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Diagnosis and Pathogenesis of Fungi F. Oxysporum and R. Solani Associated with Tomato Seed Rot and Seedlings Death in Najaf

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Abstract

This study was conducted to detect fungi associated with seed rot and seedling death of tomato in tomato growing areas in Najaf, where pathogenic fungi were isolated and purified from ten main tomato fields in the study area. The detection and isolation results showed that seed rot and seedling death of tomato in the detection areas were caused by the two pathogenic fungi Rhizoctonia solani and Fusarium oxysporum. The results showed a clear variation between the pathogenic isolates in terms of color and shape of the fungal hyphae, density and growth rate, in addition to a difference in the virulence of the fungus in causing the disease. Although R. solani showed faster growth on PDA medium, F. oxysporum showed higher pathogenicity and always resulted in higher rates of tomato seedling death. The molecular diagnosis and sequencing results showed that the isolated R. solani was completely identical to that those isolated and diagnosed in Pakistan (PQ304388.1), Turkey (PP339772.1), and Serbia (OP546633.1). Similarly, the NCBI data base showed that F. oxysporum in this study had a 100% similarity to those identified in China (PQ482025.1), USA (PQ443573.1), and India (PQ387094.1).

Keywords:

Soil-borne pathogens, fungi, molecular diagnosis, solanum.

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Introduction

Tomato (Solanum lycopersicum L.), like many vegetable crops, is affected by a wide variety of diseases and pests causing significant economic losses, such as fungal, bacterial and viral pathogens, causing plant health deterioration and reduced production (Blancard, 2012). The reduction in crop production caused by plant diseases is estimated to be between 10-40% (Ghorbanpour et al., 2018). Most of the time, Verticillium, Pythium, Rhizoctonia solani, and Fusarium oxysporum are the ones in this group that hurt plants (Gladkov & Gladkova, 2021). As per Calabi-Floody et al., 2018), people think that plant diseases, mostly those caused by fungus pathogens that live in the soil, kill 10 to 40 percent of the food that is grown (Khudhur & Aziz, 2024). These fungi (Behiry et al., 2023) cause seed rot, juvenile death, root rot, and stem base rot, which are all very serious and harmful tomato illnesses. In 2022, Asaf et al. found that the fungi R. solani and F. oxysporum cause seed rot and the death of young plants in many places around the world, including Iraq. It is possible that R. solani and F. oxysporum will kill off about 60% and 10–80% of Iraq's tomato crops each year (Ma et al., 2023; Abdelaziz et al., 2022; Halifu et al., 2019). So, this study was done to find and name the fungi R. solani and F. oxysporum that kill tomato plants and make the seeds rot in places where tomatoes are grown in Najaf (Adriani et al., 2023). To be sure that the fungus could really make people sick in the lab and in the soil for the pots (Radmanović et al., 2018; Al-Jashaami et al., 2024).

Materials and Methods

As part of the study, fungi like R. solani and F. oxysporum were looked for in the field. These fungi cause seed rot and plant death. It helped us find, sort, and check the isolates from the two mushrooms to look at their traits and genes. Scientists also used P.D.A. to test two types of invasive mushrooms, R. solani and F. oxysporum, to see if they could make people sick. As the test with the plastic pot showed, fungi could also hurt things.

Field survey

The field study looked at seven places in Najaf Governorate that are good for growing tomatoes. Ten different plants were chosen at random from each area. In each area, there were infected tomato plants and signs of injury or death in young plants. (Abdelhameed et al., 2024) When the infected plants' stems were cut down the middle, strange things were seen inside them. Plants were used to get fungi that kill seedlings and make seeds rot. The next day, each sample was checked.

Isolation, purification and identification of pathogenic fungi causing tomato seed rot and seedlings death

NaOCl (1%), which is bleach, was used to clean and put back together broken parts. Then, four of these pieces were put into a clean Petri dish that had P.D.A. growth medium in it. The plates were kept at 25 ± 2 °C for two to four days. To clean the mushrooms, a small piece was moved from the colony's edge to the middle of the P.D.A. plate. The plates were kept warm for five to seven days. Among other things, the way the colonies grew and the spores they made helped scientists figure out what kind of fungus was on the plates Spring Lesli (2006).

