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Studies on packaging materials, storage duration on seed viability and seed health in soybean (*Glycine max* L.) seeds

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Abstract

The present study elucidated that the effect of storage of soybean seeds treated with biocide [Castor oil (T₁) @ 5ml/kg and Neem cake (T₂) @ 10g/kg] and stored in types of packaging materials [vacuum polythene bag (P₁), Non-vacuum polythene bag (P₂) and Cloth Bag (P₃)] in ambient condition in the Post Graduate Laboratory, Department of Genetics and Plant Breeding, SHIATS, Allahabad for two, four and six months. Observations shows that the treatment of Neem cake @ 10g/kg of seed stored in vacuum plastic bag for 6 months displayed significant higher percentage of seed germination, seedling length, Fresh weight, Dry weight, seed moisture and Viability as compared to other treated seeds that were stored in vacuum plastic bag. But in the case of seed health Castor oil @ 5ml/kg treated seed shows better result. Germination and viability decreased with the period of storage. It is also found that vacuum polythene bag could be the best options among Non-vacuum polythene bag, Non vacuum polythene bag and Cloth bag for seed packing in terms of maintaining the seed quality as reflected in the varied parameters of the seed quality assessment indicators. Seeds are stored in vacuum polythene bags were affected due to storage but the effects were more pronounced in the Non-vacuum polythene bags as compared to Cloth bags.

Keywords: soybean seed, botanicals treatment, seed quality, storage container, storage period

Introduction

Soybean (*Glycine max* L.) is a leguminous vegetable of the Fabaceae family that grows in tropical, subtropical, and temperate climates. Soybean is an important crop with protein content of 40% protein, 32% carbohydrate, 20% unsaturated fat, 5% minerals with 3% fiber, and other trace substances. The oil contains about 0.5-1.0 per cent lecithin which is essential for building up of human nerve tissues. Soybean oil is the largest component of the world's edible oils. The world production of edible oils consists of 30% soybean (Assefa, 2008). The three countries dominate global production, accounting for 80% of the world (PPPIAD, 2015) [7]. The kind of packaging material to be used is dependent upon several inter-related factors such as kind and quantity of seed handled, type and size of packs, intended storage duration, storage environment and the geographical location of storage (Justice and Bass, 1978) [2].

One of the most reliable technique is the tetrazolium test, often referred to as a "quick test". The tetrazolium staining is an established method of testing seed viability, and most commonly practiced biochemical staining method. The TZ test remains as one of the seed industry's most rapid and useful methods to assess seed quality (Aslam *et al.*, 2010) [3].

During the storage seeds or grains that might be infected by fungi which cause the deteriorate its viability, discoloration, various biochemical changes, heating and mustiness, loss in weight, and production of toxins when it is consumed by human and animals may injurious to their health (Arya and Perello, 2010) [4].

the present research work, therefore, was undertaken with a view to determine suitable packaging material and best biocides treatment for maintaining longevity of Soybean seed quality under ambient and control conditions of Allahabad.

Materials and Methods

The soybean seed were treated with biocides [castor oil (T₁) and Neem Cake (T₂)] at recommended dosage. It were packed in Vacuum polythene bag (P₁), Non-vacuum polythene bag (P₂) and Cloth bag (P₃) and stored under ambient condition in the Post Graduate Laboratory, Department of Genetics and Plant Breeding, SHIATS, Allahabad for 2, 4 and 6th Months.

For seedling character, the germination test was conducted during four replications of 100 seeds from each sample in rolled towel paper as per procedure described by ISTA (1993) The following formulas were used in this experiment for determining seedling characters:

$$\text{Germination \%} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Seedling length} = \frac{\sum \frac{SL1}{F1} \frac{SL2}{F2}}{2}$$

Seed moisture content (ISTA 1999) ^[6]: In a set of 4 replicates seed sample of each treatment, the seed moisture content was determined by electric seed moisture meter method. It consists of a compression unit to compress the sample to pre-determined thickness. The thickness setting is very easily read on a vertical and circular scale. The seed material on test is taken in a test cup and is compressed. Then press the push type switch till the reading comes in the display. Here no temperature reading and correlated dial are required. The computer version of digital moisture meter automatically compensate for temperature corrections

Viability Test (TZ test): The tetrazolium test was performed according to the procedure devised by International Seed Testing Association. 200 seeds from each seed lot were used for this test in four replications of 50 seeds each. The seeds were soaked in distilled water for 24 hours before staining to allow complete hydration of all the tissues. This test based on the reaction of 2,3,5-triphenyl tetrazolium chloride (bromide) salt with dehydrogenase enzyme, active during respiration in living tissues of the seed and the hydrogen ions so released, reduce tetrazolium chloride to a red stable substance, resulting in the coloring of the tissues. Thus, it makes possible to distinguish the red colored living tissues of the seed from the colorless dead ones

