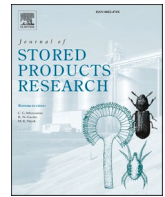




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Desiccant drying prior to hermetic storage extends viability and reduces bruchid (*Callosobruchus chinensis* L.) infestation of mung bean (*Vigna radiata* (L.) R. Wilczek) seeds

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ABSTRACT

Seeds of mung bean (*Vigna radiata* (L.) R. Wilczek) are subject to loss of viability due to aging and damage from pulse beetles (or bruchids; *Callosobruchus* spp.) infestation during storage. We investigated whether seed drying using desiccants and hermetic packaging would prevent or ameliorate these consequences of storage. Sun-dried mung bean seeds at a moisture content of 10% were subjected to further drying for 72 h using five different desiccants: Drying Beads® (a zeolite-based desiccant), silica gel, sodium aluminum silicate, activated alumina, and cow-dung ash (a traditional desiccant). Seeds were subsequently stored in hermetic plastic containers in the presence of these desiccants under ambient conditions along with sun-dried seeds stored in cloth bags or in hermetic containers. In addition, parallel samples of each treatment were inoculated with one pair of bruchid beetles (*C. chinensis* L.) and stored under the same conditions. The seed drying treatments did not affect initial seed quality (germination percentage and seedling vigor) significantly. After storage for 9 months at ambient temperatures, seeds dried using Drying Beads, silica gel, sodium aluminum silicate and activated alumina had higher germination percentages, seedling vigor indices and soil emergence, and lower electrical conductivity (leakage upon imbibition) and fungal infestation compared to other conditions. In addition, the mung bean seeds inoculated with bruchids and stored with these effective desiccants had less damage, oviposition, and insect respiratory activity in the hermetic containers and maintained higher seed germination and seedling vigor after six months of storage compared to other treatments and controls. The results demonstrate the superior ability of desiccants to quickly and safely dry seeds prior to and during storage and the benefits of such drying and hermetic storage conditions for preventing seed deterioration and insect damage during storage.

1. Introduction

Mung bean (*Vigna radiata* (L.) R. Wilczek) is a popular pulse crop cultivated throughout the world. Its seeds contain easily digestible protein (25%) and available nutrients, including calcium (124 mg/100 g), phosphorus (326 mg/100 g), iron (7.3 mg/100 g) and vitamin B, and are highly prized by vegetarians and as a source of plant-based protein (Anwar et al., 2007; Yi-Shen et al., 2018). Producing and maintaining high quality seeds for planting is an essential component of mung bean cropping systems. Seed quality is influenced by edaphic, environmental, biotic and management factors during seed production and by the

conditions and duration of storage (Bewley et al., 2013). Most of the seed quality losses occur during harvesting and post-harvest handling, including threshing, processing, transportation and storage. Maintaining the quality and integrity of the seeds during storage is critical for subsequent crop establishment and production. The most important factors affecting seed longevity in storage are seed moisture content (m. c.) and temperature (Ellis and Roberts, 1981). The high relative humidity (r.h.) and temperatures encountered in tropical climates often result in rapid seed deterioration and loss of viability of seeds stored under ambient as compared to controlled conditions (Ellis, 1988; Nagel and Börner, 2010).

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Sun drying is commonly practiced to reduce seed m.c. for storage, but as seeds are hygroscopic, they will absorb or lose water in relation to the r.h. of the ambient air. Ambient conditions in tropical and sub-tropical regions often exceed 75% r.h. and 30 °C after seed harvest and during storage, resulting in rapid seed deterioration and promoting fungal and insect damage that can make it difficult to store seeds even until the next planting season (Daniel and Ajala, 2004; Afzal et al., 2017; Bradford et al., 2018). While the use of heated air is common for drying seeds and commodities, the maximum temperatures that can be used are lower for seeds than for commodity grains in order to maintain viability, and heated-air drying is inherently less effective when the ambient air is warm and humid (Mrema, 2011). Desiccant-based drying can be more effective under these conditions (Kunusoth et al., 2012), and desiccants such as zeolite beads (Hay et al., 2012; Afzal et al., 2019; Bakhtavar et al., 2019; Bakhtavar and Afzal, 2020a, b; Kamran et al., 2020) and silica gel (Zhang and Tao, 1989; Doijode, 1995; Eklou et al., 2006; Basavegouda and Reddy, 2008) have been used for seed drying. However, if seeds are not packaged in waterproof containers after drying, water absorption from the air at high humidities can increase their m.c. during storage, reducing longevity. Longer-term maintenance of seed viability, as for germplasm storage, requires refrigeration for storage at low temperatures, which is often unavailable or dependent upon erratic power supplies, especially in tropical and sub-tropical regions. In addition, refrigeration increases the r.h. in storage rooms, resulting in elevated m.c. if the seeds are not stored in waterproof containers, partially offsetting the benefit from reduced temperatures and making the seeds vulnerable to rapid deterioration upon removal from cold storage. Hence, farmers or companies storing seeds in the tropics would benefit from low-cost techniques that can achieve and maintain low seed m.c. to extend seed quality for long periods without investments in refrigeration (Guzzon et al., 2020).

Elevated r.h. and temperature during storage not only cause seed deterioration, but also make seeds more prone to insect and fungal attack (Bradford et al., 2018). Mung bean seeds are susceptible to losses due to attack from as many as sixty-five different insect pests at both pre- and post-harvest stages (Lal, 1985). Among these pests, pulse beetles or bruchids (*Callosobruchus* spp.) are the most destructive, causing 50–60% damage in mung bean seeds in storage (Ramzan et al., 1990). Bruchids are a minor pest in the field, where they lay eggs on seeds before harvest and subsequently become serious pests in storage, causing both quantitative and qualitative losses (Casewell, 1961; German et al., 1987). They are among the most damaging pests of legume seeds, including mung beans, causing extensive grain and seed quality losses (Gahukar and Reddy, 2018; Stathers et al., 2020). Storing seeds in polythene bags or aluminum foil packets reduced pulse beetle infestation of mung beans (Singh, 1995). Purdue Improved Crop Storage (PICS) bags having low permeability to both water and oxygen effectively arrested insect damage during storage (Murdock and Baoua, 2014). Low water activity can arrest or kill insects, and at higher m.c. where insects are active, their respiration can lower oxygen levels in hermetic containers to lethal levels (Murdock et al., 2012).

