Effect of seed age and varieties on seed quality of soybean (*Glycine max* (L.) Merrill) in Dangur District Metekle Zone, West Southern Ethiopia

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Abstract: This study was conducted in Dangur District at Pawe Agricultural Research Site during 2013 cropping season. The aim of the study was to identify the effect of seed age and varieties on physical, physiological and health quality of soybean. The experiment was done on two varieties (Belessa-95 and TGX) having two different ages (year one and year two). The study employed SAS computer software and the treatments significant differences were subjected to LSD (least significant difference) test for mean separation at ($p<0.01$) and (<0.05) significant levels. Analysis of variances showed that significant ($p\leq0.01$) differences were recorded among physical purity components and germination due to the main effect of seed age. The highest mean of analytical purity (61.88 %) was obtained in year one and the lowest mean (42.63) were recorded on year two. The highest mean of germination was (72.63%) on year one and lowest mean of germination (38.62) were recorded on year two. The highest mean density (5.94%) of Pseudomonas syringe was observed on the combination of TGX by year two and the lowest mean was recorded on same varieties (0.06%) for Pythium and Botrytis. Therefore among the two different soybean varieties (Belessa-95 and TGX) having the two different storage years (year one and two) those soybean varieties with one storage year were indicated maximum seed quality performance.
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1. Introduction

Soybean [*Glycine max* (L.) Merrill] belongs to the family Fabacesae sub family Papilionoideae and genus Glycine. The crop also does well in some areas as low as 500 m and as high as 1900 m above sea level that receives a well distributed average rain fall of 550 to 700 mm throughout the growing period [1]. The need for a long growing season and satisfactory soil moisture during flowering and pod filling are very important for higher yield of soybean. It grows best under good soil conditions. A fertile, medium textured soil usually is the best for the crop to perform [2]. Soybean varies in growth habit, height and has two types, determinate and indeterminate. The big inconspicuous, self-fertile flowers are born in the axel of the leaf and are white, pink or purple [3].

Soybean was first introduced to Ethiopia in 1950`s for nutritional value, multipurpose use and wider adoptability in different cropping systems [1]. It is a crop that can play major role as protein source for resource poor farmers of Ethiopia who cannot afford animal products. Besides, it can also be used as an oil crop, animal feed, poultry meal, soil fertility improvement and more importantly as income for the country [4]. Soybean has become a crop of growing importance in the country as it has demonstrated an increase in area from 1027 ha in 2004 to 11, 261 ha in 2010 under private peasant holdings with a production of 15824.4 tons with average productivity of 1.4 t/ha [5]. Because of constraints like disease, insect- pest, weeds, soil and agronomic factors, the average yield of soybean is low. Excess plant density reduces yield due to competition for water, sunlight, nutrient and space, which cause self thinning, branches and spindly stalks. Similarly, wider spacing rendered low yields due to decreased plant populations/unit area [6]; [7]. The recommended plant spacing for early and late varieties is 40x5cm and 60x5cm respectively [8]. It can grow very well from 1300 to 1800 m above sea level; where average mean annual temperature range between 20-25^{0}c and pH varies from 5.5 to 7. Studies also showed that the yield range from these areas was between 1000 to 2900 kg/ha depending on the management [9].

Longevity of stored seed of any crops considerably depends of the stored conditions, primarily in terms of air temperature and relative air humidity in storage. The seeds of many crops deteriorate at fast rate and lose the planting value. Ageing of the seed is a serious problem associated with oxidation of lipids increase in fat acidity and membrane integrity leading to deterioration [10]. The percentage of germination is an excellent indicator of growth potential of surviving seeds irrespective of factors for loss of viability [11] and accelerated ageing is an excellent predictor of seed storability [12]. The

unfavorable storage conditions (high air temperature and high humidity of air) accelerate seed deterioration, causing seed quality losses and therein lower germinability percentage of stored seed [13], [14] and [15]. Temperature and seed moisture content are the main factors influencing seed deterioration and viability loss in storage. Lower temperature and humidity result in delayed seed deteriorative process and aging there by leading to extended viability period.

