

Effects of autoclaving (*Pleurotus osteratus*) spent mushroom substrate water extract on the minerals, elicitors, cassava yield, and the management of African cassava mosaic virus

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ABSTRACT

Farmers all over the world spend millions of dollars to partially control plant diseases that attack their crops using agrochemicals that pollute the environment. Plant diseases reduce crop yield, quality, and shelf life. Enhancing the inherent ability of plants to resist diseases using elicitors and minerals is one of the neglected, sustainable, low-cost, and environmentally friendly approaches to ensure the continued supply of food and fiber. Therefore the aim of this paper is to evaluate the composition of the elicitors and minerals in autoclaved and unautoclaved water extract of *Pleurotus osteratus* spent mushroom substrate and the effects of its application on the management of African cassava mosaic virus (ACMV), growth, and yield of cassava. The treatments for this investigation comprised *Pleurotus osteratus* water extract spent mushroom substrate (POWESMS), *Pleurotus osteratus* autoclaved spent mushroom substrate (POAWESMS), and untreated cassava plants as control. The mineral compositions and the elicitors in the treatments were evaluated using standard procedures. The data generated were subjected to analysis of variance (ANOVA) at (p=0.05). The results obtained from this investigation revealed significant differences in the values of resistant elicitors and minerals. Significant differences were also recorded for the growth, yield parameters, and disease severity evaluated at 4 weeks intervals. The results showed that unautoclaved water extract performed significantly better than the autoclaved in reducing African cassava mosaic disease (ACMD) severity which did not translate to higher crop yield.

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1. INTRODUCTION

Certain compounds and chemicals when applied to plants in low concentrations can activate genetic, physical, and biochemical defense mechanisms [1] These compounds which are known as elicitors/effectors also include certain compounds which are released by pathogens in their effort to colonize plants [1], [2]. Chitin based substances such as spent mushroom substrate have been shown in a number of studies to be potent elicitors of plant defense which in turn have allowed plants to resist or tolerate a range of diseases

including viruses [3]. These findings revealed that chitin-based products hold promise commercially to protect crops in large scale agriculture.

Recently the control of plant diseases has become increasing difficult despite the merits brought by the application of pesticides to agricultural development. The excessive use has impacted negatively on the environment and human health, as a result there is a great demand for residue-free fresh agricultural produce globally. Currently there is worldwide trend to explore new alternatives that controls pre and post harvest pathogenic diseases with emphasis on the methods that avoid negative side effect on human health.

The biodegradable nature of compounds obtained from plants and animal products have recently been of interest to plant pathologist. Therefore, the mycelia of mushrooms that are prevalent in spent mushroom substrate (SMS) are abundant sources of elicitors which are nontoxic, bioactive agents has become a useful appreciated compound due to its effect on the elicitation of defense mechanisms in plants [4], [5]. Examples of such elicitors are carbohydrate and protein elicitors that induce defense mechanism in plants are released from the mycelia of fungal pathogens [6]. Once the plants recognize these elicitors, many plants develop an enhanced resistance to further pathogen attack also in the uninoculated organs. This type of induced resistance is called systemic acquired resistance (SAR) [7]-[11]. The application of SMS to plants may be useful for the control of plant diseases. However, the study of the potential role of SMS in disease control has not received adequate attention. In the few studies that have addressed this topic, the emphasis has been on exploiting the antibiotic-producing microorganisms in SMS by applying SMS as compost [12]-[15].

Cassava mosaic disease is one of the most severe and widespread diseases that affect cassava plant as it reduces production of the crop in sub-Saharan Africa. The symptoms of the disease include a variety of foliar symptoms such as mosaic, mottling, misshapen and twisted leaflets, and general reduction in leaf and plant size. Infected plants produce few or no tubers depending on the severity and the age of the plant at the time of infection.

