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Alleviation of Peanut Seed Deterioration during Storage using Biotic and Abiotic Agents

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ABSTRACT

In order to reduce the peanut seed deterioration and improve seed quality during storage a laboratory experiments were conducted. Seeds were subjected to different storage periods (0, 2, 4 and 6 months) using biotic (*Saccharomyces cerevisiae* extract at 25, 50 and 100%) and abiotic (citric and salicylic acids at 10, 15 and 20 mM) agents. After 6 months storage period of the treated seeds the viability of peanut seeds was evaluated under greenhouse conditions. The seed health showed inhibitory effects of biotic and abiotic agents on presence of different fungal genera in all storage periods, *Saccharomyces cerevisiae* then salicylic acid were most effective. Increasing storage periods from zero to six months significantly decreased germination percentage, germination energy, germination index, seedling length, seedling vigor index, dry weight of 10-seedlings as well as dead and rotted seeds, rotted seedlings and survived healthy seedlings and seed oil, protein and carbohydrates percentages. Treating peanut seeds with 100% *Saccharomyces cerevisiae* improved germination criteria, seedling characters and seed chemical characters as well as survived seed health and minimized the died seeds%, rotted seeds% and rotted seedlings as compared with the control under all storage periods. Peanut seeds free from aflatoxin B_1 were detected in the moderate and high concentrations of both biotic and abiotic agents. The maximum growth parameter and physiological characters (chlorophylls a and b, carotenoids and total phenols) were recorded from seed treated with 100% *Saccharomyces cerevisiae* extract after six months storage under greenhouse conditions. It could be recommended to use 100% *Saccharomyces cerevisiae* extract or 15 mM salicylic acid as seed treatment to improve seed quality and reduce deterioration of peanut seeds under storage.

Key words: *Arachis hypogaea* L., storage, seed deterioration, rotted seedlings, germination, yeast, citric acid, salicylic acid, conolization

INTRODUCTION

During storage, seed deteriorates rapidly and as a result substantial loss in terms of vigor and germination ability occurs particularly under humid conditions (Reddy *et al*., 2009). Incidence of seed interacting with fungal pathogen during harvest period is higher under humid conditions which leads to rapid rate of deterioration during storage.

Peanut (*Arachis hypogaea* L.) is one of the most important fabaceous crops in Egypt for both exportation and locally consumption. Peanut seeds contain 45-53% oil that is rich in monounsaturated fatty acids and don't contain cholesterol (Higgs, 2003). It also contains a fair amount of protein I(25-32%) and its products have found wide acceptance as food through out the

world. It is available in many countries, particularly those regions where protein deficiencies exist (Passone *et al.*, 2009). Also, peanut contain certain bioactive compounds that may play a role in the reduction of the risk for development of chronic diseases such as cancer, diabetes and coronary heart diseases. These components, such as isoflavones and trans-resveratrol, have been previously identified and quantified in peanuts (Liggins *et al.*, 2000).

An important factor contributing to low yield is disease attack by different microorganisms. Peanut is prone to be attacked by more than 55 pathogens, including fungi, bacteria, viruses, mycoplasma, nematodes and parasitic flowering plants, among which fungal diseases cause the majority of economic losses of yield (Podile and Kishore, 2002). Due to presence of peanut pods in soil, it is vulnerable crop for *Aspergillus* sp. especially at preharvest and subsequently at postharvest. *A. flavus* invades peanut producing four types of aflatoxins, viz., B_1, B_2, G_1 and G_2. Of them, B_1 is the most toxic form followed by G_1, B_2 and G_2 (Sudhakar *et al.*, 2009). Harvesting peanuts that contain fungal propagules of aflatoxigenic fungi can significantly decrease the quality and economic value of the product. Peanuts are harvested with moisture contents between 18 and 25% and subsequently dried 12-14%. Sometimes the process is inefficient and the environmental conditions cause rapid aflatoxin production (Wilson, 1995). Aflatoxins are associated with the high incidence of primary liver cancer (Gong *et al.*, 2004; Jiang *et al.*, 2005; Turner *et al.*, 2007).

Passone *et al.* (2007), Passone *et al.* (2008) and Passone *et al.* (2009) pointed out that fungal population significantly affected by *Aspergillus* group flavi inoculum, tissue type, sampling period, antioxidants treatment and their interactions. *Penicillium, Aspergillus* and *Fusarium* spp. were the most common genera detected on peanut seeds. *Aspergillus flavus* was the most frequently isolated species and there was significant difference between its population in the control and treated peanuts. No aflatoxins detected in any of the control or treated samples during storage.

Temperature and moisture content are the most important factors controlling seed longevity during seed storage (Vijay *et al.*, 2009). However, both colonization of peanuts by fungi and the high seed lipid content lead to the acceleration of seed deterioration during storage by decreasing germination criteria and vigor index (Novas and Cabral, 2002).

Environmental conditioning and the use of chemicals to control the plant diseases are expensive remedy and may also reduce populations of beneficial microorganisms, thus the use of safe chemicals control has become more attractive (Cook, 1993). There are numerous reports demonstrating that resistance can be systematically induced by chemical substrates (Khalifa *et al.*, 2007). Induced disease resistance can be defined as the process of active resistance dependent on the host plant physical or chemical barriers activated by biotic or abiotic agents (Kloepper *et al.*, 1992; Walters *et al.*, 2007).

The *Saccharomyces cerevisiae* gave high control of *Aspergillus carbonarius* and *A. niger* which produce ochratoxin A in grape (Bleve *et al.*, 2006). Lassois *et al.* (2008) stated that the antagonistic activity of two yeast strains, *Pichia anomala* and *Candida oleophila* on the parasitic complex of banana crown rot. Abiotic agents are molecules that can neutralize harmful oxygen radicals. Normally the plant can handle free radicals but if antioxidants are unavailable, or if the free-radical production becomes excessive, damage can occur (Shahda, 2001).

