SEROLOGICAL DIAGNOSTIC SURVEY AND FARMER PERCEPTION OF CUCUMBER MOSAIC VIRUS DISEASE IN THE GREATER ACCRA REGION OF GHANA

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ASEM WISDOM (ID: 10806431)

B.Sc Agriculture Technology (2012)

(University for Development Studies)

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DECLARATION

This thesis is the results of research work undertaken by Asem Wisdom, at the school of Nuclear and Allied Sciences (Department of Nuclear Agriculture and Radiation Processing), University of Ghana under the supervision of DR. ANDREW SARKODIE APPIAH and DR. SAMUEL AMITEYE

Signature Date 25- 01- 2023

ASEM WISDOM

(Candidate)

Signature Date: 25 – 01 - 2023

Dr. Andrew Sarkodie Appiah

(PRINCIPAL SUPERVISOR)

Signature Date 25-01-2023

Dr. Samuel Amiteye

(Co-SUPERVISOR)

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DEDICATION

I dedicate this work to Almighty God and to my late parents. This would not have been achieved without you. May God gives you eternal rest forever.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADRRI	Africa Development and Resources Research Institute
AVRDC	Asians Vegetable Research Development Center
BBWV	Broad Bean Wilt Virus
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CMV	Cucumber Mosaic Virus
DAS-ELISA	Double Antibody Sandwich Enzyme Linked Immunosorbent Assay
DNA	Deoxyribonucleic Acid
DSMZ	Deutsche Sammlung von Milkroorganismen und Zellkulturen
ELISA	Enzyme-Linked Immunosorbent Assay

EM	Electron Microscopy
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization's Statistical Database
GAEC	Ghana Atomic Energy Commission
GDP	Gross Domestic Product
GLD	Grapevine Leafroll Disease
GLSS	Ghana Living Standards Survey
GSS	Ghana Statistical Service
ICTV	International Committee on Taxonomy of Viruses
IDM	Integrated Disease Management
IEM	Immuno-Electron Microscopy

IFPRI	International Food Policy Research Institute
IOSR	International Organization of Scientific Research
ISSAP	Index of Symptom Severity of all plants
ISSER	Institute of Statistical, Social and Economic Research.
IWMI	International Water Management Institute.
MOFA	Ministry of Food and Agriculture
MP	Movement Protein
ORF	Open Reading Frame
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PVMV	Pepper Veinal Mottle Virus

PVP	Polyvinylpyrrolidone
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribose Nucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SPSVV	Sweet Potato Sunken Vein Virus
TEM	Transmission Electron Microscopy
TMV	Tobacco Mosaic Virus
UNICEF	United Nation International Children's Emergency Fund
UV-ray	Ultraviolet ray
WEDC	Water Engineering and Development Centre.
WHO	World Health Organization

ABSTRACT

Vegetables are important in diets of practically every household in Ghana. Vegetables are essential dietary portion that provide important vitamins and minerals. In addition to providing farmers with a source of income, vegetable cultivation helps the economy of the country to grow by creating jobs and bringing in substantial amount of foreign currency. Viral diseases are one of the largest obstacles to vegetable production, and in Ghana they are regarded to be a significant factor limiting the output of vegetable production. The Cucumber Mosaic Virus (CMV), one of these viruses, is extremely devastating and infects more plant families than any other plant virus. Unfortunately, since its discovery in 1974, the host range of CMV among important vegetable crops in Ghana has gotten comparatively little research attention. Therefore, the aim of this study was to identify the prevalence, host range, and severity of CMV among the main vegetables grown in Ghana's Greater Accra region, namely in the districts of Tema West, Ga East, and Ayawaso West. A standardized questionnaire was used to conduct a survey involving 120 farmers in these districts to assess the perception of the importance of CMV in vegetable production. It was discovered that the majority of farmers had little to no knowledge about viruses and instead implicated abiotic and biotic factors for their problems. After making extensive visual observations, it was discovered that CMV symptoms were present in every farm that was visited. The presence of CMV in tomato, broccoli, lettuce, spinach, cucumber, radish, and cabbage was confirmed by Enzyme-Linked Immunosorbent Assay (ELISA) on samples taken from symptomatic plants. This is the first account of broccoli, cabbage, lettuce, spinach, and radish in Ghana testing positive for CMV. The disease is spread mechanically through sap inoculation from an infected plant to a healthy plant, according to an

ELISA confirmation test. The findings of this study will contribute to the development of efficient control methods that would help manage the disease, particularly given the new host range of CMV discovered in Ghana.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

In Ghana, vegetables play a significant role in almost every household's diet. They are crucial dietary additives that supply critical vitamins and minerals (Graham et al., 2007). Almost all of Ghana's ecological zones are used for vegetable farming, which is done either as a monoculture or in intercropping arrangements with other crops including cereals, legumes, root and tuber crops, plantation etc. The most extensively grown and economically significant vegetables are tomato, pepper, eggplant, and okra. Ghana is able to produce yields of 15,000 kg/ha for tomatoes, 30,000 kg/ha for eggplant, and 20,000 kg/ha for peppers. However, it was anticipated that the comparable national mean yields in 2016 would only be around half of these yields (MOFA, 2017). Vegetables are extremely susceptible to biotic and abiotic stressors, which contribute to low yields, as does the lack of governmental and private investment in productivity enhancing technology. Vegetable crops are frequently susceptible to a variety of diseases, which can affect both the quality and quantity of harvests. Infected plants frequently experience stunting, reduced leaf size, and leaf mottling and deformation. For instance, some tomato mosaic virus strains might result in brown dead streaks along the stem or the death of a portion of the plant, notably the tip (Lamptey et al., 2005)

Numerous bacterial, viral, and fungal infections result in financial losses for vegetable farming. Among them, viral infections stand out because they significantly lower quality and yield while having a wide range of symptoms that differ greatly from host to host. Because most farmers are unaware of this issue, specialized methods for locating and managing virus infected plants under field conditions, including identification and control methods are poorly understood. According to Biswas et al. (2016), one of the best ways to stop the spread of viral infections in the field is to identify the causal agent and practice good hygiene in accordance with the most common diseases. Although many plant viruses have been reported to affect vegetables, *Cucumber mosaic virus* (CMV), one of the oldest viruses identified, is still being studied on the basis of epidemiological reviews of viral diseases of vegetables (Gallitelli, 2000). One of the most ubiquitous plant viruses, having a very diverse host range, the virus is a member of the family Bromoviridae and the genus Cucumovirus. (Meena et al., 2022). It infects over 1000 species of plants, including grains, fruits, vegetables, and ornamentals. More than 75 species of aphids can non-persistently spread the virus from infected to healthy plants (Krenz et al., 2015).

The virus's wide range of natural hosts, non-persistent transmission by numerous aphid species, and seed transmission in some hosts may be the causes of its widespread distribution (Biswas et al., 2016). The management and monitoring of CMV infestations is challenging due to the virus's spreading properties. Thus, viral disease management and precise strategies to recognize CMV in vegetables are greatly needed. The availability of multiple CMV variants makes it challenging to recognize the isolate from the symptoms alone, which is another problem with CMV in terms of identification. Various plant viruses may be identified, according to Katoch et al. (2003), using techniques such as bioassays, indicator plants, symptom observation, host range estimation, viral particle morphology, biochemistry, and immunology. However, it is necessary to develop methods for simultaneously detecting many plant species and multiple viral diseases (James et al., 2006). The most effective strategy to tackle the cucumber mosaic virus disease would be to do an extensive study on the distribution, diagnosis, and currently popular and advanced disease management techniques. CMV is one of the most prevalent viral infections in the globe due to its extensive dispersion. (ICTV, 2018) resulting in decreased in production (Rivera-Toro et al., 2020).

Three positive sense single-stranded RNA segments make up the CMV genome (RNA1, RNA2 and RNA3). Each piece of viral RNA is packaged into an isometric particle, where it is translated into vital proteins that control viral encapsidation, replication, and transportation throughout the plant (ICTV, 2018). Two subgroups of CMV isolates, subgroup I and II, have been identified based on serology, nucleic acid hybridization, and gene sequencing (Dubey and Singh, 2008).

1.2 Statement of problem and justification of study

In Ghana, vegetable production generates income for farmers and contributes to the development of the country's economy through creation of employment and earning of foreign revenue (Kofi and Torvikey, 2018). For instance, in 2017 hot pepper contributed 4.5% of the foreign revenue generated from the sale of vegetables (Abdulai et al., 2017).

One of the biggest challenges associated with vegetable production is viral diseases (Ogundeji, et al., 2012) and in Ghana viral diseases are thought to be an important factor restricting the output of vegetable production (Appiah et al., 2014). Vegetables can be infected by over 40 viruses (Lee et al., 2018) with numerous symptoms, including mosaic, mottle, leaf deformation, vein chlorosis, and stunting which considerably affect plant vigor and yield. Plant viruses are known to reduce crop quality and yield, cause horrific, enormous losses, and other adverse effects (Rao and Reddy, 2020). One of these viruses, the Cucumber mosaic virus (CMV), is devastating and it infects more plant families than any other plant virus. The host range of CMV among Ghana's significant vegetable crops has received relatively little research since its detection in 1974. This research therefore, sought to determine the prevalence, host range and severity of CMV among the major Ghanaian vegetables. The outcome of this research will aid in the management of the disease through the formulation of effective control measures.

1.3 Objectives

The principal objective of this research was to assess the prevalence, severity and host range of Cucumber mosaic virus among vegetable crops in the Greater Accra region of Ghana

Specific objectives were to:

1. Assess farmer awareness on virus disease incidence within Greater Accra region.

2. Conduct sero-diagnostic survey for CMV in the different vegetable crops.

3. Determine the experimental host range of CMV among Ghanaian vegetables using mechanical sap inoculation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Types of vegetables and importance in human and animal health

Vegetables are horticultural crops that may be cultivated either annually or perennially. They have parts that may be consumed raw or cooked, including their roots, stalks, flowers, fruits, and leaves (Welbaum, 2015). In consideration of the plant component utilized as food and the specific nutritional value, vegetables can be categorized Khashroom et al., 2019). They can be classified as leaf (for instance, spinach, lettuce, curly lettuce, chard, purslane, and chicory), stalk (for instance, celery and asparagus), fruit, and flower vegetables (example are Broccoli, cauliflower, artichoke, tomatoes, peppers, cucumber and eggplant), Vegetables with root, bulb, and tuber (such as carrot, beet, turnip, fennel, onion, radish, and potato) and Legumes (example are peas, and soya beans) (Ülger et al., 2018). Vegetables are essential for human nutrition because they include bioactive nutritional molecules such as dietary fiber, vitamins, and minerals, as well as non-nutritive phytochemicals (phenolic compounds, flavonoids, bioactive peptides, etc.). These nutrients and non-nutrient molecules lower the risk of chronic illnesses like obesity, diabetes, some malignancies, and cardiovascular disorders (Pennington et al., 2009; September-Malaterrer et al., 2018).

Consuming a variety of food groups in the proper amounts is necessary for a healthy, high-quality diet. Globally, the number of people who experienced hunger decreased to 795 million in 2015 (Pelletier et al., 2016), demonstrating improvement in guaranteeing appropriate access to staple foods as assessed by caloric intake. However, according to McLean et al. (2009), 2.1 billion more individuals are either overweight or obese, and micronutrient deficiencies affect an estimated 2 billion individuals worldwide (Ng et al., 2014).

The important sources of the micronutrients required for healthful diets are fruits and vegetables. Vegetables provide dietary fiber, which reduces cholesterol levels in the blood, which may lessen the risk of heart disease. Maintaining a healthy blood pressure requires potassium whilst folate (folic acid) reduces the likelihood of cardiac disease and the chance of birth abnormalities. The health of the eyes and skin is maintained by vitamin A, while the teeth and gums are safeguarded by vitamin C, which also aids in the body's absorption of iron. In order to prevent chronic diseases (especially heart diseases, cancers, and diabetes) and provide essential micronutrients (especially calcium, iron, iodine, vitamin A, and zinc), the World Health Organization (WHO) recommends a minimum intake of 400 g of fruits and vegetables per day (WHO and UNICEF, 2003; WHO/FAO, 2003) . Consumers today, including those with more income, are thought to be falling short of this goal. In order to narrow this dietary gap and provide people with access to vegetables' nutritional worth, greater effort must be made.

With a rising interest in foods that encourage healthy living and the preservation of excellent health, many people have started to adapt their eating habits in recent years. Vegetables are therefore increasingly being included in diets to support the maintenance of a healthy lifestyle and boost disease resistance (Afari-Sefa et al., 2015).

2.2 Production levels and economic value of vegetables

Vegetable cultivation takes place in close to 200 countries, and in many parts of the world, vegetables make up a significant portion of the average person's diet. Three hundred and ninety-two (392) vegetable crops comprising 70 families and 225 genera were reported in a global assessment of vegetables (Silva Dias, 2010). The most often consumed category of vegetables were the leaves or young green shoots (53% of the total), followed by vegetable fruits (15%). Roots, tubers, rhizomes, corms, and stolons were the most often used below ground crop vegetable organs, making up 17% of the total (Silva Dias, 2011). Vegetables are cultivated everywhere, by both large commercial producers and small holder farmers, on good and marginal soils, in urban and rural locations, and on large and small farms. A major portion of vegetables grown globally are produced on small plots and have short production cycles, which makes it difficult to gather reliable production numbers thus hindering the understanding of the importance of these crops to the global food supply. Even with the poor dependability of FAO figures about vegetables grown in impoverished countries, global production of vegetables in 2007 was about 900 million tonnes (FAO, 2009). Asia produced 74.7% of the world's vegetables on 72.8% of the global vegetable producing land (671 million tonnes; 52.7 million ha) with China contributing significantly to global vegetable accounting for almost 50% of global vegetable production (Silva Dias 2011)

Similar to other emerging economies in Sub-Saharan Africa, the agriculture industry contributes significantly to Ghana's GDP and provides the majority of the country's jobs (ISSER, 2013). For instance, a record-high of GH¢ 8441 million (18.3%) of the nation's GDP was provided by the agriculture industry in 2017 (Ghana Statistical Service, 2018)

In Ghana, the agricultural industry and the economy as a whole continue to be dominated by the crops sub-sector in terms of GDP contribution. In 2016 and 2017, it contributed 14.6% and 14.2% of nominal GDP, respectively (GSS, 2018). Vegetable cultivation, especially non-traditional or exotic vegetables, is a significant production area within Ghana's agricultural industry's crop sub-sector, which has significant potential for both domestic as well as global markets. Vegetable production levels have expanded dramatically over the previous years, rising from 682.43 million metric tons in 2000 to about 1.09 tonnes in 2017 (Statista, 2019), and as a result, they have greatly influenced global consumption, employment, and income demands.

Growing vegetables in Ghana has the potential to reduce poverty and increase food security. Asians Vegetable Research Development Center (AVRDC) report in 2006 indicates that vegetable farming offers smallholder farmers substantially better revenue and more jobs per hectare than growing basic crops (Asravor, 2016).

According to the most recent consumption figures from the Ghana Living Standards Survey (GLSS), in homes, vegetables accounted for 12.8% of the overall food budget in 2012 to2013, with the most common vegetables being tomatoes, onions, carrots, and chillies. For example, tomatoes accounted for 35.2% of all vegetable spending, followed by onions (19.0%), chilies (9.7%), and carrots (1.3 %) (Asselt et al., 2018).

According to Badmus and Yekinni (2011), growing exotic vegetables is a lucrative industry that takes little start-up money and has turned into a source of income for the farmers involved. Urban vegetable cultivation provides people with a nutritious diet and a source of income, ensuring food security, nutrition, and better livelihoods (Hoornweg and Munro-Faure, 2008). Cabbage (*Brassica* *oleracea* var. capitata L.), lettuce (*Lactuca sativa* L.), spring onions (*Allium fistulosum* L.), bell peppers (*Capsicum annuum* L.), and cucumber (*Cucumis sativus* L.) are common urban vegetables that are not indigenous to Ghana (Cofie et al., 2003). but are cultivated by peri-urban vegetable growers.

2.3 Constrains to vegetable production in Ghana

In Ghana, Vegetable production is hindered by a variety of variables that impact negatively on production, processing, marketing, and the entire exploitation of the potential prospects that vegetables may generate (Djokoto et al., 2015; Obuobie et al., 2006; Afari-Sefa et al., 2015). The land tenure structure, limited access to water for irrigation, and crop destruction by pests and diseases are the key factors limiting productivity. The types of crop that can be cultivated, as well as the overall cost involved in producing vegetables, are determined by the land's availability, size, and location, while the water source for irrigation dictates whether the farmer can cultivate throughout both the dry and rainy seasons.

