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Investigation on the effect of cassava effluent-polluted soil on germination, emergence and oxidative stress parameters of *Telferia occidentalis*

Linus Ahuwaraeze Nwaogu[1*], Nonyelum Comfort Agha[1], Chinwe Sylvanus Alisi[1], Chinedu Emeka Ihejirika[2]

[1]*Department of Biochemistry, Federal University of Technology, Owerri, Nigeria*

[2]*Department of Environmental Technology, Federal University of Technology, Owerri, Nigeria*

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Abstract

The effect of cassava effluent-polluted soil on germination, emergence and oxidative stress parameters of *Telferia occidentalis* from soil exposed to the effluent over a long period of time was studied. *Telferis occidentalis* leaves from soil samples devoid of cassava effluent served as the control. *T. occidentalis* seeds from soil samples devoid of cassava effluent-polluted soil samples showed 100% germination and emergence while those from cassava effluent-polluted soil samples had 50% germination and emergence after fifteen days. Results showed that there was a significant ($p<0.05$) difference in all the oxidative stress parameters investigated from the aqueous leaf extracts of *T. occidentalis* from cassava effluent-polluted soil samples when compared to the control indicating that cassava effluent-pollution might have induced stress on *T. occidentalis* leaves and delayed germination and emergence of the seeds. Hydrogen cyanide in cassava effluent-polluted soil induced stress on plant when absorbed in high concentration. Cyanide has a toxic effect on the normal metabolism of plants. Plants/vegetables growing on high cassava effluent- polluted soil exhibit characteristics of unhealthy growth which effect their nutritive and market values.

***Corresponding Author:** Linus Ahuwaraeze Nwaogu ✉ nwogulinus@yahoo.com

Introduction

The threat to human animal and plant lives by industrial effluents cannot be over-emphasized. Industries have long been implicated in the discharge of effluents that are harmful in the environment. Over 10% of world rivers and soil are heavily polluted with effluent discharges from food and allied industries and users of these rivers and streams are exposed to health related risks as well as plants that grow on the soil are affected by this indiscriminate discharge of industrial effluents (Salami and Egwin, 1997).

Cassava (Manihot esculenta Crantz) belong to the family Euphorbiaecae. It is native to South America and is extensively cultivated in the tropical and subtropical regions of the world for its edible starchy tuberous roots. It is a major source of carbohydrate and it is the third largest source of carbohydrate in the world with Africa being the largest centre of production (Claude and Deris, 1990). Annual cassava production in Africa is about 84 million tones. Democratic Republic of Congo 16.8million tones and Madagasear 2.4 million tons (Nweke, 1993).

Cassava processing into garri involves several unit operations which include peeling, washing, grating, pressing and fermenting, sieving, frying and drying. Effluent from cassava processing is discharged into the environment without pollution. Cassava effluent that run freely along soil surfaces contaminate surface water, stream as it percolates into underground water and sub soil which has serious adverse human, fauna and flora (FAO, 2004; Ogundola and Laiasu, 2007). The recent encouragement by Nigerian government to grow and process more cassava for domestic and international needs has resulted in corresponding increase in production and processing thereby increasing the amount of cassava effluent and its discharge to the environment.

The potential toxicity of cassava and its effluent is related to the ability of all parts of the plant to release hydrogen cyanide from stored cyanogenic glucosides, a phenomenon known as cynogenesis (Coursey, 1973; Ayernor, 1985). The normal range of cyanogens content of cassava tubers falls between 15 and 400 mgHCN/Kg fresh weights (Coursey, 1973). Cyanide is produced during cassava processing depending on the species of cassava 40-70% of total cyanide is released in the water used to wash the disintegrated cassava (Arguedes and Cookes, 1982). Evaporation of cyanide occur either during cassava processing or after effluent discharges (Cooke and Maduagwu, 1978). The purpose of this study was to investigate the effect cassava effluent pollution might have on germination, emergence of seeds and oxidative stress parameters on *Telferia occidentalis* leaves which is the vegetable commonly consumed in the southern part of Nigeria which do not thrive well on cassava effluent-polluted soil.

Materials and methods

Soil preparation and planting

Seeds of *T. occidentalis* used for the study were purchased from Ihiagwa market Owerri, Nigeria. The cassava effluent-polluted soil sample was collected near a cassava processing effluent discharge point in Umuchima village in Ihiagwa, Owerri, Nigeria.

The garden soil sample collected 1000 metres away from cassava effluent discharge point from Umuchima village in Ihiagwa Owerri, Nigeria. Two groups of nine perforated polythene bags containing 700 grams of garden soil and cassava effluent-polluted soil samples were measured and labeled. Each group had triplicate representation respectively. Four viable seeds of *Telferia occidentalis* were planted in each bag. Water was added to the soil samples occasionally when necessary to keep the soil samples moist. After germination/ emergence, and after two leaf stage, the seeds were thinned down to two plants in each bag. The germination / emergence were recorded in all the groups. The experiment was allowed to stand for 50 days after which the leaves were harvested.

