FARMERS' PERCEPTIONS, OCCURRENCE AND TRANSMISSION OF MOROCCAN WATERMELON MOSAIC VIRUS INFECTING CUCURBITS IN SELECTED COUNTIES IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis is dedicated to my beloved mother, my entire family, and friends for their unwavering support, love, and encouragement throughout my entire study period away from home

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ABBREVIATIONS AND ACRONYMS

bp	Base Pairs
CABYV	Cucurbit Aphid-Borne Yellow Virus
cDNA	Complementary Deoxyribonucleic Acid
CMV	Cucumber Mosaic Virus
CRD	Completely Randomized Design
CTAB	Cetyltrimethylammonium Bromide
DNA	Deoxyribonucleic Acid
MWMV	Moroccan Watermelon Mosaic Virus
PABYV	Pepo Aphid Borne Yellows Virus
PCR	Polymerase Chain Reaction
PRSV	Papaya Ringspot Virus
PVY	Potato Virus Y
TSV	Tobacco Streak Virus
WMV	Watermelon Mosaic Virus
ZYMV	Zucchini Yellow Mosaic Virus

ABSTRACT

Cucurbits are important food crops worldwide due to their nutritional benefits and also contribution to the national economies. In Kenya, cucurbits are mainly produced by small scale farmers for their leaves, immature and mature fruits and seeds. However, the production in the country is constrained by viral disease infections including Moroccan watermelon mosaic virus (MWMV), a potyvirus in the family potyviridae. The virus is transmitted by several species of aphids in a non-persistent manner and also by mechanical means.The cucurbit crops can be infected by the virus at every stage of growth, with early infection resulting in total yield loss if no management measures are taken.The virus was recently reported in Kenya in two counties infecting pumpkin (*Cucurbita pepo* L). Since the virus is known to infect several cucurbit species, determination of farmers perceptions, and distribution of MWMV in the major cucurbits growing counties in the country as well as transmission to different host species are important in the development of effective management strategies. A survey was conducted in the year 2024, covering a total of 229 cucurbit fields in seven sub-counties within three counties namely; Machakos County (Matungulu and Kangundo sub-counties) Kirinyaga County (Kirinyaga East, Kirinyaga West, and Mwea East sub-counties) and Embu County (Embu West and Mbeere North sub-counties). To understand farmers' knowledge, perception and management strategies of the MWMVdisease, a total of 229 farmers'were randomly identified in seven sub-counties and a semi-structured questionnaire was administered through face to face interviews.The disease severity in the farmers' fields was determined using a scale of 0 to 4. To determine the disease incidence, ten (10) plants per field were visually inspected for symptoms that are associated with MWMV disease. Moroccan watermelon mosaic virus distribution was determined by testing samples collected from the field using polymerase chain reaction (PCR)-based approach. To determine the influence of host plant species on aphid's transmission efficiency of the virus, a greenhouse trial was carried out at the Jomo Kenyatta University of Agriculture and Technology (JKUAT). Aphids (*Myzus persicae*) were obtained from the University farm and maintained on common beans (*Phaseolus vulgaris*) plants established in the pots and placed in cages in an insect freee screenhouse. Two infected and natural host plants; pumpkin and papaya were used as source of MWMV inoculum for subsequent aphid transmissions. The experiment was laid out in a completely randomized design (CRD) with three treatments replicated (3) times. To transmit the virus, aphids were straved for one hour before feeding on the infected plants for 30 seconds to acquire the virus, The viruliferous aphids were then transferred to the healthy plants and allowed to feed for 40 seconds before spraying them with an insecticide. The plants were observed for MWMV disease symptoms development daily after transmission for a period of six weeks. Data for farmers perceptions were subjected to analysis of variance at $p < 0.05$ using (SPSS) and correlation analysis was done to determine the relationship between knowledge, perception and management of MWMV. Incidence and severity data was analysed through descriptive statistics, percentages and mean values using (SPSS). Influence of host plant efficiency data was analysed by determining the virus presence based on the amplification of the right size of fragment as observed in the agarose gel electrophoresis. The findings indicate that the farmers were aware of MWMV disease symptoms presence in their farms ranging from deformation, dark green blisters, and mosaic. Majority of the respondents 49.5% perceived the symptoms to be caused by weather changes, while 16.1% associated the symptoms with pest infestation while 11.1% did not know the cause of the symptoms in their farms. As a management measure, majority of the famers 85.5% used chemical control.The highest disease severity of 3.8 was recoded in Matungulu sub-county in Machakos county and the lowest 1.6 in Embu West sub-county in Embu county. Kangundo and Kirinyaga East sub-counties recorded the highest disease incidence of 100% while Kirinyaga West reported the lowest disease incidence of 62.5%. The number of positive samples was 12,8,4,and 3obtained from Matungulu, Embu West, Mwea East, and Mbeere North subcounties respectively. All the samples tested positive were from pumpkin crop species. Moroccan watermelon mosaic virus aphid transmission occurred from pumpkin to watermelon, Zucchini, and butter nut and not in papaya. Conversely, the virus was transmitted by aphids from papaya to zucchini crop species and not in the other cucurbit species. Although the virus was earlier reported in two counties, the presence of the virus to these counties under current study indicates that the virus is present in other counties and could probably spread to other parts of the country leading to damage cucurbit crops. Further, pumpkin crop species could be playing an important role in the epidemiology of the disease. Limited knowledge and management of MWMVsuggest farmers' training is necessary for improved disease diagnostics and management. Futher, a detailed survey across the country is recommended to determine the extent of the virus spread.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Cucurbit was named by Liberty Hyde Bailey which mean cultivated species of the family Cucurbitaceae and is now refers to any species within the family since the 20th Century (Wehhner et al., 2020). Globally, cucrbits are the second most widely cultivated vegetables after tomatoes (McCreight et al., 2013) and they are mainly cultivated in the tropical and subtropical climates worldwide (Weng and Sun, 2012), playing an significant rloe in human welfare (Weng and Sun, 2012). Cucurbits belong to the family *Cucurbitaceae* and are very significant crops globally

(Moya et al., 2023; Lecoq et al., 2014 Weng &Sun, 2012). The family *Cucurbitaceae*, order Cucurbitales with eight familie, 15 tribs, and has about 130 genera and 825 species (Renner et al., 2007; Schaefer & Renner, 2011). The most important species in this family include cucumber (*Cucumis sativus L*.), watermelon (*Citrullus lanatus* L.), Courgettes / Summer squash (*Cucurbita pepo L*.), muskmelon (*Cucumis melo L*.) and pumpkin/Squash (*Cucurbita moschata*),cucumber (*Cucumis sativus* L.),bottle gourd (*Langenaria siceraria* (Molina Standl),bitter gourd (*Mormodica charantia* L.),wax gourd (*Benincasa hispida)*. Different cucurbits are produced for different purposes. Some are produced for their fruits (watermelon, cucumber, courgette (zucchini) and others are produced for their fruits and leaves e.g. pumpkin (Stevenson et al., 2007).

Cucurbits species are source of leaves, fruits, and seeds, for consumption, as well as rich in phytonutrients and antioxidants (Salehi et al., 2019). For instance, pumpkin seeds are rich in macro elements including magnesium, phosphorus and calcium (Stevenson et al., 2007). Butternut squash is rich in phytonutrients, low in fat, and dietary fiber for the maintenance of the human body. Butternut also provides significant amounts of potassium and vitamin B6 important minerals for the proper functioning of the nervous and immune systems (Elinge et al., 2012; Badr et al., 2011; Rakcejeva et al., 2011). Watermelon is a good source of water in the diets of human beings and is used in making jams jellies, and flavoring drinks (Jensen, 2012; Kim et al., 2012) and rank in the top ten (10) fruits crops produced globally (FAOSTAT, 2020) due to its fresh nutritious fruit (Sultana and Ashraf, 2019; Vinhas et al., 2021).The consumption of cucumber reduces the risks of breast, uterus, ovary and prostate cancer (Thoennissen et al., 2009). Further, cucumbers produce fatty acids, vitamins, and amino acids that can be added at various stages of biomedicine to improve human health (Kim et al., 2012). Pumpkin is rich in carbohydrates, mineral salts, and vitamins (Chen and Huang, 2019).

Cucurbits have diverse food uses depending on the crop and the stage of maturity. They can be used in making alcohol, sweets, beverages, cake. The fruits of cucurbits species can be eaten when raw, fried, cooked, baked, dried or processed into juices and other products (Khan et al., 2012). Immature fruits and leaves are used as vegetables while the seeds have varied nutritional values (Bhat and Anju, 2013). Cucurbits are source of income to farmers due to their nutritional and economics benefits. Some cucurbit species such a bottle gourd (*Lagenaria siceraria*) used for storage and also as a decorative element in homes, gardens and other public spaces.

In Kenya, cucurbits are produced in several counties for home consumption and for export markets (HCDA, 2020; Njoroge, 2012). The major cucurbit species grown in the country include butternut/ Squash pumpkin, cucumber, watermelon and zucchini (HCDA, 2020). In the recent past, there has been an increase in production area of cucurbits leading to an increase in occurrence of epidemics, more so severe viral disease that cause danger to the long-term growth and preservation of these crops (HCDA, 2020).

Moroccan watermelon mosaic virus (MWMV), a potyvirus in the family Potyviridae (McKern et al., 1993) was reported in Kenya in the year 2021, infecting pumpkin (Mumo et al., 2021). The virus has been reported to naturally infect other cucurbits crop species in Africa and Mediterranean region (Mumo et al., 2021; Kidanemariam et al., 2019; Ibaba et al., 2016). *Carica papaya* L is also another natural host of Moroccan watermelon mosaic virus (Mumo et al., 2021). Aphid species such as *Myzus Persicae*, *Aphis gossypii*, *Aphis spiraecola*, and *Aphis umbrella* can transmit

MWMV at different growth stages in a non-persistent manner (Chatzivassiliou et al., 2016; Owolabi and Ekpiken., 2014). *Myzus persicae* and Aphid *gossypii* are the most efficient for the transmission of Moroccan watermelon mosaic virus (Chatzivassilliou et al., 2016). Moroccan watermelon mosaic virus can also be mechanically transmitted to cucurbits via sap inoculation (Mumo et al., 2021).

Cucurbits infected with Moroccan watermelon mosaic virus exhibit symptoms such as mosaic, extreme filiform, striking interveinal chlorosis, and distortion, with dark green blisters on the leaves (Davis et al., 2020, Mumo et al., 2021). The symptoms sometimes resemble those caused by other biotic and abiotic factors making it difficult for non-experts to distinguish between symptoms caused by the virus and those caused by other factors. Early infection of the virus to the crop before fruits are formed may lead to partial or entire loss of yield (Davis et al., 2020).

Farmers' knowledge and perceptions describes what people know about a problem, how they feel about it, what they perceive as the severity and cause of the problem, as well as the actions they take to address it (Mumo et al., 2020; Schreinemachers et al., 2015). Moroccan watermelon mosaic Viral disease symptoms is like any other disease that maybe difficult for farmers and non-experts to identify due to similarity of symptoms to those caused by abiotic stresses (soil nutrients or mineral deficiency), insects or pests (Mumo et al., 2020; Islam, 2017; Schreinemachers et al., 2015). Therefore, documenting what farmers in Kenya know about the disease, how they perceive it,and how they manage it is of paramount importance.

1.2 Statement of the Problem

The MWMV infects cucurbits and poses substantial production challenges in African countries and the Mediterranean regions, resulting in yield losses ranging from 60 - 100% (Kidanemariam et al., 2019; Ibaba et al., 2016). Moroccan watermelon mosaic virus causes symptoms closely resemble those caused by other biotic and abiotic factors such as nutrient deficiency, and drought (Schreinemachers et al., 2015).A key component of developing integrated disease management strategies in agriculture is understanding farmers' perspectives and knowledge of the disease and how these influences the management techniques (Schreinemachers et al., 2015). In Kenya, the virus was reported in two counties namely Kiambu and Meru, infecting pumpkins and *Carica papaya* L.(Mumo et al., 2021). Cucurbits are grown in several counties in Kenya, however, the occurrence, and distribution of the virus in the main cucurbits producing counties in the country is not documented. The Morrocan watermelon mosaic virus is transmitted naturally from cucurbit crops by several species of aphids in a non-persistent manner. The virus can also be mechanically transmitted from one cucurbit crop to other (Mumo et al., 2021). Study by Mumo et al. (2021) reported that the Moroccan watermelon mosaic virus isolates from papaya and the Moroccan watermelon mosaic virus from isolates from pumpkin in Kenya are naturally adapted to papaya and pumpkin, respectively through sap/mechanical transmission. However, transmission of the virus from the two naturally adapted species using aphids vectors to other cucurbits and papaya plant is not documented.

