DETECTION OF VIRUSES AND THE SPATIAL AND TEMPORAL SPREAD PATTERNS OF VIRAL DISEASES OF CUCURBITS (*Cucurbitaceae spp*.) IN THE COASTAL SAVANNAH ZONE OF GHANA

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DECLARATION

This thesis is the result of research work undertaken by GYAMENA ANTWI EBENEZER in the Department of Nuclear Agriculture and Radiation Processing, of the School of Nuclear and Allied Sciences, University of Ghana, under the supervision of Dr. H.M. AMOATEY and Dr. G.K. OWUSU.

I hereby affirm that except for references which have been duly cited, this work is a result of my own research and that it has not been presented in part or whole for any other degree in this University or elsewhere.

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DEDICATION

I dedicate this research to the glory of Jehovah God, All Merciful.

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LIST OF ABBREVIATIONS

AfDB	African Development Bank
AGO	Argonaute Protein
APSNET	American Phytopathological Society
BC	Before Christ
BCTV	*Beet curly top virus*
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CaMV	*Cauliflower mosaic virus*
CGMV	*Cucumber green mottle virus*
CMV	*Cucumber mosaic virus*
CRIG	Cocoa Research Institute of Ghana
CuLCrV	*Cucumber leaf curl virus*
CVYV	*Cucumber vein yellowing virus*
CYSDV	*Cucurbit yellow stunt disorder virus*
DAP	Days after planting
DAS-ELISA	Double Anti-body Sandwich-Enzyme Linked Immuno-sorbent Assay
DNA	Deoxyribonucleic Acid
DON	Deoxynivalenil
DPI	Days post inoculation
EDTA	Ethylenediaminetetraacetic Acid
EPA	Environmental Protection Agency
EPPO	European and Mediterranean Plant Protection Organization
FAOSTAT	Food and Agriculture Organization Statistics
GA	Gibberellic Acid

GAEC	Ghana Atomic Energy Commission
GBN	Ghana Business News
GNA	Ghana News Agency
HIV	Human Immuno-deficiency Virus
HR	Hypersensitive Response
HSV	Herpes Simplex Virus
IAA	Indole Acetic Acid
IDW	Inverse Distance Weighting
IgG	Immuno-ɣ-globulin
IU	International Unit
kDa	Kilo Dalton
MCMV	*Maize chlorotic mottle virus*
MDMV-B	*Maize dwarf mosaic virus-B*
NAIMA	NASBA Implemented Microarray Analysis
NMIMR	Noguchi Memorial Institute of Medical Research
NRGP	Northern Rural Growth Programme
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reactions
pRBR	Plant Retinoblastoma-related Proteins
PRSV-P	*Papaya ringspot virus-P*
PRSV-W	*Papaya ringspot virus-W*
PTGS	Post Transcriptional Gene Silencing
Q	Quadrat
RDRP	RNA Dependent RNA Polymerase
REP	Repetitive Extragenic Palindromic Proteins

RISC	RNA Induced Silencing Complex
RNA	Ribonucleic Acid
RSD	Range of Spatial Dependence
SADA	Savannah Accelerated Development Authority
SAGE	Serial Analysis of Gene Expression
SIR	Secondary Infection Rate
SiRNA	Short Interfering Ribonucleic Acids
SKP-1	S-Phase Kinase-associated Protein-1
SNF-1	Sucrose Non-fermenting-1 kinase
SqVYV	*Squash vein yellowing virus*
TEV	*Tobacco etch virus*
TMV	*Tobacco mosaic virus*
TNV	*Tobacco necrosis virus*
TRSV	*Tobacco ringspot virus*
UK	United Kingdom
USA	United States of America
USSR	Union of Soviet Socialist Republics
WMV	*Watermelon mosaic virus*
ZYMV	*Zucchini yellow mosaic virus*

ABSTRACT

Cucurbits are susceptible to over 35 plant viruses; each of these viruses is capable of causing total crop failure in a poorly managed virus pathosystem. The objectives of this study were to detect the viruses that infect six cucurbit species in the coastal savannah zone of Ghana and to describe the spatial and temporal spread patterns of virus epidemics in zucchini squash (*Cucurbita pepo* L.) by the use of mathematical and geostatistical models. Cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* Thunb.), zucchini squash (*Cucurbita pepo* L.), butternut squash (*Cucurbita moschata* Duchesne), *egushi* (*Citrullus colocynthis* L. Schrad.) and melon (*Cucumis melo* L.) were grown on an experimental field in the coastal savannah zone of Ghana and were monitored for the expression of virus and virus-like symptoms. The observed symptoms were further confirmed by Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS ELISA) and mechanical inoculation of indicator plants. The temporal spread patterns of virus disease in zucchini squash were analyzed by exponential, logistic, monomolecular and gompertz mechanistic models. The spatial patterns of virus disease spread in zucchini squash field were analyzed by semivariograms and inverse distance weighting (IDW) methods. Cucumber, zucchini squash, melon and butternut squash were infected by both *Cucumber mosaic virus* (CMV) and *Papaya ringspot virus* (PRSV-W). *Egushi* was infected by CMV but not PRSV-W. None of the six cucurbit species were infected by *Watermelon mosaic virus* (WMV) or *Zucchini yellow mosaic virus* (ZYMV). The temporal pattern of disease incidence in the zucchini squash field followed the gompertz function with an average apparent infection rate of 0.026 per day. The temporal pattern of disease severity was best described by the exponential model with coefficient of determination of 94.38 % and rate of progress of disease severity of

0.114 per day. As at 49 days after planting (DAP), disease incidence and severity factor had reached 11.82 % and 1.09 respectively. The spatial pattern of disease spread in the zucchini squash field was best described by the Gaussian model with range of spatial dependence of 0.63 m.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

The Cucurbitaceae family of crops contains many species that are of economic importance (Ng, 1993). There are over 128 genera in the cucurbitaceae family which, collectively, contain more than 989 species with numerous landraces. Many of these cucurbit species are used in medical research, the food industry and in the manufacturing of musical instruments (Schaefer and Renner, 2011). Both old and new world cucurbits of either African, Asian or American origin are very essential in combating food security and malnutrition considering their wider adaptation, nutritional status and relatively short growing period (Wehner and Maynard, 2003).

Cucurbits produce the largest of all known fruits; the fruit of *Cucurbita maxima* Duchesne can weigh as much as 911 kg (Wikipedia, 2014). Various nutritious soups, purees, salads, beverages and confectioneries are made from cucurbits such as cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* Thunb.), butternut squash (*Cucurbita moschata* Duchesne*)* and melon (*Cucumis melo* L.). Butternut squash contains as much as 2000 IU of vitamin A and melon is known for its relatively high energy content of approximately 124 calories. Cucumber, *Momordica cochinchinensis* Spreng. (gac) and *Momordica charantia* L. (bitter gourd) contain 17.4 g of carbohydrate and 88 mg of vitamin C, respectively (Rahman *et al.*, 2008).

Almost all parts of many cucurbits including, cucumbers and bitter gourds, have been used in folk medicine in treating medical conditions such as diarrhoea, excessive thirst,

dysentery, acute conjunctivitis, burns, sore throat, laryngitis and also as a detoxifying agent (Leung, 2007). Recently, various primary extracts from cucurbits, such as α- and β- momorcharins and trichosanthin, have been scientifically proven to have antiviral properties. The ribosome-inhibiting properties of trichosanthin prevent the replication of HIV in infected lymphocytes and phagocyte cells (Ananya and Raychaudhuri 2010; Ng, 1993). Other cucurbit extracts such as cucurbitacins which give many cucurbits their characteristic bitter taste are also known to exhibit anti-inflammatory activities due to their ability to inhibit cyclooxygenase (COX) enzymes which initiate anti-inflammatory processes (Dhiman *et al.*, 2012; Ananya and Raychaudhuri, 2010).

Cucumber and watermelon are the two most commonly cultivated cucurbits in Ghana; however, many other cucurbits such as various squashes and melons can also be found on the Ghanaian market. The production of cucurbits in Ghana is largely centered within the peri-urban areas, and in these areas production is all-year round and largely dependent on irrigation. Since 2011, the production of a new cucurbit, butternut squash, has been on the increase and many farmers are being attracted to the crop in the various regions of Ghana. This is due to government promotion and numerous incentives attached to butternut squash production (GBN, 2012; GNA, 2012; IFAD, 2012).

An indigenous cucurbit which has received little scientific attention but can still be found on almost every Ghanaian market is *egushi* (*Citrullus colocynthis* (L) schrad.), which is highly prized for its rich oily seeds used in soups and stews. Though watermelon and cucumber are very important fruits in Ghana, there is scarce information on their production and consumption; the little statistics on cucurbit production are almost entirely limited to the Greater Accra Region.

1.2 Statement of Research Problem

There are more than 35 known plant viruses which naturally infect cucurbits (Lecoq *et al.*, 1998). Plant viruses, unlike other plant pathogens, are extremely difficult to control and spread very fast under favourable environmental conditions. There are no known direct and efficient methods to control plant viruses. Most control measures are vector-targeted or through use of resistant varieties. Not only could these viruses hinder the growth and fruit setting in cucurbits but could also hinder their production for the local and export market.

Many of the viruses infecting cucurbits such as *Cucumber mosaic virus*, *Zucchini yellow mosaic virus* and *Papaya ringspot virus* are considered as regulated pests and their presence in cucurbits could pose a threat to the international trade of cucurbits (EPPO, 2013a).

Currently, there is limited research on cucurbits and their pathological challenges in Ghana. There are no known devised, effective strategic measures to prevent or control possible virus pandemic in cucurbit fields and there are no prediction or simulation models that can forecast possible virus disease outbreaks in cucurbit fields. It is possible that indigenous cucurbits in Ghana such as *egushi* may consist of landraces which might have developed resistance or tolerance to any of the cucurbit-infecting viruses over years of co-evolution and adaptation and, hence, could be crucial in resistance breeding programmes in transferring these traits to other susceptible cucurbit species, but not much research interest has been given to the indigenous cucurbits in Ghana.

1.3 Justification

Butternut squash (*Cucurbita moschata* Duchesne), has recently been introduced into Ghana by the Export Development and Investment Fund (EDIF) as a new non-traditional export commodity to widen the source of foreign exchange to Ghana. According to EDIF, the government has invested GHC 37,000 (18,583.5 USD) in butternut production in Ghana and has already secured market in Europe for the butternut fruits. Returns from butternut are expected to far exceed foreign exchange gained from cocoa export considering that butternut matures in just within 90 days. The African Development Bank (AfDB) and the International Fund for Agricultural Development (IFAD) have funded many pilot projects on butternut squash, especially in the northern regions through the Northern Rural Growth Programme (NRGP), to improve livelihood and reduce household poverty (GBN, 2012; GNA, 2012). Butternut squash is also to be included in the school feeding programme to mitigate widespread malnutrition, particularly, in poorer communities, due to the high nutritional value of the crop. However, these proposals may not be possible if measures are not taken to control the virus threat to cucurbit production in Ghana. Already, *Cucumber mosaic virus*, a major virus threat to cucurbits, has been detected in yam (*Dioscorea spp*) and on tomato (*Solanum lycopersicum*) in Ghana (Eni *et al.*, 2008; Lamptey *et al.*, 2013). It is possible there could be other virus threats to the increasing cucurbit investment in Ghana. A preliminary step in any plant disease management approach is the accurate detection of the pathogen through a reliable and precisive method.

1.4 Objectives

General Objective

This study seeks to detect and identify the plant viruses affecting six cucurbit species in an open-field farm in the coastal savannah zone of Ghana. It also seeks to investigate the nature and severity of the virus threats and their dynamics of spread to enhance selection of appropriate mathematical models for forecasting future virus epidemic patterns. This will also enable the identification of cucurbit species with field resistance for breeding programmes.

Specific Objectives

The specific objectives of this study are;

1. To detect viruses that infect six cucurbit species in an open-field farm in the coastal savannah zone of Ghana by means of;
    - Symptomatology
    - DAS-ELISA
    - Mechanical Inoculation and
    - Electron Microscopy
2. To describe the spatial and temporal spread patterns of virus-like disease symptoms in a zucchini squash field through Model Fitting and Parameter Estimation.
3. To assess the incidence and severity of detected viruses in zucchini squash.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Taxonomy of Cucurbitaceae

Many of the oldest cultivated crops belong to the family cucurbitaceae (Crase, 2011). The earliest use of cucurbits dates back to 5000 BC in Mexico (Botgard, undated). The monophyletic cucurbitaceae family is a distinct group of crops without any close relatives and also the single group of plants with most species used by man. Cucurbits have significant use in the food, medical, agrochemical and music industry **(**Schaefer and Renner, 2011; Botgard, undated; Wehner and Maynard, 2003). All 989 species of cucurbits are frost sensitive and are grouped into 128 genera, 19 subtribes, 7 tribes and 2 subfamilies. Most of the cultivated cucurbits are in the subfamily cucurbitodeae. Watermelon and cucumber are the most cultivated cucurbits worldwide, particularly in China and Turkey.

Cucurbits of either Africa or Asia origin are termed old world cucurbits; these include cucumber, watermelon and muskmelon. Cucurbits of American origin are called New World cucurbits and they include butternut squash and zucchini squash. Though cucurbits are cultivated in all the major continents of the world, they grow exceptionally well in tropical and subtropical climates such as in South-East Asia, West Africa, Madagascar and Mexico**.** Figure 1 and Figure 2 show all the geographical locations across the globe where cucurbits are cultivated and a phylogeny of cucurbitaceae with focus on widely cultivated cucurbits (Wehner and Maynard, 2003).

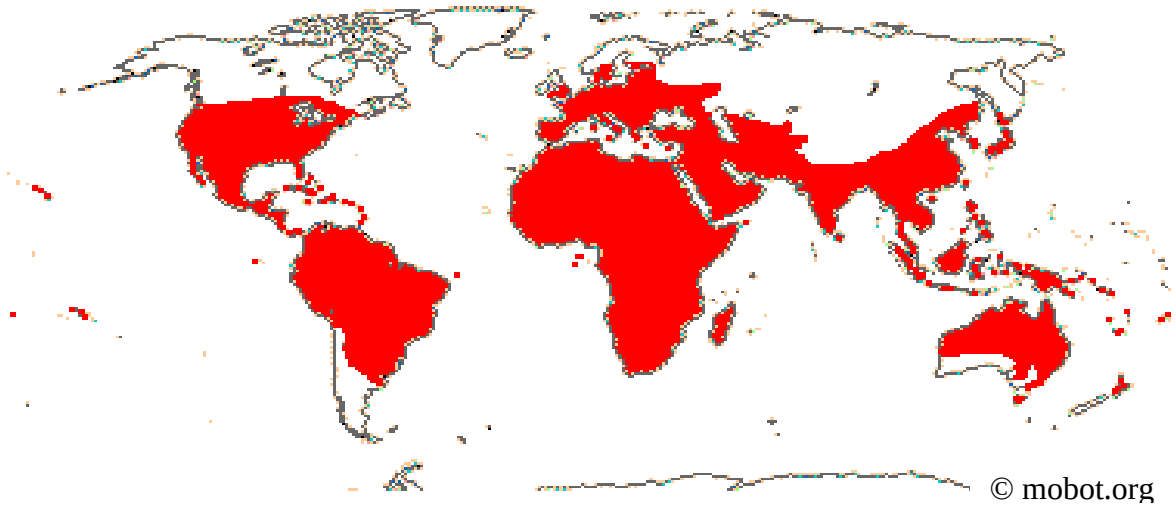

Figure 1: Cucurbit growing areas in the world

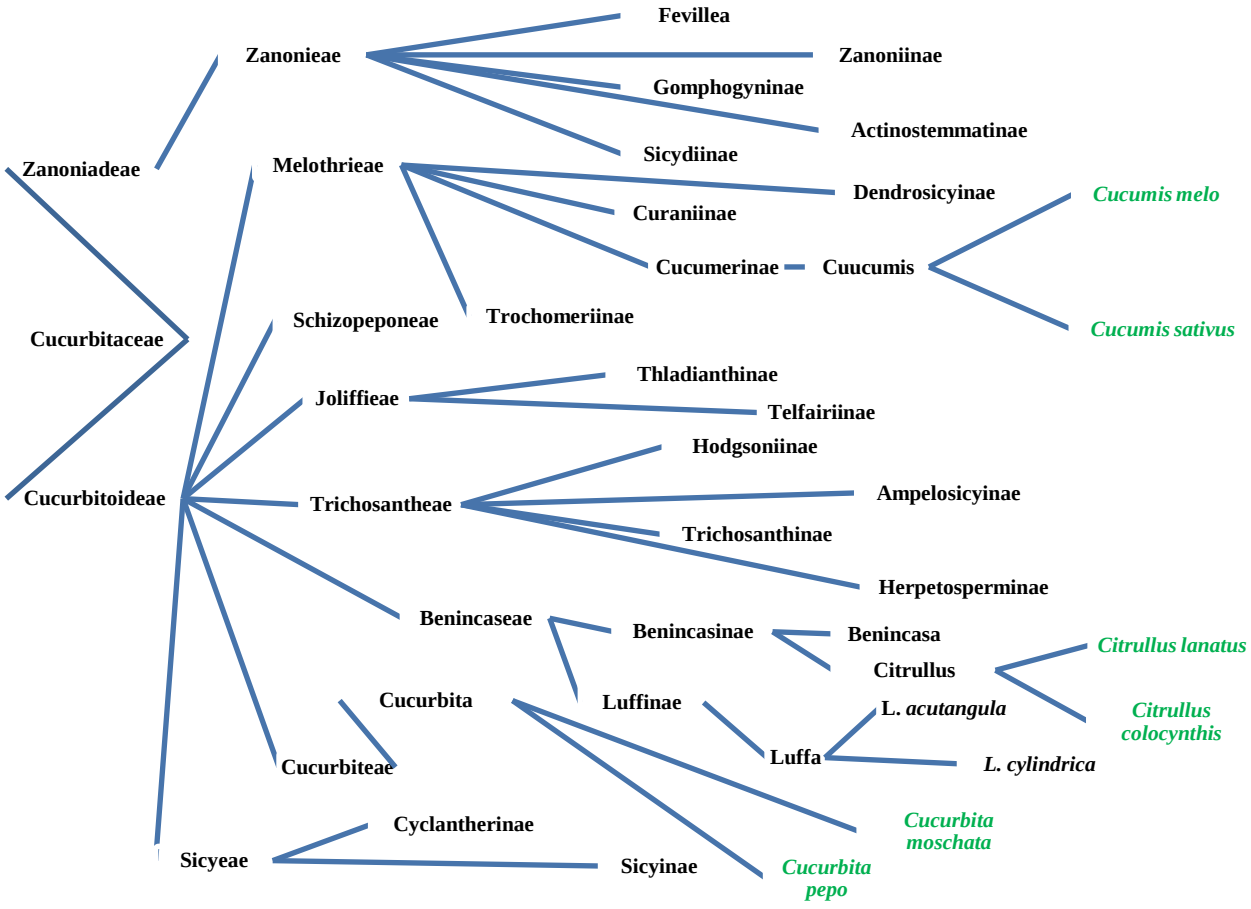

Figure 2: Phylogeny of the cucurbitaceae family with focus on widely cultivated cucurbit species

2.2 Commonly Cultivated Cucurbit Genera

2.2.1 Cucumis Cucurbits

Cucurbits of the genus cucumis belong to the tribe Melothrieae and subtribe Cucumerinae. They are scandent or creeping herbaceous annuals that have scabrous stems and branches with simple and slender tendrils. Cucumis cucurbits have almost orbicular, reniform or cordate-ovate, undivided or palmately 3-7 lobed leaf blades. Both staminate and pistilate flowers may be fascicled or solitary. The superior ovaries of the pistillate flowers develop into a polymorphic, fleshy, indehiscent fruits which may be smooth or verrucose (Anmin *et al.*, undated). The genus contains at least 511 scientific plant names. Out of these 254 scientific names are ranked for the position of species but only 50 names are accepted, 165 of the names are considered synonymous and 35 more tentative species are unassessed (The PlantList, 2010a). *Cucumis sativus* L. and *Cucumis melo* L. are the most commonly cultivated crops in the cucumis genus.

2.2.1.1 Cucumber (*Cucumis sativus* L.)

Cucumber originated in northern India. The fruits are pendulous and are in variable shapes and sizes. Many cultivars are nearly globular to oblong and elongated with scattered spinous tubercles and warts especially when young. The flesh is pale green with the characteristic cucumber odour and contains many white flat seeds except when parthenocarpic (Mendoza, 2002).

2.2.1.2 Melon (*Cucumis melo* L.)

Cucumis melo L. probably originated in Africa. The fruits are of variable sizes, shapes and rind texture. Most cultivars are globular or oblong which may be smooth or

furrowed, with glabrous and smooth to rough and reticulate rind. The rind colour ranges from pale to deep yellow to yellow-brown, or green. The mesocarp has numerous seeds which may be yellow, pink or green. Melon seeds are smooth and whitish or buff in colour. The sizes of the seeds range from 5-15 mm in length (Dadshani, 2002).

2.2.2 Momordica Cucurbits

Momordica cucurbits are of the Joliffieae tribe. They are creeping or scandent herbaceous annuals or perennials with unbranched or 2-fid tendrils that are usually with a glandular petiole. Their leaf blades are sub-orbiculalar or ovate-cordate and are either monoecious or dioecious crops that produce ovoid, oblong, elliptic or fusiform fruits that may be undivided or 3-valved which are usually verrucose or spinescent (Anmin *et al.*, undated). There are 192 scientific names recorded in the genus momordica. Of these 187 are ranked for the species level of which 19 scientific names are accepted species names and 46 names considered synonymous to accepted names. However, there are 122 scientific names ranked at the level of species which are unassessed (The Plant List, 2010b). Economically important species in the momordica genus are *Momordica charantia* L., *Momordica cochinchinensis* Spreng., *Momordica subangulata* Blume. and *Momordica basalmina* L. These perennial cucurbits are found largely in mainland and island of South East Asia, also in China and Japan.

2.2.2.1 Bitter Gourd (*Momordica charantia* L.)

Bitter gourd probably originated in China or India. It is also known for its two simultaneous domestications; in the Himayans, India and in Yunnan, China. Bitter gourd is cultivated throughout the tropics. The pendulous fruits are fusiform and ribbed with

numerous tubercles. The fruit is approximately 5-25 cm long with numerous brownish seeds with scarlet arils and are approximately 1-1.5cm long (Dahal, 2002).

2.2.2.2 Bottle gourd (*Lagenaria siceraria* (Molina) Standl.)

Bottle gourd probably originated in Africa or India. The fruits are of variable shapes and sizes ranging from 10 cm -100 cm or more in length with hard and durable rind. The fruits are usually either flattened, globular, bottle-club shaped or crook-necked or coiled, with many seeds. The white or tan seeds are compressed and ridged and are approximately 2 cm long (Kpongor, 2002).

2.2.3 Citrullus Cucurbits

Citrullus cucurbits belong to the tribe Benincaseae and the subtribe Benincasinae. The genus contains some of the most widely translocated and cultivated cucurbits since the Paleolithic era. Commonly cultivated citrullus cucurbits are watermelon, *Citrullus lanatus* (Thunb.), and *egushi*, *Citrullus colocynthis* (L.) Schrad (Wikipedia, 2013a).

2.2.3.1 Watermelon (*Citrullus lanatus* (Thunb.))

Watermelon is grown throughout the tropics. It produces globose or oblong fruits up to 60 cm or more in diameter. Its hard rind is mostly glabrous with green, cream, striped or mottled green coloration. The many seeded mesocarp is usually sweet and may be red, green, yellow or whitish. The flat and smooth seeds could be white, black reddish or yellow in colour. A gram of the seeds contain approximately 15 seeds **(**Bastas, 2002**).**

2.2.3.2 *Egushi* (*Citrullus colocynthis* (L.) Schrad.)

Egushi is native to tropical Africa and has similar characteristics to watermelon (*Citrullus lanatus* (Thunb.)). This highly xerophytic cucurbit produces extremely bitter fruits, but it is the seeds that are of economic significance. *Egushi* is cultivated throughout West African states, especially in Ghana, Togo, Benin and western Nigeria. *Egushi* seeds are larger but lighter than watermelon seeds (Abrefa, 2002).

2.2.4 Cucurbita Cucurbits

Cucurbita cucurbits are new world cucurbits that are believed to have originated from Mexico to Peru. They belong to the tribe Cucurbiteae and the subtribe Cucurbitnae. Pumpkin, *Cucurbita maxima* Duchesne, zucchini squash, *Cucurbita pepo* L. and butternut squash, *Cucurbita moschata* Duchesne are among the widely cultivated cucurbita cucurbits (Wehner and Maynard, 2003).

2.2.4.1 Zucchini Squash (*Cucurbita pepo L.*)

Zucchini squash is a new world cucurbit that originated from around the Southern USA to northern Costa Rica area. The fruit has an extended shape and it is smooth and unilocular without any central hole; Zucchini squash cultivars are in many colours. The smooth yellowish white seeds are large, about 1.5 cm wide and 0.1-0.2 cm thick and sharp pointed (Okten, 2002).

2.2.4.2 Spaghetti squash (*Cucurbita pepo* L.)

Spaghetti squash originated in northern or central America, probably in Mexico. The fruits are approximately 25 cm long and 12 cm in diameter, weighing about 1kg (Gayosso, 2002).

2.3 Economic Uses of Cucurbits

2.3.1 Medical and Pharmaceutical Uses of Cucurbits

By the 8th century the medicinal uses of cucurbits were well known; cucumber leaves were prescribed at a dose of one leaf per one year-old child to treat excessive thirst, sore throat, laryngitis, acute conjunctivitis, dysentery, diarrhea and burns and other common diseases **(**Leung, 2007) Modern advances in phytochemical research have scientifically proven the medicinal properties of cucurbits. Numerous compounds with medicinal properties in rats and humans such as Momorcharins, Momordenol, Momordicilin, Momordin, Momordolol, Charantine, Charine, Cryptoxanthin, Cucurbitins, Cucurbitacins, Cucurbitanes, Cycloartenols, Urease, Momordicine, Ascorbigen, Polypeptide-P Insulin and Insulin-Like compounds have been isolated from cucurbits (Ananya and Raychaudhuri, 2010).

