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Impact of storage conditions on seed-borne mycoflora and seed health parameters of soybean [*Glycine max* (L.) Merrill]

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Abstract

The study was conducted to investigate the changes in soybean seed health parameters that may occur during storage in different types of commonly used containers i.e., polythene bag, cloth bag and metallic bin. Seeds of two soybean varieties *viz*., Shivalik and Hara Soya were stored in these containers during December, 2022 to June, 2023, in the Department of Plant Pathology, CSKHPKV, Palampur. Lowest incidence of mycoflora as well as highest germination percentage, seedling shoot length, root length and seedling vigour index were obtained from sterilized seeds of Hara Soya stored in polythene bags for two months from fungicide Tebuconazole 1ml/L sprayed plots while highest mycoflora along with lowest germination percentage, seedling shoot length, root length and seedling vigour index were recorded in seeds of variety Shivalik stored in cloth bags for 6 months from unsprayed plots.

Key words: Soybean, seedling vigour index, storage, storage period, storage container

Soybean [*Glycine max* (L.) Merrill] is an important source of oil and protein. Earlier it was known as a pulse crop but due to high oil content soybean has now been placed in oilseed crop. Due to presence of high protein content, soybean is also known as 'Poor man's meat'. Lack of high seed vigour at the time of sowing is one of the main obstacles to soybean cultivation as it cannot remain viable for longer periods of time (Priestley *et al*. 1985). The genotype as well as storage conditions, particularly length of time, have an impact on the lifespan of seeds affecting seed viability (Shelar *et al*. 2008). Hamman *et al*. (1996) found that seed-borne infections had a negative impact on germination percentage and resulted in death of seeds, malformed seedlings and damaged seed coats. The seed moisture content, storage period, prevailing temperature and degree of invasion influence the development of seed borne diseases (Sharma *et al.* 2015). To preserve the seeds quality for a longer period of time, it is preferable to keep them in moisture and vapour-proof containers like polythene bags, aluminium foil, tins or any other sealed containers (Raikar *et al*. 2008). The objective of this research was to determine the effects of storage period and different types of storage containers on seed mycoflora and germination parameters of different soybean cultivars.

Materials and Methods

Collection of seed sample

To obtain seed samples of soybean, seeds of different varieties *viz*., Shivalik and Hara Soya were multiplied during the month of June 2021 at the experimental farm of the Department of Plant Pathology, CSK HPKV, Palampur. To assess the impact of fungicide on seed health, one spray of tebuconazole (1ml/L) was applied to various soybean cultivars sown in the field at pod initiation stage. At the time of harvest, seed samples were collected and labelled with the variety name, sample type (sprayed or unsprayed) and harvesting time. These samples were brought to the laboratory of the department where they were kept in different storage containers including metallic bins, polythene and cloth bags at room temperature until further investigations. The observations were recorded on the frequency of mycoflora and seed health parameters at an interval of 60 days. Seed samples were taken randomly from each storage container and were tested at the end of 2, 4 and

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6 months of storage period. Quality of soybean seeds was evaluated by determining seed germination percentage, root length, shoot length and seedling vigour index through blotter paper method as mentioned below:

Blotter paper method

In blotter paper method, three pieces of filter paper were soaked in sterilized distilled water and placed at the bottom of 9cm dia. Petri dish. Four hundred seeds of soybean from each sample of seed lot were used in this method. Sterilization of seeds was done by immersing seeds in sodium hypochlorite solution (1%) for 3 min. followed by three subsequent washings with distilled water. Seeds were then placed on the moist filter paper at the rate of 10 seeds per Petri dish. The Petri dishes were then incubated at 25+1°C for seven days and data pertaining to frequency of mycoflora, seed germination percentage, root and shoot lengths were recorded from random seedling samples. Fungal frequency and germination percentage and seedling vigour index were calculated by using formula as given below:

Fungal frequency

Fungal colonies were calculated and accessed by using formula:

$$\text{Total fungal colonies (\%)} = \frac{\text{No. of seeds colonized in each plate by a particular species}}{\text{Total no. of seeds in each plate}} \times 100$$

Germination percentage

$$\text{Seed germination \%} = \frac{\text{Germinated seed}}{\text{Total Seed}} \times 100$$

Seedling vigour index

Vigour index was calculated by the following formula given by Baki and Anderson (1973):

Vigour index = % seed germination x (mean root length + mean shoot length)

Results and Discussion

Effect of storage conditions on seed mycoflora

Data on detection of associated mycoflora in soybean seeds stored in separate containers for different durations is given in Table 1. Perusal of the data revealed that frequency of fungal species *viz*., *A. niger, A. flavus, Rhizopus* sp., *Penicillium* sp., *Trichoderma* sp., *Cladosporium* sp. increased as the storage period increased from 2 to 6 months while reduction in the frequency of *A. alternata, C. truncatum, Curvularia* sp., *F. proliferatum, F. equiseti, Phoma* sp. and *Pestalotiopsis* sp. was observed at the end of six months of storage period.

