ISSN No:-2456 –2165

Distribution of Cassava Mosaic and Cassava Brown Streak Diseases in Agro-Ecological Zones of Lower Eastern Kenya

I. N. Koima , C.O. Orek , S.N. Nguluu
Department of Dryland Agriculture, School of Agriculture & Veterinary Sciences,
South Eastern Kenya University, P.O. BOX 170-90200, Kitui, Kenya

Abstract: Cassava mosaic Begomoviruses (CMBs) and cassava brown streak viruses (CBSVs) respectively cause cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Transmitted by whitefly vector (Bemisia tabaci), both diseases significantly inhibit cassava production in Kenya. This study examined the prevalence and distribution of CMD and CBSD in different agro-ecological zones (AEZ) of lower Eastern Kenya through a multi-stage sampling survey. Sampling involved stopping at regular pre-determined intervals of about 15 to 20km between farmers' fields along transect in each zone. Thirty plants were randomly sampled along diagonals of each field to determine both disease incidences. General results revealed 73% CMD and 53% CBSD prevalence in lower Eastern Kenya. Specifically, both disease incidences were high in LM4 (68% CMD and 26% CBSD), followed by UM4 (55% CMD and 19% CBSD) and least in LM5 (30% CMD and 8% CBSD). Whitefly (Bemisia tabaci) infestation significantly and positively correlated with CMD and CBSD incidences further indicating considerable contribution of the vector in spreading both diseases. Molecular diagnostics performed on leaves of randomly selected plants detected ACMV that cause CMD and CBSV that causes CBSD. High distribution of CBSD and CMD in lower Eastern Kenya as assessed in this study could potentially be integrated in future CMD and CBSD resistance breeding or virus management programs within the region.

Keywords:-Agro-Ecological Zones; CMD; CBSD; Bemisia Tabaci

I. INTRODUCTION

The food crop cassava (*Manihot esculenta* Crantz) provides a cheap source of carbohydrates for over 700 million people worldwide particularly in the tropics [1]. The crop is rated fourth in importance after rice, maize and wheat in developing countries [2]. Annual cassava production is estimated at 662,405 MT fresh roots against an estimated annual demand of 301,200MT dry cassava in Kenya [3]. Cassava production is affected by many biotic constrains of which cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are of major threats each causing up to 100% economic damage on local susceptible cultivars [4].

Cassava mosaic disease is caused by cassava mosaic Begomoviruses (CMBs) of family *Geminiviridae*. Among the nine CMB species, African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV) and East African cassava mosaic virus – Uganda variant (EACMV-Ug) are the most prevalent in East Africa [5]. Across the tropics, CMD cause an estimated loss of over US $ 2.4 billion per annum [4]. These losses result from damage to the above ground parts characterized by yellow to pale green chlorotic mosaic of leaves, commonly accompanied by distortion and cramping. In several cases, the CMD infected plant becomes stunted and the petioles immediately below the shoot tip may be angled downwards and occasionally may become necrotic, shrived and abscised [6].

Cassava brown streak disease (CBSD) is caused by two distinct virus species; cassava brown streak virus (CBSV) and Uganda cassava brown streak virus (UCBSV) belong to genus *Ipomovirus* and family *Potyviridae* [7-9]. Both viruses are commonly referred to as CBSVs [10]. CBSD can cause between 70 - 100% yield loss in cassava, attributed to damage on above ground parts characterized by leaf chlorosis, and stem lesions with complete dieback as well as spoilage of roots due to dry corky necrotic rot on the starchy tissues, reducing the root size and causing spitting and constriction on roots [11-12]. The severity of both diseases depends on the host genotypes and is usually more severe on susceptible than tolerant or resistant genotypes [13].

According to reference [12], CBSD reduces yields of the most sensitive cassava cultivars by 70% as well as induce necrosis of roots which render them unpalatable and unmarketable while tolerant cultivars are less severely affected with no or little effect on the root yield or quality. CMD reduced the number of tuberous roots and the root yield by 68% and with 50% respectively in local Ugandan cultivar, Ebwanateraka,

with infected plants giving no root yield in severe infections [14]. CBSVs and CMBs interact synergistically in dual infection causing severe disease symptoms in local landraces [6]. Since the first report [15] CMD and CBSD was known to be most prevalent in coastal East Africa below 500m above sea level [16]. It was rarely observed above 1000m above sea level [17]. Recent reports indicates a wide spread occurrence of the two diseases in all cassava growing areas of countries including Kenya, Malawi, Mozambique, Tanzania and Uganda [18]. Other countries known to occur include Burundi, Democratic Republic of Congo, Rwanda and Zambia [19].

Transmission of CMBs and CBSVs from one plant to another is reported to occur through propagation of infected cuttings, grafting CMBs and or CBSV-free with infected cuttings and natural spread [20]. Both CMBs and CBSVs are transmitted by the whitefly vector, *Bemisia tabaci* [21] with other arthropods such as *Bemisia afer* and *Aleurodicus dispersus* also transmitting the viruses in low rates [22]. Transmission and distribution of CBSD and CMD in Kenya has been reported [22], however, distribution of the two viral diseases specifically in agro ecological zones within lower Eastern Kenya is unknown. The present survey was thus designed to determine prevalence and distribution of CBSD and CMD in relation to *Bemisia tabaci* population in different agro-ecological zones (AEZs) of lower Eastern Kenya. The AEZs were lower midlands 4 (LM4), lower midlands 5 (LM5) and upper midlands 4 (UM4).

II. MATERIALS AND METHODS

A. Survey Areas

The survey areas where sampling was done were systematically sampled according to the available data of cassava production, where the disease under study has caused serious problems and diversity in terms of AEZs. Multi-stage sampling survey was conducted between April and May 2017 in LM4, UM4 and LM5 to determine the CMD and CBSD incidence and prevalence as well as associated whitefly (*B. tabaci*) counts.

