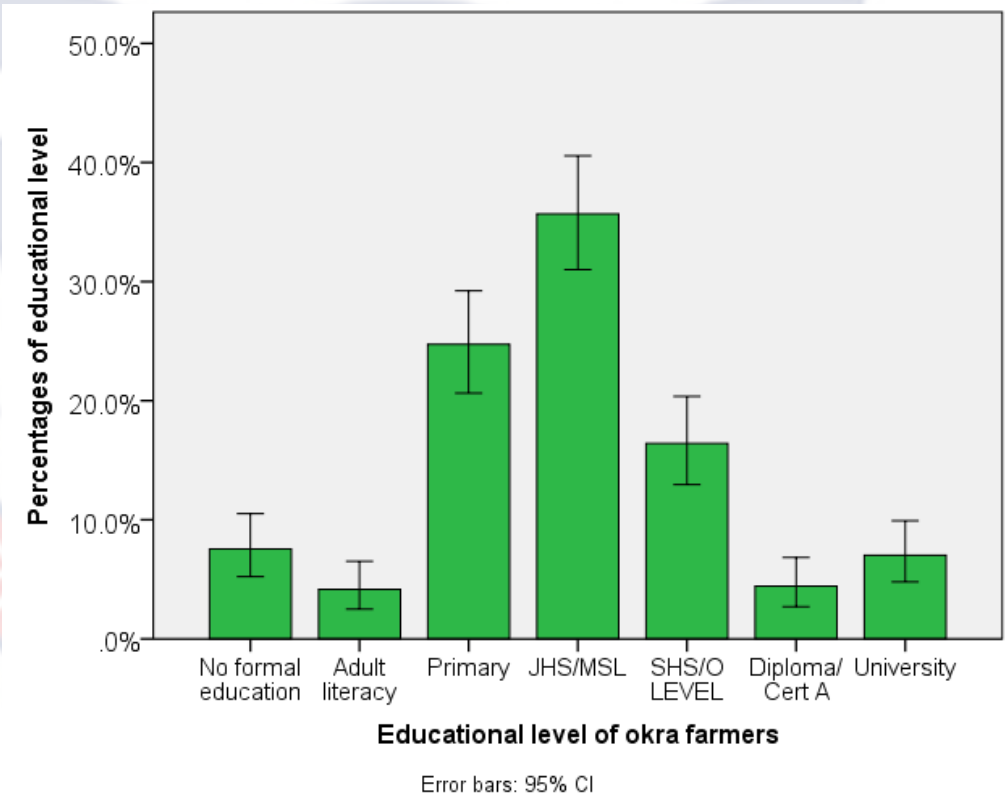


N=384

***Figure 4*: Gender distribution among respondents in the study area.**

3.4.4 Educational Level

Figure 5 shows the educational levels attained by okra farmers in the region. The vast majority of farmers (35.68%) had completed their junior high/middle school education. Primary school graduates made up 24.74% of those polled. Sixteen percent (16%) of the farmers completed Senior High School or Ordinary level, 4.43% hold a Diploma or Teacher Certificate 'A,' and 4.17% attended adult literacy classes. University education was the highest educational level attained by okra farmers, accounting for 7.03% of the total. Farmers with no formal education accounted for 7.55% of all respondents.



N=384

Figure 5: Educational level of respondents in the study area

3.4.5 Marital Status

Figure 6 shows the marital status of respondents. The majority of farmers, 271 (70.6%), were married, while 41 (10.7%) were not. Widows and separated people made up 34(8.9 %) and 7.8% (30) of the population, respectively. Farmers in cohabitation constituted at least 8 (2.1%).

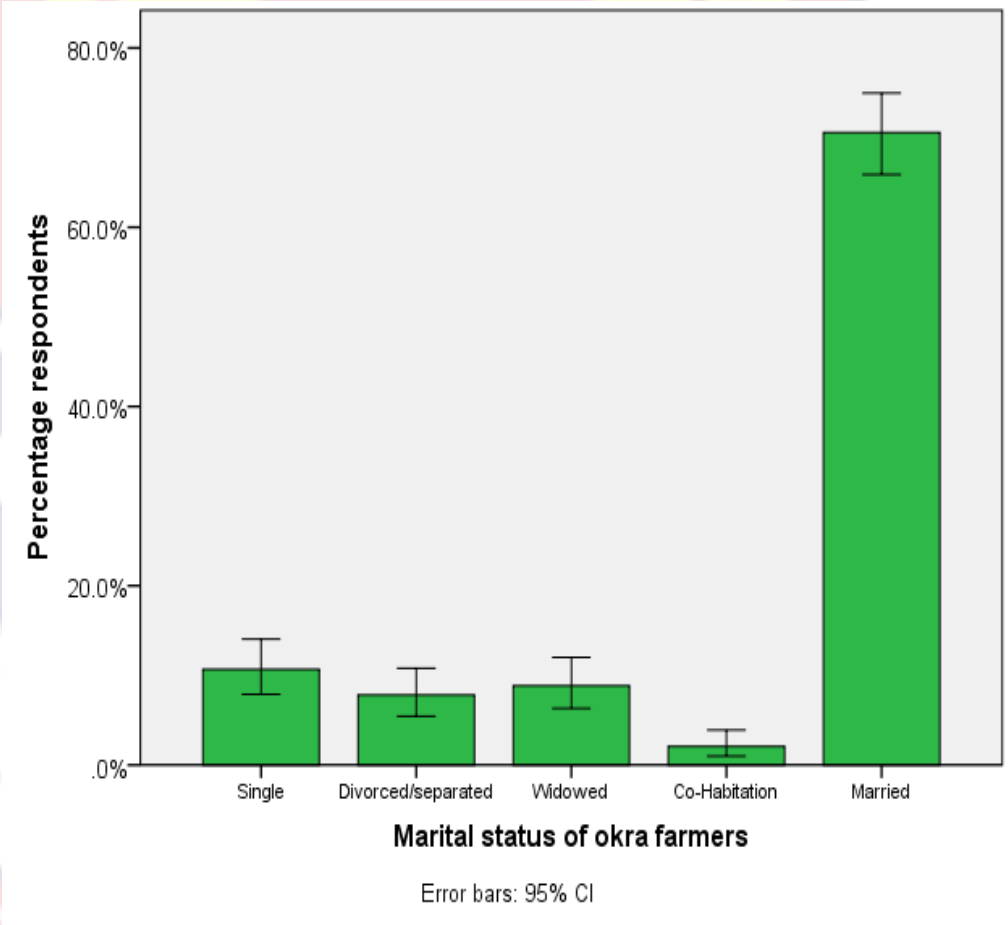


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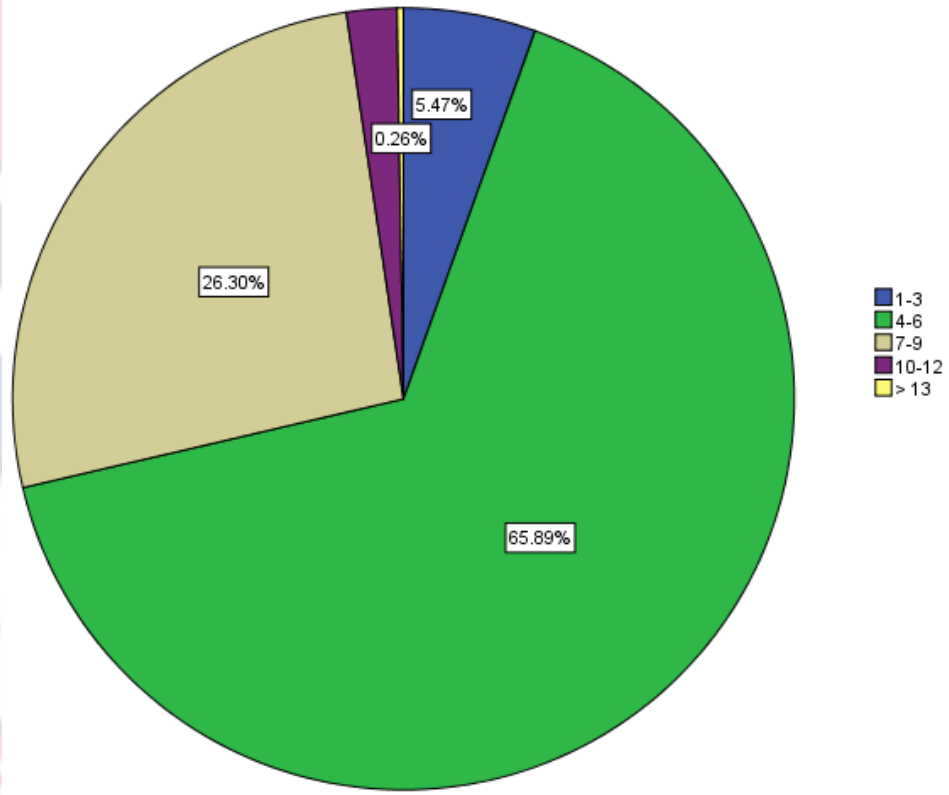
Figure 6: Marital status of respondents in the study area

3.4.6 Number of Persons in Household

Figure 7 shows the number of people in the okra farmer's household. The majority of respondents (65.9%) had 4 to 6 people in their household, 26.3% had 7 to 9 people in their household, 5.5% had 1-3 people in their household, and 2.1% had 10 to 12 people in their household. One (0.3%) respondent had the most people (>13) in their household.

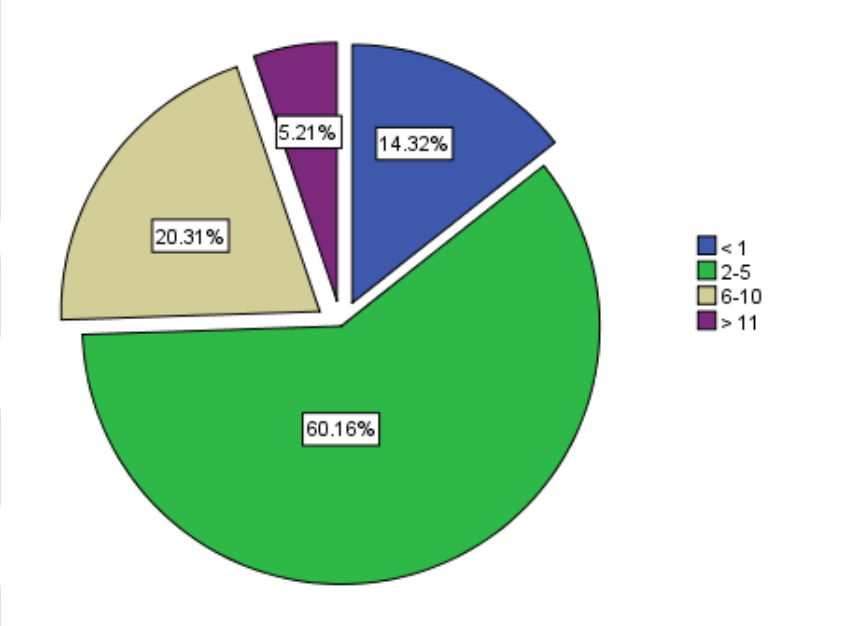


N=384

Figure 7: Number of persons in respondents' farmers household in the study area

3.4.7 Number of Hectares of Okra Cultivated by Respondents

Figures 8 shows the total number of acres of okra cultivated by okra farmers in a year. Sixty percent (60.16%) of farmers cultivated 2 to 5 acres of okra in a year, 20% cultivated 6 to 10 acres of okra, and 14.32% cultivated less than one acres of okra. 5.21% (20) of the respondents cultivated the most okra, with a total land area of more than eleven (11) acres.

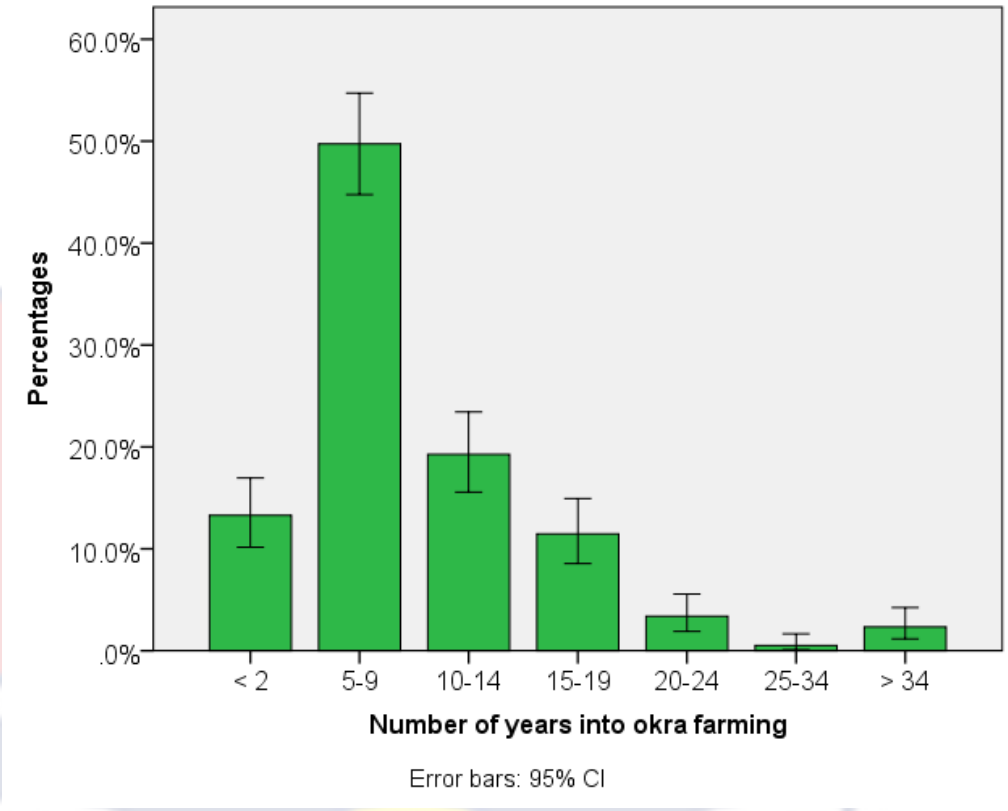


N=384

Figure 8: Number of hectares of okra cultivated by farmers in the study area

3.4.8 Number of Years Farming Okra

Figure 9 shows the number of years a farmer has been cultivating okra. According to the findings, the majority of the farmers (49.7%) had 5 to 9 years of experience in okra farming, 19.3% had 10 to 14 years. 13.3% and 11.5 % had less than two years and 15 to 19 years of experience, respectively. Farmers aged 25 to 34 years and over 34 years had the lowest percentage of okra farmers at 0.5% and 2.3%, respectively.



N=384

Figure 9: Number of years into okra farming by farmer in the study area.

3.4.9 Reasons for Cultivating Okra

Figure 10 shows multiple responses on why respondent farmers grow okra in the study area. The majority of the farmers see okra farming as a source of food (244) and as a source of income (235) to them. A total of 158 of the respondents said there was high market demand for okra, hence their reasons for cultivating it. Okra was a major crop in the area (16). Others said it was a source of employment (10). It is also an early maturing crop; ten (10) of the farmers chose to cultivate okra. Only two famers said okra easy to cultivate.

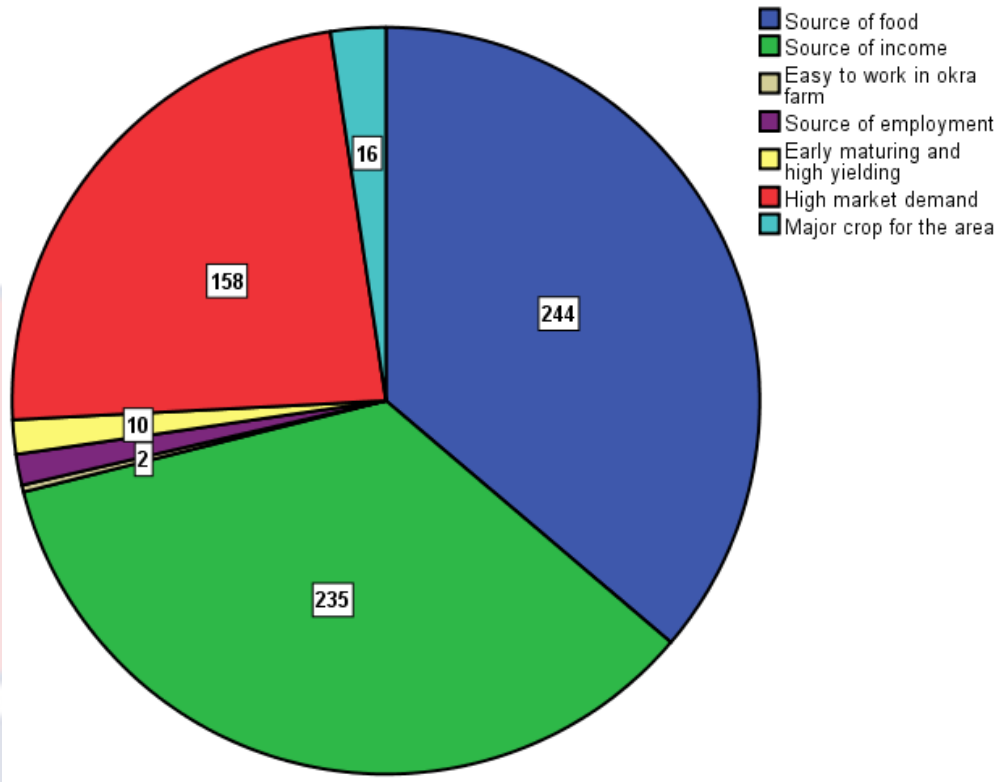


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Figure 10: Reasons for cultivating okra by respondent farmers in the study area

3.4.10 Farm Characteristics

3.4.11 The type of Farming Systems Practiced

The farming strategies employed by okra producers in the research area are depicted in Figure 11. Monocropping was done by 87.24% (335) of the respondents, while mixed farming was practiced by 12.76 % (49) of the farmers.

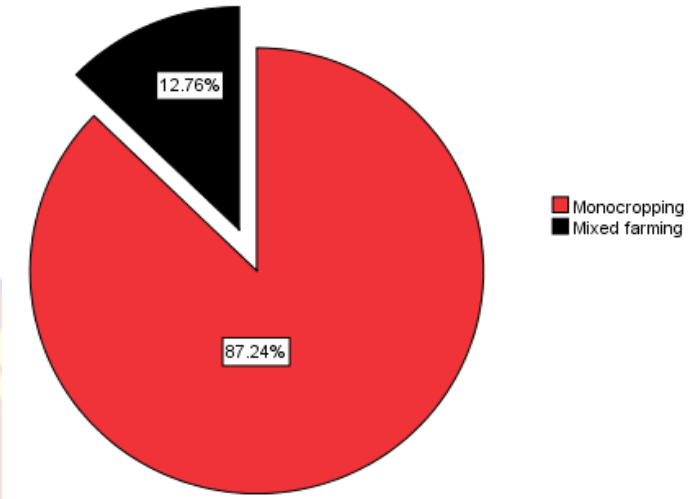

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Figure 11: Farming systems practiced by okra farmers in the study area

3.4.12 Main Source of Water for Okra Plant

Figure 12 shows the various sources of water used by respondents to cultivate okra. Rainfall was cited as the primary source of water for cultivating okra by the majority of farmers (340). Irrigation is used by 187 farmers to water their okra, especially during the dry season. Finally, 183 people used water from dugouts and dams to water their okra.

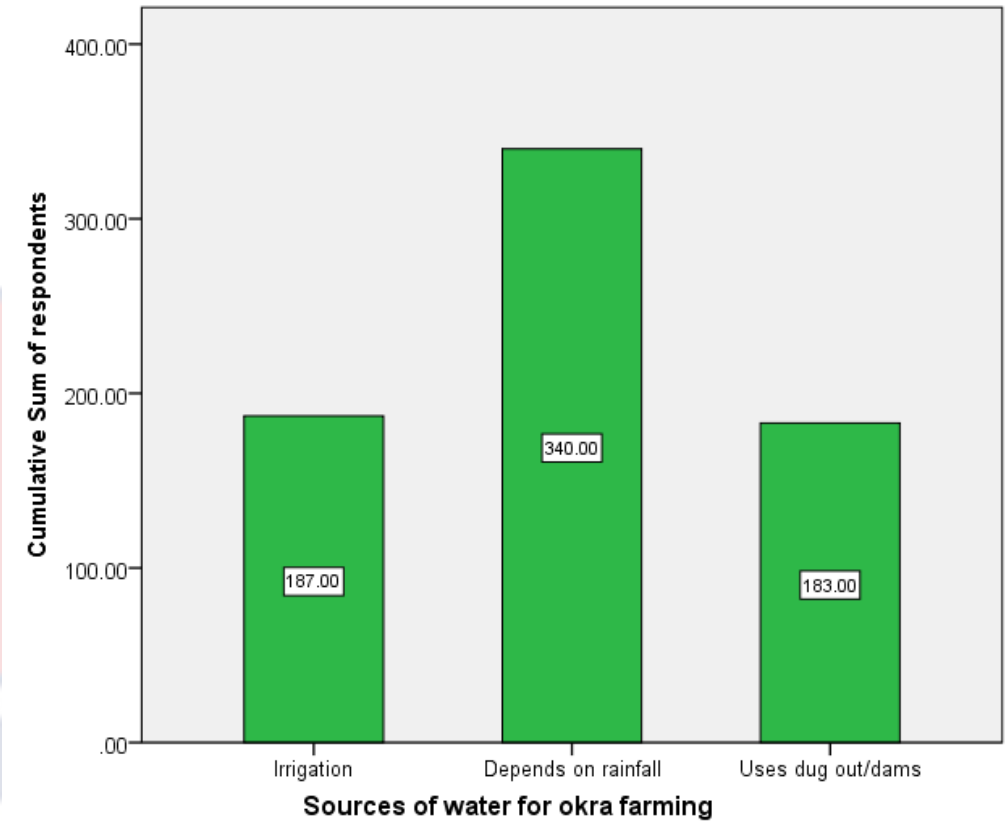

Figure 12: Main source of water for okra farming

Figure 13: A. Farmer using dug out to water B. Irrigation as source of water

3.4.13 Land Preparation

Figure 14 shows the various land preparation techniques employed by okra growers. The majority of the farmers who responded prepared their fields by using pesticides to kill weeds before sowing, followed by slashing or manual weeding and ploughing.

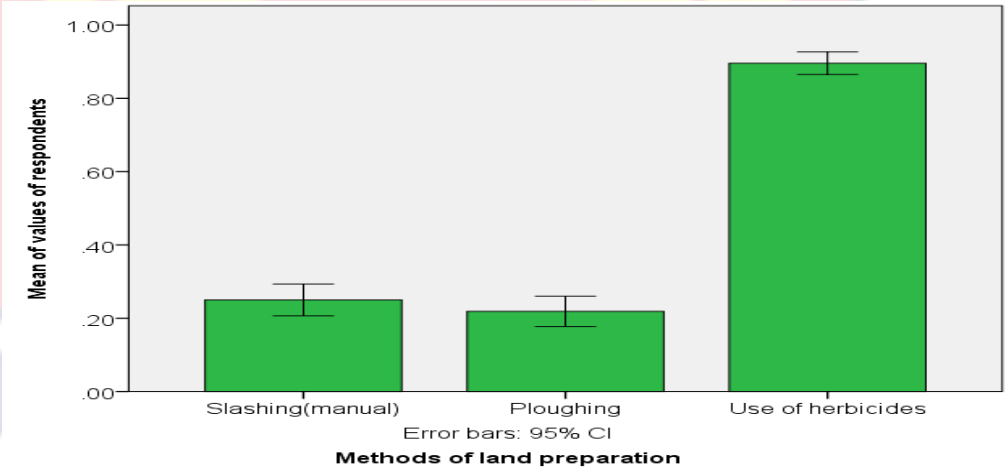

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Figure 14: Means of land preparation methods by respondent farmers in the study area.

3.4.14 Number of times Weeds are Controlled

The majority of farmers (78.65%) weed their farms twice before harvesting, 12.76% weed their farms three times, and 8.59% only weed their farms once before harvesting, as shown in Figure 15.

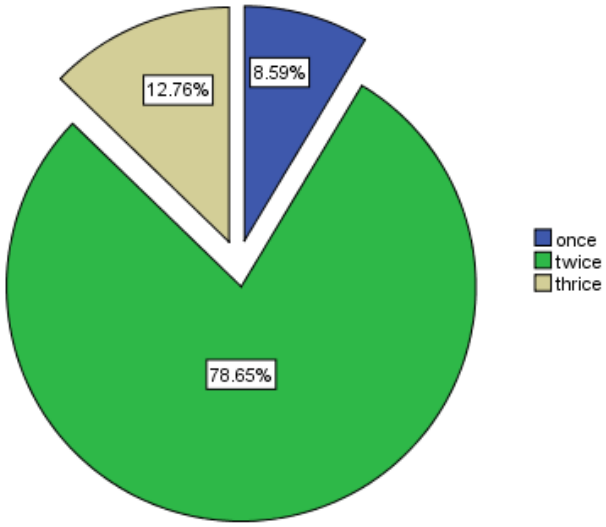

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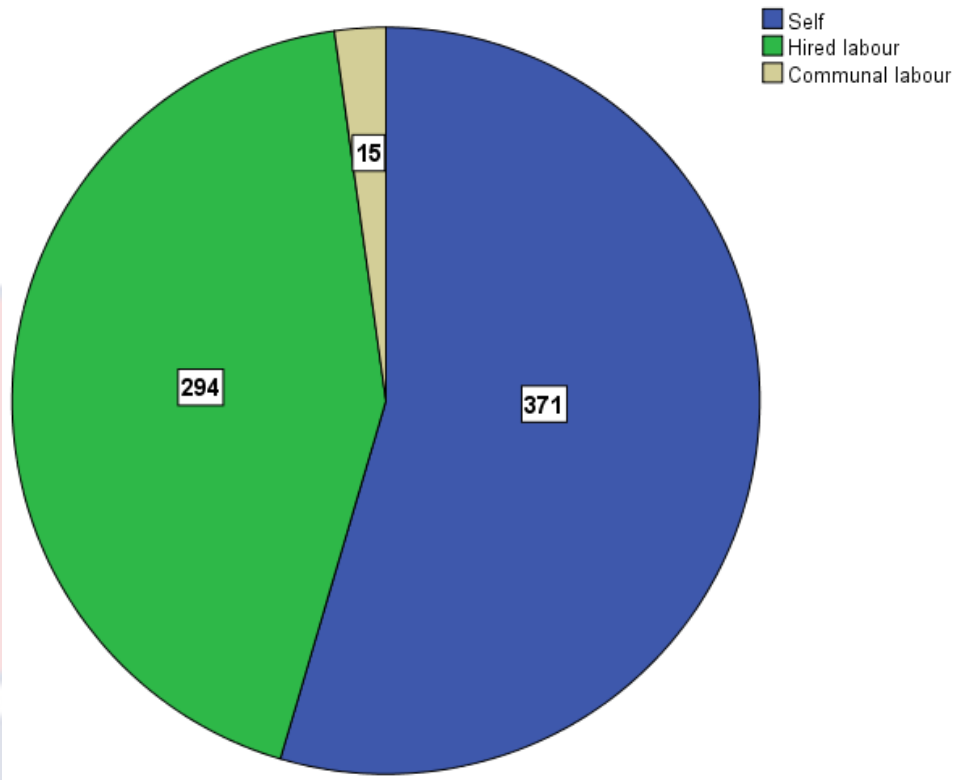
Figure 15: Number of times farmers control weeds in their farms at the study area.

3.4.15 Source of Labour

Figure 16 shows the multiple responses from okra farmers on the sources of labour for okra farming in the research area. The majority of farmers stated that they did all farming activities all by themselves (371). A total of 294 responded that they heavily depended on hired Labour. The communal labour system was the least productive source of labour (15).



N=384

Figure 16: Sources of labour for okra cultivation in the study area.

3.4.16 Sources of Okra Seed

Figure 17 shows where to get okra seeds for planting. The majority of farmers (57%) rely on seeds from previously harvested okra, 22.1% (85) obtained okra seeds from relatives, 15% purchased okra seeds from the market, and 5.2% purchased okra seeds from agro-input/certified seed stores.

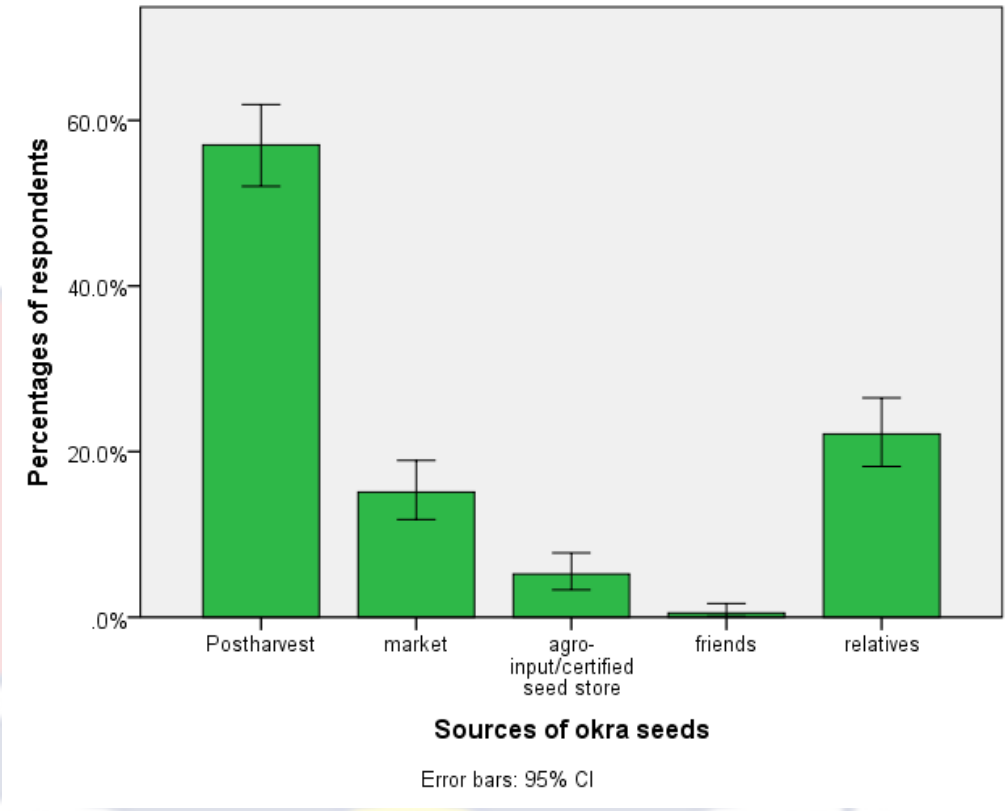


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Figure 17: Sources of okra seeds for planting by farmers in the study area

3.4.17 Variety of Okra Planted

Figure 18 shows information on the types of okra planted by the respondents. Local varieties such as Avalavi (39.8%), Awayibor (15.4%), Fetre (7.8%), Adzrale (3.6), Black (3.4%), and Short (3.4%) are used by the majority of okra farmers (3.4 percent). Clemson spineless (7.6%), Okra Indiana (2.9%), Okra hire (1.0%), and Okra OH102 were among the exotic species grown (1.2%). Farmers only cultivated Asontem (13.8%) as an enhanced okra variety.

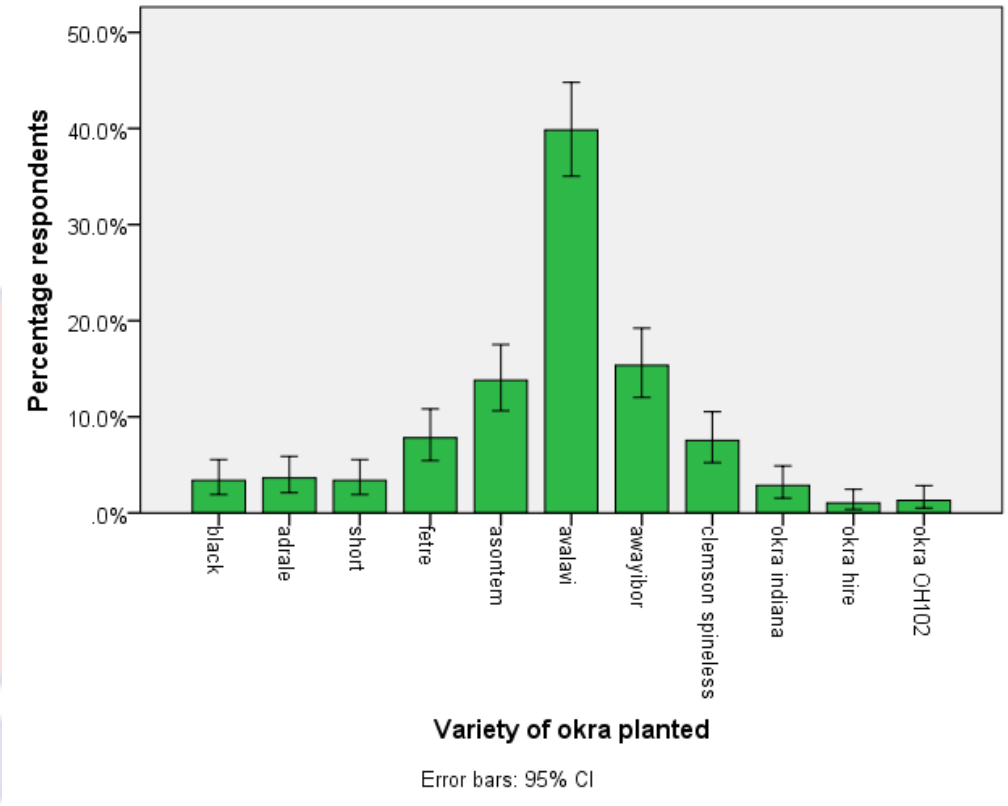

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Figure 18: Okra varieties cultivated by respondent farmers in the study area.

3.4.18 Farmer's Awareness of Okra Leaf Curl Disease

3.4.19 Observation of OLCD on farmer's farm

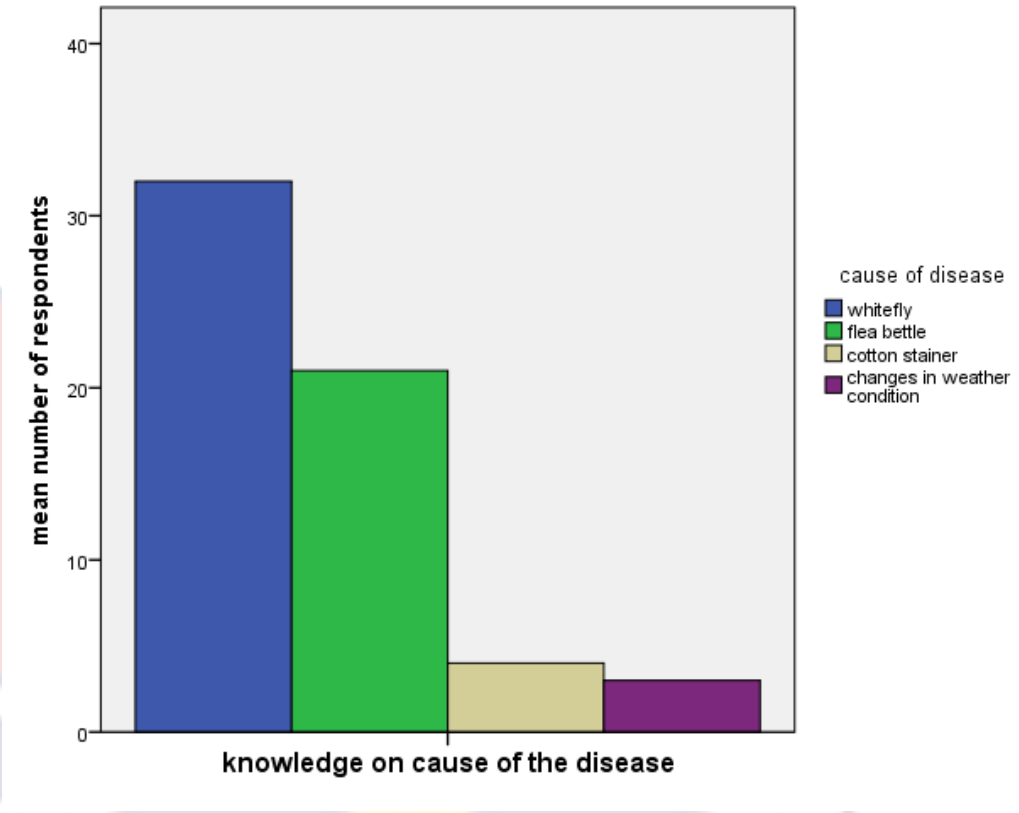
Table 1 shows farmers' reactions to disease observation and seasonal fluctuation in disease occurrence. The majority of the farmers (98.7%) observed the OLCD in their okra fields, whereas only 1.3 % did not. The disease is seen during both rainy(major) and dry(minor) seasons, according to the majority of farmers (95.8%), with the dry season (83.9 %) being the most occurs.

Table 1: Farmers' response on disease incidence occurrence in the study area

Variables	Response	Frequency	Percentage (%)
Observation of OLCD	Yes	379	98.7
	No	5	1.3
Total	**384**	**384**	**100**
Disease occurrence in both season	Yes	368	95.83
	No	16	4.17
Total	**384**	**384**	**100**
Season disease most occurs	Dry season	322	83.85
	Rainy season	62	16.15
Total	**384**	**100**	**100**

3.4.20 Knowledge of the Causes of OLCD

Figure 19 shows respondent farmers' awareness of the causes of OLCD in the research area. 64 (16.67%) of the 384 respondents were aware of the cause of OLCD, while 320 (83.33%) were not. The majority of the farmers who said they knew what caused the disease (36) mentioned whitefly, while others said flea beetle (21), cotton Stainer (4), and changes in weather conditions (3).



N=64

Figure 19: Farmer's perception of causes of okra leaf curl disease (OLCD) in the study area

3.4.21 Symptoms of Okra Leaf Curl Disease (OLCD) in Respondent Farms

Figure 20 shows the signs of okra leaf curl disease that different okra farmers have noticed in their okra farms. Most farmers (179) said most of the okra showed symptoms of top leaves curling and slightly stunting. A total of 130 farmers observed curling of a few top leaves, 97 observed that all leaves curled and slightly stunting of plants, and 30 observed severe curling of leaves, stunting of plants and proliferation of auxiliary branches. Farmers who did not see any symptoms were six.

