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EFFECT OF STORAGE CONDITION AND PACKAGING MATERIAL ON INCIDENCE OF STORAGE FUNGI AND SEED QUALITY OF MAIZE GRAINS

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ABSTRACT

In general, the highest frequency of seed-associated fungi was *A. niger* and *A. flavus,* followed by *Fusarium*spp. and *Alternaria* spp., and then *Penicillium*spp. and *Rhizopusstolonifer*. The storage temperature has no effect on the incidence of *Alternaria*sp., *Fusarium* sp., *Penicillium*sp., and *R. stolonifer*, while it has a significant effect on *A. flavus* and *A. niger*. The best storage conditions for avoiding *A. flavus* incidence on maize grains were storage in refrigerator (10ºC) in packages made from cotton cloth, high- or low-density polyethylene whereas the least incidence of this hazardous fungus was recorded (0.10%). Generally, storage in refrigerator (10ºC) in packages made from low-density polyethylene was favorable against grain contamination/infection with all storage fungi that may cause seed rot of maize. Storage of maize grain in cold conditions (10^{o}C) caused a significant increase in kernel weight and seed germination while decreased seed moisture content regardless of the packaging material (with an exception with woven polyethylene as packaging material on seed germination) comparing with storage in room conditions. In general, the paper packaging material was favored for all grain quality parameters when grains are stored in cold conditions, whereas it recorded the highest kernel weight (34.86 g) and seed germination (100%) and moderate grain moisture content (8.33%). Under room conditions, grains stored in cotton+polyethylene packages had the highest oil and protein contents (19.67 and 26.20%, respectively).

In conclusion, this study recommends storing maize grains at low temperature inside packages made of low-density polyethylene in order to preserve the vitality of seeds and keep oil content at high levels in addition to reducing the incidence of storage fungi especially those producing mycotoxins/aflatoxins to maintain public health.

Keywords: Maize, storage fungi, cool storage, package type.

INTRODUCTION

Maize (*Zea mays* L.) belongs to family Poaceae (Graminae) (Wani*et al*., 2014 and khan *et al*., 2014) is considered the third most important cereal crop all over the world (FAO, 2013). It is used mainly for human, animal and poultry feeding. Also, there are industrial uses of maize such as the industrial corn products: ethanol and hydrosorb materials, production of corn oil, dextrose and high fructose corn sweetness (Gwirtzand Garcia-Casal, 2014). Proper storage may help in alleviating problems of seed viability. Seeds of most plant species may be safely stored for several months by carful control of temperature and relative humidity (Lacerda*et al*., 2003 and Chatth*aet al*., 2012).

In some parts of the world, especially in the tropics, conditioned storage is necessary in order to maintain high viability of some seeds from harvesting to planting (Harrington, 1973; Santoso*et al.,* 2015; Tripathi and Lawande, 2014). The storage fungi, mainly comprising several species of *Aspergillus* and *Penicillium,* do not invade grains to any appreciable degree or extent before harvest (Tuite, 1961). Seed quality is a multiple criterion that encompasses several important seed attributes: which include chemical composition, physiological germination and seed vigor and presence of seed-borne pathogens. During storage, seed quality can remain at the initial level or decline to a level that may make the seed unacceptable for planting purpose, what is related to many determinants: environment conditions during seed production, pests, diseases, seed oil content, seed moisture content, mechanical damages of seed in processing, storage longevity, packaging, pesticides, air temperature and relative air humidity in storage, biochemical injury of seed tissue (Al-Yahya, 2001; Šimic*et al*., 2004; Guberac*et al*., 2003; Heatherly and Elmore, 2004). Field fungi*, Alternaria, Cladosporium, Curvularia, Fusarium* and *Helminthosporium* usually do not continue to grow in grains after harvest, but may remain alive for years in grains stored at low moisture content and low temperature. While storage fungi i.e., *Aspergillus* spp. and *Penicillium* can grow in stored grain under bad storage conditions and cause serious losses (Mehrotra, 1983).

