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Cucumber mosaic virus in African yam bean (*Sphenostylis stenocarpa*): Occurrence and management with baking soda

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Abstract

African yam bean (AYB) is an underutilized grain legume rich in protein, carbohydrate and minerals. Management of pests and diseases in this legume involves the use of chemical pesticides which have adverse effects on the consumers and the environment. Therefore, this study investigated the use of baking soda (BS) as an alternative for the management of cucumber mosaic virus (CMV) in AYB. Four genotypes of AYB (NGBO349, AYB61, TSs3 and AYB94) were planted on the experimental field of Institute of Agricultural Research and Training, Ibadan in 2017. Treatments involved spraying with 15 g of baking soda per liter of water, 20 g of baking soda per liter of water and these were compared with spraying with an insecticide (lambda cyalothrin). Data were collected on the incidence and severity of CMV in the field. Virus titers were obtained using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Results revealed that the incidence of CMV in all the genotypes ranged between 11.8% and 39.3% while the severity ranged from 2 to 3 on a scale of 1-5. Spraying with 15 g/L of BS did not control CMV in AYB genotypes except in AYB94 in which the virus was absent. Spraying with 20 g/L controlled CMV in all the genotypes except TSs3 while spraying with insecticide controlled CMV in all but one genotype (NGBO349). The study concludes that baking soda at 20 g/L which has been previously used to control fungal pathogens in plants is a good alternative for the management of viruses in plants because it competed favourably with insecticide. However, further studies using polymerase chain reaction is required to validate the findings obtained using ELISA.

Keywords: African yam bean, baking soda, biopesticide, cucumber mosaic virus, ELISA

Introduction

African yam bean (*Sphenostylis stenocarpa*) is an underutilized legume producing edible tubers and seeds containing proteins which are higher than what could be obtained from most tuberous and leguminous crops (Nwokolo, 1996). The amino acid contents of African yam bean are higher than those found in pigeon pea, cowpea and bambara groundnut (Uguru and Madukaife, 2001) while the protein profile of its tubers compares favourably with those of yam, sweet potatoes and other root crops (Norman and Cunningham, 2006).

Despite the nutritional qualities of African yam bean (AYB), grain yield is affected by pests and diseases (Ameh and Okezie, 2005). There is sparse information on the viral diseases of AYB. Cucumber mosaic virus (CMV) is an important pathogen with a wide host range infecting about 1200 plant species belonging to 100 families (Adhab

and Al-Ani, 2011). Transmission of CMV is by aphids in a non-persistent manner (Palukaitis and Garcia-Arenal, 2003). The non-persistent transmission of CMV by aphids makes the control of the virus difficult (Azizi and Shams-bakhsh, 2014). This is because the insect would have acquired the virus quickly and transmitted it to healthy plants before insecticides could kill the aphids (Nault *et al*., 2004). Apart from this fact, the use of insecticides is detrimental to the expected agricultural productivity in terms of the effect on humans and the environment. It is important to emphasize that the use of eco-friendly pesticide will in no small way improve food security situation in our country.

Baking soda, also known as sodium bicarbonate ($NaHCO_3$) is an effective antibacterial, antifungal and antiviral agent which has been used for the control of pathogens affecting agricultural crops (El-Mougy and Abdel-Kader, 2009; Kareem *et al*., 2018a; Kareem *et al*., 2018b). The aim of the study is to indirectly control CMV in AYB by controlling the aphid vector using inorganic pesticide and baking soda which is an eco-friendly biopesticide.

Materials and Methods

Seeds of African yam bean genotypes (NGBO349, AYB61, TSs3 and AYB94) were obtained from the Genetic Resources Center of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The genotypes were planted on the experimental field of the Institute of Agricultural Research and Training (I.A.R.&T), Ibadan ($7^0$22′N, $3^0$50′E 182 masl). Two seeds were sown per hill and later thinned to one after germination. Plants were arranged in a Randomized Complete Block Design with three replications and a spacing of 1 m x 1 m. At three weeks after planting, each seedling was staked.

