EFFECT OF DIFFERENT STORAGE MATERIALS AND CONDITIONS ON SEED-BORNE FUNGI ASSOCIATED WITH COWPEA, *VIGNA UNGUICULATA* L. WALP

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SUMMARY

The effect of different storage materials and conditions on seed-borne fungi associated with cowpea, *Vigna unguiculata* L. Walp was evaluated in this study. Ife brown variety of cowpea seeds of 10g were weighed into five different storage materials: jute bag, envelope, polythene bag, muslin cloth and rubber plastic. These were stored in Refrigerator and ambient temperature; subjected to 4^0C and 27 $\pm$ 2ºC respectively, and taken for seed borne fungi identification at 1, 2, 3, 4, 5 and 6 months. The effect of seed-borne fungi on viability of the seeds was determined. The experiment was laid out with 3 replicates in 2 x 5 factorial in Completely Randomized Design (CRD). Data collected were subjected to analysis of variance using SAS package and means separation by Duncan Multiple Range Test. It was observed that seeds stored in refrigerating had significantly higher germination compared to ambient condition, while incidence of fungi increase as period of storage increases in ambient condition compared to refrigerating condition that decrease as the period of storage increases irrespective of the materials. The results showed that the storage materials and conditions influenced viability and seed-borne fungi in cowpea seed. The most effective among the storage materials is polythene bag at 4^0C. Cowpea seeds were associated with many fungi in ambient condition which led to reduction in seed viability. It is therefore important to store cowpea seeds in polythene bag in a

refrigerating condition in order to maintain the seed viability and prevent establishment of seed-borne fungi on cowpea seeds.

Keywords: fungi, cowpea, storage conditions, storage materials, seed viability

COWPEA (*Vigna unguiculata* (L.) Walp) commonly known as bean in Nigeria, is an herbaceous plant belonging to the Leguminosae. Cowpea is the most important legume in the diet of the population and also the cheapest source of protein to most of the people in Nigeria. It provides a rich source of proteins and calories as well as minerals and vitamins. A cowpea seed consists of 25% protein and is low in anti-nutritional factor (18).

One major limitation to cowpea is disease attack; cowpea suffers most from diseases from its vegetative phase of its growth to its harvesting period and in storage. Cowpea production in Nigeria is constrained to a large extent by infection of a wide range of diseases. These diseases of cowpea induced by viruses, bacteria, fungi, nematodes and adverse environmental conditions have brought about the use of pesticides usually in large quantities in an effort to combat them and preserve the cowpea seed for future purposes. The cost of these pesticides is making cowpea production prohibitive.

Among the numerous pathogens affecting cowpea, fungi are the most numerous. The field fungi of cowpea invade seeds and the various parts of the plants on the field. High moisture in seeds which usually occurs as a result of wet weather before or during harvesting, lack of drying facilities and improper transportation and storage aids the proliferation of mycoflora and leads to deterioration of the seeds. However, it is observed that storage fungi affect the seeds in storage even under a very low moisture content compared with the field fungi which required high moisture content in seed. Field fungi of cowpea are *Alternaria* species, *Fusarium* species, *Aspergillus* species *and Penicillum* species. The major seed borne fungi of cowpea are *Aspergillus candidus, Aspergillus flavus, Aspergillus tamari, Aspergillus niger and Aspergillus fumigatum* (16).

Seed-borne pathogens of cowpea may be carried either inside the seed in form of mycelia, on the surface of seeds in forms of spores or mixed with seeds as modified structure such as Sclerotia or as contaminants (2). However, their role is either

unknown or significant affecting germination of seedlings (15). Seed-borne fungi pathogens may cause damaged such as seed abortion, shrunken, seed rot, sclerotisation, seed necrosis, seed discolouration and reduced germination and vigour, failure to control such diseases may result in low yield (22). Shetty (20) reported that seed borne fungi such species of *Fusarium, Aspergillus, Penicillum* and *Diplodia* cause seed decay, discolouration and reduction of seed germinability. In Nigeria, the fungi deterioration of cowpea stored under different storage materials and various conditions were assessed and it was found that *Fusarium* species and *Aspergillus* species was the most frequent and prevalent mould (16).

