ORIGINAL RESEARCH REPORT NIGER. J. PLT. PROT. 40(2):89-102(2026)

EFFECTS OF STORAGE CONDITIONS, BOTANICAL ASH AND GREEN-SYNTHESIZED NANOPARTICLE TREATMENTS ON WATER ACTIVITY, MOISTURE CONTENT AND MOULD DEVELOPMENT IN STORED COCOA BEANS VARIETIES

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SUMMARY

Cocoa bean quality is strongly influenced during storage, by environmental conditions that can promote fungal contamination and compromise product safety. This study investigated the effects of storage conditions on water activity, moisture content and mould development in dried fermented cocoa beans varieties in Nigeria. Freshly fermented and dried F3 Amazon and CRIN Tc cocoa beans varieties were separately treated with ash and green-synthesized silver nanoparticles forms of neem and scent leaf botanicals at different concentrations and copper sulphate (positive control), and stored for 120 days at 29–34 °C (under 55–65% RH) and 29–31 °C (under 85–95% RH). Moisture content and water activity were determined at the beginning and end of storage using standard procedures, while mould development was assessed at the end of the storage period. Results showed that cocoa beans stored under high humidity conditions exhibited higher moisture content (5.21 -12.09%), water activity values (0.59 – 0.73), and higher mouldiness (up to 100%), indicating strong influence of environmental humidity on moisture dynamics and mould development during storage. Positive moisture content/water activity correlations (0.369 – 0.822) were observed in both cocoa varieties, implying close relationship. Mould development ranged between 60% and 100% across treatments, with untreated control samples showing the highest levels of mouldiness. Findings from this study demonstrated that elevated moisture favoured higher water activity and fungal proliferations in stored cocoa beans. The proliferations were however brought under significant control by neem ash treatments. The inclusion of neem ash (particularly at 20 g/kg beans) as a component of integrated management of toxigenic and non-toxigenic mouldiness in stored cocoa beans varieties is therefore essential towards maintaining cocoa bean quality.

KEYWORDS: Cocoa beans, water activity, moisture dynamics, mould contamination, relative humidity

INTRODUCTION

Cocoa is a major agricultural commodity that is traded and consumed around the world. The crop is of great economic benefits to producing countries, small holder farmers cultivating the crop, and the confectionery industry by providing key raw materials (cocoa beans), which have been linked to improved cardiovascular health (1, 2, 3) and help protect against a

broad range of conditions, including respiratory issues, heart disease, cancer, obesity, diabetes, and the decline of cognitive function (4, 5).

Africa contributed 74.5% of the expected 4.82 million tonnes of cocoa produced globally in 2021-2022, while Asia and Oceania contributed 5.5% (6). Cocoa beans were reported to be the 43rd most traded product in the world in 2022, with a total trade value of $8.29 billion. Cote d'Ivoire was the biggest producer and exporter of cocoa beans in 2022 with an export value of US $3.33 billion, followed by Ghana (US $1.08 billion), Ecuador (US $937 million), Nigeria (US $489 million), and Cameroon (US $450 million) (7).

In spite of all these benefits, cocoa beans are faced with some critical challenges during post-harvest handling and storage, which in turn, largely affect the quality and/safety of the commodity. Following fermentation, cocoa beans are dried to reduce their moisture content from approximately 60 % to between 6 % and 8 %. Drying is a crucial stage in cocoa processing because excessive moisture may promote fungal growth and deterioration of bean quality (8). Moisture levels above 8 % are particularly dangerous because they favour internal mould growth, whereas excessively low moisture levels below 5 % may make the beans brittle and susceptible to breakage (9).

In tropical regions such as Nigeria, environmental conditions characterized by high relative humidity and warm temperatures often create favourable conditions for microbial contamination of stored agricultural commodities. Relative humidity levels of 70 % to 90 % and temperatures around 30 °C have been reported to support the development of storage insects and moulds in cocoa beans. Fungal contamination during storage represents one of the major quality challenges in cocoa production systems. Various fungal genera have been reported on stored cocoa beans, including *Aspergillus, Penicillium, Mucor, Rhizopus, Fusarium* and *Lasiodiplodia*. Many of these fungi are capable of producing mycotoxins that may pose serious health risks to consumers and reduce the market value of cocoa beans. Airborne spores represent a major source of contamination during storage, and fungal growth is often favoured by the availability of moisture and suitable environmental conditions (10, 11, 12).