2.3.1 Insect Pest of Vegetable grown in Ghana

The primary biotic barrier to the production of vegetables in Ghana is insect infestations. Many of them not only cause direct harm but also serve as carriers of many viral infections. Vegetable crop losses of between 30 and 40 percent have been reported (Rai et al., 2014). Pre-harvest pests globally cause the loss of 35% of potential crop output, around 45% of total food production losses of the world's total food are caused by insect pests, diseases and weeds (Oerke, 2006).

Pests on crop plants have an agricultural relevance because of the harm they do, which lowers the yield's quality or quantity (or both). The appearance of the crop, which may display certain forms of pest damage or disease signs, is frequently the first sign of the existence of a pest or disease (Hein, 2003).

According to their feeding behavior, insect pests create two basic forms of agricultural damage (Imam et al., 2010). The first is harm caused by the chewing of plant materials by insects such as grasshoppers, locusts, and crickets (Orthoptera), caterpillar (Lepidoptera) and beetles (Coleoptera) (Khan et al., 2020). The second is harm caused by sucking plant sap from tissues of leaves, roots, or fruits, as well as from the phloem (or xylem) system (Khan et al., 2020). The bugs (Hemiptera) and thrips (Thysanoptera) are the two primary groups of sucking insects (Khan et al., 2020). Crop damage caused by insect pests includes decreased yield, disease transmission, decreased market value of crops, and increased agricultural costs. The *Chenopodiaceae* and *Solanaceae* groups of plants contain both succulent and leafy plants, such as spinach and tomatoes, which are attacked by pest insects with a broad host range. Monophagous insects are only found on a small number of the host plants in the system, notably the leafy vegetables like spinach, lettuce, and kale. If all or most of the plants in a mixed system are edible to a polyphagous pest, it is likely that the pest will stay longer because of its capacity to expand its menu of food sources and subsequently multiply, causing more harm (Imam et al., 2010)

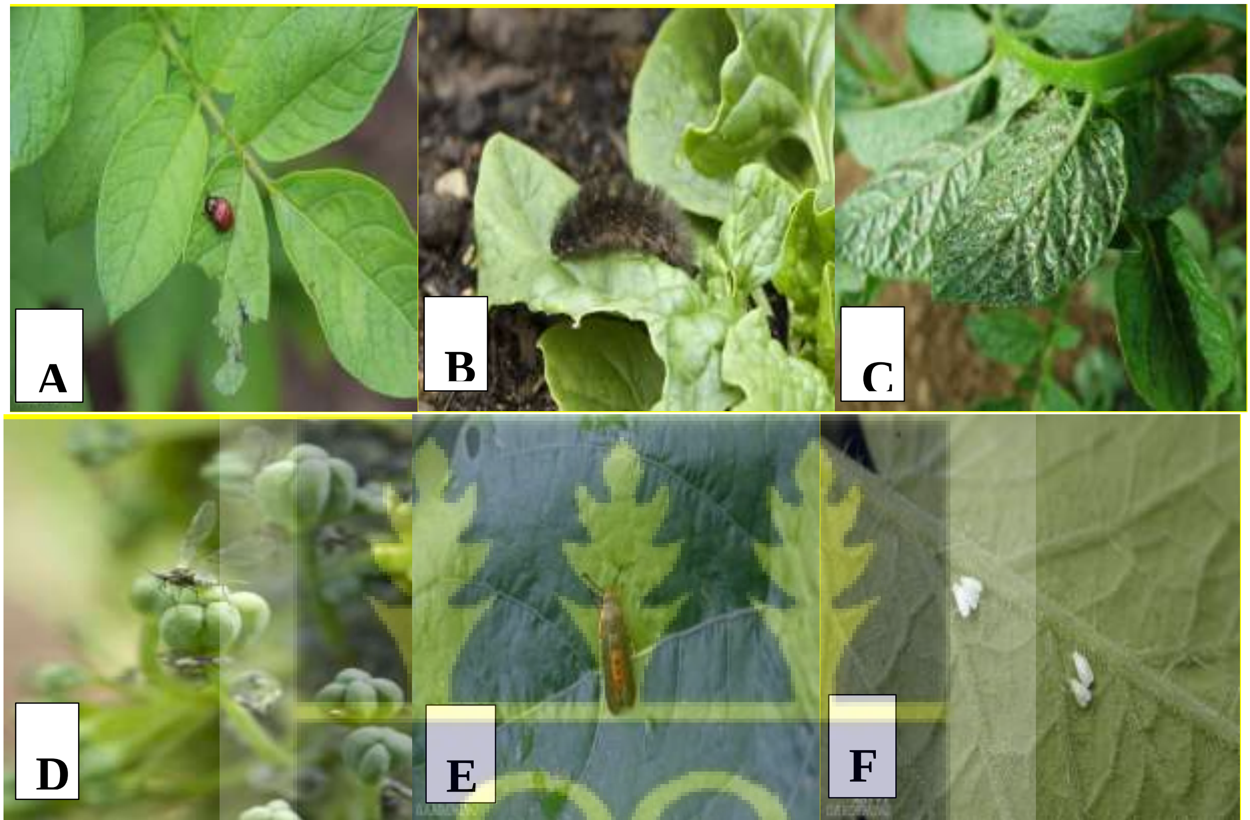

Figure 2.1 Insect pests affecting vegetable production :(A) Colorado potato beetle larvae on the leaves of potatoes (B) The woolly bear caterpillar on a spinach plant, (C) Thrips feeding causes silvering on underside of a potato leaf, (D) Aphids on broccoli (E) Squash vine borer adults (F) whitefly on a leaf.

2.3.2 Diseases of vegetables

Plants infected with bacteria, fungi, parasites, and viruses exhibit certain symptoms that are similar to those caused by insect attack. Finding the pest close to the damage on the plant typically makes it easier to identify the destructive organisms while studying crop damage and agricultural pest (Hein, 2003).

The many vegetable varieties that are often produced in Ghana are vulnerable to a variety of diseases. Both abiotic and biotic factors may contribute to the development of these disorders. The abiotic influences may include some climatic and cultural circumstances including high temperatures, nutritional deficiencies, and excessive sunshine (Roberts et al., 2004). The Food and Agriculture Organization (FAO) generally classifies the biotic disease causing organisms into three groups: bacteria, fungus, and viruses (FAO, 2004). These diseases drastically lower crop yields, raise production costs, and thus have a negative impact on farmer revenue (Manneh et al., 2016).

2.3.3 Bacterial Disease of Vegetables

Many vegetable crops are susceptible to leaf blights and spots caused by plant pathogenic bacteria. These diseases often start off as little, water-soaked patches that gradually turn brown, and they are most frequently brought on by Pseudomonas and Xanthomonas species. The bacterial diseases include bacterial canker (*Clavibacter michiganensis* subsp. michiganensis), bacterial spot (*Xanthomonas axonopodis* pv. vesicatoria), bacterial wilt (*Ralstonia solanacearum*), bacterial speck (*Pseudomonas syringae* pv. tomato) and bacterial stem rot (Erwinia spp, Pectobacterium carotovorum subsp. carotovorum and Pectobacterium carotovorum subsp. atrosepticum, Pectobacterium chrysanthemi). (Bastas, 2013).

Vegetable leaves, fruit, and stems develop lesions from the bacterial spot disease (Jones et al., 2000). The lesions prevent the leaves from performing photosynthetic processes, which ultimately results in leaf death. The fruit may also be impacted, rendering it unfit for processing or export. Although the bacteria are spread over a field by wind-driven raindrops, wounds from field work (grafting, cutting, tying, harvesting, pesticide spraying), and aerosols (Lindemann and Upper, 1985; McInnes et al., 1988), seeds are the main source of inoculum (Obradovich et al., 2004; Jones et al., 1991)

2.3.4 Fungal Diseases of Vegetables

A variety of dangerous plant diseases are brought on by fungi, which make up the majority of plant pathogens. Generally, fungi are responsible for vegetable diseases. Fungal infections are spread through infected soil, weeds, infected seed, agricultural waste, and nearby crops. They are disseminated by the movement of contaminated soil, animals, humans, tools, equipment, seedlings, and other plant material, as well as by wind and water splash. They penetrate plants through stomata, which are naturally occurring openings, as well as wounds caused by cutting, picking, hail, insects, other diseases, and mechanical damage (Tournas, 2005).

Various fungi are the cause of foliar diseases. The following are some of the most prevalent foliar diseases: White blister, Powdery mildew, and Downy mildew. Other soil-borne fungus includes Club root, Sclerotinia species, Sclerotium species, Pythium species, Fusarium species, and Rhizoctonia species.

Numerous vegetables are susceptible to some fungi-related infections. Anthracnose, Botrytis rots, Downy mildews, Fusarium rots, Powdery mildews, Rusts, Rhizoctonia rots, Sclerotinia rots, and

Sclerotium rots are some of the fungal diseases. Other examples are Red root complex in beans, Leaf blight (Alternaria dauci) in carrots, and Club root in brassicas (Plasmodiophora brassicae). Punja and Utkhede (2004).

2.3.5 Viral Diseases of Vegetables

Viral infections are emerging as a significant limitation to agricultural production in the context of changing climatic conditions. Worldwide, virus infections drastically reduce the productivity and quality of agricultural produce and are responsible for significant yield losses of up to 100%. Viral infections are often confused with other irregularities, making it difficult to select the best management approach in order to produce crops free of viruses. (Nagendran et al., 2017). Some important viral diseases in vegetables are; Tobacco mosaic virus disease, Tomato spotted wilt virus disease, Pepper veinal mottle virus disease, Cucumber mosaic virus disease, Potato virus Y disease, Cauliflower mosaic virus disease, Tomato mosaic virus disease, Tomato yellow leaf curl virus disease, Plum pox virus, and Potato virus X. (Arogundade et al., 2014).

2.4 Cucumber mosaic virus (CMV)

2.4.1 Origin and Geographical Distribution of CMV

In addition to its importance as a model for studying plant viral interactions, *Cucumber MosaiVirus* (CMV), has a significant effect on agricultural production all over the world. Among plant viruses, it is one of the most common (Palukaitis and Garca-Arena, 2003).

The first cases of CMV infections on cucumber and other *Cucurbitaceae* plants were reported concurrently by Doolittle and Jagger in Michigan and New York respectively (Garcıa-Arenal and Palukaitis, 2008). Since then, approximately 1200 species in over 100 groups of monocots and dicots, including agricultural plants, ornamental plants, and woody plants have been documented to suffer CMV infections, particularly in temperate to tropical regions. There are two subgroups of CMV isolates that have recently been identified, namely I and II. While isolates from different subgroups have only 69–77% sequence identity, the nucleotide sequences of CP genes from CMV isolates within each subgroup can share up to 95% nucleotide sequence identity (Palukaitis and Garca-Arena, 2003). Isolates of subgroup I are able to tolerate high-temperatures compared to those in subgroup II, while symptoms in subgroup II are less severe. In terms of the country's warm climate, subgroup I isolates are therefore more prone to infect Ghana's vegetable crops. Subgroup I can be further divided into subgroups IA and IB, and populations of subgroup IB is very common in East Asia (Palukaitis et al., 1992; Roossinck et al., 1999). Worldwide dissemination of CMV isolates is evident in open-field crop cultivation and greenhouses. Isolates from subgroup I are more prevalent than those from subgroup II, with the latter being mostly restricted to colder regions or times of year in temperate zones (Garca-Arenal and Palukaitis, 2008). The majority of the subgroup IB isolates found in the Mediterranean region may have been transported there from East Asia, the subgroup's supposed place of origin (Garca-Arenal and Palukaitis, 2008). In Greece, tomato plants were used to identify a severe, satellite-free CMV isolate known as CMV-G that belonged to the subgroup IB. Through further inoculation on a host with a local lesion, two distinct genotypes that infected tobacco with either moderate symptom (green mosaic) or more severe yellow mosaic were isolated (Sclavounos et al., 2006). According to Lavina et al. (1996), CMV causes significant viral reservoirs in native weed populations by infecting a variety of weed species close to horticulture crops. CMV was discovered, CMV strains from subgroup II were discovered in France (Gallitelli et al., 2004). and southern Italy (Palukaitis and Garcia-Arenal, 2003; Roossinck, 2005; Paradies et al., 2000). There are further credible reports of CMV infection from Spain (Gallitelli et al., 2012) and Tunisia (Chabbouh and Cherif, 1990). Uganda, Zambia, Tanzania, Ethiopia, Zimbabwe, Kenya, Malawi, Madagascar, Sudan, Rwanda, Ghana, and Nigeria are among the African countries where the virus has been reported. (Skelton et al., 2018; Appiah et al., 2014).

2.4.2 Transmission of *Cucumber mosaic virus*

2.4.2.1 Natural transmission

Aphids and mechanical inoculation of plant sap are the most important natural transmission of cucumber mosaic virus, and they are also the most effective (Li et al., 2020). In a non-persistent, stylet-borne method, CMV has been discovered to be transmitted from plant to plant by at least 75 species of aphids (Palukaitis et al., 1992). CMV is not passed to offspring aphids and does not multiply in its aphid carrier. According to Li et al. (2020), *Myzus persicae* and *Aphis gossypii* are the most efficient vectors for CMV transmission in vegetables. One of the main characteristics of non-persistent viral transmission is that aphids only pick up the virus for brief periods of time (often a few seconds) before losing it after their usual feeding on plants. Additionally, CMV can spread through pollen, contaminated soil particles, infected seed, infected agricultural debris, and other means (Pares and Gunn, 1989; Alvarez et al., 2003; Chen et al., 2000). Additionally, the virus can spread by the parasitic plant dodder; *Cuscuta* spp. (Palukaitis and Garca-Arenal, 2003).

2.4.2.2 Experimental transmission

CMV infection has frequently been produced in experimental settings by mechanical inoculation using sap, pure virions, or viral RNA. In addition to many other plant species, CMV principally causes mosaic and stunt diseases in the families; *Cucurbitaceae*, *Solanaceae*, *Leguminosae*, *Brassicaceae*, and *Gramineae*. Symptoms of the disease can vary based on the host species and CMV strain, and may include leaf deformity, systemic necrosis, stunting, and chlorosis. Normally utilized for CMV replication, *Nicotiana glutinosa* and *N. tabacum* are well recognized for their aggressive CMV reproduction. The mechanical inoculation of CMV causes necrotic local lesions in the test plants *Vigna unguiculata*, *Chenopodium amaranticolor*, and *C. quinoa*. Garcıa-Arenal & Palukaitis (2009) *Ipomoea setifera* has been the source of CMV isolation Loebenstein (2012) was able to mechanically inoculate *Ipomoea nil*, *Ipomoea purpurea*, *Ipomoea lacunosa*, and *Ipomoea trichocarpa* with CMV but not *Ipomoea batatas* cv. Porto Rico. CMV transmission to healthy sweet potato plants was unsuccessful, according to Cohen and Loebenstein et al. (2009). However, aphid, or graft inoculations can quickly transmit CMV to sweet potatoes in the presence of Sweet Potato Sunken Vein Virus (SPSVV); whitefly-transmitted virus (Loebenstein et al., 2003).

2.4.3 Host Range of CMV

Cucumber mosaic virus (CMV) has a very wide host range of wild and cultivated plants with more than 1,200 known hosts, comprising some monocotyledons and a large number of dicotyledonous plants (Chen, 2003). CMV has also been reported to infect cowpea, celery, cucurbits, pepper, lettuce, tomato, chilies, banana, pasture legumes, and some ornamental plants (Palukaitis et al., 1992); Flasinski et al., 1995; Davis et al., 1996; Gafny et al., 1996; Latham et al., 1999; Iqbal et al., 2011; Ashfaq et al., 2014b).

2.4.4 Alternative Hosts of CMV

Weeds or other alternate natural hosts serve as the primary hosts for the majority of plant viruses from which the economically important crop plants may contract viruses (Neeraj and Zaidi, 2008; Mathews and Dodds, 2008). Due to the biotrophic nature of plant viruses, which obviously needs alternate hosts to survive if the original crop host is unavailable, in close proximity to agricultural fields, weeds, ornamental plants, and wild plants appear to be infected with plant cultivable species' viruses. (Sivalingam and Varma, 2007). These plants also play a significant role in the development of virus disease epidemics. As a result, the epidemiology of viral infections is more complex than that of other plant diseases Budnik (1995).

Many weeds have the potential to serve as CMV reservoirs, which can aid in the virus' early-season transmission to crops. This is due to the virus's wide variety of potential hosts. Common milkweed (Asclepias syriaca), yellow rocket (Barbarea vulgaris), marsh yellowcress (Rorippa islandica), and yellow toadflax (also known as butter-and-eggs, Linaria vulgaris) are a few examples of perennial, biennial, and winter annual plants that harbor CMV in their roots, tubers, and underground organs all winter long. They were shown to be significant causes of infection in lettuce (Zitter and Murphy, 2009).

