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Genotoxicity by ear and kernel rots in three maize genotypes stored at different conditions

Samar S. A. Elsayed [1] ; *Ibrahim E. Elshahawy* *[2] ; *Salem Hamden*[3]; *Mona E.H. Abd Elgawad*[4] *and Mohamed D. Sehsah*[1]

Address:

[1] *Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt*
[2] *Plant Pathology Department, National Research Centre, Cairo, Egypt*
[3] *Botany Department, Faculty of Agriculture, Kafrelsheikh University, Egypt*
[4] *Cytogenetics Lab- National Gene Bank, Agriculture Research Center, Giza, Egypt*
Corresponding Author: Ibrahim Elshahawy*, **e-mail:** ibrahim_nrc@yahoo.com

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ABSTRACT

This study set out to determine the impact of different storage temperatures and packing materials on storage fungi, genotoxic effect, germination rate, and chemical properties of three different cultivars of maize (SC131, TWC324, and Balady) that were stored for 0, 8, and 18 months. Data show that *Fusarium verticilioides*, was the most common fungus attacking maize grains and causing kernel rots in all tested maize cultivars and under all storage conditions. It was followed by *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp. For storage conditions, keeping maize grains in refrigerators (at 10°C and -5°C) in high density polyethylene packing was the most effective technique to prevent kernel rot. Results for grain germination and chemical attributes showed that when grains were maintained in refrigerators (at 10°C, and at -5°C) in high density polyethylene packing, maize SC131 had the highest germination percentage, protein, carbs, oil, and fibre content. In contrast, the Balady cultivar that was held at room temperature in woven polyethylene containers had higher amounts of ash, free fatty acids, and acidity than normal. Increased storage periods were observed to significantly reduce germination percentage and chemical grain component, particularly at room temperature and in woven polyethylene packing. In terms of genotoxicity, maize grain cultivar SC131 had the lowest percentage of chromosomal aberrations (CA), the highest percentage of mitotic index (MI), and the lowest infection by kernel rot.

Keywords: Kernels rot, Genotoxic effects, Storage conditions, Packaging materials, maize cultivars.

INTRODUCTION

Maize (*Zea mays* L.) is the world's third most important food crop, trailed only by wheat and rice. Food consumption in developing countries alone is expected to rise by roughly 1.3% per year (International Grains Council, 2020). Maize demand in the developing world is expected to double by 2050, and maize is expected to become the grain with the highest production globally and in the developing world by 2025 (Rosegrant *et al*., 2008; International Grains Council, 2020). According to the International Grains Council (2020), fungal diseases reduce worldwide maize production by 11%. The contamination of maize grain either before harvest or after harvest results from ear rot fungi that create mycotoxins under specific environmental conditions (Mukanga *et al*., 2010). According to Tsedaley and Adugna (2016), fungi are one of the primary causes of maize grain deterioration and loss. The predominant Fusarium species present in maize grains was always *F. verticillioides*, independent of the treatment, location (plot), or sampling time. While the others *Fusarium* species found in low percentages (Farahat and El-Shabrawy, 2021; Carbas *et al*., 2021). According to Tsedaley and Adugna (2016), fungi can cause 50-80% damage to farmers' corn during storage if conditions are favorable for their growth. Numerous fungi can remain attached to maize grains during storage, either destroying them or just remaining alive to infect germinating seedlings. *Aspergillus, Penicillium, Fusarium*, and a few xerophytic species are the most prevalent fungal genera found in stored grains (Orsi *et al*., 2000; Castlellarie *et al.,* 2010). Several of these species can create toxins. *Fusarium verticillioides* (Sacc.) Nirenberg (synonym *F. moniliforme* Sheldon, teleomorph *Gibberella moniliformis* Wineland) is the most common pathogen responsible for ear or kernel rot in maize (Lanubile *et al*., 2017; Gai *et al*., 2018; Lina *et al*., 2019). The predominant *Fusarium* species present in maize grains was always *F. verticillioides*, independent of the treatment, location (plot), or sampling time. While, the others Fusarium species founded in low

percentages (Farahat and El-Shabrawy, 2021; Carbas *et al*., 2021). The discovery that *F. verticillioides* not only reduces yield but also affects seed quality and can produce the secondary metabolite fumonisins has renewed interest in the fungus (Zhou *et al*., 2018; Czembor *et al*., 2019). Paddy *et al*. (2018) found that, the combined effect of warm temperatures and unsuitable storage bags results in accelerated grain deterioration and promotes growth of insects and fungi. Storage fungi including *F. moniliforme, Aspergillus* spp., and *Penicillium* spp. can grow in stored grain and cause large losses (Mehrotra, 1983; Mesterhazy *et al*., 2022).

Maize grain quality can be significantly influenced by the handling and storage procedures utilized after harvest (Mulhanga *et al*., 2022). Chemical composition, physiological germination, seed vigor, and the presence of seed-borne diseases are all considered "seed quality." The quality of the seed may remain constant during storage or decline to the point where it is unfit for planting. Factors influencing seed quality include environmental conditions, pests, diseases, seed oil content, moisture content, mechanical damage during processing, storage longevity, packaging, pesticides, storage temperature and humidity, and biochemical injury to seed tissue (Al-Yahya, 2001; Guberac *et al*., 2003; Šimic *et al*., 2004; Fufa *et al*., 2020; Topan *et al*., 2023). The physical and chemical properties of maize can change during storage, affecting its nutritional value and acceptability for ingestion (Lacerda *et al*., 2003; Chattha *et al*., 2012; Chen *et al*., 2020). Temperature, humidity, and pest management approaches can all be considered as storage conditions. However, storage times, storage temperatures, and packaging materials all had an impact on changes in grain germination, chemical composition, acidity, and oil content of maize grains as a result of infection by various fungi that cause ear and kernel rots (Chatha *et al*., 2012; Rahraw *et al*., 2013; Timoteo and Marcos-Filho, 2013; Santoso *et al*., 2015; Kibar *et al*., 2021). Inadequate storage conditions can also foster the growth of fungi, which produces mycotoxins that pose serious health problems to both humans and animals (Basyal *et al*., 2022). At all, Elsayed *et al*. (2023) illustrated that the tested maize cultivars, i.e., SC10 and SC176, were resistant to some diseases in maize, as compared with the open pollinated variety, *i.e*., the Balady cultivar. They also added that, the lowest kernel rot diseases, and high quantitative and qualitative yields per 2 rows were obtained from the plots sprayed with insecticide and fertilized by stored animal manure (decomposing animal manure).