B. Sampling Procedure and Molecular Diagnostics

Sampling was done using procedures described by reference [22], following the existing AEZ boundaries that were chosen randomly. Farmers in each AEZ were identified using systematic sampling. This involved stopping at regular pre-determined intervals of about 15 to 20 km (to allow for wide coverage of the survey area) between farmers' fields in each sampling location. Data was collected on cassava fields or farms with 6 to 9 months old plants. This is the preferred feeding stage by *B. tabaci* and when both CBSD and CMD symptoms are clearly visible [22]. Plants were selected along representative transects of the field at the opposite ends and the center of the diagonals of the cassava fields [6]. Sampling was conducted by visually inspecting cassava plants for presence of typical virus disease symptoms.

The CMD and CBSD incidences were calculated as a percentage of symptomatic plants to the total number of plants assessed in a field [23]. Qualitative description of both disease symptoms were used to score CMD severity [24] and CBSD severity [17]. Prevalence was determined as the proportion in percentage of production unit (farmer field) in which the disease symptoms were observed [22]. A total of 30 plants were randomly chosen along the diagonals of each field to determine both CMD and CBSD incidence. Leaf and shoot severity for both diseases were visually scored based on a scale of 1 to 5 where 1 signified no symptoms and 5 indicated most severe symptoms including severe mosaic, distortion of three-thirds of leaflets, twisted and misshapen leaves for CMD [24] and defoliation with pronounced stem lesions and dieback for CBSD [17; 25]. Adult whitefly population were manually counted and averaged from the top five fully expanded leaves of a representative shoot on each of the 30 cassava plants [22; 23; 26].

Polymerase chain reaction (PCR) and Reverse transcriptase PCR (RT-PCR) were respectively applied for detection of CMBs and CBSVs. For PCR, genomic DNA was extracted as described by reference [27], while for RT-PCR, total RNA was first isolated using the modified pine tree method [28-29] and then cDNA synthesized from the RNA using Bio-Rad's iScript cDNA Synthesis Kit. Prior to diagnosis, concentration and integrity of each DNA and RNA sample was respectively confirmed on NanoDrop ND-1000 and 1% agarose electrophoresis. Primers for diagnosing CMBs (ACMV & EACMV) and CBSVs (CBSV and UCBSV) were sourced from literature [30].

C. Data Analysis

Data on incidences, severity and *B. tabaci* population were subjected to analysis of variance and means between populations separated by least significant difference (Lsd) at $P \leq 0.05$ using SAS software version 9.0. Pearson correlation coefficients were also computed using the same software. The PCR and RT-PCR conditions were adopted from reference [30] and amplicons separated on 1.5% agarose gel electrophoresis.

III. RESULTS

A. Foliar Symptoms for Cmd and CBSD

Symptoms typical of CBSD were observed on cassava leaves including yellow vein banding, expressed mainly on the lower, older leaves and chlorosis which occurred along the secondary and tertiary veins, giving a feathery appearance (Fig. 1a & b)

while CMD symptoms comprised of yellow to pale green chlorotic mosaic, stunting growth on the severely affected plants and leaf curling (Fig. 1c & d). Generally, in all cassava fields, CMD symptoms were distinct from CBSD symptoms, as leaves from CBSD infected plants had little or no distortion. Occurrence of spiraling white fly (*Aleurodicus dispersus*) was recorded during the survey (Fig. 1e & f).

B. Prevalence of CBSD and CMD

The foliar symptoms comprised CMD and CBSD indicating the presence of the diseases in different AEZs of lower Eastern Kenya. Varied prevalence per agro-ecological zone LM4, UM4 and LM5 was observed where LM4 had 73%, followed by UM4 at 59% and LM5 37% for CMD, while CBSD prevalence of 28%, 22% and 10% was respectively recorded in LM4, UM4 and LM5 (Table 1). Analysis indicated significant difference ($P \leq 0.05$) in CMD prevalence between LM4 and LM5 and between UM4 and LM5. Although CMD prevalence between LM4 and UM4 did not significantly vary, LM4 exhibited relatively higher prevalence at 73% compared to 59% of UM4 (Table 1). CBSD prevalence was significantly different ($P \leq 0.05$) between LM4 (28%) and LM5 (10%) and between UM4 (22%) and LM5 (10%) (Table 1).There was no significant difference in CBSD prevalence between LM4 (28%) and UM4 (22%). Generally CMD exhibited higher prevalence (~56%) compared to lower (20%) CBSD prevalence (Table 1).

C. Incidence, Severity & Occurrence of B. tabaci in AEZs

CMD and CBSD incidences and severity varied significantly ($P \leq 0.05$) across AEZs surveyed (Table 2). Generally, CMD, CBSD and whitefly population were found to be significantly higher ($P \leq 0.05$) in LM4, followed UM4 and least in LM5 (Table 2). Specifically, significant variation ($P \leq 0.05$) in CMD incidence was noted between LM4 and LM5. For instance, the mean CMD incidence recorded in LM4 was 68% with most of the farms surveyed recording incidences more than 60% and a severity of between 3 and 5 while, LM5 recorded mean CMD incidence of 30% with most of the farms recording CMD incidence less than 50% and severity of between 2.0 and 3.0 (Table 2).

