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Effects of late wilt disease on infection development of ear rot disease, phenolic compounds, trypsin and α-amylase inhibitors of some maize hybrids grains and quality characteristics of fortified cookies

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ABSTRACT
Under Sakha Res. Farm field conditions, ears of infected maize plants by late wilt disease more infected by ears rot disease than free ones were observed, so this study was done. Positive relation between two fungal diseases of late wilt and ears rot was subjected in the two tested successive seasons, wherever infection by the first disease led to significant increase of infection and efficiency percentages of the second disease of most tested maize hybrids, as well as, by *Fusarium verticillioides* fungus in compassion of healthy free one from late wilt disease. This positive relation resulted in significantly decrease of ears yield, length and 100 kernels weight of most tested hybrids. The infection by late wilt disease resulted in increasing of asymptomatic infection efficiency by major ears rot causal fungus of *F. verticillioides* and SCs166 and 128 were the highest and the lowest ones, respectively, in compassion of healthy ones in the two tested seasons. Climatic conditions were suitable for ears and kernels rot disease in the two tested seasons. *F. verticillioides* fungus was the most kernels deteriorative one followed by *Aspergillus niger,* while *A. flavus* was the lowest one. Moisture, crude protein, ether extract, ash, crude fiber and total carbohydrates were significantly affected by hybrids and infection status. Antifungal compounds of total phenolic compounds, antioxidant activity, trypsin and α-amylase inhibitors were recorded in high amount of healthy and resistant maize hybrids grains, especially in SC173 than healthy and infected ones of susceptible hybrids grains, especially in SC131and may have a role in resistance to late wilt disease and ears rot infection. Chemical composition of defatted maize germ flour (DMGF) is very rich in protein (23.60%), ash and fiber compared with wheat germ flour and wheat flour (72%). The cookies supplemented with defatted maize germ was considered more nutritive compared with that of control (wheat flour cookies) since it has higher contents of protein, ash and crude fiber. From the results of sensory evaluation, it should be noted that, fortification of DMGF until 20% is acceptable for the sensory evaluation of cookies.

Keywords: maize late wilt, ears rot diseases, phenolic compounds, trypsin and α- amylase inhibitors, defatted maize germ, cookies

Introduction
Maize or corn (*Zea mays* L.) was considered as a staple food, third leading crop of many parts in the world after rice and wheat (Sandhu, *et al.*, 2007 and Nwosu *et al.,* 2015). In addition, it used in many industries, *ie.* oils, starch, weaving of fibers, paper making, a bio-fuel source (Atta *et al.*, 2017). Late wilt is a vascular wilt disease of corn that caused by soilborn and seedborn fungus of *Harpophora maydis* (Samra *et al.,* 1966) W. Gams with syn.:*Cephalosporium maydis* (CABI 1999 ; El-Shafey and Claflin 1999), and considered the most economically important fungal disease of corn in 1960 in Egypt, serious economic losses up to 40% of grain yield and 100% infection disease affected 70% of plants occurs in susceptible varieties (Johal *et al.,* 2004). Late wilt is associated with infection by secondary invaders especially, *ie. Fusarium verticillioides* to present stock rot complex (El-Shafey and Claflin 1999). *F. verticillioides* syn. *F. moniliforme*, teleomorph *Gibberella moniliformis* was the most common pathogen causing *Fusarium* ear rot of maize which can infect all tissues, a disease which significantly decreases the amount and quality of its annual yield (Veenstra *et al.*, 2018). This fungus produced fumonisins, harmful mycotoxins, accumulated in kernels and toxic to humans and animals when used for food or feed. (Desjardins *et al.*, 1998 and Covarelli *et al.*, 2012). To date no reports had

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studied the pathological relationship between late wilt and ear and kernels rot diseases so, we used another studies. Scholars of Gai *et al.* (2018), Duncan and Howard (2010) and Chen (2000) found that, infection cycle from seeds to stems to ears to grains by *F. verticillioides* occurs, which infected maize plants from stalk extended to ears and infected kernels, causing systemic infection and infected kernels are also pathogenic to maize stalks. Interaction between diseases was studied by Tolba and El-Sayed (2005) and reviewed that, maize downy mildew (DM) disease was companied and related with infection by certain common smut (CS) disease which distributed in the plants infected by DM disease of susceptible maize hybrids. Moreover, (Farahat, 2008 and Fadel *et al.*, 2009) demonstrated that, positive relation between DM and certain CS diseases in maize was shown, which DM infection played an important role in spreading of CS disease by high level of indole acetic acid (IAA) content in maize plants. Moreover, Farahat *et al.* (2014) recorded that, potential and positive pathological relation between the two above diseases and increment of infection by first disease led to increment of infection and disease index of the second disease in susceptible and resistant maize hybrids. The close association between *Fusarium* and *Aspergillus* ear rots in maize has been identified by Robertson-Hoyt *et al.* (2007).In addition, Abbas *et al*. (2006) reported that, both *Fusarium* and *Aspergillus* can thrive on maize without necessary competing in highly susceptible corn. Environmental factors played a major and largely role in defense of maize against *F. verticilliodes* (Shelby *et al.*, 1994). As well as Vigier *et al.* (1997) added that, both *F. gramenarum* and *F. subglutinos* required cooler temperature than *F. verticillioides*, since mean temperature did not exceed 25°C and a higher water activity; this could explain the lower disease severity symptoms with *F. verticillioides.* Also, Miller (2001) showed that *F. verticillioides* grows well at higher temperature and ear rots are associated with drought and insect stress. Murphy and Rice (1993) noted that, *Fusarium* allowed dense colonization in heat and high humidity. Phenolic compounds are essential plant antimicrobial activity and in maize grains had been implicated in resistance to ear rots and insects of maize (Classen *et al.*, 1990 and Casati and Walbot, 2005). Enzymes inhibitors in cereals were elucidation of natural defense mechanisms to prevent microbial contamination of *F. verticillioides* (Sauders *et al.,* 2001). Alpha amylase inhibitors are proteins in cereals that possess ability to act on the pathogens amylase, inhibiting nutrient supply and restrict the development of fungi; effective as natural antifungal, antifumonisin effect and fungal diversified (Pagnussatt *et al.*, 2012 & 2013 and Mendes *et al.*, 2015). Several studies have validated the function of plant trypsin enzyme inhibitors (TI) as a means of plant defense against fungal infection. (Baker *et al.,* 2009). Furthermore, Chen *et al.* (2007) found that, antifungal trypsin inhibitor proteins was up regulated twofold or higher in resistant maize lines compared with susceptible ones.