At exactly four weeks after planting, the emerged seedlings were sprayed with the following treatments i) 15 g of baking soda per liter of water + 1 ml of liquid detergent to act as a binding agent ii) 20 g of baking soda per liter of water + 1 ml of liquid detergent and iii) Lambda cyalothrin (according to manufacturer's instruction). Subsequent sprayings were carried out at 2 weeks intervals until a total of 4 spraying regimes were reached. Data were collected at the onset of flowering on virus disease symptoms.

Determination of virus disease traits

Disease incidence was calculated by counting the number of virus infected plants and expressing it as a percentage of the total number of plants per plot. Virus severity was determined by visual observation of virus infected plants and scoring on a scale of 1 to 5 with 1 representing absence of virus infection and 5 representing very severe virus infections (Gumedzoe *et al*., 1997).

Double Antibody Sandwich Enzyme-linked immunsorbent assay (DAS-ELISA) was used for the determination of CMV load in AYB genotypes after treatment with baking soda and lambda cyalothrin. The ELISA kit was obtained from Agdia, USA. Leaves of AYB showing the symptoms characteristic of CMV were ground in Agdia's general extract buffer (GEB) and 100 µl of extracted sap was loaded into the duplicate wells of micro titre plate previously loaded with capture antibody. The plate was incubated, washed and then loaded with the enzyme-linked antibody. Absorbance values at 405nm were determined after 1 h of incubation and any value that was twice the value of the negative control was considered positive.

Statistical analysis

Data obtained on virus incidence, virus severity and virus titers were subjected to analysis of variance using SAS statistical package v 9.4. Mean separation was carried out using Least Significant Difference (LSD).

Results and Discussion

The mean square (MS) values for Cucumber mosaic virus titers were significant at 1% level of probability for genotype and treatment as well as their interactions. However, the MS value was not significant for incidence and severity (Table 1).

The occurrence of CMV in plant species has been reported by several authors (Garcia-Arenal and Palukaitis, 2008; de Breuil *et al*., 2012). The high significant effect of genotype and treatment as well as their interactions on CMV titers revealed that genotype and the biopesticide treatments imposed on AYB are important factors in determining CMV accumulation in AYB. Egesi *et al*. (2009) reported the importance of host plant resistance in disease control. The use of baking soda generally regarded as safe (GRAS) is an environmentally responsible way for the control of diseases (Afolabi and Kareem, 2018).

Results of the incidence of Cucumber mosaic virus in the four genotypes indicated that there was no significant difference in the response of all the genotypes to the virus irrespective of the type of treatment given (Table 2). Similarly, there was no significant difference in the severity of the virus in all the genotypes and values ranged from 2.0 to 2.33. The genotypes responded differently in the accumulation of the virus. NGBO349 had the highest virus titer followed by TSs3. There was no significant difference in the response of AYB61 and AYB94 and their titer values were very low (Table 2).

The reduced values of viral disease traits in some genotypes of AYB implied that genotype plays an important role in the establishment of diseases in plants and such genotypes are useful in resistance breeding program. Aliyu and Balogun (2011) reported that identification of tolerant cowpea genotypes is an important step in achieving the objective of economic control of viral diseases in agricultural crops.

Treatment did not have any significant effect on the incidence of CMV and mean values ranged between 18.03% and 26.65%. The effect of treatment was not significant on virus severity and the highest mean value was 2.25 while the least mean value was 2.13. However, treatment had significant effect on virus titers. Plants sprayed with insecticide had the highest virus titer (0.418) while plants that were sprayed with 20 g/L of baking soda had the least titer (0.317) (Table 3).