This investigation is an attempt to study the role of biotic i.e., yeast (*Saccharomyces cerevisiae*) extract and abiotic (citric and salicylic acids) in decreasing peanut seed deterioration during storage.

MATERIALS AND METHODS

Laboratory experiments were conducted at Seed Technology Research Unit in Mansoura, Dakahlia Governorate, Field Crop Research Institute, Agricultural Research Center, Egypt during

2009 and 2010 years to reduce of peanut seed deterioration under different storage periods (0, 2, 4 and 6 months) using biotic (*Saccharomyces cerevisiae* extract at 25, 50 and 100%) and abiotic (citric and salicylic acids at 10, 15 and 20 mM) agents. Also, pot experiment was carried out under greenhouse conditions for 30 days during summer season of 2010 at greenhouse of the Agriculture Directorate of Mansoura, Dakahlia Governorate, Egypt to evaluate the response of peanut to the tested previous treatments after six storage months.

Source of seeds and tested chemical: Peanut seeds (Giza 6 cultivar) were obtained from Department of Oil Crop Research, Agricultural Research Center, Egypt after harvest. Citric and salicylic acids were obtained from Al-Gomhoria Company, Egypt.

Isolation and identification of yeast: Commercial bread yeast was used as a source for the isolation of *Saccharomyces cerevisiae*. The most appropriate medium for this purpose was the modified Saborou medium (Savova and Nikolova, 2002) with the following composition (w/v); dextrose 4%, peptone 1% and agar 2%. pH was adjusted at 4.0 for griping the growth of the other microorganisms. After 72nd h cultivation at 25-28°C single, morphologically well-formed colonies were isolated. The appropriate one was re-cultivated several times for purity. The isolated strain was identified as *S. cerevisiae* 16°C according to the procedures described by Barnett *et al.* (1990) and in consultant with Department of Microbiology, Soils, Water and Environment Research Institute, ARC, Giza, Egypt.

Preparation of *S. cerevisiae* extract: Partial extraction of *S. cerevisiae* (Meyen ex E.C Hansen) was prepared by growing the yeast on plates of malt extract agar medium for 4 days at 28°C. The resulted cells were scraped gently with Pasteur pipet using 10 mL of sterilized water. The cell suspension was adjusted to 10^{11} cell mL^{-1} and kept under freezing for 24 h to allow partial breaking of the cell wall. After melting, the obtained was considered as 100% concentration of yeast extract.

Seed treatments: Samples of seeds were individually sprayed with distilled water (wet control), yeast extract (25, 50 and 100%), Citric acid (10, 15 and 20 mM) and Salicylic acid (10, 15 and 20 mM). The untreated seeds (dry seeds) served as control. All treatments were kept at laboratory temperature. After spraying, the seeds were surface-dried in an incubator with forced air circulation for 48 h on filter paper at a temperature of 25°C to return to original moisture 12-14% (on dry weight basis). The seeds were stored in cotton bags (500 g for each one) and kept in laboratory conditions for period of zero, two, four and six months.

Laboratory experiments

Seed Health Testing (SHT): Detection of seed-borne mycoflora was carried out following the procedures published by the International Seed Testing Association (ISTA, 1996). Two hundred seeds from each sample and the two controls were tested using the standard blotter. Ten seeds were plated in 11 cm diameter Petri-dish containing three layers of water-soaked blotters using sterilized tap water. The plates were incubated at 20±2°C for 7 days under 12 h alternating cycles of cool white fluorescent light and darkness. Plates were examined under a stereoscopic binocular microscope (6-50X) for the presence of seed-borne fungi and to study their habit characters. When necessary, the compound microscope was used for confirming the identification after having examined the morphology of conidia and conidiophores. Fungi presented on seeds were identified

by means of comparison with the description sheets of Commonwealth Mycological Institute, Kew, Surrey, England (CMI), Danish Government Institute of Seed Pathology (DGISP) publications as well as publications of Raper and Fennell (1965), Ellis (1971), Chidambaram *et al.* (1973) Moubasher *et al.* (1977), Booth (1985), Burrges *et al.* (1988) and Singh *et al.* (1991).

Germination criteria: Treated and untreated seeds were subjected to standard germination test as the rules of International Seed Testing Association (ISTA, 1985). Counts of germinating seeds were taken daily up to ten days after the start of germination. Germination Energy (GE) was recorded as the percentage of germinating seeds at 4th days after plantation is relative to the total number of seeds tested (Ruan *et al.*, 2002). The Germination Index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983).

Disease assessment: The dead seeds and rotted seeds% (ungerminated seed) as well as rotted seedlings% and survival seedlings were recorded for each storage period.

Seedling characters: Treated and untreated seeds were sown in sterilized sand with the same previous method to determine the germination % and seedling characters after ten days. Germination percentage was defined as the total number of normal seedlings at the end of the test. Seedling length (cm) were determined from 10 normal seedlings and then dried in a forced air oven at 70°C for 48 h to obtain seedlings dry weight (g) under laboratory conditions. The Seedling Vigor Index (SVI) was calculated according to following formula:

$$SVI = \text{Seedling length (cm)} \times \text{germination percentage}$$

Seed chemical analysis: Seed samples collected periodically from each treatment were oven dried, ground finely for chemical analysis. Seed oil percentage (%) was determined after extraction with Soxhelt`s apparatus using petroleum hexane as an organic solvent according to AOAC (1998). Seed nitrogen percentage was estimated by using micro Kjeldahel apparatus and multiplied by the converting factor (6.25) to get seed protein percentage (Jackson, 1962).