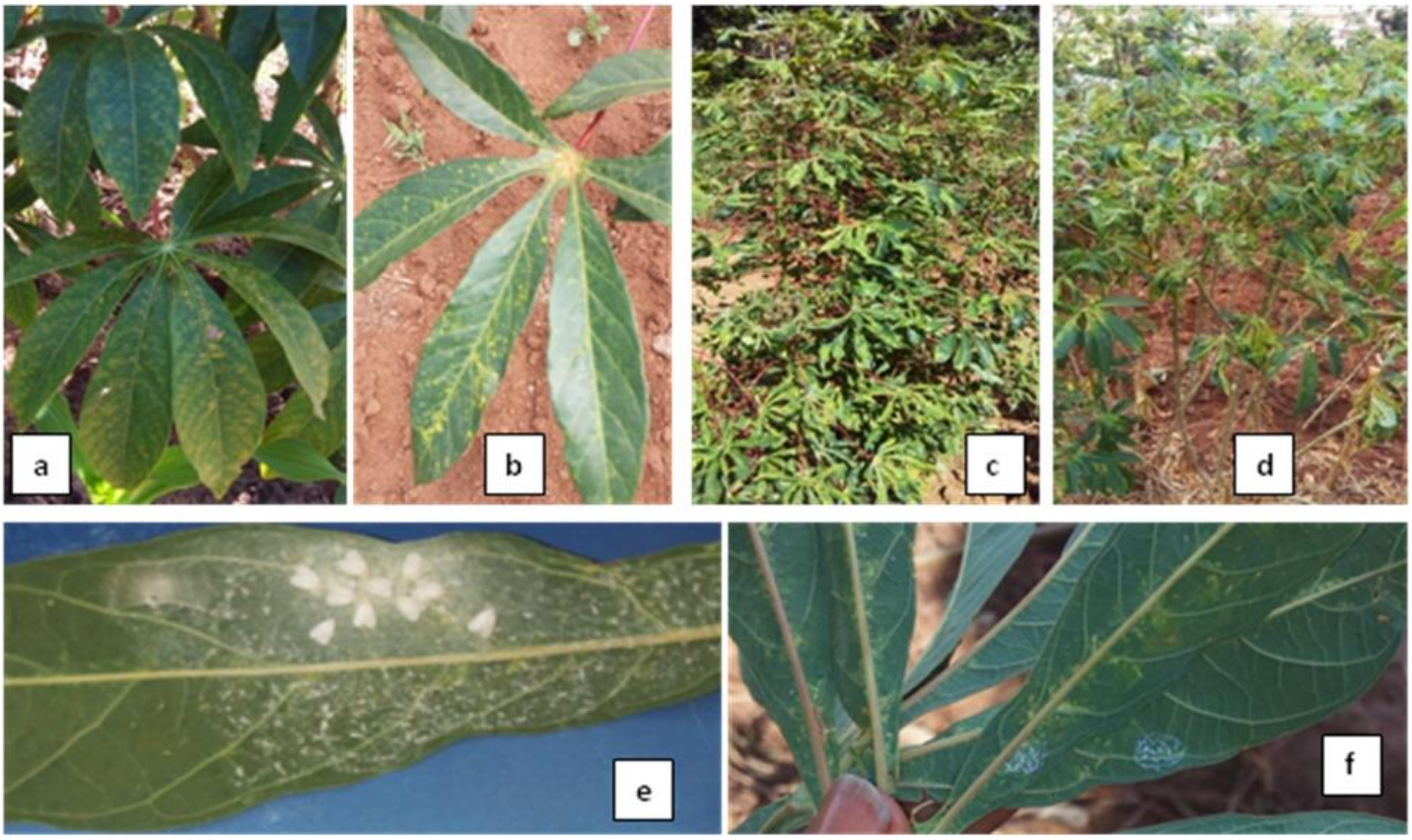


Fig. 1: Plants showing CBSD and CMD symptoms in the farmers' field: Fig. 1a & b: chlorotic spots along the secondary and tertiary vein of the leaves of a plant attacked by CBSD. Fig. 1c & d: pale green to yellow mosaic on the leaves of plants infected by CMD. Fig. 1e: Spiraling whitefly (Aleurodicus dispersus) underside the leaf of a cassava plant and Fig. 1f: white spiral mark showing evidence of presence of the spiral whitefly.

ISSN No:-2456 –2165

AEZs	Farm #	No. of plants	CMD-P (%)	CBSD-P (%)
LM4	1	30	100	80
	2	30	100	0
	3	30	80	30
	4	30	100	10
	5	30	100	50
	6	30	100	50
	7	30	100	40
	8	30	0	0
	9	30	50	20
	10	30	0	0
Mean-LM4			**73.0**	**28.0**
UM4	11	30	100	20
	12	30	50	50
	13	30	80	40
	14	30	100	50
	15	30	100	0
	16	30	40	0
	17	30	20	20
	18	30	0	0
	19	30	0	0
	20	30	100	40
Mean-UM4			**59.0**	**22.0**
LM5	21	30	50	0
	22	30	0	0
	23	30	50	0
	24	30	100	20
	25	30	10	0
	26	30	100	80
	27	30	20	0
	28	30	20	0
	29	30	20	0
	30	30	0	0
Mean-LM5			**37.0**	**10.0**
Grand Mean			***56.33***	***20***
(Lsd) at P≤0.05			***15.63***	***9.35***

Table 1: Cassava Mosaic and Cassava Brown Streak Disease Prevalence (P) in Different Agro-Ecological Zones (Aezs) of Lower Eastern Kenya.

Significant variations between UM4 and LM5 was also observed with UM4 showing on average 55% CMD incidence with most farms showing more than 80% CMD and severity of between 3.0 and 5.0 while LM5 had CMD incidence of 30% and only one farm (#24) recording 100% with severity of 5.0 (Table 2). The 68% CMD incidence in LM4 was relatively higher than 55% of UM4, although the two AEZs did not substantially vary.

The CBSD incidence was not significantly different between LM4 (26%) and UM4 (19%). Most areas surveyed in both zones recorded CBSD incidence ranging 20% to 60% (Table 2). Significant CBSD incidence difference (P≤0.05) between LM4 and LM5 and UM4 and LM5 was analyzed. Most farms in both UM4 and LM4 showed 20% to 60% CBSD incidence while 80% (8) farms under LM5 were CBSD-free i.e. showed 0% incidence and mean severity of 1.2 (Table 2). Mean CMD severity was significantly different (P≤0.001) between LM5 (2.4) and LM4 (3.4). No significant difference was noted between LM4 (3.4) and UM4 (2.8). Also, a significant difference (P≤0.05) in mean CBSD severity was recorded between LM5 with mean CBSD severity score of 1.2 and UM4 with mean CBSD severity score of 1.9 and, between LM4 (1.8) and LM5 with mean severity score of 1.2 (Table 2).

