# ELICITORS AND MINERAL CONTENT OF WATER EXTRACT FROM AUTOCLAVED SPENT MUSHROOM (*PLEUROTUS TUBER-REGIUM)* SUBSTRATE AND ITS EFFECT ON CASSAVA INOCULATED WITH AFRICAN CASSAVA MOSAIC VIRUS (ACMV)

**[1*]Okere, S. E. and [2]Ataga, A. E.**

[1]Department of Crop Science and Technology, Federal University of Technology P. M. B. 1526, Owerri, Imo State, Nigeria.
[2]Department of Plant Science and Biotechnology, University of Port Harcourt, P. M. B. 5323, Port Harcourt, Rivers State, Nigeria
*Corresponding author: E-mail: samchezo@yahoo.com; Tel.: 08037565046,

## SUMMARY

**This study investigated the effect of the water extract from spent mushroom *(Pleurotus tuber-regium*) substrate (autoclaved) on the mineral compositions and resistant elicitors and on the growth, yield and management of *African cassava mosaic virus.* The treatments for this investigation comprised of cassava plants treated with autoclaved and unautoclaved water extract from spent mushroom substrate and untreated cassava plants as control. The treatments were applied 4 months after culturing from meristem tip culture and inoculated with viral inoculum 7 days after treatment application and then transplanted. The experiment was laid out in a completely randomized block design with 3 replicates. The mineral compositions and elicitors in the treatments were analyzed using standard procedures. The data generated were subjected to analysis of variance (ANOVA) at (p=0.05). The results revealed significant differences in the values of elicitors: carbohydrate polymers (1.6-33.4%), glycoproteins (0.04-0.90%) and lipid molecules (0.2-18.0g). PTAWESMS had 15.52 mg/100g and 33.64mg/100g higher K and Na than PTWESMS which had 8.94 and 24.21mg/100g for K and Na respectively which were significantly difference. PTAWESMS had 6.3, 39.2 and 76.5 significantly higher number of stems, LAI and LAR than PTWESMS and the control while the other growth parameters were not significantly different. PTWESMS had 8.33 and 35cm significantly higher number of storage roots and storage root length when it was compared with PTAWESMS and the control, while**

**PTWESMS and PTAWESMS had 17.3mm significantly higher root girth than the control. The control had 2.5 severity index at 4WAI significantly higher than PTAWESMS and PTWESMS, while at 28 WAI, the control had 1.37 significantly higher severity index than PTAWESMS and PTWESMS. The result obtained from this investigation clearly revealed that PTWESMS and PTAWESMS significantly reduced ACMD severity when it was compared with the control. It also revealed that unautoclaved water extract SMS performed significantly better than the autoclaved in reducing ACMD severity. However, the reductions in ACMD severity did not translate to higher growth and yield of cassava. Therefore, it is recommended that ACMD management using water extract from *P. tuber-regium* should be complimented with the application of solid SMS in order to achieve the desired results.**

**Keywords:** Mineral, water extract, autoclaved, elicitors, mushroom, African Cassava Mosaic Virus

Certain chemicals and compounds when applied to plants in low concentrations have been reported to activate genetic, biochemical and physical defense mechanisms (13). These compounds which are released by pathogens as they attempt to colonize plants are known as effectors or elicitors (13,16). Elicitors represent a diverse array of bioactive molecules of either pathogen (exogenous elicitors) or of host origin (endogenous elicitors) that can induce defense response in plant tissue. Many elicitors have been described, as simple and complex carbohydrate (6, 8, 26), peptides, proteins and glycoprotein (9, 14), Fatty acids and derivatives (2, 25).

Many reports indicate that the response of plant cells to elicitors consists of a highly defined series of temporally and spatially regulated events. Electrolyte leakage, oxidative burst, production of phytoalexins and PR protein, proteins phosphorylation or dephosphorylation, membrane depolarization and increased biosynthesis of salicylic acid (SA), ethylene and jasmonic acid have been described for leaf tissue treated with non-specific or specific elicitors (23,12, 33). Thus, the resolution of biochemical and molecular processes by which the elicitors exerts its complex changes in cell metabolism is an interesting challenge.