2.4.5 Symptoms of CMV

Numerous vegetable and horticultural crops are susceptible to CMV, which affects 1200 species in more than 100 plant families and can lead to severe economic losses. In most hosts, the virus induces systemic infection; but, in particular crops, like alfalfa, it may go undetected. Depending on the crop affected and the age of the plant when infection occurs, cucumber mosaic symptoms can vary widely (Zitter and Murphy, 2009)

CMV infected cucurbits, particularly zucchini, squash, melons and cucumber, exhibit severe mosaic symptoms as well as leaf deformation and constriction. Infected plants also exhibit stunting, decreased fruit output with pitted and deformed fruits which are unmarketable. Mixed infections with different viruses are typical, which makes the situation worse. The virus, and the usual mosaic signs on and melon leaves. On fruits, it is also common to see mottling or mosaic. A quick and total wilt is seen on mature plants of several cucumber cultivars a few days following CMV infection. The symptoms of CMV in zucchini and squash are quite severe and include mosaic, yellow spots, and leaf deformities. Infected plants continue to be stunted, and fruit setting is typically significantly decreased or even stopped. Deformed fruits have little depressions all over them. Watermelon reacts with black necrotic lesions when it has CMV infection, which is an uncommon occurance (Garcia-Arnal and Palukaitis (2009).

Chlorotic local lesions between the secondary leaf veins are one of the early CMV infection symptoms in crops like tomato plants. Necrosis advances toward the base of the plant, which might die a few weeks after infection, starting with brown spots on the leaves and progressing to brown lines along petioles and stems. Fruits are misshaped, sunburned, and necrotic if they are present at all. Infected plants typically exhibit reduced growth, a bushy look, and deformed leaves when they are grown (shoestring-like leaves). Fruits shrink in size and do not ripen. Depending on a variety of variables, including the existence of satellite RNAs, plants may have normal-appearing leaves but exhibit fruit necrosis (losses are much decreased in this instance), or they may exhibit usual symptoms but with more pronounced manifestations (in this case, losses can be very high) (Bragard et al., 2013).

The viral strain and the age of the plant at the time of infection affect the CMV infection symptoms in pepper plants. CMV induces symptoms of necrosis on both the fruit and foliage of young pepper plants, including leaf yellowing, leaf constriction, and fruit necrosis. If older plants are infected, CMV signs can be subtle and show up on lower, yellowing leaves as green ring spots or oak-leaf patterns, as well as a subtle mosaic.

CMV infected celery and parsley exhibit yellowing and necrosis of the leaves. When the temperature falls below 13 °C, CMV infected lettuce exhibits strong mosaic, vein chlorosis, and commonly vein browning and necrosis. Stunting, yellowing, and mottling of the older leaves as well as deformation of the younger leaves are some of the indications of CMV infection in spinach (Bragard et al., 2013).

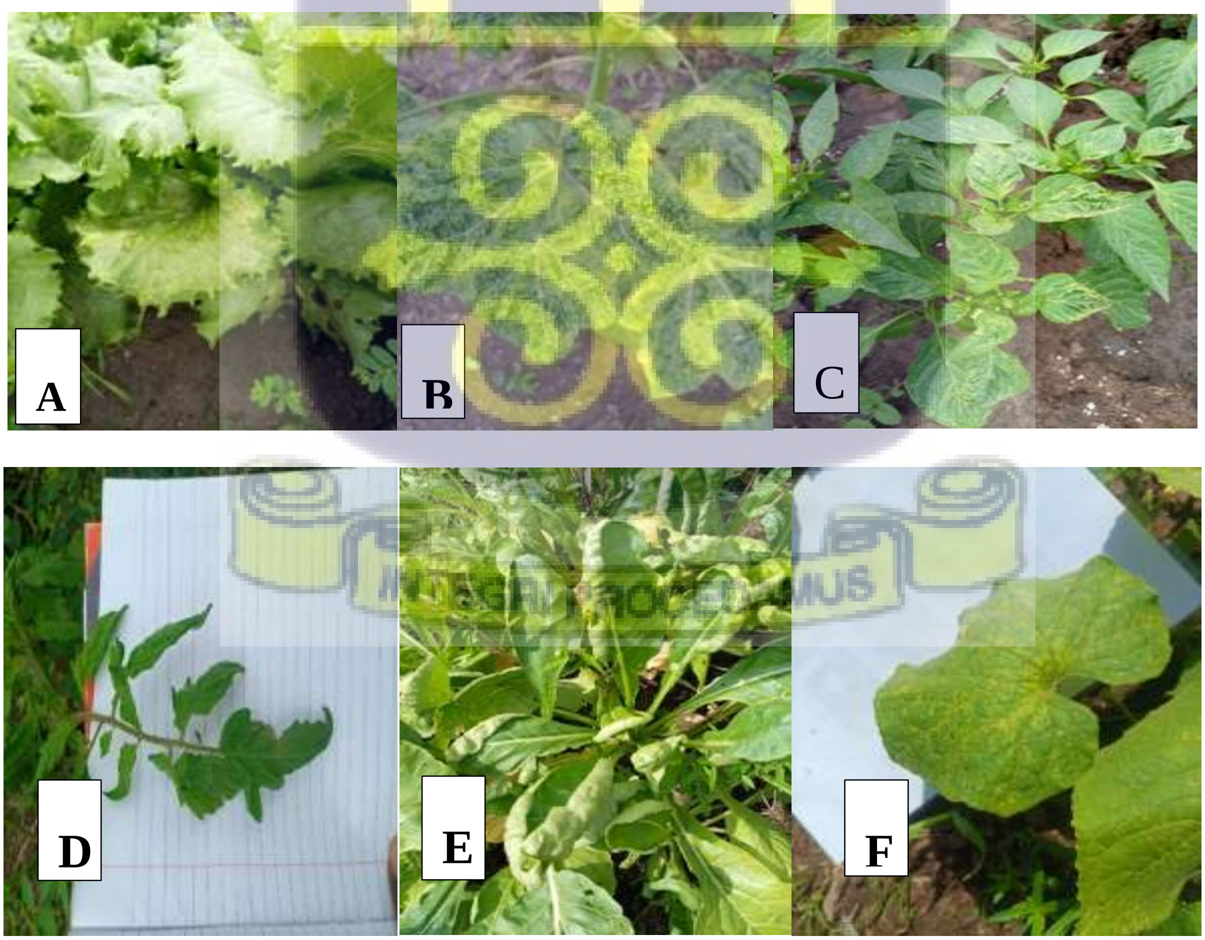

Figure 2.2 Some symptoms of Cucumber mosaic virus; A- leaf yellowing in lettuce, B- Leaf blistering in okra, C-mosaic leaf deformation in pepper, D-leaf deformation in tomato, E- leaf curl upwards in Radish, F- Yellow patches (mosaic) in cucumber.

2.4.6 Genome Organization of CMV

Three positive-sense, single-stranded RNAs packed in distinct particles may be found in the CMV genome (Palukaitis et al., 1992). Additionally, two subgenomic RNAs are present in virus particles (Palukaitis, 2003). The 1a and 2a proteins, which make up the two components of the viral replication complex, are encoded by RNAs 1 and 2, respectively (Hayes and Buck, 1990). Additionally, RNA 2 encodes the 2b protein, a multifunctional protein implicated in host-specific, long-distance migration, symptom development, and virulence determination via inhibiting gene silencing (Brigneti et al., 2015). Additionally, a recent study has shown that the 2b gene controls the choice of inter-viral recombination (Shi et al., 2008). Cell-to-cell movement protein (MP) 3a and capsid protein (CP), which is translated from a subgenomic RNA 4, are both proteins that are encoded by the CMV RNA 3. Cell-to-cell communication, virion assembly, and aphid-mediated transmission are all facilitated by CP (Perry et al., 1998).

Based on serology, nucleic acid hybridization, RT-PCR followed by RFLP, and nucleotide sequence identity, CMV strains have been divided into two major subgroups known as subgroups I and II (Roossinck, 2002). The nucleotide identity between these two groupings is 75% (Roossinck, 2002), with subgroup I being more diverse than subgroup II (Palukaitis, 2003). Subgroup I has been further divided into IA and IB, with 92–95 percent nucleotide similarity between these two A phylogenetic examination of a few CMV strains revealed that the subgrouping from CP ORF analysis is not entirely supported by the estimated trees for the distinct open reading frames (ORFs) found on the different RNAs. As a result, several RNAs may have independent evolutionary histories (Roossinck, 2002). In agricultural regions, the subgroups are not spread equally. Subgroups IA and II are distributed globally, although subgroup IB is reportedly confined mostly to Asia (Roossinck et al., 1999) Random mutation, recombination, and re-assortment are the most frequent causes of RNA virus evolution and variety, and RNA viruses are capable of undergoing fast genetic change (Garca-Arenal et al., 2001). All three CMV subgroups have been observed to exhibit RNA re-assortment (Bonnet et al., 2005; Chen et al., 2007). Recombination in the 5′ and 3′ NTR, between ORFs 3a and 3b, and in naturally occurring populations of CMV harboring satellite RNA have also been demonstrated to be additional sources of heterogeneity in the viral population (Bonnet et al., 2005; Chen et al., 2002; Pierrugues et al., 2007). As a result, CMV is a heterogenic species with a wide range of isolates. This enables the virus to quickly adapt in distinctive habitats with fluctuating selection pressures (Roossinck, 2002; Lin et al., 2004; Escriu et al., 2000; Koundal and Praveen, 2011). For a better understanding of the evolutionary mechanisms that produce variety, studies concentrating on this genetic diversity and the causes of variation in viral populations are crucial.

For many years, CMV has been prevalent in several regions of the United States. But in the 2000s, a number of viral outbreaks impacted the processing snap bean (Phaseolus vulgaris L.) in crucial agricultural areas in the upper Midwest and northeast of the US (German et al., 2004; Nault et al., 2006). The introduction of new variations in viral populations can help to partially explain the origin of such plant virus outbreaks.

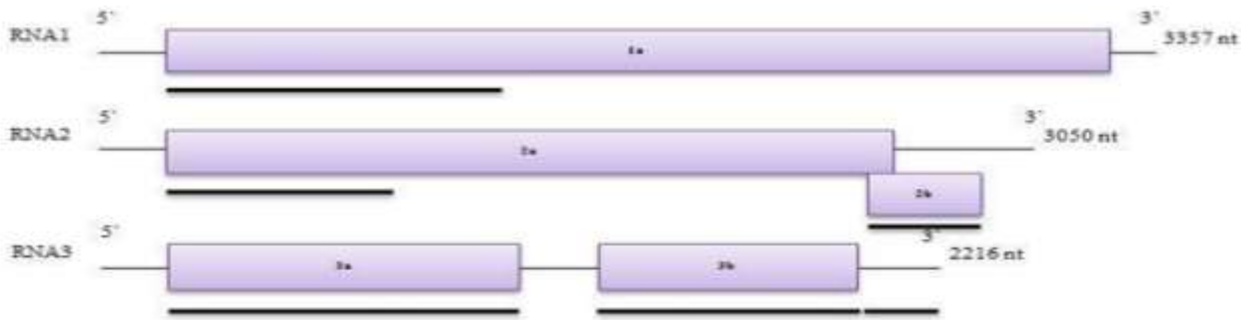

Figure 2.3 Genome organization of CMV.

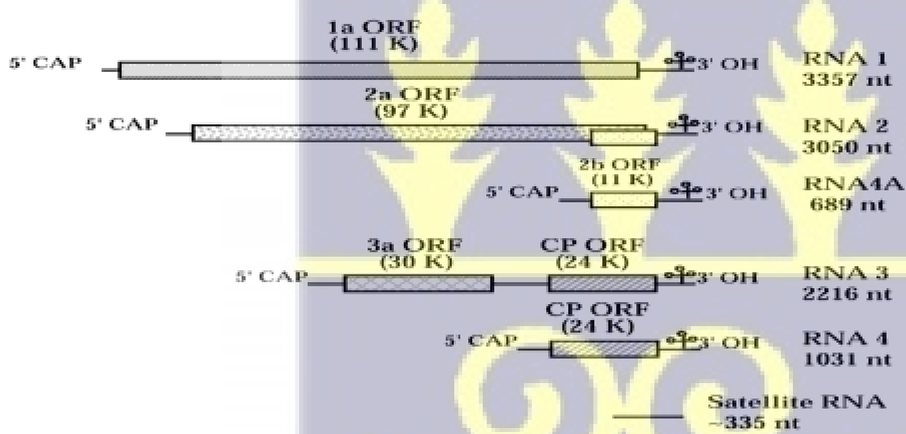

Figure 2.4 Genome organization of CMV.

The nucleotide and amino acid numbers are for the Fny strain. RNAs 1, 2, and 3 are genomic, and required for infection. RNAs 4 and 4 A are subgenomic. RNA 4 is packaged in virions of all strains; RNA4A is only packaged in subgroup II strains. The satRNA is a molecular parasite sometimes associated with the virus. It is packaged in the virions of the virus.

2.4.7 Detection of CMV

A plant that has a viral infection may show a variety of indications indicating the virus is active.

However, since viruses can induce symptoms that are identical to those caused by abiotic stressors, using symptoms alone to diagnose the presence of viruses may be misleading (Gao, 2020).

Additionally, it is uncommon for plant viruses of the cryptic viral class to ever cause any visible symptoms (Marwal et al., 2021; Lukács, 1994; Valverde and Gutierrez, 2008). Therefore, rapid diagnostic approaches must be used to enable accurate, early diagnosis and disease control due to the catastrophic impact of viral diseases on economically important plants. In situations where symptoms may be similar to those of more than one virus, molecular and serological diagnostic approaches have shown to be very helpful (Shukla, 2015). The cucumber mosaic virus has been identified using these methods, which include the Enzyme Linked Immunosorbent Assay (ELISA), Reverse-Transcription PCR, and Electron Microscopy.2.4.7.1 Serological Detection (ELISA)

One of the most commonly used immunodiagnostic techniques is the Enzyme Linked Immunosorbent Assay (ELISA), which is popular due to its simplicity, accuracy, and relatively low cost. The use of ELISA for the detection of plant viruses is widely known and has proven to be one of the most effective technologies for the identification of plant viruses

The ELISA technique relies on the sensitive detection of non-precipitates reaction, made possible by the use of enzyme-labeled antibodies. González-Garza (2017), discovered that ELISA differs from nearly all other serological techniques in plant virology that were based on the formation and detection of immune precipitates. For practical purposes, the ratio of antibodies to antigen has little effect on the effectiveness of ELISA technique. Thus, once the proper concentrations had been established, they could be used in subsequent tests to detect the virus at all concentrations, and since the reaction of enzyme-labeled antibodies was dependent on the virus concentration, the method has a high quantitative potential for figuring out how sensitive ELISA is.

The ELISA test enables the researcher to do both a quantitative and qualitative analysis and the test has been used extensively by researchers studying the *Cucumber mosaic virus* due to its usefulness and simplicity of use (Gan and Patel, 2013).

2.4.7.2 Nucleic Acid based Test

Due to its capacity to identify viruses at low titer levels or concentrations. Reverse Transcription Polymerase Chain Reaction (RT-PCR) is the most widely used technique for diagnosing plant viruses (Scagliusi et al., 2009; Mekuria et al., 2003). Several investigations have demonstrated that the RT-PCR is more accurate at detecting plant viruses than ELISA, with fewer false-negative findings (Mekuria et al., 2003; McGavin et al., 2011). By calculating the DNA concentration following each amplification step in the PCR process, the qPCR, also known as real-time PCR, can calculate the viral titer level in the samples (Taylor et al., 2010; Deepak et al., 2007).

The PCR technique employs a pair of artificial oligonucleotides or primers, each of which hybridizes to one strand of a double-stranded DNA (dsDNA) target and which spans an area that will be exponentially replicated. The DNA Taq polymerase uses the hybridized primer as a substrate, and the hybridized primer is used to sequentially add deoxynucleotides to the substrate to form a complementary strand. The Taq polymerase is most frequently produced from the thermophilic bacteria *Thermus aquaticus*. Three phases make up the process: (i) dsDNA separation at temperatures over 90 °C, (ii) primer annealing at temperatures between 50 and 75 °C, and (iii) optimum extension at temperatures between 72 and 78 °C. A programmable thermal cycler regulates the rate of temperature change, often known as the ramp rate, the duration of incubation at each temperature, and the number of times each set of temperatures is repeated. With the use of fan-forced warm air flows or electrically controlled heating blocks, modern technologies have drastically decreased ramp times. As a result, several of the best cell culture, antigenaemia, and serological techniques are being replaced by PCR (Niubo et al., 1994). In order to gather quantitative data, it has been possible to employ existing PCR and detection assay combinations (referred to as "conventional PCR"), with encouraging outcomes. However, these methods have been hampered by the time-consuming post-PCR processing procedures needed to assess the amplicon (Guatelli et al., 1989).

