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Overcome Deterioration of Soybean Seed During Storage by Using Some Plant Extracts

El-Mowafy, M. R.
Seed Technology. Res. Sec. Field Crops Res. Institute, A.R.C.

ABSTRACT

This experiments was conducted at Seed Technology Research Unit, Mansoura city and Experimental Farm at Tag El Ez, Agricultural Research Station (+ 7 m altitude,31^{o} 36" latitude and 30^{o} , 57" longitude) to study the effect of treating soybean seed with some plant extracts(Moringa, Basil and Neem) as well as fungicide Vitavax-Thiram-200(Carboxin 37.5 % + Thoram 37.5 %), before storage on soybean seed germination , seedling vigor traits and field emergence after the different storage period (0, 6 and 18 months). The main results could summerized as-follows ; Vitavax treatment recorded the highest germination percentage (85%) and field emergence (75%) compared to other treatments.Treating soybean seed with Neem and Moringa extracts improved germination parameters and field emergence compared to untreated seed. Increasing storage periods decreased gradually germination parameters and seedling growth traits. Interaction between seed treatments and storage periods had significant effects on germination percentage and field emergence. From this study soybean seed (cv Giza 111) could be treated befor storage by Neam , Moringa extract instead of vitavax fungicide for maintenance seed germination and field emergence above 75 % after 6 months from storage.

Keywords: Soybean,Vitavax, Neem extract, Moringa extract, grmination, storage, field emergence.

INTRODUCTION

Seed storage is one of the important factors affecting the seed quality. Seed deterioration starts immediately after a crop has attained the physiological maturity stage (Martins *et al* , 2012). The extent of seed deterioration during storage depends on species, seed content, seed treatment, storage environment, durations of storage and initial quality of stored seeds. Maintenance of seed quality during storage is the most important challenging in the crop due to problem of quick loss of seed quality, where Kumar *et al* (2014) reported that number of biotic and abiotic factors influenced the storage potential of seeds and resulted in gradual seed deterioration.

Many studies revealed that the decrease in enzyme activities (Azadi and Younesi, 2013), loss in integrity of cell membrans Villiers (1980) and decreasing seed germination traits (Cabrera and Lansskara, 1995) and Heatherly *et al* (1995).Thus, in order to prevent seed quality deterioration during storage, several methods are being adopted such as seed treatments with suitable chemical fungicides as vitavax. Gupta *et al* (1989) reported that seed treatment with chemicals was very effective in maintaining seed quality because it hinders the activities of storage pests and fungi. But using chemical pesticides may affect human health and caused environmental contamination, so many researchers used plant products as seed protectants from storage pests. Research activities provide that Neem leaves and seeds have the ability to kill some disease causing by fungi, viruses and parasites (Khan *et al,* 1988, Munoz *et al* 2008 and Iqbal, 2014).

Moringa leaf extract also used by many studies as pre sowing seed treatment, where its leaves extract is considered to be rich with natural plant growth regulators , macro-nutrients and improve seed germination as well as seedling vigor traits (Nwangburuka *et al* 2013 and Soliman *et al* , 2017).Pre storage seed treatment with some studies refers using Moringa leaf extract as seed protectant during storage as Ilesanmi and Gungula (2013) and Abo EL-Dahab *et al* (2016). Nwangburuka *et al*, (2012) explained that pre-treatment of seeds before storage with Moringa concentration at 2.5 % reduces the possibility of fungal infection and also maintains seed viability and vigour.

Oyekale *et al* (2012) recommended that natural botanicals could be adopted for short and medium term storage of sesame seeds; as it maintained seed viability and seedling vigour optimally among other treatments. Sanjeet *et al.* (2012),studied effect of sweet basil extracts on germination and seedling growth of commercial crop plants. They found that maximum germination inhibition was observed in leaf extract (42%) and the minimum in seed extract (15%).The aims of this study was to investigate the effect of dressing soybean seed c.v Giza 111 in leaf plant extract i,e,Moringa, Neem , Basil as well as vitavax on seed quality and field emergence during storage.