The study conducted by (21) on the effect of autoclaved spent mushroom substrate of "Hatakeshimeji" mushroom and its water extract on Anthracnose disease of cucumber

revealed that cucumber plants were protected systemically from anthracnose by spraying the whole plants with or by dipping the first true leaf in water extract spent mushroom substrate or autoclaved water extract spent mushroom substrate. These results suggest that water-soluble and heat-stable compounds in Spent Mushroom Substrate (SMS) enhance the state of systemic acquired resistance and protect cucumbers from anthracnose disease (21).

It has been proposed that the application of chitin-rich treatments activates plant defenses as they mimic those compounds that plants would normally respond to when attacked by organisms containing chitin. The cell membranes of plants contain chitin-specific receptors, which are known to activate induced defense mechanisms (5). (18) have reported the isolation of a good number of "chitin elicitor binding proteins" (CEBIP) from crops and all these glycoproteins are made up highly conserved extracellular lysin motif (LYSM ) that binds chitin directly when in contact with the plasma membrane in which it is embedded. Plants are known to contain CEBIP and respond to the application of chitin oligomers because they are structural component of many pathogens which are not produced by plants themselves (16). Therefore, chitin is classified as a microbe-associated molecular pattern (MAMP). According to (16) many elicitors of plant defense are MAMPs, such as the protein from bacterial flagella. Others act by mimicking the hormonal signal which act downstream of MAMP detection and as a result "elicitor" and "MAMP" are not interchangeable terms.

The spent mushroom substrate (SMS) is the unutilized substrate and mycelium left after harvesting its fruitbodies. As the mushroom industry is growing steadily, the volume of SMS generated on yearly basis is also increasing. Recently, the industry was faced with the challenges of storing and disposing these SMS whose solution is to explore and utilize new SMS applications. According to (27), the carbohydrates, glycoprotein and lipid elicitors that induce defense mechanism in plants are released from the mycelia of fungal pathogen. Once they are recognized by plants many of them develop an enhanced resistance to further pathogen attack including the uninoculated organs. This type of induced resistance is called systemic acquired resistance (SAR) (7,30). Therefore, the application of SMS to plants may be useful in the management of plant diseases. The potential role of SMS in disease management has not received

adequate attention. However, in the few researches carried out in this direction emphasis has been on the exploitation of the antibiotic-producing microorganisms in SMS and the application of SMS as compost (4,32,31).

## MATERIALS AND METHODS

### Study location / experimental layout

The research was conducted at University of Port Harcourt Faculty of Agriculture, Teaching and Research Farm Choba, Rivers State, Nigeria on a 14mx5m plot size, the size of each experimental plot was 2mx2m.Randomized complete block design was used for this investigation with three treatments replicated thrice.

### Source of planting materials

The cassava plantlets of variety TMS 98/0505 used for this investigation were generated through meristem tip culture collected from the Eastern Farm, National Root Crop Research Institute, Umudike Abia State and were excised and aseptically cultured at the tissue culture laboratory for viral elimination.

### Preparation of water extract from spent 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, Nigeria, after four months of fruit body production and was used immediately for water extract preparation. 200g of *P. tuber-regium* Spent mushroom substrate was homogenized in a blender with 150cl of distilled water (DW) for two minutes at 1500 rpm according to the procedure described by (21). The homogenate was filtered through two layers of calico. Half of the filtrate was used as water extract from spent mushroom substrate (WESMS) while the other was autoclaved and used as autoclaved water extract spent mushroom substrate (AWESMS). The water extract (PTWESMS) and the autoclaved water extract (PTAWESMS) from *P. tuber-regium* SMS including the control represents the treatments for this investigation. They were both sprayed profusely on the cassava plants after four months of culturing with a hand sprayer.

### Viral inoculum preparation / Inoculation

Young cassava leaves with symptoms of ACMD collected from the Farms within the University of Port Harcourt were used for the preparation of viral innoculum.10 grams of infected tissues were grinded with 100 ml buffer (0.01M) Phosphate buffer pH

7.0. in a sterilized mortar. Celite was added to the inoculums as an abrasive to create wounds on cassava leaves thereby increasing chances of infection. Sodium diethyldithiocarbamate (DIECA) in the concentration range of 0.01-0.1M was added to stabilize the inoculum. The plants were inoculated mechanically five days after treatment application by gently robbing the inoculum on the leaf surface with a cotton swab in the inoculation chamber, covered with black polyethylene bags to increase the rate of symptom expression.

**Transplanting/Post planting operations**

The plants were planted flat at a planting distance of 1x1m with 4 plants/plot, weeding and other basic agronomic practices were carried out regularly. Harvesting was carried out after nine months of transplanting after which growth and yield parameters were taken.

