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**ORIGINAL ARTICLE**

## **Biostimulation Potential of Sawdust on Soil Parameters and Cassava (*Manihot esculenta*; Crantz) Yields in Crude Oil Polluted Tropical Soil.**

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### **ABSTRACT**

The biostimulation potential of sawdust on soil parameters and yield of *Manihot esculenta*; Crantz in a crude oil polluted tropical soil were investigated. 5kg of soil polluted with 200ml of crude oil was remediated with 50g of sawdust alongside a control (polluted but no remediation) and double control (no pollution and no remediation). The biostimulated soil was allowed for 8 weeks before cassava planting. Composite soil analyses for nitrate, phosphate, total hydrocarbon content (THC), total organic carbon (TOC), pH, and conductivity; and cassava yield parameters were done at every 8 weeks and 4 weeks interval respectively. Result showed that addition of sawdust increased the soil nutrient status as well as the yield of the two cassava varieties. Nitrate increase from 12.4 - 34.89mg/kg as against 16.30mg/kg in the control while THC decreased from 675 to 150 mg/kg. pH showed no significant difference between and within treatments. Soil conductivity recorded was in the order: double control (243  $\mu$ s/cm) > control (112  $\mu$ s/cm) > biostimulation (55  $\mu$ s/cm). These invariably led to improvement in the yield of cassava varieties with increase in shoot length (36.6 $\pm$ 1.24 and 29.0 $\pm$  0.74); above-ground fresh weight (40.12 $\pm$  0.12 and 27.26 $\pm$  0.2); below-ground fresh weight (24.06 $\pm$  0.05 and 20.00 $\pm$ 0.09); above-ground dry weight (9.64 $\pm$  0.12 and 7.75 $\pm$  0.03); below-ground dry weight (8.40 $\pm$  0.10 and 9.20 $\pm$  0.18); and total dry weight (18.04 $\pm$  0.10 and 17.01 $\pm$ 0.2) for var. NR 8082 and var. TMS 30572 respectively. These values were significantly (p=0.05) higher than their respective controls. Therefore, sawdust has the potential to biostimulate crude oil polluted tropical soil for cassava cultivation especially in the Niger-Delta region of Nigeria where crude oil pollution is inevitable.

**Key words:** crude oil, sawdust; biostimulation; cassava; soil parameters; pollution. \*Correspondence author

### **Introduction**

The exploration of petroleum has resulted in the widespread contamination of water, land and air with petroleum and its by-products. Hydrocarbon products affect the soil by reducing the nutrient content of the soil [21,26]; increase the toxicity of heavy metals [3], and reduces water infiltration into the soil as a result of its hydrophobic characteristics [20].

It may also affect plants by retarding seed germination and reducing height, stem density, photosynthetic rate and biomass or resulting in complete mortality [2,14,20,16,8]. Therefore, for

effective cultivation of crops like cassava in this ecozone where crude oil pollution is inevitable, some remedial measures need to be applied.

Several remedial measures ranging from mechanical, physical and chemical methods have been adopted in the past for the remediation of crude oil polluted sites. These methods have been found to be costly and caused more ecological damage to the environment than the crude oil itself. Biostimulation (nutrient enrichment) have been proven to be an effective strategy to enhance crude oil biodegradation as it is found to be less costly and ecofriendly. Detrimental effect from nutrient enrichment have not

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been observed following full scale field operation [17,11]. Many researchers such as Odokuma and [12,25,13], Ofor and Akonye [14], Akonye and Onwudiwe [1,2], and Tanee and Kinako [23] have proven the effectiveness of biostimulation of crude oil polluted soil using inorganic and organic manures. Tanee and Akonye [22] reported an improvement in the yield of cassava in a crude oil polluted phytoremediated soil.

Several attempts have been made in seeking for ways of remediating polluted (especially crude oil) environment using local and cheap materials like sawdust hence the need for this study. Sawdust is a waste product usually discarded in carpentry and sawmills. It has high organic matter content as well as high absorptive property. Results obtained from this study will widen our knowledge on ways of bioremediating crude oil polluted soil for the improvement of crop production in Nigeria especially in the Niger delta.

