**Journal of Global Biosciences**
**ISSN 2320-1355**
**Volume 4, Number 2, 2015, pp. 1594-1599**
**Website: www.mutagens.co.in**
**E-mail: submit@mutagens.co.in**
**researchsubmission@hotmail.com**

***Research Paper***

# AGRONOMIC EVALUATION OF CASSAVA (TMS 98/0505) TREATED WITH *PLEUROTUS OSTERATUS* SPENT MUSHROOM SUBSTRATE WATER EXTRACT AS DISEASE RESISTANCE ELICITOR

[1]Okere, S.E., [2] Ataga, A.E, [2]Elenwo, E.N. and Ekpe I.I.[3]

1 Department of Crop Science and Technology,
Federal University of Technology Owerri, Imo State.
2 Department of Plant Science and Biotechnology,
University of Port Harcourt,Rivers State.
3.Department of Soil Science and Technology,
Federal University of Tecchhnology, PMB 1526, Owerrui, Imo State, Nigeria.

**Abstract**

The effect of *Pleurotus osteratus* spent mushroom substrate water extract on the growth and growth parameters of cassava propergated through meristem tissue culture was investigated. The cassava plantlet (tms 98/0505) were propergated at the Tissue Culture Laboratory ,Biotechnology unit,National Root Crop Research Institute , Umudike Umuahia Abia State before they were transfered 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 as disease resistance elicitor after 4 months of culturing in a nutrient medium. The treatments for this investigation comprised of *Pleurotus osteratus* water extract spent mushroom substrate(POWESMS),*Pleurotus osteratus* autoclaved water extract spent mushroom substrate(POAWESMS) and the control. The experiment was replicated 3 times in a completely randomized design. The data generated were subjected to analysis of variance (ANOVA). Means were separated using Fishers Least Significant Difference at P=0.05.The following plant parameters were evaluated: plant height (cm),number of leaves, stem diameter(mm),number of internode and leaf area ($cm^2$). The results showed that the mean plant height was in the range of 23.53-16.03 cm, Number of internode ranged of 18.72 to 11.56Mean number of leaves and stem diameter were not significantly different. This could probably be the first account of this discovery.

Key words: Tissue culture, *Pleurotus osteratus,* Mushroom ,Substrate, Meristem.

## INTRODUCTION

After cellulose , chitin is the second most abundant polysaccharide on the planet(1).Chitin is found in and can be sourced from a variety of different organisms,with the exception of higher plants and vertebrate. Chitin –rich animal tissue include the exoskeletons of arthropods,the beaks of Cephalopods and the eggs and gut linings of nematodes(2).Various microbes also produce chitin in cell walls, membranes and spores (3).

The chitin polysaccharide can be partially depolymerized to produce oligosaccharide derivatives(4).These oligosaccharides can be produced with varying polymer lenght or

completely depolymerized to N-acetylglucosamine.If the chitin oligomers are deacetylated, the resultant compound produced is called chitosan(4).Chitosan is soluble in weak acid and so, once the alkali is neutralized,it can be safely applied to plants /soil as an ammendment.

The cationic properties of the chitosan oligosaccharide imbue it with unique properties that can be exploited by biotechnologists;including applications in the fields of medicine(5,6,7),material science (8),and crop science.Chitin, chitosan and glucosamine have all been experimentally tried 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 plant defence and growth,stimulation of plant defence responses against biotic stress and up-regulation of plant growth ,development,nutrition and tolerance to abiotic stresses.Positive responses to chitin and its derivatives have been reported in numerous economically important crop species that themselves represent a broad coverage of the plant kingdom, including monocotyledons,dicotyledons, magnoliids and gymnosperms(9,10).

Improvements in plant growth have been reported after the application of chitin-based treatment to a range of crops, which are thought to be independent of the effects on pest and disease control.Significant improvements in growth have been reported in cabbage(9),soybean sprouts(11).The reports of chitosan treatments stimulating growth should be tempered by the findings of other trials showing no significant effect on growth ,biomass production or yield in rice and soybean(12), maize and soybean(13).