Pathogenicity of the isolated fungi on tomato seeds on PDA and potting soil

We also checked how well the two fungi could make plants sick by covering millet seeds in 250 ml plastic pots with 150 g of clean soil and 1 g of isolates of F. oxysporum or R. solani (Dewan, 1989; Ahmadi et al.,

2018). They used three of each isolate and made sure the pots had enough water and plastic bags with holes in them to cover them. Another pot was there with clean dirt that wasn't sick. After three days, ten Balqis tomato seeds were put in each pot. For three minutes, the seeds were cleaned lightly with a solution of 1% sodium hypochlorite. After that, pure water was used to wash the seeds and a lot of water was added to them. They should be watered again if they need it. After that, the pots were left alone to grow. The veggie group had the average length and weight. They also found out what made the groups of fresh and dry plants different. The 4-degree pathological scale was used to figure out how bad the sickness was. The trees are safe if they get a score of 0. Level 1: The seeds aren't alive, so they can't grow. Grade 2: Plants wilt for a short time. The third graders are having trouble with their tomato plants. To figure out what part of the virus was bad, the McKinney, (1923)

Molecular Diagnosis of Fusarium and Rhizoctonia Isolates using PCR Technology

Fusarium and Rhizoctonia were found to be the fungi that caused tomato seeds to rot and plants to die. This was proven by genetic tests. The most infectious types of Rhizoctonia and Fusarium mushrooms were used to get DNA for this study. The method came with an extract kit that was bought from Favorgen Company in Taiwan, China. The PCR was done with a kit from the South Korean company iNtRoN called Maxime PCR PreMix (i-Taq), Cat. No. 25026. We used 20µl of DNA, 1µl of each forward starter (TCCTCCGCTTATTGATATGC: ITS4) and revers GGAAGTAAAAGTCGTAACAAGG: TS5 (White et al., 1990), and 1µl of DNA that had already been taken out. The manufacturer's tube was filled with 20 µl of water that doesn't contain nuclease.

More copies of the DNA from Rhizoctonia and Fusarium samples were made with a PCR method. DNA was broken down at 95 °C for 5 minutes to start the process. Following 35 cycles, the DNA was finally broken down for 40 seconds at 95 °C, the primers were annealed for 40 seconds at 55 °C, the PCR product was first stretched for 1 minute at 72 °C, and finally it was stretched for 5 minutes at 72 °C to finish (Zhang et al., 2012). The ITS1 and ITS4 results were sent to the Korean company Macrogen along with PCR amplicons so that they could find out the nucleotide sequence of the DNA before and after it was amplified. This helped us figure out what was wrong. The Basic Local Alignment Search Tool (BLAST) was used to look at patterns of nitrogenous bases. After that, the results were matched with mushroom data from the National Centre for Biotechnology Information (NCBI).

Experimental Design and Statistical Analysis

The tests were done with Complete Randomised Design (C.R.D.), and there were three sets. We used Genstat, a tool for statistical analysis, and Excel, a tool for making shapes, to look at the numbers. To see if there was a change between the averages, the LSD and a 5% chance level were used (Al-Rawi and Khalaf Allah, 2000).

Results and Discussion

The results of the field survey (Figure 1) in seven scattered tomato fields belonging to the Al-Haidariyah Agriculture Division (H1, H2, H3 and H4) and the Central Agriculture Division (C1, C2 and C3) in Najaf Governorate showed the spread of seed rot disease and death of tomato seedlings in all survey fields with different infection rates, as the infection percentage ranged from 5% to 60%. Al-Haidariyah area recorded the highest infection rates, which showed an infection rate that did not fall below 26% in the H4 field, while the highest infection rate was recorded in the H1 field at 60% compared to that recorded in the Central Agriculture fields, where the lowest infection rate was 5% in the C3 field and the highest infection rate was 44% in the C2 field.

