Journal of Stored Products Research 64 (2015) 133–138

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

# Purdue Improved Crop Storage (PICS) bags for safe storage of groundnuts

H. Sudini [a, *], G.V. Ranga Rao [a], C.L.L. Gowda [a], R. Chandrika [a], V. Margam [b], A. Rathore [a], L.L. Murdock [b]

[a] International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India
[b] Department of Entomology, Purdue University, West Lafayette, IN, USA

ARTICLE INFO

*Article history:*
Available online 7 October 2014

*Keywords:*
Groundnut
Aflatoxin
Bruchids
Hermetic storage
PICS

ABSTRACT

Groundnut seeds are prone to quality deterioration and damage due to improper storage. Hermetic storage of pods offers a novel, sustainable and ecologically safe alternative over traditional methods. In this paper, we demonstrate the efficacy of triple-layer "Purdue Improved Crop Storage (PICS)" bags, (that comprises of two inner high density polyethylene bags and one outer woven polypropylene bag), for protecting pods from quality deterioration, damage by bruchids (*Caryedon serratus*) and aflatoxin contamination (*Aspergillus flavus*). Custom made triple-layer bags were used and pods (of cv ICGV 91114) were placed @ 2 kg/bag. Over four months of storage under ambient conditions, triple-layer bags supported retention of seed weight, germinability and oil content significantly better than cloth bags. Further, under both natural and artificial infestations with *A. flavus*, seed aflatoxins levels were lower in PICS bags compared to cloth bags. Toxin accumulation in PICS bags deliberately infested with bruchids and *A. flavus* was less compared to cloth bags under similar conditions. Bruchid damage to pods was less in PICS bags versus cloth bags in all cases. Our results suggest the superiority of triple-layer PICS bags over cloth bags in protecting seed viability, seed weight and oil content while safeguarding the groundnuts from bruchids and retarding toxin accumulation.



## 1. Introduction

Post-harvest food losses during storage are substantial and have various causes (Kitinoja and Gorny, 1999; Musa, 1984; Tindall and Proctor, 1980). Losses in stored cereals, pulses and oilseeds depend on the crop, the storage conditions and the type of post-harvest processing. Groundnut (*Arachis hypogaea* L.) is an important cash crop, rich in oil, protein and energy value. Sizeable post-harvest losses have been reported in groundnut, particularly during storage (IITA, 2000). Molds, pests, flavor changes, and rancidity are the major negative factors that affect groundnuts during storage. Physical deterioration of pods/seeds such as shrinkage and weight loss, are also common.

Groundnuts are semi-perishables and can be stored for long periods if pod/kernel moisture, temperature and relative humidity are optimized. Any deviations from optimum conditions of storage result in losses either during storage or at milling. For bulk storage of unshelled groundnuts at farmers' level up to one year, optimal conditions are 7.5% kernel moisture, a temperature of 10 deg C and a relative humidity (RH) of 65% (Pattee and Young, 1982). In general, major storage problems for groundnut include suboptimal weather and infestation by insects, rodents and toxigenic molds. The groundnut bruchid, *Caryedon serratus* (Olivier) is the primary storage pest of unshelled groundnuts in many parts of Asia, and throughout West and Central Africa (Delobel, 1995; Singal and Toky, 1990; Okeke, 1986; Misari, 1975; Davey, 1958). Pod damage by bruchids of up to 83% has been reported under ambient conditions for unprotected groundnuts following 8–13 months of storage (Dick, 1987; Okeke, 1986; Conway, 1974). For confectionary varieties that are harvested early and dried for prolonged periods under field conditions, the bruchid poses an even greater threat when the pods are kept in the open for longer durations (Conway, 1983). Bruchids can greatly reduce germinability of seeds and the quality of oil produced from them and are considered an economically important pest of stored groundnut in India (Wightman et al., 1987). On the other hand, mold growth on pods in storage is associated with high moisture content of the groundnuts. Storage molds in groundnut result in reduced levels of germination, decreased weight, kernel discoloration, and chemical and nutritional changes in addition to mycotoxin contamination (Sauer et al.,