1.3 Justification of the Study

Viruses can cause significant reduction in crop yield and quality resulting in economic losses for farmers and food shortages for consumers. Documentation of farmers' knowledge and perceptions are influential in developing management measures (Schreinemachers et al., 2015).The information is key to the development and adoption of a successful Integrated Pest Management (IPM) program (Parsa et al., 2014). Farmer's knowledge can help policy makers to develop and implement effective strategies for disease infection control, reducing the impact on crop yields and quality and protecting the economic viability of their farms. Farmers' behaviours stem from their knowledge; the information acquired can assist in modifying interventions to address specific knowledge gaps or rectify existing misconceptions (Schreinemachers et al., 2015). Understanding the distribution of MWMV within the counties of the Eastern region of Kenya will help in mapping the disease occurrence and assist in assessing the risk it poses to crops and how policy makers can intervene to mitigate their impact and improve food security. Because farmers intercrop different crops in a given piece of land, assessing the aphid's transmission efficiency of MWMV from host crop to another is crucial in understanding the disease epidemiology that will ultimately contribute to the disease management and improved production.

1.4 Objectives

1.4.1 General Objective

To determine the occurrence, farmers' knowledge and influence of host plant species on aphids transmission efficiency of Moroccan watermelon mosaic virus disease for effective management

1.4.2 Specific Objectives

I. To determine the occurrence, incidence , and severity of MWMV in cucurbit crops in selected counties in Kenya
II. To assess farmers' knowledge, perceptions, and management practices regarding MWMV
III. To evaluate the influence of host plant species on aphids transmission efficiency of MWMV

1.5 Hypotheses

I. There is no significant difference in the incidence, severity, or distribution of MWMV across the selected counties
II. Farmers' knowledge and perceptions have no significant influence on disease management of MWMV
III. Host plant species have no significant influence on aphids transmission efficiency of MWMV

CHAPTER TWO

LITERATURE REVIEW

2.1 Cucurbits

2.1.1 Origin and Taxonomy of Cucurbits

Cucurbits are among the first crops to be domesticated (NEE, 1990), with pumpkin and squash reported to have been cultivated first from seasonally arid parts of the Americas (Paris, 2016). Watermelon (*citrullus lanatus*), originated in African and has been grown in Northeastern African at least 4,000 years and it was brought to Europe about a decade ago (Paris, 2016). The cucumber (*cucumis sativus*) underwent domestication in India and was imported to Europe during the ancient Greek era. Squash (*cucurbit moschata***)** is native to America (Mexico and South America), watermelon has its origin in Africa and pumpkin (*Cucurbit maxima*) a native to South America, Uruguay and Argentina (Whitaker et al., 1975; Weng & Sun, 2012; Schaefer & Renner, 2011).

Cucurbitaceae belong to the kingdom (Plantea), phylum (Magnololphata),class (Magnoliopsida), order (Cucurbitales) and family (Cucurbitaceae) (Deyo and Malley, 2008). The Family is divided into two sub-family namely Zanonioideae and cucurbitoideae. Zanonioideae are flowering plants and cucurbitoideae are food producing plants which including the following crops cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita maxima*), squash (*Cucurbita moschata*).

In 2016, the cultivation of cucurbit crops covered an area of 11 million hectares, resulting in the production of 256 million tons of vegetables and fruits (source: http://faostat.fao.org). China, Turkey, Iran and USA produce about 50% of the world's production (29.9 million tons) of watermelon (*Citrullus lanatus*). Cucumber (*Cucumis sativus*) cultivation is the second largest of the Cucurbits (23.2 million of ton) and the leading countries in cucumber production are China, Iran, Turkey, and USA, which account for 66% of the global production. China, Turkey, Iran, and

USA produce 57% of the global production of melon (*Cucumis melo*), squash and pumpkin (*Cucurbita pepo*), amounts to 16.2 million tons (FAO, 2021). China, Ukraine, Argentina, and Turkey contribute 45% of the production of squash and pumpkin (FAO, 2021).

Cucurbit production in Kenya are used for both domestic consumption and export markets depending on their species (HCDA, 2020). Butternut (*Curcubita moschata*) is a potential food security crop due to its long shelf life accounting for 46.6% of the country's total value (HCDA, 2020). Zucchini/Courgette/Summer Squash (*Cucurbita pepo*) are used for both domestic consumption and export market accumulating Kenya shillings 139.32 million (HCDA, 2020) accounting for 68% of Kenya's total value (HCDA, 2020). Pumpkin (*Cucurbita maxima*) is a food security crop that is grown for its leaves and fruits accounting for 42.4% (HCDA, 2020). Watermelon (*Citrullus lanatus*) and Muskmelon (*Cucumis melo*) are produced for both domestic consumption and export markets are accounting for 75% of the country total value (HCDA, 2020). Cucurbits are among the major horticultural crops grown in Kenya and are mainly grown in Meru, Embu, Homabay, Kirinyaga, Muranga, Nakuru, Kiambu, Taita Taveva, Kisii, Bomet, Lamu, Migori, Makueni, Machakos, Mandera, and Tana River counties (HCDA, 2020).

2.1.2 Nutritional Benefit of Cucurbits

Cucurbits have diverse food uses depending on the crop and the stage of maturity which include being used to alcohol, sweets, beverages, cake, fried, juice, cooked, baked, dried and other products (Khan et al., 2012). Immature fruits and leaves are used as vegetables while the seeds have varied nutritional values (Bhat and Anju, 2013). Pumpkin has a high amount of beta- carotene and moderate amounts of vitamins K, and B6 and minerals like phosphorus, potassium, magnesium, selenium and iron. Pumpkin is also used for making dried products, pomade, prickles and juice (Badr et al., 2011; Elinge et al., 2012; Jacobo-Valenzuela et al., 2011; Rakcejeva et al., 2011) Watermelon is rich in minerals such as copper and Potassium and vitamins A and B (Bhat and Anju, 2013).Courgette is rich in minerals such as potassium and magnesium and vitamins such as A and C. Butternut is rich in magnesium, potassium and vitamin C (Khan et al., 2012). Cucurbits, such as pumpkin and watermelons are medicinal plants, which are beneficial for the skin. Additionally, they include phytonutrients that have been shown to lower the incidence of prostate cancer (Khan et al., 2012).

2.1.3 Constraints to Cucurbit Production

Like all agricultural products, cucurbit production is challenged by both abiotic and biotic constraints during all stages of production. Some of the constraints faced in cucurbits production are pests infections, drought, and diseases among other factors (HCDA, 2020). Cucurbits are extremely impacted by diseases and viruses that lead to both pre- and post-harvest losses (Perez et al., 2009). Drought and poor health are the most important abiotic constraints affecting cucurbit production (Khoury et al., 2020; Wien, 2020). Downy mildew, powdery mildew, anthracnose, Fusarium oxysporum, *Erwinia tracheiphila* Smith (McGrath, 2004; Jahn et al., 2002; Zuniga et al., 1997), cucumber mosaic virus (CMV), watermelon mosaic virus (WMV), Moroccan watermelon mosaic virus (MWMV) and zucchini yellow mosaic virus (ZYMV), Tobacco streak (TSV) (Sharman et al., 2009, 2015; Prasada et al., 2003),Pepo aphid borne yellows virus (PABYV) (Masika et al., 2022; Kidanemariam et al., 2019; Ibaba et al., 2017; Desbiez etal., 2016; Kone et al., 2015), cucurbit aphid-born yellow virus (CABYV) (Lecoq et al., 1998) are the most important viruses. The major pests include the following melon fruit fly, whitefly, western flower thrips, aphids, pumpkin beetle, spotted beetle, pumpkin leaf caterpillar, spider mite, golden twin spot moth and blister beetle (Sahu and Samal, 2020; Johnson et al., 2013; Abdalla et al., 2012; Sobh et al., 2012; Liu et al., 2009).

2.2 Moroccan Watermelon Mosaic Virus (MWMV)

Moroccan watermelon mosaic virus is a member of the genus potyvirus which is one of the largest genera of plant viruses infecting several economically importance cucurbit crops globally (Gibbs, 2010). The virus is non- enveloped flexuous filamentous of about 680-900 nm long and 11-15 nm wide (Gibbs, 2010). Analysis of the coat protein nucleotide sequence revealed that MWMV is a quite different potyvirus from watermelon mosaic virus (Mckern et al., 1993). Serologically and biologically, MWMV is related to papaya ringspot virus (PRSV), but there are molecular distinction between them based on species (Lecoq and Desbiez, 2012; Adams et al., 2005). MWMV primarily infect cucurbitaceae family and papaya (Mumo et al., 2021; Owolabi et al., 2012; Arocha et al., 2008).

2.2.1 Symptoms of Moroccan Watermelon Virus Infection in Cucurbit Plants

The virus infect cucurbit crops naturally through mechanical and aphid transmission in a non-persistent manner. The virus causes symptoms including fruit deformation, leaf deformation, filiform, interveinal chlorosis, leaf yellow, necrosis, stunted growth, and dark green bilsters on leaf, leaf and fruit blister (Mumo et al., 2021; Lecoq et al., 2001).

2.2.2 Distribution of Moroccan Watermelon Mosaic Virus

Morocan watermelon mosaic virus was first discovered in Morocco and documented as a strain of watermelon mosaic virus in 1972 (Fischer and Lockhart, 1974).Since then the presence of MWMV has been observed across various African countries and the Mediterranean region including Cameroon, Democratic Republic of Congo, Niger, Nigeria, Republic of South Africa, Sudan, Tanzania, Tunisia, Zimbabwe, (Ibaba et al., 2016; Owolabi et al., 2012; Arocha et al., 2008; Yakoubi et al., 2008) Kenya (Mumo et al., 2021), Ivory coast (Kone et al., 2010). Outside of Africa MWMV has been reported in the following countries Greece (Malandraki et al., 2014). Iran (Bananej et al., 2018), France (Lecoq et al., 2007), Turkey (Yesil, 2021) and Portugal (Yakoubi et al., 2008), Spain (Miras et al., 2019), and Italy (Roggero et al., 1998).

2.2.3 Transmission of Moroccan Watermelon Mosaic Virus

Moroccan watermelon mosaic virus is a an aphids-borne viral disease that is transmitted to cucurbits in a non-persistent manner. Aphids species that transmit the Moroccan watermelon mosaic virus include Myzus persicae, Myzus persicae subsp, nicotianae, Aphids gossypii and aphids spiraecola (Yakoubi et al., 2008). Aphids acquire viral particles during short feeding intervals on an infected host and transmit it by feeding on the healthy plant (Murant et al., 1988). Moroccan watermelon mosaic virus can also be transmitted to cucurbits via sap inoculation (Mumo et al., 2021).

2.2.3.1 Melon Aphid (*Aphis gossypii*)

Aphis gossypii is a little arthropod often referred to as a "greenfly". It belongs to the superfamily Aphidoidea under the order Hemiptera. The pest is prevalent in several agricultural crops belonging to the groups Cucurbitaceae, Rutaceae, and Malvaceae (Stefanie et al., 2008). Watermelons, cucumbers, squash, and pumpkin are susceptible to significant damage caused by Aphid gossypii (Stefanie et al., 2008). Furthermore, it is recognized for its ability to transmit over 50 viruses which include but not limited to the following Cucumber mosaic (CMV), family *Bromoviridae*, genus Cucumovirus, Cucurbit aphis-borne yellow virus (CABYV), family *Luteoviridae*, genus Polerovirus, Zucchini yellow mosaic virus (ZYMV), family *Potyviridae*, genus Potyvirus, Papaya ringspot virus (PRSV), family *Potyviridae*, genus Potyvirus and Cotton leafroll dwarf virus (CLRDV), family *Solemoviridae*, genus Polerovirus.

2.2.3.2 Green Peach Aphid *(Myzus persicae)*

Myzus persicae, is a green aphid belonging to the order Hemiptera. It is the most significant aphid pest of peach trees, causing decreased growth, shriveling of the leaves and the death of various tissues. It also acts as a vector for the transport of plant viruses which include cucumber mosaic virus (CMV),potato virus Y (PVY) (Capinera, 2005). This aphid, which is probably of Asian origin but is now spread worldwide, is the most important virus vector. It has been shown to vector in excess of 100 plant viruses. Although this species is normally heteroecious between prunus and secondary host plants, it is capable of an anholocyclic existence on secondary hosts in climates that permit survival over the winter period crops (Capinera, 2005).

2.3 Management of Moroccan Watermelon Mosaic Virus

There has been no effective management control for Moroccan watermelon mosaic virus (MWMV), however several strategies for the management of aphids that cause MWMV in cucurbits have been put forth and tried which include cultural, biological and integrated pest management (Barbercheck et al., 1995)

2.3.1 Cultural Control

Rotating between crop families can help minimize crop-specific insect pests, one of the well-known cultural practices, before transplanting check for aphids and remove them before planting or destroy infested plants by dropping the plant or infested plant part in a bucket of soapy water, and disposing of them away from the field. To prevent the rapid spread of the aphid population in the field, remove and bury, if possible, the few severely infested plants as they appear in spring. Aluminum foil mulches can repel invading aphid populations, reducing the numbers of seedlings and small plants, and can reduce transmission of aphid-transmitted viruses in summer to squashes, melons, and other susceptible vegetables (Barbercheck et al., 1995).

2.3.2 Biological Control

Aphids have a fast life cycle and reproduce and disperse rapidly. However, the efficacy of insecticides to lower the rates of disease damage by spraying is not very effective because of the fast spread of the virus to unaffected plants by vectors and the late manifestation of disease symptoms (Barbercheck et al., 1995). Biological control by natural enemies (predators, parasitoids, and pathogens) can have a significant impact on aphid population. Beneficial insects will be attracted to plants with moderate to heavy aphid's infestations. These natural enemies such as ladybugs, green lacewings, and hoverfly larvae may feed on large numbers of aphids which will help reduce their population (Barbercheck et al., 1995). Also IPM is another encourages natural control of the pest population, it includes enchancing natural enemy population, planting of pest resistant crops and adapting cultural control mechanism (USDA, 1993).