Total soluble carbohydrates were extracted from 2 g dry matter by ethanol (70%) for overnight at laboratory temperature (Kayani *et al.*, 1990) then filtered through filter paper Watman No. 1. Total soluble carbohydrates were determined by anthrone method (Sadasivam and Manickam, 1996) through adding 3 mL anthrone reagent to 0.1 mL filtrate, then heated for 10 min. in a boiling water bath cool rapidly and the optical density of the developed green colour was read at 630 nm by spectrophotometer.

Seed aflatoxin determination: Aflatoxin content in the seed was determined after six months of storage .The extraction of aflatoxin was conducted according to AOAC (1998). Aflatoxin B_1 was determined according to Singh *et al.* (1991) using thin layer chromatographic technique in the Mycotoxin Laboratory, Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt.

Greenhouse experiment

Morphological characters: Samples of each treatment were cultured in plastic pots (25 cm diameter) which were filled with 2 kg (2 sand: 1 clay). Ten peanut seeds were sown in 1st April 2010

at 21.3°C and relative humidity 58% in each pot. After germination (10 days) the plants were thinned to leave only three seedlings. The experiment was watered using equal amount of water per pot each time. At the end of the experiment, shoot and root length (cm) and dry weight of shoot and root (g) were measured.

Physiological characters: At 30 days from planting, the blade of the second leaf from tip (terminal leaflet) was taken to determine photosynthetic pigments (chlorophyll a and b and carotenoids) which were extracted with methanol (Robinson and Britz, 2000) and determined according to Mackinney (1941). Total phenols were determined in fresh shoot using the folin-ciocalteau reagent according to Singleton and Rossi (1965).

Statistical analysis: Statistical analysis was performed using analysis of variance technique by means of "MSTAT-C" computer software package for the factorial completely randomized design as published by Gomez and Gomez (1984). Using the Least Significant Difference (LSD) test compared differences among treatment means at the level of 5% of probability.

RESULTS

Laboratory experiments

Seed health testing: The mycological survey of peanut seed samples showed the occurrence of nine principal genera of filamentous fungi i.e., *Alternaria, Aspergillus, Botrytis, Cephalosporium, Fusarium, Penicillium, Rhizoctonia, Rhizopus* and *Verticillium* (Table 1). The fungal population of peanut seeds increased with increasing storage period from 0 to 6 months. Fungal populations lowered in check dry seeds than check moist seeds, the highest fungal colonizations occurred under check moist after 6 storage months. Generally, Aspergilli recorded the superiority in frequency followed by *Fusarium* then *Rihizopus*. *A. flavus*, then *A. niger* recorded the highest frequency in all storage periods. Inhibitory effects of biotic (*S. cerevisiae*) and abiotic (citric and salicylic acids) agents on the presence of different fungal genera were observed in all storage periods. These effects increased with increasing concentration of different agents. *S. cerevisiae* came first followed by salicylic acid while citric acid came late. There is no significant effect for both biotic and abiotic agents on total fungal population counts at 0 time of storage.

Germination criteria: Table 2 shows the effects of storage periods, biotic and abiotic agents and their interactions on germination criteria, i.e., germination%, germination energy and germination index of peanut seeds. The germination% as well as its energy and index decreased significantly with increasing storage periods. The highest reduction in these parameters occurred after six months from storage. Treating peanut seeds with tested agents significantly increased germination percentage as well as its energy and index as compared with dry and wet check. Yeast extract at 100% followed by 50%, then salicylic acid at 15 mM gave the highest values of these parameters. Meanwhile, the interactions between storage periods and any of treatments had no significant effect on germination criteria.

Disease assessment: Table 3 showed that dead and rotted seeds percentages were significantly affected by the interaction between storage periods and inducer agents. The maximum of dead and rotted seeds percentages was recorded at 6 months of storage with untreated seeds (wet

Table 1: Frequency (%) of seed-borne fungal population as affected by the tested agents at different storage periods

Treatments	*Alternaria alternata*	*A. flavus*	*A. niger*	*A. ochraceous*	*A. oryzae*	*Botrytis cinerea*	*Cephalosporin* sp.	*Fusarium moniliforme*	*Fusarium oxysporum*	*Fusarium semitectum*	*Fusarium solani*	*Penicillium* sp.	*Rhizoctonia solani*	*Rhizopus* sp.	*Verticillium* sp.
0 Month															
Check (dry)	9.50	63.50	45.50	12.70	10.90	10.02	6.37	23.43	37.37	5.60	46.40	32.90	4.80	24.07	6.87
Check (wet)	9.60	64.14	45.96	12.83	11.01	10.30	6.43	23.67	37.75	5.66	46.87	33.23	4.85	24.31	6.94
S. cerevisiae (%)															
25	9.40	62.80	45.00	12.56	10.78	10.09	6.30	23.17	36.96	5.54	45.89	32.54	4.75	23.81	6.79
50	9.22	61.60	44.14	12.32	10.57	9.89	6.18	22.73	36.25	5.43	45.01	31.91	4.66	23.35	6.66
100	9.46	63.25	45.32	12.65	10.86	10.16	6.34	23.34	37.22	5.58	46.21	32.77	4.78	23.97	6.84
Citric acid (mM)															
10	9.41	62.87	45.05	12.57	10.79	10.10	6.31	23.20	37.00	5.54	45.94	32.57	4.75	23.83	6.80
15	9.36	62.55	44.82	12.51	10.74	10.05	6.27	23.08	36.81	5.52	45.70	32.41	4.73	23.71	6.77
20	9.41	62.87	45.05	12.57	10.79	10.10	6.31	23.20	37.00	5.54	45.94	32.57	4.75	23.83	6.80
Salicylic acid (mM)															
10	9.22	61.60	44.14	12.32	10.57	9.89	6.18	22.73	36.25	5.43	45.01	31.91	4.66	23.35	6.66
15	9.12	60.96	43.68	12.19	10.46	9.79	6.12	22.49	35.88	5.38	44.54	31.58	4.61	23.11	6.60
20	9.22	61.60	44.14	12.32	10.57	9.89	6.18	22.73	36.25	5.43	45.01	31.91	4.66	23.35	6.66
2 Months															
Check (dry)	10.20	72.90	46.90	13.20	11.87	10.60	5.44	22.13	35.49	3.61	42.46	33.50	1.46	27.83	5.93
Check (wet)	11.60	74.30	48.10	13.60	12.21	11.40	5.42	22.73	36.45	2.98	42.51	34.34	1.22	28.39	5.96
S. cerevisiae (%)															
25	0.00	33.64	24.60	6.29	0.00	0.00	3.65	12.98	20.65	0.00	26.01	17.89	0.00	12.13	4.17
50	0.00	22.49	16.79	0.00	0.00	0.00	2.63	9.08	14.40	0.00	17.65	12.42	0.00	7.67	3.16
100	0.00	8.32	6.87	0.00	0.00	0.00	1.34	4.12	6.47	0.00	7.02	5.48	0.00	2.00	1.89
Citric acid (mM)															
10	3.20	52.80	38.01	9.64	8.84	0.00	5.39	19.69	31.38	0.00	40.38	27.28	0.00	19.79	5.70
15	1.90	36.20	26.39	6.73	6.37	0.00	3.88	13.88	22.08	0.00	27.93	19.14	0.00	13.15	4.40
20	0.00	15.10	11.62	0.00	0.65	0.00	1.96	6.49	10.27	0.00	12.11	8.80	0.00	4.71	2.50
Salicylic acid (mM)															
10	0.00	34.60	25.27	6.45	6.13	0.00	3.74	13.32	21.19	0.00	26.73	18.36	0.00	12.51	4.26
15	0.00	23.73	17.66	0.00	0.00	0.00	2.75	9.51	15.10	0.00	18.58	13.03	0.00	8.16	3.28
20	0.00	9.92	7.99	0.00	0.00	0.00	1.49	4.68	7.36	0.00	8.22	6.26	0.00	2.64	2.04

