International Journal of Agriculture Innovations and Research
Volume 5, Issue 3, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 02/12/2016 | Accepted on : 07/12/2016 | Published : 20/12/2016

Integrated Pest Management Options for the Cassava Mosaic Disease in Sierra Leone

Alusaine. E Samura[1], Sheku. M.Kanteh[2], Johnny. E. Norman[2] and Sahr. N.Fomba[2]
[1] Sierra Leone Agricultural Research Institute (SLARI), Njala Agricultural Research Centre (NARC), Freetown, Sierra Leone
[2] Crop Protection Department, school, of Agriculture, Njala University
Correspondence: Alusaine E Samura, Sierra Leone Agricultural Research Institute (SLARI), Njala Agricultural Research Centre (NARC), Freetown, P.O. Box 540, Sierra Leone. Tel: 232-76-731-554.
E-mail: aesamura@yahoo.com

Abstract **– Cassava cultivation in Sierra Leone is characterized by the dominance of local varieties that are susceptible to the cassava mosaic disease. The cassava mosaic disease is one of the most important diseases of cassava and disease control is mainly through the deployment of improved varieties that are resistant to the disease but is not readily adopted by farmers. This situation may be partially due to the poundable trait possessed by local varieties and the preference of mild mosaic infection on the leaves used for the preparation of the popular cassava leaf sauce. Farmers in Sierra Leone do not apply any control measure for the cassava mosaic disease. This study therefore assessed three options (identification of resistant varieties, Hot water treatment, Neem and Moringa – treated cuttings) for the development of an integrated option for the control of cassava mosaic in Sierra Leone.**

76 genotypes were selected and evaluated across major agro ecological zones. Expression of disease symptoms of the African and East African cassava mosaic viruses on hot water, neem and moringa-trested cuttings at varying concentrations or temperatures and soaking durations were assessed in a screen house. Symptomless cuttings and infected cuttings were used as checks.

High yielding cassava genotypes resistant to the cassava mosaic disease were identified for food, feed and industries. African Cassava mosaic infected cuttings subjected to 75^0C hot water treatment at 10, 30, and 60 seconds had significantly ($P_{0.005}$<0.001) lower diseased severity score. African cassava mosaic infected cuttings treated with higher concentration Neem and Moringa leaf extract had significantly the lowest disease severity score compared to all other treatments.

The treatments were effective in supressing the African cassava mosaic disease but not effective against the East african cassava mosaic disease. The implication of this study is that in areas with limited access to improved varieties or where the use of local varieties is dominant, African cassava mosaic infected cuttings can be treated with hot water, moringa and neem extract at recommended concentrations, temperatures and soaking durations to reduce symptom expression and consequently increase yield. The deployment of resistant varieties that meet farmers' desired traits however remains the most promising option in the management of the disease.

Keywords **– Cassava Mosaic Disease, Cassava Genotypes, Control Options.**

I. INTRODUCTION

The cassava mosaic disease (CMD) remains the most important disease of cassava in Sierra Leone. Several species and strains of cassava mosaic geminivirus have been described (Fauquet *et al*., 2003). Recent survey on cassava mosaic diseases indicates that the number of farms with local varieties were higher than those with improved varieties. The study also reviled that country - wide, the prevalence of cassava mosaic disease was 85.2% out of 156 sites visited with mixed infections of African Cassava Mosaic Virus and the East African Cassava Mosaic virus occurring among infected cassava genotypes (Samura *et al*, 2014). The continuous use of local infected varieties is associated with the traditional belief that mosaic infected cassava genotypes act as an indicator for the identification of poundable cassava genotypes with high dry matter. Further there is a popular demand for mild mosaic infected cassava leaves among women which is used for preparing a popular vegetable sauce using cassava leaves. It is believed that the infected leaves due to the low chlorophyll content consumes less palm oil, an expensive but crucial element in the preparation of the cassava leaf sauce compared to improved resistant varieties which consumes more palm oil. Women play a vital role in the selection of planting materials and would influence varieties of their choice which in most cases would include infected local varieties for domestic consumption. In the absence of a change in mind set, it is expected that the adoption of improved varieties resistant to the cassava mosaic disease will be low. Thermotherapy has been used directly on the cuttings from plants infected with cassava mosaic disease (CMD) in Kenya (Walter and Raymond, 1982). Temperature dramatically affects plant-virus interactions with an attenuation of virus-induced symptoms at high temperature (Szittya *et al*., 2003). Consequently, thermotherapy has been considered as a first-choice method to free vegetative material from viruses (Manganaris *et al.*, 2003).

Neem is also key ingredient in bio - pesticidal management (BPM), providing a natural alternative to synthetic pesticides. It acts as an anti-feedant, repellent, and egg-laying deterrent, protecting the crop from damage. The insects starve and die within a few days. Neem also suppresses the hatching of pest insects from their eggs Isman, M. B. (2006). Neem has anti-bacterial, anti-fungal and anti-nematicidal properties and positive effect in combating several diseases in rice cultivation, and there are many active constituents of Neem which are still to be exploited (Lokanadhan *et al.*, 2012). Moringa oleifera contain powerful antioxidants that can help prevent or delay some of the worst complications arising from viral infection. In the absence of readily available pesticide for ACMD, Neem and Moringa based bio - pesticides use is a

natural choice worth exploiting for controlling ACMD. The proceedings of the 14th International AIDS conference held in Barcelona, Spain in 2002 included a recommendation that M. oleifera powder be considered as an alternative treatment to boost the immune systems of HIV-positive patients in Africa who would otherwise not receive antiretroviral drugs or, in fact, any treatments at all (Burger *et al*., 2002). This objective of this study therefore was to identify alternative option to mitigate the effect of the cassava mosaic disease in farmers' fields. This was archived through four main interventions, firstly, a search for cassava mosaic resistant varieties for local food and industrial uses. Secondly the efficacy of hot water and infected cuttings, thirdly the use of Neem treated cutting and finally Moringa treated cassava cuttings on the symptom expression of the mosaic virus.