In the present study, we have conducted experiments to assess the feasibility and effectiveness of combining desiccant-based drying with different types of packaging to prevent deterioration of seed quality and insect infestations during storage under ambient temperature conditions. We determined the extent and speed of seed drying by different desiccants and the ability of hermetic or porous containers to maintain low seed m.c. and extend viability during storage. In addition, we evaluated the effects of these desiccants and packaging materials on pulse beetle reproduction on mung beans during storage.

2. Materials and methods

2.1. Seeds and desiccants

The study was conducted at the Department of Seed Science and

Technology, College of Agriculture, Acharya N G Ranga Agricultural University, Hyderabad, Andhra Pradesh, India during 2012–13. Freshly harvested mung bean seeds (cv. LGG-460) with 10.0% and 10.2% initial m.c. and 98% germination were used for the study. The desiccants included Drying Beads®, a zeolite-based desiccant (www.dryingbeads.org) obtained from Rhino Research (www.rhino-research.com, Phichit, Thailand), sodium aluminum silicate, activated alumina, and silica gel (Zheng et al., 2014) obtained from dealers in Gujarat, India. In addition, cow-dung ash, which is a traditional desiccant used with stored seeds (Chiranjeevi, 1991), was obtained locally.

2.2. Storage conditions and treatments

Mung bean seeds with initial m.c. of 10.0% (fresh weight basis by oven test; see details below) were used to determine the extent and speed of drying due to different desiccants under ambient temperatures and their subsequent effects on seed quality. The study was conducted with a total of 3000 g seeds per treatment in a Factorial Completely Randomized Design (FCRD) with desiccant type and duration as the main factors. The seed material (1000 g) in each of three replications was divided into 20 subsamples of 50 g each enclosed in a muslin cloth bag to facilitate quick removal of seed samples from the hermetic containers and minimize effects on the desiccants' efficiency. The seed packets were placed in hermetic plastic containers along with the desiccants. Control seeds were similarly prepared without any desiccant in the hermetic container.

The required quantities of activated desiccants were determined based on experiments measuring their moisture adsorption capacities. A weighed quantity of activated (dried) desiccant was placed over water in a sealed container for 24 h and the weight increase indicated the maximum adsorption capacity. An amount of desiccant calculated to absorb sufficient water to dry the total quantity of seeds in a container from 10 to 6% m.c. (i.e., to absorb 40 g of water per kg of seeds) was calculated for each desiccant: 200 g of Drying Beads, 159 g of silica gel, 190 g of sodium aluminum silicate, 200 g of activated alumina, and 406 g of cow-dung ash. These quantities of the appropriate desiccant were placed in the hermetic plastic containers with the seeds and sealed. The treatments were: T1: seeds stored in hermetic container without desiccant (control); T2: seeds dried over Drying Beads; T3: seeds dried over sodium aluminum silicate; T4: seeds dried over activated alumina; T5: seeds dried over silica gel; and T6: seeds dried over cow-dung ash.

The r.h. (%) and temperature (°C) were recorded at 4 h intervals throughout the drying process using Extech Hygro-thermometers enclosed in the hermetic containers. Seed samples were retrieved every 4 h for 72 h for seed m.c. (fresh weight basis) and water activity measurements. Seed m.c. was determined on 5 g of seeds by the high constant temperature (130 ± 1 °C) oven method (ISTA, 1999). A Rotronic HygroLab based on vapor pressure measurements was used to determine water activity (a_w), i.e., the thermodynamic energy of water in the seed.

2.3. Effects of desiccants on seed quality during storage

Mung bean seeds with 10% initial m.c. and 98% seed germination were used for the seed storability study with the desiccant treatments described above. The seeds and desiccants were stored in hermetic containers for nine months under ambient temperatures in the laboratory. The seeds were mixed in the container and seed samples were drawn quickly using a spoon at random from the hermetic containers at bimonthly intervals for seed quality analyses. In addition, control seeds were stored in cloth bags without desiccant under the ambient laboratory r.h. and temperature conditions (treatment T7). The experiment was organized in a Factorial Completely Randomized design (FCRD) with seven treatments, six storage periods and three replications each.

After each storage period, the seed m.c. (fresh weight basis) was determined on 5 g samples using the oven method. Germination tests

were conducted on pure seed fraction using 100 seeds in four replicates following the top of paper method at 25 °C and 90 ± 3% r.h. (ISTA, 1999). The germinated seeds were evaluated as normal or abnormal seedlings, dead and hard seeds on the 7th day of the test. The germination percentages are based on the normal seedlings. For seedling vigor index, ten normal seedlings were selected randomly from each treatment and replication at the end of a germination test. The combined shoot and root lengths of each of the seedlings were measured in centimeters and average seedling lengths were calculated. The seedling vigor index was calculated by multiplying germination (%) by seedling length (cm) (Baki and Anderson, 1973). For seedling dry weight, ten normal seedlings were dried in an oven maintained at 80 °C for 24 h, cooled in a desiccator for 30 min and weighed on an electronic balance. For soil emergence, 100 seeds were taken randomly from each replication per treatment and hand dibbled in raised seed beds in a greenhouse. Seeds were sown equidistantly and watered to maintain the optimum soil moisture for emergence. The normal seedlings that had emerged at least 3 cm above the soil surface on the 15th day after sowing were counted and expressed in percentage.

Ion leakage was evaluated on 50 seeds per replication via the conductivity test. The seeds were soaked in 25 mL of distilled water at 25±1 °C for 24 h with frequent stirring. The seed leachate was collected and conductance was measured and expressed in $\mu\text{S cm}^{-1}$.

Storage fungi present on seeds were detected using the blotter method (ISTA, 1996). Four replications of 100 seeds each were placed equidistantly in circular fashion in sterilized Petri plates containing two moist blotters. The seeds were incubated at 25 °C for seven days with alternating cycle of 12 h light and 12 h dark. On the 8th day, the plates were examined under low power stereo binocular microscope and *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp. and *Fusarium* spp. colonies were identified and expressed as a percentage of total fungal colonies.

2.4. Effects of storage conditions on bruchid beetle infestation during storage

A culture of bruchid beetles (*Callosobruchus* spp.) was obtained from infested samples at seed storage facilities. After identifying the species (with assistance of the Indian Grain Storage Management and Research Institute, Hyderabad) as *C. chinensis* L., they were reared on cowpea (*Vigna unguiculata* (L.) Walp) seeds. Unsexed bruchids (300) were allowed to oviposit for seven days on 500 g of cowpea seeds and then removed. The emerging adults were collected 30 days after oviposition for use in the study.

