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## EFFECT OF YELLOW VEIN MOSAIC VIRUS DISEASE OF OKRA ON GROWTH AND PROXIMATE ANALYSIS OF SOME OKRA CULTIVARS IN DUTSIN-MA

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### ABSTRACT

The study was conducted on okra, an important food consumed in Dutsin ma, Katsina State, Nigeria. Reports have indicates the effect of viral infections on produce quantity and quality, this study determined and document the effect of yellow vein mosaic virus disease of okra on performance and nutritional composition of some okra cultivars in Dutsin-ma, Sudan Savannah. Seedlings were inoculated with the virus at 1 week post emergence. Each cultivar was evaluated as inoculated (infected) and uninoculated (Healthy) treatments. Percent disease severity of infection (%), yield related parameters and proximate analysis of pod were measured. Data were subjected to independent *t* test and significance was determined at 5 % level of probability. Percent disease severity was highest in 'Yar Balla' (21.3%) as compared to the remaining cultivars. Reductions in plant height (8.8 %), fruit weight (37.6 %) and crude protein were significantly lower in 'Yar Balla' and 'Jikin Mutum' The present study reveal that the reduction in crude protein as a result of YVMV infection would worsen the protein deficiency problem of the populace who depend on the crop as the cheapest means of obtaining their daily protein and mineral requirement. There is therefore the need to prevent viral infection of vegetable crops in order to ensure high yield and guarantee its nutritive value.

**Keywords:** Disease severity; okra; proximate analysis; pod; yellow vein mosaic virus disease.

### INTRODUCTION

Vegetables are crop species which are not only good sources of essential vitamins and minerals (Bakhru, 2003), they are widely used as complement to starchy staple foods. In Africa, vegetables constitute the fourth largest group of commodities produced for various uses. Okra (*Abelmoschus esculentus* L. Moench) also known as lady's finger is a member of the family Malvaceae (Walker, 2012). It is cultivated in several parts of tropical and sub-tropical Africa (Saifullah and Rabbani, 2009). Okra is grown on subsistence and large-scale farms in Iran, Turkey, West Africa, many Asian countries and the southern United States. In 2016, the world total okra output was approximately 8.9 million tonnes. India was the largest producer with about 5.5 million tonnes, followed by Nigeria which produced approximately 2 million tonnes and Sudan had about 287, 300 tonnes (FAO, 2016).

Farmers usually harvest fruits at edible maturity to sell in the fresh market or dry fruit and partially processed for the market. It is rich in calcium and phosphorus, it also contains protein, carbohydrate, fibre and vitamins (Ogungbenle and Omosola, 2015). Okra contains carbohydrates and vitamins (Arapitsas, 2008, Dilruba *et al.*, 2009), and plays a vital role in human diet (Kahlon *et al.*, 2007, Saifullah and Rabbani, 2009). Mainly, the immature leaves are sometimes used for soup making and flavouring or may be added to salads and stews. Consumption of young immature okra pods is important as fresh fruits, and it can be consumed in

different forms (Ndunguru and Rajabu, 2004). Fruits can be boiled, fried or cooked (Akintoye *et al.*, 2011). The composition of okra pods per 100 g edible portion (81% of the product as purchased, ends trimmed) is: water 88.6 g, energy 144.00 kJ (36 kcal), protein 2.10 g, carbohydrate 8.20 g, fat 0.20 g, fibre 1.70 g, Ca 84.00 mg, P 90.00 mg, Fe 1.20 mg,  $\beta$ -carotene 185.00  $\mu$ g, riboflavin 0.08 mg, thiamin 0.04 mg, niacin 0.60 mg, ascorbic acid 47.00 mg (Ogungbenle and Omosola, 2015).