The experiment was conducted from April to June, 2009.

Preparation of extract

Extract of the *Telferia occidentalis* leaves was obtained using sodium phosphate buffer (pH 7.4) according to the method as described by Levine et al (1990). The aqueous extract from each group was used for the various analyses.

Reduced glutathione (GSH) concentration was determined by the method of Jollow et al, (1974). The method is based on the formation of a relatively stable chromophoric product on reacting with a sulphydryl compound with Ellman's reagent. The concentration of GSH in the extract was calculated using standard GSH. Protein thiol concentration was determined using the method of Levine et al, (1990). Total thiol concentration was determined by the summation of glutathione and protein thiol concentrations (Levine et al., 1990). Ascorbic acid (AA) concentration was determined using the method of Roe and Kuether (1961). Lipid peroxidation was detected spectrophotometrically by assessing the level of thiobarbituric acid substances and expressed as malondialdehyde (MDA) equivalent. This was measured according to the method of Albro et al., (1986). Protein content was determined using a protein test kit (Biosystem USA) that utilizes the Biuret method as described by Gornall et al, (1949) for protein determination.

Statistical analysis

Each determination was performed in triplicate. Data generated were expressed as means ± standard deviation and statistically analyzed using one way Analysis of Variance (ANOVA) with $p \leq 0.05$ taken to be significant.

Results:

Germination and emergence of *Telferia occidentalis* seeds: Germination and emergence of *Telferia occidentalis* seeds in the two groups of soil samples are shown in Table 1. Telferia occidentalis seeds grown on soil samples devoid of cassava effluent-pollution (control) had 100% germination and emergence while those from cassava effluent-polluted soil samples had 50% germination and emergence. Toxic effect of cyanide in the effluent may have influenced the germination and emergence of some of the seeds in the cassava effluent-polluted soil samples due to poor aeration.

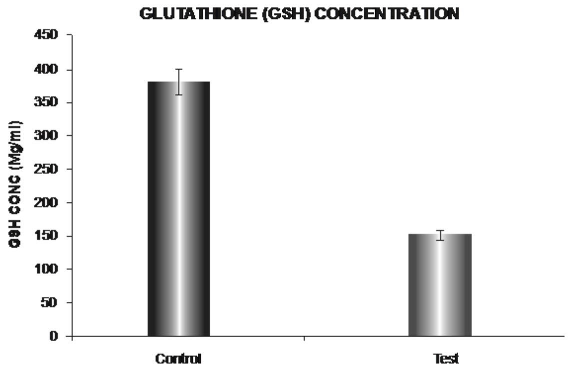

Fig. 1. Glutathione concentrations from palm oil effuent-polluted soil.

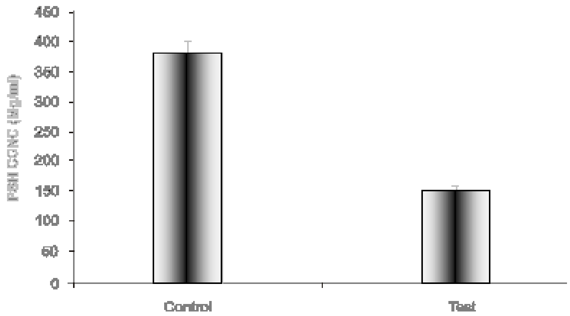

Fig. 2. Protein thiol concentrations from cassava effuent-polluted soil.

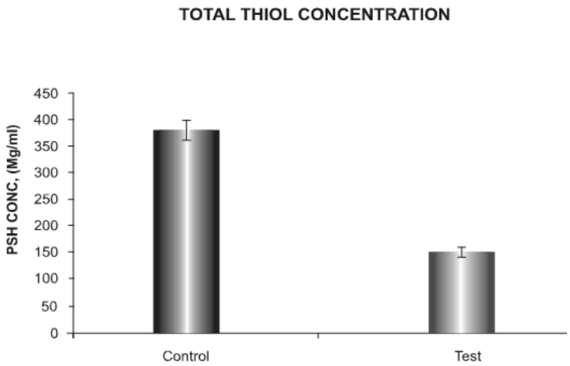

Fig. 3. Total thiol concentrations from cassava effuent-polluted soil.

Glutathione concentrations

The glutathione concentrations of leaf extract of T. *occidentalis* from cassava effluent-polluted soil samples and those from the control are presented in Fig. 1. The result indicated that glutathione concentration in aqueous leaf extract of T. occidentalis from cassava effluent-polluted soil are significantly ($p < 0.05$) reduced than those from the control. Glutathione being an antioxidant might have been used to mop up reactive intermediates generated as a result of the pollution from cassava effluent thereby reducing its concentration.

