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Hermetic technologies and grain quality of *Phaseolus lunatus* L. in the Yucatan peninsula, Mexico

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In southern Mexico, postharvest losses of grains are an important constraint in achieving food security and conservation of biodiversity. This study was conducted under field conditions to investigate the effect of prolonged storage on the grain quality of lima beans. Grain damage, moisture content, seed germination, and culinary quality were evaluated during six months of storage in hermetic containers and traditional methods. Data were subjected to analysis of variance and differences between treatments separated by Tukey. Both, the traditional storage method, and hermetic technologies allowed to limit the damage caused by insects and moulds for six months. The moisture content of grain in hermetic containers remained unchanged while polypropylene bags with lime decreased. The polypropylene bags with lime were able to maintain acceptable seed viability after six months of storage. The storage period significantly affected the culinary quality of lima beans, increasing the water absorption capacity and cooking time. Technical training on postharvest management is needed to reduce food losses and improve poverty and household food security. Further research is required to understand the effects of hermetic technologies and inert dust on stored product quality.

1. Introduction

The Maya milpa system is recognized as a globally important agricultural heritage system (GIAHS) by the Food and Agricultural Organization (FAO). This ancestral Mesoamerican farming system retains an important presence in the landscape in the tropical dry region of southern Mexico. The small-scale milpa production system is a highly diversified system relying on the sustainable use of biodiversity where a group of basic crops, such as maize, squash, and beans, provides household food security through direct consumption and income generation to poor families (Martínez-Castillo et al., 2004). Lima bean (*Phaseolus lunatus* L.), known as Ib or Ibe in Maya, represents an important crop integrated within the milpa production system. It is the fourth main crop for the Maya of the Yucatan Peninsula, with the highest morphological variation of landraces in the country. It constitutes a rich source of proteins, carbohydrates, iron, calcium, and fibre, distinguished by having low fat content (Becerra et al., 2004; López-Alcocer et al., 2016).

In this region, postharvest grain losses due to insects, rodents, and microorganisms represent a challenge for smallholder farmers. FAO recognized that post-harvest losses are an important constraint in achieving food security (García-Lara et al., 2020). Farmers require stored beans for at least six months, which is the period between the dates of harvest and sowing (January and July, respectively) nevertheless, the practices used by farmers to store grains are sometimes inappropriate and health risk (Latournerie et al., 2005). Usually, farmers store their grain using a variety of storage technologies, including traditional wooden structures and various containers, sometimes in combination with toxic insecticides (Odjo et al., 2020). Among the containers used to conserve beans, polypropylene bags are the most common (74%), also plastic containers (13.8%), and nylon bags (6.9%) among others (Latournerie et al., 2005). In addition, other additional methods to control insects are also used such as smoke (46.3%), insecticides (24.1%), and lime (14.8%) nevertheless losses are high and can reach 40% of harvests (Latournerie et al., 2005).

Precise quantitative assessment of these losses is difficult due to high year-on-year variability in pest infestation (Omotilewa et al., 2019). Among pests that damage the Lima bean are the Mexican bean weevil (*Zabrotes subfasciatus* Boheman) and the bean weevil (*Acanthoscelides obtectus* Say). *Zabrotes subfasciatus* is the main post-harvest pest in warm climate areas (Bautista-Sosa et al., 2021). The damage caused by larval feeding includes weight loss, germination and nutritive value reduction, and commercial devaluation of grains (Silva et al., 2021). It is important to have a method that allows conserving the grain and seeds in optimal conditions during storage, especially in rural communities which are vulnerable to flooding from hurricanes, heavy rains, or prolonged dry periods that destroy harvests.

Hermetic technologies are one of the most promising sustainable management tools in diverse climates in preventing food storage losses (García-Lara et al., 2020). Several studies, mostly using maize, demonstrated hermetic storage technologies' effectiveness in controlling postharvest pests in Mexico (García-Lara et al., 2020; Odjo et al., 2020). Hermetic technologies are storage structures such as metal silos, plastic containers, or recycled plastic bottles with different capacities that are sealed to create a modified atmosphere inside the containers, which results in the control of pests and grain loss reduction. This reduction is due to the combination of a closed environment with the biological respiration of seeds, insects, and fungi. Low oxygen and high CO_2 generate a lethal atmosphere highly effective for the control of insects and moulds (García-Lara et al., 2020; Odjo et al., 2020). Despite its effectiveness in decreasing losses due to insect pests, safety, and a friendly environment, several issues have limited its use in Mexico. The lack of knowledge of this technology, high cost, and low accessibility are some constraints to small-farmers (Baributsa & Njoroge, 2020). To overcome these constraints, the need for dissemination and technological transfer activities, technical training, alternative low-cost approaches, and potential subsidies for technology adoption is evident (Omotilewa et al., 2019).