Several authors have reported the use of baking soda for the control of plant diseases (Palou *et al*., 2001; El-Mougy and Abdel-Kader, 2009; Kareem *et al*., 2018a).The results of CMV titers in AYB genotypes showed that the type of treatment given to plants determines their response to disease establishment. Baking soda competed favourably with lambda cyalothrin in the management of the virus. The benefits offered by biopesticides along the food value chain and as additional options for growers are incomparable to the use of inorganic chemicals. The research of Kareem *et al*. (2018b) reported the use of baking soda (a biopesticide) for the management of Amaranthus mosaic virus (AMV) in *Amaranthus cruentus* leading to reduced incidence and severity of AMV in the vegetable.

Enzyme-linked immunosorbent assay revealed that AYB94 sprayed with either baking soda or pesticide was negative to CMV infection. AYB61 sprayed with 20 g/L of BS and pesticide also showed negative reaction to CMV. TSs3 that received baking soda treatment was positive to CMV while the plot treated with pesticide was negative to the virus. NGBO349 plot sprayed with 15 g/L of BS and the plot sprayed with insecticide were positive to CMV infection. However, the plot sprayed with 20 g/L of BS was negative (Table 4).

The detection of CMV in underutilized legumes by ELISA has been previously reported (Azizi and Shams-bakhsh, 2014). Mofunanya (2016) also reported the infection of AYB by Telfaria mosaic virus, in which the virus impacted negatively on the nutritional qualities of AYB. It is important to note that negative results obtained by ELISA can be positive if tested with molecular means of identification such as polymerase chain reaction (PCR).

Conclusion

The study revealed that baking soda competed favourably with the synthetic insecticide (lambda cyalothrin) for the control of Cucumber mosaic virus. Genotypes also played a significant role in crop susceptibility/tolerance to diseases. However, a single method cannot be efficient for disease control but a holistic approach encompassing

the use of resistant genotypes coupled with the use of eco-friendly pesticide and the use of integrated disease management (IDM) are all important in disease control.

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Conflict of interests

There is no conflict of interests among the authors

Tables, Figures and Charts

Table 1. Mean square values of the effect of baking soda on cucumber mosaic virus in African yam bean

		Mean square values		
Source of variation	**df**	**incidence**	**severity**	**Virus titer**
Rep	1	714.420 ^{ns}	2.000*	0.006*
Genotype (V)	3	273.137 ^{ns}	0.083 ^{ns}	0.130**
Treatment (T)	3	349.588 ^{ns}	0.250 ^{ns}	0.020**
V x T	9	196.206 ^{ns}	0.333 ^{ns}	0.180**
Error	15	215.550	0.267	0.000

ns = not significant, * = significant at 5% level of probability, ** = significant at 1% level of probability

Table 2. Mean effect of genotype on virus disease traits

Genotype	**Incidence**	**Severity**	**Virus titer**
NGBO349	22.98	2.167	0.578
AYB61	30.00	2.333	0.279
TSs3	20.03	2.167	0.353
AYB94	21.15	2.000	0.261
LSD	17.40	0.585	0.032

LSD = Least Significant Difference

Table 3. Mean effect of treatment on virus disease traits

Treatment	**Incidence**	**Severity**	**Virus titer**
15 g/L of BS	26.65	2.13	0.369
20 g/L of BS	18.03	2.25	0.317
Insecticide	25.95	2.13	0.418
LSD	15.07	0.51	0.027

BS = Baking soda; LSD = Least Significant Difference

Table 4. Occurrence of Cucumber mosaic virus in African yam bean as detected by Enzyme-linked immunosorbent assay (A_{405nm})

Treatment	NGBO349	AYB61	TSs3	AYB94
15 g/L of BS	0.383/+	0.408/+	0.384/+	0.302/-
20 g/L of BS	0.242/-	0.176/-	0.525/+	0.323/-
Insecticide	1.109/+	0.253/-	0.150/-	0.159/-
Healthy control	0.158	0.158	0.158	0.158

BS = Baking soda; + = Virus present; - = Virus absent; values that are twice the healthy control are positive (+)

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