**Determination of the minerals, resistance elicitors in the PTWESMS and PTAWESMS**

The mineral composition of the treatments was determined using atomic spectrophotometer at the Soil Science Department, Federal University of Technology, Owerri in conjunction with the Agronomy Department University of Ibadan, Oyo State, Nigeria.

The elicitors were determined using a spectronic 21D digital spectrophotometer. The presence of glycoprotein was determined at a wavelength of 495nm and calculated using the formula

$$\frac{\text{Absorbance of sample x gradient factor x dilution factor if any x 100}}{\text{Weight of sample taken for protein glycosylation}}$$

The total soluble carbohydrate was determined at a wavelength of 620nm. The total soluble carbohydrate was then estimated using the standard curve of Glucose according to (22).

The lipid extraction was carried out at the Biochemistry Laboratory, Federal University of Technology Owerri Imo State Nigeria. The procedure is also outlined below: two gram each of the sample was soaked in 50ml of distilled water for 24 hours. The solution was sieved using muslin cloth and 50 ml of ethanol was added while the combined solution was transferred into a separating funnel positioned with retort stand. Also 50 ml of petroleum ether was added into the separating funnel and allowed to stand for about five minutes. The waste was removed by opening the tap. The residue containing the lipids

was transferred to a pre-dried and weighed 50 ml beaker. The pre- dried and weighed beaker with the content were oven dried between (40- $70^0$ C) for a few minutes ($W_1$). The beaker and content were allowed to cool and re-weighed as $W_2$. The quantity of lipid (oil) extracted was calculated as follows: $W_2 - W_1$.

**Data Collection**

For the agronomic evaluation, the following data were taken every four weeks: Plant height (cm) was determined using a meter rule, number of leaves and number of stems were determined by counting. The stem diameter (mm) were evaluated using a Vernier calipers and leaf area ($cm^2$) as described by (10). Crop growth rate (CGR) C = ULR X LAR, where ULR is the unit leaf rate and LAR is leaf area ratio, leaf area index (LAI) $L=L_A/P$ where $L_A$ is the total leaf area, P is the ground area, leaf area ratio (LAR) $F= L_A/W$ where $L_A$ is the total leaf area and W is whole plant dry biomass, leaf weight ratio (LWR) = $L_W/W$ , Where $L_W$ is total leaf dry weight, W is the total dry biomass weight, unit leaf rate (= net assimilation rate) ULR (=NAR) $E_A=1/L_A$. dW/dT where $L_A$ is the total leaf area, W is the total dry biomass weight, and T is the duration of the plant.

**Disease Severity Index**

Cassava mosaic disease symptom development on new emerging leaves was assessed weekly. The symptoms were recorded from 1-28 weeks after inoculation (WAI). Disease symptom severity on fully expanded leaves was recorded on a scale of 0-4 as described by (35) where 0 = no symptom, 1= mild mosaic, 2 = yellow mosaic, malformation, 5-10% size reduction 3= severe mosaic, distortion, up to 50% size reduction, 4 = severe mosaic, severe distortion, leaf reduced to veins with 50-80% size reduction.

**Data Analysis**

The data generated from this investigation were analyzed using analysis of variance (ANOVA) while means were separated using Fisher's least significant difference.

**RESULTS**

**Resistance elicitors**

The result of the evaluation of resistance elicitors is presented in Table 1. The results obtained from the evaluation of the different elicitors in the spent mushroom substrate revealed that the values obtained for carbohydrate, glycoprotein and lipids molecules in PTAWESMS were 31.8%, 0.86% and 17.8g significantly higher than the values observed for PTWESMS respectively

**Table 1:** Evaluation of the elicitors in the autoclaved and unautoclaved water extract SMS

| Treatment | Carbohydrate polymers (%) | Glycoproteins (%) | Lipid molecules (g) |
|---|---|---|---|
| PTWESMS | 1.6[b] | 0.04[b] | 0.2[b] |
| PTAWESMS | 33.4[a] | 0.9[a] | 18.0[a] |
| LSD (p=0.05) | 0.288 | 0.038 | 0.67 |

Means values within the same column with the same superscript letter do not differ.

