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Survey Of *Fusarium Verticillioides* Associated With Maize (*Zea Mays* L.) In Iraq

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Abstract:
Maize (Zea mays L.) is an important food crop worldwide. Some Fusarium species cause maize ear rot leading to significant yieldlosses and, for Fusarium verticillioides, potential risk of mycotoxin contamination and produces Fumonisin mycotoxins .a survey ofpopulation composition of Fusarium species on maize in Iraq representative important task due to high infection in maize plants ineach field. The aim of this study survey the frequency of fungi associate with maize ears .The result showed that the Fusariumspecies isolates were highly frequent in all plant parts , F. verticillioides out off Fusarium spp were highest frequency found in allmaize samples, it was showed frequency percentage 45%. PCR assay are more accurate for identification Fusarium spp. compared toconventional cultural approaches. Conclusion that Fusarium species representative common pathogen on maize plants.

Key words: Fusarium verticillioides, Zea mays PCR assay. Iraq.

Methods for identification of *Fusarium* spp require considerable expertise ,special media, time consuming ,high cost and challenge contamination. The morphologically and physiologically characterize fumonisin forming *Fusarium* spp. are considered labor intensive and slow in determination(Kuiper-Goodman, , 2004).

ITS(rDNA) for the fungus *Fusarium* using a pair ITS1- Fuf: / ITS4-Fur and gave the outputs of polymerization 400 base pairs in length and got the results many of the species of the fungus(El-Fadly et al.,2008). The aim of this study was targeted isolation, and identification of *F. verticillioides* and typing 21 isolates of species by using sequencing results of PCR products amplified by primer pair ITS1- Fuf: / ITS4-Fur .

Materials and methods:

1-Samples collection

A total of 260 samples of seeds and kernel rots of maize were randomly collected from different farms of

Introduction

Fusarium verticillioides are commonly associated with maize (*Zea mays* L.) causing root, stalk, ear , s e e d s and kernel rots is one of the most common plant pathogenic fungi affecting maize (Nelson,et al., 1991 ;Leslie, 2006) and is the prevailing maize pathogen in the world (Zhang, et al 2014). It attacks roots, stems ,seeds and ears causing crop yield reductions ranging from 10% to 30%. *F. verticillioides* is a major pathogen to crop maize is the most common cultivation regions (Abbas et al., 2002).

It's affects the yellow corn cultivars , colonize this cultivars of basic parts of inflorescence (Desjardins et al.,2002).This fungus produce secondary metabolites (mycotoxins) as contaminated maize crop such as Fumonisins .This toxins production in high levels ranged from 25% to 40% of the grain in the world ,mostly associated with maize grain (Rheeder et al., 2002).Millions of dollars were lost by mycotoxins contamination in the world through harm to human, animal (Bhat, and Vasanthi, 2003).

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4- PCR assay

The primer pair that targeted the sequences site of the ITS1-5.8S-ITS2 gene was Fuf: 5`-CAACTC CCAAACCCCTGTGA-3` and Fur:5'-GCGACGATTACCAGTAACGA-3' dubitable as *F. verticillioides* isolates were used. The PCR mixture (25 µl) consisted of 12.5 µl of 20x Master Mix (Promega), 2 µl (10 pemole) of each primer and 1 µl template DNA, made up to 25 µl with molecular-grade water. The PCR mixture was amplified by the thermal cycler PCR System (Labnet, USA) .initial denaturation temperature 95ºC for 5 min, , 30 cycles, 95ºC for 30 sec , annealing temperature of 56ºC for 1.5 min , extension temperature of 72ºC for 1 min ,final extension temperature 72ºC for 10 min ,cool step by 4 ºC. (Abd-Elsalam et al., 2004).

The PCR products were run on 1.5% agarose gel (Bio Basic Canada Inc.) and electrophoresis was performed at 70 V in TBE buffer. The gel was pre-stained with 0.05% ethidium bromide. The DNA bands were detected using the Desktop Gel imager scope 21 ultraviolet transilluminator.

Results and Dissuasion:

1- Isolation and identification fungi associated with the infected parts of maize plants.

The results showed many isolates of fungi associated with the seeds , ear and kernel rots of maize taken from the areas famous for the cultivation of maize crop in the province of Babylon. 15 species of fungi were diagnostic were: *Alternaria alternata* , *Aspergillius flavus* , *A niger* , *A.oryzae* , *A. prasiticus* , *A. terreus* , *Fusarium graminearum*, *F. solani* , *F. verticillioides* and *Fusarium* spp. *Mucor* sp , *Penicillium* sp , *Rhizoctonia solani* , *Rhizopus stolonifer* and *Trichoderma harzianum*, these fungi classified morphologically based on Booth (1971) and Watanabe (2010).