2.3.3 Chemical Control of Aphids

Aphids destroy field crops by sucking, chewing, or burrowing into various plant sections, affecting agricultural food production and stored products (Singh and Kaur, 2018), and are regarded as the primary biotic restrictions on crop productivity (Bhat and Ahangar, 2018).

The most popular way to manage aphid species is using chemical insecticides, but using them leads to high levels of insecticide resistance, insecticide residues, and high costs (Saruhan, 2018).

2.4 Farmer's Knowledge, and Perceptions on the Disease

The idea of farmer's knowledge, perception, and practices depicts farmers' understanding about a problem, as well as the actions to take in mitigating it. Also it demonstrates that adjustment changes in farmers' knowledge, attitudes, and perceptions (Mumo et al., 2021; Schreinemachers et al., 2015). The identification of plant viral diseases poses challenges for both farmers and non-experts due to symptoms that similarity induced by abiotic factors such as soil nutrient deficiency, as well as by insects or pests (Schreinemachers et al., 2015).

Therefore, determining farmers' level of awareness on infection cycles and transmission is crucial to creating a management plan. Research assessing farmers' understanding of plant viral infections have shown that farmers are unsure about correctly diagnosing and treating the disease. This uncertainty led to a reduction in output or perhaps a complete loss of crop (Schreinemachers et al., 2015).

Farmer's limited knowledge of disease management is one of the factors affecting farming practice. Farmers' in developing countries have usually not attended any training program on the appropriate management of pest and disease (Fan, 2001; Matteson and Kenmore, 1993; Pontius and Bartlett, 2002). This inadequate knowledge among farmers'is associated with farmers' level of education (Olajide, 2011). It was well acknowledge during the Green Revolution period that the lack of farmers' knowledge caused unnecessary use of chemical that resulted in resistance and pesticide residue, which is currently been observed that similar misfortune may also occur in this current Gene Revolution period (Bernard and David, 2001; Yang et al., 2005). A research repot in Northern China in 2002, showed that the farmers' limited knowledge led to the used of high pesticide in field crop production (Yang et al., 2005). The choice of farmers to adopt pest management practices depends on their awareness and knowledge of the disease and the caused of the disease (Segura et al., 2004). Farmers' perceptions and understanding of integrated pest management (IPM) could help uncover the factors that influence disease management (Abdollahzadeh et al., 2015). Farmers' decision about crop protection may be influence by a variety of variables, including their familiarity with the pest, disease and the level of harm they caused to the cultivated plants, as well their awareness of the presence of natural enemies. It is widely considered that pest management extension will be more stronger when farmers perceptions and practices are taken into serious consideration (Heong et al., 2002). Although crop protection is crucial, but farmers may be more anxious with level of soil fertility, water availability, or other factors than pests in many situations (Morse and Buhler, 1997).Therefore farmers' knowledge, perceptions and pest control techniques may be beneficial in designing (IP) system that more may embrace (Matteson et al., 1984; Morse and Buhle, 1997). The lack of adequate farmer' knowledge, perceptions and practices is one the major constraints in establishing effective disease management strategies (Van Mele, 2000). The knowledge of pests and diseases does not only varies among farmers' working in the different agro-ecosystems, but also varies among those farmers' working in similar agro-ecosystem, Therefore understanding farmers' knowledge is important to determine the information gaps and to assess the impact of different information for strategies management approach (Van Mele, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Areas and Sampling Procedure

The study was conducted in selected counties in Kenya, focusing on three major cucurbits production counties. The counties included Machakos, Kirinyaga and Embu. Within the counties, the leading sub-counties in cucurbits production were selected for the study (HCDA, 2020). The sub-counties included Matungulu and Kangundo in Machakos county, Mwea East, Kirinyaga East, and Kirinyaga West in Kirinyaga county and Mbeere North and Embu West in Embu county. The counties and sub-counties within the counties were selected based on the presence of cucurbit crops (HCDA, 2020).The sampling techniques employed included stratification, purposive and snow-balling. Stratified sampling was done based on the three counties where the crops are mainly grown (HCDA, 2020), purposive was done to target specific farmers that grown cucurbit crops and snow-ball sampling techniques were used for the referral of cucurbit farmers by fellow farmers in the selected sub-counties.The surveyed sites were mapped using a global positioning system (GPS; Magellen GPS315,San Dimas, CA).

3.1.1 Determination of Severity, Incidence and Distribution of Moroccan Watermelon Mosaic Virus in Selected Counties in Kenya

In the farmer fields, an imaginary lines were drawn diagonally from both direction resulting into two transects (Sseruwagi et al., 2004). Representative plants were randomly selected during sampling along the X- shape transect in each field to reduced biases. A total of 229 cucurbits farmers were surveyed.

3.1.2 Incidence and Severity of Moroccan Watermelon Mosaic Virus in Farmer's Fields

To determine the disease incidence, ten (10) plants per field were visually examined for the presence of MWMV symptoms. Disease incidence was determined as a proportion of the plants exhibiting MWMV symptoms out of the ten plants inspected and expressed as a percentage (Ayo-John et al., 2024). The disease severity was calculated using the area or proportion of symptomatic plants. The scale of 0 to 4 (Ndunguru and Rajabu, 2004) was used (Table 3.1) (Appendix I).

Table 3.1: Scale Used in Rating MWMV Disease Severity in Cucurbit Plants During the Field Survey

Scale	Symptoms expressed in cucurbit crops
0	No symptoms
1	1- 25% Mild mosaic discoloration and mottling of leaves
2	26- 50% Slight distortion, deformation, chlorosis, yellowing puckering, and dark green blisters on the leaves, mild stunted growth
3	51-75% Severe mosaic, distortion, deformation, chlorosis yellowing dark green blister, vein clearing on leaves and moderate stunted growth
4	More than 75% Leaves severely distorted, or leaf deformation and severe stunted growth

Source: Ndunguru and Rajabu (2004)

3.2 Sample Collection and Determination of Virus Infection Using PCR

Young leaf samples were collected from 3 to 5 plants per field using sterile forceps. A total of seventy seven (77) symptomatic and forty six (46) asymptomatic plant leaf samples were collected. The number of leaf samples collected per field depended on the plants population and disease severity. The collected leaf samples were preserved in 15ml sterile falcon tubes containing 10ml of RNA preservation solution (0.5M EDTA, sodium citrate, and Ammonium Sulphate; pH 5.2) to prevent RNA degradation. The samples in the falcon tubes were put in zip lock bags and clearly labelled before putting them in a cool box containing cold ice packs and transported to Jomo Kenyatta University of Agriculture and Technology laboratory and stored at 4˚C before RNA extraction.

3.2.1 Ribonucleic Acid (RNA) Extraction

Ribonucleic acid was extracted from the collected leaf samples using a modified cetyltrimethylammonium bromide (CTAB) method (Lodhi et al., 1994). The CTAB buffer contained 2% CTAB, 100 mM Tris- HCl, 20 mM EDTA, 1.4M NaCl, freshly prepared 1% sodium sulphite, and 2% polyvinylpyrrolidone in nuclease-free water. Briefly, 100mg of leaf sample were homogenized in 2µl extraction buffer, transferred in a 1.5ml micro centrifuge tube and incubated at 65ºC for 15 minutes while mixing at 10-minutes intervals. The tubes were centrifuged at 10,000 rpm for 5 minutes, and the supernatants (700 µl) were transferred to new tubes. An equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 minutes. The aqueous layers were transferred into new tubes, and 700µl of lithium chloride (stored at 4ºC) was added while the tubes were gently inverted. After overnight incubation at 4ºC, RNA was pelleted at 14, 000 rpm for 30 minutes at 4ºC.The supernatants were discarded, and the pellets re-suspended in 200µl of TE buffer containing 1% of SDS. Then, 100µl of NaCl and 300µl of ice-cold isopropanol were added, mixed gently, and incubated at -20ºC for 20 minutes. The samples were then centrifuged at 14,000 rpm for 10 minutes at 4ºC. The salts were removed and the pellets were washed with 500µl of 70% (v/v) ethanol and then centrifuged for 4 minutes at 10,000 rpm. The ethanol was removed and the RNA pellets were air dried at room temperature for 15 minutes before resuspending in 50µl of nuclease- free water. The integrity of the extracted RNA was assessed using agarose gel electrophoresis and visualized in UVP PhotoDoc-It™ imaging System.

3.2.2 Complementary Deoxyribonucleic Acide (cDNA) Synthesis and PCR Process

The cDNA was synthesized using 5X LunaScript RT SuperMix (Biolabs). Briefly, a 25µl reaction consisting of 20µl of nuclease-free water, 4µl of 5X LunaScript RT SuperMix and 1µl of RNA sample. The mixture was incubated at 55ºC for 10 minutes, and at 95ºC for one minute. The cDNA was stored at -25ºC before the PCR process. PCR process was carried out using OneTaq 2XMaster Mix with Standard Buffer (New England Biolabs). Briefly, a 20µl PCR reaction consisted of 7.2µl of nuclease-free water (New England Biolabs), 10µl of OneTaq 2X Master Mix with Standard Buffer (New England Biolabs), 0.4µl of 10µM of forward and reverse primers, and 2µl of cDNA. Primers used were 5 ʹ-TGCTGTTGGTAGTGGCAAAT-3 ʹ and 5 ʾ- TTCTGTTCGCCCAACTTTA-3 (Macrogen), (Mumo et al., 2021) amplifying at 613 bp fragment for MWMV in the coat protein region and forward 5′-TCTCAGCTAGCACGCAACAA-3′ and reverse 5′-CGGTGTTGAGCCAAACGAAG-3′ primer pair based on papaya isolates of MWMV (Mumo et al., 2021) amplifying at 315 bp fragment for MWMV in the coat protein region. Both positive and negative controls were included in the reaction. The positive control constituted a sample infected with MWMV from a previous study (Mumo et al., 2021), maintained in the insect-free greenhouse, while the negative control contained nuclease-free water. The PCR reactions were run on a thermal cycler (BIO-RAD T100™) under the following cycling conditions: 3 minutes at 95ºC; 35 cycles of 30s at 95ºC, 30s at 55ºC and 1 min at 72ºC and a final extension at 72ºC for 5 min for the sets of primers. The amplified PCR products, alongside the 1-kb plus DNA ladder, were run on 2% (wt/vol) agarose gels and visualized under a UV transilluminator.

3.2.3 Determination of Farmers' Knowledge, Perceptions and Management of MWMV

3.2.3.1 Data Collection

To determine farmers' knowledge, perceptions and management of Moroccan watermelon mosaic virus, data was collected using a semi-structured questionnaire (Appendix 1) administered through face-to-face interviews.

The questionnaire was pre-tested with ten (10) farmers before conducting the study. Data collected included socioeconomic characteristics of the farmers, cucurbits production, farmers' knowledge, perception and management practices of the MWMV disease. To test farmers knowledge of the disease, an A4-sized photographs of cucurbit plants infected with Moroccan watermelon mosaic virus disease was used

(Khan et al., 2014; Asudi et al., 2015). The photos had no text to ensure the identification was based on visual cues by correlating the symptoms in the fields with those in the picture. When necessary, the disease symptoms were described. Farmer's knowledge of the disease was assessed by asking if they knew the symptoms in the photos and if the cucurbits in the farms displayed those symptoms. The responses to the knowledge questions were recorded in a series of binary responses (1 for yes and 2 for no). The perception of the disease and the rate of spread was captured as a categorical variable using a 4-point Likert scale rating (Khan et al., 2014; Asudi et al., 2015). The farmers were asked to rate the disease problem on a scale of 0 to 3, where 0 = no problem, 1 = moderate problem, 2 = severe problem, 3 = very severe problem. The current rate of the disease spread was scored on a scale of 0 to 3 where 0 = no spread, 1 = slow spread, 2 = fast spread, 3 = very fast spread. Information such as the purpose for cucurbits production, source of planting materials, cropping system, seasonal prevalence of the symptoms on cucurbit crops, the cause of the symptoms and the control measures was also captured.

3.3 Determination of Host Plant Species Influence on Aphid Transmission Efficiency of MWMV to Cucurbit Crops

3.3.1 Source and Rearing of Aphids

Myzus persicae, green peach aphids were obtained from JKUAT farm, the species were confirmed through microscopy, and maintained on common beans (*Phaseolus vulgaris*) plants established in the pots placed in cages in an insect proof greenhouse (Ateka, 2004). The cages were prepared using insect nets of equal size, and the plants were carefully placed in the cages for the rearing of the aphids.