Mung bean seeds were sterilized by heating in an oven at 40 °C for 6 h, then 1800 g per treatment with initial m.c. of 10.2% were divided into three replicates. Each replication was divided into six sub-replications of 100 g which were each placed in a hermetic container. Different desiccants were added to the seeds depending on their adsorption capacities in quantities estimated to bring the seed to 6% m.c. (see above). One pair of newly emerged one-day-old bruchids were released in each container, which then were sealed and maintained in storage up to six months. One sub-replication from each replicate container of a treatment was destructively sampled each month for recording bruchid population increases. The experiment was organized in a FCRD with 7 treatments and 3 replications each for a period of six months. The treatments were as described previously with the addition of one pair of bruchids to each container.

The consequences of bruchid introduction to the seed containers were assessed monthly on the sub-replicate samples. A CO₂/O₂ analyser (PBI Dansensor CheckPoint) was used to measure the CO₂ and O₂ concentrations inside the hermetic containers. The analyser was calibrated with atmospheric air (20.9% O₂ and 0.04% CO₂). The sampling needle of the analyzer was introduced into the top inlet lid of the hermetic container to obtain the sample, which was automatically analyzed by the instrument. Overall consequences of insect damage were calculated by deducting the final weight from the initial weight and expressed in

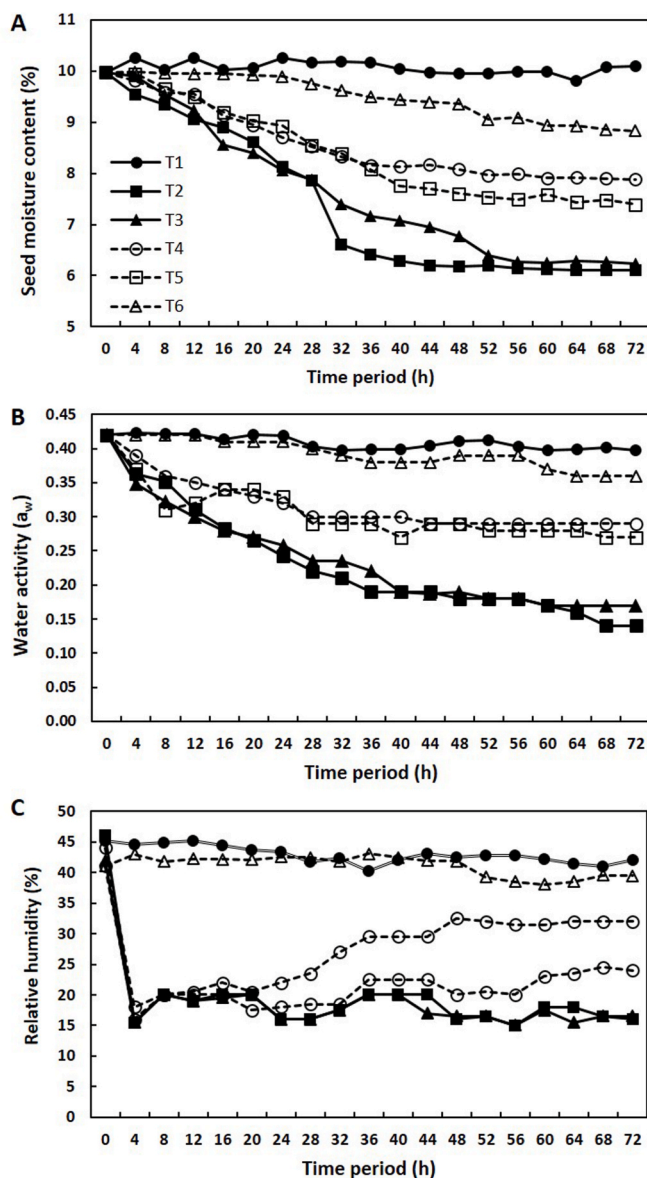


Fig. 1. Effect of different desiccants on speed and extent of mung bean seed drying based on moisture content (A) and water activity (or r.h./100) (B). The relative humidity inside of the containers is also shown (C). T1 (closed circle): Seeds in hermetic container (control); T2 (closed square): Seeds dried over Drying Beads; T3 (closed triangle): Seeds dried over sodium aluminum silicate; T4 (open circle): Seeds dried over activated alumina; T5 (open square): Seeds dried over silica gel; T6 (open triangle): Seeds dried over cow dung ash. Temperature varied diurnally between 27 and 32 °C during the experiment. Standard errors of means were between 0.03 and 0.14 for seed m.c., less than 0.01 for a_w , and less than 2% for r.h., generally smaller than the symbol sizes, so are not shown.

percent weight loss. Seed damage was assessed by counting the numbers of damaged or bored seeds in random sub-samples (10 g) and the mean number was expressed as a percentage of the total seeds in the sample. Representative 10 g samples drawn from each sub-replication were assessed for oviposition by counting the numbers of eggs laid by adult bruchids on the surfaces of the seeds with the help of a hand lens; the mean number of eggs per 10 g was calculated. In an additional experiment, five young gravid bruchids were released in each treatment condition, and the mortality of adult beetles was recorded every day for 10 days.

