EFFECT OF PACKAGING MATERIALS AND STORAGE CONDITIONS ON SEED-BORNE FUNGI IN STORED KENAF (*HIBISCUS CANABINNUS.* L) SEEDS

AKINBODE, O. A[1*]., BALOGUN, M. A[2]., KAREEM, K. T[1]. DADA A. O[1]. AND OLAGUNJU Y. A[1].

[1]Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan. [2]Federal College of Agriculture, Moor Plantation, Ibadan

**foyedeji@yahoo.com*

ABSTRACT

Despite an increase in production and utilization of kenaf products in recent years, there is a problem with low-quality seeds due to scanty information on kenaf seeds storage. This experiment aimed at determining the best material for storing kenaf seeds and to determine the best storage condition that will hinder the proliferation of fungi on kenaf seeds. Kenaf seeds (var IFE KEN) were collected from the Institute of Agricultural Research and Training, IAR&T, Nigeria. The seeds were packed in six (6) different storage materials and kept at ambient and freezing conditions of 27 $\pm$ 2°C and 4°C respectively, for 6 months, seed-borne fungi isolation and identification, and viability tests were carried out. The experiment was a 2 x 7 factorial experiment laid out using a Completely Randomized Design (CRD). Isolation of fungi was carried out using the agar method. Data collected were subjected to descriptive statistics and analysis of variance, while means were separated using the Duncan Multiple Range Test at a 5% probability level. Results showed that the polyethene bag recorded the highest germination of IFE-KEN 400 kenaf seeds (90%), followed by the envelope after being stored in the two storage conditions for 6 months. All the storage materials used to store the seeds at ambient conditions had fungal incidence throughout the period of storage. Thus, it is advised to store kenaf seeds in a polyethene bag at 4^0C to maintain the seeds' quality in storage for seed viability preservation and to reduce the establishment of fungi on the seeds.

Keywords: Storage conditions, Fungal incidence, Kenaf seeds, Seed viability

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a common warm-season annual fibre plant found in tropical and subtropical Africa and Asia. Kenaf can be processed into various products such as building materials, furniture, clothing, car components, biofuel and animal feed (Samson and Pitt, 2000). In Nigeria, kenaf is a drought-resistant crop with a relatively wide range of adaptation, which ranges from soil types, and good tolerance to salinity, water stress and logging. However, it is susceptible to climate extremes and to pests and diseases (Aimin, 2006). Kenaf is a veritable source of making strong ropes for drawing water from wells. It has the potential to become one of the main crop plants and sources of income in the country. Due to its extensive and diverse uses, kenaf has remained one of the most useful materials to both local farmers and agro-allied industrialists. Even though kenaf is resistant to many types of diseases, it is susceptible to some pathogens in the groups of fungi, bacteria, nematodes and viruses (Basri *et al.*, 2014). Previous studies on diseases of kenaf reported pathogens such as *Rhizoctonia solani, Sclerotium rolfsii, Phytopthtora parasitica, Ralstonia solanacearum,* and nematodes (Basri *et al.*, 2014) have association with Kenaf disease infections.

In Nigeria, many attractive incentives were offered to kenaf farmers to encourage them to grow the crop. However, kenaf production witnessed a setback as a result of the instability of market prices and the apparent glut in the market due to serious competition with imported products. In recent years, kenaf production, processing and utilization is on the increase, but poor seed quality is used by farmers, and scanty information is available on the storability of the seeds. Proper storage of seeds is an important process, which starts after harvest to avoid deterioration. Storage conditions with the main focus on temperature and relative humidity are essential to preserving high seed quality (Rahmawati and Muhammad, 2020).

The longevity of seeds in storage is a good indicator of seed viability and seed vigour in many crops (Adebisi *et al.*, 2008). It is already well known that seed longevity is a function of storage temperature and seed moisture content (Robert, 2003), stress before seed storage and initial seed quality (https://agriinfo.in/factors-affecting-seed-longevity-in-storage-2334/ retrieved online 15/11/2021), genetic makeup (Adebisi *et al.*,2004), pests and pathogens damage in storage. Seeds are stored for a few days, weeks or year during which it deteriorates, moving inexorably towards death (Greg *et al.*, 1994). Kenaf seed, being high in oil content, is prone to deterioration if improperly kept.

A major storage constraint to kenaf production is the poor viability due to the high oil contents of the seeds. Even in good storage, the signs of physiological deterioration in terms of slower germination and reduced seedling growth of carryover seeds are apparent (Genes and Nyomora, 2018; Mohd Sahwahid *et al*., 2012). However, when care is taken during storage in a good environment, seed deterioration occurs at a reduced rate (Pradhan and Badola, (2012). The negative cumulative normal distribution pattern of seed deterioration exists at specified storage conditions. Such differences in deterioration have been revealed in the storability of soybean (Thseng *et al*., 1996 cited by Adebisi *et al*., 2013; Akintobi *et al.*, 2004). The ability to monitor seed deterioration is important for the proper management of seed longevity in stores and germplasm repositories. It also helps in decision-making in gene bank management.

Seed-borne pathogens actively attack seeds and may be harmful due to the secretion of mycotoxins by different fungi. Seeds can be infected by pathogens that colonize the seeds both externally and internally (Adebisi *et al.*, 2008). The infection of seeds by micro-organisms (e.g. fungi, bacteria and viruses) has adverse effects on seeds, such as seed germination losses. Germination loss due to seed infection may indirectly decrease crop yields by interrupting radicle emergence from the seed coat, thereby reducing the overall crop stand (Khazanda *et al.*, 2002). Infections occurring after post-emergence (i.e. after the seedling emerges from the soil) include root rot, cotyledon rot and basal stem rot (Mithal and Mathur, 2003). Swart and Tarekegn (2007) reported that *Fusarium verticillioides*, cause damping off on Kenaf in South Africa. The damage to seedlings and the interruption of plant development results in systemic or local infections of seed-borne pathogens in the later stage (Khazanda *et al*., 2002).

Kenaf production in southwest Nigeria is hampered by the extent to which the seeds lose their viability when exposed to warm, moist air that characterizes ambient humid climate. To arrest the rate of seed deterioration under the prevailing adverse storage conditions, seeds are maintained in dry cool condition stores for conservation of genetic resources, a facility that is expensive, uneconomical and unaffordable by the poor resource farmers. This makes kenaf seeds storage an expensive venture for any resource-poor farmer. Hence, it is imperative to devise an appropriate system of storage in which the seed-borne pathogens' proliferation is hindered. As it is a known fact that the storage life and viability of kenaf under the prevailing conditions in Southwestern Nigeria are determined by good management of kenaf resources. This study was aimed at identifying the effect of different storage conditions and different storage materials on kenaf seed-borne fungi and investigating the effect on the viability of seeds packed in them.

