

Evaluation of growth responses of Cassava Treated with *Pleurotus tuber-regium* Spent Mushroom Substrate Water Extract to Elicit Disease Resistance.

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ABSTRACT

This paper investigated the effect of *Pleurotus tuber-regium* mushroom substrate water extract on the growth of cassava generated through meristem tissue culture. The cassava plantlet (tms 98/0505) were generated at the Tissue Culture Laboratory of Biotechnology unit, National Root Crop Research Institute, Umudike Umuahia Abia State before they were transferred to the screen house of the Faculty of Agriculture Teaching and Research Farm, University of Port Harcourt, Rivers State. The water extract was applied on the cassava to elicit disease resistance after 4 months of culturing in a nutrient medium. The treatments for this investigation comprised of T1; *Pleurotus tuber-regium* water extract spent mushroom substrate (PTWESMS), T2; *Pleurotus tuber-regium* autoclaved water extract spent mushroom substrate (PTAWESMS) and T3; the control. The experiment was laid out in a completely randomized design with 3 replicates. The data generated were subjected to analysis of variance (ANOVA). Means were separated using Fisher's Least Significant Difference at $P=0.05$. The results obtained revealed that there was no significant difference when the different treatments were compared with the control and when the treatments were compared with one another in their mean plant height, mean number of leaves, mean number of node and mean leaf area. However, the stem diameter and number of internode were significantly different. The result also revealed a significantly positive correlation (r) between plant height and stem diameter and a positive correlation between the number of internodes and leaf area and had a negative correlation between number of leaves and number of nodes. Number of leaves correlated negatively with plant height, stem diameter and leaf area and correlated positively with number of internode and number of nodes. Again, the stem diameter had a significantly positive correlation with plant height, number of internode, and leaf area and a positive relationship with Number of node. Number of internode correlates positively with all the parameters evaluated. Number of nodes related positively with number of leaves, stem diameter and number of internode while a negative correlation with plant height and leaf area was observed. This could possibly be the first account of this investigation.

Keywords: Tissue culture, *Pleurotus tuber-regium*, mushroom, substrate

INTRODUCTION

In recent decades, a greater knowledge of chitin chemistry, and the increased availability of chitin –containing waste materials have led to the testing and development of chitin –containing products for a wide variety of applications in the Agricultural industry. In addition to direct effects on plant nutrition and plant growth stimulation, Chitin –derived products have also been shown to be toxic to plant pests and pathogens, induce defenses and stimulate the growth and activity of beneficial microbes (Russel, 2013).

After cellulose, Chitin is the second most abundant polysaccharide on the planet (Gooday, 1990). Chitin is found in, and can be sourced from a variety of different organisms, with the notable exceptions of higher plants and vertebrate animals. Chitin – rich animals' tissues include the exoskeletons of arthropods (including insects, crustaceans and arachnids). The beaks of cephalopods and the eggs and gut linings of nematodes (Gohel, 2006). Various microbes also produce chitin in cell walls, membranes and spores, including fungi (Castro, 2012). As is the case with the cellulose in plants cell walls, the Chitin polysaccharides is combined with other compounds to produce strengthened tissues. Both polysaccharides form microfibrils which differ in length and construction depending on the species and cellular location (Bowman and Leong, 2006). In fungi this involves cross linkages to glucan polymers to create a meshed hyphal wall (Jayakumar, Prabakaran, Nair and Tamura, 2010; Towheed, Anastassiades, Shea, Houpt, Welch,

and Hochberg, 2001). Due to the involvement of other polymers, such as glucans, the chitin content of fungal cell walls ranges from 22-40% (Pillai, Paul and Sharma 2009). The cationic properties of the chitosan oligosaccharide imbue it with unique properties that can be exploited by biotechnologists and physicians (Towheed, Anastassiades, Shea, Houpt, Welch, Hochberg, 2001), Material science, and Crop Science (Ramirez, Rodriguez, Alfonso and Peniche, 2010). Chitin, Chitosan and Glucosamine have all been experimentally trialed on Crop Plants with a range of beneficial agronomical responses recorded. These include direct antibiosis against Pests and Pathogens of Crops, enhancement of beneficial microbes both in Plants defense responses against biotic stress, and up-regulation of Plant growth, development, nutrition and tolerance to abiotic stresses.