Bewley and Black (1994) defined germination as the process by which seeds begin to take up water, and that is followed by the radical penetrating the endosperm and seed coat and the embryo lengthening. Because of their great initial downward development potential and quick subsequent expansion, maize seeds have a high germination potential. For seed to attain such a high germination rate, it must be stored for the necessary length of time to maintain its quality. To maintain high viability from harvest to planting, some seeds must be kept in a controlled environment (Tripathi and Lawande, 2014; Santoso *et al*., 2015). Marco (2023) showed that, the increasing of storage period can reduce grain weight, nutritional value, and germination capacity of stored maize grains. As a result, commercial grain buyers may pay a reduced price. Proper storage can increase a seed's vitality (Garoma *et al*., 2017). On the other hand, chromosomal abnormalities and genotoxic consequences are often the consequence of exposure to chemical and physical mutagens (Gecheff, 1996). One of the primary problems with *Fusarium verticillioides* is the production of genotoxic chemicals called mycotoxins (Sekhon *et al*., 2006). Numerous studies have reported that chromosome stickiness, univalent, multivalent, laggard, chromatin bridges, micronuclei, and other abnormalities can be caused by physical and chemical mutagens (Kumar and Singh, 2004; El-Diasty *et al*., 2009). Particularly in underdeveloped countries worldwide, there are worries regarding how to safely store seeds, including maize grains, for an extended period of time without rot pathogens and compositional changes. There is a need for precise and comprehensive approaches to evaluate how storage circumstances affect maize quality, given how important it is to maintain maize quality during storage. The current study set out to determine the impact of packing materials and storage conditions on the incidence of storage fungi in maize, grain quality, and genotoxic effects on the studied cultivars of maize.

MATERIAL AND METHODS

Study area, maize cultivars and treatments:

This study was carried out at the Sakha Agriculture Research Station, Agricultural Research Centre (ARC), Egypt, in the Research Farm, the Laboratory of Seed Technology, and the Laboratory of Plant Pathology. One commercial single cross (SC), three way cross (TWC) and open pollinated variety (balady) were used. These cultivars were namely, SC131 (A1), TWC 324 (A2) and balady cultivar (A3), respectively. These cultivars were obtained from the Egyptian Agricultural Research Centre (ARC). The cultivars listed above were cultivated in clay soil during the 2021 growing season as part of a field experiment. All prescribed farming techniques were followed. During harvest, ten kg of grains from each investigated maize cultivar were randomly picked and placed in containers composed of

paper (C1), high density polyethylene (143 g/m^2), and woven polyethylene (C2). Each container contained one kg of the tested grains, which were kept at three distinct temperatures: ambient (B1), 10°C (B2), and -5°C (B3). A factorial totally randomized design experiment consisted of three replicates were used.

Grain samples and data to be collected:

Random grain samples were collected from each package at three different storage intervals: zero time storage, eight months storage, and eighteen months storage. These samples were tested for germination, grain oil properties, acidity, physical and chemical compositions, and the isolation of fungi that cause kernel rot. To assess the mitotic index (MI) and chromosomal aberrations (CA), additional samples of the previously reported samples were transferred to the Cytogenetics and Animal Cell Culture Laboratory, National Gene Bank, ARC, Egypt, for further research.

Isolation and frequency of kernel rot pathogens:

According to EL-Sayed and Tolba (2005), laboratory tests were used to isolate the kernel rot fungi. Three maize cultivars (SC131, TWC 324, and Balady) were sampled at random on and after each storage term, storage condition, and under each evaluated packaging material test. To isolate the pathogens that cause maize kernel rots, 100 kernels of each tested maize cultivar were sterilized in 1% sodium hypochlorite for 3-4 minutes, washed several times in sterilized distilled water, blotted between two sterilized filter papers, and plated in Petri dishes with 10 ml of potato dextrose agar medium (PAD). For 8-10 days, the plates were incubated at 26-27 °C. The emerging fungi were examined and purified under a microscope using the hyphal tip procedure. Purified fungi were identified at the Department of Mycology, Institute of Plant Pathology, ARC, Giza, Egypt. The acquired fungi were counted (as a percentage) in each maize cultivar and treatment based on how frequently they appeared on isolation plates (ISTA, 1985).

Standard germination:

The test was conducted under ideal conditions in accordance with international testing standards (I.S.T.A., 1999).

Chemical composition characters:

Random samples of maize grain were taken from each container and ground to a fine powder that could pass through a 2 mm mesh for chemical analysis. A.O.A.C. (1990) provided the techniques for calculating the percentages (%) of oil, crude protein, carbs, crude fiber, ash, free fatty acids (FFA), and acidic value (AV).

The genotoxicity assay:

Grains of various maize varieties were germinating on wet filter paper in Petri dishes at room temperature. Root-tips that were between one and two centimeters long were gathered, processed, and fixed in a 3:1 mixture of ethanol and glacial acetic acid. The root tips were put into 70% ethyl alcohol and preserved for 24 h. The root tip cells were stained using the aceto-orcein staining technique as reported by Sayed-Ahmed (1985) and Ismail and Abd El-Gawad (2021). Following a thorough wash with distilled water, the fixed root tips were pressed onto dry, clean slides and stained with a little drop of aceto-orcein stain. At least five prepared slides with 400 cells each were used to score cytological criteria. Using a vertical fluorescence microscope (Leica DM4 B) fitted with a cooled digital color camera (Leica DFC450C), chromosomal aberrations and the mitotic index was calculated. The number of chromosomal abnormalities overall was estimated in dividing cells, and the mitotic index represented the proportion of divided cells to the total number of cells analyzed. Micronuclei (compact and non-compact), pieces, sticky binucleate cells, and laggards were among the abnormalities (Jacobs, 1997).

Statistical Analysis:

Statistical evaluation of data was performed by Mstatc program using two-way analysis of variance (ANOVA) followed by Duncan's multiple range test at p= 0.05 were considered as significant.

RESULTS

Effect of storage temperature on kernels rot fungi:

According to the data in Tables 1 and 2, SC131 had the lowest frequency of the kernels rot disease whereas balady cultivar had the highest frequency. On the other hand, storage at ambient temperature resulted in the highest incidence of kernel rot-causing fungi, whereas storage at -5°C resulted in the opposite. After an eight-month storage period, results in Table 1 showed that the cultivars SC 131, TWC 324, and balady each had a frequency of presence of the fungus *F. verticilioides* of 28.0, 33.1, and 37.1 %, respectively. Additionally, it measured 37.2, 32.4, and 28.2 % at room temperature, at 10°C, and at -5°C, respectively. The storage temperature 10°C was also suitable and resulted in decreasing of infection by the tested fungi, being 32.4, 3.0, 9.0, 11.0, 11.2 and 7.4 % comparing with storage at room temperature which recorded 37.4, 3.4, 13.0, 14.2, 15.0 and 10.1 % for each of *F. verticilioides*, *F. semetictum*, *A. flavus*, *A. niger*, *Penicillium* spp. and other fungi, respectively. The fungus *F. ver*ticilioides recorded

the highest frequency of occurrence (%) after an 18-month storage period, with respective frequencies of 32.3, 38.2, and 41.1 % in the SC 131, TWC 324, and Balady cultivars, respectively (Table 2). Additionally, it measured 45.4, 36.2, and 30.1 % at room temperature, at 10^{o}C, and -5 at -5^{o}C, respectively. As compared to storage at room temperature, which resulted in infection rates of 45.4, 19.1, 13.0, 21.0, 22.0 and 17.0 % for each of the tested fungi (*F. verticilioides, F. semetictum, A. flavus, A. niger, Penicillium* spp., and other fungi), storage at 10^{o}C was also effective and decreased infection rates by tested fungi.

