

Effect of time of harvesting, storage and fungicide seed dressing on soybean seed health and germinability

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Abstract

A key constraint to soybean (*Glycine max*) production worldwide is the rapid loss of viability after harvest. This study examined effects of time of harvesting, moisture content, storage period and fungicide treatment on seed fungal flora infection and germinability of two local soybean cultivars (Nam 1 and Nam 2). At maturity (R8 stage), a portion of the soybean crop was randomly selected from each of six fields and divided into three sections that were harvested at 90, 97 and 104 days after planting (DAP). After harvesting, seeds were dried to two moisture levels; 9.5% and 12%. For each moisture content, the seeds were divided into 2 sub-samples, one treated with Vitavax 200 FF fungicide at a rate of 3 ml kg⁻¹, and the other was left untreated (control). Each sub-sample was then packed in polythene bags and tightly sealed and stored at room temperature (25 ± 2°C) and subsequently assayed every 30 days for three months using International Seed Testing Association procedures to determine levels of fungal flora infection and seed germinability. Results indicated that time of harvesting and fungicide treatment significantly influenced incidence of fungal flora and seed germinability, regardless of the season of growth and variety. The highest percentage germinability was recorded from the earliest harvested seeds (R8) compared to the late harvested seeds (7 - 14 days after R8). Seed dressing with Vitavax 200 FF significantly reduced levels of seed-borne fungi and improved germinability compared to the untreated seeds, although this varied with seed moisture content in storage. However, storage period had no significant effect on soybean seed germinability and incidence of most fungal micro-flora. Varietal and seasonal effects were also not significant for most fungal microflora species, except *Cercospora kikuchii* and *Penicillium* spp. The fungal microflora identified were, *Aspergillus flavus*, *A. niger*, *Alternaria* spp., *Cercospora* spp., *Cercospora kikuchii*, *Fusarium* spp., *F. equiseti*, *F. oxysporium*, *F. moniliforme*, *F. semitectum*, *Phoma* spp., *Phomopsis* spp., *Penicillium* spp., *Cladosporium*, *Curvularia* spp. and *Colletotrichum truncatum*. These results imply that soybean should be harvested at R8 stage, and seeds dressed with a fungicide so as to reduce fungal microflora incidence and increase germinability.

Key words: Fungal micro-flora infection, *Glycine max*, Uganda

Introduction

Globally, one of the most recognised problems in soybean production is the rapid loss of viability in seeds. In the United States of America, for example, farmers and seed dealers experience a lot of difficulties in maintaining viability in storage (Justice and Brass, 1978). Similarly, in Ghana, uneven seedling establishment in the field has been attributed mainly to loss of viability in storage (Nangju, 1977). In Uganda this problem has long been recognised as well (Mukasa, 1970; Leakey, 1971). Unfortunately, although several interventions were put in place to minimise the problem (USP, 1973), soybean viability continues to be low due to rapid loss of viability in storage (Kabeere, 1977; Tukamuhabwa, 1992). Furthermore, studies done elsewhere indicate that seed borne fungal inocula contribute significantly to loss of viability (Onesirosam, 1986; Mycock and Berjark, 1995). Fortunately,

studies done in Ghana have shown that time of harvesting could be manipulated to lengthen viability of soybean seeds and hence, storage duration. For example, Nangju (1977) observed that soybean seeds harvested 100 days after planting registered higher germination and longer storability than those harvested at a later date. It is suspected that delayed harvesting and long storage duration promote build-up of seed-borne inocula, thus contributing to rapid loss of seed viability.

Thus, the objectives of this study were to: 1) assess the effect of time of harvest on soybean seed health and germinability, 2) identify the fungal flora associated with soybean seed in storage and their effect on seed germinability, and 3) assess effect of seed moisture content during storage on soybean seed viability.