MATERIALS AND METHODS

Source of Seed and Storage Materials

Freshly harvested kenaf (Variety - IFE KEN) seeds were collected from the Kenaf and Jute Improvement Programme (KJIP) of the Institute of Agricultural Research and Training, Ibadan, Nigeria. The storage materials used to pack the seeds were: jute bag, aluminium can, envelope, polythene bag, muslin cloth, covered plastic (rubber) plates and were swabbed with 70% ethanol. The storage conditions used were: ambient and freezing conditions of 27 $\pm$ 2^{o}C and 4^{o}C respectively. Five (5.0) grams each of the kenaf seeds were weighed into the six different storage materials and were replicated three times. The seeds packed in different storage materials (Table 1) were thereafter kept in the refrigerator and laboratory desk at the Pathology laboratory of the Institute of Agricultural Research and Training by subjecting them to 4^{o}C and 27 $\pm$ 2^{o}C temperatures respectively. The control experiment was set up using sack material and kept on the laboratory desk. The packed seeds were stored for six months and taken for seed-borne fungi isolation and identification at 1, 2, 3, 4, 5 and 6 months after being stored. A seed viability test was also done to know the influence of seed-borne fungi on the germination of the seeds. Isolation and identification of fungi were done using the agar method. A 9.8 g of Potato Dextrose Agar (PDA) was weighed into a large conical flask and 250 ml of water was added to it. It was corked with cotton wool and wrapped with aluminium foil. The medium was sterilized for 15 minutes at 1.05 kg/cm^2 and 121^{o}C in an autoclave. The experiment was carried out at the Pathology Laboratory of the Institute. The experiment was laid out in a 2 x 7 factorial experiment fitted into a Completely Randomized Design and replicated three times.

Table 1: Arrangement of the kenaf seeds stored in different storage materials and condition

	Arrangement of Seeds in Storage Materials and Conditions
IFKJR	Seed stored in Jute bag in Refrigerator
IFKPR	Seed stored in Polythene bag in Refrigerator
IFKMR	Seed stored in Muslin cloth in Refrigerator
IFKCR	Seed stored in Aluminum can in Refrigerator
IFKRR	Seed stored in covered plastic plates in Refrigerator
IFKER	Seed stored in envelopes in Refrigerator
IFKJA	Seed stored in Jute bag in ambient temperature
IFKPA	Seeds stored in polythene bags at ambient temperature
IFKMA	Seeds stored in muslin cloths at ambient temperature
IFKCA	Seeds stored in Aluminum can at ambient Temperature
IFKRA	Seeds stored in covered plastic plates at ambient temperature
IFKEA	Seeds stored in envelopes in ambient temperature

A seed germination test was carried out to determine the viability of the seeds, this was done before storing the seeds and during storage. Here, 10 seeds were placed on a filter paper moistened with sterile distilled water inside a Petri dish, replicated three times, and left for 72 hours. Germination count was done after 48 hours (It takes 2 - 5 days for Kenaf seeds to germinate on the field and 1 - 3 days in the laboratory) and at the same interval. Germination percentage is computed as follows:

$$Germination\ percentage = \left(\frac{Total\ number\ of\ normal\ seedlings\ that\ emerge}{Total\ number\ of\ seeds\ planted\ (in\ each\ petri\ dish)}\right) \times 100$$

For the seed-borne fungi identification, 10 IFE KEN kenaf seeds were removed from each of the storage materials in the two storage conditions (4^0C and 27 $\pm$ 2^0C). The seeds were sterilized in 0.5% of NaOCl for 5 minutes and then rinsed for 2 minutes in three changes of sterile distilled water before plating. Agar solution was poured slowly to avoid bubbles into the sterile Petri dishes and allowed to form a gel. A 0.3 g of streptomycin was added to the 250 ml of agar to avoid contamination. After solidifying, the surface sterilized seeds were then placed in the Petri dishes for incubation.

The organisms were individually subcultured to get the pure cultures. The pure cultures were identified with the use of physiological and morphological features of the organisms according to the methodology of Barnet and Hunter (1972). Data collected included germination count, colony count, number of organisms found and organisms identified. These were subjected to descriptive analysis and analysis of variance using a statistical analysis system (SAS, 2010). The means were separated using the Duncan Multiple Range Test at p = 0.05.

RESULTS

The seed-borne fungi found in kenaf seeds are stored using different storage materials in two storage conditions

Different fungi were observed on the kenaf seeds stored with the six storage materials in ambient and freezing conditions. It was observed that *Aspergillus* spp occurred most at 4^0C irrespective of the storage materials used. The seeds stored in rubber plates and Aluminium can have different species of *Aspergillus: A. flavus, A. niger, A. candidus, A. parasiticus, and A. fumigatus* at 4^0C. The fungi observed in seeds stored at 27 $\pm$ 2^0C inside polythene bag, jute bag, muslin cloth, envelope, aluminum-can had *Alternaria* spp, *Fusarium* spp, *Hymenella* spp, *Tieghemiomyces* spp in them. Table 2 shows the list of seed-borne fungi isolated from kenaf seeds stored in polythene bags, jute bags, muslin cloths, aluminium cans, envelopes and rubber plates at ambient (27 $\pm$ 2^0C) and freezing conditions (4^0C). The seeds stored at 27 $\pm$ 2^0C had *Alternaria* and *Tieghemiomyces* spp in common, occurring mostly in the storage materials. *Aspergillus* species were found in the different storage materials under different storage conditions. *Aspergillus lucknowensis* was present in freezing conditions (4^oC) and absent in ambient conditions (27 $\pm$ 2^oC). It was also observed that the seeds stored in the envelope at 27 $\pm$ 2^oC had the highest number of fungi and the fungi found in it were: *Alternaria* spp, *Fusarium proliferatum*, *Aspergillus niger*, and *Aspergillus parasiticus*.

The seeds stored in polyethene bags had the highest number of fungi observed on them at 4^oC. *F. oxysporum* was the commonest fungus in jute bags and envelopes at 4^oC. Muslin cloth and rubber plates had only *Aspergillus flavus* and *Aspergillus niger*, respectively in them when stored at 4^oC. At 27^oC, the polythene bag had only two fungi observed in the seeds and had the least number of fungi. Also, the Jute bag, envelope, and muslin cloth had four (4) fungi observed on the seeds. Rubber plates and aluminium cans have 7 fungi at 27 + 2^0C. *Fusarium solani* and *Penicillium* spp occurred most in the storage materials at 27 + 2^0C. *Aspergillus parasiticus* and *F. chlamydosporium* did not occur in all the storage materials used at 27 + 2^0C, while *F. longipes, F. xyloriodes, F. anthophilous, F. solani, Colletotrichum* spp, *Hymenella* spp and *Tieghemiomyces* spp were not observed in all the kenaf seeds stored in all the materials at 4^0C (Table 2).