Health test of stored Soybean seeds (Blotter method): One hundred seeds will be tested for each variety maintaining four replications. Twenty-five seeds were placed on three layers of moist blotting paper (Whatman No.1) in each glass Petridishes. The Petridishes will be incubated at 25±1°C under 12/12 hrs light and darkness cycle for 7 days. Each seed will be observed under stereomicroscope in order to record the presence of fungal colony and bacterial ooze 7 days after incubation based on growth habit. In doubtful cases temporary slides will be prepared from the fungal colony temporary slides were prepared from the fungal colony.

Results and Discussion

The germination percentage of soybean seeds declined progressively with the enhanced storage period. On an average the germination percentage recorded at the beginning and at the end of storage period was 81.86 and 69.33 percent, respectively.

Significant differences in germination percentage due to seed treatments were observed throughout storage period except initial month. The Treatment in combination with biocide or alone recorded significantly higher germination percentage compared to untreated seeds. Significantly higher germination was recorded with seeds treated with Neem Cake @ 10 g per

kg of seed along with Castor Oil @ 5 ml per kg of seed at the end of storage period (T₂) 73.33%, followed by (T₁) 72.42%. Significantly lower seed germination was recorded throughout the storage period with untreated control (T₀) which recorded germination percentage of 62.25 at the end of 6th month of storage.

The seeds stored in vacuum polythene bag recorded significantly higher germination percentage over Non vacuum polythene bag and cloth bag throughout the storage period. The germination percentage recorded with Vacuum polythene bag, Non vacuum polythene bag and cloth bag, at the end of 6th month of storage was 71.2%, 69.50% and 67.25%, respectively.

Interaction effects due to packaging materials and seed treatments were significant throughout the storage period except at initial month of storage. Significantly higher germination percentage was recorded in (P₁T₂)75.25 and lower germination percentage was noticed in (P₃T₁)70 percent at the end of 6th month of storage period.

This indicates that along with Neem cake and Castor oil also helpful in maintaining seed quality. The better seed quality parameters observed with chemical and botanicals treatment may be because of lower insect infestation noticed with these treatments. The insects not only eat the storage food in the seed, but also eat the germ, leading to death of the seed and hence result in poor seed germination and lower vigour (Deshpande *et al.*, 2004) ^[5].

The percentage of germination declined slowly increased storage period even the presence of storage fungi and insect pests and chemicals acted as antioxidants to counteract the release of free radicals during the storage period Vacuum polythene bag deteriorates, although slowly on direct exposure to storage sunlight or ultraviolet radiation. Similar results were reported by Tripathi, P. C. and Lawande, K.E. (2014) ^[9] also observed highly effect and difference for treatment, container and storage period for germination percentage, castor oil forms a thin film over seeds and due to this processes like respiration and other metabolic activities gets inhibited which effects on seed germination process.

The germinated seedling of soybean seeds declined progressively with the enhanced storage period. On an average the seedling length recorded at the beginning and at the end of storage period was 19.84 cm and 14.98 cm, respectively.

Significant differences in germinated seedling length due to seed treatments were observed throughout storage period except initial month. The Treatment in combination with biocide or alone recorded significantly higher seedling length compared to untreated seeds. Significantly higher seedling length was recorded with seeds treated with Neem Cake @ 10 g per kg of seed along with Castor Oil @ 5 ml per kg of seed at the end of storage period (T₂) 16.17, followed by (T₁) 15.62 cm. Significantly lower seedling length was recorded throughout the storage period with untreated control (T₀) which recorded a seedling length of 13.16 cm at the end of 6th month of storage.

The seeds stored in vacuum polythene bag, recorded significantly higher germination percentage over Non vacuum polythene bag and cloth bag throughout the storage period. The germinated seedling length recorded with Vacuum polythene bag, Non vacuum polythene bag and cloth bag at the end of 6th month of storage was 15.78cm, 15.48cm and 13.69cm, respectively.

Interaction effects due to packaging materials and seed treatments were significant throughout the storage period

except at initial month of storage. Significantly higher seedling length was recorded in (P₁T₂)17.19 cm and minimum seedling length was noticed in (P₃T₁)13.47 cm. at the end of 6th month of storage period.