Seed ageing is generally marked by reduction in vigor, viability, rate and capacity of germination, increased solute leakage and susceptibility to stresses and reduced tolerance to storage under adverse conditions. Thus, in oil crops, such as soybean and sunflower, auto oxidation of lipids and increasing the content of free fatty acids during storage period are the main reasons for rapid deterioration of seed as announced by [16], [17] and [18]. In Ethiopia seeds are stored for various periods and reasons; seeds are not sold after they are produced, seeds are not planted immediately after harvested due to unfavorable growing conditions, and seed market is variable and unpredictable. In view of the above, seed get aged. However, the effect of variable seed ages by varieties and spacing have not yet well assessed on quality of seed for subsequent utilization. Hence, this study was initiated to evaluate the effect of seed age and varieties on seed quality of soybean.

2. Materials and Methods

Description of the Site

Pawe Agricultural Research Center is located in north western part of Ethiopia. It is located in BeniShangul-Gumuz Region, Metekle Administrative Zone, and 573km far Addis Ababa at altitude of 1197 meters above sea level. The climate of Pawe is tropical hot humid with annual rain fall range from 1000-1500 mm concentrated in one season from May to October. Annual minimum and maximum temperature were 16^0c and 32.4 ^{0}c respectively [19]. The soil type of the area is Nitosol. The total amount of rainfall received during the experimental period (June to October) was 1112.3 mm [19].

Treatments

The treatments consisted of the seed age (year one and two) and varieties of soybean (TGX and Belesa-95). These varieties TGX and Belesa-95 released in 2003 and adaptable to several areas including Awassa, Gutin, Baco, Dedessa and Pawe etc. were used.

Data Collection

Seed quality parameters

i) Seed moisture content analysis

Moisture content determination was best done only if the seed was in an intact moisture proof container from which as much air as possible has been excluded. Before the working sample was drawn, the submitted sample was thoroughly mixed by either stirring the sample in its container with spoon or by placing the opening of the original container against the opening of a similar container and pouring the seed back and forth between the two containers. The drying period began at the time the oven turned to temperature 103^oc +/-2 for 17 hours. At the end of the prescribed period, the container was covered and placed in desiccators to cool for 30-45 minutes. After cooling, the container was weighed with its cover and contents, and then the moisture content was calculated by the following formula:

Where, M1 is the weight in grams of the container and its cover,

M2 is the weight in grams of the container, its cover and its contents before drying

M3 is the weight in grams of the container, cover and contents after drying.

ii) Physical Quality Analysis

a) Analytical purity

The single sample from each treatments amounting to 1kg was thoroughly mixed, and then divided by seed divider until 500 g were obtained. Each sample sorted out to five components including (i) pure seed, (ii) other crop seeds, (iii) inert mater, (iv) weed seed and (v) infected seeds. After analysis, the percentage of each fraction based on weight was recorded. Then, purity was calculated by the following formula:

b) Thousand seed weight

Thousand seed weight was determined by counting from pure seed fraction and weighing eight replicates of 100 seeds and there mean weight has taken and multiplied by 10.

iii) Determination of physiological quality

a) Standard germination test

To conduct germination test, four hundred seeds were counted at random from the well-mixed pure seed. Then, 100 seeds were planted from each replication in a sterilized sand media. The planted seeds were germinated at room temperature of 25^0c for 8 days. After 8 days, germination boxes were removed and the seedlings were evaluated following principles of [20]. Germinated seedlings were counted and divided into normal and abnormal seedlings. Also dead and hard seeds were recorded and calculated based on the final count. The results were expressed as mean percentage of normal seedlings, for each seed lot. Therefore, normal germination percentage was the average number of seeds that germinated as normal over the eight days period. Then, germination (SG) was calculated by the following formula:

b) Vigor test

Seed vigor reflects potential seed germination, field emergence and seedling establishment under different environmental conditions [21]. High vigor seed lots show rapid and uniform seedling emergence, leading to the production of vigorous plants and optimum stand establishment [22]. This may improve plant performance under normal and stressful conditions. The standard germination test only distinguishes between normal and abnormal seedlings. However, variation in seedling size and vigor are likely to occur within the category of "normal seedling", since initial growth was highly influenced by the seed, vigor was determined by the following parameters.