The defatted maize germ flour (DMGF) is a by-product of the maize oil industry, which results from the separation of germ, starch and gluten. The nutrient-dense composition of DMGF offers a good potential for its use as an ingredient or fortificant in a variety of foods, such as bread, cookies, muffins, cake, etc. in spite of the excellent nutritional quality, (Siddiq *et al*., 2009). Using healthy and resistant genotypes maize grains to fungus disease avoid food chain contamination with possible mycotoxins which are so hazards for human.

Due to the limited understanding of the relationship between late wilt and ears rot infection in maize, and lack of understanding of common resistance mechanisms to this pathogens, the objective of this study was to estimate the effect of late wilt disease on ears and kernels rot disease development, *F. verticillioides* fungus infection (to minimize the confounding effects of the interaction), yield and effect of environmental conditions of ears rot disease development in the field, role of phenolic compounds, antioxidant activity, trypsin and alpha amylase inhibitors on diseases resistant and so as making of healthy cookies fortified with DFMGF from healthy and resistant hybrid of studied maize to ear rot disease.

Materials and Methods

1-Role of late wilt disease in development of ears and kernels rot disease and yield components:

The present work was carried out at the experimental farm of Sakha Agric. Res. Station during 2017 and 2018 growing seasons. Grains of pure maize single crosses (SCs),*viz*. 128, 131, 166, 173 and three way crosses (TWCs), *viz.* 314, 352 were used. Randomized complete block design with three replicates was used. Each plot included three rows, 4 m long at 80 cm distance and sown by 2-3 grains/hill, thinned to one plant/hill after three weeks. The experiment was carried out under artificially infection in field nursery of late wilt disease and natural infection for ears and kernels rot disease. All cultural practices were applied at the proper time. Late wilt disease infection was estimated as percentage after 35 days from an thesis (90-100 days from planting) as El-Shafey *et al*. (1988).

Ears and kernels rot disease was recorded after harvesting directly in the field in healthy and infected plants by late wilt disease as illustrated scale of Raid *et al.* (1996) as follows: The severity of ear rot symptoms was evaluated using a 7 class rating scale where 1= 0%, 2=1-3%,3=4-10%, 4=11-25%, 5=26-50%, 6=51-75% and 7=>75% of kernels exhibited visible symptoms of infection such as rot and mycelial growth. Yield / 3 ears (g), weight of 100 kernels (g) and ear length / 3 ears (cm) for healthy and infected plants by both of late wilt and ears and kernels rot diseases together for each maize hybrid were recorded in the two tested seasons.

2-Effect of late wilt disease of asymptomatic infection by *F. verticillioides* :

Randomized samples (3 ears) were collected form each maize hybrids healthy and infected by late wilt disease, transported to the laboratory in a separate paper bags, air dried and stored at laboratory temperature, to isolate and record frequency percentage of different fungal pathogens causing ears and kernels rot,especially *F. verticillioides*, standard blotter test method of ISTA (1993) was used.

3-Deteriorative ability of associated fungi of maize kernels:

Vigor index (VI) of maize seedlings, *cv*. balady with three main isolated fungi, *ie. F. verticillioides, A. flavus* and *A. niger* was applied according to methods adopted by Purushotham *et al.* (1996) in which:

VI= (mean root length + mean shoot length) x percentage of germination.

4-Climatic conditions of fusarium ear rot:

Effect of temperature (max. and min.) degrees, relative humidity (RH% at 7.30 and 13.30 O'clock) wind velocity(km/24 hr.) and pan evaporation (mm) on ears and kernels rot disease severity development, meteorological data of these parameters were obtained from Rice Res. &Training Center at Sakha Agric. Res. Station. The parameters were recorded during the period of July to September in the two study seasons of 2017and 2018, which the infection were done from the silk appearance until the maturation of kernels and ears as shown later in Table (5).

5-Chemical analysis:

a -Proximate chemical composition:

Maize grains samples of SCs 166, 173 and SC131, TWC314 as resistant and susceptible hybrids to both diseases, maize germ flour (MGF), defatted maize germ flour (DMGF), wheat flour 72%(WF) and cookies were subjected for next all chemical analysis. Moisture, crude protein, ether extract, crude fiber and ash contents of the samples were determined according to the method described by AOAC (2005). Total carbohydrates content was calculated by difference as follows: Total carbohydrates (%) =100–[(crude protein (%) + ether extract (%) + ash (%)] on dry weight basis. The factors, n = 5.95 for (wheat flour72 %,WF, MGF and DMGF); and n = 6.25 (for cookies) were used for conversion of nitrogen to crude protein.

b- Extraction of Total Phenolic Compounds:

According to the method defined by Hu and Xu (2011) total phenolic compounds are extracted. Phenolics of maize grains flour was extracted using 95% of methanol. The process of extraction was gone on for 24 hours, by stirring the flour in solvent at room temperature. The extracts were centrifuged for 15 minutes at 4000 rpm, and purified by filtering paper (Whatman No. 41). The solvent was removed from the extracts using a rotary evaporator at 40°C under vacuum. The extracts were further dried with a 40 ° C vacuum oven and then kept for further analysis in a dry clean, black glass bottle at 4 ° C.

c- Determination of Total Phenolic Compounds:

According to Singleton *et al.* (1999), total phenol compounds was determined. The method was based on the color reaction of Folin-Ciocalteu reagent with hydroxyl groups. A spectrophotometer was used to measure the reaction absorbance at 760 nm. The results were expressed as mg gallic acid per 100g of extract.

d- Antioxidant activity (DPPH) assay:

The DPPH method (2.2-diphenyl-1- picrylhydrazyl) described by Lee *et al*. (2003) was used to measure antioxidant activity.

e- Trypsin inhibitor (IIU/mg):

Trypsin inhibitor was determined using the method of Kakade *et al.* (1969) and expressed as the number of units inhibited per mg dry matter.

f- α- Amylase inhibitor (AIU/g) :

α- Amylase inhibitors was determined using Deshpande, *et al.* (1982) method.

6- Preparation of the experimental cookies:

Wheat flour extraction 72% and other ingredients used to prepare the other ingredients used to make the cookies were purchased from local market at Kafr El-sheikh City, Egypt. Chemicals and biochemicals of analytical grade were purchased from Gomhoria Co. for Chemicals and Drugs, Egypt.

a- Maize Germ Separation:

Based on the nutritional evaluation analyses one best germ from various hybrids, resistant and healthy maize, *cv.* SC173 to both diseases was selected for further assay and product development.