Among the physicochemical factors influencing microbial growth in stored commodities, moisture content and water activity are particularly important. Water activity reveals the amount of free water available for microbial metabolism and is therefore considered a more reliable indicator of microbial stability than total moisture content. Understanding the relationship between storage conditions, moisture content, water activity and mould development is therefore essential for improving postharvest management of cocoa beans (8; 13).

This study therefore sought to investigate the effects of storage conditions on moisture content, water activity and mould development in dried fermented cocoa beans stored under varied tropical conditions in Nigeria.

MATERIALS AND METHODS

Collection and storage of freshly fermented dried cocoa beans varieties

Freshly fermented dried cocoa beans of two different cacao varieties (F3 Amazon and the newly developed hybrid- CRIN Tc Series) were obtained from Cocoa Research Institute of Nigeria (CRIN) Fermentry Unit. The beans were sun-dried for 7 to 14 days

depending on the weather condition (14) and stored in jute bags for 120 days.

Treatment and storage of beans under varied storage conditions

One kilogramme (1.0 kg) of dried fermented cocoa beans varieties (F3 Amazon and CRIN Tc series) were separately packed in new small-size jute bags. Ash forms of the botanicals which gave highest inhibitions in the *in-vitro* study: 20 g neem, 20 g scent leaf, 40 g neem, 40 g scent leaf, 20 g (neem and scent leaf), 40 g (neem and scent leaf), and the green-synthesized nanoparticles: 75 ml (neem nanoparticles), 100 ml (scent leaf nanoparticles) and 100 ml (neem and scent leaf) were separately mixed with 1.0 kg of each of the selected dried fermented cocoa beans varieties (F3 Amazon and CRIN Tc series) in duplicates.

Another 1.0 kg of each of the varieties was treated with 50 ml synthetic fungicide (1% copper sulphate) used in the *in-vitro* experiment and also packed in separate jute bags. Similar quantities of the dried fermented cocoa beans samples were not treated with any botanical(s) or chemical.

Both the treated and untreated cocoa beans samples were kept in a store at Cocoa Research Institute of Nigeria (CRIN) Headquarters, Ibadan and the stored cocoa beans were maintained for 120 days under environmental conditions representative of typical tropical storage environments as follows, with the aid of a humidifier:

a. Dry season conditions: 55–65 % relative humidity and temperatures of 29–34 °C

b. Wet season conditions: 85–95 % relative humidity and temperatures of 29–31 °C

Samples were evaluated over a storage period of 120 days, with observations recorded at intervals including days 0, 60 and 120 of storage.

Determination of moisture content and water activity

Moisture content and water activity were determined using AOAC (15) standard procedures. Two grammes (2.0 g) of the sample was weighed into a previously weighed crucible. The crucible with the sample was then transferred into the oven set at 100° C to dry to a constant weight for 24 hours. At the end of the 24 hours, the crucible plus sample was removed from the oven and transferred to desiccator, cooled for ten minutes and weighed.

The percentage moisture content were calculated using the formula:

$$\% \text{ Moisture} = \frac{W1 - W2}{W1 - W0} \times 100$$

Weight of empty crucible = W_0;

Weight of crucible + sample = W_1;

Weight of crucible + oven-dried sample = W_2.

Water activity measurements were conducted using a digital portable water activity meter (A180WA). Approximately 2 g of each cocoa bean sample was weighed into the meter chamber and allowed to stabilize for about two minutes before readings were recorded. Mean values of moisture content and water activity were subsequently calculated and used for correlation analysis.

Assessment of mould development

At the end of the 120-day storage period, representative samples of both treated and untreated cocoa beans varieties were placed on sterile, moistened Whatman's filter papers inside sterile Petri dishes. The experimental set-up was observed for about 7-10 days at 30±2° C; the beans samples were visually observed and the number of mouldy beans were counted. The Percentage Mouldiness (PM) were thereafter calculated using the formula:

$$PM = \frac{\text{Number of mouldy beans}}{\text{Total number of beans}} \times 100$$

The Percentage Apparently Toxigenic Mouldiness (PATM) of the beans was also determined using the formula:

$$PATM = \frac{\text{Number of mouldy beans apparently toxigenic moulds}}{\text{Total number of beans}}$$

The calculated percentage mouldiness and percentage apparently toxigenic mouldiness were an indication of the effectiveness of the treatments applied (or degree of disease control) and the suitability of the storage conditions for their growth. Additionally, apparently toxigenic mouldiness was assessed to determine the potential presence of mould species capable of producing mycotoxins.

