



# Maintaining dryness during storage contributes to higher maize seed quality



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## ABSTRACT

Smallholder farmers in Pakistan store their seeds and grains in porous polypropylene (woven) and jute bags or in bulk. Seed stored in these containers is susceptible to fluctuating seasonal relative humidity and temperature, which promote mold and insect growth. The present study assessed the performance of Purdue Improved Crop Storage (PICS) bags for maize seed storage during a two-month period. Seed moisture content increased in polypropylene bags while it remained constant in PICS bags. No change in germination was observed in maize seeds stored in PICS bags while in polypropylene bags it was reduced in half when compared to the initial germination. Seed stored in polypropylene bags had higher insect damage with a weight loss of 35% while in PICS bags the infestation was minimal with a weight loss of about 3%. Higher aflatoxin contamination levels were observed in seeds stored in polypropylene than PICS bags. PICS bags are effective at preserving the dryness of maize seed in storage during high relative humidity conditions, which leads to maintenance of seed quality.

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## 1. Introduction

Maize is a high-yielding cereal crop with global production of 2525.7 million tons in 2015 (FAO, 2016). Pakistan is one of the eight major maize-producing countries in Asia with 5.2 million metric tons per year (FAO, 2017). Millions of smallholder farmers in Sub-Saharan Africa and Asia are involved in maize production and depend on it for their livelihoods. Although maize consumption as animal feed is rapidly increasing in Asia, it is still an important staple food in the hill and tribal regions of Pakistan, especially Azad Jammu and Kashmir (AJK). In the AJK, more than 82% of maize production is used for human consumption and is planted on almost 41% of farmed area in the kharif (summer) season (Qureshi et al., 2002). Rosegrant et al. (2009) predicted a double increase in the demand for maize in the developing world by 2025. Increasing maize production could help address this challenge. But preserving what is produced, especially in developing countries, is equally important and would help alleviate the growing demand for maize.

Postharvest losses along the value chain from harvest to consumption results in increased prices and lost income for resource-poor farmers. Thus, sustainable agriculture not only involves producing more grains for a growing population but also to preserve what is already produced to ensure food and nutrition security. Global annual food losses amount to 1.3 billion metric tons or enough food to feed 2 billion people (FAO, 2013). In Kenya, losses of more than 30% have been reported due to insect pest infestation during the storage season (Wongo, 1996; Tefera et al., 2011). Similar situations prevail in many developing countries in Africa and Asia including Pakistan. Several insect pests e.g. *Sitophilus zeamais*, *Prostephanus truncates*, *Tribolium castaneum* and *Rhyzopertha dominica* are known to cause damage to stored grains including maize (Adams and Schuller, 1978; Tefera et al., 2011; Ng'ang'a et al., 2016; DeGroot et al., 2017). First instar larvae of grain-boring insects such as Angoumois grain moth (*Sitotroga cerealella*) and lesser grain borer (*Rhyzopertha dominica*) are known to cause invisible damage on the germ of the seed leading to loss of viability (Prakash and Rao, 1995). The infestation of grain and seed by insect pests is exacerbated by high temperature and relative humidity, which are prevalent in many developing countries. Seeds are hygroscopic as their moisture contents change in response to the relative humidity

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in which they are exposed to and thus affect the seed longevity during storage (Ellis and Roberts, 1980). Higher moisture content in dried seeds promotes the growth of insects and microorganisms that affect seed viability in storage (Murdock et al., 2012; Bradford et al., 2016). Likewise, aflatoxin-producing molds (*Aspergillus flavus*) develop at higher relative humidity and temperature, and are prevalent in stored grains in Pakistan (Abdel-Hadi et al., 2012; Ahsan et al., 2010). In warm and humid environments, aflatoxin concentrations increase rapidly if grains are not properly dried and stored (Bankole et al., 2006). Aflatoxins cause liver cancer, renal diseases, and gastroenteritis, and have also been associated with child growth impairment esophageal cancer and neural tube defects (Wu et al., 2014).