MATERIALS AND METHODS

Two experiments were carried out at Laboratories of Seed Technology Research Unite Mansoura, Seed Technology Research Department and experimental farm of Tag-El-Ezz Research station ARC, Egypt from 2014 to 2016 year to study the effect of treating soybean seed (cv.Giza 111) with some plants extracts as Neem (*Azadirachta indica*) 3 ml/L , Moringa (*Moringa oleifera*)100g/500 ml and basil (Ocimum basilicum) 100 g/ 500 ml as will as Vitavax 2g/l on seed , seedling vigor and field emergence of soybean seed c.v. Giza 111 after storage periods 0, 6 and 18 months. Soybean seed was obtained from Legumes Crops Research Section at Field Crops Research Institute, Agriculture Research Center, Giza, Egypt.

Plant extraction

Moringa and basil leaves were dried and 100 g from each plant material was soaked in 500 ml of distilled water for 3 days befor filteration through two folds of whatman filter paper.The extarcts from each plant material was used for treating seeds.

Neemazal 2 % (Neem extract) was obtained from local market and used for treating seed with concentration of 3 ml/L.

Soybean seeds immersed for 5 minutes in plant extracts solution *i.e.* Nemazal (3 ml/L.), Moringa leaf extract (100g /500ml), basil leaf extracts (100/500ml) as

well vitavax (2 g/L), the treated seed and untreated seed (dry seed) were subjected to storage treatments (0, 6 and 18 months) in cloth bags at the first of November 2014 to May2016 under open- air condition of common storage.

Chemical composition of Moringa leaves extract according to U.S. Department of Agriculture (2013) is shown in Table (1).

Table 1. Chemical composition of Moringa leaves extract.

Nutrient	Amount in 100 grams
Energy (Kcal, MJ)	64
Protein (g)	9.4
Carbohydrates (g)	8.3
Fiber, crude (g)	2
Ca (mg)	185
P (mg)	112
Na (mg)	9
K (mg)	337
Fe (mg)	4
Zn (mg)	0.6
Cu (mg)	0.15
Thiamin (mg)	0.26
Riboflavin (mg)	0.66
Vitamin B-6 (mg)	1.2
Vitamin A (µg RAE)	378
Vitamin C (mg)	52
Vitamin E (mg)	25

The experiment was arranged in a completely randomized design with four replicates.

Germination percentage (G%): Eight replications of 50 seeds of each treatments were planted in plastic boxes of 40×20×20 cm dimensions and contained sterilized sand. The boxes were watered and kept at 25 C° in an incubated chamber for 8 days. Normal seedling (germination percentage) was counted after 8 days (final account) according to ISTA (1999).

G % = Number of normal seedling /number of seed tested X 100

Germination rate (GR): It was calculated according to the equation outlined by Bartlett (1937) .

Mean germination time (MGT): It was determined according to Ellis and Roberts (1981).

Seedling vigor traits: At the final count, ten normal seedlings from each replicate were randomly taken to measure seedling length.

Seedling length (shoot and root length cm): It was measured of ten normal seedlings 8 days after planting After then, the seedlings were dried in hot-air oven at 85 ^{0}C for12 hours according to obtain the seedling dry weight (g).

Seedling dry weight (g): Ten normal seedlings were weighted 8 days after planting according to Krishnasamy and Seshu (1990).

Accelerating Aging Test: It was carried out according to ISTA rules (1999), where one hundred seeds each in four replicates are tied in a fine muslin cloth, then placed in a jar on a wire mesh. The lower part of the jar is filled with water. There were no direct contact between water in the jar and the seed in the muslin cloth. The jar was covered with the lid and sealed with paraffin wax to make it air tight. The jar was placed in an accelerated aging chamber maintain at 40 ±2 C° for 48 hours. After this period the seed package was removed and cooled in a desecrator, then the seeds were subjected to normal germination test.

