Morphological and molecular identification of the root and stem base rot pathogen on broad bean crop and control it using some *Trichoderma* biological agents

Ahmed Hayyawi Dakhil and Usamah A.Alkarim A.Almunam Alshimaysawe

Faculty of Agriculture - University of Kufa - Republic of Iraq.

Corresponding Author Email: ahmedhayawi1314@gmail.com

DOI: https://doi.org/10.36077/kjas/2023/v15i2.10323

Received date: 2/10/2022

Accepted date:16/10/2022

Abstract

This study looked at the effects of biological control strategies for root and stem rot disease caused by *Rhizoctonia solani* using soil organisms as biocontrol agents. The results of the field survey showed the spread of root and stem rot disease on broad bean plants in some areas of Najaf Governorate, where the infection rate ranged from 32- to 89.5%. The Isolation process showed that five fungal isolates of *Rhizoctonia solani* were associated with the disease, which differed in some phenotypic characteristics in terms of growth speed and color difference. The results of the molecular diagnosis using the ITS marker sequencing for the three most pathogenic isolates showed that they were belonging to *Rhizoctonia solani*, thus were named Ahmed 1, 3, 4 and registered in the GenBank under accession numbers; ON 394595.1, ON 394597.1, and ON 394599.1, respectively. In the pathogenicity test, *R. solani* isolates displayed a difference in their virulence on broad bean plants, where *R. solani* 1 (Al-Mashkhab isolate) was the most virulent among the isolates and was selected for subsequent studies. Isolate *T. longibrachiatum* (T3) showed high antagonistic efficiency against isolate of the pathogenic fungus *R. solani* on P.D.A culture media, gave the highest inhibitory percentage 75.06% compared to isolates T1 and T2 of *Trichoderma harzianum*, which led to inhibition of the pathogen to 68.71% and 68.94%, respectively. In the culture filtrate test of isolates of the living fungi, isolate T3 had the highest inhibition in the growth of pathogenic *R. solani*, which was 5.20 cm compared to the two live fungi T1 and T2, which led to 6.20 and 5.95 cm, respectively. As for the pots experiment, the results revealed that the isolate of *T. longibrachiatum* (T3) protected the broad beans plants from root and stem rot pathogen, where the disease was not seen compared to the treatment of the pathogenic fungus *R. solani*, only in which the infection severity was 80%.

Keywords: root and stem rot disease, broad bean, *Rhizoctonia solani*

Introduction

Broad bean, *Vicia faba* L., belongs to the Fabaceae family, which ranks second after the Grass family in terms of importance, as its seeds contain high levels of protein estimated at 25-40% (1). It is also the third most important legume crop after beans and peas (2). This crop is exposed to many agricultural pests such as insect pests and fungal diseases. Root and stem-base rot disease is considered one of the most important fungal diseases that affect the broad bean crop in many cultivated lands in the world and lead to large production losses of up to 100% when conditions are suitable for the pathogen (3 and 4). This disease is caused by the pathogenic fungus *Rhizoctonia solai*, which is one of the most common fungal diseases in the world and Iraq, causing economic losses. The fungus attacks plants at all stages of their growth, causing the rotting of seeds and death of seedlings before and after emergence, and causing rotting of the roots and the stem base. Due to the importance of this disease, many management methods have been used, including chemical methods, physical methods, and biological methods. The most common and easiest – to - use methods are chemical pesticides.

Due to the danger of these materials to the environment, their high cost and toxicity to humans and animals on the one hand, and the ability of pathogens to develop resistance to many chemical pesticides on the other hand (5), the study turned to search for safe alternative methods. One of the most prominent of these methods is the use of biological control using microorganisms against plant pathogens. The most important of these organisms is the biological control agent, the fungus *Trichderma* spp. This fungus is known to possess diverse antagonistic properties against plant pathogens, involved in improving plant growth and production through root colonization and activation of defense mechanisms (7 and 6). This research was chosen due to the lack of studies on root and stem rot disease, and the great losses it causes on the broad bean crop. This study aimed to isolate the fungus causing the disease, its phenotypic and molecular diagnosis, and to evaluate the use of the biological control agent *Trichoderma* spp. to control the disease caused by the fungus *R. solani* on broad beans.

Materials and Methods

The study included conducting a field survey of root and stem rot disease caused by the pathogenic fungus *R. solani*, isolation of the pathogen, and diagnosis of isolates of pathogenic fungus *R. solani* morphologically and molecularly, and testing the pathogenicity of isolates of the pathogen *R. solani*. The study also included testing the antagonistic ability of *Trichoderma* spp. isolates against the pathogic fungus on PDA culture medium, and studying the effect of *Trichoderma* spp. isolates filtrates on pathogenic *R. solani* growth on PDA culture media.

Field survey

A survey was conducted of broad bean fields planted with a local variety during the 2020-2021 season in Najaf Governorate. Infected broad bean plants were observed in each field that showed symptoms of root and stem rot disease. Also, the pathological symptoms were observed on the roots of the affected plants, and the percentage of infection was calculated. Isolation of the pathogen from each sample was carried out on the day following sample collection (8).

As for the isolation, purification, and diagnosis of the fungi that cause root and stem rot on broad bean plants. The affected plant parts were cut and cultured on a standard PDA culture medium, four pieces were used for each plate. The plates were incubated at 2±25°C for three days. Then fungal colonies were purified by transferring the terminal piece of mycelium to new Petri dishes (PDA) and incubated at 2±25°C for 2-3 days to obtain pure isolates of the pathogen for further morphological diagnosis.

Phenotypic diagnosis of isolates of the fungus *Rhizoctonia* spp.

The phenotypic diagnosis of the fungi isolated from the pure fungi isolates to the genus level, depending on the nature of growth, colony color, and the structures formed by the fungus was done using the approved taxonomic keys (9).