In addition, several studies have demonstrated that prolonged storage for months or years causes the deterioration of the quality of the grain and diminishes its commercial value (Jacinto-Hernández et al., 2017; Fufa et al., 2020). The main changes observed are the darkening of the testa and hardening, which causes increased cooking time and a decrease in the culinary quality of the grain (Jacinto-Hernández et al., 2017). Also, prolonged storage has been reported to cause up to a 10% loss of seed viability (Fufa et al., 2020). Understanding the effects of prolonged storage on the grain quality of lima beans is important to the adoption of hermetic technologies and increased food security in Yucatan.

The main goal of this research was to examine how extended storage impacts the quality of lima beans under field conditions in the Yucatan Peninsula. The study specifically focused on assessing the influence of different storage methods on the grain quality of lima beans.

2. Materials and Methods

2.1 Site location

The experiment was conducted in three independent sites located in the milpera region of Yucatan, the municipalities of Tahdziú, Tixmehuac, and Chacsinkín, from February to July 2021 (Figure 1). The municipalities Tahdziú, Tixmehuac, and Chacsinkín have 5,854, 5,444, and 3,104 inhabitants, respectively (SE-

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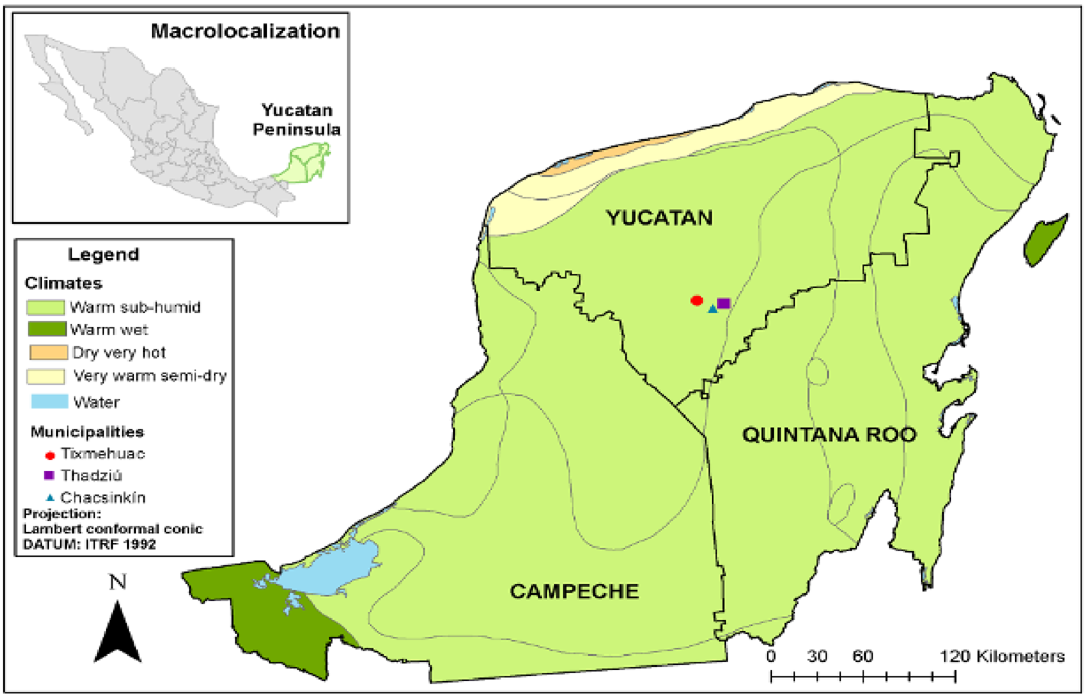

Figure 1. Sites locations and climates of the Yucatan Peninsula formed by the states of Yucatan, Campeche, and Quintana Roo.

FOET 2015). Its population is mostly indigenous Mayan with a high degree of marginalization. The main productive activities are in the primary sector such as agriculture, livestock, and hunting (CONEVAL 2020). In this region, the climate is warm-sub humid with summer rains with minimum and maximum temperatures ranging from 16.2 to 21.9 °C and 32.3 to 37.3 °C and mean daily precipitation ranging from 0.9 to 5.1 mm from February to July respectively (CONAGUA 2019). Additional environmental characteristics and agronomical management of Southern Yucatan are described in (Martínez-Castillo et al., 2004).

2.2 Grain used in the experiment

The Lima bean used was the landrace "munición" which is common in the region and characterised by an elliptical round shape, white colour, and great nutritional value, rich in protein, calcium, phosphorus, and iron (Becerra et al., 2004; Martínez-Castillo et al., 2004).