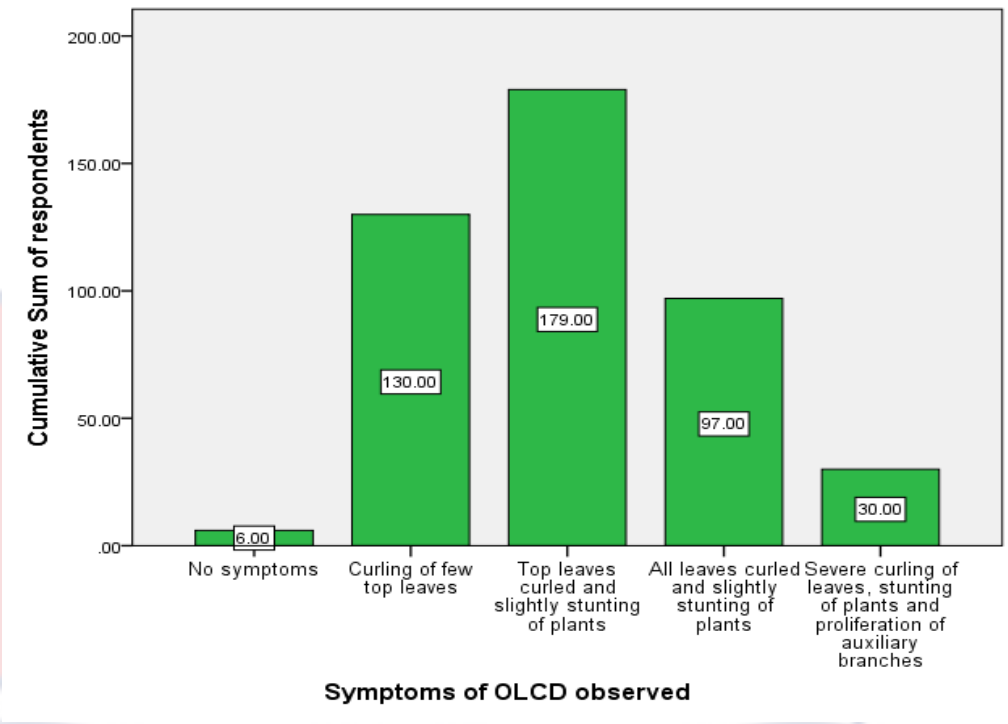


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Figure 20: Symptom types observed in okra fields by farmers in the study area

3.4.22 Proportion of Farm Infected by OLCD

Figure 21 shows the proportion of okra farms infected with OLCD in the research area as observed by respondent farmers. The majority of the farmers (194) said that 51-70% shows of their farms were infected with OLCD, while 155 indicated the disease was present in more than 71% of their farms. In addition, 26 of the farmers said that the disease incidence ranged from 31 to 50%, with the lowest incidence being 11 to 30%.

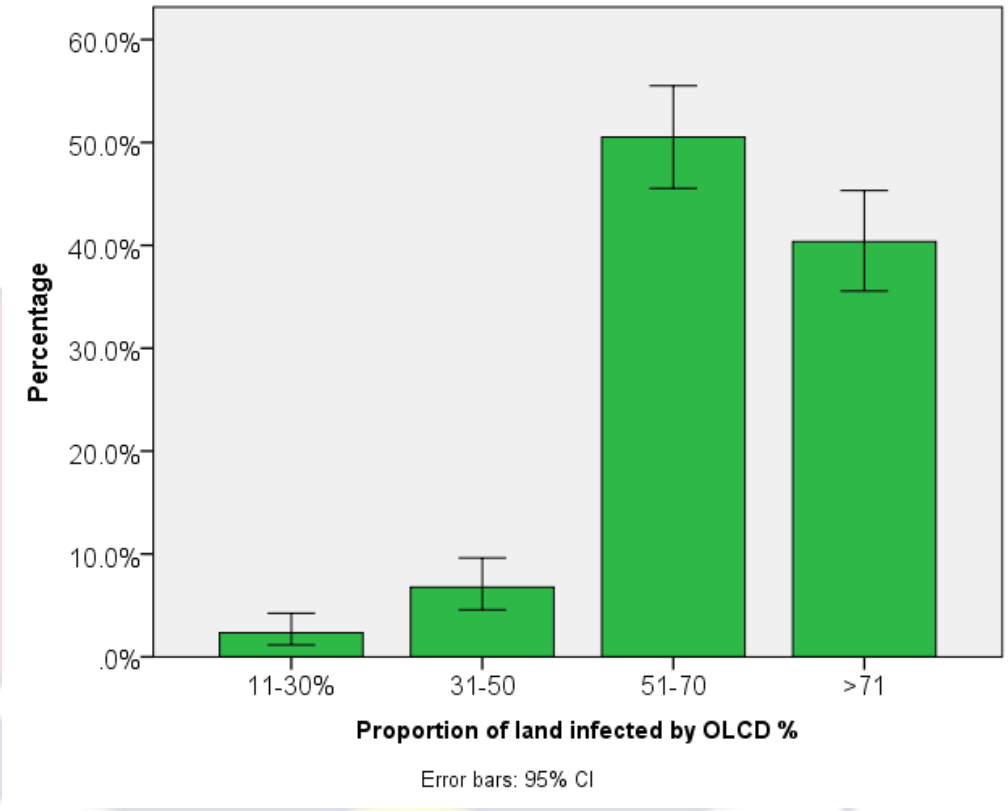

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Figure 21: Proportion of okra farm infected by OLCD in farmers study area

3.4.23 Other diseases/ Symptoms observed in Okra Farms

Farmers' responses to additional diseases or symptoms seen in their farms in the research area are depicted in Figure 22. Apart from OLCD, 63% of okra farmers reported Okra mosaic disease as a serious problem on their crops, while 31.5% reported root swollen caused by nematode. In their crops, fruit rot (5.5 %) was also discovered.

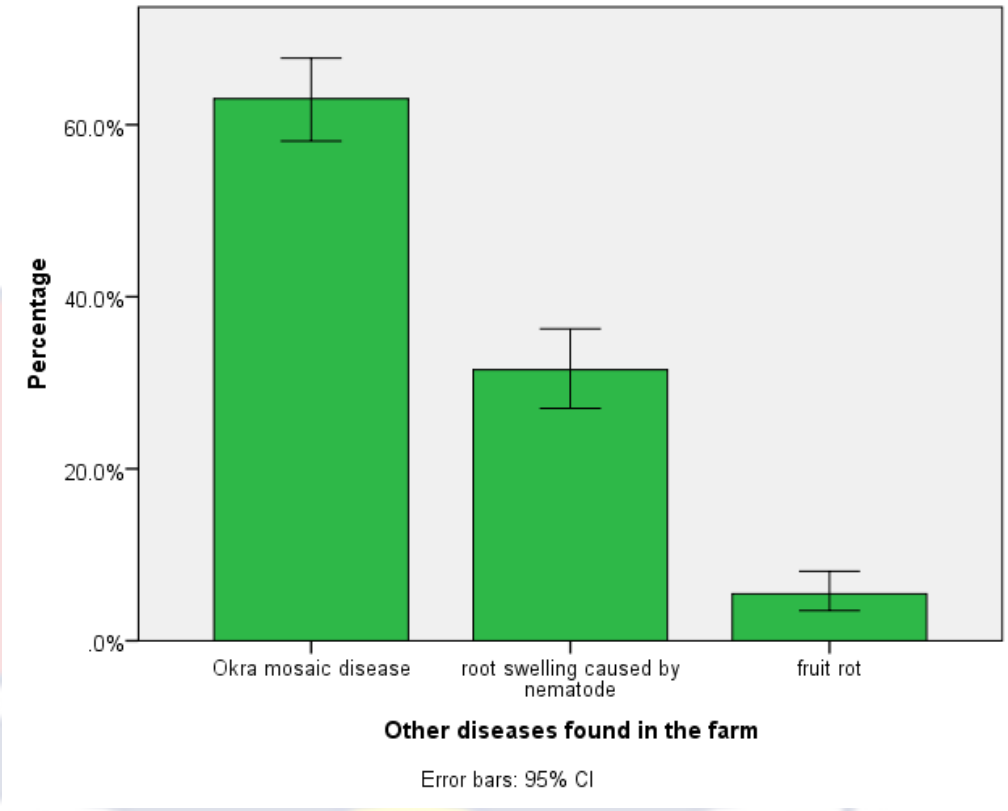

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Figure 22: Incidence of other diseases in farmers' fields in the study area

3.4.24 Management of OLCD

Figure 23 shows the cumulative multiple response from okra farmers on various management practices in the control OLCD. The findings showed that the majority of farmers rely extensively on the use of pesticide in vector management (376), 22 treated their seed with insecticide before sowing and 15 adopted change of planting date to prevent whitefly from transmitting the disease. Two of the farmers said they applied fertilizer for the plant to grow fast. Seven never adopted any management practices in managing the disease.

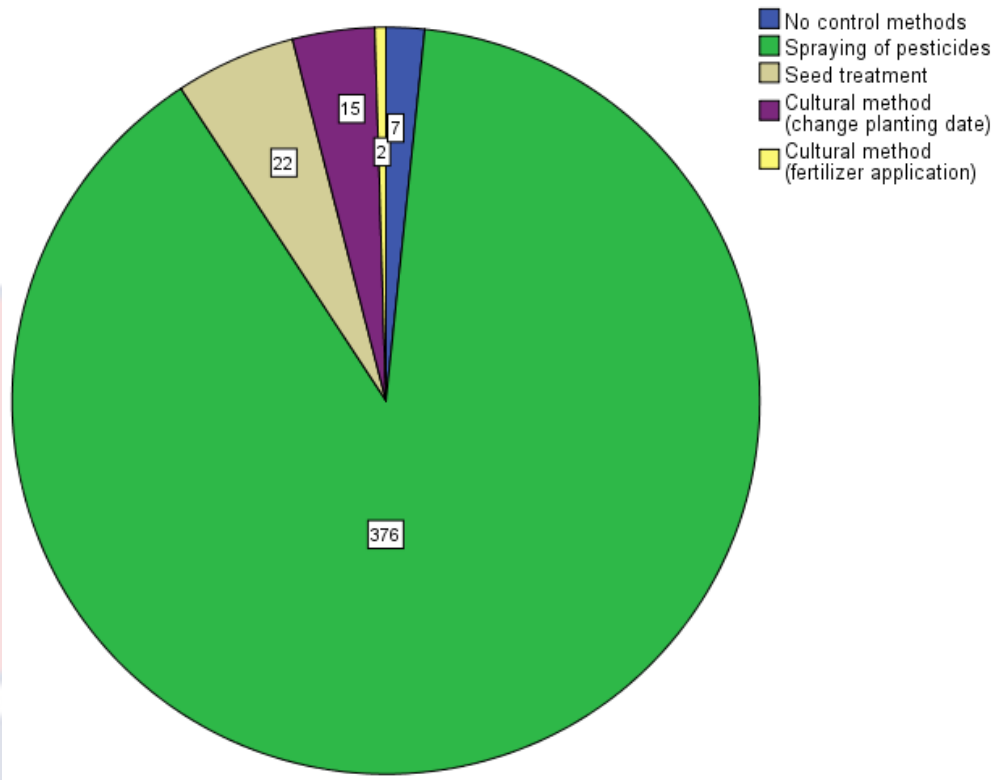

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Figure 23: Current practices used by farmers in the management of OLCD in the study area

3.4.25 Farmers' Knowledge of Whitefly Infestation on Okra

Whitefly was regarded by the majority of farmers (98.96%) as a major pest infesting okra in their fields. 1.04% indicated they have never seen it before (Figure. 24).

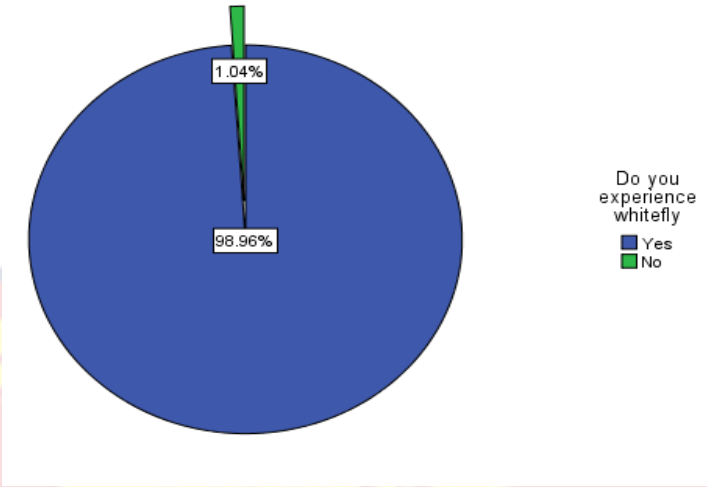

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Figure 24: Presence of whitefly in okra fields in the study area

3.4.26 Seasonal Variation in Whitefly Infestation

Figure 25 shows farmers' reactions during the peak of the whitefly season on their crops. Majority of farmers (95.05%) said the population is higher during the dry season, while 4.95% farmers said it was also seen during the wet season.

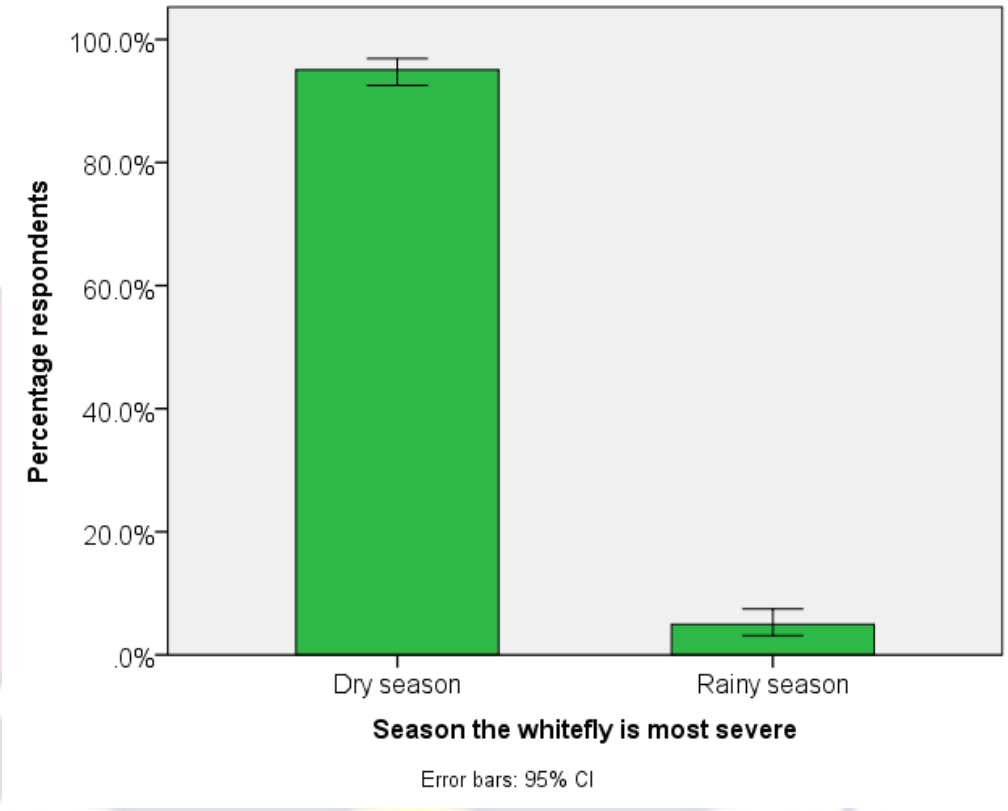

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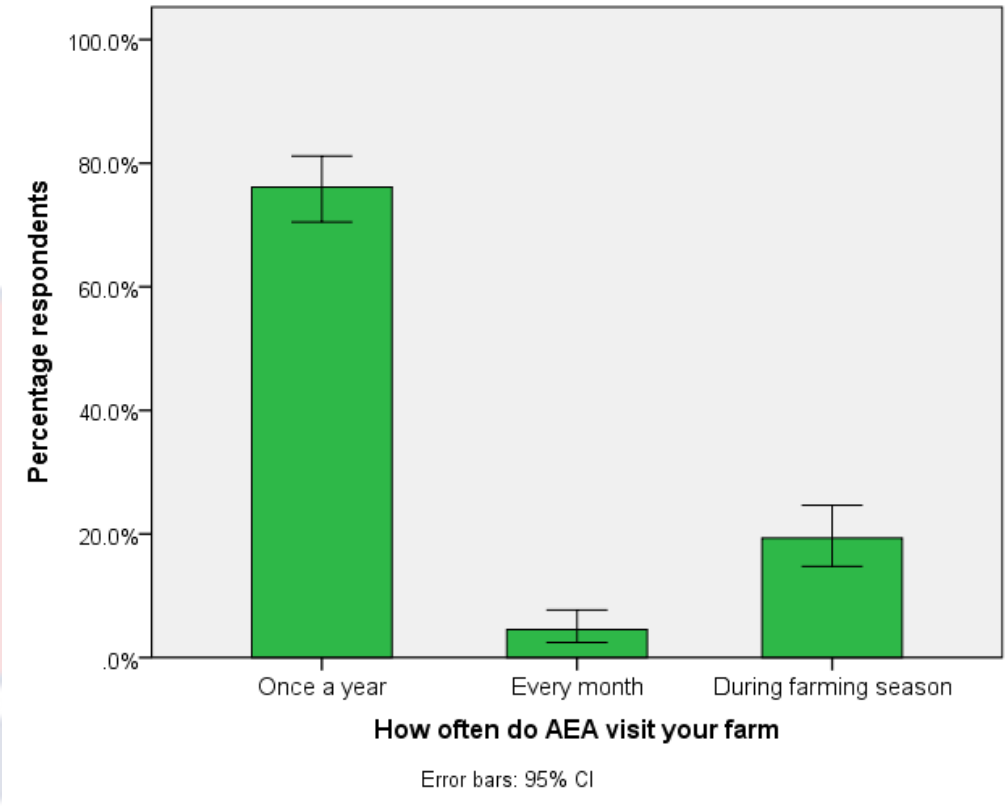
Figure 25: Okra farmers perception of whitefly population abundance during in dry and wet seasons in the study area

3.4.27 Number of times Agricultural Extension Agency (AEA) visited okra farmers

The Figure 26 shows the number of times AEA visited okra farmers to advise them on insect pest and disease management and general agronomic practices. The majority of the farmers (76%) were visited by the AEA once a year. Some further claimed that the AEA only visited during the farming season (19.3%), whereas 4.5% farmers were visited every month.



N=243

Figure 26: Visit to okra farms by AEA in the study area

3.4.28 Other Insect Pests of Okra

Other insects, according to the respondents, were also sighted on their crops. Other okra insect pests seen on okra crops in the study area are depicted in Figure 27. Apart from the whitefly, the flea beetle was regarded as a major pest of okra by majority of respondents (79.2%). The cotton Stainer (14.6%) and the green stink bug (6.3%) were discovered on their crops.

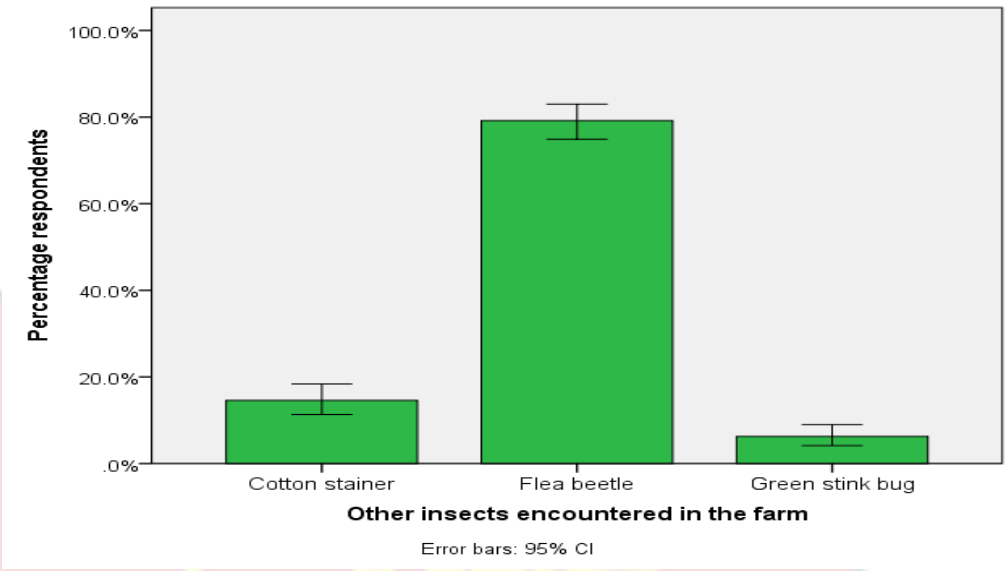


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Figure 27: Other insect pest identified in farmers' okra fields in the study area.

Figure 28: Some insect pests found on okra plants in the study area; A – Cotton stainer, B – Flea beetle and C – Whitefly.

3.4.29 Whitefly Management Methods

Figure 29 shows the various management measures employed by okra farmers to control whiteflies. The majority of farmers rely extensively on synthetic insecticides, which have a mean reaction of 0.979, while a minority utilize bio-pesticides, which have a mean response of 0.198. Okra farmers discovered the utility of water in managing whitefly throughout the research.

The employment of a yellow sticky trap was the least effective control strategy, followed by no control.

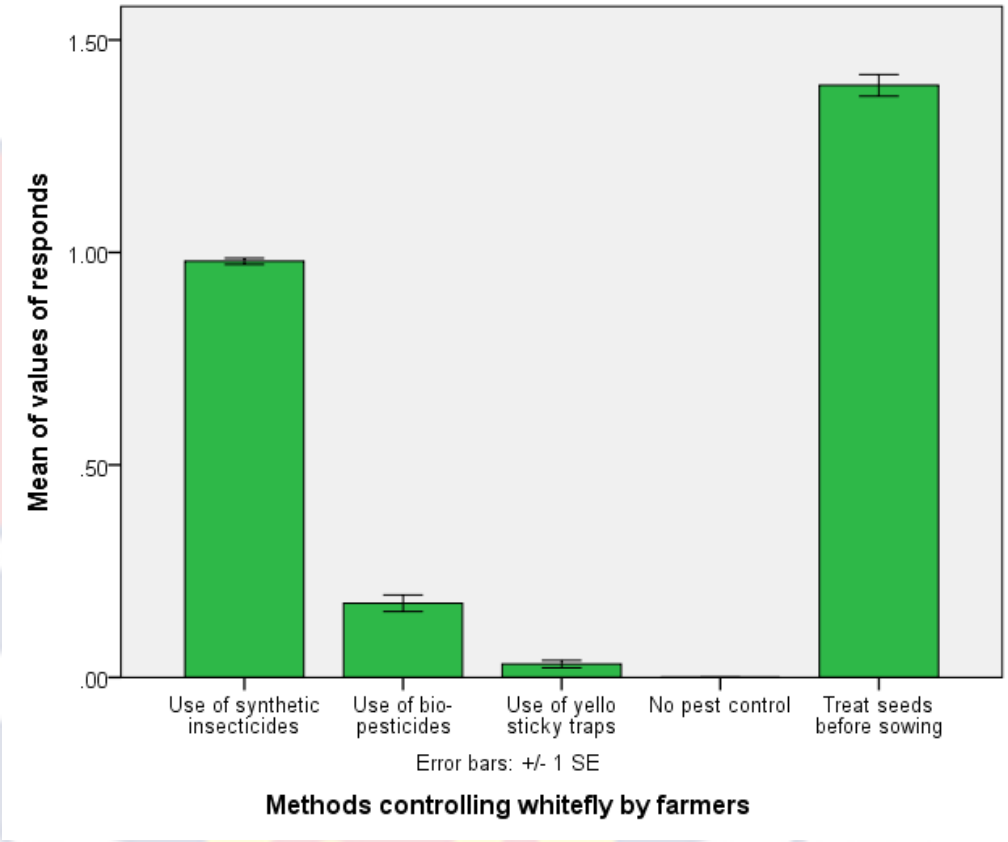


Figure 29: Different methods of controlling whitefly by respondent farmers in the study area.

3.4.30 Types of Pesticide Used in Controlling Whitefly

The type of agrochemical employed by respondent farmers to control whiteflies is represented by multiple response frequency on Table 2. The following respondent farmers accepted using the following pesticides in controlling whitefly in their farms; Sulphur 80W (25.8%), Sunpyrifos (22.1%), Confidor200SL (14.9%), Lambda (12.8%), Attack (6.8%), Durban 4E (3.9%), Champion (3.4%), Acetellic (3.4%), Karate 5EC (2.1%), Kocide (1.9%), Fokosuper 80WC (1.8%), and Golan (1.2%).

Table 2: Types of pesticides used by farmers in controlling whitefly

TYPES OF PESTICIDES[a]	Responses		Percentage of Cases (%)
	N	Percentage (%)	
Sulphur 80W	260	25.8%	67.7%
Dursban 4E	39	3.9%	10.2%
Golan 20%	12	1.2%	3.1%
Karate 5EC	21	2.1%	5.5%
Lambda	129	12.8%	33.6%
Attack	68	6.8%	17.7%
Champion	34	3.4%	8.9%
Kocide	19	1.9%	4.9%
Sunpyrifos	222	22.1%	57.8%
Acetellic	34	3.4%	8.9%
Fokosuper 80 WC	18	1.8%	4.7%
Confidor 200 SL	150	14.9%	39.1%
Total	1006	100.0%	262.0%

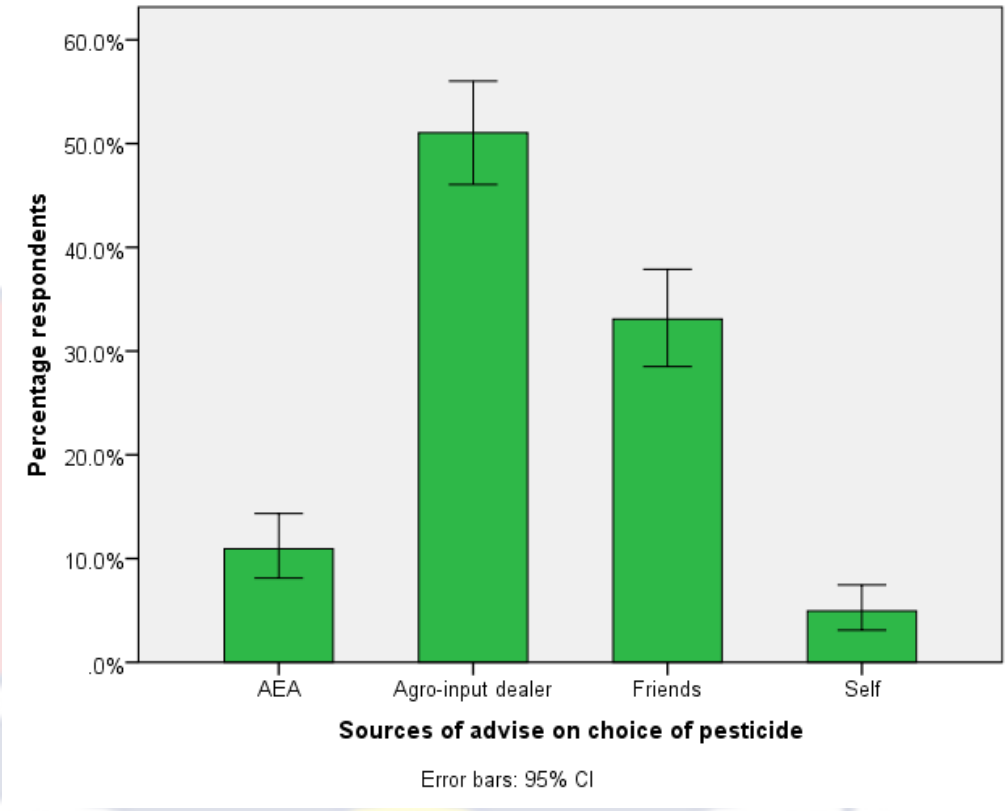
a. Dichotomy group tabulated at value 1.

Figure 30: Different types of pesticide used by respondent farmers in the the management of whitefly A-Confidor, B- C-Lambda, D Sicorin, E-Agoo(bypel), F-Bypel

3.4.31 Pesticide use and Management by Okra Farmers

3.4.32 Advice on Choice of Pesticides

Figure 31 shows information on the pesticides used by okra producers for insect management. The majority of farmers (51%) seek help from local agro-input dealers when it comes to pesticide selection. Friends in the community (33.1%) were the second source of knowledge on pesticide selection, while agricultural extension agents (10.9%) in the catchment were the third. The farmers' assessment (4.95%) provides the least amount of pesticide guidance.

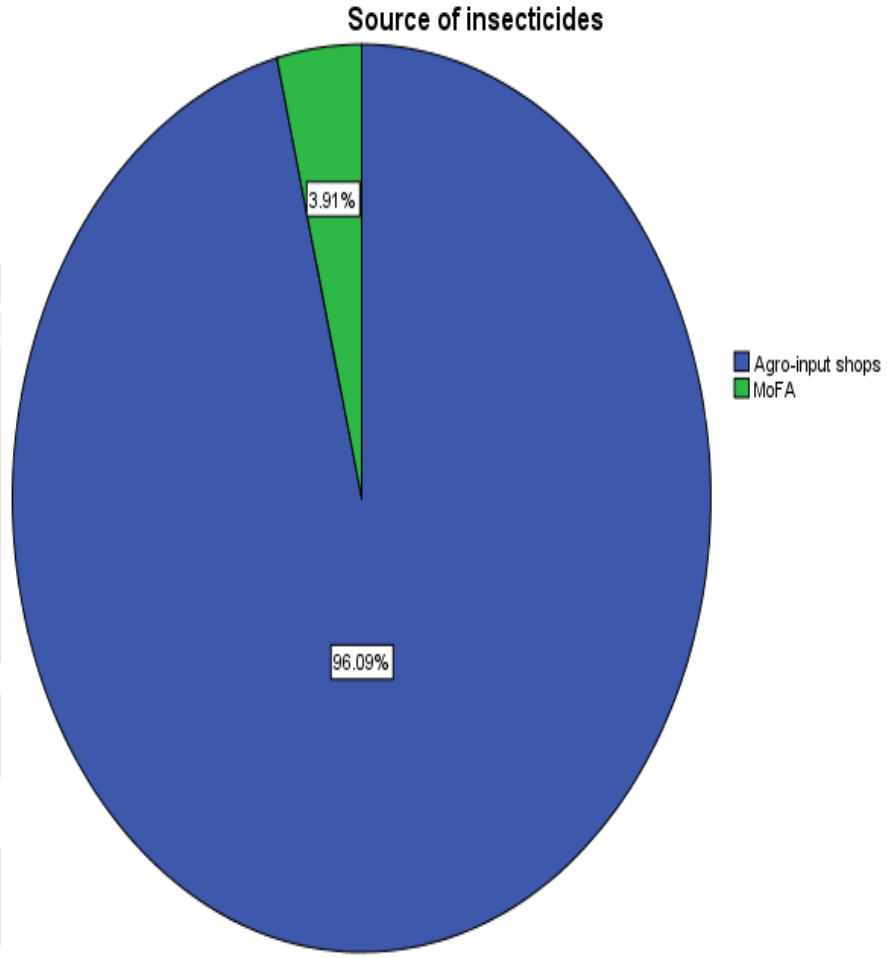


N=384

Figure 31: Respondents' sources of information on pesticide use

3.4.33 Source of Pesticides

Figure 32 shows the sources of pesticides used by farmers in the research area. The majority of respondents (96.09%) named agro-input dealers as their primary supplier of pesticides, while the Ministry of Agriculture (MoFA) provided some agro-chemicals (3.91%).

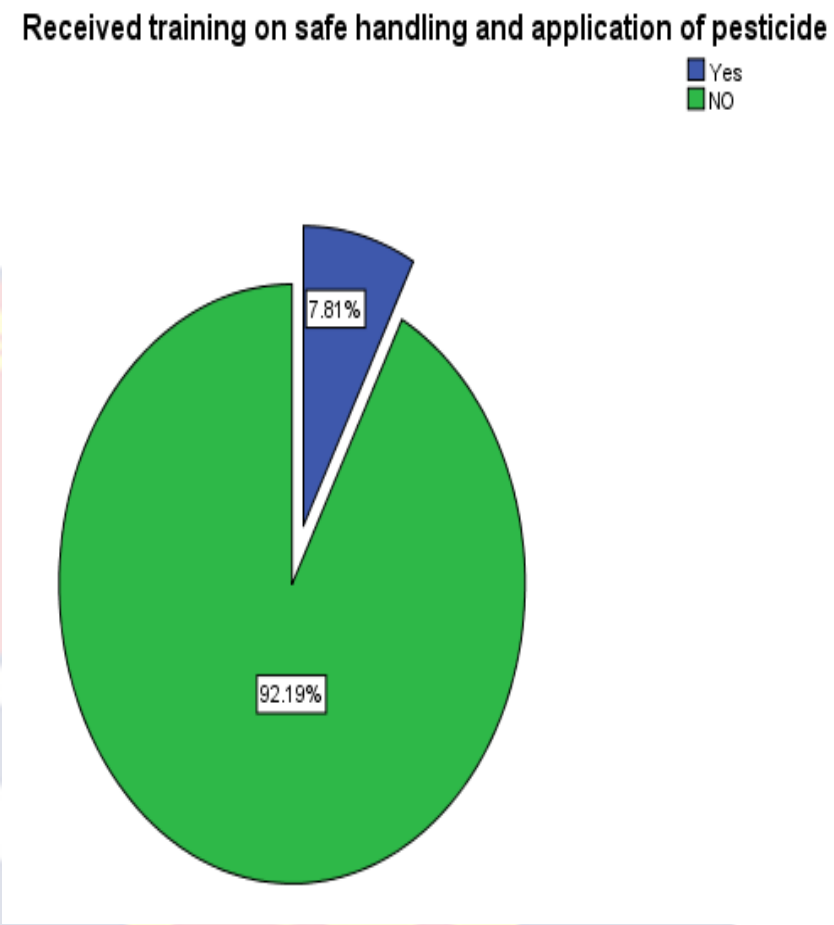


N=384

Figure 32: Sources of pesticides for okra farmers in the study area

3.4.35 Training on Safe Handling and Application of Pesticide

Figure 33 shows the farmers' responses to the question of whether or not they have received any pesticide safety instruction. According to the findings, the bulk of the okra farmers in the survey (92.19 %) had never received any pesticide safety instruction, while only a handful (7.81%) have received training from the AEA.



N=384

Figure 33: Farmer training in safe handling and application of pesticide in the study area.

3.4.36 When to Apply Pesticide

Figure 34 shows farmers' opinions on when to use pesticides in the management of OLCD and insect pests. Pesticides are used by the majority of farmers (47.7%) when diseases or insects on the farm are severe. Other farmers (40.1%) said they used pesticides when they noticed the first signs of disease or the presence of insects on the plants. 28 (7.3%) of responding farmers used pesticides on their farm crops based on expert judgment, while

others used pesticides based on AEA recommendations when they were called upon.

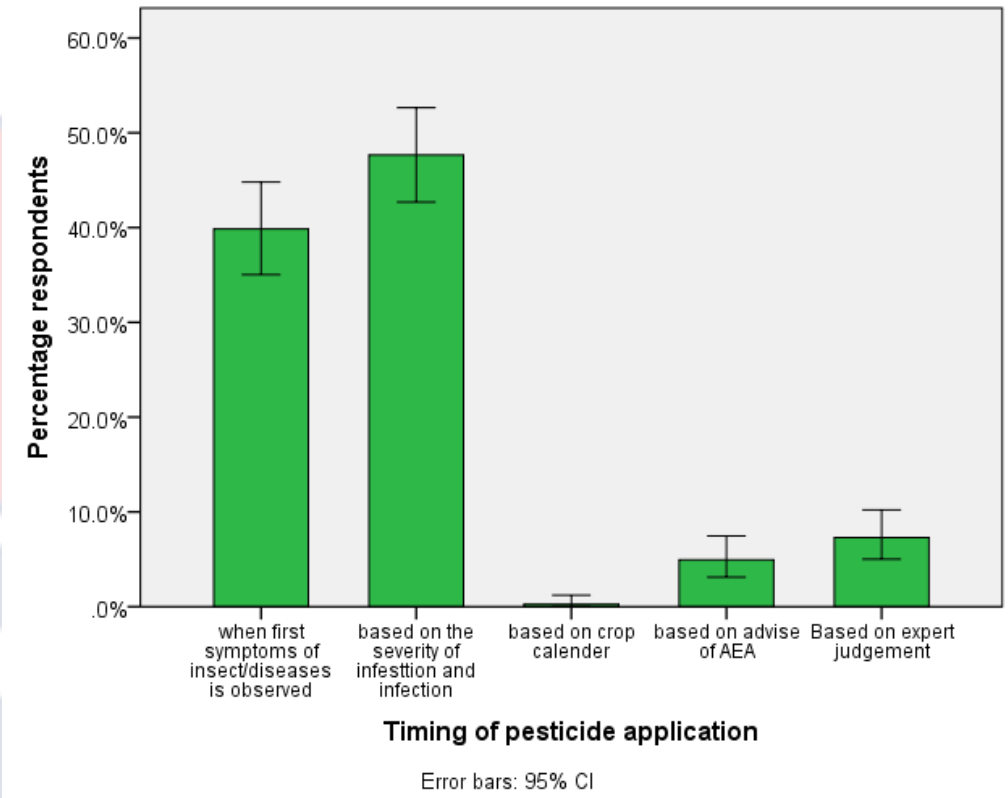


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Figure 34: Time of pesticide application by respondent farmers in the study area

3.4.37 Mixing of Pesticides

Figure 35 shows how respondents combine pesticides before applying them to their farms. After placing insecticide and water into the knapsack sprayer, the majority of farmers (82.6 %) shake it. Some of the farmers who responded (15.4%) also use a stick to swirl the chemical in the backpack sprayer. A few farmers stir using hand gloves and safety goggles (6), while two others indicated they stir with their bare hands-on occasion.