Molecular identification

The diagnosis was made using the polymerase chain reaction (PCR) for the fungi isolated in this study using two methods. The first is molecular diagnostics using the internal transcribed spacer (ITS) genetic marker. The second is a molecular diagnosis by identifying the complete genome of the isolates using Next Generation Sequencing (10).

Table 1. Primers used for the PCR technique under study

PCR product size	Sequence 5 ⟶3		Primer
500-800 bp	TCC GTA GGT GAA CCT GCG G	F	ITS1
	TCC TCC GCT TAT TGA TAT GC	R	ITS4

Nitrogenous base sequence determination and bioinformatics analysis

After performing the PCR multiplication process, the products were sent to Macrogen Corporation in South Korea to determine the nitrogenous base sequence of each fungal sample. The data received from the company was evaluated and analyzed using Chromas software. For the purpose of knowing the similarities between the studied mushrooms and the fungi registered globally, the Basic Local Alignment Search Tool (BLAST) program of the National Center for Biotechnology Information (NCBI) National Center for Biotechnology Information was used.

Study of the genetic compatibility of the diagnosed fungal isolates

The genetic tree is of importance in the study of mycology, through which the location and degree of genetic compatibility of the globally diagnosed fungal isolates can be known. The program Molecule Evolutionary Genetics Analysis (MEGA) (11). Was used. The Neighbor-joining method was also adopted to match the sequences under study using the Clustwai alignment.

Pathogenicity test of *R. solani* isolates causing root and stem rot in broad bean

The inoculum of the fungus *R. solani* was loaded onto sterilized wheat seeds for 15 days. The pathogenicity test was conducted in plastic pots (1 Kg soil) to which 5 gm of wheat seeds loaded with pathogenic fungi were added with three replicates for each treatment, with three replicates and sterilized wheat seeds only for the control treatment. The pots were planted with superficially sterilized broad beans, and 5 seeds/pot. Then the pots were watered carefully and distributed randomly under natural conditions

while maintaining soil moisture whenever needed. The percentage of germination of seeds and dead seedlings was calculated after 15 days of sowing. The lengths of the shoot and the soft root system were also calculated after 30 days. The severity of the injury was calculated according to the recommended pathological evidence for roots (12). Where: 0 = healthy roots. 1 = discoloration of secondary roots, 2= discoloration of the secondary roots and part of the main root, 3= main root discoloration but the stem base is not affected, 4 = discoloration of the main root and the base of the stem, 5=Death of the plant.

Antagonistic ability test of *T. harzianum* 1 and 2 and *T. longibrachiatum* T3 against the pathogenic fungus *R. solani*

The double culture method was used on a PDA medium in a 9 cm Petri dish. Two halves of the plate were inoculated with a 5 mm diameter disc from a 5-day-old culture on a PDA culture medium, of *R. solani* or any of the isolates of *Trichoderma* spp. The experiment was carried out with three replications, in addition to the fungus *R.solani* treatment for the (control) unit, the dishes were incubated at 25±2°C for 5 days, after which the percentage of inhibition was measured (13).

Effect of filtrate of *Trichoderma* spp. and *R.solani* filtrate on radial growth of *R. solani* on PDA medium

Filtrates of *Trichoderma* spp. isolates and the filtrate of the fungus *R. solani* were prepared. A 4 tablets of 5 mm diameter of 7-day-old fungus grown on PDA were added to 250 ml glass flasks with PSB liquid medium for each fungus separately, taking into account the shaking flasks (Shaker 150 rpm/min) to ensure the spread of the fungi. at 10 days after addition, the fungi were filtered on the liquid medium using sterile filter paper, then the filtrate was passed through (Millipore) 0.22 mm using sterile medical syringes.

A 5 ml of each filtrate was added to each Petri dish with the addition of PDA culture medium in three replications, with three replications without filtrate (control treatment). The plates were inoculated with a disc 5 mm from the edge of the plate of 5-day-old *R.solani* fungus grown on PDA. The dishes were incubated at 22±2°C for 5 days, after which the radial growth rate was calculated (15 and 14).

The study also included testing the effect of the biological fungus *Trichoderma* spp. isolates on the growth of broad bean plants in plastic pots, and evaluation the efficiency of *Trichoderma* spp. isolates in controlling the pathogenic fungus *R.solani* (Al-Mashkhab isolate) in the plastic pot soil.

Statistical analysis

Statistical analysis of the experimental data was carried out using the statistical analysis program Genstat and Excel program. The averages were compared using the least significant difference at the L.S.D probability level of 5% (16).

Results and Discussion

Field survey

The results of the field survey (Figure 1) showed that the fields of broad bean cultivation in Najaf governorate showed root and stem rot disease, with an infection rate ranging from 32 to -89.5%. The highest infection rate was recorded in the fields of Al-Mishkhab district with 89.5%, followed by Al-Hurriya district with 86%. It was also observed that the disease spread in the fields

of Abu Sakhir district 32%, Al-Hira sub-district, 40% and Al-Abbasiya sub-district, 41.4%. The wide spread of the disease may be due to the repeated cultivation of broad beans annually in these areas, and the suitability of the environmental conditions that led to the accumulation of fungal inoculum and the structures formed by the fungus as stone bodies (Sclerotia) in the soil that are resistant to environmental conditions. In addition to the lack of effective ways to control the pathogen endemic in the soil (18 and 17).