II. MATERIALS AND METHODS

2.1 Activity 1: Screening and identifying promising cassava genotypes with resistance to ACMD that meets industrial and farmers' desired traits.

2.1.1 Cassava Genotypes

Seventy one introduced and five check varieties were selected for multi-location evaluation.

Two thousand one hundred genotypes were initially multiplied, screened and promising genotypes selected at the Foya experimental and demonstration site of the Njala Agricultural Research Centre (NARC) in 2009 cropping season for establishment in five agro-ecologies in 2010/ 2011 planting season. These locations were as follows Njala (Forest Transition) in the Southern province, Kenema (Rain Forest) in the Eastern province and Kabala (Savannah highland), Kambia (Savannah lowland), Yenkessa (Coastal plain) and Makeni (Savannah lowland) in the Northern province. Multi location evaluation was conducted for two years 2010/2011 and 2011/2012 planting seasons.

2.1.2 Experimental Design

Cuttings 20 – 30 cm long of each of the 2,100 varieties were planted in single row plots. Rows were 10m long with 1m between hills and 1.5m between replications. Infector rows were randomly placed between plots to ensure the presence of the cassava mosaic virus in the field. The design was a randomized completed block (RCB) with two replications assessed in two years in five locations.

Data were collected on symptom severity of the cassava mosaic disease based on 1-5 disease severity scale (IITA, 1990) from 10 plants. Plants were assigned disease severity scores based on the standard five point scoring scale for CMD. Where there was no obvious symptom, plants was assigned a score of "1". Plants with mild chlorotic patterns or mild leaf distortion at the base were scored "2", while those with strong mosaic on the entire leaf, distortions of leaves were scored"3". Severe mosaic distortion, reductions of leaf affecting about 2/3 of the leaves were scored as "4" and plants with the most severe mosaic symptoms, with severe distortion of leaves, stunting of entire plant and about 4/5th of leaves affected were assigned a score of "5' (IITA, 1990). Plants with mean CMD scores of "1" were classified as highly resistant (HR), those with a score of "1.1 to 2" were classified resistant (R), those with a score of "2.1 to 3" were classified as Moderately susceptible (MS) and those with scores of "3.1 to 4" were classified as Susceptible (S) and "4.1 to 5" were classified as highly susceptible (HS).

2.2 Activity 2 Effect of Hot Water Treatment on infected Cassava Cuttings in the Expression of Cassava Mosaic Disease

2.2.1 Study Area

The study was conducted at the Njala Agricultural Research Centre (NARC), Njala, Kori chiefdom which is located at the eastern part of Moyamba District in southern Sierra Leone. Njala is located at an elevation of 50m above sea level on 8^{O}06N latitude and 12^{O} 06W longitude. Njala has two distinct seasons, the wet season (May to October) and the dry season (November to April) recently rainy season has been observed to extend to December which may be attributed to climate change. Mean annual rainfall at Njala is 2526mm, mean monthly maximum air temperature ranges from 29^{O} C to 34^{O} C , while mean minimum air temperature ranges from 21^{O}C to 23^{O}C. Relative humidity is very high often close to 100% for the greater part of the day and night especially during the rainy season (Odell *et al.*, 1974). During the dry season, potential evapo-transpiration exceeds rainfall while during the rainy season precipitation exceeds evapo-transpiration. This work was conducted in a Rossell screen house at NARC Njala, Kori Chiefdom, and Southern Sierra Leone.

2.3 Activities 3 (Moringa trial) and activity 4 (Neem trial) were conducted under the same condition.

2.3.1 Data Collection

Data were collected from all plants. Data included plant height measured in centimeters from the surface of the pot to the top most expanded leaf using a well calibrated meter rule. Leaf area was calculated in centimeter square (cm2) using the length and breadth method from three (3) fully expanded leaves per plant. Leaf number was calculated by counting the number of leaves at each sampling period. Cassava Mosaic Disease assessment was done using the 1-5 scale (IITA, 1990).

2.3.2 Data Analysis

Data collected on various parameters were analysed using Statistical Analysis System (SAS) and Duncan's Multiple Range Test (DMRT) at 5 % probability ($p < 0.05$) was used for mean separation with case letters. Standard errors of means was also used to estimate

2.3.3 Activity 3. Effect of Moringa - and Neem - Treated Cassava Cuttings on the Expression of the African Cassava Mosaic and the East African Cassava Mosaic Diseases (ACMD and EACMD)

2.3.3 .1 Experimental Design and Cultural Practices

The two experiments were carried out in polythene bags of 30cm x 50 cm. Well perforated polythene bags were filled with steam sterilized soil obtained from a compost pit. The Polythene bags were laid out in a randomized complete block (RCB) design with three replications. Single stem cuttings infected with the cassava mosaic

viruses (set one ACMV and set two EACMV) and treated with leaf extract solution of Neem and Moringa. Cuttings, 30 cm long were planted in polythene bags. Controls for the experiments consisted of African cassava mosaic virus (ACMV) infected cuttings and an EACMV infected plant diagnosed at IITA. Both experiment consisted of a symptomless cuttings obtained from apical meri - stem derived from three successive generations of the check varieties (Cocoa for ACMD and IFO 7003).

