

Original Article : Open Access

Improved and safe storage practices for control of bruchids (*Callosobruchus theobromae*) in seeds of Dolichos bean (*Lablab purpureus* L. var. *typicus* Prain)

K. Vanitha*, Saidaiah Pidigam**♦, Hari Kishan Sudini***, A. Geetha**** and K. Ravinder Reddy*

College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Rajendranagar-500030, Hyderabad, Telangana, India

* Department of Vegetable Science, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Rajendranagar-500030, Hyderabad, Telangana, India

** Department of Genetics and Plant Breeding, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Mojerla-509382, Telangana, India

*** Department of Plant Pathology, International Crops Research Institute for the Semi-Arid Tropics, Patancheru-502 324, Hyderabad, Telangana, India

**** Department of Crop Physiology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar-500030, Hyderabad, Telangana, India

Article Info

Article history

Received 5 December 2023

Revised 17 January 2024

Accepted 18 January 2024

Published Online 30 June 2024

Keywords

Bruchid infestation
Dolichos bean seeds
Hermetic storage
Hypercarbia
Hypoxia
Lablab purpureus L. var. *typicus*
Prain
Triple-layer purdue improved crop
storage bag

Abstract

Dolichos bean seed storage to protect from the attack of bruchid is a significant problem across the world. Bruchid attack leads to 100% damage, causing losses in terms of bean produce quantity and quality and making produce inferior for consumption. Purdue University developed Triple-layer bag-based PICS technology holding the promise for chemical-free storage on hermetic principles, which was explored for Dolichos bean storage to control pulse beetle infestation in the present investigation. Findings indicated the lowest infestation of bruchid in PICS bags at 2, 4 and 6-month periods of storage. PICS bag has not recorded any change in the temperature, whereas other jute and plastic bags recorded at par value changes according to the environment prevailing externally. PICS bags scored high relative humidity at two-month intervals time, viz., 2, 4 and 6 months of bean storage as against other storage bags tested in the experiment. The present investigation confirms the efficacy of the triple layer-based PICS bags in managing the lowest bruchid infestation compared with other types of bags and advantageous over 3 differential periods of safe, hermetic storage of Dolichos bean produce from bruchids.

1. Introduction

Dolichos bean botanically is *Lablab purpureus* L. var. *typicus* Prain, which is termed as Indian bean or hyacinth bean as well. It is an important vegetable legume crop from Fabaceae. India is the centre of origin of the Dolichos bean. It is even cultivated as an inter-crop in cereal crops, especially in south India. It is popular among the perennial vegetable crops and preferred to use its green pods and dried seeds as a vegetable and for various food preparations, respectively. The remaining parts of the plant are used as fodder to feed livestock. As per the reports of Aykroyd (1963), the Dolichos bean is considered superior in nutrition than that of the French bean. The mature seed is highly proteinaceous (40.0% protein) (Schaaffhausen, 1963).

Dolichos bean crop suffers losses due to pests and diseases in the field and in the storage due to bruchid damage. In this crop, post-

harvest losses are high due to bruchid damage and loss can account for up to 100% in severe bruchid infestation. The bruchid-infested pulses in general are inferior for human consumption due to excreta and uric acid produced by them during period of infestation, which also leads to fungal infection in produce as reported by Gowda and Kaul (1982).

Several traditional methods employed for control of bruchids are either ineffective or cost-effective and especially the use of chemical methods is detrimental to the farmers (fumigants used indoors) and consumers (residues). Mixing of beans with ash, which is used for storage of beans in smaller quantities and other indigenous methods like solarization, mixing fine dust, botanicals, oviposition deterrent, fumigation, and contact insecticides are less effective and provide variable results as there is no standard dosage and guidelines for their usage (Songa and Rono, 1998). Hence, there is a dire need for non-chemical methods for the protection of stored products; because chemical-based control methods are often not acceptable for sustainable cultivation. Once the bruchid attacks, the germination, and test weight quality decrease and make the beans unfit for use as seed to raise a new crop.

Corresponding author: Dr. Pidigam Saidaiah

Associate Professor, Department of Genetics and Plant Breeding, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Mojerla-509382, Telangana, India.

