Seed-Borne Fungi and Seedling Performance of Provitamin A Maize (*Zea Mays* L.) Stored in Different Packaging Materials and Temperatures

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Abstract

Maize (*Zea mays* L.) is widely cultivated in most agro-ecological environments in Nigeria. However, fungi are commonly associated with maize from field to storage. These organisms reduce the economic value and viability of stored maize. It is therefore essential to control post-harvest storage fungi on maize. This study evaluated the effect of cold (0 – 5ºC) storage and room temperature (25 ±5ºC) preservation of maize grains (in plastic containers, aluminium packs and tin-silos), for a 120-day storage period, on seedling properties and occurrence of fungi. Provitamin A maize varieties: PYA SYN2, PYA SYN6 and PYA SYN8 were used in the experiment. Data were collected on fungal incidence, germination percentage, root length and shoot length. Fungi species were isolated on Potato Dextrose Agar and identified. *Aspergillus niger, A. fumigatus, A. flavus, Rhizopus stolonifer,* and *Curvularia lunata* were identified in the stored maize varieties. In general, *Rhizopus stolonifera* (50.00%), *Curvularia lunata* (46.67%), and *Aspergillus fumigatus* (40.00%) had high frequency in the provitamin A maize varieties. Although, storage temperature, materials and fungal contamination of the three provitamin A maize varieties expressed no effect on the rate of germination, seedling performance (shoot and root length) significantly varied. However, the best storage conditions observed for maize, in order to mitigate against fungal contamination, were the preservation in tin silo and aluminium packages at low temperature. To limit fungal contamination in stored provitamin A maize, and improve seedling growth, maize grains should be stored in tin silos and aluminium packs, at low temperatures.

Keywords: Fungi, Maize grains, Storage conditions, Viability

Introduction

Maize (*Zea mays* L.) is a popular grain globally, this is owing to its numerous applications, as food, feed, and raw materials to produce other value-added products such as biofuel (Maitra and Singh, 2021). Asides its benefits, maize performs well in diverse agro-ecological environments, these include the forest and Sudan ecological environments in Africa (Sharifai *et al.*, 2012). Nigeria, ranked the 11th largest producer of maize globally, is one of the major producers of maize in Africa, substantively contributing to the dry grain production in the sub-Saharan Africa (Erenstein *et al.*, 2022). With an ever increasing population, especially in West Africa, increased production of maize for both domestic and industrial utilisation is imperative. The quality and application of regular maize varieties are limited, due to vitamin A (essential fat-soluble micronutrient) deficiency (Arlappa, 2023).

Vitamin A deficiency is a nutritional problem associated with abnormal growth and development, poor maintenance of healthy mucosal membranes, reproductive health, immunity, and vision impairment, especially for dark adaptation (Arlappa, 2023). Consequently, maintaining the production, supply and accessibility of provitamin A maize could significantly improve the overall health of populations with endemic diseases associated with vitamin A deficiency. In order to improve the nutrient supply from maize consumption, provitamin A maize varieties have been bred for increased nutritional content through micronutrient fortification (Zuma *et al*., 2018).

Unfortunately, vitamin A maize production is affected by several microbial infections through the chain of production, from the field to storage (Bryła *et al*., 2022). Fungi are however the most predominant spoilage microorganisms responsible for maize deterioration and quality reduction during storage (Omotayo and Babalola, 2023). When conditions are favourable for fungal growth (24–26°C and humidity exceeding 85%), incidence of spoilage fungi in stored maize grains could be as high as 86% (Bryła *et al*., 2022). With estimated damages ranging between 50-80%, spoilage fungi could increase the cost of maize production, reduce the quality of provitamin A maize, and militate against food security.