Three species of whitefly vectors were identified in most fields. These were *Bemisia tabaci, Bemisia afer and Aleurodicus dispersus* (Fig. 1e & f). Of the three, *B. tabaci* was the most abundant vector in all fields. The mean total adult *B. tabaci* per plant differed significantly (P≤ 0.01) across the agro-ecological zones with the vector being more abundant in LM4 and Um4 (2- 3 per plant), wider variations (0-3) were counted in LM5 (Table 2).

D. Correlations Among Parameters.

There was a significant (P≤ 0.05 or P≤ 0.01) and positive correlations between disease incidence, severity and whitefly population (Table 3). For example, the number of *B. tabaci* positively correlated with CMD incidence (r= 0.672), CMD severity (r= 0.684), CBSD incidence (r= 0.713) and CBSD severity (r= 0.664) (Table 3).

ISSN No:-2456 –2165

AEZs	Plant #	CMD-I (%)	CMD - S	CBSD-I (%)	CBS - S	*B. tabaci*
LM4	30	100	4	60	3	2
	30	100	4	0	1	1
	30	60	5	30	2	2
	30	60	4	20	2	2
	30	100	4	30	2	3
	30	100	4	50	2	2
	30	100	4	40	2	3
	30	0	1	0	1	1
	30	60	3	30	2	3
	30	0	1	0	1	1
Means – LM4		**68.0**	**3.4**	**26.0**	**1.8**	**2.0**
UM4	30	100	5	20	2	3
	30	50	2	60	3	2
	30	80	3	30	3	2
	30	100	5	40	3	3
	30	100	4	0	1	1
	30	20	2	0	1	0
	30	0	1	20	2	1
	30	0	1	0	1	0
	30	0	1	0	1	0
	30	100	4	20	2	3
Means - UM4		**55.0**	**2.8**	**19.0**	**1.9**	**1.5**
LM5	30	50	3	0	1	3
	30	0	1	0	1	0
	30	50	3	0	1	1
	30	100	5	20	2	3
	30	10	2	0	1	0
	30	20	2	60	2	3
	30	10	2	0	1	0
	30	30	3	0	1	1
	30	0	1	0	1	0
	30	30	2	0	1	0
Means - UM5		**30.0**	**2.4**	**8.0**	**1.2**	**1.1**
Grand Mean		**51**	**2.87**	**1.767**	**1.63**	**1.53**
(Lsd) at P≤0.05		**15.67**	**0.52**	**7.86**	**0.37**	**0.37**

Table 2: Disease (CMD & CBSD) Incidence (I) and Severity (S) and mean *B. tabaci* count in different agro-ecological zones of lower Eastern Kenya.

	CMD-I	CMD-S	CBSD-I	CBSD-S	*B. tabaci*
CMDI	1				
CMDS	.931**	1			
CBSDI	.380*	.370*	1		
CBSDS	.311	.372*	.831**	1	
B. tabaci	.672**	.684**	.713**	.664**	1

**Significant at P≤0.05 level; **Significant at P≤0.01 level*

Table 3: Correlation Analyses Among CBSD and CMD Incidence, Severity and the Number of Adult Whitefly (*B.* Tabaci)

PCR for CMBs and RT-PCR for CBSVs carried out on ten randomly selected plants from each AEZ, produced varied results. For instance of the two CMBs screened, ACMV was amplified only on two plants (20%) in UM4 with no detection under LM4 and LM5 while EACMV was not amplified in all AEZs (Table 4; Fig. 2 and 3.0). Of the two positive control plants, one exhibited multiple EACMV PCR bands (Fig. 3). For CBSD, UCBSV was not detected in all AEZs while CBSV was detected in 60% of plants under LM4, 70% plants in UM4 and 40% plants under LM5 (Table 4; Fig. 4).

AEZs	Plants tested	Positive for CMBs		Positive for CBSVs	
		ACMV	EACMV	CBSV	UCBSV
LM4	10	0	0	6 (60%)	0
UM4	10	2 (20%)	0	7 (70%)	0
LM5	10	0	0	4 (40%)	0

Table 4: Molecular detection of CMBs and CBSVs in randomly selected plants across agro-ecological zones