RESULTS

Moisture content and water activity of stored cocoa beans

The initial moisture contents of the F3 Amazon cocoa beans variety at dry and wet season storage conditions (29.10-34.10 °C, RH 55-65% and 29.00-31.00, RH 85-95%, respectively) were 6.38 and 11.94%, respectively. At the end of storage, the mean moisture content values ranged from 5.08 - 6.61% and 5.21 - 6.94% respectively. During the dry season storage, the significantly highest moisture contents ($P \leq 0.05$) were observed in the AgNP treated cocoa beans, followed by that of the 20 g/kg neem ash treated samples (5.49%), while the lowest value was recorded for 40 g/kg neem ash treatment (5.08%). This was however not significantly different ($P \leq 0.05$) from those of scent leaf ash and the control treatment values (Table 1). Highest average moisture content during the wet season storage (6.94%) was observed in the 40 g/kg neem+scent leaf ash treatment, and closely followed by that of neem+scent leaf AgNP treatment, while 20 g/kg scent leaf ash treatment (5.21%) gave the least value (Table 1).

The initial water activity values in both the dry and wet season stored F3 Amazon cocoa beans variety were 0.66 and 0.71 respectively. After the 120-day period of storage, the water activity values reduced significantly ($P \leq 0.05$) as also observed in the moisture content values, to between 0.46-0.61 and 0.61-0.67 at both dry and wet seasons respectively. Both the neem AgNP and the 20 g/kg scent leaf ash treatments with the untreated control showed highest water activity value, while the 40 g/kg neem and scent leaf ash treatment gave the lowest dry season value (0.46). Highest mean water activity value after the wet-season storage was observed in the untreated control (0.67), followed by those of the neem, and neem and scent leaf AgNP treatments (0.63 and 0.65 respectively), while the lowest water activity value (0.61) was recorded for the neem ash, 20 g/kg scent leaf ash, 40 g/kg neem and scent leaf ash, and the scent leaf AgNP treatments (Table 1).

The moisture and water activity values of the Tc series followed similar trend as observed in the F3 Amazon variety (Tables 24a and 24b). Highest average moisture contents (6.41 - 6.51%) during the dry-season storage were observed in the AgNP treatments, followed by that of the untreated control (6.32%), while 20 g/kg neem ash treatment gave the least (Table 2). During the wet season storage, highest moisture content (8.05%) was however observed in the 20 g/kg scent leaf ash treatment, followed by those of neem+scent leaf ash treatment (6.73%) and neem+scent leaf AgNP treatment (6.71%), while those of scent leaf AgNP and 40 g/kg neem ash treatments (6.28 and 6.30% respectively) were significantly lowest ($P \leq 0.05$). Significantly highest water activity values ($P \leq 0.05$) were observed in both the untreated control (0.61) and the neem AgNP treatment (0.60) during dry season storage. The lowest value (0.51) was however

recorded for the 20 g/kg scent leaf ash treatment. A similar trend was noticed in the wet season stored beans of the same variety (Table 2).

Table 1: Mean percentage moisture contents and water activity of stored F3 Amazon cocoa beans varieties at varied storage conditions

Treatment	% moisture/Season		Water activity/Season	
	Dry	Wet	Dry	Wet
FCTR (DAY 0)	6.39^{c}	11.94^{b}	0.66^{a}	0.71^{b}
FCTR	5.14^{j}	6.55^{ik}	0.61^{bc}	0.67^{c}
FAI20	5.49^{g}	6.42^{lm}	0.53^{i}	0.61^{i-k}
FAI40	5.08^{j}	6.37^{mn}	0.55^{gh}	0.61^{i-k}
FOC20	5.13^{j}	5.21^{p}	0.61^{bc}	0.61^{i-k}
FOC40	5.14^{j}	6.57^{i-k}	0.51^{j}	0.62^{h-j}
FOA20	5.43^{gh}	6.65^{f-i}	0.57^{f}	0.63^{f-h}
FOA40	5.41^{hi}	6.94^{d}	0.46^{k}	0.61^{i-k}
FNA75	6.60^{a}	6.66^{f-h}	0.61^{bc}	0.63^{e-g}
FNOC100	6.61^{a}	6.49^{kl}	0.59^{d}	0.61^{i-k}
FNAO100	6.51^{b}	6.83^{e}	0.60^{cd}	0.65^{d}
FCU	5.36^{hi}	6.32^{no}	0.58^{ef}	0.62^{g-i}