Chitosan and all its derivatives have a high nitrogen content of 6.1-8.3%(14).This is a comparable level to other organic fertilizers such as dried blood ,bone meal, and hoof and horn meal(15).Plants can access the nitrogen in chitin via microbial breakdown and the release of inorganic nitrogen, or directly taking up monomers as organic nitrogen(16,17).Spiegel *et al.* (16) demonstrated that Chinese cabbage treated with chitin-based products grew faster than plants treated with a standard mineral fertilizer.Chitin –rich edible fungi waste has a long history of use in agriculture and landscape,horticulture with spent mushroom compost used primarily to add organic matter and raise soil $p^H$.The mycelia of mushroom that are prevalent in spent mushroom substrate are abundant sources of elicitors ,and thus application of spent mushroom substrate to plants may be useful for the control of plant diseases.Parada *et al.*(18) reported that water extract spent mushroom substrate and autoclaved water extract spent mushroom substrate contain water soluble and heat –stable elicitors for inducing systemic acquired resistance (SAR) in cucumber plants which are released from the cell wall by heat treatment of spent mushroom substrate.

Cassava (*Manihot esculenta Crantz*) is a shrub 1-5m high which is cultivated for its starch –containing tuberous roots(19).One of the greatest problems confronting this all important crop in Africa is cassava mosaic Disease(CMD).In Africa,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 (19,20,21).

The most widely used method for virus elimination is Meristem Tip Culture.This technique takes advantage of the fact that many viruses fail to invade the meristematic region .The use of this method is not 100% efficient in that its efficiciency depends on the size of the meristem tip as well as the ability of the operator to excise the dome shaped meristem tip unwounded.

The primary aim of this paper is to evaluate the effects of *Pleurotus osteratus* spent mushroom substrate autoclaved /unautoclaved water extract on growth and growth parameters of cassava applied after 4 months of culturing.

## MATERIALS AND METHODS

### Study site and source of sample

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 (22) at the tissue culture laboratory ,Biotechnology Unit, 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 osteratus* 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 circle 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 proceedure described by Parada *et al.* (18).The homogenate was filtered through two layers of cloth.The filtrate was used immediately for leaf treatment as water extract from spent mushroom substrate (WESMS).Half of the WESMS were autoclaved at $121^0$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 completly 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 2weeks:Plant height (cm) with a meter rule, Number of leaves, Number of internodes, stem diameter (mm) with a vernier calipers and leaf area ($cm^2$) according to the method described by Edje and Osiru (23).

**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 proceedure outlined by Steel and Torrie(24).

## 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 treatments on the growth and growth parameters of cassava**

| Treatment | Plant Height(cm) | Number of leaves | Plant Diameter(mm) | Number of internode | Leaf Area($cm^2$) |
|---|---|---|---|---|---|
| POWESMS | 23.53[a] | 8.22[a] | 0.30[a] | 18.72[a] | 15.90[a] |
| POAWESMS | 18.84[b] | 7.61[a] | 0.33[a] | 14.67[b] | 24.72[a] |
| CONTROL | 16.03[c] | 8.12[a] | 0.26[a] | 11.56[c] | 18.62[a] |
| F-LSD(P=0.05) | 2.23 | NS | NS | 3.69 | NS |

a- Mean values within the same colume with the same superscript do not differ significantly.
b - POWESMS –*Pleurotus osteratus* water extract spent mushroom substrate.
c - POAWESMS-*Pleurotus osteratus* autoclaved water extract spent mushroom substrate.

**Table 2:Correlation matrix for the relationship between the growth parameters.**

| Plant Character | Plant Height(cm) | Number of Internode | Number of leaves | Leaf Area ($cm^2$) | Plant Diameter(mm) |
|---|---|---|---|---|---|
| Number of Internode | +0.99 | | | | |
| Number of leaves | +0.29 | +0.23 | | | |
| Leaf Area | -0.44 | -0.37 | -0.98 | | |
| Plant Diameter | +0.45 | +0.51 | -0.72 | +0.61 | |

+ positive correlation,- negative correlation

**Plant height**

The effcet of treatment on plant height showed significant difference.The results revealed that plants treated with POWESMS was 4.69 and 7.50 taller than the plants treated with POAWESMS and the control respectively. Plants treated with POAWESMS was 2.81 taller than the control, when correlated with the number of internodes, number of leaves and plant diameter, the result indicate a positive correlation( r) of 99,29,and 45% respectively and a negative correlation of 44% with leaf area. This result is however in aggreement with findings of Yen, *et al.* (14).