Cowpeas' susceptibility to diseases from its vegetative phase to storage exposes the seeds to various infections by fungi pathogens and this resultantly affect the viability of the seeds. Storage materials and conditions are major determinants of the save keeping of seeds for any planting season and for consumption. Different storage materials and conditions which include room temperature, cold room and refrigerator have been used to determine the longevity of cowpea seeds without spoilage and disease infection (13). Some species of fungi which include *Fusarium* spp, *Aspergillus* spp, *Penicillium* spp and *Drechslera* spp are able to survive in infected cowpea seed in storage and maintain its viability for over five years (10).

Therefore, the identification of the appropriate storage materials and conditions shall provide information on storage systems for high viability and prevention of the growth of seed-borne fungi associated with cowpea. The objective of this study was to determine the effects of different storage materials and different storage conditions on seed-borne pathogens of cowpea seeds. Also, to determine the viability of seeds packed in these storage materials and conditions.

MATERIALS AND METHODS

Ife brown variety of cowpea seeds which were harvested a month prior to the time of this study; were collected from the Seed store of Institute of Agricultural, Research and Training, Moor Plantation, Ibadan, Nigeria. The experiment was carried out at the Pathology laboratory of the Institute. Prior to storing the seeds in the different materials and conditions; Potato dextrose agar (PDA) of 9.8g was weighed into a conical flask containing 250ml sterile distilled water, then sterilized for 15 minutes

at 1.05 kg/cm^2 (121^{o}C) in an autoclave.

A 0.3 g of streptomycin was added to the cooling PDA and then poured into Petri dishes. Seeds were surface sterilized and 10 seeds (with average weight of 2.4g) were placed each into the four PDA Petri dishes, to determine the mycoflora on the seeds before storage. These were incubated for 4 days for the growth of seed-borne fungi. These organisms were then sub-cultured to get pure cultures of the organism. The slides were prepared for identification of the organisms.

Before subjecting the seeds to different storage conditions and materials, seed germination test was done to determine the viability of the seeds. Ten seeds (which weighed 2.4g on the average) of Ife brown cowpea were placed on moistened filter paper inside sterile Petri dishes. It was left for 24 hours and germination count was done at 24hours interval for four days. This was also repeated when the seeds were subjected to the different storage conditions and materials.

$$\text{Germination percentage} = \frac{\text{Total number of seedling that emergence}}{\text{Total number of seeds}} \times 100$$

Ife brown cowpea seeds of 10 g each were weighed into five different storage materials which include: - Jute bag, Envelope, Polythene bag, Muslin cloth and Rubber plastics. These were stored on the Cold room shelf (4^{o}C) and at ambient (room) temperature (27 $\pm$ 2^{o}C). These were replicated three times. These were stored individually in the materials and under the two conditions for 1, 2, 3, 4, 5 and 6 months (i.e. for a storage condition, each material was used to pack differently for the 6 months and replicated three times). The packed seeds in the refrigerator and at ambient temperature were stored and taken for seed borne fungi isolation and identification as well as viability test at 1, 2, 3, 4, 5 and 6 months.

Detection of Seed-Borne Fungi

Ife brown cowpea seed of 10g were removed from each of the storage materials at the two storage conditions (refrigerator and ambient temperature). The seeds were surface- sterilized in 0.5% NaOCl for 5 minutes, rinsed in 3 changes of sterile distilled water, towel dried and plated on freshly prepared PDA amended with streptomycin (of 0.3g/L) in 9 cm Petri dishes, to prevent bacterial growth and incubated for 5 days. Sub-culturing

was done to get pure culture of seed borne fungi which were examined microscopically and identified using a fungi compendium as a guide (Barnett and Hunter, 1998).

Statistical Collection and Analysis

Data were collected include: germination count, isolate(s) identification, fungi colony were collected. The experiment was laid out in three replicates in a 2 x 5 factorial in Completely Randomized Design (CRD). The data collected were subjected to analysis of variance using SAS package (2001), and the means separation was done using Duncan Multiple Range Test.