* Corresponding author. Tel.: +91 40 30713696.
*E-mail addresses:* h.sudini@cgiar.org, sudinhk@gmail.com (H. Sudini).

http://dx.doi.org/10.1016/j.jspr.2014.09.002
0022-474X/

1992). Coupled with this, the quality of fats in the groundnuts is reduced, thereby leading to a lower quality of commodity and market value (Pomeranz, 1992). Aflatoxin contamination in food stuffs due to *Aspergillus flavus* and *Aspergillus parasiticus* is severe in developing countries where handling and storage technologies are less than needed (Bulaong and Dharmaputra, 2002).

Current farmer practice is to use jute (gunny) and woven polypropylene bags for groundnut storage (Bulaong and Dharmaputra, 2002). Storage of groundnuts in pod form in jute bags is associated with bruchid infestation and mold growth, especially with *A. flavus*. Jute bags are highly porous and can easily absorb moisture, and so foster the rapid growth and multiplication of these aflatoxigenic molds. While polypropylene bags are non-absorptive, they tend to trap heat inside (Kennedy and Devereau, 1994). On the other hand, storage of food grains in polyethylene (PE) bags has several advantages. For example, storing wet corn for two weeks in PE bags either singly or in combination with polypropylene inhibited fungal growth and aflatoxin production (Siriacha et al., 1990). Groundnut seeds stored in PE bags retained germination for longer periods (up to 7 months) compared to jute bags (Reddy et al., 1992). Hermetic storage offers a new alternative to traditional storage of grains and pods, and is a sustainable practice. Hermetic storage works on the principle of creating airtight conditions in which oxygen levels are lowered through insect, fungal and seed respiration (Quezada et al., 2006). In the present study, we explored the use of triple layer "Purdue Improved Crop Storage (PICS)" bags, evaluating their performance and safety for short-term groundnut pod storage. These hermetic triple-layer bags consist of three bags, one inside the other, made up of an outer woven polypropylene layer for strength, and two inner bags composed of 80 micron thick high density polyethylene. They have been used with success for storing several crops including cowpea, maize, Bambara groundnut and others (Hell et al., 2010; Murdock et al., 2003; Murdock, unpublished). Our hypothesis was that the controlled atmospheric conditions that prevail in triple layer bags will delay insect infestation, reduce kernel damage, weight loss, mold growth, and rancidity while maintaining germinability. Our results suggest that PICS bags can provide a sustainable and ecologically safe approach to preserve groundnut pods at the farmers' household level.

## 2. Materials and methods

### 2.1. Storage bags and description

Triple-layer hermetic "Purdue Improved Crop Storage" (PICS) bags were used in the present study. Triple-layer bags were originally developed under the Bean/Cowpea Collaborative Research Support Program (CRSP) project in the late 1980s through funding from USAID (Murdock et al., 2003). These bags consist of two inner layers of 80 micron thick high density polyethylene bags surrounded by a third layer of woven nylon bag for strength. These bags are produced in 50 kg and 100 kg capacity sizes (Baributsa et al., 2010). For our experiments, we used reduced size bags created by cutting the original PICS bags and heat sealing them to form units that held 2 kg of groundnuts. PICS bags were obtained from Purdue University, USA but were originally produced by Lela Agro, Kano, Nigeria.

The non-airtight muslin cloth bags were procured from the local market and held 2 kg of groundnut pods. These cloth bags served as controls for comparison with triple-layer bags. Cloth bags were selected since they work on the same principle as that of jute/gunny bags in permitting air exchange with the surrounding environment. Triple-layer bags were carefully inspected for holes and sealing imperfections, to ensure that only good quality bags were used (Vales et al., 2014).

