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Isolate and identify the fungi associated with the roots of infected plants okra using the PCR

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ABSTRACT: This study was conducted in the laboratories of the Plant protection department – Agriculture College /University of Bagdad to isolate and identify the fungi associated with the roots of infected plants okra .Microscopic examination showed that the six genera associated with the roots of the okra plants. , These fungi, Fusarium solani , Rhizoctonia solani , Macrophomina phaseolina , Pythium sp. , Mucor sp. and Aspergillus sp were in varied incidence rates. The highest incidence percentage was 58% for Fusarium solani. The Molecular diagnosis confirmed the isolates belong to the F. solani using primer (TEF-F.S4F / TEF-F.S4R) amplified (680 bp).

INTRODUCTION

Okra is important summer vegetable in Iraq and widespread. They are grown in all regions of the country (Mohammadi and Peter. 1997). okra is opt to attack various fungal diseases Fusarium spp. Rhizoctonia solani and Macrophomina phaseolina ,Aspergillus sulphorus .Chaetomium sp and Phytophthora spp. (Alhei and others .2009).). these fungi are not invisible to humans by virtue of their presence and usually symptoms pathological clearly show the total vegetable infected by plants and after you can eliminate the root (Garrett. 1970 and Agrios. 2005) Plantings okra in each of the province of Baghdad, Babel, Karbala, 14099 and 21264 and 1475 acres, respectively, where production reached in each of the provinces of Baghdad, Babel, Karbala, 37 530 and 32 615 and 667 tons, respectively .PCR
Is a useful method for Fusarium classification.

MATERIALS AND METHODS

Isolating fungi associated with the roots of infected plants okra*.*

The samples were collected from the roots of okra deteriorating from the provinces of Baghdad, Babil, Karbala These samples were obtained from some provinces in the middle and south of Iraq 2013. Samples were cut into 0.5-1cm and washed under running tap water for 30 minute, then surface sterilized in 1% sodium hypochlorite for 2 minute and cultured on Potato dextrose agar (PDA) supplemented with 200 mg\l Tetracycline and incubated at 25±1C° for 7 days, single spore technique was made for each isolate. Isolates were identified to the species level according to their cultural and morphological characteristics The isolation frequency and relative density of genera and species were calculated according to [15] as follows:
Apperance (%)= No.of samples of occurrence
Total No.of samples X100
Frequency (%) = No.of plant segments of species occurrenceTotal No.of segments used X100

Morphological Characteristic

Fungus saved from pipeline development testing on the same center PDA under temperature 25 ± 2 Meh After 7-14 days of growth examined under a microscope and sex morphology on the center PDA either of the fungus to the genus Fusarium (CIA) carnation leaf Agar(Fisher et al. 1982, Leslie and Summerell. 2006) Isolates were identified to the species level according to their cultural and morphological characteristics (Barnett and Hunder. 1972 and Booth. 1977 and Leslie, and Summerell .2006).

Molecular Characteristics
DNA Extraction

Different Fusarium species were grown on PotatoDextrose Agar (PDA) at 25 ± 20C for 3 days. The mycelia grown were harvested and total DNA was extracted using fungal genomic DNA isolation kit from Bioneer Company

Table: 1 primer sequences

Fungus	Primers	Sequence (5 to 3)	pb
F. solani	TEF-F.s4f	-ATCGGCCACGTCGACTCT-3	658
	TEF-F.s4r	-GGCGTCTGTTGATTGTTAGC-3	

Polymerase Chain Reaction

Table: 2 reaction

Materal	Volume
Master Mix	5 µl
Primer forward	2.5 µl
Primer ReveR.se	2.5 µl
DNA	5 µl
Nuclease – Free Water	5 µl
Total	µl 20

Table 3: program set..