After two months of storage maximum frequency of fungal species was recorded in unsterilized seeds stored in cloth bags obtained from unsprayed plots of variety Shivalik (Table 1), wherein maximum frequency was of *A. niger* (35.75%) followed by *A. alternata* (30.25%) and *C. truncatum* (28.00%). However, minimum prevalence of mycoflora was in seeds stored in polythene bags obtained from fungicide sprayed plot of variety Hara Soya where, predominantly occurring seed mycoflora was *A. niger* (12.75%) followed by *A. alternata* (8.00%). After six months of storage period, maximum frequency of fungal species was recorded in seeds stored in cloth bags obtained from unsprayed plot of variety Shivalik (Table 1). Fungal frequency was highest for *A. niger* (38.75%) followed by *Cladosporium* sp. (36.25%) and *A. alternata* (25.75%). However, minimum prevalence of mycoflora was in sterilized seeds stored in polythene bags obtained from fungicide sprayed plot of variety Hara Soya (Table 1), where predominantly occurring seed mycoflora was *A. niger* (22.00%) followed by *A. alternata* (5.75%). Many other workers have also reported that as storage period advanced, field frequency of fungi *viz., A. alternata, Cladosporium* sp., *Fusarium* sp. declined whereas storage fungi *viz., A. niger, A. flavus, Penicillium* sp. and *Rhizopus* sp., increased up to 6 to 12 months afterwards their population also declined (Srivastava and Gupta 1980; Dwivedi and Shukla 1990; Lambat *et al*. 2015). Lambat *et al*. (2017) also reported that increase in the number of infected seeds was due to increase in mycoflora. They also advocated storage of seeds in polyethylene bag to preserve greater germinability and lesser seed invasion by the fungal flora during storage.

Effect on seed health parameters

Interaction between soybean cultivars and storage periods

The results (Table 2) clearly indicated that highest germination percentage, root length, shoot length and seedling vigour index were recorded after two months of storage of seeds of Hara Soya cultivar obtained from fungicide sprayed plots followed by four months of storage in the same seed sample. The lowest final germination percentage, root length, shoot length and

Table 1. Effect of storage container and storage duration on seed mycoflora of soybean varieties by blotter paper method