The conventional method for detecting amplified DNA involves electrophoresis of the nucleic acids in the presence of ethidium bromide and visual or densitometric examination of the bands that emerge after exposure to UV light (Kidd et al., 2000). Additionally, PCR-ELISA may be used to capture amplicon onto a solid phase using biotin or digoxigenin-labelled primers, oligonucleotide probes (oligoprobes), or just after the incorporation of the digoxigenin into the amplicon (Watzinger et al., 2001). An enzyme-labeled avidin or anti-digoxigenin reporter molecule can be used to detect the amplicon once it has been collected, much like a conventional ELISA format.

Real-time PCR has demonstrated its worth in labs all over the world, adding to the vast quantity of data produced by traditional PCR tests. In contrast to the traditional assays, real time PCR allows the detection of the amplicon to be seen as the amplification developed (Mackay, 2004).

The use of fluorogenic molecules to label primers, probes, or amplicon has made it possible to monitor accumulating amplicon in real time. The technique has become faster in large part as a result of shorter cycle durations, the elimination of post-PCR detection steps, the use of fluorogenic markers, and sensitive methods for detecting their emissions (Wittwer et al., 1990; Wittwer et al., 1997).

Comparatively to conventional PCR, real-time PCR has significant drawbacks, such as the inability to monitor amplicon size without opening the device, the incompatibility of some platforms with certain fluorogenic chemicals, and the relatively limited multiplex capabilities of present applications. When utilized in low-throughput facilities, real-time PCR's startup costs might also be too high. The hardware of the system or the fluorogenic dyes or fluorophores available both have certain restrictions that contribute to these flaws.

2.4.7.3 Electron Microscopy (EM)

Scientists have worked to understand the structure of viruses ever since they were identified as the disease-causing agents in the last decades of the nineteenth century (reviewed in Mettenleiter, 2017). In order to conduct investigations at the nanoscale and obtain direct pictures of viruses for use in research and diagnostics, electron microscopy (EM) has a high resolving capacity. Due to its capacity to identify contaminations, the use of TEM is crucial for quality control of reference material, viral preparations used for antibody generation, or directly for vaccination. During an unexpected regional viral outbreaks or epidemics, prompt generation of consistent and comparable data is crucial for effective diagnosis, making TEM a vital component of the procedures followed by national reference laboratories. EM image databases have just been created and made accessible by Laue and Möller (2016). The expansion of these archives will offer the ease of identification of unexpected as well as newly developing infections. For instance, plant viruses can act as markers to forecast the presence of harmful human viruses in irrigation water (Shrestha et al., 2018)

2.4.7.4 Immuno-electron microscopy

The same serological concepts that underlie ELISA are also the foundation of immuno-electron microscopy (IEM), which may be used to further identify viruses during regular Transmission electron microscopy (TEM) testing. IEM has the benefit of not requiring further immunoglobulin purification or conjugation procedures because it operates directly with raw serum. Antibody usage is minimal since only modest reaction volumes are needed. Most TEM labs maintain extensive antisera collections tailored to a variety of viral types and isolates. Antisera may be kept for a long time at 4°C with 0.05% sodium-azide without suffering a substantial loss of activity. Polyclonal antisera may respond in a heterogeneous manner depending on the antigen composition and the epitopes found in the initial viral purification and therefore can be used for a variety of purposes during regular diagnostics (Griffiths and Lucocq, 2014). While serological associations will become apparent by the intensity of antibody attachment during the decorating process, some antisera are adequate for IEM capture of several viruses within a genus. In a homologous response, the virion is shown to be firmly packed with antibodies, whereas emerging isolates or heterogonous viruses would only have a thin antibody coating (Richert-Pöggeler et al., 2019). When present, monoclonal antibodies that target single epitopes offer the best specificity and repeatability for differentiating between viral isolates (Griffiths and Lucocq, 2014).

2.4.7.5 Biological Test Using Indicator Plants

Accurate disease identification is difficult due to the fact that viral infections do not always result in immediately noticeable visual signs in the host plants. With the use of biological indexing, it is possible to validate the existence of a virus that could otherwise go undetected in some plants, identify an uncommon host plant for the virus, and quantify the virus (Smith, 1977). The sensitive plant species or kinds that often exhibit typical symptoms after exposure to the pathogenic viruses are the indicator plants (Smith, 1977).

Biological indexing is still employed in conjunction with lab-based testing techniques to effective disease diagnosis (Wolfenden et al., 2018). The main drawback in the utilization of indicator plants for virus detection is the lengthy time (weeks or months) it takes between inoculation and the appearance of disease symptoms (Legrand, 2015). Furthermore, indicator plant symptoms may change depending on the surrounding environment. Constable et al. (2013) discovered that rugose wood symptoms on Rupestris St. George indicator plants could not be seen in a cold climate but symptoms were evident in hot climate. Contrarily, Cabernet Franc in a cool location exhibited Grapevine Leafroll Disease (GLD) symptoms, but no symptom was observed in the hot climate site for the identical treatments, limiting the use of this variety as an indication (Wang et al., 2022)

2.4.8 Economic Importance of CMV

Viruses are to blame for significant quality and production losses in crops all around the world. *Cucumber mosaic virus* (CMV), one of the most significant viruses affecting vegetable crops globally, transmitted by polyphagous aphid vectors (Montasser et al., 2006; Palukaitis and Garcia-Arenal, 2003; Pakdeevaraporn et al. 2005; Biswas et al. 2013). CMV is particularly severe on chilli (Green, 1993) and up to 41% yield reductions in vegetable has been reported (Selvarajan et al., 2023).

Additionally, more than 60% yield losses resulting from CMV infection in peppers has been documented (Rahman et al., 2015). If an infection occurs early, yield losses might be as high as 100% (Rahman et al., 2015). However, the quantity of crop losses is influenced by the virus strain, vegetable type, plant age and, environmental variables, viral disease during disease development, etc (Thackray et al., 2004). One of the about 80 species of aphids that can spread CMV is the green peach aphid, Myzus persicae, Cerkauskas et al. (2004). CMV is not spread by pepper seed. Although CMV may be mechanically transferred, workers handling infected pepper plants do not readily spread it because it is less stable than TMV. Weeds serve as hosts for both the virus and the aphid vectors. The high prevalence of CMV in field plants is caused by the abundance of aphid vector species and natural host reservoirs (Rahman et al., 2015).

2.4.9 Control and Management of CMV

Several strategies are often used to reduce the prevalence and spread of CMV. The devastation brought on by viral infections has been fought against with intensive efforts that include interdisciplinary approaches to viral disease control. Aphid vector management with chemicals, cultural techniques, and the adoption of virus-resistant cultivars are some of the strategies that are commonly used.

2.4.9.1 Integrated Management Practices

Worldwide, large agricultural losses are caused by plant diseases, which are regarded as a serious biotic constraint. Particularly in the tropics and semi-tropics, where viruses and their vectors thrive, viral infections of plants result in significant economic losses. Furthermore, viral diseases have been made worse by intensive agricultural techniques that have been made necessary by the population's expanding needs and the introduction of novel genotypes and cropping patterns (Varma, 2007). To reduce the losses brought on by these diseases, a variety of innovative strategies have been tested. The main strategies involve avoidance of potential infection sources, avoiding or controlling vectors, altering cultural practices, using resistant varieties developed through conventional breeding techniques, cross-protection, systemic acquired resistance, and using transgenic plants that have been genetically modified to express alien genes that confer viral resistance (Varma, 2007). In contrast to adopting a single component method of control, integrated disease management (IDM), which integrates biological, cultural, physical, and chemical control measures, has been shown to be more successful and long-lasting (Ownley and Trigiano, 2016).

Chemical pesticides are viewed as being both uneconomical and environmentally unfriendly. Despite the fact that several studies have been done to identify non-chemical alternatives. Pesticide-related issues including pest resistance, dangers to non-target organisms, pest comeback, and pesticide residue have gotten much worse as a result of overuse of pesticides. Thus, the creation of disease-resistant cultivars or hybrids is the most efficient, long-lasting, and sustainable method of CMV management (Yao et al., 2013). Although it has been shown that using resistant cultivars is the most practical and inexpensive option, sustainable agriculture must adopt an integrated strategy for the viral infections to be effectively managed. Therefore, in order to create efficient integrated management strategies, it is essential to have a complete grasp of the ecology of viruses and their vectors (Wightman 2007).

Several methods have been used to combat CMV, including as cultural practices like weeding, eradicating infected plants, using disease-free seeds, and using chemical and biological pesticides like pyrethroids, abamectin, and carbamate to control aphids (insect vectors). Disease management is highly challenging due to the wide host range and many insect vectors. (Palukaitis et al., 1992). It is possible to increase yield by using methods to postpone early infection. Use of certified seeds and plants, screening and disinfection of infected mother stock, washing and disinfecting of hands and tools, and planting resistant cultivars are the most effective ways to manage CMV. Vegetables should also be kept out of weedy border areas and planted next to taller border plants like maize, which can serve as a non-susceptible host. (Arogundade et al., 2019).

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CHAPTER THREE

3.1 FARMER PERCEPTION OF VIRAL DISEASES ON VEGETABLE FARMS IN THE GREATER ACCRA REGION

3.2 INTRODUCTION

Vegetables are the fourth-largest commodity group produced in Africa (Salaudeen et al., 2018). They are served as a side dish with the starchy staple foods that make up the majority of the continent's diet. Vegetables are essential in Sub-Saharan Africa's ongoing fight against the effects of malnutrition and covert hunger (Abro and Sadaqat, 2010). Furthermore, many subsistence farmers in Ghana depend on vegetables for revenue and food security. Plant-virus interactions have attracted a great deal of study interest because of the destructive effects of virus infections on crop yields and nutritional quality that ultimately result in the loss of economically important crops.

The primary biotic agents that significantly impede the optimum development and yields of vegetables are virus infections and insect pests (Phophi and Mafongoya, 2017; Godwein-Egein et al., 2017). Viral diseases lower output, standards and marketability of crops, inflict severe economic harm, and substantially reduce farmer revenue. In order to effectively control and manage diseases, it is crucial to be aware of crop diseases and their accompanying symptoms (Godwein-Egein et al., 2017). The method and speed with which control measures are implemented will be greatly influenced by the farmer's knowledge and perception of the diseases that are present on the farm. Usually, farmers who don't have enough expertise or information about the diseases that are prevalent on their farms struggle to produce crops as efficiently as possible.

Dodoo (2009) stressed the significance of farmer perception and awareness of diseases related to their crops and administration of control measures. According to him the strong opposition of cocoa farmers to destroying infected trees caused by the *Cocoa swollen shoot virus* (CSSV) as part of a control program was due to the farmers' ignorance of the significance of destroying the diseased cocoa trees. In this regard, the farmers participating had received enough information, enabling them to comprehend the disease issue and the importance of the exercise to control tree damage. Furthermore, farmers' resistance to such disease control programs is typically overcome through education (Dodoo, 2009), thus farmer's expertise is an essential step in disease management and control.

Climate change indicators demonstrate that virus diseases are spreading and that they are having a more catastrophic impact on crop productivity and quality (Ghini et al., 2011). Effective disease management strategies are seriously constrained by the fact that it might be difficult for farmers to diagnose viral diseases. The justification is that symptoms of viral infections, including deformed leaves, streaking and stunting, vein clearing, or mosaic, might resemble physiological changes brought on by abiotic stresses such drought and low soil fertility. Small-scale farmers, especially those in sub-Saharan Africa, frequently lack such accurate virus symptom evaluation skills. To be able to make substantial impact on virus disease management and control, researchers must fully understand the level of farmers' knowledge of plant viruses and the diseases that they cause. Establishing farmers' views of agricultural production and quality damage on their farms brought on by viruses and other diseases is also crucial. Additionally, it is necessary to determine the justifications behind their decision to implement a particular control measure as well as their perception of its efficacy.

The goal of the study was to determine how much information vegetable growers in the Greater Accra Region had regarding the pests and viral diseases that affected their crops, as well as how that knowledge affected how they managed those crops. The results of this research are anticipated to aid in promoting a more efficient and long-lasting management of plant virus diseases, particularly *Cucumber mosaic virus*.

3.3 MATERIALS AND METHODS

3.3.1 Study area and location

The study was carried out in the Ayawaso West Municipal District, Ga East Municipal District and Tema West Municipal District of the Greater Accra region of Ghana.

3.3.2 Ayawaso West Municipal District

The Ayawaso West Municipal District was formerly part of the then Accra Metropolitan District until 15 March 2018 when small portion of the district was split off to create the Ayawaso West Municipal District. It has a population of 75,303 and covers a land area of 34.90km². This district is located 514 m above sea level on latitude 5° 36' 53'' N and longitude 0° 12' 3'' W. The district has mainly a dry equatorial climate with an average annual rainfall of about 730 mm, most of which is experienced within the two rainy seasons of Ghana. The average annual temperature recorded for the district is 26.8 °C. Approximately 32 % of the population is engaged in agriculture with a little over 77 % practicing crop farming (Ghana Statistical Service, 2021).

3.3.3 Ga East Municipal District

The Ga East Municipal District has a total population of 283,379 people. This municipality is located 546 m above sea level on latitude 5° 40' 2'' N and longitude 0° 13' 01'' W and covers a land area of about 166 km^2 of which 58.10km² of total land size is urbanized whilst 107.90 km² is for agricultural production. The Ga East District lies in the savanna agro-ecological zone and characterized by an average annual temperature with the range 25-35^{o}C with a bi-modal rainfall pattern. An estimated 55% of the population is engaged in agriculture with about 80% of that number involved in crop farming. (Ghana Statistical Service, 2021; Ministry of Food and Agriculture, 2021).

3.3.4 Tema West Municipal District

The district has a population of 177,924 (Ghana Statistical Service, 2021) and covers a land area of 87.8 km^2 and located on latitude 5° 38' 19.19" N and longitude 0° 00' 5.40" E. The Tema West Municipal district situated in the coastal savannah zone, 32 meters above sea level. The average annual precipitation and temperature recorded in the area are 798 mm and 26.7℃ respectively. The lowest amount of rainfall of about 10 mm is usually recorded in January whereas the highest rainfall for the area is recorded in June with an average of 195 mm. In the Tema West Municipal District, an estimated 3.6% of the population engages in agricultural activities with 74.7% of that number involved in crop production (Ghana Statistical Service, 2021).

3.4 SELECTION OF FARMERS

Farmers were chosen from each district using purposive sampling techniques. Interviews were conducted with a total of 120 respondents, 81 of whom were men and 39 of whom were women. Farmers who either had vegetable farms at the time of the study or had vegetable farms in the previous year were the ones who were interviewed.

3.5 SURVEY AND DATA COLLECTION

Field observation assisted by a questionnaire and organized, self-administered interviews were used to gather primary data. The questionnaire had both open-ended and closed-ended questions that were written in English and administered in both English and Twi due to some of the farmers' weak English ability. The survey's questions were centered on the respondent's socioeconomic status (sex, age, and education), farming experience, and agronomic practices (land size, crop cultivated, cropping method utilized, etc.), as well as disease awareness and management (disease incidence, causes, effects on the crop, management of the disease, etc.). The IBM Statistics SPSS version 25.0 was used to analyse the data collected.

3.6 RESULTS AND DISCUSSION

3.6.1 Demographic Characteristics of respondents

In Figures 3.1, 3.2 and 3.3, the respondent farmers' educational background, the number of years they have been involved in vegetable farming, and the size of land they cultivate are shown respectively. The total number of farmers interviewed was 120, 81 of whom were men and 39 were women. Based on the distribution of men and women, it appears that men predominate in the small-scale vegetable growing sector in the Greater Accra area. According to Zibrilla and Salifu (2004), men may typically have greater access to land and other resources than women for market-oriented farming. In addition, farming requires a lot of labor, thus males tend to work in it more often than women. Women were probably more likely to work in marketing than farming in the surveyed locations.

The current findings are in line with those of Drechsel et al. (2006), who noted that open-space farming was predominately conducted by men in Accra, Kumasi, Koforidua, and Tamale, with a male to female ratio of roughly 90 to 80 percent. The majority of urban farmers, however, appear to be women, according to research conducted in other African nations (Kenya, Mozambique, Tanzania, Uganda, Zambia, and Zimbabwe). This, according to Chancellor (2004), is due to the fact that women are still primarily responsible for ensuring the nourishment and welfare of the household.

