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Fusarium Brachygibbosum and *Fusarium Acutatum* Were First Recorded as Wilting Agents on Tomato Plants in Basra.

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ABSTRACT

In this study Fusarium wilt is considered as one of the most important disease that affecte the tomato plants (*Solanumlycopersicon* L.), The aim of this study was identified and diagnosed the *Fusarium* species that cause wilt in tomato using morphological and molecular approaches. The isolates were confirmed and genetically diagnosed according to translation elongation factor TEFα1 region and recorded in the genebank DDJB. The isolation of the species *F. brachygibbosum* LC529919ADK7 and LC527418ADK23 for *F. acutatum* was performed and the pathogenicity test of the aforementioned isolates was recorded. It reached 80% to *F. brachygibbosum* and the isolation of *F. acutatum* reached 58%, as yellowing and wilting symptoms appeared after re-infection with the aforementioned isolates on healthy tomato plants, and this confirms the ability of Fusarium isolates to heterogeneity and produce strains capable of attacking the host continuously.

Keywords: Fusarium brachygibbosum, Fusariumacutatum,tomato, TEFα1

Introduction

The tomato plant *Lycopersiconesculen*tum Mill is one of the Solanaceae family's Solanaceae crops, and it is an important crop due to its high nutritional value for humans, South America is the original home of tomato, especially Mexico, and in the sixteenth century its cultivation moved to Europe and then to South and East Asia, Africa and all parts of the world[1-3].The tomato crop is infected with many bacterial, viral, fungal and nematode diseases in addition to insect infestations. Fungal diseases are the determining factor for tomato cultivation, as they are infected with many diseases that represent a challenge for tomato cultivation and production, and there are more than 200 diseases that affect tomatoes around the world and cause great economic losses in production [4].Fusarium wilt of tomato caused by *Fusariumoxysporumf.sp. Lycopersici* is one of the most dangerous, whether in protected or open agriculture worldwide [5] The disease causes losses in the United States of 50-20% and in India 45%, and in severe cases, losses can reach 80% [6-7], and the incidence in Zubair and Safwan farms ranged from 25-40% [8], and in the Dohuk governorate, the incidence reached 70% [9] .The genus *Fusarium* is one of the most important groups of plant pathogenic fungi, which causes great losses to many agricultural crops around the world in various environments and causes diseases to humans in addition to its secretion of toxins. The genus Fusarium is very broad and includes about more than 1600 species subordinate to the genus, some of which are registered in the genebank and others are not registered, but the number is still subject to increase. [10] The fungus has three types of asexual germs 1. Microconidia 2. Macroconidia. 3.Chlamydospore. Which is the resistant phase to environmental conditions

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and can remain for several years in the soil. Identification of the host is considered vital for the onset of infection by releasing secretions from tomato roots that represent a source of carbon that the fungus needs [11] and these materials include multiple sugars and sugars. Amino acids, oleic and aromatic compounds, phenolic compounds, enzymes, vitamins, growth regulators, and other secondary compounds [12] . Fusarium is usually diagnosed phenotypically depending on the form of microconidia, macroconidia, the conidial vein, and the chlamydospores [13] Or by using specialized differential strains or by using molecular inheritance methods.The fungus is known for its genetic diversity and it is difficult to determine *Fusarium* at the species level by relying only on phenotypic traits [14].It was indicated that the TEFα1 region excelled in giving more accurate results in the identification of the fusarium species [15]. indicated that translation by TEF-α1 was very suitable for differentiating Fusarium species. It has been proven that TEFα1 is a useful genetic region for studies of the genetics of Fusarium species, so it was used as an identification tool in the Fusarium –ID database[16].Therefore, this study, which aims to diagnose Fusarium species isolated from tomato plants infected with wilt, came to be molecularly diagnosed to ensure their species

Methodology

1- Isolation and morphology of fungi

In november 2019 tomato plants was infected with wilt with symptomes were seen in Burjisia and Garma regions in Basrah, Iraq. The plants washed carefully with tap water to clean them frome soil and clay then dried on filter paper,the pieces sample werer 0.5-1cm were sterilized with 10% hypoclorite solution then it was planted in sterile Petri dishes containing the sterile PDA medium and the plates were incubated at 25 ° Cfor7 days. After that, fungal growth was purified and microscopically examined to ensure their relevance to Fusarium according to the phenotypic characteristics mentioned in [17]