Germs were separated from maize grains using quick germ milling process according to the method described by Singh and Eckhoff (1996). For 12 hours, one kg sample of maize was soaked in water, then coarse grinding to remove germs. Then floating and skimming processes were used to recover the germs. The germs thus obtained were sun dried in open air for 10-12 hrs. Then milled using a laboratory scale hammer mill, then passed from 60 mesh screen, packed in polyethylene bags and stored at -18 ^{o} C until use.

b- Preparation of defatted Maize Germ:

A maize germ mill weight was soaked for 48 hours at room temperature in an n-hexane solvent (40-60^{o}C), then filtered. This process was repeated three times using fresh solvent each time to extract most of the oils from the sample. Then the obtained solution was filtered and the solvent was removed by rotary evaporator according to Kahlon *et al.* (1992). The DMG mill was packed in bags of polyethylene and kept at -18^{o}C until use.

c- Preparation of the experimental cookies:

Method of Alobo (2001) was used to prepare the cookies samples. Blends containing 5, 10, 15 and 20 % of DMGF was used as replacement of wheat flour (72% extraction). The basic ingredients were 455g of flour blends, 200g shortening, 200g sugar, 50g of whole egg, 5g of baking powder and water variable. The dry ingredients were hand-mixed for 3 minutes in a bowl. Added and mixed other ingredients in a rotary mixer (Moulinex model Depose type 171) for 5 min, using a wooden rolling pin. The dough was sheeted on a pastry board to uniform thickness of 7 mm and cut into circular shapes of 6 cm diameter using a circular scone cutter. The cut dough pieces were backed at 210^{o}C for 12-15 min using preheated backing oven. Cookies were allowed to cool at room temperature for 30 min after removal from the oven and were then then divided into two lots. One lot was initially used for sensory evaluation measurement and the second lot for chemical analysis.

d- Sensory evaluation:

A semi-trained panel of twenty members using ten-point hedonic-scale ratings for color, taste, flavor, texture and overall acceptability in order to provide organoleptic characteristics for different prepared cookies, Watts *et al*.(1989).

Statistical analysis:

Data were analyzed statistically using the analysis of variance and the means were further tested using DMART test outlined by Steel and Torrie (1980).

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Results and Discussion

1-Role of late wilt disease in development of ears and kernels rot disease and yield components:

Interaction and relation between diseases is complex, did not obvious and / or need more study, in an attempted to study the relation between both of late wilt and ears and kernels rot diseases, results in table (1) showed that, certain and obvious relation between the two diseases is found, since the infection by first one led to significant of infection by the second one with the tested maize hybrids, *ie*. SCs 128,131,166 and TWCs314, 352, which had visible ear rot disease severity ranged from 8.66 to 23.33 % in infected plants by late wilt disease compared to 5.00 to 12.01 % in healthy ones from late wilt disease. Efficiency of late wilt disease on ears rot disease development ranged from 36.40 to 64.29 with the mentioned above treatments. This relation was not detected with SCs173 in this season (2017). The results in the second season (2018) ascertain and supported that in the first one, whereas the positive relation between the two diseases was recorded with the most tested maize hybrids. Disease severity of ears rot disease ranged from 10.33 - 17.36 with infected ones by late wilt disease in comparison of 3.01-10.66 % in free healthy ones of late wilt disease. Effect of the first disease of development of the second disease (ear rot efficiency) ranged from 38.48-67.84 %. The reverse was true in case of SC128.

Table 1: Effect of infection by late wilt disease on visible ear rot disease severity and yield weight of some maize hybrids in the field in seasons 2017 and 2018.

Maize hybrid	Late wilt disease	Ear rot*		Yield	
		Severity	Eff %*	3 Ears /g	Eff %
		Season 2017			
SC 128	Infected	10.00e	36.40e	501.66efg	45.37c
	Healthy	6.36gh	-	918.33a	-
SC 131	Infected	20.66b	41.90d	516.66ef	42.59d
	Healthy	12.01d	-	900.01ab	-
TWC 314	Infected	23.33a	64.29a	366.66g	56.86a
	Healthy	8.33f	-	850.05abc	-
SC173	Infected	7.23gh	13.69f	433.34fg	42.22e
	Healthy	6.33h	-	751.08cd	-
SC 166	Infected	8.66f	42.26c	450.07fg	27.02f
	Healthy	5.00i	-	616.66de	-
TWC 352	Infected	15.67c	53.19b	416.67fg	45.60b
	Healthy	7.33g	-	766.67bc	-
		Season 2018			
SC 128	Infected	5.66f	11.66f	557.06e	67.38c
	Healthy	5.00fg	-	932.33b	-
SC 131	Infected	17.36a	48.09d	318.66fg	75.48a
	Healthy	9.01e	-	1299.66a	-
TWC 314	Infected	17.33a	38.48e	386.67f	57.03f
	Healthy	10.66d	-	900.91bc	
SC173	Infected	11.66e	57.11c	267.33fg	65.90d
	Healthy	5.00fg	-	784.05cd	-
SC 166	Infected	10.33d	67.84a	366.45f	62.57e
	Healthy	3.01h	-	978.01b	
TWC 352	Infected	12.66b	65.79b	211.66g	68.75b
	Healthy	4.33g	-	677.33de	-

In the same colum, means followed by a common letter are not significantly different at the 5% level by DMRT.
Ear rot severity* as Raid *et al.* (1996) as 7 class rating scale where 1= 0%, 2=1-3%,3=4-10%, 4=11-25%, 5=26-50%, 6=51-75% and 7=>75% of kernels exhibited visible symptoms of infection as rot and mycelial growth.
Eff *: Efficiency.

As to effect of both diseases in yield components, results in table (1) indicated that infection by late wilt which associated by ears rot disease led to highly significant decrease in yield weight with all tested maize hybrids in the two tested seasons compared late wilt free ones.

Yield /3 ears ranged from 366.66 to 516.66 g with infected ones by the two diseases in the first season compared to 616.66 - 918.33 g of healthy ones from late wilt, ranged from 211.66 to 557.06 and

677.33 to 1299.66 g in the second season, respectively. Efficiency of the two diseases of yield reduction ranged from 42.59 to 56.86 and 57.03 to 75.4%, respectively, in the two seasons. Yield efficiency was more effective in the second season than the first one.

Concerning the effect of late wilt disease which associated by ears and kernels rot disease on 100 kernels weight, results in table (2) showed that in all cases and in the two tested seasons, infection by the two diseases together led to significantly decrease of 100 kernels weight with all tested maize hybrids, the most effected one was SC131 followed by SC173 while SC166 was the least effective one in the first seasons, recording efficiency 24.98, 21.35and 4.29 %, respectively, and ranged from 8.45 to 18.64 with other tested ones. 100 kernels weight/g ranged from 24.89 to 31.13 with the infected ones by the two diseases and ranged from 27.58 to 41.46 with free healthy ones from late wilt disease in the first season. 100 kernels weight/g was more effective in the second season than the first one and ranged from 23.66 to 32.33 with the infected by both diseases and from 36.66 to 43.05 g with healthy ones from late wilt. Efficiency of 100 kernels weight reduction was recorded in high percentages and the most effective one wasTWC352 followed by SC173 while SC128 was the lowest one, *ie*. 38.05, 35.46 and 19.17, respectively.