Table 1: Continued

Treatments	*Alternaria alternata*	*A. flavus*	*A. niger*	*A. ochraceous*	*A. oryzae*	*Botrytis cinerea*	*Cephalosporin* sp.	*Fusarium moniliforme*	*Fusarium oxysporum*	*Fusarium semitectum*	*Fusarium solani*	*Penicillium* sp.	*Rhizoctonia solani*	*Rhizopus* sp.	*Verticillium* sp.
4 Months															
Check (dry)	10.80	76.40	49.50	14.20	12.72	10.80	4.56	21.43	26.57	1.62	38.08	35.32	0.92	33.23	5.07
Check (wet)	12.30	79.10	51.20	14.90	13.32	11.10	4.76	22.08	27.93	1.06	39.11	36.51	0.95	35.31	5.27
S. cerevisiae (%)															
25	0.00	29.77	21.89	5.61	5.42	0.00	3.30	11.63	0.00	0.00	23.11	15.99	0.00	10.58	3.83
50	0.00	18.74	14.17	0.00	0.00	0.00	2.29	0.00	0.00	0.00	0.00	10.59	0.00	6.17	0.00
100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.47	0.00
Citric acid (mM)															
10	3.20	52.80	38.01	9.64	8.84	0.00	5.39	19.69	31.38	0.00	40.38	27.28	0.00	19.79	5.70
15	1.90	36.20	26.39	6.73	6.37	0.00	3.88	13.88	22.08	0.00	27.93	19.14	0.00	13.15	4.40
20	0.00	15.10	11.62	0.00	0.65	0.00	1.96	6.49	10.27	0.00	12.11	8.80	0.00	4.71	2.50
Salicylic acid (mM)															
10	0.00	34.60	25.27	6.45	6.13	0.00	3.74	13.32	21.19	0.00	26.73	18.36	0.00	12.51	4.26
15	0.00	23.73	17.66	0.00	0.00	0.00	2.75	9.51	15.10	0.00	18.58	13.03	0.00	8.16	3.28
20	0.00	9.92	7.99	0.00	0.00	0.00	1.49	4.68	7.36	0.00	8.22	6.26	0.00	2.64	2.04
6 Months															
Check (dry)	12.30	83.50	53.20	14.60	13.06	10.80	4.48	17.65	18.53	0.43	23.41	37.91	0.64	37.07	5.00
Check (wet)	13.90	86.20	56.40	15.30	13.66	11.90	4.59	19.20	20.09	0.52	25.43	40.15	0.66	39.15	5.10
S. cerevisiae (%)															
25	0.00	19.90	14.98	0.00	0.00	0.00	0.00	0.00	12.95	0.00	15.71	0.00	0.00	0.00	0.00
50	0.00	11.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100	0.00	1.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Citric acid (mM)															
10	0.00	36.54	26.63	0.00	0.00	0.00	3.91	14.00	16.27	0.00	20.19	19.31	0.00	13.29	4.43
15	0.00	23.13	17.24	0.00	0.00	0.00	0.00	0.00	14.76	0.00	18.13	12.74	0.00	7.92	0.00
20	0.00	6.09	5.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00
Salicylic acid (mM)															
10	0.00	24.44	18.16	0.00	0.00	0.00	2.81	6.76	15.50	0.00	19.11	13.38	0.00	8.45	3.34
15	0.00	14.52	11.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.52	0.00	4.48	0.00
20	0.00	1.91	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2: Germination criteria of biotic and abiotic treated peanut seeds at different storage periods