Warburg [16] reported that the first account of ACMD came from the Usambaras mountain range in Northeast Tanzania in 1894 while the disease was named as “Krauselkrankheit”, a German word that translated to ‘rippling/crinkling illness’ which described symptoms observed on infected plants. Chant [17] reported that viruses were originally believed to be the causal agent of ACMD, and its transmission occurred through the whiteflies, the viral status was not proven immediately not until the 1970s, when small quasi-isometric, germinate particles were found in symptomatic host tissues [18].

However, it would not be until many years later that the virus, African cassava mosaic virus (ACMV) was molecularly characterized [19] and Koch’s postulate fulfilled [20]. CMBs induce in cassava and the experimental host *Nicotiana benthamiana* Domin several cytological and morphological modification whose symptoms and accompanying cellular modifications depend on whether they are infected with a single virus or a concurrent infection of two or more CMBs resulting in synergistic interactions [21]. ACMD symptoms for plants infected with a single virus is characterized by a range of green to yellow mosaic in infected leaves coupled with leaf distortions. These alterations in the morphology of cassava often result in low tuber yield while significant storage root losses can occur even in resistant genotype that show only mild or no foliar symptom [21].

Overall storage root yield loss across sub-Saharan Africa has been estimated to be between 5% and 24% annually which is equivalent to 12-23 million tons or an annual loss of US \$1.2 to \$2.3 billion [22]. Therefore, the main objective of this study was to determine the effect of autoclaved spent mushroom substrate water extract on the minerals, elicitors, cassava yield and the management of African cassava mosaic virus.

2. RESEARCH METHOD

2.1. Experimental site/layout

This experiment was located at Faculty of Agriculture, Teaching and Research farm University of Port Harcourt Choba Rivers State Nigeria on a 14×5 m plot size, each plot measured 2×2 m with 8 plants per plot. Randomized complete block design was used for this investigation with 3 treatments replicated 3 times.

2.2. Source of planting materials

TMS 98/0505 used for this experiment were generated through meristem tip culture collected from the National Root Crop Research Institute, Umuahia Abia State and were excised and aseptically cultured using M & S Medium [23] for viral elimination.

2.3. Preparation of water extract from spent mushroom substrate

The spent mushroom compost/substrate was obtained from Dilomat Farms located at Rivers State University of Science and Technology, Port Harcourt, Rivers State Nigeria, after 4 months of fruit body production and was immediately used for the preparation of water extract according to the procedure described by Okere and Ataga [25]. Part of the filtrate was used as water extract from spent mushroom substrate (WESMS) while the remaining part was autoclaved as autoclaved water extract spent mushroom substrate (AWESMS) for leaf treatment. The *Pleurotus osteratus* water extract spent mushroom substrate (POWESMS) and *Pleurotus osteratus* autoclaved spent mushroom substrate (POAWESMS) from *Pleurotus osteratus* including the controls represents the treatments for this investigation. Both treatments POWESMS and POAWESMS were sprayed profusely on the cassava plants after 4 months of culturing with a hand sprayer.

2.4. Viral inoculum preparation and inoculation

Viral inoculum was prepared according to the procedure described by Okere and Ataga [25] while the plants were inoculated 5 days after application of the treatments mechanically by gently robbing it on the leaf surface with a cotton swab in the inoculation chamber covered with black polythene where temperature and relative humidity were recorded.

2.5. Transplanting/post planting operations

The plants were planted flat at a planting distance of 2×2 m with 8 plants/plot, weeding and other basic agronomical practices were carried out regularly. Harvesting was done after 9 months of transplanting with growth and yield attributes taken.

2.6. Determination of the minerals, compositions, elicitors and lipids in the various treatments

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 both in Nigeria. The elicitors and lipids were determined according to the procedure described by Okere and Ataga [25].

2.7. Data collection

The following agronomic parameters were taken every 4 weeks: number of leaves, plant height (cm), number of internodes, number of stems, number of nodes, stem diameter (mm) and Leaf area (cm²) according to the method described by Edje and Osiru [26], crop growth rate (CGR) $C = ULR \times LAR$, 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) $E_A = 1/L_A \cdot dW/dT$ where L_A is the total leaf area, W is the total dry biomass weight, and T is the duration of the plant.

