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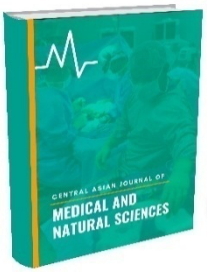

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Antimicrobial Effect of *Fusarium solani* (Endophytic fungi) Extract Associated with *Coriandrum sativum* L. plant

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Abstract: In this work, *Fusarium solani* (an endophytic fungus) was isolated from *Coriandrum sativum* L. healthy leaves. Its molecular identification was based on the ITS1 gene. Effect of endophytic fungal extract on a few pathogenic fungi and bacteria found at Tikrit University. In the current study, *Fusarium solani* was isolated from *C. sativum* leaves and showed 99% identity with genes in NCBI. Some secondary metabolites were detected in the extracts of *F. solani* and *C. sativum* which included total alkaloids, saponins, terpenoids, flavonoids (rutin 100 mg), and phenolic compounds (gallic acid 100mg). *F. solani* showed high total phenolic compounds (179.87 mg/ml) and showed significant activity against 29 isolated fungi belonging to *Candida albicans*, *C.tropicalus, C.parapsilosis, C.krusei, C.kefyr, C.glabrata*, and *C.dubliniensis*. The study showed the highest inhibition diameter of fungal isolation of *C.kefyr* was 39 mm at a concentration of 40 mg. ml and the lowest inhibition diameter was recorded in two fungi *C.glabrata* and *C.dubliniensis* with a diameter of 10.7 mm and a concentration of 20mg. ml. also the result showed that *F. solani* extract given high activity against 14 isolates of bacteria, the highest inhibition diameter of bacteria isolation of *E.faecalis* was 39.4 mm at a concentration of 40mg. ml and the lowest inhibition diameter was recorded in *K.pneumoniae* with a diameter of 10 mm and a concentration of 20mg. ml.

Key words: ITS1 gene, endophytic, *Fusarium solani*, *Coriandrum sativum*, Antimicrbial

1. Introduction:

Fusarium solani (Mart) species complex is a group of filamentous fungi from the family Nectriaceae that belongs to the phylum Ascomycota (Yu et al. 2010). That organism, Nectria haematococca, was an anamorph. It's common in soil and in plant matter. In particular, *Fusarium solani* is linked to plant diseases and is one of the most common causes of root rot in a variety of higher plants. *Fusarium* is a

globally distributed genus that comprises saprophytes and plant pathogens (Latiffah et al. 2010). The nuclear ribosomal internal spacer (ITS) region is the most often utilized genetic marker for the molecular identification of the fungus. It consists of two well-conserved variant spacers named ITS1 and ITS2, as well as the spliced 5.8S ribosomal gene (Schoch et al. 2012). A significant issue that affects health care is the evolution of resistance by pathogenic bacteria and fungi to commercial medications (Aminov 2017), and is now a major global concern , this scenario has been promoted by a wide range of factors, including the widespread and usually incorrect use of antibiotics, unhygienic living conditions, frequent travel, an increase in patients with immunocompromised states, and a delay in the diagnosis of infections (Von et al. 2006).

Exploring new niches and habitats helps with the extensive search for novel, efficient antibacterial agents that are consequently required (Zhao et al. 2011). Several microbial species colonize the inter and intracellular spaces of plant tissues without obviously causing harm and seem to be present with all plants in natural ecosystems. These organisms are known to be endophytic (Hyde et al. 2019). Fungi are endophytic microbes that have a close association with their host plants and can produce substances that encourage vegetative growth, competition, and host defense against pathogens and herbivores (Porras-Alfaro and Bayman 2011). Research on novel medications for application in medicine, industry, and agriculture frequently turns to endophytic fungi (Abdel-Azeem et al. 2019; Wen et al. 2022). They display a vast range of microbial adaptations that have developed in unique and peculiar habitats. Some examples of the advantageous secondary metabolites that these microbes are known to create include polyphenols, terpenes, steroids, quinones, coumarins, phenyl propanoids, phenolic acids, and alkaloids (Mousavi and Karami 2022). One of the possibilities under study is that endophytic fungi have a role in the ability of medicinal plants to produce some secondary metabolites, which is the reason for giving them therapeutic properties (Kaul et al. 2012). An Iraqi medicinal plant known as *Coriandrum sativum* is utilized in folk medicine for treating a variety of diseases such as gastrointestinal syndrome, irritable colon, breathing problems, laryngitis, coryza, and influenza that are not accompanied by sweating (Mobeen et al. 2022).

