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Molecular Test and Reduction of Stalk and Kernels Rots Disease through Controlling of Leaf Blight and Stem Borers in Maize

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ABSTRACT

During two growing seasons, a field experiment was performed and conducted to observe the reaction cultivars of SC10, TWC320 and Balady to maize leaf blight disease under artificial infection with the causal agent *Exserohilum turcicum*. The efficacy of leaf blight disease and corn borer insect management as importance tools to control of stalk rot complex, and ear & kernels rot diseases of maize was also investigated. The obtained data had the same trend. The reaction of the tested maize cultivars to leaf blight disease indicated that, the single cross 10 was ranged from highly resistance HR and resistance R. The three way cross 320 ranged from R to moderately resistant MR, while, the Balady cultivar ranged from MR to MS. Data of stalk rot complex showed that, the lowest infection percent was recorded in plots which sprayed against both of leaf blight disease (Dithane M45) and corn borer (Lannate 90% SP). The highest infection percent by stalk rot complex recorded in the control plots (which not sprayed by any ones). The fungus *Fusarium verticilioides* was the prevalent causing stalk rot complex, during the two seasons with all the tested maize cultivars and all tested treatments. It was significantly followed by *Magnaporthiopsis maydis*. The molecular identification reveal that the collected isolates were recognized as *F. verticilioides* and *M. maydis* based on ITS sequences analysis. Other fungi *i.e.*, *Macrophomina phaseolina*, *Alternaria* spp., *Penicillium* spp. and *Aspergillus* spp. had the lowest infection percentage of stalk rot disease. Data indicated that, positive correlation was found between infection by corn borers % and plants lodging %, in all maize cultivars. Also, the spraying against corn borer and leaf blight disease lead to the lowest infection by stalk rot disease and highest value of weight of 100 kernels as well as the highest of yield production comparing with control treatment. The fungus *F. verticilioides* also caused the highest infection percentage of ear and kernel rot disease with all the tested maize cultivars and under all tested treatments. While, other fungi *i.e*, *Aspergillus niger*, *A. flavus* and *Penicillium* spp. recorded the lowest infection percentage of ear and kernel rot disease. In generally, the lowest infection percent by ear and kernel rot disease was recorded in plots which sprayed against leaf blight and corn borer. Therefore, this study concluded that, the control both of northern leaf blight disease and insects' infection during growing season resulted in finally the lowest infection by stalk rot complex and kernels rot diseases in maize.

Keywords: Cultivars reaction, Leaf blight, Stalk rot complex,European corn borers, Maize

1. Introduction

Maize (*Zea mays* L.), is an important crop after wheat and rice, and the most important cereal crop cultivated worldwide. Diseases are major constraint in realizing the potential yield production. Among them, stalk rot disease is the most important one in globe responsible for yield losses. Stalk rot disease is caused by the complex of *Fusarium verticillioides* (Sacc.) Nirenberg (synonym *F.*

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moniliforme Sheldon, teleomorph *Gibberella moniliformis* Wineland) and the late wilt pathogen *Magnaporthiopsis maydis* (also known as *Cephalosporium maydis*) Sabet and Samra. The disease apparent in senescence phase and activity increases during late stage (grain filling). The symptoms of disease observed during post flowering, 30-35 days after flowering, and pre-harvest stage (Khokhar *et al.,* 2014; Samar *et al.,* 2023). These rotting resulted in premature drying, stalk lodging and ear damages, thus decreasing maize yields (Jackson-Ziems *et al.,* 2014; Samar *et al.,* 2023). The disease also causes rotting and discoloration of internal stalk tissues, finally, decreasing yield by reducing translocation of water and nutrients, thus lead to death and the plants lodging, and also results in reducing filled kernels, and plants lodged down which cause economic loss (Singh *et al.,* 2012; Fitriyanti *et al.,* 2023). The late wilt pathogen *Magnaporthiopsis maydis* also known as *Cephalosporium maydis* (Samra, Sabet) and also *Harpophora maydis* (Jackson-Ziems *et al.,* 2014; Citation *et al.,* 2021; Esker *et al.,* 2022). Other secondary fungi *i.e., Macrophomina phaseolina*, *Alternaria* spp., *Penicillium* spp. and *Aspergillus* spp., are also involved (Tessoa and Ejeta, 2011).

Stalk rot complex disease is endemic in maize fields affecting maize productivity in Egypt and also in other parts of the world (Gai *et al.,* 2018; Jambhulkar *et al.,* 2022; Elshahawy and Khattab 2022 a; Elshahawy and Abd El-Wahed 2022 b). The yield losses annually due to stalk rot are about 10 percentages typically and by 30–50% in high epidemic areas (Silva *et al.,* 2017). *Fusarium verticillioides* is also the important causal pathogen of maize ear and kernel rot (Lanubile *et al.,* 2017; Gai *et al.,* 2018; Lina *et al.,* 2019). The fungus (*F. verticillioides*) not only decreases yield but also reduces the quality of the grains and can produce the secondary metabolite fumonisins (Zhou *et al.,* 2018; Czembor *et al.,* 2019). Fungicides applications are not labeled for management of stalk rot complex and kernels rot diseases. Symptom development of these diseases depend on many stress factors including a poor of moisture, high and continuous cloudiness, foliar diseases, high of plant density, and infestation by corn borer (Khokhar *et al.,* 2014; Costa *et al.,* 2019; Pfordt *et al.,* 2020; Maryke *et al.,* 2020; Paul and Thomison, 2021). Stalk rot pathogen remain overwinter in infected plant residue and invasion plants across natural entry sits such as through damages created by insects or mechanical damage, or by penetration of stalk and root tissue directly (Maryke *et al.,* 2020).

