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A comparative study on qualitative loss of wheat by keeping under different way of storage

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Abstract

A Comparative study on qualitative loss of wheat grain (Variety-2643) performed through keeping under various kind of storage bags at ambient temperature storage of grains were made in four different types of bags i.e. HSGB bag, Polyethylene bags, Plastic bags and Jute Bag. The freshly harvested wheat grains were observed by bio treatment (neem) or Chemical treatment (Aluminium phosphide). Physical property like grain moisture content, colour index, water activity, damage of insect infestation (degradability) and percentage of germination were recorded for Nine months. Super Grain Bag showed least in it variation in grain moisture content (0.15), colour index (3.85) and water activity (0.085) while maximum variation were recorded in Jute sulphas i.e. 0.41, 6.98 and 0.19 respectively. Super Grain Bag showed maximum 88% germination while least 76% minimum was recorded in jute neem. Infestation of insect was found in all kind of bags except Hermetic Super Grain Bag

Keywords: Hermetic, super grain Bag, Storage, Germination, Degradability, Moisture Content, water activity, colour index

Introduction

With 2.85 % of India's geographical area and 8.07 % of population, Bihar is the third most populous state in the country (2001 census). About 80% of Bihar's population is dependent for its livelihood on agriculture. Bihar is the third largest producer of vegetables; fifth largest producer of fruits and eighth largest producer of grains in India. On one front, state of Bihar is being talked of as the next big hope for the agriculture sector, on the other this sector remains the most crucial factor for the state economy. Degree of dependence on agriculture in terms of employment as well as income is high. In spite of high volume of production and a good range of crops, the earnings from farming are poor. The value-addition in agricultural products is negligible. Owing to low literacy, small land holdings and poor infrastructure, the production practices and input usage is either less or more than recommended practices. Needless to say, if the recommended practices are followed the potential for sustainable increase in production and productivity is huge.

Hermetic storage bags is a safe, cost-effective storage method that controls insect infestations in addition to preserving the quality of grains, while allowing for pesticide-free, short-term and long-term qualitative and quantitative seed preservation, without refrigeration, maintaining seed vigor and pest control. Storage at low temperature (4°C) ensures greater safety margins between insect development time and break of dormancy, although hermetic storage, even at ambient temperatures, naturally eliminates insect development altogether. Hermetic storage is capable of maintaining relative humidity that preserves seed moisture and prevents mold growth.

Hermetic bags need to be validated for its effectiveness in hermetic storage of food grains under Bihar condition. The present study was undertaken in response to requests by farmers, traders and private seed companies to determine the effectiveness of hermetic bags for storage of wheat grain. Hence, a comparative study on storage behavior of wheat in different storage bags was made to assess the qualitative and quantitative loss and to validate the advantages of hermetic bags in wheat storage over the conventional storage bags used in the region. On-farm hermetic storage has the potential to substantially reduce these losses without the use of pesticides.

Materials and Methods**1.1 Sample preparation**

Fresh and healthy wheat seeds were procured from local market. Cleaning and grading of grain were done in seed cleaner-cum-grader and specific gravity separator of 6.50 mm top screen and 2.10 mm bottom screen. Grains from the bottom screen outlet were discarded and only top and main grain outlet was used for storage study. 50kg cleaned and graded wheat were

weighed using weighing machine (Wensar weighing scales limited, range 0.2 gm to 2000 gm with sensitivity of 0.01 gm) and stored in different storage bags.

1.2 Treatment

The experiment consisted of seven treatments using four different types of bags – Hermetic grain bags, Polythene bags, Jute bags and Plastic bags by using one chemical fumigant (Aluminium Phosphide) and dried Neem leaves.

There was no any treatment in grain stored in hermetic grain bag. For neem treatment, the fresh neem leaves were taken from the tree and dried by sun drying method. The dried neem leaves 75 g in weight was mixed with the wheat at three different layers (at 20 cm from bottom and 25 between the next consecutive layer) of the bags. The surface at the top was covered with the remaining 25 gm of neem leaves. The bag-end was closed by tightly twisting the free portion and then tying it by ropes or some suitable means.

The fumigant (Aluminium Phosphide) popularly known as sulphash were used for the chemical treatment which molecular formula is AlP , molecular weight is 57.955 gm. mole⁻¹ and density is 2.85 gm. cm⁻³. Around 0.93 gm of sulphash was placed in the centre of the bag and bag-end was closed by tightly twisting the free portion and then tying it by ropes or some suitable means.