One ton of Lima beans was obtained from farmers in Tahdziú through a local agency (ADHL-Alianzas) and used in this study. The grain was harvested during the 2020 season. Following local practices, the grain underwent prolonged periods in the field drying. Usually, between December and January, until farmers choose the right moment for harvesting (Martínez-Castillo et al., 2004). In the farmer`s house, legumes were thrashed, and beans cleaned with forced air to remove impurities and crop residues then, the bean was sun-dried and stored, free of insecticides or fungicides until used. The grains were not inoculated with insects or fungi since beans usually harbour sufficient pests that grow naturally if environmental conditions are conducive.

2.3 Treatments and sampling

We assessed low-capacity alternatives adapted to the requirements of small-scale farmers who typically cultivate maize and legumes for self-consumption and need to store different basic crops to achieve food security.

Each site contained one replica of four different stored methods as treatments: 1) the local storage method, which consists of a polypropylene bag (PB) with the capacity of 50 kg of grain with standard lime (calcium hydroxide) at a dose of 25 g kg^{-1} of grain followed local recommendations; 2) metal silo (MS) with the capacity to 60 kg, had a superior conical cup and a bronze plug, made by a local blacksmith under the principle of hermeticity following the Mexican norm (NMX-FF-123-SCFI-2015) (DOF, 2015); 3) hermetic plastic bag, SuperGrainbag Farm™ 50RZ (HB) with double safety zipper market by GrainPro Inc with the capacity to 50 kg (60 x 105 cm) and 4) recycled hermetic PET container (PC), usually used for water with capacity for 20 kg.

During the experiment, the grain's moisture content (%) and temperature (°C) were taken in triplicate and registered using a hand-held grain moisture tester (John Deere Moisture Check Plus Grain Moisture, Illinois, USA). The polypropylene bags (PB) were filled with 15 kg of grain mixed with lime (25g kg^{-1}) and closed using a plastic tie. The metal silos (MS) were filled with 60 kg of grain and sealed. After extracting the most air in the bag, the hermetic plastic bags (HB) were filled with 30 kg of grain and closed with a double safety zipper. Before usage, bags' hermeticity was verified by inflating them and searching for any perforation. Then, hermetic bags were placed inside a polypropylene bag for additional protection and tied. Plastic containers (PC) were filled with 20 kg of grain and sealed with a plastic cap and tape. Before sealing each container, initial samples of 1 kg of grain were taken.

The experiment was conducted for 6 months. At 0, 2, 4, and 6 months of storage, each container was open samples of 1 kg of grain were collected and a mean of three sub-samples was used to test grain quality. In the case of MS and PC, samples were taken using the exit opening. For PB and HB, samples were taken by inserting a cup into the middle of the grain mass.

2.4 Grain quality of stored beans

Before quantifying grain damage, the percentage of impurities, including all organic and inorganic materials other than beans was determined. Then, 100 grains were randomly selected and checked for grain damage from the pure seed fraction. Grain damage refers to physical spoilage and apparent evidence of deterioration, such as holes, cracks, crevices, and discolouration. Insects, fungi, and farmers' management are the main agents causing this deterioration (Odjo et al., 2020). The grain was separated for insect damage and fungi damage and the percentage of damaged grain was recorded (Manuel et al., 2007). When grains had more than one type of damage, these grains were counted in both damage categories. Insect damage grain was the grain that presented perforations of galleries caused by insects. Fungi-damaged grain was the grain that, on the surface, presented the development of fungi mycelium, generally characterized by discolouration (yellowish, greyish, blackish, green, or orange) (Odjo et al., 2020).

A germination test and grain moisture were carried out every two months for each grain container to assess how effective they were in maintaining the viability of the seeds over the six months. The baseline germination percentage was the initial loading sample taken. The germination test was done in the laboratory of biodiversity at the Campus of Biological and Agricultural Sciences, Autonomous University of Yucatan according to the standard procedures described elsewhere (Rao et al., 2006). We used the rolled "between paper" method with moistened paper towels. Three replicates of 100 seeds were taken at random and incubated at room temperature (26 °C ± 3 °C) under photoperiod conditions of 16/8 h, light/dark, for seven days. The germinated seeds were counted visually upon the appearance of radicle and/or plumule and percentage germination was calculated as follows: Seed germination (%) = No. of germinated seeds/Total No. of seeds planted x 100.