E-mail: saidu_genetics@yahoo.co.in; drpidigam@gmail.com

Tel.: +91-7780509322

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Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

Triple layers containing bags designed by Purdue University are named Purdue Improved Crop Storage shortly as PICS are considered effective for minimizing grain losses after harvest of produce storage to insects. Triple-layered PICS bags contain the first two inner layers made of high-density polyethylene with 80 μ thickness and polypropylene woven layer as the outer bag (Baributsa *et al.*, 2012). Several research teams such as Murdock *et al.* (2012); Baoua *et al.* (2012, 2013) reported and proven excellent protection offered by PICS bags for Cowpea grain in West Africa against bruchid seed beetles. The PICS technology works to create an airtight storage atmosphere inside the bag, which is detrimental to insect growth and replication in the stored bags. PICS bags work in line with several other hermetic storage bags or containers such as sealed drums made up of steel (Seck *et al.*, 1996), and the insects respire aerobically followed by utilizing the available oxygen in the airtight container, in which they produce stored, while increasing carbon dioxide above the normal levels. The insect population become inactive and ceases feeding as the oxygen level in the storage structure decreases significantly (Margam, 2009). Insect inactivity leads to the ceased regular anatomical growth of the insect and further development, leading to stopping the reproduction of the insect. Thereby, the multiplication of the pest population is arrested. Particularly, early instar larvae and pupal stages of insects seem to be highly vulnerable, ultimately leading to the death of bruchids in these bags. Hence, the current experiment was planned to evaluate the efficacy of different bags for hermetic storage of dried seed of Dolichos bean aiming for bruchid control.

2. Materials and Methods

2.1 Location of work

The research experiment was conducted at the Post Graduate Research Laboratory, College of Horticulture, Rajendranagar, Sri Konda Laxman Telangana State Horticultural University, Hyderabad, Telangana state, India.

2.2 Dolichos bean seeds

Approximately 180 kg of dried seeds of leading commercial variety, *i.e.*, RND-1, Dolichos bean with 10% initial seed moisture content were obtained from National Seeds Corporation (NSC). The seed was stored in different types of storage bags for the experiment.

2.3 Storage bags

Four different types of storage bags, *viz.*, jute bags, polythene bags, PICS bags and jute bags treated with Chlorpyrifos were used in the experiment to evaluate their efficacy in managing Dolichos bean from bruchid attack. Newly-made jute bags and polythene bags were used along with untreated jute bags and polythene bags for storing the dried Dolichos bean seeds, while the jute bags treated with Chlorpyrifos were turned inside out and sprayed with insecticide, which was applied @2 ml per litre of water on the inner side followed by drying them in shade. Then, they were used for the experiment. PICS bags developed by Professor Murdock (1987) at Purdue University jointly with USAID team of researchers and Cowpea Collaborative Research Support Program to combat bruchid infestation in Cameroon on Cowpea. The same PICS bags were sourced locally and used in the experiment.

2.4 Rearing of Dolichos bean bruchids, *Callosobruchus theobromae*

Initially, the bruchid-infested culture of Dolichos bean, *C. theobromae*, was collected from naturally infested dried Dolichos bean seeds purchased from the local market. The Dolichos bean bruchid population was multiplied by exploring the locally available plastic jars by releasing the naturally-grown and collected bruchids into healthy bean seeds. For good ventilation and aeration, the infested plastic jars were covered with fresh muslin cloth. The inoculums of seeds with live adults and seeds covered with creamy eggs were used in the experiment for an initial 10% infestation, *i.e.*, 4.5 kg of healthy Dolichos bean seeds mixed with 500 g of bruchid-infested Dolichos bean seed for each treatment.

2.5 Mean temperature and average relative humidity

Mean temperature and RH as well within the experimental sealed bags were recorded on hourly intervals, placing data loggers (Lascar model, EL-USB-2, White parish, Wiltshire, Great Britain) inside the bags for the entire period of six months. Meteorological data about mean minimum and maximum temperatures, relative humidity (%) and rainfall in weekly averages during the period of experiment were recorded at Agricultural Research Institute, Meteorological Station, Rajendranagar, Hyderabad.