IV. DISCUSSION

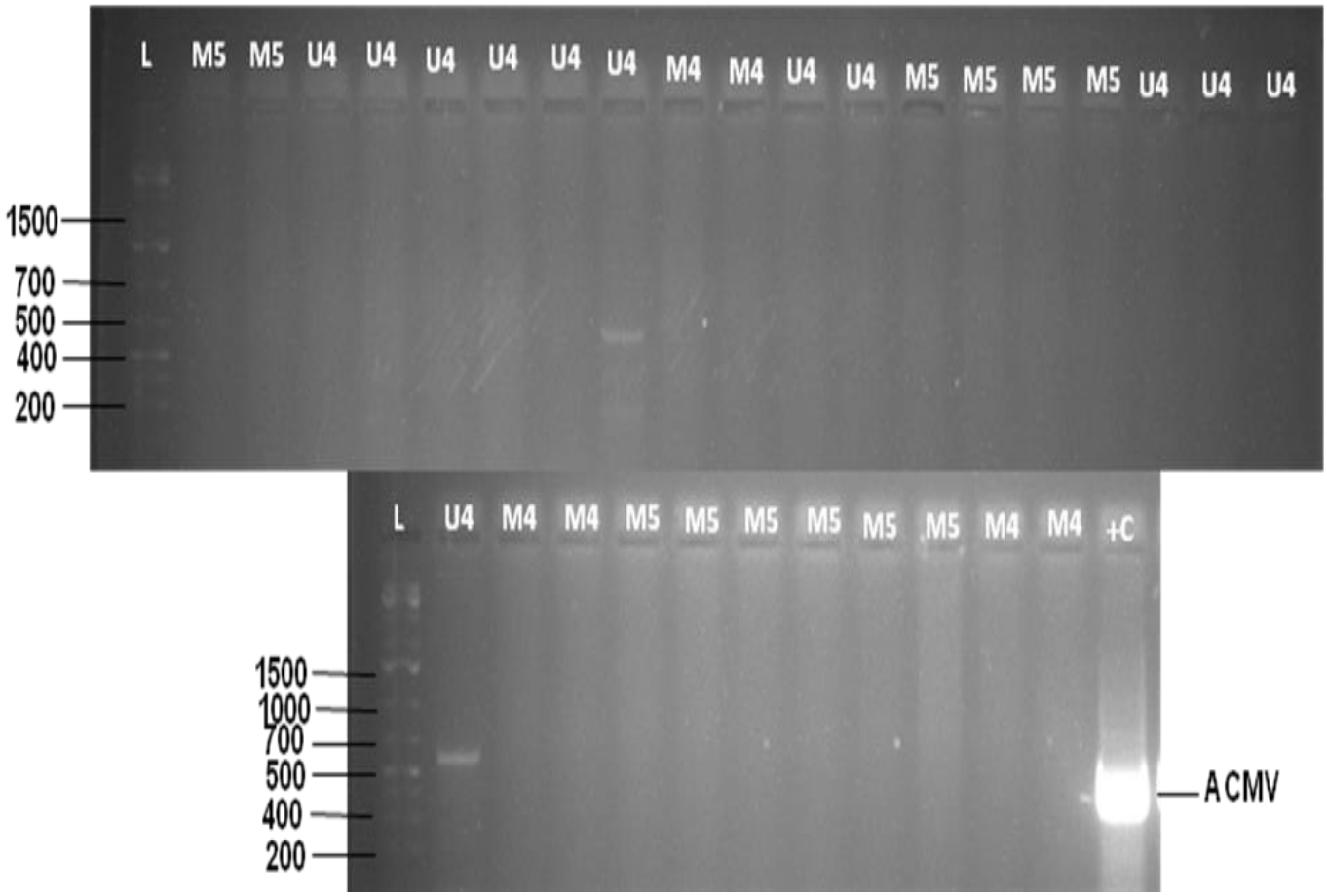
Foliar symptoms observed during the survey in the present study were characteristic of CMD and CBSD, an indication of the presence of the two diseases in lower Eastern Kenya. The CBSD symptoms Recorded were yellow veins banding expressed mainly on the lower, older leaves and chlorotic spots which occurred along the lamina similar to those reported by reference [31], while CMD included yellow to pale green chlorotic mosaic, stunting of severely affected plants, leaf curling and distortion similar to those reported by reference [32]. Cassava mosaic disease prevalence ranged 20% to 100%, while CBSD was 10 to 80% (Table 1) at mean altitude of 1175.78m above sea level. The results showed that CBSD which was previously endemic in the coastal region of Kenya has spread to higher altitudes confirming reports by reference [22]. This is contrary to previous report by reference [33] which restricted high CBSD prevalence to altitude below

ISSN No:-2456 –2165

300m above sea level and less common between 300 to 700m. The high variations and extensive spread of CMD and CBSD could probably be due to high rate of evolution among viral populations. For instance, cassava mosaic disease has been reported to be caused by nine viruses of the genus Begomoviruses [34] while cassava brown streak disease is caused by two (CBSV and UCBSV) viruses [7].

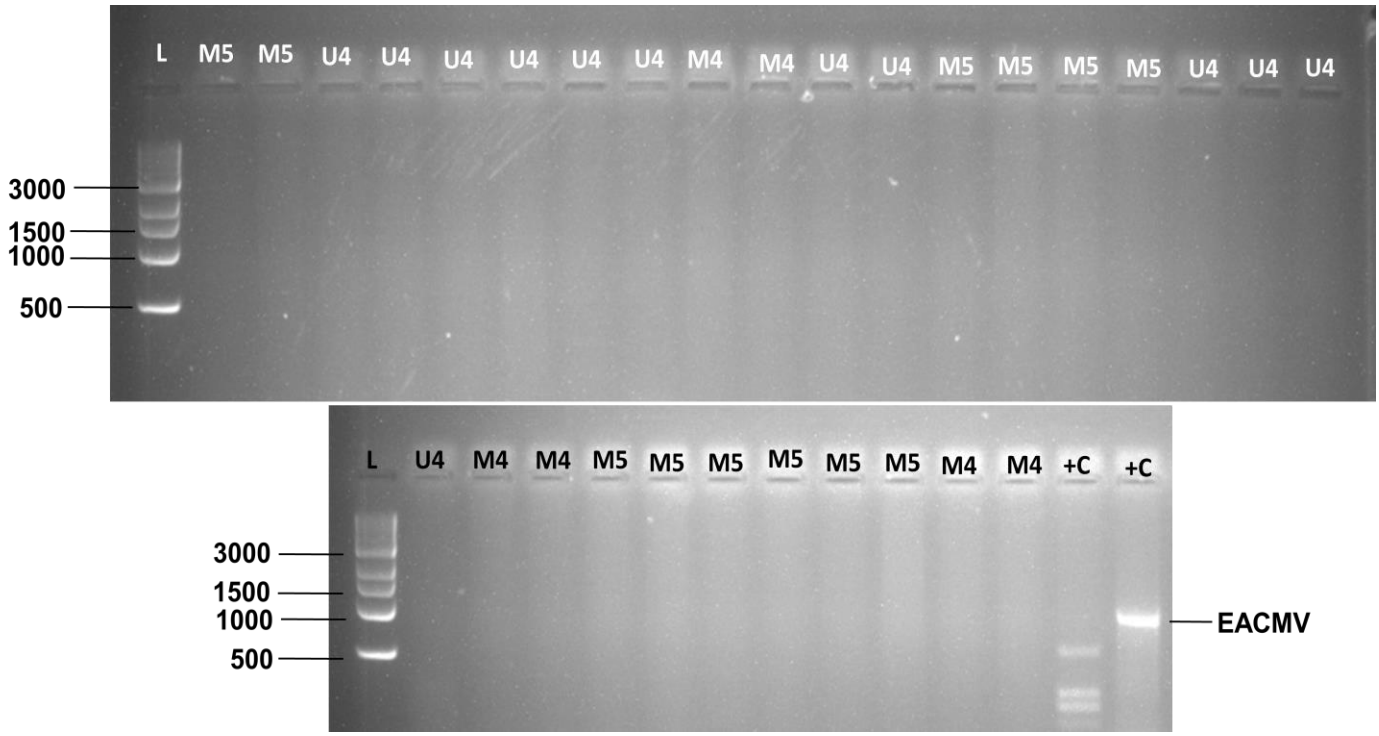
The role of whitefly vectors in propagation of CMBs and CBSVs was corroborated through identification of the three species (*Bemisia tabaci, Bemisia afer,* and the *Aleurodicus dispersus)* in the surveyed zones. The pre-dominant vector, *Bemisia tabaci*, exhibited wider distributions with an average of two flies per plant in both LM4 and UM4 and one fly per plant in LM5. This could perhaps explain the high CMD and CBSD incidences and severity observed in LM4 and UM4 compared to LM5. Similar findings have been reported in previous work in which super abundant *Bemisia tabaci* population led to increased incidence of both CMD and CBSD [19; 35]. Significantly positive correlations between CBSD and CMD incidence and whitefly count (Table 3) suggested possible contribution of whitefly to the spread of CBSVs and CMBs in surveyed region. Indeed high abundance or significant increase in whitefly number seemed to enhance spread of CBSD and CMD [19; 22].