Most of the respondents were literate with the majority (78%) having had some form of formal education. This level of literacy among the farmers is higher than that reported by IWMI (Drechsel and Keraita, 2014.) which indicated 52 % literacy level among vegetable farmers in Accra. Out of the 78 % literacy level recorded in this study, 43% have had Junior High School education, 22% Senior High School education and 13% Primary School education (Fig. 3.1). Twenty-two percent (22%) of the respondents has no formal education of any sort. This level of illiteracy is far lower than the 48 % observed among vegetable farmers in Accra Drechsel and Ketaita, 2014).

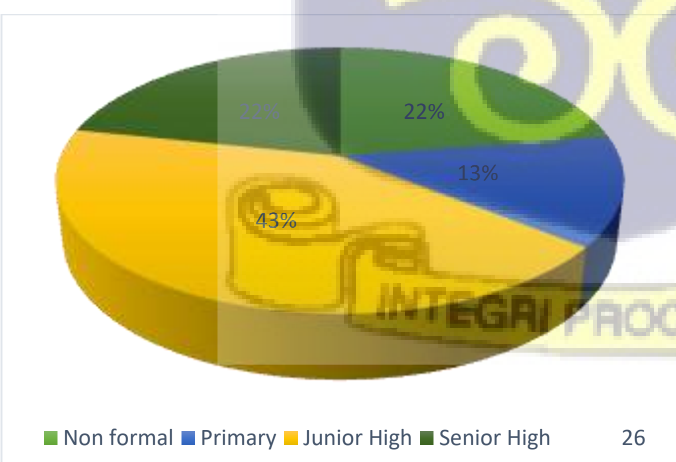

Figure 3.1 Educational level of respondent farmers in the study areas.

The majority of the farmers representing 60% have been growing vegetables for between 1 and 5 years. Von (2018) made a similar observation that 68.8 % of respondent vegetable farmers in the Central Region were less experienced, having participated in the farming between 1 and 5 years. About 38% of the farmers have been growing vegetables for over 5 years while 2% have farmed for less than a year (Fig. 3.2), implying that a lower number of the vegetable growers in the region are new entrants and this signifies a reduction in vegetable industry with potential employment opportunities. According to Von (2018), the vegetable industry in Ghana has been identified to provide quick returns to investment, generates relatively higher annual revenue and an assured way of improving the livelihoods of farmers especially women.

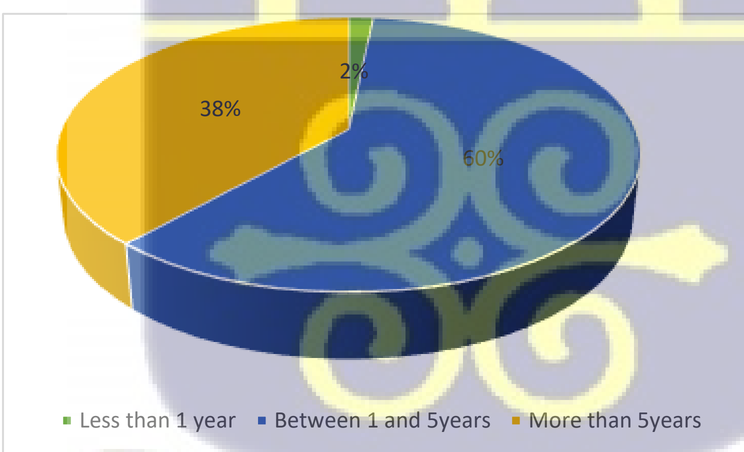

Figure 3.2 The number of years the respondent farmers have practiced vegetable farming.

Most of the farmers (58 %) have medium sized farm holdings between 1 and 3 acres while 25% have farm sizes that are more than 3 and 17% have farm sizes less than 1 acre (Fig. 3.3). The findings of this study are consistent with Obuobie et al (2006) and Mishra and Ghadei (2015) who observed that most vegetable farmers usually had land areas of less than 0.741 acres. The small sizes of the farm holdings could be attributed to the high cost of lease or non-availability of land. None of the farmers interviewed actually owned the parcels of land they were farming on. Some farmers were encroaching on the land they cultivate and were invariably being tolerated by the land owners, usually government agencies, who do not bother the farmers until they have to undertake some projects on the land, in this case the farmers are forced to relocate. Most of the farmers revealed that they do not farm at only one location but managed multiple farms in order to increase and maximize their profits. The farms visited were located either by the roadside or in the interior of the urban settlement.

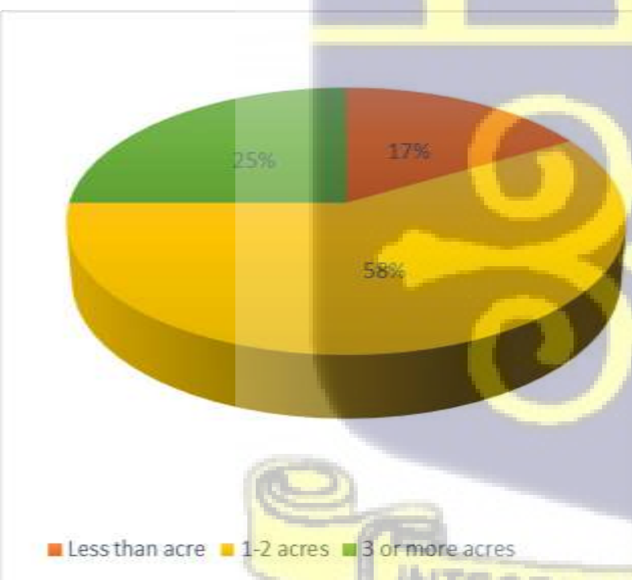

Figure 3.3 Size of farmland cultivated by respondent farmers in the study area.

3.6.2 Cropping systems and agronomic practices of respondents

Majority (94%) of farmers interviewed practiced monocropping (Fig. 3.4) while the remaining 16% of respondents practiced mainly mixed cropping system. According to Drechsel and Keraita (2014), monocropping was a common practice among vegetable farmers in Kumasi in the Ashanti Region. The farmers who practiced the monocropping system believe that they are better off concentrating resources on one type of crop at a time and harvest after which they could switch to another crop during the next planting season. Contrarily, those who practiced mixed cropping were of the view that the land is much better used if different types of crops are grown at the same time on the same land. These farmers explained that since usually crops that are mixed have different growth cycles, one could be assured of a harvest at different times of the year. The most common intercropped plants that were observed are sweet pepper with lettuce and sweet pepper with maize.

All the farmers interviewed stated that it was possible to grow their vegetables throughout the year, provided they had available a reliable source of water such as dugout pools or existing water bodies which could be tapped or from which water could be pumped for irrigation. It was observed that some of the vegetable farmers along the Tema motorway in the Tema West Municipal District irrigated their crops with water from water bodies polluted with waste from the processing factories. The unavailability of water for irrigation is one of the major limiting factors that affect vegetable production especially during the dry season. The farmers, who have no access to any source of water, usually buy water and store for use. This introduces extra cost and increases their cost of production which eventually reflects in higher selling price of the crop.

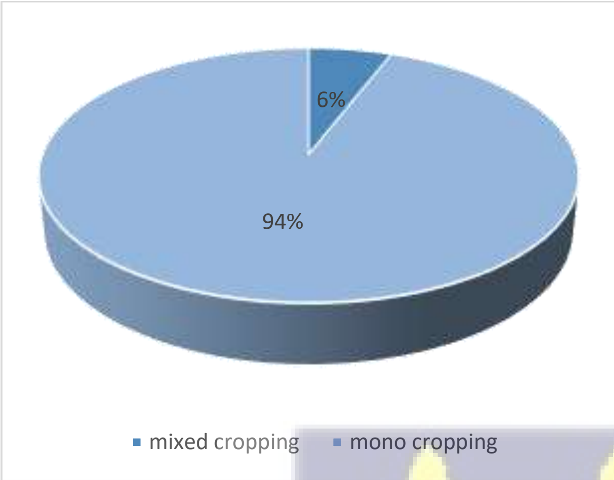

Figure 3.4. Cropping systems practiced by respondents.

Information on the main varieties and sources of seed used by the farmers is presented in figures 3.5 and 3.6. Majority of farmers (88%) preferred to grow imported exotic seeds. The use of improved crop varieties is essential for increasing agricultural productivity in Africa (Walker and Alwang, 2015). The rest of the farmers constituting 12% preferred to grow the improved varieties. Interestingly, the farmers avoid the local varieties because they are of the view that they give very low yields and are more susceptible to diseases compared to the exotic or improved varieties. Seventy-four percent (74%) of the farmers obtained their seeds from dealers like Agrimat, Technisem and Meridian seeds while the rest indicated the Ministry of Food and Agriculture as their source of planting seed materials.

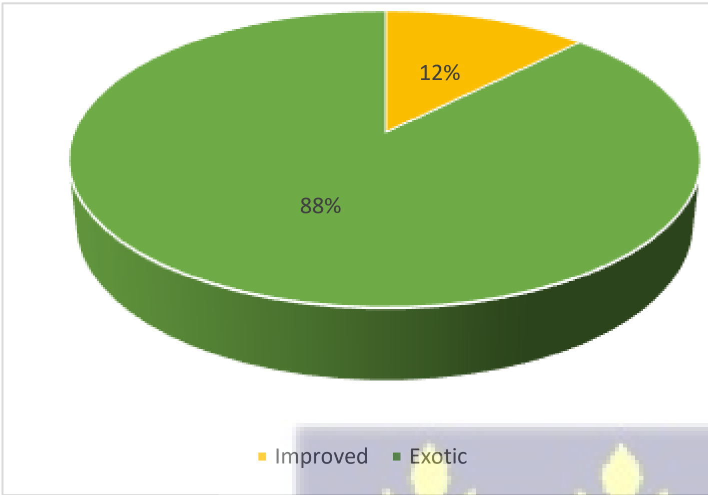

Figure 3.5 Type of seeds planted by the respondent farmers.

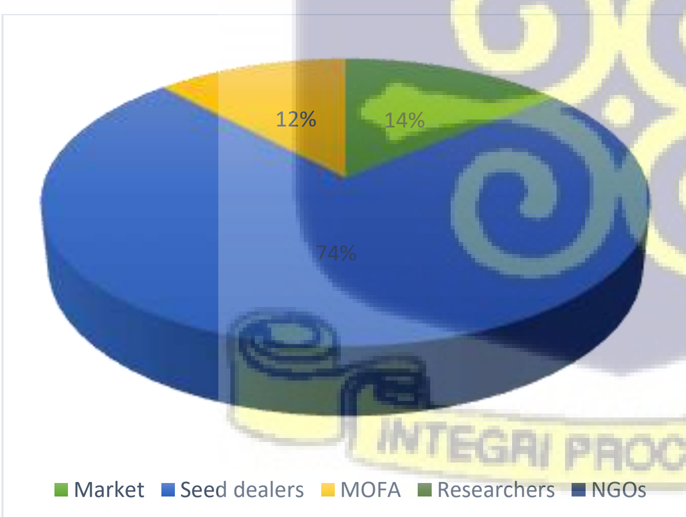

Figure 3.6 Source of planting materials used by the respondents.

3.5.4 *Cucumber mosaic virus* awareness and management by farmers

Each and every one of the 120 farmers who were questioned acknowledged that diseases existed on their fields. To find out if any had been detected on their fields, a list of the cucumber mosaic virus's symptoms was sent to each farmer.

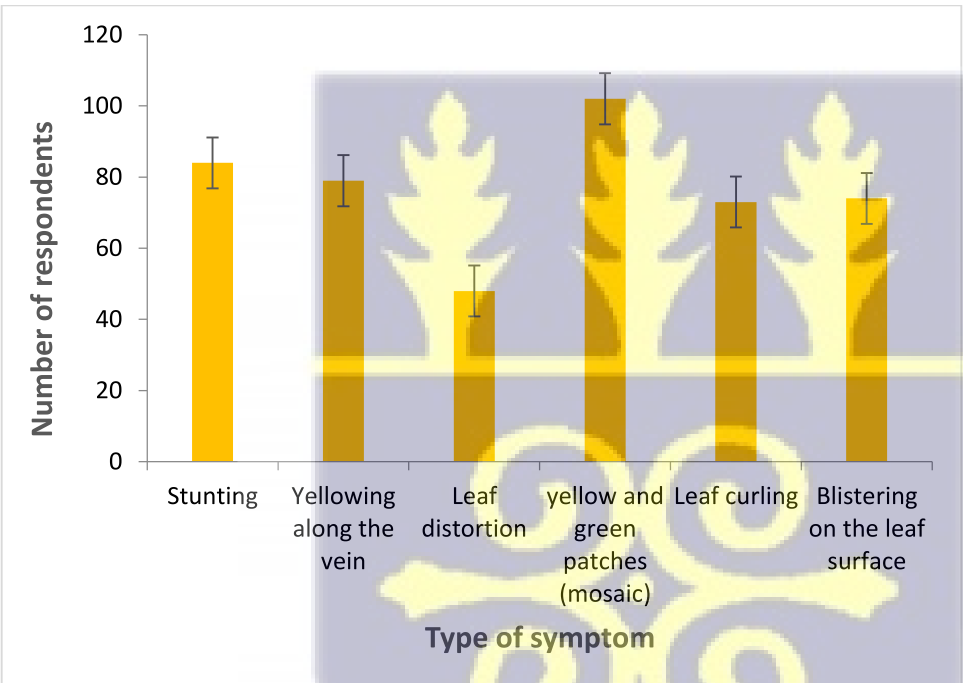

Figure 3.7 Cucumber mosaic virus symptoms observed on the farmers' fields.

The most common symptom observed by the respondents was yellow and green patches (mosaic) on leaves, followed by stunting of plant. The farmers explained the cause of the symptoms was due to the influence of the weather (55.47%), poor soil quality and fertility (28.46%) and poor seed quality (16.05%) (Fig. 3.7). On initial questioning, none of the farmers connected any symptoms they had seen to a virus, demonstrating how little they knew about viral disease. Some of the farmers later admitted that they knew that some of the diseases that affected the plants were caused by viruses. However, none of them were able to name any of the viruses. The inability of the farmers to identify the viral disease symptoms may be due to lack of extension services.

Figure 3.8 Respondents' perception on causes of abnormalities on their fields.

3.7 CONCLUSION

The findings from the study indicate that viral diseases are of a major concern to vegetable farmers in the Greater Accra region of Ghana. Although majority of the respondents were literate, having had some form of formal education, their knowledge on viral diseases of vegetables was still low.

Majority of the farmers were familiar with the various symptoms on their fields, but they did not know actual cause and hence attributed it to other abiotic factors. It has therefore, become necessary that the Extension Service Division of the Ministry of Food and Agriculture (MOFA) intensifies farmer training in disease identification, disease epidemiology and management strategies. Furthermore, in order to tackle viral infections, host resistance should be combined with other management techniques to boost production.

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CHAPTER FOUR

4.1 NATURAL HOST RANGE AND INCIDENCE OF CUCUMBER MOSAIC VIRUS IN SOME SELECTED VEGETABLE FIELDS IN THE GREATER ACCRA REGION OF GHANA

4.2 INTRODUCTION

Vegetables are a valuable component of a healthy diet since they are low in fat, soluble sugars, and calories and contain high amounts of vitamins, antioxidants, minerals, and dietary fiber. (Gruda, 2005; Głąbska et al., 2020; Rashmi and Negi, 2020). Numerous studies have shown that eating more vegetables lowers the risk of certain malignancies and cardiovascular conditions (Aune et al., 2017; Cena and Calder, 2020). The majority of recommendations, including those from the World Health Organization (WHO), call for the consumption of at least 240 g of vegetables per day and more than 400 g of fruits and vegetables per person (WHO/FAO, 2003; Miller et al., 2016; Herforth et al., 2019).

Vegetables are one of the main export goods from both Africa and Asia. The production of vegetables for both domestic use and export in Africa and Asia in 2018 was more than 81.4 and 82 billion metric tonnes, respectively. In Ghana, vegetable production is a potentially lucrative sector of the economy. According to Adombila (2014), a study identified the potential annual value of the vegetable sector as $25 million. This figure was far lower than the average of $3 million observed in annual income accruing from production. Several factors have been found to hinder the nation's vegetable sector from operating at its highest output. One of the most significant factors that severely hampers vegetable production in Ghana is viral infections with *Cucumber mosaic virus* being one of the most economically important viruses. Generally speaking, all vegetables cultivated for both local and international trade are susceptible to one or more viral infections.

