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المجلة العراقية لبحوث السوق
وحماية المستهلك

MOLECULAR DIAGNOSIS OF FUNGI CAUSING MELON *CUCUMIS MELO* L. ROOT ROT DISEASE, AND TESTING THE EFFICIENCY OF K_2HPO_4 AND TANNIC ACID IN INHIBITING THE FUNGUS *RHIZOCTONIA SOLANI* IN VITRO

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ABSTRACT

This study was conducted Plant diseases laboratory in the agricultural protection Directorate / Ministry of Agriculture for the purpose of molecularly diagnosing fungal isolates obtained by isolating them from different areas of melon *Cucumis melo* L. growing fields in Iraq and testing the chemical factors K_2HPO_4 and Tannic acid in inhibiting the growth of the fungus. The most pathogenic *R. solani* in the laboratory. The phenotypic diagnosis was confirmed by molecular diagnosis of isolates F8, F19, F23, F28, and F34 through polymerase chain reaction (PCR) technology for DNA amplification and nucleotide sequencing of the nitrogenous bases of the genetic material of the isolates. The electrophoresis results showed that bands with a molecular weight between 500 and 750bp were obtained, and the results of the nucleotide sequences of the nitrogenous bases of the genetic material of the isolates were F8 and F34, *R. solani*, F19, *Fusarium oxysporium f.sp. ciceris,* F23 *Macrophomina phaseolina* and semi fungs F28 *Pythium aphanidermatum* and have been registered in NCBI GenBank (National Center Biotechnology Information) under Accession numbers PP342522.1 (F8), PP342523.1 (F34), PP342524.1 (F19), PP342526. .1 (F23) and PP342527.1 (F28), and were compared with closely related global isolates recovered from GenBank. The isolates F8 and F34, *R. solani*, F19 is *Fusarium oxysporium f.sp. ciceris* and F28 *Pythium aphanidermatum* first registration in NCBI on Melon in Iraq. The results of testing different concentrations of K_2HPO_4 and Tannic acid showed that they inhibited the growth of the fungus *R. solani*. The concentrations were 10 g.100 ml^{-1} and 1.5 g.100 ml^{-1}, respectively, completely inhibited the growth of the fungal colony. with a significant difference from the comparison treatment for both agents. It was noted that as the concentration of K_2HPO_4 and Tannic acid increased, the percentage of inhibition increased.

Keywords: Melon, Root Rot, *Rhizoctonia solani*, K_2HPO_4, tannic acid.

* **This article is taken from the first researcher's master's thesis.**

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التشخيص الجزيئي لفطريات مرض تعفن جذور البطيخ *Cucumis melo* L. واختبار كفاءة K_2HPO_4 و Tannic acid في تثبيط الفطر *Rhizoctonia solani* مختبريا

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الخلاصة

اجريت هذه الدراسة في مختبر الامراض النباتية في دائرو وقاية المزروعات/ وزارة الزراعة لغرض تشخيص خمسة عزلات فطرية جزيئيا تم الحصول عليها من خلال عزلها من مناطق مختلفة من حقول زراعة البطيخ *Cucumis melo* L. في العراق، واختبار العوامل الكيميائية K_2HPO_4 و Tannic acid في تثبيط نمو الفطر الاشد امراضية *R. solani* في المختبر. تم تأكيد التشخيص المظهري بالتشخيص الجزيئي للعزلات F8، F19، F23، F28، F34 من خلال تقنية تفاعل البلمرة المتسلسل (PCR) لتضاعف الحامض النووي والتتابع النيوكليوتيدي للقواعد النتروجينية للمادة الوراثية للعزلات. بينت نتائج الترحيل الكهربائي الحصول على حزم بوزن جزيئي مابين 500و bp750 ونتائج التتابع النيوكليوتيدي للقواعد النتروجينية للمادة الوراثية للعزلات على انها F8 و F34 *Rizoctonia solani* ، F19 *Fusarium oxysporium f.sp. ciceris*، F23 *Macrophomina phaseolina* و F28 *Pythium aphanidermatum* وتم تسجيلها في بنك الجينات NCBI (National Center Biotechnology Information) تحت ارقام الانضمام (Accession numbers) PP342522.1(F8)، PP342523.1 (F34)، PP342524.1 (F19)، PP342526.1 (F23) و PP342527.1 (F28)، وتمت مقارنتها مع العزلات العالمية القريبة منها والمسترجعة من بنك الجينات. وتعد العزلات F8 و F34 *Rizoctonia solani*، F19 *Fusarium oxysporium f.sp. ciceris* و F28 *Pythium aphanidermatum* التسجيل الاول في بنك الجينات على نبات البطيخ في العراق. بينت نتائج اختبار تراكيز مختلفة من الـ K_2HPO_4 و Tannic acid في تثبيط نمو الفطر *R. solani* وكانت التراكيز 10غم.100مل$^{-1}$ و 1.5غم.100مل$^{-1}$ على التتابع قد منعت نمو المستعمرة الفطرية بالكامل وبفارق معنوي عن معاملة المقارنة لكلا العاملين، ولوحظ انه كلما زاد التركيز لـ K_2HPO_4 وTannic acid ازدادت النسبة المئوية للتثبيط.

الكلمات المفتاحية: البطيخ ، تعفن الجذور، *Rhizoctonia solani*، K_2HPO_4 و حامض التانيك.

INTRODUCTION

Melon, *Cucumis melo* L., is an important economic crop that belongs to the Cucurbitaceae family **(Zhuo *et al*., 2024)**. Melon is grown in wide areas of the world, including temperate, tropical, and tropical regions **(Napolitano *et al*., 2020)**, and its global production in 2022 is estimated at approximately 236 million dollar **(FAO, 2023)**. The cultivated area in 2021 of the Melon in Iraq reached 15.77 thousand hectares and productivity was 13.011 tons/ha **(Arab Organization for Agricultural Development, 2022)**.