Experiment 1 consisted of two sets of trials. This included an assessment of moringa treated cuttings on ACMV and EACMV infected cuttings. Each trial consisted of eight treatments. African Cassava Mosaic Virus and East African Cassava Mosaic Virus infected cuttings of Cocoa and IFO 7003 respectively, treated with moringa leaf extract at varying concentrations per litre as follows:

Cassava mosaic virus infected cuttings immersed in 100g of finely grinded moringa leaf suspension per 1iter for 1hr, 6hr and 12hr.Cassava mosaic virus infected cuttings immersed in 200g of finely grinded moringa leaf suspension per liter for 1hr, 6hr and 12hr. A symptomless cuttings of cocao (Local) and IFO 7003 and ACMV infected Cocoa and EACMV infected IFO 7003 varieties.

Experiment two also consisted two sets of trials. This included an assessment of neem treated cuttings on ACMV and EACMV infected cuttings. Each trial consisted of eight treatments. African Cassava Mosaic virus and East African Cassava Mosaic virus infected cuttings of Cocoa and IFO 7003 respectively, treated with neem leaf extract at varying concentrations per liter as follows:

Cassava mosaic virus infected cuttings immersed in 1000g of finely grounded neem leaf suspension per 1iter for 1hr, 6hr and 12hr.Cassava mosaic virus infected cuttings immersed in 2,000g of finely grounded neem leaf suspension per liter for 1hr, 6hr and 12hr. A symptomless cuttings of cocao (Local) and IFO 7003 and ACMV infected Cocoa and EACMV infected IFO 7003 varieties.

2.3.3.2 Preparation of Planting Materials

Before planting, cuttings were cut into 30 cm length and treated for 10 min in solution of benomyl (2.5g a.i/L of H2O). Single stem cuttings were heat treated and planted in polythene bags. Symptomless plants obtained from the apical meri- stem of Cocoa and IFO 7003 was used as symptomless plants. The two experiments consisted of the local cassava variety, Cocoa confirmed to be infected with the African Cassava Mosaic Disease (ACMD), and IFO 7003 confirmed to be infected with the East African Cassava Mosaic Disease (EACMD) through polymerase chain reaction (PCR) at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

2.3.3.3 Preparation of Plant Leaf Extract

Moringa: Moringa leaves were kept in a well-ventilated room to avoid direct sunlight for five days and finely grounded into powder. The moringa powder was mixed with water into a clean container at a ratio of 100g of moringa powder to one litre of water for concentration one. Cassava cuttings were then immersed in moringa solution at varying at varying soaking durations. A mixture of 200g moringa powder was mixed with one litre of water constituted concentration two. In the same manner, cassava cuttings were immersed in moringa solution at varying soaking duration.

Neem: Fresh neem leaf was finely grounded with a mortar and pestle and mixed with water into a clean container at a ratio of 1000g of fresh neem leaf to one litre of water for concentration one and 2,000g of fresh neem leaf to one litre of water for concentration 2. The suspension was then filtered using a clean white cloth. The cassava cuttings were then immersed in neem solution at varying concentration and soaking duration.

2.3.3.4 Cassava genotype and treatment

Assessment for ACMD infected cassava cutting treated with moringa solution consisted of the local variety, Cocoa, confirmed to be infected with the African Cassava Mosaic Virus (ACMV) and symptomless cuttings of the local variety Cocoa raised from three successive generations of cuttings derived from the upper part of the stem cuttings and treated with neem solution referred to as symptomless cuttings. Infected Cocoa varieties treated with moringa leaf extracts solution at varying soaking duration and concentrations were used as treatments to assess severity of the African Cassava Mosaic Disease (ACMD) over time.

Assessment for EACMD followed similar treatment as in the case of ACMD. However the check variety used was IFO 7003 confirmed to be infected with the East African Cassava Mosaic Disease through polymerase chain reaction (PCR) at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Symptomless plant derived from IFO 7003 in similar manner as derived for the assessment of ACMD was used to obtain symptomless cuttings of IFO 7003. Other treatments consisted of the variety IFO 7003 infected with EACMV but treated with moringa leaf extract solution for the assessment of the East African Cassava Mosaic Disease (ACMD) over time.

In a similar manner to experiment 1, assessment for ACMD consisted of the local variety, Cocoa confirmed to be infected with the African Cassava Mosaic Virus (ACMV) and symptomless but susceptible local variety, Cocoa as checks. Infected Cocoa varieties treated with neem leaf extracts solution were used to assess severity of the African Cassava Mosaic Disease (ACMD) over time. Similarly assessment for EACMD consisted of the check variety IFO 7003. Symptomless plants derived from IFO 7003 were used as symptomless cuttings. Other treatments consisted of the variety IFO 7003 infected with EACMV but treated with neem leaf extract solution for the assessment of the East African Cassava Mosaic Disease (ACMD) over time.

2.3.3.5 Data Collection

Data were collected from all plants. Data included plant height measured in centimeters from the surface of the pot to the top most expanded leaf using a well calibrated meter rule. Leaf area was calculated in centimetre square (cm2) using the length and breadth method three (3) fully expanded leaves per plant. Leaf number was calculated by counting the number of leaves at each sampling period.

International Journal of Agriculture Innovations and Research
Volume 5, Issue 3, ISSN (Online) 2319-1473

Cassava Mosaic Disease (CMD) assessment was done using the 1-5 scale (IITA, 1990).

2.3.3.6 Data Analysis

Data collected on various parameters were analysed using Genstat statistical package (Genstat release version 7.

III. RESULT

3.1 Activity 1. Screening and Identifying source of resistance to cassava mosaic diseases among Farmers' Preferred Genotypes.

Seventy one (71) genotypes and five check varieties SLICASS 1, 2, 4, 6, and the local variety Cocoa were selected by farmers and evaluated in 5 locations for resistance to cassava mosaic disease and other farmer desired traits.