2.5 Culinary quality parameters

Before and after six months of storage, the bean's culinary quality was measured with five parameters per triplicate: Weight of 100 grains (g) and volume of 100 grains (cc). Usually, larger, and denser beans are associated with better quality. Water absorption capacity (WAC) to detect whether the genotype presented a hard shell and good cooking quality, leading to desirable texture and flavour. Grain samples of 30 beans were soaked in a beaker containing 100 mL of distilled water at room temperature for 18 hours. The WAC was obtained by equation 1:

$$\%\,WAC = (Pf - Pi)\,/\,Pi \times 100 \qquad (1)$$

where Pi = sample initial weight (g); Pf = sample final weight (g).

Cooking time (CT), indicates the time required for

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the beans to reach a desirable level of doneness. Cooking time was recorded in terms of the minutes after a sample of 30 grains was placed in boiling water (100 mL) until 90 % of the grains reached a smooth granular texture determined by sensory evaluation. The percentage of solids in the cooking broth (% SB) was determined by the difference in weight when evaporating the liquid from a 10 mL aliquot of the broth (Muñoz-Velázquez et al., 2009). The solids in the cooking broth can reveal the degree of softness or firmness of the beans. Each parameter reveals different aspects of the beans' characteristics and collectively provides a comprehensive assessment of the cooking quality of the beans. Pre-evaluation, grains from PB with lime were washed with running water.

2.6 Statistical analysis

For the grain quality parameters, a complete randomized design with three replications was used with one-way ANOVA and Tukey test ($P < 0.05$).

Per cent, grain damage, moisture, and seed germination data were first square root ($\sqrt{x}$) transformed to stabilize the variances. The transformed data were analysed by one-way ANOVA to compare significant differences between storage containers with the software Infostat. Significant differences between the means were separated by the Tukey test at $P < 0.05$. However, for ease of understanding, the untransformed means are presented.

3. Results

3.1 Effect of the storage container on grain damaged

The initial physical grain quality has 99% of pure grain, 23.4 ̊C temperature, and 14.5% grain moisture. The mean total damage was 0.75% for insects and fungi.

Significant differences were observed between storage containers for insect damage during the fourth and sixth months of storage (Figure 2). At six months of storage, the highest damage was observed in the PB with 3.9 % insect damage and the least damage was similar in the hermetic containers MS, HB, and PC. Significant differences were observed between storage containers for fungi damage at six months of storage (Figure 3). At this stage, the highest damage was observed in the hermetic containers MS, HB, and PC with a mean of 1.2 % fungi damage. The PB resulted in the lowest fungi damage with 0.4 %.

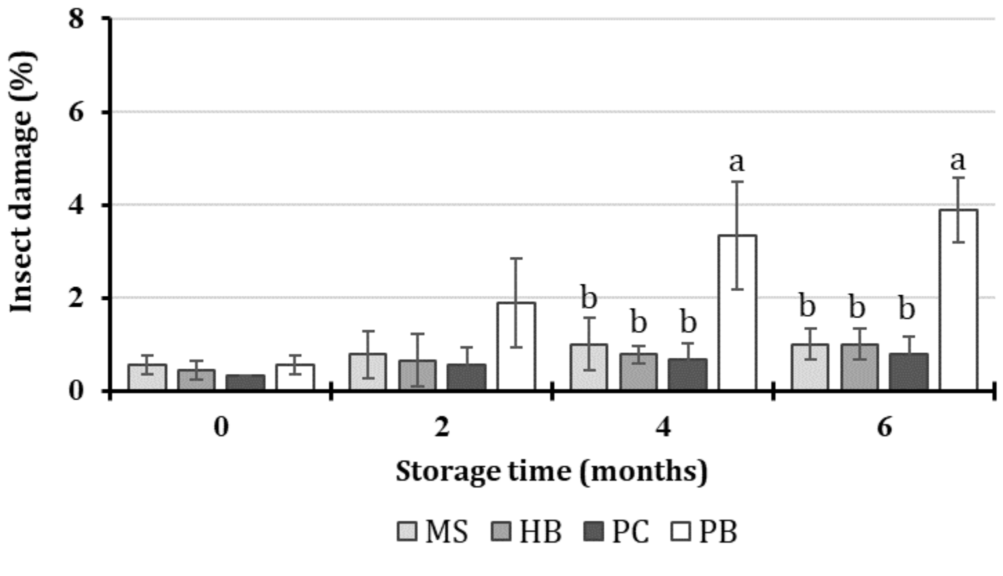

Figure 2. Average insect damage percentage (± SD) in Phaseolus lunatus at 0, 2, 4, and 6 months of storage in Yucatan. MS-Metal silo; HB-Hermetic bag; PC-Pet container; PB- polypropylene bag with lime. * Significant differences between storage containers at $P < 0.05$ at each storage time in months.