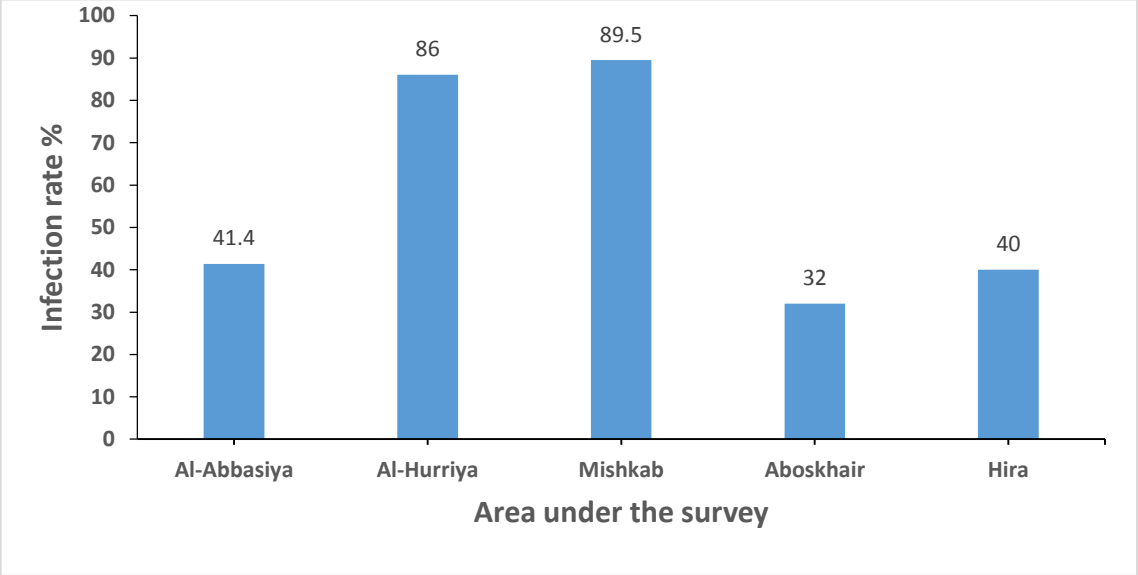

Figure 1. Incidence of broad bean infection with root and stem rot disease caused by *R. solani* in the surveyed fields within the Al-Najaf Governorate

Isolation and identification of fungi causing root and stem rot on infected bean plants

The results of isolation, purification, and microscopic examination of fungi grown from planting infected parts of the broad bean plant on P.S.A. culture media showed that five isolates belonging to the fungus Rhizoctonia were obtained. After growing on culture media, the isolates showed variation in some phenotypic characteristics in terms of growth speed and colony color, which is consistent with the results of the isolation of a previous study (19 and 17).

Phenotypic diagnosis of *Rhizoctonia solani* isolates

Isolates of *Rhizoctonia* were diagnosed to be the species it belongs to by relying on the growth of colonies and the colors that are formed during the growth of the mycelium, which ranged from light brown to dark brown. Divided by large diameter septa, it branches near the terminal septa of the fungal cells and is often at right angles and shortens the cell that branches in the phylogenetic zone (9). Figures (2, 3, and 4) show the colonies that were grown on P.S.A culture medium for the three most virulent isolates, depending on the nature of their growth and the reduction of the mycelium at the initial growth area.

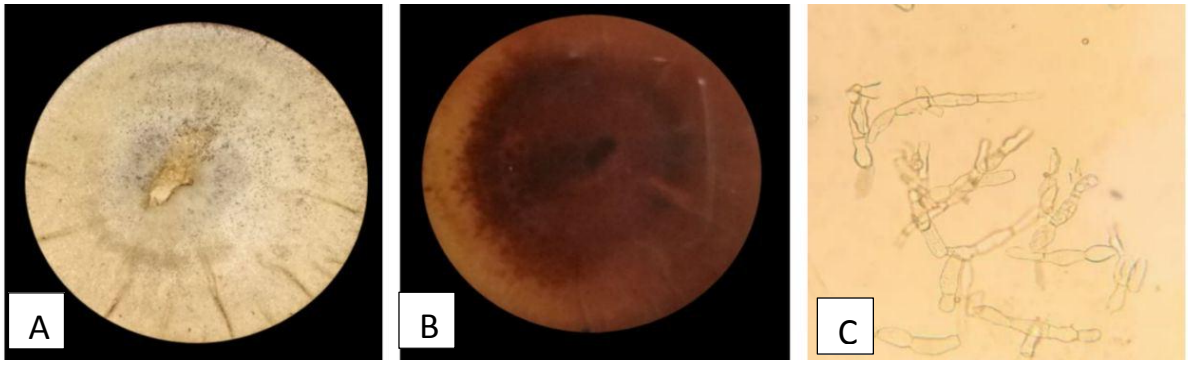

Figure 2. *R. solani* Al-Mishkab isolate, A) Colony upper face, B) Colony lower face, and C) fungal mycelium

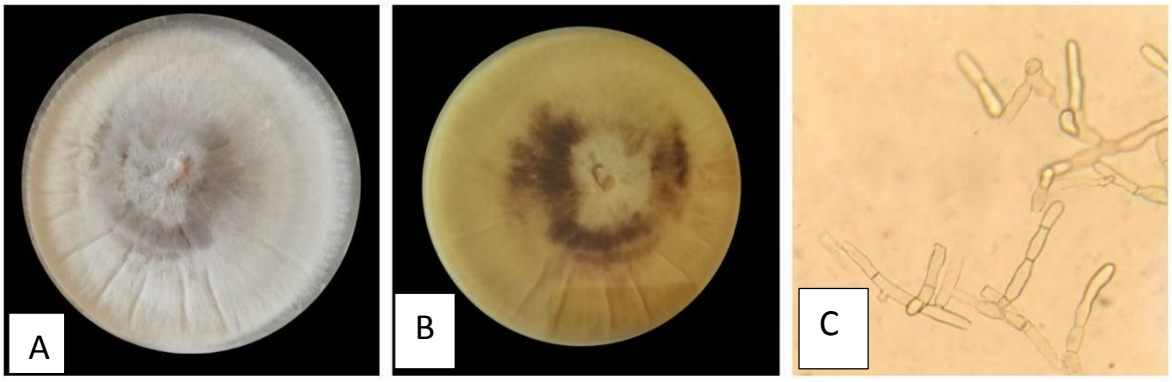

Figure 3. *R. solani* Hurriya isolate, A) Colony upper face, B) Colony lower face, and C) fungal mycelium