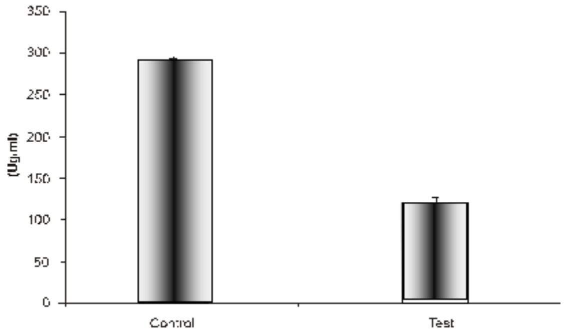

Fig. 4. Ascorbic acid concentrations from cassava effluent-polluted soil.

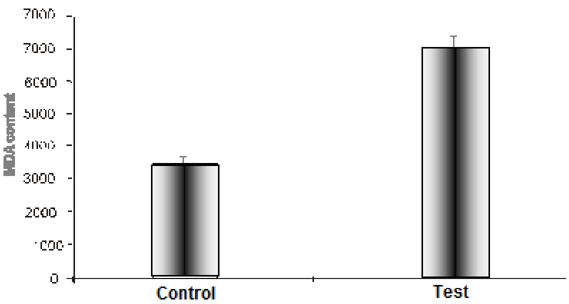

Fig. 5. Malondialdehyde concentrations from cassava effluent-polluted soil.

Protein thiol and total thiol concentrations

The stability of protein thiol and total thiol is determined by the sequential arrangement of amino acids that make of the protein. The protein thiol and total thiol concentrations of the leaf extract of *T. occidentalis* are presented in Fig. 2 and 3 respectively. The concentrations of these parameters are significantly ($p < 0.05$) higher in the aqueous leaf extract of T. occidentalis from the control soil compared to the cassava effluent-polluted soil. It was observed that the reduced concentrations in these parameters is attributed to the extent of damage the reactive intermediates has caused to the amino acid sequence of the protein due to oxidative stress from cassava effluent-polluted soil samples.

Ascorbic acid concentrations

The mean concentrations of ascorbic acid from the aqueous leaf extract of *T. occidentalis* from cassava effluent-polluted soil and those from the control are presented in Figure 4. The result indicated that ascorbic acid concentration is significantly ($p<0.05$) lower in the leaf extract of *T. occidentalis* from cassava effluent-polluted soil. This is attributed to the fact that ascorbic acid being a soluble antioxidant might have been used by the plant to scavenge free radicals generated within the plant tissues as a result of the pollution thereby reducing its concentration.

Table 1. Germination / Emergence of *T. occidentalis* seeds in cassava effluent-polluted soil and soil devoid of cassava effluent.

Groups	Soil Types	Germination / emergence 5	6	7	8	9	10	11	12	13	14	15	Total Germination	% Germination
1	Garden Soil (control)	2	0	1	1	1	1	0	0	0	0	0	6	100
2	Cassava effluent-polluted soil	0	0	0	0	1	0	0	1	1	0	0	3	50

Malondialdehyde (MDA concentration)

The result obtained for lipid peroxidation which was estimated through the production of malondaldehyde concentration is presented in Figure 5. There was a significant ($p < 0.05$) difference in the concentration of MDA from the aqueous leaf extract of *T. occidentalis*. A higher concentration of MDA was observed from aqueous leaf extract of T. *occidentalis* from cassava effluent-polluted soil when compared to the control. This revealed that free radicals from cassava effluent-pollution might have mediated a high lipid peroxidation observed in the aqueous leaf extract of *T. occidentalis* from the effluent-polluted soil samples than those from the control.

Protein contents

The protein contents from the leaf extracts of *T. occidentalis* from the two groups of soil are presented in Figure 6. The samples result indicated that the aqueous extract of *T. occidentalis* from cassava effluent-polluted soil samples had reduced protein content when compared to the control samples. This protein content reduction might have been due to toxic effect of cyanide from cassava effluent-polluted soil which affected the soil chemistry and subsequently mediated oxidation of protein thereby reducing its contents.

Discussion

Cassava production and processing in Nigeria has increased drastically in recent years as a result, environmental pollution due to the effluent is expected. Investigation on the impact of cassava effluent pollution on *Telferia occidentalis* revealed several adverse effects both on the seed germination, emergence and antioxidant parameters.

The germination and emergence of *T. occidentalis* seeds in the two groups of soil samples revealed that soil samples devoid of cassava effluent had 100% germination and emergence while those from cassava effluent-polluted soil samples had 50% germination and emergence respectively. The result showed that cassava effluent significantly ($p < 0.05$) inhibited and delayed germination of the seeds. This was attributed to poor aeration caused by hydrogen cyanide from cassava effluent which prevented oxygen from being available to the seeds (Coursey, 1973).