Electrical conductivity: It was evaluated according to the procedures outlined by Matthews and Alison (1987).The HANNA conductivity meter (Hi 80333) was used, where fifty seeds in three replications of tested samples were weighted to 2 decimal numbers and placed in 500 ml flask and 250 ml of distilled water was added. The flask was cover and placed in an incubator at a constant temperature of 20 C° for 24 hours after which the contents of the flask; were gently stirred. The electrical conductivity was measured in the solution after removing the seeds. The results were reported as (µ mhos/g seeds).

EC= Reading of replicate / weight of seeds

Field emergence: Four replications of 100 seeds of each treatments from storage seeds after 0, 6 and 18 months were sown in complete block design with three replicates reat Tag El-Eiz Research station. Sowing dates were 6 and 11 June during 2014 and 2016 seasons, respectively. All cultural practices concerning land preparation irrigation were done as recommended for growing soybean crop. Seedling emergence were recorded at 20 days from seed sowing.

All obtained data of characters were subjected to the statistical analysis according to the technique of analysis of variance(ANOVA) of completely randomized design, as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Data in Table 2 cleared that the effect of seed treatments on seed moisture content and seed germination traits. Plant extracts and Vitavax treatments had significant effect on seed moisture content and seed germination traits. Minimum values of seed moisture content was noticed with Moringa extract (10.10%) compared with non-seed treatment (10.72%). Plant extract increased germination percentage from 79% to 82%, while, Vitavax treatment recorded the highest germination percentage 85% compared to control 79% and plant extract treatments. Neem extract gave the highest values of germination rate (0.700) and speed of germination (6.2) compared to control 0.590 and 5.3, respectively. Regarding to mean germination time basil extract recorded minimum values (3.2 day), while, Vitavax treatment recorded the highest value (4.1 day). Seed treatment with some chemicals has been reported to be very effective in maintaining seed quality because it hinders the activities of storage pests and fungi (Gupta *et al*., 1989).The positive impact of Moringa is due to the presence of zeatin in Moringa, which is a natural plant hormone and belongs to the cytokinine group, involved in enhancing germination percentage (Makkar and Becker, 1996). These results are in agreement with Khatun *et al* (2010) who found that Neem treated of lentil seeds had the highest germination percentage (86.0% and 87.2%), dry weight (0.72 g and 0.78 g) and vigor index (62.3 and 68.0)in 2004 and2005 seasons, respectively. Oyekale *et al* (2012)showed that Neem leaf powder sesame seeds treatments had better mean seed germination of 89.53% compared to control (80.76). Maraddi (2002)observed that cowpea seeds treated with Neem leaf powder (5g/kg) recorded higher germination (39.5%) and vigour index (1072) compared to control (34.2% and 864,respectively) at the end of 10 months of storage period. noticed that maximum percentage of germination (86.33±0.1833%)of faba

bean seed was recorded in 2.5% MOLAE compared to control. Muhammad *et al* (2015) recorded Moringa leaf extract priming reduced time to start emergence (1.23 days) and time taken to 50% emergence (2.81 days)of soy bean seedas compared with seeds that were not subjected to any priming technique (control).

Table 2. Effect of plant extracts on moisture content and germination traits of soy bean seed quality

Characters / Seed Treatments	Seed moisture content %	Germination percentage (%)	Germination rate	Mean germination time (day)	Speed of germination
Control	10.72	79	0.590	3.6	5.3
Vitavax	10.32	85	0.670	4.1	6.1
Neem extract	10.50	82	0.700	3.4	6.2
Basil extract	10.31	79	0.680	3.2	5.7
Moringa extract	10.10	81	0.640	3.4	5.9
LSD at 5%	0.05	4	0.030	0.2	0.9