Injury which causes by feeding of corn borers, *Ostrinia nubilalis* (Hübner), is one stress that can assistance the development of stalk rot in many ways (Chiang and Wilcoxson, 1961; Gatch and Munkvold, 2002). By tunneling of stalk tissue, they create entry points for the pathogens invasion, and cause many stress and predispose maize plants to infection by stalk rot (Maryke *et al.,* 2020). This relationship between European corn borers damage and stalk rot disease is very important tools in pest management decision making, because the yield loss attributed to corn borers is resulted in subsequent fungal damages of tissue which injured by the larvae (Gatch and Munkvold, 2002; Al-Eryan *et al.,* 2019). Sabra *et al.,* (2020) found that, using some insecticide enhanced biosynthesis and accumulation of proteins are integral components of the induced chemical defense system against insects. On the other hand, the maize plants are predisposed to stalk rot due to any other stress which decreases the photosynthetic capacity of the plant when the ear developing competes because the maize stalks become poor available of carbohydrates, which were quit importance for plant defenses (Maryke *et al.,* 2020). Fungi causing foliar diseases can to be destructive, and one of respected important disease was Northern Corn Leaf Blight caused by *Exserohilum turcicum* (Passerini) Leonard and Suggs (syn. *Helminthosporium turcicum* Pass.) (Bankole *et al.,* 2023). The leaf blight causal organism survives about overwinter on infected maize residue in soil surface (Jakhar *et al.,* 2017; Samar *et al.,* 2023). At temperatures of the spring and/ or early summer, the pathogen produces their structures (spores and mycelium) on residue, and the spores are wind-blown or splashed onto leaves of new maize crop. Infection occurs within temperature of suitable (64° to 81°F), wet and high humid weather (up to 85% RH). The disease development as long, slender, greyish leaf lesions that distribution to the mid vein (Jakhar *et al.,* 2017). Lesions can expand to a very oblong or "cigar" shape. It may also founded on husks of ears. Loss of photosynthetic tissue can lead to reduced yield, and silage yield quality can be also affected (Swathi *et al.,* 2021). Effective control practices to this decrease included, selecting resistant varieties, decreasing maize residue, suitable time of planting and application foliar fungicides (Jakhar *et al.,* 2021). Because of relationship between European corn borers damage combined with maize leaf blight disease and fungal of stalk rot diseases, control of these factors are considerable importance as one considerable component of an integrated of stalk rot diseases management strategy (Pronczuk *et al.,* 2004; Jackson, 2009; Maryke *et al.,* 2020; Paul and Thomison, 2021; Esker *et al.,*

2022; Grayville, 2022). Therefore, the objectives of this study were to highlight on importance of control of Turcicum Leaf Blight disease and European corn borers injury in maize as an economical and sustainable approach to manage each of stalk rot and kernels rot diseases.

2. Materials and Methods

2.1. Experimental field site and materials

This field experiment carried out in Sakha agricultural research farm and was conducted to observe the reaction cultivars of SC10, TWC320 and balady to northern leaf blight disease, and also conducted to explain the effect of leaf blight and corn borer management on the reducing of stalk rot complex, ear and kernels rots infection as well as their consequence on yield production in tested maize cultivars. This study was carried out in a field disease nursery (this field assigned to late wilt disease and infested by the mixed races of pathogen annually to evaluation the promising materials of maize breeding programme) which was located at the Sakha Agriculture Research Station farm, during the 2020 and repeated during 2021 growing seasons. This field was artificially infested by the mixed races of stalk rot pathogen annually and that causes late wilt disease of maize and often used in Egyptian maize for breeding programs (Zeller *et al.,* 2002). Three maize cultivars (SC10, TWC320 and balady) were used in this experiment. These cultivars were taken from the Agricultural Research Centre (ARC), Egypt. The other fungi especially *F. verticilioides* were heavy founded already in all Egyptian soil naturally. The fungicide Dithane M45 was sprayed against Northern Corn Leaf Blight disease after about 40-45 days from sowing. The insecticide Lannate 90% SP was sprayed against European corn borers (ECBs) insects after about 30 days of sowing (the first fungicide was specific on fungi while the second insecticide was specific on insects). The active ingredient, rate of application and source of tested fungicide and insecticide were detected in Table 1.

Table 1: The active ingredient, rate of application and source of tested fungicide and insecticide.

Treatments	The active ingredient	Rate of application	Obtained from
Fungicide Dithane M45	Mancozeb, which related to the dithiocarbamates group of compounds	2.5 g/L	Elgamhoria company
Insecticide Lannate 90% SP	Amethomyl-based formulation, 90% active ingredient	2.0 g/L	Elgamhoria company

Split plot design and three replicates was used in this experiment, the main plot was planted by three maize cultivars (i.e. SC 10, TWC320 and balady). The sub plots were the following treatments:

T1: Pesticides application against leaf blight (Dithane M45) and corn borers (Lannate 90% SP).
T2: Insecticide application against corn borers only (Lannate 90% SP).
T3: Fungicide application against leaf blight only (Dithane M45).
T4: Non-treated plants (control).

The experimental plot size was 10.5 m^2, each plot consists of three rows 5m in long and 70 cm apart. Maize grains were planted with hand drill in about holes (two grains/hole). The seedlings of maize plants were thinned after 18 days of sowing to maintain plant per each hill, with distance of 25 cm, all recommended practices were done as recommended and in proper time. The treatment against Turcicum leaf blight disease (with the fungicide Dithane M45 at 2.5 g per each one little water) was application after 40-45 days from sowing, and the spray of insecticide against insects (Lanit 90% at 300 g / feddan) was performed after 30 days from planting. The control treatment was keep without any spray. The percentage of infected plants by insects was estimated two weeks after spraying against insects. The percentage of lodging plants for each cultivar under each treatment was estimated before harvest directly. At harvest (after 4 months from sowing), samples of late wild disease infected plants were taken to isolate the pathogens which cause stalk rot complex disease. The yield per two rows and weight of 100 kernels was also calculated for each cultivar under each treatment. In addition to 500 g of kernels from each cultivar at each treatment were taken for further analysis to isolation of kernel rots causal pathogens in the laboratory. In finally three isolated from each *F. vertecilioides* and *M. maydis* which

isolated were sending to Natural Gene Bank. National Centre for Biotechnology Information (NCBI) for molecular examination.