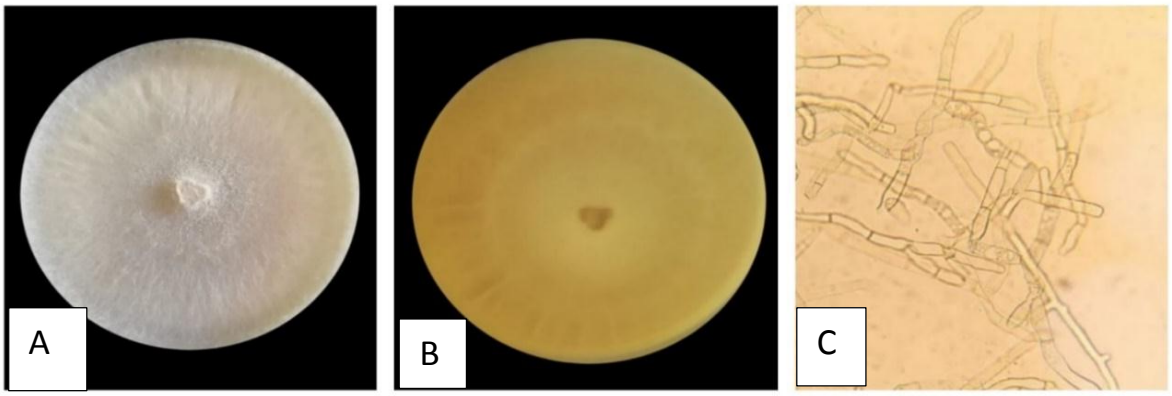

Figure 4. *R. solani* Hira isolate, A) Colony upper face, B) Colony lower face, and C) fungal mycelium

Molecular diagnosis of *Rhizoctonia solani* isolates

The *Rhizoctonia solani* isolates under study were molecularly diagnosed, then the nitrogenous base sequences of the diagnosed species were registered in the GenBank database under a specific Accession number for each isolate (Table2).

Table 2. *R. solani* isolates diagnosed and recorded in Gen Bank database with their accession numbers

No.	*R. solani* isolate name	Accession number
1	Isolate Ahmed-1*Rhizoctonia solani*	ON394595.1
2	Isolate Ahmed-3*Rhizoctonia solani*	ON394597.1
3	Isolate Ahmed-4*Rhizoctonia solani*	ON394599.1

The results of phylogenetic analysis of the sequence of nitrogenous bases confirmed the genetic relationship between the isolates of the species diagnosed in this study with a group of global fungal isolates of the same genus and species.

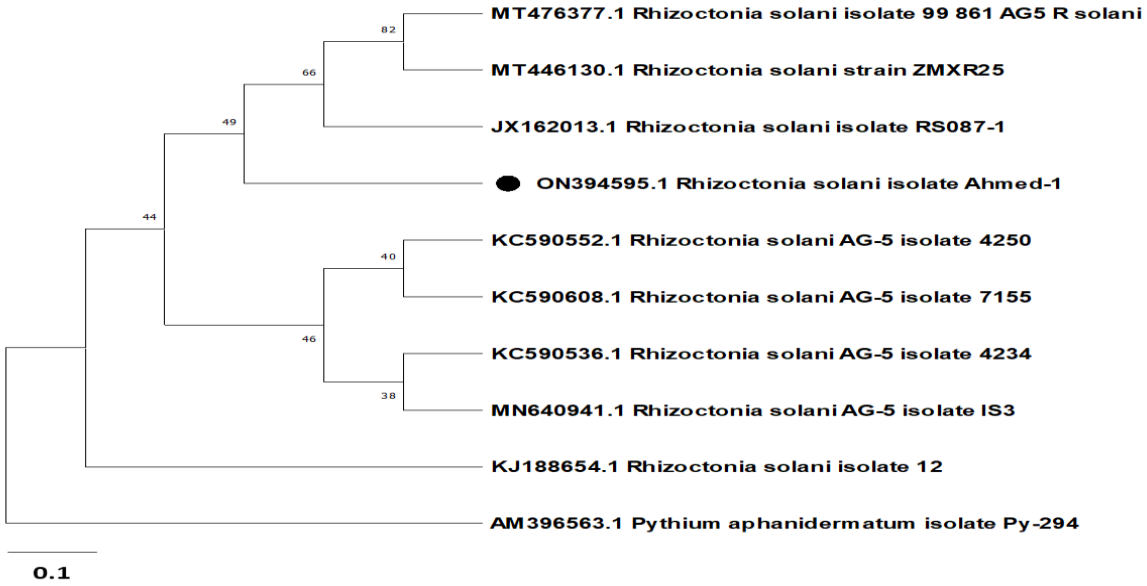

Figure 5. Genetic tree of the pathogenic fungus *Rhizoctonia solani* isolate Ahmed-1 detected in the study (marked with a black dot). The tree was built based on ITS-rDNA region sequences, compared with a number of isolates of the same fungus in Gen Bank previously registered at the National Center for Biotechnology Information (NCBI). Genetic distances were calculated using the neighbor-joining method. Use of the fungal isolate *Pythium aphanidermatum* as Out Group