The results showed that the preliminary diagnosis of dubitable as *F. verticillioides* showed based on microscopic characters included microconidia arranged in maize in Babylon province Iraq. The study period conducted in 2014-2015. Plant parts were collected in polyethylene bags labeled with leaflets included name of sample(leaves and seeds) collection sites and other essential information.

2-Isolation and purification of *F. verticillioides* isolates from inoculated maize tissue:

Adoptable infected seeds , seeds and kernel rots were sterilized sodium bicarbonate a concentration of 3.2% for two minutes and then washed triple times with sterile water distilled twice every time, then transferred by sterile forceps into Petri of Potato dextrose Agar (PDA). Tiny portion from ear , kernel rots tissue and 5 sterile seeds of maize inoculated into Petri of PDA a just 1 cm from the edge of the Petri dish and then incubated plates in the incubator at a temperature of 25 ± 2º C for five days, following fungal growths were pick up by sterile needle into new culture medium of PDA and incubate on PDA for 5 dyes at 28ºC,Slant cultures were prepared for pure cultures dubitable as *F. verticillioides* and preserved at 4 ºC for following purpose. Identification of fungi were performed based on Watanabe (2010).

3- Genomic DNA extraction

The culture media for each of the 21 pure cultures of dubitable as *F. verticillioides* were frozen for 1 h . Tiny portions of the mycelia mat were harvested into 1.5 ml tube. The harvested mats were suspended in 400 µl of lysis buffer(Promiga comp.) then vortexes for 5 min and added 10 µL protinase K to each tube, then was suspended in 200 nuclear lysis in a sterile Eppendorf tube.100 µl protein precipitate and mixed and incubated in cool temperature for 10 min. and centrifuged at 8000 rpm , the supernatant transferred to new tube ,mixture of phenol –chloriphorm mixed and centrifuged at 12000 rpm .the supernatant transferred and mixed with 500 µl isoproponal and centrifuged for 12000rpm ,discharged the isopropanol and wish the DNA pelt with alcohol and dry DNA pelt and re dehydrate with 70 µl and preserved for next molecular assays.

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reported to complex included *F. verticillioides* and *F. proliferatum* .

rosary form (Figure 1).but these characters sometimes confuse with these of *F. proliferatum* . Leslie (2004)

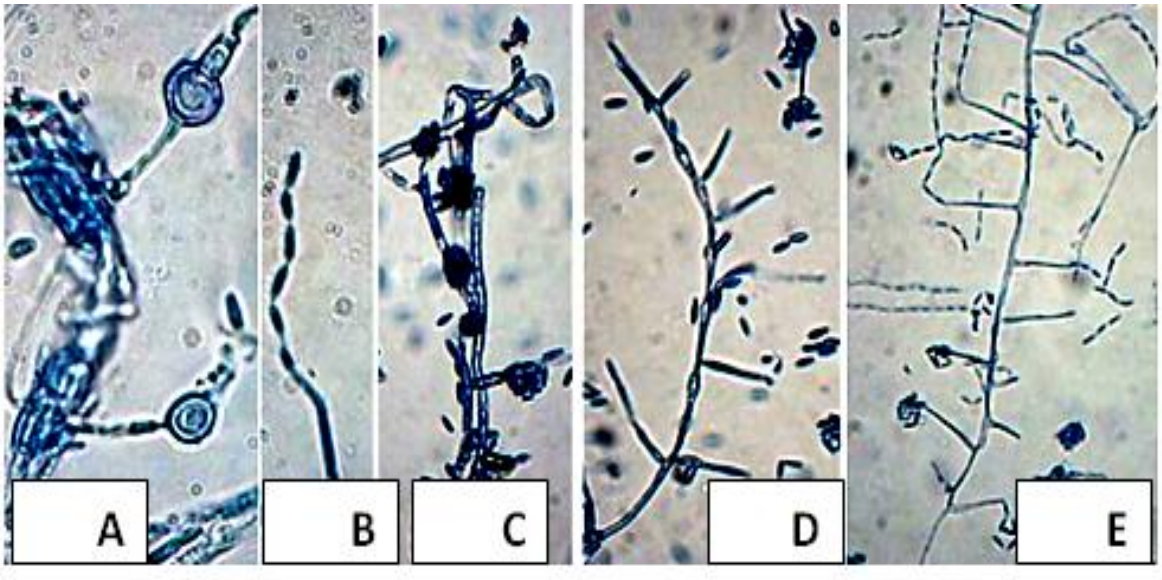

Figure 1:Microscopic feature of dubitable as *F. verticillioides* showed chlamydospores (A), phailophores(D), cluster of microconidia(C), and rosary form microconidia(B-E), Magnification power A-D (X100),E (X10).