Incidence of fungi in the different storage materials under the ambient and freezing conditions

It was observed that high percentage of incidences of fungi occurred at the ambient temperature than in the freezing condition at 4^0C, also in the control experiment. At 4 weeks of storage, the incidence (20%) of *Aspergillus* spp in the control was not significantly different from each other at both ambient and 4^0C conditions, while there were variations in the percentage incidences of the other pathogens found at 4^0C and ambient storage conditions. *Fusarium* spp had an 11.0% incidence when the seeds were stored in an envelope at ambient conditions. Polythene and jute bags had an 8.0% incidence each. The incidence of *Fusarium* spp (3.5%) occurred in the rubber plate. However, muslin cloth had the least incidence of seed-borne pathogens when kept in ambient conditions. At 4^oC, *Alternaria* spp was 11.0% in rubber plate followed by polythene bag with a 7.0% incidence. The *Fusarium* spp had a 7.0% incidence when stored inside a jute bag and envelope kept in freezing condition. Muslin cloth had 7.0% and 3.0% *Collectotrichum* spp and *Sclerotium* spp, respectively at 4^oC conditions.

Table 2: The seed-borne fungi found in kenaf seeds after storing for 6 months in different storage materials under two storage conditions

Fungi isolated	Freezing conditions						Ambient conditions					
	P. bag	J. bag	Enve	M. cloth	R. plate	Al can	P. bag	J. bag	Enve	M. cloth	R. plate	Al can
Aspergillus flavus	-	-	+	-	+	-	-	-	-	+	+	-
A. niger	-	+	-	+	-	+	-	-	-	+	-	-
A. parasiticus	-	-	+	-	-	-	-	-	-	-	-	-
A. candidus	+	-	-	-	-	-	-	-	+	-	-	+
F. oxysporum	-	+	+	-	-	-	+	-	-	+	-	-
F. longipes	-	-	-	-	-	-	-	-	-	-	+	-
F. proliferatum	+	-	-	-	-	-	-	-	-	-	-	+
F. graminum	-	-	-	-	-	-	-	-	-	-	+	+
F. xylariodes	-	-	-	-	-	-	-	-	-	-	+	+
F. antholphilum	-	-	-	-	-	-	-	+	+	-	-	-
F. chlamydosporum	+	-	-	-	-	-	-	-	-	-	-	-
F. pallidoroseum	+	-	-	-	-	-	-	-	-	-	-	+
F. solani	-	-	-	-	-	-	-	+	+	-	+	-
Alternaria spp	+	-	-	-	-	-	+	-	-	-	-	+
Penicillium spp	-	-	-	-	-	+	-	+	+	-	+	-
Tieghemiomyces spp	-	-	-	-	-	-	-	+	-	-	-	+
Hymenella spp	-	-	-	-	-	-	-	-	-	+	-	-
Colletotrichum spp	-	-	-	-	-	-	-	-	-	-	+	-

A = Aspergillus, F = Fusarium, P.bag = polythene bag, J. bag = Jute bag, Enve = Envelope, M. cloth = Muslin cloth, R. plate = Rubber plate, Al can = aluminium can, + means presence of fungus, - means absence of fungus. A = Aspergillus, F = Fusarium

Incidence of fungi at 8 weeks after storage in different materials showed that *Aspergillus* spp at 4^0C and *Tieghemiomyces* spp at 27 ± 2^0C had 35.0% incidences under control. The ambient condition had 17.0% of *Fusarium* spp and when the envelope was used as packaging material under this condition, the occurrence of *Meria* spp and *Tieghemiomyces* spp. The best material to store kenaf seed for 2 months at the ambient condition as observed in this study was in Aluminium cans, followed by polythene bags, which had *Fusarium* spp and *Meria* spp of 7.0 and 3.5% incidence respectively. The 4^0C condition had 35.0% of *Aspergillus* spp, followed by *Fusarium* spp of 16.0% in the control. Aluminium cans had a 10.0% incidence of *Meria* spp which was the only organism observed at this stage. *Meria* spp of 10.0% and 7.0% were observed in Aluminium cans and rubber plates stored under 4^oC conditions. *Aspergillus* spp incidence of 7.0% and 4.0% were observed on seeds kept in envelope and jute bags respectively. At 8 weeks of kenaf seeds' storage, the best material to use at 4^0C is polythene bags, while at ambient conditions, the Aluminium can was the best.

At 12 weeks after storage, *Aspergillus* and *Fusarium* species were the most occurred fungi under the two storage conditions with an incidence of 48.0%. Polythene bag, Jute bag and aluminium can had percentage incidence of *Fusarium* spp of 10.0%, 4.0% and 48.0%, respectively. *Penicillium spp* were observed on seeds stored in Muslin cloth with an incidence of 14.0% and rubber plate with 4.0%. Rubber plates kept in an ambient condition had multiple infections of *Sclerotium* spp (3.5 %) *Colletotrichum* spp (4.0%) and *Meria* spp (4.0%). Jute bag had the least *Fusarium* spp (4.0%), but the highest incidence of *Aspergillus* spp was observed when compared with other materials used for storage at ambient conditions. When the envelope was used as storage material, *Tieghemiomyces* spp (5.0%) and *Meria* spp (4.0%) species were observed on the seeds stored in it at ambient conditions. *Fusarium spp* of 17.2% was observed in seeds in an envelope kept at 4^{o}C. This incidence of *Fusarium* spp was followed by muslin cloth used to store seeds which had 13.7%. At 4^{o}C, *Alternaria* spp (3.5%), *Fusarium* spp (3.5%) and *Tieghemiomyces* spp (3.5%) were the multiple organisms observed on seeds stored in the aluminium can at 4^{0}C. The best storage material to store kenaf seeds for 3 months as observed in this study, was the Jute bag, which had only 3.5% incidence of *Fusarium* and *Aspergillus* spp., and closely followed by the aluminium can. However, the aluminium-can material had multiple incidences of fungi (*Fusarium* spp, *Alternaria* spp and *Tieghemiomyces* spp of 3.5% each) on the seeds stored in it.

At 16 weeks after storage, aluminum-can still had the least incidences of pathogens growing on the kenaf seeds. Although the percentage incidence of the pathogens increased with 6.9% in *Fusarium* spp and 3.5% in *Alternaria* spp, compared with the 12 weeks after storage at 4^{o}C. Storage at 4^{o}C greatly reduced the incidence of pathogens that would have occurred on the seeds if they were not stored in this condition. The control had an incidence of 34.7% of *Aspergillus* spp and *Fusarium* spp of 24.2%, but the highest incidence of *Aspergillus* spp was 13.7% in the rubber plate when stored both at 4^{0}C and 27 $\pm$ 2^{0}C.