The ACMV, EACMV and EACMV-Ug are the most prevalent CMBs in East Africa [5]. Amongst these, only two plants (20%) tested positive for ACMV under AEZ-UM4 and non in LM4 and LM5 (Table 4; Fig. 2) while EACMV was not diagnosed in all AEZs (Fig. 3). The low or no molecular diagnosis of the two CMBs (ACMV & EACMV), despite higher CMD field incidences recorded, could be attributed to a number of factors. First, plants were randomly sampled from the fields during the surveys. The non-preferential sampling could have resulted in selection of more asymptomatic plants. Secondly, other CMBs such as EACMV-Ug, EACMKV, EACMZV, EACMCV and EACMMV [36] among others could have been responsible the high CMD incidence observed. These viruses were however not subjected to molecular diagnosis in the present study due to lack of positive controls.



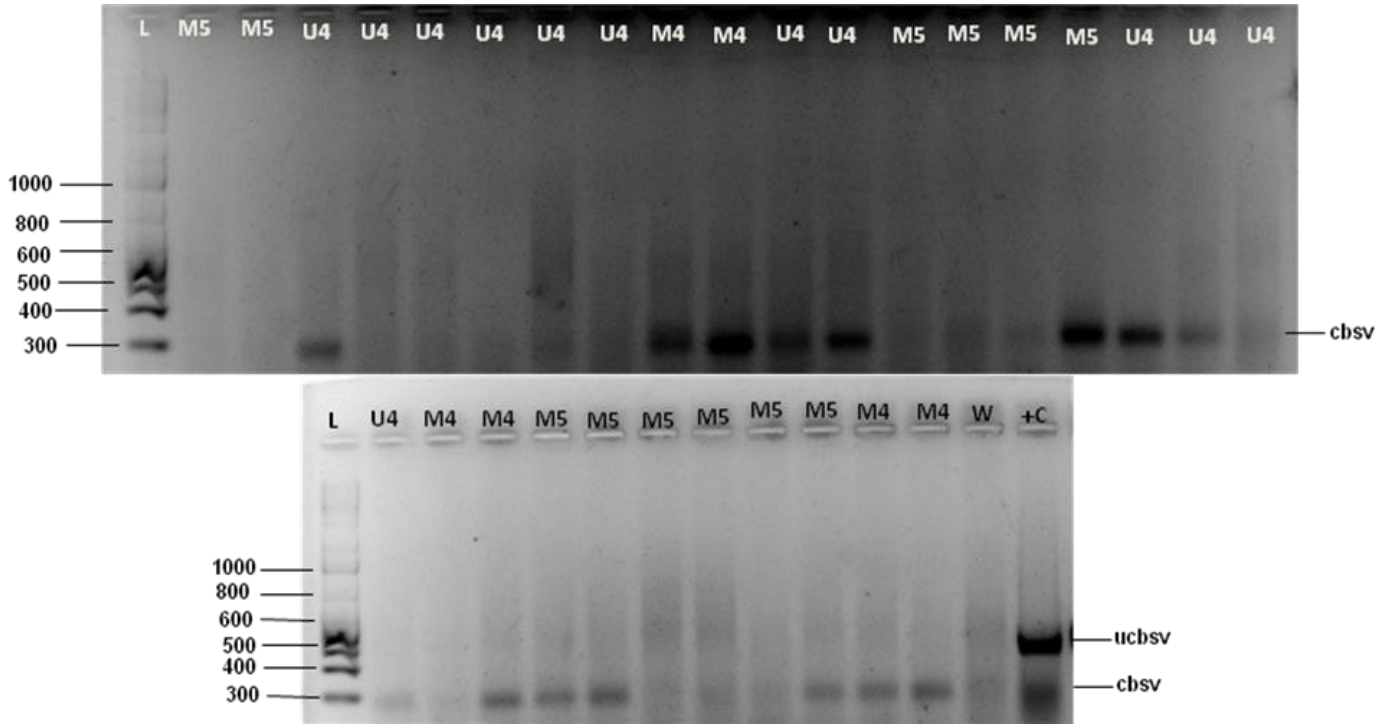
ACMV amplicon = 650 bp (Maruthi et al., 2014); M4, U4 & M5 respectively represent plants sampled from AEZ LM4, UM4 and LM5; L = DNA Ladder; +C = Positive control

Fig. 2: PCR Diagnosis for ACMV in Randomly Selected Cassava Plants Across Agro-Ecological Zones



EACMV amplicon = 1.0 kb (Maruthi et al., 2014); M4, U4 & M5 respectively represent plants sampled from AEZ LM4, UM4 and LM5; L = DNA Ladder; +C = Positive control

Fig. 3: PCR Diagnosis for EACMV in Randomly Selected Cassava Plants Across Agro-Ecological Zones