The results indicate the presence and prevalence of seedling death associated with tomato cultivation, which may be attributed in general to the fact that tomato cultivation in these areas is considered a uniform, repeated crop, which may allow the same pathogenic fungi to remain and spread between fields in the same area (Ma et al., 2023). In addition, the possibility of infection appearing in such fields due to repeated cultivation with the same host (tomato) or other plants belonging to the same family, which encourages the survival of the same pathogens associated with the crop (Amran, 2021), which may lead to the accumulation of fungal inoculum causing the infection and its survival in the soil until the weather conditions and the presence of the host are suitable (Awad, 2016 and Amran, 2021). The infection rate, which was recorded at high levels in most cases and the spread of infection in all sites, may also be attributed to the ability of pathogenic fungi to parasitize a wide range of plant hosts (Bertoldo et al., 2015).

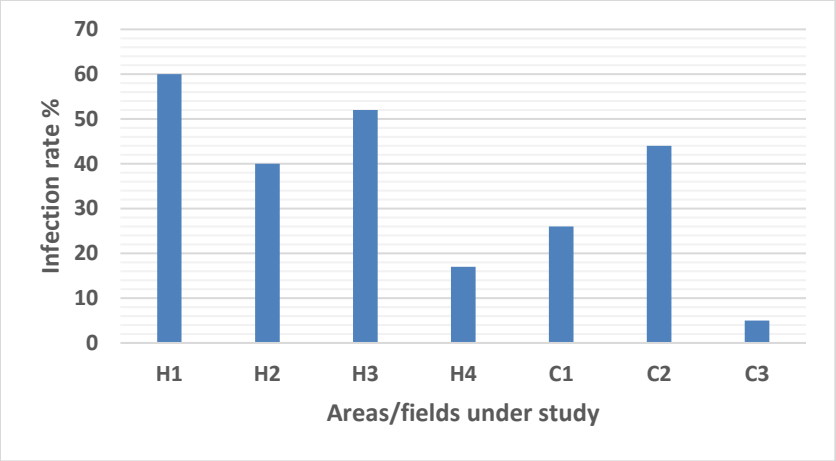


Figure 1. Infection rate (%) of tomato seed rot and seedlings death in different areas in Najaf Governorate for the 2023 winter growing season

Isolation and Identification of Fungi Causing Seed Rot and Death of Tomato Seedlings

A microscope was used to look at PDA growth plates that had been taken apart. They saw Rhizoctonia solani and Fusarium oxysporum, which are harmful fungi, on them. The first match was made based on how the fungus's mycelium grew. Five kinds of R. solani were grown with P.D.A. There were groups of light brown, medium brown, and dark brown. There was a lot of mould present. In general, the vegetative mycelium of R. solani showed the presence of multiple cells branching at right angles to the main hyphae with slight constriction at the points of contact, which are characteristic features of the fungus. The fungus is also characterized by rapid growth on the nutrient medium (Cubeta &Vilgalys, 1997).

As for Fusarium spp., it was found in two isolates on the P.D.A. medium, showing varying colors from dark pink to light. The dense growth of mycelium was observed in the form of cottony growth (Figure 2 and 3). By looking at the isolates' shape and size through a microscope, it was determined that they were from the fungus Fusarium spp. This fungus has hyphae that are split into several pieces. The large conidia are either crescent-shaped with curved ends (Macroconidia) or oval-shaped with round ends (Microconidia). Chlamydospores were also observed in a spherical appearance, large in size and with a thick, dark-colored wall (Leslie and Summerell, 2006).