Shoot and root length of seedlings

The seedlings shoot and root lengths were measured after the final count in the standard germination test by randomly selecting ten normal seedlings from each replication. The shoot and root length were measured from the point of attachment to the cotyledon up to the tip of the shoot and root of the seedlings, respectively.

Dry weight of seedlings

The seedlings dry weights were measured after the final count in the standard germination test. Ten seedlings randomly selected from each replication were cut free from their cotyledons and weighed, then placed to dry in an oven at 120 ^{0}c for about 72hrs. The seedlings were dried and weighed to the nearest milligram and the average seedling dry weight was calculated.

Vigor index I and vigor index II

Two vigor indexes for each sample were calculated. Seedling vigor index I (VIG-I) was calculated by multiplying the standard germination with the average sum of shoot length and root length after eight days of germination; and vigor index II (VIG-II) was again calculated by multiplying the standard germination with mean seedling dry weight (drying at temperature of 120 ^{0}c for about 72hrs).

Speed of germination (SG)

Speed of germination is also another indicator used for assessing the vigor of seeds. One hundred seeds were taken from each sample and divided into four replicates and kept at 20 ^{0}c -22 ^{0}c for maximum of 12 days in the seed germinator. Normal seedlings were removed each day. Then, speed of germination (SPG) was calculated [23] by the following formula:

Field emergence index

A pot experiment was conducted using well prepared soil for emergence index (EI). Four hundred seeds planted from each variety by using Completely Block Design (CBD).The seedling emergence index was calculated and correlated with the physiological test results obtained from laboratory tests by dividing the number of seedlings emerged at each day with the number of days in which they were emerged [23] was calculated by the following formula:

iv) Accelerated seed aging test

The ageing process is normally accelerated by subjecting the seeds to high temperature and relative humidity in a chamber before standard germination. Four replicates of 420 seeds were exposed to 45°c and nearly 100% relative humidity for 72 hours [24]. The seeds were placed in jars on perforated plastic plates. The lower part of the jar was filled with 250 ml of distilled water in each plastic accelerated aging (AA) box (12 cm length x 14 cm width x 4 cm depth). There was no direct contact between water and the perforated plastic plate on which the seeds were placed. The jars were covered with the lid and sealed with paraffin wax to make it air tight. The jars were then placed in the accelerated aging chamber maintained at 45 ^{0}c temperature and 100% relative humidity for 72 hrs. The jars were removed after this stress period and the seeds with perforated plastic plate were cooled in desiccators for 30 minutes. After stress period, germination was conducted as in the standard germination test described by [25]. The accelerated seed aged ones were compared with control.

Seed Health Test

Seed-borne micro-organisms were identified using agar plate method. The method was used to detect fungi pathogens that could not be detected by blotter method. Four hundred seeds from each sample were pre-treated with 1% aqueous solution of sodium hypochlorite (NaOCl) for 10 minutes and then rinsed with sterilized water. The seeds were planted on potato dextrose agar in Petri dishes and incubated at 20 ^{0}c in 12 hours of alternating cycles of day light and darkness for seven days. After seven days the seeds were further incubated for additional four days in 12 hours cycle of darkness and daylight. Colonies and fruiting bodies of the micro-organisms were detected using stereoscopic microscope (morphological characteristics such as shape, color and size) and appropriate reference materials were used. The relative intensity of infection percentage of fungal genera and species were computed according to [26] was calculated by the following formula:

Where: ns= represent number of seeds infected by pathogens, N= Total number of seeds.

Data Analysis

The analysis of variance was performed following the procedure of [27] using the SAS computer software. The treatments significant differences were subjected to LSD (least significant difference) test for mean separation at ($p<0.01$) and (<0.05) significant levels.