Management of storage conditions, to inhibit the growth and proliferation of fungi in maize grains, has become imperative to maintain the quality of maize during storage (Erasto *et al*., 2023). However, most of the recommended storage materials and conditions are inaccessible to maize grain retailers, especially, in developing countries. It is therefore essential to explore the influence of locally available storage materials and accessible storage conditions on the abundance of spoilage fungi, as well as the viability of provitamin A maize grains. Therefore, this study investigates the potentials of plastic containers, Aluminium foil packs and tin silos for the storage of provitamin A maize varieties, and their effect on viability and fungal contamination of maize stored at room and cold temperatures.

Materials and Methods

Experimental Site and Source of Provitamin A Maize

The study was conducted at the Germplasm and Seed Health Laboratory of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan Oyo state, Nigeria (7°15'N, 3°25'E). The Laboratory is located in the rainforest and savannah transition ecological zone of southwestern Nigeria. Provitamin A maize varieties: PYASYN2, PYASYN6 and PYASYN8 were acquired from the Institute of Agricultural Research and Training, Ibadan, Nigeria. Maize seeds were kept in a short term genebank at NACGRAB, Ibadan, prior to the commencement of fungal isolation and identification.

Treatments and Experimental Design

The treatment-set consisted of two factors (storage temperature and storage materials), including provitamin A maize packaged in three storage materials. The storage materials included plastic container (1 litre capacity), resealable aluminium foil pouches and table-top tin silo (200 mL), fabricated with an inner chamber of galvanized steel (Figure 1). These were used to store maize grains under room temperature (25±5ºC) and cold room (0 - 5ºC) for a period of 120 days. The storage treatments were set up for three varieties of provitamin A maize: PYASYN2, PYASYN6 and PYASYN8. The experiment, in each storage environment, was laid out in a completely randomized design (CRD), replicated three times.

Plastic Containers   Aluminium Foil   Tin Silos

Figure 1: Storage materials

Isolation and Identification of Fungi associated with the Provitamin A Maize Variety

After a 120-day storage period, maize seeds were surface sterilized with 1% NaOCl for 5 minutes before rinsing with sterile distilled water thrice, to ensure that traces of chemical sterilizer were completely removed.

Ten provitamin A maize seeds (from each treatment) were placed in sterile Petri dishes (9 cm) with five layers of germination paper. Forty milliliters of sterile distilled water was introduced to the germination paper and covered. The preparation was incubated for 7 days under room temperature (25±5ºC). Fungal growth was aseptically transferred to antibiotic-modified PDA (Robert *et al*. 1995; Horsburgh *et al.*, 2023).

Inoculated plates were incubated in a growth chamber at room temperature (25±5ºC) for fungi growth. The growing fungi were transferred to fresh PDA media to obtain pure cultures. Cultural characteristics of pure fungi was observed for preliminary identification. Microscopic identification was done by placing a drop of lactophenol cotton blue stain on a clean slide, using a mounting needle, small portion of the growing mycelium was placed in the drop of stain. The mycelium was subsequently teased and a cover slip was placed on the slide before viewing under the microscope (× 400) and compared with the original description of each fungus for morphological characterization according to Barnett and Hunter (2006). Photomicrographs of each fungus was taken using a camera attachment of a Euromex microscope (iScope 1153 PLi trono).

Assessment of Fungal Incidence in Stored Provitamin A Maize

Assessment of fungal incidence was conducted after microbial isolation and identification. Fungal incidence on provitamin A maize was evaluated through the number of infected seeds, this was presented as percentage incidence (Horsburgh *et al.*, 2023).

Total number of infected seeds per plate were counted and expressed as percentage disease incidence. (Horsburgh *et al.*, 2023). Frequency of infection was determined as follows:**Fungal incidence**:

$$Pi = n/N \times 100$$

Where:; Pi = Percentage fungal incidence

n = number of infected seeds per plate

N = Total number of seeds in each plate.

Influence of storage conditions on seed germination and seedling properties

To determine the effects of storage conditions and materials on the germination potential of maize seeds, the number of germinated seeds was recorded daily. The germination potential of stored seeds in each category was determined at the third, fifth and seventh days of *in-vitro* assessment. Percentage seed germination was reported as the number of germinated seeds of the total number of seed (10) assessed from each storage material after storage (AOSA, 2009). The seedling shoot length (cm) was measured by stretching a rope from the base of each seedling stem to the apex of plant, maintaining a precise measurement for all the plant samples. The root length (cm) was also measured using a ruler ten days after emergence (Deng *et al*., 2024).