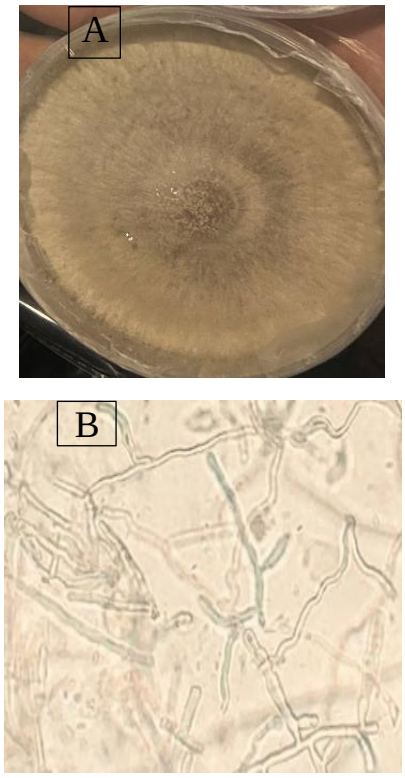
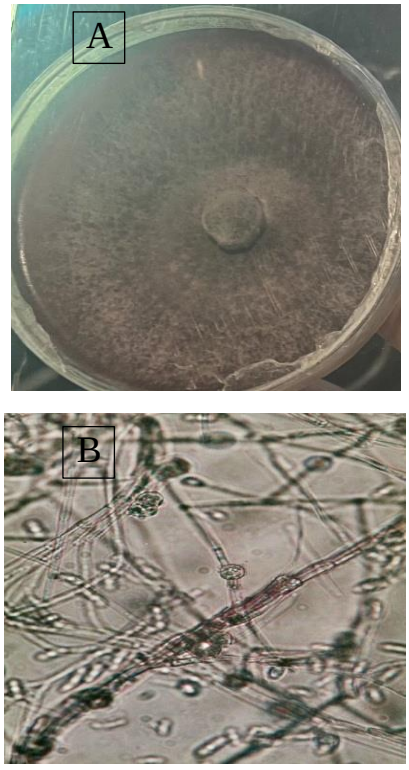


Figure 2. Rhizoctonia solani isolate R2, fungal colony (A), and fungal mycelium (B)



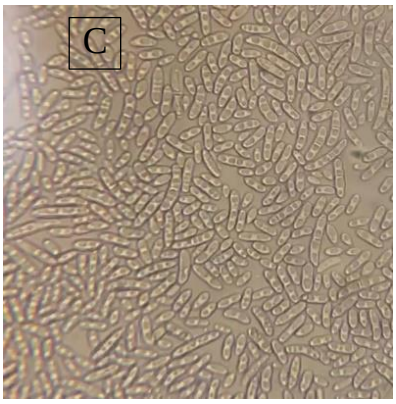


Figure 3. F2 colony of Fusarium sp. (A), Chlamydospores (B), presence of Macroconidia and Microconidia of different shapes and sizes (C)

Effect of R. solani and F. oxysporum Isolates on Tomato Seeds and Seedlings in Petri Dishes and Plastic Pots Experiments

The results of the pathogenicity of R. solani and F. oxysporum isolates on tomato seeds and seedlings showed different pathogenicity on seed germination and growth indicators of tomato seedlings (Table 1). The results indicate that R. solani isolates differed in their pattern of effect on germination indicators in Petri dishes and plastic pots, where isolate R2 showed the highest pathogenicity with a significant difference from the remaining isolates in germination percentage and seedling death percentage, while the difference was slight compared to isolate R5 in Petri dishes, while the significant difference between the same two isolates was large when tested in the pot experiment. These results were also accompanied by the percentage of seed rot, which recorded the highest value of 38.70% in plates and 83.40% in pots for isolate R2, which did not differ from R1, while the rest of the isolates recorded a percentage of rot from 10.70%-21.40% in plates, while it differed significantly from all isolates in the pot experiment. Also, isolate R2 showed the highest ability in the infection severity index of all isolates, as it recorded a percentage of 79.6% compared to the rest of the isolates, which showed an infection severity ranging from 26.30-63.30% in Petri dishes, while the significant difference between the same two isolates was large when tested in the pot experiment.