The total damage differed significantly between containers (F=5.3, p=0.0045, CV=33.3) after six months of storage (Figure 4). The lowest damage resulted in the hermetic containers MS, HB, and PC, and the highest was in the polypropylene bag with lime (PB). Insect damage was the highest source of damage in the PB. Also, the grain on the PB with lime was least susceptible to fungi damage.

3.2 Effect of the storage container on the moisture content and seed germination

Grain moisture content showed a significant difference between containers (F = 137.09, $p < 0.0001$, CV = 1.85) during the stored period.

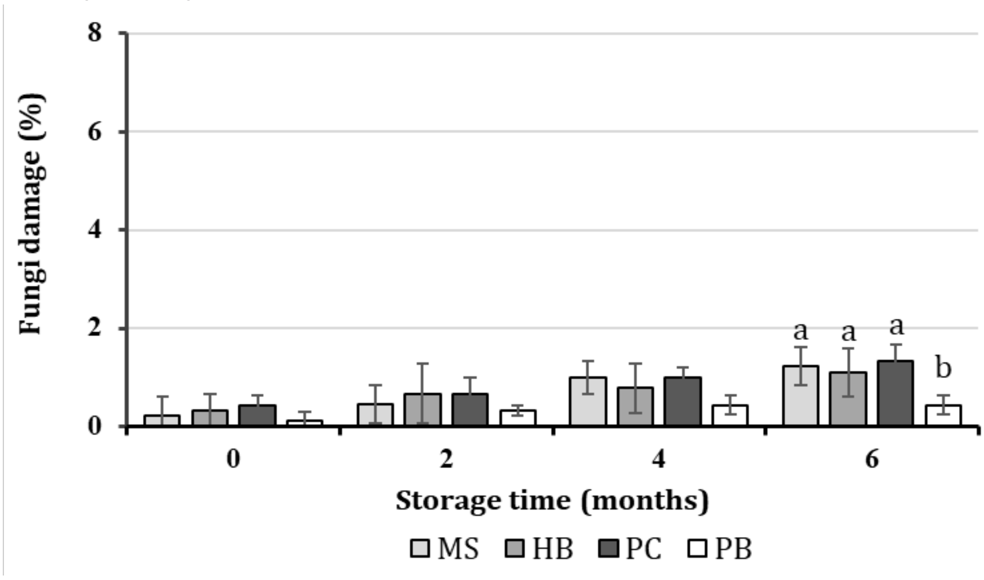

Figure 3. Average fungi damage percentage (± SD) in Phaseolus lunatus at 0, 2, 4, and 6 months of storage in Yucatan. MS-Metal silo; HB-Hermetic bag; PC-Pet container; PB- polypropylene bag with lime. * Significant differences between storage containers at P < 0.05 at each storage time in months.

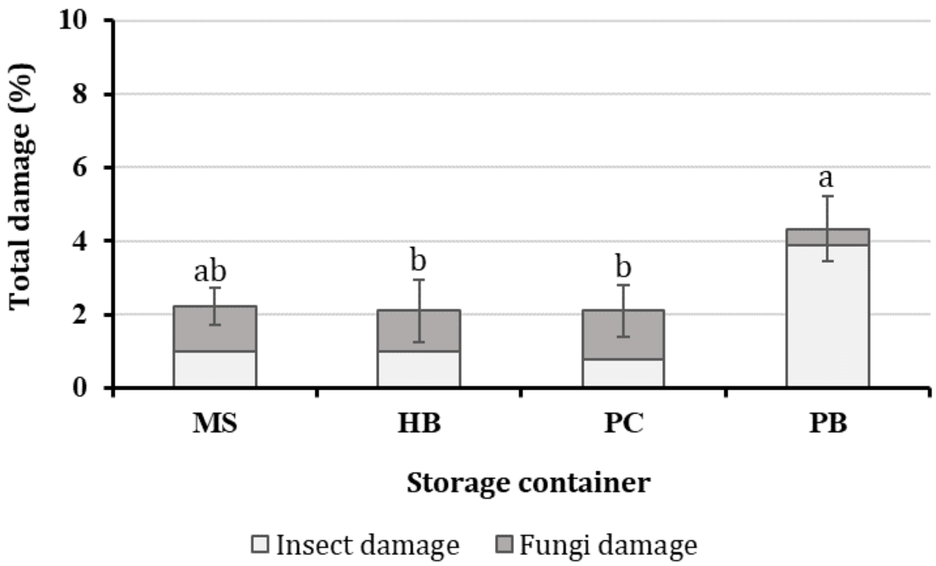

Figure 4. Total damage percentage (± SD) in Phaseolus lunatus at six months of storage in Yucatan. MS-Metal silo; HB-Hermetic bag; PC-Pet container; PB- polypropylene bag with lime. Different letters mean significant differences between storage containers at P < 0.05.

