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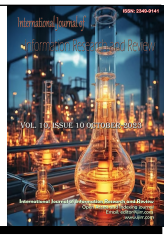

RESEARCH ARTICLE

EVALUATION OF THE EFFECTS OF DRY AND RAINY SEASONS ON CASSAVA *(MANIHOT ESCULENTA CRANTZ)* ROOTSTOXICITY AND FUNGI ASSOCIATED WITH ITS ROT

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ABSTRACT

Water stress is known to enhance the accumulation of vitamins and cyanogenic glucosides in cassava (Manihotesculenta Crantz), a food security crop in Sub-Saharan Africa. A study was carried out to evaluation of the effects of dry and rainy seasons on cassava rootstoxicity and fungi associated with its rot**.** Laboratory work was done at Imo State University's Department of Plant Science and Biotechnology, while the field experiment was conducted at Nigeria's National Root Crop Research Institute in Umudike, Umuahia, Abia State. Two native varieties, L1 and L2, and four improved cassava cultivars were used: TME419, NR87/184, UMUCAS 46, and 07/0539. There were three iterations of the experimental field's randomised whole block design. After planting, each variety of cassava was allowed to grow for either twelve or fifteen months. In July, during the wet season, and December, during the dry season, their storage roots were gathered and analysed according to conventional techniques for cyanide, vitamins A and C, and carotenoids. Improved cassava cultivars accumulated more vitamins A and C, and carotenoids, while the native varieties accumulated more cyanide than the mproved cultivars. In contrast to the wet season, the dry season showed higher quantities of cyanide, vitamins A and C, and carotenoids in all the varieties Botryiodiplodiatheorbromae, Fusariumoxysporum, Aspergillusflavus, Rhizopusstolonifer, and Sclerotiumrolfsii are some of the fungal infections linked to cassava rot. The correlation between climate change and food quality will be brought to light in the study.

INTRODUCTION

Cassava,(*Manihotesculenta* Crantz), plays a crucial role in ensuring food security, especially in the face of climate change. It is consumed by almost one billion individuals, primarily in tropical and subtropical areas across the globe (Alicia *et al*., 2016). Cassava roots are a significant provider of dietary and industrial carbohydrates. They are primarily consumed for their starch content, the roots are valuable for producing industrial starch and animal feed, making the crop economically important. However, cassava is highly susceptible to post-harvest physiological deterioration. Cassava tissues contain significant amounts of cyanogenic glucosides, which are very poisonous and pose a significant risk to both health and food safety (Alicia *et al*., 2016). These cyanogenic glucosides are released as toxic hydrogen cyanide when the leaves and tubers of cassava are crushed or chewed (Montagnac*et al*., 2009). Consuming cyanogenic substances without eliminating them can induce acute poisoning, resulting in symptoms such as headaches and vomiting.

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In severe cases, it may lead to a sort of irreversible leg paralysis or even death (Cliff, 1994; Burns *et al*., 2012). Cassava exhibits a significant ability to adapt to recurring drought conditions, both in terms of its growth and chemical makeup (Vandegeer*et al*., 2013). The adaptability of cassava underscores the significance of recognising climate change as a complex phenomenon, which has consequences for individuals who depend on cassava as their primary source of sustenance and livelihood (Nhassico*et al*., 2008). The quantities of cyanogenic glucosides in cassava are subject to variation based on agricultural practices, genotype, food preparation methods, and environmental conditions (Bokanga*et al*., 1994; Burns *et al*., 2010; Mtunguja*et al*., 2016). Fungi are linked to the occurrence of cassava root rot, resulting in average output losses of 0.5 to 1 ton/ha. However, in some cases, losses can exceed 3 ton/ha, which is comparable to a yield reduction of 15 to 20% (Bandyopadhyay*et al*, 2006). Projections for the regions in Africa where cassava is grown indicate that the average surface air temperature will rise by 3-4 °C, with seasonal rises of up to 7 °C by the year 2099, according to studies conducted by Collins *et al*. (2013) and Niang*et al*. (2014). Understanding the impact of environmental factors on the cyanogenic glucoside content of this staple food is

vital, considering its significance in human nutrition and the overall importance of food quality in achieving food security.