Table1. Pathogenicity of the fungus Rhizoctonia solani, which causes seed rot and death of tomato seedlings, on PDA culture medium and potting soil

Treatments *R. solani* isolates	Germination rate %		Seedlings death %		Seed rot %		Infection severity %	
	PDA	Pot	PDA	Pot	PDA	Pot	PDA	Pot
Control	100	100	0.00	0.00	0.00	0.00	0.00	0.00
R1	61.30	60.00	13.00	20.00	38.70	40.00	26.30	45.20
R2	69.30	16.60	66.60	10.00	30.70	83.40	79.60	80.40
R3	78.60	76.30	18.00	33.00	21.40	23.70	32.60	18.20
R4	89.30	70.60	26.60	24.00	10.70	29.40	45.00	27.30
R5	82.00	73.60	56.00	17.00	18.00	26.40	63.30	36.60
L.S.D. (P≤0.05)	16.36	34.51	18.91	15.58	25.7	24.37		

*Values are means of three replications

On the other hand, the two Fusarium isolates showed a difference in the level of effect on the previous criteria (tomato seeds and seedlings), as it was found that the F2 isolate showed a higher pathogenicity than the other isolate, as it led to the lowest percentage of seed germination and a higher percentage of seedling

death compared to F1. It also recorded the highest rate of rotten seeds 23.40%. This is also consistent with the percentage of infection severity, which recorded the highest value 45.20% in the F2 isolate compared to the infection severity 32.0% in the F1 isolate, while no infection or seed rot or seedling death appeared in any of the comparison treatments. The results of the culture plates experiment were consistent with the pot experiment, as the results showed that the F2 isolate was superior in pathogenicity to the F1 isolate, as it recorded the highest rate of rotten seeds, 43.40%. This is also consistent with the percentage of infection severity, which recorded the highest value of 54% in the F2 isolate, compared to the infection severity of 28.33 in the F1 isolate. (Table2).

Table 2. Pathogenicity of Fusarium spp., which causes seed rot and death of tomato seedlings in PDA medium and potting soil

Treatments *F.oxysporum* isolates	Germination rate %		Seedlings death %		Seed rot %		Infection severity %	
	PDA	Pot	PDA	Pot	PDA	Pot	PDA	Pot
Control	100	100	0.00	0.00	0.00	0.00	0.00	0.00
R1	76.60	73.00	6.60	23.00	23.40	27.00	45.20	28.33
R2	86.60	56.60	6.60	30.00	13.40	43.40	32.00	52.40
L.S.D. (P≤0.05)	21.17	4.382	9.77	9.14	14.79	11.24		

*Values are means of three replications

Seed rot and seedling death in general are caused by the effect of fungal hyphae of the pathogen that penetrate the plant tissue, causing the affected tissue to turn brown. Acids and poisons made by fumes can hurt the roots of young plants or seeds. A lot of people think this is what made it happen. Roman-Aviles et al., (2003) and Stack et al., (2017) say that the infection could kill the seed cells and stop them from growing. The trees could also die. Researchers discovered that various types of the fungus R. solani had various effects on the growth and flowering times of seeds. So, this backs up the idea that not all isolates were bad for plants. This is probably because genes and the surroundings have changed. The illnesses in the samples were all different, and some were worse than others. They were sometimes funny and sometimes not so funny. There is a chance that different isolates will have different genetic differences and release different amounts of hydrolytic enzymes and toxins. These enzymes and toxins might kill seeds or make them rot before they can grow. Bigger plants are more likely to have this happen (Rush et al., 1994; Ogoshi, 1996; Chen et al., 2016). This also means that the two types of Fusarium are not both very dangerous. That one isolate hurts seeds by making them rot and not letting them grow. Another isolate kills or weakens seeds before they grow, which makes it take longer for them to spread. This study backs up what other studies have found: A study by (Mwaniki et al., 2016) says that fusarium species are some of the most important fungi that kill plants. Chełkowski, (2010) A virus called fusarium can make plants sick in a number of different ways. Perincherry et al. (2019) say this is because different fungi make different amounts and kinds of chemicals, toxins, or enzymes that weaken the plant's defenses. They said in 2010 that isolates of Fusarium spp. might be able to attack plants in new and different ways. Their genes may have changed, which could have changed how they make things like enzymes that break down things and toxins that help them get into the cells of the plant host and attack it.