Preventing postharvest losses is a major challenge for small-holder farmers in developing countries. Hermetically sealed containers such as the Purdue Improved Crop Storage (PICS) bags have been reported to maintain quality of stored seeds and grains of more than 15 crops including cowpeas, maize, common beans, mungbeans, pigeon peas, sorghum, and several other crops (Baoua et al., 2012, 2014; Murdock et al., 2012; Mutungi et al., 2014, 2015; Vales et al., 2014; Murdock and Baributsa, 2015) against insect pests. Storage in PICS bags has helped to reduce aflatoxin contamination in maize (Williams et al., 2014; Ng'ang'a et al., 2016; Tubbs et al., 2016). Most of these studies have shown that PICS bags are able to maintain a constant relative humidity inside bags regardless of the outside environment. Therefore, the present study was conducted to evaluate the performance of PICS bags for safe maize seed storage under high summer relative humidity in farming communities of Pakistan.

## 2. Material and methods

### 2.1. Experimental details

This study was conducted for about two months (from June 8 to August 4, 2015) in farmers' storehouses in the Kotli district of Azad Kashmir, Pakistan. Storehouses are used by farmers to keep their produce for domestic consumption and seed for planting. Relative humidity and temperature data were hourly recorded during the experiment using a data logger (Rhino Research Thailand) placed in a corner of the storehouse. Locally produced maize seeds of cultivar "Sarhad White" were used during this study. Completely Randomized Design (CRD) was used for this experiment and each experimental unit was replicated thrice. PICS bags of 50-kg capacity were provided by the Purdue's Afghanistan Agricultural Extension Project (AAEP II) from Herat, Afghanistan. The bags were cut into smaller size due to small quantity (10-kg) of seed used in this experiment.

### 2.2. Seed storage and sampling

Maize seed (10-kg each bag) was stored in PICS and polypropylene (woven) bags at ambient storage conditions of the storehouse. Data Loggers (Centor Thai, Rhino Research Group, Thailand) were used to collect data on Relative Humidity (RH) and temperature of the storehouse for the duration of the experiment. At the end of the experiment, 1-kg seed samples were taken to assess storage losses and aflatoxin contamination.

### 2.3. Determination of seed moisture contents and germination

Seed moisture content was determined using the protocol developed by International Seed Testing Association (ISTA, 2015). Low constant temperature method was applied by drying grinded sample of 5 g maize seed in an oven at 103 °C for 17 h. Seed

germination was tested by placing four replicates of 100 seeds, from randomly drawn seed samples, in sterilized and moistened blotting paper in a germinator (SANYO Japan, MIR-254) at 25 °C (ISTA, 2015). Seeds were scored germinated when radicle protrusion was visible.

Germination index (GI) was calculated as described by the Association of Official Seed Analysts (1983) using formula

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Root and shoot lengths were measured 15 days after sowing. Germination energy (%) was recorded by counting the number of seedlings germinated the fourth day after the start of germination.

### 2.4. Assessment of losses due to insects

At the beginning of the experiment, maize seed was clean and appeared not infested. No assessment of insect infestation was done. At the end of the experiment, randomly drawn 2-kg samples of stored maize seed were sieved to separate grain and insects. Live insects were sorted by species and then counted manually for assessment of live insect populations. Damaged grains and grains with damaged embryos were counted. Percent weight losses were estimated using the equation of Adams and Schulter (1978).

$$\text{Percent weight loss} = \frac{Und - DNu}{U(Nd + Nu)} \times 100$$

where "U" represents weight of undamaged grain, "D" is the weight of damaged grain, "Nd" is the number of damaged grain and "Nu" is the number of undamaged grain.

Grains with damaged embryo (%) were estimated by separating the damaged grains from those having intact embryo using the following equation:

$$\text{Grains with damaged embryo (\%)} = \frac{\text{Grains with damaged embryo}}{\text{Total number of grains}} \times 100$$

### 2.5. Determination of aflatoxin contamination

Fifty (50) grams of thoroughly milled grains were used for aflatoxins B<sub>1</sub> and G<sub>2</sub> analysis. Aflatoxins B<sub>1</sub> and G<sub>2</sub> were purified with Vicam Afla B<sub>1</sub> and G<sub>2</sub> HPLC columns following standard procedures developed by the manufacturer and mobile phase was analyzed on Shimadzu HPLC (Shimadzu Scientific Instruments, Inc. Kyoto, Japan). Aflatoxin levels were quantified by comparing the B<sub>1</sub> and G<sub>2</sub> peaks with the standards (range 1–50 ppb) prepared by Sigma Chemical Corporation St. Louis, MO.