Table 2: Effect of infection by late wilt and associated with ear rot disease on 100 kernels weight and ear length of some maize hybrids in the field in seasons 2017 and 2018.

Maize hybrid	Late wilt disease	Season 2017			
		100 kernels		Ear length	
		Weight/g	Eff %	cm	Eff %
SC 128	**Infected**	30.69cd	18.46c	16.63de	28.62a
	Healthy	37.64b	-	23.30a	-
SC 131	**Infected**	31.13cd	24.98a	17.73bcde	23.57b
	Healthy	41.46a	-	23.20a	-
TWC 314	**Infected**	25.83gh	6.45e	15.20e	19.40d
	Healthy	27.58ef	-	18.86bcd	-
SC173	**Infected**	24.89h	21.35b	16.33de	20.72c
	Healthy	31.65c	-	20.61ab	-
SC 166	**Infected**	26.74fg	4.29f	17.16cde	14.88e
	Healthy	27.94e	-	20.16abc	-
TWC 352	**Infected**	26.08g	14.88d	15.40e	8.11f
	Healthy	30.64d	-	16.76de	-
		Season 2018			
SC 128	**Infected**	32.33e	19.17f	17.33d	21.58e
	Healthy	40.06b	-	22.10ab	-
SC 131	**Infected**	28.04f	34.36c	14.36ef	40.97a
	Healthy	42.66a	-	24.33a	-
TWC 314	**Infected**	27.56fg	25.51e	16.30de	20.98f
	Healthy	37.02cd	-	20.63bc	-
SC173	**Infected**	23.66h	35.46b	14.46ef	34.74b
	Healthy	36.66d	-	22.16ab	-
SC 166	**Infected**	26.12g	32.16d	17.46d	24.51d
	Healthy	38.33c	-	23.13ab	-
TWC 352	**Infected**	26.66fg	38.05a	13.30f	26.23c
	Healthy	43.05a	-	18.03cd	-

In the same colum, means followed by a common letter are not significantly different at the 5% level by DMRT.
Eff *:Efficiency.

Consequently, effect of both diseases together was interpreted to ears length /cm, since infection led to significantly decrease of its with all maize hybrids in the two tested seasons compared to healthy ones (free from late wilt). SC128 and SC131 were the most effective ones in the two tested seasons, respectively, recording efficiency 28.62 and 40.97% while the least ones were TWCs 352 and 314, *ie.*,8.11 and 20.98%.The other maize hybrids recorded ear length efficiency ranged from 14.88 to 34.74% in the two seasons. No significant differences of ear length between all maize hybrids infected by the two together diseases in the first season and ranged from 15.20 to 17.73 compared to 16.7 to 23.30 cm of healthy ones, while the significancy was found between healthy ones and the highest and least ear length are SC128, *ie*.23.30 and TWC352, *ie*. 16.76 cm, respectively, other hybrids ranged from 20.16 to 23.20 cm. Results in the second season supported the first one with significant differences in case of infected by both diseases and healthy ones, and TWC 352 recorded the most decrement of ear length, *ie.*13.30 cm followed by SCs 131 and 173 while SC128 was the least effected one. In healthy hybrids groups alone, ear length recorded more length than the infected by the two diseases with all tested hybrids and ranged from 18.03 to 24.33cm. The highest ear length was recoded with SC131 followed by SCs166, 173 and 128 while TWCs 352 and 314 recorded the least ear length ones. In all treatments, the lowest weight of yield, 100 kernels and ear length were recorded with infected maize hybrids by late wilt diseases and associated of infection by ears and kernels rot disease in both tested seasons, indicating that infection by both diseases were presented and led to significant decrease in this characters, while the reverse was true in healthy case from late wilt. These differences between the two cases were returned to infection by late wilt disease and associated of high infection by ear and kernels rot disease in maize. For the most tested maize hybrids in years, the highest ears and kernels rot disease severities were found in case of late wilt disease infection. The most interesting finding was the differences in ear rot disease severity between cases of infection by late wilt and /or free one, whenever late wilt disease caused increasing of percentages of infection and efficiency of ear and kernels rot disease. This could explain lower ear rot disease severity symptoms of free healthy ones from late wilt disease.

Our data confirms previous finding of *F. verticillioides* and *H.maydis* fungi are important members of stalk rot complex disease whose interaction can have substantial influence to maize plants and easement of ear rot disease infection by systemic infection by *F. verticillioides* from stalks to ears and kernels. Infection by late wilt disease led to increasing of infection by ears and kernels rot disease by attributed to chemical and physiological changes, *ie.* production of sugars and gums in xylem of maize stalks especially after an thesis which used as nutrients by *F. verticillioides* and reduce of maize plants ability to defense against ears and kerns rot causal pathogens. Similar to a previous report for Gai *et al.* (2018), Duncan and Howard(2010) and Chen (2000) showed that, systemic infection and relation between diseases were showed and *F. verticillioides* infected maize stalks extended to the ears and causing infection of kernels. Additionally, Tolba and El-Sayed (2005) found that, maize downy mildew disease was companied infection by certain common smut disease especially on tassel of susceptible maize genotypes. Moreover, Farahat (2008) and Fadel *et al.* (2009) showed that, DM and CS diseases positive relation was found in maize, which infection by DM led to spreading of CS disease in the field. Consequently, the increment of infection by DM disease led to increment of infection and disease index of CS disease in susceptible and resistant maize genotypes, recording potential and positive pathological relation between the two above diseases, Farahat *et al.* (2014). In addition to, Saxena (1983) and Khangura and Sokhi (1995) found that, infection by *Albugo cadida* fungus, *Albugo-Peronospora* (causal of white rust and downy mildew disease, respectively) had been reported in *Brassica and Eruca sativa* than healthy ones. SCMV-virus inoculation increased susceptibly to downy mildew disease *(Sclerospora sorghi* fungus) of susceptible and resistant ones (Sutabutra *et al.*, 1976).