3.3.2 Establishment of the Source of MWMV Inoculum

Pumpkin and papaya leaf samples were collected from the field and were confirmed to be infected with MWMV using PCR approaches. After the virus confirmation, the virus was isolated and propagated on pumpkin and papaya plants established and maintained in an insect-proof screenhouse. To propagate the virus, seeds of papaya variety ‘red mountain’ and gabber F1 pumpkin plants obtained from a local stockist were established in a sterilized mixture of soil, sand and manure. To prepare the viral inoculum, MWMV- infected pumpkin and papaya leaf samples were ground in 10 volumes (1g per 10ml) of the prepared cold inoculation buffer (0.01M potassium phosphate, pH 7.5) plus 40g of 600 mesh carborundum in 1.5L inoculum. Established papaya and pumpkin plants were mechanically inoculated with the viral inoculum by gently rubbing with cheesecloth on the fully grown leaves and rinsing with double-distilled water (Mumo et al., 2021). The inoculated plants were monitored daily for symptom development. Fourteen days after inoculation, viral symptoms such as leaf yellowing and dark green blistering of leaf were visible, and the presence of the virus was confirmed using primers specific to MWMV papaya and pumpkin isolates as documented (Mumo et al., 2021; Mumo et al., 2020).

3.3.3 Aphid Transmission of MWMV from Pumpkin and Papaya Plants to Papaya and Cucurbit Species

Infected pumpkin and papaya plants mechanically inoculated and confirmed MWMV infections were maintained in an insect free screenhouse were used as the source of the MWMV inoculums.

Cucurbit species used in the aphid transmission trials included the following varieties crimson sweet for watermelon, dark green for Zucchini, Pluto F1 for butter nut and red mountain for papaya. The seeds of these cucurbits were sourced from a local stockist and established in an insect free screen-house in a sterilized mixture of soil, sand and manure. For transmission, the reared aphids were placed on a moist filter paper in a petri-dish and starved for 1 hour, after which these aphids were allowed to feed for 30 seconds on infected pumpkin and papaya plants to acquire the virus. Subsequently, a group of five aphids were transferred to a two–leaf stage zucchini, pumpkin, watermelon, butter nut, and two- month’s old papaya plants maintained in an insect free screen-house. The aphids were allowed to feed on the test plants for 40 seconds to transmit the virus before killing them by spraying with an insecticide. For the control, aphids were allowed to feed on non-infected cucurbits and papaya for 30 seconds before transferring them to the test plants and allowed to feed for 40 seconds because the virus disease can be transmitted in non peresistant mood before spraying them with insecticide. The experiment was laid out in a completely randomized design (CRD) with three treatment replicated three time with thirty (30) plants per replicate. The treatments were infected aphids with MWMV from pumpkin plant, infected aphids with MWMV from papaya plant and control (non-infected aphids).

3.3.4 Monitoring and Testing of Infected Plants

Plants monitoring was done daily for symptoms development and symptoms developed were recorded from 14 days to six (6) weeks. To determine the disease incidence four weeks after transmission, five plants per each crop species were visually examined for the presence of MWMV symptoms. Disease incidence was determined as a proportion of the plants exhibiting MWMV symptoms out of the five plants inspected and expressed as a percentage (Ayo-John et al., 2024). The disease severity on the test plants were determined based on a scale of 0-4 (Ndunguru and Rajabu, 2004).

3.4 Data Analysis

Data on disease incidence and severity was analyzed through descriptive statistic (percentages and mean values) using Statistical Package for Social Sciences (SPSS). When determining disease severity, score of 0 (no symptoms) were excluded. The presence of the virus was scored based on the amplification of the 613 bp fragment following the observation in the agarose gel

For farmer’s knowledge, perception and management of MWMV, data collected was cleaned then subjected to analysis using Statistical package for the Social Science (SPSS) software version 21. Frequency analysis was used to describe the distribution of key categorical variables, providing insights into the count and percentage of responses across different categories. To assess the differences in means between multiple groups of more than two, an analysis of variance (ANOVA) was conducted. Cross- tabulation and Chi-square test were employed to explore association between two categorical variables. Statistical significance was used to determine the association between farmer's at ($P < 0.05$). Correlation analysis was also done to determine the knowledge, perception and management of MWMV.

CHAPTER FOUR

RESULTS

4.1 Incidence And Severity of Moroccan Watermelon Mosaic Virus in Selected Counties in Kenya

A total of 229 cucurbit farms were surveyed in the three counties (Figure 4.1). The number of farms surveyed in each county and sub-counties are presented in the Table 4.1

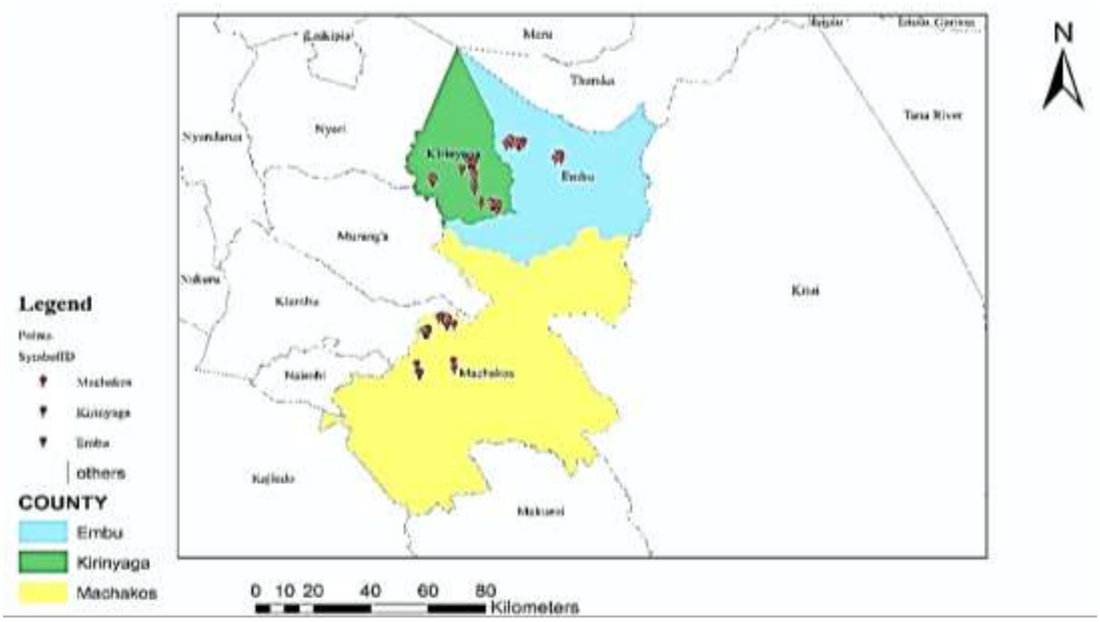

Figure 4.1: A Section of a Map of Kenya Showing Three Counties where the Survey was Conducted With The Sampling Points

Table 4.1: Number of Farmers Sampled Per Counties and Sub-Counties

Counties	Number of farmers sampled per county (N)	Sub-counties	Number of farmers sampled per sub-counties (N)
Embu	69	Embu West	55
		Mbeere North	14
Machakos	98	Kangundo	26
		Matungulu	72
Kirinyaga	62	Kirinyaga East	11
		Kirinyaga West	12
		Mwea East	39

The disease severity and incidence differed significantly between the sub-counties ($P< 0.05$).

The highest disease severity (3.8) was reported in Matungulu sub-county, followed by Kangundo sub-county 3.7, Mwea East and Kirinyaga East sub-counties 2.7, Kirinyaga West 2.2, Mbeere North 1.8, and Embu West 1.6 respectively (Table 4.2).The mean disease incidence across the seven sub-counties reported was 85.5% with the highest of (100%) in Kangundo and Kirinyaga East, followed by Matungulu (98.4), Mwea East (81.7), Mbeere North (78.2), Embu West (71.9) and Kirinyaga West (62.5) (Table 4.2).

It was observed that the disease incidence were found throughout the seven sub-counties (Table 4.2). Machakos County (sub –county Kangundo) and Kirinyaga County (sub-county Kirinyaga East) obtained 100% disease incidence, followed by Matungulu sub-county (Machakos county) 98.4%, 81% in Mwea East sub-county (Kirinyaga county), 78.2% in Mbeere North sub-county, 71.9% Embu West sub-county (Embu county), and 62.5% in Kirinyaga West sub-county (Kirinyaga county) respectively.

Table 4.2: The Mean Disease Incidence, and Severity of MWMV in Selected Counties in Kenya

County	Sub-county	Disease Incidence (%)	Disease Severity
Machakos	Matungulu	98.4	3.8
	Kangundo	100	3.7
Kirinyaga	Mwea East	81.7	2.7
	Kirinyaga East	100	2.7
	Kirinyaga West	62.5	2.2
Embu	Mbeere north	78.2	1.8
	Embu West	71.9	1.6
Mean		85.5	2.8
LSD (P = 0.05)		0.005	0.001

4.1.2 Symptoms Diversity and Incidence of MWMV Through PCR

Symptoms of MWMV observed in the fields included leaf deformation, dark green blisters of leaf, vein clearing; severity mosaic, mild mosaic, chlorosis and yellowing of leaf, stunted growth, mottling and puckering of leaf (Table 4.3) and (Figure 4.2). Out of the seven sub-counties surveyed, Moroccan watermelon mosaic virus was detected in four (4) sub-counties namely Matungulu, Mwea East, Mbeere north and Embu west (Table 4.3). MWMV was detected in 27 out of the 71 pumpkin samples tested. However, MWMV was not detected in other cucurbit plant samples tested including watermelon, zucchini, and butter nut. Presence of band size of 613 in the gel indicated presence of the virus in the sample (Figure 4.3). In terms of distribution of Moroccan watermelon mosaic virus in Machakos County, 12 samples from Matungulu sub-county were positive for the virus. In Kangundo sub-county, no virus was detected. In Embu County, 8 and 3 samples from Embu west and Mbeere North sub-counties respectively tested positive. In Kirinyaga County, 4 samples from Mwea east sub-county were infected with the virus. None of the samples collected from Kirinyaga east and Kirinyaga west tested positive for the virus infection.

The number of symptomatic samples varied with the highest obtained from Machakos County, Matungulu and Kangundo sub-counties 31 and 5 pumpkin samples respectively. In Kirinyaga County, Mwea East Sub County obtained 10 pumpkin, 4 watermelon, 1 butter and 1 zucchini symptomatic samples were collected, in Kirinyaga West, Kirinyaga East sub-counties 9 and 3 symptomatic pumpkin samples were also collected. In Embu county 8 and 6 symptomatic pumpkin samples were collected from Mbeere North and Embu West sub-counties respectively (Table 4.3).

Table 4.3: Incidence (PCR) and Diversity of MWMV Symptoms Observed During Field Survey

County	Sub-county	Cucurbit crop species	No. of samples collected	No. of symptomatic samples	No. of positive samples through PCR	Symptoms expressed
Machakos	Matungulu	Pumpkin	38	30	12	SG,LD,SM,LB,VC
	Kangundo	Pumpkin	10	5	0	LD,SM,LY,MM,LP
Kirinyaga	Mwea East	Pumpkin	15	10	4	SG,LD,SM,LB, LP
		Zucchini	1	1	0	LD,LB,
		Watermelon	7	4	0	LD, MM
		Butternut	1	1	0	LD, SM,
	Kirinyaga west	pumpkin	16	9	0	LD,SM,LB,LM
	Kirinyaga east	pumpkin	8	3	0	LD,LB, SM
Embu	Mbeere north	pumpkin	15	8	3	SG,LD,SM,LB
	Embu west	pumpkin	12	6	8	LD, LB, LM

*Symptom description: SG, stunted growth:LD, leaf distortion: SM,severe mosaic: LB, leaf blistering: MM, mild mosaic: VC, vein clearing: LY, leaf yellowing: LM, leaf Mottling: leaf puckering.

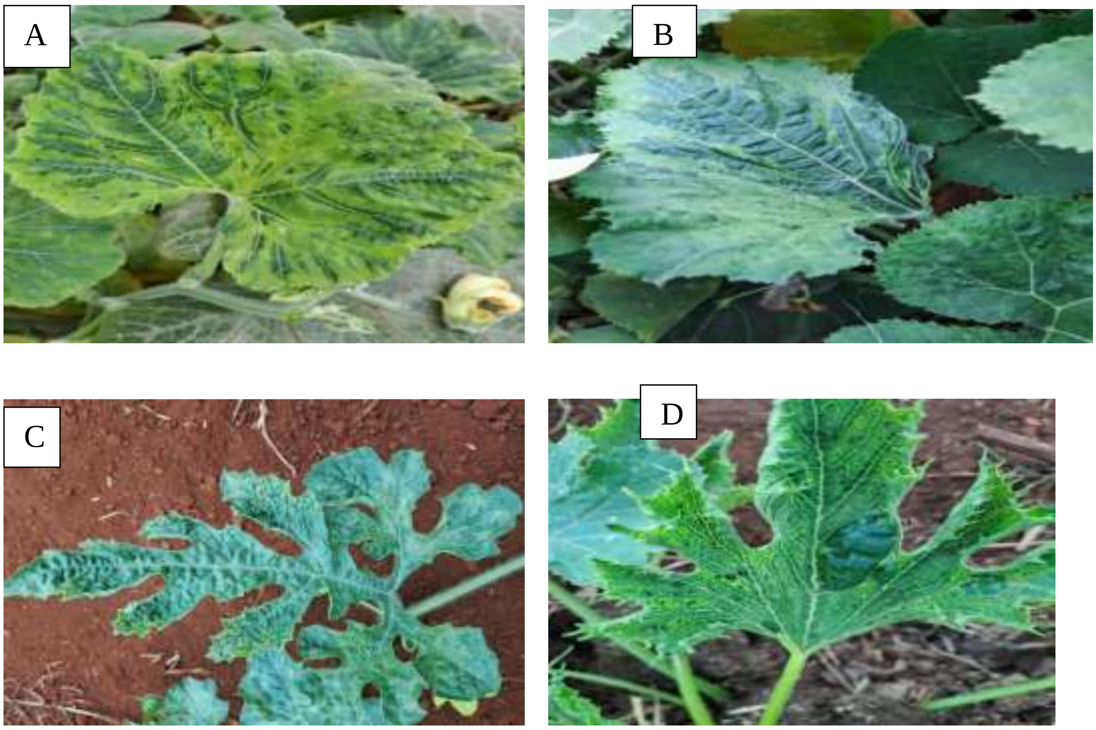

Figure 4.2: Moroccan Watermelon Mosaic Virus Disease Symptoms Observed in the Field.