Sample	Storage Container	Storage Period (months)	*Aspergillus niger*	*Aspergillus flavus*	*Rhizopus* sp.	*Alternaria alternata*	*Penicillium* sp.	*Colletotrichum truncatum*	*Trichoderma* sp.	*Curvularia* sp.	*Fusarium proliferatum*	*Fusarium equiseti*	*Phoma* sp.	*Cladosporium* sp.	*Pestalotiopsis* sp.
Shivalik (seeds obtained from sprayed plot)	Metallic bin	2	18.75	5.50	0.00	24.00	6.75	17.50	0.25	0.00	0.00	0.00	13.00	8.00	0.00
		4	26.50	7.75	0.00	10.25	8.00	15.75	0.25	0.00	0.00	0.00	11.25	10.25	0.00
		6	30.75	9.00	0.00	6.25	16.75	12.00	0.75	0.00	0.00	0.00	9.75	13.00	0.00
	Polythene bag	2	13.00	4.25	0.00	16.25	4.25	13.75	0.00	0.00	0.00	0.00	10.75	6.75	0.00
		4	18.75	7.50	0.00	8.00	6.00	9.00	0.00	0.00	0.00	0.00	7.00	10.00	0.00
		6	23.25	10.75	0.00	4.25	8.50	6.25	0.00	0.00	0.00	0.00	2.50	12.75	0.00
	Cloth bag	2	24.50	7.50	0.00	34.00	8.50	24.00	6.75	8.75	7.25	0.00	18.50	10.25	12.00
		4	28.75	15.25	0.00	24.75	13.75	20.75	8.00	5.50	0.00	0.00	15.75	13.00	8.75
		6	35.00	18.75	0.00	22.50	16.00	14.00	13.25	0.00	0.00	0.00	14.25	17.25	6.50
Shivalik (seeds obtained from unsprayed plot)	Metallic bin	2	24.50	8.25	0.00	32.00	14.50	26.75	4.00	6.75	0.00	6.00	18.25	17.00	0.00
		4	27.75	13.75	0.00	28.25	16.00	24.25	7.25	0.00	0.00	0.00	15.50	20.75	0.00
		6	36.50	11.00	0.00	25.50	18.50	23.50	11.75	0.00	0.00	0.00	12.75	27.50	0.00
	Polythene bag	2	14.50	4.25	0.00	27.00	8.75	24.25	2.00	0.00	7.75	0.00	18.25	15.00	0.00
		4	26.25	6.75	0.00	24.25	11.00	17.50	5.00	0.00	0.00	0.00	15.75	17.50	0.00
		6	35.75	9.00	0.00	20.75	13.50	15.75	7.75	0.00	0.00	0.00	12.00	23.25	0.00
	Cloth bag	2	23.75	11.75	15.00	35.00	15.00	28.25	13.00	17.25	14.75	10.00	28.00	32.50	18.50
		4	28.50	13.25	17.00	32.25	17.25	25.50	15.25	14.00	12.50	8.50	26.50	33.00	15.50
		6	38.75	21.50	20.50	25.75	20.50	18.00	17.00	12.75	9.75	6.75	23.25	36.25	12.25
Hara Soya (seeds obtained from sprayed plot)	Metallic bin	2	12.75	0.00	0.00	14.00	2.50	15.75	0.25	0.00	0.00	0.00	5.50	4.00	0.00
		4	16.00	0.00	0.00	12.75	5.75	12.00	0.00	0.00	0.00	0.00	3.75	6.75	0.00
		6	25.75	0.00	0.00	8.25	8.75	8.75	0.00	0.00	0.00	0.00	0.00	9.50	0.00
	Polythene bag	2	12.75	0.00	0.00	8.00	0.00	6.75	0.00	0.00	0.00	0.00	4.75	0.00	0.00
		4	18.75	0.00	0.00	7.00	0.00	2.75	0.00	0.00	0.00	0.00	3.50	0.00	0.00
		6	22.00	0.00	0.00	5.75	0.25	0.50	0.00	0.00	0.00	0.00	0.00	4.00	0.00
	Cloth bag	2	32.50	2.75	0.00	24.00	4.50	18.75	0.00	0.00	0.00	0.00	7.25	5.25	0.00
		4	35.75	6.25	0.00	21.50	7.25	14.50	0.00	0.00	0.00	0.00	4.50	8.00	0.00
		6	37.50	8.00	0.00	18.75	10.75	10.00	0.00	0.00	0.00	0.00	2.75	12.50	0.00
Hara Soya (seeds obtained from unsprayed plot)	Metallic bin	2	15.00	0.00	0.00	18.75	5.75	16.25	0.00	0.00	0.00	0.00	11.00	12.25	0.00
		4	24.25	0.00	0.00	15.75	7.75	14.50	0.00	0.00	0.00	0.00	10.00	15.50	0.00
		6	27.25	0.00	0.00	12.00	10.25	11.75	0.00	0.00	0.00	0.00	8.25	23.75	0.00
	Polythene bag	2	10.50	0.00	0.00	16.75	0.00	12.75	0.00	0.00	2.50	0.00	7.25	8.50	0.00
		4	15.25	0.00	0.00	12.00	0.00	10.25	0.00	0.00	0.00	0.00	5.00	12.75	0.00
		6	18.75	0.00	0.00	10.25	6.50	7.50	0.00	0.00	0.00	0.00	3.25	15.00	0.00
	Cloth bag	2	16.50	6.75	0.00	28.00	8.50	18.25	8.50	12.00	10.00	3.25	13.75	19.00	10.50
		4	23.25	9.25	0.00	26.25	9.00	15.75	10.25	9.75	7.75	0.00	10.25	22.75	6.25
		6	28.75	14.75	10.75	22.00	12.75	12.00	13.50	7.00	5.00	0.00	7.00	25.50	4.50

Table 2. Effect of storage container and storage duration on seed health of soybean varieties by blotter paper method