3. Results and Discussion

Seed moisture content

The highest mean (11.5%) moisture content was recorded in year one, whereas lowest mean values (8.63%) was obtained in year two (Table 1).

Table 1: Main effect of seed moisture content of soybean varieties tested in 2013 crop season.

Treatments	Moisture content (%)
Age	
Year one	11.50[a]
Year two	8.63[b]
LSD (5%)	1.07
Variety	
Belessa-95	9.88
TGX	10.25
LSD (5%)	NS
CV%	9.40

Mean values within a column followed by the same letter(s) are not significantly different.

Determination of physical purity

Purity

Highest mean physical purity percentages were recorded in year one (61.88 %), while the lowest mean value (42.63%) was recorded on year two. The highest mean of other seed, inert matter, weed seed and infected seed were 13.38, 14.5, 14.63 and 14.88 were on year two and the lowest mean 10.25, 9.25, 9.0 and 9.25 were recorded on year one respectively (Table 2).

Table 2: Main effect of seed physical purity components of soybean varieties tested in 2013 crop season.

Treatments	PS	OS	IM	WS	INS
Age					
Year one	61.88[a]	10.25[b]	9.63[b]	9.00[b]	9.25[b]
Year two	42.63[b]	13.38[a]	14.50[a]	14.63[a]	14.88[a]
LSD (5%)	2.52	0.93	1.31	1.34	1.14
Variety					
Belessa-95	50.75	12.5	12.25	12.25	12.25
TGX	53.75	11.13	11.88	11.38	11.88
LSD (5%)	NS	NS	NS	NS	NS
CV%	5.32	13.50	9.44	14.09	10.06

PS = Pure seed, OS=other crop seed, WS = Weed seed, IM = Inert matter, NS=Infected seed. Mean

Values within a column followed by the same letter(s) are not significantly different.

Thousand Seed weight

The highest mean value (162.7) was recorded in year one, whereas lowest mean values (104.35) was obtained in year two (Table 3).

Table 3: Interaction effect of seed age and varieties on thousand seed weight on soybean.

Variety	Thousand seed weight(g)		
	Age		
	Year one	Year two	Mean
Belessa-95	161.7[a]	92.70[c]	127.20
TGX	163.7[a]	116.00[b]	139.85
Mean	162.7	104.35	
LSD (5%)	7.22		
CV %	4.96		

Means within a column followed by the same letter(s) are not significantly different ($p \leq .0.05$).

Determination of physiological quality

Standard germination

The highest mean value (72.63) was recorded in year one, whereas lowest mean values (38.62) was obtained in year two. In addition the highest mean for abnormal seedlings, dead seed and ungerminated seed was 19.74, 21.63 and 20 recorded in year-two (Table 4).

Table 4: Main effect of seed germination parameters of soybean varieties tested in 2013.

Treatments	NG	ABS	DS	US
Age				
Year one	72.63[a]	9.00[b]	9.25[b]	9.13[b]
Year two	38.62[b]	19.75[a]	21.63[a]	20.00[a]
LSD (5%)	3.29	1.37	1.23	0.63
Variety				
Belessa-95	53.38	15.62	16.0	15.0
TGX	54.88	15.75	15.0	14.37
LSD (5%)	NS	NS	NS	NS
CV%	5.43	8.75	7.42	7.74

NG=Normal germination, ABS= abnormal germination, DS = Dead seed, US= Ungerminated seed. Mean values

Within a column followed by the same letter(s) are not significantly different at 5%.

Seed vigor

The highest mean value 8.41, 3.35, 3.34 and 137.25 was recorded in year one, whereas lowest mean values 4.90, 2.66, 2.39 and 63.05 in year two. Among varieties the highest mean value were (3.23) on TGX and the lowest mean values was (2.79) on Bellesa-95 (Table 5).

Table 5: Main effect of seed vigor parameters of soybean varieties tested in 2013 crop season.