Extracts from immature bitter gourds are known to have a hypoglycemic effect in man and have been used in the management of diabetes. Charatin, a sterol glycoside, P-$ZnCl_2$, an insulin-like protein and the pyrimidine alkaloid nucleoside vicine are known to possess diuretic properties and have all been isolated from bitter gourd. These compounds produce hypoglycemic effect in man (Ananya and Raychaudhuri, 2010). Water extract of the cucurbit is known to increase glucose uptake and adiponetin secretion in adipose cells. When the cucurbit extract was tested on tissues with streptozotocin induced impaired antioxidant status, the affected cells normalized through scavenging of the free radicals hence reducing the risk of diabetic complications (Dhiman *et al.*, 2012). Cucurbits have long been used in treating dysentery. *Escherichia coli, Salmonella paratyphi* and *Shigella dysenterae, Klebsiella pneumonia, Plasmodium*

vincicei petteri 279BY, Plasmodium falciparum, Caenorhabditis elegans, Bacillus megaterium, Bacillus subtilis, *Proteus mirabilis*, *Aspergillus niger* and *Aspergillus flavus* were found to be very responsive to bitter gourd oil extracts (Ananya and Raychaudhuri, 2010).

The use of Cucurbits is increasingly gaining attention in the development of novel antiviral drugs. When the cucurbit protein, MAP-30, was tested on Herpes Simplex Virus (HSV) propagated in human lung fibroblasts, it showed significant anti-HSV activity to the HSV. The cucurbit extracts, alpha and beta Momorcharin have also shown anti-HIV activity when tested on rabbit's reticulosyte lysate (Ananya and Raychaudhuri, 2010). The ribosome inhibiting properties of Trichosanthin, an extract from the cucurbit Trichosanthes has also been found to be effective in inhibiting the replication of HIV in infected lymphocyte and phagocyte cells (Ng, 1993). Bitter gourd fruit extracts are now known to decrease serum and liver triglyceride levels in rats. The extracts; Echinatin, Saponins, *b* and *e* Cucurbitacin, β-Sitrosterol, Echinatol α and β and Oleanolic acids protect the liver against CCl_4 induced hepatotoxicity in rats. The anti-ulcer cucurbitane type triterpenoid has been isolated from *Cucurbita pepo* L. seeds and ethanol extracts of bitter gourd also show significant dose dependent anti-ulcerogenic activity against various ulcer models. Cucurbit extracts such as cucurbitacin actively inhibit the activities of cyclooxygenase enzymes which otherwise cause inflammation (Dhiman *et al.*, 2012; Ananya and Raychaudhuri, 2010; Ng, 1993**)**.

2.3.2 Dietary Use of Cucurbits

The cucurbit family contains numerous species and cultivars that are widely used as fruits, nuts and vegetables. The largest of all fruits is produced by the cucurbit *Cucurbita maxima Duchesne* (pumpkin) a single pumpkin fruit can weigh as much as 911 kg (Wikipedia, 2014). Almost every part of most cucurbits is used for food; leaves, fruits, seeds, pulp, stems, flowers and roots (Shrivastava and Roy, 2013). They are used in soups, purees, drinks and as garnish with other food items. *Benincasa hispida* is used in preparing jams jellies and cakes. Watermelon is also used in the confectionary industry, pumpkins are commonly eaten as vegetables and also used in baking pies and making jams (Rahman *et al.*, 2008). The nutritional values of selected edible cucurbits are shown in Table 1. Aside their major use as food and in medicine, cucurbits are also used as fodder, soap substitutes, handicrafts and insecticides (Lira and Caballero, 2002).

Table 1: Nutritional values of selected edible cucurbits

Cucurbit	Moisture (g)	Vitamin C (mg)	Mineral (g)	Carbohydrate (g)	Energy (Calorie)	Vitamin A (IU)
***Cucumis sativus* L.**	96.3	7.0	0.3	2.5	13	40
***Cucurbita pepo* L.**	92	20	0.6	4.8	25	1700
***Lagenaria siceraria* (Molina) Standl.**	96.1	6	0.5	2.5	12	60
***Momordica charantia* L.**	92.4	88	0.8	4.2	25	210
***Cucumis melo* L.**	92.7	35	0.3	5.9	125	190
***Citrullus lanatus* (Thunb.)**	92.0	6.0	-	6.5	35	599
***Cucurbita moschata* Duchesne**	94.0	15	0.6	4.6	21	2000
***Cucurbita maxima* Duchesne**	92.6	2.0	0.6	4.6	13	1840

(**Source**: Compiled from Rahman *et al.*, 2008)

2.4 Production of Cucurbits in Ghana

In May, 2011, the Export Development Investment Fund (EDIF) of Ghana's Export Promotion Council introduced butternut squash as a new export commodity from Ghana and stated that it also had plans of introducing melon as another export commodity from Ghana. Among the cucurbits cultivated in Ghana, butternut receives significant investment, followed by watermelon and cucumber for both domestic and international markets. The recent investment in cucurbits is largely due to the high demand for cucurbits especially in winter when most European countries cannot grow these frost-sensitive crops. Cucurbits can be grown all year round in Ghana, especially under irrigation in the dry season. Zucchini squash production can be very productive even

with limited moisture. EDIF has initially invested GHC 37,000 (USD 18,439.7) in the production and export of butternut alone and has already secured markets; Minor, Weir and Willis Group of Companies, based in the UK for butternut export. The hybrid butternut seeds are largely imported from South Africa. Revenue from butternut production is projected to exceed that of cocoa in the near future. Through the Northern Rural Growth Programme, international donors such as the International Fund for Agriculture Development, (IFAD) and the African Development Bank, (AfDB) have funded many rural initiatives involving cucurbits, particularly butternut squash, to encourage farmers in the northern regions to go into cucurbit production for poverty alleviation and improved livelihood (GBN, 2012; GNA, 2012; IFAD, 2012).

Watermelon and cucumber have been cultivated in Ghana for a long time. However, the majority of watermelon and cucumber production centres around the urban and peri-urban markets. Zarapri Farms Limited and Balaji Agro Products Limited export watermelons and cucumber, respectively, from Ghana. Until recently, the statistics on the production of cucurbits was largely restricted to the Greater Accra Region. Due to their high demand, especially on the international market and their less intensive cultivation, the government of Ghana is encouraging more farmer involvement in the production. Over the years, cucurbit production in tonnage has not been stable; there are seasons of rapid decline and seasons of slight rise in production.

EDIF is managing four pilot butternut production farms in the Northern Region, Upper East Region, Upper West Region and in the Central Region. Through the Savannah Accelerated Development Authority (SADA) and the Northern Rural Growth Project (NRGP), over 300 farmers at Gbetuor in the Jirapa District of Upper West Region are to

benefit from a GHC 50 million capital investment in butternut production. Farmers willing to undertake butternut production are to be certified and provided with agrochemicals, hybrid seeds, land clearing implements and technical support. Certified farmers will be supplied with hybrid seeds and supervised by a group of experienced nucleus farmers. Within the three northern regions, the government of Ghana is to undertake commercial production in Gbetuor and Meto in the Upper West Region, Pwalugu in the Upper East Region and Kukobilla and Yapei in the Northern Region. These selected sites are ideal for irrigation farming, especially in the dry season. Construction of irrigation dams in these areas is feasible due to the nearness of the tributaries of the White Volta. There are also other individually initiated butternut productions which are currently ongoing in Ghana, such as the 15 acre butternut production of Kobbiman Farms Limited at Asekye in the Nkoranza North District of the Brong Ahafo Region (GhanaGov, 2012; GNA, 2012; GBN, 2011; GNA, 2011). Figure 3 shows the production levels of selected cucurbits in Ghana.

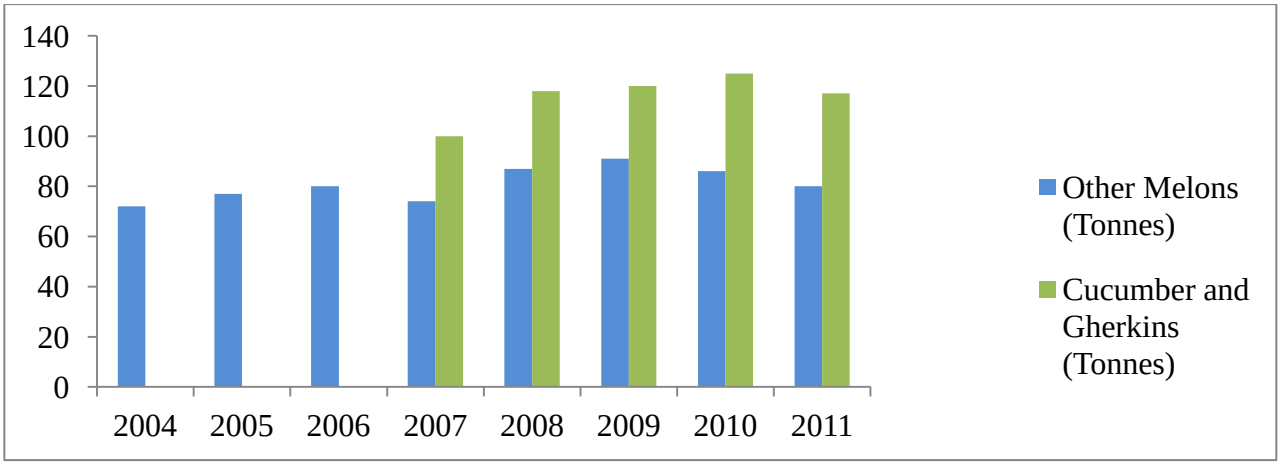

Figure 3: Production of selected cucurbits in Ghana (1000 kg=1tonne) **Source**: FAOSTAT, 2013

2.5 Cucurbit Susceptible Plant Viruses

There are more than 35 viruses that infect cucurbits. These viruses are diverse and belong to at least five different families of virus taxons; however many of the economically important viruses infecting cucurbits belong to the families *Comoviridae* and *Potyviridae* (Lecoq *et al.*, 1998).

2.5.1 *Tombusviridae*

2.5.1.1 *Carmovirus*

*Carmovirus*es are isometric particles of approximately 32 nm - 35 nm in diameter and contain a 3.88 - 4.45 kb RNA genome contained in a capsid composed of 80 copies of a single protein species of 36 – 41 kDa. *Carmovirus*es have a regular granular surface structure under the electron microscope; however, the subunit arrangement of its T=3 icosahedral symmetry is not shown in electron micrographs (Hull, 2004).

2.5.1.1.1 *Cucumber leafspot virus*

Cucumber leafspot virus causes light green to yellowish, irregularly shaped spot-like clearings with brown necrotic centers in cucurbits, especially on young leaves of infected cucumber. The virus is known to occur in Germany, Great Britain, and also in Jordan. Though the virus has no known biological vector, it is known to be soil-borne and seed transmissible in cucumber. The 28 nm single stranded RNA containing particles are easily detected in necrotic tissues of infected plants; the virus is also present as scattered or clustered particles in the cytoplasm of infected systemic hosts' cells (Weber, 1986).

2.5.1.1.2 *Cucumber soil-borne virus*

The 31 nm sized isometric virus particles are composed of a linear positive sense monopartite 5.25 kb RNA and an accompanying 0.5 kb RNA satellite. In cucurbits the virus may show no obvious symptoms except in roots only; however leaf sap may contain many infectious virus particles. Infected cells may contain 9 nm thick thin fibril inclusions. *Cucumber soil-borne virus* was first identified in Lebanon (Buchen-Osmond, 2006a; Makkouk, 1987).

2.5.1.1.3 *Melon necrotic spot virus*

Melon necrotic spot virus causes necrotic spots or large necrotic lesions on melons and cucumbers. These 30 nm isometric virus particles are soil-borne and are transmitted by the zoospores of the chytrid fungus *Olpidium radicale*. The virus is also seed transmissible through a mechanism known as vector-mediated seed transmission. Though the virus can't be found in the embryo of infected seeds it can be detected in the internal structures of the seed coat. The virus can be found as aggregates or crystalline arrays in the cytoplasm of infected cells; virus particles can also be found in the central vacuoles of infected host plants (Hibi and Furuki, 1985; Annon, undatedb).

2.5.1.2 *Necroviruses*

Necroviruses are 28 nm in diameter and are composed of 180 subunits of a 30 kDa protein constituting a capsid that contains a linear single stranded 3.7 kb RNA genome. *Necroviruses* have wide host range including both monocots and dicots. Naturally, in all its hosts, infections are limited to roots which later become systemic throughout the host

plant. *Necroviruses* are transmitted by the chytrid fungus *Olpidium brassicae* (Kassanis, 1970).

2.5.1.2.1 *Tobacco necrosis virus*

*Tobacco necrosis virus*es are isometric particles of 26 nm in diameter. They are soil-borne and are transmitted by *Olpidium brassicae*. The virus has a worldwide distribution. *Tobacco necrosis virus* does not survive in the resting spores of the vector but virus particles can be observed in the cytoplasm of infected cells. Common strains of TNV are cucumber necrosis, Stripple Streak and Urbana strain. The genome of the virus is about 19 % of the particle weight. The single stranded RNA weighs 1.3-1.6x10^6 daltons (Brunt, 1991; Kassanis, 1970).

2.5.1.3 *Tombusvirus*

*Tombusvirus*es have a 4.7 kb genome encapsidated in a 32 nm - 35 nm particle. The capsids are composed of 180 subunits of a single 41 kDa protein species. Transmission of *Tombusvirus*es is mostly soil-borne with no obvious biological vectors, though cucumber necrosis virus is transmitted by the fungus *Olpidium bornovanus*

2.5.2 *Closteroviridae*

2.5.2.1 *Crinivirus*

Criniviruses are transmitted naturally by whiteflies in a semi-persistent manner. *Criniviruses* have two modal lengths; 700 nm - 900 nm and 650 nm - 850 nm with an analogous protein at one end that gives it a “rattlesnake-like” structure. These two segments are encapsidated separately. The major coat protein weighs 28 - 33 kDa. Since

the end of the twentieth century, *Criniviruses* have emerged as a major threat to agriculture (Tzanetakis *et al*., 2013).

2.5.2.1.1 *Beet pseudo-yellows virus*

Beet pseudo-yellows virus is transmitted by whiteflies in a semi-persistent manner. The virus has a worldwide distribution and is associated with yellowing symptoms in cucumber. Symptoms appear first as chlorotic angular spots on lower leaves with interveinal areas eventually becoming chlorotic. Stunting, unthriftiness, interveinal yellowing or reddening is also observed. Symptoms may commonly be mistaken for iron, manganese or magnesium deficiency, water stress, natural aging or insect feeding damage. The viruses are long flexuous rod shaped particles approximately 12 nm wide and 1,500-1,800 nm long (Boubourakasa *et al.,* 2006; Liu and Duffus, 1990)

2.5.2.1.2 *Cucurbit yellow stunt disorder virus*

Watermelon, melon, cucumber, courgette and *Cucurbita maxima Duchesne* are susceptible to cucurbit yellow stunting disorder virus. This crinivirus occurs in Egypt and Morocco and is transmitted by *Bemisia tabaci*. In melon and cucumber, the virus is associated with severe yellowing symptoms which start as interveinal mottling on the older leaves and intensifies as the leaf ages. The veins remain green even after complete yellowing of the leaf lamina. The virus causes significant leaf drop in its host. CYSDV has been grouped as the Eastern and the Western subpopulation. The virus has flexuous particles measuring 750 nm - 800 nm in length and contains a bipartite positive sense single stranded RNA genome encapsidated in a major and minor capsid (EPPO, 2013a; Wilson, 2010).

2.5.2.1.3 *Lettuce infectious yellows virus*

Lettuce infectious yellows virus is transmitted by *Bemisia tabaci* in a semi-persistent manner and associated with yellowing of older leaves in cucurbits. As the disease progresses, the lamina becomes completely yellow but the veins still remain green. Severe interveinal yellowing or reddening may be accompanied by stunting, rolling, vein clearing and brittleness of leaves. *Cucurbita pepo* L., melons, *Cucurbita maxima* Duchesne, *Cucurbita moschata* Duchesne and *Cucurbita foetidissima* Kunth are susceptible to lettuce infectious yellows virus. The long flexuous particles measure 12 nm in width and 800 - 900 nm in length and contain a bipartite genome of sizes 8,188 and 7,193 nucleotides. The virus is not sap transmissible and is phloem limited within its host. They induce vesiculated inclusions and plasmalemma deposits (EPPO, 2013b; Falk and Tian, 1999; Brown *et al.*, 1990).

2.5.3 *Comoviridae*

2.5.3.1 *Comovirus*

Comoviruses are bipartite, linear, single stranded, positive sense RNA virions transmitted by beetles. Viruses in this genus have a narrow host range and could replicate into higher titres in infected cells. The genome is encapsidated separately into B and M particles containing RNA_1 and RNA_2 respectively (Wellink, 1998; DPV, 2014a).

2.5.3.1.1 *Squash mosaic virus*

Squash mosaic virus (SqMV) can be transmitted by coccinellid beetles, mechanical inoculation and also through embryos of infected *Cucurbita maxima* Duchesne,

Cucurbita mixta Pangalo, *Cucurbita pepo* L. and *Cucumis melo* L. seeds. As much as 94 % seed transmission has been reported in these cucurbits. In certain species, SqMV may remain symptomless but in species that show symptoms there are usually ring patterns, severe blisters, mottles, deformations and enations and intense dark green mosaic. Infected fruits may show lack of netting, chlorotic areas; deformed fruits with raised green areas are also common. SqMV disease has been reported in the western hemisphere and also in Israel (Zitter and Banik, 1984; Campbell, 1971).

2.5.3.2 *Nepovirus*

Except the capsid of tobacco ringspot virus which is made up of a single polypeptide species of molecular weight 52-60 kDa, all other *Nepoviruses* may have two or three smaller proteins composing the capsid. *Nepoviruses* largely cause ringspot symptoms in their hosts. Many *Nepoviruses* are transmitted by longidorid nematodes but most species are also transmitted by pollens and mites. There are other *Nepoviruses* that are known to infect plants, though their vectors are yet to be known. *Nepoviruses* consist of a bipartite, linear, single stranded, positive sense RNA, encapsidated in icosahedral particles. Based on their serological relations and on the length and packaging of the $RNA_{2,}$ *Nepoviruses* are classified into either of three distinct groups; Group A, Group B or Group C *Nepoviruses*. *Nepoviruses* have a wide plant host range and are associated with mosaic, mottles, ringspots and systemic necrosis symptoms (Wood, 1998; DPV, 2014b).

2.5.3.2.1 *Artichoke yellow ringspot virus*

The isometric capsids of *Artichoke yellow ringspot viruses* are 30 nm in diameter. It contains a bipartite segmented genome distributed among two linear positive sense

single-stranded RNA molecules. The viron contains a characteristic T component made up of minor non-genomic nucleic acids. A complete *Artichoke yellow ringspot virus* genome measures 11,600 nucleotides long and encodes both structural and non-structural proteins. Cucumber infected with this RNA virus shows chlorotic lesions on cotyledons which progress to mosaic, malformations and enations in non-inoculated leaves. The virus can be found in roots and shoots of infected plants and are readily transmitted by sap inoculation and through pollens and seeds, but there are no known biological vectors for *Artichoke yellow ringspot virus*. Infected cells show inclusions composing of accumulation of tangled membranes and vesicles that contain finely stranded nucleic acid-like materials. The virus is known to occur in Italy and Greece (Buchen-Osmond, 2006b; Rana *et al.*, 1983).

2.5.3.2.2 *Tobacco ringspot virus*

Tobacco ringspot virus infects a wide range of woody and herbaceous plant species. These viruses are transmitted by the *Xiphinema* species of nematodes, especially *Xiphinema americanum* and other closely related species. Thrips, tetranychus spider mites, melanoplus grasshoppers, tobacco flea beetles and possibly aphids (*Myzus persicae* and *Aphis gossypii*) can also transmit *Tobacco ringspot virus*. The virus causes ringspot diseases of cucurbits, stunting, leaf malformation and reduced fruit set. TRSV is reported to occur in North America, Japan, Australia, UK, The Netherlands, India, Nigeria, USSR, Yugoslavia and Iran. The frequency of *Nepovirus* infections increases with increasing nematode population, though a single nematode can initiate an epidemic. TRSV is seed transmitted in cantaloupe, cucumber and muskmelon. In infected cells nepoviruses are seen as files of particles occurring in tubules that pass through the

plasmodesmata and the cytoplasm of neighbouring cells. The virus can also be seen in the terminal 0.5 mm of root tips of infected plants. Common strains of Tobacco ringspot viruses include; the Tobacco Green Ringspot Strain of Valleu1938, Tobacco Yellow Ringspot Strain of Valleu 1932, Tobacco ringspot virus No. 1 of Price 1936, Anemone necrosis strain of Hollings 1965 and the satellite-like strain of Schneider 1969 (Stace-Smith, 1984).

2.5.3.2.3 *Tomato black ring virus*

This *Nepovirus* has isometric particles with hexagonal outline that measures 26 nm -28 nm in diameter with a linear bipartite single stranded 18 kb sized RNA. The virion has no conspicuous capsomere arrangement. The virus causes necrotic local lesions and systemic mottling in susceptible host species. *Cucumis melo* L., *Cucumis sativus* L., *Cucurbita pepo* L. var medullosa are susceptible to tomato black ring virus. The virus can be found in all tissues of infected plants, especially in the mesophyll, cytoplasm, vacuoles and in tubules extending through the plasmodesmata. *Tomato black ring viruses* are transmitted by *Longidorus attenuatus* and *Longidorus elongatus* (EPPO, 2013c; Harrison, 1982).

2.5.4 *Potyviridae*

2.5.4.1 *Ipomovirus*

Ipomoviruses are whitefly transmissible and are characterized by flexuous, filamentous particles of 750-950nm long and 12-15nm in diameter. An *Ipomovirus* viron has a monopartite, linear, single stranded, positive sense RNA genome (DPV, 2014c).

2.5.4.1.1 *Squash yellow leafcurl virus*

Squash yellow leafcurl virus produces small yellow spots on leaves of infected hosts. These symptoms may progress into diffused veinal yellowing and leaf curling in young leaves. These 700-750 nm long flexuous virus particles are easily transmitted through sap and in a semi-persistent manner by *Bemisia tabaci*. Infected cells show pin-wheel-like inclusions (Apsnet, 1998a).

2.5.4.1.2 *Cucumber vein yellowing virus*

Cucumber vein yellowing virus is a rod shaped 740-800 nm long and 15-18 nm wide double stranded RNA *Ipomovirus*. The virus is transmitted in a semi-persistent manner by whiteflies. It occurs in Sudan, Turkey, Israel and in Jordan. *Cucurbita maxima* Duchesne, *Cucurbita foetidissima* Kunth, watermelon, cucumber, melon and courgette are susceptible to cucumber vein yellowing virus. In cucumber, the virus is associated with profound vein clearing, chlorosis, and general necrosis. Infected fruits show light to dark green mosaic. In melon, the virus causes vein yellowing and clearing, stunting and sudden death of infected plants. In watermelon, fruit splitting may be observed and in courgette, chlorotic mottling to vein yellowing may be observed. Pinwheel inclusions may also be observed in infected cells (EPPO, 2013d).

2.5.4.2 *Potyvirus*

Potyvirus particles are 680 nm – 900 nm in length and 11 nm - 13 nm in diameter. They have a genome size of approximately 9.7 kb encapsidated by a capsid composed of multiple copies of a single 30-47 kDa protein species. *Potyviruses* and their helper components are transmitted in a non-persistent manner by aphids. Some species of

potyviruses can also be seed transmitted. The *potyvirus* genus is the largest of all the plant-infecting genera of viruses; the genus consists of 91 and 88 certified and tentative species respectively (Dolja *et al.*, 1993).

2.5.4.2.1 *Clover yellow vein virus*

Clover yellow vein virus is an RNA virus with filamentous particles measuring about 767 nm in length and 12 nm in width. They are transmitted by aphids in a non-persistent manner but can also be transmitted through sap inoculation. The virus induces mild veinal yellowing and mottling, vein clearing and mosaic symptoms in their host. Leaf malformations and reduced plant size can also be observed. Amorphous, granular intracellular inclusions, pinwheels and bundle inclusions may be seen in infected cells (Zitter and Banik, 1984; Hollings and Stone, 1974).

2.5.4.2.2 *Melon vein-banding mosaic virus*

Melon vein-banding mosaic virus is a non-enveloped monopartite linear positive sense single stranded RNA nucleocapsid with a helical symmetry and a filamentous capsid. The virus occurs in Taiwan. The virus has no DNA stage in its replication (UNIPROT, 2013a; Buchen-Osmond, 2006c).

2.5.4.2.3 *Papaya ringspot virus*

*Papaya ringspot virus*es are found wherever cucurbits and papaya are cultivated. There are two major groups of the *Papaya ringspot virus*; PRSV-P infects both cucurbits and papaya whilst the PRSV-W, which is also known as *Watermelon mosaic virus*-1, infects only cucurbits but not papaya. PRSV contains an encapsidated monopartite single

stranded positive sense RNA genome. *Papaya ringspot virus* measures 760-800 nm in length and 12 nm in width. The virus is transmitted in a non-persistent manner by several aphid species and causes severe mosaic accompanied by narrowing of leaves. In intense infection, shoestringing occurs and fruits become bumpy with green blotches. Severe stunting, puckering, blistering and distortion of leaves are also common. Apical leaves usually are reduced to the main veins. The virus is mechanically transmissible but has a narrow host range and induces amorphous inclusions in the cytoplasm of infected cells (Gonsalves *et al.*, 2010; Moran, 2001; Morgan, 2001; Purcifull *et al.*, 1984).

