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First Record of *Fusarium solani* as a Causal Agent of Root Rot and Damping-Off Disease in *Acacia mangium* and Its *In Vitro* Control in Karbala Province, Iraq

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Abstract: This study was conducted to determine the pathogens of root rot and damping-off of *Acacia mangium* in Karbala governorate, Iraq. The study included evaluating some biological and chemical methods against the pathogen. Four isolates, *Fusarium* sp (F1), *Fusarium* sp (F2), *Fusarium* sp. (F3), and *Rhizoctonia* sp (R1), were obtained. The F3 was the best isolate superiority compared to the other isolates in reducing the seed germination rate of red radish seeds on the water agar medium to inhibition rate 100%. While the seed germination, percentage in the control treatment amount 100%. The greenhouse experiment also demonstrated the same results. The F3 isolate significantly superior in decreasing the germination percentage compared to the control treatment. The morphological and molecular identification revealed that the highest pathogenicity isolate (F3) is the fungus *Fusarium solani*. The sequence was deposited in NCBI under the accession number PP319596. Moreover, the results indicated the effectiveness of the bioagent *Trichoderma koningiopsis* in controlling *F. solani*. It significantly outperformed the control treatment in controlling this pathogen an inhibition percentage of 96.29%. whereas the control treatment a mounted 0.00%. In addition to these findings, it was found that Beltanol fungicide demonstrated the highest inhibition efficacy (100%) against the isolated pathogens.

Keywords: *Fusarium solani, Acacia mangium,* root rot damping-off

Introduction

The Acacia (Acacia mangium) plant belongs to the legume family Fabaceae, a widespread tree naturally distributed in Asia, Africa, Australia, and America (Aly Hassam, 1993). It was used for ornamental purposes and was recently introduced to Iraq due to its aesthetic appeal. This plant has a long flowering period of 3-6 months, and demonstrates high tolerance to Iraqi environmental conditions. The genus Acacia includes approximately 1,350 species, some of which are shrubs, while others are characterized by the distinctive, pleasant scent of certain species. Additionally, Acacia has been utilized as a fodder crop, a source of firewood, and as a windbreak to prevent soil erosion and stabilize dunes (Dawod, 1979; Aly Hassam, 1993).

Acacia mangium plant was also used to purify the soil polluted with sewage water from heavy elements in Selangor, Malaysia. (Majid et al.,

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2012). The highest cadmium and lead contents were found in the stem tissue, chromium and copper in the roots, and zinc in the leaves (AL-Ghazali et al. 2022). Thus, this species showed promising potential in purifying polluted soils from these elements. The plant is exposed to many fungi, including the pathogenic fungi causing damping-off and root rot, such as Sclerotinia sclerotiorum, phytophthora, and Fusarium solani (Fayyadh and Al-Badran, 2017; Lahuf, 2019). Consequently, it is essential to identify these causal agents with contemporary techniques, where the polymerase chain reaction (PCR) is the most significant (Lahuf, 2019; Tami et al., 2024). Many methods have been used to control and reduce the pathogens, including chemical control, plays a crucial role in managing plant diseases (Lahuf et al., 2023). However, resistant pathogen strains have emerged due to their frequent use, in sddition to their hazrdious effects to humans and animals the bioagent Trichoderma koningiopsis considered on the best biological control agent among Trichoderma species (Jaber and Lahuf, 2020) that showed effectiveness against different pathogens due to its fast reproduction growth, parasitism, and secretion of some enzymes. It directly affects fungi and nematodes (Lahuf et al., 2020b; Matrood and Al-Taie, 2017). Due to updated knowledge about root rot and the damping–off disease of the acacia in Karbala and its repercussions, the study was conducted to isolate and identify the pathogen and control it biologically and chemically.

Materials & Methods

Isolation of the fungi associated with root rot and damping-off of Acacia plants

The pathogenic fungi associated with diseased Acacia cassin ware isolated. The disease symptoms included stunted growth, leaf chlorosis, and root decay. Symptoms of plant wilting, root death and yellowing of leaves were observed on the plant (Tarad et al.,2023) The roots were washed well in water for thirty minutes to eliminate soil they were cut into small pieces, sterilized with sodium hypochlorite solution, washed with water (D.W.), and dried with sterile blotting paper (Lahuf et al., 2018). The pieces were transferred to P.D.A. plates. The plates were then incubated for three days. At a temperature 25 °C Afterward, the fungi were purified by transmitting a 0.5 cm diameter disc of any fungus by using cork borer into Petri dishes containing medium PDA (Al-Ghazal et al., 2023). Then, they were initially identified under the microscope using morphological characteristics and taxonomic keys (Leslie & Summerell, 2006). The Pathogens isolated from red radish seeds were tested by using water agear