A: leaf deformation, dark green blisters and vein clearing on pumpkin, B: Mosaic and leaf deformation on butter nut leaf; C: mild mosaic, and leaf deformation on watermelon leaf; D: leaf deformation, and dark green blister on courgette zucchini

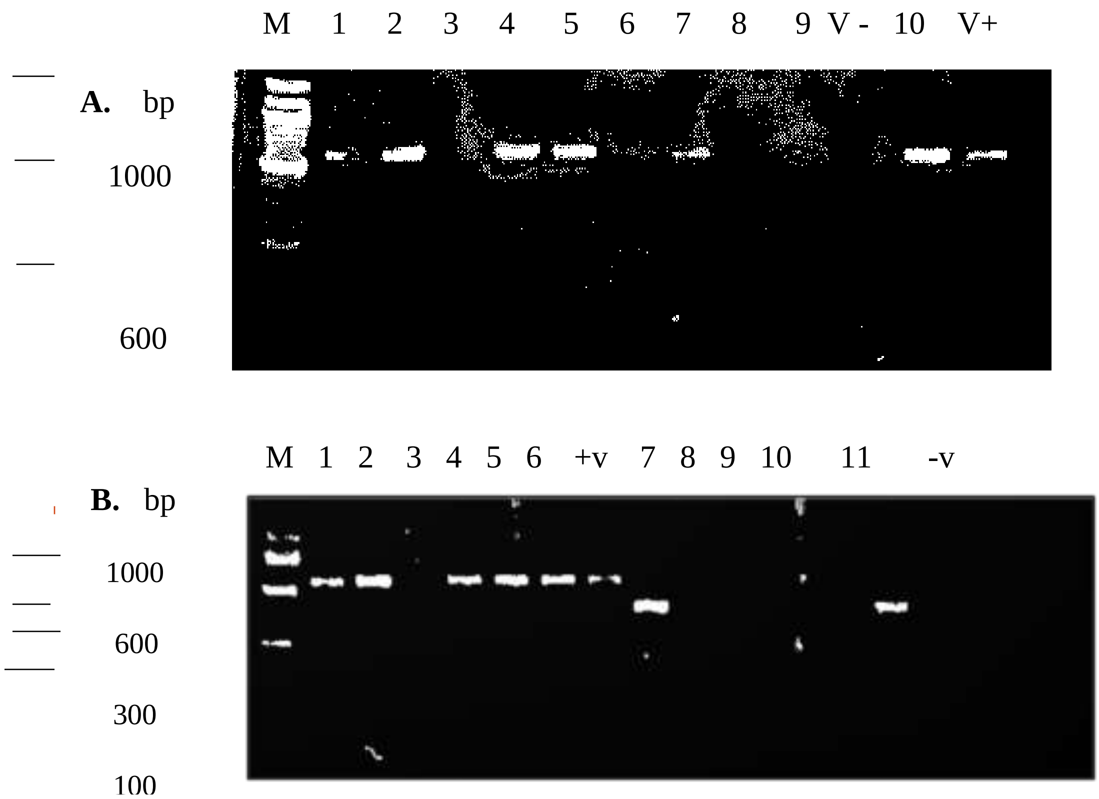

Figure 4.3: Gel Electrophoresis for Diagnostic Studies of MWMV in Collected Leaf Samples.

A. band at 613 bps indicate presence of MWMV in pumpkin, B. band at 315 indicate presence of MWMV in papaya isolate (8), and (11). At 613 indicate the presence of MWMV in pumpkin isolate samples (1-6). M indicate 1kb plus DNA ladder. +v is the positive control and –v is the negative control.

4.2 Farmers' Knowledge, Perceptions and Management of Moroccan Watermelon Mosaic Virus Disease in Selected Counties in Kenya

4.2.1 Socio-Demographic Characteristics of Cucurbit Farmers Interviewed During the Survey

About 59% of the respondents were females and 41% were males. The age of the respondent ranged between 18 to more than 66 years. There was no significant difference observed between the age group ($\chi 2 = 40.9$; df = 30; P = 0.08; Table 4.4). About 63.3% of the respondents had obtained primary education (8 years of basic education). The production purpose of cucurbits varied significantly across sub-counties ($\chi 2 = 47.5$, df = 12; $P < 0.001$; Table 4.4) with 68.1% of the respondents producing cucurbits for home consumption and selling the surpuls (Table 4.4). The land size owned by respondents varied across sub-counties, 2.83 acres in Kangundo, 2.27 acres in Matungulu, 2.16 acres in Kirinyaga East, 1.98 acres in Mbeere North, 1.88 acres in Mwea East, 1.66 acres in Embu West and 1.43 acres in Kirinyaga West (Table 4.4).The average land size in acres that was under cucurbit crops production varied across sub-counties with the highest of 1.10 acres in Matungulu, and 0.33 acres in Embu West (Table 4.4)There was a significant difference across the sub-counties in regards to current land size under cucurbits production ($\chi 2 = 4.58$, df = 6, P = <0.001; Table 4.4).

Table 4.4: Characteristic of Cucurbit Farmers in Seven Sub-Counties in Kenya

	Embu County Sub-counties		Machakos County Sub-counties		Kirinyaga County Sub-counties						
	Embu West	**Mbeere North**	**Kangundo**	**Matungulu**	**Kirinyaga East**	**Kirinyaga West**	**Mwea East**	**Total**	**Mean**	**$\chi 2$**	**P- value**
Number of farms surveyed	55	14	26	72	11	**12**	39	229			
Gender of respondents (%)											
Male	43.6	42.9	53.8	33.3	36.4	16.7	54		41	7.8^{ns}	0.16
Female	56.3	57.1	46.2	66.7	63.6	83.3	46		59		
Age (years) of respondent (%)											
18-24	-	7.1	-	5.6	-	-	7.7		3	40.9^{ns}	0.08
25-34	12.7	28.6	11.5	8.3	18.2	-	13		13		
35-44	16.4	21.4	15.4	20.8	9.1	8.3	18		16		
45-54	25.5	35.7	7.7	31.9	27.3	41.7	18		27		
55-65	30.9	7.1	61.5	25.0	18.2	41.7	31		30		
Above 66	14.5	-	3.8	8.3	27.3	8.3	13		11		
Educational level of Respondents (%)											
Primary School	45.5	50	34.6	94.4	60.0	83.3	51		60	93.3^{**}	<0.001
Drop out secondary	12.7	28.6	7.7	-	10.0	-	15		10.5		
Secondary school completed	30.9	21.4	42.3	28	30.0	-	26		22		
Technical training	9.1	-	15.4	-	-	-	-		3.5		
Undergraduate	-	-	-	1.4	-	8.3	7.7		2.4		
Post graduate	1.8	-	-	1.4	-	8.3	-		1.6		
Reason for cucurbits Production (%)											
Subsistence	47.3	50	7.7	19.4	54.5	33.3	17		28.8	47.5^{**}	<0.001
Subsistence and Market	52.7	28.6	88.5	79.2	88.5	66.7	77		68.1		
Market	-	21.4	3.8	1.4	-	-	5.1		3.1		
Average land owned (Acres)	1.66	1.98	2.83	2.27	2.16	1.43	1.88		2.05	4.00^{ns}	0.06
Land under production (Acres)	0.33	0.84	0.65	1.10	0.40	0.41	0.50		0.68	4.58**	0.001

(Chi- square test, and P < 0.05) at *P < 0.05, **P < 0.001; shows significant difference; ns means not significant, - mean no case present.

4.2.2 Cucurbits Farming Experience and Production Constraints

The farming system differed across the sub-counties ($\chi 2$ = 49.9, df = 25, P = 0.005; Table 4.5) with majority of the farmers adopting intercropping system (94.1%), Mono cropping (5.1%), crop rotation (0.4%), and shifting cultivation (0.4%).The years of cucurbits production varied significantly across the sub- counties ($\chi 2$ =56.4; df = 18; P = <0.001). Most of the farmers had more than two years of experience (89.3%) in cucurbit farming (Table 4.5).Cucurbit species grown varied significantly across the seven sub-counties ($\chi 2$ = 18.8; df = 8; P = 0.01), with majority of the respondents (97.3%), growing pumpkin, followed by watermelon (5.9%), butter nut (5.7%), and zucchini (0.37%). There were no reported cucumber cultivation during time of the survey in the sub-counties. Production constraints reported in the surveyed areas included pest infection such as melon fly, aphids, whiteflies, and beetles. The major diseases reported to infect cucurbits were mainly fungal including anthracnose, powdery and downy mildew, and blights. Diseases encountered during the survey as per respondents varied significantly across the sub-counties ranging from anthracnose with a significant difference of ($\chi 2$ = 2.55; df = 6; P = <0.001), followed by powdery mildew ($\chi 2$ = 26.1; df = 6; P = < 0.001), downy mildew ($\chi 2$ = 39.0; df = 6; P = 0.001) and blight ($\chi 2$ = 45.98; df = 25; P = 0.006), the pest (melon fly) also varied significantly across the sub-counties with a significant difference of ($\chi 2$ = 52.9; df = 24; P = 0.004 Table 4.5).

Table 4.5: Farmer's Knowledge in Cucurbit Production and Constraints

	Embu county Sub- Counties		Machakos county Sub-counties		Kirinyaga county Sub-counties						
	Embu West N = 55	**Mbeere North N = 14**	**Kangundo N = 26**	**Matungulu N = 72**	**Kirinyaga East N = 11**	**Kirinyaya West N = 12**	**Mwea East N = 39**	**Total 229**	**Mean**	**χ2**	**P-value**
Farming System (%)										49.9*	0.005
Crop rotation	-	-	-	-	3.8	1.4	-		0.4		
Mixed cropping	94.5	91.7	100	90.9	96.2	98.6	87.2		94.1		
Mono cropping	5.5	16.7	-	9.1	3.8	-	10.3		5.1		
Years of experience in cucurbit production (%)										56.4**	<0.001
<1 year	-	21.4	-	-	-	8.3	5.1		4.9		
1 year	1.8	7.1	-	-	-	-	-		1.3		
2 year	1.8	21.4	3.8	-	-	-	5.1		4.5		
More than 2 years	96.4	50	96.2	100	100	91.7	89.7		89.3		
Cucurbit species grown (%)											
Watermelon	-	14.3	-	1.4	-	8.3	17.9		5.9	18.8**	0.01
Butter nut	-	14.3	19.2	4.2	-	16.7	5.1		5.7		
Pumpkin	100	85.7	100	98.6	100	100	97.4		97.3		
Zucchini	-	-	-	-	-	-	2.6		0.37		
production constraints											
Anthracnos e	-	-	-	14.1	-	-	-		2.01	2.55**	<0.001
Powdery mildew	89.1	100	91.7	63.4	100	66.7	87.2		85.4	26.1**	<0.001
Downy mildew	50	85.7	91.7	31	72.7	50	66.7		64.9	39.0**	<0.001
Blight	85.7	-	100	68.2	25	-	50		46.9	45.9*	0.006
Melon fly	70	100	28.6	76.3	81.8	97.1	80		76.2	52.9*	0.004

(Chi square test, and P < 0.05) at *P < 0.05, **P < 0.001, shows significant difference; ns means not significant, — mean no case present

4.2.3 Farmer's Knowledge of the Disease, its Cause and Symptoms Observed in the Field

Moroccan watermelon mosaic virus disease symptoms were found in the seven sub-counties namely mosaic, severe filiform, chlorosis, dark green blisters and distortion on the leaves. The highest disease symptoms reported was leaf distortion (72.5%), followed by dark blister on leaf (64.5%), chlorosis on leaf (64.4%), mosaic (64.1%), and severe leaf filiform (4.5%) respectively (Table 4.6). The presence of the disease symptoms across the seven sub-counties ranged from 100% in Kirinyaga East and Kirinyaga West, 90.3% in Matungulu, 92% in Kangundo, 85.7% in Mbeere North, 74.4% in Mwea East and 68.8% in Embu West (Table 4.6). All the respondents in the survey field were able to recognize the presence of MWMV symptoms within their farms using an A4 –sized photographs of cucurbit plants infected by (MWMV (Table 4.6). The cause of the disease varied significantly within the sub-counties ($\chi2 = 13.6$; df = 4, P = 0.009).