	Characters											
	Germination (%)				Germination energy (%)				Germination index			
Treatments	0 month	2 months	4 months	6 months	0 month	2 months	4 months	6 months	0 month	2 months	4 months	6 months
Check (dry)	67.55	63.33	61.97	61.67	73.33	72.67	69.33	65.00	3.14	3.06	2.71	2.52
Check (wet)	78.00	76.67	75.00	66.67	83.33	78.33	75.00	71.00	3.39	3.17	2.71	2.65
S. cerevisiae (%)												
25	86.00	81.67	80.00	80.00	88.33	81.67	80.00	80.00	3.76	3.64	2.81	2.95
50	88.33	85.00	83.33	81.67	93.33	86.67	82.00	81.67	3.88	3.58	2.81	2.89
100	91.67	86.67	86.67	83.33	95.00	88.67	85.67	84.00	4.26	3.56	3.12	2.96
Citric acid (mM)												
10	78.33	76.67	75.20	68.33	86.67	80.00	76.67	73.33	3.57	3.22	2.81	2.80
15	86.67	83.33	82.33	76.67	89.33	86.67	83.33	81.33	3.98	3.44	2.98	2.87
20	83.33	81.67	81.33	75.00	88.33	83.67	80.00	76.67	3.73	3.33	2.91	2.82
Salicylic acid (mM)												
10	83.67	81.00	80.00	76.00	86.67	81.67	78.33	76.67	3.74	3.33	2.74	2.73
15	85.67	83.50	83.33	81.67	93.33	88.33	86.67	84.00	4.11	3.70	3.02	2.89
20	85.00	82.00	81.67	77.00	90.00	85.00	83.33	78.67	3.83	3.39	2.89	2.74
LSD at 5%												
Storage periods		2.10				2.29				0.17		
Agents		3.49				3.79				0.28		
Interaction		NS				NS				NS		

NS: Non significant

Table 3: Dead and rotted peanut seeds as affected by the tested agents at different storage periods

	Characters							
	Dead seeds (%)				Rotted seeds (%)			
Treatments	0 month	2 months	4 months	6 months	0 month	2 months	4 months	6 months
Check (dry)	6.11	6.53	7.69	8.61	15.57	15.70	18.17	20.03
Check (wet)	5.92	6.51	7.89	9.81	15.32	15.66	18.43	22.33
S. cerevisiae 25%	3.38	4.49	4.70	4.99	7.46	11.23	11.97	12.48
S. cerevisiae 50%	2.07	2.14	2.24	3.80	2.05	2.54	5.93	6.58
S. cerevisiae 100%	1.94	2.60	2.61	2.64	1.58	1.55	1.88	2.09
Citric acid 10 mM	5.12	6.16	6.20	6.48	12.89	14.31	14.12	15.27
Citric acid 15 mM	2.78	3.20	3.39	3.67	2.05	2.46	8.48	9.33
Citric acid 20 mM	3.81	4.19	5.12	6.48	3.91	4.31	12.74	13.24
Salicylic acid 10 mM	4.07	5.02	5.33	6.18	11.11	12.62	12.75	12.87
Salicylic acid 15 mM	2.23	2.31	2.61	2.97	1.55	2.11	4.58	5.82
Salicylic acid 20 mM	2.34	2.77	3.11	3.26	3.05	3.08	8.64	8.82
LSD at 5%								
Storage periods			NS				0.36	
Agents			0.44				0.59	
Interaction			0.88				1.18	

NS: Non significant

and dry seed). Meanwhile, storage periods had no significant effect on dead seeds. Seed treatment by any of biotic and abiotic agents at any concentration significantly decreased dead seeds, rotted

seeds. Yeast extract at 100% followed by salicylic acid at 15 mM gave the lowest dead seeds, rotted seeds as compared with the two controls (dry and wet). Concerning the interaction effects, data showed that the application of the tested agents as peanut seed treatments alleviated the harmful effect of storage periods on peanut seeds.

Table 4 indicated that rotted seedlings and survived healthy seedlings as influenced by the interaction between storage periods and inducer agents. It can be easily notice the negative correlation between storage periods and total survival seedlings. Seed treatment by any of biotic and abiotic agents at any concentration significantly decreased rotted seedlings but increased healthy survival seedlings%. Yeast extract at 100% followed by salicylic acid at 15 mM gave the lowest rotted seedlings and gave the highest percentage of survival healthy seedlings as compared with the two controls (dry and wet). Concerning the interaction effects, data showed that the application of the tested agents as peanut seed treatments alleviated the harmful effect of storage periods on peanut seeds which reflected survived healthy seedlings. The high percentage of survived seedlings occurred under high level of yeast extract (100%) followed by salicylic acid (15 mM).

Seedling characters: As for the effects of storage period, Table 5 shows that seedling length, vigor index and dry weight of 10-seedlings decreased significantly with increasing storage periods from 0 to 6 months. Data also, indicated that all treatments at any dose used significantly increased seedling length, seedling vigor index and 10-seedlings dry weight. In this respect, the highest seedling characters were obtained from seeds that were treated with yeast extract at 100%. While the lowest values of the previous traits produced from untreated seeds (control dry). Additionally, any concentration of either biotic or abiotic agents increased seedling length, vigor index and dry weight of seedlings during all storage periods. Taking into account the non significant differences between 0 and 2 months storage periods in seedling length and seedling vigor index. At 6 months storage period, yeast extract at 100% followed by 50% then salicylic (15 mM) gave the highest values of seedling length and seedling vigor index. While, seeds of dry control gave the lowest values of seedling characters.

Seed chemical analysis: Table 6 shows that, oil, protein and carbohydrate percentages in peanut seeds significantly decreased with increasing storage period. The maximum content was recorded in zero time. While the lowest content of these characters were resulted from six months stored seeds. Of biotic and abiotic agents, seed treated with any concentration of the tested agents increased significantly oil, protein and carbohydrate% in peanut seeds. Yeast extract was the most effective followed by salicylic acid then citric acid, the moderate concentration of abiotic agents was more effective than the high and low concentrations. The interaction between storage periods and different agents significantly increased protein% and carbohydrate% in peanut seeds. At six months storage period, yeast extract at 100% produced the highest values of seed protein and carbohydrates followed by 50% then salicylic acid at 15 mM.