The crop is prone to damage by various diseases caused by various insects, fungi, nematodes and viruses. But its cultivation is seriously threatened by attack of one most important Yellow Vein Mosaic Virus (YVMV) by affecting different parts of plant (Fajinmi and Fajinmi, 2010) which causes heavy losses not only in respect to the fruit yield but fruit quality (Alegbejo, 2001; Ali *et al.*, 2000) and occurred at all crop growth stages. *Yellow vein mosaic virus* of okra is transmitted by whitefly (*Bemisia tabaci* Gen.), as reported by Kumar *et al.* (2017). The disease was first described as yellow vein banding, though the disease was characterized by clearing of veins, but there was no evidence that the veins remain green and banded by stripes of yellow tissue.

The virus produces typical vein yellowing and thickening of leaves forming a network of veins and veinlets in the infected leaves. Initially, the leaves exhibit only yellow colored veins but under the severe infection, the leaves become completely

chlorotic and turn yellow. There is reduction of leaf chlorophyll and the infected plants give a stunted look and produce small sized pale yellow fruits (Kumar *et al.*, 2017) If plants were infected within 20 days after germination, their growth is retarded; few leaves and fruits are formed and loss may be about 94% (Fajinmi and Fajinmi, 2010). The extent of damage declines with delay in infection of the plants. Plants infected 50 and 65 days after germination suffer a loss of 84 and 49%, respectively (Sastry and Singh, 1974).

In the tropics, diseases caused by virus have been recognized to contribute one of the major factors limiting vegetable production (Salaudeen, 2016). In most African countries, viruses are major limiting factors to vegetable production and serves as hosts to number of other viruses (Alegbejo, 2015). In 2001, *Yellow vein mosaic virus* was reported for the time in Nigeria infecting Okra (Alegbejo *et al.*, 2001). Since then, YVMV has been found to be highly prevalent with an increased incidence rate of 25.7% in a study conducted by Abdullahi *et al.* (2016). Also, reports have also indicated the effect of virus infections on produce quality. Aliyu *et al.* (2016) reported a decreased in nutritional composition in *Amaranthus* infected with *Amaranthus mosaic virus* (AMV). There is dearth of information on the detailed nutritional composition of okra infected with viral diseases in the study area. The objective of this study therefore was to determine and document the effect of Yellow vein mosaic virus disease of okra on performance and nutritional composition of some okra cultivars in Dutsin-ma, Sudan Savannah of Nigeria

## MATERIALS AND METHODS

The growth trial was conducted in 2021 at the Teaching and Research Farm, Faculty of Agriculture and Agricultural Technology, Federal University Dutsin-Ma (12°26'N and 07°29'E and 212 m above sea level), Nigeria. Also, the proximate analysis was carried out at the Food Science Laboratory, Faculty of Renewable Natural Recourses in same institution. The climate is the tropical wet and dry type (tropical continental climate) classified by Koppen as Aw climate. Rainfall is between May and September with a peak in August. The average annual rainfall is about 700 mm. The mean annual temperature ranges from 29 °C – 31 °C. Evapo-transpiration is generally high throughout the year. The vegetation of the area is the Sudan Savanna type which combines the characteristics of both the Guinea and Sahel Savanna (Abaje *et al.*, 2014)

### Virus Inoculum and Multiplication

The *Yellow vein mosaic virus* (YVMV) isolate used was obtained from the stock in the Department of Crop Protection, Institute for Agricultural Research, Ahmadu University Samaru, Zaria, Nigeria. Virus

inoculum was multiplied in 10-day old cowpea (cv. Ife Brown) seedlings by mechanical inoculation. This was accomplished by homogenizing (1g/1mL) YVMV-infected leaves in inoculation buffer (0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine per litre of distilled water, adjusted to pH 7.2) using cold sterilized mortar and pestle. Carborundum powder (600-mesh) and 2 µL of β- mercapto ethanol were added to the extract before the extract was rubbed on the upper leaf surface. The inoculated plants were rinsed with distilled water and maintained in a screenhouse for symptom development. Symptomatic leaves were harvested at two weeks post inoculation and subjected to Antigen Coated Plate-Enzyme-Linked Immunosorbent Assay (ACP-ELISA) as described by Kumar (2009). All the reagents for ELISA were purchased from BDH Chemicals Ltd., England. Sample wells with absorbance values (at 405-nm wavelength) more than twice those of healthy soybean control wells were considered positive. Excess leaf tissues from the YVMV positive samples were preserved on silica gels and used for subsequent inoculation.