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

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

**Evaluation of the mineral compositions in PTWESMS and PTAWESMS**

The result obtained from the evaluation of the mineral compositions is presented in Table 2. The values of nitrogen, phosphorus, magnesium and calcium obtained from PTWESMS were 0.44, 17, 2.3, and 14.49 mg higher than the values obtained from PTAWESMS respectively were not significantly different. However, the values of potassium and sodium obtained from PTAWESMS were 6.58 and 9.43mg significantly higher than the values obtained from PTWESMS respectively.

**Table 2:** Mineral compositions of the autoclaved and unautoclaved water extract SMS

| Parameter | PTWESMS | PTAWESMS | LSD (P=0.05) |
|---|---|---|---|
| Nitrogen (mg/100g) | 3.43 | 2.99 | NS |
| Phosphorus (mg/100g) | 204.8 | 187.8 | NS |
| Potassium(mg/100g) | 8.94[b] | 15.52[a] | 0.817 |
| Sodium (mg/100g) | 24.21[b] | 33.64[a] | 8.20 |
| Magnesium (mg/100g) | 32.20 | 29.90 | NS |
| Calcium (mg/100g) | 105.5 | 91.31 | NS |

Mean values within the same row with the same superscript letter do not differ.

NS- Not significant

PTWESMS = *P. tuber-regium* water extract spent mushroom substrate.

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

**Effect of the PTAWESMS and PTWESMS on the growth attributes of cassava**

The results obtained from the effect autoclaved and unautoclaved water extract *P tuber-regium* SMS application on the growth and growth attributes of cassava are presented in Table 3. The control experimental plants were 1.1 and 2.9 cm taller than PTWESMS and PTAWESMS treated plants, respectively while PTWESMS treated plants were 1.8 cm taller than PTAWESMS plants. PTWESMS plants had 0.9 and 11.7 higher number of leaves than PTAWESMS and the control, respectively. However, PTAWESMS had more (10.8) leaves than the control. PTWESMS plants had 0.1 and 0.2mm thicker stems than PTAWESMS and the control, respectively while PTAWESMS treated plants had 0.1mm thicker stems than the control. The control experimental plants had 944 and 2692 $cm^2$ higher total leaf area than PTAWESMS and PTWESMS, respectively while PTWESMS plants had 1748 $cm^2$ less total leaf area than PTAWESMS treated plants. Also, the control had less (20.44) unit leaf rate than PTWESMS treated plants but higher (10.76) unit leaf rate than PTAWESMS treated plants. Again, PTWESMS treated plants had higher (1048 $x10^{-6}$) CGR than the control but, less CGR (300 $x10^{-6}$) than PTAWESMS treated plants. PTAWESMS treated plants had 0.04 higher leaf weight ratio than the control but 0.163 less leaf weight ratio when it was compared with the PTWESMS treated plants. These parameters were not significantly different. However, PTWESMS treated plants had higher (1.5) number of stems but, less (0.8) number of stems than the PTAWESMS treated plants. Also, PTWESMS treated plants had higher (11.2) LAI than the control but, less (1.7) LAI than PTAWESMS plants. Similarly, PTWESMS plants had higher (19.5) LAR than the control but, less (30.5) LAR than PTAWESMS. These values were highly significantly different.

**Effects of the autoclaved and unautoclaved water extract SMS on the yield of cassava**

The result obtained from the evaluation of the effect of autoclaved and unautoclaved water extract SMS application on the yield and yield attributes of cassava is presented in Table 4. The result presented revealed that PTAWESMS plants 1.03 more storage roots than the control but, less (2.0) number of storage roots when compared with PTWESMS plants. Also, it had 7.0cm longer storage roots than the control and 17 cm

shorter storage roots than PTWESMS treated plants. Both PTWESMS and PTAWESMS treated plants had 4.6 mm larger root girth than the control plants.

**Table 3:** Effect of the Water Extract SMS on the Growth and Growth Attributes of Cassava

| Parameter | Control | PTWESMS | PTAWESMS | LSD (P=0.05) |
|---|---|---|---|---|
| Plant height (cm) | 55.3 | 54.2 | 52.4 | NS |
| Number of leaves | 83.0 | 94.7 | 93.8 | NS |
| Number of stems | $4.0^{b}$ | $5.5^{a}$ | $6.3^{a}$ | 1.22 |
| Stem diameter (mm) | 1.6 | 1.8 | 1.7 | NS |
| Total leaf area ($cm^2$) | 10422.3 | 7730.3 | 9478.3 | NS |
| Leaf area index | $26.3^{b}$ | $37.5^{a}$ | $39.2^{a}$ | 9.87 |
| Leaf area ratio | $26.5^{b}$ | $46.0^{a,b}$ | $76.5^{a}$ | 30.21 |
| Unit leaf rate | 56.46 | 76.9 | 45.7 | NS |
| Crop growth rate($x10^{-6}$) | 2150.9 | 3198.9 | 3498.9 | NS |
| Leaf weight ratio | 0.795 | 0.998 | 0.835 | NS |

Means values within the same row with the same superscript letter did not differ significantly.