Changes in grain germination, chemical composition, acidity and oil content of three maize genotypes, due to infection by different fungi of ear and kernel rots, were affected by storage periods (6 and 18 months), storage temperature (room temperature and 10ºC) (Timóteo and Marcos-Filho, 2013) and package materials (paper, woven polyethylene and high density polyethylene) (Chatha*et al.*, 2012 and Rahraw, *et al*., 2013). The present study aimed to determine the effect of storage conditions in combination with packaging materials on the incidence of maize storage fungi and on grain quality.

MATERIALS AND METHODS

The present experiment was carried out in the centrallaboratory and the laboratory of plant pathology at Sakha Agricultural Research Station (SARS), Kafr El-Sheikh, Egypt.

The experiment was conducted during 2011. Visual symptomless seed samples (12 kg) of tested maize cultivar "Three Way Cross 324" (TWC324) were taken at the harvest time and dried using hot air dried methods to14% moisture content by moisture tester (model 8400, serial No.4, Michigan USA) and packaged in 6 types of packages made from different materials i.e., paper, cotton clothes, woven polyethylene, cotton+polyethylene, high density polyethylene, and low density polyethylene. Each package was filled with one kg of tested maize seeds and stored under two different conditions: cold room (10˚C) and at room conditions. Random seed samples with three replicates were taken from each package after 6 months of storage. The presence of storage fungi in

each treatment was assessed according to ISTA (2004): whereas 200 seeds were surface sterilized with 5% sodium hypochlorite for 5min., washed thoroughly with sterile distilled water, and then dried in a lamenter flow. The seeds were plated in potato dextrose agar (PDA) plates (5seeds/plate) and incubated for 7 days at 27ºC. The developed fungi were recorded and the incidence percentage of each fungal genera was estimated. The developed fungi were purified by using astereoscopic microscope; hyphal tips from fungi were transfer it to PDA plates and later to slants for identification. Identification of fungi was carried out using their morphological and microscopic features according to Barrant and Hunter (2004). Barron, 1968; Hunter and Barnett, 1973; Hunter *et al.,* 1978 and Alexopoulus *et al.* 1996 were provide additional information on many aspects of the morphology, sporulation, growth, ecology of imperfect fungi. Identification was reconfirmed by the help of staff at the Department of Maize and Sugar Beet Disease, Sakha Agriculture Research Station, A. R. C., Giza, Egypt. Germination percentage, 100-grain weight, grain moisture content, and oil and protein contents were determined according to methods described by (AOAC, 1999). All obtained data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the split-plot design to each experiment.

RESULTS AND DISCUSSION

Effect of storage conditions and packaging material on incidence of fungi during storage.

In general, the highest frequency of seed-associated fungi was *A. niger* and *A. flavus,* followed by *Fusarium* spp. and *Alternaria* spp., and then *Penicillium* spp. and *R. stolonifer* (Table 1). The effect of storage package types and conditions on frequency of fungi associated with stored maize grain is shown in Table (1). The obtained data clearly show that, the storage temperature has no significant effect on the incidence of *Alternaria* sp., *Fusarium* sp., *Penicillium* sp., and *Rhizopus stolonifer* (Table 1), whereas there were no significant differences among all packages types regarding these fungi under both storage conditions (at room temperature and at 10^{o}C).Thus, the storage temperature has a significant effect only on *A. flavus* and *A. niger* (Table 1).

The best storage conditions for avoiding *A. flavus* incidence on maize grains were storage in refrigerator (10ºC) in 2 tested types of packages made from cotton cloth, high- or low-density polyethylene whereas the least incidence (0.10%) of this storage fungus was recorded (Table 1).

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These results are supported with the findings of El-Sayed and Tolba (2005) who reported that storage under 10^{o}C in high-density polyethylene packages led to reducing infection of maize grains by pathogens including *A. flavus*. Storage in refrigerator (10ºC) in packages made from low-density polyethylene was favorable against grain infection with all storage fungi that cause seed rot of maize. Also, Mehrotra (1983) reported that the storage fungi i.e., *Aspergillus* spp. and *Penicillium* can grow in stored grain under bad storage conditions and cause serious losses

Effect on maize grain quality (seed weight, germination, and moisture content):