2.2. Late wilt pathogen infestation

This inoculum was performed annually in department of maize disease research in agricultural research centre, Giza, Egypt and sending to Sakha agricultural research station annually as follow: mixture of *M. maydis* isolates which collected from different location were raised on sterilized grain sorghum seeds, moistened with water, in 500 mL milk bottles. After sufficient fungal growth had been obtained, the inoculum of different isolates were thoroughly mixed and used to infest fresh Nile silt soil at the rate of 10 g/ kg soil. The infested soil was then stored outside of high temperature centigrade to promote fungal growth in the soil. In May 2020 and 2021, this infested soil was thoroughly mixed and distributed into the disease nursery at the rate of 200 kg per feddan as uniformly as possible before sowing the maize materials, as was adopted by El-Shafey *et al.,* (1988).

2.3. Leaf blight pathogen inoculation

To prepare inoculum of *Exserohilum turcicum*, the cause of Northern Corn Leaf Blight disease, heavy infected leaves were collected during previous seasons (these infected leaves sure contain the fungus structures as described by Shekhar and Kumar (2012). These collected infected leaves were stored in large cotton packages in about dry condition and protected against moisture and rodents. To preparation of inoculums, the dry infected leaves are ground to become meal about the coarseness of wheat bran. Inoculation carried out by placing about pinch (1.5-2 g) of leaf meal in whorl of each plant when plant was about 40-45 days old as described by Shekhar and Kumar (2012). This inoculation method can to be ineffective if dry weather condition prevails following inoculation by the leaf meal. Therefore, 10-12 mL of water can be sprayed or added in the whorls by using of sprayer. Therefore, high humid weather was obtained for effective inoculation and disease spread. Symptoms was detected in Figure 1.

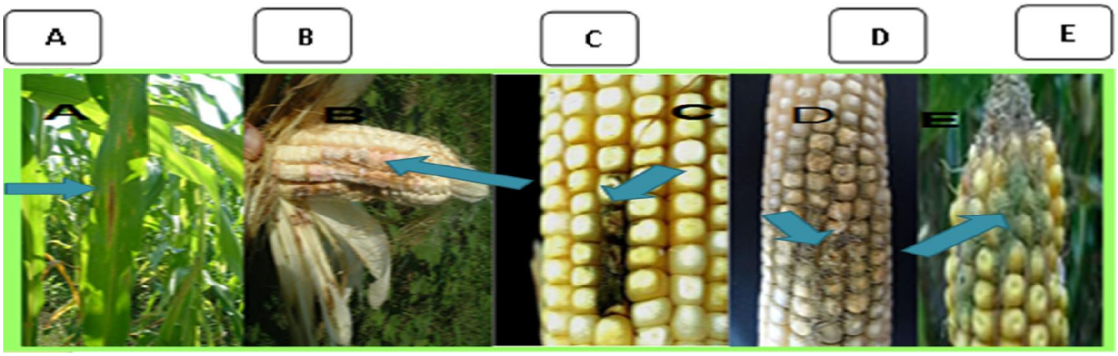

Fig. 1: Symptoms of tested diseases. A= Turcicum leaf blight disease B= *F. verticilioides* ear rot C= *Aspergillus niger* ear rot D= *Aspergillus flavus* ear rot and E= *Penecilium* spp. ear rot (showing direction line).

2.4. Determine of Leaf blight disease

Estimation of cultivars reaction to leaf blight disease was estimated 30-35 days after inoculation (after about 75-85 days from sowing) based on the disease levels scale which was reported in Table 2 (Badr *et al.,* 1999; Shekhar and Kumar 2012).

Table 2: Modified scale for estimating *Exserohilum turcicum* leaf blight (TLB) infection on maize plants.

Rating scale	Infection intensity	Severity (%)	Host response
0	No visible lesions	0.0	Highly resistance
0.5	Very slight infection: about one or two lesion on lower leaves	0-5	Resistant
1	Slight infection: about a few scattered lesion on lower leaves	6-10	Moderately resistant
2	Light infection: about moderate number of lesions on lower leaves	11-25	Moderately susceptible
3	Moderate infection: about abundant lesions on middle leaves	26-50	Susceptible
4	Heavy infection: about abundant lesions on lower and up leaves	52-75	Highly susceptible

2.5. Determine of infection% by stalk rot complex

Samples of maize plants showed typical stalk rot complex symptoms were collected from each cultivar at each treatment and transferred to laboratory for isolation procedures. Collected samples pieces were cut into very small pieces, sterilized using 0.25% sodium hypochlorite solution for about 4 minutes, then washed many times in sterilized distilled water and blotted between two sterilized filter papers. These pieces mentioned above were grown on potato dextrose agar (PDA) medium and supplemented with yeast extract. Then the cultures were incubated at 28 ± 2 ºC for 7-10 days and purified by the hyphal tip technique. The frequency (%) of each pathogen which caused stalk rot complex disease was calculated. Pure cultures of each fungus were tested microscopically (The microscopic investigation was 40×10 magnification, Otika digital camera, b-193,Germany) and maintained on PDA slants which were supplemented with yeast extract (0.1 %) at 4ºC. According to the method described by Awad (2002), the isolated fungi were also identified in department of Mycology, Institute of Plant Pathology, ARC, Giza, Egypt. Three isolates from each of *M. maydis* and *F. verticilioides* were molecular identified for their pathogenicity comparing with standard isolate for each one in National Gene Bank, National Centre for molecular identification. Symptoms were detected in Figure 2.

Fig. 2: Stalk rot complex disease symptoms on some infected plants.