Materials and methods

Six randomly selected contract growers planted two soybean seed crops, in September 2001 and March 2002. Two local cultivars of soybean (Nam 1 and Nam 2) were used in the study, each variety being planted by three farmers. The three farmers were considered as a block with each farmer as a replicate. The seeds were planted at a spacing 60 cm between rows and 5cm within rows, placing 1 seed per hill. All required agronomic practices were done as recommended by Tukamuhabwa (2000). At maturity (R8 stage), when 95% of the pods had turned dark-gray and brown, a portion of the soybean crop was randomly selected from each of the six fields and divided into three sections that were harvested at 90 (R8), 97 and 104 days after planting (DAP). After harvesting, seeds were dried to two moisture levels, 12% and 9.5%. For each moisture content, the seeds were divided into two sub-samples, one treated with the fungicide (Vitavax 200 FF) at a rate of 3ml kg⁻¹, and the other left untreated (control). Each sub-sample was then packed in tightly sealed polyethene bags and stored at room temperature (25 ± 2°C) and subsequently assayed every 30 days for three months using standard procedures outlined by the International Seed Testing Association (ISTA, 1996) to determine levels of fungal flora infection and seed germinability.

Seed health assay

A sample of 200 seeds was drawn from each sub-sample following procedures recommended by the International Seed Testing Association (ISTA, 1996). Ten seeds were plated on three layers of moist blotters, evenly placed in each Pyrex petri-plate of 9cm diameter, previously oven sterilized. For each test, the petri-plates were arranged in a completely randomised design with three replicates. The plated seeds were incubated at 21 ± 2 °C under alternating 12 hours of near Ultra-Violet (NUV) light and darkness to encourage sporulation of the fungal flora (ISTA, 1996). After incubation, each individual seed was examined for the presence of fungal flora infection under a stereo microscope (x 50 – 60 magnification). The fungal flora identity was ascertained on the basis of their habitual characters (Marthur *et al.*, 1992). The number of seeds infected was also recorded and these values were used to calculate the incidence (%) of each fungal species infection. The seed health assays were done once every 30 days on each sample for a period of 3 months.

Seed germination assay

Each sample was thoroughly mixed and a representative sample of 200 seeds taken and divided into four replicates each of 50 seeds. Each seed was surface sterilised by soaking in 1% sodium hypochlorite solution (Reckitt, Benkiser East Africa Limited, Nairobi Kenya) for two minutes, rinsed three times in sterile distilled water and dried between two sterile blotters. The samples were tested using "between paper rolled method" as described by ISTA (1996). The surface sterilized seeds were evenly placed on four layers of moist germination paper (newsprint) measuring 16.54"x11.69" and two layers of the

same type of paper were laid on top to cover the seeds. The bags were tied with a rubber band to prevent drying of the germination papers. The rolls were placed upright in a water-proof bag then put in a wire rack in a completely randomised design (CRD) with 3 replicates. The seeds were then incubated in the germination room and maintained at 23 ± 2 °C for 7 days. After incubation, the seedlings were examined and categorised as normal (seeds with well developed roots, hypocotyls and cotyledons) or abnormal (roots, hypocotyls or cotyledons when absent or rotten or malformed). Percent seed germination was calculated as the number of normal seeds that germinated over the total number of seeds plated/plate.

Moisture content assay

The moisture content of the seed samples was determined using the oven method. After threshing and cleaning each sample was transported to the laboratory in an air-tight package to minimize the change in moisture content. With a precision divider, a representative sample was portioned for moisture content testing. The seeds were ground, 4.5 gm put in a moisture content testing tin of known weight then oven dried at 103 ± 2 °C for 16 hours. Subsequently the samples were allowed to cool in a desiccator, reweighed and the moisture content (Wet basis) calculated as described by ISTA (1996).

Effect of storage duration on soybean seed germinability and fungal flora infection

The harvested seeds were stored at room temperature at 22-25 °C for 90 days. Periodically (once every 30 days) the seed samples were subjected to moisture content, seed health and germination tests to determine fungal flora types and population, and seed viability. Seed health and germination tests were done following procedures described earlier.