The bags were placed in a room made of concrete roofed and wall with suitable ventilation. All the treatments were kept under ambient conditions. The different treatments were arranged in two rows on a dunnage so as to protect the grains bags from the direct contact with ground. The temperature and relative humidity were recorded on a daily basis while the other dependent parameters were recorded on weekly basis.

1.3 Hermetic storage

Hermetic storage bags are airtight storage bags used worldwide for the prevention of post-harvest storage losses. The intrinsic advantage of the hermetic storage of dry cereal grains lies in the generation-by the aerobic metabolism of insect pests and microorganisms-of an oxygen-depleted and carbon dioxide-enriched inter-granular atmosphere of the storage ecosystem. A hermetic storage bags is a safe, cost-effective storage method that controls insect infestations in addition to preserving the quality of grains, while allowing for pesticide-free, short-term and long-term qualitative and quantitative seed preservation, without refrigeration, maintaining seed vigor and pest control.

The hermetic bags were provided by Grain Pro Inc., the manufacturer (<http://www.grainpro.com/grainpro-supergrainbag.php>) through their agent in India. Their dimensions were same size as the polyethylene bags. The super grain bags are manufactured in high density polyethylene that reduces gas exchange. Most agricultural commodities stored in these bags will develop a modified atmosphere of low oxygen and high carbon dioxide content, created by respiration of living organisms such as insects and fungi. After filling the super grain bag with grain, the free plastic portion (above the grain) was squeezed in order to remove excess air. The opening was then closed by tightly twisting the free portion and sealing it with a special strap fastener provided by the manufacturer. The top end of the bag was twisted once more, folded back and sealed with another fastener. As recommended, the super grain bags were used as a liner bag inside polyethylene bags, which provide support and ease in handling. The outer bag was also closed.

1.4 Observations on storage study

From each bags, grain samples were obtained with a compartmentalized grain sampling spear (Seed Buro Equipment Company, Chicago, USA) at one week intervals. The sampling spear was 1 m long, with five slots, 15 cm long, evenly-spaced, and separated from each other by a 2.5 cm-long wooden plug. For this study, wheat samples of about 25.27 gm per slot were taken with the bottom three slots.

1.4.1 Moisture Content

Initial moisture content of the wheat was determined for finding the dry matter as well as moisture content of the raw sample. The moisture content of sample was determined by standard hot air oven method. The samples were dried in the hot air oven at 105°C for 24 hours. The total dry materials or the initial moisture content of sample was determined in accordance with AOAC method (Anonymous, 1990) and Moisture Content (MC) was calculated using following formulae:

$$MC = \frac{W_m}{W_m + W_d} \times 100$$

1.4.2 1000 Grain Weight

1000 grain weight was measured with the help of Electrical balance. First, randomly selected 1000 seeds from each treatment of wheat samples were taken and weighed using electronic balance of 0.01 g sensitivity.

1.4.3 Water Activity

Water activity is defined as the ratio of the vapour pressure of water in a material (p) to the vapour pressure of pure water (p_0) at the same temperature. Relative humidity of air is defined as the ratio of the vapour pressure of air to its saturation vapour pressure. When vapour and temperature equilibrium are obtained, the water activity of the sample is equal to the relative humidity of air surrounding the sample in a sealed measurement chamber. Multiplication of water activity by 100 gives the equilibrium relative humidity (ERH) in percent.

$$a_w = p/p_0 = ERH (\%) / 100$$

Water activity (a_w) is one of the most critical factors in determining quality and safety of the goods you consume every day. Water activity affects the shelf life, safety, texture, flavour, and smell of foods. Water activity may be the most important factor in controlling spoilage. Water activity of wheat was measured during experimentation using water activity meter at ambient temperature.