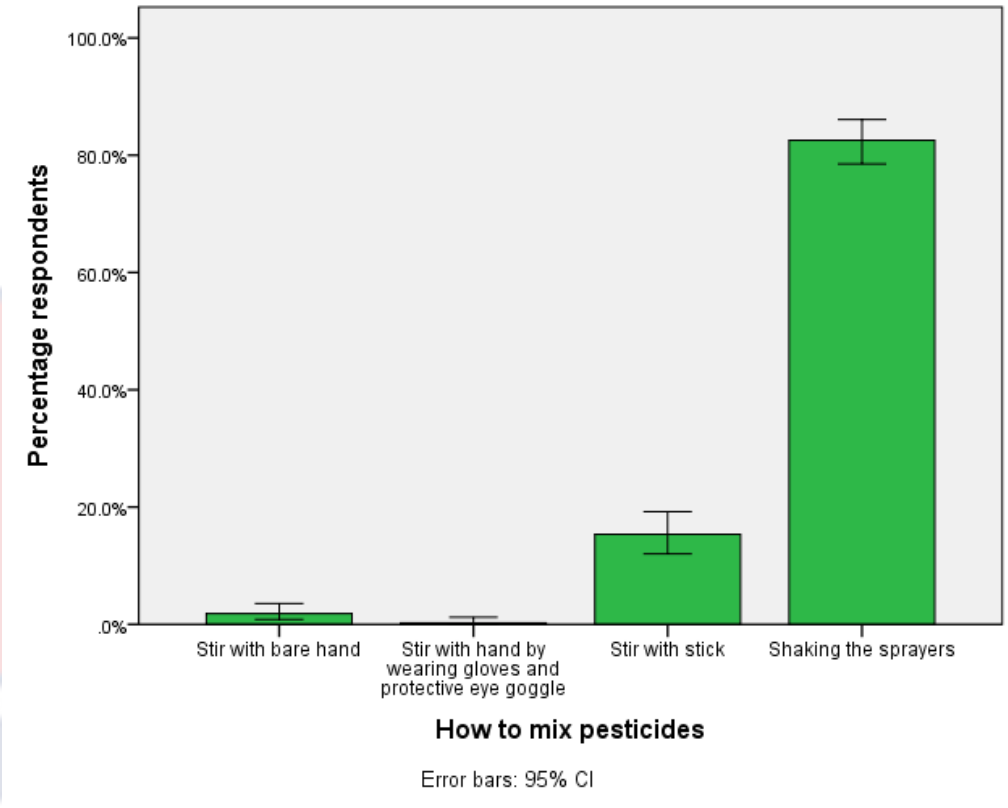

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Figure 35: How farmers mix pesticides before applying.

3.4.38 Direction of Pesticide Spray

In respondent farms, 70.31% sprayed pesticides with the wind, 16.67% did not consider the direction of the wind, 12.5% sprayed against the wind, and 0.52% sprayed perpendicular to the wind (Figure 36).

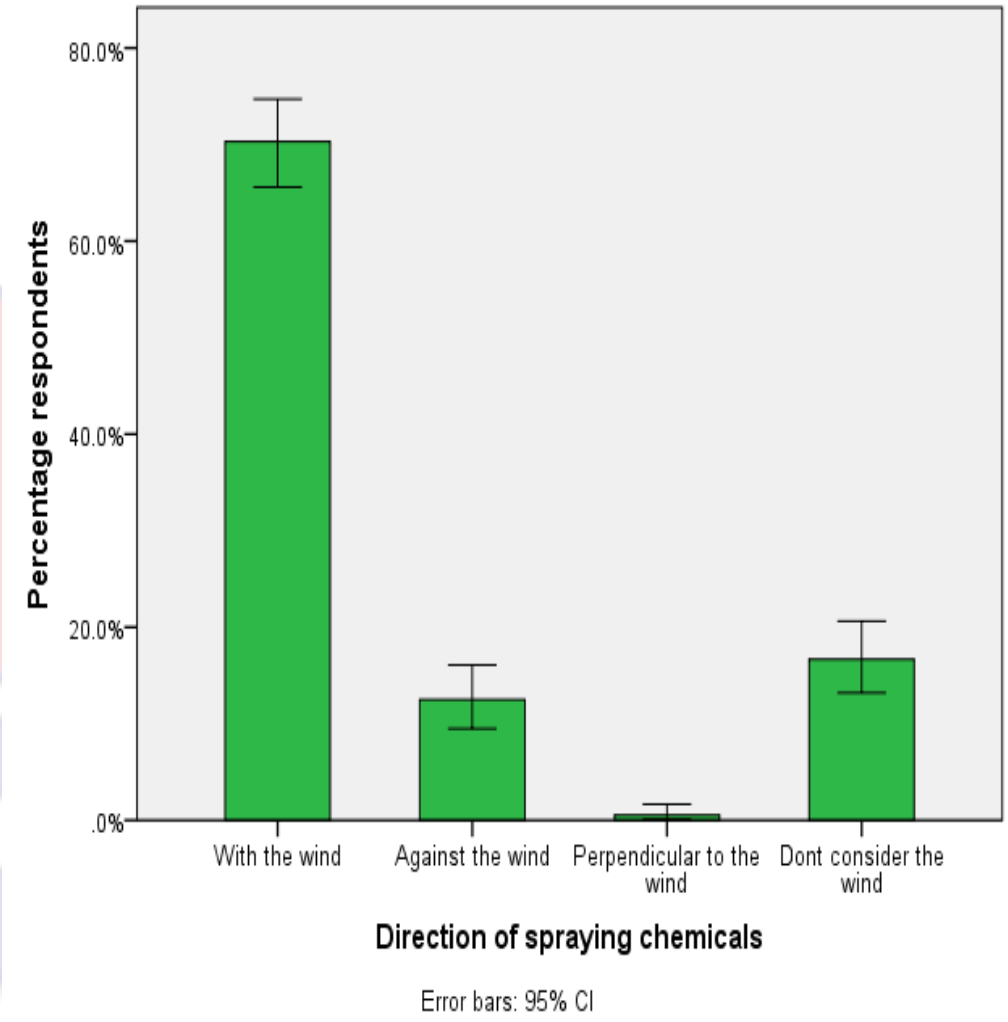

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Figure 36: Direction of pesticide spray by respondent farmers.

3.4.39 Period of time within the day to spray pesticide

The time of day when farmers apply pesticides to their fields is depicted in Figure 37. Pesticides are sprayed by the majority of farmers (92.97%) early in the morning, 3.65 % in the afternoon, and 3.39% late in the evening.

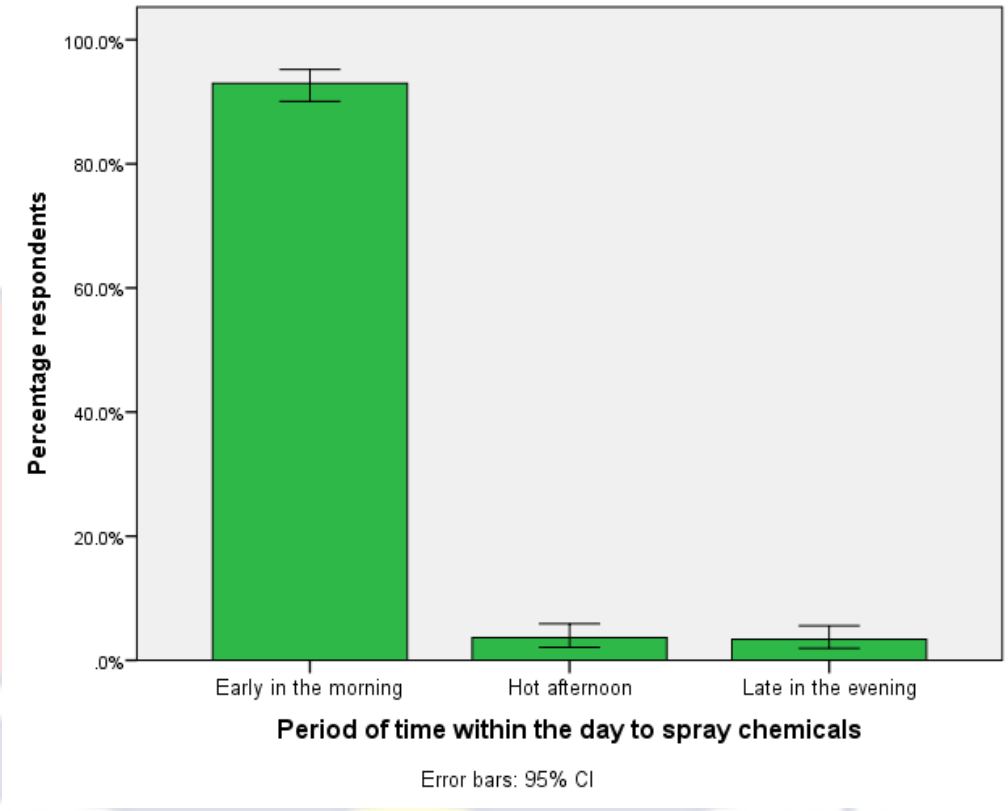

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Figure 37: Farmers response on time of day for spraying of pesticides.

3.4.40 Evidence of pesticide Application

Figure 38 shows evidence of pesticides application of respondent farmers. The vast majority of responders (92.2%) never gave any evidence that pesticides had been used on their farms, while 4.2 percent hanged empty pesticide bottles as evidence on their farms. Only a few farmers (2.1%) and (1.6%) employed sign boards and red flags as indicators of pesticide application, respectively.

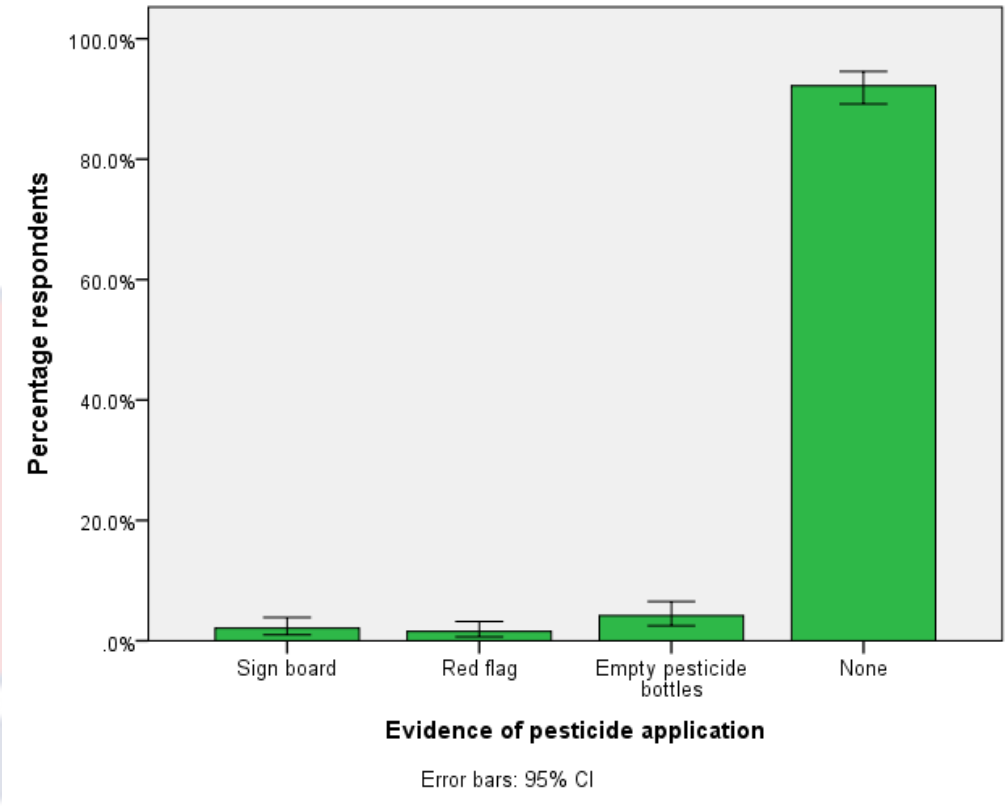

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Figure 38: Evidence of Signs to indicate pesticide application in farmers' okra fields.

3.4.41 Re-entry Period After Pesticide Application

Farmers' reactions to their re-entry time after pesticide spraying on their farms are seen in Figure 39. The majority of farmers (51.6%) re-enter their farms the same day after spraying pesticides, 45.8% enter between 1 and 3 days following chemical application, and 2.6% enter between 4 and 7 days after chemical treatment.

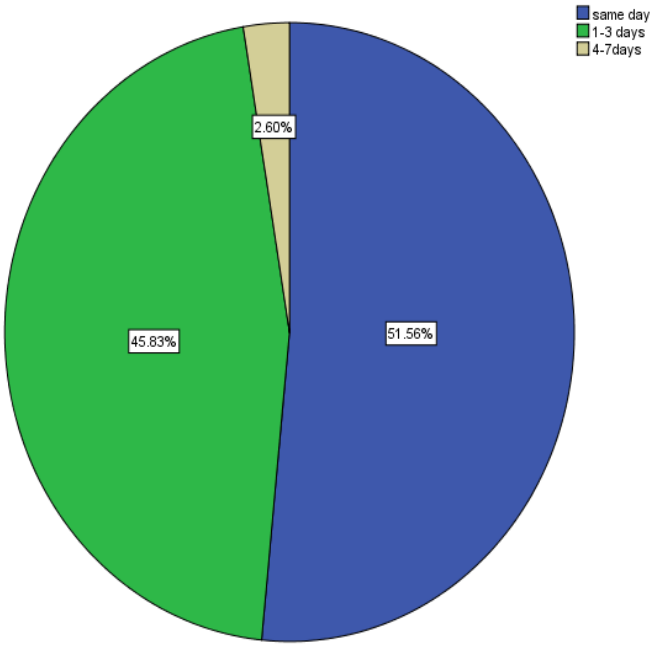

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Figure 39: Respondent farmers' re-entry period after application of pesticides in their farms.

3.4.42 Pre-harvest Interval in Days

Figure 40 below represents the farmers' response on their pre-harvest interval of okra after the last harvest. Majority of the of the farmers (94.8%) used a pre-harvest period of 1 – 3 days whilst the rest (5.2%) used a period of 3 – 7 days

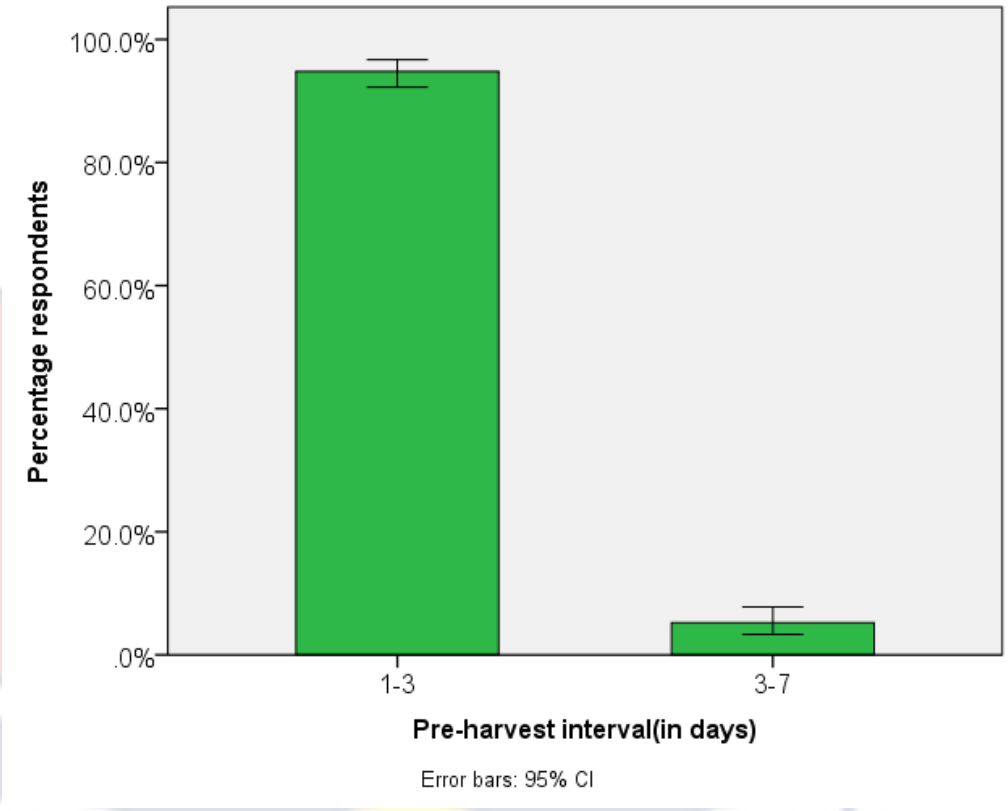

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Figure 40: Pre-harvest interval used by okra farmers in the study area.

3.4.43 Disposal of Empty Pesticide Containers

Farmers employed a variety of methods to dispose of empty pesticide cans after usage (Figure 41). The majority of people (55.2%) dump their used empty containers on the farm, 22.5% throw them away in the hamlet, 13.5% bury them, and 8.9% incinerate them.

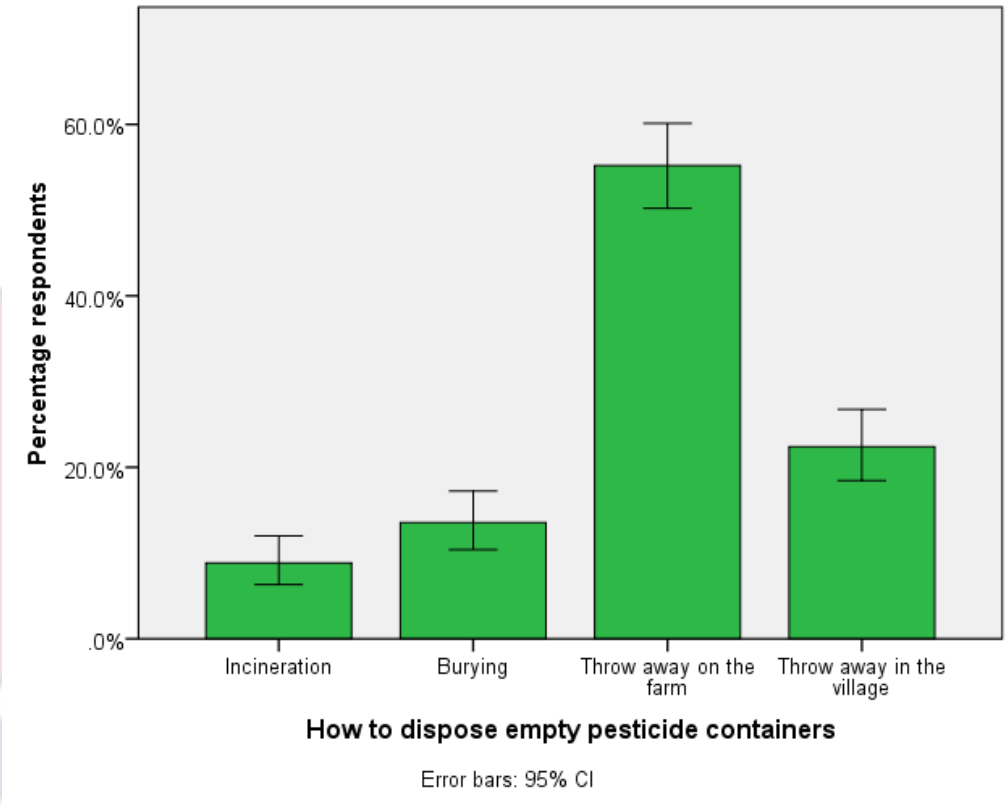

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Figure 41: Farmers' ways of disposal of empty pesticide containers in the study area.

3.4.44 Ownership of spraying equipment used

Figure 42 shows whether or not respondent farmers own pesticide sprayers. The majority of responders (93.49%) own sprayers, while only 6.51% do not.

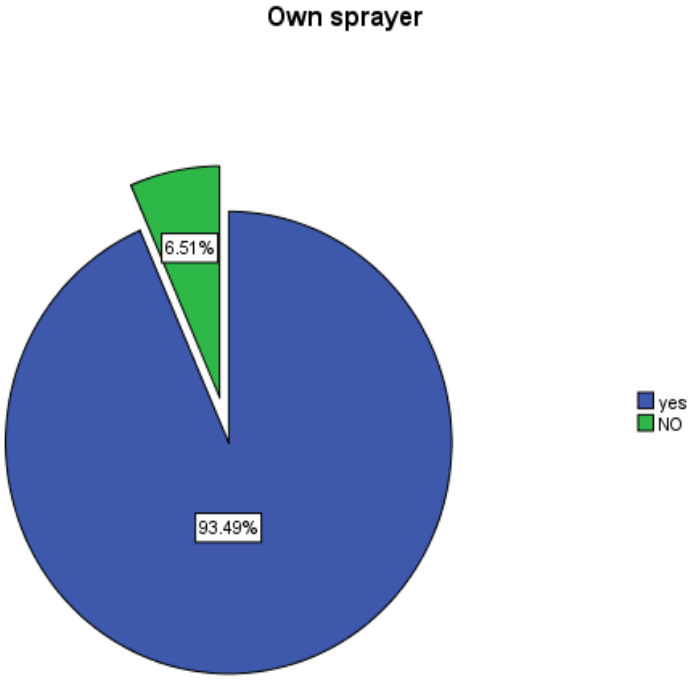

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Figure 42: Percentage of respondents who own sprayers in the study area.

3.4.45 Type of Sprayers

Motorized mist blowers were owned by (1.3%) of the 384 respondents, while knapsack sprayers were owned by (98.7%). (Figure 43).

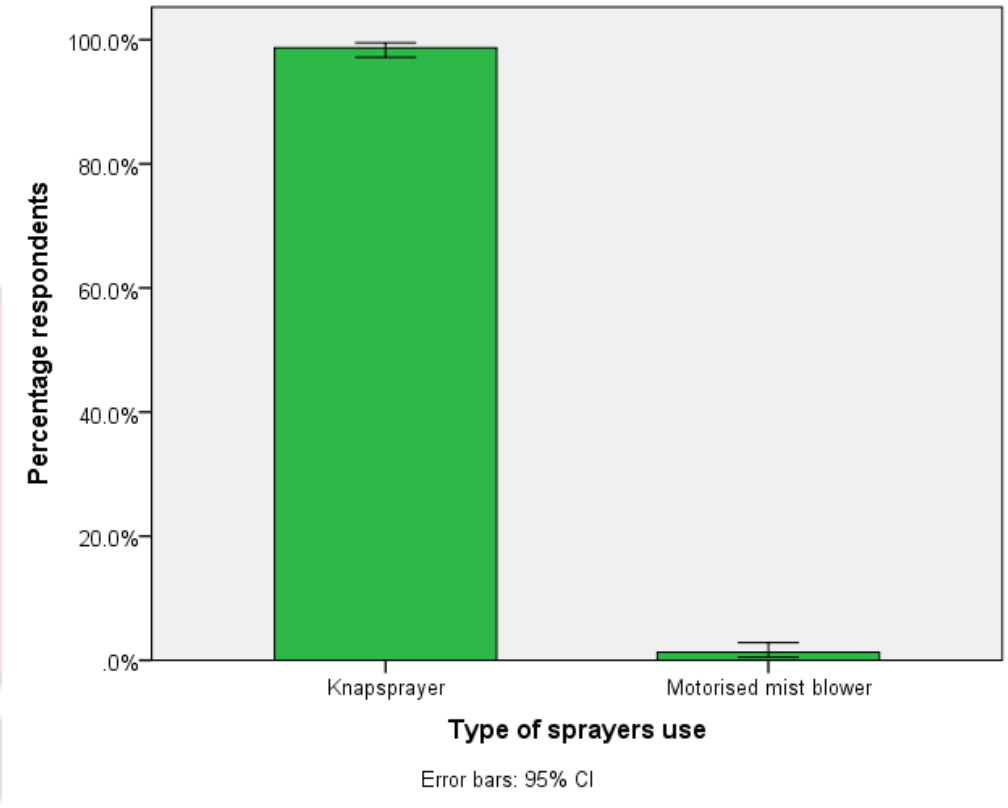

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Figure 43: Types of sprayers used by farmers for pesticide application.

3.4.46 Types of Personal Protective Equipment (PPE) used by farmers

The sort of personal protection equipment (PPE) used by okra farmers in the study area is depicted in Figure 44. A total of 339 responders (88.3%) do not have personal protective equipment (PPE) for pesticide application. Only a few farmers (1.8%) have full protective cover, while 9.9% (38) have only partial protection.

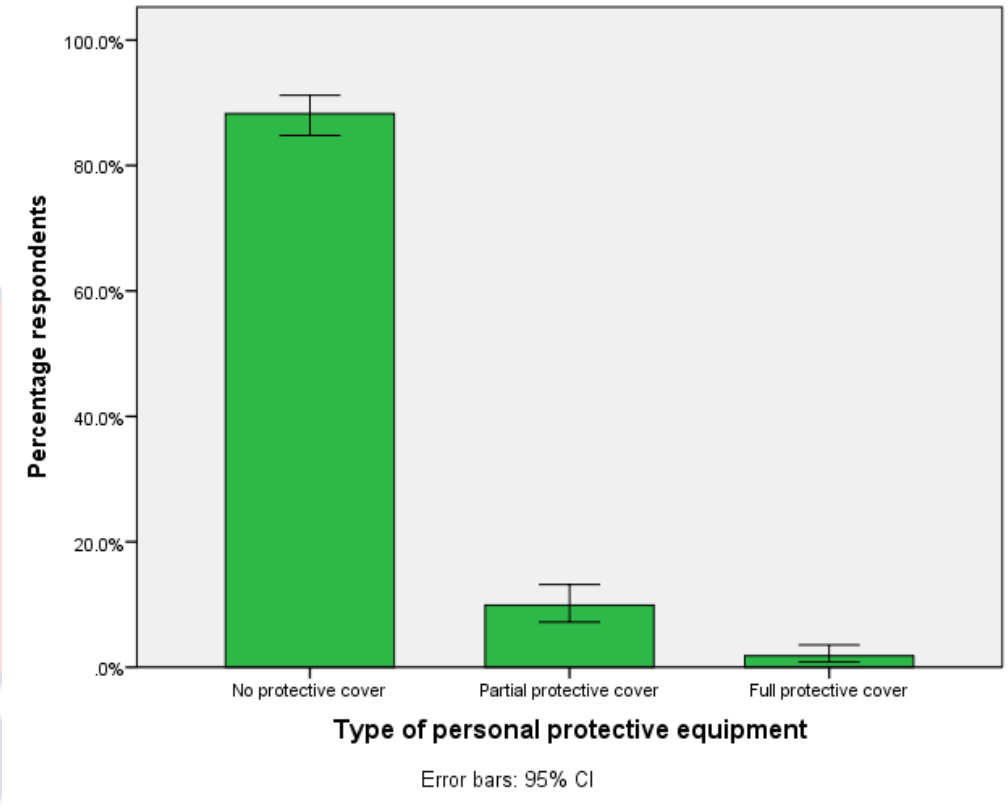

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Figure 44: Personal protective equipment used by okra farmers during pesticide application.

3.4.47 Educational Level and years of Cultivation of Okra on Farmers

Farmers' understanding of the causes of okra leaf disease was significantly influenced by their educational background (x^2=109.02; df=6; p=0.000), disease severity across seasons (x^2=30.63; df=6, p=0.000), timing of chemical application (r^2=23; df=6; p=0.000), signage display post pesticide application (r^2=89.31; 18; p=0.000), re-entry into field after pest. Farmers' knowledge of the causes of okra leaf disease (x^2=17.13; df=6; p=0.009), the timing of chemical application (r^2=289.31; df=18; p=0.000), the placement of sign posts after spraying (r^2=224.44; df=18; p=0.000), the farmers' re-entry period (r^2=36.63; df =12; p=0.000), the disposal of empty pesticide. However,

as demonstrated in Table 3, farming experience had no effect on the season in which the disease is most severe (r^2=11.32; df=6; p=0.079) or their management.

Table 3: Effect of educational level and years of cultivation of okra on farmers' perception on OLCD, whitefly identification and pesticide use.

Variables	Pearson **Chi-**square	df	P-value
Educational level*Causes of OLCD	109.02	6	.000
Educational level*Season disease most severe	30.63	6	.000
Educational level*Timing of chemical application	235.41	18	.000
Educational level*Sign to show pesticide is sprayed	89.31	18	.000
Educational level* Re-entry period	97.67	12	.000
Educational level*Disposal of empty containers	230.91	18	.000
Educational level*Type of PPE	98.48	12	.000
Years of cultivation of okra* Causes of OLCD	17.13	6	.009
Years of cultivation of okra*season disease most severe	11.32	6	.079
Years of cultivation of okra*timing of chemical application	289.31	18	.000
Years of cultivation of okra*sign to show pesticide is sprayed	224.44	18	.000
Years of cultivation of okra*re-entry period	36.63	12	.000
Years of cultivation of okra*Disposal of empty container	177.83	18	.000
Years of cultivation of okra*type of PPE	170.98	12	.000

3.5 Discussion

Farmers in the research region are aware of diseases, whitefly prevalence, and pesticide use, according to the findings. The majority of okra farmers in Volta and Oti region were male, while only a few were female, according to their background characteristics. The large proportion of men fits with a survey undertaken by Askira, (2012) on okra farmers in Nigeria, which found that exclusively males were active in okra farming, with few females, likely due to the hard nature of the occupation. The findings, however, contradict studies by Asare-Bediako *et al*., (2014) in KEEA, Central region, that the majority of okra farmers were women rather than men. The farmers' ages range from 18 to 70 years, with the majority being between the ages of 20 and 29 years. Petry (2002) divided people into three age groups: young (18–35 years old), middle-aged (36–55 years old), and older (above 55 years old) (aged older than 55 years). The results of this category revealed that the majority of the farmers in the study region were young and middle-aged people, which is beneficial for Ghana's agricultural development. Asare-Bediako *et al*. (2018) found similar results, with most respondents between the ages of 20 and 29 years. This age range also shows that the majority of respondents are active, energetic, and willing to put in the effort necessary to expand okra production in the region. In the research area, the majority of the okra growers had a formal education. The findings back up the GSS, (2013) regional literacy assessment report, which found that 70.7 % of the population in the studied area was literate.

Due to the Volta and Oti region's high literacy rates, OLCD, the whitefly vector, and pesticide use may have been more widely known. Asare-

Bediako *et al*. (2014), on the other hand, found 70% illiteracy among okra growers in four communities in the Central region of Komenda-Edina-Eguafo-Abirem (KEEA) municipality of Ghana. Asante and Ntow (2009) stated that this could be the outcome of ongoing and obvious pesticide abuse and misapplication in okra production. The majority of the farmers grew okra on plots of land ranging from 1 to 5 hectares, demonstrating that Ghanaian farmers are predominantly small-scale. The Volta Region's farmers averagely cultivate 0.4 hectares of land, according to the 2010 population and housing census (GSS, 2013). According to the findings of the current study, the majority of the youth in the region are entering into okra farming, which is a major source of food and revenue (Sugri *et al*., 2015). The survey revealed that most respondents have been cultivating okra for five to nine years and have a lot of experience with pests and disease conditions.

Okra was grown all year, in both the major and minor seasons, primarily as a monoculture, with only a few farmers practicing intercropping. Asare-Bediako *et al*. (2018) found that okra farmers in the Central area used mono-cropping. Conversely, Nayudu (2008) claimed that greater monocropping across wide areas promoted the quantity of vectors and the development of viruses, leading to a high incidence and severity of OLCD. This was undeniably obvious in the Volta and Oti regions disease incidence and severity index. However, according to Perring *et al*. (2018), some of the farmers used mixed cropping/intercropping, which helps to prevent *Bemisia tabaci* from finding host plants, and hence, the presence of numerous hosts may change the insect's behavior. Intercropping can have a positive impact on

the natural enemy complex. When comparing tomato intercropped with roselle and maize to tomato grown in monoculture.

Volta and Oti regions have an average annual rainfall of 1,168 mm and a maximum of 2,103 mm (GSS, 2013). However, according to Nyatuame *et al.* (2019), the total water requirement of okra for optimal production is 111.13mm. This indicates that the most important factor to consider when growing okra is the availability of water. According to the report, rainfall is the primary source of water for the vast majority of farmers (88.5%). Even if total rainfall in the Volta and Oti regions is sufficient, its distribution throughout the year may be uneven, resulting in low okra yields. A small proportion of okra producers (48.7%) have irrigation facilities, which could allow for multiple cropping throughout the year (Figure 12). Due to higher yields each season and the ability for multiple cropping, the potential productivity of irrigated land can be four times or higher than that of non-irrigated (or "rain-fed") land (Nyatuame *et al.*, 2019). Multiple cropping will also facilitate an increase in the whitefly population and a corresponding increase in disease incidence and severity. In terms of adequate water supply, the study found that 47.7% of respondents relied on their dug or pond for okra farming. According to okra farmers in the Volta and Oti regions, the dug-out used by okra farmers was cost-effective, but some dried up, rendering production ineffective.

One of the most important agricultural practices is weed control (El-solimany and Abd-El-Kareem, 2018). Weed management in okra farms in the study area is a problem, as observed, because the majority of the farms visited were not well maintained. According to the weed management practices used

by the respondent okra farmers, 78.7% weeded their farm twice, 12.8% weeded three times, and a few weeded once. When the farmers were interrogated, it was clear that those who slashed the land before sowing had to weed three times before harvest, but the high cost of labor compelled most of the farmers to do their work. According to the okra farmers, using herbicide to prepare the land was better because weeds were only controlled twice. El-solimany and Abd-El-Kareem (2018) discovered that weeds could have both beneficial and negative effects on crop health, and the majority of farmers were aware of the hazards involved with leaving the land weed-infested. The negative way manifests itself through competition with the main crop for essential growth elements such as light, water, and nutrients, particularly in the early stages; additionally, they act as alternative hosts for insect pests and diseases and even as vectors for plant pathogens. A large weed species serves as an alternate host for *Bemisia tabaci*, as it is possible that the whitefly's population and activities are sustained throughout the year (Langer and Sahito, 2007).

Okra cultivars grown in Ghana could be indigenous, improved, or exotic cultivars Asare-Bediako (2018). Some of the okra farmers in Volta and Oti regions knew the names of the okra varieties cultivated; however, Avalavi (39.8%), Awayibor (15.4%), Asontem (13.8%), and Fetre (7.8%) stood out as prominently cultivated local varieties. Exotic cultivars such as Okra Indiana, okra hire, okra OH102, and Clemson spineless were also grown. The majority of okra farmers interviewed obtained their okra seeds from previous harvests, while others obtained them from certified seed sellers. Farmers were seen drying their okra in order to extract the seed for the next planting. They

claimed that buying certified seed was more expensive than growing it on their own farms. On the other hand, Obeng-Ofori *et al.* (2007) found that purchasing seeds from a farmer directly adds to the spread of viral infections. Okra seeds are probably going to get infected due to the virus inoculum that is present in the field.

Majority of okra farmers (98.7%) during the survey indicated that they were aware of the disease and its presence in their farms, confirming the findings of Asare-Bediako *et al.*, (2018), who found that 98.9% of okra farmers in the Komenda-Edina-Eguafo-Abirem (KEEA) district in the Central region were aware of OLCD. The farmers also stated that the disease occurred in both the rainy and dry seasons (95.8%), but was more severe in the dry season (83.9%) and less severe in the rainy season (16.1%). To further develop efficient management measures, data on farmers' agronomic practices and an understanding of the origins of these viral infections are essential (Asare-Bediako *et al.,* 2018). The majority of farmers (83.33%) did not know the cause of the disease, while 16.67% of the farmers did. Further investigation revealed that thirty-six (36) of the farmers strongly implied that whiteflies were responsible for transmitting the viral disease, twenty-one of the respondent farmers mentioned flea beetles, and a few stated that changes in weather conditions were to blame (Figure 19). Farmers' knowledge of the disease's main cause confirms similar findings that the whitefly persistently transmits Okra leaf curl disease (*Bemisia tabaci* Gen.) (Ali *et al.,* 2012; Leke *et al.,* 2015; Asare-Bediako *et al.,* 2018). The findings clearly demonstrated that the farmers had no idea what was causing the disease; let alone how to effectively manage it.

Farmers' knowledge of the symptoms of OLCD was further questioned. According to the respondents' observations, the most common symptoms of OLCD were top leaves curled and plants slightly stunted in okra fields, followed by curling of a few top leaves and all leaves curled and plants slightly stunted. The okra farmers described the fewest symptoms as severe curling of leaves, stunting of plants, and proliferation of auxiliary branches, and some farmers did not see any symptoms to describe. The findings support other researchers' descriptions of OLCD symptoms such as leaf curling, yellowing, leaf distortion, stunted growth, and yield reduction (Krishnarreddy *et al.*, 2003; Tiendrebeogo *et al*., 2010 Asare-Bediako *et al.*, 2018)

The respondents' observations suggest that the farmers were aware of the disease's presence. According to the majority of farmers, the disease incidence ranged from 51 to 71%. Aside from OLCD, the majority of okra farmers observed okra mosaic disease as a serious disease infecting okra. The observation backs up previous findings (Alegbejo *et al*., 2008; Bi-Kusi, 2013; Asare-Bediako *et al.*, 2014, 2018) that okra mosaic virus disease is common in okra farms in Ghana and elsewhere. Other diseases reported by farmers in the study area included root swelling and fruit rot, which must be thoroughly investigated and resolved. Information on farmers' insect pest knowledge and agronomic practices is also useful in developing effective management strategies. The majority of the farmers (99%) in the area were aware of whiteflies, which were known by various names in their local dialect. Further investigation revealed that the whitefly was observed by farmers to be more severe in the dry season than in the rainy season. The majority of farmers

reported that whitefly was a problem on their farms and that controlling whitefly was a major challenge for them.