Majority of the sampled respondents (49.5%), associated the cause of the disease to be due to weather changes and 16% believed the symptoms were due to pest's infestation. About 12.9% linked the symptoms with disease infestations, 6.2% thought the symptoms were due to nutrient deficiency. However a significant number of the sampled respondents (11.1%) did not know the cause of the disease (Table 4.6).

4.2.4 Farmer's Perceptions on MWMV Disease Problem, Rate of Spread and Cause of Spread

Famer's perception of the disease problem differed significantly within the sub-counties ($\chi2 = 40.8$; df = 12; P = 0.001 Table 4.7). Majority (52.8%) of the respondents perceived it as a minor problem, and 31.6% perceiving it as a severe problem. The spread of the virus disease on cucurbit farms across the seven sub-counties varied significantly ($\chi2 = 19.6$; df = 6; P = 0.003 Table 4.7) with the respondents from Kirinyaga East and Mbeere North sub-counties reporting the highest spread of 100%, 95.5% of the farmers in Kangundo and Matungulu, 90.6%, in Mwea East, 91.7% in Kirinyaga West, and 73.3% in Embu West sub-counties reported its spread.There is a significant difference amongst factors responsible for the disease spread across sub-counties ($\chi2$ = 30.3, df = 18, P = 0.003 Table 4.7). About 64.7% of the respondents did not know what was causing the spread of the disease. 25.2% associated the disease spread with weather, 9.2% thought the disease was spread by pest while 1.1% associated the viruses to be spreading the disease (Table 4.7). The disease spread across the seven sub-counties was rated as slow by 56.5%, fast by 42.9%, and very fast by 0.6% (Table 4.7).

Table 4.6: Farmer's Awareness of MWMV in their Farm, Symptoms Present, and Cause of the Disease in your Farms

	Embu county Sub- counties		Machakos County Sub-counties		Kirinyaga county Sub-counties					
	Embu West N = 55	Mbeere North N = 14	Kangundo N = 26	Matungulu N = 72	Kirinyaga East N = 11	Kirinyag West N = 12	Mwea East N=3	Total N= 229 Mean	χ2	P-Value
Farmers' awareness of MWMV disease Symptoms in their farm (%) Yes	64.8	85.7	92	90.3	100	100	74.4	82.4	22.9**	0.001
Farmers' awareness of Mosaic symptoms in their farm Yes	64.6	50	80	52.1	81.8	66.7	53.8	64.1	10.1^{ns}	0.12
Farmers' awareness of Severe leaf filiform symptoms on yourfarm Yes	6.3	35.7	33.3	25.4		45.5	23.1	4.5	17.4*	0.008
Farmers' awareness of chlorosis leaf symptoms in their farm Yes	58.3	64.3	68	57.1	63.6	83.3	56.4	64.4	4.0^{ns}	0.67
Farmers' awareness of dark green blisters on leaf in their farm Yes	50	64.3	44	69	81.8	91.7	51.3	64.5	15.7**	0.01
Farmers' awareness leaf distortion in their farm Yes	56.3	78.6	68	60.6	100	83.3	61	72.5	11.3^{ns}	0.78
Farmer's awareness of the presence of MWMV in their farm Yes	100	100	100	100	100	100	100	100	11.3^{ns}	0.78
What is the cause of the problem										
Pest Yes	21.4	25	38.5	13.6	-	-	13.8	16.1	10.1^{ns}	0.122
Disease Yes	-	-	7.7	29.5	9.1	20	24.1	12.9	9.3^{ns}	0.157
Weather Yes	61.5	100	38.5	44.2	18.2	50	34.5	49.5	11.1^{ns}	0.86
Nutrient deficiency Yes	-	-	9.1	5.7	9.1	20	-	6.2	6.8^{ns}	0.34
Not aware	-	-	60	18.2	-	-	-	11.1	13.6*	0.009

(Chi- square test and P < 0.05) @ *P < 0.05, **P < 0.001, shows significant difference; ns means not significant, - means no cause present

Table 4.7: Farmer's Perception of MWMV Disease Rate of Spread and Prevalence

	Embu county Sub-counties		Machakos county Sub-counties		Kirinyaga county Sub-counties					
	Embu Mbeere West North N = 55	N = 14	Kangundo Matungulu N = 26	N = 72	Kirinyaga East N = 11	Kirinyaga West N = 12	Mwea East Total N =39 .229	Mean	χ2	P-Value
Is the disease problem spreading in your farm Yes	73	100	95	95.5	100	91.7	90.6	90	19.6 *	0.003
What is causing the spread of the problem										
Pest	15.2	8.3	4.8	7.7	-	-	16.1	9.2		
Virus	-	-	-	3.1	-	-	-	1.1		
Weather	9.1	-	23.8	23.1	45.5	63.6	35.5	25.2	30.3 *	0.003
Not aware	75.8	91.7	71.4 66.2		54.5	36.4	48.4	64.7		
How do you rate the disease problem in your farm										
No problem	33.3	-	9.5	5.9	9.1	8.3	21.6	15.6		
Low/ minor problem	58.8	58.3	76.2	45.6	45.5	50	45.9	52.8	40.8**	<0.001
Severe	7.8	41.7	14.3	48.5	45.5	41.7	32.4	31.6		
The rate of the disease spread										
Slow	63.6	54.5	30	66.1	63.6	72.7	37.9	56.5		
Fast	36.4	45.5	70	32.3	36.4	27.3	62.1	42.9	16.6 ns	0.16
Very fast	-	-	-	1.6	-	-	-	0.6		

(Chi- square test and P < 0.05) @ *P < 0.05, **P < 0.001, shows significant difference; ns means not significant, - means no cause present

4.2.5 Farmer's Perception on the Disease Prevalence and Management Practices

There is significant difference across the sub-counties in regards to the seasonal prevalence of the disease ($\chi2 = 54$, df = 24, P = 0.001 Table 4.8). About 56% of the respondents perceived the symptoms of the disease to be more prevalent during the cold season and 33.1% during the hot season (Table 4.8). The infection of new cucurbit crops by the disease after planting varied significantly across the sub-counties ($\chi2 = 69.8$, df = 24, P = 0.001). Majority of the respondents (41.5%) across the sub-counties were not aware of when their newly planted cucurbit crops get infected with the disease, whereas 24.4%, 16.6% and 0.5% reported noting the symptoms more than three months, three months, two months, and one month after planting respectively. The management practices of the disease varied across sub-counties with majority of the respondents spraying chemicals, with 100%, 90%, 89.7%, 81.8%, 94.1%, 72.1% in Mbeere North, Kirinyaga West, Mwea East, Kirinyaga East, Kangundo, Matungulu and Embu West respectively. Use of cultural practices such traps and ashes in the management of the disease was also reported by 40% in Kirinyaga West, 26.2% in Matungulu, 23.5% in Kangundo, 22.2% Embu West, 10.3% Mwea East, and 9.1% Kirinyaga East. Meanwhile, 23.8% respondents in Embu West, 14.3% in Kangundo, 18.2% in Kirinyaga East, 6.6% in Matungulu, and 3.6% in Mwea East did not apply any control measure (Table 4.8)

Table 4.8: Farmer's Perceptions on the Disease Prevalence, Cause and Management Practices

	Embu County		Machakos County		Kirinyaga County					
	Sub-counties		Sub-counties		Sub-counties					
	Embu West N = 55	Mbeere North N = 14	Kangundo N = 26	Matungulu N = 72	Kirinyaga East N = 11	Kirinyaga West N = 12	Mwea East N = 39	Total 229 Mean	$\chi2$	P-Value
Seasonal prevalent of the disease										
Cold season	45.7	91.7	21.7	62.3	81.8	50	58.1	56		
Hot season	54.3	-	73.9	18.8	18.2	33.3	29	33.1		
Rainy season	-	-	-	4.3	-	-	-	1.6	54.0**	<0.001
Always	-	-	-	-	-	-	3.2	0.5		
Don't know	-	-	4.3	14.5	-	16.7	9.7	8.8		
When are new plants affected										
One month	2.9	-	-	-	-	-	-	0.5		
Two months	5.7	41.7	-	13	27.3	-	32.3	15		
Three months	20	25	4.3	15.9	9.1	25	19.4	16.6	69.8**	0.001
More three months	25.7	8.3	4.3	46.4	18.2	8.3	16.1	26.4		
Not aware	45.7	25	91.3	24.6	45.5	66.7	32.3	41.5		
Control measure										
Chemical (%)	71.4	100	94.1	72.1	81.8	90	89.7	85.5	10.8^{ns}	0.09
Cultural (%)	22.2	-	23.5	26.2	9.1	40	10.3	18.7	9.27^{ns}	0.15
No control	23.8	-	14.3	6.4	18..2	-	3.6	9.2	35.5^{ns}	0.22

(Chi – square test and P < 0.05) @ *P < 0.05, **P <0.001, shows significant difference, ns means not significant, - means no cause present

4.2.6 Correlation Analysis for Cucurbits Farmer Practices and MWMV Management

There is no correlation between farmer educational level and MWMV management, this indicate that education level did not play a significant role in determining disease control. Therefore, there are other underlying factors that influence farmer’s disease management (Table 4.9).A strong positive and statistically significant correlation ($r = 0.896$, $p < 0.01$) exists between years of farming experience and years of growing cucurbits, suggesting that those with more general farming experience also tend to have more specific experience with cucurbits. There is also a moderate positive correlation between chemical control use and efforts to manage the disease ($r = 0.605$, $p < 0.01$), indicating that respondents who attempted to manage the problem were more likely to use chemical methods. Interestingly, cultural control shows a statistically significant but negative correlation with chemical control ($r = -0.357$, $p < 0.01$), which may imply that respondents tend to prefer one control method over the other rather than using both simultaneously.

Additionally, the perception that weather causes the problem negatively correlates with the belief that disease is the cause ($r = -0.207$, $p < 0.05$) and with nutrient deficiency ($r = -0.192$, $p < 0.05$), suggesting different root cause of the problem among farmers. Overall, while most correlations are weak, a few statistically significant patterns offer insight into farmers' perceptions, experiences, and management strategies related to cucurbit production challenges (Table 4.9).

Table 4.9: Correlation Analysis Indicating Farmers' Practices and MWMV Management

	Education	**Farming Experience**	**years of cucurbit production**	**Knowledge Pest**	**Knowledge Disease**	**Knowledge Weather**	**knowledge Nutrient deficiency**	**cause _I don't know**	**Perception**	**Chemical Management**	**Cultural Management**
Education	1										
Farming Experience	-.054	1									
Years of cucurbit production	-.056	.896**	1								
Knowledge –Pest	-.116	.091	.017	1							
Knowledge Disease	-.004	-.110	-.096	-.152	1						
Knowledge Weather	.017	.108	.067	-.054	-.207*	1					
Knowledge Nutrient deficiency	.077	-.054	-.047	-.106	-.128	-.192*	1				
Causes I don't know	-.016	-.089	-.089	-.130	-.179	-.147	.259	1			
Perception	-.042	.024	.036	-.183*	.107	.031	-.057	-.236	1		
Chemical Management	.126	.131	.117	0.605	.074	-.046	.109	-.208	.044	1	
Cultural Management	.028	-.077	-.096	.148	-.018	.253**	-.109	-.091	-.078	-.357**	1

** shows statistically significant

4.3 Determination of the Influence of the Host Plant Species on Aphid Transmission Efficiency of MWMV to Cucurbit Crops

4.3.1 Symptoms Observed on the Test Plants for MWMV Transmission Using Aphids

Symptoms of MWMV observed in the greenhouse included leaf deformation, dark green blisters on leaf, vein clearing, Severity mosaic, mild mosaic, leaf yellowing, and stunted growth (Table 4.10)

Table 4.10: Symptoms Expressed in Cucurbits and Papaya Plant Species after MWMV Transmission with Aphids

Source of MWMV	Recipient plants species	No. of plants inoculated No. plants displaying symptoms	Symptoms expressed
Pumpkin	Pumpkin	9/9	LD, SM, LP
	watermelon	9/9	VC, SM, LB
	Zucchini	9/9	SG, LD, LB, VC
	Butter nut	9/9	LD, SM,LP
	Papaya	9/0	No symptom
Papaya	Papaya	9/9	SM, VC. LP
	Zucchini	9/9	LD, CV, LB
	Watermelon	9/0	No symptoms
	Butter nut	9/0	No symptoms
	Pumpkin	9/0	No symptoms

*Symptom description: SG:stunted growth; LD:leaf distortion; SM:severe mosaic; LB:leaf blistering; MM:mild mosaic; VC:vein clearing; LY:leaf yellowing; LM:leaf Mottling, LP:leaf puckering .

4.3.2 Incidence and Severity of MWMV Disease from Pumpkin and Papaya to other Cucurbit Species and Papaya Plants

The disease severity differed significantly between zucchini, papaya and the rest of the other cucurbit species namely pumpkin, butter nut and watermelon ($P< 0.05$). There were no significant deference in the disease incidence among the cucurbit species. The highest disease severity (4.0) was reported in zucchini species (Table 4.10). The mean disease incidence reported among the cucurbit species and papaya was 60%. There was no disease incidence reported from pumpkin to papaya and also no disease incidence was reported from papaya to watermelon, butter nut and pumpkin (Table 4.10). The presence of band size of 613bp for pumpkin and 315bp for papaya in the gel indicated presence of the virus in the sample (Figure 4.6). The disease symptoms observed in the greenhouse during the experiment were similar to those observed in the field during the survery (Table 4.10).