1.4.4 Colour Index

The colour of stored wheat was measured with the help of hunter colour lab meter. The instrument was calibrated using standard white and black tiles as per standard procedure. Then samples were kept on the specimens port (dia. 95 mm) so as to cover the full exposed area of port emitting light and Hunter L, a and b values were noted. Hunter L-values (which denotes the degree of whiteness) was chooses to represent the colour values of samples. In addition to Hunter L-value, a new parameter called total color index (E) was developed to represent the total colour index and calculated by formula.

$$E = \sqrt{L^2 + a^2 + b^2}$$

Where,

E = total colour index

L = Hunter L-value (+ is lightness, - is darkness)

b = hunter b-value (+ yellowness,-blueness)

a = Hunter a-value (+redness,-greenness)

1.4.5 Germination percentage

100 grains of wheat were taken in 7 different petri-dishes. The disc was fully filled with sand and water. Water spraying was done regularly to keep the grain moist. After a time of 72 hrs germination of grain was counted carefully and germination percentages of 7 respective samples were collected.

$$\text{Germination Percentage} = \frac{\text{number of seeds sprouted}}{\text{total number of seeds taken}} * 100\%$$

1.4.6 Insect -pest damage in stored grain

At the end of every month of storage period random samples were drawn and each sample was visually rated for damage

by insect and pests. Although the storage period was nine months but some indication was obtained with regard to the damage under 7 different types of storage conditions.

1.4.7 Temperature humidity profile during storage

The temperature and relative humidity were recorded by portable relative humidity meter on daily basis.

Results and Discussion

2.1 Grain moisture content

The variation in grain moisture content with storage period different bag storage modes under various treatments is shown in Table 2.1 and Figure 2.1. The initial moisture content of wheat when procured was 10.90 % (w. b.). In all the treatments the moisture content of wheat showed increasing trend. This may be due increase in ambient relative humidity and dampness created by the heat of respiration of the grain.

Table 2.1: Moisture content variation in wheat grain in different storage bags

Storage Treatment	Monthly observations									
	0	1	2	3	4	5	6	7	8	9
Jute+SGB (No treatment)	10.90	10.91	10.92	10.94	10.95	10.97	10.98	11.00	11.02	11.05
Jute+poly(neem)	10.90	10.91	10.94	10.98	11.00	11.03	11.05	11.08	11.10	11.13
Jute(neem)	10.90	10.93	10.97	11.05	11.09	11.14	11.17	11.22	11.25	11.28
Plastic(neem)	10.90	10.91	10.95	11.01	11.05	11.09	11.12	11.16	11.19	11.21
Jute+poly(sulphash)	10.90	10.91	10.93	10.97	11.00	11.04	11.06	11.08	11.09	11.13
Jute(sulphash)	10.90	10.92	10.98	11.07	11.10	11.16	11.18	11.21	11.25	11.31
Plastic(sulphash)	10.90	10.91	10.96	11.01	11.04	11.07	11.12	11.17	11.2	11.23

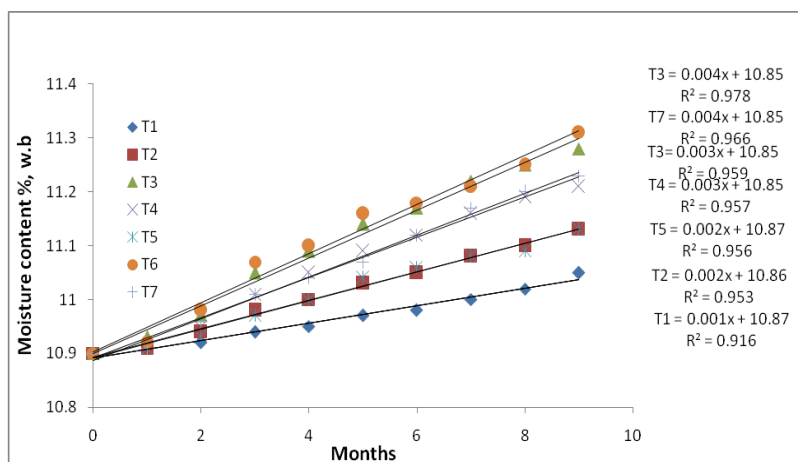


Fig 2.1: Variation in moisture content with storage duration in experimental samples

The variation in moisture content of wheat in the Jute bag with hermetic grain bag was least which ranged between 10.90% to 11.05%. Moisture in the polythene bag storage kept inside the jute bag with neem treatment increased from 10.90 % to 11.13% while moisture in plastic bag storage with neem treatment increased from 10.90% to 11.21%.

Moisture content variation in jute bag with chemical/biological treatment was maximum. Moisture content in jute bags with neem treatment increased from 10.90% to 11.28 % (w. b.) while moisture in jute bag with chemical treatment increased from 10.90% to 11.31 % (w. b.). The marginal increase in moisture content in all the treatments was due to respiration of grain and the increased relative humidity with storage duration. It was also seen that the moisture increased initially slowly up to Nine months of storage period and then it increased rapidly as relative humidity increases day-by-day.