3.1.1 Severity of Cassava Mosaic Disease on Farmers' Preferred Genotypes Assessed for Two Years.

Most of the cassava genotypes selected by farmers had high resistance to the cassava mosaic diseases. The overall mean of the genotypes assessed was 1.1. The mean severity score for most genotypes assessed were below the local check variety Cocoa with a score of 1.92 which was significantly ($P_{0.05}$<0.0001) highest score but not significantly different from SLICASS 2 with a score of 1.75. Other check varieties released by SLARI which include SLICASS 1, SLICASS 4 and SLICASS 6 exhibited no symptom of the cassava mosaic disease. Out of the 76 genotypes assessed, 59 (77.63%) showed no symptom of the disease (Table 1).

The severity of cassava mosaic disease among cassava genotypes selected was low. Yenkessa in the coastal plains had significantly ($P_{0.05}$< 0.0001) the highest severity scores of cassava mosaic disease (1.32) compared to all other locations. No significant difference was observed in disease expression among genotypes in Kabala (1.01), Kambia (1.01), Kenema (1.02), Makeni (1.01) and Njala (1.01) (Table 2).

Table 1. Some Mean Severity of Cassava Mosaic Disease on Farmer Preferred Genotypes Assessed in 2010 and 2011

CLONE	[a]ACMD severity
COCOA (Local check)	1.92a
SLICASS 2	1.75b
96/0595	1.50c
01/0025	1.33d
TME 203 (4X)	1.33d
UNKNOWN 6	1.33d
94/0069	1.33d
01/1181	1.33d
TME 1674	1.21de
90853	1.19e
07/0620	1.17e
IFO 7010	1.17e
07/0498	1.17e
05/0127	1.08ef
MM97/3069	1.08ef
SM 1406-1	1.08ef
05/1652	1.08ef
05/0303	1.00f
06/1474	1.00f
2006/004	1.00f
02/0577	1.00f

[a]Means with the same letter are not significantly different. DMRT ($P_{0.05}$ <0.0001). List not exhaustive

Table 2. Severity of Cassava Mosaic Disease on farmers' preferred varieties across six locations in Sierra Leone.

Location	[a]ACMD severity
Yenkesa	1.32a
Kenema	1.02b
Makeni	1.02b
Kabala	1.01b
Njala	1.01b
Kambia	1.01b

[a]Means with the same letter are not significantly different. DMRT ($P_{0.05}$ <0.0001).

3.1.2 Mean Tuberous Root Yield of Farmers' Preferred Genotypes

Significant difference ($P_{0.05}$<0.0001) was observed among cassava genotypes in terms of tuberous root yield. The highest mean tuberous root yield taking into account the two years of study was (MM96/7204 (4X) with a mean tuberous root yield of 47.0 t/ha followed by 06/1474 with 43.24 t/ha. CC 107 had the lowest yield of 17.43 t/ha followed by Cocoa and CC 2365 with 17.46 t/ha and 18.96 t/ha respectively (Table 3).

Table 3. Mean Tuberous Root Yield of Some Farmers' Preferred Genotypes Assessed for Two Year.

CLONE	[a]Tuberous root yield (t/ha)
MM96/7204 (4X)	47.00a
06/1474	43.24b
MM02/1807	42.82b
96/0160 (4X)	37.81c
SLICASS 1	37.72c
90853	36.38cdcde
07/0649	35.97cde
MM001/0146	35.90cde
CC 2365	18.96ab
COCOA	17.46b
CC 107	17.43b

[a]Means with the same letter are not significantly different. DMRT ($P_{0.05}$ <0.0001). List not exhaustive

3.1.3 Tuberous Root Yield (t/ha) of Farmers Preferred Cassava Genotypes across Locations for 2011 And 2012 Cropping Season.

International Journal of Agriculture Innovations and Research
Volume 5, Issue 3, ISSN (Online) 2319-1473

Significant (P0.05<0.0001) difference in tuberous root yield was also observed across each location. Kabala had the highest tuberous root yield of 35.89 t/ha significantly higher than all other locations followed by Kenema with 34.83 t/ha significantly higher than Makeni with 33.55 t/ha which in turn was significantly higher than Njala with 28.69. Kambia and Yenkesa had significantly the lowest yields of 26.29 t/ha and 18.70 t/ha respectively (Table 4).

Table 4.Tuberous Root Yield (t/ha) of Farmers Preferred Cassava Genotypes across Locations in 2011 And 2012 Cropping Season.

Location	[a]Tuberous root yield (t/ha)
Kabala	35.89a
Kenema	34.83b
Makeni	33.55c
Njala	28.69d
Kambia	26.29e
Yenkesa	18.70f

a Means with the same letter are not significantly different. .DMRT ($P_{0.05}$ <0.0001).

3.2 Activity 2 . The Effect of Hot Water Treatment on African Cassava Mosaic and the East African Cassava Mosaic Diseases.

3.2.1 African Cassava Mosaic Disease (ACMD) severity as affected by varying hot water treatment

ACMV infected cassava cuttings significantly varied in their respond to the cassava mosaic disease expression when subjected to varying treatments. The symptomless cutting (check) had no symptom expression with the score of 1.0, whiles the ACMV infected cuttings with no hot water treatment (check) had a disease severity of 2.3. Cassava cuttings subjected to 75^{0}C hot water treatment at 10, 30, and 60sec had significantly lower diseased severity score of 1.28, 1.29, and 1.06 respectively compared to treatments with lower water temperatures. For instance, there was no significant difference between the untreated cutting and those treated 25^{0}C irrespective of the soaking duration. Diseased severity scores for cassava cuttings immersed in hot water at 25^{0}C where significantly higher than those subjected to higher temperatures of 50^{0}C and 75^{0}C (Table 5).