Seed aflatoxin content: Figure 1 indicates that seed treatment with the tested agents reduced greatly the aflatoxin B_1 content in peanut seeds after 6 months of storage. Generally, aflatoxin B_1 decreased markedly with increasing the concentration of the tested agents.

The highest content of aflatoxin B_1 was recorded in wet check followed by dry check. On the other side, the best treatment in reducing aflatoxin B_1 content in peanut seeds after 6-months

Table 4: Rotted and survived seedlings as affected by the tested agents at different storage periods

	Characters							
	Rotted seedlings (%)				Survival seedlings (%)			
Treatments	0 month	2 months	4 months	6 months	0 month	2 months	4 months	6 months
Check (dry)	15.45	15.58	17.87	19.05	62.87	62.19	56.27	52.31
Check (wet)	15.23	15.54	17.88	20.64	63.53	62.29	55.80	47.22
S. cerevisiae 25%	8.56	11.02	12.31	12.47	80.60	73.26	71.02	70.06
S. cerevisiae 50%	3.77	3.07	7.34	8.16	92.11	92.25	84.49	81.46
S. cerevisiae 100%	1.39	1.54	1.79	2.32	95.09	94.31	93.72	92.95
Citric acid 10 mM	13.25	14.55	14.02	15.20	68.74	64.98	65.66	63.05
Citric acid 15 mM	5.52	5.21	9.66	10.11	89.65	89.13	78.47	76.89
Citric acid 20 mM	6.19	6.33	13.13	13.59	86.09	85.17	69.01	66.69
Salicylic acid 10 mM	12.03	12.95	13.13	12.94	72.79	69.41	68.79	68.01
Salicylic acid 15 mM	2.81	2.09	7.56	6.48	93.41	93.49	85.25	84.73
Salicylic acid 20 mM	4.24	4.35	8.98	9.98	90.37	89.80	79.27	77.94
LSD at 5%								
Storage periods			0.35				0.70	
Agents			0.57				1.17	
Interaction			1.15				2.33	

Table 5: Seedling characters of biotic and abiotic treated peanut seeds at different storage periods

	Characters											
	Seedling length (cm)				Seedling vigor index				Dry weight of 10-seedlings (g)			
Treatments	0 month	2 months	4 months	6 months	0 month	2 months	4 months	6 months	0 month	2 months	4 months	6 months
Control (dry)	19.67	19.07	17.47	13.83	13.28	12.12	10.82	8.53	5.66	3.28	3.24	2.78
Control (wet)	22.01	21.34	19.77	18.83	17.17	16.37	14.78	12.52	5.98	4.64	4.05	3.51
S. cerevisiae 25%	24.95	24.59	23.00	20.13	21.46	20.08	18.40	16.16	7.05	5.22	4.37	4.39
S. cerevisiae 50%	28.83	28.47	24.73	22.57	25.80	24.20	20.59	18.38	8.93	7.28	5.99	5.35
S. cerevisiae 100%	32.17	31.46	27.43	23.27	29.48	27.33	23.81	19.41	9.01	7.84	6.34	5.88
Citric acid 10 mM	25.30	24.83	21.70	19.50	19.82	19.04	16.26	13.40	6.13	4.84	4.23	4.11
Citric acid 15 mM	28.27	28.18	25.87	21.77	24.51	23.48	21.29	16.69	7.93	7.06	6.17	4.57
Citric acid 20 mM	26.80	26.55	24.60	19.63	22.33	21.68	20.00	14.73	6.91	6.95	4.52	4.13
Salicylic acid 10 mM	24.68	24.42	23.93	21.43	20.66	19.78	19.15	16.30	6.58	4.87	4.47	4.53
Salicylic acid 15 mM	31.83	31.71	26.60	22.17	27.27	26.48	22.17	18.10	8.49	7.27	5.99	5.57
Salicylic acid 20 mM	27.12	27.02	24.37	21.47	23.05	22.16	19.91	16.49	7.76	6.98	5.54	4.85
LSD at 5%												
Storage periods			0.64				0.72				0.32	
Agents			1.07				1.19				0.53	
Interaction			2.13				2.37				1.06	

storage was yeast extract followed by salicylic acid then citric acid. It is of special important to note that the moderate and high concentrations of both biotic and abiotic agents result in aflatoxin B_1 free seeds.

Table 6: Chemical composition of peanut seeds as affected by the interaction between storage periods and tested agents

	Characters											
	Oil (%)				Protein (%)				Carbohydrates (%)			
Treatments	0 month	2 months	4 months	6 months	0 month	2 months	4 months	6 months	0 month	2 months	4 months	6 months
Control (dry)	55.30	52.38	52.56	50.55	29.53	21.01	20.14	18.43	23.67	19.04	16.95	16.56
Control (wet)	55.20	53.23	52.92	50.49	29.67	23.58	23.11	21.03	23.62	19.89	17.91	17.37
S. cerevisiae 25%	55.41	54.41	54.06	50.76	30.03	28.01	27.65	25.66	24.33	21.56	20.53	21.23
S. cerevisiae 50%	55.83	54.63	54.30	53.05	30.72	28.01	27.97	25.73	24.64	22.46	22.26	22.07
S. cerevisiae 100%	56.26	55.07	54.98	53.42	31.28	28.54	28.01	26.27	25.11	23.47	23.16	22.62
Citric acid 10 mM	55.27	53.47	52.96	50.56	30.13	25.04	24.51	21.57	23.83	20.60	19.92	19.43
Citric acid 15 mM	55.50	54.25	53.96	52.69	30.80	26.61	26.30	24.16	24.59	22.26	21.05	21.52
Citric acid 20 mM	55.43	53.70	53.07	50.75	30.28	25.74	25.38	23.64	23.67	21.70	20.95	20.32
Salicylic acid 10 mM	55.35	54.45	54.34	51.24	29.70	26.61	26.61	22.77	23.75	21.10	20.09	19.78
Salicylic acid 15 mM	55.65	54.83	54.83	52.89	30.92	27.53	27.14	25.40	24.93	23.06	22.01	21.56
Salicylic acid 20 mM	55.56	54.54	54.57	52.87	30.40	26.90	27.00	25.10	24.17	22.44	21.19	21.23
LSD at 5%												
Storage periods		0.49				0.72				0.32		
Agents		0.81				1.19				0.53		
Interaction		NS				2.37				1.06		