The least variation in moisture content in hermetic storage bags is due to generation-of the aerobic metabolism of insect pests and microorganisms-of an oxygen-depleted and carbon dioxide-enriched inter-granular atmosphere of the storage ecosystem. The hermetic storage bags showed a safe, cost-effective storage method that controls insect infestations in addition to preserving the quality of grains.

2.2 Determination of weight of 1000 seeds

The variation between 1000 grain weight in hermetic grain bag kept inside the jute bag, polythene bag kept inside the jute bag, plastic bag, and jute bag storage with storage period are shown in Table 2.2 Figure 2.2. This parameter behaves in same manner as the change in moisture content with storage period was nine months. If the grain gained moisture, the 1000 grain weight increased and vice-versa.

Table 2.2: Variation of 1000 grain weight (in gram) with days in different storage bags for wheat grain

Storage Treatment	Monthly observations										
	0	1	2	3	4	5	6	7	8	9	
Jute+SGB(No treatment)	40.4	40.4	40.7	41.1	41.4	41.6	42	42.3	42.5	42.6	
Jute+poly(neem)	40.4	40.4	40.8	41.2	42	42.8	43.5	43.9	44.2	44.4	
Jute(neem)	40.4	40.6	41	41.5	42.6	44.1	45.6	46.2	46.9	47.2	
Plastic(neem)	40.4	40.5	40.9	41.4	42.1	43.4	44	44.3	44.6	44.9	
Jute+poly(sulphash)	40.4	40.5	40.7	42.1	41.9	42.9	43.5	43.8	44.3	44.5	
Jute(sulphash)	40.4	40.6	41.1	41.5	42.4	44	45.5	46.3	47	47.5	
Plastic(sulphash)	40.4	40.5	40.8	41.4	42.2	43.5	44.2	44.5	45	45.3	

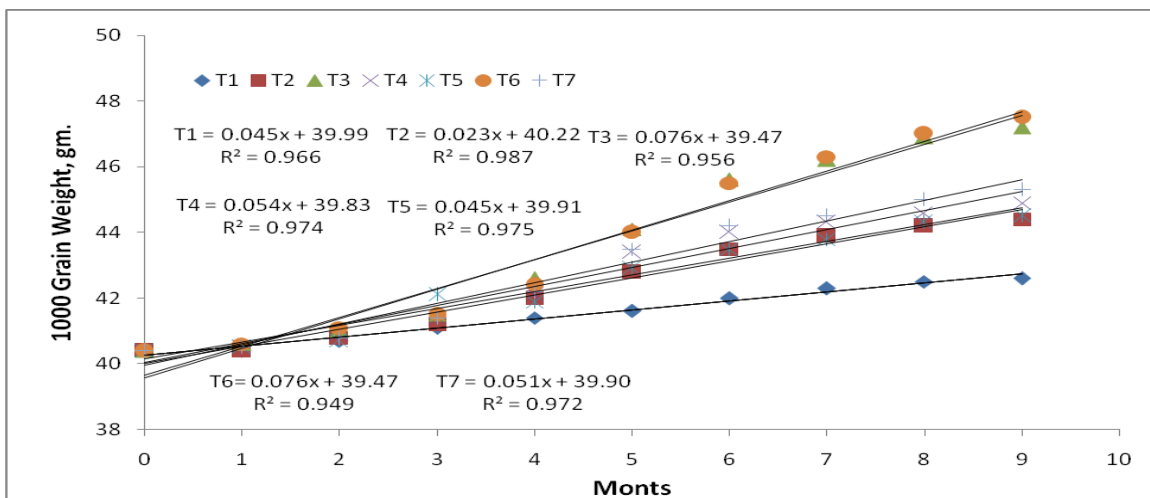


Fig 2.2: Variation in 1000 grain weight with storage duration in experimental samples

2.3 Water activity

The variation between water activity of wheat in hermetic bag kept inside the jute bag, polythene bag kept inside the jute

bag, plastic bag, and jute bag storage with storage period days are shown in Table 2.3 and Figure 2.3.