2.6. Molecular characterization of stalk rots pathogens

Genomic DNA of the phenotypically identified *Fusarium verticilioides* and *Magnaporthiopsis maydis* isolates were extracted from growing mycelium 4 days old cultures on Malt extract broth. The procedures were carried out according to manufacture instruction of QIAGEN DNeasy Plant Mini Kit adapted from (www.qiagen.com/HB-1166). According to Protocol of Total DNA Purification from fungal tissue, fungal tissue was grounded to a fine powder under liquid nitrogen using a mortar and pestle. The tissue (≤100 mg wet weight) was smashed in liquid nitrogen to an appropriately sized tube and the rest of liquid nitrogen was allowed to be evaporated. Steps of lyesis, extractions buffers and purifications in supplied column through many centrifugation intervals were added directly after the previous grinding step. In the end of the procedure, around 100 µL of extracted DNA eluted into a 1.5 ml micro centrifuge tube. Five microliter of extracted DNA were taken up for PCR amplification to be genetically identified using ITS1 (TCTGTAGGTGAACCTGCGG) & ITS4 (TCCTCCGCTTATT GATAT GC) primers (White *et al.,* 1990). Genomic DNA was amplified by mixing the template DNA (50 ng), with the polymerase reaction buffer, dNTP mix, primers and Taq polymerase. Polymerase Chain Reaction was performed in a total volume of 100 µL, containing 78 µL deionized water, 10 µL 10 X Taq pol buffer, 1 µL of 1 U Taq polymerase enzyme, 6 µL 2 mM dNTPs, 1.5 µL of 100 mM ITS1 & ITS4 primers and 1 µL of 50 ng template DNA. PCR was programmed using primer condition. The program was started with an initial denaturing at 94°C for 8 min. followed by 32 cycles of denaturation at 94°C for 1 min, annealing at 51 °C for 30 sec and extension at 70 °C for 2 min and the final extension at 72 °C for 7 min in a BIOER/Life ECO 96 advanced gradient Thermo cycler. PCR product (20 µL) was mixed with loading buffer (8 µL) containing 0.25% bromophenol blue, 40 % w/v sucrose in water and then loaded in 2% Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis (Welsh *et al.,* 1991 and McDonald, 1997). ITS PCR productes were sequenced at least

twice for one direction by the Sanger chain-termination method, primers were utilized. Sequence data were assembled and compared with databases using the BLAST server on the NCBI Web server, Mega 11 program and TreeView programmes.

2.7. Estimation of ear and kernel rots causal pathogens percentages

After harvest, grains samples (500 g) from three maize cultivars (SC10, TWC 320 and Balady) were collected randomly from each of tested treatment and each of replicate and remained under laboratory condition until beginning of examination. Samples were taken (500 g of kernels) from each treatment at each replicate for isolation of ear and kernel rots causal organisms. To isolate pathogens causing ear and kernel rots of maize, one hundred kernels of each tested maize cultivar at each treatment and replicate, were sterilized in 1% sodium hypochlorite for 3-4 minutes. Surface sterilized kernels were then washed three times in distilled sterilized water, blotted between two sterilized filter paper and plated in Petri plates containing 10 ml of potato dextrose agar medium (PDA). The plates were incubated at 26-27CO for 8-10 days. Fungi emerged from infected kernels were microscopically examined (using microscope 40×10 magnification, Otika digital camera,b-193, Germany) and purified using the hyphal tip technique. The obtained fungi were accounted (as percentage %) in each maize cultivar at each treatment and replicate according to their frequency of developing on isolation plates (ISTA, 1985). Isolated fungi were identified in department of Mycology, Institute of Plant Pathology, ARC, Giza, Egypt. Symptoms were detected in Figure 2.

2.8. Statistical analysis

Statistical analysis was performed as recorded by Gomez and Gomez (1984), the variance and the mean analysis were estimated using the least significant difference test (LSD).

3. Results and Discussion

3.1. Maize leaf blight disease

Data presented in Table (3) found that, the reaction of tested maize cultivars to infection by leaf blight disease under artificial inoculation defriended within them. Therefore, the SC10 was ranged from highly resistance (HR) to resistance (R), the TWC 320 ranged from R to MR (moderately resistance), while the balady cultivar ranged from MR to MS (moderately susceptible) under all tested treatments and under two tested seasons (this reaction estimated according the scale which detected in Table 2).

Table 3: Severity of maize leaf blight infection in three maize cultivars as influenced by different treatments during two growing seasons 2020 and 2021 under field conditions.

Maize cultivar	Treatment	Maize growing season			
		2020		2021	
		Severity (%)	Host reaction	Severity (%)	Host reaction
SC10	T1	4.33 fg	HR	3.66 g	HR
	T2	5.00 f	HR	4.33 fg	HR
	T3	3.66 g	HR	4.33 fg	HR
	T4	7.33 e	R	9.33 e	R
TWC 320	T1	4.33 fg	HR	5.00 f	HR
	T2	7.66 e	R	8.66 e	R
	T3	5.00 f	HR	4.33 fg	HR
	T4	16.33 c	MR	22.33 c	MR
Balady	T1	6.66 e	R	9.33 e	R
	T2	28.66 b	MS	30.00 b	MS
	T3	12.33 d	MR	19.33 d	MR
	T4	30.00 a	MS	35.000 a	MS
F. test		**		**	
LSD 5%		1.112		1.041	

Values are mean of three replicates for each treatment as well as maize cultivars. Means within the column followed by different letters records statistically significant differences (P = 0.05) between treatments adopted by Duncan'smultiple range test. Disease severity was estimated according to the rating scale reported by Badr *et al.,* (1999) and Shekhar and Kumar (2012). T1, pesticides application against leaf blight (Dithane M45) and corn borers (Lannate 90% SP). T2, insecticide application against corn borers only (Lannate 90% SP). T3, fungicide application against leaf blight only (Dithane M45). T4, Non-treated plants (control). HR, highly resistance. R, resistance. MR, moderately resistance. MS, moderately susceptible.