Data analysis

Data was subjected to analysis of variance (ANOVA) and means were separated using the Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Results

Effect of Storage Conditions on Percentage Germination of Provitamin A Maize Varieties

The percentage germination of PYASYN2, PYASYN6 and PYASYN8 provitamin A maize varieties, stored at different conditions, was not significantly different through the *in-vitro* assessment duration of seven days (Table 1). However, PYASYN2 grains stored in tin silo, at low temperature (0-5 ºC) had the highest percentage germination (100%) at the fifth and seventh day. Also, PYASYN6 stored in tin silo and Aluminium foil pouch, at low temperature (0-5 ºC) had the highest percentage germination (96.70%), at fifth and seventh days, while PYASYN8 grains stored in tin silo at cool temperature expressed 100.00% germination at fifth and seventh days of *in vitro* seed culture.

Table 1: Effect of storage conditions on percentage germination of provitamin A maize varieties

Storage temperature	Storage material	PYASYN2			PYASYN6			PYASYN8		
		Day3	Day5	Day7	Day3	Day5	Day7	Day3	Day5	Day7
Cool (0-5ºC)	Aluminium	93.30a	96.70a	96.70a	93.30a	93.30a	90.00a	93.30a	93.30a	93.30a
	Plastic	90.00a	93.30a	93.30a	90.00a	86.70a	90.00a	90.00a	90.00a	90.00a
	Tin silo	86.70a	100.00a	100.00a	93.30a	96.70a	96.70a	96.70a	100.00a	100.00a
Room (25±5ºC)	Aluminium	93.30a	93.30a	93.30a	96.70a	96.70a	93.30a	93.30a	93.30a	93.30a
	Plastic	90.00a	96.70a	96.70a	83.30a	96.70a	96.70a	86.70a	96.70a	96.70a
	Tin silo	96.70a	96.70a	96.70a	93.33a	92.67a	96.67a	96.70a	96.70a	86.70a

Mean values with similar letter(s) within a column are not significantly different at 5 % level of significance using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

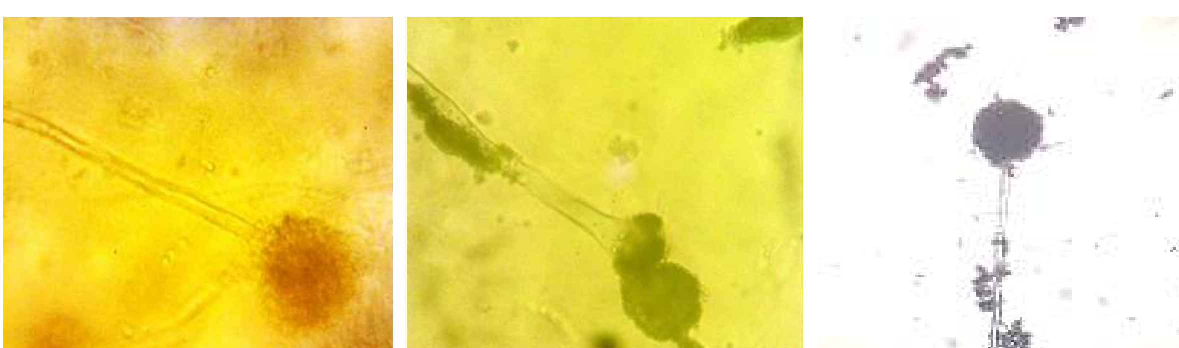
Influence of Storage Methods on Incidence of Spoilage Fungi in Stored Maize grains

