Original Research Article

~~BIOHERBICIDE EFFECT OF EFFLUENT FROM PROCESSED *MANIHOT ESCULENTUS* TUBERS~~

Comment [a1]: Screening of a Processed *Manihot esculentus* Tuber Effluent for Bioherbicidal Effect against Some Selected Weeds

ABSTRACT

Effluents from processed bitter cassava (*Manihot esculentus)* tubers as bioherbicide was applied on the leaves of Beans, Broom weed, Maize, and Itch grass and investigated. Quantitative and qualitative characterization of microorganisms (bacteria and fungi) was done both in effluent and test soil. Mineral analysis using atomic absorption spectrum (AAS), cyanide quantification in the effluent, and quantification of chlorophyll (a and b) from the leaves of test plants (Beans, Broom weed, Maize, and Itch grass) were carried out. The result from the microbial characterization of effluent, test soil, and control soil revealed the effluent had the highest microbial load. The isolated bacteria were *Staphylococcus spp, Bacillus spp, Lactobacillus,* and *E. coli*. Test soil had 16.13% *Staphylococcus*, 9.68% *Bacillus spp*, 16.13% *Lactobacillus,* and 6.45% *E. coli*. The isolated fungi were *Saccharomyces*, *Mucor,* and *Aspergillus* in the effluent, while *Saccharomyces* and *Mucor* were in the test soil and only *Aspergillus* in the control soil. The fungal count revealed a high total viable count (TVC) in the effluent (7.0×10^4 cfu/ml) followed by test soil (6.6×10^4 cfu/ml) while control soil had the least ($4.5\%\times10^4$ cfu/ml). Cyanide analysis of effluent revealed 1.0 mg/ml, while metal analysis revealed potassium (40.221 mg/kg), sodium (32.009 mg/kg), Manganese (0.057 mg/kg) and Copper (-0.004mg/kg).The chlorophyll (a and b) concentration expressed in µg/ml of the experimented plants (Beans, Broom weed, Maize, and Itchgrass) further revealed a significant ($p\leq0.05$) decrease ~~with respect to~~ the volume of effluent applied (50 ml and 25 ml). Dicotyledonous plants; beans (0.461 $\pm$ 0.025 and 0.609 $\pm$ 0.013 chlorophyll (a) compared to a control of 7.698$\pm$ 0.100. Chlorophyll (b) on the other hand revealed 5.507 $\pm$ 0.141 and 11.599 $\pm$ 0.282 when compared with control of 16.426 $\pm$ 0.016). Broom weed (0.291 $\pm$ 0.071 and 0.457 $\pm$ 0.068 for chlorophyll (a) when compared to the control with 0.595 $\pm$ 0.071 and 1.549$\pm$0.141 and 1.683$\pm$0.353 for chlorophyll (b) when compared to the control with 22.252 $\pm$ 0.282. Other plants analyzed revealed various significant ($p\leq0.05$) decreased levels of chlorophyll (a and b). All the results revealed this effluent may be selectively used as a potential bioherbicide especially when applied to the leaves.

Comment [a2]: concerning

Keywords: Bioherbicides, Effluent, *Manihot Esculentus* Fungi, and Test soil

Introduction

Weeds are seriously a threat to native species, communities, and ecosystems in many areas around the world. They can compete with and displace native plants, animals, and other organisms that depend on them changes ecosystem functions and cycles significantly, hybridize with native species and encourage the existence of other invaders (Hurd and Randall, 2001). They interfere with food and fiber production in agriculture, wherein they must be controlled to prevent lost or diminished crop yields. Weed scientists are now facing new challenges, especially in the light of the emergence of weeds resistant to herbicides and concerns and questions about herbicide residue in food, soil, groundwater, and atmosphere (Rao, 2000). To circumvent these, an alternative approach of natural product-based

management (bioherbicide) is needed, since most natural products are rapidly degraded and are environmentally friendly.

Weed control is important in agriculture in other to improve crop yield and agricultural produce and methods of control include hand cultivation with hoes, powered cultivation with cultivators, smothering with mulch, lethal wilting with high heat, burning, and chemical control with herbicides (weed killers) (https://en.m.wikipedia.org/wiki/Wikipedia, 2020).