The maize breeder can use the SC10 and TWC 320 in maize breeding program as a source of resistance to the leaf blight disease. These results cleared changes in reaction of each cultivar due to kind of treatment. The obtained results are in agreement with those reported by Jakhar *et al.* (2021), which reported that, among forty maize inbred lines which were tested, 10 resistant and 13 partial resistant to leaf blight disease under artificially inoculated and field conditions.

3.2. Maize stalk rot complex

Data presented in Table (4) showed that, the fungus *Fusarium verticilioides* was the prevalent pathogen and had the highest stalk rot infection percentage. The percentage of infection by it ranged from 2.333 to 11.676 % during season 2020, and from 1.63 to 10.17% during season 2021 at all the tested maize cultivars and at all tested treatments. It was significantly followed by the fungus *M. maydis*, here, and the percentage of infection by it ranged from 1.600 to 8.500% during season of 2020, and ranged from 0.833 to 9.166 % during season of 2021.

Table 4: Effect of leaf blight and corn borer management on the infection percentage (%) by stalk rot complex fungi in three maize cultivars during 2020 and 2021 growing seasons.

Variable	Infection Percentage (%)							
	F. verticilioides		*M. maydis*		Other fungi		Stalk rots complex	
	2020	2021	2020	2021	2020	2021	2020	2021
Cultivars (C)								
SC10	4.230c	3.195c	2.577c	2.055c	2.105c	2.377c	8.892c	7.626c
TWC 320	6.155b	5.403b	4.877b	3.715b	3.592b	3.302b	14.355b	12.423b
Balady	8.932a	8.065a	6.492a	5.692a	5.232a	4.457a	20.662a	18.217a
F. test	**	**	**	**	**	**	**	**
Treatments (T)								
T1	4.193d	3.526d	2.677c	1.731d	1.343d	1.127d	8.368d	6.387d
T2	5.925c	4.891c	4.843b	3.377c	2.865c	2.609c	13.124c	10.880c
T3	6.885b	6.189b	4.675b	4.199b	4.088b	3.870b	15.648b	14.260b
T4	8.758a	7.610a	6.383a	5.976a	6.277a	5.899a	21.412a	19.486a
F. test	**	**	**	**	**	**	**	**
C×T								
SC10 ×T1	2.333e	1.633g	1.600f	0.833g	0.000f	0.333g	3.933h	2.799j
SC10 ×T2	3.633d	2.676f	2.600def	2.300ef	1.433e	1.666ef	7.633g	6.642i
SC10 ×T3	4.656d	3.633f	2.260ef	1.933f	2.500de	2.333e	9.416f	7.899h
SC10 ×T4	6.300c	4.833e	3.833cd	3.166e	4.500c	5.166bc	14.600d	13.165f
TWC 320 ×T1	4.333d	3.116f	2.833def	1.933f	1.433e	1.116fg	8.599fg	6.165i
TWC 320 ×T2	5.766c	4.666e	3.500cde	3.000ef	2.833d	2.333e	12.099e	9.999g
TWC 320 ×T3	6.666c	6.000d	4.833c	4.333d	3.933c	4.166cd	15.432d	14.499e
TWC 320 ×T4	8.300b	7.833c	6.833b	5.599bc	6.166b	5.599b	21.299b	19.031c
Balady ×T1	6.373c	5.833d	3.600cde	2.432ef	2.600d	1.933ef	12.573e	10.198g
Balady ×T2	8.376b	7.333c	6.933b	4.833cd	4.333c	3.833d	19.642c	15.999d
Balady ×T3	9.333b	8.933b	6.933b	6.333b	5.833b	5.116bc	22.099b	20.382b
Balady ×T4	11.676a	10.166a	8.500a	9.166a	8.166a	6.933a	28.342a	26.265a
F. test	**	**	**	**	**	**	**	**

Values are mean of three replicates to each treatment, as well as, maize cultivars. T1, pesticides application against leaf blight (Dithane M45) and corn borers (Lannate 90% SP). T2, insecticide application against corn borers only (Lannate 90% SP). T3, fungicide application against leaf blight only (Dithane M45). T4, Non-treated plants (control).

Other fungi i.e., *Macrophomina phaseolina*, *Alternaria* spp., *Penicillium* spp. and *Aspergillus* spp., had the lowest infection percentage of stalk rot disease. The percentage of infection by them ranged from 0.00 to 8.166% during the season of 2020 and from 0.333 to 6.933% during the season of 2021 at all tested maize cultivars and at all tested treatments. Over all, the infection % by stalk rot complex was ranged from 3.933 to 28.342% during season 2020, and from 2.799 to 26.265% during season 2021. On the other hand, data obtained from (Table 4) showed also that, the lowest infection percentage by stalk rot complex recorded in plots which sprayed against both of leaf blight disease and insects. It recorded 3.933 and 2.799 % in SC 10, 8.599 and 6.165 % in TWC 320, 12.573 and 10.189 % in balady cultivar during 2020 and 2021 seasons, respectively. The highest infection percentage by stalk rot

complex were recorded in control plots which non sprayed against both of leaf blight disease and insects, it recorded 14.600 and 13.165 % in SC 10; 21.299 and 19.031 % in TWC 320; 28.342 and 26.265% in balady cultivar during 2020 and 2021 seasons, respectively. These results indicated the importance of spraying the maize plants against both of leaf blight disease and insects infection for obtaining the lowest infection by stalk rot complex causal organisms (*F. verticilioides*, *M. maydis* and other fungi) in maize. These results agree with which recorded by Gai *et al.,* (2018) which reported that, the most dangerous factors impacting stalk rots disease in corn are infection by foliar diseases (for example northern leaf blight disease), insect injury, and root rot infection damage. Loss of leaf area which caused by major and extensive foliar disease decrease the plant's machinery of photosynthetic that produces carbohydrates, which importance in production of defence chemicals. In the same situation, many researchers also added that, *F. verticillioides*, the pathogen of stalk rot disease, is often associated with damaged by insects, primarily due to the feeding of the European corn borer insects (Czembor *et al.,* 2019; Pfordt *et al.,* 2020; Citation *et al.,* 2021)