The current study aimed to identify endophytic fungi by using the ITS region and determine active compounds and the effect of extracts on *Fusarium solani* antifungal and antibacterial activity.

2 Materials and Methods

Salahaldin-Tikrit was the site of the plant samples' collecting. At Tikrit University in Iraq, samples were processed right away by the biology department. In order to conduct the biological and chemical studies, the *Coriandrum sativum* leaves taken from the Tikrit region in September 2022 were put into bags and brought to the lab.

2.1 Isolation of Fungal Entophytes:

To remove the dust that had been suspended on the Coriandrum sativum leaves, water was used to wash them. After that, the leaves were surface sterilized using 70% ethanol for two minutes and 5% sodium hypochlorite solution for five minutes. The leaf fragments were then dried by being placed on filter paper after being cleaned multiple times with sterile water. To isolate internal fungus, potato dextrose agar (PDA) medium (HiMedia, Bombay, India), sealed with parafilm, was utilized. In order for fungal growths to form, 3 pieces of 1 cm in length were scattered across the surface of the sterilized culture media in Petri dishes, which were then placed in a fungus incubator at 28.2 C° (Domsch et al. 1980 ; Schulz et al. 1993).Using morphological characteristics found in the plant segments, fungi growing out of them were removed and identified (Kirk et al. 2001).

2.2 Molecular diagnosis of endophytic fungus :

The CTAB approach described in was used to extract the genomic DNA of the *Fusarium solani* strain (Zhang et al. 1996). According to (White et al. 1990), the ITS domain of rDNA was amplified using the universal primers ITS1 (50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50-

TCCTCCGCTTATTGATATGC-30).Deionized water was used to prepare a final volume of 50 ml. This is how the ITS area amplification was done: After 40 cycles of 94 C for 60 s, 50 C for 60 s, 72 C for 60 s, and a final extension at 72 C for 5 min, the temperature was 95 C for 5 min. Shanghai Sangon Biologic Engineering Technology and Service Co.Ltd. sequenced the PCR products after they had been purified using the Gel Extraction Kit (TianGen, China).

2.3 ITS Sequence and Phylogenetic Analysis: NCBI-BLAST and iTOL were used to sequence the DNA for the ITS gene. The ITS gene sequence was examined online using BLAST of the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST) to analyze evolutionary relationships. According to what was said in (Kumar et al. 2004), the evolutionary connections were estimated using the MEGA version 4.0 tool.

2.4 Quantitative analysis of secondary metabolites:

The secondary metabolites were detected in *Coriandrum sativum* plants and the endophytic fungus *Fusarium solani* by measuring the amounts of total phenol content, total flavonoid content, total phenolic content, total saponin content, and total terpenoid content according to methods in (Lin and Tang 2007 ; Zhu et al. 2010 ; Herrera et al. 2010 ; Liu et al. 2011).

2.5 Antimicrobial Activity of Entophytic Fungi:

The antimicrobial effects of Fusarium solani extract was investigated using 14 pathogenic bacterial species and 29 pathogenic fungal isolates from various clinical sources. The Department of Biology at the University of Tikrit provided these isolates. According to Balouiri et al., (Balouiri et al. 2016), the cross-streak approach was utilized to identify fungus strains' hostile behavior with entophytic fungus strains. Control, 20 mg, 30 mg, and 40 mg doses of F. solani extract were used to measure the widths of the inhibitory zones between the pathogen and the entophytic fungus (Bokhari and Perveen 2012).