### Materials and methods

This research was carried out at the Botanic Garden, University of Port Harcourt, Rivers state. The study site is situated along the East-West Road, 26 km North-West of the city of Port Harcourt, on latitude 4° 43'N and longitude 7° 05'E in the south-south geopolitical (Niger-Delta) zone of Nigeria. The meteorology of the area reveals an average temperature range between 25°C to 35°C; an average rainfall of 2500 mm/yr and a relative humidity value range between 50%-96%.

Top-loamy soil collected from the premises of the Botanic Garden; thoroughly mixed to obtain a homogenous mixture were filled into black cellophane bags of diameter 25cm. 5kg of the soil were filled into each bag leaving a space of 5cm from the top to make allowance for the addition of treatments and water. The bags were also perforated at their bases and sides to ensure proper drainage and better aeration of the soil. A total of 72 bags were used for the experiment. The bags containing soil were separated into 3 sets of 24 replicates each designated as X, Y and Z. Crude oil (Bonny light) obtained from the Nigerian National Petroleum Corporation (N.N.P.C), Eleme, Rivers state was applied as the pollutant. 200ml of crude oil were applied to each bag in sets X and Y while Z was not polluted. The oil was thoroughly mixed with the soil in the bags and the set-ups were allowed to stand for one week for full acclimatization between the soil and the oil to take place.

After one week post-pollution treatment, remediation was carried out on set X. Sawdust obtained from a carpenter's workshop at Timber market, Bori, Rivers State was used as the remediation (biostimulation) material. 50g of the

sawdust was applied to each bag in set X while Y was the control (polluted but no remediation) and Z the double control (no pollution and no remediation). After two months, each set (X, Y, Z) were later separated into 2 subsets each; designated as X<sub>1</sub>, X<sub>2</sub>, Y<sub>1</sub>, Y<sub>2</sub>, Z<sub>1</sub> and Z<sub>2</sub> of 12 replicates each.

Stem cuttings of two cassava (*Manihot esculenta*; Crantz) varieties NR 8082 and TMS 30572 obtained from Agricultural Development Programme (ADP), Port Harcourt were planted in the bags. Two 25cm stem-cutting of each variety were planted in each subset. That is, variety NR 8082 was planted in X<sub>1</sub>, Y<sub>1</sub> and Z<sub>1</sub> while variety TMS 30572 was planted in X<sub>2</sub>, Y<sub>2</sub> and Z<sub>2</sub>. The stem-cuttings were planted in a slanting position at an angle of 60°C with half of the number of buds above the soil surface. The whole experiment lasted for 24 weeks. Weeds were removed from the set-ups as the need arose.

Soil Parameters such as soil pH, conductivity, nitrate, phosphate, total organic carbon (TOC), and Total hydrocarbon content (THC) were analyzed while the following plant growth parameters were measured: shoot length (plant height), fresh weight yield (below-ground and above -ground) and dry weight yield (above -ground, below-ground and total yield). Soil parameters were analysed at every 8 weeks interval while the cassava growth parameters were analysed at every 4 weeks interval.

The soil pH and conductivity were analysed using pH meter (Jennway 3015 model) and conductivity meter (model: Jennway 4010) respectively. Soil nitrate, phosphate and total organic carbon were analysed by the Kjeldahl, ascorbic acid and oxidation methods respectively (Stewart *et al.*, 1974); while the total hydrocarbon content (THC) was measured at 430nm using DR/300 HACH Spectrophotometer. The shoot length was measured with a meter tape from the base of the stem to the shoot apex in centimetres. The fresh weights in grammes were obtained by uprooting the plant from each bag; washed to remove soil particles and immediately weighed on a weighing balance (PN 163 model) to avoid water loss from the plant tissues. The dry weight were obtained by oven-drying the plants at 80°C for 48 hours and then reweighed on the weighing balance (model PN 163).