The pathogenicity test Radish seeds in the plates

To assess the pathogenicity, fungal isolates obtained from the roots of the acacia plant were cultured in Petri dishes containing a water Agar medium. The plates were inoculated with pure fungal colonies and positioned at the center of the pre-prepared medium. All plates were incubated for three days at 25 ± 2 °C. Sterilized radish seeds were then distributed at a rate of 10 seeds per plate in a circular pattern around the perimeter of the fungal colony. This procedure was repeated across three plates for each fungal isolate, with the control treatment consisting of plates planted with seeds only. All plates were incubated at 25 ± 2 °C for a week; germination percentage was calculated according to the following equation.

$$\textbf{Germination}\ \% = \frac{\textbf{No. of germinated seeds}}{\textbf{Total seeds}} \times 100\ \%$$

Also, the percentage of inhibition was calculated using the equation below.

$$\textbf{Inhibition}\ \% = \frac{\textbf{Number of germinated seeds in control} - \textbf{Number of germinated seeds in treatment}}{\textbf{Number of seeds germinated in control}} \times 100\ \%$$

Pathogenicity test in greenhouse

The test was carried out in a greenhouse in the Department of Plant Protection at the College of Agriculture, University of Kerbala. The soil was sterilized using 70% ethyl alcohol, applying 10 ml per kilogram. The soil was placed in 1 kg plastic pots, moistened, and inoculated with an isolated fungal vaccine. Then, they were applied to local millet seeds (Panicum miliacem) (Al-Ghazali et al., 2022). The pots were treated at a concentration of 1% and then covered with plastic bags for 48 hours. Afterward, they were sown with Acacia seeds superficially sterilized using a 1% sodium hypochlorite solution at 10 seeds per port density.

Molecular identification

As per the method, PCR is one of the methods used to identify the morphological characteristics of fungal isolates that cause root rot and damping off of Acacia plants (Narayanasamy, 2011).

Isolates were processed using commercial kits D Neasy plants. The ITS-rRNA region was amplified and sequenced using the primer set ITS1 and ITS4, respectively (White et al., 1990; Lahuf et al., 2019).

Morphological identification

Four fungal isolates were associated with root rot and seedling death in the Acacia plant. Three of them belonged to the genus Fusarium sp. Only one isolate belonged to the genus Rhizoctonia sp. This is according to the external morphological characteristics of the growth of fungal colonies. In addition to the microscopic characteristics of each fungus (Lesli & Summerell, 2006)

Evaluation of T. koningiopsis antagonistic activity

The antagonistic activity of the fungus T. koningiopsis was conducted in Virus Laboratory / College of Agriculture / University of Kerbala against the pathogenic fungus and was evaluated by using the dual culture method (Baker & Cook, 1974). Petri dishes containing PDA were divided into two equal parts by an imaginary line. A 5 mm diameter disk taken from the margin of a pure seven–day–old colony of the pathogenic fungus was placed in the centre of the first half of the dish. Another 5 mm diameter disk taken from the margin of the seven-day-old colony of the bioagent T. koningiopsis was placed in the center of the second half of the dish. With control dishes containing the studied fungi and the biological agent T. koningiopsis, each separately. All dishes were incubated at 25 ± 2 °C. Afterward, the biological agent's inhibitory efficiency and antagonistic capacity were calculated according to Bell et al. (1982) scale, which consists of five degrees (Table 1).

Table(1): The calculation of the biological agent's inhibitory efficiency and antagonistic capacity based on the scale of Bell *et al.* (1982).

Degree number	The description
Degree 1	The biological agent covers the entire surface of the dish area without allowing the pathogenic fungus to grow.
Degree 2	The bioagent covers two-thirds of the dish, and the pathogenic fungus covers the remaining third of the plate.
Degree 3	The bio agent covers half of the plate, and the pathogenic fungus covers the remaining half.
Degree 4	The bio agent covers one-third of the plate, while the pathogenic fungus covers two-thirds.
Degree 5	The pathogenic fungus covers the whole surface of the plant

The bioagent is considered efficient if the degree is 1 or 2. Also, the method of determining the percentage of fungal growth inhibition (Nwankiti and Gwa, 2018) was calculated according to the equation as in (Al-Ghazali *et al*., 2022).

$$\textbf{Inhibition}\ \%\ \% = \frac{\textbf{Average of growth in Control} - \textbf{Average of growth in treatment}}{\textbf{Number of germinated which seeds}} \times 100\ \%$$

The antagonistic activity of the bioagent was determined based on the percentage of inhibition and using the Sangoyomi scale (2004).

