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Impact of hermetic packaging on green gram (*Vigna radiata*) insect and microbial damage under environmental storage condition

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Abstract
Green gram (*Vigna radiata*) is an important income generation crop for millions of farmers and processing units in India. In most developing countries, the post-harvest losses associated with this crop remain a major concern. This case investigates the impact of hermetic and non-hermetic packaging material on the green gram in tropical ambient storage. The green gram was held for 180 days in hermetic bags, plastic containers, and jute bags. Calculated parameters were the amount of moisture, the infestation of insects, the weight of 1,000 seeds, and the microbial load. In hermetic bags, the moisture content decreased a little, from 13.37 to 12.60% compared to the sharp decline in the non-hermetic plastic containers and jute bags of 13.70 to 11.90% and 13.70 to 8.30%, respectively. The insect population drastically reduced in the hermetic bags 30 days onwards and reached to zero by 60 days of storage. In this study, non-hermetic plastic containers and jute bags, the population of live insects increased 30 days onwards. The weight of 1000 grains in jute bags decreased significantly from 64.50 g to 56.11 g, while the hermetically stored kernels decreased to just 63.75 g. In non-hermetic plastic containers were significantly superior over other treatments with the least bacterial count (1.55 x 10^6cfu/gm) followed by hermetic bags (1.65x 10^6 CFU/gm) and jute bags. Based on our findings, the hermetic bag is better packaging material as far as spoilage due to insects and fungi is concerned that is followed by a plastic container and jute bag. At the same time, the jute bag was having the highest bacterial load followed by a hermetic bag and plastic container. It was thus concluded that hermetic storage could be a better way than non-hermetic storage to store green gram.

Keywords: Grain pro hermetic bag, plastic container, jute bag, green gram, storage, insects, fungi, bacteria

Introduction
Pulses are a big source of food [1] which has contributed to people's nutrition for thousands of years. Green gram is believed to be native to India and Central Asia and is grown from prehistoric times in these areas [2]. Recently, it has also been introduced to Africa and the USA. In India, post-harvest losses of a green gram are very high with losses during storage being 11.83% [3]. The development of multiple toxic metabolites such as aspergillus spp is a big problem in pulses. Produces aflatoxin and ochratoxin and citrine, which contaminates beans (Phaseolus vulgaris L.) food products in Brazil [4]. The attack of Callosobruchus Chinensis, which is caused by bruchid, it is estimated to yearly damage between 20% and 50% of the stored green gram from of the Indian Continent and may reach 70%-80% in some areas. In developing countries, particularly smallholder farmers, post-harvest losses in stored products are very prevalent. Developing strategies to reduce this decline will ensure food safety and lead to rapid economic growth and nutritional improvements in sub-Saharan Africa and India [5, 3].

Environmental conditions greatly affect the quality of grain both during production as well as at the storage level. Relative humidity and temperature are the most important environmental factors, for storing seeds [6] including pulses. According to [7] studies the loss of post-harvest can vary according to the area, year, atmospheric condition, post-harvest technology available, and finally the quantity of seed harvested. Fumigation is the most common approach for reducing storage loss, but storage losses in green grams can now be managed without the use of pesticides through the advancement of simple post-harvest technology. Many storages have emerged but are often too costly to afford to tropical and subtropical smallholder farmers [8]. Moreover, most of these farmers cannot buy pesticides during storage to control pestilence and disease. While a major way remains for several smallholder farmers to solve post-harvest losses, the use of insecticides is widespread and causes environmental and health risks [9]. Hermetic storage takes place primarily in a controlled environment that decreases oxygen constantly and at the same time increases carbon dioxide due to the respiratory activity of insects in the green system of sealed storage and consequently decreases the inhabitants of insects and microbes [10]. In general, this delays sensitivity and can decrease spoilage [11]. Unfavourable changed atmospheric conditions, however, accelerate senescence, physiological disintegration, and the products become more susceptible to microorganisms from post-harvest. The current production of pulses is heavily dependent on the input of pesticides; however, multiple adverse effects on health and the environment; stresses the need for more effective pest control and agricultural techniques. Thus, this work has taken a significant step forward toward pesticide-free technology to store green gram following harvest by evaluating the impact of hermetic and non-hermetic packaging material on moisture contents, infestation, grain mass, and microbial loads of green gram grain from local farmers in Maharashtra, India which are stored in the environment.