All data collected were statistically analysed using Analysis of Variance (ANOVA), and Standard Error Mean (SEM). Means were separated using the New Duncan Multiple Range Test (NDMRT) at 95% confidence interval.

### Results and discussion

Addition of sawdust as a biostimulation material to crude oil polluted soil affected the soil parameters as well as the growth and yield of cassava (*Manihot esculenta*; Crantz).

Biostimulating the crude oil polluted soil with sawdust was found to significantly ( $p=0.05$ ) improve the nitrate content of the soil with time when compared to the control (Fig. 1). At the termination of the experiment, nitrate content of the biostimulated soil was higher than that in the control and double control. The result also showed a significant decrease in the phosphate content in the crude oil polluted soil as well as the double control (Fig. 2). Although, the decrease was more in the control than in both the biostimulated and double control soils. The increase in nitrate content may be attributed to the sawdust used; in which the sawdust supplied the nitrogen. Sawdust is known to contain nitrogen since it is of plant origin.

Fig.3 showed the effect of sawdust on the soil total hydrocarbon content (THC). Significant reduction ( $p=0.05$ ) in the THC was found to occur with time. At the 24<sup>th</sup> week the THC in the biostimulation treatment was reduced from 675mg/kg to 150mg/kg while that of the control from 675mg/kg to 470mg/kg. The result obtained for the soil total organic carbon (TOC) showed a significant decrease in both the control and biostimulated treatments with significant difference ( $p=0.05$ ) between them (Fig.4). The double control showed a constant TOC value (0.48%) throughout the study. Similar result was obtained for the soil pH (Fig.5). Slight decrease in soil pH were observed in all the treatment options with time but showed no significant difference ( $p=0.05$ ) between them. The reduction in the total hydrocarbon content in the biostimulated plots may be attributed to the high nutrient level especially nitrate which stimulates microbial population and activities. Increase in nitrogen and phosphorus in crude oil polluted soil have been reported to enhanced crude oil biodegradation through the stimulation of microbial populations [13,6]. There was an initial increase in the total organic carbon in both the biostimulated and control soils. This is understandable because of the crude oil introduced as the pollutant. Crude oil is known to have 84 -87% by weight of carbon [15]. Increase in microbial activities and population will invariably means increase in nutrient demand especially carbon by the microorganisms for their metabolic activities. This might have accounted for the subsequent reduction in the total organic carbon content with time in the different treatments. The range of pH observed in the study provides better conditions for mineralization of hydrocarbons since most bacteria capable of metabolizing hydrocarbons develop best at pH close to neutrality [9,5]. This might also be the reason for the reduction in the total hydrocarbon content especially in the biostimulated soil.

Significant increases in soil conductivity were observed in all the treatment options from the 0 – 24<sup>th</sup> weeks (Fig. 6). At the 24<sup>th</sup> week, soil conductivity recorded was in the order: double control (243  $\mu\text{s/cm}$ ) >control (112  $\mu\text{s/cm}$ ) >

remediation (55  $\mu\text{s/cm}$ ). This showed that sawdust is capable of reducing the conductivity of a crude oil polluted soil.

The result of the growth and yield parameters of the two cassava varieties are presented in Tables 1 – 6.

Shoot length (plant height) of the two cassava varieties were found to be affected by the application of sawdust as a biostimulating material in the crude oil polluted soil (Table 1). Both varieties showed significant improvement in the shoot length in the biostimulation treatment. At the 16<sup>th</sup> week, var. NR 8082 showed highest growth in terms of shoot length in both the biostimulation and double control treatments with no significant difference between them while in var. TMS 30572, double control recorded higher yield than the biostimulation treatment. Similar result pattern was obtained for the above-ground fresh weight yield for the two varieties (Table 2).

Below-ground fresh weight yield (Table 3) and above-ground dry weight yield (Table 4) showed similar result pattern. Significant ( $p=0.05$ ) increase in the yields were observed with time in the different treatment options. Although, at the 16<sup>th</sup> week highest significant yields were recorded in the double control while the control recorded the least for the both varieties.