2.6 Experimental setup

Five kilograms of dried Dolichos bean seeds with recommended (10 per cent) moisture was taken weights individually, and placed in four bags replication-wise. Each of these storage bags was infested with 10% Dolichos bean seed in which bruchids *C. theobromae* were multiplied. Then, the bags were gently moved up and down sides to ensure a uniform mixing of bruchid-infested seed with uninfested seed in the bags before tying the bags to close. The filled storage bags (starting with the innermost layer and proceeding further to other layers in the PICS bags) were sealed with a heat sealer and the outer polypropylene bag was tied tightly with a thread, which gives mechanical strength to the inner two layers. All four bag treatments were replicated and used for the experiment with identical conditions (10% moisture). As per the layout indicated, 36 kinds of storage bags as treatments were investigated. The bags were tested for bruchid infestation in the seed at 2-month intervals (2, 4 and 6-month time points).

2.7 Bruchid infestation

Randomly selected samples from the storage bags after 2, 4 and 6 months of storage were collected and pooled. From the sample, the per cent bruchid infestation was calculated using the formula:

$$\text{Bruchid infestation (\%)} = \frac{\text{No. of bored Dolichos bean seeds}}{\text{Total No. of Dolichos bean seeds taken}} \times 100$$

2.8 Statistical Analysis

A complete randomized design (CRD) following Snedecor and Cochran (1967) was explored to conduct the experiment, wherein 4 different storage bags and 3 storage periods were used as factors influencing insect population growth in the seeds. Analyses of variance followed by LSD tests at a 5% level were employed to assess bruchid damage in each treatment.

Replication 1		Replication 2		Replication 3	
Treatment	Bag type used	Treatment	Bag type used	Treatment	Bag type used
T ₁		T ₈		T ₁₀	
T ₂		T ₆		T ₉	
T ₃		T ₁₀		T ₈	
T ₄		T ₉		T ₂	
T ₅		T ₁₁		T ₁₂	
T ₆		T ₅		T ₃	
T ₇		T ₇		T ₁	
T ₈		T ₁₂		T ₁₁	
T ₉		T ₁		T ₅	
T ₁₀		T ₄		T ₆	
T ₁₁		T ₃		T ₄	
T ₁₂		T ₂		T ₇	

Lay out of the experiment

The data about bruchid infestation (%) of dried seed of the RND-1 variety of Dolichos bean in the bag of the treatment was put to statistical analysis following the model of Panse and Sukhatme (1985).

3. Results

Initial level of bruchid infestation in the seeds of RND-1 variety of Dolichos bean calculated from three replicates before the experiment initiated was noted as zero.

3.1 Analysis of variance of CRD

The CRD analysis of variance for types of bags, periods of produce storage and as well as their interactions with bruchid infestation (10%) revealed significant variations for treatments under the experiment (Table 1).

Table 1: CRD ANOVA for bruchid infestation (%) in RND-1 variety of Dolichos bean

Source of variation	Degrees of freedom	Bruchid infestation (%)
A (Bag type)	3	449.76***
B (Storage periods)	2	4.90***
A×B (Interaction)	6	0.03***
Error (B)	24	0.246
Total	35	3.203
General mean	16.108	
C. V.	4.310	

Significance level *** = <0.001

3.2 Influence of storage bags on bruchid infestation (%) of dried seeds in RND-1 variety of Dolichos bean

In the case of storage bags, all bags differed significantly ($p<0.001$) from each other. The bruchid infestation (%) of Dolichos bean seed was highest in the jute bag (28.44), followed by the polythene bag (26.984), and insecticide-treated jute bag (19.09). The lowest infestation (%) level was recorded in the PICS bag (13.33). Among all, the PICS bag performed better with the lowest percentage of bruchid damage and the quality of the seed was also good. When

comparing the sets of storage periods, maximum bruchid infestation (%) was recorded after 6-month storage periods (24), followed by 4 months (21.65) and 2 months (20.24) and were significant and different ($p<0.001$) with each other. With the increase in the storage period, bruchid infestation (%) was increased in all interactions except in the PICS bag, which has the very least infestation. Out of 12 interactions, PICS bag stored for 2 months (13.27), 4 months (13.35) and 6 months (13.38) showed the least pulse bruchid infestation and were best among all interactions and all were at par (Table 2 and Figure 1). While, the highest per cent of infestation of bruchid was reported in a jute bag for 6 months stored period (31.47), followed by polythene bag for 6 months period of storage (29.19), jute bag for 4 months stored (27.94), polythene bag for 4 months stored time (26.93), jute bag for 2 months of storage (25.91), jute bag treated with insecticide kept for 6 months (25.91), polythene bag for a period of 2 months (24.83), jute bag treated with insecticide for a storage period of 6 months (21.95), jute bag treated with insecticide with a storage period of 4 months (18.37) and insecticide-treated jute bag, when stored for 2 months (16.95) time. Among treatment combinations, the performance of triple-layer bags was outstanding followed by insecticide-treated jute bags, polythene bags and jute bags and a similar trend was noticed among almost all the storage periods. The insect pest in the PICS bag had died due to hypercarbia and hypoxia at varied set storage periods in the experiment.