Effect of seed treatment on fungal flora infection and soybean seed germinability

For each moisture content group, the seeds were divided into two sub-samples of which one was treated with Vitavax 200FF fungicide at a rate of 3 ml kg⁻¹ of seed (USP, 2001). The other half was not treated and this served as a control. Each of these sub-samples was then packed in polythene bags used by USP and stored at room temperature (25 ± 2 °C) for up to three months. All the sub-samples were assayed for seed health and germination every 30 days to determine levels of fungal flora infection and seed germinability. The laboratory assays were done following the procedure described by ISTA (1996), and as outlined earlier. Data collected for the different trials was subjected to either one or two way analysis of variance (ANOVA) using Genstat Computer program (Genstat, 1995) and standard error of the difference (SED) values obtained were used to assess the difference between two treatment means.

Results and discussion

Effect of harvesting time and seed moisture content on incidence of fungal microflora on soybean seed

Delayed harvesting significantly ($P < 0.05$) increased the incidence of the majority of fungal microflora on soybean seeds during both seasons (Table 1). On the contrary, the varietal, season x harvest time interaction and harvest time x variety interaction effects were only significant ($P < 0.05$) for a few fungal microflora. Also delayed harvesting significantly ($P = 0.001$) reduced soybean germinability in both seasons. However, variety and its interactions with harvesting time and season were not significant ($P > 0.05$). Irrespective of the harvest period, seeds stored at 12% moisture content recorded significantly higher incidences of fungal microflora than those stored at 9.5%.

The incidence of most fungal microflora on soybean seeds, especially the storage fungi were significantly influenced by high seed moisture (Table 2). However, there was no consistent trend for the effect of moisture content on seed germination (Table 3).

Table 1. Summary of main effect of time of harvesting on incidence (%) of fungal microflora on soybean seeds harvested in 2001 and 2002.

Fungal species	Time of harvesting (days after planting)												Statistics (combined seasons)	
	2001			2002			Across seasons			Across harvest times		Across seasons and harvest times		
	90	97	104	90	97	104	90	97	104	2001	2002			CV (%)
<i>Aspergillus flavus</i>	1.9	2.1	4.7	1.4	2.6	4.5	1.7	1.7	4.6	2.9	2.4	2.6	149.2	0.3
<i>Aspergillus niger</i>	2.0	2.0	5.7	1.5	3.0	5.1	1.5	1.7	5.1	3.2	2.8	3.0	150.6	0.5
<i>Penicillium spp.</i>	1.4	1.7	9.6	1.1	3.2	4.1	1.3	1.4	6.9	4.3	2.1	3.2	160.8	0.6
<i>Rhizopus spp.</i>	1.1	1.3	2.5	1.2	1.9	4.2	1.2	1.2	3.4	1.7	2.2	1.9	172.6	0.4
<i>Cladosporium</i>	2.3	1.7	5.5	1.9	2.8	4.2	2.1	1.4	4.8	3.2	2.4	2.8	144.7	0.5
<i>Fusarium oxyspor</i>	0.7	0.8	3.6	0.7	1.5	2.4	0.7	0.7	3.0	1.7	1.2	1.5	198.9	0.1
<i>Fusarium equiseti</i>	0.0	0.1	0.3	0.1	0.1	0.3	0.1	0.1	0.3	0.1	0.2	0.1	391.0	0.1
<i>Fusarium semitectum</i>	0.1	0.2	0.3	0.1	0.2	0.3	0.2	0.1	0.3	0.2	0.2	0.2	171.1	0.1
<i>Fusarium moniliforme</i>	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	267.2	0.1
Other <i>Fusarium spp.</i>	0.6	0.8	0.7	0.5	0.7	0.8	0.5	0.7	0.7	0.7	0.6	0.7	171.1	0.1
<i>Phomopsis sojae</i>	0.6	0.6	0.5	0.5	0.5	0.6	0.6	0.5	0.5	0.6	0.5	0.5	154.7	0.1
<i>Phoma spp.</i>	0.4	0.6	0.5	0.3	0.4	0.3	0.4	0.5	0.4	0.5	0.4	0.4	171.5	0.1
<i>Cercospora spp.</i>	0.0	0.4	0.4	0.1	0.3	0.3	0.1	0.4	0.4	0.2	0.3	0.3	209.9	0.1
<i>Colletotrichum truncata</i>	0.0	0.2	0.5	0.1	0.2	0.4	0.1	0.2	0.4	0.2	0.2	0.2	244.6	0.1
<i>Cercospora kikuchii</i>	0.4	0.2	0.3	0.4	0.3	0.2	0.4	0.2	0.3	0.3	0.3	0.3	209.9	0.1
<i>Alternaria spp.</i>	0.4	0.2	0.4	0.4	0.3	0.4	0.4	0.2	0.4	0.3	0.3	0.3	188.8	0.1
<i>Curvularia spp.</i>	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.3	0.3	208.9	0.1

DAP = Days after planting; CV= Coefficients of variations, SED = Standard error of difference between means.