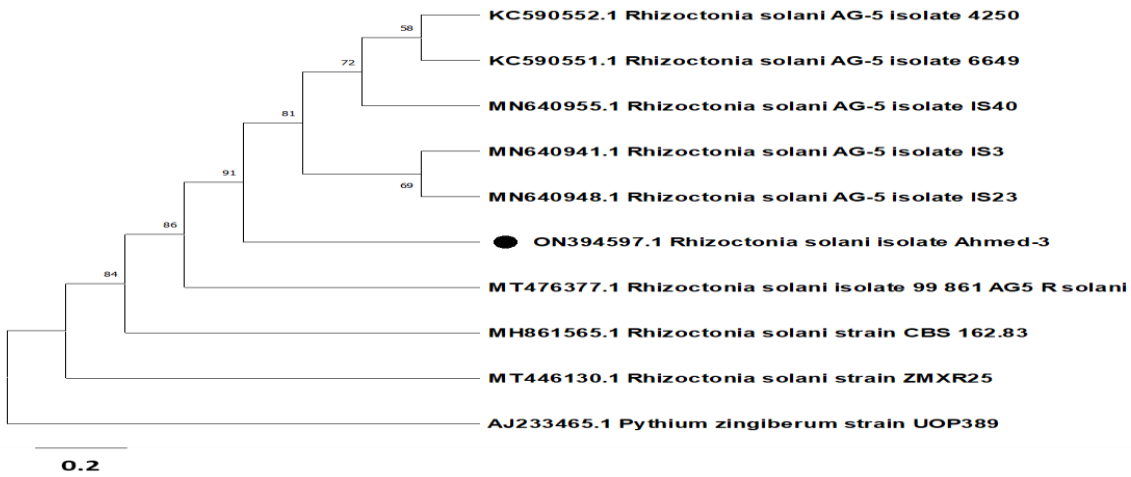

Figure 6. Genetic tree of the pathogenic fungus *Rhizoctonia solani* isolate Ahmed-3 detected in the study (marked with a black dot). The tree was built based on ITS-rDNA region sequences, compared with a number of isolates of the same fungus in Gen Bank previously registered at the National Center for Biotechnology Information (NCBI). Genetic distances were calculated using the neighbor-joining method. Use of the fungal isolate *Pythium aphanidermatum* as Out Group

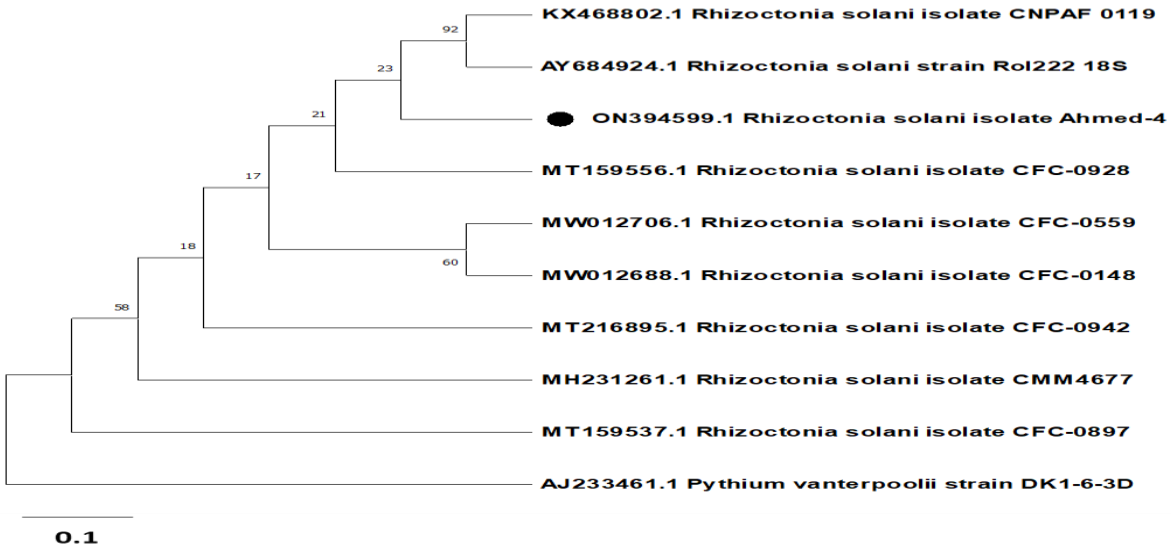

Figure 7. Genetic tree of the pathogenic fungus *Rhizoctonia solani* isolate Ahmed-4 detected in the study (marked with a black dot). The tree was built based on ITS-rDNA region sequences, compared with a number of isolates of the same fungus in Gen Bank previously registered at the National Center for Biotechnology Information (NCBI). Genetic distances were calculated using the neighbor-joining method. Use of the fungal isolate *Pythium aphanidermatum* as Out Group

Pathogenicity testing of isolates of the fungus *Rhizoctonia* spp.

The results of the study showed in Table (3) that all isolates of the pathogenic fungus *R. solani* isolated from (Hera, Mashkhab, Horreya, Abbasiya, and Abu Sakhir) had a significant effect on the germination of broad bean seeds. Germination rates ranged from

0.00% in Al-Heera isolate to 26.67% in Abu Sakhir isolate, compared to 90% in the control treatment. Most of the pathogenic fungi did not lead to the death of seedlings. It also showed a clear effect of the pathogenic fungi in a significant reduction of growth indicators in the shoot and root system compared to the treatment that was not inoculated with the pathogenic fungus.

Although all isolates led to the emergence of infection on broad beans in different degrees, the isolates of Hira, Mishkab, and Al-Horriya were the most virulent and led to infection severity of 100, 86.67, 53.33%, respectively, compared to the rest of the isolates. The pathogenicity difference between the tested isolates of *R. solani* may be due to genetic and environmental differences. The virulence of the isolates ranged from moderate to severe, and this may be due to the ability of the different isolates to secrete toxins and seed-degrading enzymes. Some isolates also cause rotting of seeds before germination more than causing the death of seedlings or large plants, or vice versa. The virulence of *R. solani* (Hira) and *R. solani* (Mishkab) isolates in increasing the severity of infection (the more pathogenic) may be due to the difference in the ability to secrete important toxic enzymes (21 and 20). In light of these results, the isolate *R. solani* (Al-Mashkhab) was selected for the subsequent studies.