Five fungal species, *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Rhizopus stolonifer* and *Curvularia lunata*, were isolated from the stored provitamin A maize grains (Figure 2). The highest incidence of *Aspergillus niger* (26.67%) (Table 2) was observed in PYASYN2 maize grains stored in Aluminium pack at low temperature; however, this was not significantly different from the incidence of the fungi observed in grains stored in other conditions. The lowest incidence of *Rhizopus stolonifer* and *Curvularia lunata* (13.33%) was observed in maize grains stored at low temperature. The highest incidence of *Aspergillus niger* (40.00%) was observed in PYASYN6 maize grains stored in plastic container at room temperature. This was significantly higher than the incidence of *A. niger* observed in grains stored in plastic and tin silo at low temperature. Highest incidence of *Rhizopus stolonifer* (50.00%) in PYASYN6 was observed in maize stored in Aluminium foil under cold temperature (0-5 ºC). However, highest incidence of *Aspergillus flavus* (16.67%) was observed in the maize stored in plastic container under cool temperature.

The highest incidence of *Aspergillus niger* (30.00%) was observed in PYASYN8 stored in tin silo at room temperature (25±5ºC) (Table 2).

Highest incidence of *Rhizopus stolonifer* (36.67%) was recorded in PYASYN8 stored in plastic container under cold temperature (0-5 ºC) and room temperature (25±5ºC). Highest incidence of *Aspergillus fumigatus* (40.00%) was recorded in the grains stored in plastic container at room temperature. However, the lowest incidence of *Aspergillus flavus* (10.00%) was recorded in PYASYN8 stored in tin silo stored under cool temperature; although, this was not significantly different from the incidence of *A. flavus* isolated from grains stored in Aluminium pack at room temperature

Rhizopus stolonifer ***Aspergillus flavus*** ***Aspergillus niger***



***Rhizopus stolonifer* (×400)** ***Aspergillus flavus* (×400)** ***Aspergillus niger* (×400)**

Figure 2: Most abundant fungal isolates associated with the provitamin A maize varieties

Effect of Storage Conditions on Seedling Properties of Provitamin A Maize Varieties

The highest root length of PYASYN2 (19.31 cm) was observed on the seedling of maize grains stored in Aluminium at cool temperature (Table 3), this was however not significantly different from the root length recorded for the seedling of maize stored in Aluminium at room temperature. Seedlings of this provitamin A maize stored at cool temperature in tin silo had the shortest root length (14.62 cm). The highest shoot length of PYASYN2 (18.32 cm) was observed on the seedling of maize grains stored in tin silo at cool temperature (0-5 ºC); although, this was not significantly different from the shoot length recorded for the seedling of maize stored in Aluminium at room temperature. The highest root length (19.30 cm) expressed by PYASYN6 was observed on seedlings of maize grains stored in plastic container at cool temperature (0-5ºC) (Table 3). This was not significantly different from the root length observed on other plant sets, except for root length recorded for the seedling of maize stored in tin silo at cool and room temperatures. The highest shoot length (18.74 cm) was observed on seedling of PYASYN6 maize grains stored in Aluminium foil at cool temperature, followed by the shoot length of seedlings from maize grains stored in tin silo. However, seedlings of PYASYN6 provitamin A maize stored at room temperature in tin silo had the shortest shoot length (14.13 cm).

Root length of seedlings associated with PYASYN8 maize stored across all storage materials was not significantly different. However, highest root length (15.38 cm) of this variety was observed on the seedling of maize grains stored in tin silo at cool temperature. Seedlings of PYASYN8 provitamin A maize stored at room temperature (25±5ºC) in plastic container had the shortest root length (14.43 cm). The highest shoot length (19.08 cm) was observed on the seedling of PYASYN8 grains stored in Aluminium foil at room temperature (25±5ºC) (Table 5). Seedlings of this the grains stored at room temperature in tin silo had the shortest shoot length (15.69 cm).