### 2.2. Groundnut pods

Pods of variety ICGV 91114 (released as "Anantha Jyothi" in Andhra Pradesh; "Devi" in Odisha of India) were used. Important characters of the variety are: it has predominantly 2-seeded pods (with occasional 3-or 1-seeded) with slight ridges, slight reticulation, slight beak and constriction. It has an average shelling turnover of 75% and the seeds are tan-colored. The average seed oil and protein contents are 48% and 27%, respectively.

### 2.3. Insect culture and maintenance

Groundnut bruchid *(C. serratus* Olivier) culture was obtained from a state agriculture university (Acharya NG Ranga Agricultural University, Hyderabad, Andhra Pradesh, India). The population was allowed to multiply under laboratory conditions at ICRISAT. Cultures were established by dispensing 200 g of unshelled groundnut into a plastic container fitted with a mesh lid. Each container was then infested with a few adult *C. serratus*. A total of ten containers were set up and all incubated at laboratory temperature that fluctuated between 26 and 30 °C and 60–75% RH. After 7 days, the original adult weevils were sieved off from the pods and the groundnuts were kept for about 10 weeks to collect emerging adults. Emergence was checked daily and new adults were kept in separate containers containing groundnut pods (Ekesi et al., 2001).

### 2.4. Aspergillus flavus inoculum

A highly virulent, toxigenic isolate of *A. flavus* (AF11-4) was obtained from the culture collection of the Groundnut Pathology Laboratory at ICRISAT, Patancheru, India. The isolate was originally isolated from freshly harvested groundnut pods at ICRISAT. The culture was maintained on potato dextrose agar (PDA) slants at ambient temperature. Inoculum of AF 11-4 was produced on PDA by transferring 3 mm core plugs aseptically on to PDA petri-dishes. Dishes were sealed with parafilm and incubated at room temperatures. Two weeks later, *A. flavus* cultures profusely sporulated on PDA and conidia were harvested in sterile distilled water (SDW) and adjusted to a concentration of $5 \times 10^5$ CFU $ml^{-1}$.

### 2.5. Experimental design and procedure

The experiment was conducted at ICRISAT, Patancheru (Andhra Pradesh, India) in a storage room at ambient temperature. The experiment consisted of six treatments with three replications. The treatments were: 1) Triple-layer bags with pods infested with *A. flavus*; 2) Cloth bags with pods infested with *A. flavus*; 3) Triple-layer bags with pods infested with *A. flavus* + Bruchids; 4) Cloth bags with pods infested with *A. flavus* + Bruchids; 5) Triple-layer bags with pods alone; and 6) Cloth bags with pods alone (resembling farmers' practice of storing in jute bags). Two kg of pods (kernel moisture of 8%) were added uniformly to the test bags. Pods were fumigated using standard procedures to prevent any field contamination from getting carried over to the experimental site. *A. flavus* inoculum (@ 3 ml) was spray inoculated onto pods in treatments wherever applicable prior to placing them in bags. Approximately 200 g of bruchid-infested pods were added to the selected treatments, ensuring approximately ten pairs of adult bruchids were added to the pods. The bruchid infested pods were gently and uniformly mixed with the remaining pods before heat sealing the two inner layers independently. Storage was for four months. Altogether, there were 18 bags in the experiment, and the

treatments were arranged in a factorial manner with two factors (triple-layer bags and cloth bags) and three levels (*A. flavus* inoculated; bruchid inoculated; and *A. flavus* + bruchid inoculated pods).

### 2.6. Data collection and equipment used

Initial data on pods such as percent germination, oil content, 100 seed weight, insect damage and aflatoxin contamination were collected one day prior to the start of the experiment. Final data was recorded on all these parameters at the end of the four month storage period. Total aflatoxin levels were estimated on samples using indirect competitive enzyme linked immunosorbant assay (ELISA) at the end of the four months (Waliyar et al., 2005). Initial and final levels of seed germination (ISTA, 1999) and oil content (Jambunathan et al., 1985) were measured using standard procedures.

