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VARIETAL RESPONSES TO STORAGE TREATMENT WITH NANOBIOTECHNOLOGY FOR REDUCTION OF POSTHARVEST LOSSES OF FRESH YAM (*DIOSCOREA SPP)* TUBER

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Abstract: Post-harvest food losses of yam constitute a huge source of food insecurity in Africa. A storage experiment was conducted in the Department of Crop Science, University of Nigeria, Nsukka to investigate the responses of three varieties of yam to application of silver nitrate and neem leaf extract (silvermeem) for reduction of postharvest losses of yam tubers in storage. It was a 3 x 4 x 3 factorial laid out in completely randomized design (CRD) experiment. The treatments comprised 3 varieties of yam; *Dioscorea rotundata*, *Dioscorea alata* and *D. cayenensis* with 4 levels of silverneem prepared as 0 mg/ml, 100 mg/ml, 200 mg/ml and 300 mg/ml solutions replicated three times. The apical sections of the yam tubers were soaked into 6 cm depth of each level of the prepared silverneem and allowed to stay for 3 min, air dried and placed on the wooden shelf in the barn. Temperature and relative humidity were monitored both inside and outside barn. Data were also collected on dormancy period, weight loss, rot incidence and sprout length. The improved yam barn reduced the temperature with ($1.5 - 4.5 \pm 1^0$C) and provided cooler environment for the yams compared to the outside barn temperature. The dormancy period of *D. rotundata* and *D. alata* were significantly ($P \leq 0.05$) extended at the concentrations of 100 mg/ml and 200 mg/ml of the silverneem. Generally, the interaction effects of silverneem with variety significantly reduced dormancy period of *D. cayenensis* at 100 mg/ml but at 200 mg/ml the dormancy significantly increased. In the same vein, weight loss, rot incidence and sprout lengths were significantly reduced in *D. alata,* and *D. cayenensis* compared to *D. rotundata.* Linear correlation analysis result showed high positive and significant relationship between sprout length and tuber weight loss (r = 0.92). Interestingly, the green metallic nanoparticle of silverneem has no toxic chemicals and no adverse effect on food.

Keywords: Post-harvest, Yam tuber, Nanobiotechnology, Yam barn, Storage, Silverneem.

INTRODUCTION

Yams *(Dioscorea species*) are major sources of carbohydrate to millions of people. Yam tubers when mature are carefully dug out of the ground for purposes of either immediate consumption, processing or fresh tuber storage. Methods of yam storage vary from delayed harvest to other methods of preservation including harvesting and heaping under trees, tying to life poles in yam barns as practiced in the yam belt of West Africa. In addition to

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addressing the food needs of people, yam is central to various ceremonies related to their importance in traditional societies of Africa and the Pacific islands (Orkwor *et al,*1998).

Post-harvest food losses of yam which sets in at 2-3 months after harvest (Eze and Ugwuoke, 2010) constitute a huge source of food insecurity in Nigeria. This post harvest losses have implicated pathological, physiological or mechanical damage (Ray et al., 2000). Although it has been difficult to quantify post-harvest storage losses, some claim that as much as 20-50% of yam tubers may be lost to pest attack in storage (FAO 1998). Thus, losses associated with yam food crops limit the potential income of the farmers, threaten food security and exacerbate conditions of poverty among rural households, whose income stream depends on the ability to store excess farm produce for a later date (FAO, 2014). Although farmers have been known to practice indigenous methods of storage of farm produce, these have been known to be less effective compared to modern storage methods. A study on traditional storage of yam by Girardin *et. al* (1998) showed that produce stored under the traditional system usually do not keep long and farmers usually suffer great losses. Storage practices have been categorized into three broad type; traditional storage techniques, improved storage techniques and advanced storage techniques (Osagie, 1992)

However, the three categories have the same principle of modifying the storage environment to elongate the shelf life of yam tuber. The advanced storage technique which involves the use of electricity is not feasible in a developing country like Nigeria where power supply is grossly inadequate or totally non-existent especially in yam producing areas. Therefore, storage treatment of yam tuber in other systems is imperative if shelf life of yam should be extended.

Synthetic chemical (Gibberellic acid, Thiaben dazol, Deltathrine) and local botanicals such as *Azadiractha indica*, *Xylopia acthiopica*, *Occimum graticimum* have been employed in controlling post harvest losses of yam (Girardin *et al*,1998). According to Eze *et al* (2013) result obtained with these chemical storage treatments showed no wholesome responses regarding yam rot, weight loss, dormancy and sprouting.

