

Dynamics of fungi associated with storage of dried fermented cocoa beans varieties in Nigeria

Ogundeji B. A.^{1*} Akintokun A. K.² Akintokun P. O.³ Afolabi O. R.² Ogundeji F. O.⁴ and Agbeniyi S. O.¹

1. Plant Pathology Section, Cocoa Research Institute of Nigeria, Ibadan, Nigeria
2. Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria
3. Department of Crop Production, Federal University of Agriculture, Abeokuta, Nigeria
4. Department of Microbiology, Federal University of Technology, Akure, Nigeria

* Corresponding author: tundeji1@gmail.com

Abstract: Fungi associated with seed coats and nibs of dried fermented F3 Amazon and CRIN Tc series cocoa beans varieties stored for 120 days at 29-34°C/RH 55-65% (low humidity condition) and 29-31°C/RH 85-95% (high humidity condition) were isolated and identified at 15-day intervals (till the end of storage) using standard methods. Percentage of occurrence of each of the isolated fungal species was also calculated. Yeasts (13.00-100.00%) and *Rhizopus* species (5.88-60.00%) were most consistently isolated throughout the storage period from both seed coats and nibs of the two cocoa varieties used in this study under high and low humidity storage conditions. Isolation of *Fusarium* spp. (4.45-37.50%) from the seed coats of F3 Amazon variety, began at the 15th day of storage, while that of *Aspergillus* spp. (7.69-19.28%) began on the 30th day on the seed coats of same variety under both storage conditions. Similar trend was noticed on the nibs of the same variety. The seed coats of Tc series cocoa beans also followed similar pattern. The conspicuous presence of yeasts, *Fusarium* spp., *Rhizopus* spp. and *Aspergillus* spp. on the seed coats and nibs of both F3 Amazon and Tc series cocoa beans varieties during both low and high humidity storage showed that the fungi were both internally and externally borne on the beans and could cause infections on the beans both during dry and wet seasons. Suitable measures therefore need to be taken at the critical storage periods for production of safer cocoa beans.

Key word: Seed coat, nibs, storage, cocoa

INTRODUCTION

Cocoa beans are of commercial, nutritional and medical importance to man. The commodity constitutes very important ingredient in foods such as cakes, biscuits, child-foods, ice-creams and sweets. It is also the source of cocoa powder and some other derivatives (Assiedu, 1991; Sánchez-Hervás *et al.*, 2008).

Since neither storage nor processing conditions of cocoa are strictly controlled in the tropics, fungal contamination of cocoa beans is possible at many critical points in the cocoa production chain (Magan and Aldred, 2005). The beans are susceptible to fungal spoilage during and after fermentation, drying, storage, and in the course of shipment to foreign countries. Fungal species belonging to the genera *Aspergillus*, *Mucor*, *Penicillium* and *Rhizopus* have been observed on mishandled or improperly dried fermented beans (Sánchez-Hervás *et al.*, 2008; Fagbohun *et al.*, 2011).

The development of filamentous fungi (moulds) on cocoa beans during storage has constituted a major challenge to cocoa production in Nigeria and other cocoa producing countries. The differing physiological requirements of implicated storage moulds suggest some changes or alterations in the fungal populations associated with the stored beans with storage time.

Although storage moulds are generally involved in the bio-deterioration of stored cocoa beans, some of them are capable of producing toxic substances known as mycotoxins (aflatoxins, ochratoxins, fumonisins, etc.) which if taken in with the infected beans or their products, may pose much threat to life (Fagbohun *et al.*, 2011; Ogundeji and Olufolaji, 2014). This study therefore sought to determine the dynamics of storage moulds associated with stored cocoa beans varieties under different storage conditions in Nigeria.

MATERIALS AND METHODS

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. Both packed cocoa beans samples were kept in a store at Cocoa Research Institute of Nigeria (CRIN) Headquarters, Ibadan and observed for about 120 days under varied storage conditions: 29-34°C/RH 55-65% and 29-31°C/RH 85-95%.

Before and during the period of storage, seed coats and nib samples of the two varieties of cocoa beans used in this study, were taken and separately inoculated into Potato Dextrose Agar (PDA) plates at 15 days intervals. The inoculated PDA plates were incubated at 30±2°C and the fungal isolates obtained were thereafter characterized accordingly. Percentages of occurrence of each of the isolated fungi were thereafter calculated.