Effect of packaging materials on kernels rot fungi:

Data given in Tables 1 and 2 demonstrated that, for all tested maize cultivars and storage settings, the highest frequency of kernel rot causal fungi were observed in cases of storage in paper and woven polyethylene package. However, the opposite was true when stored in high density polyethylene container. Regarding packaging materials, data in Table 1 showed that the fungus *F. verticilioides* recorded the highest frequency of occurrence of 32.0, 30.1 and 36.2 % when paper, high density polyethylene, and woven polyethylene package materials were used after an 8-month storage period. The reverse was true in case of infection by the fungus *Fusarium semetictum* and slightly other tested fungi. When employing paper, high density polyethylene, and woven polyethylene package materials, respectively, the infection percentage by *F. verticilioides* was the highest after an 18-month storage period, coming in at 36.4, 34.0, and 41.2 %. The opposite, however, was true when other examined fungi and the *Fusarium semetictum* causative organism were the source of the infection.

Effect of storage temperature, durations and packaging materials on kernels rot fungi:

Regarding the interactions between the tested treatments, after 8 months of storage, the obtained results in Table 1 illustrated that, the highest infection % by the tested fungi was obtained with interaction between A3×B1×C3 (balady cv × at room temperature × woven polyethylene). The frequencies of *F. verticilioides, F. semetictum, Aspergillus flavus, Aspergillus niger*, *Penicillium* spp. and other fungi were recorded as 45.0, 4.3, 18.3, 22.3, 25.0 and 15.3 %, respectively. The interaction between A1×B3×C2 (SC131× at -5^{o}C × high density polyethylene) resulted in the lowest infection rate, with frequencies of 20.3, 2.0, 3.3, 5.3, 3.3, and 3.0% for the fungi indicated. The results in Table 2 showed that, after an 18-month storage period, the interaction between A3×B1×C3 (balady cv × at room temperature × woven polyethylene) produced the highest infection percentage by the tested fungi. It was recorded frequencies of 56.0, 26.3,25.3, 30.3, 32.0 and 22.3 % for *F. verticilioides*, *F. semetictum*, *A. flavus*, *A. niger*, *Penicillium* spp. and other fungi, respectively. The interaction between A1×B3×C2 (SC131× at -5^{o}C × high density polyethylene) resulted in the lowest infection percentage, which was 23.3, 7.0, 5.0, 8.0, 4.3, and 4.3 %, respectively.

Table 1. Effect of storage conditions and different package materials on the percentage of kernels rot causal organisms after eight months storage period in three maize cultivars.

Treatment	Frequency of occurrence (%)					
	F. verticilioides	***F. semetictum***	***A. flavus***	***A. niger***	***Penicillium* spp.**	**Other fungi**
A= varieties						
SC131	28.0 c	3.0 c	7.0 c	9.0 c	8.0 c	6.0 c
TWC 324	33.1 b	3.0 b	9.0 b	11.0 b	12.0 b	7.0 b
Balady	37.1 a	3.4 a	12.0 a	13.4 a	14.4 a	9.2 a
F. test	**	**	**	**	**	**
LCD 5%	0.2	0.1	0.3	0.3	0.3	0.3
B = Storage conditions						
At RT	37.4 a	3.4 a	13.0 a	14.2 a	15.0a	10.1 a
At 10°C	32.4 b	3.0 b	9.0 b	11.0 b	11.2 b	7.4 b
At -5°C	28.2 c	3.0 c	6.0 c	8.0 c	8.0 c	4.3 c
F. test	**	**	**	**	**	**
LSD 5%	0.2	0.1	0.3	0.3	0.3	0.3
C= Package kinds						
Paper	32.0 b	3.0 b	8.4 b	10.0 b	10.0 b	7.0 b
HDP	30.1 c	2.7 c	7.2 c	9.0 c	9.0 c	6.0 c
WP	36.2 a	3.4 a	12.0 a	14.2 a	15.0 a	10.0 a
F. test	**	**	**	**	**	**
LSD 5%	0.3	0.1	0.3	0.4	0.3	0.3
A×B×C						
A1×B1×C1	32.0	3.0	10.0	11.0	10.0	8.0
A1×B1×C2	28.3	3.0	8.3	9.0	9.0	6.3
A1×B1×C3	39.0	4.0	13.0	14.0	15.0	11.0
A1×B2×C1	26.0	3.0	6.0	8.0	7.0	5.3
A1×B2×C2	25.3	2.3	4.3	7.3	5.3	5.0
A1×B2×C3	33.0	3.0	10.0	11.3	11.3	7.3
A1×B3×C1	21.3	2.3	4.0	5.3	4.3	3.3
A1×B3×C2	20.3	2.0	3.3	5.3	3.3	3.0
A1×B3×C3	28.0	3.0	5.3	7.3	7.3	4.3
A2×B1×C1	37.0	3.3	12.0	13.0	14.3	9.3
A2×B1×C2	35.0	3.1	11.0	11.3	12.3	8.0
A2×B1×C3	42.0	4.0	16.3	20.0	19.0	12.3
A2×B2×C1	32.3	3.0	8.0	9.3	10.3	7.0
A2×B2×C2	31.0	3.0	6.3	8.3	10.0	5.3
A2×B2×C3	35.3	4.0	10.3	14.3	14.3	9.3
A2×B3×C1	28.3	3.0	5.0	6.3	7.3	3.3
A2×B3×C2	27.0	2.3	3.3	5.3	6.3	3.0
A2×B3×C3	32.0	3.0	7.3	11.3	11.0	6.0
A3×B1×C1	42.0	4.0	14.0	15.3	16.0	12.0
A3×B1×C2	39.3	3.4	13.0	14.0	15.0	10.3
A3×B1×C3	45.0	4.3	18.3	22.3	25.0	15.3
A3×B2×C1	37.0	3.2	11.3	13.3	13.0	9.0
A3×B2×C2	34.3	3.0	10.3	10.3	12.0	8.0
A3×B2×C3	39.3	4.0	14.3	16.3	20.0	12.3
A3×B3×C1	32.3	4.0	8.0	10.0	9.3	5.3
A3×B3×C2	31.3	3.0	6.3	8.0	7.3	3.3
A3×B3×C3	35.0	4.0	11.3	12.3	14.3	8.3
F. test	**	**	**	**	**	**
LSD 5%	**0.8**	**0.2**	**0.9**	**1.0**	**0.9**	**0.9**

RT= Room temperature, HDP= High density polyethylene and WP= Woven polyethylene

Table 2. Effect of storage conditions and different package materials on the percentage of kernels rot causal organisms after eighteen months storage period in three maize cultivars.