According to the FAO (2008), the global production of cultivated edible mushrooms had increased from 2.26 million tons in 1998 to 3.48 million tons in 2008. Despite the evident benefits of mushrooms, the exponential increase in their consumption worldwide is also generating a high volume of Spent Mushroom Substrate (SMS). It has been reported that about 5kg of substrate are needed to produce 1kg of Mushroom (Williams, McMullan, and McCahey, 2001; Uzun, 2004; Finney, Ryu, Sharifi, and Swithenbank, 2009), and about 17 million tons of SMS are produced each year. Consequently, one of the main problems faced by Mushroom production companies is finding a way to properly dispose of the SMS without contaminating the soil and water. In fact, the lack of a sustainable waste management solution for SMS is the most significant barrier to the future development of the mushroom industry (Finney, *et al* 2009). Several studies have been carried out to demonstrate the benefits of SMS application in mushroom re-cultivation, enrichment of soil, restoring areas that have been destroyed through development, deforestation or environmental contamination (Sanchez, 2004), cultivation of vegetables, fruits and flowers in green houses and fields (Medina, Paredes, Perez-Murcia, Bustamante, and Moral, 2009; Polat, Uzun, Topcuoglu, Onal, Onus and Karaca, 2009; Ribas, Mendoca, Camelini, and Soares, 2009), and soil amendment and degradation of organopollutants (Semple, Reid, and Fermor, 2001; Lau, Tsang, and Chiu, 2003). The SMS can also be used as a potential energy feedstock (Williams *et al.* 2001; Finney *et al.* 2009), and ethanol production (Hideno, Aoyagi, Isobe, and Tanaka, 2007).

Cassava (*Manihot esculenta* Crantz) is a shrub 1-5m high which is cultivated for its starch –containing tuberous roots (Cock, 1985). One of the greatest problems confronting this all important crop in Africa is cassava mosaic disease (CMD). This disease is caused by the viruses-African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV). They are transmitted by the whitefly *Bemisia tabaci*. Presence of these viruses can cause yield losses of up to 40 -50% of total yield in cassava throughout the Continent (Cock, 1985; Thresh, Fargette and Otim-Nape, 1994; Otim-Nape, Thresh, and Fargette, 1996).

The primary aim of this research is to investigate the effect of *Pleurotus tuber-regium* Spent Mushroom substrate autoclaved/unautoclaved water extract on growth and growth parameters of cassava generated through Meristem tip tissue culture applied after 4 months of culturing.

MATERIALS AND METHOD

STUDY SITE AND SOURCE OF TEST CROP

Cassava variety (TMS 98/0505) used in this experiment were obtained from the Eastern Farm of National Root Crop Research Institute Umudike, Umuahia Abia State Nigeria. The cassava cuttings with about 3-4 nodes were planted in buckets filled with sawdust and placed in a shade and watered periodically. The cuttings were kept at room temperature for about 2 weeks until the apical buds of the sprouted shoots were excised and aseptically cultured according to the method prescribed by Murashige and Skoog (1962) at the tissue culture laboratory, National Root Crop Research Institute of Nigeria, Umudike Umuahia Abia State.

PREPARATION OF MUSHROOM SUBSTRATE

Spent mushroom substrate used for this study was obtained from Dilomat Farms and Services located at the Faculty of Agriculture, Rivers State University of Science and Technology, Port Harcourt, Rivers State. The *Pleurotus tuber-regium* mushroom spawn was inoculated in polypropylene bags containing 2 kg mixtures of sawdust, lime and rice bran in the ratio 1000:1:100. The bags were incubated at room temperature in a specially constructed chamber for 30 days and opened to initiate fruit body production. At the end of the production cycle of about 6 months, the spent mushroom substrate was used immediately for water extract preparation.

PREPARATION OF WATER EXTRACT FROM SPENT MUSHROOM SUBSTRATE

Spent mushroom substrate (400g) was homogenized in a blender with 150cl of distilled water (DW) for 2 minutes at 1500rpm according to the procedure described by Parada, Murakami, Shimomura, Egusa and Otani, (2011). The homogenate was filtered through two layers of calico cloth. The filtrate was used immediately for leaf treatment as water extract from spent mushroom substrate (WESMS). Half of the WESMS were autoclaved at 121°C for 30 minutes (AWESMS) and was also used immediately for leaf treatment. The WESMS, AWESMS and the control (zero application of the extract) which represents the treatments were replicated 3 times in a completely randomized design. The treatments were sprayed profusely on the cassava plants with a hand sprayer.