Aluminum-can had the least *Fusarium* spp (6.9%) and *Alternaria* spp 3.5% at 4^{0}C growing at 20 weeks after storage. The incidence of *Aspergillus* spp in polythene bag (17.2%), envelope (17.2%) and muslin cloth (17.2%) increased compared to when the seeds were kept at 16 weeks after storage. Aluminum-can appeared promising at 4^{0}C, with an incidence of *Aspergillus* spp (10.3%), *Alternaria* (6.9%), and *Fusarium* spp (3.5%) growth on the seeds. The worst storage material used at 4^{0}C was the jute bag which had 4 organisms growing on the seeds: these were *Aspergillus* spp (6.9%), *Alternaria* spp (10.3%), *Hymenella* spp (3.5%) and *Colletotrichum* spp (3.5%), which put the seeds at a greater risk of seed-borne pathogens. The polythene bag had only 17.2% of *Aspergillus* spp and 3.5% of *Sclerotium* spp. Also, at 27 $\pm$ 2^{0}C, the polythene bag had 6.9% of *Aspergillus* spp and 3.5% *Fusarium* spp, indicating the safe keep of the seeds at 4^{0}C. The rubber plate had an incidence of 10.3% of *Aspergillus* spp, 3.5% of *Alternaria* spp and *Tieghemiomyces* spp of 3.5% at 27 $\pm$ 2^{0}C.

At 24 weeks after kenaf seeds storage, the seeds kept as control had multiple incidences of 5 different organisms growing on them. The organisms identified on the seeds were *Aspergillus* spp (20.7%), *Alternaria* spp (20.7%), *Hymenella* spp (6.9%), *Tieghemiomyces* spp (6.9%) and *Meria* spp (3.5%). Storage with the jute bag had the least presence of organisms with only *Alternaria* spp (10.3%) identified. This was closely followed by the rubber plate with 6.9% *Fusarium* spp and 3.5% *Aspergillus* spp. The polythene bag used for kenaf seeds storage at 4^0C had a 3.5% incidence of *Alternaria* spp, 6.9% *Fusarium* spp and 10.3% *Aspergillus* spp at 24 weeks. It was observed that the percentage incidence of the fungi on the kenaf seeds in the different materials stored under the two conditions generally had an increase of up to 20.7% and at least 3.5%.

Effect of storage materials and conditions on viability of kenaf seeds

Results obtained as shown in Table 3 reveals that kenaf seeds stored with different materials at 4^0C and 27 $\pm$ 2^0C influenced the viability of kenaf seeds. At 4 WAS, the viability of the seeds in control plates is high which is not significantly different from the viability obtained from Jute bags, Muslin cloth, Envelope, and Aluminum can but had significant viability of seeds stored in the envelope which had the least viability at both storage condition. At 8 WAS, kenaf seed viability values for Envelope and Aluminium can in freezing conditions were not significantly different from each other but significantly different from those obtained from other storage materials. At 12 WAS, muslin cloth had the highest viability which differed significantly from the viability obtained with polythene bag material at 27 $\pm$ 2^0C, but this did not differ significantly ($p < 0.05$) from the control experiment. At 16 WAS, seeds stored in polythenes bag had the highest seed viability this was not significantly different from the viability obtained from the seeds stored in Envelope.

At 20 WAS, the viability of seeds in Jute bags, Envelopes, Rubber plates, Polythene bags, and Muslin cloths was not significantly different but was different from that of Aluminum-can at 4^oC. At 24 WAS, the viability of seeds in all the storage materials is not significantly different. But the viability reduced as the period of storage increased. Aluminium can and muslin cloth had the least germination at 24 weeks after storage at 27 + 2^0C with 6.33. At 4^oC had least germination count of 7.33 was observed in envelopes used to store the seeds. The aluminium can had the highest germination count of 9.00 at 24 weeks followed by the polythene bag with 8.67 germinations at 4^oC at 24 WAS.

Effect of different storage materials and conditions on the occurrence of seed-borne fungi

Table 4 shows the occurrence of fungi on kenaf seeds in ambient and freezing conditions. The control experiment was found to have the highest number of fungi occurrences than the other two storage conditions. *Aspergillus* species was found to be the mostly occurred organism in the control experiment. Several fungi, including *Fusarium* spp and *Hymenella* spp, were found to occur on Kenaf seeds at different storage conditions.

Table 3: Mean values of germination counts of kenaf seeds stored in the different storage materials and conditions

Storage materials	Storage conditions	4 WAS	8 WAS	12 WAS	16 WAS	20 WAS	24 WAS
P.bag	A	7.33^{a}	7.33^{ab}	8.00^{b}	10.00^{a}	7.00^{bc}	6.67^{ab}
	R	8.33^{abc}	8.33^{abc}	8.67^{bc}	10.00^{a}	8.00^{ab}	8.67^{ab}
J.bag	A	9.67^{a}	9.33^{ab}	7.67^{bc}	9.00^{a}	8.33^{ab}	7.33^{abc}
	R	9.00^{ab}	8.67^{abc}	9.00^{ab}	9.00^{a}	8.00^{ab}	7.67^{abc}
A.can	A	8.00^{abc}	10.00^{a}	8.00^{b}	9.67^{a}	8.67^{ab}	6.33^{bc}
	R	7.00^{bc}	7.67^{bcd}	7.33^{bcd}	9.67^{a}	7.00^{bc}	9.00^{ab}
R.plate	A	7.33^{abc}	10.00^{a}	7.67^{bc}	9.00^{a}	7.67^{abc}	8.00^{abc}
	R	9.00^{ab}	9.00^{ab}	7.67^{bc}	9.00^{a}	8.33^{ab}	7.67^{abc}
M cloth	A	1.3^{ab}	10.00^{a}	8.33^{ab}	9.67^{a}	7.67^{abc}	6.33^{bc}
	R	8.3^{abc}	7.67^{bcd}	10.00^{a}	9.00^{a}	8.33^{ab}	8.67^{abc}
Envelope	A	7.67^{abc}	9.67^{a}	7.33^{bc}	9.67^{a}	8.33^{ab}	7.00^{abc}
	R	7.33^{abc}	7.67^{bcd}	9.00^{ab}	10.00^{a}	8.67^{ab}	7.33^{abc}

Means in the same row followed by the same letter are not significantly different by Duncan Multiple Range Test at 0.05 level of significance. A = Ambient condition R= Freezing condition C = Control WAS = Weeks After Storage, P.bag = Polyethylene bag, J.bag = Jute bag, A.can = Aluminium can, R.plate = Rubber plate, M cloth = Muslin cloth

Ambient conditions increased the occurrence of *Aspergillus* species, *Fusarium* species, *Alternaria* species and other organisms' contamination on the seeds irrespective of the storage materials, whereas, freezing conditions slowed down the occurrence of fungi on kenaf seeds for different storage materials. Kenaf seeds during the period of storage under the two conditions had high significant differences. The incidence of fungi in ambient conditions increases as the period of storage increases while the incidence had a sharp decline under freezing conditions irrespective of the storage materials.