The moisture content of soybean seeds increase progressively with the enhanced storage period. On an average the moisture content recorded at the beginning and at the end of storage period was 8.9% and 11.1%, respectively.

Significant differences in moisture content of seeds due to seed treatments were observed throughout storage period except initial month. The Treatment in combination with biocide or alone recorded significantly lower moisture content of seeds compared to untreated seeds. Significantly higher moisture content of seeds was recorded with seeds treated with Neem Cake @ 10g per kg of seed along with Castor Oil @ 5ml per kg of seed at the end of storage period (T₂) 11.16 %, followed by (T₁) 11 %. Significantly maximum moisture content was recorded throughout the storage period with untreated control (T₀) which recorded a moisture content of 11.28%, at the end of 6th month of storage.

The seeds stored in vacuum polythene bag recorded significantly lower moisture content over Non vacuum polythene bag and cloth bag throughout the storage period. The moisture content recorded with Vacuum polythene bag, Non vacuum polythene bag and cloth bag, at the end of 6th month of storage was 10.85 %, 11.15% and 11.44%, respectively.

Interaction effects due to packaging materials and seed treatments were significant throughout the storage period except at initial month of storage. Significantly minimum moisture content was recorded in (P₁T₁)10.65% and maximum moisture content was noticed in (P₃T₂)11.50% at the end of 6th month of storage period.

The seed viability% of soybean seeds declined progressively with the enhanced storage period. On an average the viability percentage recorded at the beginning and at the end of storage period was 83.31 and 74.67 percent, respectively.

Significant differences in seed viability percentage due to seed treatments were observed throughout storage period except initial month. The Treatment in combination with biocide or alone recorded significantly higher viability percentage compared to untreated seeds. Significantly higher seed viability was recorded with seeds treated with Neem Cake @ 10g per kg of seed along with Castor Oil @ 5ml per kg of seed at the end of storage period (T₂) 77.67%, followed by (T₁) 76.33%. Significantly lower seed viability was recorded throughout the storage period with untreated control (T₀) which recorded a seed viability percentage of 70 at the end of 6th month of storage.

The seeds stored in vacuum polythene bag recorded significantly higher seed viability percentage over Non vacuum polythene bag and cloth bag throughout the storage

period. The viability percentage recorded with Vacuum polythene bag, Non vacuum polythene bag and cloth bag, at the end of 6th month of storage was 76.50%, 75.17% and 72.33%, respectively.

Interaction effects due to packaging materials and seed treatments were significant throughout the storage period except at initial month of storage. Significantly higher viability percentage was recorded in (P₁T₂)80% and minimum viability percentage was noticed in (P₃T₁)73 percent at the end of 6th month of storage period.

Irrespective of packaging material along with fungicide, the mean percent seed born fungi increased from 11.57 percent at initial to 9.58 per cent at the end of storage period. (Fusarium-10.50 to 12.50, Aspergillus-9.92 to 11.92, Cercospora-9.22 to 11.22 and Alternaria-8.69 to 10.67)%. Significantly minimum seed born fungi of 10.71 per cent was recorded in T₁ followed by T₂ 11.23% as compared to untreated control 12.79%, which at the end of 6th month of storage.

The seeds stored in vacuum polythene bag recorded significantly minimum seed born fungi percentage over Non vacuum polythene bag and cloth bag throughout the storage period. The seed born fungi percentage recorded with Vacuum polythene bag, Non vacuum polythene bag and cloth bag, at the end of 6th month of storage was 10.81% (Fusarium-11.75, Aspergillus-11, Cercospora-10.42, Alternaria-10.08), 11.40% (Fusarium-12.33, Aspergillus-11.75, Cercospora-11, Alternaria-10.50) and 12.52% (Fusarium-13.42, Aspergillus-13, Cercospora-12.25, Alternaria-11.42)% respectively.

Interaction effects due to packaging materials and seed treatments were significant throughout the storage period except at initial month of storage. Significantly minimum seed born fungi percentage was recorded in (P₁T₁) 9.94percent (Fusarium-10.50, Aspergillus-10, Cercospora-9.75, Alternaria-9.50) and maximum seed born fungi percentage was noticed in (P₃T₂)12.13 percent (Fusarium-13, Aspergillus-12.75, Cercospora-12, Alternaria-10.75) at the end of 6th month of storage period.