Tables 5 and 6 showed the below-ground dry weight and total dry weight yield responses to the different treatment options. Var. NR 8082 showed highest yield for both below-ground dry weight and total dry weight in the double control treatment while var. TMS 30572 showed highest below-ground dry weight and total dry weight yields (with no significant difference) in both the biostimulated and the double control treatments.

Improvement in the growth and yields of the two cassava varieties in the biostimulated soil than the control may be as a result of the increase in soil nitrate. This in line with Tane and Akonye [22] who reported an increase in yields of cassava in a crude oil phytoremediated soil. Increased soil nitrogen increases vegetative growth of cassava [18]. Reduced level of total hydrocarbon content has also been reported to stimulate growth of plant [7] as a result of the improvement in soil structure [10]. The reduction in yields in the control soil is in line with previous studies in which it was reported that crude oil polluted soil reduces the growth and yield of plants [4,20,16,24].

It can be concluded that sawdust has the potential of biostimulating crude oil polluted soil for increase cassava performance in terms of growth and yield as it is capable of increasing the soil nutrient content and reducing the soil total hydrocarbon content toxicity. It is therefore, recommended as a secondary treatment option for crude oil polluted habitat.

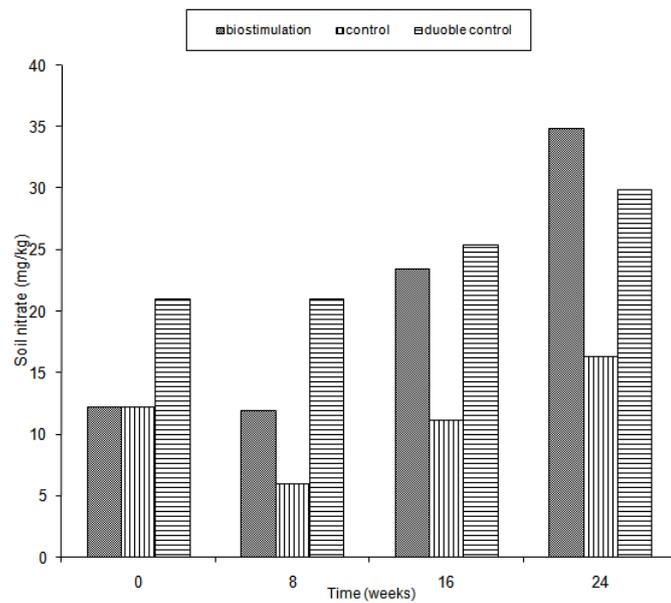


Fig. 1: Biostimulation effect of sawdust on soil nitrate (mg/kg)