**Number of internodes**

There was also significant difference when the results of the treatments were compared with the control and when the treatments were compared with one another.This result showed that plants treated with POWESMS had 4.05 and 7.16 higher number of internode than plants treated with POAWESMS and the control respectively.However plants treated with POAWESMS have 3.11 more internode than the control.The performance of POWESMS over POAWESMS may be attributed to the effect of heat on the available minerial element present in the extract as a result of autoclaving.When subjected to correlation analysis (r),the result reveal 99%,23% and 51% positive relationship between the plant height, number of leaves and plant diameter respectively and 37% negative relationship with leaf area .These results were in aggreement with the findings of Spiegel *et al.* (16).

**Plant diameter**

The plant diameter measured in millimeter was in the range 0.33-0.26 were however not significantly different.The result however showed that plants treated with POAWESMS was 0.03 thicker than plants treated with POWESMS and 0.07 thicker than the control.Plants treated with POWESMS was 0.04 thicker than the plants that received zero application (control).The result also reveal a positive relationship of 61% with the leaf area.

**Number of leaves**

The mean number of leaves in the range 8.22-7.61 which were however not significantly different.The results also revealed that plants treated with POWESMS had 0.61 higher number of leaves than the plants treated with POAWESMS and 0.1 higher than the control experiment.The result also show a negative correlation coefficient (r) of 72% between plant diameter and number of leaves indicating that increases in the number of leaves do not translate to increase in plant diameter.

**Leaf area**

The leaf area measured in $cm^2$ ranges from 24.72-15.90 were however not significantly different.The result also revealed that plants treated with POAWESMS were 8.82 and 6.1 wider than plants treated with POWESMS and the control respectively.The result also revealed a positive correlation (r) of 61% between leaf area and plant diameter and a negative correlation of 98% between number of leaves and leaf area.This indicates that increases in leaf area leads to increases in plant diameter and increase in leaf number do not translate to increase in leaf area.All these findings also agree with Spiegel *et al.* (16) which demonstrated that Chinese cabbage treated with chitin –based products grew faster than plants treated with standard mineral fertilizer.

## CONCLUSION

The major findings of this research was that water extract of spent mushroom substrate even though applied to elicit disease resistance can also enhance growth of plants and probably improve yield.This may become another form of organic fertilizer.However more work is recommended.