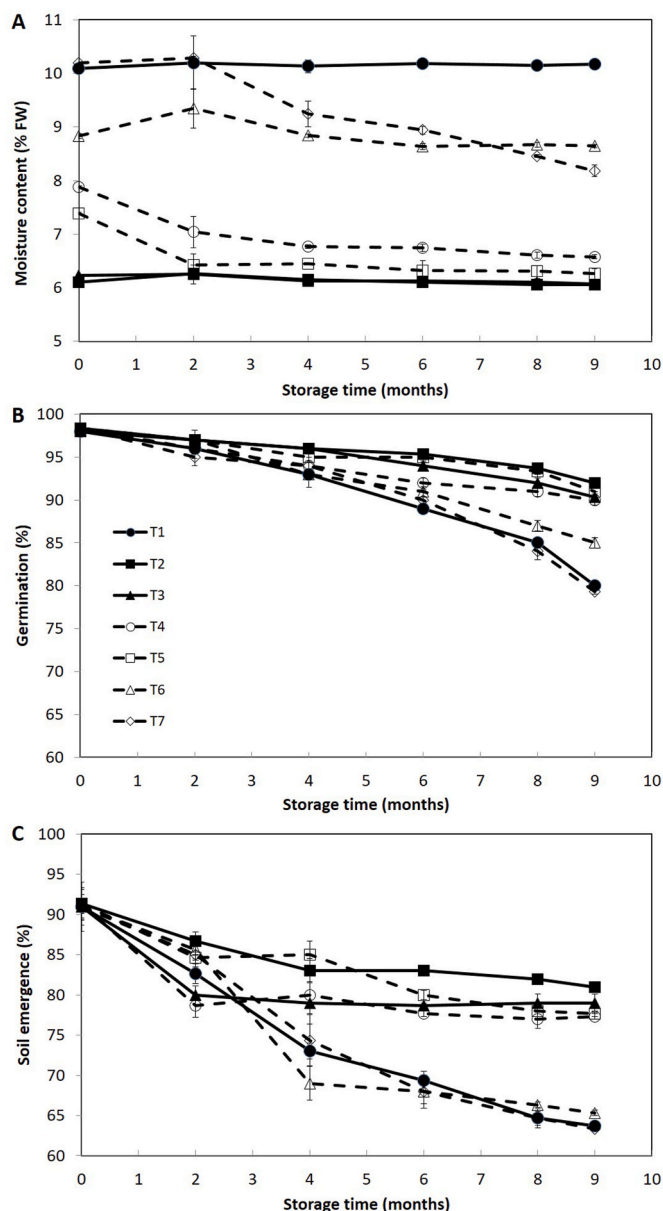


Fig. 2. Effect of different desiccants and storage containers on mung bean seed moisture content (A), laboratory germination (B) and soil seedling emergence (C). T1 (closed circle): Seeds without additional drying in hermetic container (control); T2 (closed square): Seeds dried with Drying Beads in hermetic container; T3 (closed triangle): Seeds dried with sodium aluminum silicate in hermetic container; T4 (open circle): Seeds dried with activated alumina in hermetic container; T5 (open square): Seeds dried with silica gel in hermetic container; T6 (open triangle): Seeds dried with cow dung ash in hermetic container; T7 (open diamond): Seeds without drying stored in jute bag. Temperature varied diurnally between 27 and 32 °C during the experiment. Standard errors (bars) are indicated when they exceed the size of the symbols. ANOVA tests of Treatment \times Duration interaction for moisture content, $df = 30$, $F = 7.01$, $P < 0.001$; for germination percentage, $df = 30$, $F = 3.22$, $P < 0.001$; for soil emergence, $df = 30$, $F = 5.43$, $P < 0.001$; see [Supplementary Tables S1 and S3A](#) for full ANOVA and treatment comparisons.

2.5. Statistical analysis

The data recorded were subjected to Analysis of Variance (ANOVA) (Gomez and Gomez, 1984) using INDOSTAT software. Tests for normality were conducted and angular transformation was applied when required, as for percentage data. As main factors and interactions

were highly significant, standard error of difference was calculated for each treatment effect and least significant difference (LSD) was calculated at $p < 0.05$ level to compare the mean differences among all treatments and storage times. Full ANOVA data are in [Supplementary Tables 3–5](#).

3. Results

3.1. Extent and rate of seed drying by different desiccants

We tested a number of desiccants for their ability to dry seeds quickly and to low m.c., including Drying Beads (a zeolite-based desiccant), silica gel, aluminum silicate, activated alumina, and cow-dung ash. All desiccants significantly reduced seed m.c. or water activity (a_w , equivalent to equilibrium r.h./100) compared to the control seeds, for which m.c. remained constant in the sealed container (Fig. 1A and B). Drying Beads and sodium aluminum silicate were the most effective, reducing seed m.c. from 10% ($a_w = 0.42$) to 6% ($a_w = 0.15$) within approximately 48 h, while activated alumina and silica gel reduced seed m.c. to 7.5–8% ($a_w = 0.27$ – 0.29) within the same period. Cow-dung ash was relatively ineffective, only reducing seed m.c. to 9% ($a_w = 0.36$) after 72 h (Fig. 1A). Seed germination and seedling growth tests before and after drying for 72 h showed no effects on seed quality for any of the treatments (data not shown). Thus, effective desiccants placed in sealed containers with seeds can quickly reduce seed m.c. to lower levels with no detrimental effect on seed quality.

All desiccants except cow-dung ash quickly reduced r.h. inside the containers to 16–18% (Fig. 1C). The r.h. in containers with Drying Beads and sodium aluminum silicate remained between 15 and 20% for the duration of the experiment, exhibiting a small diurnal fluctuation with daily temperature changes ranging between 26 and 32 °C (Fig. 1C). This agreed well with the final a_w (~ 0.15) of seeds with both desiccants (Fig. 1B). The r.h. in containers containing silica gel increased slowly to $\sim 25\%$ after 72 h, similar to the final a_w (~ 0.27) of seeds incubated with this desiccant. The r.h. in containers with activated alumina also slowly increased to 32% by 48 h, then remained constant; this also was close to the final a_w (~ 0.29) of seeds incubated with this desiccant (Fig. 1B). Cow-dung ash reduced seed m.c. only about 1% in 72 h (Fig. 1A), and only slightly lowered a_w and r.h. (Fig. 1B and C).

3.2. Effects of different desiccants and containers on seed quality parameters during storage

Seed m.c. and germination capacity were assessed during storage under the different treatment conditions. Moisture contents of seeds stored in cloth bags without drying fell from the initial 10.2% to 8.2% after 9 months of storage, indicating loss of moisture at the ambient humidity of the storage room as the seasons changed from rainy to dry, and germination percentage declined from 98 to 79% (Fig. 2; Table S1). Seeds initially at 10.1% m.c. and stored subsequently in hermetic containers maintained the same m.c. throughout the storage period, and germination percentage had declined to 80% after 9 months. Seeds stored in airtight containers with Drying Beads or aluminum silicate after initial drying maintained constant or only slightly declining m.c. (6.1%) throughout the storage period (Fig. 2A). Thus, they had reached equilibrium with the desiccants within the initial drying period at 6.1% m.c., and germination declined only to 92–90% after 9 months (Fig. 2B; Table S1). Seeds initially dried to 7.9 and 7.4% m.c. with activated alumina or silica gel exhibited further declines in m.c. to 6.6 and 6.3% during hermetic storage with the desiccants, indicating that they had not achieved equilibrium within the initial drying period but seeds continued to dry in hermetic storage, particularly in the first 4 months of storage (Fig. 2A). These seeds also germinated 90–91% after 9 months of storage (Fig. 2B). Moisture contents of seeds stored in hermetic containers with cow-dung ash increased slightly (8.3–8.7%) during storage, and viability declined to 85% by 9 months (Fig. 2A and B). Drying to

Table 1

Effect of desiccants on total fungal colonies and electrical conductivity of mung bean seeds during storage.