3.2.2 *EACMD Severity as Affected by Varying Hot Water Treatments.*

The results indicate there was a significant difference among hot water treatment at varying temperatures and immersion time. The symptomless cuttings showed no expression of the disease. The EACMV infected cutting had a disease severity of 3.33 (check) with significantly the highest severity score. The lowest severity score was 2.5 not significantly different that most heat treatment at 500C and 75^{0}C but significantly lower than cuttings immersed in hot water at 25^{0}C (Table 6).

Table 5 .Severity of ACMD expression as affected by hot water treatment

Treatment (infected ACMD cuttings treated with hot water at varying so duration	Weeks after sprouting(WAS)						Mean
	2	4	6	8	10	12	
Infected Cutting (Cocoa)	1	3.0	2.7	2.7	2.7	3.0	2.3
Symptomless cutting (Cocoa)	1	1.0	1.0	1.0	1.0	1.0	1.0
Cuttings immersed at 250C for 10 seconds	1	2.7	2.7	2.7	3.0	3.0	2.3
Cuttings immersed at 250C for 30seconds	1	2.7	2.7	3.0	3.0	3.0	2.4
Cuttings immersed at 250C for 60 seconds	1	2.0	2.0	3.0	3.0	3.0	2.2
Cuttings immersed at 500C for 10seconds	1	1.3	2.0	2.0	2.0	2.0	1.6
Cuttings immersed at 500C for 30 seconds	1	1.3	1.3	1.67	2.0	2.0	1.4
Cuttings immersed at 500C for 60 seconds	1	1.00	1.00	1.67	2.00	1.67	1.22
Cuttings immersed at 750C for 10seconds	1	1.33	1.33	1.67	1.67	1.67	1.28
Cuttings immersed at 750C for 30 seconds	1	1.00	1.67	2.00	1.33	1.67	1.29
Cuttings immersed at 750C for 60 seconds	1	1.00	1.33	1.33	1.00	1.67	1.06
Mean	1	1.67	1.78	2.06	2.06	2.15	
SE± treatment**	0.113						
SE± WAS**	0.084						
SE± WAS X Treatment**	0.277						
CV (%)	20.9						

Table 6. Severity of EACMD expression as affected by hot water treatment

Treatment	Weeks after sprouting (WAS)						Mean
	2	4	6	8	10	12	
EACMD infected cutting (IFO 7003)	1.0	4.0	4.0	4.0	4.0	4.0	3.3
Symptomless cutting IFO 7003)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cuttings immersed at 25^{0}C for 10 seconds	1.0	2.7	3.0	3.7	3.7	4.0	2.8
Cuttings immersed at 25^{0}C for 30seconds	1.0	3.3	3.3	4.0	4.0	4.0	3.1
Cuttings immersed at 25^{0}C for 60 seconds	1.0	3.0	3.3	3.7	4.0	4.0	3.0
Cuttings immersed at 50^{0}C for 10seconds	1.0	2.3	3.0	3.0	3.7	3.7	2.6
Cuttings immersed at 50^{0}C for 30 seconds	1.0	2.3	3.3	3.7	4.0	4.0	2.9
Cuttings immersed at 50^{0}C for 60 seconds	1.0	2.0	2.7	3.3	3.7	3.3	2.5
Cuttings immersed at 75^{0}C for 10seconds	1.0	3.0	3.0	3.7	3.3	4.0	2.8
Cuttings immersed at 75^{0}C for 30 seconds	1.0	2.0	3.0	4.0	3.3	4.0	2.7
Cuttings immersed at 75^{0}C for 60 seconds	1.0	2.0	3.0	4.0	3.7	4.0	2.8
Mean	1.0	2.5	3.0	3.5	3.5	3.6	
SE± treatment**	0.1						
SE± WAS**	0.1						

SE± WAS X Treatment**	0.3
CV%	12.9

3.3 Activity 3. Effect of Moringa treated cassava cutting on the expression the African Cassava Mosaic and the East African Cassava Mosaic Diseases

3.3.1 ACMD Severity Expression on Moringa Treated Cassava Cuttings at Varying Concentrations, Soaking Duration and Weeks After Sprouting.

Generally cassava cuttings treated with a concentration of 200g of Moringa leaf extract had significantly ($P_{0.005}$<0.001) the lowest disease severity score compared to all other treatments. Longer soaking duration for 12 hours significantly ($P_{0.005}$<0.001) reduced disease expression compared to 1 hour for both the 100g Moringa/litre H_20 but no significant difference was observed in soaking duration at higher concentrations of 200g Moringa/litre H_20. The 200g of Moringa treated cassava cuttings soaked for 1hr to 6hr had severity score which ranged from 1.60 to 1.7 and was significantly the lowest scores, compared to all other treatments. The 100g Moringa - treated cassava cutting had significantly higher severity score but was significantly lower than the ACMV infected cutting which had significantly higher severity scores of 2.8. The symptomless cutting maintained a severity score of 1.0 ACMD severity increased significantly ($P_{0.05}$<0.001) with time for all treatments. The highest severity score of 2.47 was attained at 12 weeks after sprouting (WAS). The lowest disease score was of 1.54 observed at 2 WAS. (Table 7).

Table 7. ACMD Severity Expression on Moringa Treated Cassava Cuttings at Varying Concentrations and Durations and Weeks after Sprouting.