Data collected on different parameters were analyzed using analysis of variance technique by statistical package Statistix-10 (Tallahassee, FL, USA). Least significance difference (LSD) test at 0.05 probability level was used to compare the treatment means.

## 3. Results

### 3.1. Environmental conditions during the experiment

Hourly relative humidity as well as temperature data were averaged for each day (Fig. 1). The average RH varied significantly with a minimum of 43% on June 12, 2015 and a maximum of 87% on August 2, 2015 (Fig. 1). There was an increasing trend of RH from

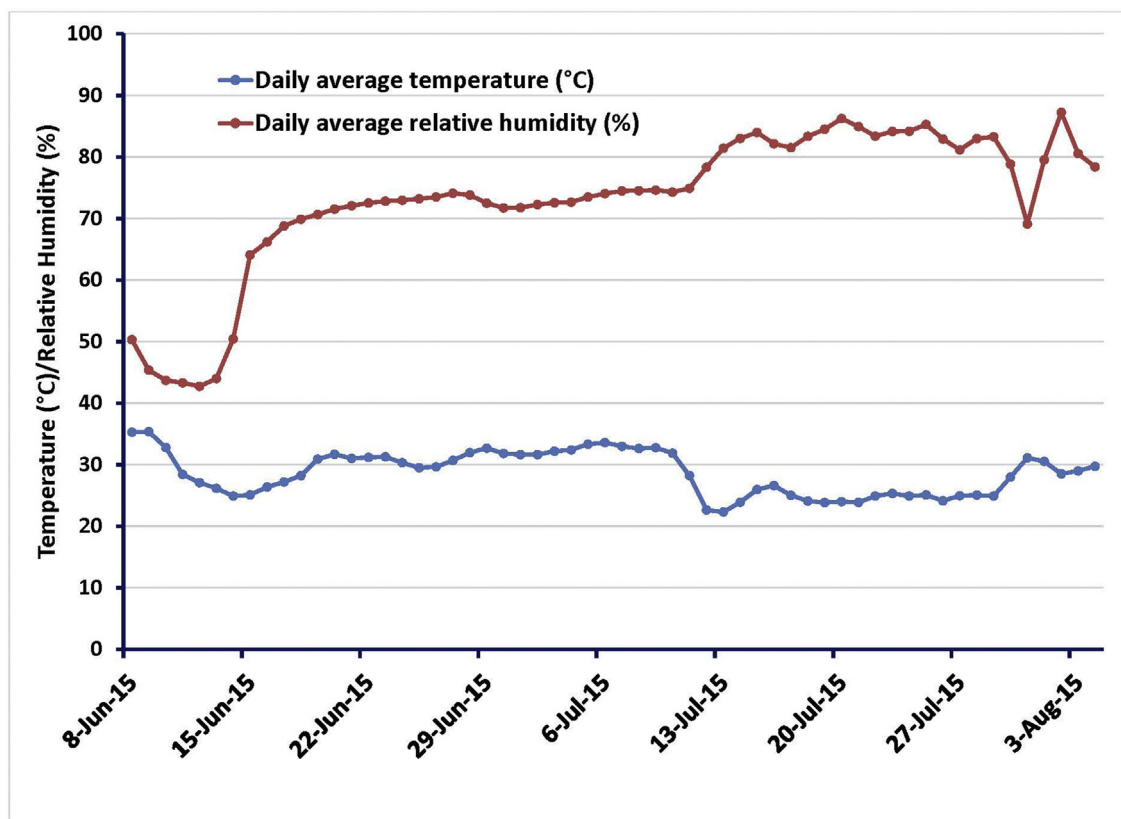


Fig. 1. Daily average relative humidity and temperature of farmers' storehouse (June 8 to August 4, 2015) during the storage of maize seed in PICS and polypropylene bags in Kotli district in Pakistan.

June to August 2015. Average temperatures ranged from a low of 22 °C in July 2015 to as high as 35 °C on June 8 and 9, 2015 (Fig. 1). Overall, the temperature decreased gradually from June to August 2015.