Treatments	SPG	VIG-II	SL	RL	DW	FI
Age						
Year one	8.41[a]	137.25[a]	3.35[a]	3.34[a]	1.83	3.14[a]
Year two	4.90[b]	63.05[b]	2.66[b]	2.39[b]	1.65	2.30[b]
LSD (5%)	0.55	12.27	0.32	0.45	NS	0.32
Variety						
Belessa-95	6.93	101.38	2.79[b]	2.86	1.80	2.63
TGX	6.38	98.93	3.23[a]	2.87	1.67	2.82
LSD (5%)	NS	NS	NS	NS	NS	NS
CV%	6.67	14.13	9.22	12.70	13.59	10.30

SPG= speed of germination, VIG-II= vigor index-I, SL=shoot length, RL=root length, DW=seedling dry weight.

Mean values within a column followed by the same letter(s) are not significantly different.

The highest mean was (505.7) recorded in year one and the lowest mean were (194.61) on year two (Table 6).

Table 6: Interaction effect of variety and age on seed vigor index one of soybean varieties in 2013crop season.

Variety	VIG-I		
	Age		
	Year one	Year two	Mean
Belessa-95	460.9[b]	191.71[c]	326.30
TGX	550.5[a]	197.50[c]	372.56
Mean	505.7	194.61	
LSD (5%)	35.31		
CV%	9.26		

Means within a column followed by the same letter(s) are not significantly different. VIG-I=vigor index-I.

Field Emergence index

The highest mean values were (3.4) field emergence index recorded in year one, whereas lowest mean values (2.30) was recorded in year two (Table 12).

Accelerated Aging

The highest mean of speed of germination, standard germination and vigor index two were 10.81,59.63, 59.25 and the lowest mean 7.72, 46.38, 41.35 were recorded in year one and two respectively. The result shows that there was difference on germination before and after aging. In the control maximum germination were (72.63) the minimum (38.63) on year one and two, respectively. While after aging seed the maximum were (59.63) and minimum mean value were (46.38) year one and year two (Table 7).

The highest mean value of vigor index one was on year one (668.24) the lowest mean were (426.05) recorded in year two (Table 8).

Table 7: Main effect of accelerated aging test of soybean varieties on SPG, SG and VIG-II in 2013 crop season.

Treatments	SPG	SG	VIG-II
Age			
Year one	10.81[a]	59.63[a]	59.25[a]
Year two	7.72[b]	46.38[b]	41.35[b]
LSD (5%)	0.91	3.45	4.1
Variety			
Belessa-95	9.09	52.75	48.58
TGX	9.44	53.25	52.03
LSD (5%)	NS	NS	NS
CV%	8.97	5.98	7.47

SPG= speed of germination, VIG-II =vigor index-II, SG=standard germination. Mean values within a column

Followed by the same letter(s) are not significantly different.

Table 8: Interaction effect of accelerated aging test on VIG-I on soybean varieties in 2013crop season.

Variety	VIG-I		
	Age		
	Year one	Year two	Mean
Belessa-95	615.1[b]	417.6[c]	516.35
TGX	721.4[a]	434.5[c]	577.95
Mean	668.25	426.05	
LSD (5%)	44.48		
CV %	7.46		

Means within a column followed by the same letter(s) are not significantly different at 5%.

Seed health

These fungi were detected using agar (PDA) plate test methods. Similarly, from two varieties of soybean, fungus and bacteria associated include *Aspergillus niger,* species of *Chaetomium, Fusarium oxisphorium, Rhyzoctonia bataticola, Penicillium, Pythium, Antracnose,* and *Botrytis* sp, *Pseudomonas syringae* were dominantly occurred on soybean cultivars. The result shows that highest density (5.94%) of seed-borne bacteria was observed and the lowest density was recorded in *Pythium, Botrytis* and *F. oxisphorium.* Among varieties, TGX-year two had highest density (22%) of anthracnose and the lowest mean were (1%) *Pythium* and *Fusarium oxisporium* that have the same result again on TGX year two (Table 9).