Table 3. Pathogenicity of different isolates of the *R. solani* causing root and stem rot in broad bean in plastic pots

Treatments *R. solani* isolates	Infection rate %	Seedlings death %	Shoot length cm	Root length	Infection severity %
Hira	0.00	0.00	0.00	0.00	100.00
Mishkab	20.00	30.00	5.33	2.23	86.67
Aboskair	26.67	0.00	10.67	7.50	33.33
Al-Abbassiya	20.00	0.00	10.00	5.67	33.33
Al-Hurriya	20.00	0.00	11.50	6.33	53.33
Control	90.00	0.00	13.67	9.00	0.00
L.S.D.$_{(P\leq 0.05)}$	8.39	37.74	5.765	1.731	16.77

Values are means of 3 replications

The results in Figure (8) indicated that the isolates of the biological fungus *Trichoderma* spp. has high antagonistic ability against the fungus *R. solani* and led to the inhibition of its growth on the PDA medium. It was noticed that *T. longibrachiatum* T3 was significantly superior to *T. harzianum* T1 and T2 isolates in inhibiting the growth of pathogenic fungus, with an inhibition rate of 75.06% compared to isolates T1 and T2, which led to 68.71 and 68.94%, respectively. It was found in previous studies the ability of different species of *Trichoderma* spp. in inhibiting the growth of the fungus *R. solani* due to its different mechanisms. One of this is the direct parasitism on the pathogenic fungal mycillium. Also, competition ability for nutrients, occupation, and production of antibiotics that inhibit many pathogenic enzymes. Also, the ability of biological fungus to produce some toxic compounds such as Trichothecin, Gliotoxin and Viridin, and enzymes that degrade the cell walls of pathogenic fungi such as Protease, β-1,3-glucanase. And Chitinase (22). The efficacy of *Trichoderma* spp., including the species

used in this study, has been shown to be antaginistic to many pathogenic fungi such as *F. oxysporum* f.sp. *lycopersici*, *F. solani* and *R. solani* (24 and 23). The results of this study agreed with Koijam and Sinha (25) for the efficacy of *Trichoderma* isolates to inhibit *R. solani* growth when tested by dual culture method.

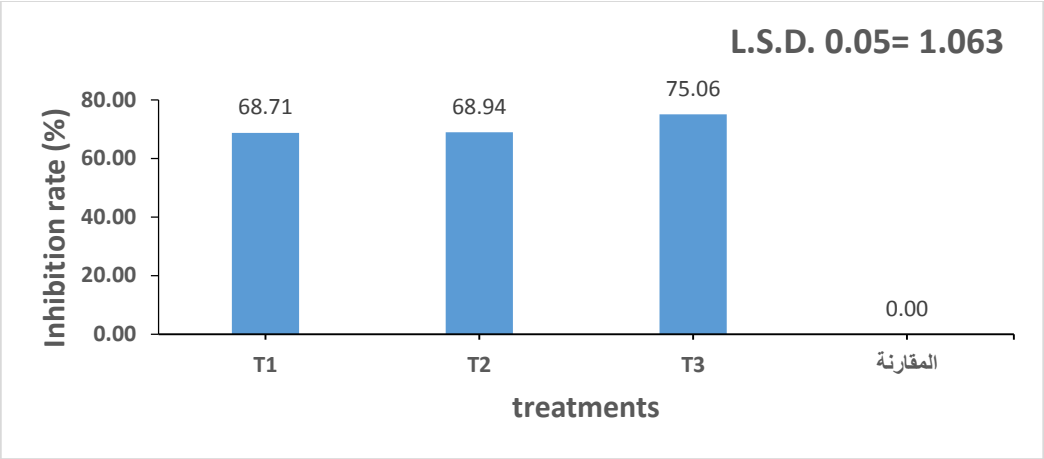

Figure 8. The antagonistic ability of *Trichoderma* spp. *T. harzianum* (T1) and (T2), *T. longibrachiatum* (T3) against the pathogenic fungus *R. solani* on PDA culture medium.

Effect of filtrate of *Trichoderma* spp. isolates on the growth of pathogenic *R. solani* on PDA culture medium

The results in Figure (9) showed that the filtrate of isolates of the biological resistance fungus *Trichoderma* spp. It led to a significant inhibition of the growth of *R. solani* when added to the nutrient media compared to the control treatment. It was observed that isolate T3 significantly inhibited the growth of pathogenic fungi to 5.20 cm compared to isolates T1 and T2, which led to a growth of 6.55 cm. Several studies indicated the efficacy of *Trichoderma* spp. In inhibiting the growth of many plant pathogens (26). The fungus *T. harzianum* secretes several antigens or secondary metabolites, including Acetaldehyde, Alkypyrones, Trichodermin, Peptaibols, Trichorzianine, Dermadine and Alamethicine. These compounds have a direct or indirect effect in inhibiting the growth of plant pathogenic soil fungi, including *R. solani* (27). In the study of Scarselletti and Faull (28) it was found that there is a relationship between the production of pyrone by *T. harzianum* and the ability of the fungus to antagonize *F.oxysporum* and *R.solani* in vitro.