No.	Steps	temperature	Time	No. cycle
1	Initial Denaturaion	94	2 min.	1
2	Denaturation	94	30 sec.	35
3	Annealing	58	1 min.	
4	Extension	72	1 min.	
5	Final extension	72	10 min.	1

RESULT AND DISCUSSION

Isolating fungi associated with the roots of infected plants okra

The results shows that the six types of fungal a Fusarium solani and Rhizoctonia solani and Macrophonina phasolina and pythium Mucore and Aspergillus associated with the root systems of infected plants okra.

Table 4

No.	province	fungi	Code
1	Baghdad	F.s+R.s+M.p	F.sB1+R.sB1+M.pB1
2	Baghdad	F.s+R.s+M.	F.sB2+R.sB2+M.pB2
3	Baghdad	F.s+R.s+As	F.sB3+R.sB3+As.B1
4	Baghdad	F.s+R.s+Py	F.sB4+R.sB4+P.B1
5	Baghdad	F.s+Mu	F.sB5+Mu.B1
6	Baghdad	F.s+M.p	F.sB6+M.pB3
7	Baghdad	F.s+Mp	F.s7+M.pB4
8	Baghdad	F.s+R.s+M.p	F.sB8+R.sB5+M.pB5
9	Baghdad	F.s	F.sB9
10	Baghdad	R.s+As+Mu	R.sB6+AS.B2+Mu.B2
11	Babylon	F.s+M.p	F.sH1+M.pH1
12	Babylon	F.s+R.s	F.sH2+R.sH1
13	Babylon	F.s+Mp	F.sH3+M.pH2
14	Babylon	M.p+As	M.pH3+AS.H1
15	Babylon	F.s+R.s	F.sH4+R.sH2
16	Babylon	F.s	F.sH5
17	Babylon	F.s	F.sH6
18	Babylon	F.s	F.sH7
19	Babylon	F.s+R.s	F.sH8+R.sH3
20	Babylon	F.s+Mu	F.sH9+Mu.H1
21	Babylon	F.s+Py	F.sH10+P.H1
22	Babylon	F.s	F.sH11
23	Babylon	F.s+M.p+R.s	F.sH12+R.sH4+M.pH4
24	Babylon	F.s	F.sH13
25	Babylon	F.s+R.s	F.sH14+R.sH5
26	Karbala	F.s+R.s	F.sK1+R.sK1
27	Karbala	F.s+R.s+M.p	F.sK2+R.sK2+M.pK1
28	Karbala	F.s	F.sK3
29	Karbala	M.p	M.pK2
30	Karbala	F.s	F.sK4
31	Karbala	F.s+R.s	F.sK5+R.sK3
32	Karbala	F.s	F.sK6
33	Karbala	F.s	F.sK7

The results show that the frequency of fungus between 3-58% .Fusarium solani highest repeat as 58%, as found in all samples except three samples (10,14,29),followed by the fungus Rhizoctonia solani rate of recurrence was 34%, as was found in 14 samples out of 33 samples and then Macrophomina phasolina came

by repeating amounted to 29%, which is the most presence in selected samples and then all of a fungus pythium and Mucore and Aspergillus by came the existence of low-lying as it was (6, 5 and 3)%, respectively. These findings are consistent with both Haidari (2007) and Karim (2012) as the isolation of each fungus F. solani and R. solani and M. phasolina also) pythium fungus from the roots of infected plants okra ,Shown in

Table 5

NO.	Fungus	isolates	Frequency heigh	Low
1	F.solani	all isolates expect 10,14,29	100%	58%
2	R.solani	1,2,3,4,8,10,12,15,19,22,25,26,27,28	100%	34%
3	M.phaseolina	1,2,6,7,8,11,13,14,22,26,27	100%	29%
4	Pythium	4,21	8%	6%
5	Mucor	5,10,25	6%	5%
6	Aspergillus	3,10,14	6%	3%

Figure 1 . F.s= Fusarium solani / R.s= Rhizoctonia solani / M.p= Macrophomina phaseolina / P=Pythium/Aspergillus As=/ M=Mucoree