RESULTS

Occurrence of Some Fungi Found in Cowpea Seeds Stored in Different Storage Materials and Conditions

It was observed that *Aspergillus* occurred in both storage conditions, but most frequently in ambient condition when compared with the refrigerating condition. The seeds stored in muslin cloth and jute bag had differences species of *Aspergillus* which include *Aspergillus flavus, Aspergillus niger, Aspergillus tamari, Aspergillus candidus, Aspergillus parasiticus and Aspergillus terreus*. While fungi observed in seeds stored in refrigerating conditions have *Aspergillus* species but not as much as in that of ambient condition. The other seed-borne found were *Alternaria* spp., *Fusarium* spp, *Tighemiomcyces*. It was also observed that the occurrence of fungi in cowpea seeds at refrigerating condition were not as much as that of ambient condition. Table 1 shows the list of seed-borne fungi isolated from cowpea seeds stored with polythene bag, jute bag, muslin cloth, rubber can and envelope at ambient and refrigerating conditions after 6 months of storage.

Table 1: Fungi present in cowpea seeds stored using different storage materials and conditions at 6 months after storage

	Refrigerating condition					Ambient condition				
Fungi	P.bag	J.bag	R.can	Enve	M.cloth	P.bag	J.bag	R.can	Enve	M.cloth
Aspergillus flavus	+	-	-	-	-	+	+	-	+	+
A, niger	+	+	+	+	-	-	-	-	-	-
A. parasticus	-	-	+	-	-	-	-	-	-	-
A. fumigatus	-	-	-	-	-	+	-	+	+	-
A. tamari	+	+	+	-	-	-	-	-	+	-
A. candidus	-	+	-	+	+	-	+	+	-	-
A. terreus	-	-	-	-	-	-	-	-	-	-
Alternaria spp	-	-	-	-	+	-	+	+	-	-
Fusarium oxysporum	-	+	-	+	-	+	+	-	-	-
F. equiseti	-	-	+	-	+	+	-	+	-	-
F. semitectum	-	-	-	-	-	-	-	+	-	-
F. solani	-	-	-	-	-	+	-	-	-	-
Tieghemimcyces	-	-	+	+	+	+	-	-	-	+

Absence of organisms = -, presence of organisms = +, P. bag = Polythene bag, J. bag = Jute bag, R. can = Rubber can, Enve = Envelope, M. cloth = muslin cloth

Incidence of Fungi in the Different Storage Materials Under the Ambient and Refrigerating Condition

The percentage incidence of fungi in the control was more than when the two storage conditions were used. *Aspergillus* species was found to occur most in the control with 45.5% incidence. Several fungi such as *Alternaria* species, *Fusarium* species, *Thallospora* and *Tieghemiomcyces* (Table 2) were identified in the cowpea seeds at different incidences. The presence of these organisms indicated that cowpea seeds are vulnerable to many infections which reduces the seeds quality thereby affecting the germinability of the seeds. The percentage of *Aspergillus* species, *Fusarium* species, *Alternaria* species and other seed-borne fungi constantly increase and became major contaminants on the seeds in

control and ambient condition while the percentage incidence of fungi on seeds at refrigerating condition decreases. Therefore, the distribution of isolated organism from cowpea seeds depend on the storage material.

Table 2: Incidence (%) of Fungi in the different storage materials at ambient and refrigerating condition

	Refrigerating condition						Ambient condition				
Fungi isolated	P.bag	J.bag	R.can	Enve	M.cloth	Control	P.bag	J.bag	R.can	Enve	M.cloth
Aspergillus species	22.7	18.2	18.2	9.1	4.5	45.5	9.1	13.6	18.2	27.3	9.1
Alternaria	0	0	0	9.1	9.1	4.5	0	9.1	4.5	0	0
Chalara	0	0	0	0	0	4.5	0	0	0	0	0
Fusarium	0	9.1	4.5	4.5	9.1	18.2	13.6	4.5	4.5	0	0
Thallospora	0	0	0	0	0	4.5	0	0	0	0	0
Tieghemiomcyces	0	0	4.5	9.1	4.5	13.6	4.5	0	0	0	4.5
Total	22.7c	27.3c	27.2c	31.5b	27.2c	90.8a	27.2c	27.2c	27.2c	27.3c	13.6d

P. bag = Polythene bag, J. bag = Jute bag, R. can = Rubber can, Enve = Envelope, M. cloth = muslin cloth

Effect of Different Storage Materials and Conditions on Viability of Cowpea Seeds

The cowpea seeds stored in Polythene bag at refrigerating condition had the best average germination percentage of 92.2% while the ones stored inside Jute bag, muslin cloth and Envelope in ambient condition had 0% germination, no germination at all at 6th month after storage (Table 3). The different storage materials at ambient condition have detrimental effect on the viability of cowpea seeds because the germination count, especially at 3months (12 WAS) obtained were not significantly different when comparing the storage materials at ambient condition, and the values reduced with increase in storage duration.