3.3. Molecular identification of stalk rots complex pathogens

Molecular tests based on the polymerase chain reaction (PCR) have been carry out as a way in genetic mapping, molecular taxonomy, evolutionary studies, and diagnosis of many fungal causal organisms (Welsh *et al.,* 1991; McDonald, 1997). In order to investigate the genetic polymorphism and confirming the identification of *Fusarium verticilioides* and *Magnaporthiopsis maydis* isolates, The PCR amplification using the ITS1 and ITS4 primers reveled on PCR products around 400 to 600 bp of each of *F. verticilioides* and *M. maydis* isolates. According to the online blast of NCBI, the selected were recognized as *F. verticilioides* and *M. maydis* based on ITS sequences analysis. These isolates were preserved on the National Gene Bank of Egypt (NGB) and registered on www.NCBI.com by the following accession numbers as showed on (Table 5). Molecular markers particularly the ITS regions have considered to be importance, both in the identification of individual varieties and in the reveal of phylogenetic relationships between fungal species (Chandra *et al.,* 2008; Mary Olowe *et al.,* 2017; Harish *et al.,* 2023). The phylogenetic analysis of the three isolates of *F. verticilioides* (Figure 3) have proved the genetic variation existence among obtained isolates in comparison with a reference isolate of *F. verticilioides* which was necessary to be registered on NCBI data base. Also, the *M. maydis* isolates were showed a genetic variation based on ITS1&4 sequences in comparison with *Magnaporthiopsis maydis* CBS.

Table 5: List of *Fusarium verticilioides* and *Magnaporthiopsis maydis* isolates NCBI accession numbers.

Isolate code	Name	NCBI accession numbers
F1- ITS1&4	*Fusarium verticilioides*	OR166195
F2- ITS1&4	*Fusarium verticilioides*	OR167767
F3- ITS1&4	*Fusarium verticilioides*	OR485023
M1- ITS1&4	*Magnaporthiopsis maydis*	OR511501
M2- ITS1&4	*Magnaporthiopsis maydis*	OR511502
M3- ITS1&4	*Magnaporthiopsis maydis*	OR511503

3.4. Corn borer infection, plant lodging (%) and maize yield

Results in Table (6) demonstrated positive correlation between infection by corn borer and percentage of lodging in maize plants. The obtained data during the two growing seasons and in case of the three cultivars i.e., SC 10, TWC 320 and balady had the same trend (Table 6). During 2020 growing season, spraying of SC 10 maize plants against infection by corn borer only resulted in 0.333% corn borer infection and 0.833% lodging. While, the infection by corn borer was 4.833% and resulted in 3.500 % lodging in control treatment. The same trend was showed in case of TWC 320 and balady maize cultivars. This indicated that, the positive correlation was founded between infection by corn borer % and lodging % in maize plants. The results in (Table 6) indicated also that the weight of 100 kernels as well as the yield per 2 rows was increased as a result of spraying against corn borer infection in maize plants. Therefore, the spraying against corn borer and leaf blight disease obtained the highest value of weight of 100 kernels (38.500g) as well as the highest of yield per two rows (10.716 kg) in SC10 cultivar.

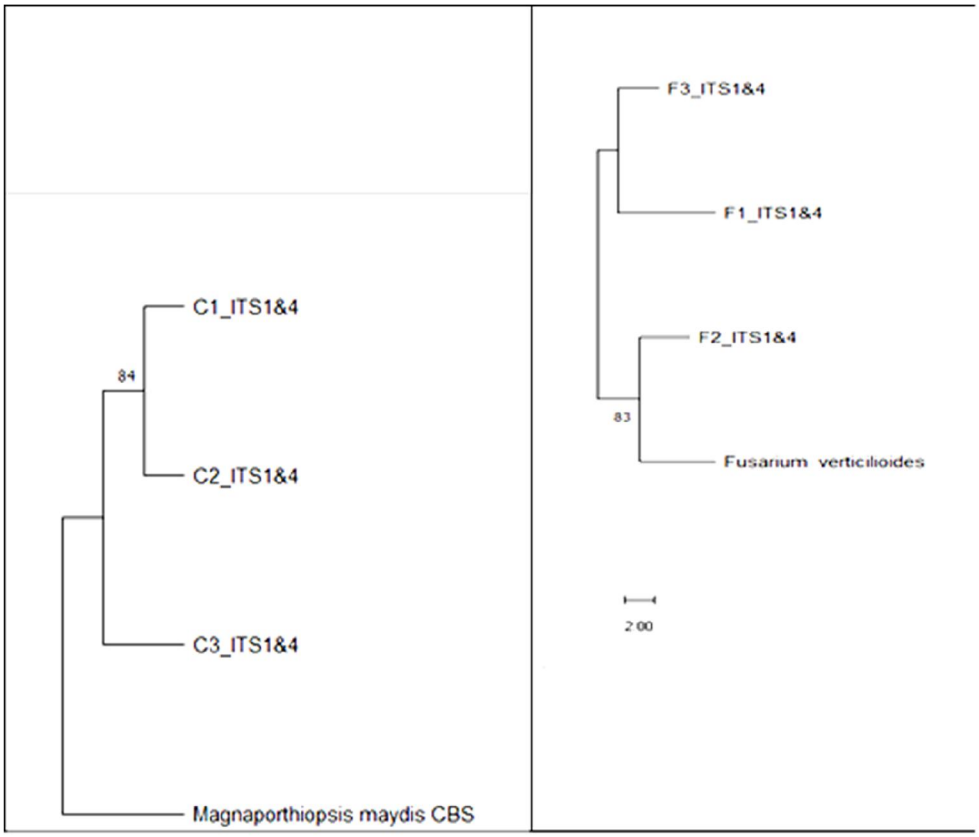

Fig. 3: Phylogenetic analysis tree showed the relationship among the three isolates of each *Fusarium verticilioides* and *Magnaporthiopsis maydis* based on ITS sequence using Mega 11.