Treatment	Frequency of occurrence (%)					
	F. verticilioides	***F. semetictum***	***A. flavus***	***A. niger***	***Penicillium* spp.**	**Other fungi**
A= varieties						
SC131	32.3 c	12.2 c	11.1 c	12.4 c	12.0 c	9.3 c
TWC 324	38.2 b	15.0 b	13.1 b	14.3 b	15.4 b	11.0 b
Balady	41.1 a	17.0 a	15.4 a	18.0 a	18.0 a	13.0 a
F. test	**	**	**	**	**	**
LCD 5%	0.2	0.2	0.2	0.2	0.2	0.2
B = Storage conditions						
At RT	45.4a	19.1 a	13.0 a	21.0 a	22.0 a	17.0 a
At 10°C	36.2 b	13.0 b	9.0 b	13.3 b	14.0 b	10.0 b
At -5°C	30.1 c	8.0 c	6.0 c	10.2 c	10.0 c	6.0 c
F. test	**	**	**	**	**	**
LSD 5%	0.2	0.2	0.2	0.2	0.2	0.2
C= Package kinds						
Paper	36.4 b	14.0 b	12.1 b	14.0 b	13.2 b	10.0 b
HDP	34.0 c	12.3 c	11.5 c	12.1 c	12.4 c	9.1 c
WP	41.2 a	18.0 a	16.0 a	19.0 a	19.1 a	14.0 a
F. test	**	**	**	**	**	**
LSD 5%	0.2	0.207	0.2	0.2	0.2	0.2
A×B×C						
A1×B1×C1	38.3	15.3	15.3	17.3	16.3	13.0
A1×B1×C2	33.0	14.3	15.0	16.0	16.0	11.3
A1×B1×C3	46.0	19.0	18.3	20.0	22.0	17.0
A1×B2×C1	32.0	12.3	10.0	9.3	9.3	8.0
A1×B2×C2	29.3	11.0	9.3	9.0	8.3	7.3
A1×B2×C3	36.3	15.0	13.3	15.3	14.0	12.0
A1×B3×C1	24.0	7.3	5.3	8.3	6.0	5.0
A1×B3×C2	23.3	7.0	5.0	8.0	4.3	4.3
A1×B3×C3	30.0	11.0	10.0	10.3	11.0	8.0
A2×B1×C1	45.3	17.3	19.0	19.0	21.3	15.0
A2×B1×C2	43.3	17.0	18.3	16.3	20.3	15.3
A2×B1×C3	53.0	23.0	23.0	26.3	25.3	22.0
A2×B2×C1	37.3	14.3	11.3	12.0	13.0	9.0
A2×B2×C2	34.0	13.0	11.0	11.3	12.3	8.3
A2×B2×C3	38.3	18.3	14.3	16.3	17.0	12.0
A2×B3×C1	31.0	10.0	7.0	8.0	9.0	5.3
A2×B3×C2	29.3	9.0	6.3	7.3	8.3	3.3
A2×B3×C3	34.0	12.3	10.0	14.0	14.0	9.0
A3×B1×C1	48.3	20.0	20.3	23.0	21.3	19.0
A3×B1×C2	46.3	18.3	19.0	20.3	20.3	18.3
A3×B1×C3	56.0	26.3	25.3	30.3	32.0	22.3
A3×B2×C1	40.0	16.0	14.0	17.0	14.0	10.3
A3×B2×C2	36.3	13.3	14.0	11.3	12.3	10.0
A3×B2×C3	43.3	21.0	18.0	20.0	24.0	13.3
A3×B3×C1	33.3	10.3	8.3	11.3	10.3	5.3
A3×B3×C2	31.3	10.0	8.0	11.0	10.0	4.3
A3×B3×C3	36.3	15.3	14.0	16.0	16.0	11.0
F. test	**	**	**	**	**	**
LSD 5%	0.7	0.6	0.6	0.6	0.7	0.7

RT= Room temperature, HDP= High density polyethylene and WP= Woven polyethylene

Effect of storage temperature and durations on kernels rot fungi and grains germination:

Data presented in Table 3 summarized that, the infection % by F*. verticilioides* was the highest on all tested maize cultivars and under all tested storage conditions and periods. It varied from 23.3 to 49.3 %, while, the reverse was true in case of infection by *F. semetictum*. The obtained results in Table 3 also showed that, the infection % by tested fungi i.e. *F. verticilioides*, *F. semetictum*, *A. flavus*, *A. niger*, *Penicillium* spp. and other fungi was significantly increased under storage at room temperature after 8 months, ranged from 37.3 to 14.4 % comparing with zero time, it ranged from 28.3 to 4.0 %, in SC131, and ranged from 42.0 to 7.0 % comparing with zero time it ranged from 33.3 to 6.0 % in TWC324, while it ranged from 45.3 to 17.0 % comparing with zero time it ranged from 38.0 to 9.0 % in balady maize cultivar. On the other hand, the germination % were decreased and the infection by tested fungi were very increased after 18 months storage period under room temperature comparing with infection at zero time, in all tested maize cultivars.

Table 3. Effect of different storage periods on grain germination (%) and kernels rot pathogens (%) in three maize cultivars.

Cultivar	Percentage of germination and ear and kernel rots pathogens (%)						
	Germination	*F. verticilioides*	*F. semetictum*	*A. flavus*	*A. niger*	*Penicillium* spp.	Others
At zero time							
SC131	94.3	28.3	4.0	9.0	7.4	8.0	5.0
TWC 324	92.0	33.3	6.0	10.0	11.0	10.0	7.0
Balady	90.0	38.0	10.0	13.0	12.4	12.2	9.0
After 8 months storage at room temperature							
SC131	91.1	37.3	15.0	13.0	15.0	14.4	11.0
TWC 324	88.0	42.0	18.0	16.4	18.0	17.0	14.0
Balady	84.3	45.3	23.0	19.2	21.0	22.0	17.0
After 18 months storage at room temperature							
SC131	89.1	39.3	18.3	16.3	18.1	18.0	14.0
TWC 324	84.4	46.3	22.0	20.0	21.0	22.0	18.0
Balady	82.0	49.3	26.3	24.0	25.0	25.0	20.1
After 8 months storage at 10^{o}C							
SC131	95.3	27.3	5.0	8.3	8.4	8.0	6.0
TWC 324	92.3	33.0	8.0	11.0	11.0	10.3	8.1
Balady	92.0	36.3	10.3	14.0	14.0	13.0	10.0
After 18 months storage at 10^{o}C							
SC131	93.0	28.3	5.3	9.0	10.0	10.0	7.3
TWC 324	90.3	36.3	9.0	11.3	12.0	13.0	10.0
Balady	90.0	39.3	11.3	15.0	14.0	13.0	10.1
After 8 months storage at -5^{o}C							
SC131	97.3	23.3	4.0	7.0	7.1	7.1	4.4
TWC 324	94.4	28.3	7.3	9.0	10.0	10.0	7.0
Balady	93.1	32.3	10.0	11.0	12.0	12.0	9.4
After 18 months storage at -5^{o}C							
SC131	95.0	25.3	5.0	8.0	8.0	8.0	5.3
TWC 324	92.3	31.0	8.0	9.3	10.3	10.3	8.0
Balady	91.3	33.3	10.3	12.0	13.0	12.0	10.0
F. test	**	**	**	**	**	**	**
LSD 5%	1.5	1.1	0.9	1.1	0.9	0.7	0.8
LSD 1%	2.0	1.5	1.2	1.5	1.3	1.0	1.0