### 3.2. Seed moisture and germination attributes

Initial seed moisture content was estimated at 12.5% using the oven-dried method. After two months, there was no increase in moisture content of seeds stored in PICS bags while in polypropylene bags seed moisture content significantly increased by 3.14% (Table 1). The initial maize seed germination was 96%. At the end of the experiment, there was a minimal damage to seed embryo stored in PICS bags (1.89%) while it was significant (33.64%) on seed stored in polypropylene bags. Germination index and energy of seeds stored in PICS bags were significantly higher than those of seeds stored in polypropylene bags. Significantly greater root and shoot lengths were recorded for seedlings produced from seeds

stored in PICS bags compared to seeds stored in traditional polypropylene bags.

### 3.3. Insect populations and weight loss

Assessment of insect pest at the end of the experiment showed a high-level infestations. After two months of storage, there were three insect species in maize seed: *Sitophilus zeamais*, *Tribolium castaneum* and *Rhyzopertha dominica* (Table 2). A significant difference of insect pest damage was observed between PICS and polypropylene bags. *Sitophilus zeamais* were found to be the major storage insect pests with 8 live adults in a 2-kg sample of maize seed stored in PICS bags, while in polypropylene bags there were 1378 live adults (Table 2). In polypropylene bags 16 live adults of *Tribolium castaneum* and 8 live adults of *Rhyzopertha dominica* were observed, whereas in PICS bags only 1 live adult of *T. castaneum* and 4 live adults of *Rhyzopertha dominica* were present (Table 2). Maize seed damage in PICS bags was 2.3% while in polypropylene bags it

Table 1

Moisture content and germination attributes of maize seed stored in PICS bags and polypropylene bags for two months (2015) in farmers' storehouse in Kotli district in Pakistan.

	Moisture content (%)	Seed with damaged embryo (%)	Germination (%)	Germination index (GI)	Germination energy (%)	Root length (cm)	Shoot length (cm)
Initial	12.5 ± 0.15 b*	—	96.00 ± 0.5 a	11.50 ± 0.2 a	82.00 ± 1.5 a	8.15 ± 0.25 a	3.0 ± 0.15 a
PICS bag after 2 months	12.74 ± 0.036 b	1.89 ± 0.41b	85.33 ± 1.45 a	11.33 ± 0.34 a	81.75 ± 1.9 a	8.10 ± 0.45 a	3.0 ± 0.24 a
Polypropylene bag after 2 months	15.64 ± 0.17 a	33.64 ± 0.82a	50.00 ± 2.89 b	6.80 ± 0.23 b	50.25 ± 2.03 b	2.67 ± 0.37 b	1.5 ± 0.24 b
LSD at P ≥ 0.05	0.49	2.54	8.97	0.88	5.80	1.16	0.31

\*Means within a column followed by the same letters are not significantly different at P ≤ 0.05.

**Table 2**  
Live insect population, weight loss, and aflatoxin contamination of maize seed stored in PICS bags and polypropylene bags for two months (2015) in farmers' storehouse in Kotli district in Pakistan.

	<i>Sitophilus zeamais</i> (Live)	<i>Tribolium castaneum</i> (Live)	<i>Rhyzopertha dominica</i> (Live)	Weight loss (%)	Aflatoxin B <sub>1</sub> (ng/g)	Aflatoxin G <sub>2</sub> (ng/g)
PICS bag after 2 months	8 ± 2b*	4 ± 1b	1 ± 1b	3 ± 0.29b	0.08 ± 0.01b	0.55 ± 0.029 b
Polypropylene bag after 2 months	1378 ± 71a	16 ± 3a	8 ± 2a	35 ± 2.89a	0.53 ± 0.04 a	1.13 ± 0.04 a
LSD at P ≥ 0.05	195.62	8.17	6.61	8.05	0.12	0.14

\*Means within a column followed by the same letters are not significantly different at P ≤ 0.05.

was 46.62% (data not shown). Only 3% weight loss occurred in PICS bags compared to 35% in polypropylene bags (Table 2).

### 3.4. Aflatoxin contamination

Assessment of aflatoxin contamination at the end of the experiment showed much lower levels in maize grains stored in PICS bags compared to polypropylene bags (Table 2). Aflatoxin B<sub>1</sub> concentration was 0.08 ng/g in maize grain stored in PICS bags whereas those in polypropylene bag had 0.53 ng/g. Aflatoxin G<sub>2</sub> level was 0.55 ng/g in the grains stored in PICS bags, whereas grains stored in polypropylene bags had 1.13 ng/g of aflatoxin G<sub>2</sub>.