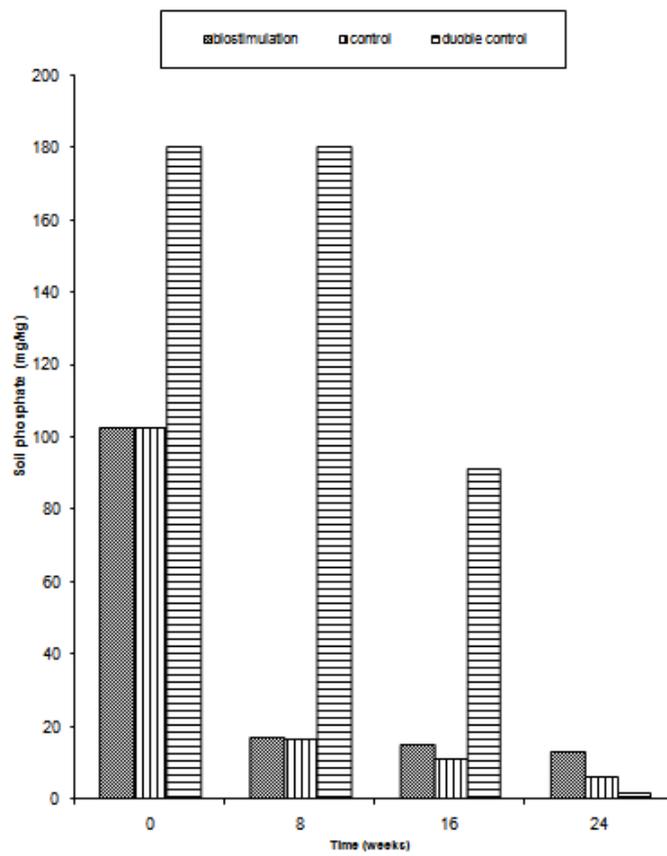
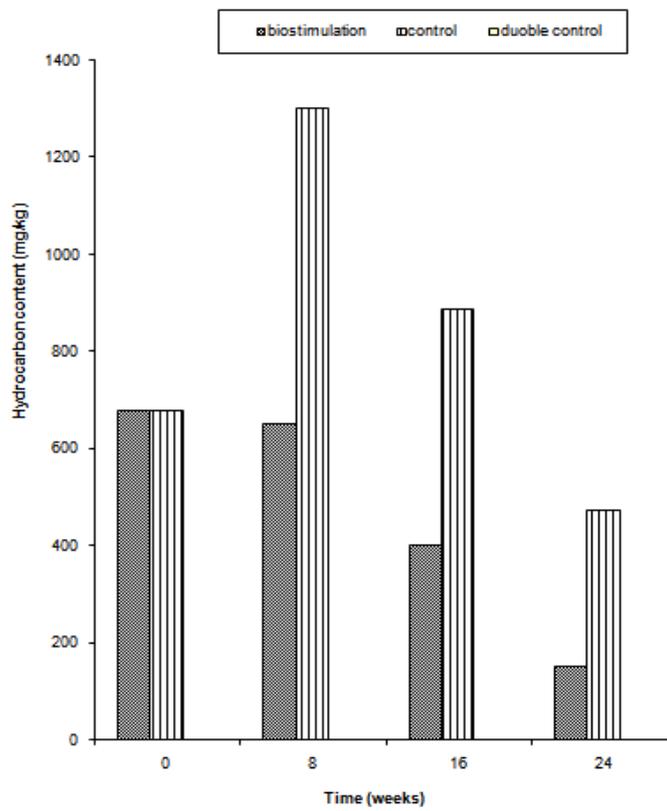
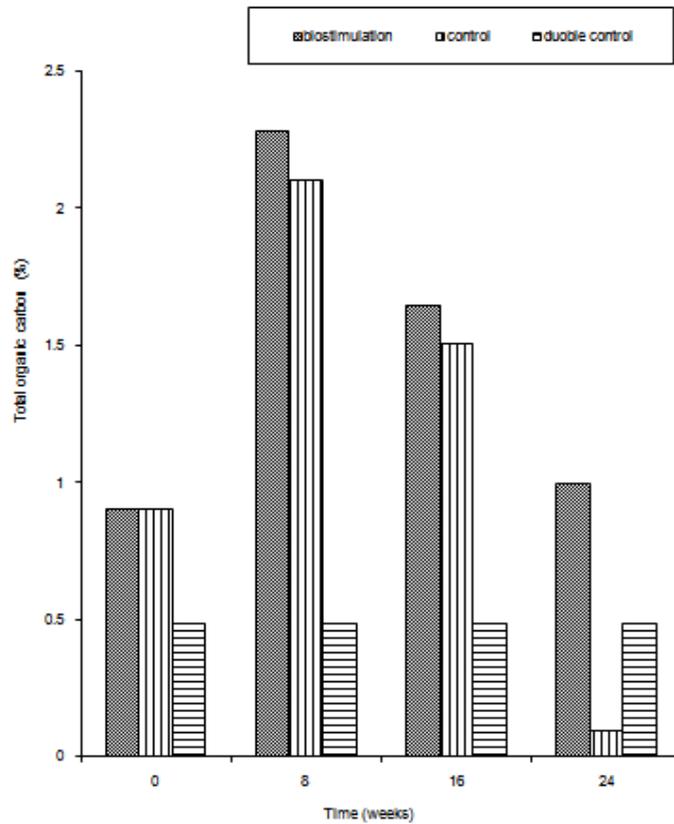