Materials and Methods

Seed sample and experimental material

A local variety of green gram called 'Utkarsha' was obtained from a local farmer in Akola district of Maharashtra state in India. The average annual temperature of the area ranges from 25 °C in November to 26 °C in February, at an annual temperature of 34 °C. The storage and laboratory experiments were conducted at the Department of Farm structure, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. Green gram was sieved through a 2 mm to exclude free insects and foreign material, including sandstones and broken grains before the experiment, was conducted. For this experiment, we have been used 'Grainpro' super hermetic bag having a thickness of 78µm its multi-layer polyethylene films (https://www.grainpro.com/en), transparent plastic containers, and jute bags for the storage of grains.

Determination of Moisture Content

The moisture content of the green gram under storage was determined by the Association of Official Agricultural Chemists (AOAC) method [12, 13]. The difference between weights before and after of hot air oven at 70°C for 24 hrs is considered as moisture and percentages were calculated as follows.

$$\text{Percentage of moisture} = \frac{W1-W2}{W1} x\ 100 \qquad (1)$$

W1= sample weight before drying in gm
W2= after drying weight of sample in gm

Determining the weight of 1000 grains

1000 grains were counted and weighed at an interval of 15 days up to 180 days of storage for measurement of the weight loss from each treatment [14].

Insect Infestation

A mesh sieve of 2 mm in diameter perforations was used to weigh 100 g in each container sample to extract all insects from stored grains. Manually the number of live bruchids dropped through the sieve openings [15].

Assessment of Microbial Load

After 6 months of storage for the microbial activities, a microbial analysis was conducted on green gram samples for fungal and bacterial loads. Martin Rose Bengal Agar (MRBA) media for fungi and Plate Count Agar (PCA) media for bacteria were used to assess microbial population by standard plate count method [16]. Dissolve 1 g of the powdered sample into 10 mL of distilled sterile water to make water blanks. One mL of 10^{-6} dilution and 10^{-3} dilution were used for counting total bacteria and fungi, respectively. 100 microliters were diluted from various samples in each Petri dish and then 20-25 mL of medium was applied. The plates may solidify and then be held for fungi and Bacteria in an incubator at 28ºC and 37ºC. Bacteria and fungi have been counted in colonies between 48 and 72 h.

The number of CFU/gm samples was calculated by applying the following formula.

$$n_c = \frac{n_m \times d_f}{w_s} \qquad (2)$$

Where

n_c = the number of colony-forming units (CFU's) per gram of the sample,
n_m = the mean number of CFU's,
df = the dilution factor and
w_s = the weight of the sample.

Statistical analysis

A completely randomized design was used with four replications. The treatments represented the storage conditions (Hermetic bags, plastic containers, and Jute bags) and sub-treatments contained the storage duration. The data were subjected to analysis of variance (ANOVA) using Web Agri Stat Package-1 (WASP-1). The experiment was performed for six months. The sample size was 5 kg in all the treatments and replications. Every sealed bag and jute bag were packed with grains, secured with rubber bands, and sealed with Teflon (also known as plumbing tape) after filling the grains, to ensure the airtight conditions performed by [17].

Results

Moisture Content

Moisture content in various treatments over the 180 days of storage is presented in Figure 1. In all three types of packaging materials, the moisture content slowly decreased during storage. A steep decrease in moisture content is observed in Jute bags in which initial moisture content was 13.37% that decreased to 8.5% on the 180th day of storage. In airtight plastic containers, this decrease was from 13.37% to 12.50%. Whereas, in hermetic bags, only a slight decrease in moisture content from 13.37% to 12.90% was recorded in 180 days. At a 5% level of the significant decrease in moisture content in non-hermetic packaging materials were statistically significant over hermetic bags.