The use of chemical weed control has been in practice just about a century ago with a few inorganic compounds, such as sulfuric acid, copper salts, and sodium chlorate (Cremlyn, 1991). The herbicidal activity of 2, 4-dichlorophenoxyacetic acid was detected in 1940 (Troyer, 2001), which was toxic to dicotyledonous, but not monocotyledonous plants. Chemical herbicides persist in the environment for a long period as they are non-biodegradable and their residues are accumulated in the farm produce thereby contaminating them. Due to increasing awareness of the effects of chemical herbicides and pesticides such as chemical residues in food, water, and the environment, bioherbicide can be adopted as an alternative, especially for integrated weed management (https://en.m.wikipedia.org/wiki/Bioherbicide, 2019). Hence, other alternative agents for weed control are imperative.

Bioherbicides are pathogens, phytotoxins, and other microbes used ~~as~~ biological weed control (Mark *et al.*, 2016). They may be compounds and secondary metabolites derived from microbes such as fungi, bacteria, or protozoa; phytotoxic plant residues, extracts, or single compounds derived from other plant species (Angélica *et al*., 2017). The significance of bioherbicides includes a high degree of specificity of target weed; no effect on non-target and beneficial plants or man; absence of residue build-up in the environment and can be effective for the control of herbicide-resistant weeds (Zvonko, 2015).

Comment [a3]: for

Manihot esculenta, commonly called cassava (Bakayoko *et al.*,2009), is a woody shrub of the spurge family, Euphorbiaceae. It belongs to the family greatly consumed by people in particular the tubers, which ~~undergoes~~ processing. It has been said according to pieces of literature, the bitter species contains high cyanide and other chemicals. It is through this light, that the effluent released during processing may have bioherbicidal properties.

Comment [a4]: undergo

This study aims to test for the herbicidal effect of effluent from processed *Manihot esculenta* tubers on some species of monocotyledonous and dicotyledonous plants.

MATERIALS AND METHODS

Sample collection

Bitter Cassava tubers for this study were obtained from Nor village, Gwer East L.G.A, Benue State and prepared according to Oyewole and Odunfa, (1992) method to obtain the effluent. The seeds of Beans, Maize, Itch grass, and Broom weed were obtained within the Federal University of Agriculture Makurdi environment.

Experimental design

This experiment was carried out in front of block B, college of the science Federal University of Agriculture Makurdi. The soil was collected at the back of the Biochemistry department into 32 polyethylene bags and weighed 2kg each and arranged in two different groups. Four different plants (Beans, Broom Weed, Maize, and Itch grass) were planted in each group and watered every morning and evening using 50ml of distilled water for 23 days. Treatment with 50ml and 25ml of cassava effluent started on the 24th day for four days using a sprayer. The second group was watered normally without applying the affluent and serves as the control. The growth activities were observed by measuring the chlorophyll content.

The chlorophyll pigments (chlorophyll a and b) were determined using a spectrophotometer at 663 nm and 645 nm respectively according to Arnon's method (1949). Quantitative and qualitative determination of microbial activities of the affluent and soil were done following the method of Cheesbrough, (2000). The method described by Kakulu and Jacob, (2006) was employed for the qualitative and quantitative determination of metals present in the effluent. Also, the alkaline picrate method of cyanide determination by Njoku *et al.*, (2014) was used to quantify cyanide in the effluent.

Statistical Analysis

The results were expressed as Mean ± SD and a test of statistical significance was carried out using one-way analysis of variance (ANOVA) where $p \leq 0.05$ was considered statistically significant. The statistical package used was the statistical package for social sciences (SPSS), version 20. (SPSS Inc., IL, USA)

Results

Morphological, cultural, and biochemical characteristics of bacteria isolated from cassava effluent, test soil, and control soil.

The bacteria isolated from the effluent, test soil, and control soil is shown in table 3 below. They were *Staphyloccocus spp* with round pinkish and spherical smooth edges colonies which test positive to Grams reaction, catalase, and citrase but negative to indole test.