2.8. Disease severity index

New and emerging cassava leaves were assessed weekly for mosaic symptom development. Data on mosaic symptoms development were taken up to 24 weeks after inoculation (WAI). Disease severity index on fully expanded leaves were recorded on a scale of 0-4 according to the procedure described by [27] as follows: 0-no symptom, 1-faint mosaic, 2-malformation, yellow mosaic, 5-10% reduction in size, 3-distortion, severe mosaic, up to 50% size reduction, 4-severe distortion, severe mosaic, leaf reduced to veins with about 50-80% reduction in leaf size.

3. RESULTS AND ANALYSIS

3.1. Resistance elicitors

The result of the resistance elicitors obtained from the treatments is presented in Table 1. The results obtained from the evaluation of the different elicitors in the spent mushroom substrate revealed that POWESMS contained 1.98% and 0.06% higher carbohydrate and glycoprotein molecules than POAWESMS respectively but 14.9g less lipid molecules than POAWESMS. However, these results were significantly different. This result suggests that autoclaving influenced the quantities of the resistant elicitors present in the water extract spent mushroom substrate due to increased solubility which is in agreement with the findings of [24] who reported that autoclaving increases the levels/quantities of resistant elicitors in the spent mushroom substrate.

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

TREATMENTS	Carbohydrate polymers (%)	Glycoproteins (%)	Lipid molecules (g)
POWESMS	19.32 ^a	0.52 ^a	0.12 ^a
POAWESMS	17.34 ^b	0.46 ^b	15.0 ^b
LSD (p=0.05)	0.288	0.038	0.67

Key:

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

POWESMS- *Pleurotus ostractus* water extract spent mushroom substrate.

POAWESMS- *Pleurotus ostractus* autoclaved water extract spent mushroom substrate.

3.2. Evaluation of autoclaved and unautoclaved mineral compositions of water extract SMS

The result obtained from the evaluation of the mineral compositions of the water extract spent mushroom substrate is presented in Table 2.

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

Parameter	POWESMS	POAWESMS	LSD(p=0.05)
Nitrogen (mg/100g)	3.17	3.40	NS
Phosphorus (mg/100g)	212.1 ^a	171.3 ^b	40.3
Potassium(mg/100g)	17.9 ^a	13.0 ^b	0.817
Sodium (mg/100g)	21.8 ^b	30.2 ^a	8.20
Magnesium(mg/100g)	58.8 ^a	46.0 ^b	10.24
Calcium (mg/100g)	111.4 ^a	75.9 ^b	20.81

Key:

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

NS- Not significant

POWESMS- *Pleurotus ostractus* water extract spent mushroom substrate.

POAWESMS- *Pleurotus ostractus* autoclaved water extract spent mushroom substrate.

POWESMS contained 0.22 mg less nitrogen than POAWESMS which were not significantly different. The result obtained also revealed that POWESMS contained 40.8, 4.9, 12.8, and 35.5 mg higher Phosphorus, Potassium, Magnesium and Calcium than POAWESMS respectively but 8.4 mg less Sodium which were highly significantly different. The result revealed that the nitrogen content of the SMS is in agreement with the findings of [28], [29]. while a non significant difference was recorded among the autoclaved and unautoclaved water extract which suggest that nitrogen may be water soluble and heat stable. The result also revealed that autoclaving influenced the quantities/availability of Phosphorus in the water extract spent mushroom substrate which suggest that they are not heat stable. The water extract from both SMS had the lowest quantities which suggest that Potassium is less water soluble but heat stable. A critical analysis of the results also revealed that lower quantities of Sodium were obtained from both the autoclaved and unautoclaved liquid extract. This suggest that Sodium appeared to be less water soluble and heat stable. Lower quantities of magnesium were obtained from the water extract and autoclaving appeared to affect its availability. as against the water extract. The result also suggest that calcium is water soluble and influenced by heat.