Melon crop is susceptible to infection with many pathogens, like any other crop. Among these pathogens are soil-borne fungi that cause root rot diseases, which are among the determinants of Melon production in the world, including the fungi *Fusarium* spp., *Macrophomina phaseolina, Rhizoctonia solani* and *Pythium. aphanidermatum* **(Infantino *et al*., 2004; Jabr & Al-Jubouri, 2014; Matloob & Al-Amri, 2017; Hashim *et al*., 2017; Silvia *et al*., 2017; Erper *et al*., 2022; Al-Enezi & Jamil, 2023)**, and the vascular wilt disease caused by the fungus *Fusarium oxysporum f.sp. melonis* **(Al-Khazraji & Ismail, 2021)** Pathogenic soil fungi such as *Fusarium solani, Fusarium oxysporium, Fusarium equiseti* and *R. solani* also

infect orchard trees such as citrus and palm trees, leguminous crops such as mung beans, and field crops such as maize, as well as ornamental plants **(Al-Juboory & Juber, 2011; Al-Karboli & Kuthair, 2016; Al-Saad *et al*., 2018; Al-Juboory *et al.,* 2020; Yasir & Almaliky, 2023**). Previous studies reported that the main pathogens of root rot and wilt disease of watermelon, cucumber, tomato, eggplant and pepper are *F. oxysporium* ، *F. solani* ، *M. phaseolina* ،*R. solani* و *Sclerotinia Sclerotiorum* **(Al-Juboory *et al*., 2016; Hassan *et al*., 2018 ; Abdullah & Al-Juboory, 2020; Hussein *et al*., 2022 ; Al-Juboory & Al-Kubaisei, 2023; AL-Aamel & AL-Maliky, 2023)**.It also causes Root rot and seedling drop disease for a number of other agricultural crops, including beans, sesame, strawberries, and Zinnia elegans Jasg **(Juber *et al.,* 2014; Juber *et al*., 2016; Saeed & Jabr, 2017; Farhan & Al-Juboory, 2018, ; Al-Juboory *et al*., 2018; Abd & Al-Juboury 2020).**

Najem & Kareem (2018) recorded the fungus *Monosporascus cannonballus* as one of the causes of root rot disease and deterioration of melon plants for the first time in Iraq in 2017, and the fungi *F. suttonianum* and *F. falciforme* were also isolated as causes of root rot disease of melon plants in its growing areas in Brazil **(Silva *et al*., 2023)**.

Despite the high effectiveness and ease of using chemical pesticides in control, they cause environmental pollution and harm to human health, as well as the accumulation of these chemicals in the food chain, their high cost, and the emergence of resistant strains of pathogens to these pesticides **(Barak, 2017; Seong *et al.,* 2017)**. Therefore, the approach was to find alternative and environmentally friendly methods in order to reduce environmental pollution caused by excessive use of chemical pesticides, as studies have tended to use biotic and abiotic factors to reduce the effect of pathogens present in the soil and as good alternatives to chemical control and environmentally friendly **(Ahmed *et al*., 2022; Naser AL- Isawi, 2022)**. Among the chemical factors are potassium salts such as K_2HPO_4, which lead to a reduction in the incidence of diseases caused by soil fungi, including damping-off disease, root rot, and wilting in beet and soybean plants **(Abdel-Monaim *et al*., 2015; Al-Mayahi & Hassan, 2021)**. It was found that K_2HPO_4 and potassium bicarbonate $KHCO_3$ have an inhibitory role on soil fungi that cause root rot and wilting of tomato plants in the laboratory, including *F. oxysporium, F. solani, R. solani, Pythium aphanidermatum* and *Verticillium dahlia*, as well as leading to a reduction in disease incidence under greenhouse conditions **(Jabnoun- Khiareddine *et al.,* 2016)**, and another chemical agent is tannic acid, which is considered an environmentally safe substance. It is one of the tannins from the phenolic acid group and consists of a central glucose unit and ten gallic acid molecules linked to it **(Aelenei *et al.,* 2009)**, and its efficiency has been proven. In controlling seedling drop disease in cotton caused by the fungus *R. solani*, by increasing the activity of peroxidase and polyphenol oxide enzymes and increasing the plant's phenolic content **(Osman *et al*., 2019).**

Melon root rot is a disease of economic importance as it affects melon cultivation in terms of cultivated areas and production. Therefore, the study aimed to do the following:

1- Molecular diagnosis of the most pathogenic fungi associated with melon roots.

2- Testing the efficiency of some agents, K_2HPO_4 and tannic acid, in inhibiting the growth of the fungus *R. solani* in the laboratory.

MATERIALS AND METHODS

Single spore method to growth of isolates

Some of the isolates to be diagnosed molecularly were grown using the single spore method. Eight 10 ml glass tubes were used, and 9 ml of distilled water were placed in them, except for one tube in which 10 ml was placed. The tubes were covered with cotton and aluminum foil and sterilized with an autoclave. They were left to cool after sterilization. Then the fungal colonies grown on the nutrient medium PDA 7 days old were scraped off separately, placed in a tube containing 10 ml of sterile distilled water and shaken well, then transferred 1 ml using a Micro Pipet to the other tube containing 9 ml (first dilution), then shook well and transferred 1 ml to another tube (second dilution), and so on until the tube. In the last (seventh dilution), 1 ml of each dilution was taken after shaking or stirring and added to a petri dish containing PDA nutrient medium. The petri dish was moved in a vortexing motion in order for the fungal suspension to be distributed in the petri dish. The petri dishes were incubated at a temperature of 25°C ±2. The spores were taken grown on the nutrient medium from the last concentrations, after 48 hours using a sterile needle and under an optical microscope, individually, and they were placed in petri dishes containing the PDA nutrient medium. The petri dishes were incubated at a temperature of 25°C ±2 for five days.