DATA COLLECTION

For the agronomic evaluation, the following data were taken every 2 weeks: Plant height (cm) with a meter rule, Number of leaves, Number of internodes, Number of nodes, Stem diameter (mm) with a vernier calipers and Leaf area (cm²) according to the method described by Edje and Osiru (1987).

EXPERIMENTAL DESIGN AND DATA ANALYSIS

The experiment was laid out in a completely randomized design. The data generated in this study were subjected to analysis of variance (ANOVA). Means were separated using Fishers Least Significant Difference at P=0.05. Means and percentages were according to the procedure outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSION

The effects of the treatment on the growth and growth parameters of cassava after 6 months of culturing and before transplanting in the field are presented in table 1, while the correlation matrix for the relationship between the growth parameters are presented in table 2.

Table 1. Effects of The Treatment On the Growth and Growth Parameters.

Treatments	Plant height (cm)	Number of leaves	Stem Diameter (mm)	Number Of internode	Number Of nodes	Leaf Area/plant (cm ²)
PTWESMS	21.31 ^a	6.59 ^a	34.67 ^a	15.20 ^a	13.83 ^a	178.7 ^a
PTAWESMS	17.98 ^a	6.95 ^a	26.33 ^b	13.95 ^{a,c}	12.33 ^a	165.4 ^a
CONTROL	16.03 ^a	8.12 ^a	26.00 ^b	11.56 ^c	12.89 ^a	173.1 ^a
F-LSD(P=0.05)	NS	NS	2.82	1.36	NS	NS

a-Figures with the same superscripts are statistically not significant.

PTWESMS-*Pleurotus tuber-regium* water extract spent mushroom substrate.

PTAWESMS-*Pleurotus tuber-regium* autoclaved water extract spent mushroom substrate.

Table 2. Correlation Matrix of the Parameters Evaluated.

Plant Character	Plant height	Number of leaves	Stem diameter	Number of internode	Number of nodes	Leaf area
Plant height	1.0	-0.264	0.788*	0.585	-0.134	0.567
Number of leaves	-0.264	1.0	-0.444	0.061	0.394	-0.601
Stem diameter	0.788*	-0.444	1.0	0.688*	0.181	0.894**
Number of internode	0.585	0.061	0.688*	1.0	0.612	0.389
Number of nodes	-0.134	0.394	0.181	0.612	1.0	-0.046
Leaf area	0.567	-0.601	0.894**	0.389	-0.046	1.0

- Negative correlation. *correlation is significant at 0.05 probability level (2-tailed); **Correlation is significant at 0.01 probability level (2 tailed).

PLANT HEIGHT

The effect of the treatment on plant height revealed that plants treated with PTWESMS was 0.73% and 24.8% taller than the plants treated with PTAWESMS and the Control respectively. Also Plants treated with PTAWESMS were 10.85% taller than the plants from the Control plots. These figures were not significantly different. This result does not agree with the results obtained by Okere, Ataga, Elenwo and Ekpe, (2015). The result further showed that there was negative percentage correlation difference of 26.4% and 13.4% when plant height was respectively correlated with number of leaves and number of nodes. This implies that increases in plant height did not necessarily lead to increase in number of leaves and number of nodes. This however does not agree with the findings of Okere *et al.* (2015). There was positive significant correlation of 78.8%, between plant height and stem diameter and 58.5% and 56.7% when plant height was correlated with number of internodes and plant height with leaf area. This result revealed that increase in

plant height lead to significant increase in stem diameter which implies that the genes controlling plant height and stem diameter may be related. Also the non-significant positive relationship between plant height, number of internodes and leaf area were in agreement with the findings of Yen and Mau, (2007).