Treatment (infected ACMD cuttings treated with moringa leaf extract	Weeks After Planting						Mean
	2	4	6	8	10	12	
100g per 1iter for1hr	1.4	2.2	2.0	2.1	2.9	3.0	2.3
100g per litre for 6hr	1.4	1.6	1.6	1.9	2.7	2.9	2.0
100g per litre for 12hr	1.6	1.8	1.9	1.9	2.9	3.0	2.2
200g per 1iter for1hr	1.4	1.2	1.3	1.6	2.2	2.5	1.7
200g per litre for 6hr	1.4	1.0	1.5	1.7	2.0	2.0	1.6
200g per litre for 12hr	1.4	1.0	1.4	1.6	2.0	2.2	1.6
ACMV infected cutting (Cocoa)	2.6	2.5	2.4	3.0	3.1	3.2	2.8
Virus free/ symptomless cutting (Cocoa)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean	1.5	1.5	1.7	1.9	2.3	2.5	
SE± (Treatment)**	0.1						
SE± (WAS)**	0.1						
SE± (WAS x treatment)**	0.2						
C V (%)	11.6						

*= Significantly different at $P_{0.005}$<0.005

**= Significantly different at $P_{0.005}$<0.001

3.3.2 EACMD Severity Expression on Moringa Treated Cassava Cuttings at Varying Concentrations, Soaking Durations and Weeks After Sprouting.

Significant difference ($P_{0.005}$<0.001) in disease severity was also observed among EACMV infected cassava cuttings treated with Moringa at varying concentrations and soaking durations. Generally 200g Moringa treatment suppressed EACMV infection significantly with a disease score 2.8, 2.7 and 2.5 respectively for 1hr, 6 hr and 12hr soaking duration, compared to the untreated EACMV infected cutting which had a severity score of 3.71. The 100g treatment also exhibited suppressive tendencies with increase in the soaking duration form 6hr to 12 hours with a severity score of 3.08 respectively and was significantly ($P_{0.005}$<0.001) lower than the 100g treatment for 1 hour with a severity score 3.44 as well as EACMD infected cutting . EACMV severity expression increased with time significantly from 2.46 at 2 WAS to 3.10 at 12 WAS (Table 8).

Table 8. EACMD Severity Expression on Moringa Treated Cassava Cuttings at Varying Concentrations Soaking Durations and Weeks After Sprouting.

Treatment (infected EACMD Cuttings) treated with moringa leaf extract	Weeks After Planting						Mean
	2	4	6	8	10	12	
100g per 1iter for1hr	3	3.3	3.3	3.3	4	3.8	3.4
100g per litre for 6hr	3	3	3	3.3	3.2	3	3.1
100g per litre for 12hr	2.9	3	3.1	3.3	3.1	3	3.1
200g per 1iter for1hr	2.3	2.5	3	3	3	3	2.8
200g per litre for 6hr	2.3	2.5	2.7	2.7	2.8	3	2.7
200g per litre for 12hr	2.3	2	2	3	2.7	3	2.5
EACMV infected cutting (IFO 7003)	3	4	4	3.3	4	4	3.7
Virus free/ symptomless cutting (IFO 7003)	1	1	1	1	1.7	2	1.3
Mean	2.5	2.7	2.8	2.9	3.1	3.1	
SE± (Treatment)	0.1						
SE± (WAS)	0.1						
SE± (WAS x treatment)	0.2						
CV (%)	6.6						

3.4. Activity 4. Effect of neem treated cassava cutting on the expression the African Cassava Mosaic and East African Cassava Mosaic Diseases.

3.4.1 ACMD Severity Expression of Neem Treated Cassava Cuttings at Varying Concentrations and Soaking Durations.

The 2,000g/litter Neem - treated cutting had a severity score 1.42, 1.29 and 1.31 for 1hr, 6hrs and 12hrs soaking duration respectively significantly ($P_{0.005}$<0.001) lower than the check (ACMD infected cutting). Generally lower treatments of 1,000g/litter Neem - treated cuttings with shorter soaking duration had significantly higher severity score compared to the 2,000g treatments with longer soaking duration. This implies that for ACMV infected cuttings, the higher the concentration (2,000g/litter) of Neem -treated cuttings immersed in Neem leaf extract for at least 6 hours was effective in suppressing the expression

of the ACMD compared to lower concentration. 1000g Neem treated cassava cuttings had a severity score of 1.82, 1.90 and 1.89 at 1hr, 6 hr and 12hrs soaking duration respectively. All Neem treated cuttings had significantly lower disease severity scores compared to the ACMV infected cassava cuttings used as a check. For each sampling period all Neem treatment were significantly lower than the ACMD infected cuttings while the 1,000g Neem treatment was significantly higher than the 2,000g Neem treatment with longer soaking duration of 6 to 12 hours. Disease severity increased with time significantly ($P_{0.005}$<0.001) with the highest severity score of 2.45 attained at 12 weeks after sprouting (Table 9).

Table 9. ACMD Severity expression on Neem treated cassava cuttings at varying concentrations and Soaking Duration and Weeks after Sprouting.

Treatment (infected ACMD cuttings treated with neem leaf extract	Weeks After Planting						Mean
	2	**4**	**6**	**8**	**10**	**12**	
1000g per 1iter for1hr	1.0	1.7	1.8	1.8	1.7	3.0	1.8
1000g per litre for 6hr	1.0	1.7	2.0	1.8	2.0	3.0	1.9
1000g per litre for 12hr	1.0	1.7	2.0	1.7	2.0	3.0	1.9
2000g per litre for1hr	1.0	1.0	1.4	1.4	1.7	2.0	1.4
2000g per litre for 6hr	1.0	1.0	1.2	1.3	1.3	2.0	1.3
2000g per litre for 12hr	1.0	1.0	1.0	1.3	1.6	2.0	1.3
ACMV infected cutting (Cocoa)	2.0	2.3	2.4	2.3	2.6	3.3	2.5
Virus free/ symptomless cutting (Cocoa)		1.0	1.0	1.1	1.2	1.4	1.1
Mean	1.1	1.4	1.6	1.6	1.8	2.5	
SE± (Treatment)	0.1						
SE± (WAS)	0.1						
SE± (WAS x treatment)	0.2						
CV (%)	14.8						

**= Significantly different at $P_{0.005}$<0.001

3.4.2 EACMD Severity Expression of Neem Treated Cassava Cuttings at Varying Concentrations, Soaking Durations and Weeks after Sprouting.