Table 3 shows thatplant extracts significantly effect on field emergence, germination after aging, electrical conductivity and seedling growth. The highest means of field emergence (76 and 73%), germination after aging (57 and 55%), seedling length (19.3 and 18.2 cm)and seedling dry weight (0.730 and 0.720 g) were noticed at Vitavax and Neem extract, on the other hand these treatments recorded minimum reading of electrical conductivity (0.075 and 0.078 mmohs\cm), respectivily compared to control and other seed treatments.Moringa leaves have high zeatin content which plays an important role in cell division and cell elongation (El Awady, 2003 ; Taiz and Zwiger, 2006). The bioactivity of Neem extracts has been attributed to various compounds found in seeds and leaves such as nimbin, nimbidin, salannin but the most important of these compounds is azadirachtin (Lale and Abdulrahman, 1999).These results are supported by Muhammad *et al* (2015)they recorded that Moringa leaf extract priming gave the highest root length (17.5 cm) and shoot length (19 cm) and highest root weight (0.19 g) and shoot weight (0.23 g) of soybean as compared with seeds that were not subjected to any priming technique (control). They also noticed that maximum root length (7.00±0.270cm) and plumal length (13.00±0.114 cm) were recorded in 2.5% MOLAE compared to control.

Table 3. Effect of plant extracts on field emergence, germination after aging, electrical conductivity and seedling growth traits of soy bean seed quality

Characters / Seed Treatments	Field emergence (%)	Germination after aging (%)	Electrical conductivity	Seeding length (cm)	Seedling dry weight (gm)
Control	56	31	0.096	17.5	0.685
Vitavax	76	57	0.075	19.3	0.730
Neem extract	73	55	0.078	18.2	0.720
Basil extract	70	45	0.087	18.0	0.721
Moringa extract	72	51	0.079	18.1	0.715
LSD at 5%	4	4	0.014	1.3	0.056

Khatun *et al* (2010) found Neem extract treated lentil seeds had the highestgermination percentage (86.0% and 87.2%), dry weight (0.72 g and 0.78 g) andvigor (62.3 and 68.0)in 2004 and2005 seasons, respectively.

Data in Table (4) showed the effect of storage periods on seed quality as measured by seed moisture content and germination traits under laboratoryconditions. Seed moisture content gradually decreased by increasing storage periods from 0 to 18 months, on the other hand, mean germination time was increased by increasing storage periods from 0 passed to 6until reach to 18 months. Stored seeds until 6 months had germination percentage above the egyptian minimum seed certification standard (75%). Prolonging storage period for 18 months affected adversely on seed germination percentage so that the seeds did not meet the minimum seed certification standard.The same trained was noticed at germination rate and speed of germination. The maintenance of seed quality during storage is the most important challenging in the crop due to problem of quick loss of seed quality. During storage, number of biotic and abiotic factors influenced the storage potential of seeds and resulted in gradual decrease seed quality deterioration and ultimately death of the seeds (Kumar *et al*., 2014). Enzyme activity are significantly decreased by increase in storage time (Azadi and Younesi, 2013).Such conclusion is in conformity with the findings of Sheidaei *et al* (2014), they found that germination percentage and germination rate significantly decreased by increasing the storage periods,the soybean seed should not be used for seed purposes more than 18 months because of descending trends of seed quality.Singh *et al* (2016), studied effect of storage period control (0 year), short term storage periods (1-3 years) and mid term storage period (4-6 year) on seed vigour and germination of soybean, they noticed that soybean seeds stored for mid term storage showed 0-15% germination while for short term stored seeds, the germination percentage was 40-70%.

Table 4. Effect of storage periods on moisture content and germination traits and field emergence of soybean seed quality

Storage periods	Seed moisture content %	Germination percentage (%)	Germination rate	Mean germination time (day)	Speed of germination
0 month	10.88	88	0.710	3.2	6.9
6 month	10.23	81	0.650	3.4	5.9
18 month	10.10	73	0.590	4.1	4.7
LSD at 5%	0.04	3	0.030	0.2	0.7