Treatments	Fungal disease colonies (%)				Electrical conductivity ($\mu\text{S cm}^{-1}$)			
	Storage period (months)							
	0	4	8	9	0	4	8	9
T1	0	11.3 c ± 0.21	15.7 ab ± 0.64	18.7 a ± 0.11	397 h ± 1.1	737 cd ± 2.9	890 ab ± 2.6	909 a ± 2.7
T2	0	4.33 e ± 0.46	4.67 e ± 0.11	5.00 e ± 0.00	397 h ± 1.8	580 g ± 3.8	658 f ± 4.3	664 f ± 3.0
T3	0	9.00 cd ± 0.48	7.67 de ± 0.11	7.00 de ± 0.00	397 h ± 0.8	600 g ± 5.5	708 de ± 2.3	711 d ± 2.7
T4	0	5.00 e ± 0.48	8.67 cd ± 0.28	9.00 cd ± 0.00	397 h ± 0.4	642 f ± 4.0	750 cd ± 2.0	752 cd ± 1.6
T5	0	7.67 de ± 0.69	8.67 cd ± 0.11	10.7 cd ± 0.11	397 h ± 0.4	610 g ± 3.8	756 cd ± 4.2	759 c ± 2.0
T6	0	11.0 c ± 0.66	14.7 b ± 0.74	15.7 a ± 0.82	397 h ± 0.7	684 ef ± 0.95	854 b ± 5.0	861 b ± 1.7
T7	0	15.7 a ± 0.69	19.0 a ± 0.66	18.7 a ± 0.82	397 h ± 0.6	730 d ± 1.64	873 b ± 1.3	883 ab ± 6.2

Mean values are presented \pm SE. Treatment mean differences (different lower case letters) are based on LSD at $P < 0.05$ level calculated from highly significant 2-factor ANOVA across all treatments and storage times (0, 2, 4, 6, 8, 9 months). Treatment \times Duration interaction for fungal colonies, $df = 30$, $F = 3.40$, $P < 0.001$; for electrical conductivity, $df = 30$, $F = 17.82$, $P < 0.001$; see [Table S4](#) for full ANOVA and treatment comparisons.

T1 - Seeds stored in hermetic container without desiccant (control).

T2 - Seeds stored in hermetic container with drying beads (zeolite).

T3 - Seeds stored in hermetic container with sodium aluminum silicate.

T4 - Seeds stored in hermetic container with activated alumina.

T5 - Seeds stored in hermetic container with silica gel.

T6 - Seeds stored in hermetic container with cow-dung ash.

T7 - Seeds stored in cloth bag without desiccant.

Table 2Effects of desiccants and containers on the activity of *Callasobruchus chinensis* during seed storage.

Treatment	Oviposition (eggs/10 g of seed)		Seed damage (% damaged seed)		Seed weight loss (%)		Germination (%)	
	Storage period (months)							
	3	6	3	6	3	6	3	6
T1	140 b ± 3.38	235 b ± 0.00	29.0 b ± 0.48	38.0 b ± 0.18	4.63 b ± 0.40	21.2 b ± 0.25	87.0 c ± 0.55	70.0 e ± 0.18
T2	10.7 c ± 0.11	9.67 d ± 0.11	5.33 d ± 0.21	9.00 c ± 0.18	4.11 b ± 0.37	4.15 c ± 0.03	96.0 a ± 0.37	91.0 b ± 0.18
T3	9.67 c ± 0.11	12.0 d ± 0.18	6.33 d ± 0.28	9.33 c ± 0.21	3.89 b ± 0.04	4.14 c ± 0.05	95.0 a ± 0.18	89.0 bc ± 0.00
T4	14.0 c ± 0.37	12.7 d ± 0.11	5.00 d ± 0.32	9.33 c ± 0.21	2.93 b ± 0.09	3.84 c ± 0.02	95.0 a ± 0.18	88.0 c ± 0.32
T5	13.0 c ± 0.95	12.7 d ± 0.11	5.00 d ± 0.37	10.0 c ± 0.18	3.12 b ± 0.01	3.97 c ± 0.11	95.0 a ± 0.48	90.0 b ± 0.00
T6	106 b ± 3.47	171 c ± 0.11	14.3 c ± 0.28	34.3 b ± 0.21	4.70 b ± 0.04	15.8 b ± 0.01	87.0 c ± 0.00	78.0 d ± 0.32
T7	233 a ± 14.0	311 a ± 2.65	38.0 a ± 0.48	57.3 a ± 0.69	11.8 a ± 0.44	80.6 a ± 0.35	74.7 d ± 0.46	42.0 f ± 0.32

Mean values are presented \pm SE. Means and SEs for germination are percentages, but lower-case letters indicating significant differences among means are based on arcsin transformed values. Treatment differences at each time point (different lower case letters) are based on LSD at $p < 0.05$ level calculated from highly significant 2-factor ANOVA across all treatments and storage times (sampled monthly for six months). Treatment \times Duration interaction for oviposition, $df = 36$, $F = 43.2$, $P < 0.001$; for seed damage, $df = 36$, $F = 46.45$, $P < 0.001$; for seed weight loss, $df = 36$, $F = 94.44$, $P < 0.001$; for seed damage, $df = 36$, $F = 83.20$, $P < 0.001$; see [Supplementary Tables S5A and S5B](#) for full ANOVA and treatment comparisons.

T1 - Seeds stored in hermetic container without desiccant (control).

T2 - Seeds stored in hermetic container with drying beads (zeolite).

T3 - Seeds stored in hermetic container with sodium aluminum silicate.

T4 - Seeds stored in hermetic container with activated alumina.

T5 - Seeds stored in hermetic container with silica gel.

T6 - Seeds stored in hermetic container with cow-dung ash.

T7 - Seeds stored in cloth bag without desiccant.

approximately 6% m.c. and hermetic storage delayed loss of seed viability at ambient temperature and maintained germination above 90%, compared to only 79% for seeds stored conventionally in cloth bags. These results were supported by seedling length, dry weight, vigor index and ion leakage measurements, with seeds stored with effective desiccants consistently having significantly higher vigour and lower

electrical conductivity values after storage ([Table 1](#); [Supplemental Tables S2 and S3](#)).