Nanoscience has been established recently as a new interdisciplinary science. A combination of silver nitrate and plant extract to generate nanoparticles is a nanobiotechnology. Silver nanoparticles have been used extensively as anti-bacterial agents in a health industry, food storage, textile coating and a number of environmental applications. As the nanorevolution in the realms of medical and technological applications unfold (Han et al, 2007), the development and application of green metallic nanoparticles in Agriculture is likely to turn a new era in the nation's efforts in solving food security problems. Against this background, we hypothesized that screening three varieties of yam with four levels of the low cost nanobiotechnology (silverneem) for reduction of postharvest losses of yam might provide reasonable decrease in yam storage losses and improve the quality of stored yam tubers. Motivation and aims of the study are the potentials, availability to rural farmers and low cost of the nanobiotechnology in the control of post-harvest losses of yam in storage.

MATERIALS AND METHOD

Location of the Experiment

The experiment was conducted at the Department of Crop Science, University of Nigeria, Nsukka. The area is located by latitude 6^0 52' N and longitude 7^0 23' E and altitude 400 m above sea level and has a humid tropical climate. Rainfall is bi-modal with an annual total of about 1500 mm and Relative humidity ranges from 70% to 80% while ambient temperature ranges from 25^oC to 30^oC during rainy season (Asadu, 1990).

Source of Yam

The three varieties of yam; *Dioscorea rotundata* (White guinea yam), *Dioscorea alata* (Water yam) and *Dioscorea cayenensis* (Yellow yam) were obtained from the farmer's field in the major yam producing area in Benue state. Arrangement for the supply of yam tubers was made prior to the harvesting period in order to ascertain the correct date of harvest. The tubers were meticulously harvested on first of February, 2014. The tubers were carefully loaded into a truck which floor was littered with rice husks in order to reduce or cushion the effects of galloping which cause bruises on yams in transit. The tubers were sorted and those without many blemishes were selected for storage.

The storage structure

The experiment was conducted in an improved yam barn at the location. The roof of the improved barn was made of corrugated aluminum sheets with ceiling of bamboo and raffia mats for heat insulation. The sides of the barn consisted of a dwarf wall (1m high) made of cement blocks and wire netting extended from the top of the dwarf wall to the roof of the building. This feature enhanced air circulation and excluded rodents. Inside the barn, wooden shelves were constructed on which the tubers were placed. Also installed inside the barn, were the gadgets for measurement of temperature and relative humidity of the storage environment.

Preparation of the solution (silverneem)

Fresh neem (*Azadiractha indica*) leaf was collected from the botanical garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The leaves were air dried on the laboratory bench for 7 days and milled in a hammer mill into powder. One and a half kilograms of the powder were extracted using Peak and Tracy (1956) method. The extraction was repeated two more times using 1200 ml in each case. The solution was heated under reduced pressure and solid materials of different weights were obtained. One gram of the dark solid material obtained after evaporation was dissolved in 4 ml of Diomethylsulphoxide (DMSO). Thereafter, it was filtered and 20 ml of the filtrate was dissolved in 2 ml of silver nitrate to obtain a solution termed silverneem. The substance was then subjected to serial dilutions to obtain three levels of concentrations thus: 100, 200, 300 mg/ml.

Application of silverneem

The primary nodal complexes at the heads of the tubers were removed to create freshness in that part of the tuber. Fresh cut was made on the tuber if the primary nodal complex disappeared during harvest or handling. This was to ensure effective movement or exchange of materials between the tuber and the prepared silverneem solution. The apical sections of the tubers were soaked to 6 cm depth for 30 minutes. After soaking, tubers were air dried and then placed on the appropriate racks in the yam storage barn according to design for subsequent observation and records. The storage was terminated after 6 months.

Measurements

Temperature (T^0C) and relative humidity (RH) of the storage environments were monitored at 10.0 am and 4.0 pm sessions daily using a thermocouple. The daily temperature and RH was obtained by average of the two sessions. Consequently, monthly temperature and RH were also obtained by average of the daily readings in each environment. The fresh weights of the tubers were taken with a top loading scale before storage and subsequently at intervals of 4 weeks during the storage. The duration of complete dormancy was determined as defined by Ireland and Passam (1985) which was given as the number of days from the start of storage to the first visible sign of sprouting.

Yam rot incidence was determined thus:

$$\text{Rot incidence } (\%) = \frac{R_0 - R_1}{R_0} \times 100$$

Where R_0 is the number of tubers with no symptom of rots before storage and R_1 is number of tubers with symptoms of rots after storage.