Similar trend in results observed in the effect of Neem on ACMD was observed for EACMD but with lesser suppressive effect. The 2,000g/litre Neem - treated cutting had severity scores of 2.79, 2.66 and 2.49 for 1hr, 6hrs and 12hrs soaking duration respectively. Overall, severity score for the 12hrs soaking duration was significantly (P0.005<0.001) lower than the 1 hour treatment. However, significant difference was observed on the 1,000g/litre treatment but no significant difference was observed with the 2,000g/litre treatments between 8 to 12 weeks after sprouting (WAS). The 1000g neem -treated cuttings had significantly higher severity score compared to the 2,000g treatments. This implies that for EACMV infected cuttings, the higher the concentration (2,000g/litter) of neem treated cuttings immersed in neem leaf extract for at least 6 hours had more suppressive ability in the expression of the disease compared to lower concentration. The 1000g/litre neem - treated cassava cuttings had severity scores of 3.44, 3.08 and 3.08 at 1hr, 6 hr and 12hrs soaking duration respectively. All Neem treated cuttings had significantly ($P_{0.005}$<0.001) lower disease severity scores compared to than the EACMV infected cassava cuttings used as a check with a severity score of 3.7. Disease severity increased with time significantly with the highest severity score of 2.9 attained at 12 weeks after sprouting (Table 10).

Table 10. EACMD Severity Expression on Neem Treated Cassava Cuttings at Varying Concentrations, Soaking Duration and Weeks after Sprouting.

Treatment (infected EACMD cuttings treated with neem leaf extract	Weeks After Sprouting						Mean
	2	4	6	8	10	12	
1000gperl1iterfor1hr	3	3.3	3.3	3.3	4.0	3.8	3.4
1000gperlitrefor 6hr	3	3	3	3.3	3.2	3	3.1
1000gperlitrefor 12hr	2.9	3	3.1	3.3	3.1	3	3.1
2000 gperl1iterfor1hr	2.3	2.5	3.0	3.0	3.9	3	2.8
2000g per litre for 6hr	2.3	2.5	2.7	2.7	2.8	3	2.7
2000g per litre for 12hr	2.3	2.0	2.0	3.0	2.7	3	2.5
EACMV infected cutting (IFO 7003)	3.0	4.0	4.0	3.3	4.0	4.0	3.7
Virus free/ symptomless cutting (IFO 7003)	1.0	1.0	1.0	1.0	1.7	2.0	1.3
Mean	2.5	2.7	2.8	2.8	3.1	3.1	
SE± (Treatment)**	0.062						
SE± (WAS)**	0.054						
SE± (WAS x treatment)**	0.153						
CV (%)	6.6						

**= Significantly different at $P_{0.005}$<0.001

IV. DISCUSSION

The use of resistant varieties remains the most effective method of managing the cassava mosaic disease in most countries. However there are other options that can be applied especially for farmers who for certain reason hold on to the cultivation of local infected varieties as observed in Sierra Leone. Fargette *et al.* (1994) reported that disease-free cuttings could be recovered from symptom-free areas of cassava plants. This suggests that doubly infected plants would have a lower proportion of disease-free cuttings than singly infected plants. Growth and severity response to ACMV and EACMV infection varied based on the soaking duration and level of exposure to varying temperature and soaking duration. To date, the

disease is managed principally by using stakes from healthy plants. According to Hansen *et al.*, 2011, heat treatment has been comprehensively studied and can be used separately in multiple forms and in combination with fire, water based and atmospheric, steam, vapour heat, dry heat, forced hot air, high temperature controlled atmosphere as well as electric field and electromagnetic energies. He further stated that hot water treatment was a simple and cost effect method of virus elimination with disadvantages such as surface heating and fuel cost. In this study, treating stakes at temperatures of more than 550C appears promising but needs adjusting to reduce losses by the consequent low germination of stakes. The study also shows that growth and development of the infected cassava plants can be influence by heat treatment.

In this study, ACMV infected cassava cuttings significantly varied in their response to the cassava mosaic disease expression when subjected to varying treatments. Cassava cuttings subjected to 75^0C hot water treatment at 10, 30, and 60sec had significantly lower diseased severity score of 1.278, 1.278, and 1.056 respectively compared to treatments with lower water temperatures. The hot water treatment applied in this study did not eradicate the cassava mosaic virus disease but significantly suppressed disease expression and the onset on the disease. Hot water treatment effect on EACMD was inconsistent with the higher heat treatment of 750C. Heat treatment did not show any significant effect at 12 WAS. Severity score of the untreated cutting was not significantly different with severity score of cutting treated at 250C and 750C at 12 WAS. This result confirms the earlier report by Storey and Nichols 1938 who recognized that the mild and severe strain of the cassava mosaic virus which can be associated with the ACMD and EACMD respectively could respond differently to hot water treatment the latter (EACMD) being more heat resistant than ACMD and could also differ in heat tolerance. The used of thermotherapy to eradicate ACMD from the tip of cuttings and the entire plant was first demonstrated in 1959 but has not been used to control the disease (Walter and Raymond, 1982). According to Chant 1959, elimination of the ACMV was attained by hot air at 370C for 28 to 42 days; Kaiser 1982 indicated that the time required for freeing cassava plants and tip cuttings less than 1.5 cm long was greater than 84 days.

It has been recommended that heat therapy and tissue culture be used in quarantine stations to free imported, vegetatively propagated plant materials of systemic pathogens such as ACMD with success being recorded with EACMD in five cassava genotypes (Kaiser and Teemba, 1979). In this study, the mosaic virus was not eliminated by immersing infected cassava cuttings in hot water for 750C but was successfully used to generated symptomless cuttings and supressed expression of the mosaic disease significantly enough to influence yield difference.