Seed health and phytosanitation also can be affected by the growth of pathogens during storage. Seed borne pathogens (*Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp. and *Fusarium* spp.) were observed at higher frequencies in mung bean seeds stored in airtight container (T1), cow-

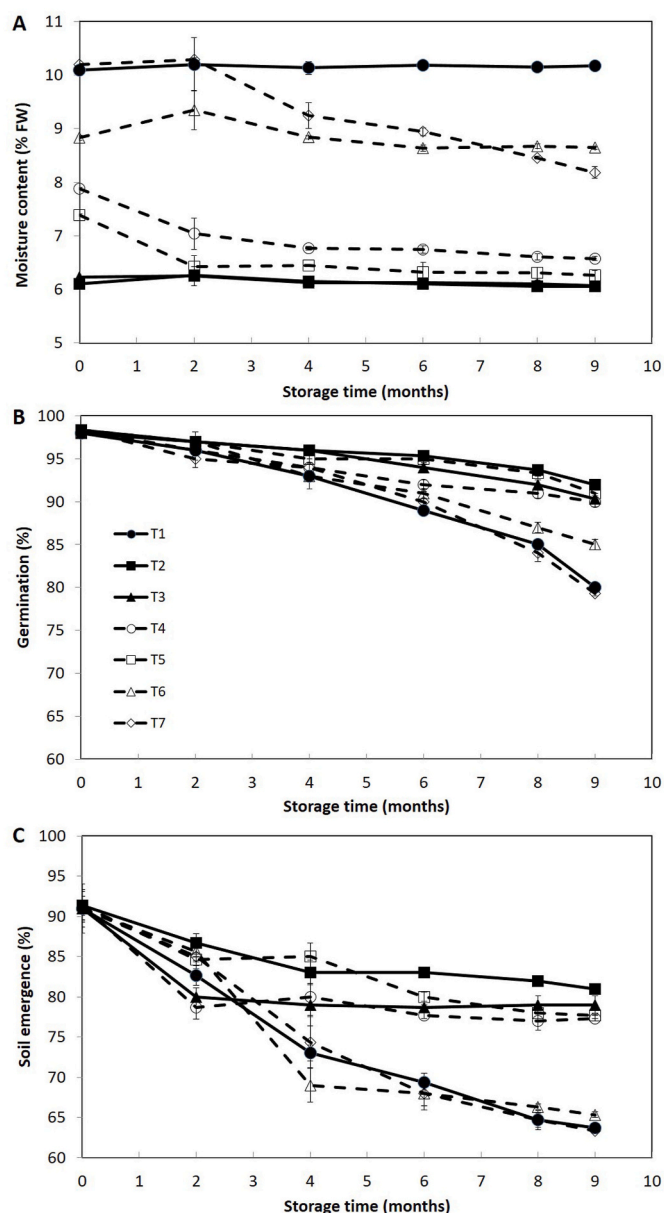


Fig. 3. Effect of desiccants and storage containers on bruchid damage (A) and germination (B) of mung bean seeds during storage. T1: Seeds in hermetic container (control); T2: Seeds in hermetic container over Drying Beads; T3: Seeds in hermetic container over sodium aluminum silicate; T4: Seeds in hermetic container over activated alumina; T5: Seeds in hermetic container over silica gel; T6: Seeds in hermetic container over cow-dung ash; T7: Standard errors (bars) are indicated when they exceed the size of the symbols. ANOVA tests of Treatment \times Duration interaction for seed damage, $df = 36$, $F = 46.45$, $P < 0.001$; for seed germination, $df = 36$, $F = 83.20$, $P < 0.001$; see Table 2 and Supplementary Tables S5A and S5B for full ANOVA and treatment comparisons.

cow-dung ash (T6) and cloth bag (T7) (Table 1), which were at higher m.c. compared to the treatments containing desiccants (Fig. 2A).

The maintenance of seed germination and vigour in laboratory tests was confirmed in soil emergence trials, which showed greater reductions in seedling emergence after storage compared to laboratory germination tests, but the same relative rankings among treatments (Fig. 2C; Table S1). Seeds stored in hermetic containers with effective desiccants had higher soil emergence after nine months of storage (78–81%) as compared to seeds that were stored at higher m.c. in an airtight container, with cow-dung ash or in a cloth bag (63–65%).

3.3. Effects of different desiccants and containers on bruchid beetle infestation during storage

Seed samples dried and stored as described above were also prepared and inoculated with one pair of bruchid beetles. These samples remained sealed and independent replicates were assayed at monthly intervals for the gas (oxygen and carbon dioxide) composition of the internal atmosphere and assessed for bruchid reproduction and damage to the seeds. Seeds stored with Drying Beads, sodium aluminum silicate, activated alumina and silica gel recorded the lowest oviposition (10–13 eggs/10 g of seeds) after six months (Table 2). Many more eggs were observed on seeds stored in airtight containers either without desiccant or with cow-dung ash (235 and 171, respectively), while seeds stored in a cloth bag had 311 eggs/10 g of seeds (Table 2). This indicates that seed m.c. was the primary factor limiting oviposition, rather than whether the storage container was hermetic. The seeds with the lowest egg numbers were all at ~6% m.c., while those with the much higher numbers were at 8–10% m.c. Storage at the higher m.c. in hermetic containers significantly reduced egg numbers compared to porous bags, but these were still 15- to 20-fold greater than for seeds at 6% m.c. in hermetic containers. The same relative ranking of treatments was also the case for percentage of damaged seeds (Fig. 3A). For all measures, the highest infestations and damage were in the seeds stored in cloth bags, followed by seeds stored hermetically at 10% and 8% m.c., with minimal damage to seeds at 6% m.c. (Figs. 3A and 4; Table 2).

Seed germination percentages also declined in association with the increase in damage to stored seeds inoculated with bruchids (Fig. 3B; Table 2). The lowest germination was recorded for seeds stored in cloth bags, i.e., only 42% after six months of storage. Seeds stored in hermetic containers at 8–10% m.c. declined to 70–80% germination, while germination of seeds stored at ~6% m.c. in hermetic containers was comparable to that of seeds stored without bruchids (~90%). Seed vigor index values were consistent with the germination percentages (Table S2).

As the efficacy of some storage containers (such as the PICS bags) has been attributed primarily to their impermeability to oxygen (Murdoch and Baoua, 2014), we measured the CO_2 and O_2 percentages in the airtight containers at monthly intervals. All treatments in which seeds were at ~6% m.c. (Fig. 2A), exhibited minimal increases in CO_2 or decreases in O_2 in the containers over the storage period compared to ambient conditions (Fig. 5). In contrast, CO_2 increased and O_2 decreased continuously in the containers with no desiccant (T1, 10.1% m.c.) or with cow-dung ash (T6, 8.6% m.c.), reaching $>15\%$ CO_2 and $<5\%$ O_2 by six months (Fig. 5). These results confirm that the hermetic containers were gas-tight, and that insect respiration (consuming O_2 and releasing CO_2) continued in parallel with increasing seed damage (Fig. 3A). However, lack of respiration and damage indicates that low m.c. due to desiccants either killed or prevented activity of bruchid beetles.