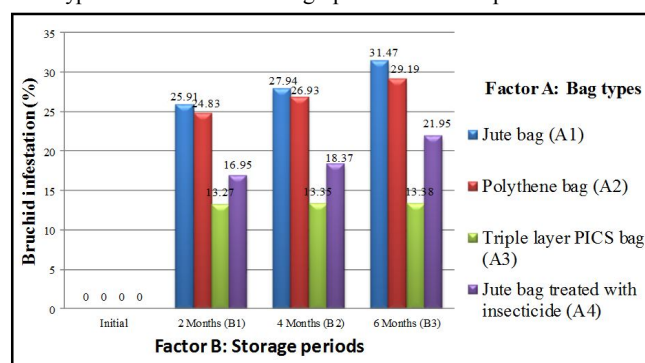


Figure 1: Influence of storage bags on bruchid infestation (%) of dried seeds in RND-1 variety of Dolichos bean.

Table 2: Influence of storage bags on bruchid infestation (%) of dried seeds in RND-1 variety of Dolichos bean

Factor (A): Different types of storage bags	Bruchid infestation (%) before storage	Factor (B): Different periods of storage			
		Bruchid infestation (%) at 2 months (B ₁) of storage	Bruchid infestation (%) at 4 months (B ₂) of storage	Bruchid infestation (%) at 6 months (B ₃) of storage	Bruchid infestation (%) as Mean A
Jute bag (A ₁)	0	25.91 ^e	27.94 ^c	31.47 ^a	28.44 ^a
Polythene bag (A ₂)		24.83 ^f	26.93 ^d	29.19 ^b	26.98 ^b
PICS bag (A ₃)		13.27 ^j	13.35 ^j	13.38 ^j	13.33 ^d
Jute bag treated with Chlorpyrifos (A ₄)		16.95 ⁱ	18.37 ^h	21.95 ^g	19.09 ^c
Mean B		20.24 ^c	21.64 ^b	24.00 ^a	
Factors			S.E. (m)	C. D. at 5%	C.V.
Factor A			0.06	0.17	3.07
Factor B			0.05	0.15	
Factor (A×B)			0.10	0.30	

Values followed by the different letters are significantly different.

The bruchid infestation levels in the PICS bag were less and the seed was good in quality compared to other treatments, which have a heavy infestation of pests. The present results are in line with the earlier report of Sudini *et al.* (2015) in Groundnut storage against bruchids, and in Rice grain storage against adult *Sitophilus oryzae* (Marco *et al.*, 2014; Njoroge *et al.*, 2018).

3.3 Influence of storage bags on temperature (°C) of dried seeds in RND-1 variety of Dolichos bean

The data loggers placed in the different types of bags recorded the temperature (°C) inside each of the treatment combinations on a 12 hour interval basis. The data when plotted following bimonthly intervals, does not qualify for statistical validity on comparison. Hence, temperature was noted as a trend only.

The data recorded in data loggers showed a temperature of 27°C initially in all types of storage bags. In triple-layer bags, the temperatures have constant at 27°C in 2 months, 4 months and 6 months storage periods, respectively, *i.e.*, same as that of the initial storage period but in the case of other bags the temperatures were reduced in 4 months storage period compared to 2 months storage period, then again increased to maximum in 6 months storage period according to the change in the season. However, the PICS bag did not show any effect of season on the changes in temperature inside the bag (Figure 2).