### 2.7. Oxygen and carbon dioxide levels

The $O_2$ and $CO_2$ levels were measured using a Mocon PAC Check Model 325 headspace analyzer (Mocon, Minneapolis, MN, USA). The first set of readings were taken a week after the experiment was set up, and thereafter at weekly intervals. The $O_2$ and $CO_2$ levels were measured on selected days by inserting the needle probe of the Mocon analyzer near the center of the middle and inner layers of the triple layer bags; the puncture hole in the outer bag was sealed using plastic adhesive tape; no sealing was done in the case of the cloth bags.

### 2.8. Statistical analysis

Data was analyzed by using the analysis of variance (ANOVA) procedure in GENSTAT statistical package (version 14.0; Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK) and the treatment means were differentiated by a least significant difference (LSD) at $P = 0.05$. Correlations were drawn between percent bruchid damage to pods with both $O_2$ and $CO_2$ contents at monthly intervals during the course of the experiment.

## 3. Results

The greatest loss of seed weight (39.6%) was observed when pods infested with *A. flavus* were stored in cloth bags and artificially infested with bruchids. This loss was significantly greater than with any of the other treatments (Table 1). The percent decrease in seed weight was comparatively less in triple-layer bags (0.8%) over cloth bags (7.2%) under natural storage conditions with no significant differences. However, with artificial infestations of *A. flavus* and bruchids, the percent decrease in seed weight was only 1.4% in triple-layer bags when compared to 39.6% in cloth bags with significant differences.

Though not significant, seed germinability was comparatively more in triple-layer bags (89.3%) over cloth bags (81.3%) under natural conditions (Table 1). However, under artificial infestations of *A. flavus* and bruchids; triple-layer bags offered more seed germinability (92.3%) over cloth bags (10.0%) with significant differences between them. The kernel oil content was significantly less over other treatments (9.5%) for pods stored in cloth bags after artificial infestations with *A. flavus* and bruchids. However, no significant differences in oil content were noticed for other treatments (Table 1).

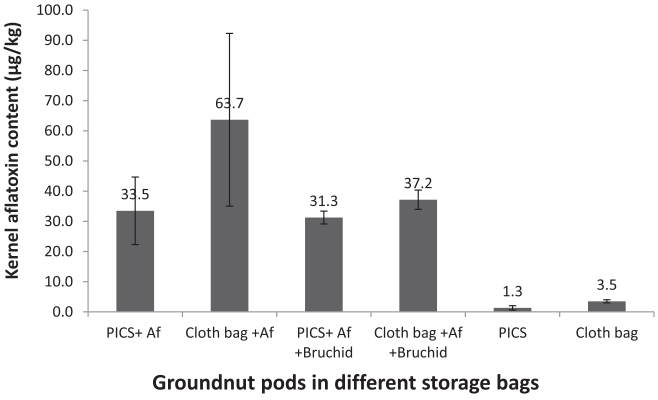
The mean final aflatoxin content of the seeds varied significantly in the different treatments. For pods stored in cloth bags the mean aflatoxin content was 3.5 μg $kg^{-1}$. However, the aflatoxin levels were significantly higher (63.7 μg $kg^{-1}$) for pods stored in cloth bags in the presence of *A. flavus* inoculum. For pods stored in triple layer bags, it ranged from 1.3 to 33.5 μg $kg^{-1}$ either under direct storage or with additions of *A. flavus* and bruchids, with no significant differences among them. The pods in cloth bags in the presence of *A. flavus* and bruchids exhibited aflatoxin levels of 37.2 μg $kg^{-1}$ (Fig. 1). Aflatoxin accumulation in triple layer bags in presence of bruchids and *A. flavus* was less (31.3 μg $kg^{-1}$) compared to that in cloth bags with added bruchids and *A. flavus* (Fig. 2).