RESULTS

The percentages of occurrence of fungi isolated from seed coats of F3 Amazon and Tc series cocoa beans varieties stored in jute bags for 120 days 29 - 34°C and relative humidity 55 - 65% are presented in Table 1a and 1b. Besides yeasts and *Rhizopus* species which occurred throughout the storage period with reducing percentages of occurrence (in the F3 Amazon beans), eight different mould species were isolated from the seed coats of the cocoa varieties. At storage day 0, only *Rhizopus* spp. (44.44%) and the yeasts (normally associated with cocoa beans fermentation) occurred on the seed coats. The number of moulds isolated from the seed coats, however increased with storage time after each fifteen-day sampling interval.

Fusarium spp. with 10.00, 37.50, 20.00, 15.38 and 14.29% of occurrence were observed on the seed coats on days 15, 45, 60, 75 and 120, respectively while *A. niger* with 7.69, 14.29, 19.28, 14.29% of occurrence were observed at days 75, 90, 105 and 120 of storage, respectively. *Aspergillus flavus* (12.50%) and *Botrytis*

spp. (28.57%) only occurred once (at Days 30 and 120, respectively). *Aspergillus ochraceus* and *A. terreus* occurred only twice in the samples between Days 75 and 105. *Aspergillus* spp. however became more prominent in the seed coat samples from storage Days 75 to the last day (Day 120) of the experiment (Table 1a).

As shown in Table 1b, nine moulds in addition to yeasts were isolated from the Tc series cocoa seed coats during storage. *Rhizopus* spp. were isolated on days 0, 30, 60, 90 and 105 with the 5.88, 40.00, 10.00, 16.67 and 21.67% occurrences, respectively. *Fusarium* spp. were isolated four times (on days 0, 15, 45, and 75) throughout the period of storage with the highest percentage of occurrence (33.33%) observed on Day 45. *Fusarium oxysporium* was however isolated on days 15, 90 and 105. Its highest occurrence on the seed coats (21.67%) was however noticed on the 105th day of storage. *Aspergillus fumigatus* (6.67%), *Pythium* (20.00%), *A. flavus* (28.57%) and *Neurospora* spp. (28.57%) were however isolated once from the Tc series cocoa seed coat throughout the 120-day storage period. *Aspergillus flavus* and *Neurospora* sp. were however isolated on the last day (Day 120) of storage.

The percentage occurrence of fungi associated with dried fermented cocoa beans nibs of F3 Amazon and Tc series cocoa varieties stored for 120 days during the dry season period of the year are shown in Tables 2a and 2b. Similar to the observation in Tables 1a and 1b, yeasts were isolated from cocoa beans nibs throughout the period of storage, but with consistent reductions in the percentages of occurrence. *Fusarium* spp. (12.50 – 50.00%) and *Penicillium* spp. (10.00 - 25.00%) were isolated five to six times, from F3 Amazon nibs throughout the storage period. *Fusarium* spp. were isolated on Days 15, 45 and 60, and consistently isolated on Days 90 (25.00%), 105 (20.00%) and 120 (25.00%) (Table 2a).

Unlike the case with the seed coats of same variety (Table 1a), *Rhizopus* spp. (10.00 - 50.00%) were only isolated five times

throughout the storage period. *Aspergillus niger* (9.00 - 25.00%) was isolated three times after each 15-day interval of storage. The mould however had highest occurrence at storage Day 120. *Aspergillus ochraceus* (37.5%) was isolated once (Day 75), while *Trichoderma* spp. (12.50%) were also interestingly isolated once from same sample, 75 days after storage (Table 2a).

Besides yeasts (25.00-75.00%) which were consistently isolated from the nibs of the Tc series varieties, *Rhizopus* spp. (10.00-50%) showed some consistent, though reducing prominence from Day 60 to Day 120 of storage. *Fusarium* spp. (9.09-27.27%) which were isolated six (out of the nine) times however showed some consistent but generally increasing prominence from Day 45 to the 105th day of storage. This was slightly similar to what was observed on the seed coat of same variety, but not in a consistent manner (Table 1b). *Aspergillus ochraceus* (10.00%) was consistently isolated twice on Days 105 and 120 of storage, while *A. flavus* (20.00%), *Mucor* spp. (20.00%) and *Botrytis* spp. (10.00%) were only isolated once on the 120th day of storage (Table 2b).