Effect of storage temperature, durations and packaging materials on grains components:

The maize cultivar SC131, according to the data in Table 4, was the best hybrid and had the largest percentage of grains containing oil, protein, carbohydrates and fiber, being 6.1, 10.0 , 80.0 and 5.0 % , respectively. In terms of free fatty acids, ash, and acidic value, this cultivar had the lowest concentrations (0.2, 1.4 %, and 4.3, respectively). The reverse was true in case of balady cultivar. The best storage conditions, on the other hand, were obtained at -5°C and 10°C; storage at these temperatures led to high grain concentrations of protein, carbohydrates and fiber, being 6.1 & 6.0, 9.0 & 9.0, 79.1 & 76.3 and 5.0 & 5.0 %, under -5°C and 10°C, respectively. According to data from Table 4 regarding storage packaging materials, high density polyethylene packages were the best and kept the grain components. The oil, protein, carbohydrates, and fiber contents of maize grains stored in high density polyethylene packages were high being 6.0, 9.0, 79.0 and 5.0, respectively, while the bad compounds, such as free fatty acids, ash, and acid value, were at their lowest levels being 0.5, 1.2 % and 5.0, respectively. In the case of storage in woven polyethylene packaging materials, the opposite was true.

Table 4. Effect of storage conditions and different package materials on chemical properties of maize grains after eighteen months storage period in three maize cultivars.

Treatment	Chemical properties of corn grains						
	% oil	Free fatty acids	% protein	% carbohydrates	% ash	% fiber	Acidic value
Cultivars							
SC131	6.1 a	0.16 c	10.0 a	80.0 a	1.4 c	5.0 a	4.3 b
TWC 324	6.0 b	0.17 b	9.0 b	78.0 b	1.6 b	4.2 b	4.2 b
Balady	6.0 c	0.19 a	8.4 c	76.0 c	2.0 a	3..7 c	5.0 a
F. test	**	**	**	**	**	**	**
LCD 5%	0.1	0.05	0.4	1.2	0.2	0.3	0.3
Storage conditions							
At RT	5.2 c	1.0 a	8.2 c	73.2 c	2.0 a	4.1 c	9.0 a
At 10°C	5.6 b	0.8 b	8.6 b	76.3 b	1.7 b	4.5 b	7.0 b
At -5°C	6.1 a	0.6 c	9.0 a	79.1 a	1.4 c	5.0 a	5.2 c
F. test	**	**	**	**	**	**	**
LSD 5%	0.3	0.1	0.3	1.6	0.2	0.3	0.9
Package kinds							
Paper	5.6 b	0.7 b	8.6 b	76.3 b	1.6 b	4.6 b	6.3 b
HDP	6.0 a	0.4 c	9.0 a	79.0 a	1.2 c	5.0 a	4.9 c
WP	5.1 c	1.0 a	8.2 c	74.0 c	2.0 a	4.3 c	8.0 a
F. test	**	**	**	**	**	**	**
LSD 5%	0.3	0.1	0.3	1.5	0.2	0.2	1.1

RT= Room temperature, HDP= High density polyethylene and WP= Woven polyethylene

Genotoxicity of storage conditions on maize grains:

Table 5 displays the results of the mitotic index (MI) and chromosomal aberrations (CA) analysis of the three maize cultivars (SC131, TWC 324, and Balady) that were kept for eight months in three distinct environments (at room temperature, at 10°C, and below -5°C). Of particular interest is the comparison between the storage conditions of high-density polyethylene at -5°C and room temperature. For SC131, the MI and CA values were 20.3 and 8.9, respectively, when stored at 10°C, and 26.0 and 2.1, respectively, when stored in high-density polyethylene at -5°C. The MI value for SC131 was significantly higher under both 10°C and high-density polyethylene at -5°C compared to room temperature (19.0). The CA value for SC131 was significantly lower in high-density polyethylene at -5°C compared to room temperature (6.0 vs. 2.1). For TWC 324, the MI and CA values were 20.0 and 9.0, respectively, when stored at 10°C, and 24.1 and 3.0, respectively, when stored in high-density polyethylene at -5°C. The MI value for TWC 324 was significantly higher in high-density polyethylene at -5°C compared to room temperature (18.0 vs. 19.0). When compared to room temperature, the CA value for TWC 324 in high-density polyethylene was much lower at -5°C (5.2 vs. 3.0). The MI and CA values for Balady were 19.0 and 10.2 while held at 10°C and 23.7 and 3.1 when stored in high-density polyethylene at -5°C, respectively. In high-density polyethylene, the MI value for Balady was much greater at -5°C than it was at ambient temperature (17.5 vs. 19.0). At -5°C, high-density polyethylene had a much lower CA value for Balady (8.0 vs. 3.1) than it did at room temperature. Table 5 displays the results of the mitotic index (MI) and chromosomal aberrations (CA) analysis of the three maize cultivars (SC131, TWC 324, and Balady) that were kept under various temperatures for 18 months (at room temperature, at 10°C, and under -5°C). The contrast of high-density polyethylene storage conditions at -5°C and room temperature is very

fascinating. The MI and CA values for SC131 were 14.3 and 17.0 while held at 10°C and 20.2 and 3.8 when stored in high-density polyethylene at -5°C, respectively. When compared to high-density polyethylene at -5°C, the MI value for SC131 at 10°C was significantly higher (14.3 vs. 20.2). In high-density polyethylene, the CA value for SC131 was much lower at -5°C than it was at ambient temperature (17.0 vs. 4.0). The MI and CA values for TWC 324 were 13.0 and 18.0 while held at 10°C and 18.6 and 4.6 when stored in high-density polyethylene at -5°C, respectively. The MI value for TWC 324 was significantly higher at 10°C compared to high-density polyethylene at -5°C (13.0 vs. 19.0). At -5°C, high-density polyethylene had a substantially lower CA value for TWC 324 than it did at ambient temperature (18.0 vs. 5.0). The MI and CA values for Balady were 11.2 and 18.0 while held at 10°C and 15.4 and 6.2 when stored in high-density polyethylene at -5°C, respectively. The MI value for Balady was significantly higher at 10°C compared to high-density polyethylene at -5°C (11.2 vs. 15.4). The CA value for Balady was significantly lower in high-density polyethylene at -5°C compared to room temperature (18.0 vs. 6.2). The data suggest that storing maize genotypes in high-density polyethylene at -5°C is a better storage method compared to room temperature for maintaining the quality and viability of the seeds, even over a longer period of 18 months. This is evidenced by the higher MI values and lower CA values observed in the former. It is important to note that the storage conditions for 18-months at room temperature led to much lower MI values and significantly higher CA values for all three genotypes, showing a clear drawback of utilizing this approach for long-term storage.