Some fungal isolates that do not form spores were grown by taking a small part of the tip of the fungal colony using a sterile needle and placing it in a petri dish containing the PDA nutrient medium. It was incubated for five days at a temperature of 25°C ±2.

Molecular diagnostics

Diagnosis of some isolates of fungi associated with melon roots using polymerase chain reaction (PCR) technology.

Fungal colonies were grown for five isolates of fungi, namely F8, F19, F23, F28 and F34 (they were isolated from different regions of Iraq in the research of a master's student's thesis, and they were diagnosed phenotypically, and the pathogenicity test proved their high efficiency), which were intended for molecular diagnosis on the liquid nutrient media Potato Dextrose Broth (PDB). The medium was prepared in 250 ml glass flasks and 100 ml of liquid nutrient medium was placed in it. After sterilization in the incubator, the flasks were left to cool down in temperature. Three discs with a diameter of 0.5 cm were taken using a sterilized cork borer from five-day-old fungal colonies, which It was grown using the single-spore method, or it was grown by taking a small part of the fungal colony individually and placing it in the flasks. The flasks were incubated at a temperature of 25°C ±2 for 7 days, then the growing fungal colony was taken on the surface of the liquid nutrient medium and washed with sterile distilled water. To get rid of the remaining nutrient medium, it was dried with sterile filter papers, then placed in sterile plastic tubes and kept in the freezer at a temperature of -20°C until the DNA was extracted.

DNA extraction

The DNA of five fungal isolates F8, F19, F23, F28 and F34, which are the most pathogenic, was extracted from Jisr Al-Musayyab Company. The extraction kit produced by Geneaid Biotech (South Korea) was used, and the extraction process was carried out according to the method prepared by the company.

Polymerase Chain Reaction (PCR).

DNA amplification was performed using a primer that targets the specific sequence of ITS, which is the interstitial dividing region of the rRNA gene, to complete the amplification reaction of the ribosomal RNA chain. The general primer ITS1-ITS4 manufactured by BioNeer (South Korea) was used in Table (1).

Table (1): Sequence of the nitrogenous bases in the primer used to amplificated the DNA of fungal isolates.

Primer	Sequence of the nitrogenous bases of the primer
Forword ITS1	5-TCCGTAGGTGAACCTGCGG -3
Reverse ITS4	5-TCCTCCGCTTATTGATATGC -3

A volume of 5µl of the extracted DNA of the fungal isolates was added to 1.5 ml tubes of the Thermal Cycler device containing Master mix in a volume of 5 µl, to which 1µl of Forword ITS1 primer, 1µl of Reverse ITS4 primer, and 13µl of Deionized distilled water were added, then the tubes were placed in the Expense device for the purpose of mixing the materials by centrifugation and shaking (15 seconds centrifugation + 5 seconds shaking). Then the tubes were transferred and placed in the thermal rotary device, and the device was set (programmed) according to the program for the general starter as in the table (2) **(White *et al.*, 1990)**.

Table (2): Program of the thermal polymerase device for DNA amplification of fungal isolates.

No.	Steps	Temperature	Time	Cycles number
1	Pre-denaturation	95°C	5 minutes	1
2	Denaturation	95°C	30 second	30
3	Annealing	55°C	30 second	
4	Extension	72°C	45 second	
5	Final extension	72°C	5 minutes	1

Electrophoresis of DNA on agarose gel:

Electrophoresis was performed to detect polymerase chain reaction (PCR) products and in the presence of standard DNA (Ladder) to characterize band size (White *et al*., 1990). Prepare an agarose gel with a density of 1.5% by dissolving 2 grams of agarose in 100 ml of TBE 1X (Tris-Borate EDTA) (prepare TBE 1X by taking 10 ml of TBE 10X + 90 ml distilled water), then put the mixture of agarose and TBE 1X in the oven until it boils. Dissolve the agarose, then add 5µl of Ethidium Bromide and leave it to cool at a temperature of 45-50°C. Then gently pour the gel into the special mold after installing the comb that makes a hole to place the DNA samples in it and leave it to cool for 30 minutes until it solidifies. The comb was carefully removed after the gel hardened and the gel was immersed in 1X TBE solution (prepare 1000 ml of it by taking 100 ml of 10X TBE with 900 ml distilled water) in the electrophoresis bath, then the PCR products were injected into the gel pits at a rate of 5µl for each hole and the standard solution was also placed in the ladder. Then it was electrophoresed at 130 volts for one hour, and after the migration process, the agarose gel was transferred to a UV imaging device to take a picture of the gel.

Reading sequences of nitrogenous bases amplified from the DNA of fungal isolates:

The products of PCR amplification of the genetic material DNA were placed in special plastic tubes and sent to Macrogene Company in South Korea in order to determine the sequence of the nitrogenous bases, which were compared in the NCBI GenBank. Two genetic affinity trees were drawn, one for the fungus *R. solani* and the other for the fungus isolates. *M. phaseolina* and the fungus *Fusarium oxysporium f.sp. ciceris* and the fungus-like *Pythium aphanidermatum* using the Molecular Evolutionary Genetics Analysis (MEGA-X) program **(Kumar *et al*., 2018)** and comparing it to what was recorded in the GenBank with the help of Prof. Dr. Nawras Abdul-Ilah Al-Kuwaiti - Department of Plant Protection - College of Agricultural Engineering Sciences.