Morphological characters

Morphological characters show that the F. solani white to gray and Microconida ,Macroconida in large numbers and are oval-shaped , Chlamydia spores with rough a wall (Both .1971) R. solani colored light brown and vary in the speed of growth and composition of the bodies of the stone with a dark color and intensity of the innate spinning which fork is a right angle and containment mycelium ,formation of barriers in the branches of an area near the emerging area (Carling. 2002 and required 0.2012) .M. phasolina characterize the composition of the stone objects black and marked colony. Pythium growth like cotton and be fast and mildew growth of fungal hyphae thin undivided transparent many minutes forking (Abu Hila.1987

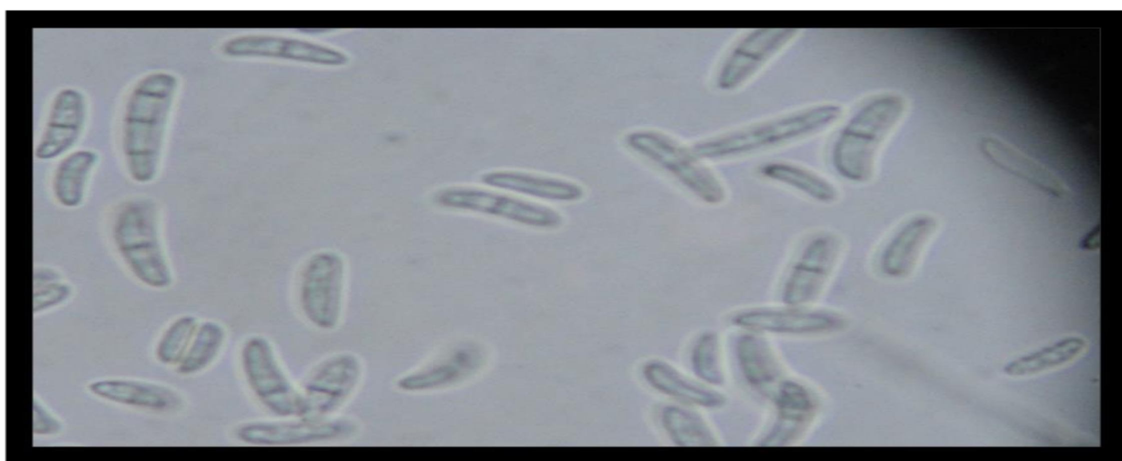
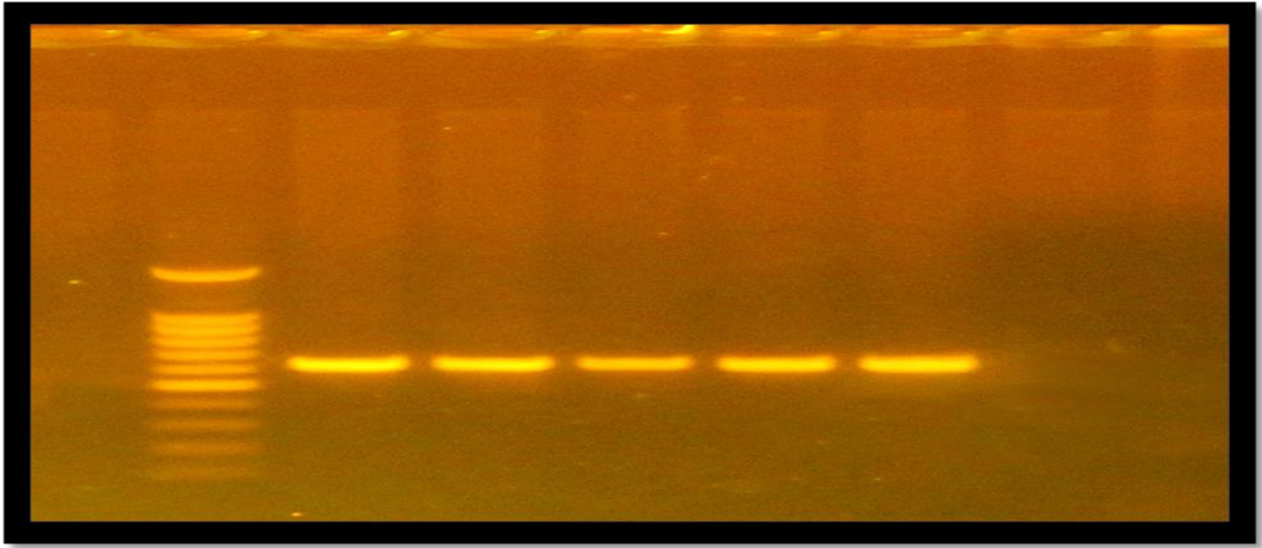
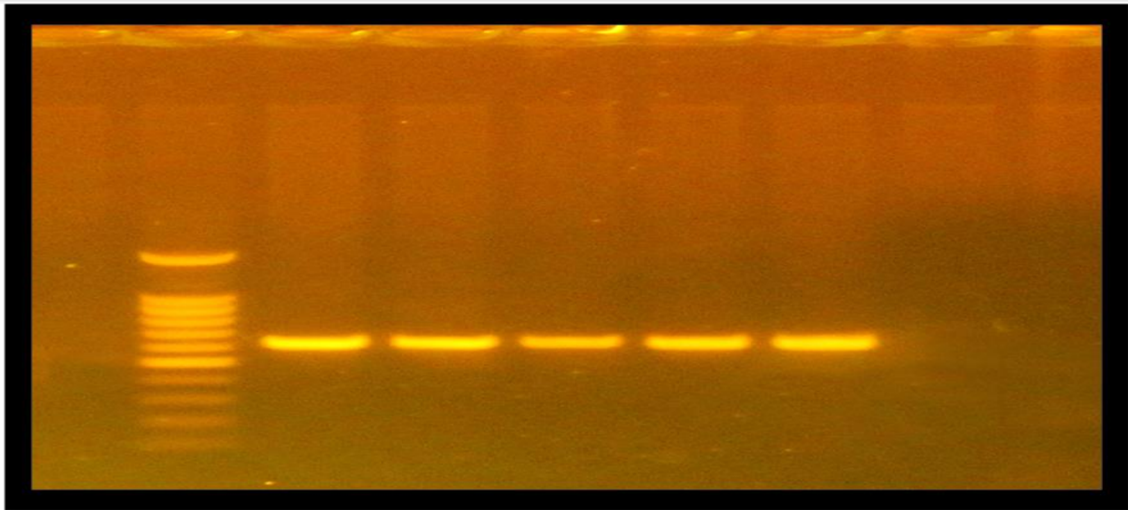