In the same respect, the spraying against corn borer only resulted in high value also of weight of 100 kernels (37.166 g) as well as high value of yield per two rows (10.283 kg) comparing with 34.166 g as well as 10.166 kg in control treatment at SC10 maize cultivar, respectively. The results from this study cleared that, the positive correlation was founded between infection by corn borer % and lodging % in maize plants. Therefore, the spraying against corn borer and leaf blight disease obtained the lowest values of lodging %, highest values of weight of 100 kernels as well as the highest values of yield per two rows. The European corn borer insects can contribute and preparing to lodging and stalk rot incidence by made creating entry entrance and by also enhancing as a vector of some pathogens which causes stalk rot diseases, particularly *F. verticillioides*. These results are in agreement with findings by Gatch and Munkvold (2002) which reported that, the European corn can contribute to lodging and stalk rot development by creating many entry wounds sites and by also serving as a vector of many stalks rot causal organisms. Stalk rot pathogen exist in infected plant residue and invasion plants through natural entry sites such as through damages or wounds created by insects or mechanical wounds, or by direct penetration of stalk and root tissue directly (Maryke *et al.,* 2020). Injury which causes by feeding of European corn borers which named *Ostrinia nubilalis* (Hübner), is one considerable that can enhancing the development of stalk rot in many ways (Chiang and Wilcoxson 1961; Gatch and Munkvold 2002). By tunnelling of stalk tissue, they create entry points for fungal pathogens invasion, and cause physiological changes that can promote maize plants to stalk rot development (Maryke *et al.,* 2020). This interaction between corn borers wounds and stalk rot disease is an considerable factor in pest management decision making, because the yield reduction attributed to corn borers usually is due to in part to subsequent fungal damages of tissue injured by the larvae (Gatch and Munkvold, 2002).

Table 6: Effect of leaf blight and corn borer management on the percentage of plant lodging and yield production in three maize cultivars during 2020 and 2021 growing seasons under field conditions.

Variable	Corn borer infection, plant lodging (%) and maize yield (kg/two rows)							
	Corn borer		Lodging		Weight of 100 kernels (g)		Yield	
	2020	2021	2020	2021	2020	2021	2020	2021
Cultivars (C)								
SC10	2.247c	1.541c	1.541b	1.012b	36.333a	36.556a	10.421a	10.531a
TWC 320	3.166b	2.382b	1.715b	1.291b	35.747b	35.965b	9.495b	9.598b
Balady	5.458a	4.315a	3.583a	2.695a	33.497c	33.816c	7.462c	7.577c
F. test	**	**	**	*	**	**	**	**
Treatments (T)								
T1	0.611d	0.444c	0.555d	0.222d	37.833a	38.055a	9.393a	9.514a
T2	0.944c	0.499c	1.111c	0.722c	36.166b	36.477b	9.238b	9.316b
T3	5.777b	4.288b	2.977b	1.911b	34.166c	34.394c	9.022c	9.138c
T4	7.166a	5.755a	4.477a	3.811a	32.610d	32.899d	8.852d	8.977d
F. test	**	*	**	**	**	**	**	**
C×T								
SC10 ×T1	0.000g	0.000e	0.000h	0.000d	38.500a	38.800a	10.716a	10.811a
SC10 ×T2	0.333g	0.000e	0.83fgh	0.333d	37.166b	37.566bc	10.566a	10.616ab
SC10 ×T3	3.833e	2.666c	1.83def	1.116cd	35.500c	35.650d	10.283ab	10.416ab
SC10 ×T4	4.833d	3.500c	3.500bc	2.600b	34.166de	34.333ef	10.166ab	10.283bc
TWC 320 ×T1	0.333g	0.000e	0.333gh	0.000d	38.333a	38.500ab	9.716bc	9.866cd
TWC 320 ×T2	0.666g	0.333de	0.333gh	0.333d	37.000b	37.200c	9.583c	9.666d
TWC 320 ×T3	5.166d	3.600c	2.600cd	1.500c	34.500cd	34.666de	9.400c	9.500d
TWC 320 ×T4	6.500c	5.600b	3.600bc	3.333b	33.166ef	33.500fg	9.283c	9.366d
Balady ×T1	1.500fg	1.333d	1.33efg	0.666cd	36.666b	36.866c	7.750d	7.866e
Balady ×T2	1.833f	1.166d	2.166de	1.500c	34.333d	34.666de	7.566de	7.666ef
Balady ×T3	8.333b	6.600b	4.500b	3.116b	32.500f	32.866g	7.383de	7.500ef
Balady ×T4	10.166a	8.166a	6.333a	5.500a	30.500g	30.866h	7.150e	7.283f
F. test	**	**	**	**	**	**	**	**

Values are mean of three replicates for each treatment as well as maize cultivars. T1, pesticides application against leaf blight (Dithane M45) and corn borers (Lannate 90% SP). T2, insecticide application against corn borers only (Lannate 90% SP). T3, fungicide application against leaf blight only (Dithane M45). T4, Non-treated plants (control).