### Treatments and Experimental Design

Five popularly adopted okra cultivars ('Yar Balla', 'Kamar Rumbu', 'Kahon Balewa', 'Yar Maradi' and 'Jikin Mutum') constituted the treatments. The okra cultivars as identified were among the early maturing types (40 days). Each cultivar was laid out separately as infected and uninfected plots using Randomised Complete Block Design (RCBD) with three replicates. Gross plots size was 11 m × 17.25 m (189.75 m<sup>2</sup>) and the net plot size was 8 m × 14.25 m (114 m<sup>2</sup>), containing 20 rows of 2 m long ridges each. An alley of 1 m was left between replicates and 20 m between infected and uninfected (Healthy) plots. The okra cultivars evaluated were obtained from local farmers' fields, Dutsin- Ma, Nigeria. They were selected among the commonly grown cultivars in the study area.

### Field Establishment, Management and Inoculations

Sowing was done on 2 m long ridges at the rate of 3 seeds per hole, using an inter-and intra-row spacing of 75 × 10 cm. Seedlings were thinned to one plant per stand at one week after sowing (WAS). The seedlings were infected with the YVMV at 10 days after sowing using the same procedure for inoculation as described above. Five plants were tagged across the treatments for data observations and collection. Weeds were manually controlled using hoe at 3 and 6 WAS. Fruits/pods were harvested at immature stage from the five tagged plants across the treatments. Each sample about 50 g was packed in a sterile polyethylene bag, placed in an isothermal box equipped with ice packs and

transferred to the laboratory for proximate and minerals analysis.

### Observations and Data Analysis

Data was collected from the 1<sup>st</sup> to the 6<sup>th</sup> weeks post inoculation (WPI) on plant height, number of leaves per plant and number of leaves with virus disease symptoms from the five tagged plants across the treatments. The percentage disease severity was measured by the number of diseased leaves relative to the number of leaves on any given plant and this value was expressed as percentage (Aliyu *et al.*, 2016).

**Proximate analysis:** The standard methods of the Association of Official Analytical Chemists (AOAC, 2005) were used to determine moisture, ash, crude fat and crude protein content. Moisture content was obtained by heating three 5.0 g portions of the okra samples in an oven (Gallenkamp QC, England) at 110°C until a constant weight was obtained. Ash determination was obtained by the incineration of three 3.0 g samples in a muffle furnace at 600°C for 3 h when a light-grey ash was produced. Crude protein (CP) was obtained using three 3.0 g portions of the samples. The CP was calculated by a multiplying factor (%N × 6.25). The crude fat (CF) was determined by extraction procedure using three 5.0 g samples in a Soxhlet apparatus using petroleum spirit (bp 40 - 60°C) as the solvent.

### Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using the General Linear Model (PROC GLM) procedure of SAS (2008) at  $p < 0.05$ . For significant *F* tests, means were separated using Least Significant Difference (LSD).

### Results

The severity of YVMV disease varied significantly among crop cultivars as shown in Table 1. Symptom expression was generally insignificant at 1<sup>st</sup> and 2<sup>nd</sup> WPI. The effect of the treatments became apparent from the 3<sup>rd</sup> WPI. Significantly highest percent disease severity was observed in ‘Yar Balla’ okra cultivar (13.66) followed by ‘Jikin Mutum’ (12.82) and Kahon Barewa (12.78). At 4<sup>th</sup> WPI, the significantly highest percentage disease severity of (18.66) was found in ‘Yar Balla’, 17.33% was observed in ‘Jikin Mutum’ whereas the lowest value of 13.78 was reported on ‘Yar Maradi’ cultivar. A consistent trend was observed such that at 6<sup>th</sup> WPI, among the cultivars, disease severity was consistently highest in ‘Yar Balla’ (21.33%), followed by ‘Jikin Mutum’ (19.33%), while ‘Yar Maradi’ consistently elicited the lowest symptom severity of 17.00%. The healthy okra plants which serve as control also showed some level of infection although very minimal (Table 1).