Data in Table 5 shows that storage period significantly affected by field emergence, germination after seed aging, electrical conductivity of seed leakage and seedling growth. Field emergence, germination after seed aging, seedling length and seedling dry weight gradually decreased by increasing storage period from 0 to passed to 6 untile rech to 18 months, on the other hand electrical conductivity of seed leakage was increased. The decrease of germination and seedling growth traits during storage may be number of biotic and abiotic factors influenced the storage potential of seeds and resulted in gradual decrease in seed quality , also deterioration and ultimately death of the seeds (Kumar *et al*., 2014). Enzyme activity are significantly decreased by increase in storage time (Azadi and Younesi, 2013).Similar observation were reported by Singh *et al*. (2016) they reported that soybean seeds showed significant increase in electrical conductivity (172.22 ± 4.1 µS cm-1 g-1 for 0 year to 470.34 ± 4.8 µS cm-1 g-1 for 6years).Sheidaei *et al*. (2014) found that germination percentage, seedling length and seedling vigor index significantly decreased by increasing the storage duration,the soybean seed should not be used for seed purposes more than 18 months because of descending trends of seed quality which reach lower than Egyptian minimum seed certification stanadard(75 %).

Table 5. Effect of storage periods on field emergence, germination after aging, electrical conductivity and seedling growth traits of soy bean seed quality

Storage periods	Field emergence (%)	Germination after aging (%)	Electrical conductivity	Seeding length (cm)	Seedling dry weight (g)
0 month	79	56	0.038	20.7	0.869
6 month	72	49	0.072	18.5	0.768
18 month	58	38	0.142	15.9	0.729
LSD at 5%	3	3	0.012	1.1	0.044

Data in Table 6 cleared that the interaction between seed treatments and storage period signficantly effect on germination percentage under laboratory and field conditions. The highest means of germination percentage (92, 95 and 78%) and (89, 82 and 76%) andfield emergence (85, 77 and 67%) and (83, 76 and 62%) noticed with Vitavax and Neem leaf extract treatments at 0, 6 and 18 months, respectevily compared to control. Neem leaf extract recorded germination percentage at 18 months 76%, on the other hand,Neem and Moringa leaf extract gave the highest germination percentage under field conditions 76 and 78% at 6 monthsabove Egyptian seed certified75%.The bioactivity of Neem extracts has been attributed to various compounds found in seeds and leaves such as nimbin, nimbidin, salannin but the most important of these compounds is azadirachtin (Lale and Abdulrahman, 1999).The nature of the inhibitory effect of allelochemical to seed germination could be attributed to inhibit water absorption which is a precursor to physiological processes that should occur in seed before germination is triggered (Oyerinde *et al*., 2009; El Awady 2003; Taiz and Zwiger, 2006) pointed out that Moringa leaves have high zeatin content which plays an important role in cell division and cell elongation.The present results were similar to those previously reported by Maraddi (2002) who observed that cowpea seeds treated with Neem leaf powder (5g/kg) recorded higher germination (39.5%) and vigour index (1072) compared to control (34.2% and 864, respectively) at the end of 10 months of storage period.Oyekale *et al* (2012) showed that NEEM LEAF POWDER sesame seeds treatments had better mean seed germination of 89.53% compared to control (80.76).

Table 6. Effect of interaction between seed treatments and storage periods on germination percentage and field emergence of soy bean seed quality

Characters	Germination percentage (%)			Field emergence (%)		
Seed treatments	0 Month	6 month	18 month	0 month	6 month	18 month
Control	87	80	69	69	60	38
Vitavax	92	85	78	85	77	67
Neem extract	89	82	76	83	76	62
Basil extract	87	80	70	77	69	66
Moringa extract	89	80	74	81	78	57
LSD at 5%		4			5	

Data in Table 7 show that the interaction between seed treatments and storage periods priming had significant effects on germination seed after aging and germination speed index. Neem leaf extract recorded the highest means of germination after aging (67, 58 and 48%) and germination speed index (7.3, 6.3 and 5.1) at 0, 6 and 18 months of storage, respectively compared to all seed treatments and non seed treatments. Decrease of germination percentage in aged seeds may be due to reduction of α-amylase activity and carbohydrate contents (Bailly, 2004). Akhter *et al*. (1992) suggested that decreasing in germination percentage was related to chromosomal aberrations that occur under long storage conditions. Such conclusion is in conformity with the findings of Babariya *et al* (2016) who reported that stored mungbean seed in normal condition with seed treatment (Neem seed kernel powder at 5 to 10 g/kg seed or Neem Cake at 5-10 g/kg seed) for years without deterioration in germination and seedling vigour.