Table 4.11: MWMV Disease Incidence and Severity from Pumpkin to other Cucurbit Species and Papaya Plants

Source plant species	Recipient plant species	Disease incidence (%)	Disease severity
Pumpkin	Pumpkin	100	3
	Butter nut	100	3
	Zucchini	100	4
	Papaya	0	0
	Watermelon	100	3
Papaya	Papaya	100	3
	Zucchini	100	4
	Watermelon	0	0
	Pumpkin	0	0
	Butter nut	0	0
Mean		60	2.00
LSD (P = 0.05)		1.24	0.006

* Severity was computed by total number of score per the symptoms divided by total number of plants expressing symptom. Scale (0-4) Ndunguru and Rajabu (2004).

One month two weeks old cucurbit plants infected with MWMV from pumpkin source plant through aphid's transmission (Figure 4.4)

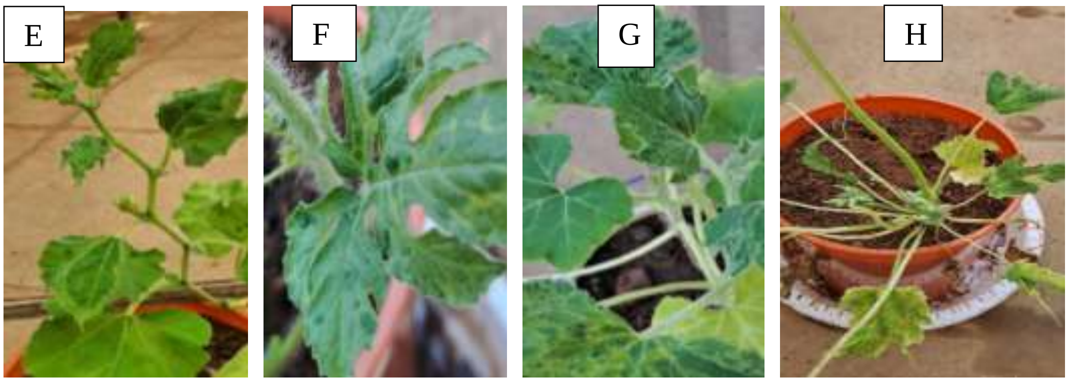

Figure 4.4: Moroccan Watermelon Mosaic Virus Disease Symptoms Observed One Month Two Weeks, from Pumpkin Isolate to Other Cucurbit Species.

E: leaf deformation, dark green blisters and leaf yellowing on butter nut leaf. **F**: leaf yellowing and dark green blisters on watermelon leaf. **G**: leaf deformation, leaf yellowing and dark green blisters on pumpkin leaf. **H**: stunted growth, and leaf deformation on zucchini leaf.

Papaya and zucchini plants infected with MWMV from papaya source plant through aphid’s transmission (Figure 4.6)

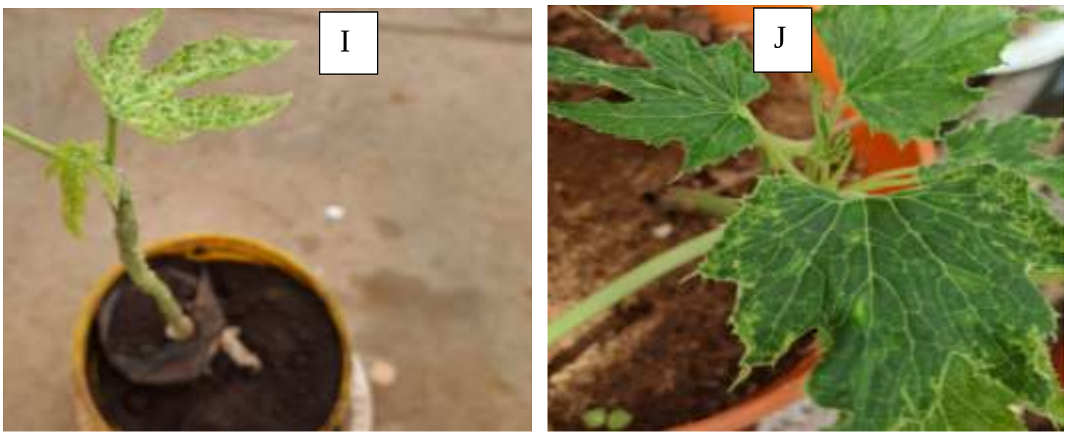

Figure 4.5: Symptoms Induced by Moroccan Watermelon Mosaic Virus Isolates from Papaya to Papaya and Zucchini.

M: leaf yellowing and vein clearing on papaya leaf. **N:** leaf yellowing and dark green blisters on zucchini leaf.

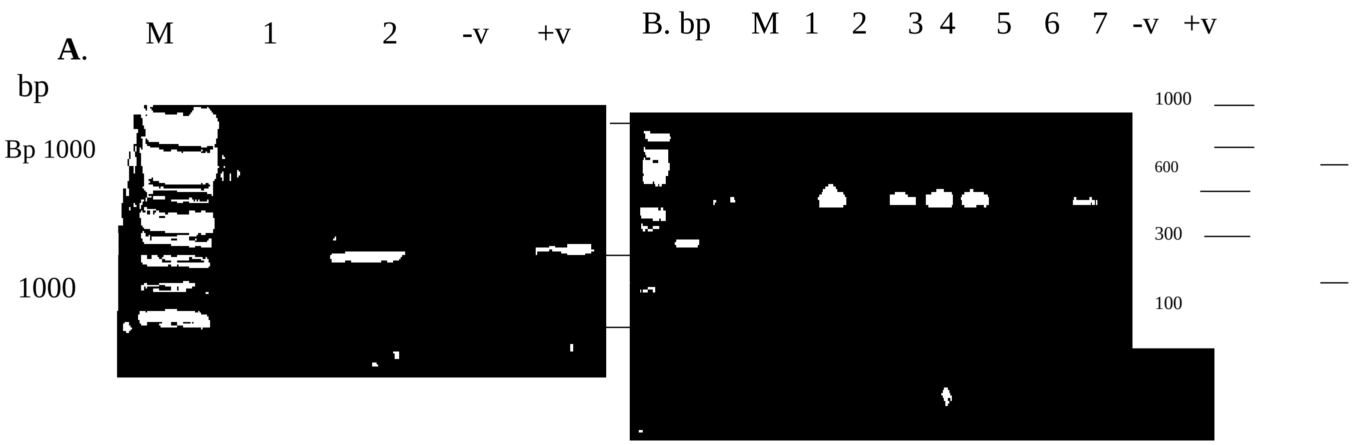

Figure 4.6: Gel Electrophoresis for Diagnostic Studies of MWMV in Collected Leaf Samples.

A, at 315 bp indicate presence of MWMV isolate from papaya to zucchini (2), B, at 315 bp indicate the presence of MWMV from papaya isolate from to papaya (1), 613 bp indicate presence of MWMV isolate from pumpkin to watermelon (4), from pumpkin to zucchini (5), butter nut (6), pumpkin (7), M indicate the 1kb plus DNA ladder. +v is the positive and –v is the negative control

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Viral diseases rank second to fungal diseases in terms of economic losses, posing a significant threat to plant growth and development, which ultimately leads to decreased crop yields and quality, thereby impacting food security (Godfray et al., 2010).

This report presents surveys conducted to determine the incidence, severity, and distribution of Moroccan watermelon mosaic virus disease affecting cucurbit crops in selected Counties in Kenya. The disease incidence and severity differed significantly between the sub-counties ($P < 0.05$).The high disease incidence may be due to mono cropping of pumpkin on extensive fields for market purposes, potentially facilitating rapid disease dissemination due to the elevated host density and expansive cultivated area as observed during the survey in Machakos county (Kangundo sub-county) and Kirinyaga county (Kirinyaga East sub-county) (Kumar et al., 2010; Piper et al., 1996). Also mono cropping enable rapid movement of vector between plants during their migratory flights while searching for an appropriate host a condition that may exacerbate the high disease incidence and severity (Kumar et al., 2010).

The high disease incidence could also be attributed to plant growth, which indicate that plant susceptibility to virus infection increases with age, as observed during the survey (Kone et al., 2017).This is in contrast to the cassava-ACMV pathosystem, which found that ageing cassava plants were less susceptible to virus infection (Fargette et al., 1993).Farmers' practices have a significant impact on viral infections in many crops; therefore, farming practices like weed presence, and volunteer cucurbit plants in and around the fields may be contributing to the rise in disease incidence (Anderson et al., 2004; Jones, 2009). Also farmers limited knowledge regarding the disease and its occurrence as observed during the survey could attributed to the increase in the disease incidence (Abdurahman et al., 2017; Gorfu and Woldegiorgis, 2013).Cropping seasons that overlap also influence disease incidence because the old maturing crop serves as a virus reservoir for the newly planted crop (Thresh, 2003).

The disease severity ranged from 3.8 in Matungulu sub-county to 1.6 low in Embu west sub-county, this may be attributed to farmers' lack of understanding concerning the causes and mechanisms of the disease spread which may impact the severity (Abdurahman et al., 2017; Gorfu and Woldegiorgis, 2013).The extensive distribution of MWMV confirmed through the PCR process clearly indicates its significant presence in pumpkin. Also several pumpkin samples obtained from Embu West sub-county exhibited no viral symptoms and were initially presumed to be virus-free; however, 2 out of 12 samples tested positive for infection, indicating a latent infection or maybe attributed to the plants being in the early stage of infection and yet to exhibit symptoms during the time of the survey (Kumar et al., 2010; Carrere et al., 1999).

In contrast, cucurbit crops such as butternut, zucchini and watermelon displayed MWMV symptoms yet the virus was not detected, additionally, pumpkin samples from Kirinyaga east and Kangundo sub counties displayed 100% disease symptoms while Kirinyaga west sub county exhibited 62.5% MWMV symptoms but the disease was not also detected. Similarly the lower number of samples detected in Matungulu, Mwea east, and Mbeere north sub-counties as compared to symptomatic samples could be as a result of other virus or an existence of a variant that may not have been detected by the primers used (Barbedo, 2016; Ghoshal and Sanfacan, 2015; Sokhandan et al., 2006; Bos, 1999).

Moroccan watermelon mosaic virus cause substantial damage to cucurbits crops globally, posing a serious production risk and early infection ultimately result in complete loss of yield leading to food insecurity (Davis et al., 2020). This study has shown that all the respondents were able to recognize the presence of MWMV disease symptoms in their various farms but were unable to identify the actual cause there by associating the symptoms to be caused by either pest, disease, weather and nutrition deficiency which validate previous report about African farmers lacking precise knowledge about diseases affecting crops (Auwal et al., 2015; Abang et al., 2014).The lack of knowledge could be a major hindrance to MWMV management in the county. There is no correlation between farmers’ knowledge and management practices of MWMV, in contrast to other study that have found correlation between farmers’ knowledge, and management practices (Lwin et al., 2012). Due to the limited knowledge, farmers' relied on their own or other farmers' experience to identify disease symptoms which could be a significant barrier to plant disease management. Therefore, training of farmers’ on simple techniques for distinguishing biotic factors from other abiotic factors will improve management (Birithia and Kuria, 2023). The lack of farmer’s knowledge about the disease may have influenced the disease management as 85% of the respondents control the disease by applying chemicals (Khan and Damalas, 2015).

There is a significant correlation between years of farming experience and years of growing cucurbits, suggesting that those with more general farming experience tend to have more specific experience with cucurbits. Majority of the respondents 64.7% were not aware about MWMV spread mechanisms there by associating the spreading with viruses and changes in the weather which may influence the disease spread (FAO, 2016; Strange and Scott, 2005). Majority of the respondents (64.7%) were not aware about the factors responsible for the spread of the disease symptoms in their farms therefore, improper identification of the spread agent could increase the disease level of spread and impede the control mechanism there by causing yield losses (Serge et al., 2019; Strange and Scott, 2005).

This study results indicate that majority of the respondents (85.5%) control the disease by spraying of chemical insecticides while (18.7%) used cultural control method. The application of insecticide to control plant diseases is one of the integrated management practices in controlling MWMV since it reduces the aphids’ population and distribution there by reducing the level of damage cause (Roy et al., 2014). Meanwhile, the application of chemical insecticides as a management strategy may be ineffective without comprehension of the role of aphids or other vectors in the transmission of the virus responsible for MWMV disease (Kalleshwaraswamy and Kumar, 2008). Moreover, effective insect control necessitates implementation prior to the manifestation of disease symptoms, which demands farmers' understanding of disease epidemiology (Schreinemachers et al., 2015).A significant proportion of respondents (41.5%) were unaware of the age at which their plants exhibited symptoms of MWMV disease, as the virus can infect the crop at any growth stage, with early infection potentially resulting in partial or complete yield loss (Davis et al., 2020). Majority of the respondents (56%) perceive MWMV symptoms to be more prevalent during the cold season there by attributing the disease to be caused by changes in the weather.