Pod damage by bruchids was highest in cloth bags initially supplemented with bruchids and *A. flavus* (92.7%), and is significantly greater than that seen with the other treatments. Pod damage in triple layer bags supplemented with initial bruchids and *A. flavus* was about 21.3%. Bruchid damage for pods stored without any extra bruchid supplementation in cloth bags was about 6.3% and was significantly greater than in triple layer bags, which exhibited 3.3–3.7% damage with or without *A. flavus* infestation. In general, cloth bags allowed greater bruchid population expansion and damage than did triple layer bags in all the treatment combinations (Fig. 3). Overall, the damage to pods in triple layer bags as a result of initial and supplemented bruchid populations was comparatively lower (21.3%) to pods stored in cloth bags under the same levels of bruchid supplementation (92.7%) (Fig. 3).

**Table 1**
Kernel quality of groundnut stored for four months in triple-layer and cloth bags.

| Treatments | % Decrease in seed weight | Germination (%) | Oil content (%) |
|---|---|---|---|
| Pods in Triple layer bag + *A. flavus* | 3.5 | 92.3 | 50.0 |
| Pods in Cloth bag + *A. flavus* | 2.8 | 88.7 | 50.1 |
| Pods in Triple layer bag + *A. flavus* + Bruchids | 1.4 | 92.3 | 49.7 |
| Pods in Cloth bag + *A. flavus* + Bruchids | 39.6 | 10.0 | 9.5 |
| Pods in Triple layer bag | 0.8 | 89.3 | 51.6 |
| Pods in Cloth bag (Farmers' practice) | 7.2 | 81.3 | 45.6 |
| SEM | 6.14 | 13.22 | 6.70 |
| LSD (5%) | 7.10 | 8.94 | 6.628 |

Observations were recorded at 4 months after storage.
Initial germination of seeds (ICGV 91114) was estimated to be 93%.
*A. flavus* was spray inoculated to pods in treatments wherever applicable @ $5 \times 10^5$ CFU $ml^{-1}$.
Bruchids were added to bags in treatments wherever applicable @ 15 adult pairs/bag.
% Decrease in seed weight was calculated based on initial weight and final weights after four months of storage.




Initial aflatoxin content of the groundnut kernels prior to storage was 1.0 μg $kg^{-1}$

**Fig. 1.** Aflatoxin contamination after four months of storage in triple-layer and cloth bags with and without supplemental bruchids and *A. flavus* inoculum.

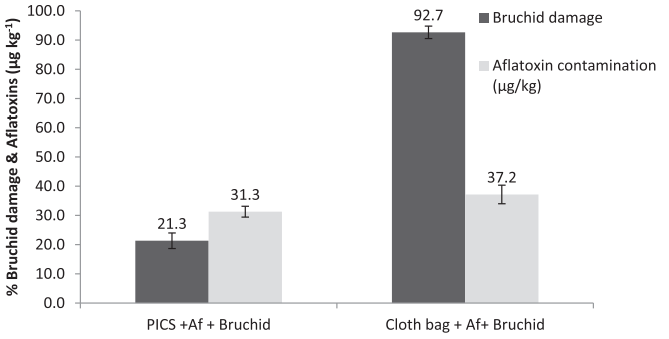



**Fig. 2.** Evaluation of triple-layer bags in minimizing bruchid damage of pods and aflatoxin contamination in groundnut after four months of storage.

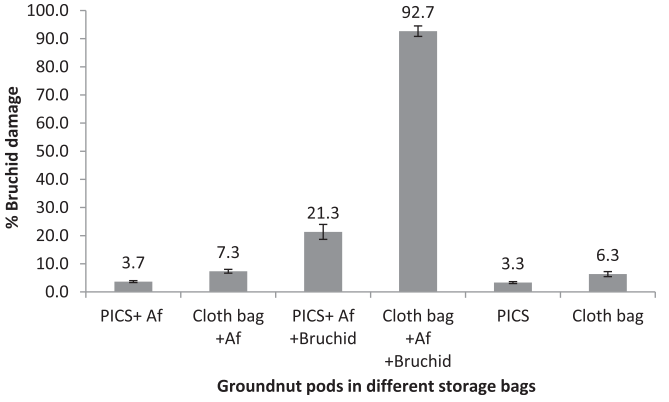



**Fig. 3.** Evaluation of different storage methods in groundnut on final bruchid damage to pods at four months after storage.