2.5.4.2.4 *Telfairia mosaic virus*

Telfairia mosaic virus is a non-enveloped monopartite linear positive sense single stranded RNA virus with a helical symmetry. The flexuous and filamentous particles measure 806 nm in length and 13 nm in width. The virus is transmitted through mechanical inoculation, grafting, seeds but not pollens. Aphids, especially *Aphis spiraecola,* are the biological vectors of *Telfairia mosaic virus* and the virus is transmitted in a non-persistent manner. Watermelon, melon, cucumber, *Cucurbita pepo* L. and *Telfairia occidentalis* Hook.f. are susceptible to *Telfairia mosaic virus* (Buchen-Osmond, 2006d).

2.5.4.2.5 *Watermelon mosaic virus*

Watermelon mosaic virus (WMV) causes mottling in watermelon, muskmelon, squash, pumpkin and cucumber hosts. Reduced yield and quality in squash have also been associated with *Watermelon mosaic virus*. WMV occurs in Australia, Chile, Iran, Italy, Japan, New Zealand, Mexico, Yugoslavia, Israel, Hungary, Venezuela and USA. WMV

is transmitted in a non-persistent manner by aphids; including *Myzus persicae, Aphis gossypii, Aphis craccivora* and *Aphis citricola*. WMV-infected plants are associated with cylindrical inclusion bodies and laminated aggregates (Purcifull *et al.,* 1984; Purcifull, 1981).

2.5.4.2.6 *Zucchini yellow fleck virus*

Zucchini yellow fleck virus is transmitted by aphids in a non-persistent manner; they can also be transmitted through inoculation and contact. The virus has a helical symmetry with filamentous capsid that measures about 750 nm. The capsid contains a monopartite, single linear positive sense single stranded RNA genome. The virus elicits symptoms that include yellow pin-point symptoms on leaves which later become completely necrotic. Severe mosaic, vein banding, stunting, fruitlessness and fruit deformations are also associated with severe infections. Zucchini squash, *Luffa acutangula* (L.) Roxb, cucumber, melon, *Cucurbita maxima* Duchesne, *Cucurbita pepo* L., watermelon and *Lagenaria siceraria* (Molina) Standl. are susceptible to *Zucchini yellow fleck virus* (Buchen-Osmond, 2006e; Gilbert-Albertini *et al.*, 1995).

2.5.4.2.7 *Zucchini yellow mosaic virus*

Zucchini yellow mosaic virus is currently reported to occur on all five continents of the world in a total of over 22 countries. The virus is associated with yellow mosaic, severe malformation, blistering, extreme reduction in leaf lamina, necrosis, severe stunting, shoestringing, prominent fruit deformities and fruit cracks. Squash, melon and watermelon are largely affected by the virus. There are two prominent strains of ZYMV; these are the ZYMV-FL and ZYMV-CT. They are both transmitted in a non-persistent

manner by aphids but can also be sap transmissible. The virus is rod shaped and flexuous and measures 750 nm in length. In infected cells, cytoplasmic pinwheels and scrolls can be observed. Accumulations of endoplasmic reticulum and vesicles containing fibrillar materials are also observed (EPA, 2013; Provvidenti, 2013; Lisa and Lecoq, 1984).

2.5.5 *Bunyaviridae*

2.5.5.1 *Tospovirus*

Tospoviruses have three single stranded RNA species, two of which have an ambisense strategy, the largest single stranded RNA species is negative sense. *Tospovirus* genome is contained in a membrane-bound particle. One of the gene products of *Tospoviruses* is involved in cell- to- cell movement of the virus. This distinguishes *Tospoviruses* from other genera of the *Bunyaviridae* family.

2.5.5.1.1 *Watermelon silver mottle virus*

Watermelon silver mottle virus naturally infects watermelon and melons. It causes severe loss in watermelon production in Japan and Taiwan. The virus is transmitted by mechanical inoculation and by several species of thrips, particularly *Thrips palmi*. Infected plants show silver mottle on leaves, and malformed fruits with chlorotic mottling. Crinkling, yellow spotting, narrowed leaf lamina, stunting, shortened internodes, upright growth of younger branches, tip necrosis and blight are also associated with the virus. The virus contains a negative-sense single-stranded RNA genome (UNIPROT, 2013b; EPPO, 2013e; Tomassoli and Meneghini, 2006).

2.5.6 *Bromoviridae*

2.5.6.1 *Cucumovirus*

Cucumoviruses are isometric in form with a diameter of 30 nm. These isometric particles are stabilized by a protein:RNA interaction that makes them salt labile. Their capsids are made up of a 24 kDa 180 copies of a single protein species. All members in the *Cucumovirus* genus are transmitted by aphids in a non-persistent manner.

2.5.6.1.1 *Cucumber mosaic virus*

Cucumber mosaic virus (CMV) has a worldwide distribution and is transmitted by aphids. More than 80 species of aphids in 33 genera are known to transmit CMV in a non-persistent manner. The virus is also seed transmissible in more than 20 plant species. About 10 species of *Cuscuta* are known to transmit CMV. Over 1,200 species of monocot and dicot species in 100 plant families are susceptible to CMV. These isometric viral particles are 30 nm in diameter and are made up of 180 subunits of pentamer-hexamer clusters with a hollow centre. CMV causes mosaic symptoms in cucurbits. Virus particles can be observed in the cytoplasm, nuclei and vacuoles of infected plant cells but not in mitochondria and chloroplasts of these infected cells. CMV is relatively unstable in sap and loses infectivity in less than 10 days. The most common strains of CMV are: Y strain, LS strain, M strain, WL strain, NT9 strain, B strain, O strain, Tfn strain, D strain, Q strain, No. 6 yellow strain, S strain and Fny strain (Francki *et al.*, 1979).

2.5.6.2 *Ourmiavirus*

2.5.6.2.1 *Melon ourmiavirus*

Melon ourmiavirus causes chlorotic spots, irregular ringspots and puckering in infected plants. The virus is also associated with mosaic symptoms in melon. The virion measures approximately 30 nm in width and 30-37 nm in length with a sharply triangular ends. *Melon ourmia virus* is made up of a linear single stranded tripartite RNA genome of molecular weights 3.20 * 10^5, 3.50 *10^5 and 9.1 *10^5 (Brunt 1990; Lisa *et al*., 1988).

2.5.6.3 *Tobamovirus*

Tobamoviruses are rod-shaped and measures approximately 300 nm-310 nm in length and 18 nm in diameter. The 6.3-6.6 kb positive and single-stranded RNA genome of *Tobamoviruses* is encapsidated in a capsid made up of multiple copies of a single 17-18 kDa polypeptide species. *Tobamoviruses* are very stable in purified preparations and leaf materials can retain infective virus for over 50 years in storage. Due to their high infectivity, most *Tobamoviruses* are effectively transmitted mechanically. Tobamoviruses infect a wide range of angiosperms are associated with stunting, chlorotic mosaics, mottles or ringspots (Chapman, 1998).

2.5.6.3.1 *Cucumber green mottle mosaic virus*

Cucumber green mottle mosaic virus (CGMV) infects watermelon, cucumber and melon crops. It is known to be distributed only in Europe, India and Japan. Though it has no known biological vector, the virus spreads by foliar contact, seed transmission and soil contamination. Certain strains of CGMMV show no foliar symptoms but they can result

in significant yield loss. Common CGMMV symptoms are leaf mottling, blistering, distortion and stunted growth. About six different strains of CGMMV are identified and they are: the type strain, cucumber aucuba mosaic strain, Japanese cucumber strain, watermelon strain, yodo strain and the Indian strain C. CGMMV is transmissible through seeds in cucumber, watermelon and bottle guard, though seed contaminations are mostly external and can be eliminated through heat treatment of seeds for three days at 70 ^{o}C. Pollens are also known to contain viable virions. *Cuscuta subinclusa*, *Cuscuta lupuliformis* and *Cuscuta campestris* are known to transmit CGMMV. CGMMV is extremely stable in *Cucumis sativus* L. sap. Infected cells show mitochondrial vesiculation (Hollings *et al.*, 1975).

2.5.7 *Rhabdoviridae*

2.5.7.1 *Rhabdovirus* (*Cytorhabdovirus*)

Cytorhabdoviruses are believed to replicate in the peri-nuclear space or in the endoplasmic reticulum and mature through budding into the cytoplasm of its host cell. Many *Cytorhabdoviruses* have a diameter of approximately 60 nm.

2.5.7.1.1 *Cucumber toad-skin virus*

Cucumber toad-skin virus is an enveloped nucleocapsids with a helical symmetry. The elongated bacilliform particle contains a non-segmented single linear single-stranded RNA genome. Cucumber is susceptible to the virus which occurs in France and in Greece. The virus causes vein clearing, severe leaf crinkling and stunting. (Buchen-Osmond, 2006f; Brunt, 1995).

2.5.8 *Luteoviridae*

2.5.8.1 *Luteovirus*

Luteoviruses were previously known to infect only plants in the grass family, but it is now known to infect a diversity of plants, including cucurbits.

2.5.8.1.1 *Cucurbit aphid-borne yellows virus*

Cucurbit aphid-borne yellows virus causes interveinal mottling, yellowing, thickening and brittleness of older cucurbit leaves. The virus is transmitted in a persistent manner by *Myzus persicae* and *Aphis gossypii* but cannot be transmitted by mechanical inoculation. *Cucurbit aphid-borne yellows virus* causes significant reduction in the yield of melon and cucumber by reducing the number of fruit set per plant. The 25 nm spherical virus has no DNA stage in its replication and contains a single positive stranded RNA genome (UNIPROT, 2013c; Mnari-Hattab *et al.*, 2009; Tomassoli and Meneghini, 2006; Lecoq *et al.*, 1992; Jones *et al.*, 1986).

2.5.9 *Geminiviridae*

2.5.9.1 *Geminivirus*

2.5.9.1.1 Subgroup II: *Beet curly top virus*

Beet curly top virus is transmitted by phloem feeding leafhoppers *Circulifer tenellus* and *Circulifer opacipennis*. In Africa the virus occurs in Egypt. *Beet curly top virus* can cause over 13% economic losses when only 72% of plants are showing symptoms (EPPO, 2013f).

2.5.9.1.2 Subgroup III: *Squash leaf curl virus*

Practically, *Squash leaf curl virus*es are restricted to cucurbits though other families of crops may be susceptible to the virus. The virus causes severe systemic stunting and leaf curl in cucurbits; this significantly affects the yield and quality of squash, melon and other cucurbits. *Squash leaf curl virus*es are transmitted in a persistent manner by *Bemisia tabaci* and also through mechanical sap inoculation. Common strains of *Squash leaf curl virus* are watermelon curly top mottle virus and Squash leafcurl virus-CA. The geminate virus particle measures 22*38 nm in size and is associated with maturation of phloem sieve tube elements. Fibrillar rings and viral particles can be found in the cytoplasm and nuclei of infected cells respectively (EPPO, 2013g; Duffus and Stenger, 1998).

2.5.9.1.3 *Watermelon chlorotic stunt virus*

Watermelon chlorotic stunt virus is transmitted by *Bemisia tabaci* and is associated with crinkling, chlorotic mottling on younger leaves, severe stunting, systemic mosaic ringspotting, necrosis and yellowing of veins in infected hosts. The non-enveloped geminate particle is 19 nm in length and 35 nm in width. The particle contains a two segmented copies of equal size circular ambisense single stranded DNA genome. Watermelon, *Citrullus colocynthis* (L.) Schrad., melon, cucumber and *Cucurbita pepo* L. are susceptible to the virus. The virus is also graft transmissible in watermelon. *Watermelon chlorotic stunt virus* occurs in Saudi Arabia and in Southern and Northern Yemen (Ali-Shtayeh *et al.*, 2012; Buchen-Osmond, 2006g; ICTVdB, 2006).

2.5.10 *Tymoviridae*

2.5.10.1 *Tymovirus*

2.5.10.1.1 *Chayote mosaic virus*

Chayote mosaic virus is an isometric virus particle of about 28 nm in diameter with a single stranded RNA genome of approximately 6.4 kb in length. The virus can be transmitted through seeds, beetles and also through mechanical inoculation. The natural host of *Chayote mosaic virus* is chayote (Bernal *et al.*, 2000; Juan, 2000).

2.5.10.1.2 *Melon rugose mosaic virus*

Melon rugose mosaic virus is a non-enveloped encapsidated particle with an icosahedral symmetry that measures 32 nm in diameter. The virus has a round or hexagonal outline and contains a linear monopartite single stranded RNA genome of about 2,100 nucleotides long weighing about 2.1 x 10^6 Daltons. The virus is associated with yellowing, rugose mosaic, leaf malformation, chlorosis, changes in leaf texture and puckering of leaf surface. It is mechanically transmissible but also seed transmissible in melon. Watermelon, sweet melon and squash are susceptible to *Melon rugose mosaic virus* (Buchen-Osmond, 2006h; Mahgrub *et al.*, 1997; Jones *et al.*, 1986).

2.6 Pathological and Non-Pathological Interactions among Plant Viruses and Their Hosts

2.6.1 Effects of Virus Infection on the Physiology of Plants

Viruses are biotrophic pathogens that require living cells for their replication; and without suitable host cells viruses cannot multiply (Pallas and Garcia, 2011). Hence at the infection-defense equilibrium, viruses may not induce any significant pathological

responses in plants. This is evidenced by the vast majority of wild plants that are symptomless even when infected by most strains of viruses. In certain cases, viruses are even known to confer comparative advantage to their host against other pathogens so as to ensure the survival of their host and their replication and survival. However, viruses may also induce severe pathological responses in their host which often are lethal and may cause the death of the host. It has been observed that viruses interfere with the normal metabolism, hormone synthesis and signaling pathways in cells (Pallas and Garcia, 2011). Plant viruses are known to modify their host so as to efficiently exploit their host for their survival. Symptoms observed in infected plants are due to the interference or the competition for host resources which disrupt the normal physiology of the host.

Gibberellin is an important hormone involved in cell cycle regulation and plant growth. Since viruses require actively dividing cells for their replication and accumulation, they develop mechanisms to alter the cell cycle to the phase that will ensure optimum virus replication. The REP proteins of geminiviruses are able to initiate a negative cell cycle regulation by interacting with a group of plant proteins known as retinoblastome-related proteins, Prbr. The viral proteins possibly inhibit the PrBr protein activity which makes infected cells enter the S phase of the cell cycle where double stranded host DNA is produced. The double stranded DNA replication mechanism of the S phase is then exploited by the infecting virus for its replication. Gibberillins are also crucial in stem enlongation. The ent-kauren oxidase protein plays a crucial role in the biosynthesis of gibberellins. Plants infected with rice dwarf virus show a dwarf phenotype and are characterized by low concentrations of endogenous gibberellins GA_1; the application of

exogenous gibberellins GA_3 to infected plants reverted their dwarf phenotype. It was observed that the P_2 protein encoded by the rice dwarf virus interferes with the ent-kauren oxidase protein activities which also affect the production of phytoalexins and facilitate the efficient replication of the rice dwarf virus (Pallas and Garcia, 2011)

Suppressor proteins encoded by viruses are also known to cause the misregulation of the miR167 target auxin response factor. This results in developmental abnormalities in the host plant. The L_2 protein encoded by *Beet curly top virus* (BCTV) deactivates the adenosine kinase and the sucrose non-fermenting-1 kinase (SNF-1). This BCTV-induced deactivation contributes to suppression of plant viral RNA silencing factors and also influences the metabolic status of the infected host. The sucrose non-fermenting-1 kinase is a universal regulator of plant metabolism. Deactivation of the SNF-1 kinase increases host susceptibility to BCTV infection. TMV replicase is known to deactivate the auxin/IAA protein synthesis; this virus-induced cell reprogramming ensures that the cellular environment is ideal for optimum viral replication. The gene product VI from CaMV is known to interfere with the ethylene hormone-signaling pathway. Mutant Arabidopsis that suppress the phenotype induced by the expression of the CaMV gene are known to be less susceptible to CaMV infection and are less sensitive to ethylene (Pallas and Garcias 2011). The clink of Faba bean necrotic yellow virus encodes an F-box protein which interacts with a protein homologous to the SKP-1 and PrBr proteins. It has been observed that when the clink protein binds with the PrBr protein, virus replication is greatly enhanced. It has been suggested that the clink protein may interfere with the natural cell cycle repression in the infected host in order to stimulate the virus replication (Ratcliff *et al.*, 1999; Voinnet *et al.*, 1999; Ruiz *et al.*, 1998).

2.6.2 Defensive Mechanisms Elicited by Plants against Virus Infection

There are more than 1,000 viruses that can infect plants; however, infection of a plant by a virus is largely a rarity. Many viruses are not successful in infecting plants. Only few viruses can infect few plants. In many instances, plants are altogether not susceptible to many of these viruses; in the few cases where the virus has the potential to infect the plant, the virus has to overcome the numerous defense mechanisms developed in plants. Most plants are not susceptible to many plant viruses; this may suggest that many plants are able to defend themselves against many infecting viruses. A genome-wide expression of *Arabidopsis thaliana* genes shows that certain plant genes are triggered by the introduction of plant viruses into the plant. These genes are involved in cell detoxification, cell wall remodeling, primary and secondary metabolism and diverse defense mechanisms. It has been observed that plants have generally two broad mechanisms by which they defend themselves against plant viruses these are: RNA mediated mechanisms and Protein mediated mechanisms. The commonly used RNA mediated mechanism used by most plants is the Post Transcriptional Gene Silencing (PTGS) (Ratcliff *et al.*, 1999). Plant viruses can both induce or be targets of gene silencing in plants. Virus induced gene silencing takes place if there is sequence similarity between the infecting virus and either a transgene or endogenous nuclear genes of the host plant. Double stranded RNA is a well known intermediate of the replication of most plant infecting RNA viruses. The plant recognizes the viral double stranded RNA intermediate and uses it as a template to produce antisense RNAs using a host encoded RNA dependent RNA polymerase. This indirectly produced antisense RNA transcript may further be processed into a specificity determinant for the degradation of the infecting virus. Virus transformed transgenic plants may also directly

produce antisense RNA against invading viruses by transcribing the introduced silencer transgene which now forms part of the genome of the genetically modified plant (Mandadi and Scholthof, 2012).

The mechanism of RNA silencing is a broadly conserved response against many viruses that infect plants. RNA silencing components such as Dicers, double stranded RNA binding proteins, HUA ENHANCER-1, Argonaute (AGO) and RNA- dependent RNA polymerase programme virus-specific RNA-induced silencing complexes to ensure the degradation of the invading viral particles. The RNA silencing mechanism cleaves the double stranded RNA viral template into small interfering RNAs, this prompts RNA induced Silencing Complex (RISC) to either degrade viral RNAs or hinder the translation of viral RNAs. PTGS spreads systemic information throughout the entire plant system ensuring the sequence specific degradation of viral particles (Mandadi and Scholthof, 2012; Senshu *et al.*, 2011; Pallas and Garcia, 2011).

Plants infected by viruses are also able to induce a hypersensitive response (HR) through the production of hormones such as salicyclic acids which hinders the movement of the invading virus from primary infected cells and also induce a systemic response throughout the host to prevent a systemic spread of the virus through the host (Kim and Palukaitis, 1997).

This hypersensitive response or plant suicide response is triggered by gene products encoded by a group of endogenous plant genes known as R genes. These R genes confine a broad range of pathogens, including viruses, to the initially infected cells

through the induction of programmed cell death, hence halting or limiting the systemic spread of the invading virus (Pallas and Garcia, 2011).

Almost all viral gene products are HR inducers in the infected host cells, though not all viral gene products are capable of complete viral replication or degradation. For instance, just the p50 helicase domain and the 30 K movement protein from tobacco mosaic virus and tomato mosaic virus respectively are able to induce a hypersensitive response in plants. Induction of systemic necrosis in *Nicotiana tabacum* and *Arabidopsis thaliana* Ler by the b RNA replicase from Potato Virus Y nuclear inclusion and double stranded RNA-mediated resistance suppression respectively are as a result of incomplete restriction of the invading virus to the initially infected cell by the triggered hypersensitive response mechanism. As the virus moves systemically, the hypersensitive response also follows systemically, creating a systemic necrosis along the path of the invading virus (Ratcliff *et al.*, 1999; Ruiz *et al.*, 1998).

2.6.3 Counter-Defensive Mechanisms Elicited by Viruses against Host Defenses

Plant viruses are able to identify and evade the numerous plant defense mechanisms through well known processes such as the encoding of PTGS suppressor proteins and rapid replication to make void the plant defense mechanisms. The virus encoded suppressor proteins interfere with the RNA mediated defense mechanisms of plants. Potyviruses and *Cucumber mosaic virus* (CMV) are known to encode suppressor proteins from the HC-Pro viral sequence. Proteases encoded from this viral sequence are known to interfere with a branch of RNA silencing involving microRNAs. A protein encoded by CMV is now known to facilitate the cell-to-cell movement of virus, and a

suppressor protein that interferes with the natural anti-viral defense mechanism in plants. The 2b protein encoded by CMV is known to promote the extensive movement of co-infecting viruses, especially *Zucchini yellow mosaic virus* (ZYMV) in their systemic hosts. This synergistic interaction of ZYMV and CMV that results in increased symptom expression is partly due to the suppression activity of the 2b protein encoded by CMV. A constructed virus that had its 2b sequence removed still replicated as the wild type CMV in the protoplast of infected cells; however, the CMV-Δ2b mutant virus was restricted in its movement in a tobacco host. The 2b protein encoded by CMV is known to harness the severity of symptoms in cucurbits. Squash plants infected with wild type fny-CMV show severe systemic symptoms in only 5-7 days post inoculation (dpi); however, when squash plants are inoculated with fny-CMV-Δ2b mutant virus, there was only very mild mosaic symptoms on the first systemic leaf, the upper leaves showed a recovery-like phenotype (Wang *et al.*, 2004; Ratcliff *et al.*, 1999; Voinnet *et al.*, 1999).

2.6.4 Synergistic Interactions among Plant Viruses

Since there are numerous plant viruses, it is common for more than one virus to infect a single plant at the same time. Plant viruses are known to interact with each other in different interactive ways such as cross protection, helper dependence, replacement, mutual suppression and synergism. These interactions usually result in unpredictable symptoms, usually enhanced vector transmissibility, virus accumulation, cellular tropism and altered host range. Synergistic viral interactions are caused by at least two different co-infecting viruses in the same host species in a particular time. Different strains of the same virus may also be involved in synergistic interactions. There are different forms of synergistic interaction observed in plants; these include neutral synergy, unilateral

synergy and mutual synergy. In neutral synergy, the co-infecting viruses interact so that the symptoms exhibited by the interacting viruses far exceed that exhibited by each of the viruses alone; however, such synergy does not result in the accumulation of any of the viruses involved in the synergistic interactions. This is the form of synergy observed when both tobacco mosaic virus (TMV) and *Cucumber mosaic virus* (CMV) co-infect a single susceptible host; the symptoms are greatly increased, but none of the viruses increase in concentration beyond what was possible if both viruses had infected the same host individually (Martin and Elena, 2009).

In mutual synergistic viral interaction, both the titre and the severity of symptoms are greatly enhanced than if both viruses infected the host individually. The Cameroonian strain of African cassava mosaic virus is known to be involved in a mutual synergistic interaction with the Cameroonian strain of *East african cassava mosaic virus*. When both viruses infect a common host, both the severity of symptoms and the titre of both viruses are greatly increased than would have occurred if both viruses infected their host individually.

In unilateral synergy, both the severity of the symptoms and an enhanced accumulation of one of the co-infecting viruses occur. In an experiment, it was observed that when both *Maize chlorotic mottle virus* (MCMV) and *Maize dwarf mosaic virus-B* (MDMV-B) co-infect a single host simultaneously, the concentration of the MCMV increased 5.4-fold than would have occurred if both viruses infected the host individually. However, the concentration of MDMV-B remained unaffected by the synergistic interactions (Zhang *et al.*, 2001).

The P1/HC-Pro sequence of tobacco etch virus is known to be a principal factor in the synergistic interaction between CMV and TEV. CMV has a multipartite genome with a satellite component in some strains. In a TEV-transformed host, the concentration of CMV genomic RNA 3 and 4 increased tenfold compared to the wild type host, with longer exposure, RNA 1 and 2 also increased in the TEV-transformed hosts. However, the titre of the dependent satellite RNA was undetectable in the TEV-transformed host though its titre was equally high in the wild type host as the other CMV genomic RNA. This suggests that the HC-Pro sequence of TEV disrupts the balance between the CMV genome and satellite replication to favour the accumulation of CMV genomic RNA (Pruss *et al.*, 1997).

Viral synergistic interactions are known to neutralize resistance of a plant to viruses which it may have previously been resistant to. The M strain of CMV does not infect zucchini squash systemically due to limited long distance movement of the virus in zucchini as a result of the virus inability to exit the sieve elements. However, co-infection of melons and zucchini by both ZYMV and CMV overcomes the resistance of zucchini to CMV-M and results in high accumulation of CMV genomic RNA and coat proteins. The ZYMV may have encoded a movement protein required by the CMV-M strain to assist in its long distance systemic movement and hence, enhanced the pathological responses in the infected host. CMV is also known to neutralize the resistance of pepper to pepper mottle virus (Choi *et al.*, 2002).

Mixed virus interactions may also either result in additive effects of both viruses or even lesser symptoms than even when both viruses infected the host separately. When at least three of the novel strains of tomato leaf curl virus infect their host simultaneously, the

symptoms observed is equivalent to the sum of all the symptoms that would have been exhibited by each of the co-infecting virus strains.

Mixed virus interactions could also result in an altogether new symptom which is not exhibited by any of the co-infected viruses. For instance, an interaction between TMV and potato virus-X causes a new disease symptom known as Leaf Drop Streak which is often lethal. Co-infecting MCMV and MDMV-B or WSMV is known to cause corn lethal necrosis which may not be caused by any of the co-infecting viruses individually (Goldberg and Brakke, 1987; Zhang *et al*., 2001).