UCBSV amplicon = 441 bp & CBSV amplicon = 345 bp (Maruthi et al., 2014); M4, U4 & M5 respectively represent plants sampled from AEZ LM4, UM4 and LM5; L = DNA Ladder; W = water (-ve) control; +C = Positive control

Fig. 4: RT-PCR Diagnosis for CBSV and UCBSV in Randomly Selected Cassava Plants Across Agro-Ecological Zones

previously reported differential interaction between the two viruses where CBSV was considered a more aggressive virus inducing more rapid and severe CBSD symptoms compared to UCBSV [37]. CBSD symptoms recorded in the present study could therefore majorly be attributed to CBSV. One plant that was positive for ACMV under UM4 (Fig. 2) was also positive for CBSV (Fig. 4) indicating a possible dual infection of CMBs and CBSVs in a cassava plant as previously reported [38; 6]. Although 80% of farms in AEZ-LM5 showed no (0%) CBSD incidence and 1.0 severity (Table 2), nonetheless 40% of plants sampled from these farms tested positive for CBSV under RT-PCR. This potentially indicated CBSV latency (at the time of sampling) where some infected plants could remain symptomless and some varieties express the symptoms in roots and not in leaves [11; 19].

V. CONCLUSIONS & RECOMMENDATIONS

Findings from this study have shown low cassava viral disease (CBSD and CMD) prevalence and *Bemisia tabaci* whitefly species in lower midlands 5 and a moderate to high prevalence of both diseases in lower midlands zones 4 and upper midlands zones 4 of Lower eastern Kenya. For an exhaustive molecular diagnosis, both symptomatic and asymptomatic cassava plants should be sampled during surveys and where possible all virus variants especially CMBs should be tested. Further cassava breeding programs should include different agro- ecological zones in cassava viral disease management strategies.

VI. ACKNOWLEDGMENT

This study was made possible through funding from Directorate of Research, Innovation & Technology of the South Eastern Kenya University. Diagnostics was carried out at molecular lab of Dr. Evans Nyaboga from the department of Biochemistry at University of Nairobi. The authors are grateful. Contribution of research assistants who made data collections successful and small scale cassava farmers in lower eastern Kenya who allowed us to collect primary data from their farms are also recognized.

REFERENCES

[1]. Fregene, M., and Puonti-Kaerlas, J.,(2002) Cassava biotechnology. In *Cassava: Biology, Production and Utilization*, pp. 179-207. Edited by R. J. Hillocks, J. M. Thresh and A. Bellotti. Wallingford, NY: CABI Publishing.

[2]. Food Agricultural Organization (FAO) of the United Nations(2009)http://www.faostat.fao.org/faostat/servlet/xteservlet3 Retrieved on 8th Aug.2010.

[3]. FAOSTAT (2014) https://www.farmconcern.org/our-work/programmes-highlights/smallholders-beans-yield-doubles-after-embracing-e-warehouse-programme/27-programmehighlights/190unearthedbusinessopportunities-in-cassava-for-industrial-use-animal-feeds-human-foods-starch-and-ethanolinkenyaandtanzania.html

[4]. Legg, J.P., and Okao-Okuja, G. (1999) Progress in diagnosis and epidemiological characterization of cassava mosaic geminiviruses in East Africa. Proceedings of VIIth International Plant Virus epidemiology Symposium, 11-16 April 1999. Aguadulce (Almeria), ES. Abstract, 74-75

[5]. Legg J.P., Lava Kumar P., Makeshkumar T., Tripathi L., Ferguson M., Kanju E., Ntawuruhunga P., and Cuellar, W. (2015) Cassava virus diseases: biology, epidemiology, and management. Adv. Virus Res. 91:85–142

[6]. Irungu, J. (2011) Prevalence and co-infection of cassava with cassava mosaic geminiviruses and cassava brown streak virus in popular cultivars in Western Kenya. MSc. thesis, Kenyatta University.

[7]. Winter, S., Koerbler, M., Stein, B., Pietruszka, A., Martina, P., and Butgereitt, A. (2010) The analysis of cassava brown streak viruses reveals the presence of distinct virus species causing cassava brown streak disease in East Africa. *Journal of General Virology* 91:1365.

[8]. Monger, W. A., Alicai, T., Ndunguru, J., Kinyua, Z. M., Potts, M., Reeder, R. H., Miano, D. W., Adams, I. P., Boonham, N.et al. (2010) The complete genome sequence of the Tanzanian strain of Cassava brown streak virus and comparison with the Ugandan strain sequence. Arch. Virol. 155, 429–433.

[9]. Mbanzibwa, D.R., Tian, Y.P., Tugume, A.K., Mukasa, S.B., Tairo, F., Kyamanywa, S., Kullaya, A., and Valkonen, J.P.T. (2011) Simultaneous virus-specific detection of the two cassava brown streak associated viruses by RT-PCR reveals wide distribution in East Africa, mixed infections, and infections in *Manihot glaziovii*. Journal Virology Methods, Vol. 171: pp.394.400

[10]. Alicai T., Ndunguru J., Sseruwagi P., Tairo F., Okao-Okuja G., Nanvubya R., et al. (2016) Cassava brown streak virus has a rapidly evolving genome: implications for virus speciation, variability, and diagnosis and host resistance. Sci. Rep. 6:36164. 10.1038/srep36164.

[11]. Ntawuruhunga, P., and Legg, J.P. (2007) New spread of cassava brown streak virus disease and its implications for the movement of cassava germplasm in the East and Central African region. Brief 3 Crop Crisis Control Project (C3P)

[12]. Hillocks, R. J., Raya, M. D., Mtunda, K. and Kiozia, H. (2001) Effects of brown streak virus disease on yield and quality of cassava in Tanzania. *Journal of Phytopathology* 149, 389-394.

[13]. Thresh, J. M., Fargette, D., and Otim-Nape, G. W. (1994) Effects of African cassava mosaic virus on the yield of cassava. Trop. Sci. 28, 34-37.

