STORAGE FACTORS INFLUENCES SEED BORNE FUNGAL INCIDENCE ON SOYBEAN SEED

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Abstract: Oilseeds are grown in an area of about 20 million hectares of which nearly 84 per cent area is rainfed in India. Soybean is one of the major oilseed crop grown in India and in Maharashtra. Seeds of three different variety of soybean were investigated for the incidence of mycoflora. Aspergilli were the first colonizers and were followed by *R. stolonifer*, *Penicillium* and *F. moniliforme*. Percentage of occurrence of fungi goes on increasing linearly up to 120 days and at decreasing rate thereafter. At 10°C its occurrence was minimum while maximum at 30°C and then goes on decreasing as the temperature in increased. Their was rapid increase in occurrence of fungi during storage of soybean from 10°C to 30°C and any further increase on temperature resulted in decrease in percent of occurrence of fungi. The highest percent of fungi occurrence was recorded at 80% RH with lowest at 40%. A combination of the three factors providing unfavorable conditions for mold development, such as low product moisture, low temperature, and short storage period are desirable.

Keywords: *Glycine max* L., seed borne fungi, storage factors.

Introduction: India is the third largest producer of oilseeds in the world. It ranks first in the production of groundnut and sesamum and second in the rapeseed/mustard. Oilseeds are grown in an area of about 20 million hectares of which nearly 84 per cent area is rainfed. The vegetable oil is obtained from nine cultivated oilseed crops like groundnut, rapeseed/mustard, seasamum, safflower, nigerseed, soybean, sunflower for edible whereas linseed and castorseed for non-edible proposes.

Seed-borne fungi cause losses in terms of seed quality and quantity in all oilseed crops. These fungi also reduce the germination and storability of the seed. They are responsible for seed rot, seedling blight, root/stem rot, foliar infection as well as pod blight diseases [1]-[3]. The losses of oilseed and grains in our country account for 10% of total production. The losses can be attributed the unhygienic storage condition and high moisture levels of seeds or absorption of moisture during storage. Normally the seed storage facilities with the agencies and farmers are meager.

Thus moisture or RH at the storage place affects the incidence of fungi on seeds. Similarly temperature also plays an important role in the association of fungi on the stored seeds. The unhygienic storage conditions of oilseeds make them susceptible to the attack of fungi.

Soybean (*Glycine max* Linn.) is important oil seed crop cultivated over several parts of the world, both in the tropics and subtropics. Marathwada region of Maharashtra is a leading region in the hecterage and production. Soybean oilseeds are affected by number of diseases caused by fungi, bacteria, viruses etc. Among these fungal diseases are more prevalent. Many of the diseases are borne in nature and transmitted in field in a considerable proportion. The farmer stores the grains of soybean in their houses and used these grains as seeds in subsequent years for sowing. So this worked aimed to bring out the relationship among seed fungi and storage factors that could affect the health of the seed viz. time, temperature and humidity during storage.

Material And Methods:

Source of cultivars: Three cultivars of soyabean were obtained from the Oilseed Research Station (Marathwada Agricultural University, Latur, Maharashtra). The seeds were stored at 22°C in cloth bags and used whenever needed.

Glycine max: Linn cv. JS 335, cv. Prasad, cv. Puja

Assessment of seed mycoflora*:* Seed fungi isolation: Three methods were used for isolation of externally and internally seed-borne fungi.

Standard blotter test: Seeds were equidistantly spaced on moist sterile blotters in petriplate moist chambers. 10 petriplates of 9" diameter each containing 10 seeds were incubated at 27±2°C for seven days. Observations were made for fungi appearing on seeds every 24 hours and growth was carefully transferred to PDA slants for further studies. A minimum of 400 seeds were observed in each case. Untreated seeds were used for mapping external seed mycoflora whereas seeds disinfected externally by treating with 10% sodium hypochlorite solution for 10 minutes. Where used for internally seed mycoflora.

Effect of storage period on seed mycoflora*:* The effect of storage period on seed mycoflora was studied. The seeds of all three oilseeds and their varieties were stored in gunny bags at room temperature (27±2°C) for nine months storage periods. Seed mycoflora of the stored seeds was detected at the end of every 30 days by moist blotter method.

Effect of temperature of storage on seed

mycoflora: The effect of temperature on the seed mycoflora was studied the seeds were stored in gunny bags at various temperature (10-40°C) in BOD incubator separately up to nine months. After regular time interval of 30 days seeds were taken and mycoflora was determined by the three stated method.

Effect of relatively humidity (RH) of storage on seed mycoflora: The effect of RH on the seed mycoflora was studied the seeds were stored in gunny bags at various RH (40-80) in humidity chamber separately up to nine months. After regular time interval of 30 days seeds were taken and mycoflora was determined.

Results: Seeds of three different variety of soybean were investigated for the incidence of mycoflora. The seeds were incubated in moist blotters and on agar plate at 27±2°C for a period of seven days. The observations of fungi for percentage incidence and growth characters were recorded.