Virus infections had significant effects on the heights of the okra plants irrespective of the cultivar. The healthy plants of all the evaluated cultivars exhibited normal and rapid growth contrary to those infected with YVMV. The virus restricted plant height at varying levels among the crop cultivars (Table 2). YVMV infected plants of ‘Kamar Rumbu’ variety exhibited the highest height reduction at 6 WPI of study (24.5 %), this was followed by ‘Kahon Barewa’ with 11.96% whereas the lowest in height reduction was reported from ‘Jikin Mutum’ (11.3%). At 9 WPI indicated that plant height was severely impaired in the infected plants of ‘Kahon Barewa’ (31.3 %), Kamar Rumbu had 21.5% reduction and the least of 13.4% was observed from the ‘Jikin Mutum’ cultivar. The healthy plants produced broad leaves with normal shape while the leaves of YVMV infected plants were narrow and twisted (Table 2). The difference in number of leaves per plant between healthy and infected plants was not significant in ‘Yar Balla’ cultivar at 6 WPI. However, there was a significantly different amongst the remaining okra cultivar on number of leaves produced by plants that received the virus treatment from those produced by the healthy okra plants (Table 2). The highest percent leaf numbers per plant reduction was most conspicuous in infected plants of ‘Jikin Mutum’ cultivar (41.6) at 9 WPI and the remaining okra varieties had minimal leaf numbers per plant reduction of 40.0% each.

The number of branches produced by plants that received the virus treatment was significantly different from those produced by the healthy okra plants (Table 3). The YVMV infected okra plants resulted in the production of fewer branches, which were significantly different from those of the healthy plants, especially in Jikin Mutum cultivar which had no branch, while ‘Yar Balla’, ‘Kahon Barewa’, ‘Yar Maradi’ and ‘Kamar Rumbu’ cultivar gave one branch each at 9 WPI (Table 3). Also, maximum of four branches were reported from the healthy ‘Kahon Barewa’ cultivar, Yar Balla, Yar Maradi and Jikin Mutum had 3 branches each whereas Kamar Rumbu produced only 2. Infection had significantly affected all the okra cultivar investigated on days to 50% flowering of the plants. While the healthy plants flowered early within 45 – 45 days after planting (DAP), the infected okra plants had delayed flowering which ranged from 51 – 55 DAP (Table 3). Infection by YVMV also had adverse effected on fruit/pod weight in the five okra cultivar investigated. However, reduction in fruit weight was more severe in the plants of than those of Jikin Mutum (10.09 g) followed by “Yar Maradi” (8.4 g) and Kamar Rumbu cultivar recorded 7.7 g weight reduction as compared to their healthy counterparts. Pods from the infected plants of “Kahon Barewa” were heavier than the remaining cultivar.

**Table 1: Percent disease severity Score of Okra yellow vein mosaic virus infection and healthy okra varieties**

WPI	Okra cultivars														
	'Yar Balla'			'Kahon Barewa'			'Jikin Mutum'			'Yar Maradi'			'Kamar Rumbu'		
	Healthy	Infected	LSD	Healthy	Infected	LSD	Healthy	Infected	LSD	Healthy	Infected	LSD	Healthy	Infected	LSD
1st	0.33	1.00	0.92	0.00	1.00	0.00	0.33	0.66	1.3.0	0.33	1.00	0.90	0.00	1.00	0.00
2nd	1.00	1.33	0.92	1.66	1.66	1.30	1.33	1.66	1.3.0	1.33	1.33	1.30	1.00	1.30	0.92
3 rd	3.33b	13.66a	3.46	3.33b	12.78a	2.77	2.66b	12.82b	2.04	2.33b	9.49a	4.10	2.66b	11.45a	5.22
4th	4.33b	18.66a	3.81	2.86b	17.33a	2.06	3.66b	15.52a	2.98	3.00b	13.78a	0.80	3.00b	15.33a	0.92
5th	4.33b	20.33a	4.43	3.33b	18.66a	3.46	3.66b	16.33a	2.06	3.00b	15.00a	1.60	3.33b	16.33a	1.30
6th	4.33b	21.33a	2.61	3.33b	19.33a	3.81	4.00b	18.00a	0.00	3.33b	17.00a	1.90	4.00b	19.00a	3.20