Understanding vector transmission efficiency is essential from an epidemiological perspective for comprehending pathogen dissemination and for formulating disease management models and control strategies. Aphids are significant pests that inflict direct damage to various crops by extracting plant sap and transmitting viral diseases (Singh and Singh, 2016) and they are the most important vectors transmitting about 30% of all plant viruses (Brault et al., 2010). Transmission of MWMV was done in four species of cucurbit crops namely pumpkin, watermelon, butter nut, zucchini and papaya crop from host plants pumpkin and papaya by aphids (*Myzus persicae*). The results of this study revealed the transmission of Moroccan watermelon mosaic virus from pumpkin to watermelon, butter nut, zucchini and pumpkin except papaya while transmission from papaya occurred to papaya and zucchini plants. This results confirmed pervious study of MWMV transmission from pumpkin source plant to watermelon, butter nut, zucchini, and pumpkin, this also validate the transmission of Moroccan watermelon mosaic virus from papaya to only papaya and zucchini (Mumo et al., 2021). The disease severity differ significantly with zucchini obtaining the highest 4.0. This indicate that MWMV symptom development is more severe in zucchini as compared to the other cucurbit species. Also since MWMV can be transmitted from pumpkin to other cucurbit species, through aphids transmission, controlling aphid population will help avoid the spread of the viral diseases in other cucurbit crops. Furthermore, intercropping of pumpkin to other cucurbit species should be avoided to help limit the disease spread.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study aimed to investigate the incidence, severity distribution and transmission efficiency of MWMV infecting cucurbit crops in selected counties in Kenya. It also assessed farmers knowledge, perception and management of Moroccan watermelon mosaic virus. This current study has reported the incidence of the virus in all the three counties and seven sub-counties; namely Machakos county (Matungulu and Kangundo sub-county), Kirinyaga county (Mwea East, Kirinyaga East, and Kirinyaga West counties), and Embu county (Mbeere North and Embu West sub-counties). This indicates that the virus is gradually spreading in the country due to the confirmation of MWMV presence in the three counties through the PCR process. The disease severity was higher in Matungulu and Kangundo sub-counties (Machakos county) as compared to the other two sampled countites and sub-counties. MWMV was found to be distributed in four sub-counties with Matungulu obtainting the highest followed by Embu West. This indicate that the virus is affecting cucurbits production more in these sub-couinties as compared to the other sub-counties. The difference between the symptomatic samples collected from the field and the number of positive samples recorded indicate that those negative samples symptoms were caused by diferent diseases.

This study demonstrated that farmers were knowledgeable about MWMV disease symptoms present in their farms, perceived it to be a problem and majority associated the disease symptoms to be caused by changes in the weather. However, there was a significant number who did not know the cause of the symptoms of the disease in their farms. Farmers also perceived the disease symptoms to be more prevalent during the cold seaseon with majority of them managing it by spraying chemicals. Moroccan watermelon mosaic virus was found to be transmitted efficiently from papaya to zucchini through aphids and not to the another cucurbit species. Additionally, alphids transmission efficiency of Moroccan watermelon mosaic virus from pumpkin to other cucurbit crop species such as watermelon, butter nut, zucchini and pumpkin was confirmed.

6.2 Recommendations

To futher understand the incidence, severity, and distribution, of MWMV, farmer's knowledge, perceptions, management and aphids transmission efficiency, the study makes the following recommendation.

- Extensive survey in the country to determine the level of incidence and distribution of Moroccan watermelon mosaic virus
- Farm-level training to enhance farmers' knowledge and management of plant viruses diseases
- Since MWMV can be transmitted from papaya to zucchini, intercropping of papaya with zucchini crop should be avoided. Further determination of aphids transmission of MWMV from zucchini to papaya is recommended in future.

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APPENDICES

Appendix I: Disease Severity and Incidence Data Form

LOCATION				DATE			
County				FIELD No.			
Sub-county				No. of cultivars			
Longitude				Dominant cultivar			
Latitude				Source of seed			
Altitude (m)				No. of Plants			
Plant No.	MWMV disease severity (0-4 scale) 0: no symptoms 1: mild mosaic 2: Slight deformed leaf 3: Sevrel mosaic / deformation 4: Stunted growth	Symptoms on leaves	Symptoms on fruits	Symptoms on stem (deformation, and Mosaic)	Symptoms on petioles (Mosaic, and deformation)	Plant vigour Size and growth	Sample No.
1							
2							
3							
4							
5							
6							
7							
8							
9							
Mean							

Appendix II: Sample Questionnaire used to Determine Farmers Knowledge, Perception and Management of MWMV

A researcher's team from Jomo Kenyatta University of Agriculture and Technology (JKUAT), undertook Moroccan watermelon mosaic virus infecting cucurbits research. The study seek to assess farmer's knowledge, perception and management of MWMV in selected counties in Kenya. The study is also trying to map the occurrence of the disease for improved disease management strategies. This is intended to deepen our understanding of the disease and deployed evidence-based control and management measures for improved cucurbits production.

We would like to emphasize that your participation is voluntary. If you consent and later decide that you would like to withdraw, you are free to do this at any point.

I confirm that I have read (or someone has read for me) this form, and I have understood the purpose of the research, and what my participation will involve. I agree to participate in the project described.

Name of participant…………………………

Signature………………………..Date……………

If No, what are your reservations?

………………………………………………………………………………………

………………………………………………………………………………………

…………………………

Name ……………………………………………..Sign………………………

SECTION 1: CHARACTERISATION OF THE HOUSEHOLD

2.4 Age of respondent: [1] Less than 18 years ( ) [2] 18-24 years ( ) [3] 25-34 years ( ) [4] 35-44 years ( ) [5] 45-54 years ( ) [6] 55-65 years ( ) [7] above 66 years ( )

2.5 Highest educational level of respondent: [1] school up to 5 years (1-4) ( ) [2] school up to 6-9 years (class 5-9) ( ) [3] Drop out secondary ( ) [4] Secondary school ( ) [5] Technical training ( ) [6] Undergraduate ( ) [7] post graduate ( ) [8] Other ________________________

SECTION 2: CUCURBIT CROP PRODUCTION

3.1 Land size owned ____Acres

3.2 Land size rented (If any) ___________________________________Acres

3.3 Total land size ____Acres

3.4 What is the area currently under cultivation of cucurbits _____________ Acres?

3.5 Which cucurbit crop do you grow? (Tick all applicable): [1] Cucumber ( ) [2] watermelon ( ) [3] Butternut ( ) [4] Pumpkin ( ) [5] Zucchini (Courgette) ( ) [6] Other1 _________________________

3.6 Reason for growing the cucurbit? [1] Subsistence ( ) [2] Subsistence and market ( ) [3] Market ( )

3.7 If for market what percentage of the cucurbits do you sell? [1] 0-24% ( ) [2] 25-50% ( ) [3] 51-75% ( ) [4] 76- 100% ( )

3.8 Age of the crop (months): [1] One month ( ) [2] Two months ( ) [3] Three months ( ) [4] Three months and above ( )

3.9 What are some of the cropping systems you often carry out in the cucurbits? (Tick all applicable [1] Crop rotation ( ) [2] Mixed cropping ( ) [3] Mono cropping ( ) [4] Shifting cultivation ( ) [5] Others

____

3.10 If for intercrops/ mixed cropping name the crops intercropped with cucurbit? [1] _________________ [2] ____________________ [3] __________________ [4] _____________

3.11 How many years have you been growing this cucurbit? [1] Less than a year ( ) [2] One year ( ) [3] Two years ( ) [4] More than two years ( )

3.12 Source of planting materials: [1] Own production ( ) [2] Agro- stockiest ( ) [3] Local trader ( ) [4] Fellow farmer/ neighbor ( ) [5] County/ National government ( ) [6] NGOs/ religious organization ( ) [7] Other _____________________________________

3.13 How much income is generated after sale of your cucurbit crops: [1] 100- 1000 ksh ( ) [2] 1001- 2000 ksh ( ) [3] 2001- 3000 ksh ( ) [4] 3001- 4000 ksh ( ) [5] 4001-5000 ksh ( ) [6] 5000 above

3.0 FARMER'S KNOWLEDGE OF MOROCCAN WATERMELON MOSAIC VIRUS DISEASE ON CUCURBIT CROPS

4.4 Have you ever observed the following symptoms in your cucurbits (please refer to the provided photos and tick all which apply): [1] Mosaic on leaf ( ) [2] Severe filiform on leaf ( ) [3] Striking interveinal chlorosis on leaf ( ) [4] Raised dark green blisters on leaf ( ) [5] Leaf distortion ( ) [6] Leaf deformation ( )

4.5 Do you have any of the above problem in your farm now? [1] Yes ( ) [2] No ( )

4.6 If yes, what do you think causes the problem? [1] Pest ( ) [2] Disease ( ) [3] Weather ( ) [4] Nutrient deficiency ( ) [5] I don't know ( ) [6] Moroccan watermelon mosaic virus ( ) [7] Other ____________________________

4.7 If the problem is associated with a pest, please name them: [1] ________________________ [2] __________________________ [3] _____________________ [4] _____________________

4.8 If the problem is associated / caused by disease, please name them: [1] ________________ [2] ________________________ [3] ______________________ [4] ______________________

4.9 If the problem is associated/ caused by weather, name the type of weather:

[1] Drought ( ) [2] Floods ( ) [3] _______________ [4] ______________

4.10 If the problem is associated/ cause by nutrient deficiency name the nutrients:

[1] ___________________ [2] _________________ [3] ________________ [4] ______________

4.11 During the year, when are the symptoms/problem most prevalent? [1] During cold season ( ) [2] During hot season ( ) [3] During rainy ( ) [4] Always ( ) [5] I don't know ( )

4.12 When do you usually observe the symptoms above on newly sown plants? [1] Within one month ( ) [2] Within two months ( ) [3] Within three months ( ) [4] More than three months ( ) [5] I don't know

4.13 Are there other crops in your farm with these symptoms? [1] Yes ( ) [2] No ( )

4.14 If yes, name the crops: [1] ___________________________ [2] ___________________

[3] ________________________ [4] _________________________

<u>4.0 FARMER PERCEPTION OF MOROCCAN WATERMELON MOSAIC VIRUS</u>

5.1 On a scale of 1-3, how do you rate the problem caused by MWMV in cucurbit production on your farm? [1] Not a problem ( ) [2] Moderate problem ( ) [3] Severe problem ( )

5.2 2 Is the disease spreading in your farm? [1] Yes ( ) [2] No ( )

5.3 If yes, what is the rate of spread? [1] Slow ( ) [2] Fast ( ) [3] Very fast ( )

5.4 What do you think is spreading the disease? [1] Pest ( ) [2] Virus ( ) [3] Weather changes ( ) [4] Others ________________________________ [5] I don't know ( )

5.5 What is the proportion of your cucurbits fields affected by this disease? [1] None ( ) [2] One quarter (25%) ( ) [3] One half (50%) ( ) [4] Three quarter (75%) ( ) [5] Entire field (100%) ( )

5.6 How do you rate cucurbit yield losses associated with the problem? [1] 0-5 % ( ) [2] 6-25% ( ) [3] 26-50 % ( ) [4] 51- 75% ( ) [5] 76- 100% ( )

5 .7 Have symptoms of this problem been reported in other farms in your area? [1] Yes ( ) [2] No [3] I don't know

5.0 MANAGEMENT OF MOROCCAN WATERMELON MOSAIC VIRUS IN CUCURBITS

6.1 If you know or you have seen the problem, have you tried to manage /control it? [1] Yes ( ) [2] No ( )

6.2 If yes, what control measures do you / have you used? [1] Chemical ( ) [2] Cultural ( ) [3] Biological ( ) [4] Other ______________________________

6.3 If cultural control, which of the following methods do you use? [1] Crop rotation ( ) [2] Resistant varieties ( ) [3] Weed control ( ) [4] Sanitation (removal of plant debris and infected plant) ( ) [5] Removal of alternative host (other crop that the pathogen can feed/ live on) ( ) [6] Others ___________________________________

6.4 If biological control, which of the following methods do you use? [1] Predators (other insect that are harmless to your crop) ( ) [2] Bio-pesticide (the used foliar spray, pheromone traps) ( ) [3] Others ________________________________

6.5 If chemical control, name the chemicals that you used: [1] ___________________________ [2] __________________________________ [3] ______________________________________

6.6 What is the most effective method of controlling the problem? [1] Chemical ( ) [2] Cultural ( ) [3] Biological [4] Resistant variety [5] Biological and Resistant variety ( ) [6] Chemical and Cultural

6.7 Have you spoken to any agricultural extension officer or any agricultural expert about the symptoms? [1] Yes ( ) [2] No ( )

6.8 If yes, what was their recommendation?

___

6.9 If yes, have you ever tried the recommendation from the extension officer/ agricultural expert? [1] Yes ( ) [2] No ( )

6.10 If yes, how effective was the recommendation? [1] Very effective ( ) [2] Less effective ( ) [3] Not effective ( )

6.11 Is there any other information you would like to share? [1] Yes ( ) [2] No ( )

6.12 If yes, what is the information?

___

___

___

Thank you for taking your time to respond to these questions