Zucchini squash is a host to cucurbit yellow stunting disorder virus (CYSDV); however, infection is not associated with any symptoms in zucchini squash. *Cucumber vein yellowing virus* (CVYV) can also infect zucchini squash and infected plants show less profound vein-clearing symptoms. When both CYSDV and CVYV co-infect zucchini, interveinal mottle on intermediate leaves are observed by 45 to 60 dpi, these symptoms later evolve to complete yellowing of the leaf lamina excluding the leaf veins; rolling and brittleness are also observed. This shows the synergistic interaction between the ipomovirus and the crinivirus (Gil-Salas *et al*., 2011).

A triply mutual synergistic virus interaction has been observed between co-infecting beet mosaic virus, beet yellows virus and beet western yellows virus. When all three viruses simultaneously infect sugar beet, there is a significant increase in virus titre and symptom severity than would have occurred by either or any of both viruses (Garcia-Cano *et al*., 2006).

2.7 Plant Disease Epidemiology

2.7.1 Introduction to Plant Disease Epidemiology

Disease epidemiology is the science that studies disease progress in a defined host population. It focuses on the disease causing organism, the host population dynamics and the environment within which the pathogen and host interact to produce disease. It is a multidisciplinary field of study that combines biological, statistical, agronomic and ecological perspectives in identifying the cause and effects of disease and measures to adopt to manage the disease situation. An epidemic can be assessed in either time or space or both; due to the physical quantities of time and space in epidemiology and the immobility of plants, various statistical models can be used in quantifying and describing the rather complex plant pathosystems in simple mathematical terms. A major goal of epidemiology is to provide guidelines for forecasting and controlling disease outbreak (Madden, 2012; Kleczkowski and Gilligan, 2007; Real and McElhany, 1996).

Geostatistical models, Regressions, Probability distributions and interpolation techniques such as Kriging and Inverse Distance Weighting are gaining more attention in plant epidemiology. Geostatistical models in plant epidemiology focus on the analysis of spatially distributed variables and the prediction of unsampled locations as well as forecasting the probability of future disease occurrence and the intensities at which they may occur. These models create surface maps which correspond to the diseased field. Such disease maps make it more interactive and easy to understand the nature and spread of the disease in the field (Esker *et al.*, 2008; Nelson *et al.*, 1999).

Knowledge of the spatial and temporal dynamics of a disease situation is essential for making cost effective and accurate management decisions. For example, a reliable spatial spread analysis of a diseased field may be able to predict the critical areas of the field where attention is needed most so as to tailor the control measures largely to these predicted spots rather can applying control measures uniformly on the field which may be costly and ineffective (Workneh and Rush, 2010).

The ultimate goal of agriculture production is to maximize yield and maximize profit. Disease is one of the significant threats to increased yield and profit maximization in agriculture production. The impact of plant disease and its associated losses are a function of the progress of the disease. Though disease may not be easily eliminated but the factors that enhance its progress could be identified and minimized. Quantifying the factors that enhance the progress of the disease such as susceptibility of the cultivar, favorable weather conditions, and availability of inoculum make it possible to know the magnitude of reduction to ensure economic production. Plant epidemiology provides the tools and theories essential to quantifying disease so as to quantify the magnitude of damage due to disease incidence in a plant population. Epidemics is mainly quantified as disease incidence or severity. Disease incidence measures the proportion of plants or plant units that are diseased in a sample or a population of host plants. It can be measured in real terms or in proportions. At the population level, incidence is a count variable with a natural denominator; a discrete variable. Incidence is also a binary variable since it has only two possible values i.e. either a plant is diseased or healthy. In principle a discrete variable has a countable number of possible values. However, for a large population size with a wide range of possible values, incidence can be

approximated as a continuous variable. For continuous variables the variance of the observed data may or may not be dependent of the estimated sample mean. The relationship of the variance to the estimated mean is very crucial in certain forms of statistical analysis such as those involving a description of the spatial and temporal pattern of disease progress; hence it is very important to select a sampling method that will permit the estimation of the variance as well as the sample mean. Knowledge of the distribution of diseased plant units in a field is also essential to efficiently sample for diseased plants. The nature of sampling adopted for monitoring epidemics is largely influenced by the objective of the sampling and the availability of resources. The sampling unit may be defined as the portion of the host habitable space from which pathological observations are made; it is characterized by a definite size (Apsnet, 2013b; Madden, 2012; Hughes, 1999; Rouse, 1988).

2.7.2 Sampling Methods in Plant Disease Epidemiology

The common sampling methods used in many life sciences include simple random sampling, systemic random sampling, stratified random sampling, random cluster sampling, stratified cluster sampling random multi-stage sampling and hierarchical sampling. Depending on the use and the statistical procedure that may be used in analyzing the collected data, simple random sampling, stratified sampling and systematic sampling may commonly be encountered in plant epidemiology. For instance, for spatial and temporal dynamics of disease progress, simple random sampling is an inappropriate sampling method for data collection. Only cluster sampling can be used to collect data for spatial and temporal pattern analysis. Once the appropriate sampling method is chosen the precision associated with the collected data must also be

estimated to ensure that decisions based on collected data can be reliable. Precision is influenced by the number of plants in a sampling unit and also the size of the sampling units. Precision can be quantified by estimating either the standard error or the confidence interval associated with the estimated parameter. Standard errors are directly proportional to the square root of the size of the sampling unit. Due to this reciprocal square root relationship between the standard error and the sampling unit size, the standard error is most significantly influenced by changes in sampling unit size, especially when the sampling unit size is not greater than 20. With smaller sampling unit size, increasing the sampling unit significantly reduces the error associated with the estimated parameter such as disease intensity. Precision is proportional to the inverse function of the sampling unit size. The number of sampling units required to achieve a specified precision in estimating a parameter can be calculated before sampling (Madden, 2012; Hughes *et al.*, 2002).

2.7.3 Classification of Plant Disease Epidemics

Plant disease is a cyclical process. The pathogen enters the susceptible host and begins to reproduce new pathogens, which are then dispersed to new susceptible host where they also initiate new infections. The period elapsing between a successful inoculation into the time where new infectious inoculum are produced constitute the infection cycle. Pathogens that produce only one infection cycle per crop season are termed as monocyclic pathogens. Pathogens that undergo more than one infection cycle within a crop cycle are termed as polycyclic pathogens. Epidemics that involve monocyclic and polycyclic pathogens are termed as monocyclic and polycyclic epidemics respectively (Apsnet, 2013c).

2.7.3.1 Polycyclic Epidemics

Polycyclic epidemics are characterized by the production of first type inoculum where new infections are initiated by the newly produced inoculum, which further produces new inoculum. In polycyclic epidemics there are multiple disease cycles within a single epidemic and the epidemic increases with time at an increasing rate. Polycyclic epidemics are started by primary infections and progresses with primarily secondary infections; the sources of the inoculum that accelerates the initiated disease are largely found in the epidemic itself. Typical polycyclic epidemics are those that involve plant viruses such as virus epidemics in cucurbits. Polycyclic epidemics usually increase at an increasing rate during the early part of the epidemic (Apsnet, 2013c; Apsnet, 2013d; Madden, 2012).

2.7.3.2 Monocyclic Epidemics

Monocyclic epidemics are characterized by the production of second type inoculum where inoculum that can progress the epidemic are not produced by pathogens or plants that were previously infected in the current epidemic. Though the pathogen or infected host may still produce the inoculum but they cannot cause disease in the current epidemic. Second type inoculum produces one disease cycle per crop season. These epidemics are common with pathogens that require long disease cycles and may be able to complete only a single cycle in the host's lifecycle. Post harvest diseases and those caused by soil borne pathogens are mostly associated with monocyclic epidemics. Usually, monocyclic epidemics progress roughly linearly at the start of the epidemics (Apsnet, 2013c; Madden, 2012).

2.7.3.3 Statistical Approaches to Plant Disease Epidemiology

For simplicity, suppose the number of plants that are diseased in a population is random, i.e. all plants in the population have equal probability of being infected, then the frequency of infected plants in such population can be described by a binomial distribution statistic. However, in most cases disease spread in a population is not random since they are influenced by several factors such as insect flight patterns and preferences and other factors. Arriving insects usually land first on border plants; hence, the probability of the border plants being infected will be higher than plants situated within the midst of the field. In such instance binomial distribution statistics may either underpredict or overpredict incidence and the predicted variance is usually overdispersed. When the probability that a plant is diseased is not constant but rather a random variable represented by the beta probability function x then the proportion of diseased plants in a population is best described by the beta binomial probability statistic. The beta binomial distribution estimates two parameters, the rho, which is the average or expected probability that a plant is diseased, and theta, which measures the spatial heterogeneity of diseased plants. When theta is greater than zero then there is aggregation of diseased plants within the host population. Theta increases with the level of disease plant aggregation (Madden, 2012).

2.7.3.4 Modeling the Temporal Spread Pattern of Disease Epidemic

Models are essential in quantifying disease progress over time. Accurately quantifying disease progress over time in a host population is essential in comparing epidemics. Host cultivar, pathogen genotype and environment affect the progress of disease and are very

useful in predicting epidemics and planning effective control strategies to manage the epidemic below a specified threshold.

In polycyclic epidemics such as those caused by cucurbit viruses, new infections result in new inoculum which can also initiate new infections in the current epidemic, resulting in multiple disease cycles within a single epidemic. Polycyclic epidemics consist of both primary infections which initiate the epidemic and secondary infections which are new infections resulting from transfer of inoculum in the current epidemic. The disease intensity y, in an absolute unit scale can be either a continuous variable or a discrete variable, however for model fitting discrete variables are approximated as a continuous variable. Continuous time deterministic models in which time is considered as continuous are often used in characterizing the temporal spread of disease. In such models it is assumed that disease intensity is continuously changing, though, in reality disease intensity may only change when conditions are favourable for the disease pathogens to cause infection. Parameters that are estimated from these temporal disease progress models represent the effects of host-pathogen interactions in the environment that results in pathological responses. In principle, these parameters are used in characterizing the epidemics. The most commonly used models in characterizing the temporal spread of disease are exponential models, monomolecular models, logistic models and Gompertz Models (; Apsnet, 2013c; Madden, 2012).

2.7.3.4.1 Exponential Models

The exponential model is associated with Thomas Robert Malthus and is one of the simplest and easy to use mathematical models in plant epidemiology. Only a single

parameter is estimated and they are ideal for polycyclic epidemics and other epidemics with low disease intensities. The model assumes that the absolute rate of disease increase $\frac{\partial y}{\partial t}$ is directly proportional to the disease intensity;

$$\frac{\partial y}{\partial t} \propto y \quad (1)$$

$$\frac{\partial y}{\partial t} = rEy \quad (2)$$

That is, increase in disease intensity results in a more increase in disease intensity. The parameter rE termed the rate parameter represents all the factors and variables that affect disease increase other than the disease intensity itself. The rate parameter measures the secondary infection rate in "per time". The magnitude of the rate parameter is affected by the host susceptibility, the environment, and infection efficiency. The length of the disease cycle, infectiousness of diseased hosts and the aggressiveness of the infecting pathogens also influence the rate parameter.

To relate field observed data to the exponential model, the differential equation is solved further to its integrated form;

$$y = y0e^{rEt} \quad (3)$$

where e is the natural log system. $y0$ is a constant, a parameter known as the constant of integration, it represents the initial disease intensity as a result of primary infections that initiated the epidemic.

In order to simplify the statistical analysis and also to visualize the effects of different rate parameters on initial disease progress the non linear integration exponential

equation is natural log transformed into a linear equation, this makes it easier to estimate the rate parameter with as few data as possible, such as from just two assessment times.

$$\ln y = \ln(y0) + rEt \quad (4)$$

The independent variable, time (t) occurs in an exponential form and the function increases without any upper bound to the right. The model assumes a constant environment over time and space and the availability of unlimited resources; hence exponential models are most ideal for modeling events with unconstrained growth. Exponential models are particularly useful in modeling nuclear chain reactions, heat transfers and human population growth. However, biological growth is usually constrained due to increased competitions as a result of limited resources available for growth. In the unlikely event of neither environmental nor physiological factors that slow the rate of disease progress, the exponential model may be the appropriate model to use (Anon, 2013a; Anon, 2013b; Wkipedia, 2013b; Madden, 2012; Paine *et al.*, 2012; Sharov 1997a; Madden, 1980).

2.7.3.4.2 Monomolecular Models

The monomolecular model also termed as negative exponential model is most appropriate for monocyclic epidemics but it can also be used in characterizing polycyclic epidemics with low disease intensities especially when $y = <$ 0.05 - 0.1 The model has been used in modeling plant response to nutrients, cell expansion and first order chemical reactions. For monomolecular models, the absolute rate of disease increase is not only proportional to disease intensities as in exponential models, but it is also proportional to the disease free intensity (1- y) because it is assumed that any

inoculum produced by the infected individuals in the current epidemic doesn't lead to additional infections in the current epidemic i.e. the infected host population produces second type inoculum. Unlike the exponential model, the monomolecular model also assumes a carrying capacity of one.

$$\frac{\partial y}{\partial t} \propto 1 - y \tag{5}$$

$$\frac{\partial y}{\partial t} = rM(1 - y) \tag{6}$$

$$\therefore \frac{\partial y}{\partial t} = rM - rMy \tag{7}$$

When incidence, y, is low it can be approximated as 0

$$\therefore 1 - y = 1 \tag{8}$$

$$\frac{\partial y}{\partial t} \cong rM \tag{9}$$

There is no point of inflection and its curve is always concave down. The rate parameter is fastest in the early part of the epidemic and slows down toward the end of the epidemic. Monomolecular models are largely suited for log transformed data. To fit a monomolecular model to the observed data the differential equation is integrated into the forms;

$$y = 1 - (1 - y0)e^{-rMt} \tag{10}$$

$$\ln({}^{1}/_{1-y}) = \ln({}^{1}/_{1-y0}) + rMt \tag{11}$$

The absolute rate, $\frac{\partial y}{\partial t}$ declines toward 0 since there are less and less disease-free individuals as y increases unlike in exponential models where there is no limit to $\frac{\partial y}{\partial t}$ increase. The parameter rM represents secondary transmission rate; it is equivalent to rE in exponential modeling (APSNET, 2013c; Madden, 2012; Paine *et al.*, 2012; Madden, 1980).

2.7.3.4.3 Logistic Model

The logistic model was developed by the mathematician Pierre Verhulst who proposed a limit to the rate of increase in population growth. Currently, the logistic model is among the most widely used models in pathology particularly in modeling polycyclic epidemics. The model best describes events with slow initial and final growth rate with characteristic moderate growth rate in the mid season of the event. In logistic modeling the absolute rate of disease increase, $\frac{\partial y}{\partial t}$ is proportional to disease intensity, y and healthy intensity (1- y). The model assumes that more disease intensities results in even more disease, however, more disease also decreases the proportion of susceptible healthy plants hence the rate of disease increase increases at a decreasing rate. As the disease progresses, more of the inoculum reach already diseased but not healthy plants which do not add up to disease increase.

$$\frac{\partial y}{\partial t} \propto y, (1 - y) \quad (12)$$

$$\frac{\partial y}{\partial t} = rLy(1 - y) \quad (13)$$

rL is the rate parameter that represents secondary infection rate. The integrated and linearized forms of the differential logistic equation are written as follows respectively;

$$y = \frac{1}{1+\exp\left(-(In(\frac{y0}{1-y0})+rLt)\right)} \quad (14)$$

$$\ln y/(1-y) = \ln(\frac{y0}{1-y0}) + rLt \quad (15)$$

The parameter $y0$ is the constant of integration that represents primary infection rate. At low y, exponential and logistic models may produce similar results and at high y, logistic and monomolecular models may also produce very similar results. The integrated logistic curve has a sigmoid form and the point of inflection occurs at y =0.5 and $\frac{\partial y}{\partial t}$ increases until it reaches y =0.5 after which $\frac{\partial y}{\partial t}$ decreases. The logistic model is ideal for modeling polycyclic epidemics (Anon 2013a; APSNET, 2013d; Sharov 1997b; Berger, 1981; Madden, 1980).

2.7.3.4.4 Gompertz Model

The Gompertz model is named after Benjamin Gompertz. The function is suited for modeling time series events with slow initial and final growth rates such as the growth of tumours. The model has been frequently used by ecologists to model biological events. Currently the gompertz model has wider application including its use in plant epidemiology. The Gompertz model is particularly ideal for polycyclic epidemics. Just like the logistic model, the integrated gompertz model is sigmoid, however it is asymmetrical about its point of inflection which occurs early, at approximately 37% of the asymptotic mass, K, also $\frac{\partial y}{\partial t}$ depends on $\ln(\frac{1}{y})$ and y. In gompertz functions the epidemic reaches at peak $\frac{\partial y}{\partial t}$ earlier compared the logistic model (APSNET, 2013b;

Wikipedia, 2013c; Paine *et al.*, 2012; Berger, 1981). The differential, integrated and linearized forms of the gompertz function are written as follows;

$$\frac{\partial y}{\partial t} = rGy\{\ln(1) - \ln(y)\} \quad (16)$$

$$y = e^{\ln(y0)e^{-rGt}} \quad (17)$$

$$-\ln(-\ln(y)) = -\ln(-\ln(y0)) + rGt \quad (18)$$

2.7.4 Criteria for Selecting Mathematical Models to Quantify Disease Epidemics

There are numerous models developed with varying assumptions that suit various kinds of field data class. The nature of the experimental data and its possible use may require that a particular model is used over other models; hence it is very essential to always select the most appropriate model for data analysis for a more precise data interpretation. There are various statistical and graphical methods used in selecting models for interpreting virus epidemic data.

Among the various graphical methods, the use of the estimated coefficient of determination (r^2) from $\frac{\partial y}{\partial t}$ versus t data is commonly employed. The higher the r^2 value of the data plot, the more appropriate the model for data fitting and parameter estimation. Observed y can also be transformed by a group of selected models and plotted against t, the graph that gives the straightest line is the most appropriate model to choose. Statistical approaches such as ordinary least squares regression can also be used in choosing the appropriate model for data fitting. The MSE and residuals are also used in selecting the most appropriate models. The best fit will correspond to the highest r^2,

smallest MSE and the straightest line. After the selection of the most appropriate model, the required parameters and their associated statistics such as standard error and confidence interval can be estimated (Madden, 2012).

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CHAPTER THREE

3.0 STUDY ONE: DETECTION OF VIRUSES IN SIX CUCURBIT SPECIES AT AN EXPERIMENTAL FIELD IN THE COASTAL SAVANNAH ZONE OF GHANA

3.1 INTRODUCTION

In the early years of plant virus studies, grafting, mechanical inoculation, vector transmissibility, X-ray crystallography, microscopy and various forms of centrifugation played a major role in the detection of viruses (Hull, 2004). Recently, more advanced techniques and effective tools have been developed for virus detection. These include DNA microarray, NASBA implemented microarray analysis (NAIMA), serial analysis of gene expression (SAGE), DNA barcoding, and many more multiplex and non-multiplex virus detection techniques which facilitate accurate detection of many viruses (Barba and Hadidi, 2011).

However, the use of these modern techniques requires advanced technical knowledge and equipment and generally associated with prohibitory high usage cost. Low cost, but effective virus detection techniques such as observation of field symptoms, bioassay, polymerase chain reaction (PCR) and various forms of enzyme linked immunosorbent assays (ELISA) provide reliable means of detecting viruses in resource limited countries. For a more accurate detection of viruses, at least two of these techniques may be used together. The use of inadequate detection methods mostly result in false diagnosis of virus infection; for instance, a study conducted by Mukasa, (2003) showed that Ugandan sweet potato farmers often associate virus and virus-like symptoms with sun scorching, low soil fertility or an effect of soil borne pathogens. Diagnosis of virus

infection solely based on observed field symptoms could be misleading due to the crucial role the environment plays in the development of symptoms. However, observed symptoms could be confirmed by either ELISA or PCR.

3.1.1 Objective of Study

The objective of this study was to detect viruses involved in the pathosystem of six cucurbit species through;

1. Observation of field symptoms of virus infection.
2. Detection of *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* (PRSV-W), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) by DAS ELISA.
3. Mechanical inoculation of indicator plants to further confirm infection of field cucurbits.
4. Detection of virus particles in cucurbit sap by electron microscopy.

3.2 MATERIALS AND METHODS

3.2.1 FIELD OBSERVATION OF VIRUS-LIKE SYMPTOMS IN SIX CUCURBIT SPECIES

3.2.1.1 Experimental Site and Weather Conditions

The experiment was conducted from 9th August to 16th November, 2012 at the research fields of the Biotechnology and Nuclear Agriculture Research Institute, (BNARI) of the Ghana Atomic Energy Commission which is within the Coastal Savannah Agro-ecological zone of Ghana. During this period, the average minimum and maximum temperatures were 23.82 ^{o}C and 30.07 ^{o}C respectively, with a maximum daily temperature peak of 33.22 ^{o}C. The average precipitation for the research period was 0.909 mm but there were days of high precipitation such as 10.19 mm and 6.92 mm. The average maximum and minimum relative humidity were 91.75% and 65.68% respectively.

3.2.1.2 Field Preparation, Layout and Experimental Design

A plot of land previously cultivated to *Jatropha sp.* was ploughed, harrowed, pegged and lined in preparation for the planting of cucurbits. The plot demarcated for the cucurbit experiment was 36 m in length and 30 m in width and was bordered on the north and the east by a *Moringa olerifera* Lam. plantation and a Neem field, respectively, and on the south by the remnants of a *Jatropha sp* field. A narrow strip of weeds, largely elephant grass, separated the western border of the field from a manure processing facility. Each of the four adjacent replications of the experiment measured 16 m x14 m with a path 2.0 m wide between the replications. The treatments, comprising the six species of cucurbits namely: muskmelon (*Cucumis melo* L.), zucchini squash

(*Cucurbita pepo* L.), watermelon (*Citrullus lanatus* (Thunb.)), *egushi* (*Citrullus colocynthis* (L.) Schrad.), cucumber (*Cucumis sativus* L.) and butternut squash (*Cucurbita moschata* Duchesne) were assigned randomly to each of the replications in double rows. Within a replication, plants of the same cucurbit species were spaced at 1.0 m apart and cucurbits of different species were spaced at 2.0 m. Seeds were sown manually.

3.2.1.3 Planting Materials

Except for *egushi*, all the seeds used as planting material in this experiment were purchased at Agriseed, Aglow Agric Production Limited and Agrimat shops, all in the Greater Accra Region. *Egushi* seeds were purchased from the open market in Techiman. Table 3.1 shows the essential specifications of the planting materials.

Table 3.1: Sources and specifications of cucurbit seeds used in the study

Common Name	Botanical Name	Variety	Quality Control	% Germ	Treatment
Melon	*(Cucumis melo* L.*)*	F1 Caluil	Norms EC	N/A	Topsin
Cucumber	(*Cucumis sativus* L.)	Darina	N/A	85	N/A
Zucchini	(*Cucurbita pepo* L.)	Nadita + F1	EC Norm	85	Topsin
Squash					
Butternut Squash	(*Cucurbita moschata* Duchesne)	Waltham	IST Norm	85	N/A
Watermelon	(*Citrullus lanatus* (Thunb.))	Kaolack	Norms EC	85	Topsin
Egushi	(*Citrullus colocynthis* (L.) Schrad.)	Landrace	N/A	N/A	N/A

3.2.1.4 Cultural Practices

There was no fertilizer, herbicide or pesticide application.

3.2.1.5 Data Collection

The date of appearance of virus-like symptoms and the pattern of spread of the observed symptoms were monitored and recorded for each cucurbit species. Photographs of the symptomatic cucurbits were taken.

3.2.2 DETECTION OF FOUR CUCURBIT VIRUSES IN SIX CUCURBIT SPECIES BY DOUBLE ANTIBOBY SANWICH ELISA

3.2.2.1 Study Location

The study was conducted at the virology laboratory of the Cocoa Research Institute of Ghana (CRIG), Akim Tafo, and at the Molecular Biology laboratory of the Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC), Accra.

3.2.2.2 Preparation of Cucurbit Leaf Samples for Virus Detection

Leaf samples from all six cucurbit species were collected in the field and kept in a styrofoam box containing ice packs to keep fresh. At the Molecular Biology laboratory, GAEC, 0.5 g of leaf tissue was taken from each of the six samples and homogenized in a PBS buffer. The resulting cucurbit sap extracts were then kept in eppendorf tubes and transported to the virology laboratory of CRIG, Akim Tafo.

3.2.2.3 DAS ELISA Protocol for Plant Virus Detection in Six Cucurbit Species

Purified IgG was diluted in coating buffer at the recommended dilutions (for 100 tests 20 µl in 20 ml buffer at a recommended dilution of 1:1000; 40 µl in 20 ml buffer at a recommended dilution of 1:500). An aliquot of 200 µl of the diluted antiserum was added to each well of the ELISA microtitre plate and incubated at 37 ^{o}C for 2- 4 hours. The microtitre plates were then washed with PBS-Tween and soaked with the same PBS-Tween for 3 minutes and repeated two more times. The plates were then blotted and filled with 200 µl aliquot of the cucurbit sap extracts. Sap extracts were dispensed to duplicate wells on the microtitre plate and incubated overnight at 4 ^{o}C. After the incubation, the plates were washed three times as earlier described.

An aliquot of 200 µl diluted anti-virus conjugate was added to each well and incubated at 37 ^{o}C for 4 hours. The plates were washed again as before with PBS-Tween and filled with 200 µl aliquot of freshly prepared substrate (10 mg p-nitrophenyl phosphate in 10 ml substrate buffer). The content of the plate was incubated at room temperature for approximately three hours, or until clear colour change was observed. Plate absorbance was read at the Chemistry Department of GAEC, with ELISA Reader Multiskan Ascent version 1.25 354-90824 in a stepping mode at 405 nm filter. Virus-positive controls for CMV, PRSV-W, ZYMV and WMV were also included in the test.