Figure 2. Microscopic characteristics of F. solani Growth of the fungal colony on CLA)

Molecular identification

Fusarium solani is the most frequent among the fungi isolated from the roots of infected okra.PCR product show that the presences all bands to five isolates for F. solani amplified (680 bp). This results agreement with (Arif 2012).





Figute 3. PCR amplification of 680 bp fragment with primer TEF-F.s4F / TEF-F.s4R PCR

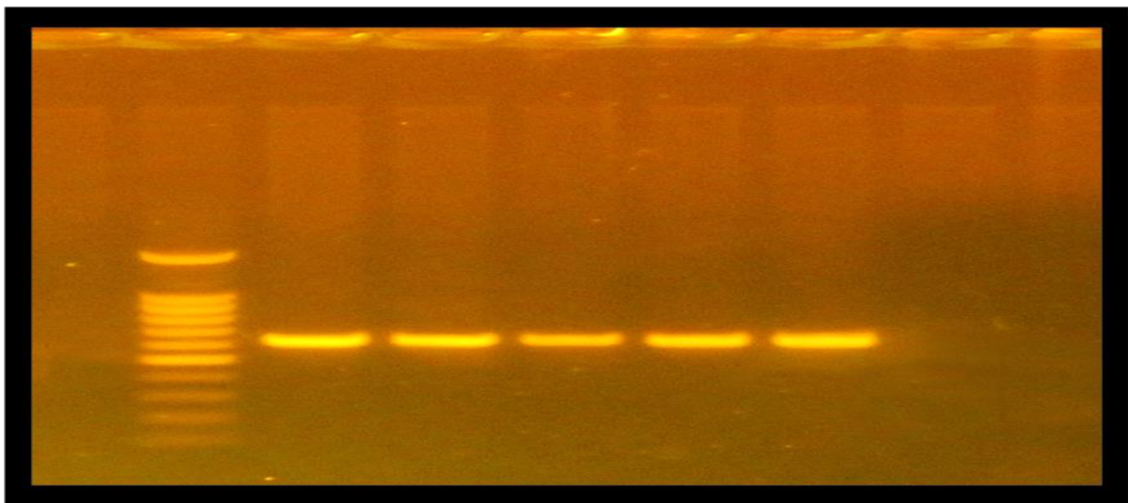
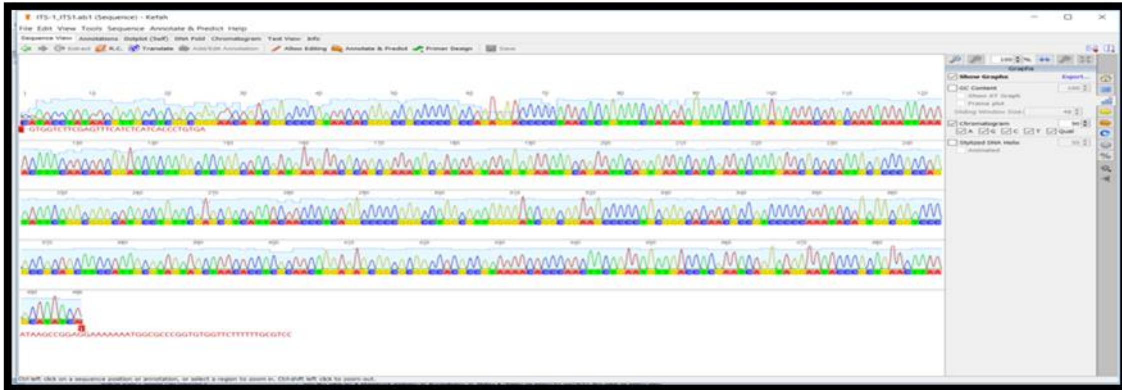
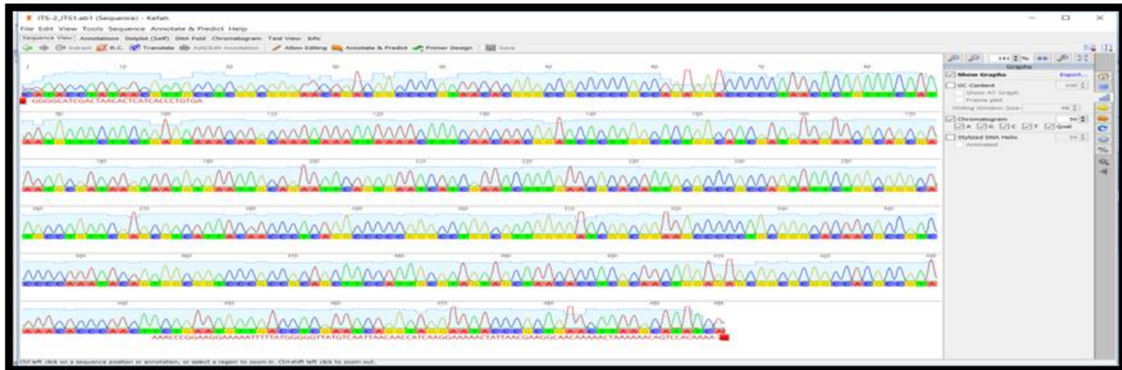
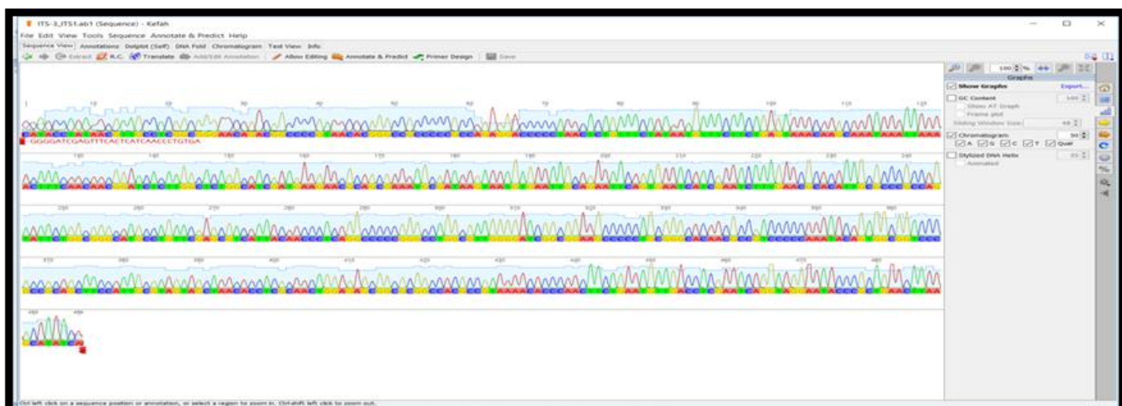