Table 2.3: Variation in water activity in different bag storage conditions

Storage Treatment	Monthly observations										
	0	1	2	3	4	5	6	7	8	9	
Jute+SGB(No treatment)	0.506	0.511	0.52	0.535	0.541	0.559	0.567	0.576	0.583	0.591	
Jute+poly(neem)	0.506	0.514	0.525	0.542	0.549	0.57	0.581	0.603	0.611	0.627	
Jute(neem)	0.506	0.523	0.531	0.548	0.553	0.582	0.612	0.652	0.668	0.687	
Plastic(neem)	0.506	0.518	0.528	0.545	0.551	0.578	0.59	0.621	0.635	0.656	
Jute+poly(sulphash)	0.506	0.513	0.524	0.544	0.551	0.571	0.583	0.606	0.617	0.63	
Jute(sulphash)	0.506	0.526	0.533	0.547	0.556	0.589	0.615	0.657	0.669	0.696	
Plastic(sulphash)	0.506	0.52	0.529	0.546	0.558	0.581	0.592	0.622	0.637	0.661	

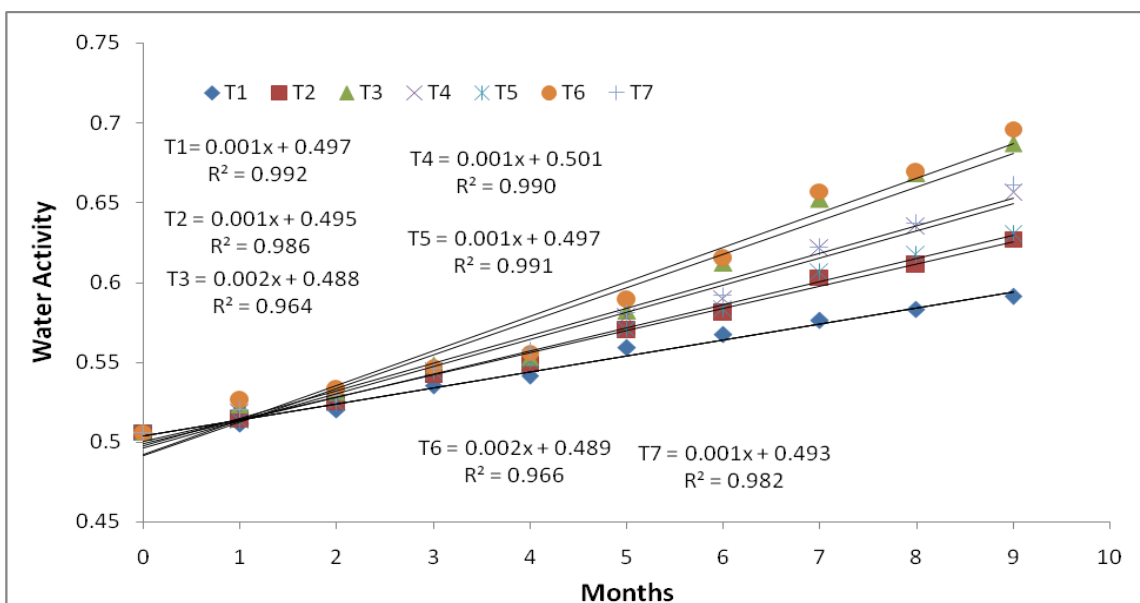


Fig 2.3: Variation in water activity with storage duration in experimental samples

The water activity of the grain was initially at 0.506 for all untreated wheat samples under study. The highest water activity value was 0.688 in case of jute bag while lowest water activity was 0.589 in case of hermetic grain bag which was kept inside the jute bag while storing at room temperature after nine months of storage period. The water activity of untreated wheat seed in the hermetic grain bag kept inside the jute bag increased from 0.506 to 0.591, water activity in the polythene bag storage kept inside the jute bag with neem treatment increased from 0.506 to 0.627, water activity in plastic bag storage with neem treatment increased from 0.506 to 0.656 and water activity in jute bag with neem treatment increased from 0.506 to 0.687. Water activity in the polythene bag storage kept inside the jute bag with Aluminium

phosphide treatment increased from 0.506 to 0.630, water activity in plastic bag storage with Aluminium phosphide treatment increased from 0.506 to 0.661 and water activity in jute bag with Aluminium phosphide treatment increased from 0.506 to 0.696 after nine months of storage period.

The elevation in water activity with storage period may be due to increase in moisture content owing due to increased in relative humidity during storage period.

2.4 Colour index

The colour index of the wheat grain was initially recorded to be 54.88 for all storage treatments. The colour index does not showed an increasing trend showing increase in values of colour index in all the storages modes (Table 2.4 and Figure 2.4).