Bacillus spp which has a rod-like shape appears as bluish and spherical colonies with smooth edges. It was confirmed positive by Gram reaction, Catalase, and Citrate but negative to Indole test, found in effluent test soil and control soil as shown in table 3

Lactobacillus spp has the shape of a rod that appears smooth, creamy long, and large flat colonies, which tested positive ~~to~~ Grams reaction, Catalase but negative to Citrate and Indole test. It was found in affluent, test soil, and control soil as shown in table 3

Comment [a5]: for

E. coli has a rod-like morphology that forms a pinkish and shiny colony which tested positive ~~to~~ Catalase, Citrate, and Indole but negative to Grams reaction. It was only present in the effluent and test soil as indicated in table 3 below

Comment [a6]: for

Distribution of bacteria isolates from cassava effluent, test soil, and control soil

The isolated organisms from their respective samples are shown in Table 4 below. *Staphylococcus spp* was present in the cassava effluent, test soil, and control soil with test soil having the highest percentage of occurrence 5(16.13%). *E. coli* was present in the

cassava effluent and test soil but was not present in the control soil. *Lactobacillus* was in all the samples, with test soil having the highest percentage of occurrence 5(16.13%). Also, *Bacillus spp* was present in all the samples but the highest percentage (3(9.68%) of occurrence was observed in the test soil

Table 1. Total bacterial counts for effluent, test soil, and control soil

Sample	Growth medium	Number of colonies	Dilution factor	Total Viable Count (cfu/ml)
Effluent	Nutrient agar	90	10^{-3}	9.0×10^{4}
	Potato ~~dextro~~ agar	76	10^{-3}	7.6×10^{4}
	McConkey agar	180	10^{-3}	$18.\times10^{4}$
Test soil	Nutrient agar	86	10^{-3}	8.6×10^{4}
	Potato dextro agar	70	10^{-3}	7.0×10^{4}
	McConkey agar	120	10^{-3}	12.0×10^{4}
Control soil	Nutrient agar	72	10^{-3}	4.5×10^{4}
	Potato dextro agar	45	10^{-3}	7.2×10^{4}
	McConkey agar	47	10^{-3}	4.7×10^{4}

Comment [a7]: dextrose

Comment [a8]: dextrose

Table 2. Morphological, Cultural, and Biochemical characteristics of bacteria isolated from cassava effluent, test soil, and control soil.

Sample	Organism	Morphology	Biochemical test				Cultural characteristics
			Gram's reaction	Catalase	Citrate	Indole	
Effluent, Test soil, and control soil	*Staphylococcus spp*	Cocci	+ve	+ve	+ve	-ve	Pinkish colony, spherical smooth edges
Effluent and Test soil	*Bacillus spp*	Rods	+ve	+ve	+ve	-ve	Bluish colony,spherical and smoothedges
Effluent, Test soil, and control soil	*Lactobacillus spp*	Rods	+ve	+ve	-ve	-ve	Smooth, large dry flat creamy long
Effluent, Test soil, and control soil	*Escherichia coli*	Rods	-ve	+ve	+ve	+ve	Pinkish colony fermenting plate shiny surface

Key +ve = positive
-ve=negative

Table 3. Distribution of bacteria isolates from cassava effluent, test soil, and control soil

Sample	Organisms and their percentage of occurrence in the samples			
	Staphylococcus spp	*Bacillus spp*	*Lactobacillus spp*	*E. coli*
Cassava effluent	4(12.90%)	2(6.45%)	4(12.90%)	2(6.45%)
Test soil	5(16.13%)	3(9.68%)	5(16.13%)	2(6.45%)
Control soil	2(6.45%)	1(3.23%)	1(3.23%)	0(0.00%)

The number outside the bracket represents the number of organisms isolated and the number inside the bracket represent the percentage of occurrence in a specific sample

Table 4 Fungi isolated from cassava effluent, test soil, and control soil

Sample	Organism	Hyphae	Vegetative cell	Colony morphology
Effluent, test soil, and control soil	*Saccharomyces*	Septate hyphae	Canidia occurs in long-chain on the canidiophore.	Moderate growing colony.
Effluent and test soil	*Mucor*	Mycelium stain blue using lactophenol cotton blue.	Spores stained green	Whitish and wooly colonies
Test soil and control soil	*Aspergillus*	Septate	Canidia occurs in long-chain on the canidiophore .	Moderately growing colony.

Fungal count and isolates from cassava effluent, test soil, and control soil.