2-Isolation and molecular diagnosis of fungi

After purification of the fungal isolates, they were grown at 5 days of age on PDA medium at 25 ° C. The growing fungal yarn was scraped over the medium and placed in liquid nitrogen for the purpose of freezing the fungal yarn. After 5 minutes, the fungal yarn was crushed with a ceramic mortar and the samples were placed in Apandroff tubes and DNA was extracted using GeneaidTiawanKitt for fungi and yeasts, according to the protocol attached with the Kitt. The extracted DNA was doubled first by preparing the sample, which was done using the Mastermix dye manufactured by Bioneer and ready in tubes and using the specialized primer TEF-α1 for Fusarium [18] in both directions. 4 µl of DNA sample was added to each fungus at a rate of 2 µl per direction, after which 42 µl of sterile deionized water was added, then the tubes were placed in the Vortex for one minute, after which the samples were entered into the PCR device of Korean origin Bioneer according to the following program mentioned in [19]. Primer pairs and PCR conditions were mentioned in table 1 and 2.

Table 1. Primer pairs sequences

Primer	Sequence 5-3
Forword	ATG GGT AAG GA(A/G) GACAAG AC

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Riverse	GGA(GlA)GT ACC AGT (GlC)AT CAT GTT

Table 2. Primer pairs sequences

PCR condition	**Cycles number**	**°C**	**Time**
Initial Denaturation	1	94°c	5Min
Denaturation	35	94°c	30S
Annealing		59°c	30S
Extension		72°c	1Min
Final extension	1	72°c	7 Min

3-Propagation of Fusarium isolates

The seeds of local millet, *Panicummliaceum* L., were used as a carrier to multiply the isolates of *Fusarium*, as the seeds were washed well with water to remove the soil from them and then soaked in distilled water for 6 hours, after which they were filtered from water by gauze and placed in glass flasks capacity of 250 ml in each 100 beaker Grain seeds were then sterilized with a closed autoclave for 45 minutes, after which they were left to cool down, and then inoculated with 4-5 tablets of the colony of each fungus separately and shaken well to ensure the uniformity of the distribution of the inoculum and incubated at 25 ± 2 ° C for 10-14 days, taking into account its shaking Every 3 days to aerate them and ensure uniform growth of the fungus on the seeds [20].

4-pathogenicity of Fusarium isolates

A mixture of sandy soil and peat moss in a ratio of 1: 2 was prepared and sterilized with formalin, as formalin was added to the soil at a concentration of 6%. Volume: the weight of the soil was placed in plastic bags and added to the appropriate volume of 50 ml per 10 kg of soil. The bags were closed tightly and left for a week afterwards. The bags were opened and the soil was spread on clean plastic and left in the outside atmosphere for three days in order to get rid of the smell of formalin. After that, the soil was distributed in pots of 800 g capacity, and the fungal vaccine was added to each isolate in three replicates at a rate of 10 g vaccine per pot [21] Three days later, six-week-old tomato seedlings were planted with two seedlings for each pot. The pots were left for 6 weeks until the symptoms appeared and the severity of the injury was calculated according to the injury severity scale.

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1 Fading rate 0-24%

2 The percentage of wilt is 25-49%.

3 The percentage of withering is 50-74%

4 The percentage of withering is 75-100%.

Injury severity was calculated according to Mckinney's equation (1923).

$$\text{Injury severity\%} = \frac{\text{Total [Number of Infected Plants} \times \text{Their Pathological Index]}}{\text{Total of the tested plants} \times \text{the highest value of pathological evidence}} \times 100$$