Table 2.4: Variation of colour index with days in different storage bags for untreated and treated Wheat grain

Storage Treatment	Monthly observations									
	0	1	2	3	4	5	6	7	8	9
Jute+SGB(No treatment)	54.88	55.22	57.75	59.27	58.99	59.08	59.08	58.27	57.65	58.73
Jute+poly(neem)	54.88	55.66	56.44	57.36	57.61	57.75	57.95	58.88	58.9	59.04
Jute(neem)	54.88	55.01	56.69	56.55	56.87	58.22	58.32	61.22	60.30	60.34
Plastic(neem)	54.88	55.21	55.39	57.04	57.22	57.68	58.02	58.34	58.44	59.68
Jute+poly(sulphash)	54.88	55.73	57.06	55.91	55.99	57.24	57.34	57.66	58.07	58.94
Jute(sulphash)	54.88	54.97	55.03	55.62	57.73	58.92	58.97	59.59	59.63	61.86
Plastic(sulphash)	54.88	55.96	56.09	56.82	58.03	58.09	58.10	58.41	59.03	60.50

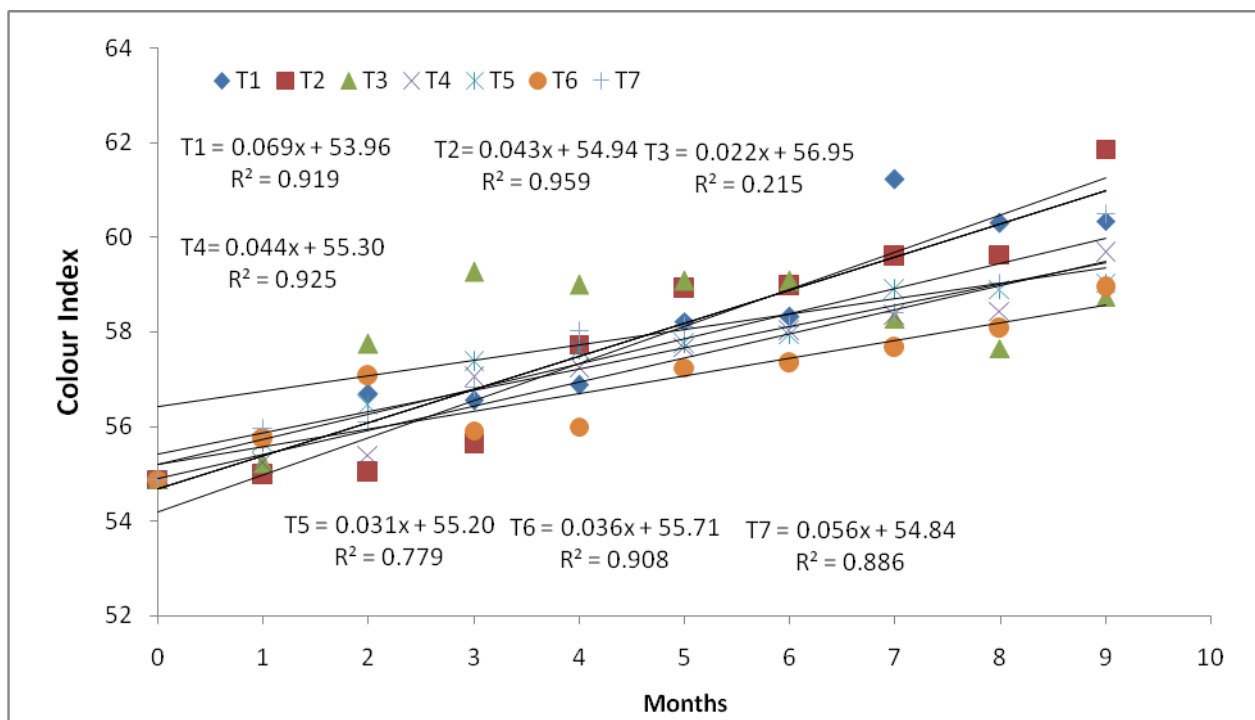


Fig. 2.4: Variation in colour index with storage duration in experimental samples

The total colour index of wheat in the hermetic grain bag kept inside the jute bag changes from 56.10 to 56.55, total colour index in the polythene bag storage kept inside the jute bag with neem treatment changes from 56.10 to 58.73, total colour index in plastic bag storage with neem treatment changes from 56.10 to 55.86 and total colour index in jute bag with neem treatment changes from 56.10 to 56.44. Total colour index in the polythene bag storage kept inside the jute bag with Aluminium phosphide treatment changes from 56.10 to 55.78, total colour index in plastic bag storage with Aluminium phosphide treatment changes from 56.10 to 57.04 and total colour index in jute bag with Aluminium phosphide treatment changes from 56.10 to 55.07, after nine months of storage period.