Fig. 2: Biostimulation effect of sawdust on soil phosphate (mg/kg)



**Fig. 3:** Biostimulation effect of sawdust on Total Hydrocarbon content (mg/kg)



**Fig. 4:** Biostimulation effect of sawdust on soil total organic carbon (%)

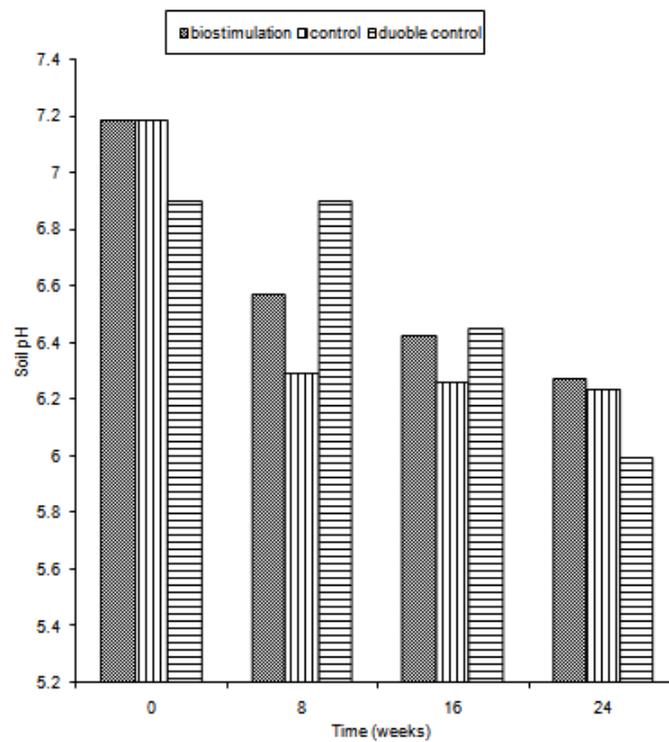


Fig. 5: Biostimulation effect of sawdust on soil pH

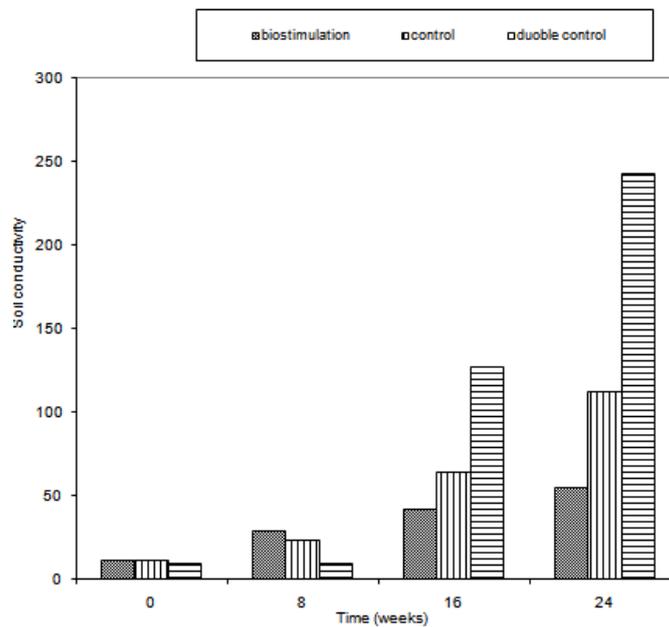


Fig. 6: Biostimulation effect of sawdust on soil conductivity (us/cm)

Table 1: Biostimulation effect of sawdust on Shoot length (cm) on the two cassava varieties.