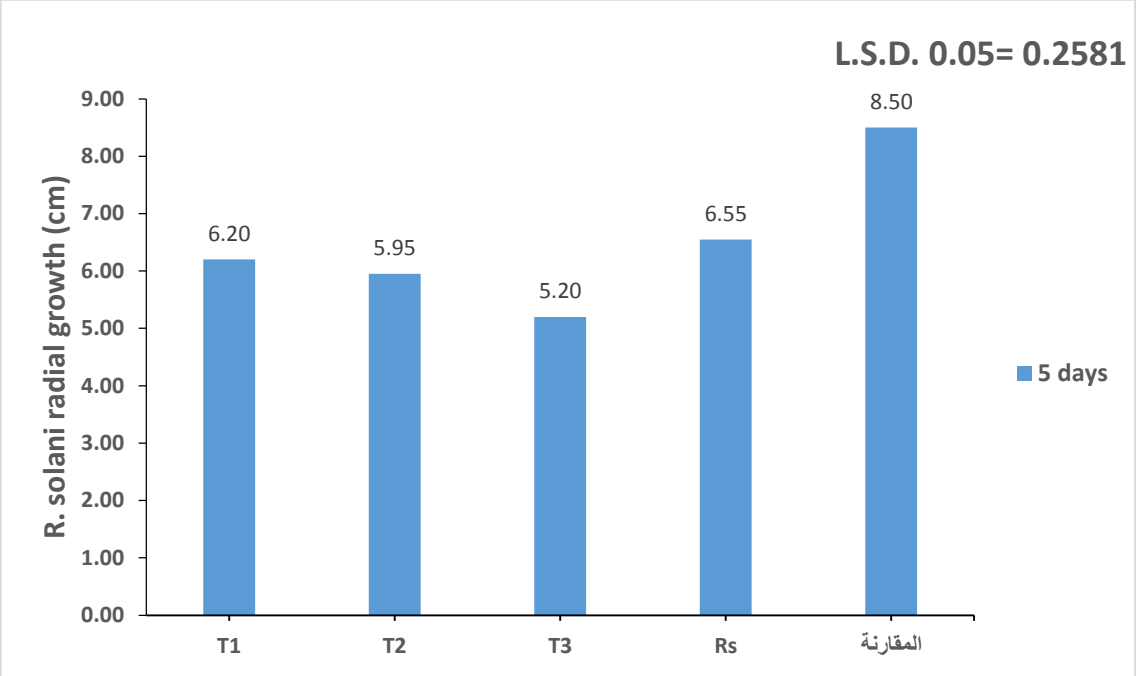

Figure 9. Effect of filtrate of *Trichoderma* spp. isolates on the radial growth of pathogenic *R. solani* on PDA media. *T. harzianum* (T1), *T. harzianum* (T2), *T. longibrachiatum* (T3), *R. solani* (Rs)

The effect of isolates of the biological fungus *Trichoderma* spp. in severity of infection and growth indicators of broad beans in plastic pots

The results of the study in Table (4) showed that all isolates of *Trichoderma* spp. used against the pathogenic fungus *R. solani* had an effect on increasing the growth indicators under study compared to the treatment infected only with the pathogenic fungus. In general, the highest growth parameter values were in the combinations containing the T3 isolate. The results also showed that the percentage of infection severity in treatments containing *Trichiderma* isolates was significantly reduced compared to untreated infected with *R. solani* ones. Only The reason may be due to the production of the biological fungus *Trichoderma* spp. of some peptides, proteins, and enzymes as peroxidase enzymes as well as some low molecular weight compounds that contribute to stimulating defense mechanisms in plants. This is resulting in an increase in the production of some phenolic and alcohol compounds that have an inhibitory effect on pathogens. Also, "the reason may be due to the increased secretion of some proteins produced by *Trichoderma* spp. upon sensitivity to the presence of the pathogen and stimulating the plant defenses against the pathogen (30, 31 and 29).

Table4. The effect of isolates of *Trichoderma* spp. on the percentage of infection severity and growth indicators of broad bean plants in plastic pots

Treatments	Plant height Cm	Shoot weight		Root weight		Infection severity (%)
		Fresh	Dry	Fresh	Dry	

Rs+ T1	18.30	10.50	1.10	6.50	0.74	5
Rs+ T2	17.50	10.50	1.14	6.53	0.72	20
Rs+ T3	22.88	11.93	1.38	7.23	0.85	0
Rs	14.13	7.50	0.68	5.05	0.41	80
Control	16.75	9.50	1.15	5.98	0.73	0
L.S.D.=0.05	2.504	2.898	0.1891	1.379	0.0691	16.96

Conflict of Interest

The authors have no conflict of interest.

References:

1- **Abbas, A.; D. Jiang and Fu, Y. 2017.** *Trichoderma* spp. as antagonist of *Rhizoctonia solani*. J Plant Pathol Microbiol .8(3): 1-9.doi: 10.4172/2157-7471.1000402

2- **Al-Fadhal, F. A.; A. N. AL-Abedy and Al-Janabi, M. M. 2018.** Molecular identification of novel isolates of *Rhizoctonia solani* Kühn and *Fusarium* spp. (Matsushima) isolated from petunia plants (*Petunia hybrida* L.). Plant Archives, 18(1):703-711.

3- **Al-Rawi, K M. and A. A. M. Khalaf-Allah. 2000.** Design and Analysis of Agriculture Experiment. Second Edition. University of Mosul. Ministry of Higher Education and Scientific Research. The Republic of Iraq. pp.487.

4- **Alshimaysawe, U. 2018.** Factors affecting biocontrol of *Rhizoctonia* diseases and growth promotion of potato by *Trichoderma* species. Ph.D. dissertation. University of New England. USA.

5- **AL-Taee, A. K. and Z. A. Taha. 2018.** First Record of *Fusarium* poae, Wheat Root Rot Fungus in the North of Iraq. Rafidain Journal of Science., 27(4):162-168.

6- **Bisen, K.; C. Keswani; J. S. Patel; B. K. Sarma and Singh, H. B. 2016.** *Trichoderma* spp.: Efficient Inducers of Systemic Resistance in Plants. (In. Microbial-Mediated Induced Systemic Resistance in Plants. Springer. Singapore. pp.185-195.) doi:10.1007/978-981-10-0388-2_12

7- **Chen, L.; P. Ai; J. Zhang; Q. Deng; S. Wang; S. Li and Zheng, A. 2016.** RSIAD, a collective resource for genome and transcriptome analyses in *Rhizoctonia solani*, AG1. IA. Database, 28.2016. https://doi.org/10.1093/database/baw031

8- **El-Gali, Z. I. 2015.** Antagonism capability in vitro of *Trichoderma harzianum* against *Alternaria alternata* on Ceratonia siliqua. EJPMR, 2(2):30-44.