Theoretically, no oxidative processes should be supported under vacuum. To recall some facts, the term vacuum is defined as space without matter in it. Vacuum packaging literally vacuums the air out of the bags or storage containers and so slows down the process of deterioration. A perfect vacuum has never been accomplished. The best man made vacuum had less than 100,000 gas molecules per cc, compared to about 30 billion molecules for air at sea level. It has also been reviewed that it is not possible to evacuate all the air ~ 0.3 – 3% (may remain after sealing) by way of vacuum packing. Oxygen supports the growth of aerobic microorganisms; thus, the removal of oxygen from the modified atmosphere will extend the microbial shelf life (Sanjeev and Ramesh, 2006)^[8].

Table 1: Effect of Packaging materials and seed treatment on the seed germination percentage and seedling length of soybean seeds during storage

Treatments	Germination %				Seedling length			
	Initial reading	2 nd month	4 th month	6 th month	Initial reading	2 nd month	4 th month	6 th month
Packaging materials (P)								
Vacuum (P ₁)	82.92	80.25	76.58	71.25	22.19	20.82	18.93	15.78
Non Vacuum (P ₂)	81.50	79.00	75.17	69.50	19.44	18.58	16.29	15.48
Cloth bag (P ₃)	80.58	75.67	72.33	67.25	17.90	16.61	15.04	13.69
SEm	0.41	0.50	0.57	0.54	0.06	0.07	0.09	0.05
CD @5%	1.19	1.46	1.65	1.57	0.16	0.19	0.27	0.14
F-test	**	**	**	**	**	**	**	**
Seed treatment (T)								

Control (T ₀)	78.83	73.50	70.08	62.25	16.20	15.88	14.42	13.16
Castor Oil (T ₁)	82.75	80.50	76.33	72.42	20.59	18.96	17.04	15.62
Neem cake(T ₂)	83.42	80.92	77.67	73.33	22.75	21.17	18.80	16.17
SEm	0.41	0.57	0.57	0.54	0.06	0.07	0.09	0.05
CD @5%	1.19	1.46	1.65	1.57	0.16	0.19	0.27	0.14
F-test	**	**	**	**	**	**	**	**
Interactions (P×T)								
P ₁ T ₀	79.25	75.00	70.50	64.50	16.37	16.06	14.76	13.31
P ₁ T ₁	84.00	82.75	79.25	74.00	24.00	21.67	19.74	16.85
P ₁ T ₂	85.50	83.00	80.00	75.25	26.20	24.72	22.30	17.19
P ₂ T ₀	78.75	73.50	70.00	61.50	16.20	16.00	14.43	13.18
P ₂ T ₁	82.75	81.50	76.75	73.25	20.19	18.89	16.82	16.55
P ₂ T ₂	83.00	82.00	78.75	73.75	21.94	20.84	17.63	16.71
P ₃ T ₀	78.50	72.00	69.75	60.75	16.03	15.57	14.08	13.00
P ₃ T ₁	81.50	77.25	73.00	70.00	17.58	16.33	14.56	13.47
P ₃ T ₂	81.75	77.75	74.25	71.00	20.10	17.94	16.48	14.60
Grand mean	81.67	78.31	74.69	69.33	19.84	18.67	16.75	14.98
SEm	0.71	0.87	0.99	0.94	0.10	0.11	0.16	0.08
CD @5%	2.05	2.53	2.86	2.72	0.28	0.33	0.46	0.24
F-test	NS	NS	NS	NS	**	**	**	**

* = 5% level of significance; ** = 1% level of significance respectively; ns = non-significant

Table 2: Effect of Packaging materials and seed treatment on the seed moisture percentage and seed viability of soybean seeds during storage