At refrigerating condition, there were no significant differences in germination counts among the storage materials except at 16 and 24

WAS. All storage materials were significantly higher than control except rubber can and Polythene bag at 16WAS and Jute bag at 24 WAS. Seeds stored in all storage materials at refrigerating condition had significantly higher values of germination as from 12 WAS except rubber can at 16 WAS.

Table 3: Effect of storage material and conditions at varying storage duration on germination count (%) of cowpea seeds

Storage materials		Germination percentage					
		4WAS	8WAS	12WAS	16WAS	20WAS	24WAS
Polyethylene bag	(A)	100.0a	90.0a	70.0b	0.00c	83.3c	83.3a
	(R)	100.0a	96.7a	100.0a	90.0ab	96.7ab	93.3a
Jute bag	(A)	100.0a	93.3a	0.00c	0.00c	0.00d	0.00d
	(R)	100.0a	93.3a	96.7a	100.0a	100.0a	70.0b
Rubber can	(A)	90.0a	100.0a	63.3b	100.0a	86.7bc	00.0d
	(R)	96.0a	100.0a	93.3a	86.7b	100.0a	96.7a
Muslin cloth	(A)	90.9a	90.0a	00.0c	00.0c	00.0d	00.0d
	(R)	100.0a	100.0a	100.0a	100.0a	100.0a	53.3c
Envelope	(A)	100.0a	100.0a	0.00c	0.00c	0.00d	0.00d
	(R)	96.7a	100.0a	100.0a	100.0a	96.7ab	76.7b
	(C)	80.0b	90.0a	70.0b	83.3b	70.0c	70.0b

Means in the same column followed by same letter are not significantly different by Duncan Multiple Range Test at 0.05 level of significance.

A = Ambient condition, R= Refrigerating condition, C = Control (seeds kept in an open tray at ambient temperature), WAS= Weeks after storage

Frequency of Seed-Borne Fungi Due to Each Storage Material and Condition of Cowpea

The frequency of seed-borne fungi on cowpea in response to the storage materials and conditions were taken at 1, 2, 3, 4, 5 and 6 months separately. At 6 months, irrespective of the storage conditions, *Aspergillus* spp occurred most in control with 100% incidence (Table 4), followed by. Several fungi including species of *Fusarium, Alternaria, and Tieghemiomcyces* were identified on cowpea seeds occurred at different

incidences. Incidence of *Aspergillus* species, *Fusarium* species, *Alternaria* species and other organisms constantly increase and became contaminants on the seeds at both conditions while the occurrence of fungi in seeds in refrigerating condition decreases (Table 3).

In refrigerating condition, A. flavus occurred only in the polythene bags while *A. niger* occurred in all the storage materials used except in the muslin cloth. No incidence of *A. fumigatus* at refrigerating condition but was found in all the storage materials used except in jute bag and muslin clot. For the prevention of any specie of Aspergillus, it is better to store cowpea seeds in muslin cloth especially at ambient condition. Envelope had the highest number of species of Aspergillus especially when stored at ambient condition. *F. semitectum* and *F. solani* were not observed at refrigerating condition in all storage materials, but present at ambient condition.

Aspergillus species had the highest incidence both at refrigerating and ambient conditions. No Fusarium specie was found on the cowpea seeds when stored in polythene bags at refrigerating condition.