Furthermore, when farmers were asked if they had reported the pest to Agriculture Extension Agents, 141 of the farmers said No, while 243 said Yes, they reported it to Agriculture Extension Agents. Farmers used various methods to control whiteflies in their okra farms; the majority of farmers used synthetic pesticides to control whiteflies, while others used water to spray on the plant to drop the eggs and adult whiteflies, others used bio-pesticides such as neem extract, and a few used traps. In many cropping systems, the primary method of pest control is the use of insecticides (Lenli, 2003). The majority's use of synthetic pesticides confirms that chemical pesticides are commonly used in the management of pests and diseases in Ghanaian vegetable production, which affects the health of the farmer, the environment and the development of resistant strains of the pest (Afari-Sefa *et al.*, 2015). None of the farmers used biological control agents such as fungi, parasitoids, and predators to manage whiteflies, which is a very effective method for preventing the development of resistance strains in whiteflies to synthetic pesticides (Gerling *et al.*, 2001). However, the use of biological control agents in the management of pests has not been introduced to most farmers. The development of resistance strains by whitefly makes pesticide management difficult. Fewer farmers used botanical insecticides, which frequently work in different ways than synthetic insecticides. They may be viable alternatives to *B. tabaci* management because they do not persist in the environment, have low mammalian toxicity, and no resistance has been reported against them (Lenli, 2003). The yellow sticky trap is one of the most widely used methods

for sampling and controlling whiteflies (Perring *et al.,* 2018). However, in the study area, farmers have not been introduced to this technology. Ekbom and Rumei (1996) observed that trap counts provided a good estimate of whiteflies at times but not at others. Water was used by some of the farmers to manage whitefly infestations on their farms, confirming that irrigation methods are also effective against whitefly infestation and virulence (Perring *et al.,* 2018). Abd Rabou and Simmons (2012) compared different irrigation systems (drip, furrow, and sprinkler irrigation) and discovered that daily drip irrigation reduced whitefly densities and virus incidence. According to other researchers, excessive use of both water and nitrogen fertilizer can exacerbate damage from *B. tabaci* infestations by increasing whitefly numbers (Bi *et al.,* 2001). The following pesticides were used based on farmers' average responses to the type of pesticide used in managing whitefly and okra leaf curl diseases in okra farms: Sunpyrifos (Chlorpyrifos-ethyl), Confidor 200SL (Imidacloprid), Lambda Super 2.5EC (Lambda-cyhalothrin), Attack (Emamectin Benzoate), Dursban 4E (Chlorpyrifos), Champion 80WP (Copper hydroxide), Actellic 50EC (Pirimiphos-methyl), Karate5EC.

According to the methods used by Asare-Bediako *et al.* (2018), nine (9) of the agro-chemicals were insecticides, while three (3) were fungicides. Some of the pesticides used were not registered, according to the results of the checks. The outcome of the survey reveals that agrochemicals such as Aceta Star EC (Bifenthrin+Acetamiprid) and Confidor 200SL (Imidacloprid) are only registered for use in cocoa production and are classified as highly hazardous by WHO Hazard Category III, but farmers in the study area were using them on okra (Amoako *et al.,* 2012; Afari-Sefa *et al*., 2015; Asare-

Bediako, 2018). This is a serious situation that necessitates immediate action to prevent harmful, toxic public health hazards and environmental pollution (Afari-Sefa *et al*., 2015). Throughout the study, it was discovered that farmers were misusing agrochemicals. Fungicides, for example, were used to control insect pests, and insecticides were also used to control fungal and viral diseases. Misapplication has resulted in an overdose of chemical application, which has resulted in the pests and diseases not being controlled. For instance, Asare-Bediako *et al.* (2018) observed that farmers incorrectly applied fungicides (Mancozeb, Copper hydroxide, and Cupric hydroxide) to combat viral diseases and insect vectors.

The majority of okra farmers, as revealed during the study, relied on agro-chemicals (synthetic) to manage the OLCD and whitefly. Knowledge of pesticide use and management should not be a source of worry to farmers. Reading instructions on chemical labels should not be a problem because the majority of okra farmers in the study area can read and write. It is encouraging to note that the majority of farmers (51%) obtained knowledge on the use and application of agro-chemicals from agro-input dealers, and the majority of them obtained their agro-chemicals from agro-input dealers (96%). Aside from chemical vendors, Agricultural Extension Agents (10.9%) advised and educated okra farmers on pesticide use and application. In contrast, Mattah *et al*. (2015) found that 43.3% of pesticide use recommendations in Ashiaman came from extension officers, 39% from agrochemical dealers, and 10% (10%) from colleague farmers. Similarly, Asare-Bediako *et al.* (2014) found that agricultural extension agents (80%) advised okra farmers in KEEA's Central region on which chemical pesticides to use, while the rest relied on

agro input dealers (20%). The current study confirmed previous findings that pesticide dealers were the primary source of information on pesticide recommendations (Ntow *et al*., 2006; Jamali *et al.*, 2014; Meenambigai and Bhubaneswar, 2017).

Despite the fact that the majority of the farmers in the study area are literate, it was clear that the majority of them did not read the labels on the instructions before using them. This assertion was evident in their awareness report on how relying on agro-input dealers, agriculture extension agents, and friends for information rather than reading chemical labels had resulted in poor chemical handling, frequency, and timing, which could lead to serious health consequences for farmers, the environment, and consumers. There is a risk of forgetfulness on the part of agro-input dealers, which could lead to misinformation and misapplication of the correct agro-chemical to use in pest and disease management.

Afari-Sefa *et al.* (2015) confirmed that farmers were unable to distinguish between different pest and disease and control measures such as insecticides and fungicides due to misinformation from agro-input dealers and reliance on their information and advice. Unsurprisingly, the study revealed how poorly the majority of farmers disposed of their empty pesticide containers on the farm, in villages, and in rivers. Farmers in the survey really abused pesticide use and management. The pattern and frequency of pesticide usage by farmers may contribute to the development of resistance in insect vectors and crop pests, in addition to the negative effects of pesticide misuse on farmers' health (Ntow, 2001).

According to the study in the Volta and Oti regions, 92.2% of farmers had not received training on pesticide safe handling and application, while 7.8% had training (Figure 33). Afari-Sefa *et al.* (2015) discovered that (56.8%) of vegetable farmers in Ghana's Ashanti and Western regions had received training on safe pesticide handling and application, while 43.2% had received no training. Whether farmers had received training or not, their behavior was consistent in the misuse and misapplication of agrochemicals to the detriment of their health, the environment, and the development of pest and disease-resistance strains. This confirms that agriculture in Ghana, particularly vegetable farming, is fraught with pesticide misuse, abuse and overuse, leading many farmers to use chemical pesticides despite receiving no training in application techniques (Asante and Ntow, 2009). According to Obeng-Ofori *et al.* (2007), the presence of an insect or discoloration on a plant does not imply that the agent is a pest that must be controlled; rather, we must ensure that the specific agent is causing economic damage. A farmer was asked when he/she used pesticide on their okra. The response of the study found that 40% of farmers used pesticides at the first sighting of a pest or disease on the okra, and 48% used pesticides when the pest or disease was severe. This finding is consistent with Afari-Sefa *et al.* (2015) and Amoako *et al.*, 2012). It is critical to determine crop losses attributed to the pest because crop damage may appear severe to the naked eye, but actual yield losses may be insignificant enough to warrant control. Farmers must also recognize that some levels of pest damage can be tolerated by many crop plants with no discernible effect on crop yield or quality (Obeng-Ofori *et al.*, 2007). According to the study, 83% of farmers poured pesticides into their knapsack

sprayers before shaking to ensure total mixing before spraying, while 15% used a stick to stir the chemical in the sprayer. Some of the respondents (2) admitted to mixing the chemical with their bare hands before spraying, which can result in poisoning. It was discovered that after stirring the chemicals with a stick, the farmers left them on the farm for their children to pick up and play with, which could be harmful to the children's health. Farmers' awareness of pesticide spraying direction in relation to wind direction was tested. Although most farmers (70%) sprayed pesticides windward, 13% also noticed spraying against the wind. Only a few said they did not consider wind direction, which contradicts a report by Devi, (2009) that rice farmers in Kerala, India, did consider wind direction when applying pesticide. According to Afari-Sefa *et al.* (2015), most vegetable farmers applied pesticides in the direction of the wind. Amoako *et al*., (2012). Discovered that the majority of farmers were also unaware of the impact of wind direction and spray drift on their health.

Okra farmers confirmed using pesticide early in the morning, while others used it late in the evening. Farmers were observed applying pesticides in the hot sun, which was hazardous to their health. Early morning is a safer time to avoid chemical drift on the applicant's body and protect against dermal irritation (Afari-Sefa *et al.*, 2015). As a precaution, the majority of respondents did not eat or smoke during pesticide application. During spraying, it is easy to contaminate food and cigarette sticks. Pesticide poisoning kills approximately 20,000 people each year, according to the World Health Organization (WHO), and at least 3 million suffer acute health effects (Barabara, 1993). Such deaths are likely to occur as a result of pesticide misapplication and poor pesticide handling. The majority of

respondents (92%) did not post a warning sign on the field after spraying with agrochemicals to deter family members and others from entering the field and to warn of the dangers of pesticide poisoning. The Volta and Oti region's extended family nature exacerbates the problem because other members feel free to enter and harvest okra from friends' and family farms.

It is worth noting that ninety-five percent (95%) of the respondents harvested okra every three (3) days, while 5% harvested between 3 and 7 days. Asare-Bediako *et al. (*2018) also reported that 39.8% of okra had a three-day pre-harvest interval. According to Amoako *et al.* (2012), vegetable farmers sprayed pesticides during produce harvesting, so no waiting period is observed, exposing consumers to a high risk of pesticide residue levels. During a field survey, a farmer was observed applying pesticides and harvesting the following day. Further questioning revealed that farmers were unable to wait any longer to harvest due to pressure from middle women who sponsored them to harvest. Harvesting within a short period of time after spraying will result in higher risk of chemical residual levels in the okra, which is harmful to consumer health (Amoako *et al.*, 2012).

Further questioning revealed that the majority of farmers (51.6%) returned to the farm the same day after applying pesticides or agro-chemicals, while others returned three (3) days later, and three percent (3%) returned on the fourth day. After entering the same day, the majority of the farmers' faces, hands, and legs were bathed in chemicals that had settled on the surfaces of the leaves. The study also looked at how farmers disposed of empty pesticide containers after using them. According to Mattah *et al.*, (2015), disposal methods are also of concern because they can have an impact on the larger

community due to pesticide leaching into water bodies, which can lead to accidental ingestion. According to the study, fifty-five percent (55%) of the okra farmers threw empty containers on the farm, as confirmed by Ntow (2008) and Afari-Sefa *et al.* (2015), and twenty-two percent (22%) threw them anywhere in the village. During the survey, it was discovered that empty containers were found close to river bodies, with some floating on them, posing a risk to human and animal health.

Despite the fact that the majority of respondent farmers own sprayers (94%), knapsack sprayers (99%) constituted the majority of sprayers owned in the study area. According to Ntow *et al*., (2006) and Afari-Sefa *et al.*, (2015), most farmers own and use knapsack sprayers; however, the use of this type of sprayer poses some risk to the user because it is prone to leakage, especially as the spray equipment ages. Farmers who did own sprayers were discovered to have borrowed or rented them from other farmers in their community. The agrochemicals discovered in the study area are toxic to humans and the environment. It is, therefore, recommended that farmers wear appropriate personal protective equipment that covers the entire body when applying such chemicals. However, 88% of farmers did not wear total protective cover such as nose guards, boots, gloves, and goggles and came into direct contact with the chemicals; 10% dressed partially during their spraying operation, and only 2% protected themselves fully during spraying operations. However, according to Amoako *et al.* (2012), 33% of farmers at an Ejisu cabbage farm did not utilize safety precautions, including nose respirators and protective clothes when spraying pesticides, whereas the majority (67%) said they did. Some researchers around the world noticed that none of the farmers were

wearing the recommended protective gear, which included a face mask with replaceable filters, goggles, a head cover, rubber gloves, long-sleeved shirts and pants, and boots (Murphy *et al.,* 1999; Salameh *et al.,* 2004; Atreya, 2007; Devi, 2009; Owusu-Boateng and Amuzu,2013). According to Devi (2009), the cost factor (which caused farmers to be hesitant to adopt the recommended gadgets and instead opt for cheaper substitutes), general lethargy, and the discomfort associated with use (in the hot and humid climate and under puddled paddy land conditions) were reported as reasons for non-adoption of proper protective gadgets. According to Afari-Sefa *et al.* (2015), respondents reported that chemicals came into contact with their hands, back, feet, and face, resulting in food poisoning. During the study, some farmers had chemicals poured on their faces, hands, and bodies, according to observations.

As shown in Table 3, the relationship between farmers' educational level and farming experience on knowing the cause of disease and insect, knowledge on season when disease is severe, best time to apply pesticide, placing signs on the farm after spraying, farmers re-entry period, disposal of an empty chemical container, and adherence to proper use of personal protective equipment. According to the study, farmers' knowledge of disease causes and insects, the best time to apply pesticides, when to spray after a disease, when to re-enter the field, how to dispose of empty chemical containers, and when to wear personal protective equipment were all significantly unaffected by their educational background and farming experience. These findings contradict the findings of Asare-Bediako *et al.* (2018), who found that education level and farming experience had a significant influence on okra farmers' awareness and management of okra

mosaic disease. Despite the fact that over 92.4% of farmers in the study area are literate, it is clear that the majority of them did not adhere to good and appropriate pesticide use. Despite the fact that education and experience can be used to obtain information about insect, disease, and pesticide use, adherence to rules and pesticide use was lacking. However, based on the Pearson correlation coefficient, farming experience had a significant influence on farmers knowledge on the season when the disease is most severe (table 2). This finding is consistent with the findings of Nagaraju *et al.*, (2002), who reported that formal education as well as farming experience or number of years in farming can serve as a means for farmers to become informed.

CHAPTER FOUR

4.0 INCIDENCE OF OKRA LEAF CURL DISEASE AND INFESTATION LEVELS OF ITS WHITEFLY VECTOR IN THE VOLTA AND OTI REGIONS

4.1 Introduction

Okra production is estimated to be 4 million tonnes across tropical, subtropical, and Mediterranean climates, accounting for about 4% of total vegetable consumption in most developing countries (Ahmad *et al.*, 2015). Under good management, yields of 10 to 15 tons per acre can be obtained (NARP, 1993). Despite the importance of okra, farmers face extremely low yields. For example, the average productivity of okra in West Africa (2.5 t ha^{-1}) is very low when compared to East Africa (6.2 t ha^{-1}) and North Africa (8.2 t ha^{-1}) (Cudjoe *et al.*, 2005). Ghana is no exception when it comes to the decline in okra productivity.

The low yield is partly due to the prevalence of pests and diseases, which has become a major biotic constraint to Ghanaian okra production (Asare-Bediako *et al.*, 2014). Flea beetles (*Podagrica* sp.), cotton stainer (*Dysdercus superstitus*), white fly (*Bemisia tabaci*), and green stink bug (*Nezera viridula*), among others, have been reported to infest okra in Ghana (Obeng-Ofori and Sackey, 2003; Bi-Kusi, 2013; Senjobi *et al.*, 2013). Okra is susceptible to 19 different viral infections, according to Asare-Bediako *et al.* (2014), with okra leaf curl virus and okra mosaic virus being the most prevalent viruses that infect okra in Ghana. The National Agricultural Research Project (NARP, 1993) reported that okra is primarily grown in Ghana's Brong Ahafo, Ashanti, Northern, Greater Accra and Volta regions,

and Central regions. All stakeholders in the agricultural value chain should be concerned with determining the prevalence and severity of okra leaf curl disease and whitefly in various districts throughout the Volta and Oti region. In order to implement effective management strategies for okra leaf curl disease in the Volta and Oti regions, it is necessary to assess the disease incidence, severity, and whitefly population estimation, as well as the impact of climatic factors. As a result, the goal of the research was to investigate disease incidence and severity, as well as the whitefly vector population.

4.2 Study Objectives

The purpose of this study therefore is to determine the incidence and severity of okra leaf curl disease in the Volta and Oti regions over two growing seasons.

Specific objectives are to;

1. Determine the prevalence/incidence and severity of okra leaf curl disease in the three agro-ecological zones in the Volta and Oti regions.
2. Monitor the whitefly population in the three agro-ecological zones of Volta and Oti regions.
3. Determine the relationship between weather parameters and disease, as well as the population of whiteflies.

4.3 Materials and Methods

4.3.1 Study area

The research was carried out in three agro-ecological zones that cover the Volta and Oti regions (coastal savanna, forest, and transition). The Volta region of Ghana is located between the Volta Lake to the west, the Republic of Togo to the east, and the Atlantic Ocean to the south, between latitudes 5° 45'N and 8° 45'N. The region encompasses 20,570 square kilometers, accounting for 8.6% of Ghana's total land area. The Region stretches for about 500 Km from the Atlantic coast in the south to the Pacific coast in the north, encompassing all of the country's vegetation zones. Coastal strand Mangrove Swamps, woodland Savannah, Savannah Grassland, mangrove Swamps, and deciduous forest are the vegetation types. The region has a tropical climate with moderate temperatures ranging from 12 to 32 ^{o}C for the majority of the year. The rainfall pattern is bimodal, which means that it has two rainfall regimes throughout the year, one from March to July and the other from mid-August to October. Rainfall figures vary greatly across the region, but are highest in the central highlands and forest zone, and lowest in the Sahel-savannah zone in the north. The annual precipitation ranges between 513.9 mm and 1099.88 mm (GSS, 2013; GTA, 2020).

4.3.2 Experimental design

A field survey was conducted to determine the incidence and severity of okra leaf curl diseases in the Volta and Oti regions. In each of the agroecological zones, two districts were selected: Keta, South Tongu, Ho West, Jasikan, Krachi East and Nkwanta South. Based on population density and the amount of okra produced, four communities were purposefully chosen for the study from each district. A total of 96 farms were selected across the study area where in each community, four okra farms were selected for the data collection.

A simple random sampling technique was used to select twenty (20) okra plants in each farm for determination of incidence, severity of okra leaf curl disease and population of whitefly in each of the farms. Visual observation and documentation of the presence or absence of okra plants exhibiting disease symptoms were used to calculate the incidence of the viral diseases. The symptoms shown by each plant were then ticked on the score sheet. Disease incidences (DI) per field were estimated as the percentage of plants displaying disease symptoms. A score sheet was developed using a method used by Alegbejo *et al.* (1997) for data collection on disease incidence and severity. Score sheet was also developed for white fly population collection. The plants were scored for severity of OLCD based visual scale 0 - 7 scale developed by Alegbejo *et al.* (1997)

Table 4: Visual scale for rating severity of okra leaf curl disease on farmers' okra fields

Disease score	Description
0	No symptom
1	curling of few top leaves
3	Top leaves curled and slight stunting of plant
5	All leaves curled and slight stunting of the plant
7	Severe curling of leaves, stunting of plant and proliferation of auxiliary branches

Incidence of OLCD based on a 0-7 visual scale, which was developed by Alegbejo *et al.* (1997)

Data on the whitefly population was also determined by a method used by Ellsworth *et al.* (1995) and Khan (2019), known as the "leaf turn" technique, was adopted for whitefly estimation. The whiteflies were sampled early at 6:30am to 8:30 am. The okra plants were carefully approached, and three leaves on the upper part of the plant were turned to count the number of adult whiteflies on the leaves.

Data on weather parameters such as rainfall, wind speed, temperature and relative humidity were obtained from the three meteorological stations in each agro-ecological zone. The stations are Krachi West station (Transition zone), Ho station (forest zone) and Akatsi station (Coastal) at the Ghana Meteorological Agency at Ho head office. The weather parameters were then correlated with the whitefly population in each of the zones.

4.4 Data Processing and Analysis

The disease incidence and severity were estimated using the formulae

$$DI(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants sampled}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots 1$$

$$Disease\ severity(\%) = \frac{\sum n}{\text{N X M}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Where; N = Total number of plants assessed.

$\sum$ n = Sum total of individual rating.

M = Highest score on the severity scale.

Arcsine transformations were used to homogenize the variation in the disease incidence and severity data before an analysis of variance (ANOVA) was performed. Using the IBM "Statistical Package for the Service Solutions (SPSS) Software Package Version 20," the other quantitative data (OLCD severity scores) were subjected to an ANOVA with the means separated by the least significance difference (LSD) at 5%. The relationship between the whitefly population and weather parameter were correlated using Pearsons's correlation coefficient using the IBM 'Statistical Package for the Service Solutions (SPSS) Software Package Version 20

4.5 Results

4.5.1 Disease Incidence for both major and minor seasons in the three agro-ecological zones in the Volta and Oti regions.

The disease was observed in all the agro-ecological zones across the districts, as shown in table 4 for both minor and major seasons in years one and two. The arcsine transformed mean disease incidence for the coastal savannah (84.58%) was significantly different from transition (72.16%) and the forest (70.14%) zones minor season. However, in the major season, the disease incidence for the coastal zone (69.71%) incidence was not significantly different from that of forest (69.49%) and transition (70.53%) zones in year one.

In year two, the mean disease incidence for coastal grassland was 82.44%, which was not significant from transition (81.31%) and forest (78.10%) in the minor season, whiles in the major season, the coastal grassland recorded 72.94%, which was not significantly different from in the mean of disease incidence in the forest (69.66%) and transition (66.24%). Generally, the minor season (78.12%) had a much higher disease incidence than the major season (69.76%).

Table 5: Arcsine transformed mean incidence of okra leaf curl disease during major and minor seasons for two years

	Disease incidence (year one) (%)		Disease incidence (year two) (%)	
Agro-ecological zones	**Minor season**	**Major season**	**Minor season**	**Major season**
Coastal	**84.58[a] (97.81)**	**69.71[a] (86.69)**	**82.44[a] (95.63)**	**72.94[a] (87.56)**
Transition	**72.16[b] (89.34)**	**70.53[a] (87.75)**	**81.31[a] (95.16)**	**66.24[a] (82.03)**
Forest	**70.14[b] (86.84)**	**69.49[a] (86.88)**	**78.10[a] (92.47)**	**69.66[a] (85.53)**
Mean	**75.62**	**69.91**	**80.62**	**69.61**
CV	**9.97**	**7.45**	**13.82**	**16.02**
P-value	**0.000**	**0.784**	**0.394**	**0.063**

Means in the same column bearing different letters are significantly different ($P<0.05$)

4.5.2 Mean OLCD incidence in Districts for major and minor Seasons in year one and two

The incidence of okra leaf curl disease across the various districts was also compared in order to assess the disease's prevalence. The findings showed that the minor season recorded the highest incidence across the district in year one, as shown in Figure 45 below, where Keta Municipal (98%) with the highest, closely followed by South Tongu District (97.63%), remained higher than Ho West (90.81%), Krachi East (90%), Nkwanta South (88.69), and Jasikan district (82.88%). During the major season, Nkwanta South had

the highest mean incidence of 89.31%, followed by Keta (87.5%), Ho West (87%), Jasikan (86.75%), Krachi East (85.94%), and South Tongu (85.88%).

In year two, the following mean incidences were recorded during the minor season: Krachi East (97.19), Keta (96.56%), South Tongu (95.69%), Jasikan (95.19%), Nkwanta South (93.13%) and Ho West (89.94%). During the major season, Keta (89.94%), Krachi East (86.38%), Jasikan (85.88%), Ho West (85.19%), South Tongu (85.19%) and Nkwanta South (77.69%) as shown in figure 46 below.

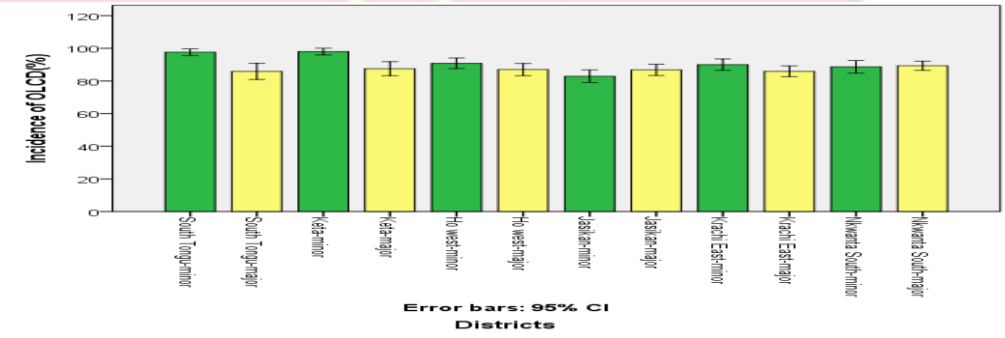

Figure 45: Incidence of OLCD in each district for year one in the minor and major season

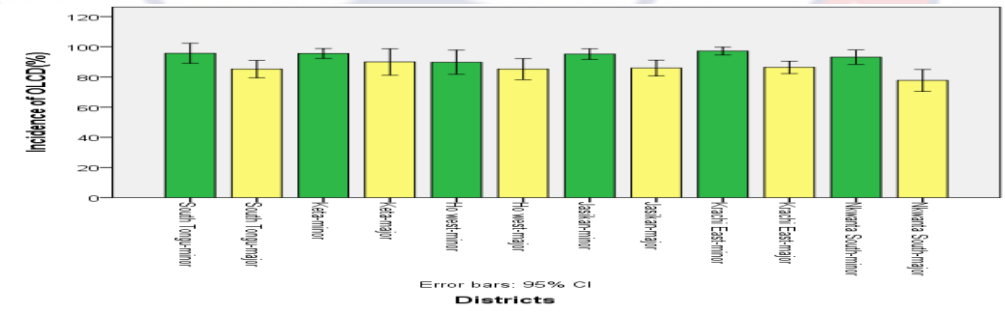

Figure 46: Incidence of OLCD in each district for year two during minor and major season

4.5.3 Severity of Okra leaf curl disease for years one and two in each of the three Agro-Ecological Zones

The mean severity index of okra leaf curl disease in the agro-ecological zones for both major and minor seasons for the first and second years of okra seasons in the study area is shown in Table 6. The mean severity index of OLCD ranged from 1.18 to 1.44. The highest mean of disease severity was observed in the second year during the minor season of 1.44 and the major season of 1.33. The severity of OLCD in the minor and major seasons in year one was 1.18 and 1.26, respectively.

There were variations mean severity index of the disease across the agro-ecological zone; the highest severity was recorded in the coastal grassland with the severity of 1.27 and 1.29 for minor and major seasons in year one. The forest zone recorded 1.14 and 1.29 in minor and major seasons, respectively whiles the transition zones had with least severity of 1.13 and 1.19 in minor and major seasons, respectively.

For the two years, the coastal grassland recorded severity values of 1.54 in minor and 1.38 in major seasons. The transition zone also recorded a disease severity of 1.56 in the minor season and 1.26 in the major seasons. In the forest zone, the disease severity was 1.27 and 1.35 in the minor and major seasons, respectively.

Table 6: The mean severity index of OLCD for major and minor season in the three agro-ecological zones for year one and two

	Disease Severity year one		Disease Severity year two	
Agro-ecological zones	**Minor season**	**Major season**	**Minor season**	**Major season**
Coastal	**1.27[a](6.46)**	**1.29[a](6.51)**	**1.54[a](7.10)**	**1.38[a](6.71)**
Forest	**1.14[b](6.12)**	**1.29[a](6.50)**	**1.27[b](6.46)**	**1.35[a](6.66)**
Transition	**1.13[b](6.10)**	**1.19[b](6.26)**	**1.51[a](6.02)**	**1.26[a](6.44)**
Mean	**1.18**	**1.26**	**1.44**	**1.33**
CV	**11.19**	**14.65**	**23.18**	**16.51**
P-value	**0.000**	**0.048**	**0.003**	**0.093**

Means in the same column bearing different letters are significantly different ($P<0.05$)

4.5.4 Population of Whiteflies in Agro-Ecological Zones

During two years of okra cropping seasons, a total of 38,504 whiteflies were counted. In the first year, a total of 21,893 and 16, 611 whiteflies were counted in both the first and second okra seasons. The minor season count was highest in the first year (15,046), while the major season count was 6847. Similarly, in year two, the minor season count was 10, 932, while the major season count was 5,679(major). The estimated square root transformed population mean of whitefly varied across the agro-ecological zones, with the coastal grassland showing a significant difference between the forest and transition zones for both minor and major seasons in year one, as shown in Table 6.

During the minor season of the second year, a mean of 10.20 was recorded, with no significant difference in the transformed mean of whitefly population in the agro-ecological zones, but a significant difference existed in the major season between the coastal grassland and the forest, but not with the transition zone.

Table 7: Square root transformed mean population of whitefly for major and minor season in the three agro-ecological zones for year one and two.

Agro-ecological zones	Whitefly mean population year one		Whitefly mean population year two	
	Minor season	Major season	Minor season	Major season
Whitefly pop.	**15,046**	**6, 847**	**10, 932**	**5,679**
Transition	**11.40^{a}(135.56)**	**7.763^{ab}(64.75)**	**9.97^{a}(108.09)**	**6.97^{a}(52.09)**
Forest	**11.41^{a}(135.56)**	**7.014^{a}(59.84)**	**9.43^{a}(96.50)**	**7.08^{a}(56.94)**
Coastal	**13.56^{b}(255.63)**	**8.928^{b}(89.38)**	**11.20^{a}(137.03)**	**8.18^{b}(68.44)**
Mean	**12.12**	**7.88**	**10.20**	**7.41**
CV	**49.76**	**36.22**	**28.38**	**26.99**
P-value	**0.006**	**0.041**	**0.070**	**0.034**

4.5.5 Relationship Between Okra Leaf Curl Disease and the Population of Whiteflies

Figures 47 and 48 show the relationship between okra leaf curl population and whitefly population during the first and second years of the study, respectively. Pearson's correlation coefficient test results revealed a significant negative linear correlation relationship between okra leaf curl

disease incidence and whitefly population (r (191) =-0.176, p=0.015) for both major and minor seasons in the first year. This indicated that the relationship between disease incidence and whitefly population was weak.

The outcomes for the second major and minor seasons of the year Pearson's correlation coefficient test reveals that there is no significant linear positive correlation between the incidence of okra leaf curl disease and the population of whiteflies (r (191) =0.109, p=0.133). The association between disease incidence and the whitefly population was weak but positive.

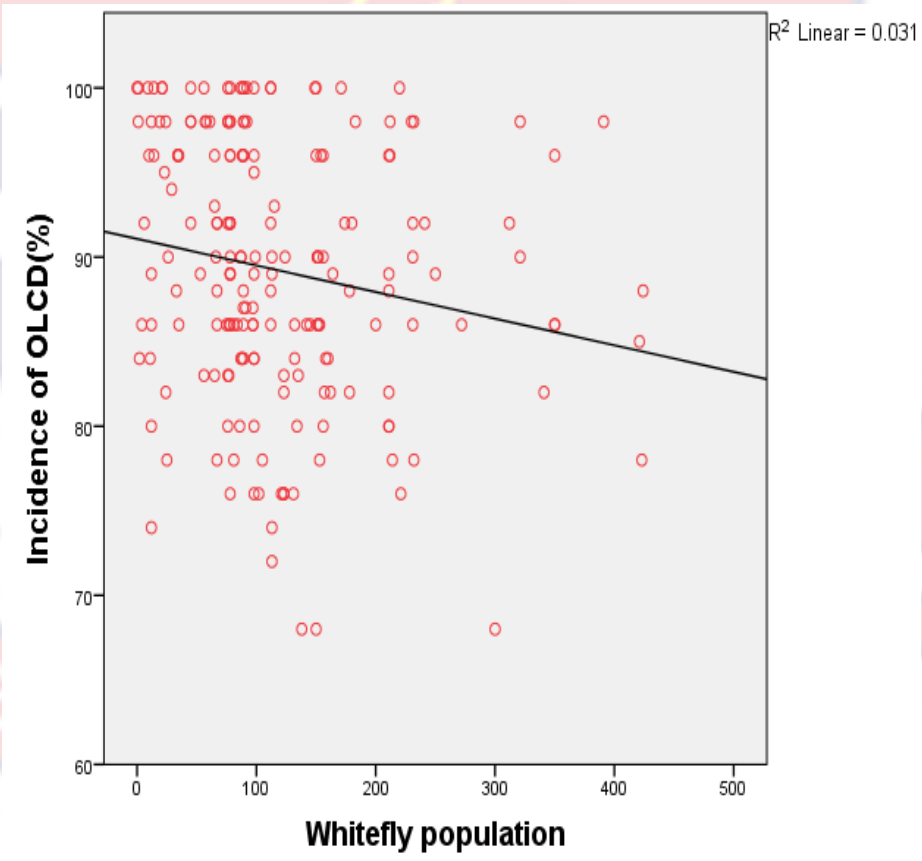

Figure 47: Relationship between disease incidence and whitefly population for year one.

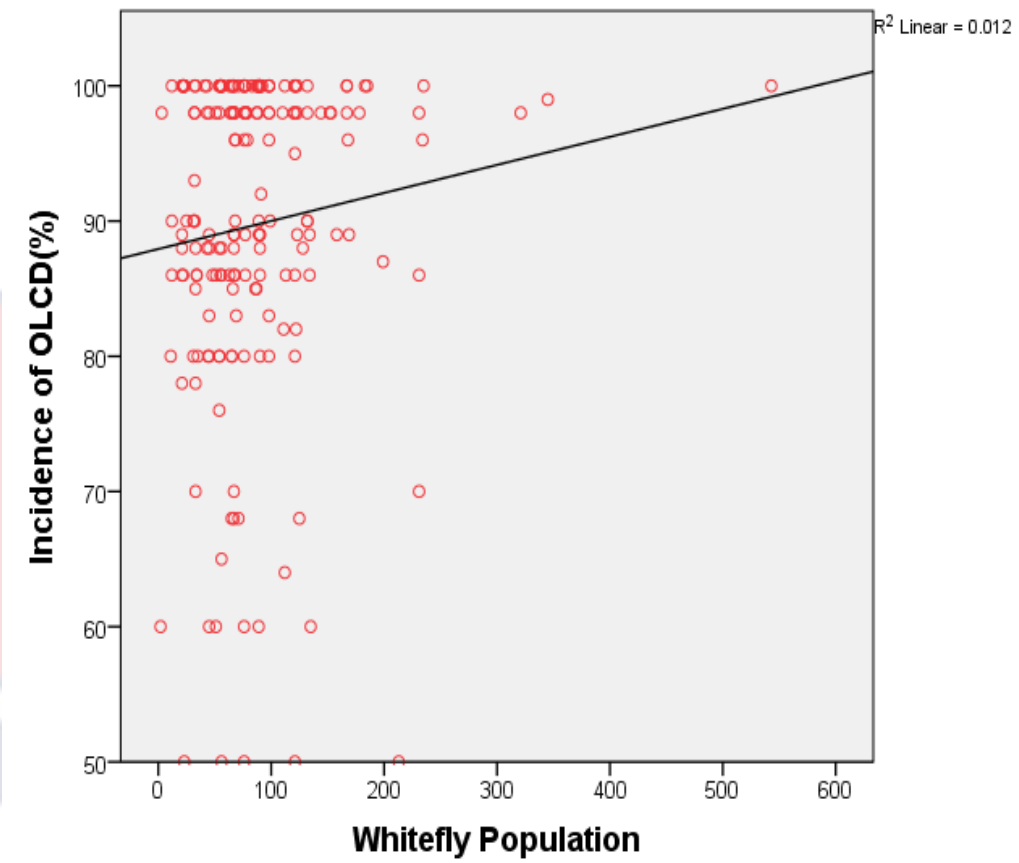

Figure 48: Relationship between disease incidence and whitefly population for year two

4.5.6 Disease Incidence and Insect Vector (Whitefly) Correlation in Three Agro-Ecological Zones

Figures 49, 50, 51, 52, 53 and 54 show the relationship between disease incidence and cumulative whitefly population identified in each agro-ecological zone for both wet and dry seasons in years one and two. In the first year, there was no significant but positive correlation between disease incidence and whitefly population in the coastal grassland (r (63) =.030, p >0.815), but there is a highly significant negative correlation in the forest zone (r (63) =-.322, p0.009) and no significant but negative correlation in the transition zones (r (63) =-.168, p>0.183) as shown in table 8.

Similarly, there was no significant but positive correlation between disease incidence and whitefly population in the coastal grassland (r (63) =0.159, p>0.208), no significant positive correlation in the forest (r (63) =.175, p>0.167), and no significant negative correlation in the transition (r (63) =-0.075, p>0.556) in the second year of okra production as shown in table 9.