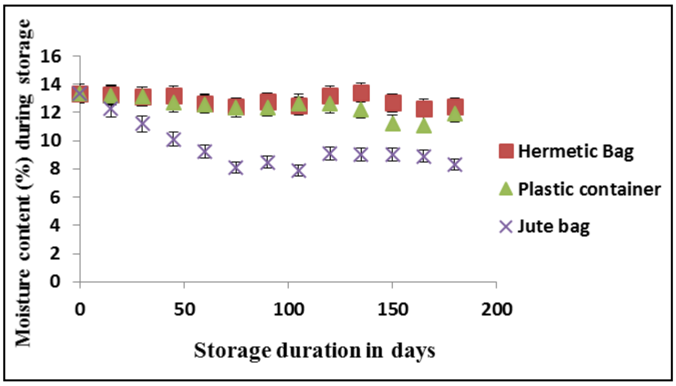

Fig 1: Deterioration of green gram moisture content in hermetic bags, non-hermetic plastic containers, and jute bags during storage. Data used are means ± standard error of three replications.

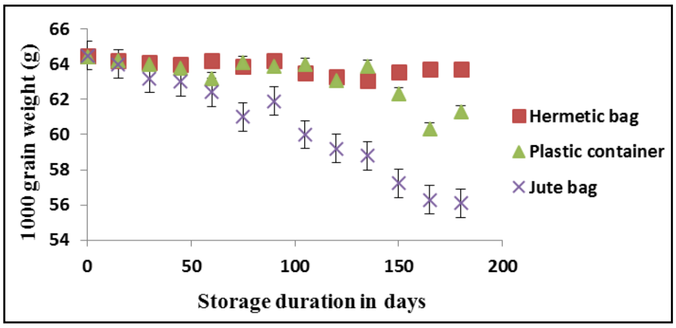

Fig 2: Decline in 1000 grain weight of green gram in hermetic bags, non-hermetic plastic Containers, and jute bags during storage. Data used are means ± standard error of three replications.

1000 grain weight

The weight of grains for both hermetically and non-hermetically stored materials decreased, although the decrease in mass in the grains not stored hermetically was higher. It was recorded that the mass of grains in the non-hermetically stored grains decreased from 64.10 g to 61.21 g. The hermetically packed grains, however, observed a reduction from 64.95 g to 63.09 g. A statistically significant difference between the three storage systems was observed during 180 days of storage by the study of variance (ANOVA) in the grain mass ($p < 0.05$).

Insect infestation

During the initial 30 days, the hermetically-sealed bag infestation of insects increased from 0-6 to 500 g of grain, but from 60 days to 180 days decreased to about 0. Green gram grains in the present study were infested by *Callosobruchus Chinensis* of the Bruchidae family. Infestation in jute bags was higher as compared to hermetic bags and plastic containers. The amount of grain contained in the non-hermetic container continuously increased to 83 living insects/500 g of grain on the 180th day. Among the 3 storage materials, non-hermetic materials have resulted in an increase in the number of insects over 12 weeks of storage over a statistically significant amount compared to the hermetically packed kernels ($p<0.05$).

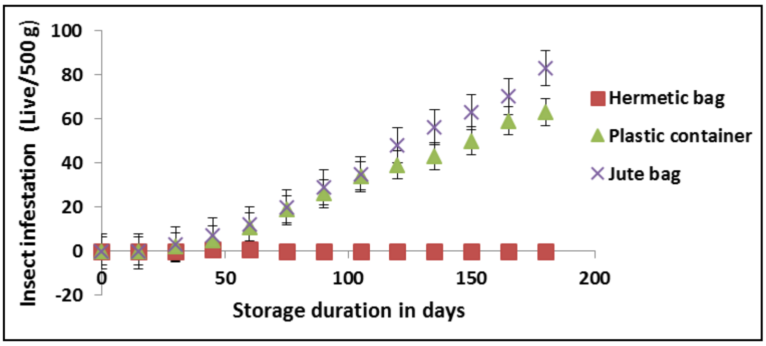

Fig 3: Mean% of live insects/500 g of green grams in hermetic bags, non-hermetic Plastic storage containers and jute bags. Data used are means ± standard error of three replications.