Record were made on for the characteristic of the colony, morphology of the spore bearing organs, associations between fungi on seeds and occurrence of fungi with time (succession). The surface sterilized and unsterilized seeds were subjected to the tests. Pure cultures of fungi were made from the growth for identification.

Seed Mycoflora Of Soybean: Three seeds lots belonging to three varieties of soybean were investigated for the incidence of mycoflora. The seeds were incubated in moist blotters at 27±2°C for a period of seven days. The observations of fungi for percentage incidence and growth characters were recorded using stereomicroscope (50X). 400 seeds from each lot were examined. The detail records were made on appearance, association and characteristic of associated fungi with the seeds. The pure cultures of fungi were made from the growth for identification and further study.

Fungi associated with seeds of Glycine max Linn. Cv: Eight fungi appeared on seeds of cultivar JS 335 (Table I). *Aspergillus niger* (39) and *R. stolonifer* (35) were dominant. Eight fungi were isolated by blotter method. *Cladosporium herbarium* and *A. terreus* appeared on blotter method only. In all 12 fungi were found to be associated with the seeds of cultivars Prasad. Observations of non-germinated seeds gave seven fungi. Aspergilli developed colonies early and were followed by *R. stolonifer*, *A. tenuis F. moniliforme* and *C. herbarum*. Most of the fungi isolates by blotter method were common to the earlier variety. Eleven fungi were found to be associated with the seeds of cultivar Puja. *A. niger* (60) gave highest percentage of incidence followed by *A. fumigatus* (55), A. *flavus* (49) and *Fusarium moniliforme* (35). Non-geminated seeds gave seven fungi. Aspergilli were the first colonizers and were followed by *R. stolonifer*, *Penicillium* and *F. moniliforme*.

Table I: Fungal incidence (%) isolated from seeds of different Soybean cultivars

Name of the fungus	cv. JS 335		cv. Prasad		cv. Puja	
	EX	IN	EX	IN	EX	IN
Aspergillus niger	39	19	59	42	60	47
Rhizopus stolonifer	35	11	50	36	55	39
Mucor mucedo	-	-	38	23	49	35
Chaetomium sp.	-	-	30	13	35	20
Aspergillus flavus	27	10	17	9	36	22
Aspergillus terreus	21	7	-	-	24	17
Penicillium sp.	11	5	2	1	-	-
Alternaria tenuis	7	3	12	6	11	8
Cladosporium herbarum	8	5	-	-	5	3.5
Verticillium albo-atrum	-	-	13	8	2	1
Fusarium moniliforme	3	1.5	-	-	2	1
Rhizoctonia sp.	-	-	4	2.5	1	1

Storage Fungi associated with Soybean:

cultivars With Relation To Storage Time: Effect of storage period on the percent of occurrence of fungi in soybean was studied. Soybean seeds were stored up to the 9 month period and observation on the percentage of occurrence of fungi was taken at 30 days interval regularly. It was found that percent of fungi occurrence on oilseed goes on increasing throughout the storage period in all the 3 varieties. Percentage of occurrence of fungi goes on increasing linearly up to 120 days after that it goes on increasing but at decreasing rate.

Table II: Effect of storage period on % incidence of fungi in soybean cultivars (EX-external IN-internal)

Time period in days	cv. JS 335		cv. Prasad		cv. Puja	
	EX	IN	EX	IN	EX	IN
30	3	1.5	3.5	1	5	2
60	10	5	11	5	11	5
90	30	19	31	17	39	18
120	57	33	59	35	61	41
150	69	47	66	39	68	45
180	72	49	69	43	70	48
210	73	50	72	45	71	48
240	74	50	73	46	71	49
270	74	50.5	73.5	46	72	50

In var. JS 335 the maximum percent of fungi occurrence was 83 externally in agar plate media at 270 days while its percent in seed blotter method was 74 and in seed wash was 64.5 while at same period percent of fungi occurrence internally was 56 in agar plate where it was 50.5 in seed blotter (Table II). In var. Puja maximum growth rate was observed up to 150 days while maximum occurrence of fungi was at 270 days which was 73.5% for external fungi while it was 46% in seed blotter for internal fungi.

Incidence Of Storage Fungi In Soybean With Relation To Storage Temperature: Change in temperature has marked effect on the percent of occurrence of fungi on soybean. At 10°C its occurrence was minimum while maximum at 30°C and then goes on decreasing as the temperature in increased.

In var. JS 335 minimum occurrence of fungi was recorded at 10°C which was just 1% in case of internal fungi. As the temperature increase percent of occurrence also goes on increasing up to 30°C and after that further increase in temperature caused reduction in the percent of occurrence of storage fungi. In var. Puja just 1% of internal storage fungi were found in seed blotter method at 10°C. At 40°C occurrence of storage fungi on seed was decreased and it was 59% for external fungi and 40% for internal fungi in seed blotter method (Table III). In var. Prasad percent of fungi occurrence 2 at 10°C while maximum percent of its occurrence was noticed at 30°C for external fungi, thus their was rapid increase in occurrence of fungi during storage of oilseed from 10°C to 30°C and any further increase on temperature resulted in decrease in percent of occurrence of fungi .