2-Effect of late wilt disease of asymptomatic infection by *F. verticillioides* fungus:

Data in table (3) studied effect of late wilt disease of infection by maize kernels rot expressed as asymptomatic infection by *F. verticillioides* fungus, the main causal of ears and kernels rot in the world, seed deterioration and produced FBs toxins groups. Results showed that, in all cases of maize hybrids infected by late wilt disease led to significantly increase asymptomatic infection by *F. verticillioides* fungus and recorded in high percentages in comparing of same healthy ones in the two season,especially with SC173 which recorded the highest infection, *ie.*45.02 followed by SCs128 and 166*, ie.*41.09 and 41.06 %, this results are parallel with those obtained by El-Naggar and Sabry (2011) they reported that, high asymptomatic infection of late wilt disease in maize plants and asymptomatic infection by *F.monilifome* can develop to infect kernels, Munkvold *et al.* (2007),while TWC314 and SC131recorded the lowest ones in the first season. TWC352 and SC166 as healthy ones from late wilt recorded the least infection, *ie.* 15.71 and 15.70%, while SC128 was the highest healthy one, *ie*. 38.01%.

As infection efficiency, SC166 recorded the highest percentage as a result of infection by *F. verticillioides*, *ie*. 61.85 followed by TWC 352, *ie.* 56.55, other treatments ranged from 7.45 to 39.55 %, while the lowest one was SC128.Results in the second season recorded the same trend as first one with exception of SC128, which the infection by late wilt did not effect of infection by *F. verticillioides* in comparing to healthy status,*ie.* 25.16 and 26.16 %, respectively. Other cases showed significantly differences and positive relation of infection by *F. verticillioides* and infection by late wilt compared to free ones,*ie*. 61.10, 56.06, 50.06, 44.06 and 30.16 compared to 58.10, 49.03, 36.03, 28.10 and 18.12% for healthy ones of hybrids TWC352, SC131, TWC314, SC173 and SC166, respectively. *F. verticillioides* infection efficiency in the second season ascertain the positive relation between the two diseases, which recoded in high percentage and ranged from 14.39 to 40.31 with the tested maize hybrids.Generally, high infection efficiency of *F. verticillioides* was noticed, especially in the first seasons resulted to infection by late wilt disease compared to healthy free ones from this disease. The results are parallel with those obtained Vigier *et al.* (1997), Desjardins *et al.* (1998), White (1999) and Igawa *et al.* (2007) they found that, *F. verticillioides* was the most wide spread and a major causal of maize kernels rot and produced toxins, which accurately toxic and carcinogenic to animals and human consumers of maize grains (Bullerman 1996). Some scholars had reported *F. verticillioides* infected maize plants from stalk and extended to the ears and infected kernels, causing systemic infection and infected kernels are also pathogenic to maize stalks, which occurs infection cycle from seeds to stems to ears to grains, Chen (2000), Duncan and Howard (2010) and Gai *et al.* (2018). Moreover, *F. verticillioides* was an important members of the *Fusarium* salk rot complex whose interaction can have substantial influence to the pathogensis in plants (Tesso *et al*. 2009). Consequently, late wilt pathogen is often associated by infection by. *F. verticillioides* to present stalk rot complex (El-Shafey and Claflin 1999). Robertson-Hoyt *et al*. (2007) found that, strong correlation between *Fusarium* and *Aspergillus* ear rots in maize. Meanwhile, both fungus can thrive on maize without necessary competing in highly susceptible corn, Abbas *et al*. (2006).

Table 3: Effect of infection by late wilt disease on asymptomatic infection by *F. verticillioides* fungus of some maize hybrids in the field in seasons 2017 and 2018.

Maize hybrids	Late wilt disease	Season 2017		Season 2018	
		Infection %	Efficiency%	Infection %	Efficiency%
SC 128	Infected	41.09b	7.45f	25.16i	0.00f
	Healthy	38.01c	-	26.16i	-
SC 131	Infected	31.10e	19.51e	56.06c	14.39e
	Healthy	25.03g	-	49.03d	-
TWC 314	Infected	32.10e	31.27d	50.06d	28.02d
	Healthy	22.06h	-	36.03f	-
SC173	Infected	45.02a	39.55c	44.06e	36.22b
	Healthy	27.20f	-	28.10h	-
SC 166	Infected	41.06b	61.85a	30.16g	40.31a
	Healthy	15.70i	-	18.12j	-
TWC 352	Infected	36.16d	56.55b	61.10a	32.73c
	Healthy	15.71i	-	58.10b	-

In the same colum, means followed by a common letter are not significantly different at the 5% level by DMRT.

In contrast, Zummo and Scot (1992) found that, infection by *Fusarium spp.* able to inhibit *Aspergillus flavus* infection in maize, due to competition relationship and a negative correlation between *Fusarium*, *Aspergillus* pathogens in maize ears, Marin *et al. (*1998). The present results supported by the finding of infection by *U. maydis* fungus was distributed and related in the plants infected by DM disease of maize, Tolba and El-Sayed (2005). Sutabutra *et al.* (1976) added that, maize SCMV–virus increased susceptibly to *Sclerospora sorghi* fungus. Consequently, Farahat *et al.* (2014) recorded that, infection by *P. sorghi* causal of DM disease led to increase of infection and disease index of common smut incited by *U. maydis* fungus in maize. Farahat (2008) and Fadel *et al*. (2009) demonstrated that, DM infection played an important role in spreading of common smut disease infection.

3-Deteriorative ability of associated fungi of maize kernels:

From previous isolation of fungi which associated with maize kernels, *F. verticillioides* was the main fungus infected maize ears and kernels and recorded the highest percentage of all treatments and control. *A. flavus, A. niger* were isolated with low frequencies. So, data in table (4) studied the effect of this fungi in deterioration of maize kernels wherever, *F. verticillioides* and *A. niger* caused significantly reduction of maize shoot / cm followed by *A. flavus* in comparison of healthy one of control. Root length was affected by infection of this fungi and the most effective one in reducing of root length /cm was *F. verticillioides* followed by *A. niger* and *A. flavus* in comparing healthy control. As to germination, *F. verticillioides* caused the highest reduction of germination percentage, *ie.* 77.14 while *A. niger* and *A. flavus* were recorded 96.29 and 98.10%, respectively. Efficiency was ranged from 24.31 to 30.82 with shoot length, 1.67 to 34.12 % with root length, 1.90 to 22.85% with germination and 20.56 to 53.48 with vigor index. Nature infection by *F. verticillioides*, *A. flavus* and *A. niger* play serious role in deterioration of maize kernels causing significantly decrease of shoot and root length, germination percentage and seedlings vigor index. *F. verticillioides* was the most deteriorative one followed by *A. niger* and *A. flavus.* This fungi caused serious damage of maize kernels, the deteriorative ability of this fungi may be due to production of different types of secondary metabolites, *ie. F. verticillioides* produced fuminsins (FBS) groups with other metabolites, *A. niger* and *A. flavus* produced aflatoxins and this toxins compounds may be the main reason of damage of kernels and reduction of germination as adopted by Purushotham *et al*. (1996). In addition, Woloshuk *et al.* (1997) added that, *A. niger* and *A. flavus* were contributed to seed deterioration by stimulating of alpha amylase enzyme activity which causing break down the starch in endosperm. Furthermore, Farahat *et al. (*2001) detected that, from three species of fusarium, *F. verticillioides* was the most infected and deteriorative one of kernels. Degraeve *et al.* (2016) reported that*, F. verticillioides* can deteriorate the yield and quality of maize grains due to associated fumonisin toxins contamination.