Effect of K_2HPO_4 and Tannic acid on the growth rate and percentage of inhibition of *R. solani* in vitro:

Test the effect of both potassium salt K_2HPO_4 (Alpha Chemika, India) and tannic acid (Alpha Chemika, India) on colony growth of the fungus *R. solani* in Petri dishes containing PDA nutrient medium. Prepare 100 ml of PDA nutrient medium in each capacity glass flask 250 ml. Isterilized it in the incubator, left the flasks to cool, and added K_2HPO_4 to each flask in concentrations (0.6, 1, 2, 3, 4, 5, 6, 8 and 10 g.100ml^{-1}) each separately **(Abdel-Monaim *et al.,* 2015; Jabnoun-Khiareddine *et al.,* 2016)**. Tannic acid was added in concentrations (0.060, 0.080, 0.1, 0.6, 0.8, 1, 1.2 and 1.5 g.100 ml^{-1}), each separately **(Wu *et al*., 2010; Osman *et al*., 2019)**. After adding the concentrations, the flasks were shaken well and then poured into Petri dishes with three replicates for each treatment. After the nutrient medium had hardened, the plates were inoculated with a 0.5 cm diameter disc taken with a sterilized cork borer from a *R. solani* colony grown on PDA nutrient medium at 5 days old. Three replicates were made as a comparison (PDA nutrient medium was inoculated with the pathogenic fungus without any

additives. (Any concentration) The petri dishes were incubated at 25°C ±2 and were monitored until the colony growth was complete. In the comparison treatment, the growth rate was calculated by measuring the perpendicular diameters of the colonies and calculating the percentage of inhibition according to the equation:

$$Percentage\ of\ inhibition = \frac{Colony\ diameter\ in\ comparison - Colony\ diameter\ in\ treatment}{Colony\ diameter\ in\ comparison} \times 100$$

Complete Randomized Design (CRD) was used to implement the experiment, with three replications for each treatment. The Genstate program was used for statistical analysis, and the significant differences between the means were compared with the least significant difference test at the 0.05 probability level.

Results and Discussion

Molecular diagnostics

Diagnosis of some isolates of fungi associated with melon roots using polymerase chain reaction (PCR) technology:

The results of the polymerase chain reaction (PCR) showed the amplification of the DNA sequence from five isolates of the most pathogenic fungi (F8, F19, F23, F28 and F34) using primers ITS1 and ITS4, which were demonstrated by electrophoresis of the PCR results using an agarose gel with the appearance of DNA fragments. It has a molecular weight between 500 - 750 base pairs (bp) (Figure 1), The ITS region is one of the best genetic regions in determining genetic variations between isolates. This is due to the fact that this genetic region is fixed for species and strains, so it can be used to distinguish Genetic variations between species of the same genus and strains within the same species with a high degree of accuracy **(Cettul *et al.,* 2008)**. Molecular diagnosis is an important tool in diagnosing fungal diseases by diagnosing the fungi that cause them, as rapid detection of the disease is an important tool in disease management. Molecular diagnosis is more accurate than traditional diagnosis, and one of the most important molecular technologies used is Polymerase Chain Reaction (PCR) technology, which Through it, the fungal DNA is duplicated, as it has been used to diagnose many fungi based on the genetic structures as well as the difference in the nucleotide sequence of a specific location on the chromosome, according to which it is possible to distinguish between phenotypically similar individuals **(Barboza *et al*., 2013; Hariharan & Prasannath, 2021)**.

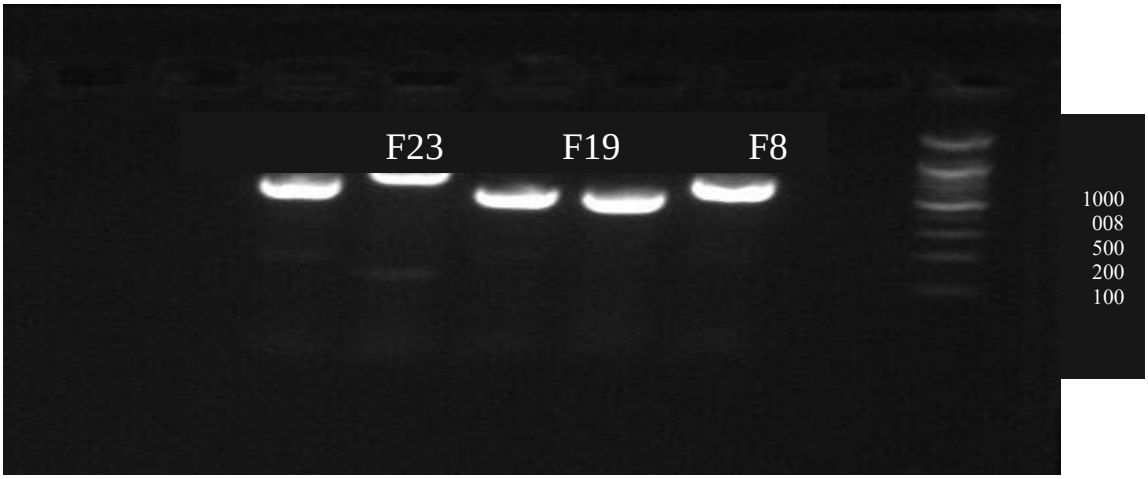


Image (1): the electrophoresis result of the polymerase chain reaction (PCR) products of the genetic material (DNA) of the five fungal isolates (F8, F19, F23, F28, F34) on agarose gel medium.