At high concentrations, reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be important mediators of damage to cell structures, nucleic acids, lipids and proteins (Valko, 2004). Exposure to free radicals from a variety of sources has led organisms to develop a series of defence mechanisms against free radical-induced oxidative stress (Cadenas, 1997).

Glutathione (GSH) being one of the important antioxidant in living organisms is highly abundant in cytosol (1-11mM), nuclei (3.15mM) and mitochondria (5-11 mM) and is the major soluble antioxidant in these cell compartments (Masella et.al, 2005). The main protective roles of glutathione against oxidative stress are: (i) It is a cofactor of several detoxifying enzymes against oxidative stress; (ii) It participates in amino acid transport though the plasma membrane, (iii) It scavengers hydroxyl radical and singlet oxygen directly, detroxyfying hydrogen peroxide and lipid peroxides by catalytic action of glutathione peroxidase; Glutathione is able to regenerate the most important antioxidants, vitamins C and E back to their active forms (Pastore et al, 2003).

The concentration of glutathione from aqueous leaf extract of *T. occidentalis* from cassava effluent-polluted soil was significantly ($p<0.05$) lower than those from normal soil samples. The significant reduction was expected as glutathione in the leaf extract of *T. occidentalis* from cassava effluent-polluted soil samples might have been used for its various protective roles against oxidative stress as a result of cassava effluent-pollution.

Mechanisms involved in the oxidation of proteins by ROS and RNS were elucidated by studies in which amino acids, simple peptides and proteins were exposed to ionizing radiations under conditions where hydroxyl radicals or a mixture of hydroxyl radicals are formed (Stadtman, 2004; Dalle-Donne et al, 2005). Oxidation of cysteine residues may lead to the reversible formation of mixed disulphides between protein thiol groups and low molecular weight thiols. Fig. 2 and 3 showed the result obtained for protein thiol and total thiols respectively from the groups of soil samples. There were significant reduction in the concentration of these parameters in aqueous leaf extracts of *T. occidentalis* soil samples when compared to the control. This reduction in concentration is attributable to increased oxidative stress from free radicals from cassava effluent-polluted soil.

Ascorbic acid is a water-soluble antioxidant molecule which scavenges free radicals in the soluble cytoplasm. It readily donates electrons to free radicals thereby scavenging their effects in the cellular compartments. Cassava effluent pollution significantly ($p < 0.05$) reduced the mean concentration of ascorbic acid (Figure 4) in *Telferia occidentalis* leaves from cassava effluent-polluted soil when compared to those from the control. These significant reduction in ascorbic acid concentration due to cassava effluent in the soil could be due to the involvement of ascorbic acid in scavenging for reactive intermediates thereby reducing its concentration in leaves of *Telferia occidentalis*.

It is known that generation of free radicals mainly ROS results in an attack not only on DNA but also on other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Siems et al, 1995). Once the peroxy radicals are formed, can be rearranged via a cyclisation reaction to endoperoxides (precursors of malondialdehyde) with the final product of peroxidation process being malondialdehyde (Marhett, 1999). There was high concentration of malondialdehyde from the aqueous leaf extract of *T. occidentalis* from cassava effluent-polluted soil when compared to the control. The observed significant ($p<0.05$) difference is accounted for by the high concentration of cyanide in the effluent which generated free radicals in the tissues of the *T. occidentalis* leaves grown on the polluted soil. The reverse was the case in normal (control) soil sample where there were lower concentration of MDA. Fedtke et al, (1990) observed that free-radicals from pollution mediate increased peroxidation in membranes of living organisms: plants and animals.

Oxidative modification of proteins increase their susceptibility to proteolytic attack, protelytic degradation is executed mainly by proteasomes. Proteolysis was estimated to increase more than 10 times after exposure to superoxide radical or hydrogen peroxide. It should be noted that proteins significantly vary in their susceptibility to oxidative damage. For example intact proteins are less sensitive to oxidation than misfolded proteins.

The protein contents from aqueous extract of *T. occidentalis* from both groups of soil samples indicated that there was reduced protein content in leaf extract from cassava effluent-polluted soil samples when compared to the control. Similar observations were made by Nwaogu and Onyeze, (2010) in aqueous leaf extract *T. occidentalis* from spent engine oil-polluted soil samples. The reduction in protein content might be attributed to interference of cyanide in the effluent to the soil physical and chemical properties which subsequently affected plant physiology and hence affect the protein content.

Conclusion

Our investigation revealed that cassava effluent pollution affected adversely the germination/ emergence of the seeds of *T. occidentalis*, and induced oxidative stress on the plants. Those involved in the processing of cassava should be educated on the environmental hazards this untreated effluent from cassava processing posses to plants and animals. This can be achieved through massive enlightenment and media campaigns.

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