Effect of periods of storage on the incidence of fungi on Kenaf

Kenaf seeds during the period of storage under the two conditions had highly significant differences (Table 5). The incidence of fungi in ambient conditions increases as the period of storage increases while the incidence has a sharp decline under freezing conditions irrespective of the storage materials. The results also showed the negative values of estimates of slopes of the seed fungi data for all seed lots irrespective of the materials.

The highest incidence of *Aspergillus* sp was obtained in the control material (30.88%) followed by Muslin cloth (8.32%) and then rubber plate used (6.31%). The least incidence of *Aspergillus* sp was obtained when jute bag was used. The incidence of *Fusarium* sp was very high when the control material was used compared with other storage materials. The least significant incidence of *Fusarium* sp was recorded with a storage rubber plate. The highest incidence of *Alternaria* sp was obtained when jute bag was used followed by polyethylene bag and then aluminium can. The incidence of *Alternaria* sp in the control was not significantly different from rubber plate (Table 5). As shown in Table 6, it was generally observed that as the period increased, the incidence of the different mycoflora also increased but *Aspergillus* spp showed reduction in the incidence at 24 weeks of storage.

The result of the ANOVA revealed that the variation due to the main effects of period, storage condition and storage materials was significant ($p \leq 0.01$) on the incidence of all the mycoflora. Similarly, the interactive effects of period, storage condition and storage materials on all the mycoflora was significant at 1% level of probability (Table 7).

DISCUSSION

It was observed from this study that *Aspergillus spp* occurred most at 4^{o}C. *Aspergillus* species are known to be responsible for various plant and food secondary rot with an associated characteristic accumulation of mycotoxins such as aflatoxin and ochratoxinogenic. These mycotoxins are usually secreted by *A. niger, A. ochraceus, A. parasiticus* and *A. flavus* which are common in contaminating agricultural products at pre and post-harvest stages (Perrone *et al*., 2007). The kenaf seeds were discoloured, pigmented and with the obvious contamination of the seeds lot as also reported by Perrone *et al*. (2007).

Polyethene bags used to store kenaf seeds at 4^{o}C and 27$\pm$ 2^{0}C had 4 and 2 different organisms respectively growing on the seeds stored with it. *Alternaria spp* was common in both conditions indicating its potential to survive in different conditions. Though, it is obvious that storage fungi normally do not play a role in disease development in the field, but play a major role in seeds deterioration during storage. So *Alternaria spp* plays a significant role in the deterioration of Kenaf seeds. Unlike the polyethene bag, the envelope had the highest number of fungi at 27 $\pm$ 2^{o}C.

Alternaria spp owing to their growth even at low temperatures are well-known post-harvest pathogens, responsible for spoilage of food during refrigeration transport and storage (Ostry, 2008). It was equally observed in abundance at 27^{o}C in this study. It is an established fact, as seen in this study, that *Alternaria* spp. is a cosmopolitan mould fungus and can be found in soils, plants, food, feed and indoor air. The pathogen was observed in all the storage materials used at 27^{o}C. Thomma (2003) stated that the genus *Alternaria* includes both saprobes and plant pathogens which have been reported worldwide, infecting crops in the field and causing post-harvest decay of many plant products. The species of *Alternaria* are found in abundance on small grains, causing great yield losses in production and processing. Economical losses are mainly related to quality reduction due to decreased nutritive value, discolouration and insipidness (Kosiak *et al*., 2004). The occurrence of Alternaria toxins in small grains and cereal-based products is a global issue of high concern, due to their potential health risks for humans and/or livestock. The presence of *Alternaria spp* in small grains poses a serious threat as some toxins produced by it have carcinogenic tendencies (Ogunade *et al*., 2018).

Table 4: Effect of different storage materials and conditions on the frequency of occurrence of seed-borne fungi on stored kenaf seeds at 24 weeks

Fungi isolated	Freezing condition							Ambient condition						
	Control	P. bag	J. bag	Env	M. cloth	R. plate	Al can	Control	P. bag	J. bag	Env	M. cloth	R. plate	Al can
A. flavus	6	0	0	2	0	2	0	6	0	0	0	3	4	0
A. niger	6	0	0	0	1	0	2	6	0	0	0	4	0	0
A. parasiticus	6	0	0	2	0	0	0	6	0	0	0	0	0	0
A. candidus	2	1	0	0	0	0	0	2	0	0	2	0	0	4
F. oxysporum	0	0	2	0	0	0	0	0	3	0	0	2	0	0
F. longipes	2	0	0	0	0	0	0	2	0	0	0	0	2	0
F. proliferatum	0	2	0	0	0	0	0	0	0	0	0	0	0	4
F. graminum	2	0	0	0	0	0	0	2	0	0	0	0	3	3
F. xylariodes	0	0	0	0	0	0	0	0	0	0	0	0	5	5
F. antholphilum	2	0	0	0	0	0	0	2	0	2	4	0	0	0
F. chlamydosporum	1	1	0	0	0	0	0	1	0	0	0	0	0	0
F. pallidoroseum	0	1	0	0	0	0	0	0	0	0	0	0	0	4
F. solani	0	0	0	0	0	0	0	0	0	5	5	0	5	0
Alternaria spp	0	2	0	0	0	0	0	0	3	0	0	0	0	4
Penicillum spp	0	0	0	0	0	0	1	0	0	4	6	0	2	0
Tieghemiomyces	0	0	0	0	0	0	0	0	0	7	0	0	0	5
Hymenella	2	0	0	0	0	0	0	2	0	0	0	1	0	0
Colletotrichum	0	0	0	0	0	0	0	0	0	0	0	0	6	0

P.bag = polythene bag, J. bag = Jute bag, Env = Envelope, M. cloth = Muslin cloth, R. plate = Rubber plate, Al can = aluminum-can

Table 5 : Effect of storage materials on the incidence of prevalent fungi during the period of 24 weeks of storage

Storage material	*Aspergillus* spp	*Fusarium* spp	*Alternaria* spp	*Tieghemiomyces* spp	*Sclerotium* spp	*Meria* spp	*Hymenella* spp
P. bag	5.75d	5.46b	5.17b	0.58c	0.29a	0.00c	0.00c
M. cloth	8.32b	5.46b	2.31f	0.29d	0.00b	0.29b	0.00c
J. bag	4.64f	4.31d	5.28a	0.29d	0.00b	0.00c	0.29b
A. can	5.75d	4.08e	4.56c	1.46a	0.00b	0.00c	0.00c
R. plate	6.31c	3.74f	3.73d	0.29d	0.00b	0.00c	0.00c
Env.	5.59e	4.88c	3.61e	0.29d	0.29a	0.00c	0.29b
Control	30.88a	17.66a	3.78d	1.31b	0.00b	0.66a	1.31a

P.bag = polythene bag, J. bag = Jute bag, Env = Envelope, M. cloth = Muslin cloth, R. plate = Rubber plate, Al can = aluminum-can. Means in the same column followed by the same letter are not significantly different by Duncan Multiple Range Test at 0.05 level of significance.