Means with same superscripts along same column are not significantly different at $P \leq 0.05$ using Duncan's Multiple Range Test

Key:

FAI20: 20g neem ash/1kg beans
FAI40: 40g neem ash/1kg beans
FOC20: 20g scent leaf ash/1kg beans
FOC40: 40g scent leaf ash/1kg beans
FAO20: 20g neem+scent leaf ash/1kg beans
FAO40: 40g neem+scent leaf ash/1kg beans
FNA75: 75ml neem AgNPs/1kg beans
FNO100: 100ml scent leaf AgNPs/1kg beans
FCu: 50ml $CuSO_4$ solution/1kg beans
FCTR: Control (no treatment)
FNAO100: 100ml neem + scent leaf AgNPs/1kg beans

Table 2: Mean percentage moisture contents and water activity of stored Tc series cocoa beans varieties at varied storage conditions

Treatment	% moisture/ Season		Water activity/ Season	
	Dry	Wet	Dry	Wet
TCTR (DAY 0)	6.51^{b}	12.09^{a}	0.66^{a}	0.73^{a}
TCTR	6.32^{d}	6.44^{lm}	0.61^{bc}	0.66^{c}
TAI20	5.08^{j}	6.58^{h-j}	0.54^{hi}	0.62^{h-j}
TAI40	5.37^{hi}	6.30^{no}	0.59^{de}	0.60^{k}
TOC20	5.13^{j}	8.05^{c}	0.51^{j}	0.59^{l}
TOC40	5.91^{f}	6.54^{jk}	0.54^{hi}	0.63^{f-h}
TOA20	6.03^{e}	6.73^{f}	0.56^{g}	0.61^{i-k}
TOA40	5.37^{hi}	6.59^{g-j}	0.53^{i}	0.62^{h-j}
TNA75	6.51^{b}	6.43^{lm}	0.60^{cd}	0.64^{d-f}
TNOC 100	6.45^{bc}	6.28^{o}	$0..59^{d}$	0.61^{i-k}
TNAO100	6.41^{c}	6.71^{f}	0.59^{d}	0.64^{de}
TCU	5.34^{i}	6.67^{fg}	0.55^{gh}	0.61^{jk}

Means with same superscripts along same column are not significantly different at $P \leq 0.05$ using Duncan's Multiple Range Test

Key:

TAI20: 20g neem ash/1kg beans
TAI40: 40g neem ash/1kg beans
TOC20: 20g scent leaf ash/1kg beans
TOC40: 40g scent leaf ash/1kg beans
TAO20: 20g neem+scent leaf ash/1kg beans
TAO40: 40g neem+scent leaf ash/1kg beans
TNA75: 75ml neem AgNPs/1kg beans
TNO100: 100ml scent leaf AgNPs/1kg beans
TCu: 50ml $CuSO_4$ solution/1kg beans
TCTR: Control (no treatment)
TNAO100: 100ml neem + scent leaf AgNPs/1kg beans

Correlation between moisture content and water activity

Relationships between the moisture contents and water activity values of the two cocoa beans varieties showed various statistically significant interactions (Tables 3 and 4). The linear correlation matrix of the moisture and water activity values of the two stored cocoa beans varieties are as shown in Tables 3 and 4. The Tc series cocoa beans variety showed strong positive moisture content/water activity correlations ($r = 0.760$, $P < 0.00$ and $r = 0.725$, $P < 0.00$) during the dry and wet season storage periods respectively. The F3 Amazon variety however only showed strong positive correlation ($r = 0.822$, $P < 0.00$) during the wet season storage, but somewhat weak positive correlation ($r = 0.49$, $P < 0.014$) during the dry season storage condition (Tables 3 and 4).

Table 3: Linear correlation matrix of the moisture contents and water activity values of F3 Amazon stored cocoa beans varieties

	aw	% moisture	aw_1
% moisture	0.494**		
aw_1	0.678*	0.369*****	
% moisture_1	0.403****	0.423***	0.822*

*P < 0.000, **P < 0.014, ***P < 0.039, ****P < 0.051, *****P < 0.076
aw: Water activity of dry season cocoa beans samples
aw_1: Water activity of wet season cocoa beans samples
% moisture: Percentage moisture of dry season cocoa beans samples
% moisture_1: Percentage moisture of wet season cocoa beans samples