Results

Morphological identification

The phenotypic diagnosis showed the presence of *Fusarium* in all isolates taken from the infected plant parts of tomato plants, as the diagnosis showed the emergence of spores of the fungus, which are microconidia that was unicellular and transparent and in large numbers and fusiform macroconidia, which contains 2-3 cells, as well as the emergence of chlamydospores of the isolate of *F. brachygibbosum* In the dishes and in chains, Picture (3), the color of the fungal mycelium of *F. brachygibbosum* isolate was white in Picture (1), while if the fungal mycelium was white yellowing to cream in *F.acutatum* isolate, picture (2)

Molecular identification

The analysis of the results of the nitrogen base sequences of the studied isolates through the Japanese Genebank database with translation elongation factor showed that the isolates belong to the fungus *F. brachygibbosum* and were given a specific A nuccession number LC529919ADK7 and the other isolate belonged to *F.acutatum*, and a specific Accession number was given to it LC527418ADK23

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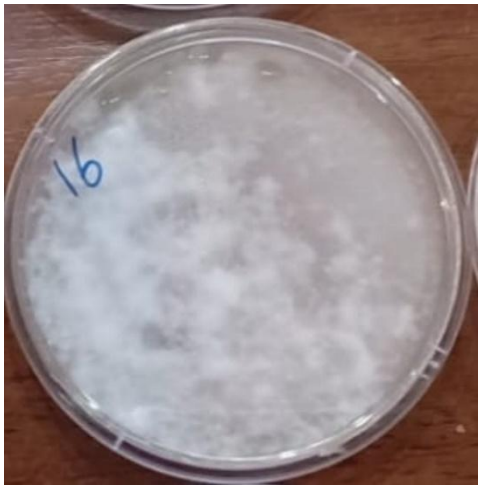

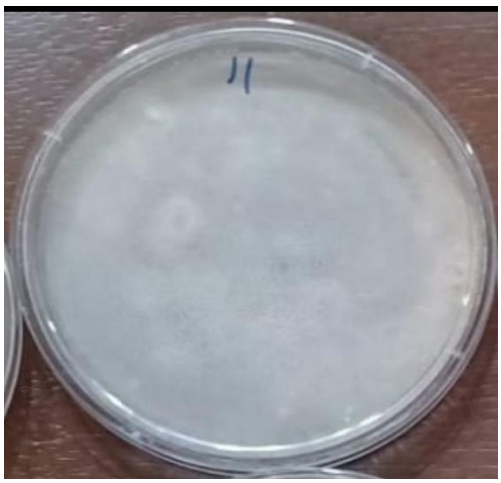

Fig1. *F. brachygibbosum*Fig2. *F. acutatum*

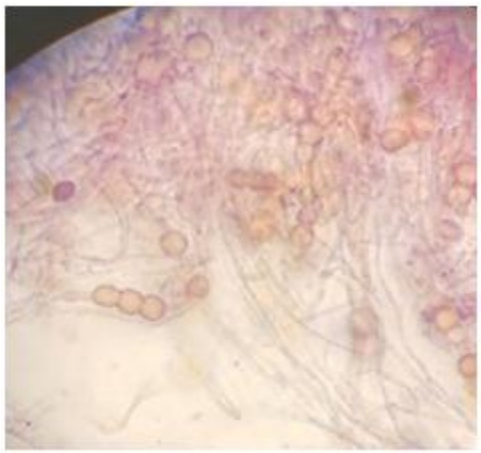

Fig3. Chlamydospores of F.brachygibbosum

Pathogenic ability

A study of the pathogenic potential of *F. brachygibossum* and *F. acutatum* demonstrated the ability of the fungi to cause wilt disease on healthy tomato plants by 80% and 58%, respectively. *F. brachygibbosum* showed an ability to cause wilting represented by coloring in the vessels and yellowing of the leaves, as shown in picture 4. As for the fungus *F. acutatum*, it showed clear symptoms of yellowing on the leaves of healthy tomato plants, as in picture 5, and a slight discoloration in the wooden vessels.

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Fig4.symptoms of *F. brachygibbosum* Fig5.symptomes of F. *acutatum*

Discussions

The recording of the two fungi F.brachygibbosum and F. acutatum the first recored in Iraq and Basrah, The genes responsible for the pathogenic events of the plant [22] and that diagnosis using different diagnostic regions can reveal this genetic variation, including the variation of pathogenicity [23]

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