In each row, means followed by same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

**Table 2: Effect of Okra yellow vein mosaic virus infection on plant height and leaf numbers of some locally adopted okra cultivar in Dutsin-ma during the 2021 rainy season**

Parameters/Treatment	Okra cultivar				
	Yar Balla	Kahon Barewa	Jikin Mutum	Yar Maradi	Kamar Rumbu
Plant height (cm) 6 WPI					
Healthy	22.13a	20.66a	21.93a	22.0a	25.53a
Infected	18.20b	17.76b	19.46b	19.23a	19.26b
LSD	3.43	1.91	2.61	2.93	2.3
Plant height (cm) 9 WPI					
Healthy	49.30a	52.23	46.66	48.03a	49.53a
Infected	40.41b	35.86	40.41	38.90b	38.90b
LSD	4.14	NS	NS	7.65	7.01
Leaf numbers 6 WPI					
Healthy	8	8a	9a	8a	9a
Infected	5	5b	5b	4b	5b
LSD	1.28	1.3	3	1.85	1.3
Leaf numbers 9 WPI					
Healthy	10a	11a	12a	11a	12a
Infected	6b	7b	7b	7b	8b
LSD	1.85	2.44	2.92	1.3	1.85

Means followed by same letter within the same column for each parameter are not significantly different according to Least Significant Difference (LSD) at  $p=0.05$

**Table 3: Effect of Okra yellow vein mosaic virus infection on plant branches and yield components of some locally adopted okra cultivars in Dutsin-ma during the 2021 rainy season**

Parameters/Treatment	Okra cultivar				
	Yar Balla	Kahon Barewa	Jikin Mutum	Yar Maradi	Dan Rumbu
Branches 6 WPI					
Healthy	2	3a	2aob	2a	2a
Infected	1	1b	0.92	10b	1a
LSD	NS	1.3	1.3	1.3	1.85
Branches 9 WPI					
Healthy	3a	4a	3a	3a	2a
Infected	1b	1b	0b	1b	1a
LSD	1.3	0.92	1.3	2.77	1.3
Days to 50% flowering					
Healthy	45b	44b	45b	44b	45b
Infected	53a	55a	52a	54a	51a
LSD	5.85	0.26	5.23	3.33	3.81
Fresh pod weight (g)					
Healthy	19.8a	23.4a	20.75a	21.80a	20.10a
Infected	13.6b	17.7b	10.66b	13.43b	12.40b
LSD	2.35	0.52	2.6	1.77	1.16

Means followed by same letter within the same column for each parameter are not significantly different according to Least Significant Difference (LSD) at  $p=0.05$

**Table 4: Proximate analysis of Okra yellow vein mosaic virus (Okrymv) and buffer infected okra varieties**

	'Yar Balla'			'Kahon Barewa'			'Jikin Mutum'			'Yar Maradi'			'Kamar Rumbu'		
	Healthy	Infected	LSD	Healthy	Infected	LSD	Healthy	Infected	LSD	Healthy	Infected	LSD	Healthy	Infected	LSD
Ash	5.5	5.1	2.68	5.2	4.5	0.78	5.6	5.2	2.68	5.3a	4.3b	0.1	5.6	5.3	2.68
Crude fat	6.2	5.7	0.87	5.8	5.5	0.52	6.4	5.7	0.89	6.7a	5.7b	0.6	6.2	5.7	0.91
Crude fibre	11.4	8.3	5.16	11.3	8.6	2.77	11.4	8.5	5.22	9.4	8.5	3.9	11.4	8.6	5.15
Moisture	13.1	13.3	1.32	13.4	13	0.58	13.6	13.4	0.46	13.7	13.4	0.8	13.7	13.6	0.77
Crude protein	23.3a	11.9b	4.19	23.1a	13.0b	1.36	23.3a	11.9b	3.92	23.4a	13.2b	1.3	23.4a	11.9b	3.94
Carbohydrate	40.5b	56.3a	5.28	42.1b	56.0a	5.28	43.8b	56.3a	7.31	41.9b	55.5a	3.1	42.3b	56.3a	5.36