Table 4: Linear correlation matrix of the moisture contents and water activity values of the Tc series stored cocoa beans varieties

	aw	% moisture	aw_1
% moisture	0.760*		
aw_1	0.811*	0.636**	
% moisture_1	0.513***	0.222****	0.725*

*P < 0.000, **P < 0.001, ***P < 0.010, ****P < 0.298
aw: Water activity of dry season cocoa beans samples
aw_1: Water activity of wet season cocoa beans samples
% moisture: Percentage moisture of dry season cocoa beans samples
% moisture_1: Percentage moisture of wet season cocoa beans samples

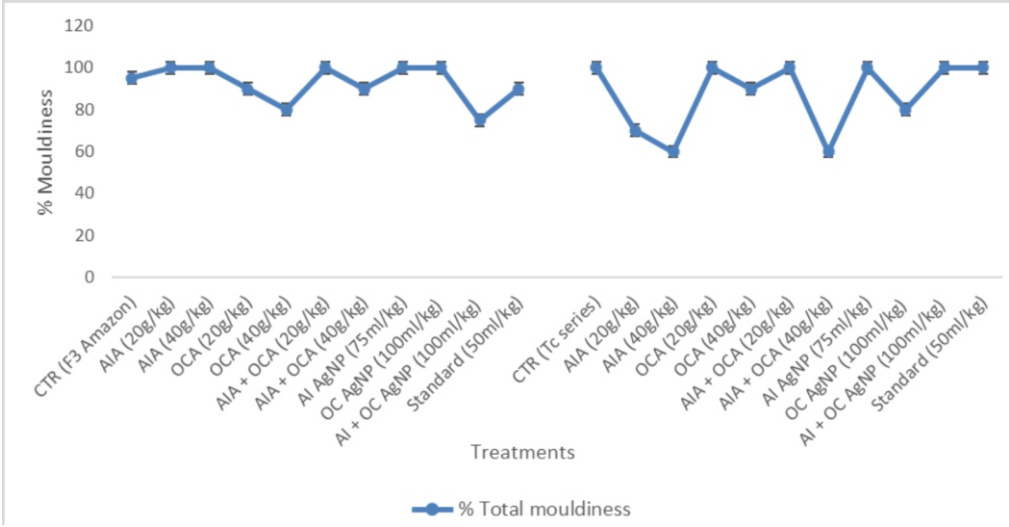
Mould development in the stored cocoa beans

Figure 1a and 1b show the percentage mouldiness and apparently toxigenic mouldiness on the treated F3 Amazon and Tc series beans varieties stored under dry season condition (29-34°C, RH 55-65%) for 120 days. The percentage mouldiness observed on F3 Amazon and Tc series varieties ranged from 75.00-100.00% and 60.00 - 100.00% respectively across treatments. While F3 Amazon untreated control showed 95.00% mouldiness, its Tc series counterpart gave 100.00%. The neem ash treatments as well as the 20g/kg neem and scent leaf ash treatments and the untreated control of F3 Amazon variety showed significantly highest percentage mouldiness, while the neem and scent leaf AgNP (100 ml/kg) treatment showed significantly lowest mouldiness (75.00%) among the F3 Amazon treatments.

Untreated Tc series control as well as the variety's scent leaf ash, neem and scent leaf ash (20 g/kg), neem AgNP, neem and scent leaf AgNP and the copper sulphate solution (positive control) treatments showed significantly highest percentage mouldiness ($P \leq 0.05$) among the Tc series treatments. Neem ash (40 g/kg), and neem ash and scent leaf (40 g/kg) treatments however gave significantly lowest percentage mouldiness of 60.00% ($P \leq 0.05$) on the Tc series cocoa beans variety used in this study (Figure 1a).

Neem AgNP treatments of both varieties showed significantly highest percentage apparently toxigenic mouldiness ($P \leq 0.05$), 75.00% followed by scent leaf ash: 20 g/kg (on F3 Amazon) and 40 g/kg (on Tc series) with 62.50% apparently toxigenic mouldiness. Neem and scent leaf ash