Table 7. Effect of interaction between seed treatments and storage periods on germination after aging and germination speed index of soy bean seed quality

Characters	Germination after aging (%)			Germination Speed index		
Seed Treatments	**0 Month**	**6 month**	**18 month**	**0 month**	**6 month**	**18 month**
Control	62	53	38	7.1	6.0	4.8
Vitavax	67	54	42	7.2	6.3	4.8
Neem extract	67	58	48	7.3	6.3	5.1
Bsil extract	51	48	35	6.9	5.7	4.5
Moringa extract	33	32	27	6.3	5.3	4.3
LSD at 5%		4			1.1	

CONCLUSION

This study has shown that natural plant extracts are better alternatives for seed preservation during post-harvest. Among the tested plant extracts, Neem extract gave a best resultes for all tested parameters under field and laboratory at all storage periods followed by Moringa. All plant extracts are organic and safe in terms of human health and environment compared to the inorganic Vitavax. In view of this, we recommend use of Neem or Moringa extracts in preserving soybean seeds.

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التغلب علي تدهور تقاوي فول الصويا أثناء التخزين بإستخدام بعض المستخلصات النباتية

محمد رضا عبد السميع الموافي

قسم بحوث تكنولوجيا البذور- معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية

بهدف دراسة تأثير معاملة تقاوي فول الصويا (صنف جيزة 111) قبل التخزين بالمبيد الفطري فيتافاكس (2جرام/لتر) وبعض المستخلصات النباتية (مستخلص لأوراق المورنجا، النيم والريحان) على جودة التقاوي والتكشف الحقلي لها بعد فترات تخزين مختلفة(صفر,6، 18 شهر).أجريت تجربة حقلية لقياس نسبة التكشف الحقلي بمحطة البحوث الزراعية بتاج العز وتجربة معملية بوحدة بحوث تكنولوجيا البذور بالمنصورة- معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعيةخلال الفترة من نوفمبر 2014حتي مايو2016م . ويمكن تلخيص اهم النتائج كما يلي: سجلت معاملة تقاوي فول الصويا قبل التخزين بمبيد الفيتافاكس أعلي القيم لنسبة الإنبات في المعمل ونسبة التكشف الحقلي مقارنة بالمعاملات الأخري. أدت المعاملة بمستخلص أوراق النيم والمورينجا الي الحصول علي أعلي القيم لمعدل الإنبات، سرعة الإنبات، التكشف الحقلي، نسبة الإنبات بعد التخزين وكذلك طول البادرة والوزن الجاف للبادرات ,بينما سجلت تلك المعاملات أقل القيم من محتوي البذور من الرطوبة، متوسط زمن الإنبات و التوصيل الكهربي لمنقوع البذور مقارنة بالبذور الغير معاملة. ادت زيادة مدة التخزين من صفر الي6 شهر إلى إنخفاض تدريجي في نسبة الانبات وقوة البادرات وزيادة في متوسط زمن الإنبات ودرجة التوصيل الكهربي لمنقوع البذور.كان التفاعل بين معاملات التقاوي وفترة التخزين تاثيرمعنوي علي كل من نسبة الإنبات، نسبة التكشف الحقلي، نسبة الإنبات بعد التدهور (إختبار الشيخوخة) ودليل سرعة الإنبات . من خلال هذه الدراسة يمكن التوصية بمعاملة تقاوي فول الصويا صنف جيزة 111 قبل التخزين بمستخلص اوراق النيم أو المورينجا بدلاً من الفيتافاكس للمحافظة علي جودة التقاوي والوصول لتكشف حقلي اعلي من 75% بعد فترة تخزين 6 شهور.