Table 6: Effect of period of storage on the incidence of mycoflora on stored kenaf seeds

Storage period	*Aspergillus* spp	*Fusarium* spp	*Alternaria* spp	*Tieghemiomyces* spp	*Sclerotium* spp	*Meria* spp	*Hymenella* spp
4 weeks	5.34f	4.69fe	2.46e	0.25c	0.00b	0.00b	0.00c
8 weeks	7.45e	5.24d	3.06d	0.00d	0.00b	0.00b	0.00c
12 weeks	11.21c	10.35a	1.49f	0.25c	0.00b	0.00b	0.00c
16 weeks	12.33b	7.15b	4.62c	0.00d	0.00b	0.00b	0.00c
20 weeks	13.66a	5.15e	5.12b	1.50b	0.50a	0.00b	0.25b
24 weeks	8.19d	6.75c	7.54a	1.86a	0.00b	0.82a	1.37a

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

Table 7: Analysis of variance for Individual and interactive effects of period of storage, storage conditions and materials used to store the kenaf seeds

Source of variation	**DF**	**Mean square values**										
		Aspergillus spp	*Fusarium spp*	*Alternaria spp*	*Tieghe*	*Sclerotium spp*	*Chaetomium spp*	*Meria spp*	*Varicose spp*	*Hymenella spp*	*Collecto*	*Penicillium spp*
Rep	2	0.41**	0.56**	0.04**	0.03**	0.00ns	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Storage condition (SC)	1	25.52**	51 .15**	0.08**	3.94	1.76**	0.44**	0.43**	0.44**	1.74**	0.44**	0.44**
Period (P)	5	438.49**	184.99**	183.77**	25.89**	1.75**	0.44**	4.02**	0.44**	10.44**	0.44**	0.44**
Storage material (SM)	5	3343.73**	968.84**	41.10**	8.56**	0.73**	0.44**	1.93**	0.44**	6.51**	0.44**	0.44**
SC x P	5	49.21**	203.87**	15.79**	9.16**	1.75**	0.44**	0.44**	0.44**	0.69**	0.44**	0.44**
SM x P	30	134.87**	90.97**	86.56**	10.07**	0.73**	0.44**	2.05**	0.44**	7.89**	0.44**	0.44**
SC x SM	6	3.92**	61.64**	32.89**	1.89**	0.73**	0.44**	0.44**	0.44**	0.73**	0.44**	0.44**
SC x SM x P	30	35.80**	41.07**	23.03**	2.24**	0.73**	0.44**	0.44**	0.44**	0.91**	0.44**	0.44**
Error	167	0.006	0.024	0.007	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000

DF = Degree of Freedom, ** Significant at 1% level of Probability, ns = Not significant, *Tieghe* = *Tieghemiomyces* spp, *Collecto* = *Collectotrichum*

All the grains stored irrespective of the storage material were found positive for *Fusarium* species. A total of 9 strains were isolated and identified using morphological characteristics. Of all the 9 strains of *Fusarium* spp: *F. oxysporum, F. longipes, F. proliferatum, F. graminum, F. xylariodes, F.antholphilum, F.chlamydosporum, F. pallidoroseum* and *F. solani*. *F. solani* was the most prevalent species in all the storage materials at 27^0C. The profusion of Fusarium species at 27^0C in the storage materials buttresses the fact that most crops (especially from field to store) are invaded by one or more species of Fusarium, the members of this group are deleterious to health due to the mycotoxin secreting potential (Bacon and Williamson,1992; Marin *et al*., 1998).

The number of fungi found was reduced when the seeds were stored in polyethene bags. *Aspergillus niger* and *Rhizopus oryzae* were found to persist on all the seeds stored, even after storage for 6 months. This agrees with the findings of El-Sayed and Tolba (2005) who reported that storage under 10^0C in high-density polyethene packages led to reducing infection of maize grains by pathogens including *A. flavus*. Storage in the refrigerator (10^oC) in packages made from low-density polyethene was favourable against grain infection with all storage fungi that cause seed rot of maize. Mehrotra (1983) reported that the storage fungi i.e., *Aspergillus spp.* and *Penicillium spp* can grow in stored grain under bad storage conditions and cause serious losses. The high percentage incidence and several fungi on seeds were observed under the ambient condition which is responsible for the reduction in seed viability. The species of *Aspergillus, Penicillium, Fusarium, Rhizopus* and *Alternaria* were commonly occurring post-harvest moulds in storage conditions (Chavan, 2011). Among different species of fungal infection, *A. flavus* was the most preponderant and play a vital role in the seed biodeterioration in groundnut (Ibiam and Egwu, 2011), just as it was observed on the seeds stored.

Aspergillus species had the highest percentage incidence of 48.3% in the control experiment. Several fungi including *Alternaria, Fusarium, Sclerotium, and Colletotrichum* were identified on the kenaf seeds at a different incidences of occurrence. The presence of these organisms indicates that kenaf seeds are susceptible to many infections which reduce the quality of the seeds (Mohammedi, 2011). The percentage of *Aspergillus* spp*, Fusarium* spp*, Alternaria* spp and other species of organism constantly increases and became heavy contamination on the seeds at the ambient and control experiment while the percentage incidence of fungi on seeds at 4^0C condition decreases. These showed that the distribution of isolated organisms on kenaf depends on the storage conditions. It was observed that the germination/seed viability fluctuated as the period of storage increased. The storage fungi produce adverse effects on the seeds where they are growing, such as discolouration of seed or embryo and reduction in seed germination, which were all observed in this study.