Nucleotide sequence analysis and registration in GenBank:

The nucleotide sequences of the DNA bands that were duplicated for the ITS region of the five fungal isolates were determined and found that they belong to the fungi (F8 and F34) *R. solani*, (F19) *Fusarium oxysporium f.sp. ciceris*, (F23) *M. phaseolina* and the fungus-like (F28) *Pythium aphanidermatum*. The sequences of the five isolates were deposited in the National Center Biotechnology Information GenBank (NCBI) under the accession numbers PP342522.1 (F8), PP342523.1 (F34), PP342524.1 (F19), PP342526.1 (F23) and PP342527.1 (F28), and the isolates F8 and F34, *R. solani*, F19 is *Fusarium oxysporium f.sp. ciceris* and F28 *Pythium aphanidermatum* first registration in NCBI on Melon in Iraq.

The genetic ancestry tree of the *R. solani* isolates of the connective type included the Neigbor-Joining Phylogenetic Tree, which was built from the nucleotide sequences of the ITS region of the isolates RS1-IRQ and RS2-IRQ under study, and the isolates from Turkey, India, and Japan, as the isolates RS1 and RS2 were from Iraq, which In this study, they were isolated close to the Turkish isolate MN872377.1 Turkey, as they were in one group and were far from the Turkish isolate MHI72618.1 Turkey. They were separated from the fungus *F. solani* NR163531.1 recovered from GenBank, which was included in the analysis for the purpose of comparison. (Out Group), MEGA-X program was used for nucleotide analyses, Figure (1).

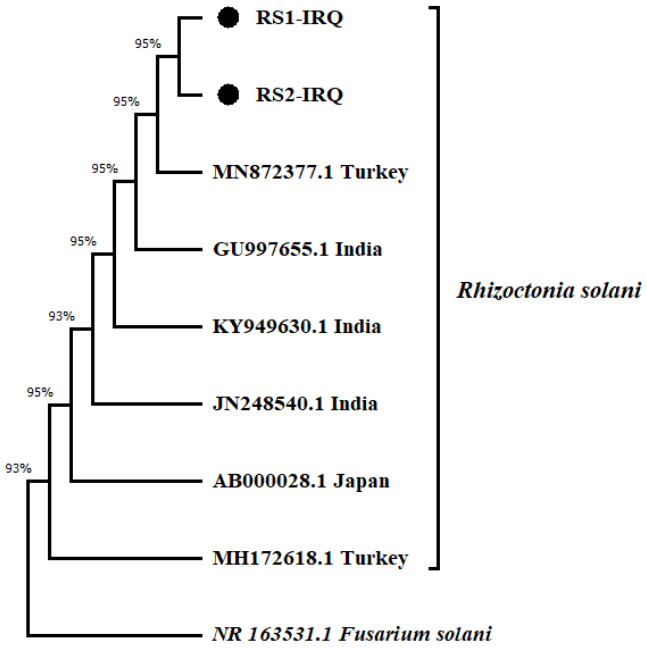


Figure (1): The genetic tree of the *R. solani* isolates RS1 and RS2 and their relationship to the isolates recovered from GenBank The isolates RS1 and RS2 were closer to the Turkish isolate MN872377.1 and far from the Turkish isolate MHI72618.1, and they separated from the *F. solani* isolate NR163531. 1 retrieved from GenBank and included in the tree for comparison.

The isolates of *R. solani* F8 (RS1) and F34 (RS2), which were isolated from Iraq, were compared with their counterparts from the global isolates of *R. solani* present in GenBank. The nucleotide identity rates of the ITS region were compared and it was found that they match by 91% with the Turkish isolates. MN872377.1, Hindi GU997655.1, KY949630.1 and JN248540.1, Japanese AB000028.1 and Turkish MHI72618.1. The nucleotide sequence of the fungus F. solani was included for the purpose of comparison. Nucleotide analyzes were conducted using the Sequence Demarcation Tool (SDT v1.2) program1 .2, Figure (2).

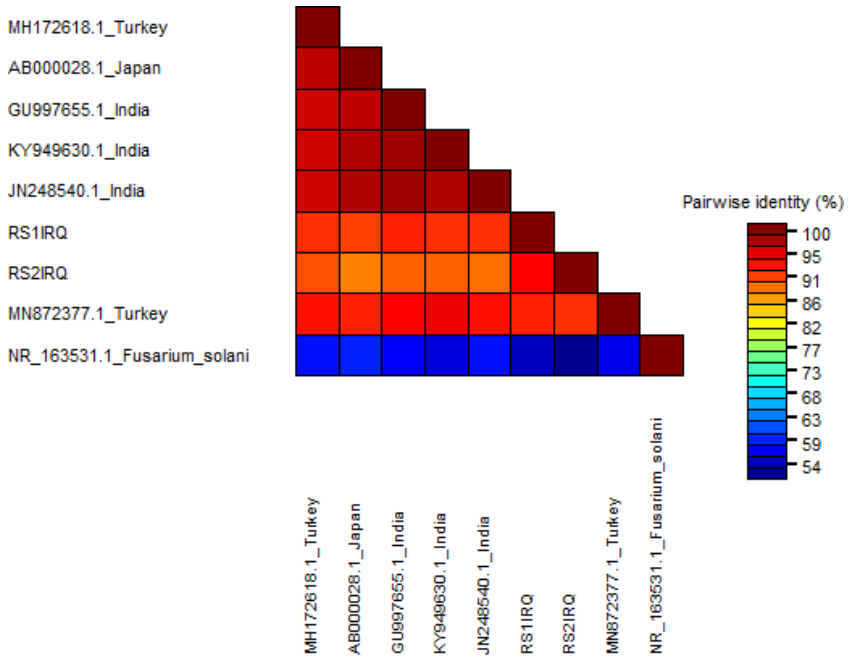


Figure (2): shows the genetic similarity values for the two isolates of the fungus *R. solani* RS1 and RS2, which were isolated and diagnosed in this study in Iraq and registered in GenBank. The figure shows comparisons of the nucleotide identity percentages of the ITS region of isolates RS1 and RS2 with their counterparts in GenBank, with the percentages of identity between them shown by the colors in the figure.