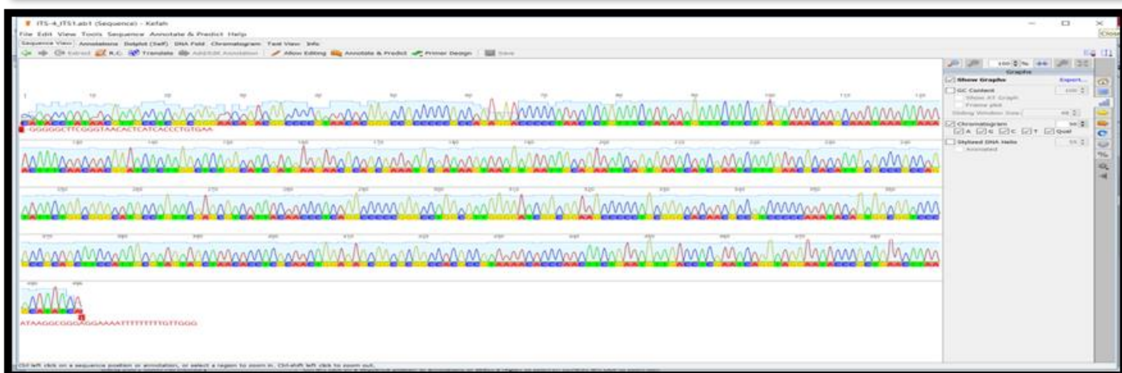
Figure 4. 3-Detrmine of DNA Sequencing

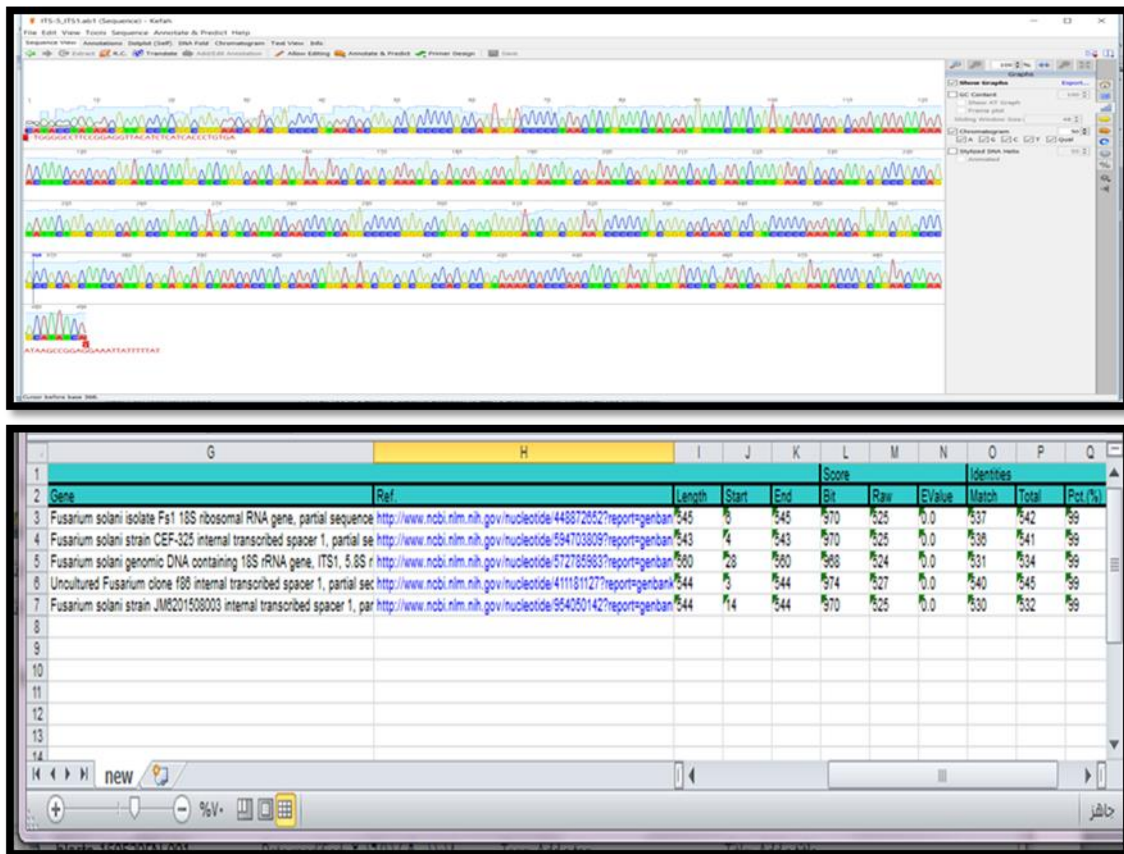
The results showed that Fusarium solani isolated agreement with Sequences company percentage 99% .











Gene	Ref.	Length	Start	End	Score Bit	Score Raw	EValue	Identities Match	Identities Total	Identities Pct.(%)
Fusarium solani isolate Fs1 18S ribosomal RNA gene, partial sequence	http://www.ncbi.nlm.nih.gov/nucleotide/448872652?report=genban	545	6	545	970	525	0.0	537	542	99
Fusarium solani strain CEF-325 internal transcribed spacer 1, partial se	http://www.ncbi.nlm.nih.gov/nucleotide/594703809?report=genban	543	4	543	970	525	0.0	538	541	99
Fusarium solani genomic DNA containing 18S rRNA gene, ITS1, 5.8S r	http://www.ncbi.nlm.nih.gov/nucleotide/572785983?report=genban	560	28	560	968	524	0.0	531	534	99
Uncultured Fusarium clone f86 internal transcribed spacer 1, partial sec	http://www.ncbi.nlm.nih.gov/nucleotide/411181127?report=genbank	544	3	544	974	527	0.0	540	545	99
Fusarium solani strain JM8201508003 internal transcribed spacer 1, par	http://www.ncbi.nlm.nih.gov/nucleotide/954050142?report=genban	544	14	544	970	525	0.0	530	532	99

Figure .5 Sequencing of Fusarium solani isolates

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