NS = Not significant. PTWESMS = *P. tuber-regium* water extract spent mushroom substrate.

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

**Table 4:** Effect of the autoclaved water extract on the yield and yield attributes of cassava

| Parameter | Control | PTWESMS | PTAWESMS | LSD(p=0.05) |
|---|---|---|---|---|
| Harvest index (%) | 34.0 | 49.0 | 46.0 | NS |
| Number of roots | $5.3^{b}$ | $8.33^{a}$, | $6.33^{a,b}$ | 2.318 |
| Root length (cm) | $11^{c}$ | $35^{a}$ | $18.0^{b}$ | 4.1 |
| Fresh root weight (tonha$^{-1}$) | 13.9 | 20.3 | 15.0 | NS |
| Fresh biomass weight(tonha$^{-1}$) | 25.3 | 41.7 | 29.3 | NS |
| Whole shoot weight(tonha$^{-1}$) | 12.3 | 21.3 | 14.3 | NS |
| Stem weight (tonha$^{-1}$) | 6.7 | 13.3 | 10.0 | NS |
| Leaf weight (tonha$^{-1}$) | 5.7 | 8.0 | 4.33 | NS |
| Root girth(mm) | $12.7^{b}$ | $17.3^{a}$ | $17.3^{a}$ | 4.5 |
| Whole dry biomas (tonha$^{-1}$) | 0.475 | 0.789 | 0.556 | NS |

Means values within the same row with the same superscript letter did not differ significantly.

NS = Not significant. PTWESMS= *P. tuber-regium* water extract spent mushroom substrate.

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

These values were highly significantly different. However, the control had 1.1 and 6.4 tonsha$^{-1}$ less fresh root weight when compared with PTAWESMS and PTWESMS, respectively while PTWESMS plants had 5.3 tonsha$^{-1}$ higher fresh root weight than PTAWESMS plants. PTAWESMS treated plants recorded 4 tonsha$^{-1}$ higher fresh biomass weight than the control but, 12.4 tonsha$^{-1}$ less fresh biomass than the PTWESMS treated plants. Also, PTAWESMS treated plants had 2

tonsha$^{-1}$ higher whole shoot weight than the control but, 7 tonsha$^{-1}$ less whole shoot weight than PTWESMS plants while the control had 3.3 and 6.6 tonsha$^{-1}$ less stem weight than PTAWESMS and PTWESMS treated plants, respectively. PTWESMS plants produced 2.3 and 3.67 tonsha$^{-1}$ higher than the control and the PTAWESMS treated plants while the control had 1.37 tonsha$^{-1}$ higher stem weight than the PTAWESMS treated plants. The control had 2.3 tonsha$^{-1}$ less leaf weight than the PTWESMS plants but had 1.37 tonsha$^{-1}$ higher leaf weight than the PTAWESMS treated plants. However, PTWESMS treated plants had 0.314 and 0.233 tonsha$^{-1}$ higher whole dry biomass than the control and the PTAWESMS treated plants, respectively while PTAWESMS treated plants had 0.081 tonsha$^{-1}$ higher whole dry biomass than the control. These values were not significantly different.

**Effect of the treatments on African cassava mosaic severity**

The result obtained from the evaluation of the effect of the treatments on the African cassava mosaic disease severity is presented in Table 5. From the result presented the control experimental plants had 0.39 and 0.34 higher severity index than PTWESMS and PTAWESMA at 4WAI, and 0.84 and 1.18 higher severity than PTWESMS and PTAWESMA at 8WAI. At 12 and 16 WAI the control had 1.06, 1.03 and 0.33, 0.41 higher severity than PTWESMS and PTAWESMS respectively. However, the control had 0.44 and 0.37 and 0.35 and 0.18 higher severity than PTWESMS and PTAWESMS respectively at 20 and 24 WAI. While at 28 WAI the control had 0.87 higher severity index than PTWESMS and PTAWESMS. These values were highly significantly different.