Table 4: Effect of isolated fungi from maize, *cv.* balady kernels of seedlings shoot, root length and vigor index.

Fungi	Shoot /cm	Efficiency (%)	Root/ cm	Efficiency (%)	Germination (%)	Efficiency (%)	Vigor index (%)	Efficiency (%)
F. verticillioides	2.02c	30.82a	2.76d	34.12a	77.14d	22.86a	36.79d	53.48a
A. flavus	2.21b	24.31c	4.12b	1.67c	98.10b	1.90c	62.83b	20.56c
A. niger	2.04c	30.13b	2.80c	33.17b	96.29c	3.71b	46.41c	41.16b
Control	2.92a	-	4.19a	-	100.00a	-	79.10a	-

In the same column, means followed by a common letter are not significantly different at the 5% level by DMRT.

4-Climatic conditions of Fusarium ear rot:

High recording percentages of visible ear rot and the main causal pathogen (*F. verticillioides* fungus) which associated with certain infection by late wilt disease of tested maize hybrids in the two tested season, that is reflect the suitable conditions to cause infection by ears rot and *F. verticillioides*. Data in table (5) recorded high Min air temperature / ºC (hot), low RH% at 7.30 o'clock (in morning) and pan evaporation (dry) and high RH% at 13.30 o'clock (humid at mid-day) in the first and second seasons recording average 24.00 ºC, 81.40 %, 501.19 mm and 57.27 % compared to 22.06 ºC, 87.72 %,750.31 mm and 53.60 %, respectively, in months disease distribution, *ie.* July, August and September.

Table 5: Grand mean of climatic parameters during the growing season 2017-2018 from July to September at Sakha Agriculture Research Station.

Season	Month	Air Temperature ºC		RH% at time		PE	Wind
		Max.	**Min.**	**7:30 O*.**	**13.30 O.**	**(mm/day)**	**Km/24hr.**
2017	**July**	32.32	24.31	79.57	54.70	610.93	110.99
	Aug.	33.79	24.76	83.63	60.52	512.92	90.24
	Sept.	32.50	22.93	81.00	56.60	381.97	87.6
	Average	32.87	24.00	81.40	57.27	501.19	96.27
2018	**July**	33.15	23.64	83.19	55.11	772.92	97.90
	Aug.	34.10	21.80	92.40	53.50	813.50	99.03
	Sept.	32.49	20.76	87.57	52.20	664.53	89.17
	Average	33.25	22.06	87.72	53.60	750.31	95.36

Max. = Maximum, Min. = Minimum, RH% = Relative humidity, PE = Pan evaporation, O*:o'clock

Wind more active in July especially in first season (time of an thesis and infection by ear rot disease) and helped to distribution of *F. verticillioides* inoculum, *ie.* 110.99 compared to 97.90 Km/24hr in the second season, wherever the fungus penetrate though silk channels, so infection was done. This results is at odds with many studies in the literature that associate fusarium ear rot severity with excessively hot and dry conditions. These results concur with our previous finding, in which environmental factors play a major

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and largely uncharacterized role in indefence of maize against *F. verticilliodes* infection, Shelby *et al.* (1994).The results were supported by the findings of White (1999) and Miller (2001), they showed that *F. verticilliodes* grows well at higher temperature (warmer limits) and ears rot are associated with drought. Moreover, the results in the same line of Murphy and Rice (1993) and Reid *et al.* (2002) they showed that, *Fusarium* allowed heavy colonization in heat and high humidity. This finding is similar to our observation in which, equerries determine high min temperature degrees and low RH% in morning were suitable for maize ears and kernels rot disease development followed high fungi frequency of Farahat *et al*. (2010 and 2017). Vigier *et al.* (1997) and Marin *et al. (*1996) added that, lower disease severity symptoms observed with *F. verticilliodes* in cooler rain conditions (high water activity).

5-Chemical analysis:

a- Proximate chemical composition of maize grains:

Chemical compositions of maize and maize products are generally affected by environmental and genetic factors (Awad *et al.*, 2011).

Data in table (6) showed that, moisture, crude protein, ether extract, ash, crude fiber and total carbohydrates were significantly affected by both hybrids and infection status. The moisture content of maize grains were ranged from 10.31 to 13.56%. However, infected susceptible maize SC131 by late wilt contained the highest content of crude protein (12.31%) followed by the susceptible TWC314 (11.96%), while healthy resistance SC173 (9.52%) was the lowest. The increment of total protein in the count of infection could be attributed to the contribution of the causal agent. On the other hand, the increase in total protein may be due to the consumption of sugars and/or carbohydrates of the host by pathogen (Tolba and El-sayed 2002).The data in the same table revealed that, healthy resistant maize SC166 had the highest ether extract (4.54%) compared with the other maize hybrids. on the other hand, infected susceptible maize SC131 contained the lowest content of ether extract (3.61%) may be due to accumulation of free fatty acids content, acid value and acidity were increased in the infected seeds as reported by Prasad *et al.* (1988) ; El-sayed *et al*. (2004) ; El-sayed and Tolba (2005) and Nwosu (2016).

Table 6: Chemical composition percentage of maize grains healthy and infected by late wilt disease and associated by ears rot infection.

Hybrids	Resistant				Susceptible			
	SC166		SC173		SC131		TWC314	
Constituents	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**
Moisture	10.31e	11.70c	10.45e	11.64c	11.45c	13.56ab	10.86d	12.72b
Protein	10.30 f	11.11d	9.52g	10.30f	11.46c	12.31a	10.80e	11.96b
Ether extract	4.54a	4.14c	4.35b	3.95d	4.21bc	3.61e	3.90 d	3.27f
Ash	1.56cd	2.14b	1.63c	2.22b	1.48d	2.16b	1.29e	2.98a
Total carbohydrates	83.60c	82.61d	84.50a	83.53c	82.85 d	81.92e	84.01b	81.79e

In the same row, means followed by the same letter are not significantly different at P<0.05.
Each value was an average of three determinations.