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At the beginning of the experiment, grain moisture was similar between containers (Figure 5). After two months of storage, grain moisture was significantly lower in the PB with a mean of 12.8, 11.5 and, 12.6% moisture at two, four and, six months of storage, respectively. Minimal variations were observed in moisture content for the hermetic containers MS, HB, and PC during the experiment.

Seed germination showed a significant difference between containers (F=5.27, p= 0.0046, CV=4.64) during the stored period. The initial germination was 95.6% for all containers. At two months of storage, seed germination ranged from 92% in the MS to 95% in the HB. At four months of storage, seed germination was lower in the MS at 85% and higher in the PB at 91%. At the end of the experiment, the seed germination ranged from 69 to 71 % in the HB, PC, and MS, and reached 88% in the PB (Figure 5).

3.3 Culinary quality parameters

Differences between treatments were found for WAC, Cooking time, and Solids in the cooking broth (Table 1). The weight and volume of 100 grains were similar between treatments and ranged from 30.2 to 32.4 g and 22.4 to 24.6 cc respectively. PB and HB showed higher values for WAC than MS, PC, and the initial sample before storage. The initial sample before storage needed less time to cook than samples with a storage period. Treatments with a storage period needed 121 to 143 minutes of cooking time, 72% plus than before storage. The percentage of solids in the cooking broth showed statistical differences between treatments and ranged from 0.8% in MS, HB, and PC to 1.2 % in the initial sample before storage.

