

# Effect of Threshing Method, Drying Temperature and Storage Condition on Microbial Deterioration of Soybean Seeds

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## ABSTRACT

Post harvest operations have an effect on the development of storage fungi in seeds of tropical legumes. This study evaluated the effects of two methods of storage, four drying temperatures and two conditions of storage on the development of fungal infections on soybean seeds. The fungi were identified by culture on potato dextrose media. Seeds threshed mechanically and dried at higher temperatures indicated a higher percentage of infection. This was most prominent when the seeds were stored at high humidity, and was reflected by the rapid development of fungal infection leading to the rapid loss of germinability. Evaluation of fungi indicated the prominent presence of *Aspergillus* and *Phomopsis* spp. In contrast, hand threshed seeds dried at lower temperatures retained their germinability 12 weeks after storage and were relatively free of fungal infection. Possible methods of retaining the quality of seed under tropical farming conditions are presented.

## INTRODUCTION

Grain legumes occupy an important place in food and nutrition of the world due to their high protein content (FAO, 1977). Hence, over 40% of the global production is from the developing world, where they play a vital role in alleviating nutritional problems (NAS, 1984).

Most legumes are harvested after a period of field drying and the pods are exposed to many hazards during this period. This lowers seed quality prior to harvest (NAS, 1984). After harvest, grain legumes are more difficult to store than cereals (TPI, 1977), and their storage life is determined to a great extent by post harvest operations (Adams, 1977). Thus, estimates indicate a loss ranging between 5-30% of total legume production due to improper harvest operations (NAS, 1984).

Post harvest operations determine the quality of legume seeds available for consumption and more importantly for seed purposes. Thus, a major constraint to the expansion of tropical legume production is the lack of good planting material (Gregg, 1985). This can be attributed to improper post harvest operations and storage, as such conditions can cause rapid loss of seed viability

through microbial infection and seed death (DeLouche and Rodda, 1976; Gregg, 1982). Studies (Christensen, 1957; Onesirosan, 1982) report the deterioration of legume seeds during storage by fungi.

Among the many grain legumes cultivated in the tropics, soybeans (*Glycine max* L.) is considered to be the most vulnerable species to pre- and post-harvest operations. Justice and Bass (1978) classified soybeans into the least storable group in their relative storability index, as these seeds cannot be stored satisfactorily under ambient tropical conditions.

Availability of seeds for field establishment of soybeans is limited in Sri Lanka, and post harvest storage conditions are considered to be a causal factor. Hence, a study was conducted to evaluate the effect of three major post harvest operations on fungal infection and seed quality of soybeans, when stored for approximately three months, which correspond to an average growing season of a short term legume. The post-harvest operations selected were drying temperature, threshing method and storage conditions (especially relative humidity) as they are considered the more important factors affecting seed germinability after harvest (Adams, 1977).

## MATERIALS AND METHODS

**Post harvest drying.** Seeds of soybean (cv. Pelican) were obtained by harvesting plants which had mature yellow pods. These mature yellow pods with seeds (having a moisture content of  $26\% \pm 1.6\%$  (wet wt. basis) were manually removed and stored in open containers at  $15^{\circ}\text{C}$  and a relative humidity of  $70\% \pm 2.5\%$  until processing.

Four selected lots of pods, each containing 5 kg were obtained from the harvest. These lots were dried to 10% moisture content (wet wt. basis) using thermal air flow ovens set at  $15^{\circ}$ ,  $30^{\circ}$ ,  $45^{\circ}$  and  $60^{\circ}\text{C}$ . One lot of seeds was dried at one temperature.

**Threshing.** Each of the four lots of dried pods were divided into two halves using the modified halving technique (Anon., 1985). Each half was further divided into four uniform lots in the same manner. From these, two were selected randomly for machine threshing and the balance used for hand threshing. Machine threshing was carried out by passing the predetermined pods through a

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rubber roller revolving approximately at 500 rpm, and cleaning thereafter. Hand threshing was achieved by placing the selected pods in trays and removing the shells manually. Winnows were used to clean the seeds. These cleaned seed lots were used for storage.

**Storage and fungal infection.** Random samples of 50 seeds were obtained from the seed lots dried at different temperatures, and threshed by hand or machine. These were examined under a binocular microscope to determine the presence of cracks on the seed coats. Three replicates each containing 600 seeds were obtained from each treatment and placed in cloth bags. These seed bags were divided into two and stored at two humidities (High -  $90\% \pm 1.5\%$  RH and Low -  $40\% \pm 1.2\%$  RH) at  $20^{\circ}\text{C}$  for 12 weeks.