This fungi showed highest frequency fungal species out of total isolated species. dubitable as *F. verticillioides* out of *Fusarium* spp were highest frequency found in all maize samples, it was showed frequency percentage 45% (Table 1).Our results coincidence with Chulze *et al*. (1996) they reported the widely infection with *F. verticillioides* in different habitats.

Table 1:The common fungi isolated from ear and kernel rots of maize were collected from different farms of maize (*Zea mays* L.) in Babylon province

Isolated Fungi	**Frequency percentage**
Alternaria alternata	**1.43**
Aspergillus flavus	**17.85**
A. niger	**5.00**
A.oryzae	**1.43**
A .prasiticus	**2.14**
A. terreus	**1.43**
Fusarium graminearum	**4.28**
F. solani	**3.57**
F. verticelloides	**45**
***Fusarium* spp .**	**4.28**
***Mucor* sp .**	**1.43**

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***Penicillium* sp .**	**5.00**
Rhizoctonia solani	**3.45**
Rhizopus stolenifer	**1. 45**
Trichoderma harzianum	**2.14**
Total	**≈100 %**

Our results agreed with the earlier studies about the issue and the rule of the *F. verticillioides* with maize (Marasas, et al., 2001). The reason for this may be due to the ability of *Fusarium* to produce huge microcondia, which can transmitted through air to infect yellow corn plant parts.

Our results was coincidence with previous studies performed in Iraq referred to the domain of *F. verticillioides* with maize samples) high frequency of fungi in Iraq may correlated with growth conditions of high temperature and a low moisture (Rheeder et al., 2002).

2-Molecular diagnosis of dubitable as *F. verticillioides*

Molecular diagnosis of dubitable as *F. verticillioides* was performed by used universal primer pair (ITS1- Fuf / ITS4-Fur) the amplification results of the target region (including the primer pair) amplicon size 400bp (Figure 2) .These findings are consistent with those findings of the study of the fungus *Fusarium* sp give molecular weight of 400 base pairs. Our result coincidence with Patiño et al. (2006).

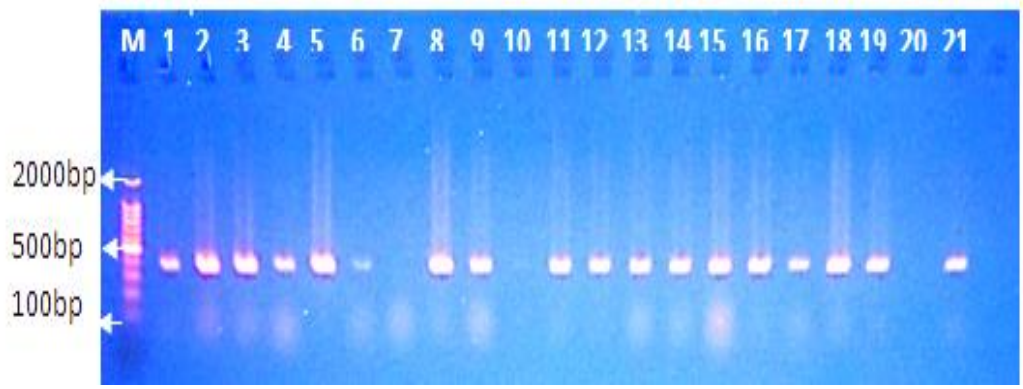

Figure 2.Gel electrophoresis of ITS PCR products amplified for 21 isolates of *Fusarium* spp dubitable as *F. verticillioides* or *F. proliferatum* with ITS1- Fuf: / ITS4-Fur) primers. Lanes 1-21, DNA amplified from dubitable as *F. verticillioides* or *F. proliferatum* M, molecular-weight markers (100 bp DNA ladder, Promega).

The universal fungal primers ITS1- Fuf: / ITS4-Fur amplified part of ITS1, 5.8S, and ITS2 regions for 21 isolates and in each case,. Species identification, non determined by phenotype-based methods . Conclusion that the result showed that *Fusarium* species isolates were highly frequent in all plant parts . PCR assay are more accurate for identification *Fusarium* spp. compared to conventional cultural approaches.The *Fusarium* species representative common pathogen on maize plants.

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