3.2.2.4 Data Collection and Analyses

The extinction values were used in determining the presence or absence of CMV, PRSV-W, ZYMV and WMV in the six cucurbit species. Sample extinction values which were equal or greater than their corresponding positive control were recorded as positive reactions. Samples with extinction values that were not significantly lower than the extinction value of their corresponding positive control were also recorded as positive reactions. Sample extinction values that were significantly lower than their corresponding positive control extinction values were recorded as negative reactions.

3.2.3 MECHANICAL INOCULATION OF INDICATOR PLANTS TO TEST FOR VIRUS INFECTION OF FIELD GROWN CUCURBITS

3.2.3.1 Study Location

Sap extracts of the various cucurbits used in the mechanical inoculation were prepared at the Molecular Biology Laboratory of the Biotechnology and Nuclear Agriculture Research Institute (BNARI), GAEC.

3.2.3.2 Source of Indicator Plants and Inoculum

The indicator plants used in the mechanical inoculation were imported as seed from DSMZ laboratories, Germany. They were zucchini squash (*Cucurbita pepo* L.), muskmelon (*Cucumis melo* L.) and cucumber (*Cucumis sativus* L.). Leaves from watermelon (*Citrullus lanatus* (Thunb.)), zucchini squash (*Cucurbita pepo* L.), *egushi* (*Citrullus colocynthis* (L.) Schrad) and butternut squash (*Cucurbita moschata* Duchesne) from the mixed cucurbit field were harvested for the preparation of the sap inoculum.

3.2.3.3 Preparation of Inoculum from Sampled Cucurbit Species

Approximately 2.0 g leaves from each of the cucurbit species were weighed and put into separate mortars. An aliquot of 20 ml inoculation phosphate buffered saline (PBS) was added to the contents of each mortar and then ground into a fine homogenate. Celite 545 was added to the homogenate and mixed thoroughly. All leaves, including the cotyldenous leaves on the 17 days after planting (DAP) indicator plants were inoculated uniformly with the cucurbit sap, labelled and the leaves rinsed with tap water. Inoculations were done in the late afternoon so as to enhance recovery overnight and to reduce plant injuries. Inoculated indicator plants were kept in a netted cage inside a plant barn for observation. Table 3.2 shows the number of indicator plants inoculated.

Table 3.2: Plan for the inoculation of cucurbit indicator plants

Indicator Plant	**Source of inoculum/Number of Indicator Plants Inoculated**				
	Watermelon	***Egushi***	**Butternut**	**Squash**	**Total**
Melon	0	2	4	3	9
Squash	2	5	4	8	19
Cucumber	2	4	5	4	15
Total	**4**	**11**	**13**	**15**	**43**

3.2.3.4 Observation of Inoculated Indicator Plants

Inoculated plants were monitored periodically, first, to record the time of earliest symptom appearance, then to monitor and record the type and the progression of symptom(s). Symptoms on the indicator plants were compared with the non-inoculated indicator plants which showed none of the symptoms expressed by the inoculated indicator plants. The indicator plants were then categorized as systemic, local or asymptomatic host to the cucurbit viruses being studied.

3.2.4 EXTRACTION OF SUSPECTED *CUCUMBER MOSAIC VIRUS* FROM ZUCCHINI SQUASH PLANTS AND EXAMINATION OF CUCURBIT SAP EXTRACTS FOR VIRUS PARTICLES BY ELECTRON MICROSCOPY

3.2.4.1 Study Location

The extraction of suspected *Cucumber mosaic virus* (CMV) was done at the Virology laboratories of the Cocoa Research Institute of Ghana, Tafo, and the examination for virus particles was carried out at the Electron Microscopy Department of the Noguchi Memorial Institute of Medical Research (NMIMR), Accra.

3.2.4.2 Extraction of Suspected *Cucumber mosaic virus* from Symptomatic Leaves of Zucchini Squash

Approximately 140 g of leaves of field zucchini squash with virus-like symptoms were extracted with 100 ml 0.5 M sodium citrate buffer, pH 6.5, containing 5 mM EDTA and 0.5 % thioglycollic acid. The sap extract was emulsified with 1.0 v (w/v) of chloroform and centrifuged at 12,000 g for 10 min. The supernatant was collected and 10 % polyethylene glycol (molecular weight 6,000) was added and stirred gently for 30-40 min at 4^{o}C. The extract was centrifuged again at 12,000 g for 10 min and the formed precipitate was re-suspended in 40-50 ml of 5 mM sodium borate buffer pH 9.0 containing 0.5 mM EDTA. Approximately 2 % Triton X-100 was added to the suspension and stirred for 30 min. The suspension was clarified by centrifuging at 19,000 g for 15 min (Francki *et al*., 1979). A sample of the partially-purified preparation was tested by ELISA with CMV and *Papaya ringspot virus* (PRSV) IgG. The PRSV IgG was used to assess the purity of the partially purified CMV preparation.

3.2.4.3 Examination of Cucurbit Sap by Electron Microscopy for Virus Particles

3.2.4.3.1 Collodion and Carbon Coating of Electron Microscope Copper Grids

Three hundred mesh copper grids were placed in acetone and gently picked unto a microscope slide placed under over 40 ^{o}C hot water in a small metallic basin. After the required number of copper grids was arranged on the microscope slide, 50 µl of collodion was pipetted gently unto the surface of the hot water. The collodion was allowed to spread thinly over the surface of the hot water to cover the area of the microscope slide containing the arranged copper grids. After the collodion had formed a thin plastic cover over the hot water in the metallic basin, the water was gently drained through the basal valve from the metallic basin, permitting the plastic layer of collodion to directly cover the copper grids. The collodion coated copper grids were then kept in an oven to dry. The vacuum carbon evaporator was thoroughly cleaned and fitted with carbon rods. The microscope slide containing the collodion coated copper grids was then placed in the vacuum carbon evaporator and sputtered unto with fine carbon particles for less than a minute. The carbon coated grids were then removed from the vacuum carbon evaporator and stored for use.

3.2.4.3.2 Preparation of Cucurbit Samples for Electron Microscopy

Sap extracts from the cucurbits were centrifuged for 15 minutes at 4,300 rpm. An aliquot of 200 µl of the supernatant was pippetted into new eppendorf tubes and mixed with 20 µl each of the diluted IgG-Conjugate mixture prepared from the CMV, PRSV, *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV) IgGs and

conjugates. The anti-sap was centrifuged for 50 minutes and 20 µl of the anti-sap was collected and diluted in 400 µl PBS buffer in a dilution ratio of 1:20.

3.2.4.3.3 Application of Cucurbit Samples and Negative Stain to Carbon Coated Copper Grids

A 5 µl volume of the re-diluted anti-sap was pipetted and placed directly on the coated copper grid and allowed to settle for a minute. Excess anti-sap was drained off with strips of filter paper. A volume of 3.5 µl 1% uranyl acetate stain was pipetted unto the grid and allowed to stain for 30 seconds. Excess stain was drained with strips of filter paper. The grids were allowed to dry and then examined in a JEOL JEM 1010 Transmission Electron Microscope at NMIMR, University of Ghana at a magnification of 30,000x or 50,000x.

3.3 RESULTS

3.3.1 VIRUS-LIKE SYMPTOMS OBSERVED IN SIX CUCURBIT SPECIES IN THE FIELD

3.3.1.1 Zucchini Squash

By 28 days after planting (DAP), yellowing and stunting were observed in zucchini squash plants, particularly in replication two of the experiment, but the majority of the zucchini squash plants in replication three were relatively healthy and vigorous in growth. Leaf curling (Figure 3.1; 1b), rugosity, severe mosaic (Figure 3.1; 1e) and various forms of leaf malformation (Figure 3.1; 1c) became more evident by 63 DAP, especially in replication three which initially had healthy zucchini squash plants. By the 84 DAP almost all the zucchini squash plants in all the replications showed some form of mosaic and leaf malformation symptoms. Shoestringing (Figure 3.1; 1f), leaf roll ups and leaf curling were also observed. The leaf curling was not necessarily associated with mosaic symptoms but the leaves that showed roll up/folding symptoms also largely showed mosaic symptoms. Though the zucchini squash plants flowered and fruited, none of the developed fruits were marketable due to fruit rot, particularly in the mixed cucurbit field.

3.3.1.2 Butternut Squash

Mild, dark-green spotting, necrosis and yellowing (Figure 3.1; 2c), interveinal clearing-like symptoms were observed in butternut squash plants by 42 DAP. Fruit rot and stunting symptoms were also observed, however, the majority of the butternut squash plants were symptomless. By 111 DAP, mild to severe mosaic symptoms (Figure 3.1; 2b) were observed. Mild rugosity (Figure 3.1; 2a) and reduced leaf area were associated

with the mosaic symptoms in young and tender butternut squash leaves, particularly among leaves in the apical zone.

3.3.1.3 *Egushi*

Clear mosaic symptoms with associated reduced leaf area (Figure 3.1; 3a) in *egushi* plants started as late as 101 DAP but reduced leaf area were observed by 48 DAP. *Egushi* plants in replications three and four showed necrotic spot symptoms and later developed into shot holes by 101 DAP; otherwise the *egushi* plants were symptomless and vigorous in growth. Later in the epidemics less than a half of the *egushi* plants in replication three showed mosaic symptoms (Figure 3.1; 3a) with an associated reduced leaf area.

3.3.1.4 Cucumber

Throughout the epidemic in the mixed cucurbit field (field one) no obvious foliar virus-like symptoms were observed in the cucumber plants. However, there was significant fruit malformation ranging from reduced fruit size, fruit scabs, constricted fruits, to curved fruits (Figure 3.1; 4a). Many of the formed fruits were therefore not marketable.

3.3.1.5 Watermelon

Stunting and severe rugosity (Figure 3.1; 5b) were common among watermelon seedlings by 42 DAP. By 77 DAP, leaf curling, reduced leaf area, yellowing (Figure 3.1; 5a, 5b) and necrosis were also observed. Figure 3.1 5c shows observed fruit malformations in watermelon by 111 DAP.

3.3.1.6 Melon

Severe downward leaf-rolling and curling (Figure 3.1; 6a), rugosity, yellowing and stunting were observed. Some of the melon leaves were rough to the touch, with a sandpaper-like texture and were heavily fed on by beetles. Aphids, black ants and mealybugs (Figure 3.1; 6b) heavily infested the melon plants, particularly in replication one. There were not much of virus-like symptoms in melon plants.

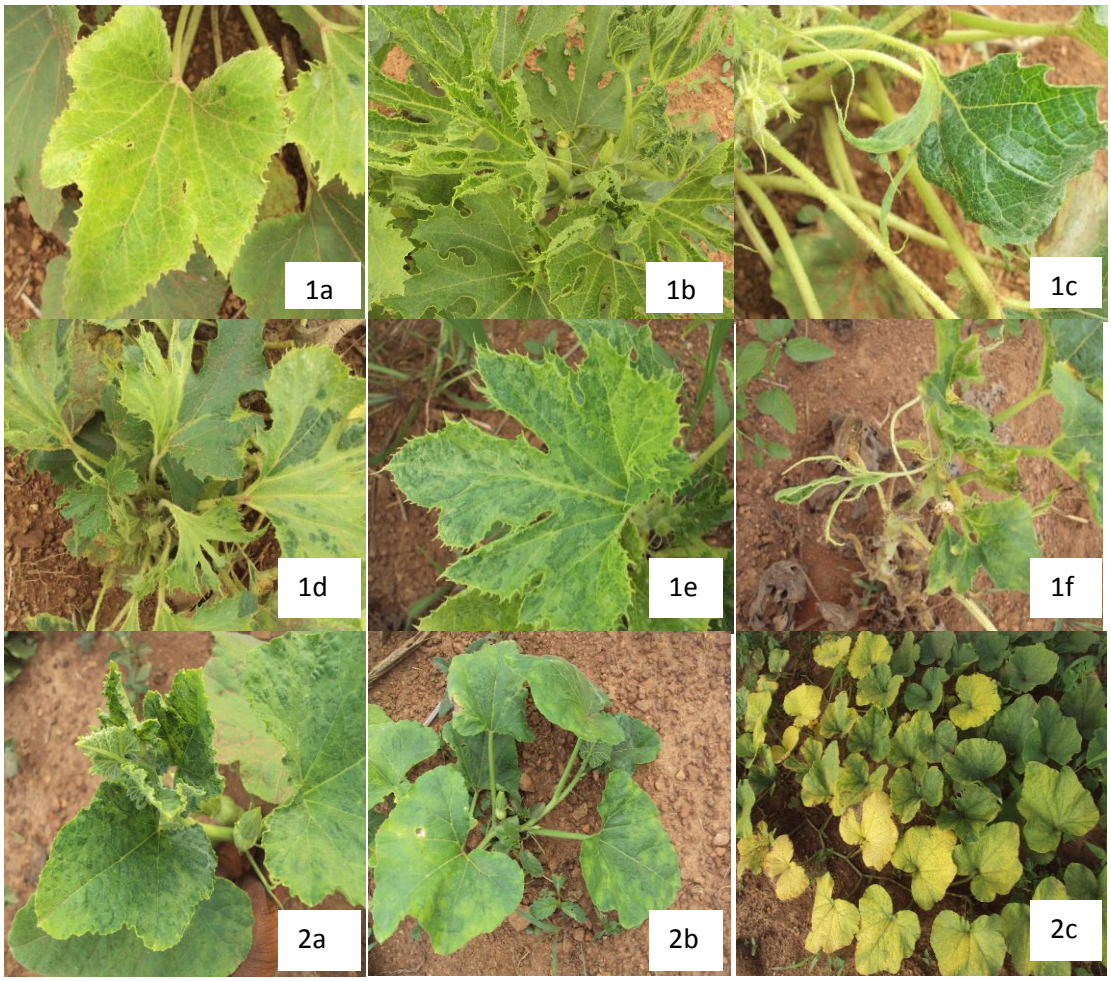

Figure 3.1 (i): Virus-like symptoms observed in two cucurbits. (1) Zucchini squash plant showing; (a) leaf chlorosis; (b) Leafroll up; (c) Partial leaf lamina malformation; (d) Partially chlorotic and healthy leaf lamina; (e) Leaf mosaic; (f) Shoestringing of leaves. (2) Butternut squash plant showing; (a) Rugosity; (b) Leaf mosaic; (c) Leaf yellowing.

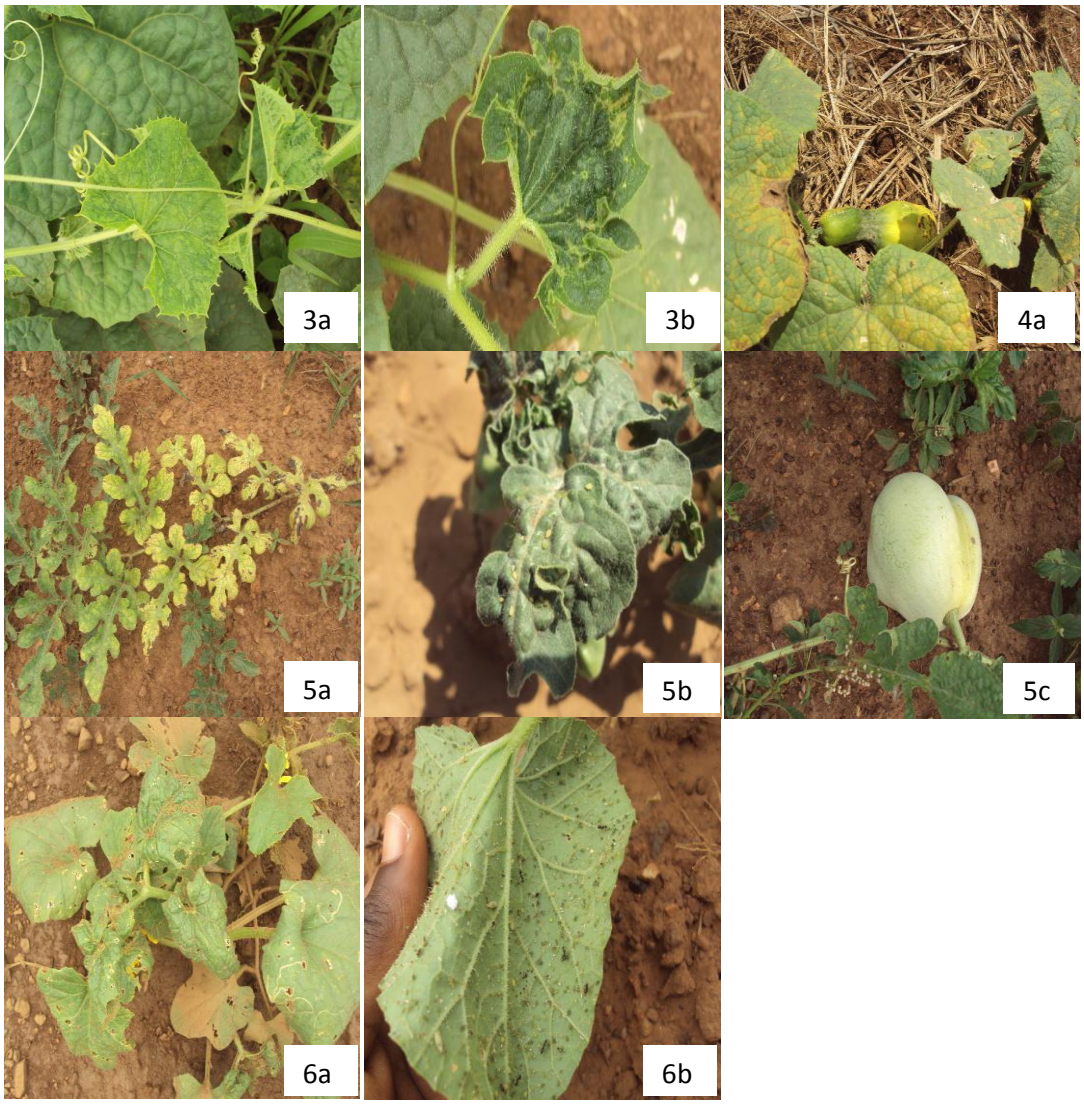

Figure 3.1(ii): Virus-like symptoms observed in four cucurbit species: (3) *Egushi* plant showing; (a) Leaf mosaic and reduced leaf area; (b) Leaf distortion. (4a): Cucumber plant showing leaf yellowing and fruit malformation. (5) Watermelon plant showing; (a) Leaf yellowing and necrosis; (5b) Leaf rugosity; (c) Fruit malformation. (6): Melon plant showing; (a) Leaf roll up (b): Aphids on a melon leaf.

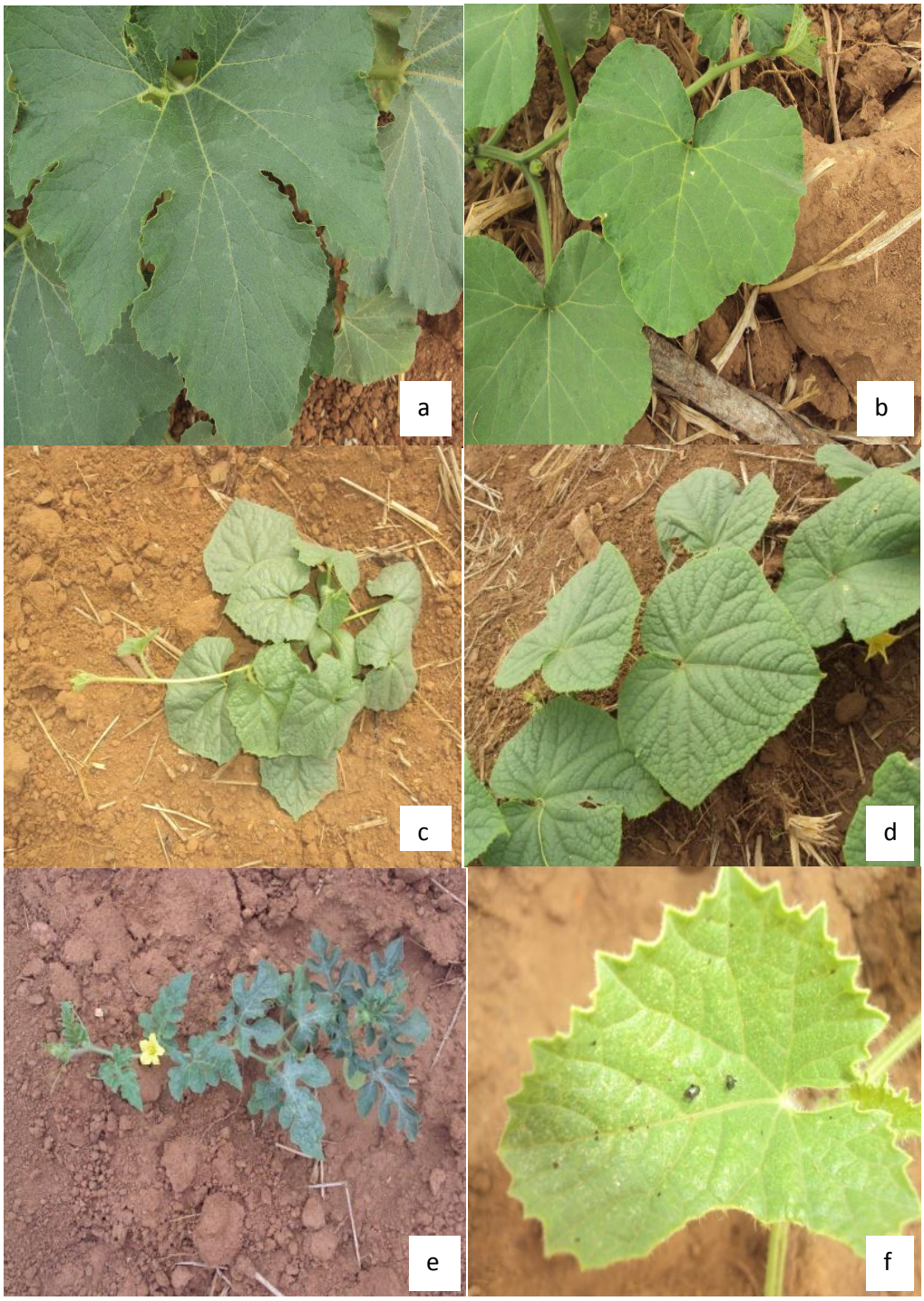

Figure 3.1(iii) Healthy (a): Zucchini squash plant (b): Butternut squash plant (c): *Egushi* plant. (d): Cucumber plant. (e): Watermelon plant. (f): Melon plant.

3.3.2 LABORATORY DETECTION OF FOUR CUCURBIT VIRUSES IN SIX CUCURBIT SPECIES

3.3.2.1 Virus Detection by ELISA

Six cucurbits, zucchini squash (*Cucurbita pepo* L.), melon (*Cucumis melo* L.), *egushi* (*Citrullus colocynthis* (L.) Schrad.), butternut squash (*Cucurbita moschata* Duchesne), cucumber (*Cucumis sativus* L.) and watermelon (*Citrullus lanatus* (Thunb.)) were tested by DAS-ELISA for *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Papaya ringspot virus* (PRSV-W) and *Cucumber mosaic virus* (CMV) which are usually reported on cucurbits. None of the six cucurbits was infected with *Watermelon mosaic virus* or *Zucchini yellow mosaic virus*. However, *egushi*, melon, zucchini squash, butternut squash and cucumber were infected with CMV in, at least, one of the four replications.

Table 3.3 shows that all the four replications of butternut squash and *egushi* were infected with *Cucumber mosaic virus*. Watermelon was not infected by CMV.

Table 3.3: Detection of *Cucumber mosaic virus* by DAS ELISA in five of the six cucurbit species

Cucurbit Species	Replication 1	Replication 2	Replication 3	Replication 4
Egushi	**0.1525 (++)**	**0.1960 (++)**	**0.1475 (++)**	**0.1390 (++)**
Melon	0.0870 (-)	0.0880 (-)	**0.2555 (++)**	**0.2270 (++)**
Watermelon	0.0850 (-)	0.1060 (-)	0.0685 (-)	N/A
Zucchini Squash	**0.2605 (++)**	**0.2845 (++)**	0.0755 (-)	**0.1115 (+)**
Butternut Squash	**0.1225 (+)**	**0.1855 (++)**	**0.2625 (++)**	**0.1295 (++)**
Cucumber	**0.1895 (++)**	**0.1625 (++)**	0.1005 (-)	**0.1425 (++)**

("++": Sample extinction value significantly higher than positive control (**0.1255**). "+": Sample extinction value not significantly lower than positive control. "–": Sample extinction value significantly lower than positive control. Absorbance was measured at 405 nm. NA = Data not available)

Papaya ringspot virus (PRSV-W) was only weakly detected in melon, zucchini squash, cucumber and butternut squash samples. PRSV-W was neither detected in *egushi* nor watermelon. Except for zucchini squash, PRSV-W infection was found in plants on only one of the four replications (Table 3.4).

Table 3.4: Detection of PRSV-W by DAS ELISA in four of the six cucurbit species

Cucurbit Species	Replication 1	Replication 2	Replication 3	Replication 4
Egushi	0.146 (-)	0.147 (-)	0.143 (-)	0.156 (-)
Melon	0.133 (-)	**0.166 (+)**	0.156 (-)	0.151 (-)
Watermelon	0.143 (-)	0.146 (-)	0.153 (-)	N/A
Zucchini Squash	**0.182 (+)**	0.174 (-)	**0.188 (+)**	0.160 (-)
Butternut Squash	**0.183 (+)**	0.155 (-)	0.145 (-)	0.126 (-)
Cucumber	0.159 (-)	0.136 (-)	0.141 (-)	**0.175 (+)**

("++": Sample extinction value significantly higher than positive control (**0.1925**). "+": Sample extinction value not significantly lower than positive control. "–": Sample extinction value significantly lower than positive control. Absorbance was measured at 405 nm. NA=Data not available)

3.3.3 MECHANICAL INOCULATION OF INDICATOR PLANTS TO VERIFY INFECTION OF FIELD GROWN CUCURBIT PLANTS

3.3.3.1 Symptom Development on Indicator Plants

Virus-like symptoms were observed at 4 days post inoculation (dpi) on zucchini squash indicator plants. Symptoms then progressed steadily throughout the assessment period. All the inocula used produced very clear virus-like symptoms on the indicator plants except the watermelon inoculum which did not produce clear virus-like symptoms. The non-inoculated control indicator plants did not show any virus-like symptoms as at the last day of assessment (36 DAP). Details of the symptoms on the indicator plants are given below:

Zucchini Squash

The first observed symptoms on zucchini squash indicator plants were tiny chlorotic spots which appeared in 4 dpi on a newly developed leaf. By 6 dpi mild leaf mottling, chlorotic spots, tiny necrotic spots, yellowing and crinkling were observed. At 14 dpi the indicator plants showed different virus-like symptoms (Figure 3.2) ranging from vein clearing, severe mosaic, chlorosis, severe leaf malformation, crinkling and reduced leaf area.