The fungal count and specific organisms isolated from the samples are shown in table 6 below, the number of colonies counted from the plate inoculated with cassava effluent was70, 66 for test soil, and 45for control soil. The total viable counts for the samples were expressed in the colony-forming unit (cfu) per ml. The total viable count (TVC) for the effluent was 7.0×10^4which was the highest, for test soil was 6.6×10^4 and 4.5×10^4 for control soil.

Table 5. Fungal count for effluent, test soil, and control soil

Sample	Number Of Colony	Dilution factor	TVC (cfu/ml)	Fungi isolate
Effluent	70	10^{-3}	7.0×10^{4}	*Saccharomyces*, *Mucor*, and *Aspergillus*
Test soil	66	10^{-3}	6.6×10^{4}	*Saccharomyces* and *Mucor*
Control soil	45	10^{-3}	4.5×10^{4}	*Aspergillus*

Table 6 Effect of the effluent from processed cassava tubers on chlorophyll concentration in Beans, Broom weed, Maize, and Itch grass.

Plant (ml)	Volume of effluent (µg/ml)	Chlorophyll a (µg/ml)	Chlorophyll b
Beans	50	0.461 ± 0.025 [a]	5.507 ± 0.141 [a]
	25	0.609 ± 0.013 [a]	11.599 ± 0.282 [b]
	Control	7.698± 0.100 [b]	16.426 ± 0.016 [c]
Broom weed	50	0.291 ± 0.071 [a]	1.549 ± 0.141 [a]
	25	0.457 ± 0.068 [a]	1.683 ± 0.353 [a]
	Control	0.595 ± 0.071 [b]	22.252 ± 0.282 [b]
Maize	50	1.393 ± 0.221 [a]	6.602 ± 0.212 [a]
	25	2.421 ± 0.141 [b]	9.472 ± 0.071 [b]
	Control	4.585 ± 0.212 [c]	16.458 ± 0.354 [c]
Itch grass	50	0.262 ± 0.028 [a]	6.702 ± 0.354 [a]
	25	1.354 ± 0.028 [b]	6.767 ± 0.706 [a]
	Control	3.604 ± 0.212 [c]	9.252 ± 0.283[b]

Results are expressed as mean ± S.D; n=2. Mean values having different lower-case letters as superscripts from top to bottom of the column are considered significant ($p \leq 0.05$).

Table 7 Metal analysis of effluent from processed bitter Cassava tubers

Metal	Concentration (mg/kg)
Potassium (K)	40.2210
Sodium (Na)	32.099.5
Magnesium (Mg)	1.509.2
Manganese (Mn)	0.0 57.1
Copper (Cu)	-0.004.6

Discussion

Bioherbicides are compounds and secondary metabolites derived from microbes such as fungi, bacteria, or protozoa; or phytotoxic plant residues, extracts, or single compounds derived from other plant species used as biological weed control (Angélica *et al*., 2017). These compounds are of great significance such as high degree of specificity of target weed; no effect on non-target and beneficial plants or man; absence of residue build-up in the environment and can be effective for the control of herbicide-resistant weeds (Zvonko, 2015). In this study, the bioherbicidal effect of effluent from processed cassava tubers was tested on Beans, Maize, Broom weed, and Itch grass through microbial characterization of the effluent, test soil and control soil, chlorophyll concentrations, cyanide content, and metal analysis. Table 1 shows that the microbial load was higher in the effluent due to the involvement of these organisms in cassava fermentation through the production of amylase and cellulase that hydrolyzed starch and cellulose to sugars. This agrees with the earlier work of Arotupin (2007). When effluent was applied to the plants, some were deposited in the test soil, which accounted for microbial load increased but with fewer activities as compared to the effluent, this was because the effluent contains nutrients but in a decreased concentration and by-products like cyanide which affect the growth of some microorganisms. The breakdown of organic matter in the effluent was exothermic (Agwaranze *et al*., 2018), which caused the increase in temperature resulting in heat stress on the plants and eventually death. The isolation of *Staphylococcus spp* and *E. coli* as shown in table 3 was attributed to human activities as they are known to be flora of humans, this was in line with the work of Agwaranze *et al*.,(2018). Table 2 revealed the specific organisms present in the samples (effluent, test soil, and control soil) as *Staphylococcus spp, Bacillus, Lactobacillus,* and *E. coli*, and their percentages of occurrence in the sample is shown in table 4. *Staphylococcus spp* has the highest percentage of occurrence in the test soil and the least percentage of occurrence in the control soil, this was because it resides in the soil and when nutrients (carbohydrates and other organic matter) were available from the applied cassava effluent, its activities increased through the released of enzymes amylase to breakdown starch to glucose which it utilizes for energy. *Bacillus spp* shows the same level of activity in both cassava