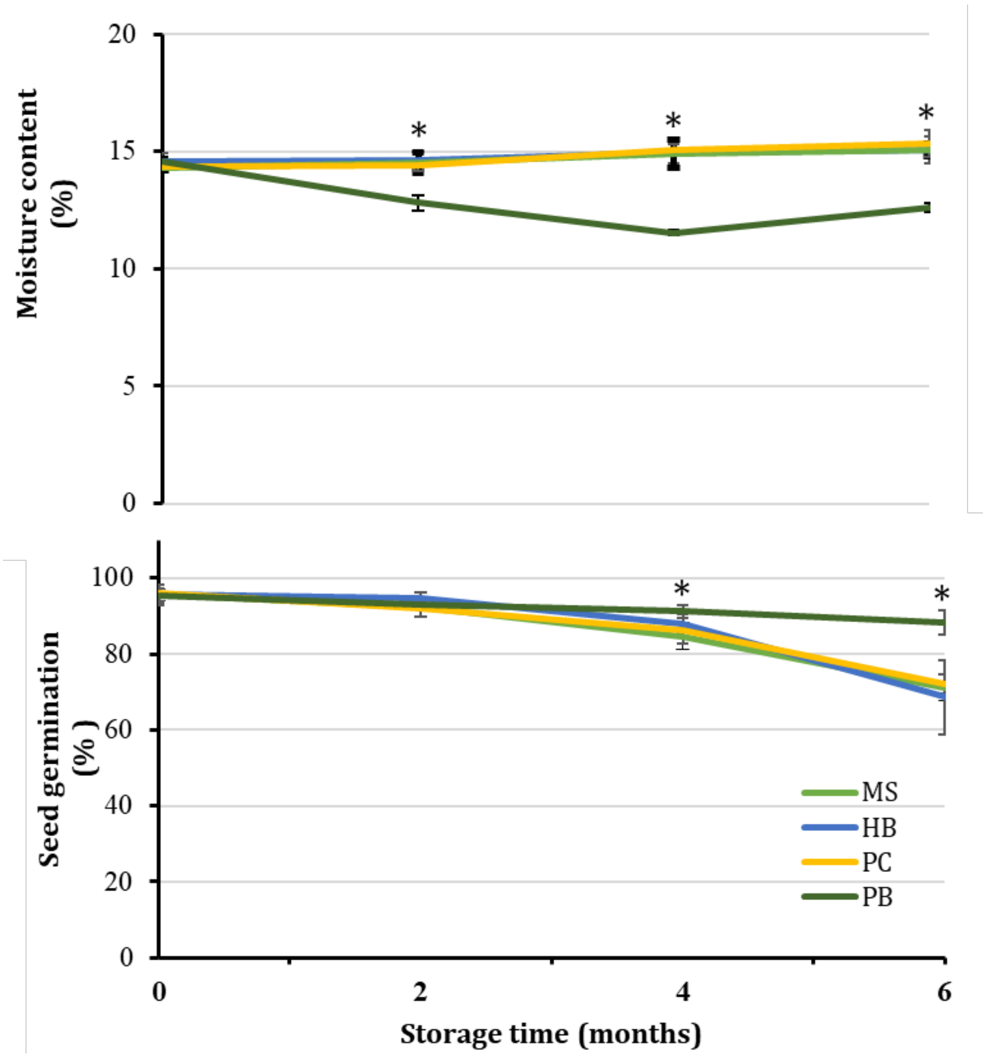

Figure 5. Moisture content percentage (± SD) and seed germination percentage (± SD) of Phaseolus lunatus at 0, 2, 4, and 6 months of storage in Yucatan. MS-Metal silo; HB-Hermetic bag; PC-Pet container; PB-polypropylene bag with lime. * Significant differences between storage containers at P < 0.05 at each storage time in months.

Table 1. Culinary quality parameters (mean ± SD) of *Phaseolus lunatus* before and after six months of storage in different containers

Treatments	Weight of 100 grains (g)	Volume of 100 grains (cc)	Water absorption capacity (%)	Cooking time (minutes)	Solids in the cooking broth (%)
Before storage	30.3 ± 1.0	22.5 ± 0.05	79.3 ± 2.1b	76.7 ± 7.6d	1.2 ± 0.2a
Metal silo	32.0 ± 1.7	22.4 ± 0.24	91.9 ± 8.5ab	133.8 ± 2.6b	0.8 ± 0.1c
Hermetic bag	32.4 ± 0.7	22.5 ± 0.29	94.9 ± 4.0a	130.0 ± 4.1b	0.8 ± 0.0bc
Pet container	30.8 ± 1.5	23.4 ± 0.15	89.4 ± 6.3ab	143.0 ± 2.5 a	0.8 ± 0.0c
Polypropylene bag with lime	30.2 ± 0.9	24.6 ± 0.05	98.0 ± 7.2a	121.3 ± 3.0c	1.0 ± 0.1b
P value	0.0530	0.3227	0.0174	<0.0001	<0.0001

Means with a different letter in each column are statistically different according to Tukey's test (P< 0.05). *N* = 3.

4. Discussion

4.1 Effect of the storage container on grain damaged

To our knowledge, this is the first study that explored hermetic storage technologies to reduce postharvest bean losses and assessed bean grain quality in Mexico. All storage technologies were efficient in reducing grain damage during six months of storage. The effectiveness of hermetic technologies in reducing postharvest losses has been widely demonstrated in maize studies (Fufa et al., 2020; García-Lara et al., 2020; Odjo et al., 2020), nevertheless, polypropylene bag (PB), still is the most common grain storage technology used in the tropical region of Mexico (Latournerie et al., 2005). Low-cost hermetic containers such as PET containers resulted equally efficiently in reducing grain damage as hermetic bags and silos and can be a good option for poor farmers that cannot afford to buy a metal silo. In this regard, Omotilewa et al. (2019) address the question of whether subsidizing a new agricultural technology for smallholder farmers can aid its adoption early in the diffusion process. The authors mentioned that a one-time use of subsidy can allow farmers to experiment with the technology and learn from the experience before investing in it. In this study, grain from PB with lime resulted in the highest grain damage, mostly caused by *Zabrotes subfasciatus* a primary grain pest in this area (Bautista-Sosa et al., 2021). Nevertheless, the level of damage was minimal compared to other studies. Recently, Odjo et al. (2020) reported that maize grain stored in PB with micronized lime had 17% and 42% less damage than grain stored in PB without lime, in two years of evaluation in Peto, Yucatan. Data on the use of lime to preserve grain are scarce, as this practice seems to be limited to Mexico and Central America and results are highly variable. In the work of Odjo et al. (2022) exists evidence that when insect infestation is high, lime control against insect decreases. Mineral powders such as lime or ash have been successfully used by Maya farmers to protect postharvest grain against insects since pre-Hispanic (Latournerie et al., 2005). Mineral powders or inert dust present a physical control method and dehydration. Have an abrasive effect or absorb the lipids of the epicuticle, facilitating the loss of water that leads to the death of insects due to dehydration (Ziaee et al., 2019).

4.2 Effect of the storage container on the moisture content and seed germination

Moisture content and temperature are the most critical factors affecting grains' quality during storage. In warm climates with high relative humidity, grains are usually harvested with high moisture content, then sun dries to reduce moisture to around 13–15% and stored. Grain is therefore stored with relatively high moisture content resulting in accelerated deterioration of the physiological grain quality (Quezada et al., 2006; Likhayo et al., 2018). The recommended value to store grains is less than 12% moisture (Aguirre 1990; Manuel et al., 2007).