Molecular Diagnosis

People checked to see if the DNA from the mushrooms matched with information from the National Centre for Biotechnology Information (NCBI). Isolate R2 comes from the fungus R. solani, as shown in Figure 4, and isolate F2 comes from the fungus F. oxysporum. We looked at the nucleotide sequences and saw that the R. solani fungus's double-stranded gene area sequence was the same as sequences from other samples of the

same fungus that had already been sent to the NCBI. Some of these isolates were found and named in Serbia (OP546633.1), Pakistan (PQ304388.1), and Turkey (PP339772.1). The analysis results showed that the isolation of F. oxysporum and other isolates of the same fungus previously registered in the

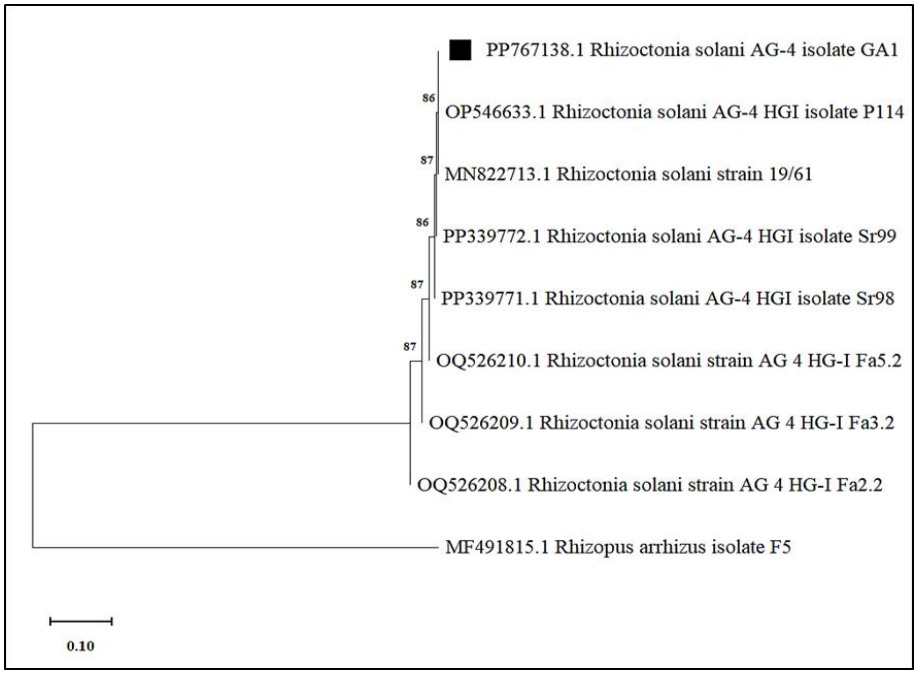


Figure 4. The phylogenetic tree of Rhizoctonia solani AG-4 isolate GA1 (marked with a black box) was constructed using the ITS-rDNA sequences and the sequences of global strains of the same pathogen obtained from the GenBank data repository. Genetic distances were calculated using the neighbor-joining method.

There are a lot of them. The National Centre for Biotechnology Information (NCBI) got all of them. These are the ones that were found and called in India (PQ387094.1), China (PQ482025.1), and the US (PQ443573.1). The isolates of R. solani and F. oxysporum that we got from this study were put into the National Centre for Biotechnology Information (NCBI) under the ticket numbers PP767138.1 and PP911388.1. As you can see in Figure 5, the genetic tree for the isolate target fungus T. longibrachiatum is marked with a star.4. The tree was made from parts of its nitrogenous roots in the ITS-rDNA region. From the GenBank data set, they were also used to make sequences of different types of the same dangerous fungus found around the world. It was 99% the same as other isolates of the same fungus that the NCBI already had on file. Some of these isolates were found and named in India (JN039070.1) and China (KX357838.1).