Table 4: Occurrence of fungi observed on the cowpea seeds stored in different materials and conditions at 6 months of storage

Fungi isolated	Refrigerating condition							Ambient condition					
	P.bag	J.bag	R.can	Enve	M.cloth	Total	Control	P.bag	J.bag	R.can	Enve	M.cloth	Total
Aspergillus flavus	1	0	0	0	0	1	2	1	2	0	2	1	6
A. niger	2	2	1	2	0	7	2	0	0	0	0	0	0
A. arasiticus	0	0	2	0	0	2	2	0	0	0	0	0	0
A. fumigatus	0	0	0	0	0	0	0	1	0	2	1	0	4
A. tamari	1	1	1	0	0	3	2	0	0	0	2	0	2
A. candidus	0	1	0	1	1	3	1	0	1	1	0	0	2
A. erreus	0	0	0	0	0	0	1	0	0	1	0	1	2
Subtotal	4	4	4	3	1	16	10	2	3	4	5	2	16
Alternaria spp	0	0	0	0	2	2	1	0	2	0	0	0	2
Fusarium oxysporum	0	1	0	1	0	2	2	1	1	0	0	0	2
F. equiseti	0	1	1	0	2	4	0	1	0	1	0	0	2
F. semitectum	0	0	0	0	0	0	1	0	0	1	0	0	1
F. solani	0	0	0	0	0	0	1	1	0	0	0	0	1
Subtotal	0	2	1	1	2	6	5	3	1	2	0	0	6
Tieghemimcyce	0	0	1	2	1	4	1	1	0	0	0	1	2

P. bag = Polythene bag, J. bag = Jute bag, R.can = Rubber can, Enve = Envelope, M. cloth = Muslin cloth

Effect of Time of Storage in the Different Materials and Conditions on the Incidence of Fungi on Cowpea Seeds.

There were significant differences on the fungi isolated after varying time of storage of cowpea seeds at the two storage conditions. The incidence of fungi in ambient condition increased generally with the time of storage, particularly the seeds stored in jute bag (Table 5). At refrigerating condition, the incidence of fungi decreased with storage time irrespective of the storage materials. There was decrease in seed-borne fungi as the storage time progressed in the refrigerator for all seed lots irrespective of the materials. Ife brown cowpea seeds stored in Muslin cloth recorded 10.00 value at 24 weeks after storage compared to other seed lots.

Table 5: Effect of storage materials and conditions at varying storage period on incidence of fungi on cowpea seeds.

Storage material		Incidence of fungi on cowpea seeds					
		4WAS	8WAS	12WAS	16WAS	20WAS	24WAS
Polythene bag	(A)	9.00a	5.33bcd	6.33b	9.67a	7.67c	10 00a
	(R)	5.0a	4.67cd	4.67ab	3.00c	4.67bcd	2.33bc
Jute bag	(A)	7.67a	8.33ab	8.67c	9.67a	10.00a	10.33a
	(R)	7.00a	7.33bc	6.67a	4.33c	4.0d	2.67bc
Rubber can	(A)	8.00a	7.33a	7.33b	8.00b	8.67ab	9.00a
	(R)	6.33a	3.33b	3.33a	8.67b	3.67d	3.33d
Muslin cloth	(A)	8.67a	10.00a	7.33a	10.67a	10.00a	10.00d
	(R)	5.33a	5.67bcd	4.67ab	3.00c	2.67d	2.33bc
Envelope	(A)	9.0a	8.00ab	6.33a	8.67b	9.00a	9.33a
	(R)	5.67a	6.00bcd	5.67ab	5.33ab	4.33ab	2.67bc
	(C)	6.00a	9.00a	7.00ab	8.33b	9.33a	9.00a

Means in the same column followed by same letter are not significantly different by Duncan Multiple Range Test at 0.05 level of significance. A = Ambient condition R= Refrigerating condition, C = Control, WAS= Weeks in storage.

DISCUSSION

Several authorities in the field of Seed Science have identified seed viability and seed-borne pathogens as components of any assessment of seed quality. In this study, considerable variations occurred among the storage materials and between storage conditions for seed viability and associated seed-borne fungi. The significant differences observed in the storage materials and conditions were mostly due to the fact that Cowpea seeds undergo various modifications from germination to development on the field, maturation and storage.