Data in Table (2) show that storage of maize grain in cold conditions (10^{o}C) caused a significant increase in kernel weight and seed germination while decreased seed moisture content regardless of the packaging material (with an exception with woven polyethylene as packaging material on seed germination) comparing with storage in room conditions (25$\pm$2^{o}C).In general, the paper packaging material was favored for all grain quality parameters when grains are stored in cold conditions, whereas it recorded the highest kernel weight (34.86 g) and seed germination (100%) and moderate grain moisture content (8.33%) (Table 2). Similar results were obtained by El-Sayed and Tolba (2005) who found that seed germination and weight of 100 kernels were affected by storage temperature and packaging material. They found that germination percentages and the weight of 100 grain were significantly decreased under warehouse conditions. Our results were also supported by those of El-Sayed*et al.* (2004) who reported that germination decline was more rapidly at warehouse, but less at low temperature, while the lowest value of the weight of 100 grain was recorded at warehouse storage conditions. The results in Table (2) showed clearly that, germination percentage, weight of 100 kernels Malaker*et al*. (2008) who tested types of containers [viz., 'dole' (bamboo made), earthen pitcher, tin container, polyethylene bag] and storage conditions [cool (10 C) and room temperature] for their effect on quality of wheat seeds during storage. They found that highest germination percentage was observed under storage in refrigerator and polyethylene bag followed by tin container and earthen pitcher. The results also similar with the obtained by Mettananda *et al*., 2001. Who found that the maize grain stored in woven poly-ethylene under cold room have good quality during storage period comparing with maize grain stored poly-ethylene under room temperature.

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Effect on oil and protein contents

Concerning the effect of storage packages and conditions on oil content of stored maize grain, data presented in Table (3) show that, under room conditions, grains stored in cotton+polyethylene packages had the highest oil and protein contents (19.67 and 26.20%: respectively). No significant difference was found among theother differentpackages stored in refrigerator. Similar results were obtained by El-Sayed and Tolba (2005) who reported a significant reduction of oil and protein contents in grains stored at warehouse conditions, while storage under 10^{o}C produced the highest values of oil content. Also they found that crude protein decreased in grains stored at 10ºC while increased in case of storage at laboratory conditions.

In conclusion, this study recommends storing maize grains at low temperature inside packages made of low-density polyethylene in order to preserve the vitality of seeds and keep oil content at high levels in addition to reducing the incidence of storage fungi especially those producing mycotoxins/aflatoxins to maintain public health.

Table (3): Effect of packaging material and storage conditions on oil and protein contents of maize grains (TWC.324 *hybrids cv.*) after six months storage.

Fungus	Storage conditions	Packaging material type						Mean
		Paper	Cotton clothes	Woven polyethylene	Cotton+ polyethylene	High-density polyethylene	Low-density polyethylene	
Seed oil content (%)	At room Temperature (25±2°C)	6.20 a	3.46 a	5.04 a	19.67 a	4.12 a	3.46 a	6.99 a
	In refrigerator (10°C)	4.65 a	4.21 a	5.12 a	5.48 a	4.96 a	5.11 a	4.92 a
Mean		5.43 a	3.84 a	5.08 a	12.57 a	4.54 a	4.29 a	5.96
Seed protein content (%)	At room Temperature (25±2°C)	17.30 d	17.46 d	20.98 c	26.20 a	23.77 b	21.28 c	21.17 a
	In refrigerator (10°C)	14.26 e	17.34 d	14.38 e	14.90 e	10.63 f	11.10 f	13.77 b
Mean		15.78 d	17.40 b	17.68 b	20.55 a	17.20 bc	16.19 CD	17.47

***Values for each fungus followed by the same letter(s) are not significantly different, according to LSD test at *P* = 0.05.**

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تأثير ظروف التخزين ونوع عبوات التعبئة على فطريات التخزين وجودة الحبوب فى الذرة الشامية