In each row, means followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

The virus inocula caused significant reductions in the crude protein content of the pods harvested from infected plants. Crude protein content of such pods ranged from 11.9 to 13.2% while those from healthy plants ranged from 23.1 to 23.4% (Table 4). Generally, pods from ‘Yar Balla, ‘Jikin Mutum’ and ‘Kamar Rumbu’ infected plants had significantly lowest crude protein content (11.9%) as compared to the other cultivars tested whereas healthy okra pods from ‘Yar Maradi’ and ‘Kamar Rumbu’ had significantly highest percent crude protein content of 23.4 than the remaining varieties (Table 4). The carbohydrate content of pods from virus infected okra plants were higher (41.9-56.3%) than those from their healthy counterparts (40.5-56.3%). Pods harvested from virus infected okra cultivars gave significantly highest ( $p < 0.05$ ) carbohydrate content than all the healthy cultivars. Also, the Ash and crude fat contents of pods from the healthy Yar Maradi cultivar gave significantly highest mean values (5.3 and 6.7 %) than those of the infected plants, while the remaining cultivars did not differ (Table 4).

## DISCUSSIONS

Infection by yellow vein mosaic virus disease resulted in a greater reduction in the growth factors and the nutritive content of the okra pods. These results agree with reports of previous studies which indicate that viral infections resulted in a greater reduction in growth and yield parameters of infected susceptible crops (Agrios, 2005). The significant reduction in plant height was due to impairment of growth hormones. Thus in a plant susceptible to virus infection, synthesis of chlorophyll is greatly reduced and this ultimately results in chlorosis and necrosis. A plant which cannot produce sufficient chlorophyll is prone to malnutrition as a result of inability to carry out photosynthesis. This probably explains the reduced leaf numbers and growth of the diseased plants. Low level of photosynthesis in the diseased plants also affected plant height. However, the observation that the plants of ‘Yar Maradi’ and ‘Yar Balla’ were drastically affected by the disease revealed its vulnerability to the pathogen. The result indicated that healthy plants were taller corroborates the findings of Balogun *et al.* (2007) when some okra lines were challenged with *Cucumber mosaic virus*. Plants inoculated with the virus generally produced tiny and fewer fruits which were of poor market value probably owing to the cumulative effect of disease severity and growth retardation. The higher fruit weight obtained from ‘Kahon Balewa’ implies that the variety combined YVMV tolerance with desirable yield (Salaudeen, 2016). Fruit weight is one of the important traits for selection in breeding programmes (Ali *et al.*, 2000). This is due to the fact that varieties that are capable of producing appreciable yield under disease pressure are more likely to be adopted by farmers. The low protein values in the infected plants may be due to changes in the metabolic activities of the plants as a result of the virus infection since the pathogen (virus) depends on the protein synthesis machineries of host cell for survival.

This is in agreement with the findings of Aliyu *et al.* (2016) while determining the effect of BMV and AMV on the proximate analysis of virus and infected *Amaranthus hybridus*. This study has once again confirmed the susceptibility of the five locally adopted cultivars to the virus used in this trial.

## CONCLUSION

The infection of the virus on the five cultivars investigated resulted in impairment of growth parameters. ‘Kahon Barewa’ cultivar appeared to be the susceptible amongst the five cultivars in respect to infections. The infection of the cultivars resulted in depletion of the nutrient in the pods; this would worsen the nutrient deficiency problem of the populace who depend on the crop as the cheapest means of obtaining their daily nutrient requirement. There is therefore the need to prevent virus infection of vegetable crops in order to ensure high yield and guarantee its nutritive value. Presently, the use of resistant varieties is the only practicable means of controlling the viruses.

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