This was confirmed in separate experiments in which five young gravid bruchids were released in each treatment and insect mortality was recorded every day for 10 days. Bruchids released in treatments with Drying Beads and sodium aluminum silicate died within three days, whereas with activated alumina and silica gel, mortality was observed on the fourth day. In airtight containers with or without cow-dung ash, the life span of bruchids was extended to seven days, compared to nine days in porous cloth bags.

4. Discussion

Mung bean seeds generally are harvested at relatively high m.c. to reduce mechanical damage due to harvesting and threshing and must be dried to a safe m.c. for storage to prevent loss of seed quality (McDonald and Copeland, 1997). It has long been known that storage life decreases exponentially as m.c. (or a_w) increases (Roberts and Ellis, 1989). However, the climatic conditions during seed harvest and storage can make it difficult to dry seeds sufficiently for safe storage in many locations

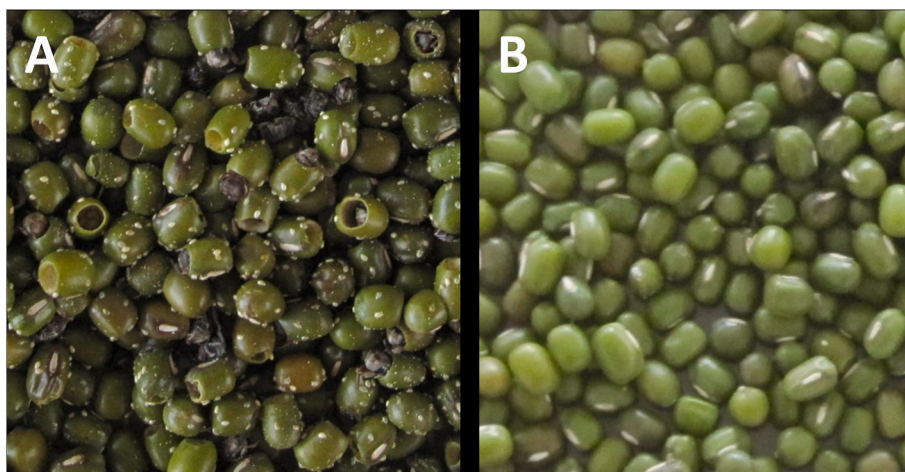


Fig. 4. Mung bean seeds stored for six months after inoculation with bruchid beetles in (A) cloth bag or (B) hermetic container with Drying Beads. Images used with permission from Kunusoth et al. (2012).

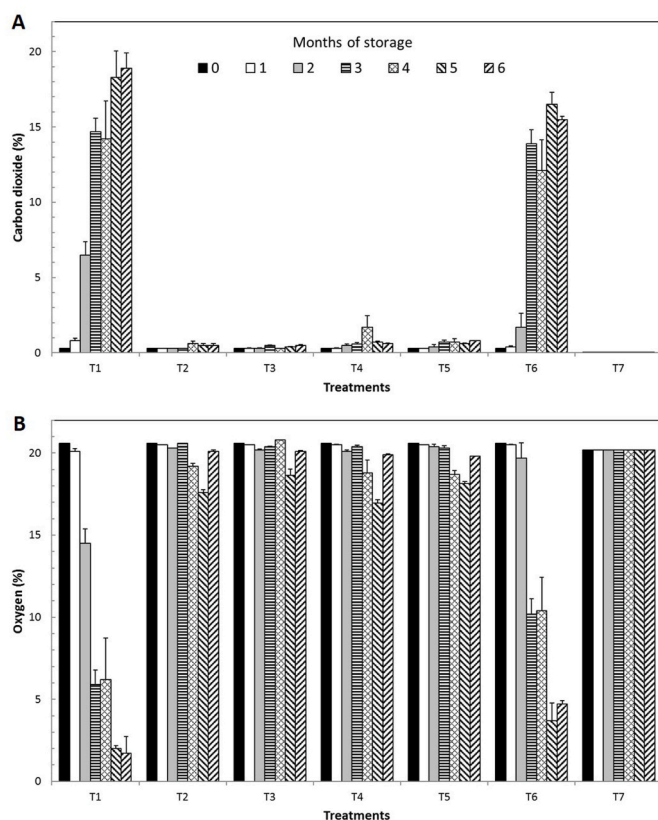


Fig. 5. Effect of desiccants on the CO₂ (A) and O₂ (B) percentages in the air inside hermetic containers during mung bean seed storage with bruchids for six months. T1: Seeds in hermetic container (control); T2: Seeds in hermetic container over Drying Beads; T3: Seeds in hermetic container over sodium aluminum silicate; T4: Seeds in hermetic container over activated alumina; T5: Seeds in hermetic container over silica gel; T6: Seeds in hermetic container over cow dung; T7: Ambient conditions as for seeds in cloth bags. Standard errors (bars) are indicated when they exceed the size of the symbols. ANOVA tests of Treatment × Duration interaction for Available CO₂, df = 36, F = 33.71, P < 0.001; for Available O₂, df = 36, F = 23.87, P < 0.001; see [Supplementary Table S5B](#) for full ANOVA and treatment comparisons.

around the world. In general, seeds must be dried to equilibrium with <70% r.h. to prevent fungal growth and to ~35% r.h. to prevent insect development (Roberts, 1972; Fontana, 2007; Bradford et al., 2018). In

many tropical climates, ambient air- or sun-drying does not dry seeds sufficiently to prevent fungal or insect damage or loss of viability due to ageing during storage (Kunusoth et al., 2012; Bakhtavar et al., 2019; Bakhtavar and Afzal, 2020a; Guzzon et al., 2020). In addition, heated-air drying, which is widely used to dry seeds in temperate climates, is less efficient when the incoming air is warm and humid, limiting the extent of drying that can be achieved (Bradford et al., 2018).