Melon

At 14 dpi interveinal chlorosis, leaf puckering and reduced leaf size were observed on seven melon indicator plants, and by 22 dpi mosaic symptoms (Figure 3.3) were observed. The symptoms observed on the melon indicator plants were mainly mosaic and reduced leaf size. There was no severe leaf malformation.

Cucumber

Leaf yellowing was observed at 7 dpi. By 14 dpi leaf mosaic was also observed. At the 22 dpi veinal and interveinal chlorosis and severe mosaic (Figure 3.4) were present.

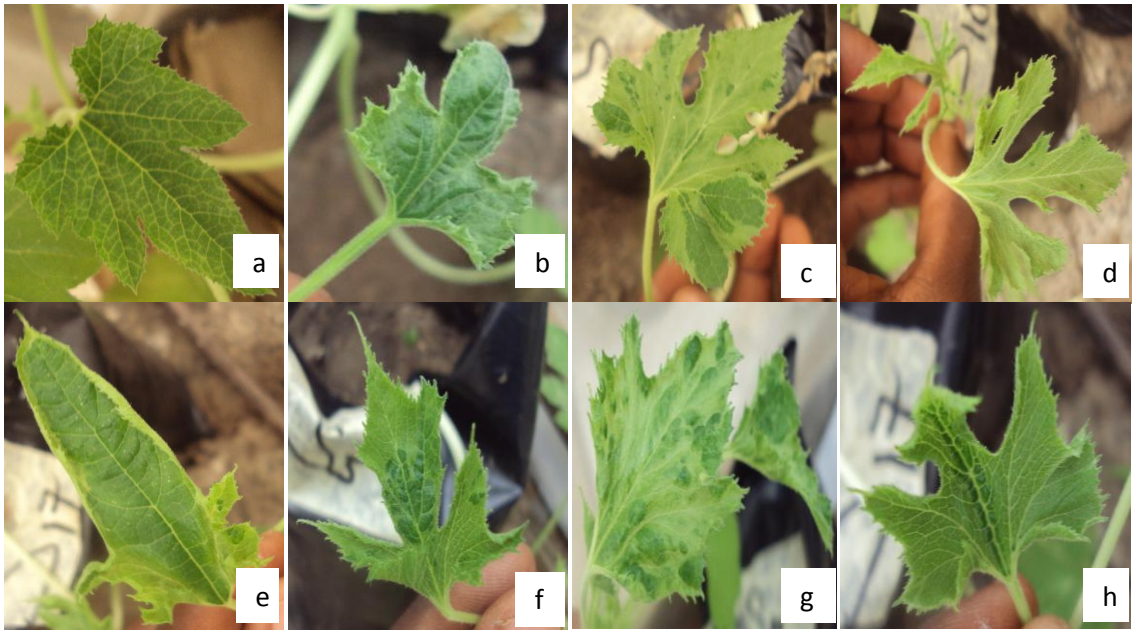

Figure 3.2: Observed symptoms on inoculated zucchini squash indicator plants showing: (a) vein clearing 8 dpi; (b) Leaf malformation 8 dpi; (c) Partly chlorotic and healthy leaf lamina 14 dpi; (d) Leaf chlorosis 14 dpi; (e) Leaf malformation 14 dpi; (f) Partly chlorotic and healthy leaf lamina 14 dpi; (g) Severe leaf mosaic, rugosity and malformation 14 dpi; (h) Reduced leaf area, chlorosis and midrib vein banding 14 dpi. (See Figure 3.5 for uninoculated plants)

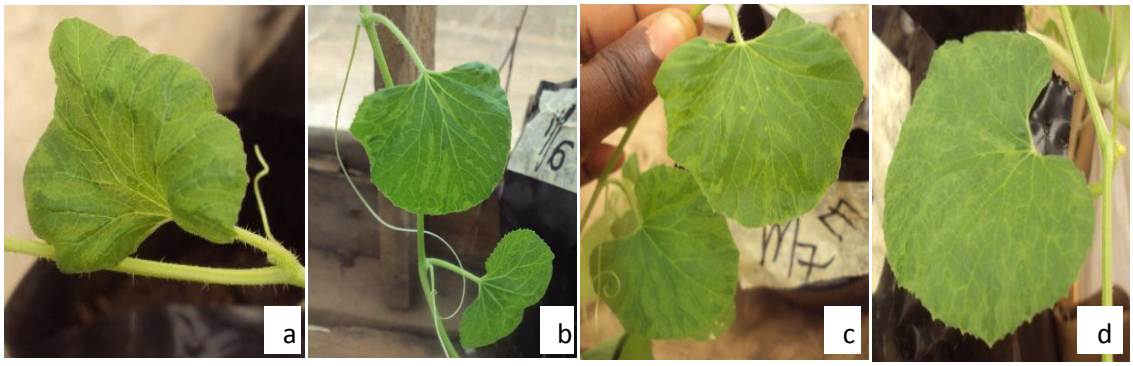

Figure 3.3: Observed symptoms on inoculated melon indicator plants showing: (a) vein banding 14 dpi; (b, c and d) leaf mosaic 14 dpi. (See Figure 3.5 for uninoculated plants)

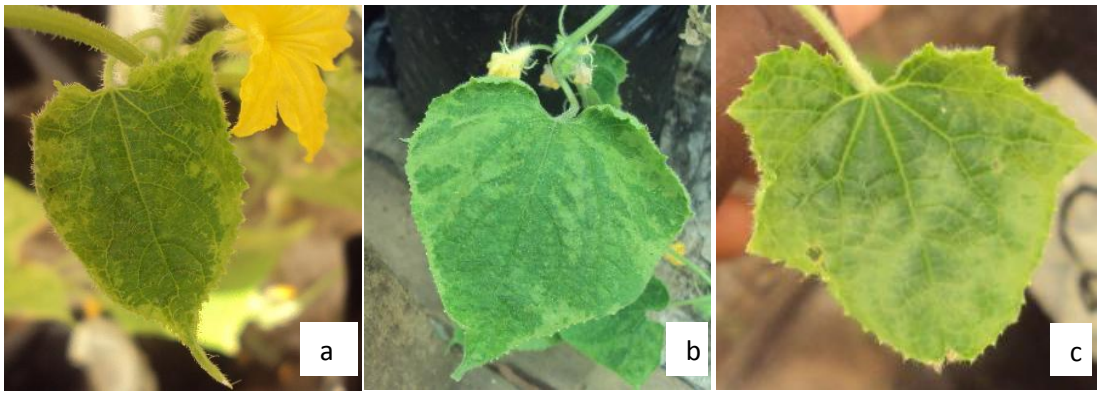

Figure 3.4: Observed symptoms on inoculated cucumber indicator plants showing: (a) leaf mosaic 36 dpi; (b) Leaf mosaic 35 dpi; (c) Leaf mosaic 14 dpi.

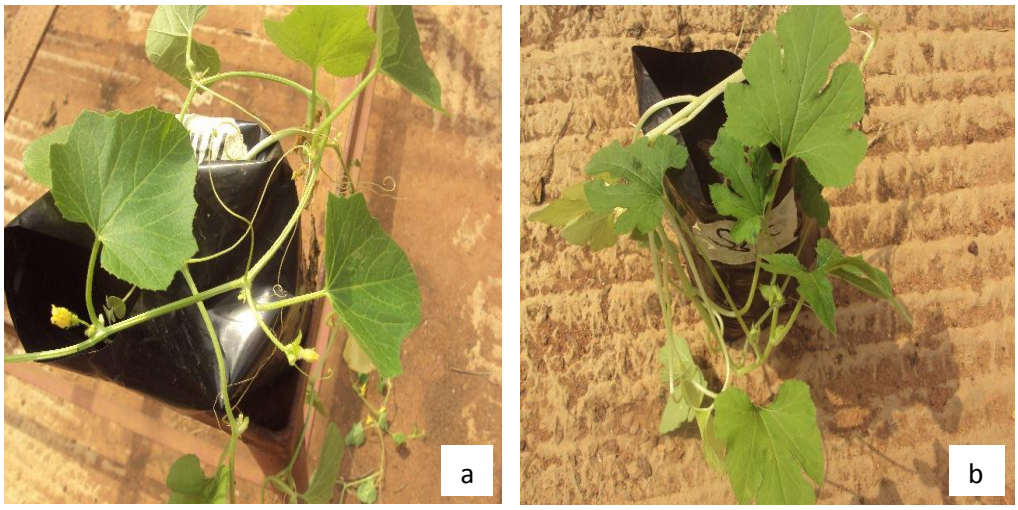

Figure 3.5: Healthy non-inoculated indicator plants: (a) non inoculated melon indicator plant 53 days after planting; (b) non inoculated zucchini squash indicator plant 36 days after planting.

3.3.4 OBSERVATION OF VARIABLE STRUCTURES IN CUCURBIT SAP BY ELECTRON MICROSCOPY

Figure 3.6 shows electron micrographs of various structures of host and non-host origin observed in cucurbit sap extracts. These structures included: (a) near spherical and (b) square crystals which usually occurred in pairs. There were also (c) agglutination of tiny spherical and (d) threadlike structures surrounded by dark areas. However, no virus-like particles were seen.

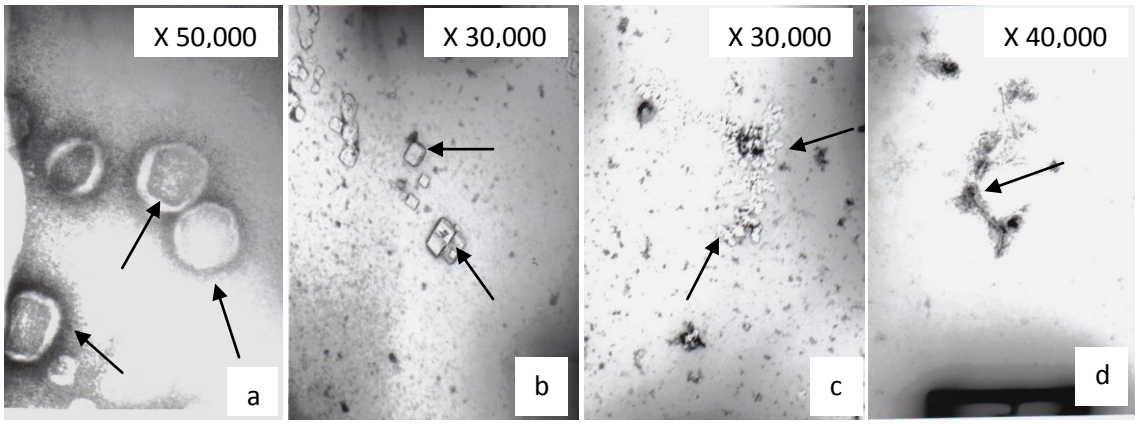

Figure 3.6: Electron micrographs of spherical, crystalline and thread-like structures observed in cucurbit sap extracts.

3.4 DISCUSSIONS

Over 42 % of all crop losses in Africa are due to plant disease. Inaccurate disease diagnosis and management lead to increased food prices, food insecurity, and in severe cases, the destruction of an entire ecosystem (Orlandini, undated). Therefore, it is essential to accurately identify the key pathogens that cause a disease of interest so as to effectively manage the disease to generate profit from farming. In this study, six cucurbit species were grown in the coastal savannah zone where they were monitored for virus and virus-like symptoms. This was successfully followed by DAS ELISA and mechanical inoculation of indicator plants to further confirm virus infection of the six cucurbits. An attempt to further confirm infection by electron microscopy was not successful.

Butternut squash (*Cucurbita moschata* Duchesne), zucchini squash (*Cucurbita pepo* L.), *egushi* (*Citrullus colocynthis* (L.) Schrad.), cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) were infected by *Cucumber mosaic virus* and *Papaya ringspot virus*. Watermelon was heavily infested by aphids but it was not infected by any of the four viruses tested. The majority of zucchini squash plants in both the mixed cucurbit field (field one) and the zucchini squash field (field three) and less than half of butternut squash and *egushi* plants in field one showed a variety of mosaic symptoms and severe forms of shoestringing as well as leaves with green blisters on largely chlorotic lamina. Such symptoms made it possible to rightly determine that these cucurbits were infected by viruses. However, in cucumber and melon plants, particularly in field one, no obvious virus-like symptoms were observed though they tested positive for *Cucumber mosaic virus* (CMV) and *papaya ringspot virus* (PRSV-W).

In the cucumber field (field two), cucumber showed mosaic symptoms only briefly; however, within a few days after a heavy rainfall, the plants appeared free of the mosaic symptoms. The lack of symptoms and the apparent recovery from symptoms in field plants indicate that the expected symptoms were masked. Masking of symptoms in virus infected plants is common and in plant species such as peach, symptoms may not appear in infected plants for three or more years of infection (Cornell, 2008). Symptom masking is one of the major phenomena that make diagnosis of virus infection by observation of field symptoms unreliable. Symptoms could be masked but the destructive effect of the virus on the economic yield of the crop may still be present. Almost all the cucumber fruits in field one were malformed and fruit setting was significantly low in melon plants. These effects could be due to the virus infection. Additionally, when sap was prepared and used in mechanically inoculating melon, zucchini squash and cucumber indicator plants, all the indicator plants showed mosaic symptoms.

Genetic and environmental factors such as the susceptibility of the various species to virus infection, drought, and temperature probably accounted for the masking of symptoms in melon and cucumber and the severity of the symptoms in zucchini squash in the field. All the field experiments were conducted in the minor growing season which is characterized by low rainfall and high ambient temperatures. The inoculated indicator plants were however, kept in a screened cage in a plant barn and supplied regularly with water. This accounted for the differences in temperature and moisture in both the field and mechanical inoculation experiments.

Generally, mosaic symptoms develop in cool weather (Huntin, 2013) and extremes of temperatures are known to mask virus symptoms. In an experiment to verify the effect of

temperature on symptom development in cocksfoot infected with a streak virus, it was observed that mosaic symptoms developed when infected plants were kept in a room with a temperature of 19 ^{o}C – 23 ^{o}C but when the symptomatic cocksfoot plants were taken into a 6 ^{o}C-10 ^{o}C cold room, there was progressive loss of symptoms, particularly in newly formed leaves (Emecz, 1963). In another study, the effect of temperature on symptom development was confirmed; Chellappan *et al.*, (2005), found that increasing the temperature from 25 ^{o}C to 30 ^{o}C enhanced the geminivirus induced gene silencing which resulted in the development of fewer symptoms in newly formed leaves irrespective of the nature of the virus, and they also observed that non-recovery type geminiviruses behaved as recovery type viruses. They proposed that at high temperatures, the ability of these viruses to induce post transcriptional gene silencing (PTGS) is enhanced through increased production of short interfering RNA (siRNA). The increased PTGS triggered by increased temperature attenuates symptoms in newly developed leaves and causes recovery from symptoms in symptomatic leaves. They suggested that increased temperatures may enhance the activities of RNA-dependent RNA polymerase (RDRP) and dicers which are directly involved in the production of siRNA from long dsRNA molecules. The cooler temperatures under the plant barn may have contributed to the development of mosaic symptoms in the melon and cucumber indicator plants which were not observed in the field, possibly due to the warmer field temperatures.

The effect of moisture on disease severity has been studied by Nita *et al.*, undated. They observed that increased moisture during anthesis in wheat resulted in significant increase in the intensity of *Fusarium* head blight disease and enhanced deoxynivalenil (DON)

production. Limited moisture or drought is known to reduce the susceptibility of plants to virus infection. Prolonged drought slows growth and in cassava, reduced plant growth rate has been associated with reduced susceptibility of plants to virus infection. This phenomenon has also been observed in many other virus-host combinations and is believed to account for the high susceptibility of young actively growing leaves as compared to older leaves to virus infection (Mandal *et al.*, 2007). The near drought conditions in field one which contained the watermelon plants may account for the non-infection of watermelon plants though they were heavily infested with aphids. Reduced growth rate of the watermelon plants due to the moisture stress could have reduced their susceptibility to any of the four tested viruses, including *Watermelon mosaic virus*. The brief appearance of mosaic symptoms in the cucumber field plants after the heavy rains may also suggest the role of drought in masking of virus symptoms. The increased moisture in the cucumber plants after the rains may have enhanced the replication and systemic translocation of the viruses in the host.

The non-detection of *Zucchini yellow mosaic virus* and *Watermelon mosaic virus* in all the six cucurbits and the low detection of *Papaya ringspot virus* could be due to the absence or low titres of these viruses in the cucurbits in the study area. A disproportionate distribution of these viruses in the host could also result in non-detection or low detection of the viruses. The titre of viruses in their hosts could vary with plant organs and structures depending on the plant species and the type of virus. Generally, *Cucumber leaf curl virus* (CuLCrV) is evenly distributed in watermelon with about 10% more probability of detection in the growing tip, but *Squash vein yellowing virus* (SqVYV) and *Papaya ringspot virus*-W (PRSV-W) are distributed

disproportionately in their hosts. There is 70% and 23% probabilities of detecting SqVYV at the base and growing tips of the watermelon vine, respectively. The distribution of PRSV-W is similar to that of SqVYV but with an increased probability of 20% higher chance of detecting the virus in the growing tip of a watermelon vine (Turechek *et al.*, 2010).

The preferential settling of insect vectors on certain cucurbit species as compared to others was also observed in the field. In field one aphids were more likely to be found on watermelon and melon plants than on the other cucurbits. *Egushi* and zucchini squash plants were also preferentially settled on by squash bugs whilst melon leaves were preferentially fed on by orange cucumber beetles. Butternut squash plants were not particularly associated with any of these insects except the larva forms of the ladybug insects. The preferential settling of insect vectors on specific crops is well known, however recent studies have shown that the preference of an insect, particularly a virus vector to a particular host, is also influenced by whether the vector is viruliferous or not. Ingwell *et al.*, (2012) observed that when an aphid successfully acquires the luteovirus, *Barley yellow dwarf virus*, it preferentially settles on non-infected wheat plants but non-infective aphids prefer to settle on infected wheat plants. They suggested that the preferential settling of non-infective aphids on infected wheat plants promoted acquisition of the virus whilst the preferential settling of infective aphids on non-infected wheat plants also promoted their transmission. They proposed the "Vector Manipulation Hypothesis" to explain the behavioral change in insect vectors that promotes virus acquisition and transmission.

Substantial capital resource is being invested in the production and marketing of butternut squash in Ghana (GhanaGov, 2012; GBN, 2011) without adequate attention to the threat of viruses, particularly *Cucumber mosaic virus* and *Papaya ringspot virus*. In this study, butternut squash was shown to be infected by the two viruses. Samples from all four replications of the butternut squash experiment were infected with *Cucumber mosaic virus* as opposed to only two replications in melon. This shows how susceptible butternut squash is to *Cucumber mosaic virus* and the urgent need to develop effective control strategies to keep the disease below economic threshold in order to realize the targeted profit from butternut squash production.

Resistance breeding and enhanced field sanitation measures could mitigate the susceptibility of butternut squash to *Cucumber mosaic virus* and *Papaya ringspot virus*. The ELISA test showed that *egushi* plants were infected by *Cucumber mosaic virus* but not by *Papaya ringspot virus*. If further studies confirm that the *egushi* plant is resistant to *Papaya ringspot virus*, then it could play a key role in the possible transfer of PRSV-W resistance genes to butternut squash plants.

3.5 CONCLUSIONS

Diagnosis of virus diseases solely by symptoms could be inaccurate; high temperatures, drought, reduced susceptibility of a plant species to virus and other factors could mask symptoms in an infected plant. In this study, observation of virus and virus-like symptoms in the field were confirmed by DAS ELISA and mechanical inoculation of indicator plants. Electron microscopy to further confirm infection could not detect virus particles in the crude leaf extracts used.

Butternut squash (*Cucurbita moschata* Duchesne), melon (*Cucumis melo* L.), zucchini squash (*Cucurbita pepo* L.) and cucumber (*Cucumis sativus* L.) were all infected by *Cucumber mosaic virus* (CMV) and *Papaya ringspot virus* (PRSV-W) in the coastal savannah zone of Ghana. *Egushi* plants were infected by CMV but not by PRSV-W. These viruses are a major threat to cucurbit production worldwide and should be effectively managed in Ghana for profitable investment in cucurbit production.

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CHAPTER FOUR

4.0 STUDY TWO: ASSESSMENT OF VIRUS DISEASE INTENSITY AND THE SPATIO-TEMPORAL DYNAMICS OF DISEASE SPREAD PATTERN IN ZUCCHINI SQUASH

4.1 INTRODUCTION

Accurate disease forecasting plays a key role in the effective management of plant diseases (Kleczkowski and Gilligan, 2007). A reliable and useful disease forecast depends on precise quantification of the essential factors that influence disease progress (Esker, *et al*, 2008). Various mathematical and geostatistical functions and techniques are used in epidemiology to describe disease spread and its interaction with the environment. Data obtained from the initial disease description are further used as the basis for forecasting and estimating important disease parameters (Workneh and Rush, 2010).

For most types of diseases, a mere observation of a diseased plant in a field may not require any significant economic investment in controlling the disease. Due to limited economic resources and the profit oriented nature of commercial crop farming, significant economic disease control measures are undertaken only after the disease reaches a specific threshold. The accurate use of mathematical models to analyze the spatial and temporal patterns of an epidemic makes it possible to forecast when these critical disease thresholds will occur. It also ensures that control measures are tailored to the dynamics of the pathogen and are applied only in critical periods of the pathogen where the most effective results are attainable.

The choice of method used in assessing disease intensities significantly affects the use and reliability of the data obtained and how the results are explained. It is therefore essential to accurately measure disease intensity. Disease intensities are usually measured as incidence, severity or as count (Madden, 2012). The choice of disease intensity measurement adopted depends on the use to which the data will be put; however, disease intensities are mostly measured as either incidence or severity. Incidence assesses disease as the proportion of plant units diseased out of an observed number of plant units. At the plant unit scale, incidence is a binary variable with only two possible values, thus, a plant is either diseased or not diseased. Severity, on the other hand, is a continuous random variable that measures the relative or absolute area of a plant that is diseased (Madden, 2012).

Field observations and mechanical inoculation experiments in study one showed that zucchini squash are readily infected by cucurbit viruses. Unlike in cucumber and melon where symptoms could be masked, symptoms in virus infected zucchini squash are readily shown. This characteristic of zucchini squash plants makes them the ideal model crops for monitoring the spatial and temporal spread patterns of virus disease in the field by observation of field symptoms.

4.1.1 Objectives of Study

The objectives of this study were;

1. To assess the incidence and severity of virus disease in zucchini squash field.

2. To assess and quantify the spatial and temporal spread patterns of virus disease in zucchini squash field through mathematical modeling and parameter estimation.

4.2 MATERIALS AND METHODS

4.2.1 Study Location and Weather Conditions

The study was conducted at the research fields of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), within the coastal savannah agro-ecological zone of Ghana. The experiment was conducted from 13th December, 2012 to 24th January, 2013. During this period, the average minimum and maximum temperatures were 24.95 ^{o}C and 33.01 ^{o}C respectively. The average precipitation was 0.087 mm but there were days of relatively high precipitations such as 2.7 mm. The average maximum and minimum relative humidity were 90.47 % and 48.53 % respectively. Average maximum wind speed and solar radiation were 3.95 m/s and 5464.55 wh/m^2 respectively.

4.2.2 Field Preparation, Experimental Layout and Design

A plot of land previously cultivated with Kersting groundnut was hand weeded, pegged and lined to map out a total field area of 24 m by 20 m. The field was divided into two sections of equal area and dimensions and planted with the same cultivar of zucchini squash. Each section had a dimension of 24 m by 9 m with a 2 m distance separating the adjacent sections of the field. Each of the two sections of the field contained approximately 250 squash plants at 1m x 1m. In all there were approximately 500 squash plants for monitoring the spread of cucurbit viruses. On the western border of the squash field was an old cucumber field; the eastern and northern borders of the squash field were cultivated with eggplants, but there were weeds as well. The southern border was largely occupied by weeds.

4.2.3 Planting Material

Certified zucchini squash seeds were procured from Agriseed Limited, Accra, Ghana.

4.2.4 Cultural Practices

All zucchini squash rows within a replication were spaced by a distance of 1.0 m and a path of 2.0 m separated the field sections. The entire field was sub-divided into quadrats to enable cluster sampling. There were, in all, 10 quadrats of equal area with approximately 50 plants in each quadrat. There was no fertilizer or pesticide application to the experimental fields.

4.2.5 Observation of virus symptoms in the zucchini squash

Every single plant in the field was examined for symptom development and insect pest attack on weekly basis throughout the experimental period. Virus-like symptoms were recorded together with the time of symptom occurrence and the location of the symptomatic plant.