Plant age (wks)	Var. NR 8082			Var. TMS 30572		
	Biostimulation	Control	Double control	Biostimulation	Control	Double control
4	11.7±0.60 <sup>b</sup>	10.1±0.18 <sup>a</sup>	17.4±0.28 <sup>c</sup>	9.9±0.70 <sup>a</sup>	9.1±1.28 <sup>a</sup>	13.9±0.44 <sup>b</sup>
8	15.9±0.19 <sup>a</sup>	15.4±1.00 <sup>a</sup>	27.5±1.34 <sup>b</sup>	13.7±0.41 <sup>a</sup>	17.3±0.96 <sup>b</sup>	21.8±1.58 <sup>c</sup>
12	20.7±0.72 <sup>b</sup>	17.3±0.66 <sup>a</sup>	36.6±0.12 <sup>c</sup>	17.8±0.65 <sup>a</sup>	22.0±1.03 <sup>ab</sup>	24.2±0.46 <sup>b</sup>
16	36.6±1.24 <sup>b</sup>	15.0±2.11 <sup>a</sup>	39.5±0.73 <sup>b</sup>	29.0±0.74 <sup>b</sup>	15.5±0.40 <sup>a</sup>	35.0±2.79 <sup>c</sup>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

**Table 2:** Biostimulation effect of sawdust on Above-ground fresh weight yield (g) of the two cassava varieties

Plant age (wks)	Var. NR 8082			Var. TMS 30572		
	Biostimulation	Control	Double control	Biostimulation	Control	Double control
4	19.74±0.75 <sup>a</sup>	18.80±0.91 <sup>a</sup>	28.91±0.49 <sup>b</sup>	16.44±0.06 <sup>a</sup>	13.80±0.42 <sup>a</sup>	25.81±2.99 <sup>b</sup>
8	24.56±0.73 <sup>b</sup>	16.14±0.26 <sup>a</sup>	31.05±0.49 <sup>c</sup>	20.06±0.50 <sup>a</sup>	16.32±0.34 <sup>a</sup>	26.11±0.01 <sup>b</sup>
12	24.04±2.22 <sup>b</sup>	17.64±0.42 <sup>a</sup>	39.93±0.71 <sup>c</sup>	25.85±0.15 <sup>b</sup>	20.50±0.50 <sup>a</sup>	31.80±0.79 <sup>c</sup>
16	40.12±0.12 <sup>b</sup>	16.75±0.51 <sup>a</sup>	45.24±1.33 <sup>b</sup>	27.26±0.20 <sup>b</sup>	14.05±1.97 <sup>a</sup>	41.03±0.52 <sup>c</sup>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

**Table 3:** Biostimulation effect of sawdust on Below-ground fresh weight yield (g) of the two cassava varieties

Plant age (wks)	Var. NR 8082			Var. TMS 30572		
	Biostimulation	Control	Double control	Biostimulation	Control	Double control
4	9.53±0.74 <sup>a</sup>	9.50±0.27 <sup>a</sup>	10.27±1.15 <sup>a</sup>	13.02±0.86 <sup>b</sup>	8.20±0.24 <sup>a</sup>	16.75±0.55 <sup>c</sup>
8	19.91±0.05 <sup>b</sup>	12.40±1.19 <sup>a</sup>	24.37±0.68 <sup>c</sup>	17.04±0.12 <sup>b</sup>	13.79±0.47 <sup>a</sup>	18.77 ± 0.63 <sup>b</sup>
12	21.62±0.36 <sup>b</sup>	17.55±0.52 <sup>a</sup>	28.46±0.33 <sup>c</sup>	18.99±0.25 <sup>a</sup>	19.90±0.15 <sup>a</sup>	21.58±0.93 <sup>a</sup>
16	24.06±0.05 <sup>b</sup>	10.97±0.61 <sup>a</sup>	31.42±1.29 <sup>c</sup>	20.00±0.09 <sup>b</sup>	10.84±1.03 <sup>a</sup>	23.67±0.33 <sup>c</sup>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