Discussion

In this study, fungal contamination of three varieties of maize seeds were compared with the rate of seed germination, it was observed that provitamin A maize grains were associated with fungal pathogens and spoilage organisms (during storage), which did not affect the viability of maize grains after the storage period. This observation is different from the report of Mirsam *et al*. (2021), on the investigation of indigenous fungi from maize as a potential plant growth promoter and its role in *Fusarium verticillioides* suppression in maize. The pathogenic fungi associated with maize grains were reported to cause abnormal seed germination or inability of contaminated seeds to germinate. In a similar study, certified and farmer-saved maize varieties were reported to be contaminated with toxigenic fungi, which could contribute to phytotoxic effects, such as poor seed germination, and inhibition of root or hypocotyl elongation, as well as poor seedling performance (Erasto *et al*., 2023).

Due to the need to store maize grains for subsequent cultivation, it has become essential to screen for superior provitamin A–biofortified hybrids (Maazou *et al*., 2022), which will adapt to various environmental conditions, especially, biotic pressure

Table 2: Influence of storage conditions on incidence of fungi associated with provitamin A maize varieties

		Fungal incidence (%)														
		PYASYN2					**PYASYN6**					**PYASYN8**				
Storage temperature	**Storage material**	***Aspergillus niger***	***Rhizopus stolonifer***	***Aspergillus fumigatus***	***Aspergillus flavus***	***Curvularia lunata***	***Aspergillus niger***	***Rhizopus stolonifer***	***Aspergillus fumigatus***	***Aspergillus flavus***	***Curvularia lunata***	***Aspergillus niger***	***Rhizopus stolonifer***	***Aspergillus fumigatus***	***Aspergillus flavus***	***Curvularia lunata***
5ºC	AL	26.67a	43.33a	30.00a	13.33a	40.00a	30.00a	50.00a	33.33a	13.33a	23.33a	13.33ab	23.33ab	23.33ab	13.33a	26.67a
	PL	20.00a	23.33ab	23.33a	13.33a	46.67a	16.67b	23.33ab	26.67ab	16.67a	26.67a	23.33a	36.67a	26.67ab	13.33a	13.33b
	TS	16.67a	13.33b	36.67a	13.33a	13.33b	20.00b	20.00b	30.00a	13.33a	10.00b	20.00a	23.33ab	30.00a	10.00a	26.67a
±5ºC	AL	13.33a	13.33b	26.67a	10.00a	30.00ab	23.33ab	13.33b	26.67ab	13.33a	26.67a	13.33ab	13.33b	33.33ab	10.00a	30.00a
	PL	13.33a	20.00b	20.00a	10.00a	13.33b	40.00a	23.33ab	33.33a	10.00ab	10.00a	16.67a	36.67a	40.00a	13.33a	13.33b
	TS	13.33a	20.00b	26.67a	16.67a	13.33b	26.67ab	30.00ab	20.00ab	10.00ab	10.00b	30.00a	16.67b	36.67a	10.00a	16.67b

Mean values with similar letter(s) within a column are not significantly different at 5 % level of significance using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

AL: Aluminium; PL: Plastic; TS: Tin silo

Table 3: Effect of Storage conditions on seedling properties of provitamin a maize varieties

		PYASYN2		PYASYN6		PYASYN8	
Storage temperature	**Storage material**	**Root length (cm)**	**Shoot length (cm)**	**Root length (cm)**	**Shoot length (cm)**	**Root length (cm)**	**Shoot length (cm)**
Cool (0-5ºC)	Aluminium	19.31a	16.30bc	17.09ab	18.74a	15.09a	18.42ab
	Plastic	14.73c	14.37c	19.30a	16.92ab	14.84a	16.53bc
	Tin silo	14.62c	18.32a	16.81bc	17.52a	15.38a	16.01bc
Room (25±5ºC)	Aluminium	17.59ab	17.31ab	17.91ab	17.08ab	15.80a	19.08a
	Plastic	16.19bc	16.08bc	18.13ab	16.90ab	14.43a	17.83bc
	Tin silo	16.43bc	14.93c	14.67c	14.13b	15.06a	15.69c

Mean values with similar letter(s) within a column are not significantly different at 5 % level of significance using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Maize varieties used in the current study were susceptible to fungal contamination during seed storage, however, the seedling emergence was not significantly affected. This could have been as a result of the ability of selected hybrid seeds to coordinate nutritional crosstalk in presence of microbial contaminants. Assimilation, transport, concentration and remobilization of nutrients in plants have been associated with the ability of host plants to manage microbial infection and nutritional crosstalk (Aluko *et al.*, 2023).