The other genetic tree of the isolates included *M. phaseolina* and *Fusarium oxysporium f.sp. ciceris* and the fungus-like *Pythium aphanidermatum*, a Neigbor-Joining Phylogenetic Tree that was built from the nucleotide sequences of these isolates and some international isolates, as the isolates from Iraq were PP342526.1 MP-IRQ, PP342524.1 FOC-IRQ, and PP342527.1 PA-IRQ. The isolates in this study are close to the global isolates MK447884.1 Argentina, EU442590.1 India and MN744684.1 Sri Lanka, respectively, and were separated from the fungus JF795580.1 *Synchytrium endobioticum* recovered from GenBank, which was included in the analysis for the purpose of comparison (Out Group). Nucleotide analyzes were performed using the MEGA-X program, Figure (3).

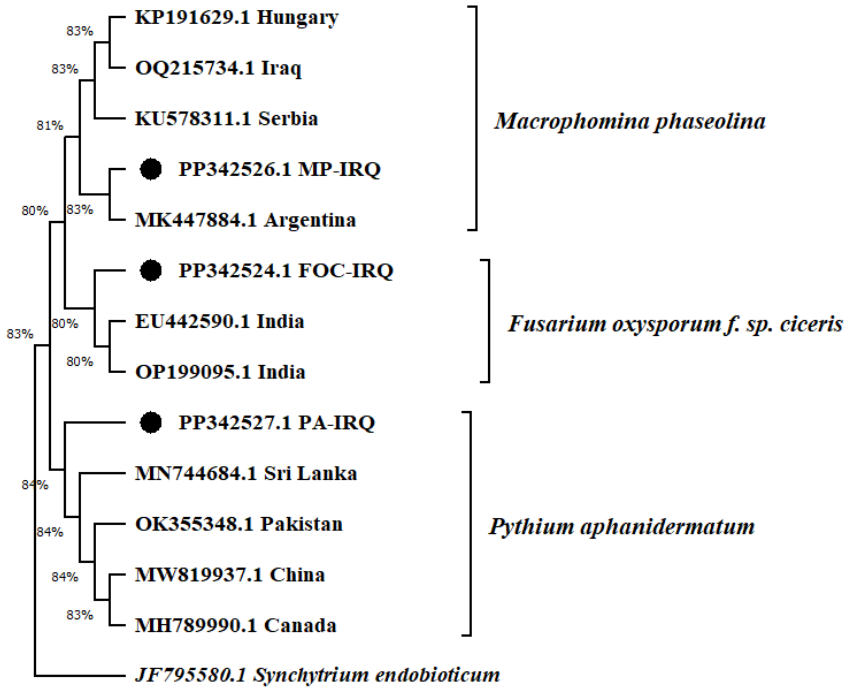


Figure (3): Genetic tree of the fungal isolates PP342526.1 MP-IRQ and PP342524.1 FOC-IRQ and the fungal parasite PP342527.1 PA-IRQ from Iraq and its relationship to the isolates recovered from GenBank, as these isolates were closest to the global isolates MK447884.1 Argentina. EU442590.1 India and MN744684.1 Sri Lanka respectively were separated from the fungal isolate JF795580.1 *Synchytrium endobioticum* recovered from GenBank and included in the tree for comparison.

Isolates of the two fungi PP342526.1 MP-IRQ and PP342524.1 FOC-IRQ and the fungus-like PP342527.1 PA-IRQ, which were isolated from Iraq, were compared with their counterparts from the global isolates present in GenBank. The nucleotide identity rates for the ITS region were compared, and it was found that PP342526. 1 MP-IRQ matched 100% to global isolates MK447884.1_Argentina, KU578311.1_Serbia, KP191629.1_Hungary and OQ215734.1_Iraq. Isolate PP342524.1 FOC-IRQ matched 100% to isolates EU442590.1_India and OP199. 095.1_ India One The fungus isolate PP342527.1 PA-IRQ is 95% identical to isolates MN744684.1_Sri_Lanka, OK355348.1_Pakistan, MW819937.1_China and MH789990.1_Canada. The nucleotide sequence of the fungus JF795580.1 *Synchytrium endobioticum* was included for comparison, and the SDT v1 program was used. 2. To conduct Nucleotide analyses, Figure (4).