3.5. Ear and kernels rot diseases

The presented data in Table (7) illustrated that, *F. verticilioides* was the precedent pathogen and caused the highest infection percentage of ear and kernel rot disease with all the tested maize cultivars and under all tested treatments. Moreover, the reverse was true in case of *Aspergillus niger*, *A. flavus* and *Penicillium* spp. The obtained data during the two growing season and in case of the three cultivars i.e., SC 10, TWC 320 and balady had the same trend (Table 7). During 2020 growing season, the lowest infection percentage by *F. verticilioides* was recorded in case of spraying against leaf blight and against corn borer, being 23.00% , 24.666 % and 29.333% in SC10, TWC 320 and balady maize cultivars, respectively. The highest infection percentage by *F. verticilioides* was recorded in case of no spraying (control treatment), being 28.333%, 33.666% and 38.333% in SC10, TWC 320 and balady maize cultivars, respectively. On the other hand, the infection percentage by the other tested fungi ranged from 0.000 to 7.666 % in all tested maize cultivars and under all tested treatments. These results summarized that, *F. verticillioides* causal agent of ear and kernels rot disease is one of the importance and very major fungal diseases on maize in the world. The disease is manufacture for considerable to losses of yield quantitative and quality. These results in the same with Czembor *et al.,* (2019) which reported that, *F. verticillioides* is the most major and common pathogen which causing kernels and stalk rot diseases in maize. In the present study the lowest infection percentage by *F. verticilioides* was obtained in case of spraying against leaf blight and against insects. This is due to the importance role which corn borer played it to preparation and predisposing maize plants to invasion by mentioned pathogen (Czembor *et al.,* 2019). This results are also in agree with those obtained by Lina *et al.,* (2019) which they provided a positive correlation between stem rot and ear rot diseases infection. Gai *et al.,* (2018) added that, *F.*

verticillioides which isolated from either stalk rot and /or ear rot diseases on maize often infect the maize plants by gaining access within plant through invasion the radicle and then infecting the tissues of stalk upward through various ways to the ear. Therefore, *Fusarium verticillioides* is also the major causal agent of maize ear and kernel rot (Lanubile *et al.,* 2017; Gai *et al.,* 2018; Lina *et al.,* 2019). The fungus (*F. verticillioides*) not only decreases yield but also reduces the quality of the grains and can produce the secondary metabolite fumonisins (Zhou *et al.,* 2018; Czembor *et al.,* 2019). Fungicides applications are not labelled for management of stalk rot complex, ear and kernels rot disease.

Table 7: Effect of leaf blight and corn borer management on the infection percentage (%) by ear and kernels rot causal organisms in three maize cultivars during 2020 and 2021 growing seasons under field conditions.

Variable	Infection Percentage (%)							
	F. verticilioides		*A. niger*		*A. flavus*		*Penecillium* spp.	
	2020	2021	2020	2021	2020	2021	2020	2021
Cultivars (C)								
SC10	25.165c	22.998c	3.248c	1.915c	2.416c	2.083c	1.247c	0.915c
TWC 320	28.748b	26.248b	4.915b	4.248b	4.333b	3.665b	3.915b	3.424b
Balady	33.915a	31.248a	6.415a	5.415a	5.333a	5.498a	5.083a	4.583a
F. test	**	**	**	**	**	**	**	**
Treatments (T)								
T1	25.666d	22.444d	2.444d	1.333d	1.777d	1.444d	1.333d	1.111c
T2	27.555c	25.332c	3.999c	3.221c	3.110c	2.888c	2.555c	2.110c
T3	30.444b	28.333b	5.555b	4.666b	4.444b	4.444b	4.111b	3.555b
T4	33.444a	31.221a	7.444a	6.221a	6.777a	6.221a	5.666a	5.110a
F. test	**	**	**	**	**	**	**	**
C×T								
SC10 ×T1	23.000l	19.666i	1.333f	1.000i	0.666g	0.000g	0.000f	0.000f
SC10 ×T2	23.666k	21.666h	2.666e	1.666hi	1.666fg	1.333f	0.333f	0.000f
SC10 ×T3	25.666i	23.333g	3.666d	2.333gh	2.666ef	2.666e	1.666e	1.333e
SC10 ×T4	28.333g	27.33de	5.333c	3.666ef	4.666bc	4.333cd	3.000d	2.333d
TWC 320 ×T1	24.666j	21.333h	2.333e	1.666hi	1.666fg	1.000fg	1.666e	1.333e
TWC 320 ×T2	26.666h	24.666f	4.000d	3.333fg	3.333de	2.666e	3.000d	2.666d
TWC 320 ×T3	30.000e	28.333d	5.666c	5.333cd	5.000bc	4.333cd	4.666c	4.000c
TWC 320 ×T4	33.666c	30.666c	7.666b	6.666b	7.333a	6.666ab	6.333b	5.666b
Balady ×T1	29.333f	26.333e	3.666d	2.333gh	3.000e	3.333de	2.333de	2.00de
Balady ×T2	32.333d	29.666c	5.333c	4.666de	4.333cd	4.666c	4.333c	3.666c
Balady ×T3	35.666b	33.333b	7.333b	6.333bc	5.666b	6.333b	6.000b	5.333b
Balady ×T4	38.333a	35.666a	9.333a	8.333a	8.333a	7.666a	7.666a	7.333a
F. test	**	**	**	**	**	**	**	**

Values are mean of three replicates for each treatment as well as maize cultivars. T1, pesticides application against leaf blight (Dithane M45) and corn borers (Lannate 90% SP). T2, insecticide application against corn borers only (Lannate 90% SP). T3, fungicide application against leaf blight only (Dithane M45). T4, Non-treated plants (control).

4. Conclusions

In the present study, maize cultivars *i.e.,* SC10 and TWC320 were recorded HR and R against maize leaf blight disease. Consequently, these maize cultivars can be employed for enhancing resistance in promising maize genotypes in maize breeding programs. The obtained data also revealed that, the most important factors impacting stalk rot disease in maize are leaf blight disease and corn borer damage, because the loss of leaf area which resulted by major and extensive foliar disease (leaf blight disease) and insects invasion decrease the plant's machinery of photosynthetic that produces carbohydrates, which importance in production of defence chemicals. Stalk rot complex & ear and kernels rot infections were high in plots which untreated against both of leaf blight disease and corn borer infection. The results indicated the importance of chemical spraying of maize plants against both of leaf blight disease and corn borer as management tools to obtain the lowest infection by both of stalk rot complex disease and /or ear and kernels rot disease in maize. The phylogenetic analysis of the three isolates of each of *Fusarium verticilioides* and *M. maydis* proved the molecular identification of obtained isolates in comparison with a reference isolates registered on NCBI data base.

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