All the three varieties (PYASYN2, PYASYN6 and PYASYN8) screened for fungal contamination were susceptible to fungal contamination, with percentage incidence range from 10 to 50. Although, storage conditions and seed contamination of the three provitamin A maize varieties expressed mild, insignificant effect on the rate of germination, differences observed in seedling performance were significant. Begum *et al*. (2024) extensively reviewed how stressors could exert selective actions on seed germination and performance. Chemicals, either released by contaminants or induced by storage conditions could modulate signaling pathways and cellular processes, thereby disrupting essential cellular functions. Such disruptions sometimes lead to diverse germination and seedling performance outcomes (Begum *et al*., 2024). Consequently, depending on biochemical alterations, influence on enzyme activity and challenges on physiological pathways induced by biotic and abiotic stressors, developmental stages in host plant may be differentially affected.

After the storage period of 120 days, maize varieties were contaminated with *Aspergillus niger, A. fumigatus, A. flavus, Rhizopus stolonifer,* and *Curvularia lunata*. This myco-contamination spanned across the maize varieties, storage materials and temperatures. In addition to their possible effect on maize quality, fungi could produce mycotoxins in maize, which is retained in maize products, even with the removal of toxigenic fungal source (Bryła *et al*., 2022). In addition to mycotoxin production by fungi in stored maize, grain kernels may be affected by other compounds produced directly by fungi, these include trichothecenes, beauvericin, enniatins and other derivatives (De Boevre *et al*., 2012). With an increasing demand in the use of maize, both as direct food source or raw materials for several industries, grain contamination with fungi (during storage) is worthy of note.

Tin silo and Aluminium packages at low temperature appeared to have inhibited the proliferation of fungal contamination in stored grains of the three maize varieties (PYA SYN2, PYA SYN6 and PYA SYN8). Tefera *et al*. (2011) and Worku *et al*. (2022) investigated the extended storage of maize grains in flexible hermetic bags, as well as in metal silo at self-generated modified atmosphere, and reported the inactivation of insect pests and molds by the storage materials. The use of metal silo was recommended to prevent storage losses, and safeguard maize quality and nutrients during storage (Tefera *et al*., 2011). This was proposed to enhance food security in developing countries. Several methods, including the use of Aluminium packages, polythene, and raffia bags, have been investigated for postharvest storage of grains, especially soybean seeds (Baributsa and Baoua, 2022). These packaging materials were reported as effective for the extension of grain shelf-life and seed viability. Studies on the storage conditions of commercially significant crops like beans, soybeans, and rice, revealed that reduction in storage temperature decreased the rate of metabolic and biochemical reactions, as well as improved grain viability and quality characteristics (Srikaeo *et al.*, 2016).

CONCLUSION

AluminiumThe study revealed that all provitamin A maize varieties stored at cold temperature and room temperature in plastic container, Aluminium foil and tin silo were contaminated with seed-borne fungi. *Rhizophus stolonlfer* expressed the highest incidence in this study, confirming it as the most dominant fungi affecting provitamin A maize grain, followed by *Aspergillus* spp. Although, storage temperature, materials and seed contamination of the three provitamin A maize varieties expressed no effect on the rate of germination, differences observed in seedling performance (shoot and root length) were significant. The best adoptable storage conditions observed for the maize varieties, in order to mitigate against fungal contamination and improve seedling performance, was the preservation in tin silo or Aluminium packages at low temperature (0 – 5°C). However, taking cognizance of the susceptibility, viability and performance of infected maize, it is essential to investigate the influence of biotic (especially, fungi species) and abiotic stressors on vitamin A content of provitamin A maize varieties during storage.

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