An alternative is to use desiccants to remove moisture from seeds. Various desiccant-based dryers of different scales have been designed, most using silica gel as the water absorbent to produce dry air for seed drying (Gill et al., 2014). A zeolite-based desiccant, called Drying Beads, has also been employed for use in drying seeds and agricultural commodities (Van Asbrouck and Taridno, 2009; Hay et al., 2012; Kunusoth et al., 2012; Hay and Timple, 2013; Bakhtavar and Afzal, 2020a, b; Kamran et al., 2020). Other water-absorbent chemicals (sodium aluminum silicate, activated alumina) and traditional materials (cow-dung ash) also have been used as desiccants. As mechanical drying equipment is seldom available to small farmers, desiccants for seed drying can be utilized by enclosing the desiccant with the seeds inside of a moisture-proof container. As the desiccant absorbs water from the air in the container, the r.h. decreases and water evaporates from the seeds until the desiccant capacity is saturated or the system comes to equilibrium. An advantage of Drying Beads is that water is held very tightly in its pores, rapidly lowering the r.h. to very low levels. In addition, its water holding capacity is essentially the same regardless of the r.h. In contrast, silica gel has a larger water absorption capacity at high r.h., but is less effective in reducing m.c. to very low levels. The adsorbent properties of other traditional desiccants (e.g., cow-dung ash) are unknown. Thus, the selection of desiccant requires information on the effectiveness, capacity, and cost relative to the quantity and value of the seeds or commodities to be dried.

For these reasons, we compared a number of desiccants with respect to their ability to dry mung bean seeds to m.c. levels that extend seed longevity and prevent growth of fungal and insect pests that can develop during storage. When used in amounts potentially capable of absorbing sufficient water to reduce seed m.c. to the desired level, Drying Beads, silica gel, sodium aluminum silicate and activated alumina were all capable of drying the seeds from 10% to near 6% m.c. Drying occurred within 72 h for Drying Beads and silica gel, but sodium aluminum silicate and activated alumina were slower to equilibrate. Cow-dung ash only reduced seed m.c. to 8.8% even after 4 months, which was too high to prevent significantly greater loss of viability than for the other desiccants (85 vs 90–92%). Thus, while inexpensive, this material was relatively ineffective as a desiccant. Drying Beads and other effective desiccants consistently maintained the highest seed quality across all

quality and vigor parameters measured. In addition, the relatively close correspondence between the measured air r.h. within the containers and the final seed a_w and m.c. supports the use of r.h. or a_w as convenient indirect measures of seed m.c. (Bradford et al., 2016; Thompson et al., 2017).

An additional advantage of using desiccant drying is that it encloses the seeds in a hermetic container. Research with the Purdue Improved Crop Storage (PICS) bags has demonstrated that storing commodities inside of water- and oxygen-proof bags can prevent insect infestations during storage (Murdock and Baoua, 2014). This is attributed to insect respiration inside the bags that reduces the oxygen in the air to lethal levels; as long as the bags are not opened frequently, insect activity will eventually be prevented by oxygen restriction. At higher m.c., fungal respiration may also be involved in reducing oxygen levels, while at lower moisture contents, desiccation can also kill the insects (Murdock et al., 2012). We therefore investigated the effects of desiccants and hermetic containers on the viability of bruchid beetles in stored mung beans. The results were consistent with the effects on seed viability in the absence of insects: seeds dried to near 6% m.c. exhibited little damage or insect growth, seeds at 8.6 or 10% exhibited greater damage, and seeds stored in porous cloth bags suffered the most damage and loss of viability (Figs. 2–4; Table 1). Prior reports have also noted improved field emergence of soybean (*Glycine max* L. Merr.) (Girase et al., 2006), chilli (*Capsicum annum* L.) (De Silva and Peiris, 1994), peanut (*Arachis hypogaea* L.) (Tripathy et al., 1996) and maize (*Zea mays* L.) (Afzal et al., 2017) seeds stored at lower m.c. and in waterproof packaging.

Measurements of O_2 and CO_2 levels inside the storage containers revealed the importance of low m.c. for reducing insect damage. In all of the treatments in which m.c. was near 6%, little CO_2 accumulation or O_2 consumption was measured over six months in samples inoculated with bruchids (Fig. 5), indicating that neither seeds, fungi nor insects were respiring significantly at this a_w (~0.15) in the containers. However, at 8.6 or 10% m.c. (0.34 or 0.44 a_w), CO_2 levels increased and O_2 levels decreased over storage time (Fig. 5), and insect damage to seeds was extensive (Figs. 3 and 4; Table 2). The decrease in O_2 was much slower than has been reported previously (Murdock and Baoua, 2014), but was due solely to insect respiration as neither seeds nor fungi can respire at those water activities (Bradford et al., 2018).

These findings have important implications for the utilization of PICS or similar crop storage bags in humid climates. If the seed or commodity a_w is above about 0.7 (= 70% equilibrium r.h.), both fungi and insects can grow. This will rapidly deplete O_2 levels inside the bags, and as long as the bags are not opened, will suppress further fungal or insect growth. Seed viability will decline relatively rapidly over time due to the high m.c., but this will be counteracted to some extent by the reduced O_2 levels (Schwember and Bradford, 2011; Groot et al., 2015). At a_w between 0.70 and 0.35, fungal growth will be suppressed, but insects can still be active (Roberts, 1972). This includes the treatments here at 8.6 and 10% m.c., which enabled insect respiration and attendant damage during storage (Figs. 3–5). At a_w of 0.15 (~6% m.c.), insects did not survive and seed quality was maintained at the highest level (Fig. 2), despite the high O_2 levels in the containers (Fig. 5). This is consistent with previous reports that pulse beetle activity is negligible below 8–9% m.c. (Christengen, 1972), and that their development is impaired below 7% m.c. (Girish, 1983).

These results indicate that if drying is sufficient, whether by sun-drying if conditions permit or by artificial drying if needed, an oxygen-impermeable barrier is not required for storage bags to preserve seed quality. At a low a_w , respiration will not occur inside the bags and O_2 levels would not be lowered, while at higher a_w or m.c., insects may be active and cause damage until they reduce the internal O_2 to restrictive levels. If the containers are not opened, this may occur sufficiently rapidly to limit damage. If containers are opened, such as to consume or market some of the seeds or commodity, oxygen will re-enter the bags and the process will resume again, with additional damage occurring each time the containers are opened. At high a_w

(>0.70), both fungi and insects can be active, and PICS-type bags will likely be better than open storage, as O_2 depletion will occur more rapidly, but damage to seeds from both fungi and insects will accompany their activity. Thus, even when oxygen-impermeable bags are available, the better practice would be to dry seeds or commodities to low a_w prior to storage in hermetic bags or containers to preserve maximum quality. If sufficient drying is possible, whether via mechanical dryers or desiccants, impermeability to oxygen is not necessary, and only waterproof containers are needed, potentially lowering their cost and increasing their availability to small farmers.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jspr.2021.101888>.

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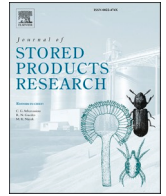
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