Table 2. Summary of effect of seed moisture content on the incidence of fungal microflora on soybean seed stored during 2001 and 2002 seasons

Fungal species	Moisture content (%) : 9.5 and 12 %						Statistics (combined seasons)	
	2001		2002		Across moisture levels			
	9.5	12	9.5	12	9.5	12	CV (%)	SED
<i>Aspergillus flavus</i>	1.7	4.0	1.8	3.1	1.7	3.5	152.8	0.4***
<i>Aspergillus niger</i>	2.2	4.3	1.7	3.8	1.9	4.1	156.7	0.5***
<i>Alternaria spp.</i>	0.2	0.4	0.3	0.4	0.3	0.4	189.6	0.1*
<i>Cercospora kikuchii</i>	0.3	0.4	0.3	0.3	0.3	0.3	210.0	0.1n.s
<i>Cladosporium spp.</i>	2.3	4.0	1.6	3.2	2.0	3.6	151.4	0.4***
<i>Colletotrichum truncatum</i>	0.2	0.3	0.2	0.3	0.2	0.3	253.1	0.1*
<i>Curvularia spp.</i>	0.2	0.3	0.3	0.2	0.3	0.3	208.3	0.1n.s
<i>Fusarium equiseti</i>	0.2	0.1	0.2	0.1	0.2	0.1	394.4	0.1n.s
<i>Fusarium moniliforme</i>	0.2	0.1	0.2	0.1	0.2	0.2	268.4	0.1n.s
<i>Fusarium oxysporium</i>	1.4	2.0	0.8	1.6	1.1	1.8	212.4	0.3*
<i>Fusarium semitectum</i>	0.1	0.2	0.2	0.2	0.1	0.2	266.1	0.1n.s
Other <i>Fusarium spp.</i>	0.6	0.8	0.5	0.7	0.6	0.7	171.0	0.1n.s
<i>Penicillium spp.</i>	3.6	5.0	1.3	2.9	2.4	4.0	182.9	0.6**
<i>Phoma spp.</i>	0.5	0.5	0.4	0.3	0.4	0.4	175.0	0.1n.s
<i>Phomopsis sojae</i>	0.5	0.5	0.5	0.5	0.5	0.6	153.6	0.1n.s
<i>Rhizopus spp.</i>	1.2	2.1	1.4	2.9	1.3	1.2	179.5	0.3***

***, ** = means significant at 5%, 1% and 0.1% level, respectively; n.s = not significant at 5% level; CV = Coefficient of variation; SED = standard error of difference between means.

Effect of seed treatment with fungicide on incidence of seed-borne fungi

Fungicide treatment significantly ($P \leq 0.05$) reduced incidences of all the fungal flora species more especially in 2001 season (Table 4). Seed dressing significantly influenced germinability in 2002, but not in 2001 (Fig. 1).

Effect of storage duration on incidence of microflora on soybean seeds and subsequent seed germinability

Storage duration significantly ($P \leq 0.05$) influenced the incidence of only two fungal species namely *Fusarium semitectum* ($P = 0.023$) and *Rhizopus* spp. ($P = 0.019$). The incidence of *Fusarium semitectum* was higher on the seeds stored for 30 and 90 days, than on seeds stored for 60 days. On the contrary, the incidence of *Rhizopus* spp. was significantly higher on the seeds stored for 90 days (2.6%) compared to those stored for a shorter duration i.e., 30 or 60 days after harvest.

Pooled data for both seasons revealed that germinability of soybean seeds declined with storage duration averaging 86.3, 85.1, and 83.0% for seeds stored for 30, 60, and 90 days after harvest, respectively.