3 Results

The current study identified entophytic fungi isolated from *Coriandrum sativum* leaves that showed the type of substitution Gap in location 77, nucleotide G, transversion in two locations 366 and 386, nucleotide C/G and G/T, respectively, the percentage identity is 99% with genes in NCBI, as shown in Table 1.

Table (1): Entophytic fungi Isolated from *Coriandrum sativum* leaves

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Gap Transversion Transversion	77 366 386	G C/G G/T	ID: MZ021578.1	*Fusarium solani* isolate F26 internal transcribed spacer 1, partial sequence	99 %

In this study, the gene sequences for the 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, large subunit ribosomal RNA gene, partial sequence, Sequence ID: MZ021578, and Fusarium solani isolates from leaves of Coriandrum sativum were presented.1,562 words long Number of matches is 1, and the first range is 16 to 444.

3.1 Phylogenetic trees for the identification of *Fusarium solani* :

The current study compared a sample of *Fusarium solani* isolated from leaves of *Coriandrum sativum* after comparing it with the gene bank (NCBI) in submission OP939924 with other isolates from the world region. This research demonstrated the presence of a similarity in gene sequencing rRNA (ITS1) of 99%, as shown in Fig (1). showed the identification of *Fusarium solani* by using gene sequencing

ITS1 in NCBI from 17 countries isolated from multiple sources and a percentage compatibility of 98%–99%.

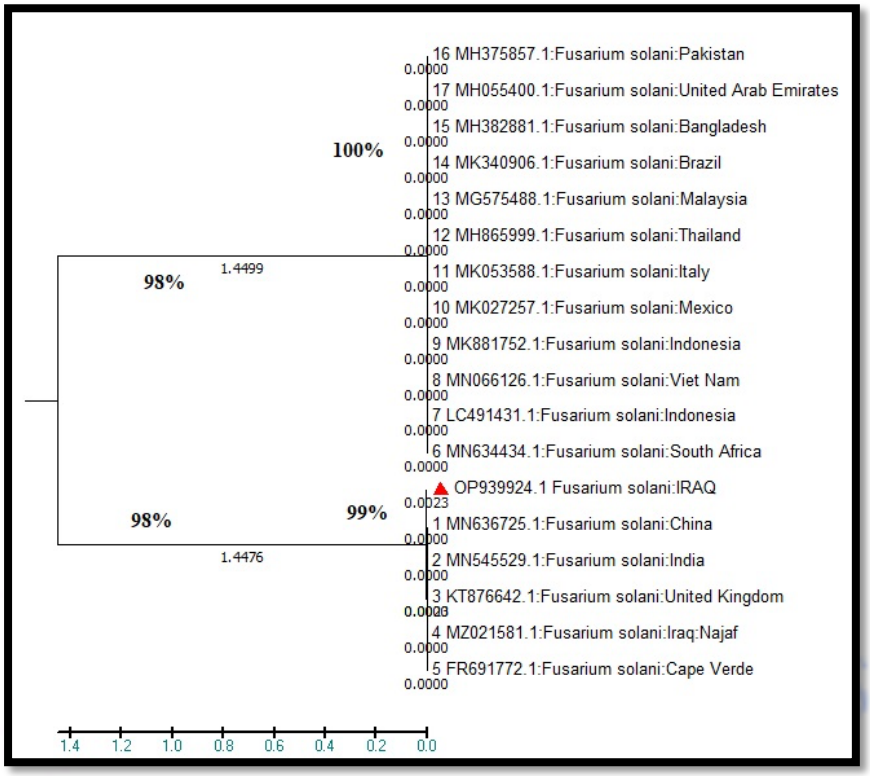


Figure (1): Phylogenetic tree for the gene (ITS1) in *Fusarium solani* isolated from leaves of *Coriandrum sativum*

The UPGMA algorithm was used to infer evolutionary history. The ideal tree, with a branch length total of 2.90220117, is displayed. The branch lengths on the tree are the same unITS as the evolutionary distances used to construct the phylogenetic tree, and the tree is rendered to scale. The evolutionary distances, which are expressed in the unITS of the number of base substitutions per site, were calculated using the Maximum Composite Likelihood technique. There were 18 nucleotide sequences in the study. First, second, third, and noncoding codon locations were covered. Positions with holes and incomplete data were all removed. The final dataset had 426 locations altogether. In MEGA6, evolutionary analyses were carried out (Balouiri et al. 2016).