Microbial load

Based on fungal as well as bacterial load it is apparent that hermetic bags followed by plastic containers had the least microbial load. Microbial load in jute bags was significantly higher than the other packaging materials under study.

Table 1: Effect of hermetic and non-hermetic packaging material on total fungal count on Martin Rose Bengal Agar (MRBA) media

SN	Treatment	Fungal Load (cfu/gm)				
		R_1	R_2	R_3	R_4	Avg.
1.	Hermetic Bag	1.6 x 10^3 (0.204)*	1.4 x 10^3 (0.146)	1.3 x 10^3 (0.113)	1.5 x 10^3 (0.176)	1.45 x 10^3 (0.161)
2.	Plastic container	2.5 x 10^3 (0.397)	4 x 10^3 (0.602)	3.2 x 10^3 (0.505)	3.3 x 10^3 (0.518)	3.25 x 10^3 (0.511)
3.	Jute Bag	2.74 x 10^3 (0.437)	3.80 x 10^3 (0.579)	3.96 x 10^3 (0.597)	2.94 x 10^3 (0.468)	3.36 x 10^3 (0.526)
	Level of significance	0.01				
	SE	0.279				
	CD	0.761				

Note: * indicates $\log_e$ transformation of data

Hermetic bags were significantly superior over other treatments with the least fungal count (1.45 x 10^3) followed by non-hermetic plastic containers and jute bags. Thus, it is clear that hermetic bags are better packaging material as far as spoilage due to fungi is concerned that is followed by plastic containers and then jute bags.

Table 2: Effect of hermetic and non-hermetic packaging material on total bacterial count on Plate Count Agar (PCA) media

SN	Treatment	Bacterial Load (cfu/gm)				
		R_1	R_2	R_3	R_4	Avg.
1.	Hermetic Bag	1.7 x 10^6 (0.230)*	1.5 x 10^6 (0.176)	1.8 x 10^6 (0.255)	1.6 x 10^6 (0.204)	1.65 x 10^6 (0.217)
2.	Plastic container	1.1 x 10^6 (0.04)	1.8 x 10^6 (0.255)	1.6 x 10^6 (0.204)	1.7 x 10^6 (0.176)	1.55 x 10^6 (0.190)
3.	Jute Bag	4.0 x 10^6 (0.602)	4.0 x 10^6 (0.602)	3.8 x 10^6 (0.579)	3.9 x 10^6 (0.591)	3.92 x 10^6 (0.593)
	Level of significance	0.01				
	SE	0.050				
	CD	0.137				

Note: * indicates loge transformation of data

Results indicate that non-hermetic plastic container was significantly superior over other treatments with least bacterial count (1.55 x 10^6 CFU/gm) followed by hermetic bags (1.65 x 10^6 CFU/gm) and jute bags. Thus, it is clear that plastic container is better packaging material as far as spoilage due to bacteria is concerned that is followed by hermetic bags.

Discussion

Moisture Content

Loss of moisture in all the types of storage materials over the storage duration confirms the findings of [18] those have reported the decrease in the moisture content of Soybean in various containers over the storage period as compared to the initial moisture content in a study conducted at Southern Nigeria. The free exchange of moisture between the grains and their atmosphere results in a greater loss of moisture in unsealed jute bags.