In this study, hermetic containers (MS, HB, and, PC) were efficient and maintained moisture content constant all over the six months. In contrast, the PB with lime showed significantly lower moisture content than other methods during the storage period. The permeability of polypropylene bags allows grains to lose moisture through seed transpiration in response to ambient relative humidity and March, April, and May are the hottest and dry months in the region. Similar results were observed by Likhayo et al. (2018) in the tropical region of sub-Saharan Africa with maize grain stored in polypropylene bags at different moisture content. Also, lime causes grain dehydration, in both cases, it helped to lower moisture content and maintain germination (Figure 5).

The initial mean germination was high for all containers, meaning that the grain was in good physiologic condition at the beginning of the experiment. After 6 months of storage, only PB reached the minimum seed quality established by the SNICS, which is 85% germination (Manuel et al., 2007). In other studies, it has been seen that germination decreased during storage under hermetic and non-hermetic conditions and decreased as the moisture content increased (Quezada et al., 2006). With 18% moisture content and above the germination percentage decreased to zero after 35 days of storage (Fufa et al., 2020). Aguirre (1990) reported that *P. vulgaris* seeds with grain moisture of less than 12% can be stored in hermetic containers for up to 8 months with little loss of physiological quality. The study done by Fufa et al. (2020) observed that the germination percentage of maize stored in a hermetic bag with 10% moisture, dropped ten points to 88.6% after the 6th month of storage. With adequate technical support for their appropriate use, hermetic technologies have the potential to reduce postharvest losses and maintain the grain quality of lima beans during storage.

4.3 Culinary quality parameters

Soaking beans is important for bean preparation and consumption. Soaking lima beans in water for 12 h at room temperature reduces the cooking time and improves cooked texture, enhanced digestibility and reduced flatulence, some culinary traditions may prefer specific methods of preparation based on regional or cultural preferences. (Munthali et al., 2022). In Yucatan, the practice of soaking lima beans is not clear in the rural areas, several women indicated (woman's that live in Tahdziú, Tixmehuac, and Chacsinkín, personal communication, February 2021) that did not presoak lima beans before cooking, and it is common to directly cook them in boiling water with firewood.

The ability of the grain to absorb water (WAC) is an indicator of the ease with which the grain will soften during cooking, so is a routine test in genetic improvement programs for grain quality (Jacinto-Hernández et al., 2017). Beans with higher WAC may absorb more water during cooking improving texture and flavour and reduction in cooking time.

In this study, grains stored for six months increased their WAC between 13 to 23% compared to grains before storage (Table 1). Similar results were observed by Jacinto-Hernández et al. (2017) with grains of *P. vulgaris* with accelerated ageing, nevertheless, prolonged storage superior to two years may decrease WAC and cause a "hard shell" problem. Two types of defects in bean grain are known that can cause slow or poor cooking. The "hard shell" describes a physical state in which the seeds have a limited capacity to imbibe enough water, and the "hardness to cooking" refers to a texture defect, which causes the seeds to require more time to cook to achieve the appropriate smoothness during cooking (Jacinto-Hernández et al., 2017). The "hardness to cooking" developed during ageing, occurs to an association between the denaturation of the reserve proteins and limited hydration of the starch during cooking. In addition, the cell wall is not softened due to a decrease in the solubilisation of pectin (Njoroge et al., 2015). In our study, the cooking time before storage was similar to four out of five lima beans improved lines evaluated in Nigeria by Owusu et al. (2018) with 81 to 84 minutes of cooking. After six months of storage, bean quality decreased, and the effect of "hardness to cooking" increased the cooking time by around 72% (Table 1). Giami (2001) tested the quality attributes of three improved lines of lima bean and reported similar values to our study before storage in the improved line Tpl 1 for the attributes, seed weight, seed volume, WAC, and solids in the cooking broth with 33.3 g, 23 cc, 94% and 0.88 %, respectively.

5. Conclusion

The traditional storage method and hermetic technologies allowed to limit the damage caused by insects and the development of fungi for six months. Under the conditions evaluated, the polypropylene bag with lime was able to maintain germination in the acceptable conditions. The storage period significantly affected lima beans' culinary quality, increasing WAC and cooking time. Maintaining a grain moisture content of 12% or lower is crucial, but challenging in regions with high relative humidity environmental conditions. Technical training on postharvest management is needed to reduce food losses and improve poverty and household food security. In this study, the inclusion of a control treatment without lime was restricted by the risk of grain loss due to pests, preventing an accurate assessment of the real damage inflicted by insects on the grain. Further research is required to understand the effects of hermetic technologies and inert dust on stored product quality.

Conflict of interest

The authors declare no conflict of interest.

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