The use of neem and moringa leaf extract as a bio pesticide in crop protection is not a new concept. However most studies have concentrated on insect pest, bacteria and fungi (Tijjani *et al.,* 2014). In this study, the use of moringa - treated cassava cuttings showed promising result which can be used as part of an integrated control option for the African cassava a mosaic disease in Sierra Leone. Despite the fact that the treatment did not eliminate the African cassava mosaic virus result indicate that moringa - treated cuttings immersed for about 12 hours suppressed the expression of mosaic smptoms and exhibited growth enhancing capabilities in terms of plant height, leaf number and leaf area. This cannot be said in the case of the East African Cassava Mosaic virus disease infection which exhibited very severe symptoms and did not respond to moringa treatments irrespective of the concentration and soaking duration.

Neem leaves contain fungicidal and antibacterial ingredients (Alzohairy, M. A, 2016).Studies with dried neem leaf extracts have shown that it can be used against several skin disease such ringworm, eczema and scabies in humans as well as the control of hyperlipidemia in a group of malarial patients severely infected with Plasmodium falciparum (Biswas, 2002). In trials, positive results have been obtained for significant analgesic, antipyretic, and anti-inflammatory effects. In this study Neem was used to control or supress the expression of the cassava mosaic disease on infected cassava cuttings. Neem has also been used to treat patients infected with HIV/AIDS with promising result. Neem treated cassava cuttings with a concentration of 2,000g per liter of water immersed between 6 to 12 hours exhibited greater potential in suppressing the African cassava mosaic virus and to a lesser extent the East African cassava mosaic virus disease. Generally neem treatment did not affect leaf production and plant for both EACMV and ACMV infected varieties however higher concentration and soaking duration of neem treated cassava cuttings performed better that ACMV and EACMV infected cuttings.

According to Tijjani A, (2014), the effectiveness of plants extracts depend on the nature and amount of biologically active ingredients it contains. Increase in the concentrations of the plant seed/bulb/leaves extracts correspondingly decreased radial growth of Aspergillus flavus and weight loss of tomato fruit. Increasing concentrations of these extracts implied an increase in the active ingredients of the solutions which act on the fungus thereby affecting its physiological processes and consequently lowering the growth of the fungus. The same inference can be said with respect Azadirachtin in the case of neem and Saponins 10% and Saponins 20% pterygospermin in the case of moringa which produce positive resulted when cutting were immersed in higher concentration of the active ingredient for a period of 6 to 12 hours.

V. CONCLUSION

The result from integrated pest management studies indicates that three options for the management of cassava mosaic disease (CMD) can be introduced in Sierra Leone. Firstly, High yielding varieties resistant to the cassava mosaic disease were identified. Secondly, the study shows that African cassava mosaic disease (ACMD) can be

influenced by heat treatment at 50 ^{0} C to 75^{0}C. The hot water treatment applied in this study did not eradicate the cassava mosaic virus disease but significantly ($P_{0.005}$<0.001) suppressed disease expression and the onset of the disease. Hot water treatment effect on the EACMD infected cuttings was inconsistent and may not be effective under severe infection of the East African cassava mosaic virus (EACMV) irrespective of the hot water temperature or soaking duration.

Thirdly, cassava cuttings treated with a concentration of 200g of dried Moringa leaf extract for the 6 and 12 hour soaking duration was effective in suppressing the African cassava mosaic disease but was not effective with the East African cassava mosaic disease.

Finally, the study shows that for ACMV infected cuttings, the higher the concentration (2,000g of fresh Neem leaf/litre) of Neem - treated cuttings immersed in neem solution for at least 6 hours was effective in suppressing the expression of the ACMD. Neem treated cuttings was more effective than hot water and the Moringa. Neem treatment was not as effective with EACMD but also exhibited suppressive tendencies.

ACKNOWLEDGMENT

The authors wish to acknowledge the Government of Sierra Leone through the Ministry of Agriculture Forestry and Food security (MAFFS), The World Bank and The West African Agricultural Productivity Programme (WAAPP) for the financial and technical support provided for this project. I wish to acknowledge staff of Njala University, for their technical input and supervision throughout this study. I wish to thank all colleagues who participated in the preparation of this manuscript.

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AUTHORS' PROFILES

Bio Data
Name: Alusaine Edward Samura
Place of Birth: Freetown Sierra Leone
Date Of Birth: 24th October 1974
Nationality: Sierra Leone
Religion: Christianity
Marital Status: Married With Two Children
Residential Address: 26a Spur Road, Lumely, Freetown
Contact Address: Njala Agricultural Research Centre Njala
Contact Numbers: 033-402-821/076-731-554 / 230370
E – Mail: aesamura@Yahoo.Com
Educational Background/ Academic Qualification

Year	Institution	Certificate/ Degree
2005	Njala University	M.Phil. Crop Protection
1998	Njala University	B.Sc Agriculture General

Bio Data
Name : Sheku Max Kanteh
Place of Birth: Pendembu, Kailahun District, Eastern Sierra Leone
Date of birth: December 22, 1962
Nationality: Sierra Leonean
Marital status: Married, with six children
Contact Address: Crop Protection Dept., Sch. of Agriculture, Njala Campus, Njala University, P. M. B., Freetown, Sierra Leone
E-mail: shekukanteh@yahoo.com OR sheku.kanteh5109@gmail.com
Mobile: +232-076-247-335 OR +232-088-288-483
Specialization: Crop Protection/Entomology
Education/Academic Qualification
Njala University
M.Phil. Crop Protection [Entomology] [USL/NUC]—2003
B.Sc. Agric. General [Div. II] [USL/NUC]--1996