3.2 Extraction of secondary metabolites:

The results showed five groups of secondary metabolites extracted by chemical methods for two organisms, *Fusarium solani*, isolated from the leaves of *Coriandrum sativum*. These active compounds are alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds. In the extract of *Fusarium solani*, the high percentage of phenolic compounds (gallic acid 100mg) was 179.87%, while the low percentage of alkaloids was 1.25%. The total alkaloids were 2,18% in *C. sativum*, while the total phenolic content in 100 mg of gallic acid was 81.32%. The other active compounds were demonstrated in Table (2).

Table (2): Detection of total secondary metabolites from the extract of *Fusarium solani* isolated from the leaves of *Coriandrum sativum.*

Extract	Total alkloid %	Total Flavonoid % (Rutin 100mg)	Total Phenolic % (Gallic 100mg)	Total Saponin %	Total Terpenoid %
Fusarium solani	1.25	98.93	179. 87	71.18	111.03
Coriandrum sativum	2.18	54.91	81.32	25.56	8.19

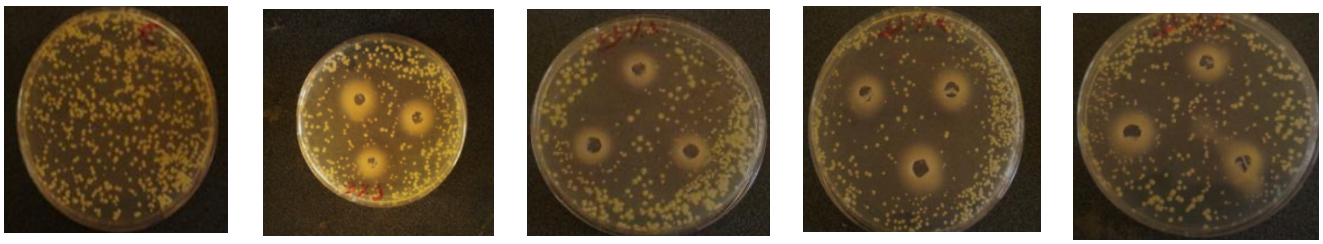
3.3 Antifungal and antibacterial activity of *Fusarium solani*:

The current study tested the extract of *Fusarium solani* isolated from the leaves of *C. sativum* on some fungi and bacteria. The extract of *F. solani* was used in three concentrations (20, 30, and 40 mg/ml) and compared by control to 29 isolated fungi, as shown in Table (3). The high inhibition diameter zone in *C.kefyr* at a concentration of 40 mg/ml was recorded at 39.3 mm, and the low inhibition diameter zone in two species *C.glabrata* and *C.dubliniensis* at a concentration of 20 mg/ml was recorded at 10.7mm, shown at in Table (3), Fig (2). The current study used the extract of *F. solani* isolate from leaves of *C. sativum* on some bacterial pathogen. The extract of *F. solani* was used in three concentrations 20,30 and 40 mg/ml and compared by control towards 14 isolated bacteria as shown in Table (4).

The high inhibition diameter zone in *E.faecalis* at a concentration of 40 mg/ml was recorded at 39.4 mm, and the low inhibition diameter zone in one species, *K.pneumoniae* at a concentration of 20 mg/ml was recorded at 10 mm, as shown in Table (3).

Table (3); Antifungal activity of *Fusarium solani* isolated from the leaves of *Coriandrum sativum* on some isolated fungi.