Tables 3a and 3b show the percentages of occurrence of fungi isolated from seed coats of F3 Amazon and Tc series cocoa beans varieties stored in jute bags for 120 days during the wet season period of the year at temperature range of 29-31°C and relative humidity, 85-95%. A total of six storage moulds were isolated from the seed coats of F3 Amazon variety within the 120-day storage period. Yeasts and *Rhizopus* spp. were however consistently isolated throughout the period of storage, and at generally decreasing percentages of occurrence (Table 3a). This was similar to what was observed on seed coats of the same variety during the dry-season storage. The trend was also similar to that of Tc series seed coats during the wet season storage, but with the exception of Day 60. *Aspergillus flavus* (9.09-16.67%) and *A. ochraceus* (14.29-16.69%) were isolated six and four times respectively within the storage period.

Both mould species were however consistently isolated from Day 90 to Day 120 storage period at somewhat increasing percentages of occurrence. While *Fusarium* spp. (4.45-13.75%) were isolated on Days 15, 45, 60 and 120, *A. fumigatus* (16.67%) was only isolated on the 120th day of storage (Table 3a).

Fusarium spp. were isolated on Days 15 (9.00%), 105 (20.27%) and 120 (27.27%) of storage from seed coats of Tc series variety, while *A. flavus* (17.00-25.00%) and *A. niger* (8.33-25.00%) were consistently isolated on Days 30-60 and Days 60-90 respectively. *Aspergillus ochraceus* and *Fusarium* spp. were however consistently isolated from the samples on Days 105 and 120 (Table 3b). The consistencies of isolation of *A. ochraceus* from seed coats of the two varieties of cocoa towards the last days of the storage were however similar. *Aspergillus niger* isolated from Tc series seed coats were conspicuously absent in F3 Amazon. Similar number of storage moulds were also isolated from seed coats of both varieties (Tables 3a and 3b).

Percentages of occurrence of storage fungi isolated from cocoa beans nibs of F3 Amazon and Tc series varieties are as shown in Tables 4a and 4b. As was the case with other samples, yeasts were consistently isolated throughout the storage period of F3 Amazon cocoa nibs. *Rhizopus* was also consistently isolated throughout the storage period as noticed on the seed coats of same variety. *Fusarium* spp. (8.50-30.00%), were isolated on the Days 15 (8.50%) and 30 (10.00%), and more consistently isolated from the nib samples from Day 75 to Day 120 (Table 4a). *Aspergillus niger* was isolated twice (Days 45 and 60) while *A. ochraceus* (10.00%) and *Pythium* sp. (11.11%) were isolated only once (Day 120 and Day 0 respectively) from the nib samples.

The occurrence of yeasts and *Rhizopus* spp. on the Tc series cocoa nibs showed similar trend to those of seed coats of same variety. *Fusarium* spp. (8.20-12.30%) were isolated from the nib samples on Days 15, 60, 105

and 120 of storage, while *A. flavus* which was also consistently isolated from seed coat of same variety on Days 30-60 (Table 3b), was only isolated on Days 105 and 120 of storage under same condition (Table 4b). Also, *A. niger* (7.05-16.67%) which was consistently isolated from the Tc series cocoa nibs on the 45th to 90th storage days

was also occurred consistently on the seed coats of same variety on Days 60 to 90. *Aspergillus fumigatus* (14.29%) and *A. ochraceous* (14.29%) were however isolated once (Day 120) from the cocoa nibs. The latter was also isolated from the seed coat of same variety on Days 105 and 120 (Tables 3b and 4b).

Table 1a: Percentage occurrence (%) of fungi associated with dried fermented cocoa beans seed coat (F3 Amazon) with storage time (29-34°C, RH 55-65%)