Cucumber mosaic virus is one of the most common viruses present around the world in both temperate and tropical regions, infecting a variety of agricultural and horticulture crops. It is one of the most devastating plant viruses that result in major financial loss in many horticultural and vegetable crops (Palukaitis et al., 1992). CMV has a wide host range with more than 1200 distinct types of plants found to be susceptible to the virus. The virus is spread through non-persistent mode by more than 60 aphid species. Due to CMV's wide variety of natural hosts, it is challenging to manage, and commercial fresh-market tomato cultivars for instance do not have genetic sources for CMV resistance (Sikora et al., 1998). To identify effective CMV control techniques, many methods are currently being used. Traditional breeding attempts to develop CMV resistance have mostly failed (Watterson, 1993). Carrot, celery, cucumbers, legumes, lettuce, onion, pepper, spinach, tomatoes are among the vegetables that serve as hosts of the virus. In order to effectively control the virus, identification of its host among the most common vegetables crops in the Ghana should be established.

4.3 PRINCIPAL OBJECTIVE OF THE STUDY

The principal objective of this research is to determine the natural hosts of the *Cucumber mosaic virus* among the most economically significant vegetable crops in Ghana to aid in the development of control measures.

4.4 SPECIFIC OBJECTIVES OF THE STUDY

1. To determine the distribution and disease incidence of CMV on selected vegetable farms in the Greater Accra region.
2. To test for the presence of the virus in symptomatic and asymptomatic plant samples of different vegetable crops
3. To identify the potential natural hosts of CMV among Ghanaian vegetable crops

4.5 MATERIALS AND METHODS

4.5.1 Study area and location

The study was carried out in the Ayawaso Municipal District, Tema West Municipal District and Ga East Municipal District of the Greater Accra region of Ghana. The prevailing weather conditions in the study areas are the same as described in chapter 3.

4.5.2 Materials and Methods.

An extensive field study was carried out to evaluate the prevalence and severity of CMV disease on 16 vegetable farms spread across 3 districts in Ghana's Greater Accra area namely Tema West Municipal District, Ayawaso West Municipal District, and Ga East Municipal District. The number of farms visited in each district was determined by the number of farmers who were available at the time of the survey.

4.5.3. Data collection

In each district, an average of six farms were visited. Fifteen (15) plants were randomly chosen from each farm and were visually examined for symptoms of CMV infection, such as chlorosis, stunting, leaf deformities, blistering, and necrotic patches. Using the method of Imran et al. (2012), the disease incidence on the farms surveyed was determined as:

$$\text{Disease Incidence} = \frac{\textit{number of infected plants}}{\textit{total number of plants}} \times \mathbf{100}$$

Disease symptom severity was assessed visually and scored on a 1-5 point scale based on Karungi et al. (2013)

Table 4.1 Five-point scoring scale for determination of severity index of Cucumber Mosaic virus disease

DISEASE SCORE	DESCRIPTION
1	No symptoms (apparently healthy plant)
2	Slightly mosaic (10- 30%)
3	Mosaic (31- 50%) leaf distortion
4	Severe mosaic (51- 70%), leaf distortion and stunting
5	Very severe mosaic (> 70%), stunting and death of plants

Disease severity index (DSI) was estimated based on the method by Chomdej et al. (2007)

Disease severity index = $\frac{\textit{Disease rating} \times \textit{number of plants}}{\textit{Highest rating} \times \textit{total number of pants}} \times \mathbf{100}$

The Index of Symptom Severity of all plants ($\boldsymbol{ISS_{AP}}$) and Index of Symptom Severity of diseased plants only ($\boldsymbol{ISS_{DP}}$) were estimated using the method by Njock and Ndip (2007):

Index symptom severity of all plants ($\boldsymbol{ISS_{AP}}$) = $\frac{\Sigma\,(SXs)}{\Sigma\,(Xs)}$

Where; S = severity score (1 – 5); X = Number of plants given severity score 1 – 5

Index symptom severity of diseased plants ($\boldsymbol{ISS_{DP}}$) = $\frac{\Sigma\,(\boldsymbol{XSs})}{\Sigma\,(\boldsymbol{Xs})}$

Where; S = severity score (2 – 5); X = Number of plants given severity score 2 –5

4.5.4 Sample collection during survey

Samples from both symptomatic and asymptomatic plants were collected. The samples were carefully cut apart, sealed in zip-lock plastic bags, and kept on ice in an ice box. The samples were taken to the molecular biology laboratory of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) for analysis.4.5.5.1 Enzyme-Linked Immunosorbent Assay (ELISA) detection of CMV

The serological method used to test if CMV was present or not in the samples was the enzyme-linked immunosorbent assay (ELISA). The test kit used for the virus detection was bought from DSMZ Plant Virus Collection in Braunschweig, Germany. As directed by the manufacturer, the double antibody sandwich ELISA (DAS-ELISA) assay was utilized to identify CMV. However, the wash buffer contained 2% skimmed milk to minimize non-specific binding.

4.5.5.2 Preparation of samples

The leaf extract was prepared by pulverizing 0.5 g of the leaf tissue in 5 ml of general ELISA extraction buffer in a sterile mortar (See appendix for composition of extraction buffer). Positive and negative controls (also obtained from the DSMZ) were also diluted in the coating buffer and included in the test.

The antiserum (IgG) was diluted in a coating buffer at a dilution of 1: 1000 (10µl in 10 ml). Hundred microlitres (100µl) of the diluted *Cucumber mosaic virus* antiserum was applied to each of the 96 wells of the microtiter plate. The buffer was used to fill in the blanks in the remaining wells. The microtiter plate was then covered with cling film and incubated for four hours at 37°C in an oven. To get rid of any unbound antibodies, the microtiter plate was washed three times at the intervals of 5 minutes with wash buffer (Phosphate Buffered Saline (PBS) PH 7.4 + 0.5 ml Tween 20 + 2% skimmed milk powder) using a wash bottle. The plate was pat dried on a paper towel. Hundred microliter (100µl) aliquots of the test samples and the controls (positive and negative) were loaded in the coated wells of the plate in duplicates.

The microtiter plate was carefully covered in cling film and incubated at 4°C overnight. The plate was washed as described previously and then pat dried on a paper towel. Hundred microliter (100µl) aliquots of diluted enzyme conjugate (1: 1000) in conjugate buffer was added to each test well and incubated at 37°C for two hours. The plate was washed three times after the incubation to make sure that each unattached enzyme conjugate was removed from the wells.

One milligram of para-nitrophenyl phosphate (pNPP) in one milliliter of substrate buffer consisting of hundred microliter (100µl) aliquots of freshly produced substrate was added to each well. The plate was carefully wrapped once again in cling film to ensure a clear response, and it was incubated at 37°C until color development in complete darkness. A spectrophotometer (Mindray MR-96A) was used to measure the absorbance values at 405 nm, and samples were deemed positive for the virus if they had values that were twice those of the negative control.

4.6 RESULTS AND DISCUSSION

4.6.1 Symptoms of CMV observed on the field

Different visible symptoms of CMV infection were evident on the farmers' fields. Although many of the symptoms were mostly found on the young leaves, older leaves also displayed symptoms in some instances. Leaf chlorosis, mosaic, leaf blistering, necrotic patches, leaf deformation, distortion, and leaf yellowing were among the symptoms observed on the vegetable crops (Fig. 4.1). These signs and symptoms were similar to those reported on vegetable crops by Nono-Womdim (2003), Pratap et al. (2008), Zitikait and Urbanaviien, (2010) and Ashfaq et al. (2014).

Figure 4.1 Symptoms of CMV observed on the vegetable farms (A) Okra leaves showing leaf blistering and yellowing, (B) Pepper leaves showing leaf deformation, (C) Pepper leaves displaying chlorosis (D) Cucumber leaf showing patches of mosaic, (E) Lettuce leaves showing leaf yellowing **(**F) Tomato leaves showing yellowing and Shoestring.

4.6.2 Viral disease incidence and distribution across the three districts

4.6.2.1 Disease incidence

The sample collection sites were Haatso in Ga East Municipal, Dzorwulu in the Ayawaso West Municipal and along the Tema Motorway in the Tema West Municipal districts.

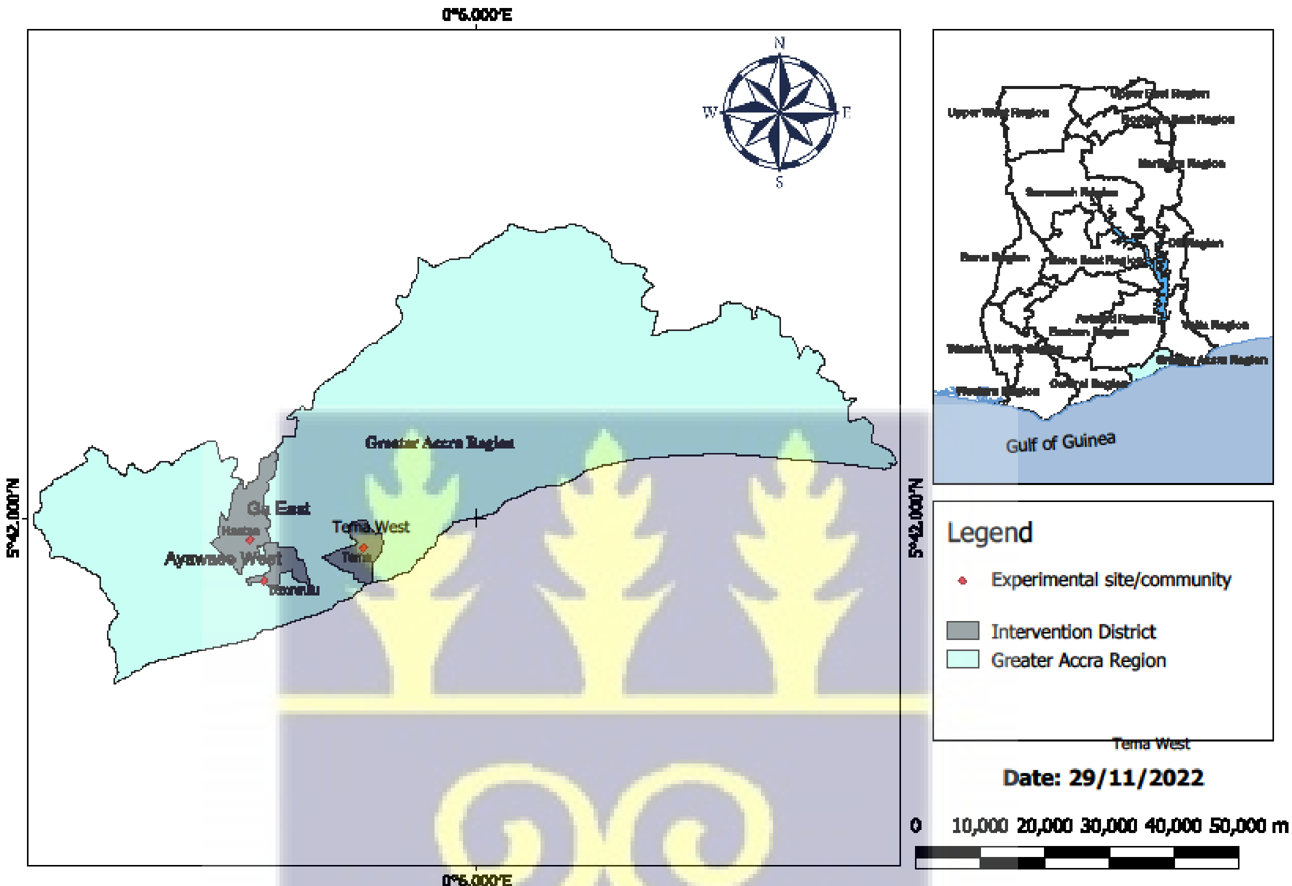

Figure 4.2 The map showing the districts within Greater Accra region as well as sample collection sites.

The presence of viral disease in all three districts was observed by visual examination of the symptoms on vegetables at the various farms. However, the incidence varied among the districts based on the location and the type of crop as shown in Figures 4.3 to 4.5.

Cucumber plants consistently had the greatest incidence of viral symptoms in each district while lettuce plants in Haatso and Tema Motorway had the lowest frequencies. In Dzorwulu, spinach similarly had the lowest incidence viral symptoms compared to the other vegetable types. The lettuce plants in Haatso were found to exhibit mild, if any, signs of viral infection. Since most of the lettuce plants were young compared to those from Dzorwulu, where the viral infection was found in more mature plants, it is possible that the lack of symptomatic lettuce plants in Haatso could be due to the absence of the virus or infection at early stages and symptoms have yet to manifest. The cucumber plants had indications of viral infection and this could be due the crop's great vulnerability to viral infections or the presence of large numbers of insect vectors which may have increased the inoculum pressure. According to Palukaitis et al. (1992), the high vector population seen on the cucumber plants may be a result of the vectors' predilection for feeding on hosts or a lack of effective vector management. This may possibly be a reference to the fact that CMV originated in the cucumber plant (Doolittle, 1916), which serves as the virus's mother host.

A farm along the Tema Motorway in the Tema West Municipal District had 87% incidence of viral disease symptoms in sweet pepper, which was consistent with Deloko et al. (2022). This high disease incidence was found in sweet pepper plants, which supported the findings of Zitter and Murphy (2009) that young pepper plants exhibit more CMV symptoms. In a farm in Dzorwulu, cabbage plants showed 84% incidence of viral disease symptoms. This contradicts the result obtained in other studies by Sevik (2016) which found no viral disease incidence in cabbage plants. Significant incidence of viral disease symptoms was also observed on radish plants in Dzorwulu. Generally, the farms visited were poorly managed, with weeds, thus this observation could be as a result of uncontrolled weeds in the farm since weeds are alternative host of CMV as reported by Kazinczi et al. (2004) and Ouattara et al. (2020). Alternative hosts of CMV include many species of weeds, such as *Carex vulpine, Solanum nigru*, and *Datura stramonium*.

Figure 4.3 Incidence of viral diseases on vegetable farms at Haatso.

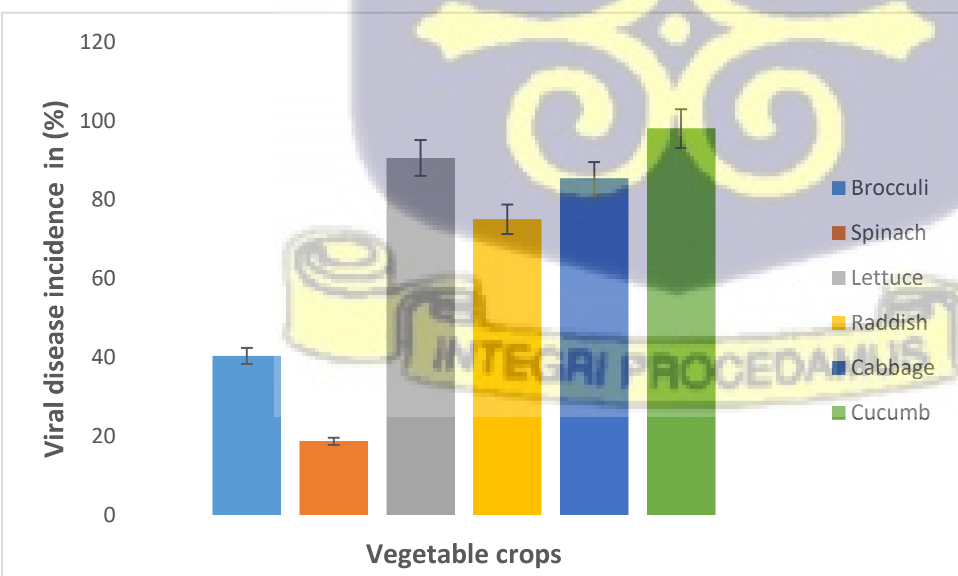

Figure 4.4 Incidence of viral diseases on vegetable farms at Dzorwulu.