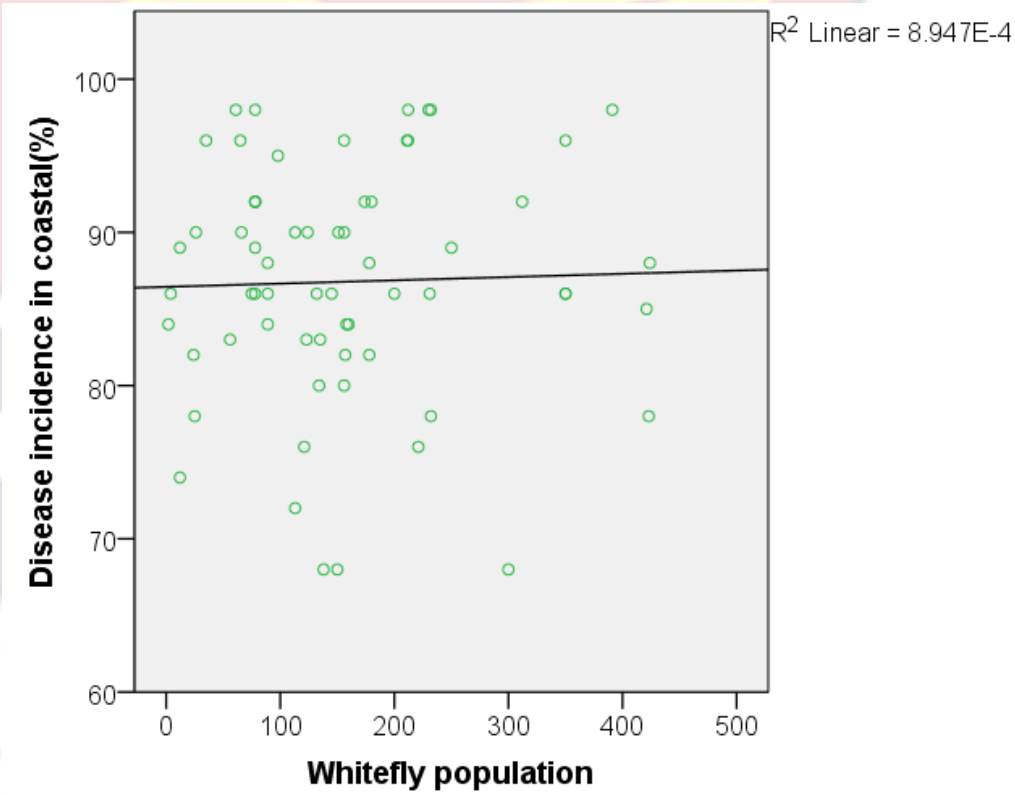

Figure 49: Relationship between disease incidence and whitefly in coastal zone in year one

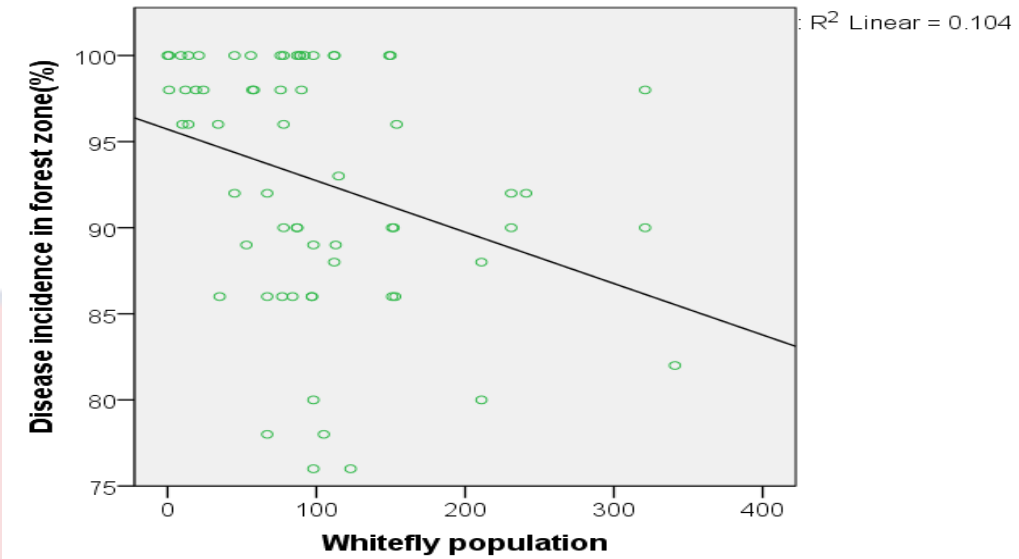

Figure 50: Relationship between disease incidence and whitefly in forest zone in year one

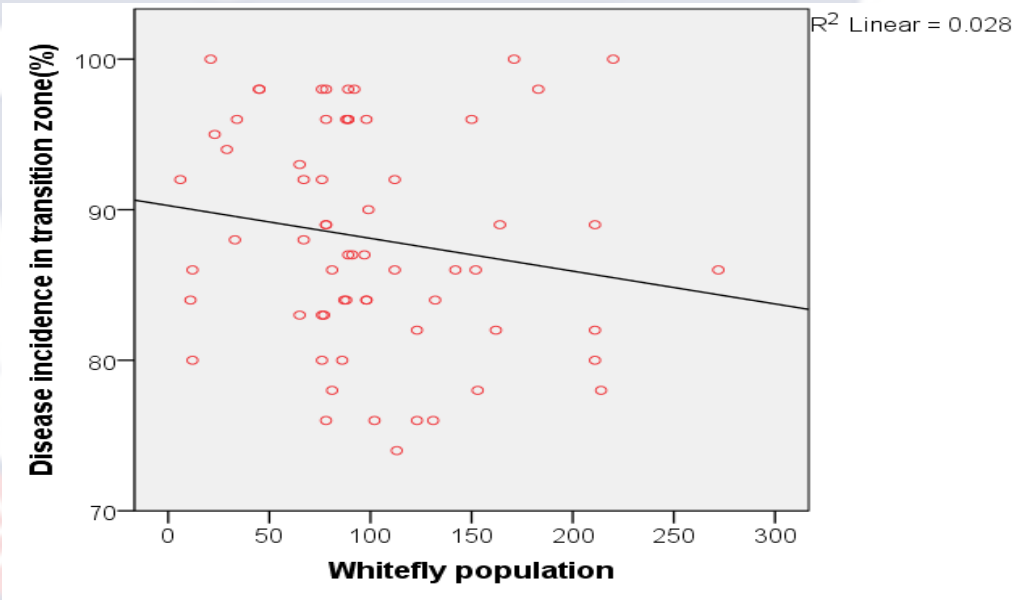

Figure 51: Relationship between disease incidence and whitefly in transition zone in year one

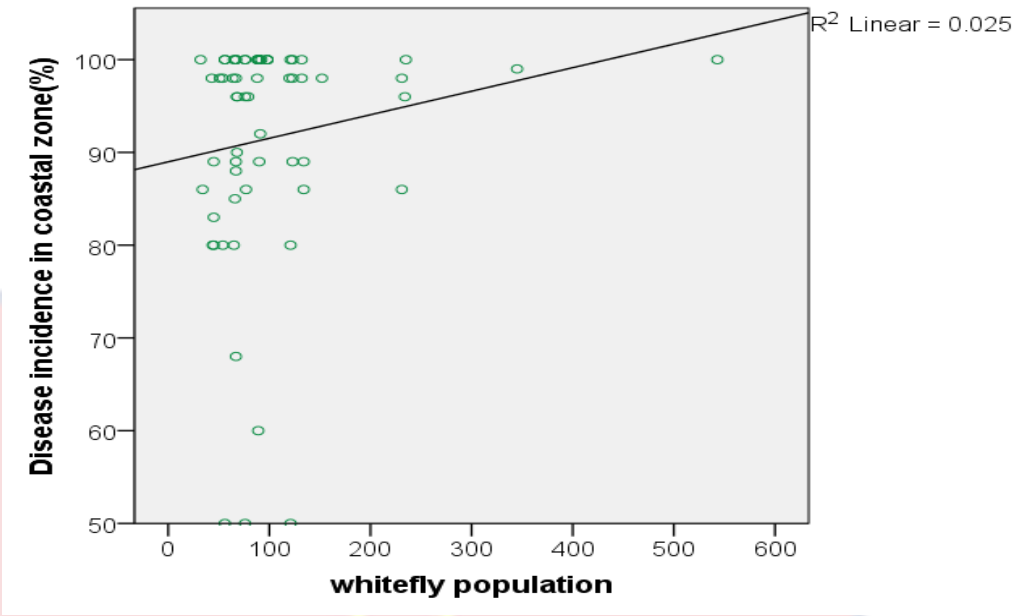

Figure 52: Relationship between disease incidence and whitefly in coastal zone year two

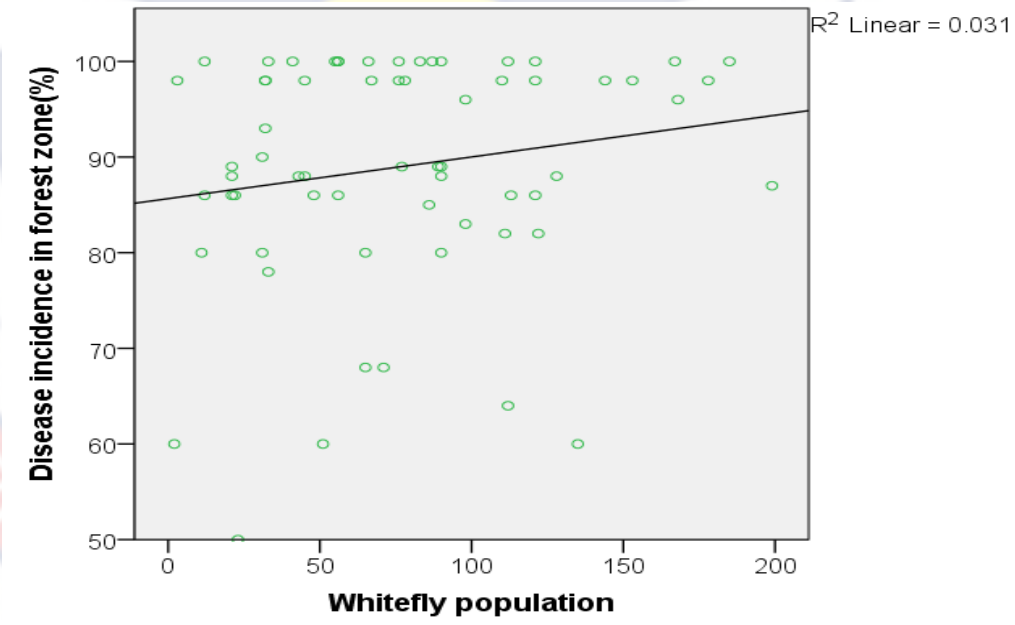

Figure 53: Relationship between disease incidence and whitefly in forest zone in year two

Figure 54: Relationship between disease incidence and whitefly in transition zone in year two

4.5.7 Relationship Between Whitefly Population and Weather Parameters in Three Agro-Ecological Zones in Year one.

Figure 55a below shows the relationship between the whitefly population and temperature and relative humidity in the first year in the coastal grassland zone. The population of whiteflies had a moderately negative and linear relationship with maximum temperature (r = -0.385), and its contribution to regression (R2=0.148) was 15%, whereas relative humidity had a non-significant positive Pearson correlation (r = 0.097), and its contribution to regression (R2=0.009) was 0.9 percent. Rainfall was significantly positively correlated (r=0.729) with whitefly population and

regression (R2=0.532) was 53%, while wind speed was non-significantly negatively correlated (r = 0.465) as shown in 55b.

The relationship between temperature and whitefly population in the forest zone is shown in Figure 56. The whitefly population had a significantly positive relationship with temperature (r= 0.210), and its contribution to regression (R2= 0.044) was 4.4 percent. The relationship between relative humidity and whitefly population, as shown in Figure 57 below, has a significantly positive Pearson correlation (r=0.195) and a 3.8 percent contribution to regression (R2=0.038). Similarly, rainfall exhibited a positive Pearson correlation (r=0.737), and its contribution to regression (R^2=0.643) was 64 percent in the same agro-ecological zone (Fig. 58), and wind speed exhibited a negative Pearson correlation (r= -0.201) and its contribution to regression (R^2= 0.040) was 4 percent, as shown in Figure 59 below.

Figures 60, 61, 62 and 63 show the relationship between the whitefly population and weather parameters in year one in the transition zone. Temperature, relative humidity, and wind speed all had a negative Pearson correlation with whitefly population, whereas rainfall had a positive correlation.

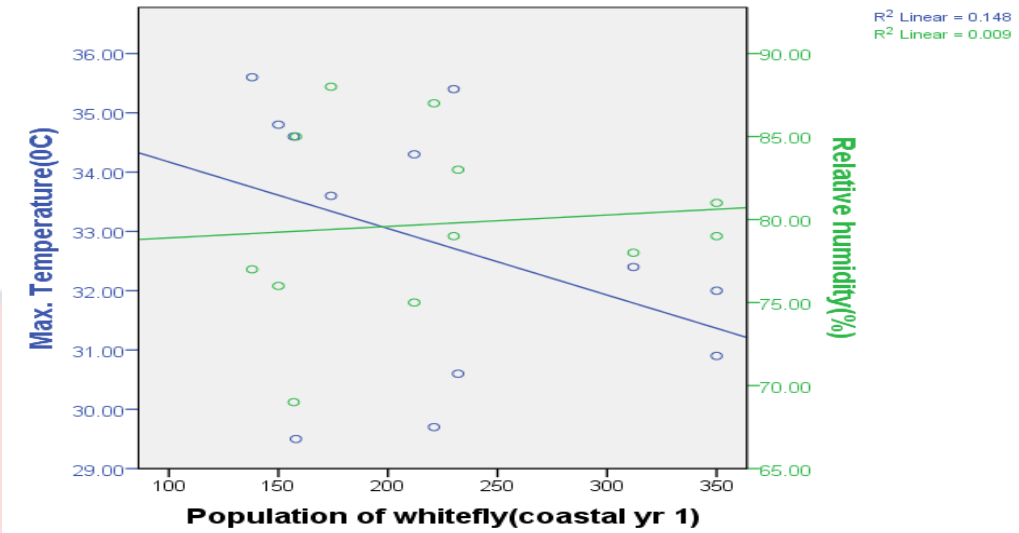

Figure 55a: Relationship between population of whitefly with relative humidity and temperature in the coastal zone in year one

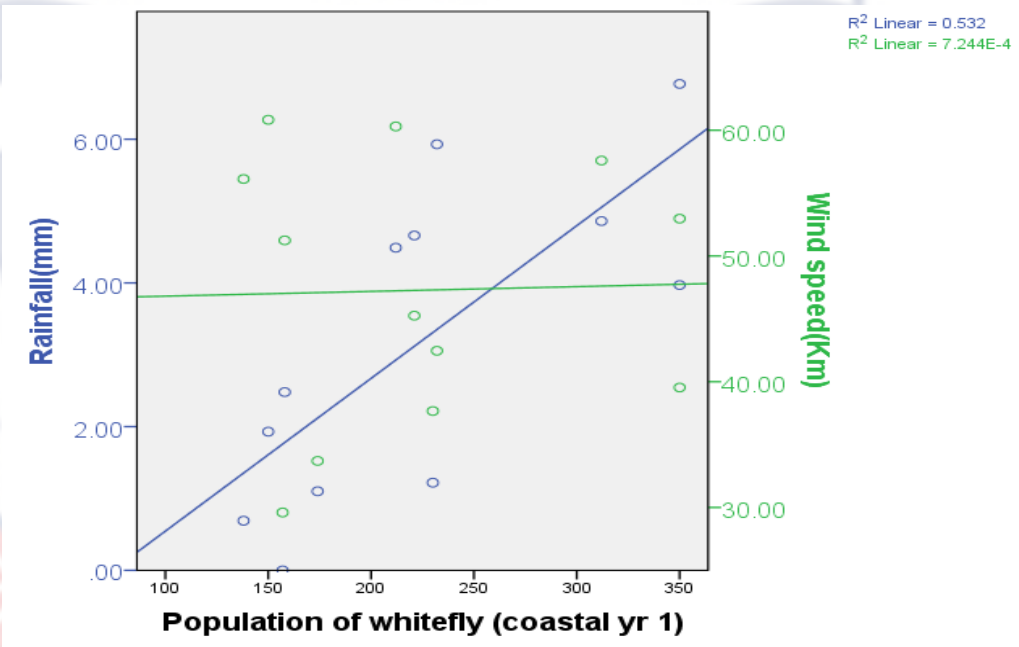

Figure 55b: Relationship between population of whitefly with rainfall and wind speed in the coastal zone in year one

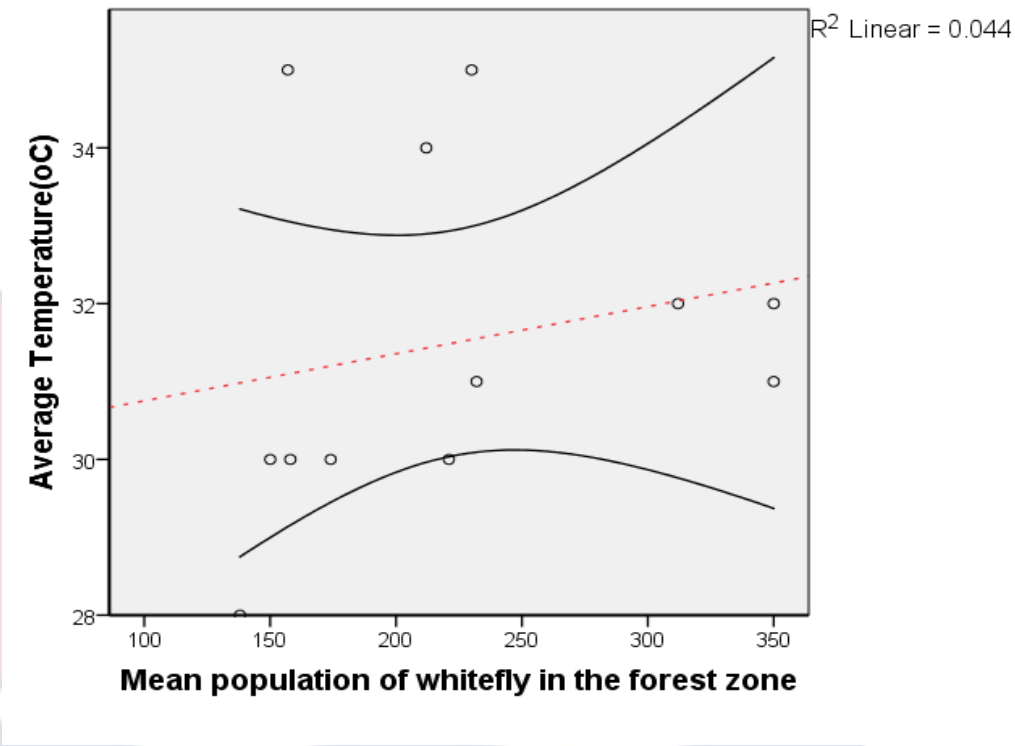

Figure 56: Relationship between population of whitefly with temperature in the forest zone in year one

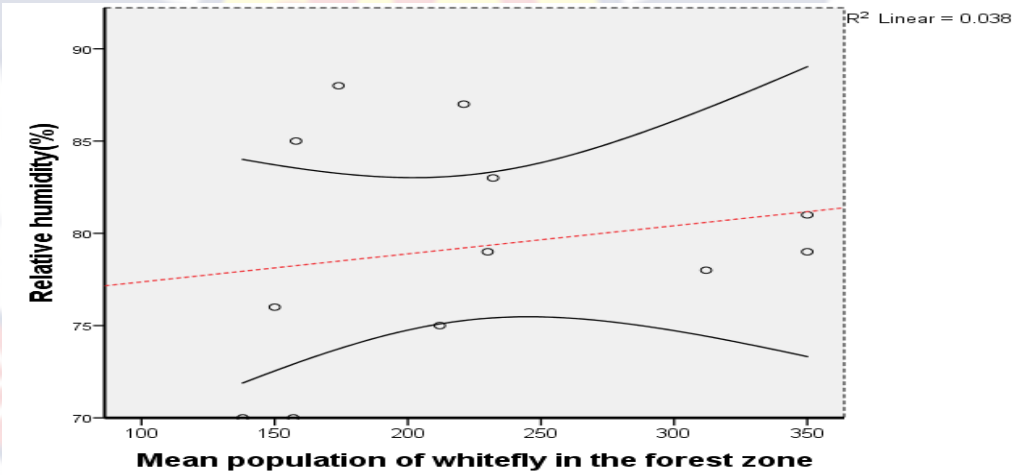

Figure 57: Relationship between population of whitefly with relative humidity in the forest zone in year one

Figure 58: Relationship between population of whitefly with rainfall in the forest zone in year one

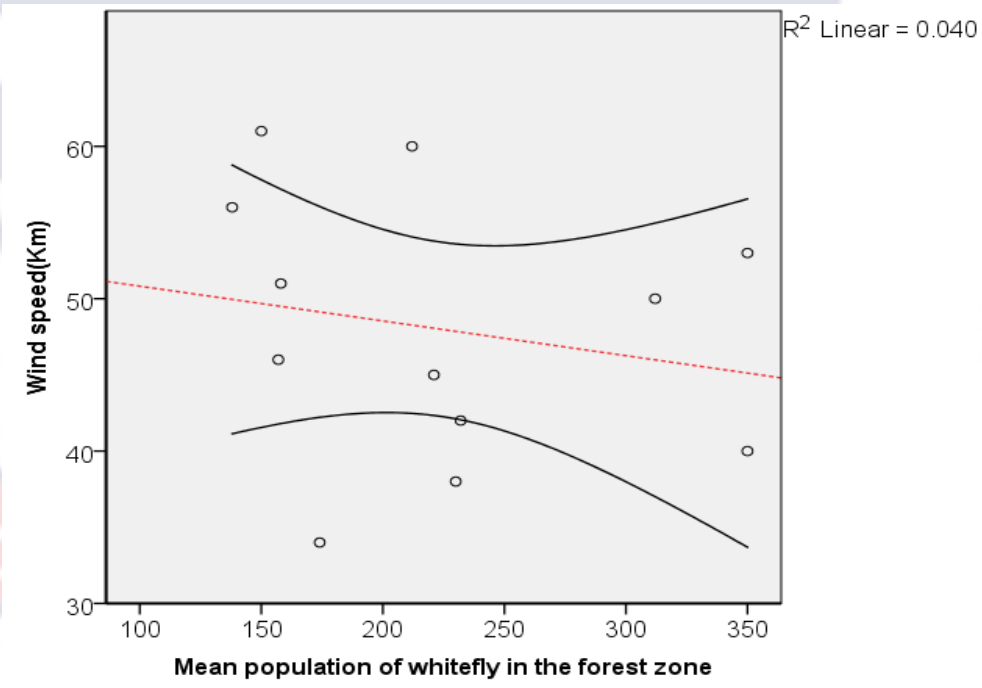

Figure 59: Relationship between population of whitefly with wind speed in the forest zone in year one

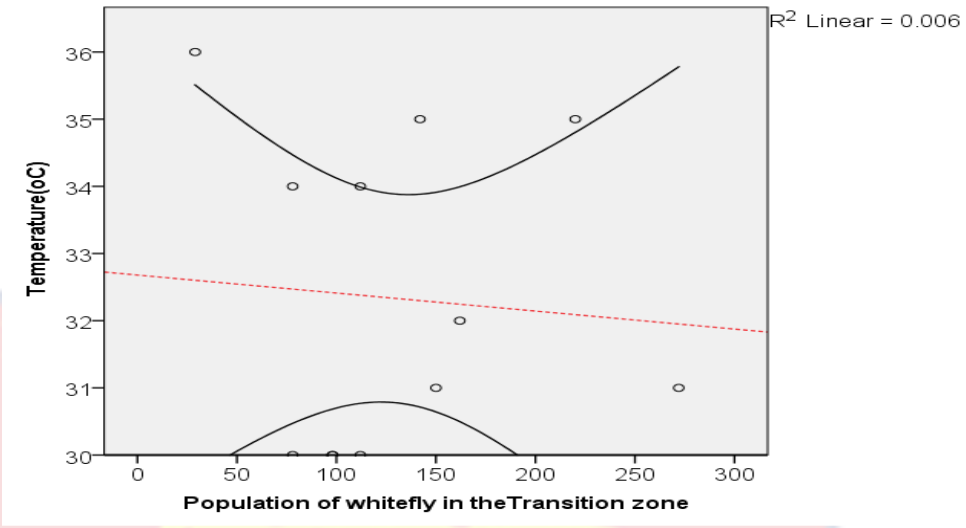

Figure 60: Relationship between population of whitefly with temperature in the transition zone in year one

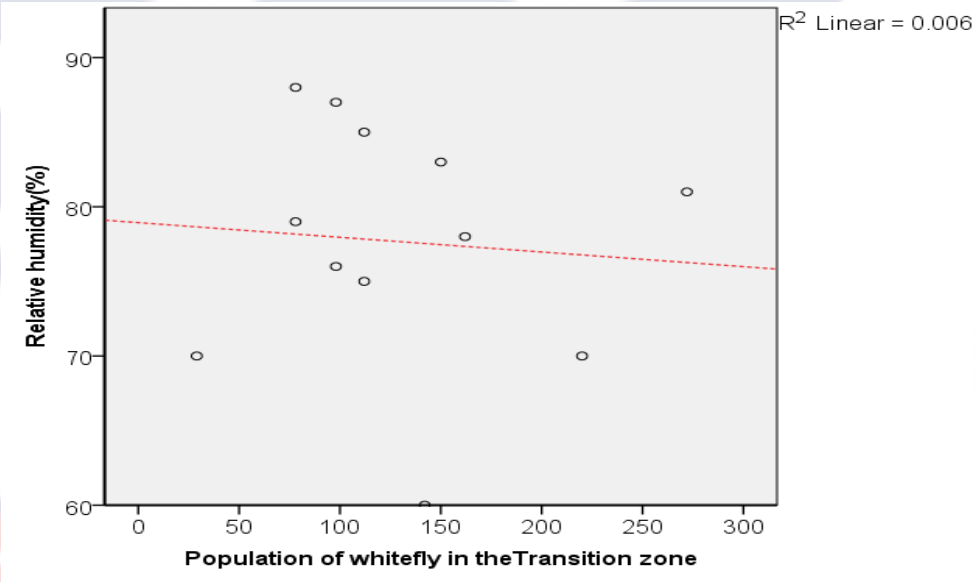

Figure 61: Relationship between population of whitefly with relative humidity in the transition zone in year one

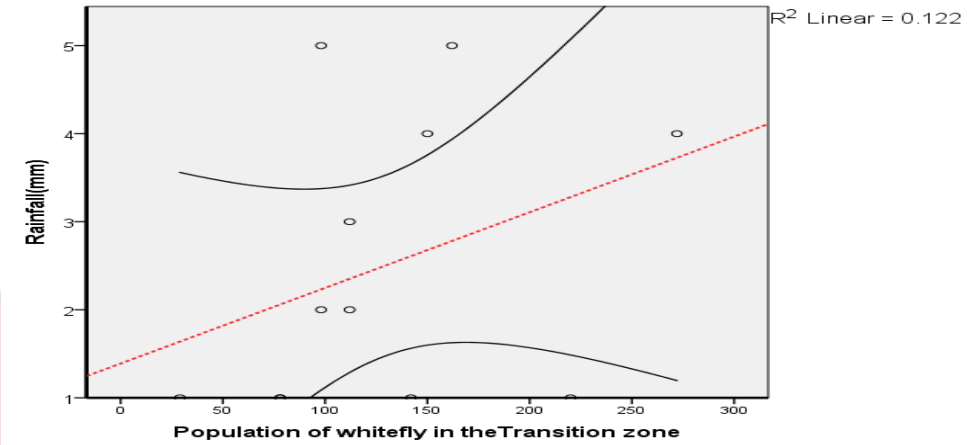

Figure 62: Relationship between population of whitefly with rainfall in the transition zone in year one

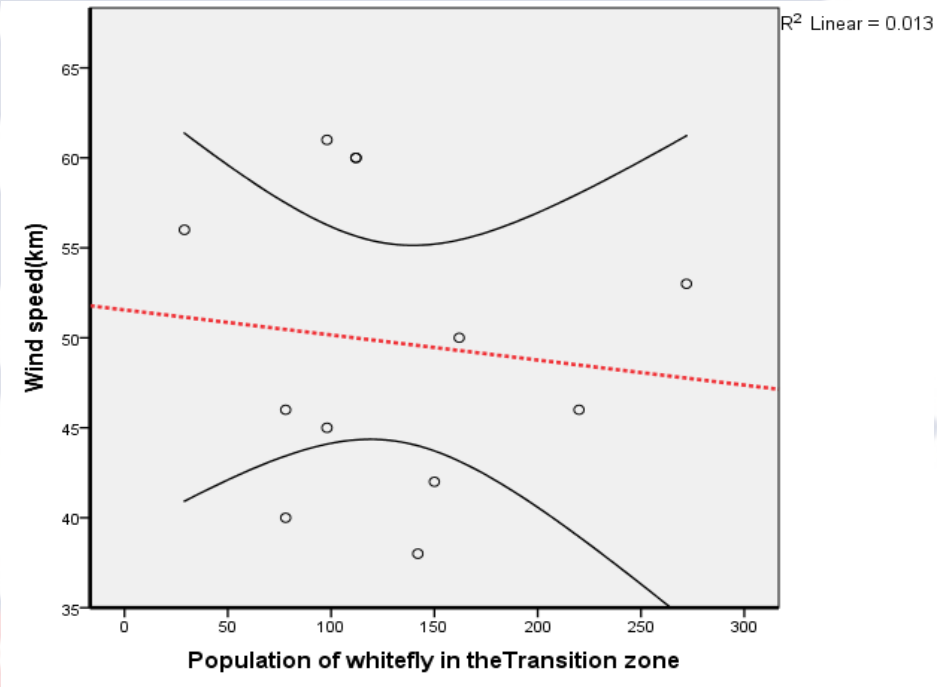

Figure 63: Relationship between population of whitefly with wind speed in the transition zone in year one

4.5.8 Relationship between Whitefly and Weather Parameter in year two

Figure 64a shows that rainfall was significantly positively correlated (r=0.131) with whitefly population and regression (R^2=0.017) was 1.7% while wind speed also had a positive correlation relationship with whitefly population in the coastal grassland. In the coastal grassland zone, the population of whitefly exhibited a significantly weak negative and linear relationship with maximum temperature (r = -0.154), and its contribution to regression (R^2 =0.024) was 2.4% while relative humidity had a non-significant negative Pearson correlation (r = -0.191), and its contribution to regression (R^2=0.036) as shown in Figure 64b.

Temperature had a negative Pearson(r=-0.158) correlation with whitefly population in the forest zone, and its contribution to regression (R^2=0.025) was 2.5%, as shown in Figure 65 below. Similarly, relative humidity had a positive Pearson correlation (r=0.123) and contributed 1.5% to regression (R^2=0.015) (Figure. 66). Rainfall, on the other hand, had a positive Pearson correlation (r=0.422) and a 17.8% contribution to regression (R^2=0.178) (Figure 67), whereas wind speed had a negative Pearson correlation (r=-0.342) and an 11.7 percent contribution to regression (R^2=0.117) as shown in Figure 68.

Figures 69, 70, 71, and 72 show the relationship between whitefly and weather parameters in the transition zone. Temperature exhibited a weak positive Pearson correlation (r=0.041) in the study, and its contribution to regression (R^2=0.002) was 2% with the whitefly population. Relative humidity also had a weak positive Pearson correlation (r= 0.193) and a 3.7% contribution to regression (R^2= 0.037). Rainfall also had a very weak positive

Pearson correlation with whitefly population, whereas wind speed had a positive Pearson correlation (r= 0.334) and contributed 11.2% to regression (R^2=0.112).

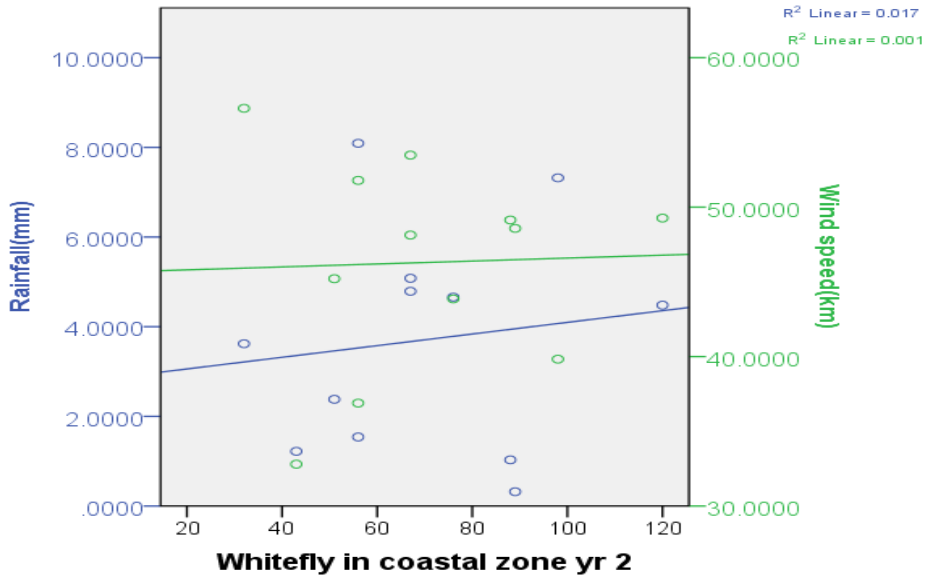

Figure 64a: Relationship between population of whitefly in coastal zone with rainfall and wind speed in year two.

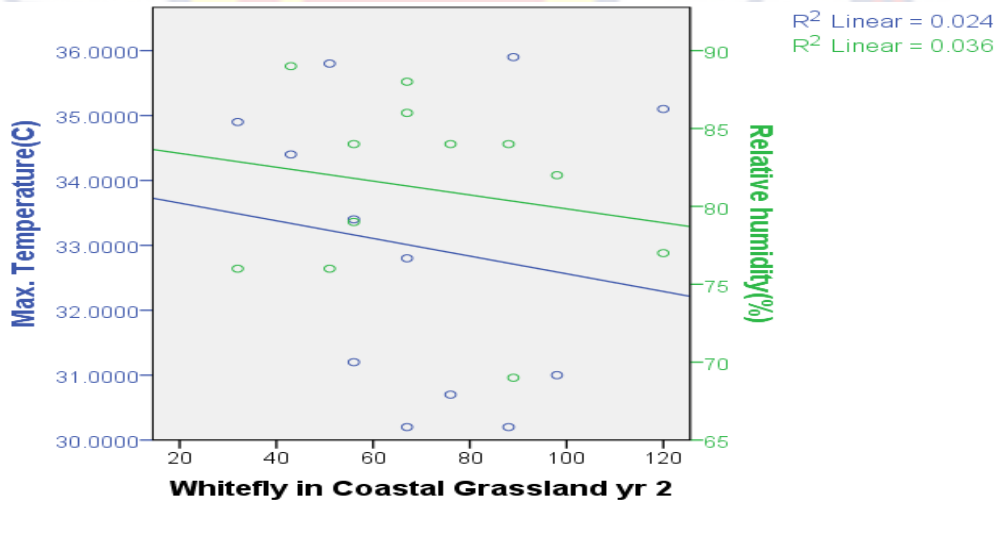

Figure 64b: Relationship between population of whitefly in coastal zone with max temperature and relative humidity in year two.

Figure 65: Relationship between populations of whitefly with temperature in the forest zone in year two.