effluent and test soil and less activity in the control soil this could be as a result of its involvement in fermentation. *Lactobacillus spp* has its highest percentage of occurrence in the test soil followed by cassava effluent and lesser activities in the control soil. *E. coli* shows higher activities in the test soil and fewer activities in the cassava effluent but no activities in the control soil, this could be as a result of its wide varieties of substrates (organic matter such as carbon sources, example sugars, starch) and uses mixed acid fermentation in anaerobic conditions producing lactic acid, succinic acid, ethanol, acetic acids, and carbon dioxides. The absence of *E. coli* from the control soil agreed with the work of Russell and Jarvis (2001).

The isolation of *Aspergillus* from the sample as shown in table 4 is similar to the work of Ogeihor *et al*.,(1990) and its herbicidal activity has been shown by the report of Saeed *et al*., (2014) on *Silybum marianum* L. Herbicidal activities of Bacillus has been shown by the work of Chellaram and Alex,(2015) using epibiotic strain, *Bacillus kochii* against *Lemna minor* L. (duckweed). *Mucor* which is a saprotrophic fungus was isolated from the effluent and test soil, showing that the decaying cassava tubers were the substrate on to which it fed. According to *Ciegler et al, (2016), mucor* produces oxalate or oxalic acid as its metabolic product which has a local corrosive effect and affinity for positively charged metals like calcium. The ability of oxalate to chelate metals shows that it contributes to the herbicidal properties of the cassava effluent since these metals like potassium and magnesium are needed by plants for normal growths. This could be responsible for the decreased concentration of chlorophylls ~~of~~ plants treated with the effluent when compared with chlorophylls of control since these plants need magnesium for chlorophyll formation. The presence of these microbes revealed that the herbicidal potentials of this effluent are contributed by phytotoxins with the growth-suppressive effect produced by these organisms. The chlorophyll concentration in all the plants experimented which shows a great significant ($p \leq 0.05$) decrease concerning the volume of effluent applied as seen in table 6. Broom weed was the most effected plant in all, with the lowest concentration of chlorophyll a and chlorophyll b, follow by Beans while Itchgrass and maize showed less effect. This may be attributed to the fact that beans and broom weeds are dicotyledonous plants

Comment [a9]: in

From the AAS result obtained, there was a high concentration of sodium in the effluent which could result to stress on plant photosynthesis thereby causing plant death. The mechanism of this process is explained by Chen *et al*., (2010) and Białasek *et al*., (2017), according to which salt stress could inhibit photosystem II reaction center in plant leaves, through the reduction of oxygen-evolving complex (OEC) activity at the donor side of photosystem II and degrading D1 protein on the acceptor side of the PSII. The rate of electron transfer is decreased, which in turn leads to the accumulation of excess electrons from the electron transfer chain resulting in electron leakage. These leaked electrons would attack the free oxygen molecule in the cell leading to the outbreak of reactive oxygen species (ROS) causing damage in the PSII reaction center or causing peroxidation or dissociation of the thylakoid membranes

In all the plants experimented with, there was a decrease in the concentrations of chlorophyll a and chlorophyll b when compared with the controls. The presence of cyanide in the effluent was

also believed to contribute to the herbicidal potentials of this effluent, as cyanide has been shown by Cereda and Mattos, (1996) to be an inhibitor of oxidative phosphorylation pathway that combines with cytochrome-oxidase and inhibits electron transportation, and consequently, the ATP (adenosie triphosphate) formation.

Conclusion

The bioherbicidal potentials of effluent from processed bitter cassava tubers have been examined on Beans, Broom weed, Maize, and Itch grass. In all the plants treated with cassava effluent, there was a significant ($p \leq 0.05$) decrease in the concentrations of chlorophyll **a** and chlorophyll **b** when compared against controls. The effluent worked best on broom weed than on other tested plants, hence the choice of weed is important when considering the bioherbicidal effect of this effluent. Therefore, further investigations should be done using high throughput practical technologies to confirm this effluent as an effective bioherbicide.

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