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أجريت هذه الدراسة لتقدير تأثير درجة حرارة التخزين (درجة حرارة الغرفة ٢٥±٢°م، والتخزين البارد بالثلاجة على ١٠°م)، وكذلك نوع عبوات التخزين (الورق، القطن، البولى إيثيلين المنسوج، القطن+البولى إيثيلين، البولى إيثيلين عالى الكثافة، والبولى إيثيلين منخفض الكثافة) لحبوب الذرة الشامية لمدة ستة أشهر وذلك على الإصابة بفطريات الأعفان (الفيوزاريوم، أسبرجلس فلافوس، أسبرجلس نيجر، بنيسيليم، ألترناريا، الريزوبس) وصفات جودة الحبة (نسبة الإنبات، وزن ١٠٠ حبة، الرطوبة النسبية فى الحبوب، نسبة البروتين، ونسبة الزيت فى الحبوب) وذلك باستخدام الصنف الهجين الثلاثى ٣٢٤ خلال الفترة من ١ سبتمبر ٢٠١١ وحتى ١ مارس ٢٠١٢، ويمكن تلخيص النتائج كالآتي:

دلت النتائج المتحصل عليها أنهبشكل عام، كان أعلى معدل تلوث بالفطريات المصاحبة لبذور الذرة الشامية كان للفطرأسبرجللس نيجر والفطر أسبرجللس فلافوس، تلاها فطريات الفيوزاريوم والألترناريا، ثم البنسليوم والرايزوبس. وأوضحت الدراسة أن درجة حرارة التخزين لم يكن لها تأثير معنوى على الإصابة بفطريات ألألترناريا والفيوزاريوم والبنسليوم والرايزوبس، في حين كان لديها تأثير معنوى على الإصابة بفطرى اسبرجللس فلافوس وأسبرجللس نيجر. كانت أفضل ظروف التخزين لتجنب الإصابة بفطر أسبرجللس فلافوس هى التخزين في الثلاجة في عبوات مصنوعة من قماش القطن، أو البولي إثيلين عالى أو منخفض الكثافة حيث سجلت أقل حالات الإصابة بهذا الفطر الخطير (٠.١٠٪). وبصفة عامة، كان التخزين في الثلاجة في عبوات مصنوعة من البولي إيثيلين منخفض الكثافة مناسبا لحماية حبوب الذرة الشامية منالإصابة بفطريات أعفان البذور. وقد أدى تخزين حبوب الذرة في الثلاجة إلى زيادة كبيرة في وزن المائة حبة وكذلك نسبة إنبات البذور،كما أدى إلى انخفاض نسبة الرطوبة فى البذور بغض النظر عن نوعية مادة عبوات التخزين (باستثناء البولي اثيلين المنسوج على إنبات البذور) مقارنة مع التخزين في ظروف الغرفة. بشكل عام،كان التخزين في عبوات ورقيةفى الثلاجة مناسبا لصفات الجودة فى الحبوب، حيث سجلت أعلى وزن للمائة حبة (٣٤.٨٦ جم) وأعلى نسبة إنبات للبذور (١٠٠٪) ومحتوى معتدل للرطوبة فى الحبة (٨.٣٣٪). وفي ظل ظروف الغرفة، سجلت الحبوب المخزنة في أكياس من القطن + البولي إيثيلين أعلى نسبة من محتوى الزيت والبروتين (١٩.٦٧ و٢٦.٢٠% على التوالي).

توصى الدراسة بتخزين حبوب الذرة فى درجات حرارة منخفضة داخل عبوات مصنوعة من البولى إيثيلين منخفض الكثافة وذلك حفاظا على حيوية التقاوى ومحتواها من الزيت بالإضافة إلىخفض نسبة الإصابة بفطريات التخزين خاصة المنتجةللسموم الفطرية للحفاظ على الصحة العامة.

Table (1): Effect of storage package material and storage condition on incidence of fungi associated with maize grain (TWC.324 *hybrids cv.*) after six months of storage.