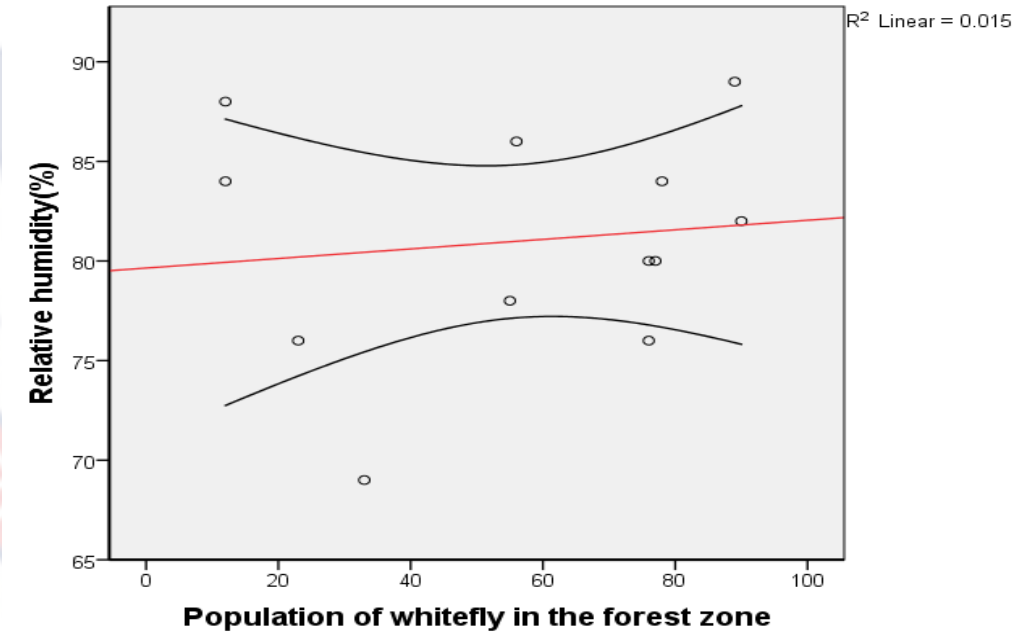

Figure 66: Relationship between populations of whitefly with relative humidity in the forest zone in year two

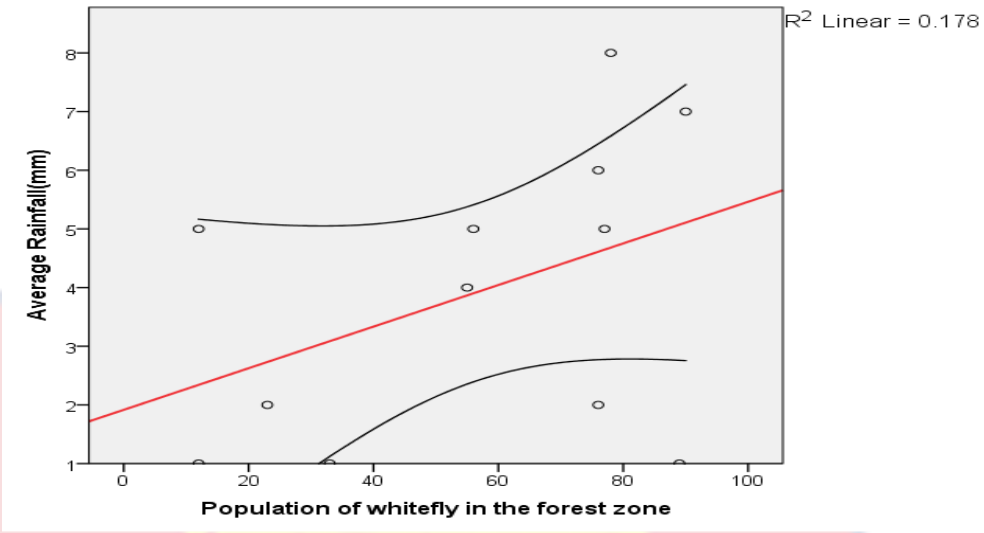

Figure 67: Relationship between populations of whitefly with rainfall in the forest zone in year two

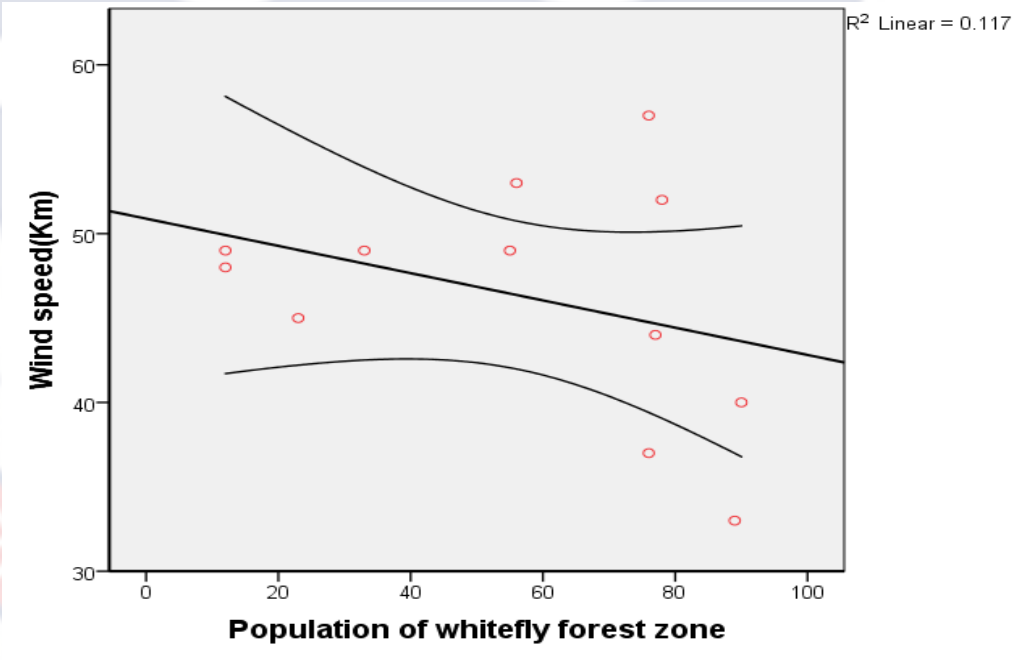

Figure 68: Relationship between populations of whitefly with wind speed in the forest zone in year two

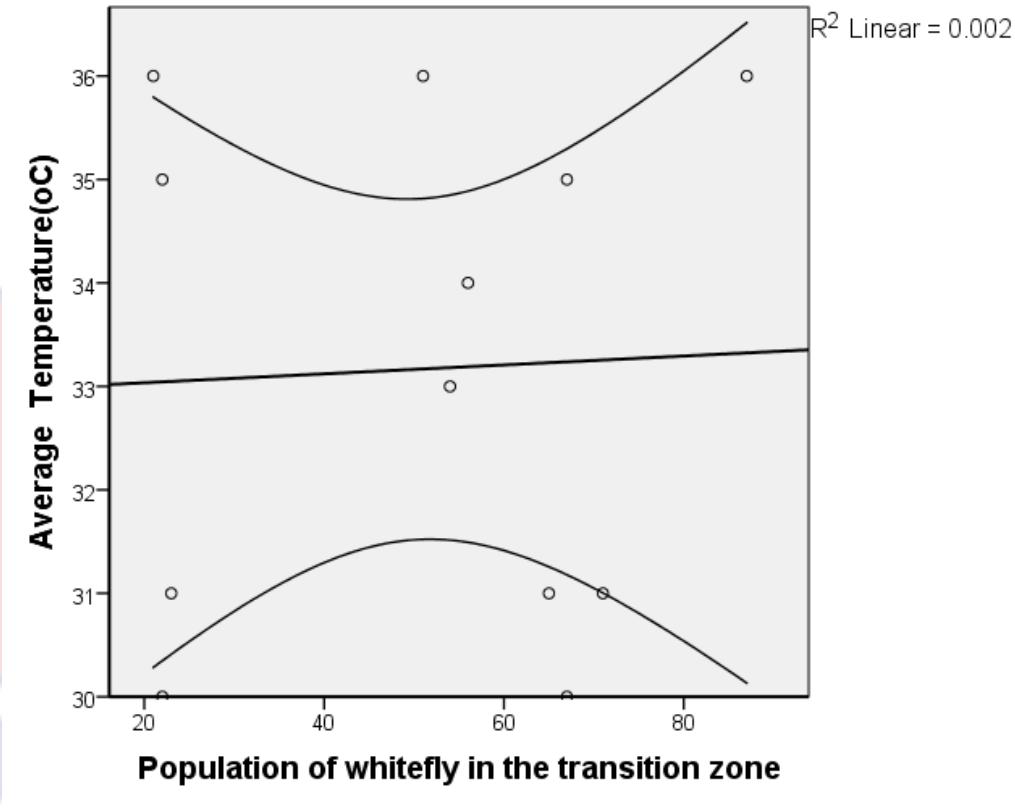

Figure 69: Relationship between populations of whitefly with temperature in the transition zone in year two

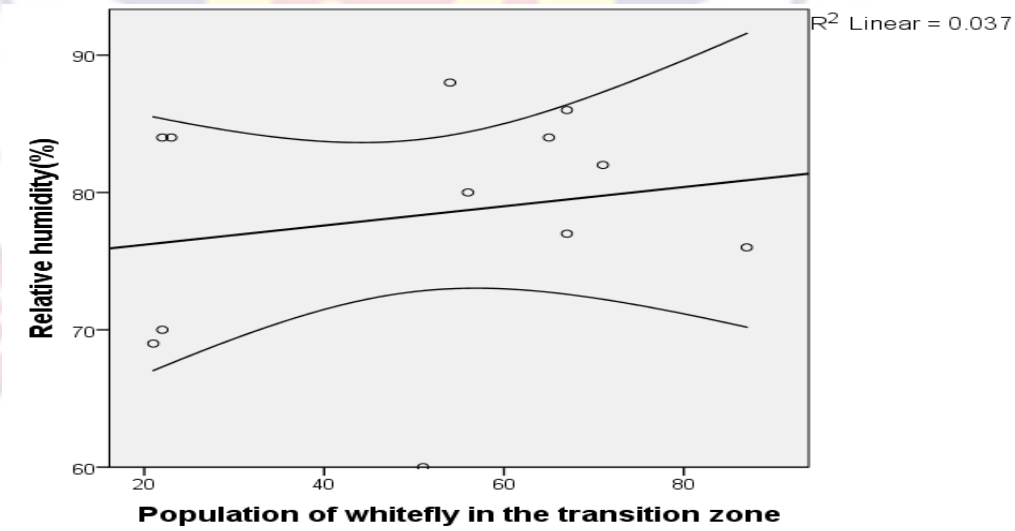

Figure 70: Relationship between populations of whitefly with relative humidity in the transition zone in year two.

Figure 71: Relationship between populations of whitefly with rainfall in the transition zone in year two

Figure 72: Relationship between populations of whitefly with wind speed in the transition zone in year two.

Table 8: Correlations between whitefly and weather parameters in three agro-ecological zones in year one

AGRO-ECOLOGICAL ZONES		Average Temperature	Relative humidity	Rainfall	Wind speed
Coastal	Pearson Correlation	-.385	.097	.729	.027
	Sig. (2-tailed)	.217	.765	.007	.934
	N	12	12	12	12
Forest	Pearson Correlation	.210	.195	.737**	-.201
	Sig. (2-tailed)	.512	.543	.006	.531
	N	12	12	12	12
Transition	Pearson Correlation	-.077	-.808	.350	-.113
	Sig. (2-tailed)	.813	.416	.265	.726
	N	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Table 9: Correlation between whitefly and weather parameters in three agro-ecological zones in year two

AGRO-ECOLOGICAL ZONES		Average Temperature	Relative humidity	Rainfall	Wind speed
Coastal	Pearson Correlation	-.154	-.191	.131	.036
	Sig. (2-tailed)	.634	.553	.684	.913
	N	12	12	12	12
Forest	Pearson Correlation	-.158	.123	.422	-.342
	Sig. (2-tailed)	.623	.704	.172	.276
	N	12	12	12	12
Transition	Pearson Correlation	.041	.193	.049	.334
	Sig. (2-tailed)	.901	.548	.880	.288
	N	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

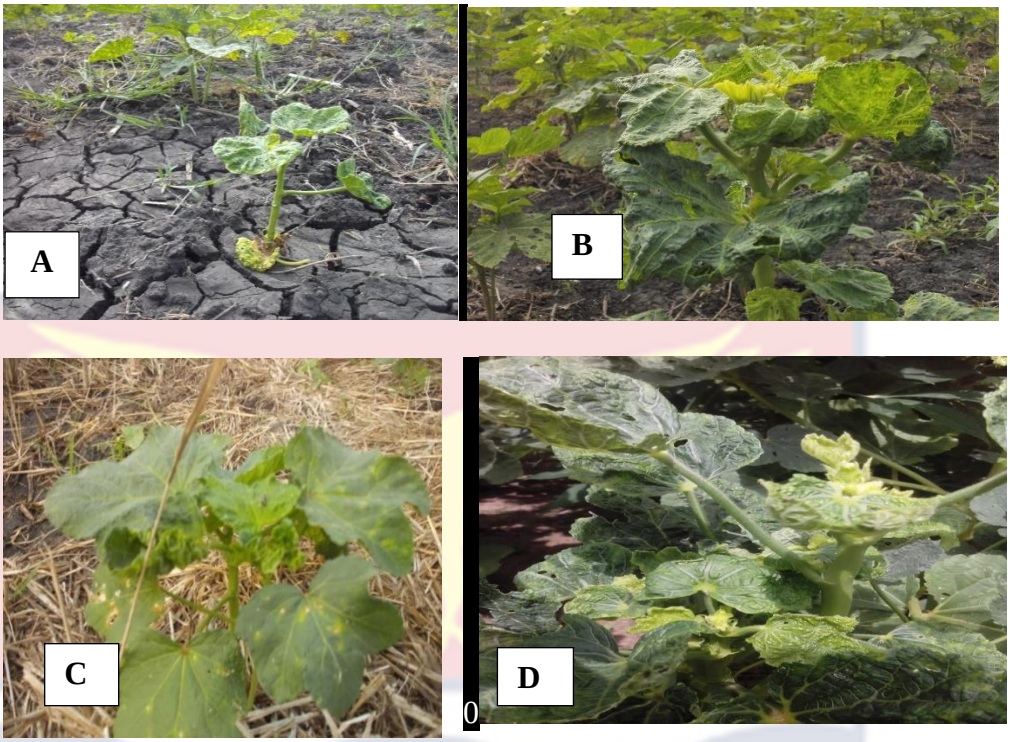

Figure 73: Symptoms of common diseases observed in the study area A. Severe leaf curling, plant stunting, and auxiliary branch proliferation B. All leaves are curled, and the plants are slightly stunted. C. A few top leaves have curled. D. All leaves are curled, and the plants are slightly stunted. Field data: Adzim (2019)

4.6 Discussion

The disease incidence and severity varies across the agro-ecological zones is high. Although it was prevalent in both the dry and wet seasons, incidence and severity were more severe in the minor season than in the major season, as reported by Asare-Bediako *et al.* (2018). The coastal grassland recorded the highest disease incidence and severity in the various districts than the forest and transition zones. For instance, the mean disease incidence recorded in the coastal savannah agro-ecological was 77.15%, followed by the transition and the forest zones with a mean incidence of 71.35% and 69.82%, respectively, for the first year. Similarly, in the second year, the coastal savannah recorded the highest mean disease incidence of 77.69% followed by the forest and transition zone with 73.78% and 73.88%, respectively. This result agrees with Asare-Bediako *et al.* (2018), who observed high disease incidence and severity in the coastal savannah compared to forest and transition zones, with a mean incidence of 69.7, 64.7 and 36.4, respectively, in the Central region of Ghana. Despite the high level of the disease observed in the coastal area, there were variations in the level of infection with respect to individual farms, villages and districts. Symptom types observed during the field survey were curling of a few top leaves, curling of top leaves and slight stunting of plants, all leaves curled and slightly stunting of plants and severe curling of leaves, stunting of plants and proliferation of auxiliary branches. The most common symptoms of observed in the field survey on okra across the agro-ecological zones for the major and minor season was the curling of a few top leaves of the okra, while stunting of plants and proliferation of auxiliary branches were the least symptoms observed (Figure 73). These

symptoms were also reported by Sayed *et al.* (2014) in okra leaf curl disease. Similarly, Askira (2012) and Asare-Bediako *et al*., (2018) also observed such symptoms as common features exhibited by virus-infected okra.

The disease incidence ranged from 69.91% to 80.62%, while the mean severity index ranged from 1.18 to 1.44 for the three agro-ecological zones. The disease prevalence supports the findings of Asare-Bediako *et al*., (2014), which identified okra leaf curl disease as a major viral disease of okra in Ghana. The mean disease incidence recorded across the agro-ecological zones in the Volta was generally higher than those reported by Asare-Bediako *et al*. (2018) in the three agro-ecological zones in the Central region, which recorded mean disease incidence in the range of 36.4% to 69.7%. The disease incidence recorded is consistent with other findings where high disease incidences have been recorded (Asare-Bediako *et al*., 2014; Asare-Bediako, 2018; Alegbajo *et al*., 2008; Ghanem, 2003). However, the mean disease severity index of 1.18 to 1.44 recorded in the study areas is lower compared to 0.68 to 2.0 reported by Asare-Bediako *et al*. (2018) in the Central region. The high disease incidence recorded in this study is consistent with Atiri and Ibidapo (1989) and Askira (2012). The high disease incidence observed across the three agro-ecological zones may be attributed to the monocropping system of farming practiced by the farmers. It was also observed that the farmers, after harvesting, do not clear their fields, leaving behind infected plants which serve as a source of inoculum for the next planting season, agreeing with the results of Aliyu (2014), where variations in mean disease incidence were observed in a rain forest area of Nigeria within the same location and agroecology.

The study has also shown high population of whitefly (*Bemisia tabaci*) vectors of 38,504 throughout the study area; their population is largely influenced by agronomic practices and weather conditions which contribute significantly to their occurrence (Jatav *et al*., 2018; Byrne and Devonshire 1991). According to Byrne (1991), weather parameters such as temperature, wind speed, rainfall and relative humidity play important roles towards the population dynamics of whiteflies. The population of whiteflies observed in the dry season was 25,978 whiles that of the wet season was 12, 526 this suggests that their population was higher during the dry season than the wet season. The high incidence of whiteflies in the dry season has also been recorded by Asare-Bediako *et al.* (2018). This observation could be due to high temperatures and low relative humidity during such periods high temperature which makes the whitefly very active (Azizi *et al*., 2008). High temperature and low relative humidity have been found to increase the whitefly population, culminating in an increase in okra leaf curl disease incidence and severity (Oyelade and Ayansola, 2015; Singh *et al*., 2013; Azizi *et al*., 2008). Additionally, the lack of rainfall in the dry season could have contributed to the low numbers of whitefly population since high rainfall and high humidity have been found to be responsible for a negligible population of whiteflies (Thriveni, 2019)

According to Venkataravanappa *et al*. (2014), the minimum number of whiteflies required to induce 100% infection is 10/plant, although a single whitefly can transmit the okra leaf curl virus effectively. The mean population of whiteflies in the coastal grassland zone was higher than forest and transition zone, which could have resulted in a higher incidence and severity of OLCD.

The study showed that there is a significant and negative linear correlation between the incidence of okra leaf curl disease and the whitefly population in the first-year cropping season for both the major and minor seasons. In the second year, however, there was a non-significant and positive correlation between okra leaf curl disease and whitefly position. These present findings are in agreement with N'guessan (2001), who reported non- non-significant and positive correlation between the whitefly population and okra leaf curl disease.

In this study a simple correlation between whitefly population and average temperature showed that population had a no significantly moderate negative and linear relationship with maximum temperature in year one and two seasons at the coastal grassland zone. However, in the forest agro-ecological zone, it was observed that in the first year, there was a positive correlation and a negative relationship between whitefly and temperature. In the transition zones, the relationship was negative in the first year but positive in the second year. The negative correlation of temperature and whitefly population indicates that, the population of the whitefly had a significant impact when the temperature was changed. High temperatures, therefore, reduced the population of whiteflies. This finding does agree with the report of several authors (Khan, 2019; Vedika and Abhishek, 2019) in which temperature had a negative and linear relationship with the whitefly population. This could possibly be due to the fact that the immature stages of *B. tabaci* were desiccated when the temperature was high as suggested by Khan (2019). In the study area, the mean maximum temperature was 32.8^{o}C, which is above the maximum temperature of 29.14^{o}C required for effective

whitefly population development, as indicated by Vedika and Abhishek (2019). The positive correlation of whitefly and temperature in forest and transition zones may suggest that when there is an increase in temperature, there is a corresponding increase in the population of whiteflies in these agro-ecological zones. The population of whiteflies was highest in the coastal grassland with a mean of 157.50 and a negative relationship with temperature, whiles the forest zone recorded 100.36 and in the transition zone 100.16 with positive and negative relationships respectively in the first cropping seasons.

Relative humidity exhibited a non-significant influence on *B. tabaci* population and there was positive correlation, that is, as relative humidity increases *B. tabaci* population also increases in the coastal grassland and forest zones. During the second year, relative humidity had a non-significant influence on whitefly population, but a negative correlation (as relative humidity increased, the whitefly population decreased) was observed in both coastal and transition zones. The correlation in the forest zone is positive, as it was observed in the first year. According to Kaushik (2012), the relative humidity gradient had a positive influence on the whitefly population. Similar research by Ali *et al*. (2005) indicated that the whitefly population decreased with increased relative humidity, suggesting that increased relative humidity inhibits the activities of whiteflies (Aktar *et al*., 2008).

Rainfall is another abiotic factor that has a relationship with the whitefly population. In this study, rainfall had a significant positive influence on whitefly population in the coastal and a non-significant positive influence in the transition zone. This implied that increased rainfall increased the whitefly population. In the forest zone, there was a negative correlation in

year one. However, a non-significant positive correlation was observed in coastal, forest and transition zones with whitefly population and rainfall during the second year of the study. The positive correlation is in contrast with findings by Henneberry *et al*. (1995) that the adult whitefly population declined after rain flashes. In addition, during rain, whitefly eggs and nymphs were reduced as a result of rainfall, as reported by Castle (2001).

The temperature had a non-significantly weak negative correlation with the whitefly population in the coastal, forest and transition zones. This implies that the percentage whitefly population was less dependent on temperature. When there is an increase in temperature, there is a decrease in the population of whiteflies across the entire agro-ecological zone. The high-temperature results in a decrease in the pest population, possibly due to the fact that the immature stages of *B. tabaci* were desiccated when the temperature was high (Khan, 2019).

CHAPTER FIVE

5.0 PHENOTYPIC SCREENING OF OKRA ASSESSIONS RESISTANCE TO OKRA LEAF CURL DISEASE UNDER FIELD CONDITIONS

5.1 Introduction

Okra (*Abelmoschus esculentus* L.) is a popular vegetable crop in tropical and subtropical areas around the world (Oppong-Sekyere *et al.*, 2012). Okra is widely grown in Ghana during both the rainy and dry seasons, primarily by small-scale farmers who rely on it for income (Asare-Bediako *et al.*, 2016). After analyzing several okra genotypes, Sugri *et al.* (2015) estimated yields ranging from 1.27 to 9.5 tons per acre. Other assessments estimate that national production is roughly 120,000 Mt on 19,500 ha of arable land with a 5.5 Mt/ha yield potential (Oppong-Sekyere *et al*. 2012). However, the crop's yield is low due to a lack of better cultivars, biotic and abiotic stressors (Asare-Bediako *et al.*, 2016). When compared to East Africa (6.2 t ha^{-1}) and North Africa (8.2 t $ha^{-1)}$, the average productivity of okra in West Africa (2.5 t ha^{-1}) is quite low (Cudjoe *et al.*, 2005). The most significant obstacles to attaining the okra yield potential in Ghana are insect pest infestation and diseases (Asare-Bediako *et al.*, 2014). Of the several diseases that infect okra, viral diseases, particularly those caused by Begomoviruses (Familiy: Geminiviruses), are of paramount importance. These Begomoviruses cause okra leaf curl disease are transmitted by the whitefly (*Bemisia tabaci*) (Brown, 2007). The disease presents a major challenge to okra production in Ghana and West Africa in general (Tiendrébéogo, 2010). A yield loss of up to 100% has been reported as a result of OLCD (Atiri, 1984). The damaged

plant's quantity of marketable fruits, fruit length, fruit diameter, and fruit weight are all substantially reduced (Asare-Bediako *et al.*, 2018). Insecticide-based management of whitefly is difficult and ineffective, host resistance is the most preferred technique (Atiri, 1984).

Ayam *et al.* (2018), identified several okra varieties which were moderately to highly resistant to enation leaf curl virus. Udengwu and Dibua (2014), on the other hand, examined 23 okra genotypes in Nigeria for resistance to okra leaf curl and okra mosaic virus disease, finding yield decreases of less than 10% in the nine varieties (Ebi Ogwu, Ojo Ogwu, Tongolo, VLO, Oru Ufie, and Ogolo) indicating some level of resistance. Similarly, some okra genotypes of Ghanaian origin (GH3760, GH2052, GH5332, UCC6, GH5302, GH5793, and GH2063) had minor symptoms during both the wet and dry seasons' trials (Asare *et al*., 2016). The genotype GH5332 with moderate symptoms had the best fruit production of 11.88 t ha-^{1}. Disease severity and yield were higher in the minor season than in the major season in all okra genotypes. Furthermore, Oppong-Sekyere *et al*. (2012) identified three okra cultivars (Atuogya, Wun mana, and Sheo mana) with high resistance to mosaic and leaf curl diseases. The inability of farmers to manage the disease with pesticides and inefficient management of the vector is a leading factor to the problems of okra farming. This study screened different okra accessions and their resistance to OLCD and its whitefly vector under field conditions in order to identify additional sources of cultivar resistance to boost okra production.

5.2 Main Objective of Study

The study evaluated the resistance of okra accessions to okra leaf curl disease and associated whitefly vector. Specific objectives include to;

1. screen 21 okra varieties for resistance to okra leaf curl disease in/during two seasons.
2. evaluation of whitefly infestation resistance in the host
3. determine the disease progression curves for the okra varieties.
4. determine the yield of each okra variety

5.3 Materials and Methods

5.3.1 Study Location and Land Preparation

The field trial was conducted at Dabala Senior High Technical School Agriculture Demonstration Farm in the Volta region of Ghana from October 2020 to January 2021 for the minor rainy season and February-May 2021 for the major season. Twenty-one (21) okra accessions were screened for viral disease incidence and severity, and associated major insect pests. The study area was the coastal grassland agro-ecological zone, which is known to be an okra leaf curl disease endemic area (Personal communication, MoFA). With the help of a tractor, the field was ploughed to a beautiful tilt and harrowed.

5.3.2 Experimental setup and Design

The okra accessions were pre-soaked with lambda before being planted in a Randomized Complete Block Design (RCBD) with three replications on a 13m × 56m plot. Based on the treatments and accessions combinations, each replication had 21 plots. A 1-meter space was left between and within plots, and a 2-meter space was left between blocks/replications. Four okra seeds

were sown for each genotype. On each treatment plot, ten plants of each accession were planted. The plants were reduced to two plants per hill, with a spacing of 0.6 m x 0.6 m and a planting depth of not more than 0.5 cm. Watering and weed control were done as needed, with no insecticide application.

5.3.3 Data Collection and Analysis

The severity and incidence of OLCD in 21 okra varieties were assessed at 2, 6, and 10 weeks after sowing (WAS) based on disease symptoms as described by Asare-Bediako *et al* (2018). The okra varieties are; Adzreley, Fetre, Shortee, Kobinami, 1056, 2025, 1052, 2003, 1097, 1014, 1020, 1090, 2033, 1069, 1093, 1001, 1077, 1048, 1012, 2031, and Avalavi. The mean ordinal scores were established by observing all ten plants from each genotype and scoring them. To measure the disease incidence, severity, fruit yield, percentage disease index (PDI) of the OLCD and whitefly population, observations was made on ten plants in each plot from each replication.

Fruits were harvested every three days intervals from each variety and weighed using a very sensitive weighing scale. The average weight was determined and the yield calculated in tons per hectare.

Disease symptoms were observed exactly 2 weeks after sowing as described in the table 10 below (Alegbejo, 1997): A five-point scoring scale used in the assessment of OLCD severity.

Table 10: Description of symptoms of OLCD

SCORE	DESCRIPTION OF SYMPTOMS
0	No symptoms
1	Top leaves curled
3	Top leaves curled and slight stunting of plant
5	All leaves curled, twisting of petioles and slight stunting of plants
7	Severe curling of leaves, twisting of petioles, stunting of plants and proliferation of auxiliary branches

The score sheet was cleaned after data collection by revising estimates for the incidence and severity scores. The incidence of diseases was determined using the equations.

$$DI(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants sampled}} \times 100 \ldots\ldots\ldots\ldots 1$$

$$Disease\ severity(\%) = \frac{\sum n}{\text{N X M}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Where;

N = Total number of plants assessed.

$\sum$ n = Sum total of individual rating.

M = Highest score on the severity scale.

Using the disease severity score of OLCV, the Percent Disease Index (PDI) was calculated as a proportion of all 30 plants in the replication. The formula was used to determine the PDI.

$$PDI(\%) = \frac{\text{Numerical rating X 100}}{\text{Total no. of obsevation x Max. disease grade}}$$

Resistance/ susceptibility rating of the okra genotypes to the disease was done according to Ali *et al*., (2005) where 0% = immune, 1% – 10% = highly resistant, 11% – 25% = moderately resistant, 26% – 50% = tolerant, 51% – 60% = moderately susceptible, 61% – 70% = susceptible, 71% – 100% = highly susceptible.

The area under the disease progress curve, a method developed by Campbell and Madden (1990), was used to calculate the disease's relative spread among the different kinds

$$AUDPC = \sum_{i-1}^{n-1} \frac{yi + y_{i+1}}{2} x(ti +_1 - ti$$

Where yi is an assessment of the disease (percentage, proportion, ordinal score etc) at the ith observation, ti is the time (in days, hours, etc) at the ith observation, and n is the total number of observations.

5.3.4 Estimation of whitefly numbers

The technique employed by Ellsworth *et al*. (1995) known as the "leaf turn" methodology to estimate the whitefly population was used. The whiteflies were sampled early in the morning, between 6:30 and 8:30 a.m., using Khan's (2019) approach. The okra plants were gently approached, and three leaves on the plant's upper section were flipped to count the number of adult whiteflies on the leaves, as described by Nagar *et al* (2017). The mean whitefly populations of okra cultivars recorded were classified using the method developed by Nagar *et al*., (2017) as follows: $\bar{x} \pm \sigma$

Where, $\bar{x}$ = Mean of peak population

σ = Standard deviation of insect population

Table 11: Determination of level of okra plants susceptibility to whitefly infestation

Mean insect population (Per plant/three leaves)	Category
Below $\bar{x}$ - σ	Less susceptible
$\bar{x}$ - σ to $\bar{x}$ + σ	Moderately susceptible
Above $\bar{x}$ + σ	Highly susceptible

5.4 Data Analysis

To homogenize the variances, data on disease incidence and cumulative average number of adult whiteflies per plant were transformed using angular and square root transformations, respectively, before analysis of variance (ANOVA).

DPI, AUDPC, disease severity scores, average fruit weight, and yield were analyzed using ANOVA, with the mean separated using the least significant difference (LSD) approach at a 5% level of probability. GenStat Discovery version 12 (VSN International) and IBM SPSS Statistics version 20 were used for all statistical analyses.

5.5 Results

5.5.1 Incidence of okra leaf curl disease among okra varieties

The incidence of okra leaf curl disease in 21 cultivars for both the dry and rainy seasons are shown in Table 12. The mean incidence of OLCD ranged from 11.35-78.13 %, with the disease being more prevalent during the minor season (78.13 %) compared to the major season (77.11 %). In the dry

season, the total mean incidence for 2WAS, 6 WAS, and 10WAS were 11.35 %, 30.53 %, and 78.13 %, respectively. In 2 WAS, a few of the okra did not display symptoms of OLCD, and there were no significant differences between the varieties in the dry season for 2 WAS (df=60, P=0.108), 6WAS (df=60, P=0.653), and 10 WAS (df=60, P=0.598). At the 10 WAS, the variety kobinami had the lowest incidence of 68.86%, which was not significantly different from varieties 1069, fetre, and 1093, which had mean incidences of 71.57 %, 71.75 %, and 71.57 %, respectively. Varieties 1012 and shortee had the highest incidence of 90 %, which was significantly different from Avalavi (83.86 %), Adzreley (75 %) and 1097 (77.71 %). During the wet season, however, mean incidence varied by variety and increased as development progressed from 2 to 10 weeks following sowing. In 2 WAS, there were significant differences (P=0.016) between the varieties, however there were no significant differences observed in 6 WAS (df=60, P=0.750) and 10 WAS (df=60, P=0.678). The variety shortee had the highest mean incidence (90.00%), while 1097 had the lowest incidence (66.64 %) at the 10th WAS.

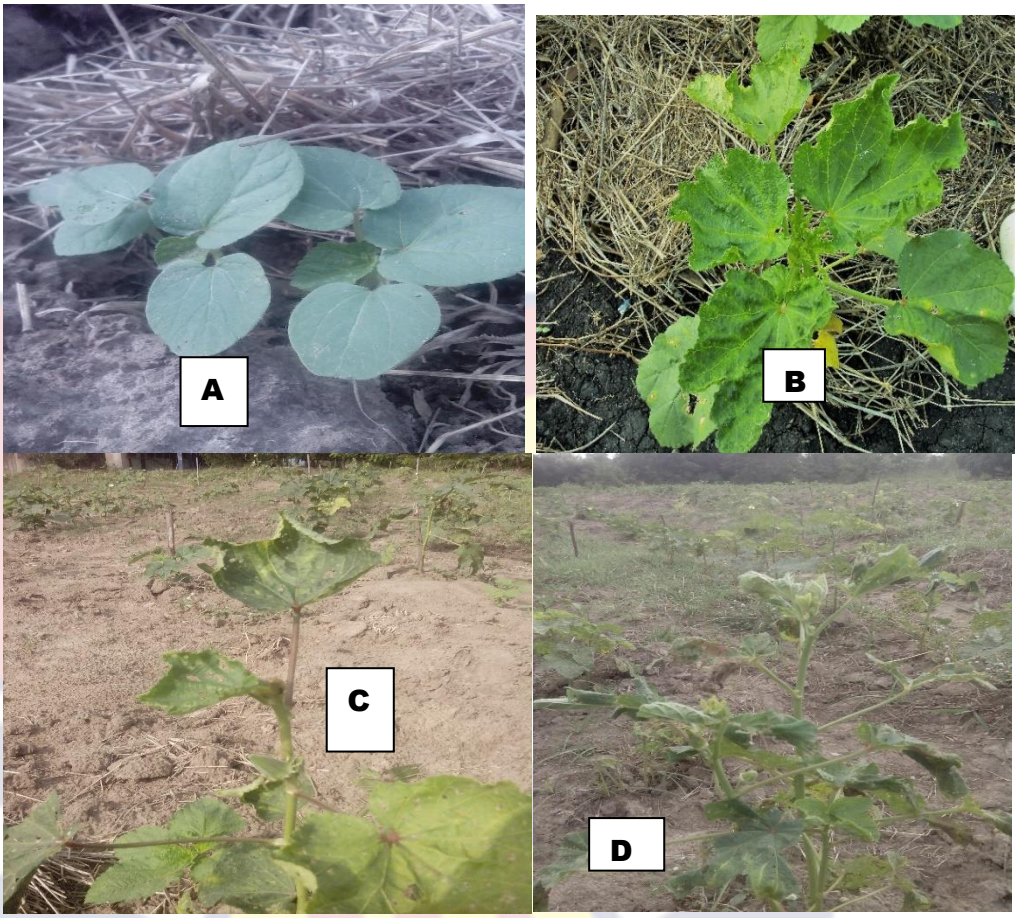

Figure 74: Symptoms of OLCD at various stages of okra plant growth in the field trial. A-No symptoms, B- Leaf curling, C- Leaf cupping, D-all leaves curled.

Table 12: Mean incidences (%) of OLCD on 21 okra genotypes under field conditions over two planting seasons

Okra variety	Mean incidence in minor season				Mean incidence in major season			
	2 WAS	6 WAS	10 WAS	Host resistance	2 WAS	6 WAS	10 WAS	Host resistance
1012	0.00[a]	27.99[ab]	90.00[b]	HS	0.00[a]	49.14[ab]	75.00[ab]	HS
1020	0.00[a]	28.78[ab]	83.86[ab]	HS	0.00[a]	36.85[a]	72.78[ab]	HS
2003	0.00[a]	42.29[b]	77.71[ab]	HS	0.00[a]	57.29[b]	77.71[ab]	HS
2033	0.00[a]	8.86[ab]	77.71[ab]	HS	0.00[a]	46.92[ab]	68.86[a]	S
shortee	0.00[a]	27.78[ab]	90.00[b]	HS	0.00[a]	59.00[b]	90.00[b]	HS
1052	6.14[ab]	25.78[ab]	83.86[ab]	HS	0.00[a]	54.99[ab]	83.86[ab]	HS
1069	6.14[ab]	38.07[ab]	71.57[a]	HS	11.07[abc]	61.22[b]	77.71[ab]	HS
1090	6.14[ab]	49.22[b]	77.71[ab]	HS	11.07[abc]	45.00[ab]	83.86[ab]	HS
Adzreley	6.14[ab]	17.22[ab]	75.00[ab]	HS	15.00[abc]	51.85[ab]	75.00[ab]	HS
1056	8.86[ab]	27.99[ab]	78.93[ab]	HS	13.08[abc]	44.92[ab]	78.93[ab]	HS
1093	8.86[ab]	30.00[ab]	71.57[a]	HS	0.00[a]	53.15[ab]	71.57[a]	HS

1014	11.07^{abc}	32.22^{ab}	77.71^{ab}	HS	8.86^{ab}	43.29^{ab}	77.71^{ab}	HS
2031	13.08^{abc}	15.00^{ab}	77.71^{ab}	HS	13.08^{abc}	52.86^{ab}	77.71^{ab}	HS
1001	15.08^{abc}	15.08^{ab}	75.00^{ab}	HS	8.86^{ab}	57.70^{ab}	75.00^{ab}	HS
2025	15.08^{abc}	45.79^{b}	77.71^{ab}	HS	15.00^{abc}	45.79^{ab}	77.71^{ab}	HS
1097	19.22^{abc}	27.29^{ab}	77.71^{ab}	HS	0.00^{a}	57.00^{ab}	66.64^{a}	S
1048	19.93^{abc}	25.78^{ab}	77.71^{ab}	HS	24.15^{bcd}	57.70^{ab}	83.86^{a}	HS
Avalavi	21.93^{abc}	45.00^{b}	83.86^{ab}	HS	24.15^{bcd}	49.22^{ab}	83.86^{a}	HS
1077	23.86^{bc}	49.22^{b}	75.00^{ab}	HS	41.07^{d}	49.22^{ab}	75.00^{a}	HS
fetre	26.07^{bc}	40.78^{b}	71.75^{a}	HS	28.78^{bcd}	53.07^{ab}	71.57^{a}	HS
Kobinami	33.00^{c}	36.14^{ab}	68.86^{a}	S	32.71^{cd}	53.07^{ab}	75.00^{ab}	HS
Mean	11.35	30.53	78.13		11.76	51.39	77.11	
LSD	22.053	40.483	16.944		23.703	20.196	17.778	
P-value	0.108	0.653	0.598		0.016	0.750	0.678	

Means in the same column bearing different letters are significantly different (P<0.05). Host resistance status was based on the final disease incidence; 0% = immune(I), 1% – 10% = highly resistant (HR), 11% – 25% = moderately resistant (MR), 26% – 50% = tolerant(T), 51% – 60% = moderately susceptible (MS), 61% – 70% = susceptible(S), 71% – 100% = highly susceptible (HS) (Ali *et al.*, 2005)

5.5.2 Severity of Okra Leaf Curl Disease among the Okra Varieties

The final mean severity index of the disease varied from season to season among the many okra cultivars. The final mean severity for the minor season was 1.666, while that of the major season was 0.891. During the minor season, the mean severity for OLCD varied among the okra varieties, with variety 2031(2.100) recording the highest severity, while varieties 1020 (1.367) and Shortee (1.367) had the least severity.

Similarly, during the major season trial, the final mean severity for the okra varieties varied, with the highest being 1077 (1.2433) and the least being varieties 1056 (0.8867) and 1020 (0.8667)

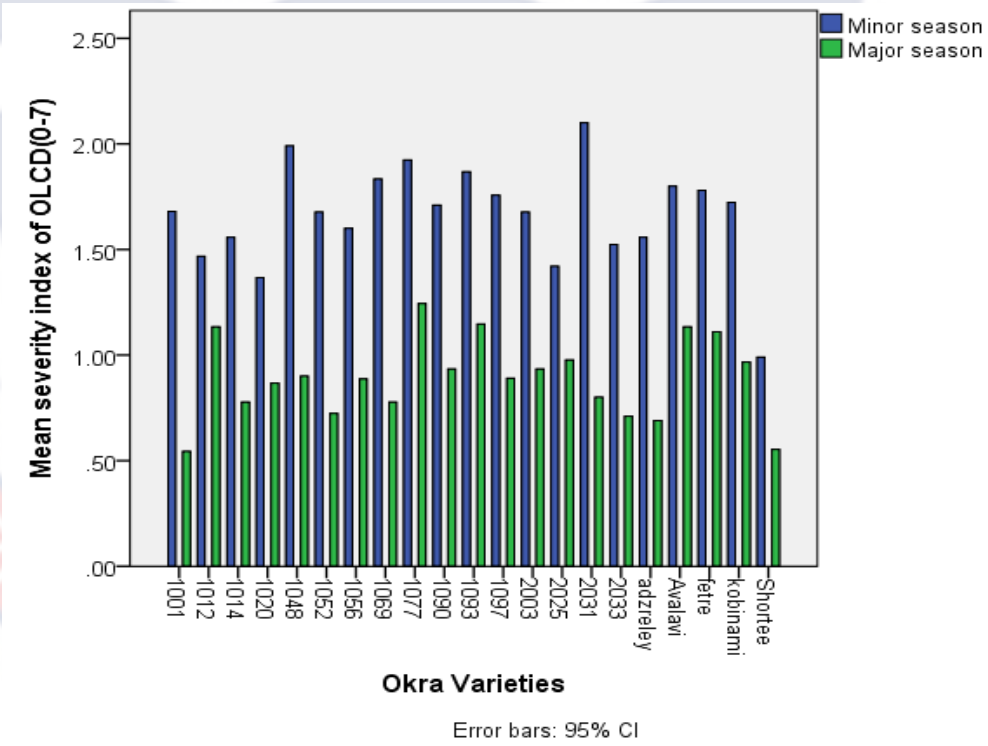

Figure 75: Okra leaf curl severity index among the 21 okra varieties tested in both the dry and wet season in the Volta and Oti region of Ghana.