**Table 5:** Effects of the autoclaved and unautoclaved water extract on ACMD severity

| Treatment | 4 WAI | 8WAI | 12 WAI | 16WAI | 20WAI | 24 WAI | 28 WAI |
|---|---|---|---|---|---|---|---|
| PTWESMS | 2.11 | 1.06$^{b}$ | 0.44$^{c}$ | 1.0$^{b}$ | 0.58$^{b}$ | 0.50$^{b}$ | 0.50$^{b}$ |
| PTAWESMS | 2.16 | 0.72$^{b}$ | 0.47$^{c}$ | 0.92$^{b}$ | 0.67$^{b}$ | 0.67$^{b}$ | 0.50$^{b}$ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Control | 2.50 | 1.90[a] | 1.50[a] | 1.33[a] | 1.22[a] | 1.25[a] | 1.37[a] |
| LSD (p=0.05) | NS | 0.47 | 0.27 | 0.30 | 0.26 | 0.36 | 0.37 |

Means values within the same column with the same superscript letter did not differ significantly.

NS = Not significant. PTWESMS = *P. tuber-regium* water extract spent mushroom substrate.

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

WAI= weeks after inoculation

## DISCUSSION

The result presented revealed that autoclaving significantly improved the quantities of the resistance elicitors present in the water extract of *P. tuber-regium* water extract. This is similar to the findings of (21) who reported that SMS contained water-soluble and heat-stable compounds (elicitors) that can enhance the state of systemic acquired resistance and protect plants from diseases. Also autoclaving significantly increased the quantities of potassium and sodium in the water extract from *P. tuber-regium*, suggesting that these minerals are water soluble and heat stable unlike the other minerals evaluated in this study.

Also, the result obtained from this investigation further revealed that autoclaved water extract from *P. tuber-regium* improved the growth attributes of cassava especially the number of leaves, leaf area index and leaf area ratio. This could be attributed to the high levels of the elicitors and minerals present in the water extract. Also, the result revealed that autoclaved water extracts significantly improved the number of storage roots, root length and girth which is in agreement with the findings of (29) who reported that Chinese cabbage treated with chitin–based products grew faster than plants treated with standard mineral fertilizer.

The results further suggest that the control plants with higher severity index had slightly lower growth and yield attributes than PTWESMS and PTAWESMS treated plants. This could be attributed to increased respiration rates required to provide energy for resistance to ACMD which was responsible for the reduced growth and yield attributes observed in this study when compared with the control. This result is in agreement

with the findings of (28) and (34). The result also agrees with the findings of (15) who reported that resistance includes allocation costs arising from the diversion of metabolites and energy from growth and other processes toward defense.

The result of the effect of the treatments on African cassava mosaic disease severity (presented Table 5) is in line with the findings of (17) who reported that application of chitin and its derivative has been used to control viral diseases in plants through the disruption of the transfer of viral particle and the induction of the hypersensitivity responses ( 3, 11, and 24). This result is also in agreement with the findings of (1) who demonstrated that application of water extract from spent mushroom substrate induced systemic resistance on tomato by significantly enhancing the expression of the PR-la and Gle A genes in WESMS- treated tomato plants when compared to water-treated control plants.

## CONCLUSION

The result obtained from this study revealed that autoclaving significantly influenced the levels of the resistance elicitors in PTAWESMS. Application of autoclaved (PTAWESMS) and unautoclaved (PTWESMS) water extract from *P. tuber–regium* was found to significantly reduce ACMD severity when it was compared with the control. Interestingly this did not significantly translate to higher yield. Again, the result also revealed that plants are naturally endowed to resist pathogen attack but this however needs to be activated or enhanced. Therefore, it is recommended that application of water extract SMS in the management of ACMD should be incorporated with the application of the necessary plant nutrients in order to achieve the desired yield increases.

## AKNOWLEDGEMENT

The authors are grateful to TETFUND for funding this research under Academic Staff Training and Development.

## REFERENCES

**1. Amin Kwak, Kyeong, Jin Min, Sang Yero lee, and Hee Wankang. 2015**. Water extract from SMS of Hericiunerianceus suppresses bacterial wilt Disease of Tomato *Mycobiology*, 43 (3) 311-318.