1000 grain weight

The findings show that during storage, hermetic containers have preserved and decreased weight loss. Due to the infestation by insects in initial stages of storage, there were slight losses in the weight of grains stored in hermetic containers seen in figure 2. The rapid weight loss was mainly because the material provided the insects with favorable conditions to survive and then bore the grain in the non-hermetical systems. Analysis of variance showed that the weight loss of the three packaging materials was statistically significant. The results show that in hermetically sealed bags with a maximum weight of 1000 grain (63.75 g) was registered at 13% moisture while in the non-sealed plastic bag of 13% moisture was the other container at 61.31 g 1000 grain weight. In jute bags containing 9 percent, grain moisture and the lowest 1000-grain weight of 56.10 g was observed. Increased insect infestation and moisture desorption in the environment were the lowest 1000 grain weight found in the jute bags. Such findings are consistent with the results [19] which found that in the population growth of Insect Pests the moisture content played a significant role, with the percentage loss of weight and percent grain loss. Likewise, the maximum changes in the quality of green gram seed in gunny (jute) bags compared with hermetic bags were seen in [20, 8].

Insect infestation

The findings of the present study suggest that green gram infestations in various types of hermetic and non-hermetic materials increased as the storage time progressed. After 30 days of storage, the number of insects increased due to the presence of a certain quantity of oxygen that contributed to their survival. But the grains contributed to the rapid mortality of the insects from this time onwards. According to the study of [21], bruchids are susceptible to an infestation when green grams are stored. Similar findings were recorded in 4 weeks of cowpea weevils (Callosobruchus Chinensis) by [22, 17]. The population was significantly decreased to zero and the oxygen intake and carbon dioxide production increased, resulting in the deaths of insects. The grain stored in a hermetic bag had fewer infestations, followed by plastic containers and jute bags, which made it easy to reach and multiplied easily, due to the pore nature of jute bags. During the storage, the hermetic bag was found to be the best packaging material for preventing insect infestation in green gram with 13.2% moisture content. According to [23] concludes that the best protection for green grams stored on the farm level in the hermetic container that was opened under sunlight without insecticidal treatment. The population of live insects in non-thermal storage was also continuously growing. This is primarily due to its continuous non-hermetic storage as described above, which also corresponds to [24] analysis, providing the conditions required for their rapid growth and development.

Microbial load

From the current findings, it is clear that hermetic bags are better packaging material as far as spoilage due to fungi is concerned that is followed by plastic containers and then jute bags. Although, less then jute bags but hermetic bags and plastic containers were observed to have a certain amount of fungal growth. This is because the atmosphere inside the hermetic bags has high relative humidity, which favours microbial growth and warm temperatures. These results confirmed the findings of [25] that the hermetic bags favoured the growth of fungi and the development of toxins by observing the pigeon pea storage pattern because of the high relative moisture and warm temperature. Jute bags produced even greater microbial load than other packing materials under analysis, and this can be attributed to their sensitivity to damp because of the permeable nature of the bag. These results confirm the findings of [26] that have reported a similar trend during the storage of wheat. Thus, it is clear that hermetic bag is better packaging material as far as spoilage due to fungi is concerned that is followed by non-hermetic material like plastic containers and jute bags. Results indicate that non-hermetic plastic container was significantly superior over other treatments with least bacterial count followed by hermetic bags and jute bags. Thus, it is clear that plastic container is better packaging material as far as spoilage due to bacteria is concerned that is followed by hermetic bags.

Conclusion

The research compared the effect on grain infestation and moisture, weight loss, and microbial loads in green gram grains under the ambient store for six months of the hermetic and non-hermetic storage systems. Observations show that the hermetic storage system has been able in comparison with the non-hermetic system, to better maintain the moisture content of the processed green gram. In the hermetic storage over non-hermetic systems, the number of live insects has also been significantly reduced, when insect populations have increased with the period of storage. While the weight loss was not significant, the hermetic storage packaging content raising the weight loss significantly compared to the non-hermetic packaging. Nevertheless, fungal and bacterial development in the hermetic system was much less than the conventionally used non-hermetic jute bags. The parameters calculated in the hermetic and non-hermetic storage systems indicate that green gram grained grains can be processed in an eco-friendly way without using chemical products or any fumigation in the hermetic system for up to 6 months.

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