5.5.3 Area under disease progress curve (AUDPC) for the different okra varieties

Reactions of different genotypes in terms of PDI values of ELCV differed at different DAS of okra. Okra varieties showed no severity of ELCV disease at 20 DAS (Figures 76 & 77). As the growth of the plant progresses in both major and minor seasons, the PDI values gradually increased from 30 DAS, which varied from 0.00% to 10.71% and then showed variable responses from 42 DAS onwards up to 70 DAS. During both major and minor seasons, no disease infection was observed after 70 days after sowing. Among the genotypes the PDI values were lower for 2033 and 1097 hence were categorized as susceptible. All the genotypes had higher PDI values and were categorized as highly susceptible. The range of area under disease progress curve (AUDPC) of the different genotypes varied greatly between susceptible and highly susceptible groups

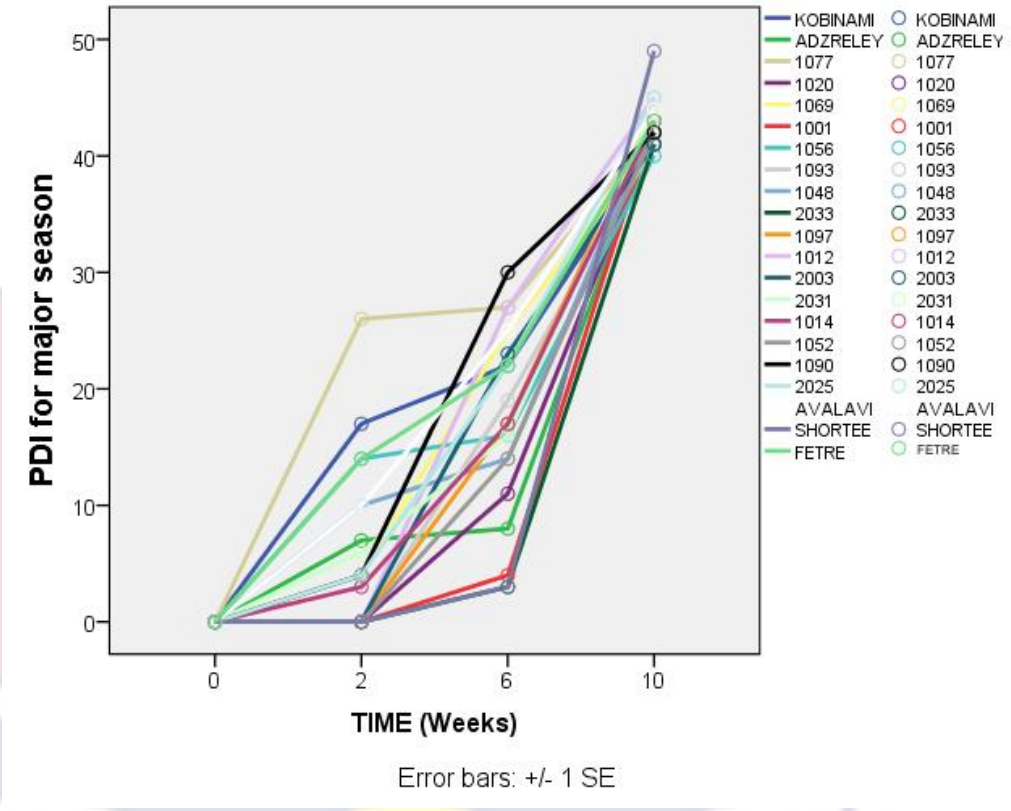

Figure 76: Disease severity of OLCV at periodic interval among twenty-one genotypes of okra in the wet season

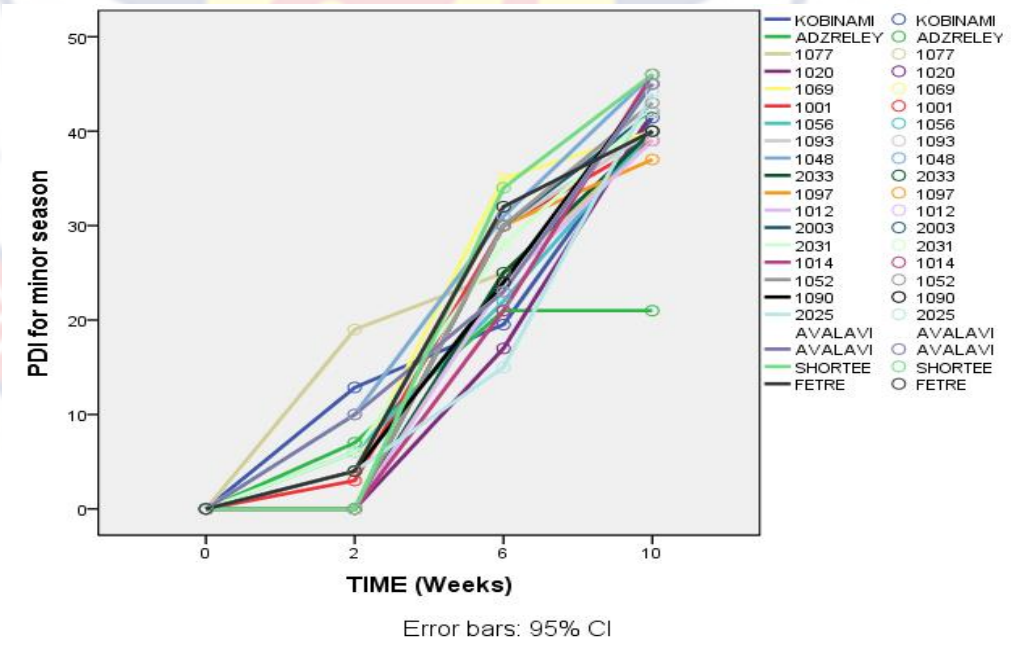

Figure 77: Disease severity of OLCV at periodic interval among twenty-one genotypes of okra in the minor season

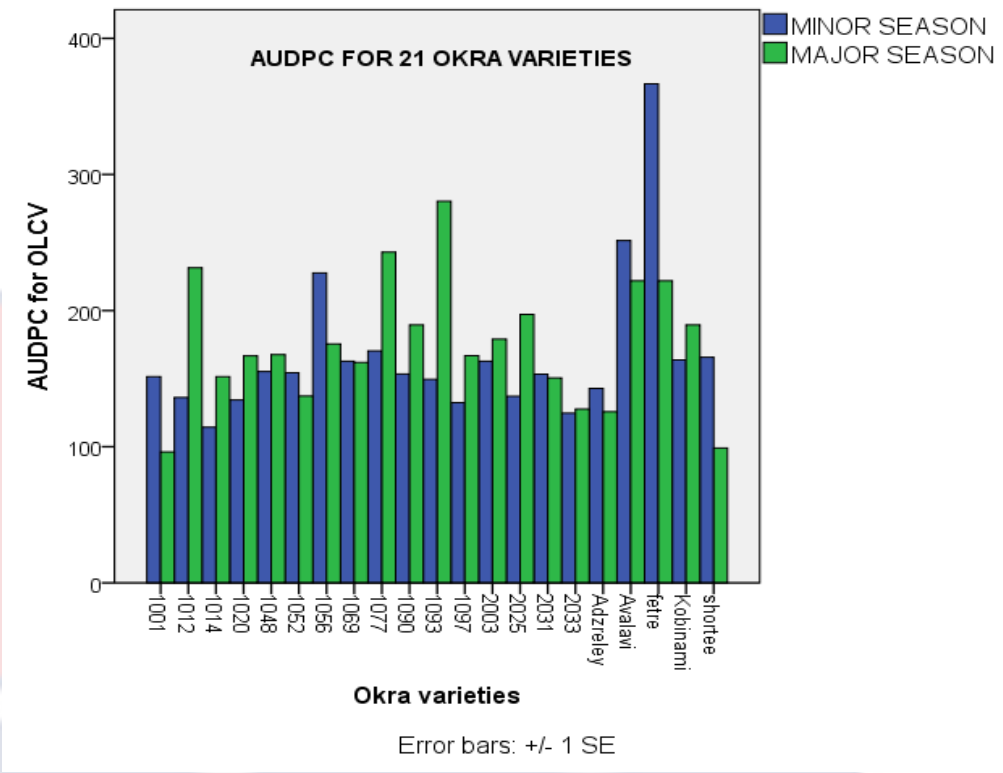

Figure 78: Comparative study on OLCD progression for 21 different okra varieties during the wet and dry seasons

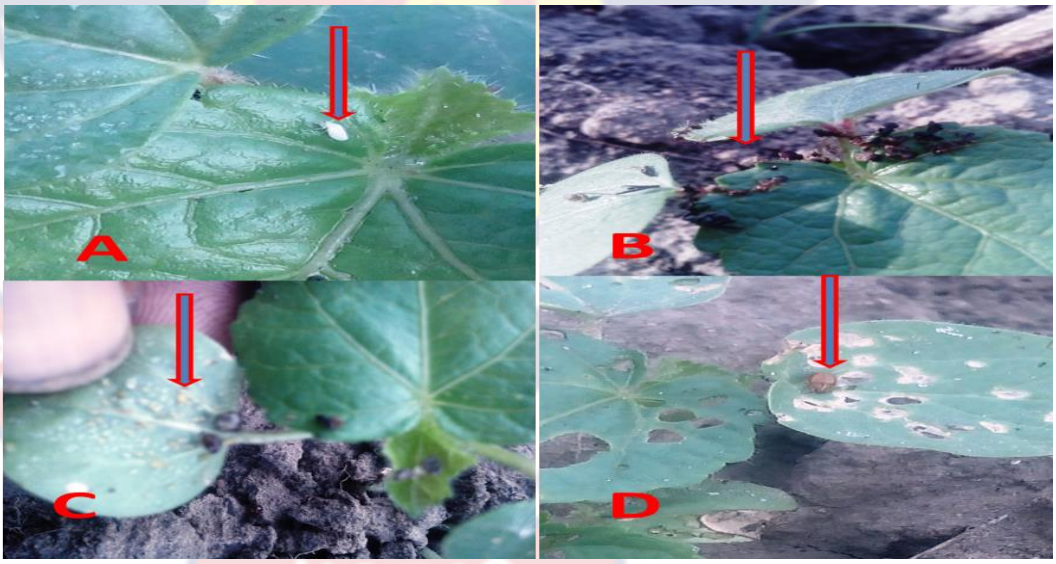

Figure 79: Major insect pests of okra found in the field on okra two weeks after sowing. A= Whitefly, B= Ants, C=Mealybug, D=Flea beetle

Source: Field data, Adzim (2020)

5.5.4 Screening of 21 Different okra varieties for resistance against whiteflies

Whitefly populations were counted during the minor and major seasons of the study. In all, the cumulative average number of whiteflies was 1388 whiteflies during the study. The minor season recorded the highest population of whiteflies (863) whiles the major season had the lowest (525). The mean whitefly population for the minor season was 10.34 whiles major season was 8.41. During the minor season the varieties 1077(14.61), Avalavi (14.57), 2025(14.39) and 1001(14.39) recorded the highest whitefly population whiles Shortee (7.73), 1069(8.47), 1093(8.74) and 2003(9.35) recorded the least whitefly population. Also, in the major season the varieties with highest infestation of whitefly were; 2025(14.39), Adzreley (14.39), 1077(13.77) and 2031(13.75) and lowest infestation was recorded on Shortee (6.54), 1069(7.33), 1001(7.33) and 1093(7.34). Variety shortee, 1069, and 1093 were least infested with whitefly for both major and minor season. Avalavi and 1077 were highest infested okra variety with whitefly. All okra varieties were moderately susceptible to whitefly infestation for minor season and major season; however, Shortee was less susceptible to whitefly infestation.

Table 13: Mean population of whitefly on 21 okra genotypes under field conditions during the two planting seasons

Genotype	Mean population of whitefly in dry season				Mean population of whitefly in wet season			
	2WAS	6WAS	10WAS	HR	2WAS	6WAS	10WAS	HR
1056	1.913^{a}	17.11^{bc}	9.73^{a}	MS	4.623^{abc}	8.130^{ab}	9.73^{abc}	MS
2025	1.913^{a}	16.98^{bc}	14.39^{bc}	MS	3.826^{abc}	15.105^{b}	14.39^{c}	MS
1052	2.710^{a}	14.56^{abc}	11.74^{abc}	MS	5.238^{abc}	12.515^{ab}	10.15^{abc}	MS
2003	2.710^{a}	11.06^{ab}	9.35^{a}	MS	2.710^{abc}	5.739^{a}	9.35^{abc}	MS
1097	3.325^{ab}	17.10^{bc}	12.51^{bc}	MS	4.623^{abc}	9.466^{ab}	10.87^{abc}	MS
1014	3.846^{ab}	18.45^{bc}	11.90^{abc}	MS	2.710^{ab}	12.032^{ab}	10.48^{abc}	MS
1020	3.846^{ab}	10.86^{ab}	11.48^{abc}	MS	1.913^{a}	9.727^{ab}	11.48^{abc}	MS
1090	4.623^{abc}	11.37^{ab}	9.65^{a}	MS	3.826^{abc}	11.366^{ab}	7.15^{ab}	MS
2033	4.623^{abc}	14.15^{abc}	13.30^{bc}	MS	4.623^{abc}	13.209^{ab}	11.23^{abc}	MS
1069	5.238^{abc}	7.15^{a}	8.47^{a}	MS	5.238^{abc}	7.151^{ab}	7.33^{ab}	MS
1093	5.238^{abc}	9.73^{a}	8.74^{a}	MS	3.826^{abc}	10.342^{ab}	7.34^{ab}	LS
1001	5.420^{abc}	10.44^{abc}	14.39^{c}	MS	4.623^{abc}	6.536^{ab}	7.33^{ab}	LS

1077	5.420^{abc}	14.33^{abc}	14.61^{c}	MS	4.623^{abc}	7.152^{ab}	13.77^{c}	MS
1048	5.759^{abc}	10.95^{abc}	12.42^{bc}	MS	4.623^{abc}	8.552^{ab}	12.42^{bc}	MS
1012	7.151^{abc}	11.38^{abc}	12.12^{bc}	MS	5.739^{abc}	13.715^{ab}	7.95^{ab}	LS
Fetre	7.151^{abc}	13.47^{abc}	11.48^{abc}	MS	5.739^{abc}	7.672^{ab}	11.48^{abc}	HS
Shortee	7.333^{abc}	13.15^{abc}	7.73^{a}	LS	7.420^{abc}	6.524^{ab}	6.54^{a}	LS
Adzreley	7.948^{abc}	20.10^{c}	13.78^{bc}	MS	8.563^{abc}	11.668^{ab}	14.39^{c}	MS
Kobinami	7.948^{abc}	16.00^{bc}	11.70^{abc}	MS	7.433^{bc}	8.930^{ab}	11.70^{abc}	MS
2031	10.761^{bc}	9.36^{a}	12.75^{bc}	MS	6.536^{abc}	9.360^{ab}	12.75^{bc}	MS
Avalavi	11.668^{c}	20.74^{c}	14.57^{c}	MS	6.636^{abc}	12.176^{ab}	10.15^{abc}	MS
Mean	5.55	13.73	11.75		4.99	9.86	10.38	
Sum	98	473	292		68	229	228	
LSD	7.580	16.154	7.381		5.227	8.774	5.679	
P-value	0.489	0.977	0.832		0.682	0.754	0.132	

Means in the same column bearing different letters are significantly different ($P<0.05$). According to mean whitefly population okra varieties were categorized as; less susceptible (mean whitefly below 7.12/three leaves), moderately susceptible (7.12 to 15.02/three leaves) and highly susceptible (above 15.02/three leaves). (Nagar *et al* 2017) (Based on 11.07±3.95)

5.5.5 Yield of Okra Varieties

Table 14 below shows the yield of okra during the field trial of 21 different varieties. The result shows that the varieties 1090(0.72 t/ha), 1052(0.71 t/ha), shortee (0.69 t/ha) and 1097(0.69 t/ha) recorded the highest yield of minor seasons and the major season was 1052(0.93 t/ha), shortee (0.92 t/ha), 1097(0.91 t/ha) and 1090(0.67 t/ha). The lowest yield was recorded by varieties 2031(0.27 t/ha), Kobinami (0.31 t/ha), fetre (0.33 t/ha) and 1056(0.35 t/ha) during the minor(dry) seasons, whiles varieties Kobinami (0.42 t/ha), 1001(0.43 t/ha), 1093(0.47 t/ha) and 2031(0.48 t/ha) were recorded in the major seasons. The yield of okra was high in the major season, with a mean yield of 0.639 t/ha while the minor yield was 0.486 t/ha.

Figure 80: Determination of the yield of okra using weighing scale.

Table 14: Yield of okra varieties for two seasons

Varieties	Average fruit weight(g)		Mean fruit yield(t/ha)	
	Minor season	Major season	Minor season	Major season
2031	19.58	35.00	0.27	0.48
Kobinami	22.25	30.22	0.31	0.42
Fetre	24.25	40.00	0.33	0.60
1056	25.69	37.45	0.35	0.51
2033	26.75	45.00	0.37	0.62
Adzreley	27.84	40.12	0.38	0.55
1093	29.28	34.52	0.40	0.47
1001	31.12	31.23	0.42	0.43
2025	31.52	36.00	0.43	0.50
1014	32.87	45.00	0.45	0.61
1069	34.22	36.20	0.47	0.49
1077	34.25	44.76	0.47	0.61
Avalavi	34.31	56.00	0.47	0.77
1048	36.88	39.34	0.51	0.54
1012	39.35	45.00	0.54	0.62
1020	40.93	59.20	0.56	0.81
2003	44.27	66.00	0.61	0.91
1097	50.36	66.45	0.69	0.91
Shortee	50.71	67.00	0.69	0.92
1052	51.80	68.00	0.71	0.93
1090	52.75	50.00	0.72	0.67
Total	740.97	972.49	10.15	13.37
CV	28.41	27.10	28.31	26.82
$\bar{x} \pm \sigma$	85.29±10.03	46.31±12.53	0.48±0.14	0.64±0.17

Table 15: Correlation coefficients between insect vector (whitefly), OLCD incidence and severity yield of okra for wet and dry seasons (2020-2021)

		CANW-WET	DI-WET	DS-WET	CANW-DRY	DI-DRY	DS-DRY
CANW-WET	Pearson Correlation		.			*	
	Sig. (2-tailed)						
	N						
DI-WET	Pearson Correlation	.312**					
	Sig. (2-tailed)	.000		.			
	N	189					
DS-WET	Pearson Correlation	-.035	-.383**				.
	Sig. (2-tailed)	.787	.002				
	N	63	63				
CANW-DRY	Pearson Correlation	.797**	.217**				.
	Sig. (2-tailed)	.000	.003	.137			
	N	189	189	63			
DI-DRY	Pearson Correlation	.235**	.521**	.013	.142		.
	Sig. (2-tailed)	.001	.000	.921	.052		.
	N	189	189	63	189		
DS-DRY	Pearson Correlation	.056	-.345**	.892**	.226	.028	
	Sig. (2-tailed)	.664	.006	.000	.075	.827	
	N	63	63	63	63	63	

**. Correlation is highly significant at the 0.01 level (2-tailed)., *Significant (P < 0.05), CANW(major & minor season)-cumulative average number of whitefly, DI - disease incidence, DS = Disease severity

5.6 Discussion

In order to formulate disease management strategies, it is necessary to understand the extent of the disease in the field and to have an understanding of virus-host interactions. In order to effectively manage OLCD in Ghana, various okra cultivars were assessed in the field for resistance to OLCD and its whitefly vector resistance. In all the varieties, severe indications of okra leaf curl disease were evident throughout the field with moderate to severe symptoms. Symptomatic plants were found in both the dry and wet seasons. Leaf curling, stunted development, yellowing, thickening of the veins, and curling of the leaf margin were some of the common signs, which led the leaves to curve downward. The symptoms were similar to those documented in Ghana on okra by Asare-Bediako (2018), in Saudi Arabia by Ghanem (2003), and in India by Krishnareddy *et al.* (2003). In the current research, disease incidence ranged from 11.35 % to 78.13 % for both the major and minor seasons, respectively. The minimum mean disease incidence is higher than the 25 % reported by Asare-Bediako (2018).

The presence or absence of virulent strains of the viruses, the population of viruliferous vectors, environmental conditions and the load of inoculum can influence the ELCV disease tolerance reaction of okra. The combination and cumulative effect of these factors have significant influence on spread of this disease (Ayam *et al*., 2018). Roy *et al.*, (2009) stated that the advantage of using AUDPC values over the single severity assessment is that the AUDPC reflects disease progress throughout the entire growth period. The higher the AUDPC values, the higher the susceptibility of the genotype to the disease (Ayam *et al.*, 2018). In this investigation, the cumulative progress of

disease using AUDPC which varied greatly between susceptible and highly susceptible groups, indicating low cumulative disease progress in tolerant genotypes. The disease incidence and severity increased rapidly from week two to week ten after sowing, with a variable disease progression curve among the genotypes. The okra genotypes showed mild symptoms in the early stages of growth, which worsened as the whitefly population and transmission efficiency rose. Among the genotypes, the PDI values were lower for 2033 and 1097; hence were categorized as susceptible, whiles the rest of genotypes had higher PDI values and were categorized as highly susceptible. According to Venkataravanappa *et al*. (2014), the minimal number of whiteflies necessary to produce 100% infection is 10/plant, despite the fact that a single whitefly can efficiently transmit the YVMV. Similar observations have been made by Asare-Bediako *et al.* (2018) and Ogbe *et al*. (2001). However, there were disparities in the severity of disease symptoms, which could be attributed to the genetic makeup of the okra cultivars, virus strain and possible infection with other viruses (Ghanem, 2003; Sherwood *et al*., 1986). The mean incidence and severity of okra leaf curl disease observed throughout the major and minor seasons were remarkably similar. Infected plants left in the field, according to Hilje *et al.* (2001), serve as a source of inoculum and allow the whitefly to infect the okra the next planting season. The host resistance test done in this study revealed that all cultivars were very susceptible to okra leaf curl disease in both the major and minor seasons. Varieties Kobinami, 1097 and 2033 were found to be the most susceptible to the okra leaf curl disease. Susceptibility of some Ghanaian okra cultivars to OLCD has been reported by Asare-Bediako *et al.* (2018),

In the minor and major seasons, the population of whitefly on the various okra cultivars changed dramatically. During the field survey, it was observed that the whitefly vector population was significantly higher in the minor season than in the wet season. A total of 1,388 whiteflies were counted, with a minor season population of 863 and a major season population of 525. The average number of whiteflies per plant in the minor season was 10.34, but in the major season, it reduced to 8.81. This observation is consistent with those of Asare-Bediako *et al. (*2018), who reported a higher whitefly population per plant in the minor season compared to the major season, although the cumulative average number of whiteflies per plant was the same for both seasons. The variation in susceptibility of the genotypes to the leaf curl disease could be attributed to differences in genotypes and vector preference, as well as the biotypes of whiteflies that were present (Azizi *et al.*, 2008).

Furthermore, the whitefly population and disease severity could primarily be controlled by weather conditions (Javar *et al.*, 2018). According to Singh *et al*. (2013), high temperature and low relative humidity could contribute to whitefly population growth during the minor season, which is consistent with the higher population of whiteflies observed during the minor season in this study. Contrarily, a higher population of whiteflies was observed during the major season. This could be a result of rainfall and high relative humidity, which is normally associated with typical rainfall period, as reported by Zeshan *et al.* (2015). Whitefly, a key insect pest known to transmit the viral diseases to healthy plants, was found on every okra variety throughout the study period. However, some varieties were less infested than

others, a situation which has also been reported by Nagar *et al.* (2017). The number of whiteflies and the severity of the damage to the okra varieties differed significantly. Whitefly infestations were first noticed one week after sowing, although data on their population was collected two weeks later. In the major season, whitefly population on variety Shortee was the lowest (7.73/three leaves), although it was not statistically different from the numbers recorded for cultivars 1056, 2003, 1090, 1069, and 1093. The varieties 1097, 2025, 2033, 1048, 1012, Adzreley, and 2031 had no significant differences in whitefly population. Variety 1077 had the largest population (14.61/three leaves), which was not significantly different from varieties 1001 and avalavi. During the dry season, the cumulative average number of whitefly ranged from 5.55 to 13.73 /three leaves, which is far lower than the 94.12 reported by Asare-Bediako (2018). The result of the study shows that all okra varieties, with the exception of variety shortee, were moderately susceptible to whitefly infestation. Whitefly population increased steadily from one week after germination to an average of about 10.38whiteflies/three leaves after ten weeks. Varieties Shortee, 1012, 1093 and 1001 were found to be less vulnerable to whitefly infestation during the wet season, while varieties 1052, 2003, 1097, 1014, 1090, 2033, 1069, 2025, 1020, 1077, 1048, Fetre, Adzreley, Kobinami 2031, and Avalavi were found to be moderately susceptible.

In the minor season, there was a significantly positive correlation between the whitefly population and disease incidence. In the major season, however, the positive correlation was highly significant. This implies that when the population of whiteflies increases, the incidence and severity of OLCD also disease increases. The total yield obtained in this study for the

various okra cultivars varied for both seasons. This finding is consistent with previous research by Asare-Bediako (2018) and Udengwu and Dibua (2014), who found that okra yield varied according to the growing season. The major season yield was 0.639 t/ha, which was greater than the minor season yield of 0.486 t/ha. In a comparable study by Asare-Bediako (2018), the yield for rainy season being higher (2.394 t/ha) than that of the minor season (1.225 t/ha). For both seasons, the cultivars Shortee, 1052, and 1090 yielded the most, while the varieties Kobinami, 2031, and Fetre yielded the least. The results of the study revealed that Shortee was less susceptible to the vector, resulting in a higher yield, while Fetre was extremely susceptible to the vector, resulting in a low yield. The differences could be related to host-virus interactions and the age of the affected plants (Sastry and Singh, 1974). However, the average okra production recorded is lower than the West African average of 2.5 t/ha and is quite low in comparison to East Africa (6.2 t $ha^{-1)}$ and North Africa (8.2 t ha^{-1}) (Cudjoe *et al.*, 2005; FAOSTAT, 2008). Despite their susceptibility to the virus, cultivars Shortee, 1052, and 1090 produced appreciable yields and, therefore, could be exploited in further breeding programme.

CHAPTER SIX

6.0 MOLECULAR DIVERSITY OF OKRA YELLOW CRINKLE VIRUS; THE CAUSAL AGENT RESPONSIBLE FOR OKRA LEAF DISEASE IN THE VOLTA AND OTI REGIONS OF GHANA

6.1 Introduction

Okra is a warm-weather annual crop that may be found in practically every African market (Schippers, 2000). Brong Ahafo, Ashanti, Northern, Volta, Greater Accra, and Central regions are Ghana's major okra producing regions (NARP, 1993). Despite the industry's health and economic benefits, leaf curl disease caused by Begomoviruses present a major constraint to production. Begomoviruses (family Geminiviridae) are widely recognized as a hazard to tropical and subtropical vegetable production (Rojas *et al.*, 1993). They are transmitted to dicotyledonous plants by the whitefly vector *Bemisia tabaci* (Fauquet and Stanley, 2003) and induce symptoms such as upward or downward leaf curling, stunting, leaf deformation and thickening of the veins as described by Sayed *et al.* (2014). There are already over 200 geminivirus species officially recognized (Fauquet *et al.*, 2008). Mastrevirus, Curtovirus, Topovirus, Becurtovirus, Eragnovirus, Turncurtovirus, and Begomovirus are the seven genera of the family geminiviridae based on their genome arrangement and biological features (Stanley *et al.*, 2005; Osei *et al.*, 2017). Begomovirus is the largest genus in the Geminiviridae family, with 196 different species (Brown *et al.*, 2012) and are regarded as a rapidly emerging and commercially significant type of germinivirus (Fauquet *et al.*, 2008; Brown *et al.*, 2012). Several studies have identified many species of the Begomovirus based on their morphology, with Fauquet *et al.* (2008) reporting

over 200 species. Brown *et al.* (2015) found 388 species that infect dicot plants and are spread by the *Bemisia tabaci* cryptic species complex whiteflies. Begomoviruses are either bipartite (DNA-A and DNA-B) or monopartite (only DNA-A-like components) (Lazarowitz, 1992). Some monopartite begomoviruses are also linked to circular ssDNA molecules that are roughly half the size of DNA-A, such as betasatellite or alpha satellite (formerly known as DNA-1). Pathogenicity has been linked to betasatellites, whereas alphasatellites have no known function and are not implicated in symptom induction (Mansoor *et al.*, 1999). Alphasatellites have only been seen in combination with betasatellites in plants infected with monopartite begomoviruses (Briddon *et al.*, 2002).

Asare-Bediako *et al*. (2014) stated that okra is susceptible to 19 viral diseases, with the okra leaf disease being the most common disease of okra in Ghana. OLCD can result in yield losses of up to 80% (Brown and Bird, 1992). In okra, Singh (1996) reported yield losses ranging from 30% to 100% owing to Okra Leaf Curl Virus. It was discovered that OLCD is associated with a group of begomoviruses in Africa: Cotton leaf curl Gezira virus (CLCuGV), Okra yellow crinkle virus (OYCrV), and Hollyhock leaf crumple virus *(HoLCrV)* (Shih *et al*., 2007; Idris *et al*., 2002). This has enhanced the requirement for reliable viral identification (Rojas *et al.*, 1993). In Burkina Faso, a single begomovirus species and a complex of beta and alpha satellite species have been reported to cause OLCD (Tiendrebeogo *et al.*, 2010). The first and most crucial stage in any crop management system is accurate virus disease diagnosis. In the last three decades, breakthroughs in molecular biology and biotechnology have been used to develop rapid, specific, and

sensitive approaches for detecting plant viruses, as compared to biological techniques that were too slow and inappropriate for large-scale deployment in prior decades. As a result, plant viral infections have been detected using such techniques as microscopic observations, serological and molecular technologies (Makkouk and Kumari, 2006). Serology has been used to detect plant viruses for more than half a century (Torrence and Jones, 1981) using methods such as enzyme-linked immunosorbent assay (ELISA), tissue blot immunoassay (TBIA), and quartz crystal microbalance immunosensors (QCMI) (Jeong *et al.,* 2014). However, the polymerase chain reaction has become the new gold standard for detecting a wide range of pathogens (Mullis and Faloona, 1987). Cloning, gene manipulation, gene expression analysis, genotyping, sequencing, mutagenesis, and disease detection are all examples of applications where PCR has been applied (Makkouk and Kumari, 2006; Schaad and Frederick, 2002). Plant viruses are currently detected in the laboratory by PCR using virus-specific primers and genus-specific primers (Makkouk and Kumari, 2006).

In Ghana, OLCD has frequently been diagnosed using symptomatology; molecular detection and viral genetic diversity determination are largely limited (Asare-Bediako *et al.*, 2014). Thus, the actual causal virus for the leaf curl disease of okra in Ghana is unknown. The information obtained from the study will inform the development of control measures to minimize disease spread to boost okra production in the country.

Therefore, the objective of this research was to identify Begomoviruses associated with okra leaf curl disease in the Volta and Oti

regions of Ghana and their relationships with other Begomoviruses at the molecular level.

6.2 Materials and Methods

6.2.1 Study Location

The samples were sent to the Biotechnology Centre Laboratory at the University of Ghana for DNA extraction and sequencing was done at the Inqaba Biotech, Pretoria, South Africa.

6.2.2 Collection of Samples

A total of 20 okra leaf samples with okra leaf curl disease symptoms were sampled from farmers' fields throughout the Volta and Oti regions. Three to four leaves (both symptomatic and asymptomatic) were taken from the plant's upper portion, placed in a Ziplock bag, labelled. and stored on ice in a cooler box. The samples were then transferred to a laboratory for DNA extraction.

6.2.3 DNA extraction of Okra Leaves

A modified CTAB procedure was used to extract total DNA from the leaf tissues (Doyle and Doyle, 1987). In a 1.5 mL screw cap tube (Assist, Japan), 200 mg of okra leaves were weighed from each sample, then ground for 20 seconds at 3000 r/min in a multi-bead shocker (Yasui Kikai, Japan). 400 µL extraction buffers were added and vortexed, and then the mixture was kept in an Eppendorf tube. The samples were centrifuged for 10 minutes at 10,000 xg. A clean Eppendorf tube was used to transfer the supernatant. 300 microliters of ice-cold isopropanol were added, along with 100 microliters of 5 mol/L NaCl, and the mixture was gently inverted to mix the ingredients.

The samples were incubated in a B.O.D incubator at -20°C for 1 hour before the upper layer was gently transferred to a fresh tube without disrupting the polysaccharides. At room temperature, the supernatant was centrifuged at 10,000 xg for 10 minutes. The pellet was washed with analytical-grade ethanol at 75% (v/v). The pellet was dried before being dissolved in 100 microliters of TE buffer. 1 mL RNaseA (Promega) (10 mg/mL) was also added and incubated at 37°C to degrade the RNA. The mixture was then mixed with an equal volume of phenol-chloroform (1:1, v/v) and centrifuged at 10,000 xg for 5 minutes at room temperature. After that, one volume of chloroform-isoamyl alcohol (24:1, v/v) was added to the supernatant in a fresh tube. The supernatant was transferred to a 1.5 mL Eppendorf tube after centrifugation at 10,000 xg for 5 minutes at room temperature. Then, 2.5µL volumes of absolute ethanol and 0.1 µL volume of 3 mol/L sodium acetate were added and left at -2°C for 20 minutes. It was then centrifuged for 10 minutes at 4°C at 10,000 xg, with the supernatant discarded and the pellet rinsed with 75 percent (v/v) ethanol. After 30 minutes of drying, the pellet was dissolved in 50 liters of TE buffer.

A 5% agarose gel electrophoresis was used to assess the purity or quality of the isolated DNA, and the bands were photographed using a gel documentation system. Before utilizing PCR to detect begomoviruses, a NanoDrop ND-1000 Spectro photo-meter (USA) was used to quantify DNA yield and quality at 260 nm.

6.2.4 Polymerase Chain Reaction

PCR was carried out in a 20 µL reaction volume containing 10 µL of HotStarTaq Plus Master mix (1-unit HotStartTaq Plus DNA polymerase, 1x PCR buffer and 200 µM of each dNTP), 1 µL each of forward and reverse primers (10 µM) and 1 µL of template DNA. Degenerate primer pair PAL1v1978 (5' - GCATCTGCAGGCCCACATYGTCTTYCCNGT -3') and PARc496 (5' - AATACTGCAGGGCTTYCTRTACATRGG - 3') was used for the DNA amplification. The PCR programme used was an initial denaturing step at 94^{o}C for 3 min, followed by 30 cycles of denaturing at 94^{o}C for 1 min, annealing at 50^{o}C for 1 min, and extension at 72^{o}C for 2 min, and a final extension at 72^{o}C for 10 min. The resulting PCR products were visualized under high performance ultraviolet transilluminator (UVP, Cambridge, UK) and images captured with the aid of UVP Life Sciences Software (Doc-It LS Image Acquisition).

6.2.5 Amplicon Sequencing

The PCR products were purified with QIAquick PCR purification kit (Qiagen) and then directly sequenced in both directions using the forward and reverse primers (Degenerate primer pair PAL1v1978(5'- GCATCTGC AGGCCCACATYGTCTTYCCNGT -3') and PARc496 (5' – AATA CTGCAGGGCTTYCTRTACATRGG - 3') as sequencing primers at the Inqaba Biotech, Pretoria, South Africa.

6.3 Sequence Data Analysis

Chromatograms were checked manually to avoid base miscalls and then trimmed low-quality sequences at the 5' and 3' ends. Forward and reverse sequences were compared, ensuring complete homology. The

sequences were then edited, aligned and compared with other published isolates of Okra yellow crinkle virus (OYCV) using Geneious 8.0 (Biomatters), ClustalW (Thompson *et al.* 1994) and BLAST (Altschul *et al.* 1990). Cluster dendrograms were generated with the Geneious Tree Builder using the Neighbor-joining method (1000 bootstrap replicates) and the graphic phylograms were viewed using the TreeView.

6.4 Results

Symptoms of OLCD was prevalent in every farm surveyed. Most field affected plants showed leaf curling, stunting, blistering, yellowing of leaf margins and mosaic symptoms typical viral disease (Fig. 81). The symptoms were most severe in the young leaves.

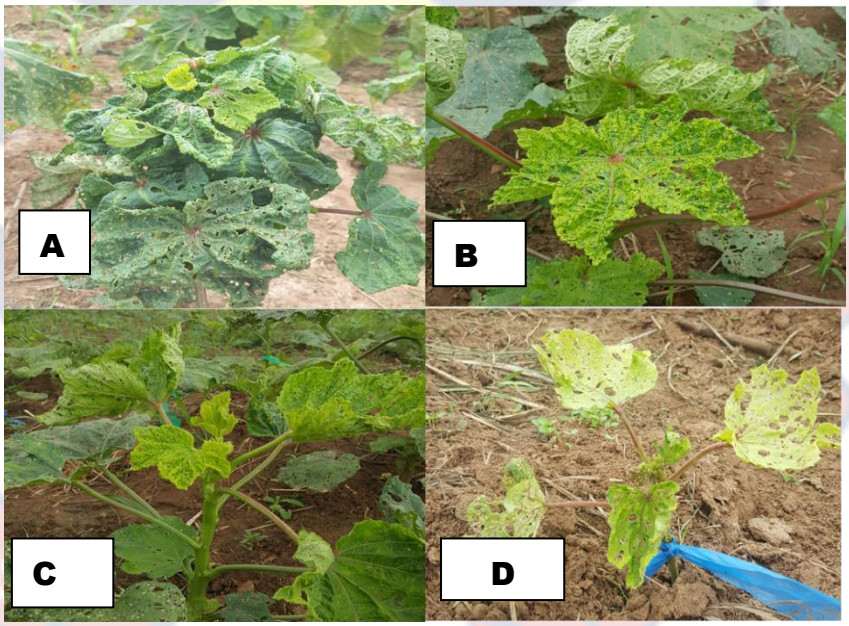

Figure 81: Symptoms observed on okra plants during sample collection. included; A mosaic, B- leaf cupping, C-leaf curling and deformation, D-leaf yellowing and stunting

6.4.1 Polymerase Chain Reaction

Gel electrophoresis showed good-quality DNA samples that were used in PCR. OYCV was successfully detected in isolate OkYVF2 and OkYVF17 which showed leaf curl symptoms. PCR with degenerate primer pair PAL1v1978 and PARc496 yielded amplicons in the range of 1212 bp and 1145 bp for the replication-associated protein (Rep) gene (Figure. 82).