Table 5. Effect of storage conditions and different package materials on the percentage of mitotic index (MI) and chromosomal aberrations (CA) of maize grains after 8- and 18-months storage period in three maize cultivars.

Package	Cultivar	% mitotic index (MI) and chromosomal aberrations (CA)							
		Control (zero time)		At RT		At 10°C		At -5°C	
		MI	CA	MI	CA	MI	CA	MI	CA
After 8 months storage									
Paper	SC131	25.7	2.1	19.0	6.0	19.3	5.0	20.3	3.4
	TWC324	25.0	2.4	18.0	6.7	19.0	5.2	19.4	4.5
	Balady	25.1	3.0	17.5	7.5	18.5	5.7	19.0	5.0
HDP	SC131	25.7	2.1	20.3	8.9	23.8	3.2	26.0	2.1
	TWC324	25.0	2.4	20.0	9.0	21.7	4.0	24.1	3.0
	Balady	25.1	3.0	19.0	10.2	20.0	4.5	23.7	3.1
WP	SC131	25.7	2.1	12.0	12.5	18.2	8.0	15.8	6.7
	TWC324	25.0	2.4	10.3	13.6	16.0	8.7	14.5	8.0
	Balady	25.1	3.0	10.1	15.1	15.4	9.0	14.0	9.0
LSD 0.05		0.4	0.7	1.2	0.8	0.7	0.6	1.7	0.8
After 18 months storage									
Paper	SC131	25.7	2.1	10.2	10.2	10.2	8.1	18.2	6.0
	TWC324	25.0	2.4	9.6	12.5	10.1	11.1	15.0	8.2
	Balady	25.1	3.0	9.0	16.0	10.0	12.3	11.2	11.0
HDP	SC131	25.7	2.1	14.3	17.0	14.5	7.2	20.2	3.8
	TWC324	25.0	2.4	13.0	18.0	13.6	9.0	18.6	4.6
	Balady	25.1	3.0	11.2	18.0	11.9	10.0	15.4	6.2
WP	SC131	25.7	2.1	8.2	20.2	12.3	13.2	11.2	8.0
	TWC324	25.0	2.4	7.0	21.3	11.4	15.0	10.0	11.2
	Balady	25.1	3.0	6.0	24.1	10.0	17.0	9.1	15.3
LSD 0.05		0.4	0.7	1.4	1.1	0.6	0.7	1.3	1.5

RT= Room temperature, HDP= High density polyethylene and WP= Woven polyethylene

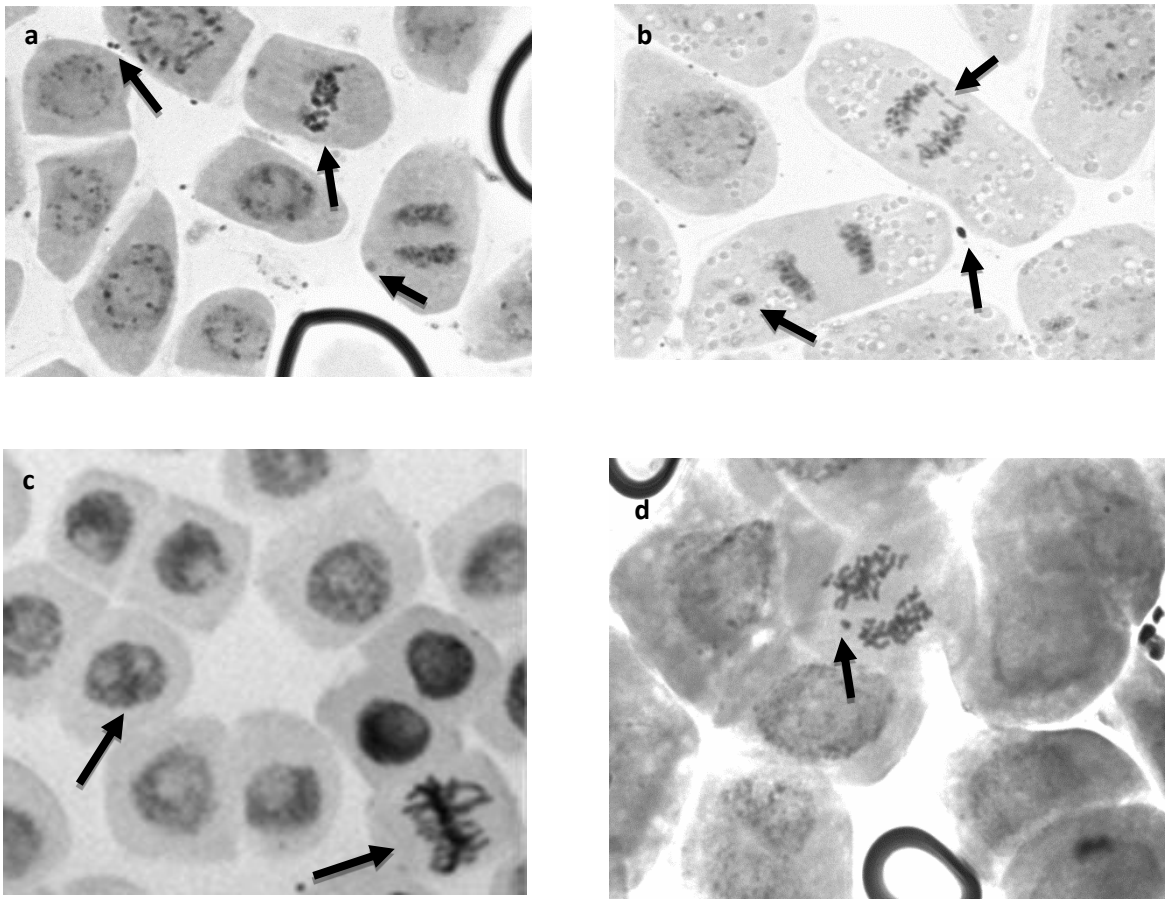

Fig. 1. The chromosomal aberrations, a: Non-Compact micronuclei, Compact micronuclei and Stickiness, b: Fragments, Compact micronuclei and Laggard, c: Binucleate cells and Stickiness, d: Laggard.