Storage conditions influence viability rates. Viability reduced as the period of storage increased; such as at 24 weeks after storage. Aluminium can and muslin cloth had the least viability, germination at 27°C with 6.33 while 4°C had the least germination of 7.33 in the envelope used to store the seeds. The Aluminium can had the highest germination of 9.00 at 24WAS at 4°C. 20%, the highest incidence of Aspergillus. Among different species, *A. niger* and *Fusarium spp* were serious in reducing the germination of seed by about 20% after six months of storage. There was a progressive increase in the *A. niger* in groundnut with the increase in storage period as reported by Raju and Krishnamurthy (2003). Kenaf, being an oily crop had *A. niger* in abundance, especially under the ambient condition which resulted in deteriorating changes in the seeds due to the presence of the oil. There were notable biochemical changes due to storage fungi in this study. The higher and better germination of kenaf seeds were observed when stored in polyethene bag stored in freezing conditions. Malaker *et al*. (2008) found that the highest germination percentage was observed under storage in freezing conditions and polyethene bags followed by tin containers and earthen pitchers. Mettananda *et al*., 2001 observed that maize grain stored in woven polyethene in a cold room has good quality during the storage period compared to maize grain stored with polyethene under room temperature.

The temperature at which the kenaf seeds were stored had positive and negative effects on the germination of the seeds, depending on the storage materials used. El-Sayed and Tolba (2005) found that seed germination and weight of 100 kernels were affected by storage temperature and packaging material. This is buttressed by El-Sayed *et al*. (2004) who reported that germination decline was more rapidly at the warehouse, but less at low temperatures. Ramamoorthy and Kariyarataraju (1989) noticed that there was a progressive decrease in germination percentage, oil and protein content and an increase in free fatty acids in the stored kernels than in the pods because of the invasion of storage fungi to kernels. Shelar (2008) also reported a higher percentage of mycoflora with the groundnut seeds that had lost their viability and had higher electrical conductivity and leaching of sugars. Bhattacharya and Raha (2002) opined a decrease in carbohydrate, and oil content and an increase in free fatty acid content with a gradual loss followed by a small increase in protein content of maize, groundnut and soybean seeds during storage due to storage fungi.

The percentage of *Aspergillus spp* increases as the storage period increases irrespective of the storage materials. The best material to use at 4°C is the polyethene bag, at 27 ± 2°C is the Aluminum can. *Fusarium* and *Aspergillus* species occurred most at the ambient condition. Actually, the storage in freezing and ambient conditions reduced the incidence of pathogens that would have occurred on the seeds if they were not stored in any of these conditions when compared with the control. The worst storage material used was a jute bag at 4°C. Rubber plates and Aluminium can have 7 fungi at 27±2°C. *Fusarium solani* and *Penicillium spp* occurred most in the materials while *A. parasiticus and F. chlamydosporium* did not occur in all the storage materials used at 27±2°C, *F. longipes, F.graminum, F. xylorides, F. antholphilum, F. solani, Colletotrichum spp, Hymenella spp* and

Tieghemiomyces spp were not found in seeds stored in all the materials stored at 4°C.

CONCLUSION

IFE-KEN 400 variety of kenaf seeds stored in freezing condition with polythene bag had the highest germination than the other storage materials. The incidence of fungi increases as the period of storage increases under ambient conditions irrespective of the storage materials while the incidence of fungi. At 4°C freezing conditions favours the increased period of storage for the safe keeping of the seeds. There was no significant difference in the incidence of fungi in the control experiment at the ambient condition.

The most effective among the storage materials was the polyethene bag. The result also showed that ambient conditions have an adverse effect on the incidence of fungi on kenaf seeds. Kenaf seed is associated with many fungal organisms under ambient conditions thereby losing its viability easily and causing a decrease in production as a result of poor germinability. It is hereby important and necessary to store kenaf seeds in the polyethene bag at 4°C or ambient conditions to maintain the seeds' moisture in storage, not to lose the seed viability and to reduce the establishment of fungi on the seeds.

REFERENCES

Adebisi, M. A., Daniel, I. O. and Ajala, M. O. (2004). Storage life of soybean seed after seed dressing. *Journal of Tropical Agriculture*, 42: 3-7.

Adebisi, M. A., Ola, J. A., Akintobi, D. C. A. and Daniel, I. O. (2008). Storage life of sesame (*Sesamum indicum* L.) seed under humid tropical conditions. *Seed Science and Technology*, 36: 379 – 387.

Adebisi, M. A., Esuoroso, O. A., Adetumbi, J. A.., Abdul-Rafiu, A. M., Kehinde, T. O., Ajani, O. O., and Agboola, D. A. (2013). Shelf life of Kenaf (*Hibiscus cannabinus* L.) seed stored under humid tropical conditions. *Plant Breeding and Seed Science*, 67: 75 – 86. DOI 10.2478/v10129-011-0071-5.

Aimin, R. I. (2006). Making pulp and paper from kenaf. Retrieved online on 14/12/2018 via https://siwafarms.wordpress.com/opportunities-for-kenaf-in-egypt/paper-making-with-kenaf/making-pulp-and-paper-from-kenaf-by-dr-aimin-liu/

Akintobi, D. C, Adebisi M. A. and Ola, J. A. (2004). Effects of storage conditions and period of storage on the maintenance of seed quality of sesame. *Asset Series* A, 6: (2): 275-285.

Bacon, C. W. and Williamson, J. W. (1992). Interactions of *Fusarium moniliforme*, its metabolites and bacteria with corn. *Mycopathologia*, 117: 65-71.

Bhattacharya, K. and Raha, S. (2002). Deteriorative changes of maize, groundnut and soybean seeds by fungi storage. *Mycopathologia*, 155: 135-141.

Barnet. J. P. and Hunter, S. (1972). Producing high quality pine seeds. *Journal of Forest Service*, 2: 52 – 56.

Basri, M. H., Abdu, A., Junejo, N., Abdul-Hamid, H. and Ahmed, K. (2014). Journey of kenaf in Malaysia: A Review. *Scientific Research and Essays*, 9: 458 – 470. 10.5897/SRE12.471.

Chavan, A. M. (2011). Nutritional changes in oilseeds due to *Aspergillus spp. Journal of Experimental Sciences*, 2: 29 – 31.

El-Sayed, S. A., Kineber, M. A. and El-Kady, E. A. (2004). Effect of storage environment and package material on storability, yield and its quality of flax seeds. *Journal of Agricultural Research*, Tanta University 30(3): 616 – 638.

El-Sayed, S. A. and Tolba, S. A. E. (2005). Effect of storage conditions and package on germination, chemical composition, infection by ear and kernel rot disease and oil quality in some maize grain genotypes. *Alexandria Journal of Agricultural Research*, 50(2): 23 – 33.

Genes, F. and Nyomora, M. S. (2018). Effect of storage time and temperature on germination ability of *Escoecaria bussei*. *Tanzania Journal of Science* 44(1): 123 – 133. ISSN 0856-1761, e-ISSN 2507-7961.