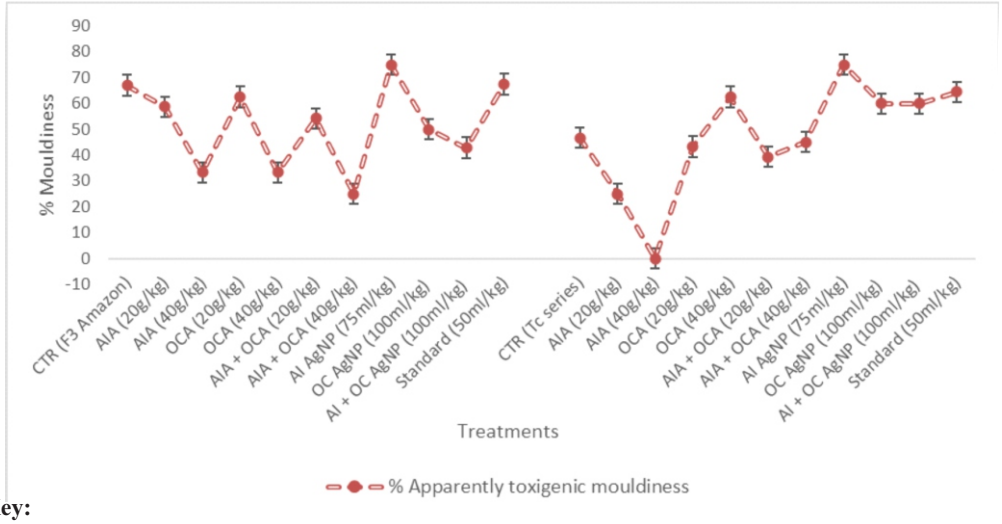
treatment (40 g/kg) however gave the least percentage apparently toxigenic moulds occurrence (25.00%) on F3 Amazon variety, while similar concentration of neem ash treatment alone gave the least figure (0.00%) on the Tc series beans variety (Figure 1b).



Key:

AIA – Neem ash OCA – Scent leaf ash AI AgNP – Neem extract silver nanoparticles
OC AgNP – Scent leaf extract silver nanoparticles CTR – Control (No treatment).

Figure 1a: Effects of botanicals and nanoparticle treatments on total mouldiness in dry season stored cocoa beans varieties (29-34°C, RH 55-65%)



Key:

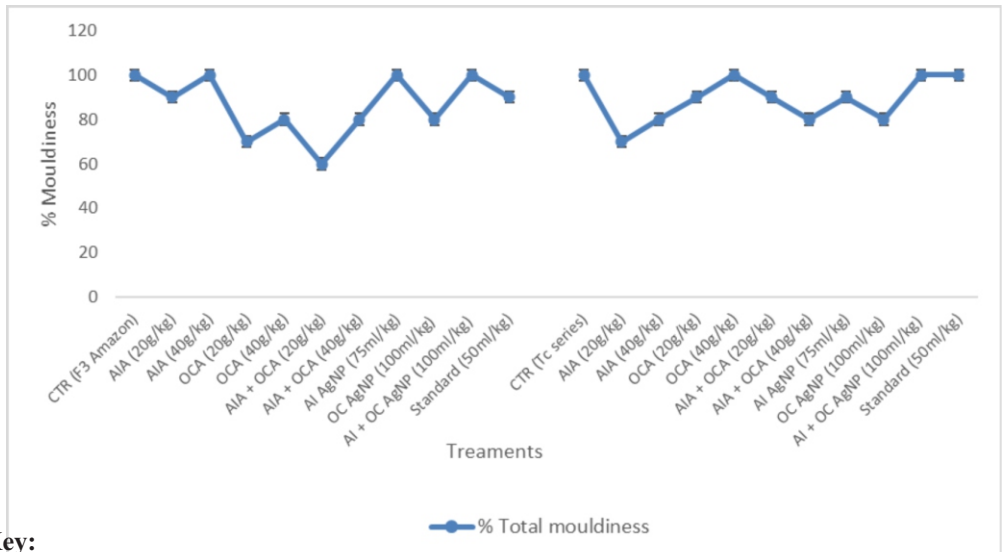
AIA – Neem ash OCA – Scent leaf ash AI AgNP – Neem extract silver nanoparticles
OC AgNP – Scent leaf extract silver nanoparticles CTR – Control (No treatment).

Figure 1b: Effects of botanicals and nanoparticle treatments on apparently toxigenic mouldiness in dry season stored cocoa beans varieties (29-34°C, RH 55-65%)

As shown in Figure 2a below, the percentage total mouldiness observed on F3 Amazon and Tc series varieties ranged from 60.00 - 100.00% and 80.00 - 100.00% respectively across treatments, under wet season storage (29-31°C, RH 85-95%). Untreated controls of both varieties revealed 100.00% mouldiness. Similar results were obtained on the F3 Amazon neem ash (40 g/kg), 75 ml/kg neem AgNP, and combination of the neem and scent leaf AgNP treatments, as well as Tc series variety's scent leaf ash (40 g/kg), combination of neem and sent leaf AgNP (100 ml/kg), and copper sulphate treatments (Figure 2a).