NS: Non significant

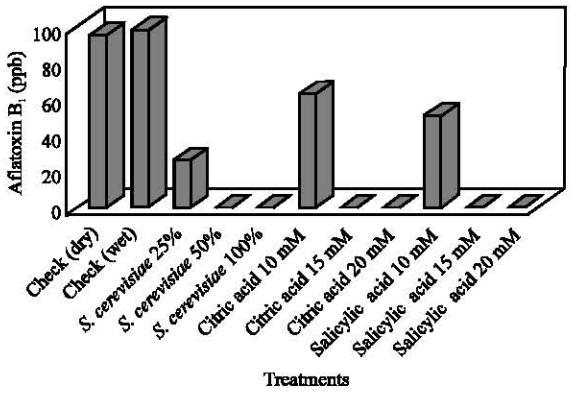

Fig. 1: Effect of biotic and abiotic agents on aflatoxin B_1 in peanut seeds after 6 months of storage

Greenhouse experiment

Seedling morphological characters: Table 7 indicated that the application of both biotic and abiotic agents significantly increased seedling shoot length, root system depth and dry weights of shoot and root systems. Generally, yeast extract was the most effective followed by salicylic acid then citric acid. The moderate level (15 mM) of both citric and salicylic acids improved the morphological characteristics than high concentration (20 mM). The maximum values of shoot length (14.67 cm) and root depth (19.60 cm) were obtained from seeds that were treated with yeast extract at 100% followed by 50% then salicylic acid at 15 mM. Moreover, yeast extract (100%) followed by salicylic acid (15 mM) gave the highest dry weights of both shoot and root system.

Table 7: Variation in morphological characters of seedling under greenhouse conditions as a function of the tested agents on peanut seeds

	Characters			
Treatments	Shoot length (cm)	Root depth (cm)	Dry weight of shoot (g)	Dry weight of root (g)
Check (dry)	7.92	12.50	0.18	0.08
Check (wet)	9.83	13.27	0.21	0.10
S. cerevisiae 25%	12.57	16.03	0.46	0.14
S. cerevisiae 50%	13.90	18.13	0.55	0.15
S. cerevisiae 100%	14.67	19.60	0.58	0.17
Citric acid 10 mM	10.33	13.83	0.27	0.12
Citric acid 15 mM	11.93	17.23	0.40	0.15
Citric acid 20 mM	11.73	16.60	0.30	0.14
Salicylic acid 10 mM	11.00	15.57	0.35	0.13
Salicylic acid 15 mM	13.67	17.27	0.55	0.17
Salicylic acid 20 mM	12.17	16.33	0.44	0.15
LSD at 5%	0.54	1.18	0.05	0.01

Physiological characters: Data presented in Table 8 show the significant increase in chlorophyll a and b and carotenoids as well as total phenols at any concentration of biotic and abiotic agents as compared with control (dry and wet) was the only exception total carotenoids which showed non significant effect at the low concentration of citric acid (10 mM) as compared with the two control treatments. Yeast extract (100 and 50%) followed by salicylic acid at 15 mM were the most effective in increasing chlorophyll a and carotenoids in the leaves of 30 days old seedling. Similar trend was observed in total phenols but citric acid (15 mM) occupied the third position after yeast extract (100 and 50%). Moreover, chlorophyll b recorded its highest values with the application of 100% yeast extract then 15 mM salicylic acid.

Table 8: Variation in physiological characters of seedling under greenhouse as a function of the tested agents on peanut seeds

	Characters			
Treatments	Chlorophyll A (mg g^{-1} f. wt)	Chlorophyll B (mg g^{-1} f. wt)	Carotenoids (mg g^{-1} f. wt)	Total Phenols (mg catechol 100^{-1} g f. wt)
Check (dry)	1.03	0.89	0.19	90.73
Check (wet)	1.06	0.99	0.25	91.24
S. cerevisiae 25%	1.31	1.11	0.42	129.60
S. cerevisiae 50%	1.44	1.27	0.72	142.33
S. cerevisiae 100%	1.55	1.43	0.83	145.57
Citric acid 10 mM	1.11	0.99	0.25	122.00
Citric acid 15 mM	1.29	1.10	0.41	137.17
Citric acid 20 mM	1.27	1.07	0.37	132.70
Salicylic acid 10 mM	1.07	1.07	0.35	124.03
Salicylic acid 15 mM	1.38	1.30	0.57	136.57
Salicylic acid 20 mM	1.35	1.14	0.55	132.30
LSD at 5%	0.09	0.05	0.08	12.10

DISCUSSION

Seed treatment has become one of the important practices in plant protection. The most widely traditional application used of seed treatment is the protection of the germinating seedling against

seed and soil-borne fungi, immediately, after planting. Seed treatment also protects seeds especially oil seeds during storage period or post harvest hence, decrease the seed deterioration. The present study showed that peanut seed treatment with both biotic (*S. cerevisiae*) and abiotic (salicylic acid and citric acid) agents inhibited the different seed-borne fungal genera and disease development under storage conditions up to 6 months. In addition to the improvement in germination criteria, seed quality and morphological and physiological characters of seedlings.