2.5 Germination percentage

The variation in germination percentage during the storage of the wheat in different storage modes are shown in Table 2.5 and Fig 2.5. In the beginning, the germination of wheat was 92%.

The germination percentage was good during the initial days. In the end, the germination was least in jute bag both treated with neem and Aluminium phosphide. The germination percentage of wheat seed in the hermetic grain bag kept inside the jute bag decreased from 92% to 88%, germination percentage in the polythene bag kept inside the jute bag with neem treatment decreased from 92% to 82%, germination percentage in plastic bag storage with neem treatment decreased from 92% to 80% and germination percentage in

jute bag with neem treatment decreased from 92% to 76%. Germination percentage in the polythene bag kept inside the jute bag with Aluminium phosphide treatment decreased from 92 % to 84%, germination percentage in plastic bag storage

with Aluminium phosphide treatment decreased from 92% to 82% and germination percentage in jute bag with Aluminium phosphide treatment decreased from 92% to 78%.

Table 2.5: Germination percentage of wheat grain in different storage bags.

Storage Treatment	Monthly observations									
	0	1	2	3	4	5	6	7	8	9
Jute+SGB (No treatment)	92	92	92	92	90	90	88	88	88	88
Jute+poly(neem)	92	92	90	90	88	86	84	84	82	82
Jute(neem)	92	90	90	88	84	82	80	78	76	76
Plastic(neem)	92	92	90	88	88	86	84	82	82	80
Jute+poly(sulphash)	92	92	92	90	88	88	86	84	84	84
Jute(sulphash)	92	92	90	88	86	84	82	80	78	78
Plastic(sulphash)	92	92	92	90	88	86	84	82	82	82

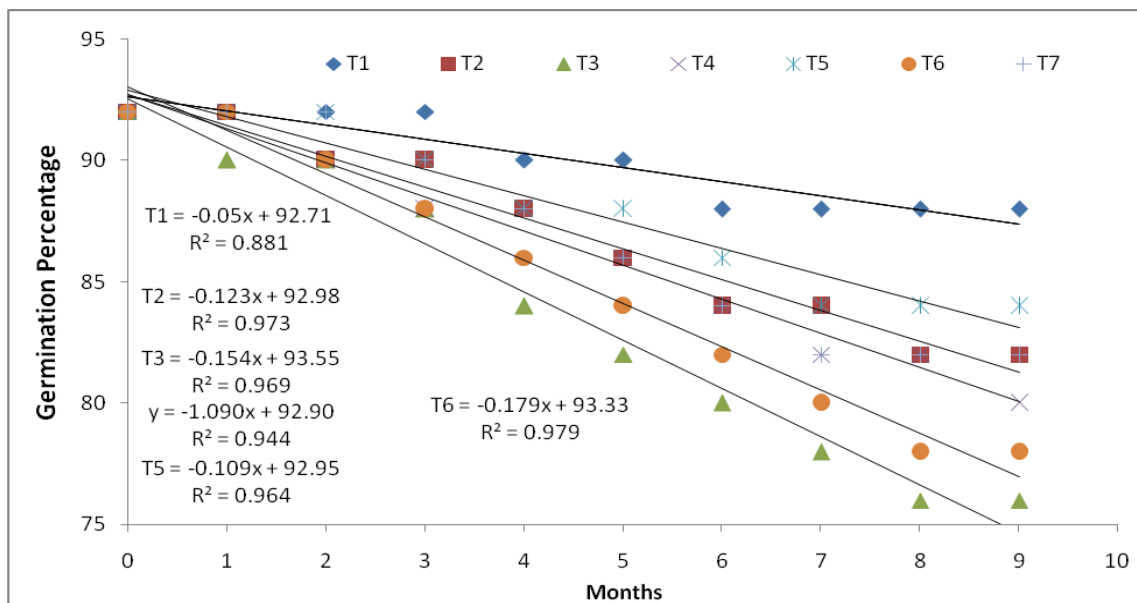


Fig 2.5: Variation germination percentage with storage duration in experimental samples

The variation between water activities of wheat in hermetic bag kept inside the jute bag, polythene bag kept inside the jute bag, plastic bag, and jute bag storage with storage period in nine months are shown in Table 2.5 and Figure 2.5. The elevation in water activity with storage period may be due to increase in moisture content owing due to increased in relative humidity during storage period

Conclusions

All the treatments were kept under ambient conditions. The temperature and relative humidity were recorded on a daily basis while the other dependent parameters were recorded on weekly basis.