**2. Bostock, R.M.; Laine, R.A.; Kuc, J.A. 1982.** Factors affecting the elicitation of sesquiterpenoid phytoalexin accumulation by eicosapentaeonic acids in Potato. *Plant Physiology*. 70, 1417-1424.

**3. Chirkov, S. N. 2002.** The Antiviral Activity of Chitosan (review). *Applied Biochemistry and Microbiology.* 38,1-8.

**4.Cronin, M. J., Yohalem, D. S., Harris, R.F., and Andrews, J.H. 1996.** Putative Mechanism and Dynamics of Inhibition of the Apple Scab Pathogen *Venturi inequalis* Compost Extracts. *Soil Biology and Biochemistry*, 28,1241-1249.

**5. Day, R.B.; Okada, M.; Ito, Y.; Tsukada, K.; Zaghouani, H.; Shibuya, N.; Stacey, G. 2001.** Binding site for chitin oligosaccharides in the soybean plasma membrane. *Plant Physiology 26*, 1162–1173.

**6. Dixon, R.A.; Dey, P.; Lamb,C.J. 1983.** Phytoalexin; enzymology and molecular biology. *Advances in Enzymology and Related Areas of Molecular Biology* 55, 1-135.

**7. Durrant, W.E., and Dong, X. 2004.** Systemic Acquired Resistance. *Annual Review of Phytopathology*. 42, 185-209.

**8. Ebel, J. 1986**. Phytoalexin synthesis: The biochemical analysis of the induction process.*Annual Review of Phytopathology*. 24, 235-264.

**9. Ebel, J.; Cosio, E.G.; 1994.** Elicitors of plant defense response. *International Review of Cytology* 148, 1-36.

**10. Edje, O.T., and Osiru, D.S.O. 1987.** Methods for Determining Leaf Area in Some Crop Plants. In workshop of Cassava-Based Cropping Systems Research IITA, Ibadan, Nigeria 16-19Nov.1987 pp. 237-245.

**11. Faoro, F., Sant, S., Iriti, M., and Appiano, A. 2001**. Chitosan-elicited Resistance to Plant Viruses: A Histochemical and Cytochemical Study. In Chitin Enzymology; Muzzarelli, R.A.A., Ed., Atec: Grottammare, Italy, pp. 57- 62.

**12. Felix, G.; Grosskope, D.G.; Regenass, M.;Basse, C.W.; Boller, T. 1991.** Elicitors-induced ethylene biosynthesis in tomato cells. *Plant Physiology* 97, 19-25.

**13. Garcia-Brugger, A., Lamotte, O., Vandella, E., Bourque, S., Lecourieux, D., Poinssot, B., Wendehenne, D., Pugin, A. 2006.** Early Signaling Events Induced by Elicitors of Plant Defenses. *Molecular Plant-Microbe Interactions*. 19, 711-724.

**14. Grosskope, D.G.; Felix, G.; Boller, T.1991.** A yeast derived glycopeptide elicitor and chitosan ordigitonin differentially induce ethylene biosynthesis, Phenylalanine ammonialyase and callose formation in suspension cultured tomato cells. *Journal of Plant Physiology* 138, 741-746.

**15. Heil, M., and Baldwin, I.T. 2002.** Fitness Costs of Induced Resistance: Emerging Experimental Support for a Slippery Concept. *Trends in Plant Sciences*.**7**:61-67.

**16.Kaku, H., Nishizawa, Y., Minami, N.I., Tomiyama, C.A., Dohmae, N., Takio, K., Manami, Shibuya, N. 2006.** Plant Cells Recognize Chitin Fragment for Defense Signaling through a Plasma E. Membrane Receptor. *Proceedings*

*of the National Academy of Sciences of USA.* 103, 11086-11091.

**17. Kulikov, S. N., Chirkov, S. N., M'ina, A. V., Lopatin, S. A., and Varlamov, V.P. 2006.** Effect of the Molecular Weight of Chitosan on its Antiviral activity in Plants. *Prikladnaia Biokhimiia Mikrobiologiia* 42, 224-228.

**18. Miya, A.; Albert, P.; Shinya, T.; Desaki, Y.; Ichimura, K.; Shirasu, K.; Narusaka, Y.; Kawakami, N.; Kaku, H.; Shibuya, N. 2007.** CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. *Proceedings of the National Academy of Sciences of USA*, 104, 19613–19618.