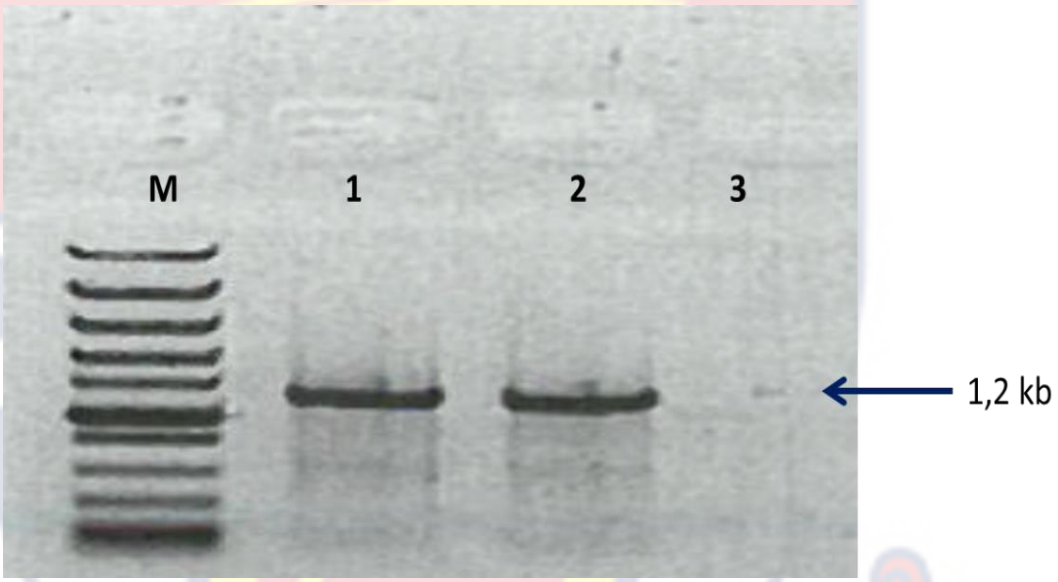

Figure 82: Amplification of OYCrV DNA using the primer pair PAL1v1978 and PARc496 which yields ~1.2 kb of Begomovirus replication-associated protein (Rep) gene. Lane M – DNA Ladder, 1 = isolate OkYVF2, 2 = OkYVF17 and 3= negative control.

6.4.2 Sequencing of PCR Amplicons

PCR amplicons of 1212 nt were obtained from the Rep gene of 2 Ghanaian OYCrV isolates. After trimming, sequences of 815 nt were compared. The sequence alignment indicates a high degree of similarity (94.1 %) among the two Ghanaian OYCrV isolates for both nucleotide and predicted amino acids. BLAST analysis of sequence data for an 815 bp

fragment of the Rep gene amplicon revealed sequence identity in the range of 83.03-96.9% to the published sequences of OYCrV isolates present on the GenBank database for both nucleotide and deduced amino acid sequences. The OYCrV isolates of closest homology originated from Ivory Coast (KX100572.1).

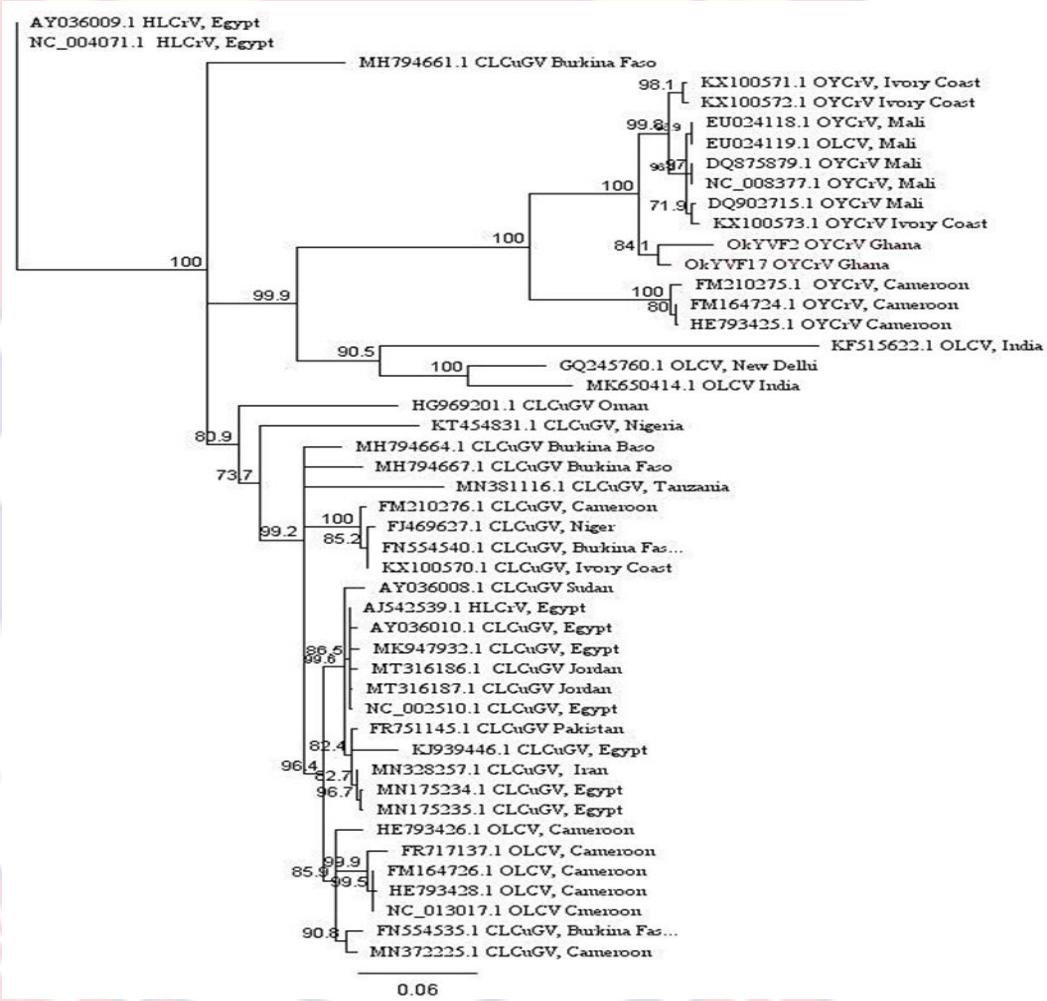

Figure 83: Phylogenetic tree (neighbor-joining) of an ~815 nt fragment of Okra yellow crinkle virus Rep gene of viral isolates from Ghana, Egypt, Cameroon, Ivory Coast, Burkina Faso, Iran, India, Jordan, Niger, Tanzania and Mali. Numbers at the nodes denote the percentage of 1000 bootstraps iterations supporting the branches. Nodes with <70% bootstrap support were collapsed. The potato leafroll virus was used as an outgroup.

6.4.3 Phylogenetic Analysis

Phylogenetic analysis of the aligned sequences for nucleotide and amino acids are shown in Fig. 83 and 84, respectively. The analysis reiterated the similarities among the Ghanaian isolates of OYCrV and those available on the GenBank. The isolates clustered according to the Begomovirus species, which are known to cause okra leaf curl disease, namely Cotton leaf curl Gezeira virus, Okra leaf curl virus, Okra yellow crinkle virus and Hollyhock leaf crumple virus. However, the two Ghanaian isolates grouped closely together with Okra yellow crinkle virus isolates from Ivory Coast, Mali and Cameroon for both nucleotide and deduced amino acids.

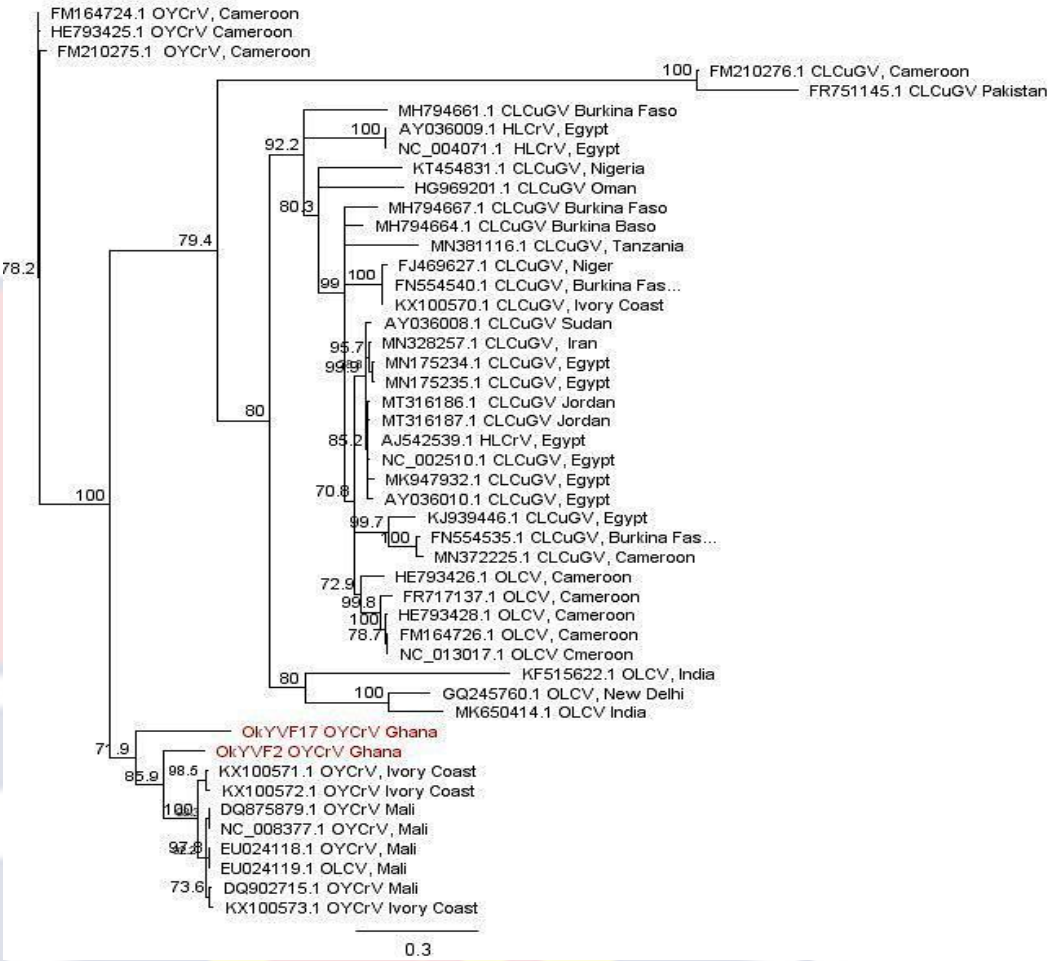

Figure 84: Phylogenetic tree (neighbor-joining) of deduced amino acid (aa) sequences of Okra yellow crinkle virus Rep gene of viral isolates from Ghana, Egypt, Cameroon, Ivory Coast, Burkina Faso, Iran, India, Jordan, Niger, Tanzania and Mali. Numbers at the nodes denotes the percentage of 1000 bootstraps iterations supporting the branches. Nodes with <70% bootstrap support were collapsed. Carrot mottle virus was used as outgroup

6.5 Discussion

Okra is widely available in African markets (Schippers, 2000). Despite the industry's positive effects on human health and the economy, it is challenged with several constraints including viral diseases. Leaf curl disease caused by Geminiviruses has been a major constraint responsible for significant yield losses. During viral isolate collection in this study, several okra plants were seen with symptoms such as leaf curling, severe stunted development with apical leaf curl upward or downward, leaf deformation, and thickening of the veins, similar to those described by Tiendrébéogo *et al.* (2020) and Asare-Bediako, (2018). The security of the world's food supply is seriously threatened by geminivirus, which is possibly the most harmful plant viruses in existence (Leke *et al.*, 2015). A field study on geminivirus responsible for okra leaf curl disease revealed high disease incidence and severity in the three agro-ecological zones in the Volta and Oti region of Ghana. These findings imply that the OLCD is highly prevalent in the study area. The high disease incidence and severity across the study is a serious threat to food security. Cotton leaf curl Gezira virus (CLCuGV) and Okra yellow crinkle virus (OYCrV) are the two major Begomovirus species that are associated with okra leaf curl disease (OLCD) in Africa (Tiendrébéogo *et al.*, 2010; Shih *et al.*, 2007; Idris and Brown, 2002). In Togo, Okra Yellow Crinkle Virus was detected in symptomatic samples and, therefore, has been implicated in OLCD development (Tiendrébéogo *et al.*, 2020). Similarly, Séka *et al.* (2016) identified Cotton leaf curl Gezira virus and Okra yellow crinkle virus associated with okra leaf curl disease in Cote d'Ivoire.

Furthermore, the two viruses have been identified in symptomatic okra samples from Cameroon (Leke *et al.*, 2006). In Ghana, however, the identity of the virus(es) responsible for OLCD had not been determined prior to this study. Twenty symptomatic okra leaf curl diseases were collected across three agroecological zones in the Volta and Oti regions. Total DNA was extracted, and quality was determined. Polymerase Chain Reaction conducted on symptomatic okra leaf samples using begomovirus-specific degenerate primer pair (PAL1v1978/ PAR1c715) tested positive for the begomovirus two of samples. Shin *et al.* (2007) previously used this primer combination to identify Begomoviruses in symptomatic okra leaf samples from Mali. The identity of the virus responsible for OLCD in the Volta and Oti region of Ghana was confirmed by amplicon sequencing as the Okra yellow crinkle virus.

Analysis of an 815 bp fragment of the replication-associated protein gene indicates a high degree of similarity among the Ghanaian isolates (OkYVF2 and OkYVF17), which did not differ greatly from isolates previously described from Ivory Coast, Mali and Cameroon. The Okra yellow crinkle virus isolate of closest identity to the Ghanaian isolates originated from Ivory Coast (KX100572.1) (Seka *et al.*, 2016), sharing nucleotide identity of 96.9%. Phylogenetic analysis reiterated the similarities among the West African isolates of Begomoviruses responsible for OLCD. The isolates clustered according to the virus species which are known to cause okra leaf curl disease namely Cotton leaf curl Gezeira virus, Okra leaf curl virus, Okra yellow crinkle virus and Hollyhock leaf crumple virus (Tiendrébéogo *et al.*, 2010). For both nucleotide and deduced amino acids, the two Ghanaian isolates clustered closely with isolates of the Okra yellow crinkle virus from

Ivory Coast, Mali and Cameroun. The very close proximity and the seemingly identical environmental circumstances present in these nations may be the cause of the Ghanaian isolates' tighter sequence identity to other West African isolates. It is possible that the whitefly that carries Begomoviruses from one location to another carried the virus to Ghana.

Additionally, potential cross-country trade in goods and services could result in the spread of viral inoculum. The predominant strain(s) of the virus can be identified using the virus's sequence diversity, which can also be used to screen germplasm for the virus and aid in the selection of cultivars with stronger resistance. Effective resistance breeding requires an understanding of the variety of virus species that cause OLCD in Ghana. To my knowledge, this is the first report of OYCrV linked to okra leaf curl disease in Ghana, specifically in the Volta and Oti regions. The current discoveries regarding the agent responsible for okra leaf curl disease will aid in the swift, accurate, and effective detection of a novel emerging virus strain responsible for okra leaf curl disease in other regions of Ghana. The recent discovery of OYCrV in Ghana may offer a clue to the complex etiology of the disease that causes okra leaves to curl there and may call for additional research.

CHAPTER SEVEN

7.0 SUMMARY, GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Summary

The first objective of the study was to assess farmers' perceptions of the occurrence/prevalence and management of both okra leaf curl disease and its vector, the whitefly respectively in six districts (Keta and South Tongu) in the Coastal grassland, (Ho West and Jasikan) in the forest zone and (Krachi East and Nkwanta South) in the transition zones of the Volta and Oti regions of Ghana. Out of 384 respondent okra farmers 379 said OLCD was prevalent in their farms. However, 320 were not aware of causative agent of the disease. The remaining 64 farmers who claimed to know what causes the disease, only 36 mentioned whitefly whiles 21 stated Flea beetles, 14 Cotton Stainer and 3 said changes in the weather condition most especially temperature. All the 384 farmers used insecticide in the management of whitefly as well as used it wrongly to manage the OLCD. Out of the 384 farmers, 339 do not have personal protective equipment during pesticide applications. Only 38 were partially dressed, with seven fully protected against pesticide spillage

The second objective of the study was a survey to assess the incidence and severity of okra leaf curl disease and whitefly population in the Volta and Oti regions over two growing seasons in the six selected districts across the three agroecological zones. A field survey to determine the incidence and severity showed that in all the farms visited, OLCD was endemic, with some farms recording 100% incidence with severe symptoms. However, the mean disease incidence of OLCD in the study area ranged 69.69% to 80.62% with

mean severity index of 1.18 to 1.44. The disease was higher in the coastal zone with a mean incidence of 77.42%, transition zone recorded 72.56% and the forest zone was 71.85%. According to the whitefly's spatial distribution during field survey, there were a total of 38,504 whiteflies in three agro-ecological zones, 25,978 of which were present during the minor season and 12,526 during the major season. There was a positive correlation between whitefly population and disease incidence in the coastal grassland during year one and two okra growing season, whiles the relationship between them in the transition zone was negatively correlated. Meanwhile, the forest zone showed a positive and negative relationship between whitefly and disease incidence. Weather parameters such as temperature, rainfall, wind speed and relative humidity correlated positively and negatively with whitefly population across the agroecological zones.

The third goal of the study was to evaluate the performance of 21 okra varieties resistance to the whitefly vector and the okra leaf curl disease in the field. The 21 okra cultivars were gathered from various areas and planted in the coastal grassland zone in the major and minor seasons (2020–2021). The okra varieties' resistance to the disease and its whitefly vector, data on disease incidence, severity, and whitefly population were analyzed two weeks after sowing, six weeks after sowing, and ten weeks after sowing. The percentage disease index from day 0 to 10 weeks after sowing was used to calculate the area under the disease progressive curve (AUDPC), which represents how the OLCD progresses over the course of the full growth period of the okra plant. The susceptibility of the okra variety to the disease on each variety increased with increasing AUDPC levels. The mean disease incidence ranged from

11.35% to 78.13% for all okra types, and the mean severity index for the major and minor growing seasons was 0.89 and 1.67, respectively. The mean incidence of OLCD for 2WAS was 11.35%, 6WAS was 30.53%, and 10WAS was 78.13%. A total of 19 out of the 21 okra types were highly susceptible to the OLCD, whereas just 2 were susceptible overall. The mean whitefly population during the minor season was 10.34/plant, whereas it was 8.41/plant during the major season resulting in an average yield of 0.49 t/ha and 0.64 t/ha, respectively.

The final objective was to identify Begomoviruses associated with okra leaf curl disease in the Volta and Oti regions of Ghana and their relationships with other Begomoviruses at the molecular level. A total of 20 symptomatic okra plants showing signs of OLCD were collected across the six districts of the Volta and Oti regions zipped, and brought to the laboratory for DNA extraction, polymerase chain reaction, sequencing and building of phylogeny tree to find diversity of the begomovirus causing the disease. Degenerate primer pair PAL1v1978 (5’ – GCATCTGCAGGCCCACATY GTCTTYCCNGT -3’) and PARc496 (5’ – AATACTGCAGGGCTTYCTR TACATRGG - 3’) were used for the DNA amplification. Out of the 20 isolates, isolates OkYVF2 and OkYVF17 tested positive to PCR. PCR amplicons of 1212 nt were obtained from the Rep gene of 2 Ghanaian OYCrV isolates. OYCV was successfully detected in isolates OkYVF2 and OkYVF17, which showed leaf curl symptoms. However, the two Ghanaian isolates grouped closely together with Okra yellow crinkle virus isolates from Ivory Coast, Mali and Cameroon for both nucleotide and deduced amino acids. To my

knowledge, this is the first report of OYCrV linked to okra leaf curl disease in Ghana, specifically in the Volta and Oti areas.

7.2 General Conclusion

1. Majority of farmers are young and have had formal education, making basic agronomic methods, pest and disease management, and pesticide use easier to comprehend.
2. Most farmers were aware of the prevalence of OLCD and reported seeing whitefly and OLCD symptoms on their farms during both the major and minor seasons, with the minor season being the worst. However, most of okra growers were not aware that whitefly was responsible for OLCV transmission and disease infection.
3. Majority of farmers used synthetic pesticides that are not approved for vegetable cultivation to control whiteflies, which led to significant misuse and improper application. The majority of okra farmers purchased and applied pesticides from agrochemical suppliers who were frequently uninformed about the science behind insecticides, thus polluting the farmers. The issue grew worse when it was discovered that most farmers had never been instructed in the safe handling and application of pesticides. This was demonstrated by the fact that some okra farmers disregarded instructions for mixing pesticide, posting warning signs after pesticide applications, maintaining spraying equipment, throwing empty chemical containers onto the farm, spraying pesticides at specific times of day, and wearing personal protective equipment.

4. The overall mean difference in OLCD incidence was higher in the minor seasons when compared to the major season. The mean incidence of OLCD ranged from 69.69% to 80.62% for the three agro-ecological zones in the Volta area, while the mean severity index of OLCD ranged from 1.18 to 1.44.
5. The mean OLCD incidence in year one and two major seasons was significantly higher in the coastal savannah (90.9%) than in the forest (86.0%) and transition (87.7%) zones whiles during the minor season, the coastal zone OLCD incidence (96.7%) was much higher than the forest (89.7%) and transition (92.3%) zones' rates. Whitefly populations were 25,978 in the minor season and 12,526 in the major season, indicating that the dry season had a higher population than the wet season.
6. Okra leaf curl disease and whitefly population showed a significant negative linear relationship in the first year, but only a non-significant positive association in the second. Temperature and whitefly population showed a slight, non-significant negative correlation in the coastal, forested, and transitional zones. Rainfall showed a non-significantly positive, and negative impact on the coastal, transitional, and forest zones.
7. All cultivars were highly susceptible to okra leaf curl disease in both the major and minor seasons, according to the host resistance test conducted in this study. The most susceptible varieties to okra leaf curl disease were discovered to be Kobinami, 1097, and 2033.

8. In the minor season, there were 10.34 whiteflies per plant, but in the wet season, there were only 8.81. With the exception of variation shortee, all okra genotypes were moderately susceptible to whitefly infestation, whereas variety fetre was extremely susceptible.
9. The okra yield during the major season was 0.639 t/ha, which was higher than the 0.486 t/ha produced during the dry season.
10. The begomovirus species responsible for okra leaf curl in the Volta region is Okra yellow crinkle virus which is the first report of the disease.

7.3 Recommendations

It is therefore recommended that:

1. The incidence and severity in the other regions should also be assessed to determine the overall intensity of the disease in the country.
2. Ministry of Food and Agriculture and Farmer Based Organizations should hold frequent, in-depth training sessions for farmers on pest and disease detection, control, and usage of pesticides
3. Plant breeders should use intense breeding techniques to create/develop okra cultivars that are more resistant to whitefly infestation, which will reduce the spread of the Begomoviruses.
4. Further research should be undertaken to perform a pathogenicity test on the virus found on okra through grafting.
5. Further molecular works should be undertaken across all regions in Ghana to identify viral species causing okra leaf curl in Ghana.

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APPENDICES

Appendix 1. Questionnaires on farmers' perception on okra leaf curl disease

A. Background information on farmers

1. Age > 18 [ ] 20-29[ ] 30-39 [ ] 40-49[ ]50-59[ ] 60-69[ ] >70[ ]
2. Gender Male [ ] Female [ ]
3. Educational level No formal education [ ]

   Non-formal Education/ Adult literacy [ ]

   Primary [ ]

   JHS/MSL [ ]

   SHS/O LEVEL [ ]

   Diploma/Cert A [ ]

   University [ ]
4. Marital status

   Single [ ]

   Divorced/separated [ ]

   Widowed [ ]

   Co-habitation [ ]

   Married [ ]

5. How many people are in your household 1-3 [ ] 4-6 [ ] 7-9 [ ] 10-12[ ] > 13[ ]

6. How many hectares of okra do you cultivate in a year < 1 [ ] 2-5 [ ] 6-10[ ] >11[ ]

7. How long have you been farming okra?

< 2 years [ ]

5-9 [ ]

10-14 [ ]

15-19 [ ]

20-24 [ ]

25-34 [ ]

>34 [ ]

8. Why do you cultivate okra?

Source of food [ ]

Source of income [ ]

Easy to work in okra farm [ ]

Main source of employment [ ]

Early maturity and high yielding plant [ ]

High market demand for okra [ ]

Major crop for the area [ ]

B. Farm characteristics

9. What is your average estimate of harvested okra in kg per hectare?

1-200 [ ]

201-400 [ ]

401-600 [ ]

601-800 [ ]

10. What type of farming do you do?

Mono cropping [ ] Mixed cropping [ ]

11. What is your source of water for the okra plants

Irrigation [ ]depends on rainfall [ ] uses dug out/dams [ ]

12. How do you prepare your land before planting?

Slashing (Manual) [ ] Ploughing [ ] Use of herbicides [ ]

13. How many times do you control weeds in your farm

Once [ ] twice [ ] thrice [ ] none [ ]

14. What is the source of labour on your farm?

Self [ ] Hired labour [ ] Communal work [ ]

15. Where do you get your okra seeds from?

Previous harvest	[ ]
Market	[ ]
Agro-input shops /Certified seed store	[ ]
Friends	[ ]
Relatives	[ ]
Research and allied institutions	[ ]

16. Which varieties do you usually plant?

C. Farmers' awareness of okra leaf curl disease (olcd)

17. Have you experienced OLCD in your okra farms?

a. Yes [ ], b. No [ ]

18. If yes, please describe the symptoms

Curling of leaves	[ ]
Stunting of leaves	[ ]
Plant stunting	[ ]

Others [ ], please describe

19. Does the disease occur in both rainy and dry seasons?

a. Yes [ ], b. No [ ]

20. If yes, which season is it very severe?

a. Rainy Season [ ], b. Dry Season [ ]

21. At What growth stage of the okra do you normally encounter the disease?

a. 4 weeks after sowing [ ], b. 6weeks after sowing [ ], c. 8 weeks after sowing [ ] ,d. others [ ], please specify…….

22. At what growth stage of okra is the disease very severe?

a.4 weeks after sowing [ ] , b. 6weeks after sowing [ ], c. 8 weeks after sowing [ ] , d. Others, [ ] please specify……..

23. Do you know the causes of the disease? A. Yes [ ], b. No [ ]

24. If yes, list the causes…….

25. Do you know how the disease is transmitted within and between okra farms?

26. If yes, please state the mode of transmission

27. How severe is okra leaf curl disease in your okra farm?

No symptoms [ ]

Curling of few top leaves [ ]

Top leaves curled and slightly stunting of plant. [ ]

All leaves curled and slightly stunting of plants. [ ]

Severe curling of leaves, stunting of plant and proliferation of auxiliary branches [ ]

28. How does the OLCD affect yields of your okra crops?

No yield loss [ ], <10% yield loss [ ], Between 10 and 50% yield loss [ ], >50% yield loss [ ]

29. What proportion of your farm does the disease normally affect?

[Disease incidence<10% [ ], 10-30% [ ], 30-50% [ ], 50-70% [ ], >70% [ ]

30. Apart from OLCD, what other diseases / symptoms do you encounter at your okra farm? You may describe the symptoms

31. How do you manage OLCD in your farm?

a. No control method [ ]
b. Spraying of pesticides
c. Seed treatment
d. Planting resistant varieties
e. Cultural method (Change planting date to avoid high infection)
f. Cultural method (Application fertilizer to ensure healthy plant growth)
g. Others. Please specify

D. **Farmers' knowledge of whitefly infestation**

32. Do you experience whitefly infestation of okra plants in your farm?

a. Yes [ ], b. No [ ]

33. Which season is the infestation more severe?

a. Dry season [ ], b. Rainy season [ ]

34. How does the whitefly infestation affect okra production?

a. Transmits viral diseases

b. Affect growth of okra plants / Causes stunting of plants

c. Affect yields of okra fruits

d. Others, please specify

35. Have you ever reported whitefly infestation in your farm to Agric Extension Agents (AEA) in your area? Yes [ ] No [ ]

36. If yes, how often do you visit or invite AEAs to your farm?

Once a year [ ] every month [ ] never visited [ ] during farming season[ ]

37. Apart from whitefly, what other insects do you encounter in your okra farms?

Cotton Stainer [ ] Flea beetle [ ] Green Stink Bug [ ] others [ ]

38. How do you control these insect pests in your farm?

Use of synthetic insecticide [ ]

Use of bio-pesticide [ ]

Use of yellow sticky traps [ ]

Use of water [ ]

No pest control [ ]

39. Others [ ], please specify……

40. Do you treat your seeds with insecticide before sowing?

No treatment [ ] treatment [ ]

41. Do you apply chemicals to control okra leaf curl disease in your farm?

Yes [ ] No [ ]

E. Pesticides use practice by the okra farmers

42. Who advices you on the choice of pesticides?

AEA [ ], Agro input dealers [ ], Friends [ ], Self [ ]

43. Where do you get the insecticides from?

Agro-input shops [ ], MoFA / AEAs [ ], Neigbours [ ] [ ]

recommended by AEA[ ]

44. Have you ever received training on the safe handling and application of pesticides? Yes [ ] No [ ]

45. What is the sources of your knowledge on pesticide application rates

Pesticide dealers [ ]

Fellow farmers [ ]

Agricultural extension officers [ ]

Media (radio, TV, Newspapers [ ]

Pesticide label [ ]

COCOBOD [ ]

NGO's [ ]

School [ ]

Government [ ]

46. What is the timing of pesticide application?

When first symptoms of pest/diseases are observed [ ]

Based on the severity of pest infestation / disease infection [ ]

Based on crop calendar/date of transplanting [ ]

Based on advice from Agricultural extension agents [ ]

Based on expert judgment of the situation [ ]

47. Do you mix different kinds of pesticides?

Yes [ ] No [ ]

48. How do you mix the pesticides/fungicides?

Stir With bare hands [ ]

Stir with hands by wearing gloves and protective eye goggles [ ]

Stir With stick [ ]

Shaking the sprayer [ ]

Wear hand gloves and protective eye goggles [ ]

49. What direction do you spray?

With the wind [ ]

Against the wind [ ]

Perpendicular to the wind [ ]

Don't consider the wind [ ]

50 What time of the day do you spray your chemicals?

Early in the morning [ ]

Hot afternoon [ ]

Late in the evening [ ]

50. Do you eat or smoke while spraying?

Yes [ ] No [ ]

51. What sign do you indicate to people the field has being sprayed with pesticides/chemicals?

Sign board [ ]

Red flag [ ]

Empty pesticides bottle [ ]

None [ ]

52. When do you re-enter the farm after spraying?

Same day [ ]

1-3 days [ ]

4-7days [ ]

8-14 days [ ]

>14 days [ ]

53. What is your pre-harvest interval (in days)?

1-3 [ ]

4-7 [ ]

8-14 [ ]

>14 [ ]

54. How do you dispose the empty pesticides container?

Incineration [ ]

Burying [ ]

Throw away on the farm [ ]

Throw away in the town or village [ ]

Reuse the container in the house [ ]

55. What type of sprayers do you use?

Knapsack sprayer [ ]

Motorized /mist blower [ ]

Hand held applicator [ ]

56. Do you own a sprayer?

Yes [ ] No [ ]

57. What type of protective cover / personal protective equipment (PPE) do you use during spraying?

No protective cover [ ]

Partial protective cover [ ]

Full protective cover [ ]

ppendix 2: Age of respondents

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	> 18	12	3.1	3.1	3.1
	20-29	163	42.4	42.4	45.6
	30-39	77	20.1	20.1	65.6
	40-49	79	20.6	20.6	86.2
	50-59	34	8.9	8.9	95.1
	60-69	2	.5	.5	95.6
	> 70	17	4.4	4.4	100.0
	Total	384	100.0	100.0	

Appendix 3: Sex of respondents

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	239	62.2	62.2	62.2
	Female	145	37.8	37.8	100.0
	Total	384	100.0	100.0	

Appendix 4: Highest Educational level

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No formal education	29	7.6	7.6	7.6
	Adult literacy	16	4.2	4.2	11.7
	Primary	95	24.7	24.7	36.5
	JHS/MSL	137	35.7	35.7	72.1
	SHS/O LEVEL	63	16.4	16.4	88.5
	Diploma/ Cert A	17	4.4	4.4	93.0
	University	27	7.0	7.0	100.0
	Total	384	100.0	100.0	

Appendix 5: Respondents number of years into okra farming

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	< 2	51	13.3	13.3	13.3
	5-9	191	49.7	49.7	63.0
	10-14	74	19.3	19.3	82.3
	15-19	44	11.5	11.5	93.8
	20-24	13	3.4	3.4	97.1
	25-34	2	.5	.5	97.7
	> 34	9	2.3	2.3	100.0
	Total	384	100.0	100.0	

Appendix 6: The type of cropping system practiced

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Monocropping	335	87.2	87.2	87.2
	Mixed farming	49	12.8	12.8	100.0
	Total	384	100.0	100.0	

Appendix 7: Whether respondents use pesticide

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	40	10.4	10.4	10.4
	Yes	344	89.6	89.6	100.0
	Total	384	100.0	100.0	

Appendix 8: Respondents number of times weed is control

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	once	33	8.6	8.6	8.6
	twice	302	78.6	78.6	87.2
	thrice	49	12.8	12.8	100.0
	Total	384	100.0	100.0	

Appendix 9: Respondents sources of acquiring okra seeds

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Postharvest	219	57.0	57.0	57.0
	market	58	15.1	15.1	72.1
	agro-input/certified seed store	20	5.2	5.2	77.3
	friends	2	.5	.5	77.9
	relatives	85	22.1	22.1	100.0
	Total	384	100.0	100.0	

Appendix 10: Respondents types variety of okra planted

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Black	13	3.4	3.4	3.4
	adrale	14	3.6	3.6	7.0
	Short	13	3.4	3.4	10.4
	Fetre	30	7.8	7.8	18.2
	asontem	53	13.8	13.8	32.0
	avalavi	153	39.8	39.8	71.9
	awayibor	59	15.4	15.4	87.2
	clemson spineless	29	7.6	7.6	94.8
	okra indiana	11	2.9	2.9	97.7
	okra hire	4	1.0	1.0	98.7
	okra OH102	5	1.3	1.3	100.0
	Total	384	100.0	100.0	

Appendix 11: Experience OLCD in your farm

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	5	1.3	1.3	1.3
	Yes	379	98.7	98.7	100.0
	Total	384	100.0	100.0	

Appendix 12: Disease's occurrence in both seasons

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	368	95.8	95.8	95.8
	No	16	4.2	4.2	100.0
	Total	384	100.0	100.0	

Appendix 13: Season disease is most severe

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Rainy season	62	16.1	16.1	16.1
	Dry season	322	83.9	83.9	100.0
	Total	384	100.0	100.0	

Appendix 14: Who advise you on choice of pesticide

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	AEA	42	10.9	10.9	10.9
	Agro-input dealer	196	51.0	51.0	62.0
	friends	127	33.1	33.1	95.1
	self	19	4.9	4.9	100.0
	Total	384	100.0	100.0	

Appendix 15: Received training on safe handling and application of pesticide

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	30	7.8	7.8	7.8
	NO	354	92.2	92.2	100.0
	Total	384	100.0	100.0	

Appendix 16: Sign to indicate pesticide has being sprayed

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	sign board	8	2.1	2.1	2.1
	red flag	6	1.6	1.6	3.6
	empty pesticide bottles	16	4.2	4.2	7.8
	none	354	92.2	92.2	100.0
	Total	384	100.0	100.0	

Appendix 17: How to dispose empty pesticide containers

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	incineration	34	8.9	8.9	8.9
	burying	52	13.5	13.5	22.4
	throw away on the farm	212	55.2	55.2	77.6

	throw away in the village	86	22.4	22.4	100.0
	Total	384	100.0	100.0	

Appendix 18: Type of personal protective equipment

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No protective cover	339	88.3	88.3	88.3
	partial protective cover	38	9.9	9.9	98.2
	full protective cover	7	1.8	1.8	100.0
	Total	384	100.0	100.0	

Appendix 18: Incidence of okra leaf curl disease in minor season for year one

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro_ecological_zone	2	2115.02	1057.51	26.31	<.001
Residual	93	3738.31	40.20		
Total	95	5853.33			

Appendix 19: Incidence of okra leaf curl disease in major season for year one

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro-ecological zone	2	684.00	342.00	7.32	0.001
Residual	93	4344.50	46.72		
Total	95	5028.50			

Appendix 20: Incidence of okra leaf curl disease in minor season for year two

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro_ecological_zone	2	185.65	92.82	0.97	0.384
Residual	93	8925.69	95.98		
Total	95	9111.33			

Appendix 21: Incidence of okra leaf curl disease in major season for year two

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro-ecological zone	2	243.6	121.8	0.83	0.438
Residual	93	13611.4	146.4		
Total	95	13855.0			

Appendix 22: Severity of okra leaf curl disease in minor season for year one

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro-ecological zone	2	0.72133	0.36066	5.33	0.006
Residual	93	6.29846	0.06773		
Total	95	7.01978			

Appendix 23: Severity of okra leaf curl disease in major season for year one

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro-ecological zone	2	0.4522	0.2261	1.96	0.146
Residual	93	10.7062	0.1151		
Total	95	11.1584			

Appendix 24: Severity of okra leaf curl disease in minor season for year one

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro-ecological zone	2	1.3900	0.6950	6.34	0.003

Residual	93	10.1942	0.1096
Total	95	11.5842	

Appendix 25: Whitefly population in minor season for year one

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro-ecological zone	2	98.994	49.497	5.49	0.006
Residual	93	838.428	9.015		
Total	95	937.422			

Appendix 26: Whitefly population in major season for year one

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro-ecological zone	2	57.463	28.732	3.21	0.045
Residual	93	833.587	8.963		
Total	95	891.050			

Appendix 27: Whitefly population in minor season for year two

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro-ecological zone	2	52.528	26.264	2.74	0.070
Residual	93	891.204	9.583		
Total	95	943.732			

Appendix 28: Whitefly population in major season for year two

Analysis of variance

Variate: whitefly population year 2 minor season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Agro_ecological_zone	2	28.542	14.271	3.51	0.034
Residual	93	378.371	4.069		
Total	95	406.913			

Appendix 29: Incidence of OLCD 2WAS in the dry season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	284.4	142.2	0.69	
block.*Units* stratum					
Variety	20	11415.3	570.8	2.78	0.003
Residual	40	8221.7	205.5		
Total	62	19921.4			