The seed lots were examined at two week intervals for fungal infection, by obtaining 30 seeds from each replicate. The seeds were surface sterilized with 1% sodium hypochlorite solution for 3 minutes and washed with distilled water. The seeds were thereafter placed in potato dextrose agar in 10 cm plates with 10 seeds per plate, and incubated at room temperature ( $25^{\circ}\text{C} \pm 2.1^{\circ}\text{C}$ ). The fungal development was observed after 5 days and the number of infected seeds counted to determine the percentage of infection. A binocular microscope was used to determine the presence of the fungi and identify them by morphological characters as described by Agarios (1978), once 100% infection was reached or at the final sampling if 100% infection was not observed.

Fifty seeds were also removed from each replicate at two week intervals to determine changes in seed germinability. These seeds were placed on moist towels, rolled and incubated at  $25^{\circ}\text{C}$  as recommended for soybeans. Germination percentages were taken at the seventh day to determine the quality of seeds for use as planting material. The experimental procedure used was a completely randomized design with sub samples, as described by Steel and Torrie (1981).

## RESULTS AND DISCUSSION

**Seed damage due to drying and threshing.** Observations made on the seeds dried at the four temperatures and threshed either by hand or machine illustrate the damage caused to the seed coats (Table 1). The presence of cracks on the seedcoats decreased significantly ( $P=0.05$ ) when dried at lower temperatures. This, as indicated by Tanner and Hume (1978) is a resultant feature of rapid removal of moisture at high temperatures, which causes stress effects on seedcoats, resulting in cracking. The presence of cracks were also significantly greater when seeds were machine threshed, and was a constant feature irrespective of drying temperature. Thus, machine threshing especially if carried out rapidly

causes mechanical damage to seeds even though seeds are dried at optimal temperatures. This makes such seeds unsuitable for planting.

**Table 1. Effect of drying temperature and threshing method on percentage cracked seed in soybeans.**

Drying Temperature ( $^{\circ}\text{C}$ )	Threshing Method	Cracked Seed (%)
15	Hand	4.4
	Machine	7.5
30	Hand	9.8
	Machine	14.25
45	Hand	18.6
	Machine	27.1
60	Hand	36.5
	Machine	59.7

**Fungal infection.** All machine threshed seed indicated the presence of fungal infection much earlier than hand threshed seeds (Table 2). Fungal infection was more extensive on machine threshed seeds. This too was uniformly evident at both humidities, and in seeds dried at the four temperatures. This could be directly related to the presence of a greater degree of seedcoat cracking due to machine threshing. Thus, as reported by Neegaard (1979), mechanical threshing develops conditions that substantially favor infection by fungi, if not carried out properly. In addition, the relative thinness of the soybean seedcoat (Justice and Bass, 1978) makes it more susceptible to mechanical damage, and thereby to fungal infection than other legumes.

Drying temperature and storage humidity also had a significant effect on the development of fungi, irrespective of the method of threshing. High drying temperatures indicate fungal infections earlier (Table 2). This again is caused by the cracks due to rapid removal of moisture from seeds, which act as points of entry for fungi.

Storage of seeds at higher humidities cause rapid decay of most seeds through fungal infection and metabolic processes (Harrington, 1973). This again is evident in soybeans in the present study and seeds stored at 90% humidity developed fungi rapidly. This is compounded by the higher drying temperatures and methods of threshing. Thus, it can be concluded that fungal infection is most prominent when legumes, especially soybeans are dried at higher temperatures, machine threshed at high speeds and stored at high humidities.

The presence of field and storage fungi (*Phomopsis* and *Aspergillus*) on the infected seeds are presented in

Table 2. Effect of post-harvest operations on fungal infestation of soybean seeds after two to ten weeks storage.