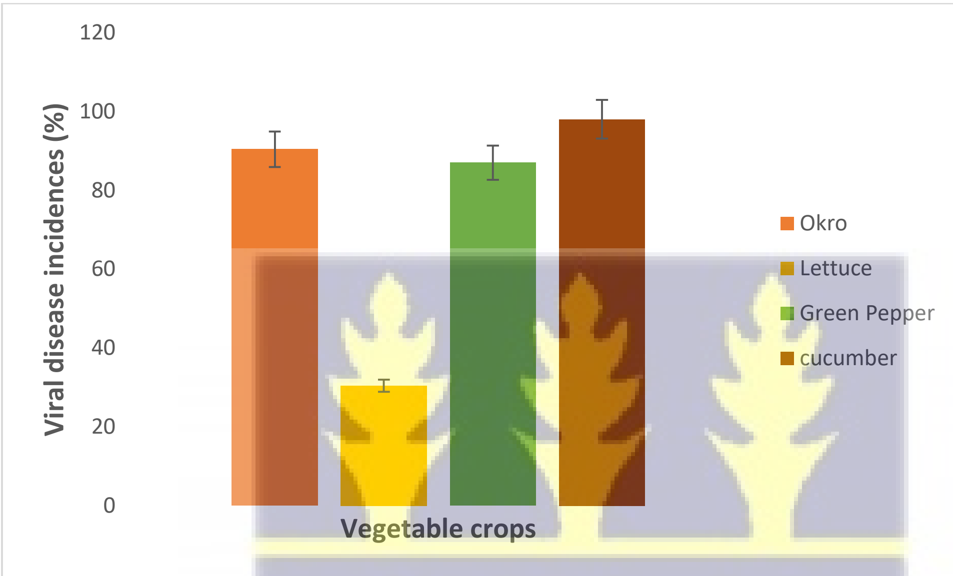

Figure 4.5 Incidence of viral diseases on vegetable farms along the Tema motorway.

4.6.3 Severity of viral disease symptom among vegetables crops

The index of symptom severity for disease plants (ISS_{DP}) only and the index of symptom severity for all plants (ISS_{AP}) varied significantly among the districts. The average scores for the index of symptom severity for all plants (ISS_{AP}) and for diseased plants (ISS_{DP}) only are shown in Figs. 4.5 and 4.6. Okro had the highest ISS_{AP} (3.46), whereas lettuce from a farm along Tema motorway had the lowest (2.0). The index of symptom severity for disease plants only varied significantly and was highest in cabbage from a farm in Dorwulu (4.08) and the least was found in lettuce from a farm along Tema motorway (2.5). Based on disease incidence and severity, both lettuce and spinach were least ranked. The two vegetable crops may have exhibited some degree of tolerance to viral diseases. There was no significant difference in the ISS_{AP} and ISS_{DP} observed among the three districts in the Greater Accra region (P > 0.05). However, cabbage (4.08) and okra (3.46) had high ISS_{DP} and ISS_{AP} scores compared to all the other vegetables that were observed in the three districts. This could be due to large number of aphids found on them, which may have resulted in higher inoculum and more severe symptoms. According Fajinmi et al. (2011), disease incidence and severity were significantly influenced by the number of aphids present on each leaf. Large insect vector populations are more likely to result in increased transmission of viruses, which in turn would lead to an increase in the incidence and severity of diseases. The highest ISSDP scores in Dzorwulu were found in cabbage, as shown in Fig 4.6. It was observed that the farm wasn't adequately maintained. It is possible that the lack of sufficient agronomic techniques may have contributed to the severe viral disease symptoms observed in the cabbage plants.

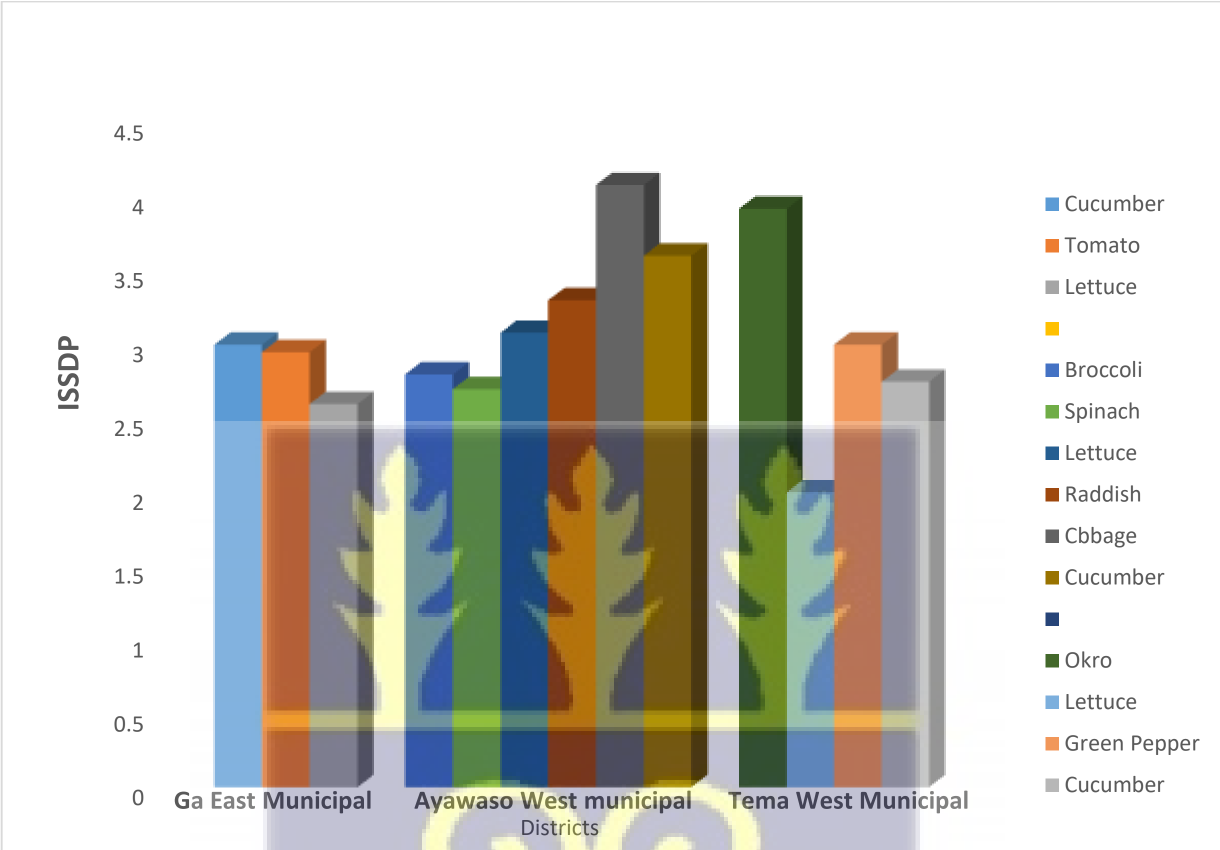

Figure 4.6 Index of severity symptoms for diseased plants only assessed within the three districts of Greater Accra Region.

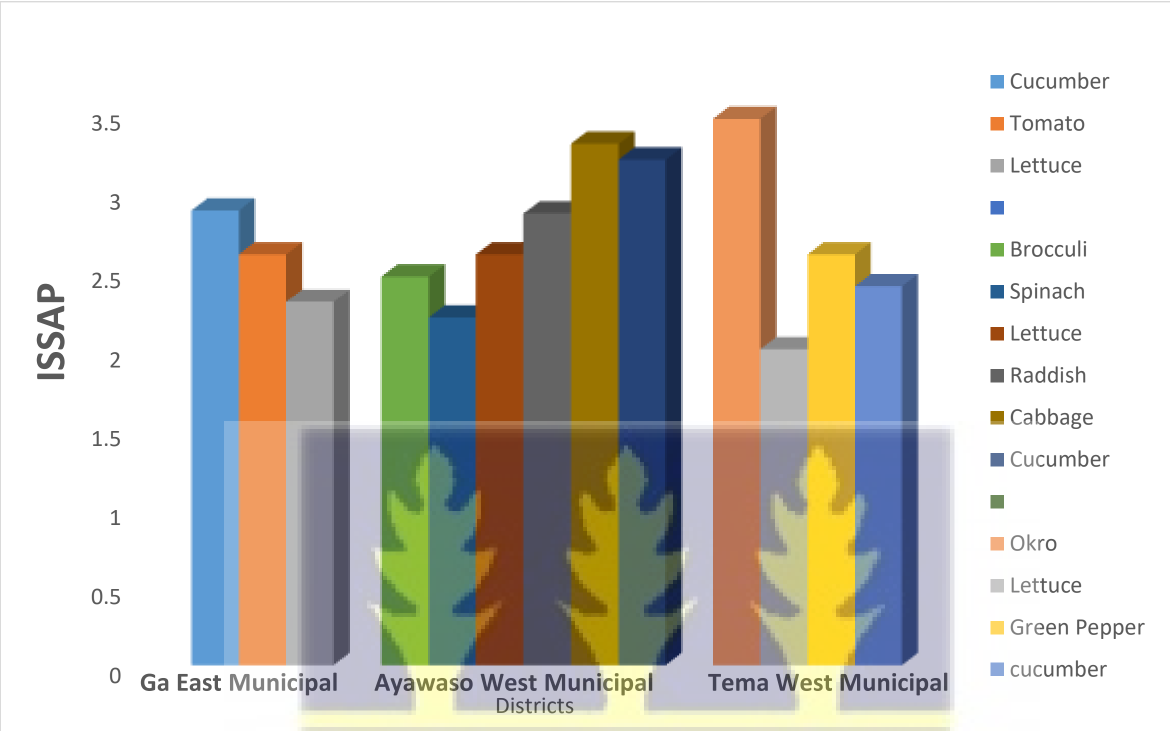

Figure 4.7 Index of severity of symptoms for all plants assessed within the three districts of the Greater Accra Region.

4.6.4 ELISA detection of CMV in leaf samples collected from the field

The ELISA test was conducted to confirm the presence of CMV in samples collected from the field. Tables 2 and 3 show the results of the ELISA testing. The test detected CMV in all three districts evaluated. In terms of absorbance, spinach had the highest value (0.094) while green pepper had the lowest (0.009). The test results corroborate the findings of Walsh and Jenner (2002), Qiaoling et al. (1995), Crescenzi et al. (1993), Palukaitis and Garca-Arenal (2003) and Arogundade et al. (2012), that CMV is among the most serious viruses affecting vegetables cultivated in fields. Only lettuce plants collected from Haatso and Dzorwulu did not show positive results for CMV.

Therefore, it is possible that the symptoms exhibited on the lettuce plants could be due to infection by other viruses such the *Lettuce mosaic virus* (LMV), which exhibits symptoms identical to those seen on plants infected with CMV (Cock, 1968; Bruckart and Lorbeer, 1975). However, a lettuce sample from a field near Tema Motorway was positive for CMV.

Furthermore, the virus was detected in cabbage, radish, spinach and broccoli. The presence of CMV in Broccoli (Sevik, (2017), lettuce, celery and radish (Bruckart and Lorbeer, 1975; Rist and Lorbeer, 1991; Swanepoel and Nel, 1995) has been reported. The findings of this study indicate a first report of CMV infection in lettuce, cabbage, radish, spinach and broccoli in Ghana. Additionally, cucumber and tomato plants were found to have the virus. These plants have previously been recognized as the virus' hosts (Al-Ali et al., 2013; Akbar et al., 2015). Despite having *Cucumber mosaic virus*-like symptoms, green pepper tested negative to the virus. According to Tsai et al. (2010), *Pepper veinal mottle virus* exhibits viral symptoms that are comparable to those of CMV, thus the green pepper may have been infected with the *Pepper veinal mottle virus* (PVMV) or another related virus(es).

CMV infection was not found in okra suggesting that okra may not be a host for the virus. According to Fajinmi and Fajinmi (2010), CMV has not been documented to infect okra. Thus, the *Cucumber mosaic virus*-like symptoms observed on the okra plants could be due to infection by other viruses. The findings of this study could serve as a guide for the development of management strategies for the control of CMV disease.

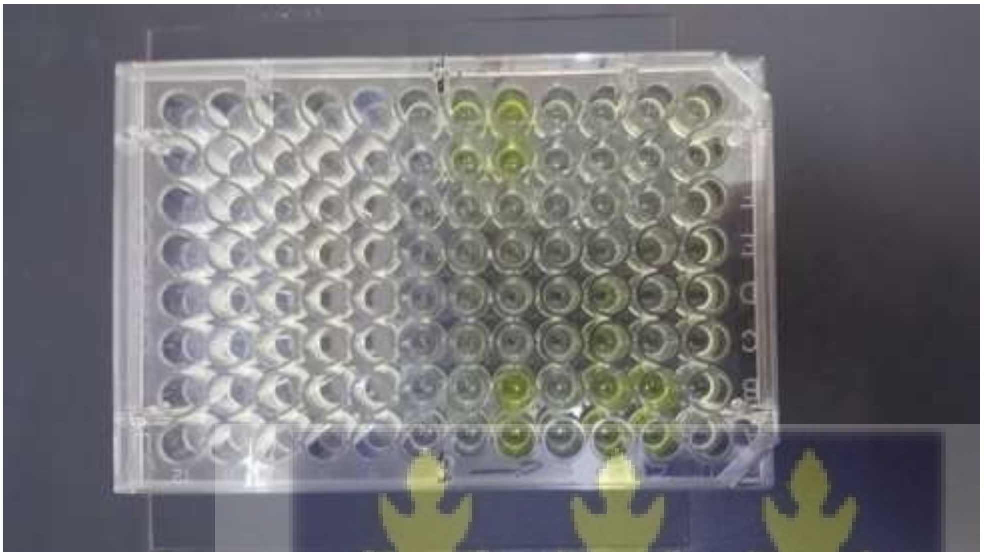

Figure 4.8 Microliter plate showing positive CMV samples.

Area	Crop	Farm	Mean absorbance @ 450 nm	ELISA test reaction (+ve/-ve)
Dzorwulu	Broccoli	3	0.045	+ve
	Spinach	2	0.094	+ve

	Lettuce	3	0.017	-ve
	Raddish	1	0.045	+ve
	Cabbage	2	0.030	+ve
	Cucumber	4	0.043	+ve
Haatso	Tomato	3	0.033	+ve
	Lettuce	2	0.013	-ve
	Cucumber	3	0.033	+ve

Table 4.2 ELISA detection of Cucumber mosaic virus in different vegetable crops in Dzorwulu and Haatso. Absorbance values more than twice the value of the negative control are considered positive.

- Mean absorbance values are averages of four tests
- Negative control value = 0.03

Area	Crop	Farm	Mean Absorbance @ 405nm	ELISA test reaction (+ve/-ve)
Tema Motorway	Cucumber	4	0.046	+ve
	Okro	2	0.016	-ve
	Lettuce	3	0.042	+ve
	Green pepper	3	0.009	-ve

Table 4.3 ELISA detection of Cucumber mosaic virus in different vegetable crops in Tema motorway area.

Absorbance values more than twice the value of the negative control is considered positive

- Mean absorbance values are averages of four tests.

- Negative control value = 0.03.

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CHAPTER FIVE

5.1 DETERMINATION OF HOST RANGE OF CUCUMBER MOSAIC VIRUS AMONG SOME SELECTED VEGETABLES IN GHANA

5.2 INTRODUCTION

Pathogens that are infectious and too small to be seen under a light microscope are viruses, yet they are still capable of wreaking havoc. The nucleic acid of viruses contains genetic information that frequently encodes three or more proteins. Every virus is an obligate parasite that must use their hosts' cellular machinery to replicate. Any living organism can be infected by viruses, including bacteria, plants, fungi, and mammals, although most viruses only infect one kind of host. In all regions of the world, viruses cause significant plant diseases that result in decreased agricultural production and quality losses (Gergerich and Dolja 2006).

Virtually all kinds of domesticated and wild plants can become infected by viruses. However, individual viruses' host range can range from extremely limited to quite expansive. For instance, the Cucumber mosaic virus affects more than 1000 species from 85 different plant families, but just a few species of the Citrus genus are infected by the Citrus tristeza virus. In essence, whether a species or cultivar is susceptible to or resistant to a virus depends on the genotype of the plant. Plants exhibit both active and passive defense mechanisms against viral invasion (Gergerich and

Dolja 2011). The inability of the plant to create one or more host components necessary for viral multiplication and spread within the host is the cause of passive defense. Active defenses also entail the detection and eradication of virus-infected cells because of how particular resistance genes function in the plant. Additionally, plants have a general immune system that is comparable to that of animals. The primary distinction between the two is that, whereas an animal's immune system targets the proteins of a pathogen, a plant's RNA silencing defensive mechanism finds and eradicates viral RNAs (Wassenegger and Pélissier 1998).