9- **El-Mougy, N. S.; M. D. Aly; E. I. Imbabi and Abdel-Kader, M. M. 2011.** First record of Sclerotinia Foliage Blight disease on pepper under protected cultivation system in Egypt. Egyptian Journal of Phytopathology., 39(2): 209-210.

10- **Gutierrez, N.; C. M. Avila; M. T. Moreno and Torres, A. M. 2008.** Development of SCAR markers linked to zt-2, one of the genes controlling absence of tannins in faba bean. Australian Journal of Agricultural Research., 59(1): 62-68. https://doi.org/10.1071/AR07019

11- **Harman, G. E. 2000.** Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma*

harzinum T-22. Plant Disease., 84(4):377-393.

12- **Icarda. 2003.** Faba bean Pathology Progress Report. Food Legume Improvement Program. ICARDA, Aleppo. Syria.

13- **Jassim, N. S. 2007.** Study of the root and stem rot disease of fava bean crop caused by the fungus *Rhizotonia solani* in Basra Governorate and its biological control, Ph.D. thesis. College of Agriculture. University of Basra. Republic of Iraq.

14- **Kasumbwe, K.; K. N. Venugopala; V. Mohanlall and Odhav, B. 2014.**Antimicrobial and antioxidant activities of substituted halogenated coumarins. Journal of Medicinal Plants Research, 8(5), 274-281. doi: 10.5897/JMPR2013.4419.

15- **Koijam, K. and B. Sinha. 2018.** Antagonistic potential and molecular characterization of *Trichoderma* spp. against *Rhizoctonia solani* infecting ghost pepper in Manipur. Int. J. Curr. Microbiol. App. Sci., 7(2):2085-2093. https://doi.org/10.20546/ijcmas.2018.702.248.

16- **Kumar, S.; G. Stecher; M. Li; C. Knyaz and Tamura, K. 2018.** MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution., 35(6): 1547. https://doi.org/10.1093/molbev/msy096.

17- **Leonetti, P.; M. C. Zonno; S. Molinari and Altomare, C. 2017.** Induction of SA-signaling pathway and ethylene biosynthesis in *Trichoderma harzianum*-treated tomato plants after infection of the root-knot nematode Meloidogyne incognita. Plant Cell Reports., 36(4): 621-631. https://doi: 10.1007/s00299-017-2109-0.

18- **Matloob, A. A. and M. I. Alkaif. 2015.** Isolation and identification of some Fungi Caused Broad root and Crowan root disease by some plant extracts and Bioagents *Trichoderma viride*. Karbala Journal of Agricultural Sciences, 2(4): 88-114.

19- **McKinney, H. 1923.** Influence of Soil Temperature and Moisture on Infection of wheat Seedlings by Helmin. Journal of Agricultural Research, 26: 195.

20- **Parmeter, J. P. and H. S. Whitney. 1970.**Taxonomy and Nomenclature of the Imperfect State in *Rhizoctonia solani*, Biology and Pathology (In.J. R. Parmeter Jried. Uni. of Calit. Press. PP: 7-19.

21- **Rahate, K. A.; M. Madhumita and Prabhakar, P. K. 2021.** Nutritional composition, anti-nutritional factors, pretreatments-cum-processing impact and food formulation potential of faba bean (*Vicia faba* L.): A comprehensive review. Lwt, 138:1107-1196.

22- **Sáenz-Mata, J. and J. F. Jiménez-Bremont. 2012.** HR4 gene is induced in the Arabidopsis-*Trichoderma* atroviride beneficial interaction. International Journal of Molecular Sciences, 13(7):9110-9128. https://doi.org/10.3390/ijms13079110.

23- **Scarselletti, R. and J. L. Faull. 1994.** *In vitro* activity of 6-pentyl-α-pyrone, a metabolite of *Trichoderma harzianum*, in the inhibition of *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. lycopersici. Mycological Research., 98(10): 1207-

1209. https://doi.org/10.1016/S0953-7562(09)80206-2

24- **Singh, A.; M. Shahid; M. Srivastava; S. Pandey; A. Sharma and Kumar, V. 2014.** Optimal physical parameters for growth of *Trichoderma* species at varying pH, temperature and agitation. Virol. Mycol., 3(127): 2161-0517.doi: 10.4172/2161-0517.1000127.

25- **Suleiman, S. A.; S. M. Gambo and Sunusi, M. 2019.** An in vitro antagonistic effect of *Trichoderma* spp .against *Fusarium oxysporum* f. sp. lycopersici. Fudma Journal of Sciences, 3:369- 374.

26- **Swami, C. S. and S. K. Alane. 2013.** Efficacy of some botanicals against seed-borne fungi of green gram (Phaseolus aureus Roxb.). Bioscience Discovery, 4(1):107-110.

27- **Tariq, M.; S. Yasmin and Hafeez, F. Y.2010.**Biological control of potato black scurf by rhizosphere associated bacteria. Brazilian Journal of Microbiology., 41: 439-451. https://doi.org/10.1590/S1517-83822010000200026.

28- **Van Leur, J. A. G.; R. J. Southwell and Mackie, J. M.2008.** Aphanomyces root rot on faba bean in northern NSW. Australasian Plant Disease Notes, 3(1):8-9.

29- **Whipps, J. M.1987.** Effect of media on growth and interactions between a range of soilborne glasshouse pathogens and antagonistic fungi. *New Phytologist.*, 107(1): 127-142. doi: 10.1111/j.1469-8137.1987.tb04887.

30- **White, T. J.; T. Bruns; S. J. W. T. Lee and Taylor, J. 1990.** Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications, 18(1):315-322.

31- **Bashah, G. M. and Mohammed A. E. 2023.** The effect of *Trichoderma longibrachiatum* Against Damping-off Pathogens on Different Varieties of Cucumber in Vitro. Kufa Journal for Agricultural Sciences 15(1)): 93-104. DOI: https://doi.org/10.36077/kjas/2023/v15i1.10320