MATERIAL AND METHODS

Study area and Experimental Design: The research was carried out at the National Root Crop Research Institute in Umudike, Umuahia, Abia State, Nigeria. The laboratory work was undertaken at the Department of Plant Science and Biotechnology in Imo State University, Owerri. The region is situated in the humid rainforest agroecological zone of Nigeria, with coordinates ranging from longitude 3° E to 12°E and latitude 4°N to 9°N (Akinsanola *et al*., 2016). It is known for being the leading producer of cassava. The experimental field was structured as a randomised complete block design, consisting of three repeats. Each iteration encompassed an area of 50 square metres, with 50 cassava plants evenly distributed at a spacing of 1 metre apart. The enhanced cultivars consist of TME419, NR87/184, UMUCAS 46 or 07/0539, as well as two indigenous types, L1 and L2.The population of Imo State strongly values the L1 and L2. All cassava cultivars were cultivated for a duration of either 12 or 15 months after being planted. Their store roots were collected in July (during the rainy season) and December (during the dry season), respectively, in order to measure all the parameters. The cassava cultivars were harvested 12 months after planting (12MAP) during the dry season and 15 months after planting (15MAP) during the rainy season.

Quantification of carotenoids: The extraction of total carotenoid and its components was conducted using a modified version of the method outlined by Meléndez-Martínez *et al*., (2007). The samples were mixed thoroughly using a mixer. Then, 5 g of the resulting mixture was carefully measured and placed into a centrifuge tube. The extraction process was carried out using high-performance liquid chromatography (HPLC) grade solvents, namely 25 mL of an extraction solution consisting of hexane, acetone, and methanol in a ratio of 50:25:25, with the addition of 0.1% butylatedhydroxytoluene. The mixture was homogenised and thereafter subjected to centrifugation for a duration of 10 minutes at a speed of 4000 revolutions per minute and a temperature of 4 degrees Celsius. The liquid portion, known as the supernatant, was utilised for measuring absorbance at a wavelength of 450 nm using a spectrophotometer called Bio Tek Power Wave.

$$\text{Total carotenoids content } (\mu g/g) = \frac{A \times V\,(mL) \times 10^4}{2505 \times P\,(g)}$$

where A = Absorbance; V = Total extract volume; P = sample
weight; 2505 (β-carotene Extinction Coefficient in petroleum ether).

Vitamin C Content Measurement: The quantification of Vitamin C was conducted using a phosphomolybdic acid colorimetry kit (Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China), in accordance with the guidelines provided by the manufacturer.

Analysis of vitamin A: The vitamin A concentration was directly determined using the Trichloroacetic acid (TCA) method. This involved adding TCA solution to dichloromethane (DCM) and dissolving it in oil. The measurement of the absorbance of the blue hue is done at a wavelength of 620 nm [6-9]. The quantification of vitamin A was achieved using visible spectrophotometry, which relies on the total antioxidant capacity (TAC) to reduce metal ions via electron transfer mechanism (Apak*et al*., 2013).

Isolation of Fungi that Cause Rot: Cassava tissues affected by disease were collected at random from various varieties and harvesting periods. A sterile knife and forceps were used to collect the tissues from spoiled cassava. The collected tissues were then cultured on potato dextrose agar (PDA), which was supplemented with 0.1 ml of Chloramphenicol to prevent bacterial growth. The cultures were incubated for 48 hours at room temperature. Isolates were collected from the dry season to the rainy season. The selected sampling locations were based on the high production of diverse Cassava types in those areas. In the laboratory, the diseased tissues were fragmented into small pieces of approximately 0.5 cm. These fragments were immersed in a 70% ethanol solution for a duration of 2 minutes, followed by sterilisation in a 0.5% sodium hypochlorite solution for 2 minutes. Finally, the fragments were rinsed three times with sterilised distilled water. The pieces were arranged on sterile filter paper to remove moisture, and subsequently transferred onto potato dextrose agar (PDA) and subjected to incubation at a temperature of 24 °C for a duration of 5-7 days, with a 12-hour light exposure each day.