A plant's reaction to an infection might range from a symptomless to a more severe disease and possible plant death, depending on the specific virus and host combination, as well as environmental factors. Local lesions, which are tiny areas of necrosis or chlorosis, can occasionally appear when an infection has already taken hold. (Gergerich and Dolja 2006)

Tobacco mosaic virus (TMV) is one of the rare viruses that depends on passive mechanical transmission from plant to plant and long-term (up to decades) persistence in the environment (Ford and Evans 2003). However, the majority of plant viruses are actively spread from diseased to healthy plants by vectors such as arthropods, nematodes, and fungi (Walkey 1991). Aphids and whiteflies are the vectors with the ability to spread the most virus species. In a matter of seconds, hours, or days, vectors actively transmit the majority of viruses to healthy plants. Additionally, certain viruses are spread vertically through contaminated plant seeds or pollen when infected plants are vegetatively reproduced, such as by grafting or the use of tubers (Gergerich and Dolja 2011). A crucial step in the investigation of viral disease is the viral spread from diseased to healthy tissues. This is frequently accomplished in the laboratory by a procedure called transmission by mechanical means or sap, in which infectious sap from the infected plant is rubbed onto the leaf of a healthy plant. The method is used in laboratories to assay for virus infectivity, subculture viruses, transfer viruses to test indicator plants and research viral signs in several host species (Gergerich and Dolja 2011) Without assistance, plant viruses cannot penetrate the host plant's cuticle and reach its inner cells. In order to infect the host, the virus has to get into the tissues through a non-fatal wound (Gergerich and Dolja, 2011)

This is often achieved in experimental mechanical transmission using mild abrasives that cause damage to the plant's cuticle and epidermis by rubbing the host's surface with the infected sap. These wounds then allow the virus to enter cells. Many different mechanisms exist in nature for the entrance of viruses into the host tissues. Any of the other methods may be checked out, despite the fact that mechanical sap transfer is the method that is most frequently used to spread viruses in a laboratory environment (Alconero, 1973). The donor plant's infected portions can be used to make the viral inoculum for sap transmission, although often fresher leaf material has a greater virus concentration than older woody plant parts.

5.3 MAIN OBJECTIVE OF THE EXPERIMENT

The principal objective of this research is to find experimentally susceptible hosts of the *Cucumber mosaic virus* in among selected vegetable crops produced in Ghana.

5.4 SPECIFIC OBJECTIVES OF THE EXPERIMENT

1. To mechanically inoculate selected healthy vegetable crops with CMV-infected sap.
2. To observe and record the symptom types produced on the mechanically inoculated plants.
3. To confirm the transmission of CMV to the inoculated plants using ELISA tests.

5.5 MATERIALS AND METHODS

The experiment was carried out in a screen house at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) between May 2022 and October 2022

5.5.1 SELECTION OF POTENTIAL HOST CROP

A total of eleven potential host vegetable crops were used in the study. These are carrot (*Daucus carota)*, cucumber (*Cucumis sativus)*, cabbage (*Brassica oleracea var. capitata)*, eggplant *(Solanum melongena)*, okra (*Abelmoschus esculentus)*, sweet pepper (*Capsicum annuum)*, radish (*Raphanus sativus*), tomato *(Solanum lycopersicum)*, lettuce (*Latuca sativa)*, squash (*Cucurbita moschata)* and hot pepper *(Capsicum frutescens)*. These were purchased from Agriseed limited, Adabraka, Accra.

Ten (10) plants each per crop were nursed in poly bags, about half a centimeter deep with six seeds sown per hole. After germination and plant establishment, the seeds were watered every other day. Two weeks following germination, the seedlings were thinned out, leaving two seedlings per stand in the poly bags, yielding twenty plants per each crop. Weed elimination was also carried out as necessary.

5.5.2 Mechanical sap inoculation of CMV

Five grams (5g) of leaf samples from an ELISA-confirmed field infected CMV plant was ground in a sterilized mortar with 0.05 M phosphate buffer containing 2% PVP, 0.65g KH_2PO4, and 4.05 Na_2H. In order to allow the virus to penetrate the leaf surface, Celite 545 was added to the inoculation buffer. Amoatey et al. (2015) provided an updated description of the inoculation procedure. The plant's leaves were covered with black plastic bags for four hours prior to the inoculation in order to deprive the plant of sunlight and increase its susceptibility to the virus. Six plants from each crop, were used as test plants. The remaining four were inoculated with buffer containing a healthy leaf sample to serve as controls. Each leaf was gently rubbed with the sap, which was followed by a rinse with water from a wash bottle. The plants were given labels after inoculation and housed in a screen house, and had frequent symptom expression checks and records made. The plants showed no signs of CMV after three weeks of observation. Another inoculation was done by using the same procedure as described earlier. After two weeks of the second inoculation, some plants showed symptoms of viral infection. Leaf tissues from the test plants were taken after another three weeks and used in an ELISA test.

5.5.3 Confirmation of CMV transmission by ELISA test

The Double Antibody Sandwich ELISA (DAS-ELISA), with some slight modifications, was the technique utilized for post-inoculation confirmation of the CMV transmission to the test plants (Clark and Adams, 1977). To avoid non-specific binding, 2% skimmed milk was used in the wash buffer. The ELISA test kit used for the test was acquired from the DSMZ Plant Virus Collection, Braunschweig, Germany. As previously reported, sample preparation and the DAS-ELISA test for presence or absence of CMV were carried out as described in Chapter 4, section 4.6.4.

5.6 RESULTS AND DISCUSSION5.6.1 Mechanical sap inoculation of Cucumber mosaic virus

A crucial step in the investigation of plant viral diseases is the transfer of an intact virus from an infected plant material to healthy plants. In the laboratory, this is often performed by grinding the diseased plant's leaf and applying the contagious sap to the leaf of a healthy plant (Walkey (1991). According to Santhoshinii et al. (2021), sap transmission is the most practicable method for physiologically researching plant viruses. The results for the mechanical sap inoculation is shown in Table 4. The results show that the mechanical inoculation of successfully transferred CMV to tomato, hot pepper, eggplant, cabbage, and radish, demonstrating the efficacy of this virus transmission method.

Crop/Variety	No. of plants Tested	ELISA Reaction (+/-)	Proportion of infected plants
Solanum lycopersicum), (Tomato)	6	+	3/5
Capsicum frutescens (Hot pepper)	6	+	3/5
Brassica oleracea var. capitata, (Cabbage)	6	+	2/5
Capsicum spp (Lettuce)	6	-	0/5
Abelmoschus esculentus (Okro)	6	-	0/5
Cucumis sativa (Cucumber)	6	-	0/5
Daucus carota (Carrot)	6	-	0/5
Capsicum annuum (sweet pepper)	6	-	0/5
Solanum melongena, (Eggplant)	6	+	2/5
Raphanus sativus (Radish)	6	+	2/5
Cucurbita moschata (Squash)	6	-	0/5

Table 5.1 Mechanical sap inoculation of selected vegetable crops with CMV

The results provided in this study agrees with the findings of Kiranmai et al. (1997), Akhtar et al. (2010), Mahjabeen et al. (2012) and Mohamed (2010), that mechanical inoculation of viruses to healthy plants is a quick and simple method for studying viruses *in vitro* (Maramorosch et al., 2003). Because it is done under controlled circumstances, it also enables appropriate research of infections and symptom manifestation. The mechanical inoculation has also confirmed prior research findings that cabbage (*Brassica oleracea* var. capitata) and radish (*Raphanus sativus*) are also hosts for the virus (Chapter 4, section 4.6.4).

Some inoculated plants showed signs of CMV infection two weeks after the second inoculation when compared to the controls, displaying signs of vigour reduction (Figures 5.1- 5.4). After two weeks, the plants continued to develop, but there were clear signs of chlorosis, mosaic, stunted growth, and leaf deformation, pointing to an acute viral infection stage.

Figure 5.1 (A) Inoculated pepper plant showing symptoms of mosaic and (B) uninoculated healthy pepper plant.

Figure 5.2 (A) Inoculated cabbage plant showing yellow patches of mosaic and (B) uninoculated healthy cabbage plant.

Figure 5.3 (A) Healthy uninoculated okro plant and (B) Inoculated okro plant showing leaf curl and blistering

Figure 5.4 (A) Asymptomatic inoculated eggplant and (B) uninoculated eggplant

However, some of the symptoms were seen to disappear four to five days after disease expression. According to Bos (1971), a viral infection goes through an acute stage during which symptoms are much severe, and then a chronic period during which symptoms may be muted or disappear entirely. However, this does not imply that the virus is no longer present, even though the plant may not be showing any symptoms, it still has the virus and may serve as a source of inoculum for the transmission of disease. Some of the inoculated plants exhibited minor symptoms with severity indices ranging from 1 to 2 and with delayed disease progression after 18–30 days. This observation is consistent with the conclusions reached by Akhtar et al. (2010). Furthermore, despite the fact that the virus was first found in cucumber plants, (Doolittle 1916), inoculated cucumber plants tested negative following the ELISA test. The combination of three resistance genes confers CMV resistance to several cucumber cultivars, according to (Keinath et al. 2017;

Zitter and Murphy, 2009). The negative results showed by the cucumber plant may be attributed to the fact that, the cucumber cultivar (Cucumber Olympic) used in the test might have contained the three resistance genes to the CMV as reported by (Keinath et al. 2017; Zitter, and Murphy 2009). Bos (1999) and other researchers have concurred that plants have internal defensive mechanisms that are engaged in response to an infection to lower the viral load (Pennazio et al., 1999; Bos, 1999). Given that farmers' agronomic practices on their farms may be facilitating the development of the disease, the discovery of viral transmission through sap has consequences for them. This may help to explain why some farmers who were interviewed for the study claimed to never have seen aphids or consistently manage vectors, nonetheless have viral disease symptoms on their farms.

In the present study, even though ELISA test confirmed the presence of CMV in eggplant *(Solanum melongena)* and tomato (*Solanum lycopersicum*) via mechanical sap transmission, they showed no visual symptoms. Symptomless virus infection is common in plants and has been reported in healthy tomato plants (*Solanum lycopersicum*) (Fukuhara et al., 2020).

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CHAPTER SIX

6.1 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.2 GENERAL CONCLUSIONS

The following are the conclusions drawn from the findings of this research work

1. One of the major problems affecting the production of vegetables ln Greater Accra region of Ghana is the incidence of viral diseases.
2. Generally, farmers in the survey area are aware of the incidence of viral diseases on their vegetable farms.
3. The results of the study reveal that respondents have inadequate knowledge in the management of the viral diseases on their farms.
4. *Cucumber mosaic virus* is prevalent on farms in the three districts of the Greater Accra region of Ghana based on ELISA tests.
5. The cultivars of tomato, cabbage, eggplant, radish and hot pepper being grown in the Greater Accra region of Ghana were susceptible to the *cucumber mosaic virus* under experimental sap transmission.
6. The sweet pepper cultivar used in this study was not susceptible to infection by the Cucumber mosaic virus both under natural and experimental conditions.
7. Cabbage and radish cultivars used in this study were identified as hosts for the *Cucumber mosaic virus* under both natural and experimental conditions.

8. The spinach and lettuce plants have been identified as host for the Cucumber mosaic virus under the natural conditions but tested negative under the experimental conditions.

6.3 RECOMMENDATIONS

Based on the results of this study the following recommendations have been made regarding any future studies on the virus.

I. The host status of cabbage, lettuce, radish, and spinach to the CMV should be subjected to further investigation.

II. To ascertain the prevalence and incidence of the virus among Ghanaian vegetable growers, a more extensive national survey involving other vegetable crops should be conducted.

III. To enable future breeding for resistance using biotechnological approaches, gene sequencing of Ghanaian isolates of CMV is warranted.

IV. Further studies are needed to confirm the apparent resistance of the sweet pepper and cucumber cultivar (Cucumber Olympic) cultivar to the virus.

APPENDICES

A. ANOVA TABLES

Error	10	2.23077	0.22308
Total	12	2.31877	

Grand Mean 2.6815 CV 17.61

Index of severity symptoms for all plants (ISSAP)

Completely Randomized AOV for Distri~01

Source	DF	SS	MS	F	P
Districts	2	0.08800	0.04400	0.20	0.8241

LSD All-Pairwise Comparisons Test of Distri~01 by Districts

Districts	Mean	Homogeneous Groups
Ayawaso west munucipal	2.7700	A
Tema west municipal	2.6150	A
Ga east munucipal	2.5933	A

Alpha 0.05 Standard Error for Comparison 0.3049 TO 0.3607
Critical T Value 2.228 Critical Value for Comparison 0.6793 TO 0.8038
There are no significant pairwise differences among the means.

Index of symptom severity for disease plants (ISSDP)
Completely Randomized AOV for Distri~01

Source	DF	SS	MS	F	P
Districts	2	0.54822	0.27411	1.18	0.3461
Error	10	2.31921	0.23192		
Total	12	2.86743			

Grand Mean 3.0123 CV 15.99

LSD All-Pairwise Comparisons Test of Distri~01 by Districts

Districts	Mean	Homogeneous Groups
Ayawaso west munucipal	3.2333	A
Ga east munucipal	2.8500	A
Tema west municipal	2.8025	A

Alpha 0.05 Standard Error for Comparison 0.3109 TO 0.3678
Critical T Value 2.228 Critical Value for Comparison 0.6926 TO 0.8195
There are no significant pairwise differences among the means.

B. BUFFERS

BUFFERS USED FOR THE DOUBLE ANTIBODY SANDWICH ELISA

Coating buffer (pH 9.6)

1.59 g sodium carbonate (Na2CO3)

2.93 g sodium bicarbonate (NaHCO3)

0.20 g sodium azide (NaN3)

Dissolve in 900 ml H2O, adjust pH to 9.6 with HCl and make up to 1 l.

PBS (pH 7.4) phosphate buffered saline

8.0 g sodium chloride (NaCl)

0.2 g monobasic potassium phosphate (KH_2PO_4)

1.15 g dibasic sodium phosphate (Na_2HPO_4)

0.2 g potassium chloride (KCl)

0.2 g sodium azide (NaN_3)

Dissolve in 900 ml H_2O, adjust pH to 7.4 with NaOH or HCl and make up to 1 l.

PBS-Tween (PBST)

PBS + 0.5 ml Tween 20 per liter

Sample extraction buffer (pH 7.4)

PBST + 2% PVP (e.g. Serva PVP-15 polyvinyl pyrrolidone)

Sample extraction buffer (pH 8.5) for Begomoviruses

0.05 M Tris containing 0.06 M sodium sulfite, pH 8.5

Conjugate buffer

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

Substrate buffer

97 ml diethanolamine

600 ml H2O

0.2 g sodium azide (NaN3)

Adjust to pH 9.8 with HCl and make up to 1 liter with H2O

B. QUESTIONNAIRE

This survey asks you questions regarding any diseases (CMV) that arise during the cultivation of your crop. Therefore, I would like to ask you a few questions concerning your production procedures, notably your disease control strategies.

NAME OF COMMUNITY……………………………………

Please provide information about your 2022 farm management practices

1. Gender 1) Male 2) Female
2. Age

   18 – 25 ☐ 26 –35 ☐ 36- 50☐ Above 50 ☐

3. Highest level of education

   a)Non-formal b)Primary c)JHS d)SHS e) Tertiary

1. How long have you been growing your crop?

a) < 1year b) 1 –5 years c) above 5 years

2. What was the size of your land?

   a) < acre b) 1– 2 acres c) above 3 acres

3. What farming methods did you use?

   a) Mixed cropping b) Monocropping c) others

4. What crops do you intercrop?

   a) Tomato b) Garden eggs c) Cassava d) Others……………………………….

5. What time did you plant your crop?

   a) Minor season b) Major season

6. What variety did you plant?

   a) Local b) Improved c) Exotic

7. From what source did you get your seeds?

   a) Market b) Farmers c) Seed dealer d) Research e) MoFA f) N.G.O g) Others

8. Did you come across diseases?

   a) Yes b) No

9. If yes, did you control?

   a) Yes b) No

10. How did you control the disease in your field?

    a)Pesticide application b) Removal of infected plant c) Others

11. Did the control work? 1) Yes 2) No

12. If no, why?

a) High cost of pesticide
b) No effect after use
c) Not sure

13. Have you seen any of the symptoms listed below?

a) Stunting 1) Yes 2) No
b) Yellowing along the veins 1) Yes 2) No
c) Leaf distortions 1) Yes 2) No
d) Yellow and green patches (mosaic) 1) Yes 2) No
e) Leaf curling 1) Yes 2) No
f) Blistering on leaf surfaces 1) Yes 2) No

14. In your opinion, what may be the origin of these anomalies?

a) Weather
b) Soil
c) Seeds
d) Not sure
e) Others

15. What losses did the disease cause?

a) Less than half the crop
b) About half the crop
c) The whole crop

16. Have you observed aphids on your crop?

a) Yes b) No

17. Did you control them?

a) Yes b) No

18. If yes, how did you control them?

……………………………………………………………………………..
……………………………………………………………………………..

19. At what stage of development did the disease first manifest?

a) Seedling b) Flowering c) Fruiting

20. How has the disease affected your livelihood?

………………………………………..