Weight loss was determined thus:

$$\text{Weight loss }(\%) = \frac{W_1 - W_2}{W_1} \times 100$$

Where W_1 is the weight of tubers before storage and W_2 is the weight after storage

Sprout length (m) = Length of the sprout at 16 weeks of storage

Temperature use efficiency of the storage barn (TUE) was calculated thus: T_0 - T_i where T_0 is the temperature reading value outside the barn and T_i is the temperature inside barn.

Relative humidity use efficiency (RHUE) of the storage barn was also calculated thus:

RH_i – RH_0 where RH_i is relative humidity inside barn and RH_0 is the relative humidity outside barn.

Data analysis

All the data collected were subjected to analysis of variance (ANOVA) according to the procedure for a randomized complete block design using the SAS statistical software (SAS, 1999). Treatment means were tested using least significant difference (LSD) of the means at 5% probability level.

RESULT

The temperature and relative humidity of the study area both outside and inside the storage barn are shown in Table 1. The improved yam barn reduced the temperature with (1.5 – 4.5 ± 1^0C) and provided cooler environment for the yams compared to the outside barn temperature. The reduction was high in the months of January to April, as shown in the temperature use efficiency (TUE). Conversely, the relative humidity inside the storage barn was high with relative humidity use efficiency (RHUE) of (12.5- 25.5 ±1%).

The dormancy period of yam was significantly ($P \leq 0.05$) extended by the silverneem at the three levels of concentration compared to the control (Table 2.). Weight loss was not affected at any level of silverneem concentration but rot incidence and sprout lengths were significantly ($P \leq 0.05$) reduced.

Dormancy period of the three varieties varied with *D. rotundata* having significantly ($P \leq 0.05$) longer dormancy while *D. cayenensis* had the least dormancy period (Table 3). Similarly, weight loss, rot incidence and sprout length were significantly higher with *D.rotundata*. These parameters also varied between *D. alata* and *D.cayenensis* but differences were not significant.

The interaction effects of silverneem on the physical characteristics of the stored yam generally varied (Table 4). The dormancy period of *D. rotundata* was significantly ($P \leq 0.05$)

extended as well as that of *D. alata* at the concentrations of 100 mg/ml and 200 mg/ml of the silverneem. Generally, the interaction effects of silverneem with variety significantly reduced dormancy period of *D. cayenensis* at 100 mg/ml but at 200 mg/ml the dormancy significantly increased. In the same vein, weight loss, rot incidence and sprout lengths were significantly reduced in *D. alata,* and D. cayenensis compared to *D. rotundata.*

The linear correlation analysis result as shown in Table 5 indicates high positive and significant relationship between sprout length and tuber weight loss (r = 0.92). Conversely, high negative and significant relationship existed between weight loss and dormancy period (r = -0.88).

Table 1.Temperature and relative humidity variations in the storage environments in the study area (inside and outside the storage barn)

DISCUSSION

The improved yam barn used in this study had roof and insulated material as ceiling which probably helped to reduce the barn temperature while higher RH probably resulted from moisture conservation due to less air movement inside the barn. This is similar to a report by Girardin *et al.* (1998) who compared storage in pits and shades, and found that the pits had lower temperature and higher RH than the shades.

Although this study did not consider the improved yam barn as a factor, we adopted the use of the barn as a standard in our system given that it has advantage every other storage structure by reducing the barn temperature where facilities for advanced storage is not tenable. In the present study, yam tuber losses due to rot ranged from 44 -51.6% with or without chemical treatment for a period of 6 months but in another study that compared storage under modern (underground) and traditional (yam barn) technologies, Okorji and Ezeike (2003) reported 45% and 80% storage losses respectively.

Significant differences in the dormancy periods of tubers exhibited by the yam varieties used in this study probably arose from genetic differences. Girardin *et al* (1998) earlier noted that in yam, length of dormancy was under genetic control being both variety and species dependent. It was therefore likely that the variability in dormancy periods, weight loss and rotting of tubers might also have been affected by the time of harvest as earlier reported by Okwuowulu *et al* (1995). They also reported that age at harvest affected storability showing that late harvesting done at 8 months after planting was associated more with losses than those harvested 6 and 7 months after planting. In the present study, planting had been done when the researchers approached the farmer for this collaboration but harvesting was done in

February indicating that the tubers may have stayed up to 9-10 months in the soil.