Table 3. The effect of seed moisture content on the germinability of soybean seeds grown during 2001 and 2002.

Seed moisture content (%)	Seasons		
	2001	2002	Mean
9.5%	86.81	91.02	88.90***
12%	73.56	78.93	76.26***
Mean	80.22***	85.00***	82.61ns
LSD 0.05	2.00		
CV (%)	10.9		

*** = significant at 0.1%.

Table 4. Summary of the effect of seed treatment with fungicide (Vitalax 200FF) on the incidence of fungal microflora on soybean seeds grown during the 2001 and 2002 seasons.

Fungal species	2001		2002		Across seasons		Across vitalax treatment		Across seasons and fungicide treatments		Statistics for combined seasons	
	+ Vit	-Vit	+ Vit	-Vit	2001	2002	+ Vit	-Vit			CV (%)	SED
	<i>Aspergillus flavus</i>	0.0	5.8	0.2	4.6	2.9	2.4	0.1	5.2	2.7		122.1
<i>Aspergillus niger</i>	0.0	6.5	0.2	5.3	3.2	2.8	0.1	5.9	3.0		126.9	0.4
<i>Alternaria</i> sp.	0.0	0.6	0.0	0.6	0.3	0.3	0.0	0.6	0.3		163.3	0.1
<i>Cercospora</i> sp.	0.0	0.6	0.0	0.4	0.3	0.2	0.0	0.5	0.3		219.3	0.1
<i>Cercospora kikuchii</i>	0.0	0.7	0.0	0.6	0.3	0.3	0.0	0.6	0.3		182.9	0.1
<i>Cladosporium</i> sp.	0.0	6.3	0.2	4.6	3.2	2.4	0.1	5.4	2.8		118.3	0.3
<i>Colletotrichum truncatum</i>	0.0	0.5	0.0	0.4	0.2	0.2	0.0	0.4	0.2		230.9	0.1
<i>Curvularia</i> sp.	0.0	0.5	0.0	0.5	0.3	0.3	0.0	0.5	0.3		182.9	0.0
<i>Fusarium equiseti</i>	0.0	0.3	0.0	0.3	0.1	0.2	0.0	0.3	0.1		110.2	0.1
<i>Fusarium moniliforme</i>	0.0	0.3	0.0	0.3	0.2	0.2	0.0	0.3	0.2		250.3	0.0
<i>Fusarium oxysporum</i>	0.0	0.4	0.1	2.4	0.7	1.2	0.1	2.9	1.5		188.1	0.3
<i>Fusarium semitectum</i>	0.0	0.4	0.0	0.3	0.2	0.2	0.0	0.4	0.2		247.1	0.0
Other <i>Fusarium</i> sp.	0.0	1.4	0.0	1.5	0.7	0.6	0.0	1.3	0.7		139.7	0.1
<i>Penicillium</i> sp.	0.0	8.5	0.2	4.0	4.3	2.1	0.1	6.3	3.2		151.5	0.5
<i>Phoma</i> sp.	0.0	1.0	0.0	0.7	0.5	0.4	0.0	0.8	0.4		142.7	0.1
<i>Phomopsis sojae</i>	0.0	1.1	0.0	1.0	0.6	0.5	0.0	1.1	0.5		117.2	0.1
<i>Rhizopus</i> sp.	0.0	3.3	0.2	4.2	1.7	2.2	0.1	0.7	1.2		140.1	0.2

Discussion

In this study, time of harvesting and fungicide treatment significantly influenced levels of fungal flora infestation and soybean seed germinability. The highest germinability was recorded on the earlier harvested seeds (R8) compared to the late harvested seeds (7-14 days after R8). Similarly, seed dressing with Vitavax 200 FF significantly reduced levels of seed-borne inoculum and improved germinability compared to the untreated seeds, although this depended on the level of seed moisture content in storage. Thus, the results support the hypothesis of the study that time of harvesting influences levels of seed infection by fungi and that seed dressing significantly reduces levels of the fungal infection. The low germinability associated with the delay in harvesting has been attributed to weather as well as fungal infection and accumulation of carbon dioxide (Mondragon and Potts, 1974; Pascal and Ellis, 1978).