DISCUSSION

The obtained data show that *Fusarium verticilioides*, was the most common fungus attacking maize grains and causing kernel rots in all tested maize cultivars and under all storage conditions. It was followed by *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp. and other *Fusarium* species. These results are in the same with findings of Carbas *et al*., (2021). They illustrated that, the predominant *Fusarium* species present in maize grains was always *F. verticillioides*, independent of the treatment, location (plot), or sampling time. While, the others *Fusarium* species founded in low percentages. In general. the results also showed that: 1) The maize single cross hybrid 131 was recorded the lowest infection (%) by the storage fungi compared to other tested maize cultivars. This result in the same line with which reported by Samar *et al*. (2023). They illustrated that, the tested maize cultivars, *i.e.*, SC10 and SC176, were resistant to ear and kernel rots disease in maize, as compared with the open pollinated variety, i.e., the Balady cultivar. The tested maize hybrids were also had the highest quantitative and qualitative yields per 2 rows as compared with Balady cultivar. 2) The best storage condition which resulted in lowest infection (%) was storage under -5^{o}C followed by storage at 10^{o}C compared with storage at room temperature for all tested fungi. 3) The best packaging material which resulted in lowest kernels rot infection (%) was high density polyethylene package followed by paper package, comparing with storage in woven polyethylene package one. These findings followed a similar pattern to those published by (El-Sayed and Tolba, 2005; Shabana *et al*., 2015; Czembor *et al*., 2019; Paddy *et al*., 2018). They demonstrated that decreasing the storage duration reduced kernel rot disease infection of maize grains. Storage at temperatures below 10^{o}C resulted in a decrease in the infection of maize grains by the pathogens that cause kernel rot (*F. verticilioides*, *A. niger*, *A. flavus* and *Penicillium* spp.). They further said that, especially when stored at 10°C, high density polyethylene packaging was the optimum material for storage at various times and resulted in a reasonable proportion of germination and the lowest percentage of infection by kernels rot disease. The findings were also consistent with those of Shabana *et al*.

(2015), who found that maize grains should be stored at low temperatures (in a refrigerator at 10°C) in low-density polyethylene bags in order to preserve the vitality of the seeds and maintain high oil content, as well as to reduce the prevalence of storage fungi, particularly those that produce mycotoxins and aflatoxins. Therefore, Paddy *et al*. (2018) found that, the combined effect of warm temperatures and unsuitable storage bags results in accelerated grain deterioration and promotes growth of insects and fungi.

In the current study, increasing storage period from eight months to eighteen months in all tested maize types, storage conditions, and all evaluated storage packing materials resulted in a modest rise in the infection % by the kernels rot fungi. When compared to storage at 10°C and under -5°C conditions, with the use of high density polyethylene and paper packages material, the rate of infection kernels rot fungi was highest when packaging was made of woven polyethylene and stored at ambient temperature. For all evaluated maize cultivars, storage at room temperature and use of woven polyethylene packaging material resulted in the highest frequency of the organisms that cause kernel rot. The opposite was true when high density polyethylene packaging material was used and storage temperatures of 10°C and -5°C. These findings can be summed up as follows: 1) By increasing storage time from eight months to eighteen months, the infection percentage by kernels rot fungi was slightly increased. 2) The maize single cross hybrid 131 was recorded as having the lowest infection (%) by the kernels rot fungi compared to other tested maize cultivars. 3) In comparison to storage at room temperature, storage under -5°C, followed by storage at 10°C, resulted in the lowest infection percentage. 4) Likewise, high density polyethylene packaging outperformed paper packaging in terms of infection percentage when compared to storage in woven polyethylene packaging. These findings followed the same pattern as those reported by Shabana *et al*. (2015). Moreover, Marco (2023) added that, the increasing of storage period can reduces grain weight, nutritional value, and germination capacity of stored maize grains. As a result, commercial grain buyers may pay a reduced price.

It's crucial to comprehend the physiological and molecular mechanisms that cause seed germination to be delayed and even fail when seeds are stored for a prolonged period of time. Natural seed ageing processes and physiological changes would occur as seed was stored for a longer period of time (Sisman and Delibas, 2004; Morda Shaba, 2013; Fufa *et al*., 2020). In the current study, maize grains stored inside high density polyethylene containers, particularly under 10^{o}C and at -5^{o}C storage conditions, yielded the maximum grain viability and germination %. According to a research report, seeds kept for longer periods of time are more likely to have chromosomal aberration, DNA damage, and/or protein degradation, which can reduce their ability to germinate and grow new seedlings (Whittle, 2006). These could be the causes of certain seeds in the current study's prolonged seed storage conditions failing to germinate and emerge. Due to the seasonal variations in usual warehouse conditions, seeds in storage are exposed to low temperatures and humidity during the winter, moderate temperatures and humidity during the spring and autumn, and high temperatures and humidity during the summer. According to Volenik *et al*. (2006), under these conditions, seeds maintained in cloth or paper bags are temperate and easily exchange moisture with ambient air of defined relative humidity and temperature. If there is a rise in seed moisture due to moisture equilibration between the air surrounding the seeds and the seeds themselves, the deteriorative process and corresponding temperature increase within the seeds speed up, which ultimately reduces germination and vigor. The best hybrid overall, maize cultivar SC131 had the largest percentage of grains composed of oil, protein, carbohydrates and fiber. The balady cultivar was the exception to this rule. On the other hand, -5°C and -10°C were shown to be the ideal storage conditions. These storage conditions led to increased grain oil, protein, carbohydrate, and fiber concentrations. These findings follow a similar pattern to those of (Tolba and EL-Sayed, 2002; El-Sayed and Tolba, 2005; Garoma *et al*., 2017). They provided evidence that by carefully regulating temperature and relative humidity, seeds of the majority of species may be securely stored for a number of years. Additionally, they noted a positive correlation between the percentage of fungal infection in maize grains and the percentages of free fatty acid (FFA), acidic value (AV), acidity, and crude protein. The proportion of fungal infection in the revers was adversely linked with the percentage of endosperm in the maize grains. *F. verticilioides*, *F. semetictum*, *A. niger*, *A. flavus* -infected maize grains stimulated seed respiration, which reduced the seed's dry weight and viability as seen by poor germination. According to Labuschagne *et al*. (2014), storage of maize grains at 3.6°C and 18.5°C resulted in some loss in the amylose and starch content, however the reduction in starch was not significant. On the other hand, after 6 and 12 months of storage, storage at 30^{o}C dramatically decreased the starch and amylose concentration. In a similar vein, Shabana *et al*. (2015) reported that, storing maize grains at low temperature (in refrigerator at 10^{o}C) inside packages made of low-density polyethylene was found to preserve the vitality of seeds and maintain high oil content in addition to reducing the incidence of storage fungi, especially those producing mycotoxins/aflatoxins. Changes in grain germination, chemical

composition, acidity and oil content of three maize genotypes, due to infection by different fungi of kernels rot, were affected by storage periods (6 and 18 months), storage temperature (room temperature and 10°C) (Timoteo and Marcos-Filho, 2013) and package materials (paper, woven polyethylene and high density polyethylene (Chatha *et al*., 2012, Rahraw *et al*., 2013 and Santoso *et al*., 2015); Topan *et al*., 2023).