Variety	DOS	Fungal isolates												
		YST	RHS	FUS	MUC	FOX	AF	AFM	PYT	AO	AN	AT	BTR	NRS
F3 Amazon	0	55.56	44.44	-	-	-	-	-	-	-	-	-	-	-
	15	40.00	25.00	10.00	25.00	-	-	-	-	-	-	-	-	-
	30	37.50	50.00	-	-	-	12.50	-	-	-	-	-	-	-
	45	37.50	12.50	37.50	-	-	-	-	12.50	-	-	-	-	-
	60	20.00	60.00	20.00	-	-	-	-	-	-	-	-	-	-
	75	15.38	53.85	15.38	-	-	-	-	-	7.69	7.69	-	-	-
	90	14.29	57.14	-	-	-	-	-	-	-	14.29	14.29	-	-
	105	13.00	45.14	-	-	-	-	-	-	8.29	19.28	14.29	-	-
	120	14.29	28.57	14.29	-	-	-	-	-	-	14.29	-	28.57	-

Key: DOS: Days of storage; YST: Yeasts; RHS: *Rhizopus* spp.; FUS: *Fusarium* spp.; MUC: *Mucor* spp.; FOX: *F. oxysporium*; AF: *A. flavus*; AFM: *A. fumigatus*; PYT: *Pythium* sp.; AO: *A. ochraceous*; AN: *A. niger*; AT: *A. terreus*; BTR: *Botrytis* spp.; NRS: *Neurospora* sp.; -: Not present

Table 1b: Percentage occurrence (%) of fungi associated with dried fermented cocoa beans seed coat (Tc series) with storage time (29-34°C, RH 55-65%)

Variety	DOS	Fungal isolates												
		YST	RHS	FUS	MUC	FOX	AF	AFM	PYT	AO	AN	AT	BTR	NRS
Tc series	0	76.47	5.88	17.65	-	-	-	-	-	-	-	-	-	-
	15	75.00	-	12.50	-	12.50	-	-	-	-	-	-	-	-
	30	53.33	40.00	-	-	-	-	6.67	-	-	-	-	-	-
	45	66.67	-	33.33	-	-	-	-	-	-	-	-	-	-
	60	70.00	10.00	-	-	-	-	-	20.00	-	-	-	-	-
	75	42.86	-	21.43	21.43	-	-	-	-	-	-	-	14.29	-
	90	50.00	16.67	-	16.67	16.67	-	-	-	-	-	-	-	-
	105	40.00	21.67	-	16.67	21.67	-	-	-	-	-	-	-	-
	120	14.29	-	-	-	-	28.57	-	-	-	-	-	28.57	28.57

Key: DOS: Days of storage; YST: Yeasts; RHS: *Rhizopus* spp.; FUS: *Fusarium* spp.; MUC: *Mucor* spp.; FOX: *F. oxysporium*; AF: *A. flavus*; AFM: *A. fumigatus*; PYT: *Pythium* sp.; AO: *A. ochraceous*; AN: *A. niger*; AT: *A. terreus*; BTR: *Botrytis* spp.; NRS: *Neurospora* sp.; -: Not present

Table 2a: Percentage occurrence (%) of fungi associated with dried fermented cocoa beans nibs (F3 Amazon) with storage time (29-34°C, RH 55-65%)

Variety	DOS	Fungal isolates								
		YST	RHS	FUS	PEN	AN	BPC	AO	AF	TCS
F3 Amazon	0	90.00	10.00	-	-	-	-	-	-	-
	15	33.33	-	50.00	16.67	-	-	-	-	-
	30	31.00	50.82	-	-	18.18	-	-	-	-
	45	16.67	-	33.33	16.67	-	33.33	-	-	-
	60	28.50	50.00	12.5	-	9.00	-	-	-	-
	75	25.00	-	-	12.50	-	12.50	37.5	-	12.50
	90	25.00	50.00	25.00	-	-	-	-	-	-
	105	25.00	45.00	20.00	10.00	-	-	-	-	-
	120	25.00	-	25.00	25.00	25.00	-	-	-	-

Key: DOS: Days of storage; YST: Yeasts; RHS: *Rhizopus* spp.; FUS: *Fusarium* spp.; PEN: *Penicillium* spp.; AN: *A. niger*; BPC: *Bipolaris cinerea*; AO: *A. ochraceous*; AF: *A. flavus*; TCS: *Trichoderma* spp.; -: Not present

Table 2b: Percentage occurrence (%) of fungi associated with dried fermented cocoa beans nibs (Tc series) with storage time (29-34°C, RH 55-65%)