NUMBER OF LEAVES

There was no significant difference in the number of leaves when the results of the treatments were compared with the control and when the treatments were compared with one another. Nevertheless, this result showed that plants treated with PTWESMS had 5.18% and 18.84% less number of leaves compared to the plants treated with PTAWESMS and the control respectively. Also plants treated with PTAWESMS had 14.41% less number of leaves compared with the control. This result is in agreement with the findings of Okere *et al* (2015). There was negative correlation of 26.4%, 44.4% and 60.4% between the number of leaves and plant height, stem diameter, and leaf area respectively. This indicated that as plant height and stem diameter increased the number of leaves decreases. This is because as cassava plants grow they tend to shed the older leaves

STEM DIAMETER

The effect of the treatment on stem diameter were however significantly different. The result revealed that plants treated with PTWESMS was 24.1% and 32.9% thicker than the plants treated with PTAWESMS and the control respectively. While the plants treated with PTAWESMS was 1.3% thicker than the control. These results also agree with the findings of Spiegel Kafafi, and Pressman, (1988). There was significantly positive correlation of 78.8, 68.8, and 89.4% respectively between the stem diameter, plant height, number of internode and leaf area. The genes controlling these parameters may be related. Also number of nodes had a positive correlation of 18.1% with the stem diameter which implies that an increase in stem diameter led to increase in the number of nodes which also agree with the findings of other researches (Okere *et al.* (2015) ; Spiegel *et al.* (1988)) which demonstrated that Chinese cabbage treated with chitin – based products grew faster than plants treated with standard mineral fertilizer.

NUMBER OF INTERNODE

The number of internodes did not reveal any statistically significant difference but the values ranged from 15.20 to 11.56. This result notwithstanding the plants treated with PTWESMS had 8.22 and 17.1% higher number of internodes than the plants treated with PTAWESMS and the control respectively while plants treated with PTAWESMS had 17.13% higher number of internodes compared with the control. The performance of PTWESMS over the other treatments may be attributed to the effect of heat on the available mineral elements present in the extract as a result of autoclaving. This result also agrees with the result obtained by Okere *et al.* (2015). When subjected to correlation analysis, the result reveals a significantly positive correlation of 68.8% between the number of internode and stem diameter. Also a positive relationship of 58.5, 61.6, 61.2, and 38.9% exists between the number of internode and the plant height, number of leaves, number of nodes and leaf area respectively. This implied that as the number of internode increases, plant height, stem diameter, number of leaves and number of nodes also increased.

NUMBER OF NODES.

The effect of the treatment on the number of nodes were not significantly different. However, the result revealed that plants treated with PTWESMS had 10.85 and 9.62% higher number of nodes than the plants treated with PTAWESMS and the control respectively while the plants treated with PTAWESMS had 1.36% less number of nodes when compared with the control. The correlation analysis result reveals a positive correlation of 39.4, 18.1 and 61.2% between the number of nodes and number of leaves, stem diameter and number of internode respectively. This result revealed that as the number of leaves, stem diameter and number of internode increased the number of nodes decreased. However, a negative relationship of 13.4 and 4.6% exist between the number of nodes and the plant height and leaf area respectively. This implied that as the number of nodes increased, the plant height, leaf area decreases which reveal that increases in the number of internodes do not necessarily lead to increase in plant height and leaf area.

LEAF AREA

The leaf area per plant was not significant but the values ranged from 165.4-178.7. However, plants treated with PTWESMS had 6.03% and 7.4% more leaf canopy than the plants treated with PTAWESMS and the Control respectively. Further, plants treated with PTAWESMS had 1.47% more leaf canopy than the control. There was a significantly positive relationship of 89.4% between leaf area and stem diameter which probably indicated a linkage between the genes controlling these parameters. Also a positive relationship of 56.7 and 38.9% respectively exist between the plant height, number of internode and leaf area. which imply that increase in leaf area also led to increases in plant height, stem diameter, number of internode. This can be attributed to the fact that increase in leaf area translates

to increase in radiation interception which affect the quantity of food manufactured during photosynthesis which also affect other growth parameters evaluated. However, a negative relationship of 60.1 and 4.6% exist between the leaf area, number of leaves and number of nodes respectively which reveal that increase in leaf area canopy would lead to increase in the number of leaves (table 1).

CONCLUSION

The result obtained from this investigation revealed that PTWESMS was superior to PTAWESMS and the control. This can be attributed to the fact that while autoclaving the liquid extract releases the resistance elicitors, it however reduced the available nutrient necessary to encourage plant growth and development.

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