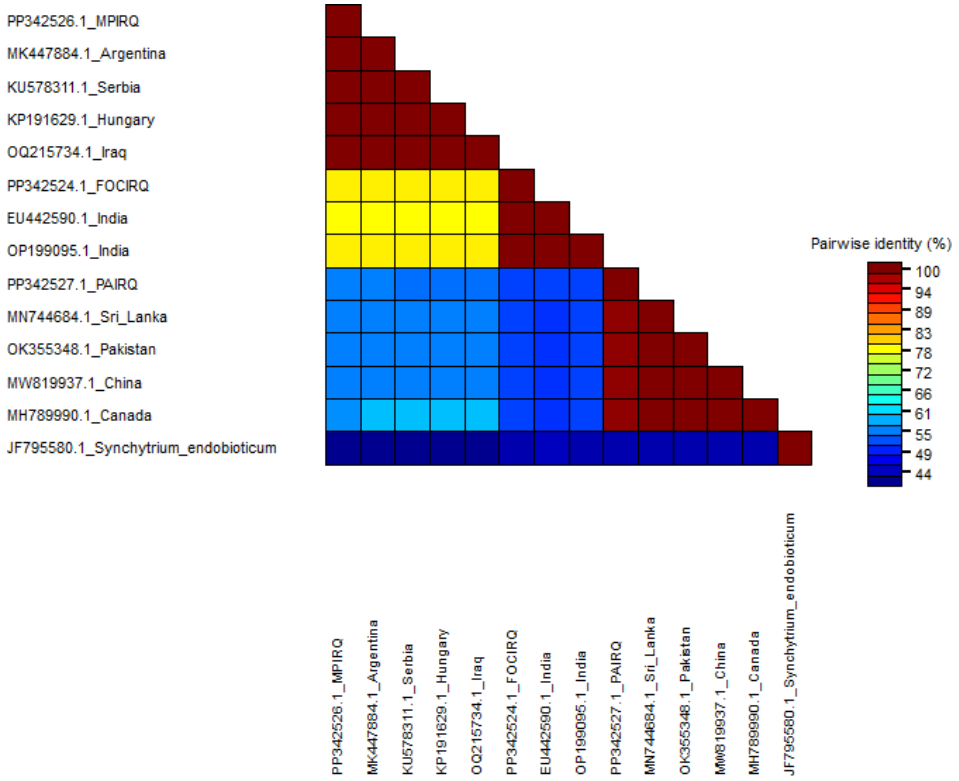


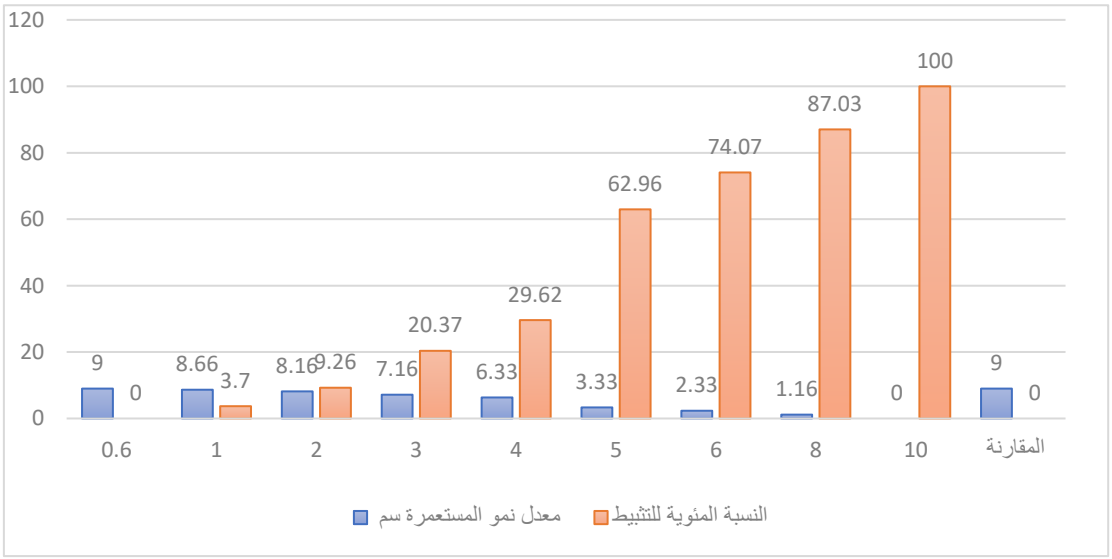
Figure (4): shows the identity values of the nucleotide sequences of the fungal isolates PP342526.1 MP-IRQ and PP342524.1 FOC-IRQ and the fungal isolate PP342527.1 PA-IRQ, which were isolated and diagnosed in this study in Iraq and registered in GenBank. The figure shows comparisons of the nucleotide identity ratios of the ITS region of these isolates with their counterparts in GenBank, along with the match ratios between them, which are shown by the colors in the figure.

Effect of K_2HPO_4 and Tannic acid on the growth rate and percentage of inhibition of R. solani in vitro:

The results of testing the effect of K_2HPO_4 on the growth rate of the fungus *R. solani* (Figure 5) showed that the concentrations used ranged from 1 to 10g.100 ml^{-1} had a significant effect in reducing the growth rate of the fungus and increasing the rate of inhibition compared to the comparison treatment, as the growth rate ranged between 0.0 - 8.66 cm, while the rate of inhibition ranged between 3.70 - 100%. The concentration exceeding 10 gm completely prevented the growth of the fungus, and the rate of inhibition reached 100%. Followed by the concentration of 8 gm and 6 gm, it reduced the growth rate by 1.16 and 2.33 cm, respectively, and raised the inhibition rate by 87.03 and 74.07%, respectively.

A number of researches have indicated the role of K_2HPO_4 in inhibiting a number of pathogens, including the fungus *R. solani* in the laboratory. **Jabnoun-Khiareddine, *et al.* (2016)** reported that concentrations of 1.8, 7.0, and 10.5 g. 100 ml^{-1} reduced the growth rate of the fungus, as it ranged from 4.70, 4.27 and 1.18 cm, respectively. **Abdel-Monaim, *et al.* (2015)** indicated that K_2HPO_4 inhibited the growth of the fungus *R. solani* when used at concentrations of 0.1, 0.2, and 0.4 g. 100 ml^{-1}, and the inhibition rates reached 25.36, 32.25, and 36.47%, respectively. Use K_2HPO_4 at concentrations of 0.04, 0.08 and 0.16 g. 100 ml-1 inhibited the growth of the fungus *R. solani*, and the rates of inhibition of the fungus reached 25, 29.63, and 69.77%, respectively **(Ghoniem *et al.*, 2023)**.