4.2.6 Estimation of Disease Incidence and Severity in the zucchini squash

Based on the observed symptoms in the mixed cucurbit field established earlier, a key was developed to assess the incidence and severity of symptoms in the squash field. For disease incidence assessment, plants that showed no symptoms at the time of disease assessment were scored 0 and recorded as healthy while plants that showed symptoms were scored as 1. For symptom severity, symptomatic plants were scored on a scale of 1 to 5. Disease incidence and severity were calculated, first, for each quadrat and then for all the quadrats by the following mathematical relations;

Disease Incidence = Number of symptomatic plants in a quadrat/field

Total Number of plants in the quadrat/field

Disease Severity Factor = $\sum {a1}/{b} + {a2}/{b} \ldots + {an}/{b}$

where a is the severity score for plant 1, 2…n (range: 0-5) and b is the upper limit for disease severity score (5).

Incidence and severity data were compared at the quadrat level as well as at the replication level and also over time to test for the level of variability and aggregation in the disease incidence and severity data. Figure 4.1 displays the range of symptoms developed in the zucchini squash plants used as a pictorial scale.

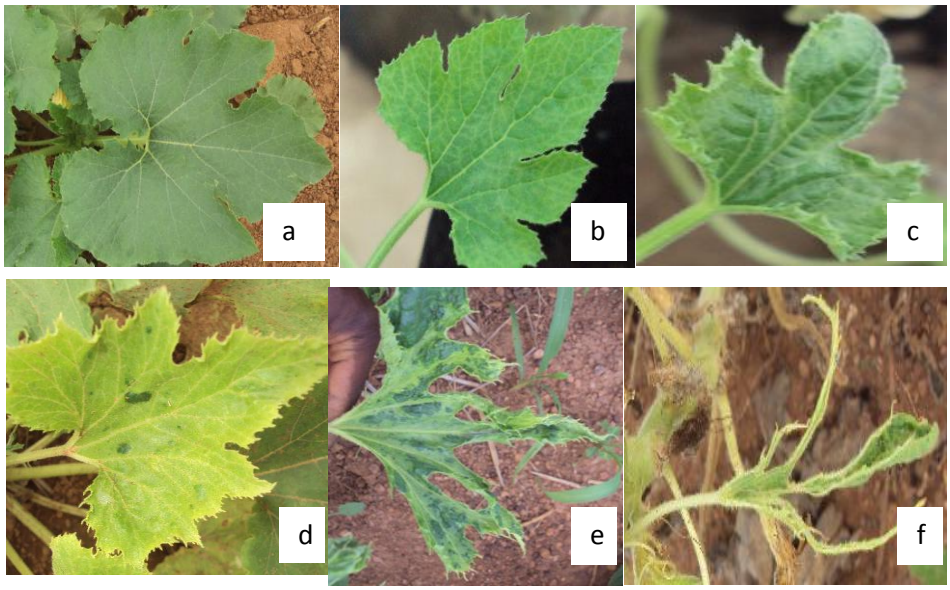

Figure 4.1: Symptoms developed in zucchini squash: (a) Healthy plant (Score: 0); (b) symptomatic plant (Score 1); (c) symptomatic plant (Score 2); (d) symptomatic plant (Score: 3); (e) Symptomatic plant (Score: 4); (f) symptomatic plant (Score: 5)

Table 4.1 gives the description of the symptom severity scale. Disease severity scores were based on Figure 4.1.

Table 4.1: Description of symptoms in zucchini squash (scored on a scale of 0-5).

Score	Description	Reference to Figure 4.1
0	Healthy plant, No obvious symptoms	a
1	Mild leaf mottling or vein clearing, no leaf malformation	b
2	Mainly non-chlorotic tissues but mild leaf malformation	c
3	Chlorotic leaf lamina with dispersed blister-like areas of non-chlorotic tissue. Leaves may be malformed	d
4	Severe leaf malformation, chlorotic leaf lamina with dispersed blister-like areas of non-chlorotic tissue	e
5	Leaves reduced to chlorotic string-like structures surrounding major veins	f

2.4.7 Analysis of the Temporal Spread Pattern of Disease Intensities

Incidence data for all the ten quadrats of the squash field were compiled and uploaded into the database of Microsoft Excel, 2007 and Minitab version 15 Statistical software. A scatterplot was generated for each quadrat and then for the average of all quadrats, with disease intensities assigned as the dependent variable and the time of assessment as the independent variable. First, linear regression functions were fitted to describe the variability observed in the raw disease intensity data. Disease intensity data were then transformed by the linearized forms of the non-linear exponential, logistic, gompertz and monomolecular mechanistic models as shown in Table 4.2. The model that gave the highest coefficient of determination (r^2), was selected as the most appropriate model for

describing the temporal pattern of the epidemic in a particular quadrat or the entire field (Madden, 2012). Parameters such as the y-intercept and the slope which represent primary infection and apparent infection (secondary transmission) rates of the epidemic respectively were estimated from the selected models for each quadrat and for the entire field. Tables of estimated parameters from the selected empirical and mechanistic models were generated for all the quadrats and the entire field.

Table 4.2: Linearized forms of the non-linear mechanistic growth models used in transforming the disease intensity (y)

Model	Linearized Form
Exponential	Exponit $(y*) = \ln y$
Monomolecular	Monopit $(y*) = \ln(\frac{1}{1-y})$
Logistic	Logit $(y*) = \ln y/(1-y)$
Gompertz	Gompit $(y*) = -\ln(-\ln(y))$

(Source: Madden, 2012; Madden, 1980)

4.2.8 Analysis of the Spatial Spread Pattern of Disease Intensities

In order to analyze the spatial spread pattern of disease symptoms, semivariogram and inverse distance weighting interpolation (IDW) methods were used. Toward these, data for all symptomatic plants were weighted by assigning a temporal value to each squash plant which corresponded with the time of symptom appearance on the particular plant in relation to the last day of disease assessment. Plants that became symptomatic earlier in the epidemic received higher weightings compared with plants that became infected around the period of final field disease assessment. Each of the plants in the field was also assigned X and Y coordinate values in a Cartesian X-Y plane based on the specific

location of the plant in the field. The 2-dimentional spatial and temporal weighting data were then uploaded unto GS+ version 9 Gama Design Software for semivariogram and interpolation analyses.

The variogram was estimated with an active lag distance of 12.78 and a lag class distance interval of 1.00. Both isotropic and anistropic variograms were estimated. For the anistropic variogram, the anistropic axis orientation was first at 0^{o} north of the principal axis with an offset tolerance of 180^{o}, then further variograms with anistropic axis orientations of 45^{o}, 90^{o} and 135^{o} north of the principal axis with an offset tolerance of 90^{o} were also performed to verify if the disease field was anistropic. The autofitted models were then selected as the appropriate models describing the semivariogram. Parameters such as nugget, sill and range of spatial dependence were estimated based on the selected semivariogram models. Interpolations were performed using the inverse distance weighting method (Roumagnac *et al.*, 2004). A map of the disease field was then generated that showed the forecasted estimated disease status of all squash plants on the field including all asymptomatic plants as well.

4.3 RESULTS

4.3.1 Assessment of Virus Disease Incidence in Zucchini Squash

Virus symptoms were first recorded in quadrat five (Q5) as early as the first week of disease assessment; incidence then progressed at a relatively slower rate until the third week when incidence in all the quadrats collectively increased exponentially. At the quadrat level the highest mean incidence of 0.0933 (s.e. 0.0441) was recorded in quadrat one (Q1), the least mean incidence of 0.00779 (s.e. 0.00510) was recorded in Q4. In quadrat four, non-zero incidence value was recorded only in the 28 days after planting (DAP) and 49 DAP. By the last week of incidence assessment, Q1 had the highest incidence of 0.09327 and the least incidence of 0.007787 was recorded in Q4. Figure 4.2 shows the proportions of disease incidence in the zucchini squash field.

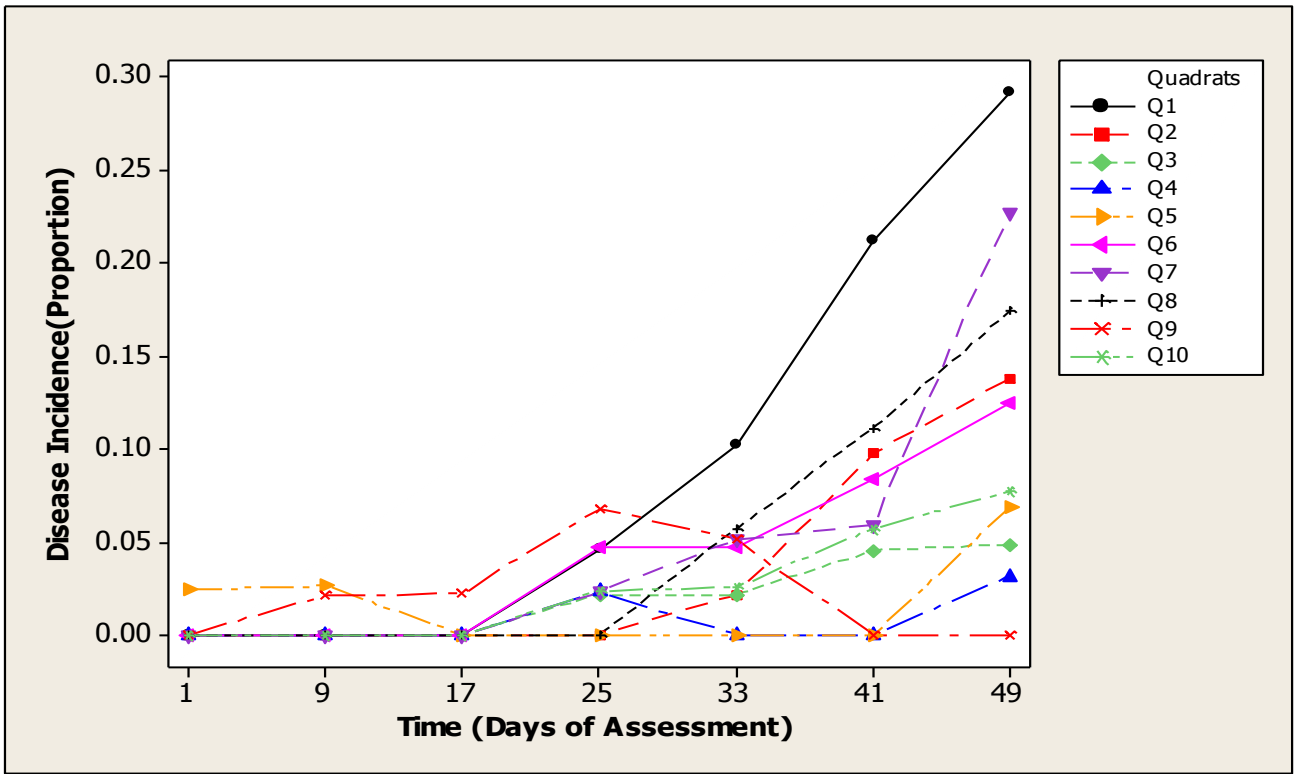

Figure 4.2: Proportions of disease incidence in the zucchini squash field

4.3.2 Assessment of Virus Disease Severity in Zucchini Squash

The severity factor started at a value of 0.02 by 7 DAP. At 14 DAP, the severity factor had risen to 0.04 and by 21 DAP the severity factor was still 0.04. From 28 DAP to 49 DAP the factor increased exponentially from 0.23 to 1.09. At the quadrat level, the severity factor started at 0.2 at 7 DAP in Q5. By 49 DAP, the severity factor had reached a high value of 1.9 in Q1 and its lowest value of 0 in Q9. The highest mean severity factor was recorded in Q1 at a value of 0.686 (s.e. 0.307) and the least mean severity factor was 0.1429 (s.e. 0.0841) in both quadrats four and five each. Figure 4.3 shows the severity of symptoms observed in all ten quadrats of the squash field.

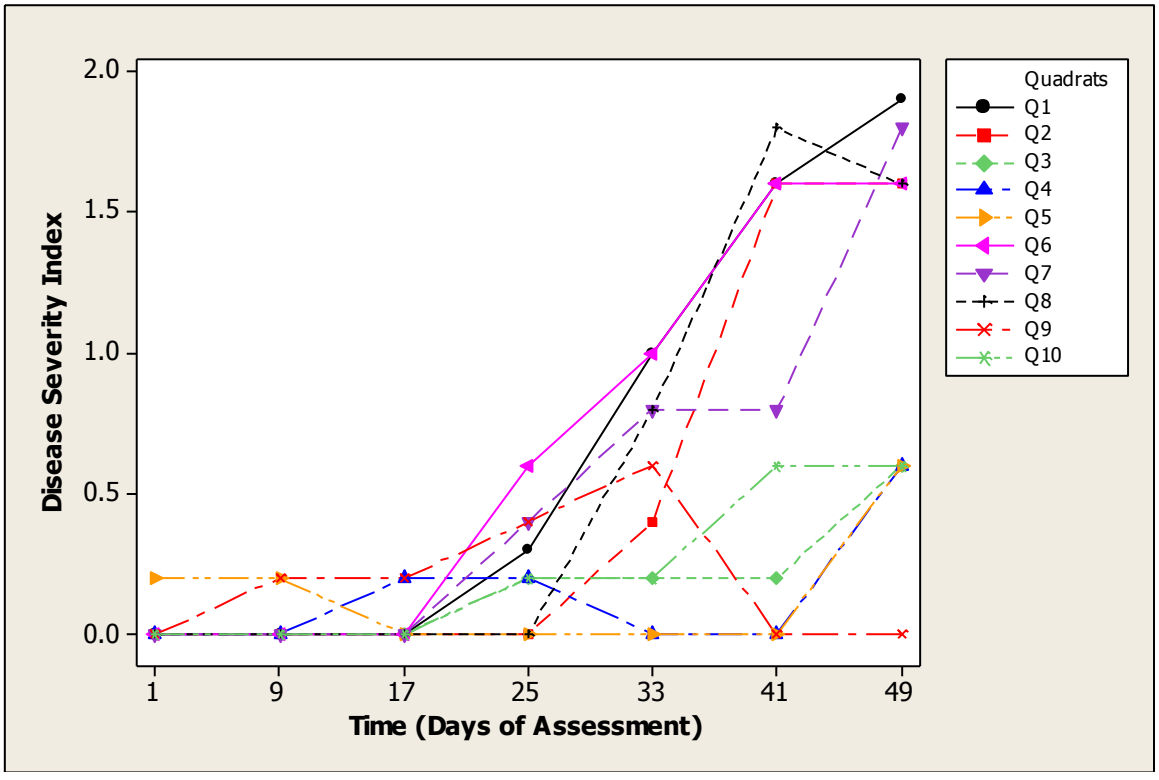

Figure 4.3: Levels of disease severity observed in ten quadrats of a zucchini squash field.

4.3.3 Description of the Statistical Structure of Disease Intensity Data and the Estimation of Parameters from Fitted Empirical and Mechanistic Models

4.3.3.1 Statistical Structure of the Disease Intensity Data and Estimated Parameters from Fitted Linear Regression Functions

Disease incidence data from eight of the ten quadrats were normally distributed based on test of Standard Kurtosis and Standard Skewness. The two quadrats with asymmetrically distributed data were quadrats five and seven (Q5 and Q7), with Standard Kurtosis and Standard Skewness values of 2.58 and 2.17 respectively, which were outside the expected range of -2 to +2 for a normally distributed data. Among the quadrats that were well fitted to the empirical models, Q1 had the highest secondary infection rate (SIR) of 0.050 (p-value 0.003). The quadrat with the lowest SIR was Q10, with SIR of 0.00222 (p-value, 0.002). Quadrats five and nine were not fitted by the empirical model at 95% confidence interval. Quadrat four was not significantly described by the fitted model at 95% confident interval (p-value, 0.215).

Disease severity data from seven of the ten quadrats were normally distributed. Severity data from quadrats three, four and five (Q3, Q4 and Q5) however, were asymmetrically distributed with Standard Kurtosis values of 2.71, 3.23 and 3.23 respectively. Among the quadrats that were significantly fitted to the empirical model, Q1 had the highest SIR of 0.3537 (p value, 0.002) whilst Q10 had the least SIR of 0.1143 (p value, 0.004). Data from Q5 and Q9 could not be described by the empirical model. Severity data from Q4 was poorly described by the empirical model with a non significant p value of 0.196.

In all, many of the temporal patterns of disease spread in terms of incidence and severity both at the quadrat level and the entire field level were significantly fitted to the

empirical model with high r^2 and low p-values. Figure 4.4 shows a graph of the average disease incidence and severity for all the ten quadrats.

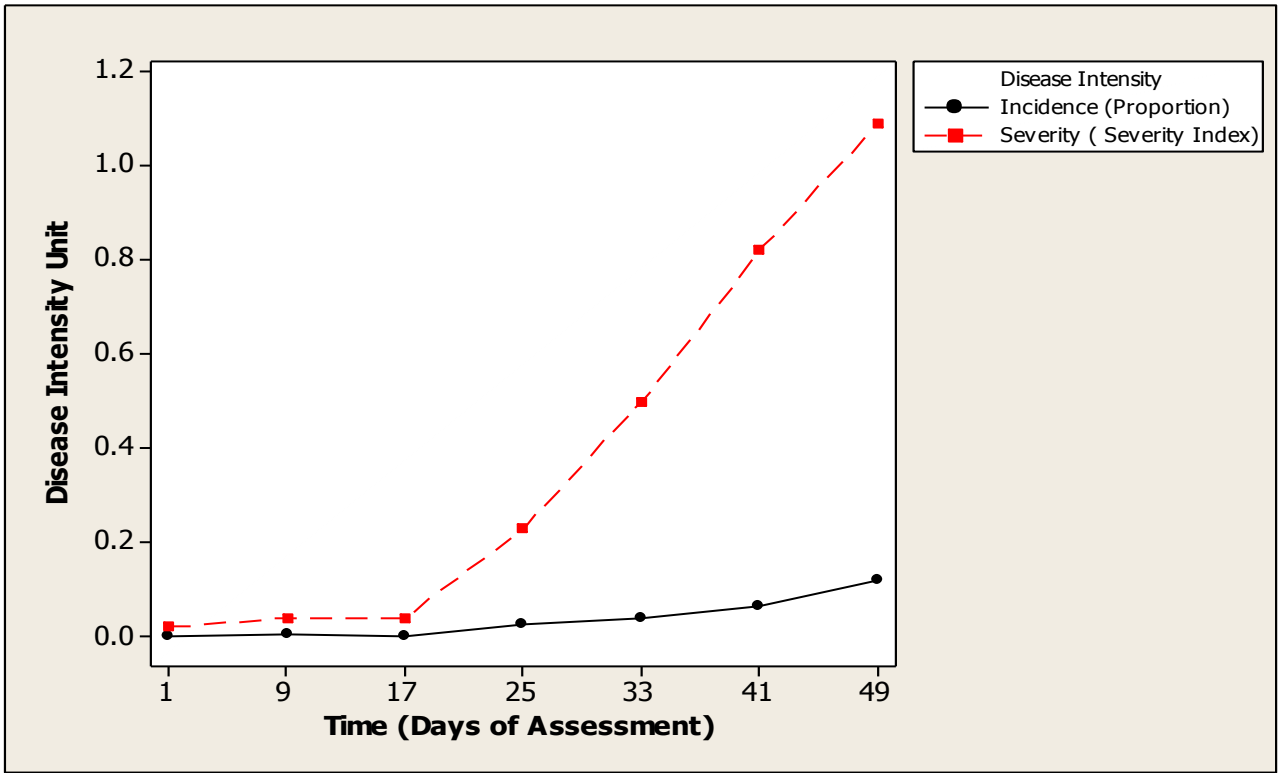

Figure 4.4: Average disease intensities observed in the entire zucchini squash field

Table 4.3 shows the list of essential disease parameters estimated from the fitted empirical models. In all ten quadrats, the apparent infection rates for the disease incidence was smaller compared with that of disease severity.

Table 4.3 Estimated parameters from the fitted empirical model (Linear Regression Function)

	Incidence						Severity					
Q	a	SE (a)	b	SE(b)	p	r^{2*}	a	SE(a)	b	SE (b)	p	r^{2*}
1	-0.107		0.050	0.00913	0.003	82.9	-0.7286	0.2586	0.3537	0.0578	0.002	85.8
2	-0.053	0.0277	0.0225	0.00619	0.015	67.1	-0.6857	0.3603	0.3000	0.0806	0.014	68.2
3	-0.0173	0.0064	0.0092	0.00143	0.001	87.2	-0.1714	0.0990	0.0857	0.02213	0.012	-
4	-0.0056	0.0106	0.0033	0.00236	0.215	14.5	-0.0857	0.1714	0.0571	0.03833	0.196	16.9
5	0.0055	0.0232	0.0029	0.00518	0.6	0.0	0.0286	0.1979	0.0286	0.0443	0.547	0.0
6	-0.0408	0.0148	0.0210	0.00331	0.001	86.8	-0.6000	0.2055	0.3214	0.0460	0.001	88.9
7	-0.0700	0.0445	0.0304	0.00995	0.028	58.1	-0.5714	0.2519	0.2786	0.0563	0.004	79.6
8	-0.0656	0.0298	0.0286	0.00666	0.008	74.4	-0.7143	0.3580	0.3286	0.0801	0.009	72.5
9	0.0252	0.0251	-0.0005	0.00562	0.935	0.0	0.2000	0.2138	0.0000	0.04781	1.0	0.0
10	-0.0267	0.0099	0.0132	0.00222	0.002	85.2	-0.2286	0.0990	0.1143	0.02213	0.004	81.1

(a is the y-intercept; b is the apparent infection rate and r* is the adjusted coefficient of determination)

4.3.3.2 Fitting of Disease Intensity Data to Linearized Forms of Non-Linear Mechanistic Models and Estimation of Essential Disease Parameters

For disease incidence, all the fitted mechanistic models gave a better fit compared to the untransformed data except data fitted to monomolecular model. The gompertz model explained best, the observed variability in the incidence data with 90.86 % agreement between field-observed and model-predicted disease incidence data. This model estimated the apparent infection rate to be 0.026 per day and predicted the doubling time (t_D) with reference to the last day of disease assessment to be 15 days. The exponential and logistic models predicted t_D of 28 days and 8 days respectively. In terms of disease severity, the exponential model best described the temporal pattern of the disease spread with a high coefficient of determination (r^2) of 94.38 %. Only the logistic and exponential model transformations described the pattern of disease severity better than the untransformed data. Table 4.4 shows the parameters estimated from the selected mechanistic models used in fitting disease intensity data.

Table 4.4: Estimated disease parameters from the zucchini squash virus epidemics from fitted mechanistic models

	Incidence			Severity		
Model	r^2	a	b	r^2	a	b
Exponential	87.75	-7.0027	0.1008	94.38	-4.9079	0.1139
Logistics	88.14	-7.0407	0.1035	93.75	-5.5111	0.1573
Monomolecular	81.96	-38.013	2.7274	73.22	-0.6032	0.0435
Gompertz	90.86	-2.0877	0.026	87.13	-2.3803	0.0828
Untransformed	82.98	-0.0356	0.0026	89.26	-0.3557	0.0267

Figure 4.5 and Figure 4.6 show the spread of the model transformed disease incidence and severity data points, respectively, around the fitted regression functions. None of the primary infection parameters were positive values for both disease incidence and severity.

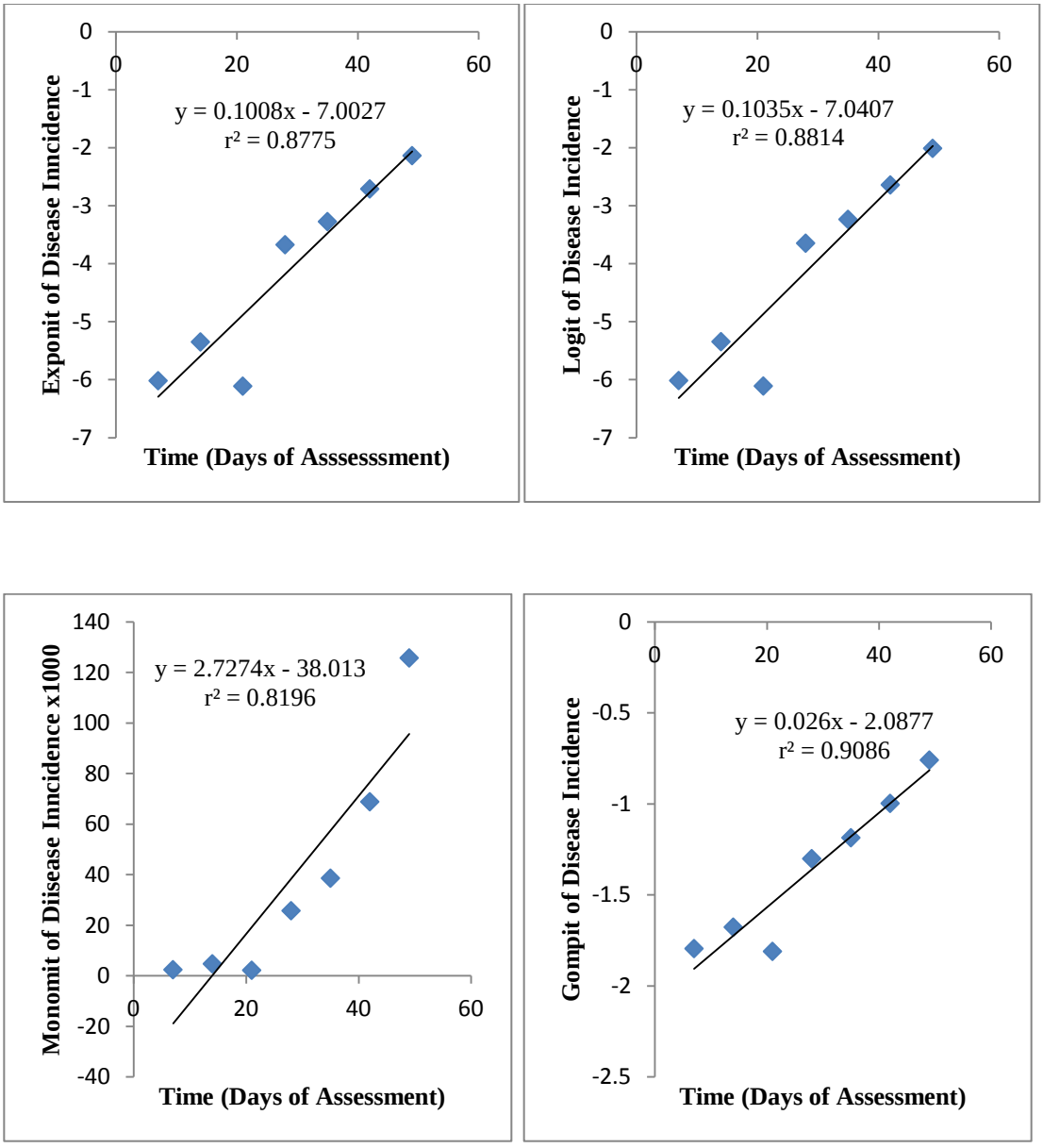

Figure 4.5: Scatterplots of mechanistic model transformed disease incidence data from zucchini squash epidemics.