## REFERENCES

(1) Gooday,G.W.(1990). The ecology of chitin .Adv.Micro.Ecol.11,387-419.
(2) Gohel,V.,Singh,A., Vimal,M.,Ashwini,P., Chhatpar,H.S.(2006).Bioprospecting and antifungal potential of chitinolytic microorganisms.Afr.J.Biotechnol.5,54-72.
(3) Castro,S.P.M.,Paulin,E.G.L.(2012).Is chitosan a new panacea?Areas of application. In the complex world of Polysaccharide :Karunaratne.D.N.,Ed.,InTech:Rijeka. Croatia.doi:10.5772/51200.Available online :http://www.intechopen.com/ Books/the –complex –world –of –polysaccharides/is-chitosan-a-new-panace -areas-of-application(accessed on 4 july 2013).
(4) Ramirez,M.A.,Rodriguez,A.T.,Alfonso,L.,Peniche,C.(2010).Chitin and its derivative as biopolymers with potential agricultural applications.Biotecnol.Apl.27,270-276.
(5) Bowman,k.,Leong,K.W.(2006).Chitosan nanoparticles for oral drug and gene deli- very. Int.J.Nanomed.1.117-128.
(6) Jayakumar,R.,Prabaharan,M.,Nair,S.V.,Tamura,H.(2010).Novel chitin and chitosan Nanofibers in biomedical applications.Biotechnol.Adv.28.142-150.
(7) Towheed,T.E.,Anastassiades,T.P.,Shea,B.,Houpt,J.,Welch,V.,Hochberg,M.C.(2001).Glucosa mine therapy for treating Osteoarthritis.Cochrane Database Syst.Revie- ws.doi 10.1002/14651858.CD 002946.pub.
(8) Pillai,C.K.S.,Paul,W.,Sharma,C.P.(2009).Chitin and chitosan Polymers:Chemistry,Solubility and fiber formation.Prog.Polym.Sci.34,641-678.
(9) Hirano,S.,Kitaura,S.,Sasaki,N.,Sakaguchi,H.,Sugiyama,M.,Hashimoto,K.,Tanatani,A. (1996).Chitin biodegradation and wound healing in tree bark tissues.J.Envinon. Polym.Degrad.4,261-265.
(10) Croteau,R.,Gurkewitz,S.,Johnson,M.A.,Fisk,H.J.(1987).Monoterpene and diterpe- ne biosynthesis in lodgepole pine saplings infected with Ceratocystis claviger-aor treated with carbohydrate elicitors.Plant Physiol.85,1123-1128.
(11) Lee,Y.S.,Kim,Y.H.,Kim,S.B.(2005).Changes in the respiration ,growth,and vitamins C content of Soybean sprouts in response to chitosan of different molecular Weights.HortScience. 40 1333-1335.
(12) Chibu,H.,Shibayama,H.,Arima,S.(2002).Effects of chitosan application on the Shoot growth of rice and soybean.Jpn.J.Crop Sci.71,206-211.
(13) Khan,W.,Prithiviraj,B.,Smith,D.L.(2002).Effects of foliar application of chitin app- Lication and chitosan oligosaccharides on photosynthesis of maize and soyb-ean.Photosynth.Res.40,621-624.
(14) Yen,M.T.,Mau,J.L.(2007).Selected physical properties of chitin prepared from Shiitake Stipes.Food Sci.Technol.40,558-563.
(15) White,R.E.(2006).Principles and Practice of Soil Science:The soil as a Natural Resource,4th ed.,Blackwell:Oxford,UK.
(16) Spiegel,Y.,Kafkafi,U.,Pressman,E.(1988).Evaluation of a protein –chitin derivat-Ive of crustacean shells as a slow –release nitrogen fertilizer on chinese ca- bbage .J.Hortic.Sci.63,621-628.
(17) Roberts ,P.,Jones,D.L.(2012).Microbial and Plant uptake of free amino sugars In grassland soils .Soil Biochem.49,139-149.
(18) Parada, R.Y., Murakami, S., Shimomura, N., Egusa, M., Otani, H. (2011). Autoclaved Spent substrate of hatakeshimeji Mushroom(Lyophyllum decastes Sing.) And its water extract protect cucumber from anthracnose.J.Crop protection30,443-450.

(19) Cock, J.H. (1985).Cassava :New Potential for a neglected Crop.Wetview Press,Boulderl London.
(20) Thresh , J.M., Fargette, D., Otim-Nape, G.W.(1994).Effects of cassava mosaic gem- inivirus on the yield of cassava.Tropical Science 34,26-42.
(21) Otim-Nape, G.W., Thresh, J.M., Fargette,D.(1996).Bemisia tabacci and cassava Mosaic virus disease in Africa. In: Gerling, D. and Meyer, R.T. (eds)Bemisia 1995:Taxonomy,biology,damage,control and management.Andover,pp.319 -350.
(22) Murashige,T ., Skoog,F.(1962).A revised medium for rapid growth and bioassayWith tobacco tissue culture:Physiol.Plant 15:473-494.
(23) Edje,O.T.,Osiru,D.S.O.(1987).Methods for determing leaf area in some crop Plants.In workshop of cassava-based cropping systems Research IITA, Ibadan, Nigeria 16-19 Nov.1987 pp 237-245.
(24) Steel,G.D. and Torrie, J.H.(1980).Principles and procedures of statistics. Biometrical approach 2nd edition. Mc Grain Hill book company inc. New York 63 pp.