Table III: Effect of storage temperature on % incidence of fungi in soybean cultivars (EX-external IN-internal)

Temperature	var. JS 335		var. Puja		var. Prasad	
	EX	IN	EX	IN	EX	IN
10	3	1	3	1.5	6	2
15	14	6	15	5	19	7
20	33	17	33	19	33	15
25	50	31	55	36	60	41
30	69	50	71	52	75	55
35	60	45	64	45	67	49
40	54	41	59	40	60	42

Incidence Of Storage Fungi In Soybean With Relation To Storage Relative Humidity: Effect of relative humidity (RH) on percentage of occurrence of storage fungi in soybean crop and its 3 varieties were studied. The general observed effect of RH on occurrence of fungi was that its goes increasing with increase in RH. The RH was from 40 to 80% and observations were recorded at 40, 50, 60, 70 and 80%.

Table IV: Effect of RH on % incidence of fungi in soybean (EX-external IN-internal)

RH	cv. JS 335		cv. Prasad		cv. Puja	
	EX	IN	EX	IN	EX	IN
40	21	13	23	12	20	11
50	31	17	34	19	36	21
60	43	27	50	33	50	32
70	61	44	65	48	71	54
80	73	58	73	57	77	61

The highest percent of fungi occurrence was recorded at 80% RH with lowest at 40%. In var. JS 335 minimum occurrence of fungi was observed for internal fungi in seed blotter method at 40% RH which was 13% as the RH % goes on increasing percentage of fungi occurrence also increased. (Table IV). In var. Puja minimum fungi occurrence was 12% for internal seed borne fungi. In var. Prasad percentage of occurrence of storage fungi was highest at 80% RH. Its was 77% for external seed borne fungi.

Discussion: Seed health conceptually is a process proportionately successful to the degree of optimization of conditions of growth and function [4]. The accompanying spectrum of harmful micro-organisms can be regarded as one of the contributory factor in unhealthy condition of the seed. Sometimes inconspicuous inoculum associated with the seed lot may lead to the epidemic situation and it is pertinent to ascertain the mycoflora of the seed of economic crop plants and to have knowledge about its impact on the yield potential and several other deleterious factors.

Three soybean varieties subjected to isolation for fungal flora associated with the seeds. The fungal flora isolated were Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Rhizopus stolonifer, Alternaria tenuis, Penicillium sp., Mucor mucedo, Chaetomium sp., Cladosporium herbarum, Curvularia lunata and Verticillium albo-atrum.

It is revealed from Table I that various fungal flora were associated with the seeds of different varieties indicating that presence of pathogenic and non pathogenic fungi on the healthy looking seeds. The appearance of fungi on the seeds varied with the fungal flora and also with the varieties. However, specifically abundant species of *Aspergillus* were observed in large proportion. Ectophytic presence of *Aspergillus* species and its frequently association with the different oil seed crops was reported by Reference [5] and it was further stated that *Aspergillus niger* and *Rhizopus* sp. were pathogenic to number of oil seed crops which affected seed germination and also increased pre and post emergence mortality of the seedlings.

Reference [6] also reported that prevalence of the *Alternaria* sp., *Aspergillus flavus*, *Aspergillus sydowii*, *Aspergillus niger* and *penicillium* sp. on the seed and these fungi were a major case of spoilage of seed and reduction in the plant stand.

The three major factors affecting the storability of oilseeds are moisture content, temperature, and duration of storage. Storage of oilseeds in the tropics and subtropics is mostly traditional; the methods used presently have been used for many years with slight or no modifications. These methods achieve varying degrees of success in applying the basic principles involved in the safe storage of food grains; the variations observed are often related to climate, but local natural resources and customs also influence the choice of storage methods. Oilseeds are stored in ordinary gunny bags, or loose in bulk in a variety of storage structures made from locally available materials.

Traditional storage structures used in rural areas are neither rodent-proof, nor secure from fungal and insect attack. The major factors causing grain spoilage are storage fungi, insects, and rodents, were temperature and humidity being the main determinants. High temperature from the development within the grain of certain molds, can lead to chemical deterioration of the grains. The ageing process in oilseeds stored inadequate can cause a major loss in oil quality in terms of colour, free fatty acids, iodine and other biological values. High moisture of grains, relative humidity temperature, and mold infection are the main reasons for deterioration oilseeds in storage. The storage life of seeds is almost halved for each increase in moisture content and for every 5°C rise in storage temperature. Other factors influencing quality of seeds during storage include initial quality, damage caused by rough handling, and the extent of cleaning of crop produce. Considerable losses of oilseeds such as groundnut, linseed, safflower, castor, rapeseed/ mustard during storage have reported.

Deterioration of oilseeds in storage is a combined

function of its moisture content, temperature, and duration of storage. Therefore, for safe storage, a combination of the three factors providing unfavorable conditions for mold development, such as low product moisture, low temperature, and short storage period are desirable.

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