The R. solani that was found was the same as the ones that were named in Turkey (PP339772.1), Pakistan (PQ304388.1), and Serbia (OP546633.1). This was shown by genotyping and sequencing. The NCBI database also showed that the type of F. oxysporum found in this study was the same as types found in China (PQ482025.1), the USA (PQ443573.1), and India (PQ387094.1). At the National Centre for Biotechnology Information (NCBI), we gave the R. solani and F. oxysporum isolates we got for this study the file numbers PP767138.1 and PP911388.1.

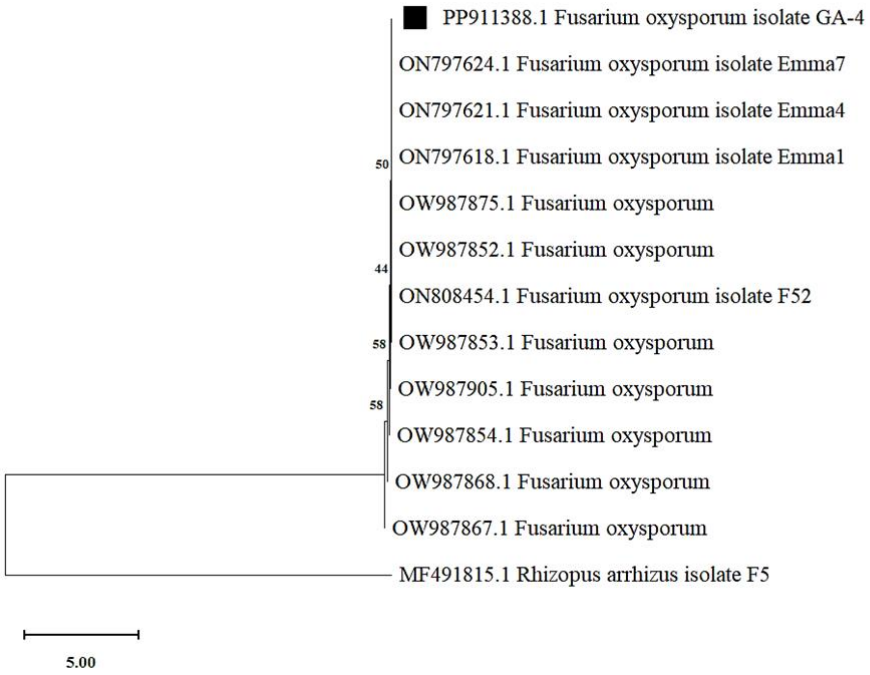


Figure5. The phylogenetic tree of Fusarium oxysporum isolate GA4 (marked with a black box) was constructed using the ITS-rDNA sequences and the sequences of global strains of the same pathogen obtained from the GenBank data repository. Genetic distances were calculated using the neighbor-joining method.

You need to get the ITS gene area right if you want to tell the difference between mushrooms species or strains from different species. It also helps us figure out the ancestral link (Bedine Boat et al., 2020; Matas-Baca et al., 2022). Every living thing has a different ITS gene area. One thing that is different is the deoxyribonucleic acid (rDNA). It is now much easier to tell the difference between living things like Fusarium spp., Trichoderma spp., R. solani, Cladosporium spp., Alternaria spp., and Pythium spp. (Al-Fadhal et al., 2018; Al-Abedy et al., 2018; Al-Sharmani et al., 2019; Al-Abedy et al., 2020; Sebumpan et al., 2022).

Conclusion

In this study, findings showed that the pathogenic isolate of R. solani had faster growth on PDA medium than that recorded in case of F. oxysporum. The latter had much higher pathogenicity than R. solani on tomato seeds and seedling. The molecular diagnosis and sequencing revealed that the R. solani pathogenic isolate was identical to those recorded in Pakistan (PQ304388.1), Turkey (PP339772.1), and Serbia (OP546633.1). Similarly, the NCBI data base showed that F. oxysporum pathogenic isolate had a 100% similarity to those identified in China (PQ482025.1), USA (PQ443573.1), and India (PQ387094.1).

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