Seed samples	Storage container	Germination (%) * Time interval (months)			Root length (cm) Time interval (months)			Shoot length (cm) Time interval (months)			Seedling Vigour Index Time interval (months)		
		2	4	6	2	4	6	2	4	6	2	4	6
Shivalik (seeds obtained from sprayed plot)	Metallic bin	92.33 (74.23)	88.75 (70.54)	86.33 (68.28)	10.32	10.27	10.24	3.75	3.72	3.70	1299	1242	1204
	Polythene bag	94.50 (76.63)	92.33 (74.05)	89.75 (71.37)	10.35	10.32	10.29	3.76	3.75	3.73	1333	1299	1258
	Cloth bag	90.50 (72.13)	87.00 (68.86)	85.33 (67.54)	10.34	10.31	10.28	3.77	3.74	3.72	1277	1222	1194
Shivalik (seeds obtained from unsprayed plot)	Metallic bin	88.33 (70.18)	85.00 (67.19)	85.00 (67.21)	9.23	9.20	9.17	1.81	1.78	1.76	975	933	929
	Polythene bag	91.33 (73.10)	87.67 (69.44)	85.33 (67.50)	9.25	9.22	9.19	1.82	1.80	1.78	1011	966	936
	Cloth bag	90.33 (72.03)	87.50 (69.29)	82.67 (65.40)	9.19	9.18	9.15	1.78	1.75	1.74	991	956	900
Hara Soya (seeds obtained from sprayed plot)	Metallic bin	95.00 (77.39)	94.67 (77.24)	91.33 (72.89)	10.54	10.52	10.47	4.06	4.03	4.01	1387	1378	1323
	Polythene bag	97.50 (81.61)	95.00 (77.29)	92.33 (74.04)	10.58	10.55	10.52	4.10	4.07	4.05	1432	1389	1345
	Cloth bag	93.33 (75.01)	89.67 (71.23)	85.33 (67.53)	10.51	10.48	10.45	4.03	4.98	4.67	1357	1386	1290
Hara Soya (seeds obtained from unsprayed plot)	Metallic bin	94.33 (76.39)	92.67 (74.26)	90.50 (72.21)	10.41	10.38	10.35	3.93	3.99	4.07	1352	1332	1305
	Polythene bag	96.67 (79.73)	94.33 (76.78)	92.00 (73.55)	10.44	10.42	10.38	4.06	4.05	4.02	1402	1365	1325
	Cloth bag	91.33 (73.00)	87.50 (69.41)	83.67 (66.14)	10.40	10.37	10.34	3.95	3.94	2.61	1311	1252	1084
CD (P = 0.05)													
Seed sample(A)		1.52			0.13			0.22					
Storage container (B)		1.32			NS			NS					
Interaction (A x B)		2.63			NS			0.37					
Storage condition (C)		1.32			NS			NS					
Interaction (A x C)		NS			NS			NS					
Interaction (B x C)		NS			NS			NS					
Interaction (A x B x C)		NS			NS			NS					

*Figures within parentheses are arc sine transformed values, NS= Non significant

seedling vigour index were recorded from seeds of cv. Shivalik obtained from unsprayed plots after 6 months.

Interaction between storage period and storage containers

The results also clearly indicated that germination percentage, root length, shoot length and seedling vigour index were significantly affected due to the interaction between storage periods and storage containers. It was noticed that highest germination percentage, root length, shoot length and seedling vigour index were obtained after two months of storage in polythene bags followed by soybean seeds stored in polythene bags for 4 months. However, the lowest germination percentage was obtained from soybean seeds stored in cloth bags for 6 months.

Interaction between soybean cultivars and storage containers

The results (Table 2) showed that highest germination percentage, root length, shoot length and seedling vigour index were observed in seeds of Hara Soya stored in polythene bags obtained from fungicide sprayed plots. The lowest germination percentage, root length, shoot length and seedling vigour index were obtained from seeds of cv. Shivalik stored in cloth bags obtained from unsprayed plots.

Interaction between storage periods, soybean cultivars and storage containers

The results clearly indicated that highest germination percentage (97.50%) root length (10.58 cm), shoot length (4.10 cm) and seedling vigour index (1432) were recorded in seeds of Hara Soya after 2 months of storage in polythene bags obtained from fungicide sprayed plots. The data revealed that at the end of 6 months of storage period, highest seed germination (92.33%), root length (10.52 cm), shoot length (4.05 cm) and seedling vigour index (1345) was given by seeds of variety Hara Soya obtained from fungicide sprayed plot stored in polythene bag followed by seeds of variety Hara Soya obtained from unsprayed plot stored in polythene bag with seed germination (92.00%), root length (10.38 cm), shoot length (4.02 cm) and seedling vigour index (1325) and differed statistically from each other. However, the lowest germination percentage (82.67%), root length (9.15cm), shoot length (1.74cm) and seedling vigour index (900) were obtained from seeds of cv. Shivalik stored in cloth bags for 6 months obtained from unsprayed plots.

Similar results were observed by Ram *et al*. (2020), who reported that during storage, the highest germination percentage was maintained by using polythene bags. Verma and Verma (2014) also reported that after 8 months of storage, the seeds kept in polythene bags exhibited significantly higher germination rates and vigour indices compared to seeds stored in cloth bags. Singh and Dadlani (2003) reported that soybean seeds stored in cloth bags maintained satisfactory germination for only up to 4 months of storage. The decline in germination percentage observed during the storage period, however, can be attributed to the process of seed aging and the depletion of food reserves. Additionally, factors such as reduced synthetic activity of the embryo, fungal invasion, insect damage, decreased dry matter accumulation in seedlings, fluctuations in temperature and relative humidity, and the choice of storage containers can all contribute to the deterioration of seed health during storage, as discussed by Beedi *et al*. (2018).

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