The following consideration is drawn from the study:

- The variation in grain moisture content, water activity and germination percent of wheat was least in the hermetic grain bag while maximum in Jute bag.
- Germination percentage of wheat stored was maximum in super grain bag throughout the storage period than other storage modes/treatments.
- The 1000 grain weight of the grain is showed similar trend showed by moisture content.
- The variation in colour index was least in super grain bag showing the minimal effect of ambient condition to the stored wheat grain.
- Wheat can be stored under ambient conditions up to 3 months in hermetic storage bag with minimum qualitative and quantitative loss. There is no requirement of any

chemical treatment of grains stored in hermetic super bags as the micro environment developed is sufficient enough to restrict the growth of pests, insects and micro-organism.

References

1. Agrawal NS. Desing on storage structure. Seminar on post-harvest technology of cereals and pulses. I.N.S.A., I.C.A.R., C.S.I.R. and F.C.X., 21-23 December, New Delhi, 1972.
2. Basavaraja H, Mahajanashetti SB, Udagatti NC. Economic Analysis of post-harvest losses in food grains in india: A case study of Karnatka. Agricultural Economics Research Review 2007; 20(6):117-126.
3. Debashri M, Tamal M. A Review on Efficacy of Azadirachta Indica A. juss Based Biopesticides: An Indian Perspective. Research Journal of Resent Sciences. 2012; 1(3):94-99
4. Gough MC. Physical changes in Large-scale Hermetic Grain Storage. Journal of Agricultural Engineering Research 1985; 31(1):55-65.
5. Groote HD, Kimenju SC, Likhayo P, Kanampiu F, Tefera T, Hellin J. Effectiveness of hermetic systems in controlling maize storage pests in Kenya J Stored prod. Res. 2013; 53:27-36.
6. Grover DK, Singh Jasdev, Singh Satwinder. Assessment of marketable and marketed surplus of major food grains in Punjab. AERC study no. 32. Agro-economic Research

- centre, Department of Economic and sociology, Punjab Agricultural University, Ludhiyana, 2012.
7. Krishnamurthy K. Post-harvest losses in food grains. *Bulletin of Grain Technology* 1975; 13(1):33-49.
 8. Kumar V, Kumari A, Rajak D, Sharma PD, Shrivastava M. Qualitative Loss of Maize Under Different Bag Storage Modes. The First International Congress on Postharvest Loss Prevention, Rome, Italy, 2015, 96-97.
 9. Kumari A, Kumar V, Rajak D, Shrivastava M. Comparative Study on Storage Behavior of Food Grain in Different Storage Bag. A project report submitted to college of agricultural engineering, RAU, PUSA, 2015.
 10. Majumdar SK, Natarajan CP. Some aspects of the problems of bulk storage of food grains in India. *World. Rev. Pest Control* 1963; 2(2):25-35.
 11. Mookherjee PB. *et al.* Studies on incidence and extent of damage due to insect pests in stored seeds-I. Cereal seeds. *Indian J Ent.* 1968; 30:61-65.
 12. Mukherjee PB, Jotwani MG, Sircar P, Yadav TD. Studies on the incidence and extent of damage due to insect pests in stored seeds. *Indian Journal of Entomology.* 1968; 30(1):61-65.
 13. Murdock LL, Baributsa D, De Boer JL. Hermetic Storage of Grain in Developing Nations. *Journal of stored products research* 2014; 58:1-2.
 14. Nanda SK, Vishwakarma RK, Bathla HVL, Rai Anil, Chandra P. Harvest and post-harvest losses of major crops and live stock produce in India. A report published by All India Coordinated Research Project on Post-Harvest Technology (ICAR), 2012.
 15. Sawant SD. Modern grain storage for reducing storage losses. *Agricultural Engineering Today* 1994; 4(1):12-20.