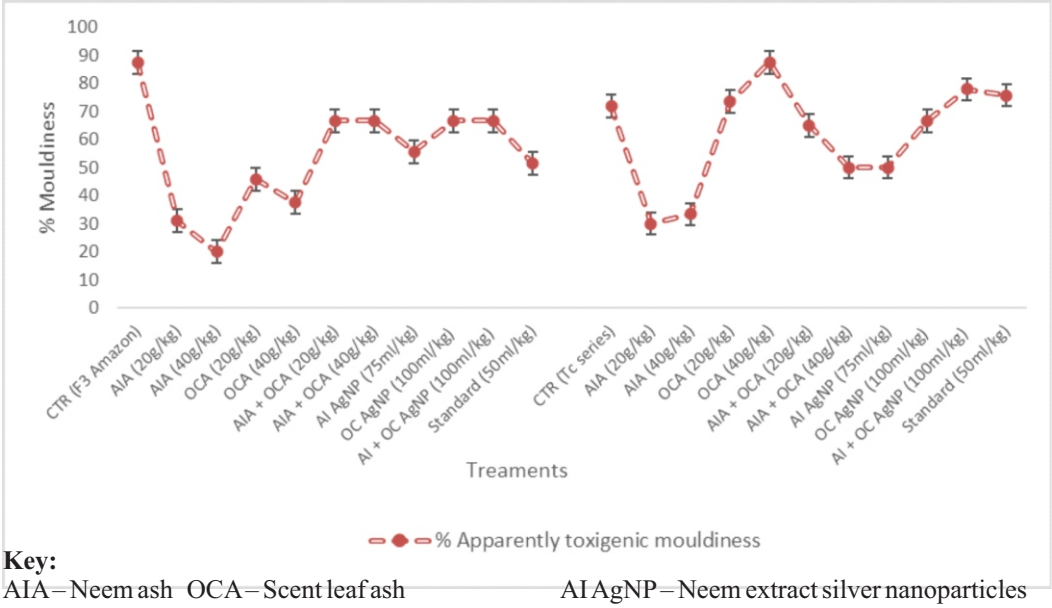
Exactly 80.00 - 90.00% total mouldiness were observed on the 40 g/kg scent leaf, 40 g/kg neem and scent leaf combination, and scent leaf AgNP treatments on the F3 Amazon stored beans variety. Six out of the eleven Tc series treatments (neem ash 40 g/kg, scent leaf ash 20 g/kg, neem and scent leaf ash treatments, as well as neem and scent leaf AgNP treatments) however showed percentage mouldiness within the said range. Significantly lowest percentage mouldiness ($P \leq 0.05$) were however 60.00% (neem and scent leaf ash 20 g/kg) and 70.00% (neem ash 20 g/kg) on the F3 Amazon and Tc series beans varieties respectively (Figure 2a).

Percentage apparently toxigenic mouldiness observed on the F3 Amazon and Tc series varieties after the 120-day wet season storage ranged from 20.00 - 87.50% and 30.00 - 87.50% respectively. While the percentage apparently toxigenic mouldiness observed on the F3 Amazon untreated control was 87.50%, that of Tc series stood at 71.70%. Scent leaf ash (40 g/kg) treatment gave the highest apparently toxigenic mouldiness on the Tc series variety. Similar to what was observed on the dry season stored beans, significantly lowest percentage apparently toxigenic moulds occurrences (20.00 - 33.33%) were obtained on the neem ash treated F3 Amazon and Tc series beans varieties (Figure 2b).



Key:
AIA – Neem ash OCA – Scent leaf ash AI AgNP – Neem extract silver nanoparticles
OC AgNP – Scent leaf extract silver nanoparticles CTR – Control (No treatment).

Figure 2a: Effects of botanicals and nanoparticle treatments on total mouldiness in wet season stored cocoa beans varieties (29-31°C, RH 85-95%)



Key:
AIA – Neem ash OCA – Scent leaf ash
AI AgNP – Neem extract silver nanoparticles
OC AgNP – Scent leaf extract silver nanoparticles
CTR – Control (No treatment).