Tuber weight loss is an important parameter in fresh yam tuber storage. In the present study, significant variations existed in tuber weight loss among the cultivars with the greatest losses occurring in *D. rotundata.* Okwuouwulu *et al* (1995) made a similar observation and related the result to the differences in physical and chemical nature of the tubers such as the skin and the internal tissue textures. Amongst the varieties of yam used, *D. cayenensis* with thin and smooth skin relative to the skins of *D. alata* and D. *rotundata* also probably have fine tissue textures with tiny air spaces that reduced moisture loss from the tubers during storage.

The dormancy period of yam was significantly ($P \leq 0.05$) extended by the silverneem at the three levels of concentrations. The use of chemicals in reducing postharvest losses of fresh yam tuber in storage is limited by availability and cost. However, given that the use of herbs in traditional medicine is widespread among farmers, use of neem leaf extract with silver nitrate (silverneem) for storage of yam will be attractive.

CONCLUSION

Evidence in this study showed that combining silver nitrate and neem leaf extract that resulted in a new product termed silverneem has potentials in controlling postharvest losses of fresh yam tuber. Most importantly, the new method of biosynthesis of green metallic nanoparticles of silverneem had no toxic chemicals and no adverse effect on food (Furno *et al*, 2004). However, further research is needed to test the toxicity or allergy potential of the silverneem in human or animal system. Again, silverneem is a combination of nanoparticles of organic (neem leaf extract) and inorganic (silver nitrate), its shelf life or degradability is yet to be determined.

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Table 1. Temperature and relative humidity of the storage environment

	Temperature			Relative humidity		
Months	Outside barn	Inside barn	TUE	Inside barn	Outside barn	RHUE
Jan.	32.0	28.4	3.6	90.1	65.4	24.7
Feb.	33.5	29.0	4.5	92.5	68.2	24.3
March	34.0	29.4	4.6	89.6	72.1	17.5
April	33.0	28.9	4.1	85.7	74.4	11.3
May	31.0	28.8	2.2	84.3	75.4	8.9
June	29.2	27.9	1.3	81.9	70.0	11.9
July	28.5	28.3	0.9	77.8	65.9	11.9

TUE = Temperature use efficiency of the storage barn

RHUE = Relative humidity use efficiency of the storage barn

Table 2. Main effects of varying concentration of silverneem on dormancy, weight loss, rot incidence and sprout length of yam in storage

Conc. Mg/ml	Dormancy Period (Days)	Weight loss (%)	Rot incidence (%)	Sprout length (m)
300	92.0	33.0	44.0	104.2
200	94.0	33.2	47.6	110.4
100	93.4	34.2	47.8	134.2
0	84.8	32.5	51.6	130.4
LSD $_{0.05}$	1.57	ns	1.50	2.23

Ns = Not significant

Table 3. Main effects of cultivar on the dormancy, weight loss, rot incidence and sprout length of yam tuber in storage

Cultivar	Dormancy (Days)	Weight loss (kg)	Rot incidence (%)	Sprout length (cm)
D.rotundata	89.0	34.6	5.3	135
D.alata	84.3	29.9	35.2	108
D. cayenensis	82.23	27.45	32.23	102
LSD $_{0.05}$	3.35	2.89	2.53	4.50

Table 4. Interaction effects of silverneem and cultivar on the dormancy, weight loss, rot incidence and sprout length of yam tuber in storage

Silverneem (mg/ml	Cultivar	Dormancy period (Days)	Weight loss (kg)	Rot incidence (%)	Sprout length (cm)
100	*D.rotundata*	90.2	35.0	40.4	135
100	*D. alata*	84.3	30.6	38.7	123
100	*D.cayenensis*	79.12	33.23	26.45	112
200	*D.rotundata*	93.8	30.2	36.5	129
200	*D. alata*	88.7	29.4	33.6	120
200	*D.cayenensis*	91.9	32.5	39.1	134
300	*D.rotundata*	87.20	34.67	40.0	130
300	*D. alata*	86.6	30.3	37.8	105
300	*D.cayenesis*	89.98	32.21	39.50	110
$LSD_{0.05}$		2.54	1.23	1.55	5.36

Table 5. Linear correlation matrices describing the relationship among the physical characteristics of yam tuber in storage

	Dormancy period	Weight loss	Rot incidence	Sprout length
Sprout length	.-453	.920**	.48	1
Rot incidence	-.278	.530	1	
Weight loss	-.880*	1		
Dormancy period	1			

*. Correlation is significant at 5% probability level (2-tailed), **. Significant at 1% probability level.