Seed deterioration during storage by fungi is determined by a range of factors which can be classified into four groups, namely (a) intrinsic nutritional factors, (b) extrinsic factors (c) processing factors and (d) implicit factors (Magan *et al*., 2004). These factors affect fungal population that could change throughout the storage period. *A. flavus* recorded the highest frequency than other *Aspergillus* sp. These results are in agreement with Mahmoud *et al*. (2006), they stated that *A. flavus* is more invasive than *A. parasiticus* and often dominated in peanut seeds than sheels. This is because due to *A. flavus* is more aggressive than other *Aspergillus* spp. (Horn *et al*., 1994), in addition to the biochemical composition of seeds which makes peanut seeds a good substrate for its growth and subsequent aflatoxin production (Holbrook *et al*., 2000; Xue *et al*., 2003).

The beneficial effects of yeast extract may be due to the antifungal activity of its metabolites (Hassanein *et al*., 2002) so the application of yeasts as plant pathogens control is recommended where, it was found to produce proteinaceous killer toxins lethal to fungal strains (Hodgson *et al*., 1995; Abranches *et al*., 1997; Marquina *et al*., 2002; Santos *et al*., 2004). Moreover, Elwakil *et al*. (2009) stated that disease severity of pre-and post emergence damping-off caused by *Cephalosporium* sp., *F. verticillioids*, *F. oxysporum*, *F. solani*, *R. solani* and *V. dahliae* reduced significantly when seeds of faba bean were coated with water suspension (10^9 CFU mL^{-1}) of *S. cerevisiae* before sowing due to activity in disease suppression. Also, Youssef *et al*. (2001) and Bleve *et al*. (2006) reported that a microorganism that colonizes roots is ideal for use as a biocontrol agent against soil-bore diseases and consequently improving plant growth. Additionally, El-Mehalawy *et al*. (2004) stated that the growth increament by rhizosphere microorganisms depends mainly on the ability of those microorganisms to survive and develop in the rhizosphere. Several plant-microbe interactions were developed which enhanced plant growth due to production of plant regulators, siderophores, phosphate solubilization, nutrient uptake and availability (Hoflich and Kuhn, 1996; Bowen and Rovira, 1999).

The role of salicylic acid in decreasing total fungal population on peanut seeds and inducing resistance to damping-off may be by induction of synthesis of pathogenesis related proteins, partial resistance to pathogens (White, 1979) and increasing the activity of oxidative enzymes (peroxidase and polyphenol oxidase) and content of phenol compounds (Mahmoud *et al*., 2006). The biochemical changes in oxidative enzymes and phenolic contents induce the resistance due to the role of peroxidase activity in disease development which correlated with the expression of resistance in different host-pathogen system (Cadena-Gomez and Nicholson, 1987; Reuveni *et al*., 1992). Phenolics are well known as antifungal, antibacterial and antiviral compounds occurring in plants. The first step of the defense mechanism in plants involves a rapid accumulation of phenols at the infection site which restricts or slows the growth of the pathogen (Martern and Kneusel, 1988; Gogoi *et al*., 2001). Chen *et al*. (1995) reported that Salicylic acid is accumulated at high concentrations in the immediate vicinity of incompatible infection sites and is considered a key endogenous regulator of defense responses, being involved both in localized defenses and systemically acquired resistance. Salicylic acid has also been shown to act as a one-electron-donating substrate that shifts the catalytic activity of catalase from dismutation to peroxidation,

thereby trapping the enzyme in a less active state and determining a slowing down of H_2O_2 removal (Durner and Klessig, 1996). Galal *et al.* (2000) reported that the reduction of infection under storage conditions seems to depend on prevention of the decline of the natural mechanisms of plant resistance and modulation of the structure of some natural phytoalexins in order to stabilize possible radical intermediates and to give them antioxidant properties.

The antimicrobial action of salicylic acid and citric acid also may be due to inhibition of functions of several enzymes by oxidized compounds, dissolve in membrane lipids and interfere with membrane functions including transport of nutrients and interfere with protein, RNA and DNA synthesis (Nesci *et al.*, 2003).

Seed treatment with salicylic acid improves the morphological characters of peanut seedlings due to the effect on hormonal status which affects ion uptake, cell elongation, cell division, enzymatic activation and protein synthesis (Shakirova *et al.*, 2003; Amin *et al.*, 2007). Similar results have been reported by Novas and Cabral, 2002. On contrast, the high concentration of salicylic acid in this study (20 mM) was less effect than moderate concentration (15 mM). This may be back to the damage effects of high concentration on the plant physiological processes. It caused the collapse of the transmembrane electrochemical potential of mitochondria which effects on ATP-production and inhibits phosphorus uptake and potassium absorption (Glass, 1974; Harper and Balke, 1981; Macri *et al.*, 1986), as well as reduces transpiration by effect on stomatal behavior (Larque-Saavedra, 1979).

Generally, seed storage caused a decrease in oil, protein and carbohydrates contents for all treatments. The lowest values of these compounds were found after six months of storage. The pattern of reduction of these characteristics may be due to oxidation of the amino acids, the increase in respiratory activity and advance in the deterioration process of the stored seeds. Thus, prolonged seed storage would increase the metabolic activity of the seeds and consequently decrease the reserve substance content and reduce the dry material weight of seeds (Bewley and Black, 1994). Since the present investigation showed increase in seed viability of peanut under storage by biotic and abiotic agents through decreasing the total fungal population which led to decrease the aflatoxin B_1 and increase of seed oil, protein and carbohydrate contents.

CONCLUSION

It could be recommended to use 100% *Saccharomyces cerevisiae* extract or 15 mM salicylic acid as seed treatment to improve seed quality and reduce deterioration of peanut seeds under storage.

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