3.3. Effects of the autoclaved and unautoclaved water extract SMS on the growth of cassava

The result obtained from the evaluation of the effect autoclaved and unautoclaved water extract SMS application on the growth attributes of Cassava is presented in Table 3. The control experimental plants were taller than POWESMS and POAWESMS treated plants by 6.1 and 6.5 cm respectively. However, the POWESMS and POAWESMS treated plants had 34.1 and 48.1 less unit leaf area, 5479.6 and 3455.3 cm² less total leaf area and 36.4 and 23.4 less unit leaf rate than the control experimental plots respectively which were also significantly different. POWESMS treated plants had 9.79 and 24.5 higher LAI, 87.6 and 162.6 LAR than POAWESMS treated plants and the control plants respectively which were significantly different. POAWESMS had 15.7 and 18.7 higher number of leaves than POWESMS and the control while POWESMS and POAWESMS had equal number of stem and stem diameter by 1.0 and 0.1 mm less stem number and stem diameter than the control respectively. Again, POWESMS had 6.5×10^{-6} and 1077.7×10^{-6} higher CGR and 0.165 and 0.448 higher LWR than POAWESMS and the control plants respectively which were not significantly different.

Table 3. Effect of the water extract SMS on the growth attributes of cassava

Parameter	Control	POWESMS	POAWESMS	LSD(p=0.05)
Plant height (cm)	55.3 ^a	49.2 ^b	48.8 ^b	5.53
Number of leaves	83.0	86.3	102.0	NS
Number of stems	4.0	5.0	5.0	NS
Stem diameter (mm)	1.63	1.73	1.73	NS
Unit leaf area (cm ²)	149.5 ^a	115.4 ^b	101.4 ^b	16.75
Total leaf area (cm ²)	10422.3 ^a	4942.7 ^b	6967.0 ^b	2279.7
Leaf area index	26.3 ^c	50.8 ^a	41.01 ^a	9.87
Leaf area ratio	26.5 ^c	188.9 ^a	101.3 ^b	30.21
Unit leaf rate	56.46 ^a	20.06 ^b	33.03 ^{a,b}	31.59
Crop growth rate(x10 ⁻⁶)	2150.9	3228.6	3222.13	NS
Leaf weight ratio	0.795	1.243	1.078	NS

key

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

NS- Not significant

POWESMS- *Pleurotus ostractus* water extract spent mushroom substrate.

POAWESMS- *Pleurotus ostractus* autoclaved water extract spent mushroom substrate.

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

The result obtained from the evaluation of the effect autoclaved and unautoclaved water extract SMS application on the yield attributes of Cassava is presented in Table 4.

Table 4. Effect of the autoclaved water extract on the yield attributes of cassava

Parameter	Control	POWESMS	POAWESMS	LSD (p=0.05)
Harvest index (%)	34.0	29.0	49.0	NS
Number of roots	5.3	3.7	4.7	NS
Root length (cm)	11 ^b	27 ^a	24 ^a	4.1
Fresh root weight(ton/ha)	13.9	6.0	11.7	NS
Fresh biomass weight(ton/ha)	25.3	16.3	22.0	NS
Whole shoot weight(ton/ha)	12.3	10.3	10.3	NS
Stem weight (ton/ha)	6.7	8.33	7.67	NS
Leaf weight (ton/ha)	5.7	3.67	4.0	NS
Root girth(mm)	12.7 ^b	12.3 ^b	20.0 ^a	4.5
Whole dry biomass(ton/ha)	0.475	0.313	0.414	NS

Key

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

NS-Not significant

POWESMS-*Pleurotus ostractus* water extract spent mushroom substrate.

POAWESMS-*Pleurotus ostractus* autoclaved water extract spent mushroom substrate.