Perhaps the reason for the inhibition of fungal growth by K_2HPO_4 is that most salts work to pull water from the cells and thus lead to their shrinkage, the collapse of the fungal hyphae and their failure to grow, and the shrinkage and drying of the spores, thus inhibiting the fungal colony **(Palmer *et al.*, 1997; Fallik *et al.*, 1997)**.



Each number in the figure represents the average of three replicates

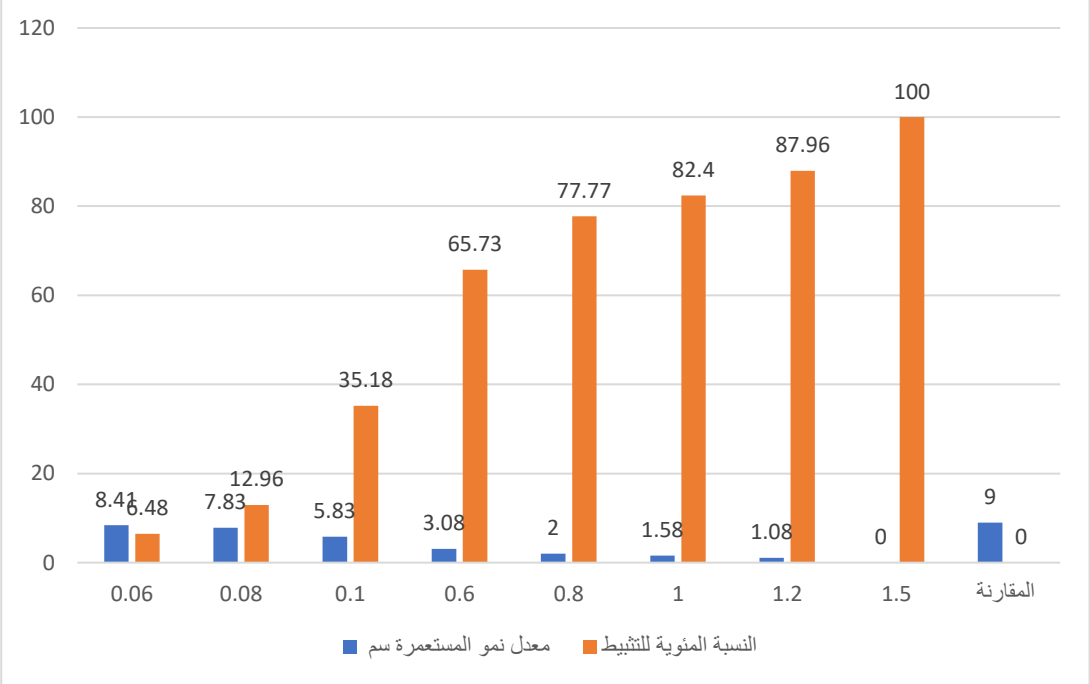
Figure (5): The effect of K_2HPO_4 on the growth rate and rate of inhibition of the fungus *R. solani* on the PDA.

The results, as shown in Figure (6), showed that tannic acid was used at concentrations of 0.06, 0.08, 0.1, 0.6, 0.8, 1, 1.2 and 1.5 g. 100 ml^{-1} PDA nutrient medium led to a significant reduction in the growth rate and increased the rate of inhibition of the fungus *R. solani* by varying degrees, as the concentration reached 1.5 g. 100 ml^{-1} significantly reduced the growth rate of the fungus and the percentage of inhibition, as it reached zero and 100%, respectively, followed by the concentration of 1.2 g and 1 g, which reached 1.08 cm, 87.96%, and 1.58 cm, 82.40%, respectively, while the growth rates and inhibition percentages for the concentrations

ranged from 0.06. 0.08, 0.1, 0.6 and 0.8 g between 8.41 - 2 cm and 6.48 - 77.77%, respectively. It was noted that the percentage of inhibition increased with increasing concentration.

Wu *et al.* (2010) reported that tannic acid has the ability to inhibit the fungus *F. oxysporium*, which causes wilt on rosehip plants in the laboratory. Osman *et al.* (2019) found that tannic acid has an effect in inhibiting the fungus *R. solani*, which causes seedling drop disease in cotton plants, on the PDA nutrient medium, at concentrations of 0.85, 1.70, and 3.40 g. 100 ml^{-1}, as the inhibition rates reached 47.44, 55.89 and 68.11%, respectively.

Perhaps the reason for the effect of tannic acid in inhibiting the growth of the fungus R. solani is that it is an organic acid that works to cause changes in large parts of the cell, which affects the process of cell respiration. It may inhibit or hinder the formation of free radicals such as OH, NO, and NO2, and the effect on iron ions and antioxidants. Which affects the germination of conidia of the fungus **(Andrade *et al.*, 2006; Omar *et al.*, 2018).**



Each number in the figure represents the average of three replicates

Figure (6): Effect of tannic acid on the growth rate and rate of inhibition of the fungus *R. solani* on the PDA.

CONCLUSION

Melon root rot is one of the important diseases on melon, as many pathogens were found accompanying the infected melon roots, such as *R. solani*, *F. oxysporium f.sp. ciceris, M. phaseolina* and *P. aphanidermatum* those that were molecularly diagnosed and found to be similar to some international isolates through drawing the genetic tree, as well as the extent to which these isolates match the global isolates through the color histogram. It was found that chemical induction agents K_2HPO_4 and Tannic acid inhibited the growth of the fungus *R. solani* through the use of different concentrations. It was found that the higher the concentration of the agent, the greater the rate of inhibition.

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