Identification of the fungi: The fungi that were separated from other organisms were classified by observing the overall physical characteristics of their colonies on Potato Dextrose Agar (PDA medium). The slide culture technique was used to study the microscopic features, following the guidelines provided in the Manual of Atlases of Fungi.

Pathogenicity Test: The diseased samples were extracted and underwent pathogenicity assessments to verify the presence of decay, employing the techniques outlined in (Ezeonu*et al*. 2018 and Dania *et al*. 2019) for the fungus.The tubers were rinsed with sterile distilled water and subsequently sterilised with 70% ethanol. Using a sterile 4 mm cork borer, cylindrical discs were extracted from the disinfected tubers. These discs were then inoculated with test moulds. The cylindrical discs were substituted and the inoculation locations were sealed with Petroleum jelly. The process of incubation was carried out for a duration of fourteen days.

Percentage incidence: During each monitoring period, Cassava tubers were picked randomly according to the

protocols outlined by Anjorin *et al*. in 2014. The disease assessment relied on the ocular signs that were seen. The percentages of rot incidence on the examined cassava samples were computed as,

$$\text{Percentage rot incidence (\%)} = \frac{\text{Total number of diseased samples per month}}{\text{Total number of examined samples per month}} \times 100.$$

Statistical analysis: The acquired data underwent statistical analysis via analysis of variance (ANOVA). The statistical significance of mean differences was evaluated utilizing Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$) through the utilisation of MINITAB 19 software.

RESULTS AND DISCUSSION

Concentration of cyanide in cassava: A significant difference was observed in the Cassava cyanide concentration when comparing different cassava varieties and monitoring periods.

conditions, as observed by Bokanga *et al.* (1994) and Simon Terver*et al*. (2015). Quantification of carotenoid levels in cassava. An evident and substantial disparity in the content of Carotenoids was discovered when comparing different types of cassava and monitoring intervals.The carotenoid concentration was higher during the dry season compared to the rainy season,(Table 1).Collectively, these findings indicate that the carotenoid content in cassava varies among different cassava cultivars and over different monitoring seasons. Carotenoids are hydrophobic pigments present in the chromoplasts of plants. They serve as precursors for provitamin A and have significant roles in enhancing drought tolerance in higher plants (Zhang *et al.*, 2021). The variations in carotenoid levels among different cassava types align with the findings of Zhang *et al*. (2021), who documented variations in carotenoid levels among three distinct carrot cultivars of varied colours.

Cassava Vitamins C and A contents: The ANOVA analysis revealed a significant variation in the content of Vitamins C and A in different cassava types and monitoring periods. The dry season exhibited a greater accumulation of Vitamins C and A compared to the wet season (Table 1).

Table 1. Effects of climatic factors on cassava vitamin and cyanide contents

Variety	Carotenoid(µg/100g)		Vitamin A		Vitamin C		Cyanide	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
T419	555.12 (0.63) c	515.28(0.61)c	456.817 (0.34) c	498.583 (0.3) c	13.813 (0.17) c	14.99 (0.04) b	0.56 (0.03) c	0.6733 (0.01) d
NR 87	560.73 (0.18) b	520.773 (0.031)b	488.067 (0.21) b	501.347 (0.045) b	14.75 (0.23) b	15.18 (0.023) b	1.56 (0.02) b	1.67 (0.02) c
CA 539	1507.25 (0.67) a	1406.18 (0.03)a	1384.89 (0.12) a	1399.82 (0.03) a	18.72 (0.25) a	20.67 (0.035) a	1.55 (0.04) b	1.60 (0.05) c
LOC 1	49.560 (0.21)d	48.077 (0.469)d	45.9067 (0.08) d	55.51 (0.031)e	3.133 (0.09)d	4.17 (0.28) d	3.25 (0.19) a	3.53 (0.03) b
LOC 2	41.413 (0.31) e	40.280 (0.43)e	5.33 (0.49) d	56.8667 (0.14) d	3.580 (0.08) d	4.81 (0.17)c	3.36 (0.08) a	3.66 (0.015) a
F- value	5213028.11	5933481.40	10717252.87	1.8297908	4578.94	6903.17	463.57	5285.83
P- value	0.001	0.002	0.001	0.003	0.001	0.002	0.000	0.001

Means that do not share a letter within a column in a treatement are significantly different (Turkey's HSD test ($p < 0.05$). Numbers in brackets are ± standard deviation.