Greg, B. Wanus, S. A. E., Bishaw, Z. and Van-Caste A. J. G. (1994). Safe seed storage WANPA Seed Network Publication No. 5/4 ICARDA Aleppo. Syria.

https://agriinfo.in/factors-affecting-seed-longevity-in-storage-2334/ retrieved online 15/11/2021. Factors affecting seed longevity in storage. *In*: Principles of Seed Technology published online April 3, 2017.

Ibiam, O. F. A. and Egwu, B. N. (2011). Post-harvest seed-borne diseases associated with the seeds of three varieties of groundnuts, (*Arachis hypogaea* L). *Nwakara, Kaki and Campalla Agriculture and Biology Journal of North America*, 2: 598 – 602.

Khazanda, K. A., Rajput, M. A., Shah, G. S., Lodhi, A. M. and Mehboob, F. (2002). Effect of seed dressing fungicides for control of seed borne mycoflora of wheat. *Asian Journal of Plant Sciences*, 144: 441 – 444.

Kosiak, B., Torp, M., Skjerve, E., and Andersen, B. (2004). Alternaria and Fusarium in Norwegian grains of reduced quality – A matched pair sample study. *International Journal of Food Microbiology*, 93(1): 51 – 62. DOI:10.1016/j.ijfoodmicro.2003.10.006

Malaker, P. K., Mian, I. H., Bhuiyan, K. A., Akanda, A. M. and Reza, M. M. A. (2008). Effect of storage containers and time on seed quality of wheat. *Bangladesh Journal of Agricultural Research*, 33(3): 469 – 477.

Marin, S., Sanchi, V., Rull, F., Ramos, A. J. and Magan, N. (1998). Colonization of maize grain by *Fusarium moniliforme* and *Fusarium proliferatum* in the presence of competing fungi and their impact on fumonisin production. *Journal of Food Protection*, 61: 1489 – 1496.

Mehrotra, B. S. (1983). The impact of fungal infestation of cereal grains in field and storage. *In*: *Recent Advances in Plant Pathology. Husain, A., Singh, K., Singh, B. P. and Agnihotri, V. P. (eds,)*, Lucknow Print House. India. Pages 185-200

Mettananda, K. A., Weerasena, S. L. and Lianage, Y. (2001). Effect of storage environment, package material and seed moisture content on storability of maize (*Zea mays* L.) seeds. A*nnals of the Sri Lanka Department of Agriculture*, 3: 131 – 142.

Mithal, R.K. and Mathur, S.B. (2003). Tropical Seed Manual. Available at:http:www.mgt.net/Reforestation publication/TTSM. Retrieved online 15/11/2021.

Mohammed, A. (2013). An overview of distribution, biology and management of Common bean anthracnose. *Journal of Plant Pathology and Microbiology*, 4(8): 193 – 197.

Mohammadi, H., Soltani, A., Sadeghipour, H. R. and Zeinali, E. (2011). Effect of seed aging on subsequent seed reserve utilization and seedling growth in soybean. *International Journal of Plant Product*, 5(1): 65 – 70.

Mohd Sahwahid, H. O., Amira, A.M. and Norfaryanti, K. (2012). Critical factors of farmer's participation in kenaf cultivation: Malaysian context. A*frican*

Journal Agricultural Research, 7: 3485 – 3489.

Ogunade, I. M., Martinez-Tuppia, C., Queiroz, O. C. M., Jiang, Y., Drouin, P., Wu, F., Vyas, D. and Adesogan, A. T. (2018). Silage review: Mycotoxins in silage: Occurrence, effects, prevention, and mitigation. *Journal of Dairy Science*, 101(5): 4034 – 4059.

Ostry, V. (2008). Alternaria mycotoxins: An overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin Journal* 1(2):175-188 DOI:10.3920/WMJ2008.x013.

Perrone, G., Susca, A., Cozzi, G., Ehrlich, K., Varga, J., Frisvad, J. C., Meijer, M., Noonim, P., Mahakarnchanakul, W. and Samson R. A. (2007). Biodiversity of aspergillus species in some important agricultural products. S*tudies in Mycology* 59: 53 – 66. DOI: 10.3114/sim.2007.59.07 PMCID: PMC2275197 PMID: 18490950).

Pradhan, B. K. and Badola, H. K. (2012). "Effect of Storage Conditions and Storage Periods on Seed Germination in Eleven Populations of *Swertia chirayita*: A Critically Endangered Medicinal Herb in Himalaya". *The Scientific World Journal*, 2012, Article ID 128105, 9 pages https://doi.org/10.1100/2012/128105

Rahmawati, Y. and Muhammad, A. (2020). The effect of temperature and humidity of storage on maize seed quality. *IOP Conference Series Earth and Environmental Science*, 484: 012116.

Ramamothy, J. B. (1999). Seed invigoration treatment for germination and seedling vigour of stored okra seeds. *B. Agriculture Project. Department of Plant Breeding and Seed Technology, University of Agriculture, Abeokuta, Nigeria*. 36p.

Ramamoorthy, K. and Karivaratharaju, T.V. (1989). Influence of pod and kernel treatments on the field performance of groundnut (*Arachis hypogeal*) cv. Pol.2 after twelve months of storage. S*eeds Farms.* 15: 15-19.

Raju, R.B.M. and Krishnamurthy, K.V.M. (2003). Studies on collar rot of groundnut (*Arachis hypogaea* L.) caused by *Aspergillus niger v*. Teighem. *Journal of Research Angrau*, 31: 115 – 116.

Robert, E. H. (2003). Storage environment and the control of viability of seeds. E.H Roberts (ed) Chapman and Hall, London. pg14-58.

Samson, R. A. and Pitt, J. I. (2000). *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. CRC Press. ISBN 9789058231598. 524p.

Statistical Analysis System (SAS) 2010, Users Guide Statistics. Version 5 edition, Cary, NC: SAS Institute Inc.

Shelar, V. R. (2008). Role of mechanical damage in deterioration of soybean seed quality during storage. A review. *Agricultural Reviews*, 29 (3) : 177 – 184.

Swart, D. C. and Tarekegn, G. S. (2007). Seed borne disease and Health testing of wheat. Danish Government Institute of Seed Pathology for developing countries. Denmark: Jordbugs for larget, Fredericksberg, Denmark 168p.

Thomma, B. P. (2003). *Alternaria* spp from general saprophyte to specific parasite. *Molecular Plant Pathology*, 4: 225 – 236.

Thseng, F. S., Hou, F. F. and Wu, S. T. (1996). Varied differences in germination rate, storability and flooding tolerance of soybean. seed produced from different cultural seasons and location. *Japanese Journal of Crop Science*, 65: 525 – 531.