Variety	DOS	Fungal isolates								
		YST	RHS	MUC	FUS	PYT	BPC	BTR	AO	AF
Tc series	0	100.00	-	-	-	-	-	-	-	-
	15	72.73	-	-	27.27	-	-	-	-	-
	30	66.67	33.33	-	-	-	-	-	-	-
	45	75.00	-	-	8.33	8.33	8.33	-	-	-
	60	45.45	45.45	-	9.09	-	-	-	-	-
	75	25.00	50.00	-	25.00	-	-	-	-	-
	90	35.00	40.00	-	25.00	-	-	-	-	-
	105	40.00	20.00	-	20.00	10.00	-	-	10.00	-
	120	30.00	10.00	20.00	-	-	-	10.00	10.00	20.00

Key: DOS: Days of storage; YST: Yeasts; RHS: *Rhizopus* spp.; FUS: *Fusarium* spp.; MUC: *Mucor* spp.; PYT: *Pythium* sp.; BTR: *Botrytis* spp.; BPC: *Bipolaris cinerea*; AO: *A. ochraceous*; AF: *A. flavus*; -: Not present

Table 3a: Percentage occurrence (%) of fungi associated with dried fermented cocoa beans seed coat (F3 Amazon) with storage time (29-31°C, RH 85-95%)

Variety	DOS	Fungal isolates						
		YST	RHS	FUS	AF	AFM	AO	AN
F3 Amazon	0	69.23	30.77	-	-	-	-	-
	15	65.55	30.00	4.45	-	-	-	-
	30	63.64	27.27	-	9.09	-	-	-
	45	58.25	28.00	13.75	-	-	-	-
	60	58.33	16.67	8.33	16.67	-	-	-
	75	53.33	22.45	-	-	-	-	-
	90	50.00	21.43	-	14.29	-	14.29	-
	105	50.00	21.43	-	14.29	-	14.29	-
120	33.33	8.33	8.33	16.67	16.67	16.67	-	

Key: DOS: Days of storage; YST: Yeasts; RHS: *Rhizopus* spp.; AF: *A. flavus*; AFM: *A. fumigatus*; AO: *A. ochraceous*; AN: *A. niger*; -: Not present

Table 3b: Percentage occurrence (%) of fungi associated with dried fermented cocoa beans seed coat (Tc series) with storage time (29-31°C, RH 85-95%)

Variety	DOS	Fungal isolates							
		YST	RHS	FUS	AF	AFM	AO	AN	
Tc series	0	72.72	27.27	-	-	-	-	-	-
	15	65.56	25.44	9.00	-	-	-	-	-
	30	61.54	23.08	-	18.18	-	-	-	-
	45	60.00	23.00	-	17.00	-	-	-	-
	60	50.00	-	-	25.00	-	-	-	25.00
	75	66.67	20.00	-	-	-	-	-	13.33
	90	66.67	25.00	-	-	-	-	-	8.33
	105	45.45	16.09	20.27	-	-	-	11.00	-
	120	45.45	9.09	27.27	-	-	-	9.09	-

Key: DOS: Days of storage; YST: Yeasts; RHS: *Rhizopus* spp.; AF: *A. flavus*; AFM: *A. fumigatus*; AO: *A. ochraceous*; AN: *A. niger*; -: Not present

Table 4a: Percentage occurrence (%) of fungi associated with dried fermented cocoa bean nibs (F3 Amazon) with storage time (29-31°C, RH 85-95%)

Variety	DOS	Fungal isolates							
		YST	RHS	FUS	AF	AFM	PYT	AO	AN
F3 Amazon	0	66.67	22.22	-	-	-	11.11	-	-
	15	62.00	30.50	8.50	-	-	-	-	-
	30	70.00	20.00	10.00	-	-	-	-	-
	45	60.45	25.55	-	-	-	-	-	14.00
	60	40.00	40.00	-	-	-	-	-	20.00
	75	58.55	22.20	20.25	-	-	-	-	-
	90	77.78	11.11	11.11	-	-	-	-	-
	105	65.40	20.60	15.40	-	-	-	-	-
	120	50.00	10.00	30.00	-	-	-	10.00	-

Key: DOS: Days of storage; YST: Yeasts; RHS: *Rhizopus* spp.; FUS: *Fusarium* spp.; AF: *A. flavus*; AFM: *A. fumigatus*; PYT: *Pythium* sp.; AN: *A. niger*; -: Not present