The triple layer bags supported accumulation of $CO_2$ at relatively higher levels than cloth bags. The final $CO_2$ levels reached 7.91% in triple layer bags versus 0.18% in cloth bags. Pronounced reduction in $O_2$ concentrations was noticed as well in triple layer bags over cloth bags during storage (Table 2). The bruchid damage was found to be positively correlated with $O_2$ content ($r = 0.230$); and negatively correlated with $CO_2$ content ($r = -0.205$) of bags. Further, the $O_2$ and $CO_2$ levels in bags have a direct negative relationship ($r = -0.988$).

**Table 2**
Effect of Triple Layer Bags on oxygen and carbon dioxide levels in groundnut storage.

| Treatments | Oxygen concentration (%) | | Carbon di oxide concentration (%) | |
|---|---|---|---|---|
| | Initial[a] | Final | Initial | Final |
| Pods in Triple layer bag + *A. flavus* | 19.73 | 19.25 | 0.03 | 0.16 |
| Pods in Cloth bag + *A. flavus* | 20.17 | 19.74 | 0.01 | 0.04 |
| Pods in Triple layer bag + *A. flavus* + Bruchids | 11.51 | 3.36 | 4.01 | 7.91 |
| Pods in Cloth bag + *A. flavus* + Bruchids | 19.85 | 19.70 | 0.10 | 0.03 |
| Pods in Triple layer bag | 15.95 | 3.45 | 2.01 | 6.11 |
| Pods in Cloth bag (Farmers' practice) | 19.97 | 19.63 | 0.03 | 0.18 |
| SEM | 0.79 | 1.85 | 0.38 | 0.83 |
| LSD (5%) (Trt × Time) | 1.026 | | 1.508 | |

[a] Initial readings were taken at one week after experimentation and final readings at 4 months after storage.

## 4. Discussion

Overall, our results demonstrated that hermetic triple layer bags are superior to cloth bags in protecting groundnut quality parameters such as germinability, oil content and seed weight. Further, we observed that mold development and subsequent aflatoxin accumulation were retarded significantly by the triple layer bags. Pod damage by bruchids was likewise less in triple layer bags compared to cloth bags. Hermetic storage of groundnuts is becoming even more attractive in view of accumulation of free fatty acids (FFA) and mold development during improper or unprotected storage (Villers et al., 2006). Maintenance of seed germinability and vigor during groundnut storage is certainly also a key issue (De Bruin, 2005). Unprotected storage or improper storage of groundnuts and storage in jute bags can lead to reduced germination (Basave Gowda and Nanja Reddy, 2008; Van Chin, 2005).

Use of hermetic storage systems for a wide range of commodities is becoming common in Africa, Asia, and South and Central America. Recent studies indicate that hermetic storage of unshelled groundnuts for eight months is useful in maintaining constant moisture and germination rates and is comparable to refrigerated storage (Villers et al., 2006; Van Chin, 2005). The principle of hermetic storage is that it relies on the metabolic activity of molds and insects present in agricultural commodities to generate the low $O_2$ atmosphere that prevents the development of mycotoxins (Villers et al., 2006). Depleted $O_2$ levels may delay rancid flavor development (Slay et al., 1985). In our studies, the sealed storage system offered by triple layer bags presumably induced direct respiration effects on the molds via low $O_2$ and high $CO_2$ (Table 2) that reduced the aflatoxin accumulation in seeds (Fig. 1). Enrichment of $CO_2$ in the storage conditions hinders aflatoxin formation (Clevstrom et al., 1983). For short-duration storage of non-cured groundnuts with high moisture content, high $CO_2$ is the factor that limits growth of *A. flavus* (Moseley et al., 1971). On the other hand, relatively free atmospheric air-flow like that which obtains in cloth bags could not have supported the conditions that retard the mold and toxin accumulation seen in triple bags used in our study. Moreover, aeration, which occurs in cloth bags, is the most critical factor for production of aflatoxins (Hesseltine et al., 1966). However, there was also aflatoxin accumulation of 1.3 µg $kg^{-1}$ in pods stored in triple-layer bags without any artificial infestation with *A. flavus* (Fig. 1). We attribute this to natural infestation of pods harvested from fields and carrying either toxigenic strains of *A. flavus* or aflatoxin itself. Mechanical/physical damage to groundnut shells is a major contributing factor in aflatoxin formation by *A. flavus* (Bampton, 1962). In our studies, though apparently healthy pods were selected and fumigated prior to experimentation, latent and hidden infection by *A. flavus* on pods coming from the field must have been responsible for toxin accumulation. The two most important favorable factors for *A. flavus* growth and multiplication in groundnuts are a temperature of 30 °C and relative humidity of 80–85% (Spensley, 1963).