**19.Murashige, T., and Skoog, F. 1962.** A Revised Medium for Rapid Growth and Bioassay with Tobacco Tissue Culture: *Physiologia Plantarum* 15: 473-494.

**20. Okere, S.E. 2017**. Studies on the Microbial Metabolites in Spent Mushroom Substrate and the Management of Cassava Mosaic Disease. A Ph.D. Thesis Submitted to the Post Graduate School University of Port Harcourt, Rivers state, Nigeria.

**21. Parada, R.Y., Murakami, S., Shimomura, N., Egusa, M., and Otani, H. 2011.** Autoclaved Spent Substrate of Hatakeshimeji Mushroom (Lyophyllumdecastes Sing.) and its Water Extract Protect Cucumber from Anthracnose. *Journal of Crop Protection* 30, 443-450.

**22. Pearson, D., Melon, H. K., and Ronald, S. 1976.** Chemical Analysis of Food, 8th. Edition. Churchill Livingstone pp. 5-63.

**23. Peever, T.L.; Higgins, V.J. 1989.** Electrolyte leakage, lipoxygenase and lipid peroxidation induced in tomato leaf tissue by specific and non-specific elicitors from *Cladosporium fulvum. Physiology* 90, 867- 875.

**24. Pospieszny, H., Chirkov, S.,and Atabekov, J. 1991.** Induction of Antiviral Resistance in Plants by Chitosan. *Plant Science* 79, 63-68.

**25. Preisig, C.L.; Kuc, J.A. 1985.** Arachidonic acid–related elicitors of the hypersensitive response in potato and enhancement of their activities by glucans from Phytophthora infestance (Mont) deBary. *Archives of Biochemistry and Biophysics.* 236, 379-389.

**26. Sharp, J.A.; McNeil, M.; Albershem, P. 1984.** The primary structure of one elicitor active and seven elicitor – inactive hexa (B-D-glucopyranosyl)- D- glucitols isolated fromthe mycelial walls of *Phytophthora megasperma* f.sp. *glycinea*. *Journal of Biological Chemistry*. 259, 11321-11326.

**27. Shibuya, N., and Minami, E. 2001.** Oligosaccharide Signaling for Defense Responses in plants *Physiological and Molecular Plant Pathology*. 59, 223-233.

**28. Smedegaard- Peters, V., and Stolon, O. 1981.** Effects of energy-requiring defensesreaction on yield and Grain quality in a powdery

mildew –resistant cultivar.*Phytopathology* 71:396-399.

**29. Spiegel,Y., Kafkafi, U., Pressman, E. 1988.** Evaluation of a Protein -Chitin Derivative ofCrustacean Shells as a slow – Release Nitrogen Fertilizer on Chinese cabbage. *Journal of Horticultural Sciences* 63, 621-628.

**30. Vallad, G. E., and Goodman, R.M. 2004**. Systemic Acquired Resistance and Induced Systemic Resistance in Conventional Agriculture. *Crop Sciences*. 44, 1920-1934.

**31.Viji, G., Uddin, W., Romaine, C.P. 2003.** Suppression of Gray Leaf Spot (blast) of Perennial Rye Grass Turf by *Pseudomonas aeruginosa* from Spent Mushroom Substrate, *Biological Control* 26, 233-243.

**32. Yohalem, D.S., Harris, R.F., Andrews, J.H. 1994.** Aqueous Extracts of Spent Mushroom Substrate for Foliar Disease Control. *Compost Science & Utilization* 2, 67-74.

**33. Yu, L.M.; Lamb, C.J.; Dixon, R.A. 1993.** Purification and biochemical characterization of proteins which bind to the H-Box Ciselenine implicated in transcriptional activities of plant defense genes. *The Plant Journal*. 3, 805-816.

**34. Zavala, J.A., Patankan, A.G., Gase, K., Hui, D.Q., and Baldwin, I.T. 2004**. Manipulation of endogenous trypsin proteinase inhibitor production in Nicotianaattenuata demonstrates their function as anti- herbivore defense. *Plant Physiology*. 134; 1181-1190.

**35. Zhang, P., Vanderschuren, H., Futterer, J., Gruissem, W. 2005.** Resistance to Cassava Mosaic Disease in Transgenic Cassava Expressing Antsense RNAs targeting virusreplication genes. *Plant Biotechnology Journal* 3:385-397.