The highest content of ash was recorded for infected susceptible maize TWC314 (2.89%), while the healthy status of SC166 had the lowest value (1.29%). These results are in line with those (Prasad *et al*., 1986; El-sayed and Tolba 2005; Nwosu *et al.,* 2015 and Nwosu, 2016). They reported that, the percentage of protein, acidity, free fatty acids (F.F.A.) and ash % increased by increasing infection with the tested fungi. Results also revealed that, the highest carbohydrates content was found in healthy resistant maize hybrid, *cv.* SC173 (84.50%) compared with the infected susceptible maize SC131 which had the lowest content (81.92 %). These results may be due to colonization of seed rot fungi which led to decrease carbohydrate content in most cases, (Prasad *et al*., 1988; Purushothem *et al.*, 1996; El-sayed and Tolba 2005 and Nwosu 2016).

b- Total phenolic compounds, antioxidant activity DPPH (%), trypsin and α-amylase inhibitors of some maize hybrids grains.

Plants contained many compounds that providing resistance to many pathogens pre and /or post infection, *ie.* phenolics, flavonoids and peptides these compounds has attracted action as alternatives to control plant pathogens, (Soares and Machado 2007; Pagnussatt *et al.*, 2013 and Perlikowski *et al.,* 2014).

Total phenolic compounds (TPC) extracted from different maize grains were determined and the results are recorded in Table (7), it was observed that, total phenolic compounds of different maize hybrids varied from (340.30 to 208.90 mg GAE /100 g) of dry weight (DW). The difference might be attributed to the hybrid of maize. Furthermore, resistant maize, *cv.* SC173 had the highest among of total phenolic compounds (340.30 mg GAE /100g), while the lowest total phenolic compounds was (208.90 mg GAE /100g) from infection susceptible maize, *cv*. SC131 compared with the other maize hybrids. In addition, susceptible maize hybrids contained lower total phenolic compounds than that of resistant ones. Meanwhile, infection maize hybrids contained lower total phenolics than that of healthy maize ones. The obtained results are in agree with those reported by (Garcia-Lara *et al.,* 2004; Zhao *et al*., 2005 and Sheng *et al.,* 2018). In addition, Nwosu *et al*. (2015) and Nwosu (2016) showed that, the bases for maize resistance to the weevil are significant increase in grain phenolic acid and trypsin inhibitor. Free phenolic acid are evidently involve in resistant mechanisms toward maize ear rot and mycotoxin contamination under field conditions, Giordano *et al.* (2017).

Table 7: Total Phenolic Compounds, DPPH, trypsin and α-amylase inhibitors of some maize grains healthy and infected by late wilt disease and associated by ears rot infection (on dry weight basis).

Hybrids	**Resistant**				**Susceptible**			
	SC166		**SC173**		**SC131**		**TWC314**	
Constituents	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**
TPC (mg GAE 100 g)	325.70b	290.41d	340.30a	310.22c	250.65f	208.90h	280.50e	239.80g
DPPH%	73.81b	67.90d	76.90a	71.0c	55.90f	48.40h	61.50e	53.20g
Trypsin inhibitor (IIU/mg)	0.62b	0.35f	0.75a	0.52c	0.39e	0.21g	0.47d	0.22g
α- Amylase inhibitor(AIU/g)	11.50c	10.65d	13.23a	12.47b	7.72f	5.60h	8.95e	6.80g

In the same row, means followed by the same letter are not significantly different at P<0.05.
Each value was an average of three determinations.

The antioxidant capacities of the maize samples were measured by the radical scavenging activities of the DPPH (%) (Table 7). Resistant healthy maize of SC173 presented highest DPPH scavenging activity (76.90%), followed by resistant healthy one SC166 (73.31%), while infected susceptible SC131 presented lowest DPPH scavenging activity (48.40%). Apparent also from this table that, healthy and /or infected resistant maize hybrids contained higher DPPH scavenging activity than ones of susceptible maize hybrids. Meanwhile, infected maize hybrids contained lower DPPH scavenging activity than of healthy maize ones. These results are in line with those of Zhao *et al.* (2005), Ramos-Escudero *et al.* (2012) and Bacchetti *et al.* (2013) and Das and Singh (2015). In addition, Zilic *et al.* (2012) reported that, total antioxidant capacities of corn cobs are affected by many factors as cultivars and growing conditions

As for trypsin and α- amylase inhibitor it could be noticed that, there are significant differences between resistant, susceptible, healthy and infected of maize hybrids. The healthy resistant maize, *cv.* SC173 had the highest amount of trypsin and α- amylases inhibitors (0.75 TIU/mg and 13.23 AIU/g) respectively, while the lowest value was found in infected and healthy susceptible SC131 (0.21 TIU/mg and 5.60 AIU/g), respectively compared with the other maize hybrids. Results are in agreement with those of Baker *et al.* (2009); Bruggera *et al.* (2015) ;Nwosu *et al.* (2015) and Shekhvatova *et al.* (2019), detected that, grains of resistant maize hybrids to aflatoxin accumulation exhibited a higher level and increased in trypsin inhibitor accumulation and reduced in susceptible ones by ratio ranged from 0.56 to 1.87 mg/g dry matter. Also, Chen *et al.* (2007) detected that, antifungal trypsin inhibitor was higher in resistant maize hybrids than susceptible ones, and decreasing the infection by ear rot fungi especially, *F. verticillioides*, Farahat *et al.* (2010). Furthermore, Blanco-Labra (2000) and Cortez-Rocha *et al.* (2009) suggested that α-amylase inhibitor could be a grain protective agent (not detected in susceptible maize hybrids), and inhibited *F. verticillioides* amylase and play important roles as part of plant defense mechanisms against fungal protease and ranged from 5.5 to 16.0 units per gram of corn (AIU/g) in the Master and AG5011 hybrids, respectively (Figueira *et al.*, 2003). The highest content of antioxidant activity; trypsin inhibitor activity and α-amylase inhibitors had a role in reducing of *F. verticillioides* maize ears and kernels rot disease, Farahat *et al.* (2018).

From the data given in Tables (6 and 7) it should be concluded that, the resistant maize hybrid *cv.*, SC173 has higher nutritional value compared with the other ones. Therefore, the defatted maize germ flour (DMG) was chosen at 5, 10, 15 and 20% substitution of wheat flour to prepare the biscuit.

c- Gross chemical composition of flours:

The gross chemical composition of wheat flour 72% (WF), maize germ flour (MGF) and defatted maize germ flour (DMGF) are recorded in Table (8). The obtained results show that DMGF contains a significant high content of crude protein (23.60%) compared with that of (WF) (10.25%) and MGF (16.63%). Maize germ flour (MGF) contains highest content of fat (26.30%) followed by DMGF (1.20%), than WF 72% (0.62%).

Data in this respect were in agreement with the findings of numerous of investigators (Johnston and Singh 2004; Montgomery *et al.,* 2005; and Barnwal *et al.*, 2013). The utilization of DMG for human consumption was an additional nutritional source and profitable (Sharma *et al.*, 2012).