**Table 4:** Biostimulation effect of sawdust on Above-ground dry weight yield (g) of the two cassava varieties

Plant age (wks)	Var. NR 8082			Var. TMS 30572		
	Biostimulation	Control	Double Control	Biostimulation	Control	Double Control
4	3.49±0.36 <sup>a</sup>	3.83±0.14 <sup>a</sup>	5.78±0.06 <sup>b</sup>	3.12±0.02 <sup>b</sup>	2.76±0.18 <sup>a</sup>	5.33±0.64 <sup>c</sup>
8	5.25±0.15 <sup>c</sup>	3.06±0.03 <sup>a</sup>	6.27±0.06 <sup>b</sup>	4.11±0.07 <sup>a</sup>	3.18±0.01 <sup>a</sup>	5.48±0.27 <sup>b</sup>
12	5.26±0.63 <sup>b</sup>	3.45±6.17 <sup>a</sup>	9.48±0.18 <sup>c</sup>	5.79±0.9 <sup>b</sup>	4.13±0.05 <sup>a</sup>	6.96±0.04 <sup>c</sup>
16	9.64±0.12 <sup>b</sup>	5.18±0.20 <sup>a</sup>	11.05±0.25 <sup>c</sup>	7.75±0.03 <sup>b</sup>	3.42±0.44 <sup>a</sup>	10.03±0.03 <sup>c</sup>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

**Table 5:** Biostimulation effect of sawdust on Below-ground dry weight yield (g) of the two cassava varieties

Plant age (wks)	Var. NR 8082			Var. TMS 30572		
	Biostimulation	Control	Double Control	Biostimulation	Control	Double control
4	1.06±0.08 <sup>b</sup>	0.46±0.19 <sup>a</sup>	1.13±0.06 <sup>b</sup>	1.36±0.12 <sup>b</sup>	0.94±0.02 <sup>a</sup>	1.54±0.04 <sup>c</sup>
8	6.46±0.32 <sup>b</sup>	1.89±0.06 <sup>a</sup>	7.63±0.07 <sup>c</sup>	6.55±0.09 <sup>b</sup>	2.57±0.22 <sup>a</sup>	6.91±0.22 <sup>b</sup>
12	7.49±0.10 <sup>b</sup>	4.58±0.02 <sup>a</sup>	6.87±0.47 <sup>b</sup>	7.55±6.40 <sup>b</sup>	3.85±0.03 <sup>a</sup>	7.86±0.37 <sup>b</sup>
16	8.40±0.10 <sup>b</sup>	4.57±0.06 <sup>a</sup>	10.62±0.72 <sup>c</sup>	9.20±0.18 <sup>b</sup>	4.17±0.45 <sup>a</sup>	9.85±0.28 <sup>b</sup>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

**Table 6:** Biostimulation effect of sawdust on total dry weight yield (g) of the two cassava varieties

Plant age (wks)	Var. NR 8082			Var. TMS 30572		
	Biostimulation	Control	Double control	Biostimulation	Control	Double control
4	4.56±0.40 <sup>a</sup>	4.30±0.29 <sup>a</sup>	6.91±0.11 <sup>b</sup>	4.47±0.11 <sup>a</sup>	3.71±0.18 <sup>a</sup>	6.87±0.60 <sup>b</sup>
8	11.71±0.43 <sup>b</sup>	4.95±0.04 <sup>a</sup>	13.09±0.12 <sup>c</sup>	10.66±0.09 <sup>b</sup>	5.75±0.22 <sup>a</sup>	12.40±0.45 <sup>c</sup>
12	12.75±0.58 <sup>b</sup>	8.04±0.16 <sup>a</sup>	16.35±0.60 <sup>c</sup>	13.34±0.11 <sup>b</sup>	7.98±0.09 <sup>a</sup>	14.82±0.40 <sup>c</sup>
16	18.04±0.10 <sup>b</sup>	7.75±0.64 <sup>a</sup>	21.67±0.85 <sup>c</sup>	17.01±0.21 <sup>b</sup>	7.59±0.89 <sup>a</sup>	19.89±0.29 <sup>b</sup>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

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