Table 9: Mean percentage occurrence of seed-borne pathogens on seeds of soybean varieties

Occurrence of seed-borne pathogens (%)

Variety	Age	Rhy	Asper	Oxis	Chet	Antr	Py	Bot	Fus	P.S
Bellesa-95	year1	5	0	0	0	10	0	0	0	0
Bellesa-95	year2	0	0	0	0	0	1	0	0	8
TGX	year1	8	0	0	0	0	0	0	0	4
TGX	year2	0	4	0	0	0	0	0	0	4
Bellesa-95	year1	6	0	0	0	0	0	0	0	2
bellesa-95	year2	0	0	0	0	0	0	1	0	16
TG	year1	0	0	0	0	0	0	0	0	9
TGX	year2	0	0	0	0	22	0	0	0	3
Bellesa-95	year1	4	0	0	0	0	0	0	0	4
Bellesa-95	year2	0	0	0	0	0	0	0	0	11
TGX	year1	0	0	1	0	0	0	0	0	5
TGX	year2	0	0	0	2	0	0	0	2	3
Bellesa-95	year1	0	0	0	0	0	0	0	0	2
Bellesa-95	year2	0	0	0	0	0	0	0	0	15
TGX	year1	0	0	0	0	0	0	0	0	4
TGX	year2	4	0	0	1	0	0	0	0	5
Mean		2	0.25	0.06	0.19	2	0.06	0.06	0.13	5.94

Rhy=Rhyzoctonia spp, Aspr = Aspergillus niger, Oxis =oxisphorium spp, *Chet= Chaetomium* spp, *Antr =Antracnose* spp, *Py = Pythium* spp, *Bot = Botrytis* spp, *Fus=fusarium, P.S,=psedomonace syringae.*

4. Discussion

Seed moisture content

Analysis of variance showed that main effect of seed age was significant (P≤0.05) on seed moisture content. While there was no significant effect due to main effect of varieties, intra row spacing, two way interaction and three way interaction. The highest mean (11.5%) moisture content was recorded in year one, whereas lowest mean values (8.63%) was obtained in year two (Table 1). These result will not agree with an increase in moisture content were due to disorganization of the cell membranes of aged seed observed by [28] and [29]. Considering the Ethiopian standards, however, all varieties meet the minimum moisture content requirement of 12 %.

Determination of physical purity

Purity

Analysis of variance showed that main effects of seed age significantly (p≤0.01) affected physical purity. The interactions effect of seed age and varieties have no significant effect on physical purity. The main effects of seed age significantly (p≤0.01) affected physical purity components such as (other seed, inert matter, weed seed and infected seed). Highest mean physical purity percentages were recorded in year one (61.88 %), while the lowest mean value (42.63%) was recorded on year two. The highest mean of other seed, inert matter, weed seed and infected seed were 13.38, 14.5, 14.63 and 14.88 were on year two and the lowest mean 10.25, 9.25, 9.0 and 9.25 were recorded on year one respectively (Table 2). These may be due to improper handling of seed during harvesting and storing of seed. Considering the Ethiopian seed quality standards, however, varieties were below the minimum physical purity requirement (97%).

Thousand Seed weight

The two way interactions effects of seed age and varieties significantly (p≤0.01) affected thousand seed weight, where as no significant difference by the main effect of seed age and varieties. The highest mean value (162.7) was recorded in year one, whereas lowest mean values (104.35) was obtained in year two (Table 3).

Determination of physiological quality

Standard germination

Considering the Ethiopian seed quality standards, however, varieties were below the minimum Standard germination requirement (85%). The result is in agreement with those of [30], [31], [32], [33] and [34]. Failure of aged seeds to germinate might be due to lipid peroxidation, mitochondrial dysfunction and less ATP production [35] and [36]. Many studies have shown that peroxidative changes in fatty acid composition of membrane lipids lead to massive dysfunction of cellular membranes associated with increasing viscosity and permeability of bilayers. Changes in membrane lipids therefore could account for the increase in solute leakage. Lipid peroxidation results in the loss of intact membranes in the mitochondrial cristae there by reducing ATP production during germination process. In short, reductions in germination were due to mitochondrial membrane leading to reduction in energy supply necessary for germination [35].