Unlike results of other studies on groundnuts (*Arachis hypogea*) (Rao *et al.*, 1996), sorghum (*Sorghum bicolor*) (Gupta *et al.*, 1996) and onions (*Allium cepa*) (Singh *et al.*, 1996), the germinability of soybean seeds in this study was not significantly influenced by storage period. This may have been due to the short storage duration (90 days) as well as the fact that the seeds were promptly well dried soon after harvest. Similarly, the incidence of most fungal microflora on soybean seeds was not significantly affected by storage duration, varietal and seasonal differences. This is in agreement with Bankole (1993) who also observed that in melons, one of the varieties (*Citrullus lanatus*) maintained higher germination percentage for 4 months while the other (*Citrullus vulgaris*) only for 2 months. In the present study, variety did not affect the incidence of most fungal microflora indicating that the conditions which affect soybean seed viability are similar regardless of variety being handled. However, it must be noted that these recommendations refer to Nam 1 and Nam 2 soybean varieties.

The use of fungicides in seed dressing is a widely recognized control strategy against many seed-borne fungi. According to Gay (1970), fungicide treatment of seeds is a necessary requirement for the

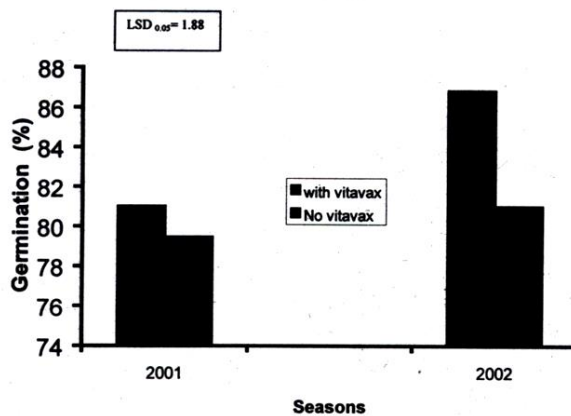


Figure 1. Effect of seed dressing with fungicide on soybean seed germinability. Only the main effect was significant.

protection of seedlings against seed-borne fungi before adult plant resistance develops. Although in this study fungicide treatment improved germinability and reduced the occurrence and the levels of fungal flora infestation, the effects were dependent on the seed moisture content.

As observed in many previous studies (e.g., Murrthy and Raveesha (1996) on soybean, Sachan and Agarwal (1994) on rice and Prokinova and Buresova (1996) on pea and barley), a number of fungal and bacterial pathogens associated with seeds of many crop species are responsible for the low germinability as well as poor quality seeds. The detrimental effects of seed-borne pathogens in soybean seeds are accentuated by long storage period under especially high moisture levels. The practical implication of our results therefore is that in order to produce soybean seeds of high quality or viability capable of long term storage, the seeds must be harvested at R8 growth stage, dried to 9.5% moisture content and dressed with appropriate fungicide before storage.

Conclusions

The major findings of this study are that: harvesting time influences the germinability of soybean seeds irrespective of the cultivar grown. Seed harvested at physiological maturity appear to store longer and exhibit higher germinability than those harvested after R8 stage. The R8 stage would of course depend on the maturity period of the variety, in which case harvesting date would be adjusted accordingly. Since high seed moisture content in storage resulted in increased fungal infection and reduced seed viability of soybean seeds, the seeds should be dried to the required moisture content soon after harvest. The following fungi were found associated with soybean seeds; *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* spp., *Cercospora* spp., *Cercospora kikuchii*, *Fusarium* spp., *Fusarium equiseti*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Phoma sojae*, *Phomopsis* spp., *Penicillium* spp., *Cladosporium* spp., *Curvularia* spp., *Fusarium semitectum* and *Colletotrichum truncatum*. Their incidence can be reduced significantly by harvesting soybean at harvest maturity (R8), proper seed drying and seed dressing with appropriate fungicides.

Arising from the above results it appears that in order to produce seeds of high quality capable of long storage, the seeds must be harvested at R8, dried to 9.5% and dressed with an appropriate fungicide.

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