Treatments	Seed moisture				Seed viability			
	Initial reading	2 nd month	4 th month	6 th month	Initial reading	2 nd month	4 th month	6 th month
Packaging materials (P)								
Vacuum (P ₁)	8.66	9.75	10.08	10.85	84.33	81.58	78.58	76.50
Non Vacuum (P ₂)	8.82	10.03	10.42	11.15	83.25	80.92	77.50	75.17
Cloth bag (P ₃)	9.23	10.53	11.24	11.44	82.33	79.00	76.25	72.33
SEm	0.036	0.052	0.048	0.048	0.38	0.41	0.54	0.56
CD @5%	0.105	0.150	0.141	0.138	1.09	1.19	1.56	1.63
F-test	**	**	**	**	**	**	*	**
Seed treatment (T)								
Control (T ₀)	8.30	10.52	10.44	11.28	81.67	77.50	74.00	70.00
Castor Oil (T ₁)	9.07	9.74	10.40	11.00	83.83	81.50	78.75	76.33
Neem cake(T ₂)	9.33	10.06	10.89	11.16	84.42	82.50	79.58	77.67
SEm	0.036	0.052	0.048	0.048	0.38	0.41	0.54	0.56
CD @5%	0.105	0.150	0.141	0.138	1.09	1.19	1.56	1.63
F-test	**	**	**	**	**	**	**	**
Interactions (P×T)								
P ₁ T ₀	8.13	10.38	10.30	11.15	82.00	78.25	75.00	70.25
P ₁ T ₁	8.85	9.33	9.88	10.65	85.00	82.75	80.00	79.25
P ₁ T ₂	9.00	9.55	10.05	10.75	86.00	83.75	80.75	80.00
P ₂ T ₀	8.28	10.43	10.38	11.25	81.75	78.00	74.25	70.00
P ₂ T ₁	8.98	9.55	10.13	10.98	83.75	81.75	78.50	76.75
P ₂ T ₂	9.20	10.13	10.75	11.23	84.25	83.00	79.75	78.75
P ₃ T ₀	8.50	10.75	10.65	11.45	81.25	76.25	72.75	69.75
P ₃ T ₁	9.38	10.35	11.20	11.38	82.75	80.00	77.75	73.00
P ₃ T ₂	9.80	10.50	11.88	11.50	83.00	80.75	78.25	74.25
Grand mean	8.9	10.1	10.6	11.1	83.31	80.50	77.44	74.67
SEm	0.062	0.090	0.084	0.082	0.65	0.71	0.93	0.97
CD @5%	0.181	0.260	0.243	0.239	1.89	2.06	2.70	2.83
F-test	*	**	**	NS	NS	NS	NS	*

Table 3: Effect of Packaging materials and seed treatment on seed health of soybean seeds during storage period

Treatment	<i>Fusarium</i>		<i>Aspergillus</i>		<i>Cercospora</i>		<i>Alternaria</i>	
	Initial	6 th month	Initial	6 th month	Initial	6 th month	Initial	6 th month
Packaging Material (P)								
Vacuum P ₁	9.75	11.75	9.00	11.00	8.42	10.42	8.08	10.08
Nonvacuum P ₂	10.33	12.33	9.75	11.75	9.00	11.00	8.58	10.50
Cloth bag P ₃	11.42	13.42	11.00	13.00	10.25	12.25	9.42	11.42
SEm	0.515	0.515	0.241	0.241	0.329	0.329	0.300	0.317
CD@5%	1.495	1.495	0.698	0.698	0.954	0.954	0.872	0.919
F-test	NS	NS	**	**	**	**	*	*
Seed Treatment (T)								
Control (T ₀)	12.42	14.42	11.25	13.25	10.17	12.17	9.42	11.33
Castor oil (T ₁)	9.25	11.25	9.00	11.00	8.50	10.50	8.08	10.08
Neemcake(T ₂)	9.83	11.83	9.50	11.50	9.00	11.00	8.58	10.58

SEm	0.515	0.515	0.241	0.241	0.329	0.329	0.300	0.317
CD@5%	1.495	1.495	0.698	0.698	0.954	0.954	0.872	0.919
F-test	**	**	**	**	**	**	*	*
Interactions (P×T)								
P ₁ T ₀	12.00	14.00	10.75	12.75	9.50	11.50	9.00	10.80
P ₁ T ₁	8.50	10.50	8.00	10.00	7.75	9.75	7.50	9.50
P ₁ T ₂	8.75	10.75	8.25	10.25	8.00	10.00	7.75	9.75
P ₂ T ₀	12.25	14.25	11.00	13.00	9.75	11.75	9.25	11.00
P ₂ T ₁	9.00	11.00	8.75	10.75	8.25	10.25	8.00	10.00
P ₂ T ₂	9.75	11.75	9.50	11.50	9.00	11.00	8.50	10.50
P ₃ T ₀	13.00	15.00	12.00	14.00	11.25	13.25	10.00	12.00
P ₃ T ₁	10.25	12.25	10.25	12.25	9.50	11.50	8.75	11.50
P ₃ T ₂	11.00	13.00	10.75	12.75	10.00	12.00	9.50	10.75
Grand Mean	10.50	12.50	9.92	11.92	9.22	11.22	8.69	10.67
SEm	0.892	0.892	0.417	0.417	0.569	0.569	0.520	0.549
CD@5%	2.589	2.589	1.209	1.209	1.652	1.652	1.510	1.592
F-test	NS							

Conclusion

It is concluded from the present study that seed were treated with neem cake and stored in vacuum polythene bag shown significant results for seedling characters followed by germination%, fresh weight, dry weight, seedling length, viability even after 6th months of storage period. But in the case of seed moisture and seed health castor oil is best for control of storage fungi.

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