[14]. Owor, B., Legg, J.P., Okao-Okuja, G., Obonyo, R., Kyamanywa, S., and Ogenga-Latigo, M.W. (2004) Field

Studies of Cross Protection with Cassava Mosaic Geminiviruses in Uganda. Journal of Phytopathology, Vol. 152, (4), pp. 243 – 249.

[15]. Storey, H. H. (1936) Virus diseases of East African plants. VI. A progress report on studies of the disease of cassava. East Afr. Agric. J. 2: 34–39.

[16]. Nichols, R. F. J. (1950) The brown streak disease of cassava: Distribution, climatic effects and diagnostic symptoms. East African Agricultural Journal 15, 154-160.

[17]. Hillocks, R.J., Raya, M.D., and Thresh, J.M. (1996) The association between root necrosis and above-ground symptoms of brown streak virus infection of cassava in southern Tanzania. Int. J. Pest Manag. 42, 285-289.

[18]. Hillocks, R.J., and Jennings, D.L. (2003) Cassava brown streak disease: A review of present knowledge and research needs. International Journal Pest Management 49:225–234.

[19]. Alicai, T., Omongo, C. A., Maruthi, M. N., Hillocks, R. J., Baguma, Y., Kawuki, R., Bua, A., Otim-Nape, G. W. and Colvin, J. (2007) Re-emergence of Cassava Brown Streak Disease in Uganda. *Plant Disease* 91, 24-29.

[20]. Lister, R. M., (1959) Mechanical transmission of cassava brown streak virus. *Nature* 183, 1588-1589.

[21]. Legg, J. P., Somado E. A., Barker I., Beach L., Ceballos H., Cuellar W.,… Fauquet, C. (2014) A global alliance declaring war on cassava viruses in Africa. Food Security, 6, 231–248.

[22]. Mware, B., Narla, R., Amata, R., Olubayo, F., Songa, J., Kyamanyua, S., and Ateka, E.M. (2009) Efficiency of cassava brown streak virus transmission by two whitefly species in coastal Kenya. J. Gen. Mol. Virol. 1, 040-045.

[23]. Legg, J.P., Ndalahwa, M., Yabeja, J., Ndyetabula, I., Bouwmeester, H., Shirima, R. and Mtunda, K. (2017) Community phytosanitation to manage cassava brown streak disease. *Virus Res.* In press.

[24]. Hahn, S. K., Isoba, J. C. G., and Ikotun, T. (1989) Resistance breeding in root and tuber crops at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. *Crop Protection* 8, 147-168.

[25]. Hillocks, R.J. and J.M. Thresh, (2000) Cassava mosaic disease and cassava brown streak diseases in Africa: A comparative guide to symptoms and aetiologies. Roots-Newsletter of the Southern African Root Crops Research Network (SARRNET) and the East Africa Root Crop Research Network (EARRNET), Vol. 7.1, Special Issue, pp: 12.

[26]. Kamau, J.P., Sseruwagi, P., and Aritua, V. (2005) Whiteflies as vectors of plant viruses in cassava and sweetpotato in Africa: Kenya, La mosaïque du manioc. Compte-Rendu de la Société de Biologie Belge 109: 1146–1148.

[27]. Osena G., Nyaboga, E., and Amugune, N.O. (2017) Rapid and Efficient Isolation of High Quality DNA from Cassava (Manihot esculenta Crantz) Suitable for PCR Based Downstream Applications. Annual Research & Review in Biology 12(2), pp. 1-10

[28]. Chang, S., Puryear, J., and Cairney, J. (1993**)** A simple and efficient method for isolating RNA from pine trees. Plant Molecular Biology Reporter, Vol. 11, pp. 113-116.

[29]. Moreno, I., Gruissem, W. and Vanderschuren, H. (2011) Reference genes for reliable potyvirus quantitation in cassava and analysis of Cassava brown streak virus load in host varieties. J. Virol. Methods, 177, 49–54

[30]. Maruthi, M.N., Whitfield, C.W., and Legg, J.P., (2014) Virus-indexing, chemo- and thermo-therapies, and micro propagation for generating virus-free cassava plants. A laboratory manual.

[31]. Munga, T. L. (2008) Breeding for Cassava Brown Streak resistance in Coastal Kenya. University of KwaZulu Natal, SA

[32]. Were, H. K., Winter, S., and Maiss, E. (2004) Variations and taxonomic status of begomoviruses causing severe epidemics of cassava mosaic disease in Kenya, Uganda, and Democratic Republic of the Congo. Journal of General Plant Pathology 70, 243-248.

[33]. Hillocks, R.J., Thresh, J.M., Tomas, J., Botao, M., Macia, R., and Zavier, R. (2002) Cassava brown streak disease in Northern Mozambique. Int. J. Pest Manag. 48, 179-182.

[34]. Fregene, M., Matsumura, H., Akano, A., Dixon, A., and Terauchi, R. (2004) Serial analysis of gene expression (SAGE) of host-plant resistance to the cassava mosaic disease (CMD) Plant Molecular Biology 56:563-591.

[35]. Njoroge, M.K., K.N., D.C, Kilalo, D.W, Miano and Mutisya, D.L. (2016) Whiteflies species distribution and abundance on cassava crop in different agro-ecological zones of Kenya. Journal of Entomology and Zoology studies; 4 (3): 258-262.

[36]. Fauquet, C.M., Briddon, R.W., Brown, J.K., Moriones E., Stanley, J., Zerbini M., and Zhou, X., (2008) Geminivirus strain demarcation and nomenclature. Archives of Virology 153: 783-821.

[37]. Ogwok, E., Muhammad, Ilyas., Titus Alicai., Marie E.C. Rey., and Nigel J. Taylor. (2016) Comparative analysis of virus-derived small RNAs within cassava (Manihot esculenta Crantz) infected with cassava brown streak viruses. Virus Research 215 (2016) 1 – 11.

[38]. Masinde, E. A., Ogendo, J. O., Maruthi, M. N., Hillocks, R., Mulwa, R. M. S., and Arama, P. F. (2016) Occurrence and estimated losses caused by cassava viruses in Migori County, Kenya. African Journal of Agricultural Research, 11(24), 2064-2074.