Control of bruchid reproduction and damage by triple layer (PICS) bags has been reported in several crops in the recent past (Vales et al., 2014; Baoua et al., 2012; Murdock et al., 2012). In our present study, pod damage by bruchids was less in triple layer bags compared to cloth bags under all conditions (Figs. 2 and 3). Reduced pod damage in triple layer bags can be attributed to low $O_2$ levels that prevailed in the storage bags. Insect infestation was generally less under depleted $O_2$ levels in storage (Slay et al., 1985). The control of insects under low $O_2$ atmospheres is due to suppression of feeding activity and eventually death by desiccation resulting from the inadequate supply of $O_2$ (Murdock et al., 2012). Correlation studies also suggested a negative relationship between $CO_2$ levels and bruchid damage ($r = -0.205$).

It has been speculated that bruchids play a role in enhancing infection by aflatoxigenic molds (Harish et al., 2012). In our studies, bruchid damage of pods was less in triple layer bags (21.3%) compared to cloth bags (92.7%) under artificial inoculation with *A. flavus* and with bruchid supplementation. A parallel trend was also observed with respect to aflatoxins, wherein the toxin accumulation was greater in cloth bags compared to triple layer bags (Fig. 2). Our results with aflatoxins and pod damage were limited to four months of storage. Studies utilizing additional storage times and conditions are merited.

It is useful to remember that hermetic storage containers that have finite gas transfer rates across their surfaces perform best when the container surface area is small compared to the volume stored. In other words, the larger the storage bags the better. The lesser the surface area relative to the volume, less oxygen can diffuse into the contained volume and resupply any $O_2$-consuming organism, e.g., molds, insects. With this in mind it is useful to point out that the small bags used in our experiments had an unfavorable surface to volume ratio compared to full size 100 kg PICS or even 50 kg PICS bags. Use of the small bags in our experiments was a practical necessity, but it very likely caused underestimation of the protective effect of the triple layer bags vis-a-vis insect and mold development.

Preventing mold growth in groundnuts during storage is key to minimizing aflatoxins from entering the food chain. For bulk storage of groundnuts stored in shell form, the moisture content should be <10% to prevent mold growth (Diener and Davis, 1977). Based on our results, hermetic storage using triple layer bags (PICS) offers superior safety for groundnuts over the traditional packaging systems using jute bags. This technology of storing groundnut pods hermetically in triple layer bags is a viable alternative among pesticide free storage methods and offers ecologically safer methods of storage with minimal pod damage. Further investigations are necessary to test the robustness of the technology and for working out its cost-effectiveness versus other available storage practices.

## 5. Conclusions

Hermetic storage of groundnuts using triple layer PICS bags is a viable and sustainable storage alternative to existing farmers' practice.

## Acknowledgments

The authors would like to thank “Purdue University” (Agreement No. 4301-202413/11098443) and “CGIAR Research Program-Grain Legumes” for providing financial support to this experiment.

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