Figure 2b: Effects of botanicals and nanoparticle treatments on apparently toxigenic mouldiness in wet season stored cocoa beans varieties (29-31°C, RH 85-95%)

DISCUSSION

The reductions in moisture contents of the ash treated beans compared with the untreated control counterparts may be due to starch hydrolysis, evaporation losses and/or incursion of (storage related) microorgnisms into the beans samples. The noticeably increased moisture on the AgNP treated beans however showed a tendency for shorter shelf life (16). The increased amounts of crude fat, protein and dry matter contents of the wet season stored cocoa beans with storage time disagree with the findings of Yin *et al.* (17) which revelaed the effects of storage time on the physico-chemical/nutritional characteristics of stored cereal.

Generally higher ash contents noticed in the AgNP treated beans also agrees with the findings of Ogundeji and Olufolaji (18). Significantly higher ash contents in the AgNP treated samples was however an indication of lower inorganic (mineral) contents which may retard microbial growth. Higher moisture contents observed in the cocoa beans stored during wet season might be due to the higher environmental relative humidity which favoured reduced rate of desiccation and evaporation.

The significant reductions in the moisture contents of both treated and untreated stored cocoa beans varieties both during the dry and wet season storage suggests that the commodity normally loses some water to the environment particularly during storage. The use of jute bags for storage in this study could also play a major role in the moisture content reduction towards maintaining desirable storage physicochemical properties (19). Also, the generally higher moisture and water activity values recorded on both treated and

untreated wet season stored cocoa beans varieties could largely be due to higher environmental moisture, which indicates a higher chance of storage fungal spoilage/contamination (20).

Findings from this study suggest that neem as well as scent leaf AgNP and ash treatments can be used to maintain the moisture contents of the stored cocoa beans varieties at low levels over a long period of time. The unique ability of the ash treatments to help initially reduce and subsequently keep the beans moisture contents low during the wet season storage when relative humidity was much higher clearly agrees with the findings of Fashanu *et al.* (21) which revealed that wood ash treatments were able to significantly reduce the moisture contents of matured green tomatoes, while still keeping their soluble solids high through 28 day storage at average relative humidity of 67%.

Higher water activity recorded for both stored cocoa beans varieties during the wet season (high humidity) storage, than in the dry season (lower environmental humidity) suggests some strong interrelationship between the water activity and moisture content of the stored beans. This seems to be confirmed by the positive correlation results obtained on the two critical parameters in this study. The water activity, unlike moisture content, however determines the cocoa beans shelf stability and resistance to microbial (storage fungi) infection (22).

Findings from this study also showed that the ash and AgNP treatments of the stored cocoa beans varieties produced varying (antimicrobial) impacts against storage mouldiness. While neem, and neem and scent leaf ash, as well as the neem and scent leaf AgNP treatments turned-out to be most effective against overall as well as toxigenic mouldiness of the cocoa beans varieties during the dry season, only neem ash treatments showed reasonable effectiveness against the storage fungi during wet season storage. Scent leaf ash treatments however showed some degree of effectiveness against the storage moulds on F3 Amazon variety only.

The above discoveries are in line with the findings of Wahedi *et al.* (23) that neem and wood ashes could be used as effective biopesticides and as good, safer alternatives for effective management of crop pests. The findings of Merghache *et al.* (24) and Muazu *et al.* (25) which discovered noticeable antifungal activities in green oak wood ash against pathogenic microorganisms even at low concentrations, agree with the results of this study.

CONCLUSION

This study showed that higher moisture contents favoured higher water activity, which in turn favoured total and toxigenic fungal proliferations in the stored cocoa beans varieties. The positive relationship established between moisture content and water activity in both cocoa bean varieties, indicated that increases in bean moisture were generally accompanied by increases in water activity. Since water activity reflects the amount of biologically available water required for microbial metabolism, this relationship provides a clear explanation for the observed mould development in both the treated and untreated stored beans. The relatively higher water activity values recorded during wet season storage created conditions more favourable for the proliferation of storage fungi and the occurrence of apparently toxigenic moulds. Despite all this, neem ash treatments stood-out in significantly reducing toxigenic mould occurrences under humid and dry

environmental conditions in both cocoa varieties

Although different treatments influenced the extent of mould development to varied extents, findings from this study revealed that the integration of neem ash component (particularly at 20 g/kg beans), with the maintenance of good storage environments, is critical in reducing the risk of total and toxigenic fungal contaminations in stored cocoa beans varieties and thus, preserving cocoa bean quality.

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