Yeast isolates	**Control**	**inhibition diameter zone (mm)**		
		20 mg/ml	**30 mg/ml**	**40 mg/ml**
C.albicans	0	16.91±3.65	20.58±3.59	26.92±5.81
C.tropicalus	0	13.83±3.05	20.08±1.89	28.8±4.96
C.parapsilosis	0	17.1±1.87	20.5±4.42	25.47±2.50
C.krusei	0	14.35±2.97	19.09±3.69	28.23±6.48
C.glabrata	0	15.15±4.75	22.75±4.79	33.97±4.63
C.dubliniensis	0	11.55±1.20	15.3±2.40	24.8±4.81



A **B** **C** **D** **E**

Figure (2): Antibacterial activity of *Fusarium solani* isolated from leaves of *Coriandrum sativum*. A. control, B. *E. coli* , C. *Streptococcus pneumonia*, D. *E.faecalis*, E . *K.pneumonia*

Table (4): Antibacterial activity of *Fusarium solani* isolated from the leaves of *Coriandrum sativum* on some bacterial pathogens.

Bacterial species	control	Concentration of extract		
		20 mg/ml	30 mg/ml	40 mg/ml
E. coli	0	17.58±4.63	20.78±5.88	26.08±8.52
Streptococcus pneumoniae	0	20.08±2.01	26.37±5.71	35.75±4.59
E.faecalis	0	18.6±1.35	21.05±3.14	29.5±7.01
K.pneumoniae	0	17.58±5.29	22.45±7.47	29.1±7.83

4 Discussions

Fusarium species live in all environments and cause diseases to many plants, especially weak and non-resistant plants (Palmerand Kommedahl 1969). The fungus *F. solani* was isolated from the leaves of the plant *C. sativum* could be due to the fact that the plant is resistant to the pathogen or that the fungal isolate obtained is non-pathogenic or weakly pathogenic.The plant possesses many secondary metabolite compounds, as indicated by this study. These effective compounds are distinguished by their various therapeutic and pharmacological properties, including being anti-cancer, anti-arthritic, and some of them are anti-microbial (Khan et al. 2018). According to several research, *F. solani* was recognized based on morphological traits and nuclear ribosomal DNA in ITS sequence analysis.

In this study, *F. solani* was isolated for the first time as an endophytic fungus of a plant was isolated from some plants as a type of endophyte *Coriandrum sativum* that does not cause any disease symptoms in the plant . several studies recorded isolated this genus from another plants such as tomato and banana (Vu et al. 2006), *Zea mays* (Bacon et al. 2008), grasses *Sorghum interjectum and Sorghum leiocladum* (Walsh et al. 2010) , *Mentha longifolia* L. (Ibrahim et al. 2018)

Fusarium in contrast to the plant from which it was isolated, the fungal isolate from *F. solani* demonstrated anti-microbial activity against pathogenic fungi and bacteria. This is because the isolate can create a variety of secondary metabolite chemicals in high concentrations. F. oxysporum has the capacity to generate several substances that are regarded as anti-bacterial, including Beauvericin, methicillin, and others, according to a research by (Wang et al. 2011) Another investigation revealed that Javanicin and 3-O-methylfusarubin, two substances with antimycobacterial capabilities produced by *Fusarium* sp. (Kornsakulkarn et al. 2011)[33]. Fusarium species shown a wide range of significant biological activities, including action as biocontrol agents for many plant diseases as well as antibacterial, antiviral, anticancer, antiparasitic, antioxidant, and immunosuppressive properties (Toghueo 2020).

Conclusions

The results of the study showed that *F. solani* is the only fungus that was isolated from *Coriandrum sativum*, and this was confirmed by the results of the molecular diagnosis. The results of the detection of the active compounds showed that both the fungus and the plant have the same effective compounds, but with different concentrations, where the concentrations of the active compounds in the fungus extract were higher than in the plant, and this was reflected in the antimicrobial activity of the fungus, as all concentrations of the fungus extract showed its ability to inhibit the growth of 14 isolates. Pathogenic bacteria and 29 pathological fungal isolates, which shows the promising future of endophytes as one of the available medicinal sources throughout the year.

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