Table 8: Chemical composition (% on dry weight base) of wheat 72%, maize germ and defatted maize germ, *cv*. SC173 flours.

Components %	Wheat flour*	MGF	DMGF
Moisture	11.30a	6.20 c	8.91b
Crude protein	10.25c	16.63b	23.60a
Lipids	0.62c	26.30a	1.20b
Crude fiber	0.77c	3.85b	8.42a
Ash	0.70c	4.61b	6.49a
Total carbohydrates	87.66c	48.61b	60.29a

Each value was an average of three determinations.
Values followed by the same letter in row are not significantly different at $P < 0.05$.
MGF = maize germ flour, DMGF = defatted maize germ flour. Wheat flour 72%.
Furthermore, DMGF is very rich in protein, ash and fiber compared with (WF) 72% and WGF. This means, the DMGF when is added to bakery products would be improved the nutritional quality.

d- Chemical composition of cookies:

The results of chemical composition of cookies made of different levels of DMGF were recorded in Table (9). The obtained results manifested that, the protein, ash and crude fiber contents increased, in contrast, carbohydrates value decreased gradually with increasing the substitution levels of DMG. These results are in a harmony with the findings of Barnwal *et al.* (2013). It could be noted that, cookies supplemented with defatted maize germ was considered more nutritive.

Table 9: Chemical composition (% on dry weight base) of cookies made of different substitution levels of defatted maize *cv*. SC173 germ flour.

Samples	Moisture	Crude protein	Lipids	Ash	Crude fiber	T.C.	Total caloric Values (k.cal.)/100 g
Control	6.43 d	11.50e	7.83a	0.55e	0.61e	80.12a	436.95
DMG 5%	6.79c	12.03d	7.47ab	0.79d	0.97d	79.71a	434.19
DMG10%	8.25b	12.68c	7.13c	1.13c	1.40c	79.06ab	431.13
DMG15%	8.81 a	13.22b	6.81cd	1.45b	1.62b	78.52ab	428.25
DMG20%	9.10a	13.88a	6.46d	1.78a	1.90a	77.88b	425.18

Values followed by the same letter in column are not significantly different at $P < 0.05$.
Control: Wheat flour cookies, DMG = defatted maize germ., A.C. and T.C.: total carbohydrates, * Energy was calculated as Kcal/100g on wet weight basis.

The protein, ash and crude fiber of cookies contained 20% of defatted maize germ recorded the highest values (13.88, 1.75 and 1.90 %, respectively) compared to those of control (11.50, 0.55 and 0.61 %). The increase of these constituents ascribed to their high contents in DMG as shown in Table (9). The increased moisture content can be explained by the higher content of protein which also increases the water binding capacity of dough with higher levels of defatted maize germ cookies flour (DMGCF). It is also reported that, moisture content of biscuits increased slightly resulted in addition of mustard flour (Tyagi, 2007). Similarly, increasing levels of millet flour in soy millets biscuits resulted in an increase in moisture content (Kumar *et al*., 2010). Thus, it can be concluded that, fortification had a positive effect on nutritional quality of cookies since proteins and ash increased significantly while total caloric were decreased as shown in table (9).

The consumption about 100g of the sample contained 20% DMG would provide about 50% of the recommended daily requirement protein (25-30g/day) as recommended by (FAO/WHO, 1973) for children aged between 5 and 19 years. This fact suggests that cookies supplemented with DMG may be useful as food supplements for the alleviation or prevention of protein malnutrition in developing countries.

The proteins in DMGF mostly consist of albumin and globulin, and are balanced in most of the essential amino acids; lysine, a major limiting amino acid in wheat, accounts for 5-6% of the total proteins in DMGF, which is more than twice than that in wheat flour (Parris, *et al*., 2006).

e- Sensory evaluation:

Table (10) describes the effects of adding DMGF on the sensory quality of cookies. There were a significantly ($p<0.05$) difference in score values of taste, flavor, texture and over all acceptability on contrast not significantly ($p<0.05$) difference in score values of color between control cookies and all samples cookies prepared using DMGF. Sensory characteristics of samples prepared using DMGF until 5% substitution ratio had nearly similar scores compared with those of control. Using of DMGF at levels more than 5% led to decrease the scores for sensory characteristics of biscuits especially texture of sample contained 20% DMGF which recorded 6.70 compared to 8.12 in control. The low overall acceptability of the cookies of blends containing more than 15% DMGF was attributed by the panelists. From the results of sensory evaluation, it should be noted that, supplementation of DMGF until 20% is acceptable for the sensory evaluation of cookies.

Table 10: Sensory evaluation of cookies made of different substitution levels of defatted maize germ.

Samples	Color (10)	Taste (10)	Flavor (10)	Texture (10)	Overall acceptability
Control	8.73a	9.41a	8.40a	8.12a	8.67a
DMG 5%	8.80a	9.32a	8.41a	7.95a	8.62a
DMG 10%	8.34a	8.50b	7.50b	7.44b	7.95b
DMG 15%	8.52a	8.40b	7.35b	7.41b	7.92b
DMG 20%	8.44a	7.70c	7.00b	6.70c	7.31c

Values followed by the same letter in colum are not significantly different at $P < 0.05$.
Control = Wheat flour cookies, DMG = defatted maize germ.

These obtained results are in accordance with those from Nasir *et al.* (2010) and Barnwal *et al*. (2013) demonstrated that cookies made with up of 15% DMG flour exhibited sensory scores within an acceptable range. In another studies of (Arshad *et al.,* 2007 ; Abd El-Hady 2012 and Salem *et al.,* 2014) found that, cookies made with 15% supplementation of defatted wheat germ flour in wheat flour showed sensory scores in the acceptable range. The general composition of defatted wheat germ is similar to DMG (Arshad *et al.,* 2007).

Conclusion

These results have implicated for maize breeding program and further revealed certain pathological relation between late wilt and ears and kernels rot diseases in the field. This result not was demonstrated earlier by other authors. This fact reinforces that knowledge about pathological relation between late wilt and ears and kernels rot diseases to applied genetic improvement techniques for production of resistant hybrids contain of phenols, antioxidants, trypsin and alpha amylase inhibitors enzymes compounds in high contents.

Furthermore, the study demonstrated that DMGF, a by-product of the maize oil industry, has a great potential to be used for value-addition in cookies. Wheat flour cookies supplemented with healthy defatted (resistant to both disease) MGF until 20% was accepted for sensory characteristics and it has high and balanced nutritional value. In addition, these cookies can be covered protein and minerals nutritional requirements of schoolchildren in developing countries from inexpensive and available sources. New prepared cookies could be recommended as food aid in institutional feeding programs for pupils in different school stages and adults as well.

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