The result of the storage root length revealed that POWESMS treated plants had 0.33 and 16 cm longer storage root length than POAWESMS and the control plants respectively while POAWESMS treated plants had 7.7 and 7.3 mm longer storage root girth than POWESMS treated plants and the control plants respectively which are highly significantly different. The control plants had 1.6 and 0.6, 7.9 and 2.2, 9.0 and 3.3, 2.0, 2.03 and 1.7 and 0.162 and 0.061 higher number of roots, storage root weight, fresh whole plant biomass, fresh shoot weight, leaf weight and dry biomass than POWESMS and POAWESMS treated plants respectively while POAWESMS had 20 and 15% higher harvest index than POWESMS treated plants and the control plants respectively which were not significantly different. The results obtained from Tables 3 and 4 suggest that the control plants with higher severity index recorded higher growth and yield attributes than POWESMS and POAWESMS treated plants which could be attributed to increased respiration rates required to provide the required energy for resistance against ACMD infection which could have been responsible for the poor growth and yield observed in this study. This result is in agreement with the findings of [30] and [31]. The result also agrees with the findings of [32] who reported that resistance includes allocation costs arising from the diversion of metabolites and the required energy from growth and other metabolic processes toward defense.

3.5. Effect of the treatments on ACMD severity

The result obtained from the evaluation of the effect of the treatments on the African cassava mosaic disease severity is presented in Figure 1. From the figure the Control experimental plants had equal severity index with POAWESMS but lower severity of 0.9 than POWESMS at 4WAI, again it had 0.1 and 0.7, 0.6 and 1.0, 0.4, 0.4 and 0.2, 0.1 and 0.2, 0.2 and 0.9 at 8, 12, 16,20,24 and 28 WAI higher severity index than POAWESMS and POWESMS treated plants respectively which were also highly significantly different. This

result is in line with the findings of [33] who reported that application of chitin and its derivative have been used to control viral diseases in plants through the disruption of the transfer of viral particle and the induction of the hypersensitivity responses [34]-[37]. This result is also in agreement with the findings of [38] in which they demonstrated that application of water extract from spent mushroom substrate induced systemic resistance on tomato by significantly enhancing the expression of the PR-1a and Gle A genes in tomato plants treated water extract spent mushroom substrate (WESMS) when it was compared to the control plants treated with water.

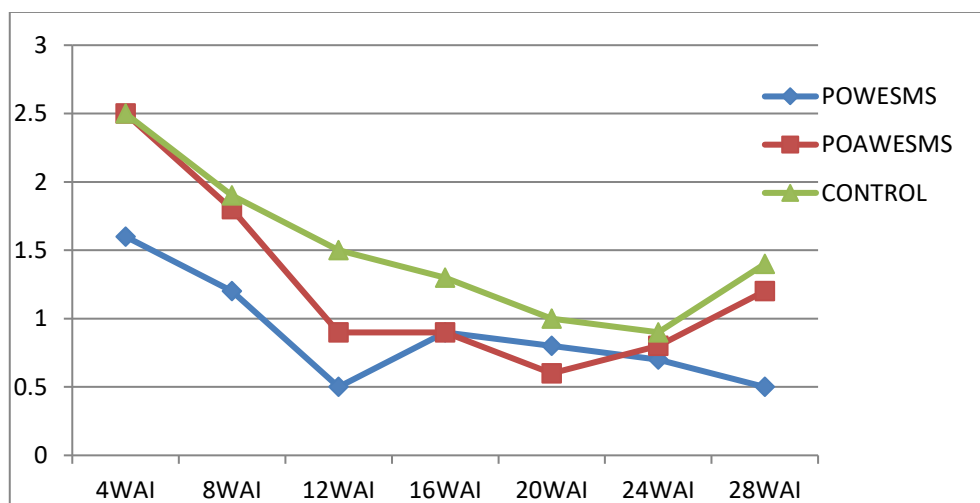


Figure 1. Effect of the treatments on ACMD severity

4. CONCLUSION

The result obtained from this study revealed that autoclaving significantly influenced the levels of the lipid molecules unlike the carbohydrate polymers and the glycoproteins. Application of POWESMS to plants significantly reduced ACMD severity than POAWESMS and the control but interestingly this did not translate to higher yield. Again, the result also revealed that plants are naturally endowed to resist pathogen attack but this however need to be activated or enhanced. Therefore, it is recommended that the application of water extract from spent mushroom substrate (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. However further studies are recommended specially to determine the impart of the treatments on viral concentration molecularly.

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