Storage Condition	Drying Temperature (°C)	Threshing Method	Fungal Infestation (%)					
			2	4	6	8	10	12
High Humidity	15	Hand	38	54	65	84	100	--
		Machine	55	100	--	--	--	--
	30	Hand	48	74	100	--	--	--
		Machine	78	100	--	--	--	--
	45	Hand	62	70	100	--	--	--
		Machine	100	--	--	--	--	--
60	Hand	89	100	--	--	--	--	
	Machine	100	--	--	--	--	--	
Low Humidity	15	Hand	--	--	--	--	--	3
		Machine	4	9	24	39	48	61
	30	Hand	--	--	--	--	2	7
		Machine	14	26	45	69	78	97
	45	Hand	--	3	9	17	29	39
		Machine	72	91	100	--	--	--
60	Hand	9	24	35	48	56	72	
	Machine	100	--	--	--	--	--	
CV %			8.3	4.2	9.6	11.2	18.3	7.8

Table 3. Effect of post-harvest operations on the development of fungi on soybean seeds at 100% infection or at 12 weeks of storage.

Storage Condition	Drying Temperature (°C)	Threshing Method	Seed Infection (%)	
			<i>Phomopsis</i>	<i>Aspergillus</i>
High Humidity	15	Hand	03	97
		Machine	05	95
	30	Hand	12	88
		Machine	15	85
	45	Hand	18	82
		Machine	21	79
60	Hand	27	73	
	Machine	31	69	
Low Humidity	15	Hand	--	100
		Machine	02	98
	30	Hand	--	100
		Machine	05	95
	45	Hand	01	99
		Machine	14	86
60	Hand	08	92	
	Machine	23	77	
CV %			8.5	7.3

Table 3. There was a significant increase in the presence of *Phomopsis* spp. when seeds were dried at higher temperatures and when machine threshed. This was evident at both conditions of storage. However, the incidence of field fungi was significantly less when seeds were stored at lower humidities. This suggests that infection by field fungi is greater when seeds are dried at higher temperatures and machine threshed, which can be associated with the greater degree of cracking, thus making them more prone to fungal infestation, especially at the higher humidity.

The lesser degree of infection observed under low humidity conditions and especially when hand threshed is attributed to storage fungi *Aspergillus* spp, and not to field fungi *Phomopsis*. The presence of *Aspergillus* under conditions of low humidity irrespective of the threshing method can be associated with its adaptability to a wide range of storage conditions (Christensen and Kaufmann, 1965). In addition, the data suggests that in the presence of field fungi, the occurrence of storage fungi are reduced, which may be an attribute of competitive relationships between the microorganisms.

**Germination.** The results observed in the germination counts are presented in Table 4. These results directly correlate ( $r=0.85$ ) with fungal infestations (Table 2). Seeds dried at higher temperatures, machine threshed and stored at high humidities lose their ger-

minability quickly. In contrast, seeds dried at low temperatures, hand threshed and stored in dry conditions retain their viability and thus seed quality for a longer period of time, although some fungal infection is observed. These observations emphasize the value of proper post-harvest operations in maintaining seed quality.

**Practical implications.** Tropical farmers generally report the lack of good quality planting material for expansion of agricultural programs. Several causal factors are attributed to this phenomenon, and pre- and post-harvest operations play a deterministic role in the availability of seeds for the next season. This is due to the hot humid climates that prevail in the tropics suitable to legume production.

The value in adopting a few simple precautions in order to maintain seed quality is shown by this study. Seeds harvested at maturity and gradually dried, preferably in the shade, retain their germinability. As the seed requirement per individual farmer is small due to the size of his landholding, his seed requirement could easily be obtained by hand threshing the dried pods. Thereafter, seeds placed in dry cool places retain their germinability for long periods of time. Thus, the value of these simple operations is evident in the provision of good quality seed material of soybeans and also other legumes at no extra cost to the farmers of the developing world.

Table 4. Effect of post-harvest operations on germination of soybean seeds after two to ten weeks storage.

Storage Condition	Drying Temperature (°C)	Threshing Method	Germination (%)					
			2	4	6	8	10	12
High Humidity	15	Hand	74	55	36	19	9	3
		Machine	28	14	3	--	--	--
	30	Hand	56	34	21	5	--	--
		Machine	22	15	--	--	--	--
	45	Hand	31	19	11	4	--	--
		Machine	11	2	--	--	--	--
60	Hand	11	3	--	--	--	--	
	Machine	4	--	--	--	--	--	
Low Humidity	15	Hand	98	96	97	94	95	95
		Machine	95	90	83	62	59	51
	30	Hand	95	96	96	95	90	90
		Machine	91	86	74	58	32	28
	45	Hand	93	93	94	86	75	69
		Machine	55	47	36	27	14	2
60	Hand	81	74	68	61	47	41	
	Machine	39	28	15	4	--	--	
CV %			13.8	8.9	17.6	16.8	19.7	12.2

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