On the other hand, our study compared the chromosomal aberrations (CA) and mitotic index (MI) of three maize genotypes (SC131, TWC 324, and Balady) maintained for 8 months under various settings (zero time, at room temperature, at 10°C, and under -5°C). The contrast of high-density polyethylene storage conditions at -5°C and room temperature is very fascinating. According to the findings, high-density polyethylene storage of maize genotypes at -5°C is preferable to room temperature storage for preserving the quality and viability of the seeds as shown by the former's greater MI and lower CA values. Moreover, use airtight containers such as plastic bags or sealed containers can help prevent moisture buildup and reduce the risk of fungal contamination (Ortiz *et al*., 2010; Topan *et al*., 2023). The information in Table 5 also demonstrated that seed quality and viability were higher when stored in high-density polyethylene bags as opposed to other types of containers. Airtight containers are necessary for preventing moisture buildup, but it's also crucial to provide proper ventilation to avoid the accumulation of gases like carbon dioxide that might encourage fungal growth. When compared to storage in paper bags, storage in woven polyethylene bags (which offer some ventilation) was related with lower MI and CA values, indicating a lower risk of fungal contamination. As shown by the lower MI and CA values seen in the Table5, storing maize seeds in high-density polyethylene bags at -5°C is the most efficient way to lower the risk of fungal infection. To further lessen the danger of contamination during storage, airtight containers and appropriate ventilation are crucial concerns. It's crucial to remember that it's advised to regularly check the viability and quality of seeds in order to maintain the best storage conditions and avoid fungal contamination. The finding implies that chromosome abnormalities are produced when a fungal infection is present. According to findings from Agar and Alpsoy (2005) and El-Diasty *et al*. (2009), Castlellarie *et al*., (2010), Chen *et al*. (2020) this consequence of the fungal infection may be attributable to the impact of mycotoxin.

CONCLUSION

Conditioned storage facilities are required in various regions of the world, particularly in the tropics, in order to preserve high viability of some seeds from harvest to planting. The obtained results showed that, 1) The maize single cross hybrid 131 was recorded the lowest infection (%) by the tested fungi comparing with other tested maize cultivars. 2) The best storage condition that resulted in lowest kernels rot infection (%) was storage under at -5°C followed by storage at 10°C comparing with storage at room temperature. 3) When compared to storage in a woven polyethylene package one, high density polyethylene packaging performed the best and had the lowest infection percentage. 4) The highest grain concentrations of oil, protein, carbohydrates and fiber were obtained when grains were stored under -5°C and under 10°C, respectively. 5) Chromosome abnormalities are produced when a fungal infection is present in maize grains during poor storage conditions and in subpar packaging materials. The impact of mycotoxin may be responsible for this result of the fungi infection.

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تأثير ظروف التخزين ونوع عبوات التعبئة على مرض عفن الحبوب , انبات الحبوب , الخصائص الكيميائية وتأثير السمية الجينية لثلاثة أصناف من الذرة الشامية

سمر صبحى السيد[1], إبراهيم السعيد الشهاوى[2]* , سالم حمدان[4] ,منى ابراهيم عبدالجواد[3] , محمد درغام صحصاح [1]

[1]معهد بحوث أمراض النبات، مركز البحوث الزراعية، الجيزة، مصر

[2]قسم أمراض النبات، المركز القومي للبحوث، القاهرة، مصر

[3]قسم النبات الزراعى - كلية الزراعة، جامعة كفر الشيخ، مصر

[4]معمل الوراثة الخلوية - البنك القومي للجينات، مركز البحوث الزراعية، الجيزة، مصر

* بريد المؤلف المراسل ibrahim_nrc@yahoo.com

تؤثر ظروف التخزين غير المواتية على جودة حبوب الذرة، مما يسبب تغيرات مرضية وكيميائية تؤدي إلى خسائر كمية ونوعية كبيرة في المنتج. كان الهدف من هذه الدراسة هو تقييم النسبة المئوية لمسببات الأمراض التي تسبب تعفن الحبوب، وإنبات الحبوب، والخصائص الكيميائية، والتأثير السمي الجيني لعينات حبوب الذرة المخزنة في درجات حرارة مختلفة ومع مواد التعبئة والتغليف المختلفة لمدة 0 و 8 و 18 شهرا. أجريت هذه الدراسة على ثلاثة أصناف SC131 :، TWC324، وبلدي. تشير البيانات إلى أن *Fusarium verticilioides* ، *Aspergillus niger*، *Aspergillus flavus* و*Penicillium* spp. كانت أكثر الأنواع شيوعًا التي تهاجم حبوب الذرة وتسبب تعفن الحبوب في جميع أصناف الذرة المختبرة وتحت جميع ظروف التخزين. وفقًا لإحصائيات إعدادات التخزين، فإن حفظ حبوب الذرة في الثلاجات (عند 10 درجات مئوية و-5 درجات مئوية) في عبوات من البولي إيثيلين عالي الكثافة كان أفضل طريقة لمنع حدوث تعفن الحبوب. أظهرت نتائج إنبات الحبوب والخواص الكيميائية أنه عند حفظ الحبوب في الثلاجات (عند درجة حرارة 10 درجة مئوية، ودرجة حرارة -5 درجة مئوية) في عبوات من البولي إيثيلين عالي الكثافة، تم الحصول على أفضل نسبة إنبات ونسبة بروتين وكربوهيدرات وزيت وألياف في الذرة .SC131 على العكس من ذلك، كانت أصناف الذرة، وخاصة البلدي، التي تم تخزينها في درجة حرارة الغرفة في عبوات البولي إيثيلين المنسوجة تحتوي على مستويات عالية من الرماد، ومستويات من الأحماض الدهنية الحرة وحمضية أعلى من المعتاد، وكلها ساهمت في تدهور وفقدان جودة حبوب الذرة المخزنة. تبين أن زيادة أوقات التخزين تؤدي إلى تقليل نسبة الإنبات ومكونات الحبوب الكيميائية بشكل كبير، خاصة عند القيام بذلك في درجة حرارة الغرفة ومع التغليف المنسوج من البولي إيثيلين. من حيث التأثير السمي الوراثي، أظهر صنف حبوب الذرة SC131 أقل نسبة للانحرافات الكروموسومية (CA) وأعلى نسبة للمؤشر الانقسامي(MI) ، وكذلك أقل إصابة بعفن الحبوب. علاوة على ذلك، كان للصنف البلدي أعلى نسبة CA وأدنى نسبةMI ، وكان لديه أعلى نسبة إصابة بتعفن الحبات.

الكلمات المفتاحية: تعفن الحبوب، ظروف التخزين، انبات الحبوب، الانحرافات الكروموسومية.