S/no.	Isolates	Average no. of each colony	Percentage incidence
Rainy season			
1	*B. theorbromae*	8.0	30.0
2	*F.oxysporium*	6.0	22.2
3	*A. flavus*	4.0	15.0
4	*R. stolonifer*	6.0	22.2
5	*S.rolfsii*	3.0	11.10
Total no. of colonies		27.0	
Dry season			
1	*B. theorbromae*	6.0	35.3
2	*F.oxysporium*	4.0	23.5
3	*A. flavus*	2.0	11.76
4	*R. stolonifer*	3.0	17.64
5	*S.rolfsii*	2.0	11.76
Total no. of colonies		17.0	

The cyanide levels were higher during the dry season compared to the rainy season, (Table1). Specifically, the findings indicate that the quantity of cyanide in cassava differed among different varieties, and the local varieties exhibited the highest accumulation of cyanide during the monitoring periods. The rise in tuber cyanide concentration during the dry season aligns with the findings of Jarvis *et al*. (2012), who observed that drought led to an increase in tuber cyanide concentration. The study conducted by Bokanga *et al*. (1994) and Simon Terver *et al*. (2015) found that several types of cassava differ in their ability to accumulate cyanide due to variations in the production, regulation, and translocation of cyanogenic glucosides. Furthermore, the accumulation of cyanide in different cultivars is influenced by factors such as soil composition, weather patterns, and geographical

Specifically, the findings indicate that the concentrations of Vitamins C and A in Cassava varied among different kinds, and local varieties exhibited the greatest levels of cyanide over the monitoring periods. Under conditions of chronic drought stress, an excessive amount of reactive oxygen species (ROS) is produced, leading to oxidative damage in several cellular components (Mittler, 2020; Abogadallah, 2010).Vitamins C and A possess antioxidant properties and play a vital role in mitigating oxidative damage caused by drought stress in plants (Zhu *et al*., 2020). Elevated concentrations of vitamins C and A may enhance the ability of several cassava genotypes to withstand drought-induced stress. According to *Fu et al*. (2016), the levels of Vitamins C and A in cassava leaves were shown to rise in order to

eliminate superoxide free radicals and regulate the extent of membrane lipid peroxidation during drought stress circumstances.

Pathogenicity test: In rainy season *B.theobromae* recorded highest percentage incidence (30.0%), while *S.* In rainy season *B.theobromae* recorded highest percentage incidence (30.0%), while *S. rolsfii* had lowest percentage incidence (11.10), (Table 2). In dry season *B.theobromae* recorded highest percentage incidence (35.3), while *S. rolsfii* and *A. flavus* had lowest percentage incidence (11.17%), (Table 2). The isolated cassava root pathogens in the present study was consistent with those reported by Msikita*et al*.,2005; Bandyopadhyay *et al*., 2006). The high incidence of cassava fungal pathogens during rainy season collaborates with (Crowl *et al*. 2008; Eastburn *et al*., 2011) who reported that changes associated with global warming may affect the incidence and severity of plant disease and influence the further coevolution of plants and their pathogens. The cassava root pathogens identified in this investigation were in agreement with those previously reported by Msikita *et al*. (2005) and Bandyopadhyay *et al*. (2006). The prevalence of cassava fungal pathogens is significantly higher during the rainy season, as supported by the findings of Crowl *et al*. (2008) and Eastburn *et al*. (2011). These studies suggest that the alterations caused by global warming can impact the occurrence and intensity of plant diseases, potentially influencing the ongoing coevolution between plants and their pathogens.

CONCLUSION

The study demonstrates that the cyanide, carotenoid, and vitamins A and C compositions of cassava are influenced by factors such as age, harvest season of the storage roots, and variety. The fungal pathogens responsible for cassava rot are Botryiodiplodiatheorbromae, Fusariumoxysporium, Aspergillusflavus, Rhizopusstolonifera, and Sclerotiumrolfsii.

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