Fungus	Storage conditions	Packaging material type						Mean
		Paper	Cotton clothes	Woven polyethylene	Cotton+ polyethylene	High-density polyethylene	Low-density polyethylene	
*Alternaria*sp.	At room Temperature (25±2°C)	13.33 a	6.70 a	3.40 a	10.07 a	10.07 a	6.70 a	8.37 a
	In refrigerator (10°C)	6.70 a	16.67 a	10.00 a	23.37 a	13.33 a	3.40 a	12.24 a
Mean		10.02 a	11.68 a	6.70 a	16.71 a	11.70 a	5.05 a	10.31
A. flavus	At room Temperature (25±2°C)	13.37 de	43.33 abc	3.40 de	60.00 a	36.67 bc	50.00 ab	34.46 a
	In refrigerator (10°C)	23.33 cd	0.100 e	13.33 de	3.40 de	0.10 e	0.10 e	6.73 b
Mean		18 35 ab	21.72 ab	8.37 b	31.70 a	18.38 ab	25.05 a	20.60
A. niger	At room Temperature (25±2°C)	23.37 bc	30.03 b	10.03 bc	30.00 b	66.67 a	30.03 b	31.69 a
	In refrigerator (10°C)	3.40 c	13.33 bc	13.37 bc	23.33 bc	6.70 bc	6.73 bc	11.14 a
Mean		13.38 b	21.68 ab	11.70 b	26.67 ab	36.68 a	18.38 b	21.42
*Fusarium*sp.	At room Temperature (25±2°C)	20.00 a	10.03 a	10.03 a	6.70 a	0.10 a	6.70 a	8.93 a
	In refrigerator (10°C)	23.33 a	23.33 a	16.67 a	16.67 a	13.37 a	6.73 a	16.68 a
Mean		21.67 a	16.68 ab	13.35 ab	11.68 ab	6.73 b	6.72 b	12.8
Penicillium sp.	At room Temperature (25±2°C)	0.10 a	3.40 a	6.70 a	0.10 a	0.10 a	3.40 a	2.30 a
	In refrigerator (10°C)	6.70 a	10.03 a	0.10 a	0.10 a	3.40 a	3.40 a	3.96 a
Mean		3.40 a	6.72 a	3.40 a	0.10 a	1.75 a	3.40 a	3.13
Rhizopus stolonifer	At room Temperature (25±2°C)	0.10 a	10.10 a	0.10 a	0.10 a	0.10 a	3.40 a	2.31 a
	In refrigerator (10°C)	0.10 a	0.10 a	0.10 a	0.10 a	10.10 a	0.10 a	1.76 a
Mean		0.10 a	5.08 a	0.10 a	0.10 a	5.08 a	1.75 a	2.04

***Values for each fungus followed by the same letter(s) are not significantly different, according to LSD test at *P* = 0.05.**

Table (2): Effect of storage packaging material and storage conditions on someseed quality parameter of maize grain (TWC.324 *hybrids cv.*) after six months storage.

Fungus	Storage conditions	Packaging material type						Mean
		Paper	Cotton clothes	Woven polyethylene	Cotton+ polyethylene	High-density polyethylene	Low-density polyethylene	
Weight of 100 kernels (g)	At room Temperature (25±2°C)	33.13 bc	31.61 d	31.50 d	31.08 de	30.13 ef	29.48 f	31.16 b
	In refrigerator (10°C)	34.86 a	34.03 ab	34.88 a	31.36 de	32.15 cd	34.56 a	33.64 a
Mean		33.99 a	32.82 bc	33.19 ab	31.22 d	31.14 d	32.02 cd	32.40
Germination (%)	At room Temperature (25±2°C)	40.00 c	0.100 e	90.00 b	23.33 d	0.100 e	0.100 e	25.61 b
	In refrigerator (10°C)	100.100 a	96.67 ab	90.00 b	100.00 a	96.67 a	90.00 b	96.11 a
Mean		70.00 b	48.38 d	90.00 a	61.67 c	50.05 d	45.05 d	60.86

Moisture content (%)	At room Temperature (25±2°C)	14.10 c	14.17 c	14.10 c	14.23 c	15.43 a	15.73 b	14.63 a
	In refrigerator (10°C)	8.33 e	7.27 g	7.57 f	7.73 f	9.83 d	9.77 d	8.42 b
Mean		11.22 b	10.72 d	10.83 cd	10.98 c	12.63 a	12.75 a	11.53

***Values for each fungus followed by the same letter(s) are not significantly different, according to LSD test at *P* = 0.05.**