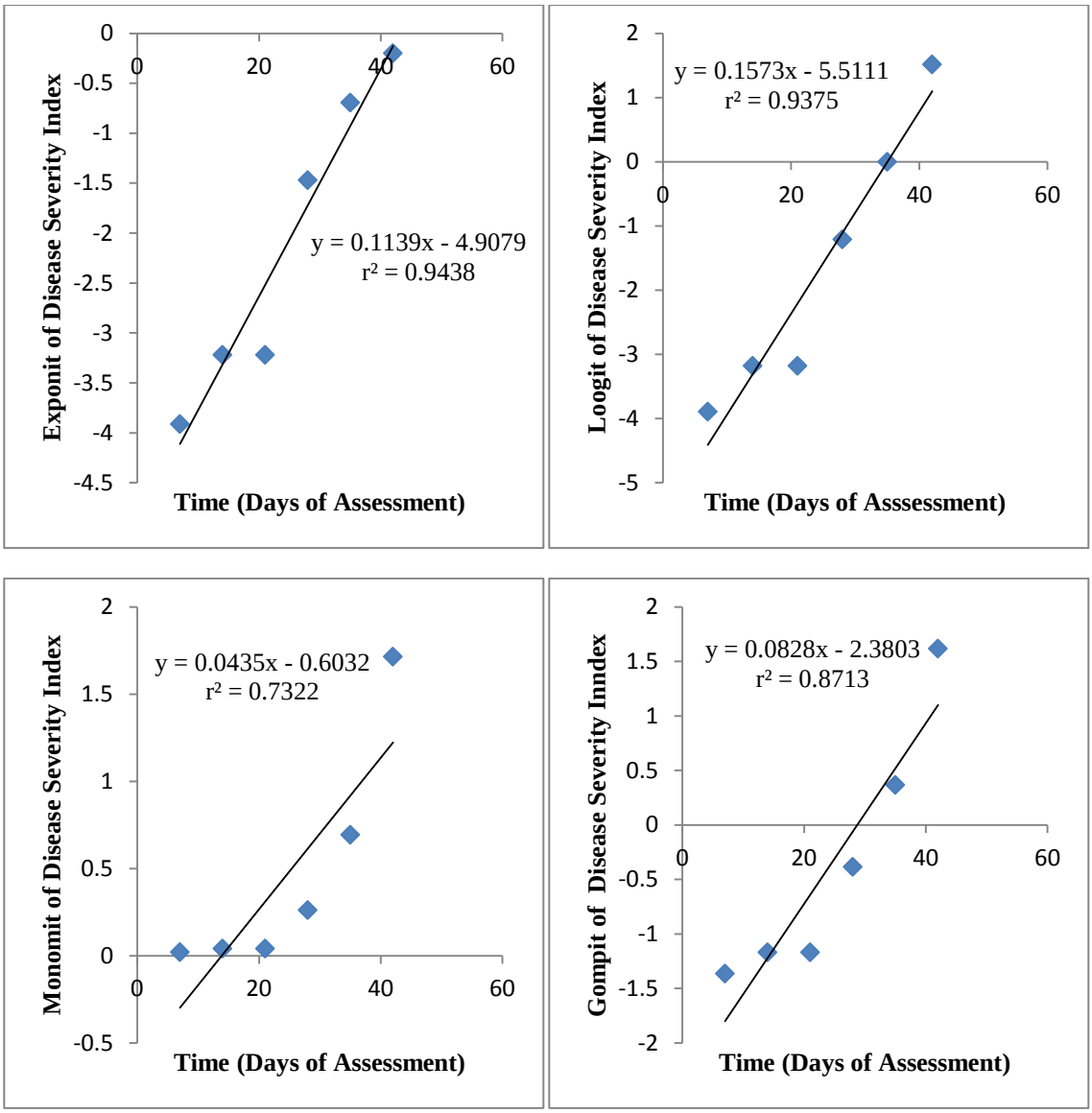

Figure 4.6: Scatterplots of mechanistic model transformed disease severity data from the zucchini squash field.

4.3.4 Analyses of Spatial Spread Pattern and Estimation of Parameters from a Virus Epidemic in Zucchini Squash Field

4.3.4.1 Semivariance Analyses

When an autocorrelation was performed with an angle of 0^o north relative to the principal axis at a tolerance limit of 180^o, a Gaussian model was fitted to the data with a residual sum of square (RSS) value of 1.12. The nugget, which is a representation of the localized discontinuity, was estimated to be 0.07300. The range of spatial dependence (RSD) and the sill were also estimated to be 0.63 m and 1.91400, respectively. When the angle relative to the principal axis was tilted from the initial 0^o to 90^o, the nugget variance increased to 1.58300 but the sill and the RSD remained unchanged. Anistropic variograms were also performed which were fitted to linear models. However, the associated r^2 were very low with relatively high RSS indicating a poor fit to data. Other estimated parameters are shown in Table 4.5.

Table 4.5: List of selected variogram models and associated parameter estimates

Variogram	Sill (C_0 +C)	Nugget (C)	RSD (A^o)	Model	Residual SS
Isotropic	1.91400	0.07300	0.63	Gaussian	1.12
Anistropic (0^o- 135^o)	4.98364	1.72500	*A Major* 120.40 *A Minor* 119.70	Linear	4.09
Anistropic (0^o- 135^o)	4.01796	1.58300	*A Major* 57.75 *A Minor* 57.74	Linear	4.95
Isotropic	1.91400	1.58300	0.63	Gaussian	1.12

4.3.4.2 Inverse Distance Weighting Interpolation and IDW Map

The inverse distance weighting (IDW) interpolation model forecasted large clusters of possible infection around the primary infection foci which largely occurred in quadrats five and nine. The majority of the forecasted clusters of higher incidences were toward the eastward and south-eastern-ward direction of the disease field, particularly around the borders of these directions. The forecasted clusters of least incidences were also estimated to be in the mid-portion of the field, with few patches on the middle section of the western border. There were also isolated clusters of smaller sized incidence peaks toward the western section of the squash field. Figure 4.7a and Figure 4.7b show 2-dimensional and 3-dimensional maps of the virus epidemic, respectively, in the zucchini squash field generated through IDW interpolation.

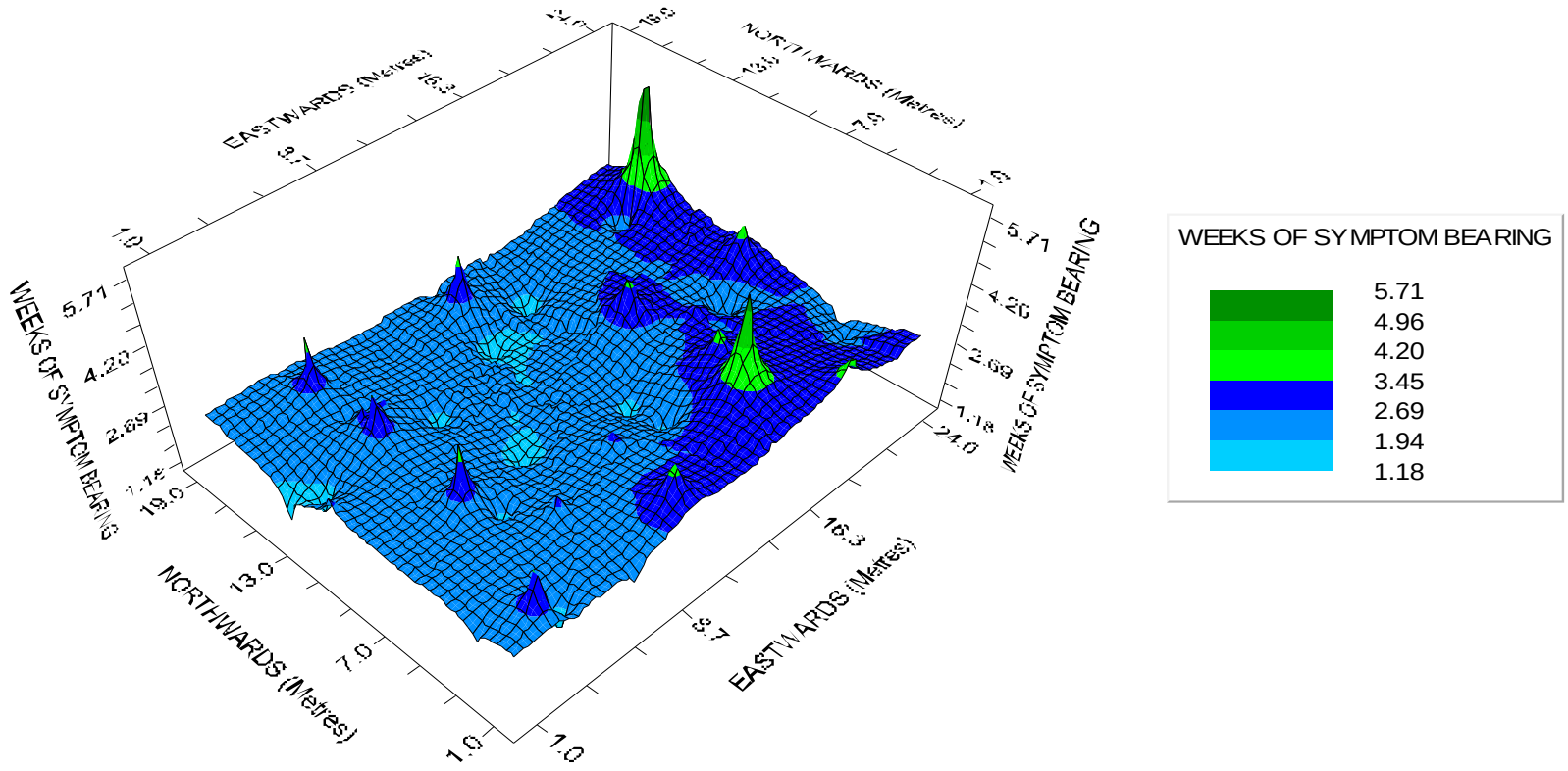

Figure 4.7a: 3-D Inverse Distance Weighting (IDW) Map with peaks and colour-coded regions corresponding to the level of disease intensity across the zucchini squash field.

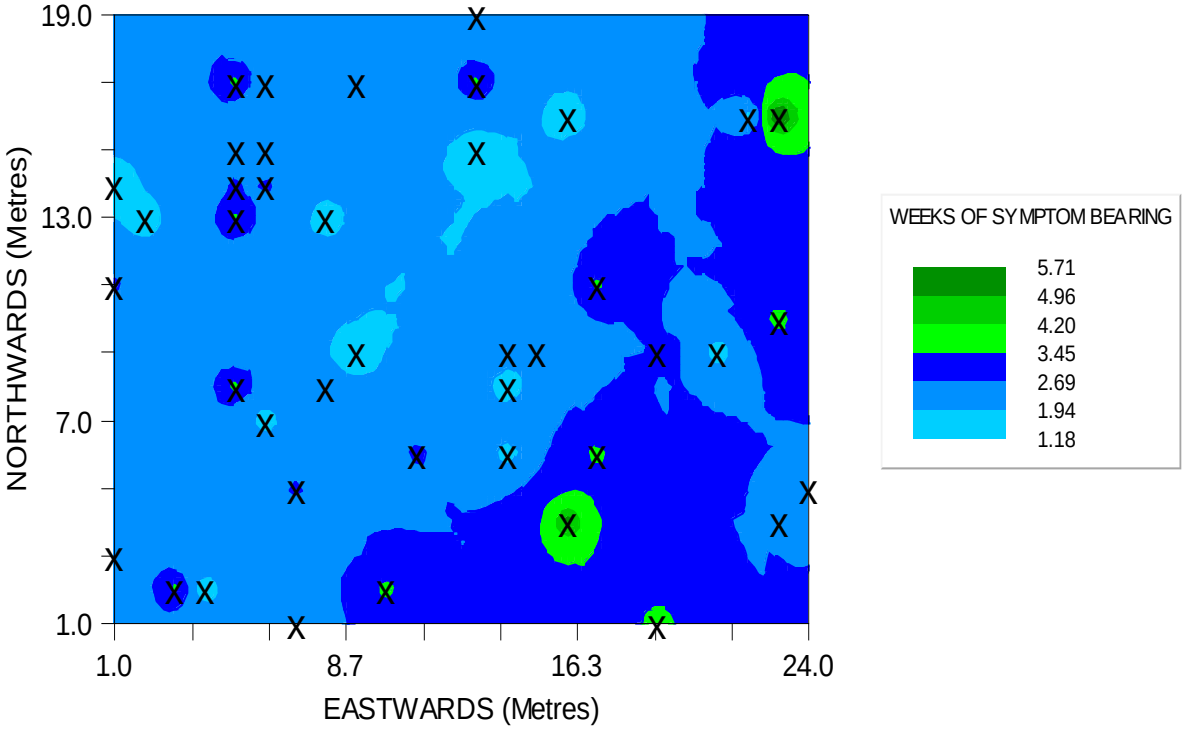

Figure 4.7b: 2-D Inverse Distance Weighting (IDW) Map showing the locations of symptomatic zucchini squash plants across the zucchini squash field.

4.4 DISCUSSIONS

4.4.1 Temporal Spread Pattern of Virus Disease in Zucchini Squash Field

A major objective in any disease management strategy is to contain disease intensities below economic injury thresholds so as to reduce the effect of disease on yield to ensure profit maximization. Hence, quantitative knowledge of the various factors that contribute to disease progress is essential in developing cost effective approaches to disease management (Nutter, 2007). Foremost of the quantification techniques applied in plant epidemiology is the use of mathematical models (Teng, 1985). Mathematical and statistical models provide the bases and theories essential in quantifying disease progress curves in order to bring out relations which may otherwise not be obvious from the raw disease intensity data (Madden and Campbell, 1990). There are many mathematical models that could be used in quantifying and analyzing disease progress curves but non-linear forms of asymptotic mechanistic models such as exponential model, logistic model, Weibull model, Richards model and Gompertz model are generally used in analyzing polycyclic epidemics such as virus pathosystems in cucurbits (Fekedulegn *et al.*, 1999). Linearized forms of the non-linear gompertz, monomolecular, logistic and exponential mechanistic models were used in transforming disease incidence and severity data from the squash field.

In this study, it was observed that in terms of incidence, the exponential model, logistic model and the gompertz model provided a superior description of the field data as compared to the untransformed data. The monomolecular model provided the least fit to the incidence data and this is consistent with the fact that the monomolecular model by Van Der Planks is best used in describing monocyclic epidemics and not polycyclic

epidemics (Van Der Planks, 1963). Among all the four models, the gompertz model provided the best fit to the incidence data, with a coefficient of determination of 90.86 %. Earlier workers (Vuori *et al.*, 2006; FAO, 2013 and Berger, 1981) have indicated the satisfactory use of the gompertz model in describing disease progress curves. The gompertz model is noted for its consistent and stable parameter estimates in pathosystems of oats, potato, celery, bean, rice and several others (Mohapatra *et al.*, 2008). The gompertz curve exhibits a decline in apparent infection rate as disease intensity increases and this is actually observed in practice (Fleming, 1983). As more and more plants become infected, the probability of new infections decline due to decline in the proportion of healthy uninfected plants in the field. This accounts for the decline in the apparent infection rate as the disease intensity increases. The gompertz model is also known to be ideal for modeling time series events such as disease progress where the initial growth and growth toward the end of the time series is slowest with an exponential growth in the mid-season of the growth (Wikipedia, 2013b). This nature of disease progress was also observed in the zucchini squash field. Disease incidence started at a very low value of 0.24 % and by 21 DAP, due to loss of few symptomatic plants, incidence had reduced to 0.22 %; however, incidence increased exponentially from 28 DAP at a value of 2.5 % to 11.82 % by 49 DAP. As at the last week of disease assessment, incidence was still increasing at an increasing rate. This shows that though the squash plants had fruited and were mature for harvest, the disease progress was still in its mid-season.

The Gomp-Exp law was postulated to explain the exponential growth observed in growth curves that are best described by gompertz functions and hence supposed to

follow a gompertz law and not an exponential law. The Gomp-Exp law attributes the exponential growth to limited competition as a result of large host population size. However, as the disease reaches a critical size threshold, the effect of the competition on growth becomes more evident, pushing back the growth to follow the Gompertz law (Wikipedia, 2013b). As at the last week of assessment, the critical size threshold had not been reached since incidence was only 11.82 %. This explains why the rate of apparent infection was still increasing at an increasing rate from the 42 DAP to the 49 DAP.

Supposing, to gain profit from the zucchini squash production, the disease incidence should not exceed 25 % by the 60 DAP: the gompertz function estimated a maximum seed incidence threshold of 2.7 % that could be tolerated so as not to exceed the 25 % incidence threshold by the 60 DAP. Now, supposing a farmer obtains cucurbit seeds whose incidence rate is 50 % less of the 2.7 % threshold: the farmer could save 7 days more, prolonging the time needed for the 25 % incidence to occur to 67 DAP instead of the 60 DAP. The logistic model and the exponential model were also consistent in forecasting the 25 % incidence threshold to occur in 67 DAP, instead of the initial 60 DAP after the sanitation measure was not adopted.

Though the gompertz model was more superior in fitting the incidence data, in terms of estimating time saved due to the sanitation measure, all three mechanistic models were consistent in their estimation. Again the logistic model, the gompertz model and the exponential model were used in estimating the time required for the last week assessment incidence of 11.82 % to double. According to the gompertz function, the incidence observed at 49 DAP would have doubled by 64 DAP. The exponential model predicted a much longer doubling time of 77 DAP but the logistic model predicted the

incidence observed in the 49 DAP to double in just 57 DAP which is only eight days from the last day of assessment. Both the logistic and exponential models predicted an average apparent infection rate (r) that was four times larger than that of the gompertz model. The apparent infection rate, also termed as the secondary infection rate is the parameter that quantifies all the factors that influence disease increase with the exception of initial disease. The estimated r value of 0.026 from the gompertz model is the sum expression of the magnitude of the susceptibility of the zucchini squashes to virus infection, the effects of environmental factors such as temperature and precipitation, the efficiency of the insect vectors and other factors on the progress of the epidemic in a physical quantifiable term. The effects of seed infection rate, quarantine measures, crop rotation schemes and vertical resistance genes in the host plants are not quantified by the secondary infection rate but rather by the initial disease parameter (y_o) (Madden, 2012).

4.4.2 Spatial Spread Pattern of Virus Disease in Zucchini Squash Field

The spatial pattern of disease progress can reveal much detail on the nature of spread of the epidemic and assist in planning effective measures to manage the disease (Sparks *et al.*, 2008). Spatial Autocorrelations and Inverse Distance Weighting methods are a few of the mathematical functions that permit the investigation of spatial dependence of disease intensities among neighbouring plant units. Based on the residual sum of squares, the semivariogram produced from the disease incidence data fitted relatively well to the Gaussian model compared to the Spherical and Linear models. Estimated parameters from the Gaussian model and a subsequent inverse distance weighting form

of mapping showed that there was significant variability in disease incidence and the level of aggregation of diseased plants across the quadrats.

The almost 22 fold increase in the nugget variance due to the tilting of the principal axis through an angle of 90^o confirms the variability of aggregation of diseased plants across the field. The nugget variance, also termed as localized discontinuity measures the extent of aggregation of diseased plants across the field in a given perspective. The lower the nugget variance, the more diseased plants are aggregated. The initial 0.0730 nugget variance showed that from that 0^o perspective of the field, more of the diseased plants were closer to each other compared to the level of aggregation from the 90^o perspective of the field. This phenomenon is termed as anistropy; thus, on the same field, the level of closeness of diseased zucchini squash plants was not uniform but rather there existed a directional difference in the level of spatial associations among the diseased plants. If the level of aggregation of diseased plants were uniform across the field, then the nugget variance would have remained constant irrespective of the angle of rotation of the principal axis.

The range of spatial dependency (RSD) which was estimated to be 0.63 m measures the maximum distance at which diseased plants show spatial dependence. The low RSD indicated the magnitude of closeness of diseased zucchini squash plants in the field. The variability of incidence and level of aggregation of diseased squash plants were also visualized and confirmed from the IDW map. The IDW analysis and its subsequent map forecasted two major primary disease foci which were all toward the eastern section of the field and in accordance with the theories underlying IDW mapping, the model predicted clusters of possible high levels of incidence around the two major disease foci.

However, a significant number of the symptomatic zucchini squash plants did not fall within the model predicted areas of high incidences. The early loss of one of the initially diseased plants in the eastern border region of the field, the close proximity of the already established cucumber field to the western border of the zucchini squash field and the behavioral pattern of insect vectors in the field are proposed to account for the observed variability in incidence and the level of disease aggregation as well as the significant departure of symptomatic plants from the model predicted zones of high incidence. Viruses depend on vectors for their transmission from plant to plant due to their inability to break the physical barriers, such as cuticle and the cellulose cell walls of their hosts (Esker *et al.*, 2007). Therefore, the behaviour of insect vectors particularly, in relation to virus transmission significantly influences the pattern of observed disease in a field (Jerger *et al.*, 2004). The estimated closeness of symptomatic plants with an RSD of just 0.63 m may suggest that insect vectors fed from clusters of plants that were within a smaller circumference, moving from one plant to another nearby plant rather than long distances of flight within an infinitesimally small amount of time in search of hosts.

If such proposed pattern of insect feeding is true, then the loss of one of the initially diseased plants in the eastern border region of the field and the not-more-than-2 metre proximity of the cucumber field to the western border of the squash field may explain why a significant number of plants did not fall within the model predicted zones of high incidence in the eastern part of the field and the high level of aggregation of symptomatic plants in the western part of the field. Particularly in the upper section of the eastern part of the field, since the initially infected plant no longer did exist from the

early part of the epidemic, it could not have served as a source of inoculum to infect zucchini squash plants in its vicinity of which the model had predicted higher incidence. In the western section of the field, the close proximity of the cucumber plants may have served as ready source of inoculum to infect nearby squash plants in the western section of the field.

4.5 CONCLUSIONS

Quantification of disease progress curves through mathematical modeling revealed more insight into the virus epidemic which could otherwise not have been possible. The description of the temporal pattern of disease spread by the gompertz model enabled a more detailed explanation of the nature of the epidemic in the perspective of time, based on the sophisticated but biologically applicable gompertz model and the Gomp-Exp law. The spatial model and Inverse Distance Weighting maps also forecasted areas, particularly toward the eastern border of the field, where disease intensities were expected to be high. A significant number of symptomatic plants did not fall within these critical zones; this could have been as a result of the proximity of the already established cucumber field to the western border of the zucchini squash field.

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CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Cucumber (*Cucumis sativus* L.), *egushi* (*Citrullus colocynthis* (L.) Schrad.), zucchini squash (*Cucurbita pepo* L.) and butternut squash (*Cucurbita moschata* Duchesne) were infected by both *Cucumber mosaic virus* and *Papaya ringspot virus*. *Egushi* was infected by *Cucumber mosaic virus* but not *Papaya ringspot virus*. Symptoms observed in the field and subsequent mechanical inoculation and electron microscopy studies confirmed that these cucurbits cultivated in the coastal savannah zone of Ghana were infected by viruses. *Watermelon mosaic virus* and *Zucchini yellow mosaic virus* could be absent in the experimental area since none of the six cucurbits were infected by these viruses.

The temporal pattern of disease incidence in the zucchini field followed the gompertz function with an average apparent infection rate of 0.026 per day. Though all the mechanistic models provided a good fit to the incidence data, the fit to gompertz model resulted in the highest coefficient of determination. The temporal pattern of disease severity was best described by the exponential model with a coefficient of determination of 94.38%. The severity of disease in the zucchini squash field was increasing at 0.114 per day. As at 49 DAP incidence and severity factor had reached 11.82% and 1.09 respectively.

The spatial pattern of disease spread in the zucchini squash field was best described by the Gaussian model. The model predicted an extent of aggregation of diseased zucchini squash plants with an RSD of 0.63 m. However, the level of aggregation of diseased

plants was not uniform across the field but there existed directional differences in the extent of spatial association among diseased zucchini squash plants across the field.

5.2 RECOMMENDATIONS

The following recommendations are made for consideration;

1. Since cucurbits are susceptible to over 35 viruses, the extent of the virus threat to cucurbit production in Ghana could not be based on the detection of only four (i.e. 11.00%) of these viruses. There is the need to detect the presence of other cucurbit viruses in Ghana particularly those known to occur in Africa such as *Telfairia mosaic virus* which occurs in cucurbits in Nigeria.
2. The absence of *Watermelon mosaic virus* and *Zucchini yellow mosaic virus* in the coastal savannah zone of Ghana could be verified with PCR or other advanced virus detection methods such as DNA microarray.
3. The potential of *egushi* to be used in resistance breeding programmes in transferring PRSV-W resistant genes to other susceptible cucurbits, particularly, butternut squash should be explored.
4. Crops, weeds and other wild plants in the study location should be tested for cucurbit susceptible plant viruses.
5. The use of mathematical and statistical models in describing and comparing disease progress curves should be encouraged to enhance effective disease management.