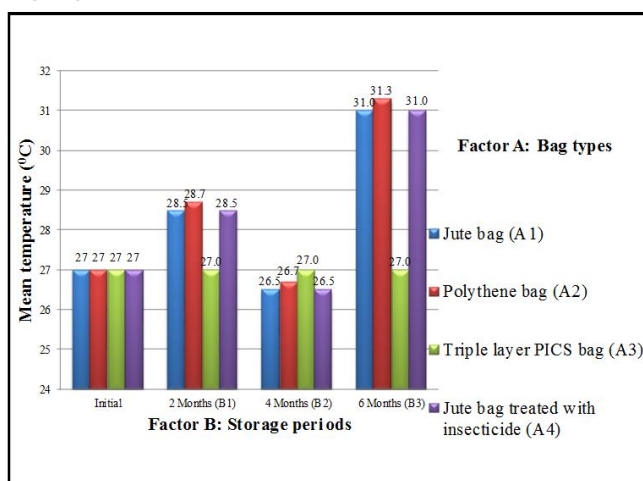


Figure 2: Influence of storage bags on temperature (°C) of dried seeds in RND-1 variety of Dolichos bean.

3.4 Influence of storage bags on relative humidity (%) of dried seeds in RND-1 variety of Dolichos bean

The data loggers placed in the different types of bags recorded high relative humidity in the PICS bag (61.20%), followed by the polythene bag (51.30%), jute bag and jute bag treated with insecticide, which recorded the same RH (50.80%). Maximum RH was recorded in the PICS bag at 10% seed moisture after 6 months period of storage (62%), followed by 4 months period of storage (61%) and 2 months period of storage (60.50%), which were at par and higher than other storage bags (Figure 3).

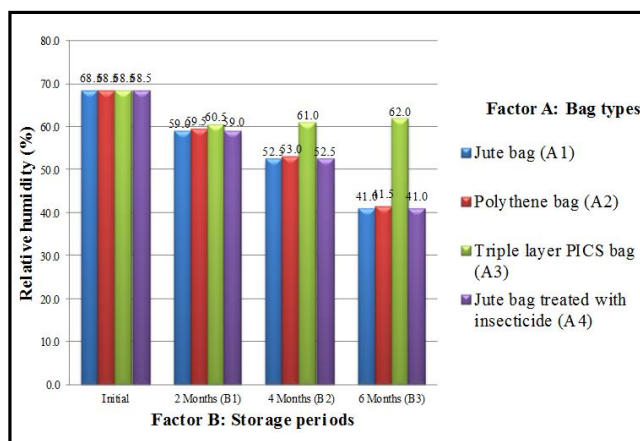


Figure 3: Influence of storage bags on relative humidity (%) of dried seeds in RND-1 variety of Dolichos bean.

4. Discussion

Dolichos bean or lablab bean is one of the profitable crops belonging to the Leguminosae family, with net profits to the farmer. More than seven states are cultivating this crop. However, the crop suffers losses both in the field and in the storage. Out of which, bruchids/pulse beetle led to significant damage to economic produce in the field conditions and storage conditions as well in addition to both quantitative and qualitative bean losses. Bruchid damage noticed is higher in Dolichos bean at post-harvest storage handling. Damage accounts up to 100% are also reported, if proper care is not taken to control bruchid damage during the storage. Farmers practice different commercial and traditional methods for bruchid control but with restricted value the methods have, as they are costly, potentially toxic and labour intensive in nature. Purdue University-designed PICS bags are easy to use, based on effective technology for safe handling of post-harvest produce, which is already demonstrated by Baributsa *et al.* (2017). Hence, the present experiment investigated in statistical design, FCRD with 2 factors, *viz.*, four bag types with their combination with three storage periods. Uninfested, fresh Dolichos bean seed with 10% of seed had an artificial infestation of bruchid (*C. theobromae*) formulated in each treatment combination. Combination-wise treatments were tested for further damage after storage.

On analysis of CRD ANOVA for a 10% level of bruchid infestation in different treatment combinations, the treatment variances were significant and high, which indicates that there is a sufficient amount of variation among bag types, storage periods and their interactions and are different from each other. They have their individual effects on bruchid population survival and other quality parameters of stored dry Dolichos bean seeds.

The biotic organisms like insects and fungi, when infests the crop produce during storage, they consume oxygen, while respirations, reducing it to 1 to 2% from near about 21% that is available in the air. At the same time, insects release CO₂ raising it to an alarming level of nearly 20% from an ambient 0.035% (White and Jayas, 2003), which creates an unfavourable environment for their living. The mortality of insects happens, when there is less than a 5% level of O₂, which leads to metabolic stress in organisms due to aggregated respiration rate (Emekci *et al.*, 2004).