Seed vigor

The rate in decline is conditioned by several factors, including genetic constitution of the cultivar, condition of the seed, storage condition, and uniformity of seed lot. Loss of vigor can be thought as an intermediate stage in the life of the seed, occurring between the onset and termination of death. [37] related seed deterioration during storage to the expression of cotyledon necrosis and the performance of seedlings with cotyledon necrosis during germination. As seed vigor declined during storage, the level of cotyledon necrosis increased with the rate of deterioration.

Field Emergence index

This is due to loss of viability and vigor during storage. This result was in agreement with that of [38].

Accelerated Aging

These indicate that aging seed may absorb water that has high relative humidity and temperature so that these may deteriorate the seed when planting. The seed has already disorganized cell membrane during aging. Similar results were described by [39] who report that ageing slowed down the process of germination on pea seeds.

Seed health

Seeds are basic input of agricultural production and hence should be free from seed borne diseases. Improper storage conditions make the soybean seed vulnerable to storage fungi. According to [40], [41], [42] and [43]. Soybeans are susceptible to the attack of different pests and diseases during their growing season. Approximately one hundred bacterial, fungal, viral and nematode pathogens are known to attack soybeans. Within the major fungal diseases could be found brown leafspot (*Septoria glycines* Hemmi), frogeye leafspot (*Cercospora sojina* Hara), phytophthora root rot (*Phytophthora megasperma* Drechs), stem canker (*Diaporthe aseolorum*), purple seed stain (*Cercospora kikuchii* T. Tomayasu) and stem blight (*Phomopsis soja* Lehman) [41]. In addition, the major bacterial diseases are bacterial blight (*Pseudomonas syringae subsp. glycinea*), pustule (*Xanthomonas campestris pv glycinea*), and wildfire (*Pseudomonas syringae subsp. tabaci*). Similarly, several pathologists have reported the associated mycoflora of soybean during storage.

Storage fungi such as *Aspergillus niger, Phomopsis sp, Curvularia lunata*, *Colletotrichum sp, Fusarium oxysporum*, *Fusarium solani* and *Penicillium sp*. were found to be associated with soybean seeds according to [44]. Generally, seed borne microorganisms may either deteriorate seed in storage or transmit diseases to new crop early in the season. There is an established fact that contaminated and/or infected seed introduce foci of primary infection in the field which may cause disease out breaks. In this study, eight fungi and one bacteria were found to be associated with soybean seed.

These fungi were detected using agar (PDA) plate test methods. Similarly, from two varieties of soybean, fungus and bacteria associated include *Aspergillus niger,* species of *Chaetomium, Fusarium oxisphorium, Rhyzoctonia bataticola, Penicillium, Pythium, Antracnose,* and *Botrytis* sp, *Pseudomonas syringae* were dominantly occurred on soybean cultivars. The result shows that highest density (5.94%) of seed-borne bacteria was observed and the lowest density was recorded in *Pythium, Botrytis* and *F. oxisphorium*. Among varieties, TGX-year two had highest density (22%) of anthracnose and the lowest mean were (1%) *Pythium* and *Fusarium oxisporium* that have the same result again on TGX year two (Table 9). Hence, variety TGX was most susceptible to seed borne pathogen than variety Bellesa-95. Besides, in year two of TGX a maximum density (22%) of Anthracnose was recorded. This implies that when an increase in seed age there may be an increase in seed borne pathogen of soybean.

In general, production and proper storage of high vigor seeds are necessary for satisfactory crop production, particularly under stressful conditions. Sowing of aged seeds reduces quality parameters and the overall performance of the plant hence, use of fresh seed for planting is imperative. In any case seed sown should not exceed more than one year for soybean, therefore, so seed should be stored at optimum level moisture content for not more than one year. However, this tentative generalization should be validated by testing more spacing, different storage life and more varieties for more than one season to give a valid recommendation.

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