## 4. Discussion

This study evaluated the performance of PICS bags for preserving maize seed quality during storage under high summer relative humidity. PICS bags maintained higher seed germination and its attributes compared to polypropylene bags. Greater germination losses in polypropylene bags were due to increased moisture content in prevailing high RH storage conditions. It is well known that every 1% increase in seed moisture content reduces seed shelf life by half (Harrington, 1972). Germination in PICS bags was reduced by 11% while it was significantly reduced by as much as 49% for maize seed stored in polypropylene bags. Despite a reduction in germination of maize seed stored in PICS bags, germination index and energy, and root and shoot length were similar to those observed at the beginning of the experiment. Increasing moisture content of grain has shown to be detrimental to seed viability and germination (Tubbs et al., 2016). Bewley et al. (2013) emphasize that seed quality is at greatest risk at high moisture content during storage. However, there is lack of awareness among many farmers in Pakistan and other developing that moisture content of seed is the main factor for preserving its quality. The oxygen barrier combined with maintenance of low moisture content of seed stored in PICS bags resulted in higher seed quality during storage. Properties in PICS bags that lead to low oxygen barrier may have also contributed to low permeability of humidity inside the PICS bags. As our trial was set up before the start of Monsoon season during which ambient relative humidity was quite high that raised up moisture content of seeds stored in polypropylene bags. Polypropylene (woven) bags are porous and essentially influenced by ambient prevailing environmental conditions such as temperature and relative humidity (Martin et al., 2015; Mutungi et al., 2014). Williams et al. (2017) showed strong correlations when comparing polypropylene bag RH to room RH (84%) and polypropylene bag temperature to room temperature (up to 99%). However, the correlation comparing PICS bag RH to room RH was about 42% though the temperature correlation was not different of that observed in polypropylene bags.

Maintenance of low moisture content (MC) is important to preserve seed viability (Bewley et al., 2013; Tubbs et al., 2016). In this study, maize seed at 12.5% MC was in equilibrium with about

65% RH, while at 15.6% MC was in equilibrium with over 80% RH. Drying before storage and preserving seed in PICS bags will minimize fungal and insect growth in waterproof packages (Kunusoth et al., 2012). The results corroborated other findings (Tubbs et al., 2016; Williams et al., 2014, 2017) that PICS bags provide an effective barrier that hindered moisture entry from environment into grain stored inside bag.

Maize seed assessed at the end of the experiment showed that *Sitophilus zeamais* was the predominant insect species (98%). Live insects in PICS bags after 2 months of storage represented less than 1% of the total population. Better performance of hermetic technologies such as PICS bags in protecting grains against insect pests is well documented (Baoua et al., 2012, 2014). Low oxygen and higher carbon dioxide environment inside bags created by insect respiration stop the development of insects (Murdock and Baoua, 2014). This increased level of CO<sub>2</sub> at low level of O<sub>2</sub> has proved fatal for the insect pests (Zhou et al., 2000). Insect mortality is the result of disrupted water supply due to O<sub>2</sub> deficit because insects meet majority of their water needs from aerobic respiration (Murdock et al., 2012). Only a small number of live insects were found in PICS bags in present study though they did not cause much damage to the seed. Live insects have been reported in PICS bags after several months of storage (Baoua et al., 2014).

Preventing the growth of aflatoxigenic molds is an important quality and safety consideration during seed and grain storage. Increased aflatoxin contamination level in polypropylene bags was due to change in seed moisture content, which augmented to 15.6%. It is well documented that high grain moisture content will lead to growth of molds and result in aflatoxin contamination (Brewbaker, 2003; Bewley et al., 2013; Tubbs et al., 2016; Williams et al., 2014). Moisture content of maize seed stored in PICS bags was maintained below 13% and thus mold growth was relatively less (Quezada et al., 2006). Lesser contamination in PICS bag might be due to hypoxia/hypercarbia that reduces the growth of pathogen (Ellis et al., 1994) and furthermore reduce pest infestation, which play a role in spreading mold.

Our results suggest that storing dry maize seed in PICS even for two months will maintain its moisture content, viability and vigor even in high relative humidity and temperature conditions. In addition, the bags will stop mold growth and insect infestation.

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