Bruchid damage observed in the PICS bag (13.33%) was low and at par with that of the jute insecticide-treated bag (10.09%) in the present investigation. While, with the same level of artificial pest infestation, Dolichos bean seeds stored in non-hermetic storage bags had severe insect damage along with egg masses on the seed coat, with clear visibility. The quality of the seed assessed post-experiment from a jute bag, the combination, jute bag treated with insecticide, in addition to polythene bag was poor in quality compared to the beans stored in the PICS bag. Due to hypoxia and hypercarbia, the death of pests occurred in the PICS bags and bruchid damage was controlled. PICS bag not only controlled the infestation in the bag but also prevented further infestation of pests on remaining healthy seeds in the bag as it has triple layers. The present findings comply with the reports of Sudini *et al.* (2015) in Groundnut, Mutambuki *et al.* (2014) in Maize and Vales *et al.* (2014) in Pigeon pea with PICS storage bags. As concluded by Marco *et al.* (2014), the required storage to favour O₂ depletion and CO₂ built up for eliminating common bean weevil, *Acanthoscelides obtectus* is provided by the triple-layered bags. The same is true in the present experiment. The reduced O₂ levels and elevated CO₂ levels lead to the death of the bruchids. The PICS technology is effective due to its airtight storage conditions. The cleaned seeds were put into high-density polyethylene bags with walls of 80 µ thick, without any air pockets, leaving a neck space (20 to 30 cm) length. The PICS technology works to create an airtight storage atmosphere inside the bag, which is detrimental to insect growth and replication in the stored bags. PICS bags work in line with several other similar hermetic storage bags or containers like sealed drums made up of steel (Seck *et al.* 1996) and as the insects respire aerobically and exhaust the available O₂ in the airtight storage container, increasing CO₂ levels (Anankware and Ire, 2013). The insect population becomes inactive and ceases feeding as the O₂ level in the storage container falls significantly low (Margam, 2009). Insect inactivity leads to ceased growth of the insect, thereby developing and in turn stops the reproduction of the insect, finally. Thereby, the multiplication of the pest population is arrested. Particularly, early instar larvae and pupae stages of insect growth seem to be highly vulnerable ultimately leading to the death of bruchids in these bags. The combination consisting of two liners inside coupled along with one woven bag provides a robust composite storage structure continue to function effectively even after handling (Mbata *et al.*, 1996; Moussa *et al.*, 2012; Baributsa *et al.*, 2014).

The mean temperature (°C) recorded in the triple layer PICS bag at 2, 4, and 6 months of storage periods remained the same as the initial temperature recorded (27°C). While, in all other types of storage bags, the temperature changed according to the seasonal variations. Further, the relative humidity (%) recorded in the PICS bag was highest (61.20%), irrespective of storage periods, compared to the RH in other storage bags. Other types of bags such as jute bags, insecticide-treated jute bags and polythene bags offered only partial restriction of air movement across the walls of these bags due to porosity. Overall, the hermetic structures of the bags created an atmosphere devoid of oxygen as a consequence of the respiration of seeds, which caused mortality of insect population without any need for chemicals or fumigants. Thus, PICS bag technology has evolved as a safe alternative storage method and is more effective in comparison to other types of storage bags and methods in managing lowest levels of bruchids in Dolichos bean seeds.

5. Conclusion

The present investigation proposes the PICS-based hermetic bags as an efficient technology in protecting Dolichos beans from bruchid infestation compared to other methods of storage. The PICS-based hermetic bags for bean storage can be used as an improved storage practice, which works on the principle of creating hypercarbia and hypoxia condition in the bag, which is highly efficient over months of storage period in preventing the infestation of bruchids. The PICS bag is a chemical-free technology and, therefore, recommended for long-term hermetic storage of dry bean produce of Dolichos bean.

Acknowledgements

The authors are grateful to Sri Konda Laxman Telangana State Horticultural University for its support and encouragement in conducting the research in the field and lab.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation

K. Vanitha, Saidaiah Pidigam, Hari Kishan Sudini, A. Geetha and K. Ravinder Reddy (2024). Improved and safe storage practices for control of bruchids (*Callosobruchus theobromae*) in seeds of Dolichos bean (*Lablab purpureus* L. var. *typicus* Prain). *Ann. Phytomed.*, **13(1):1238-1244. <http://dx.doi.org/10.54085/ap.2024.13.1.134>.**