The occurrence of *Aspergillus spp* in the two storage conditions showed that the storage conditions along with the materials used to store the seeds may not be the idea for the healthy preservation of the seeds. This is supported by the report of (8) who stressed that due to bad storage conditions and structures, cowpea attracts a number of fungi during storage, under hot and humid conditions, a number of storage fungi mainly belonging to *Aspergillus* and *Penicillum* species get associated with the grains. The predominant fungi storage species also mentioned by (18) include *Aspergillus niger, Aspergillus fumigatum, Aspergillus flavus, Aspergillus sadowl, Aspergillus tamari, Pencillium islandicum* and *Pencillium cycloplum.*

When rubber plastic can was used to store the seeds in refrigerating and ambient conditions, it had highest number of seed-borne fungi which indicates that the material used is not ideal while the seeds stored in polythene bag in the refrigerator had the least number of fungi. This implies that to store cowpea seeds under refrigeration, they must be kept inside polythene bags. At ambient condition, muslin cloth and envelopes are better to use as the incidence of fungi in the two materials were not significantly different from each other. It is important to note that polythene bags and rubber can should not be used to store seeds at ambient condition. The heat built up in these materials may have made the seeds wet and this is conducive for the growth of seed-borne fungi such as *Aspergillus spp, Alternaria spp* and *Fusarium spp.* Bretag *et al*., (6) reported *Aspergillus flavus* as a common fungus from mouldy legume and found *Alternaria alternaria* as the predominate fungus followed by *Aspergillus flavus* and *Fusarium* Species.

Storage fungi are usually not present in large quantities before harvest. Contamination occur through small

quantities of spores infecting the cowpea grain as it is going into storage from the harvest handling and storage equipment or from spores already present in storage structures under high temperature and moisture this small amount of inoculum can increase rapidly (1). It was observed that as from 12 WAS at ambient condition, seeds stored inside envelope, jute bags and muslin cloth lost their viability while the seeds kept under refrigerating condition irrespective of materials used to store the seeds had above 70% germination and least germination was observed in the rubber cans used. This buttresses the observation that rubber can used in this study was not suitable for storing cowpea seeds in the refrigerator because it had the highest number of fungi growing on the seeds. These fungi contaminated the seeds and hindered its viability. Lopez and Christensen (11) reported that infected seed samples lost their ability to germinate within few weeks or months especially in case of unhealthy seed usually invaded by storage fungi. The natural contamination of seeds with seed-borne fungi plays a vital role in deterioration of seed quality (1). All species of *Fusarium* genera reduced emergence and root growth move than shoot growth, whereas *Fusarium oxysporum* reduced shoot growth more than emergence (6, 9). *Fusarium equiseti* was the most sever pathogen among the fusaria and was also very common in seed samples.

At 24WAS, polythene bags and rubber cans were the best materials for storing seeds, these maintained the viability of the seeds under refrigerating condition. Christensen (7) reported that viability of mould free grain was not at all affected by the length of storage, the temperature and moisture content. At 4 weeks after storage there are no significant differences in seeds viability and seed-borne fungi compared to 24 weeks after storage in certain materials and conditions. It was review from the study that cowpea seeds differ in their seed viability response to period of storage and conditions.

Incidences of fungi on seed gradually increase with increased in storage days at ambient condition irrespective of the material used for storage. A sharp decline in viability and incidence of fungi after 24 weeks of storage was observed suggesting that Cowpea seed stored under ambient conditions should not exceed 6 months for the maintenance of maximum seed viability irrespective of the storage materials. Variation occurs in germination among the cowpea seeds at each

storage conditions and materials at ambient condition. Superior germination was obtained at refrigerating conditions irrespective of the materials.

It was observed that seed stored in refrigerating condition have higher germination comparing to ambient condition while the incidence of fungi increase as the period of storage increases in ambient condition comparing to refrigerating condition that decrease as the period of storage increases irrespective of the materials.

The results suggested that the storage materials and conditions influence viability and seed-borne fungi in cowpea seed. The most effective among the storage materials is polythene bag at both conditions. Cowpea seeds are associated with many fungi in ambient condition which leads to reduction in seed viability and caused increase in infestation of seed-borne fungi.

CONCLUSION

Irrespective of the storage conditions and materials used to store cowpea seeds, the storage fungus *Aspergillus* species were found in abundance in all. Polyethylene bag is better storage material than rubber plastic at refrigerating and ambient conditions though with incidence of fungi while in ambient condition, muslin cloth and envelopes had lowest fungi incidence. Seeds stored in refrigerating condition had higher germination than the ambient, while the incidence of fungi increases in the ambient condition but decreases wit the period of storage.

It is important to store cowpea seeds in polythene bag in a refrigerating condition in order to maintain the viability and minimize or eliminate the establishment of seed-borne fungi.

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