Table 4b: Percentage occurrence (%) of fungi associated with dried fermented cocoa bean nibs (Tc series) with storage time (29-31°C, RH 85-95%)

Variety	DOS	Fungal isolates							
		YST	RHS	FUS	AF	AFM	PYT	AO	AN
Tc series	0	58.33	25.00	-	-	-	16.67	-	-
	15	70.00	21.80	8.20	-	-	-	-	-
	30	77.78	22.22	-	-	-	-	-	-
	45	66.67	-	-	-	-	16.67	-	16.67
	60	60.50	23.45	9.00	-	-	-	-	7.05
	75	70.00	20.00	-	-	-	-	-	10.00
	90	70.00	20.00	-	-	-	-	-	10.00
	105	55.70	15.00	12.30	17.00	-	-	-	-
	120	42.86	7.14	7.14	14.29	14.29	-	14.29	-

Key: DOS: Days of storage; YST: Yeasts; RHS: *Rhizopus* spp.; FUS: *Fusarium* spp.; AF: *A. flavus*; AFM: *A. fumigatus*; PYT: *Pythium* sp.; AO: *A. ochraceous*; AN: *A. niger*; -: Not present

DISCUSSION

Yeasts and *Rhizopus* species were most consistently isolated throughout the 120-day storage period from both seed coats and nibs of the two dried fermented cocoa beans varieties (F3 Amazon and Tc series) used in this study and kept under two different

storage conditions. These isolates must have been carried-over from fermentation through drying, to storage (Copetti, 2009; Copetti et al., 2011).

The yeast isolates from seed coats and nibs of cocoa beans samples stored under much higher humid storage condition (wet season)

generally had higher percentages of occurrence than their *Rhizopus* spp. counterparts. The reverse was generally the case on the seed coats and nibs of dry season (lower humid condition) stored cocoa beans samples. This observation suggests the ability of *Rhizopus* spp. to thrive under drier environmental conditions (xerophiles) than yeasts on the stored cocoa beans (Delgado-Ospina *et al.*, 2021).

The conspicuous presence of yeasts, *Fusarium* spp., *Rhizopus* spp. and *Aspergillus* spp. on both seed coats and nibs of the F3 Amazon and Tc series cocoa beans varieties during both dry and wet season storage showed that the fungi were both internally and externally borne on the beans under both dry and moist storage conditions. This shows that the implicated storage moulds can cause both internal and external infections in dried fermented cocoa beans stored under both environmental conditions (Khatun *et al.*, 2020). The isolation of *A. flavus* and *A. niger* from seed coats and nibs of cocoa beans varieties used in this study also agrees with the findings of Khatun *et al.* (2020).

Discoveries on the various storage moulds isolated from the two cocoa beans varieties used in this study agree with the findings of Fagbohun *et al.* (2011), who isolated *A. flavus*, *A. niger*, *Mucor* spp., *Neurospora* spp. and *Rhizopus* spp. from stored cocoa beans across different locations in Ekiti State, Nigeria. The isolation of *Phytophthora palmivora* by the authors from the same set of stored cocoa beans samples is however at clear variance to the findings

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of this study. Results obtained from this study also agreed with the report of Ogundeji and Olufolaji (2015) which showed that members of the *Aspergillus*, *Fusarium* and *Penicillium* genera were storage moulds of both healthy and infected cocoa beans.

Higher number of storage fungi were isolated from seed coats of the stored cocoa beans varieties compared with their nibs during low humidity storage, while reverse was the case during high humidity storage. This suggests an apparently easier ability of storage fungi propagules to penetrate the cocoa beans varieties' seed coats under a humid storage environment than in a drier condition.

CONCLUSION

This study revealed that *Aspergillus* spp. and *Rhizopus* spp. dominated storage mould populations in dried fermented cocoa beans both in the dry and wet seasons of the year. *Aspergillus* spp. growth were however observed in the beans from 30 days after storage, while *Fusarium* and *Penicillium* were found in the dried fermented cocoa beans varieties from the 15th day of storage. Yeasts, *Fusarium*, *Rhizopus* and *Aspergillus* spp. were both externally and internally borne in dried fermented F3 Amazon and Tc series cocoa beans varieties. The implicated moulds could however cause infections on the cocoa beans varieties during storage. Suitable preventive and curative measures therefore need to be taken at the critical storage periods for production of safer dried, fermented cocoa beans.

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