Table (2). Antibiotic activity of *T. koningiopsis* biological agent

Inhibition percentage	Effectiveness
0 %	Not effective
More than 0 to 20%	Poorly effective
More than 20 to 50%	Average effective
More than 50 less than 100%	Effective
100%	Very Effective

Evaluating the efficiency of the beltanol in controlling *Fusarium solani*

Fungicide was added to the sterile PDA using two concentrations (1 and 0.75Ml/L). Each concentration was shaken and poured into sterile Petri dishes. After the medium had solidified, a 5 mm diameter disc taken from the edge of a seven-day-old colony of the pathogenic fungus was inoculated at the center of each dish. Three replicates were performed for each fungal isolate and concentration, with a control treatment that contained no fungicide. The plates were then incubated at 25°C for seven days. Afterward, the diameters of the fungal growth were measured, and the inhibition percentage was calculated.

Statistical analysis

The experiment was carried out using a completely randomized design. Treatment means showing significant differences were compared using L.S.D.

Results & Discussion

Isolation of the fungi associated with root rot and damping-off of Acacia plants

The isolation and morphological diagnosis process results showed that four fungi

isolates were associated with root rot and damping off on acacia plants. Three of them belonged to the genus *Fusarium* sp, and only one isolate belonged to the *Rhizoctonia* sp. Based on the external morphological characteristics of colony growth and microscopic characteristics (Leslie & Summerell, 2006).

The in vitro pathogenicity tests

Table (3) and Figure (1) Indicated isolation F3 significantly has the highest effect on the germination percentage of red radish seeds, achieving a 0% germination rate and a 100% inhibition rate, in contrast to the comparison treatment. However, the R1 was the isolate with the least effect on the germination and inhibition rates; they reached 3.33%, and 96.66% respectively.

Table (3) The in vivo pathogenicity of isolated fungi on the Water agar medium.

Treatments	Germination %	Inhibition %
***Fusarium* sp. (F1)**	6.66	93.33
***Rhizoctonia* sp. (R1)**	3.33	96.66
***Fusarium solani* (F2)**	6.66	93.33
***Fusarium solani* (F3)**	0.00	100.00
Control	100.0	0.00
L.S.D 0.01	1.2524	1.3952

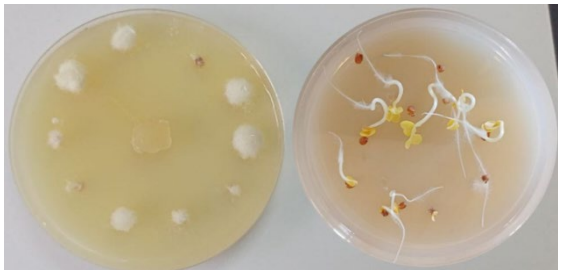

Fig. (1): in vitro Evaluating the pathogenicity of *Fusarium solani*, responsible for damping –off and root rot in Acacia plants, using red radish seeds on a water agar media.

The isolates significantly affected the germination rate, which was reduced to 0.00% with a 100% inhibition rate when using red radish seeds on water agar media. The MB1 isolate exhibited the lowest germination (46.66%) and inhibition (53.33%) rates, respectively.

This study also agreed with Berg et al. (2017) and Lahuf et al., (2023), who found numerous isolates, Fusarium spp, including F. solani, from seed and root rot diseases in various plants. This is due to extensive fungal hyphae proliferation within vascular tissues, obstructing the transport of salts and water to the leaves and producing toxins that facilitate the degradation of cell walls.

The in-situ pathogenicity test

The results (Table 4, Fig. 2) show that F3 significantly reduced the germination of reached 0.00%; the inhibition percentage reached 100% compared to the control treatment, which reached 100% and 0.00, respectively. In contrast, the results showed that the F1 isolate had a minor impact on seed germination and inhibition, which reached 40% and 60%, respectively. The other treatments were graded between them. These results were consistent with Soleha et al. (2022), who found that Fusarium solani caused the germination of pepper seeds, as the seed germination percentage reached 16.67% compared to the control treatment, which had a germination percentage of 100%. Based on the results obtained from the laboratory and field experiments, the F3 isolate was selected for molecular diagnosis and completion of laboratory experiments.

Table (4) Evaluating the pathogenicity of fungal isolates in germination.

Treatments	Germination %	Inhibition %
Fusarium sp (F1)	40.00	60.00
Rhizoctonia sp (R1)	23.33	76.66
Fusarium sp (F2)	13.33	86.66
Fusarium solani (F3)	0.00	100.00
Control	100.00	0.00
L.S.D 0.05	1.9593	2.1514

Fig. (2) Effect of fungal isolates on germination of acacia seeds in plastic pots under greenhouse.

In vitro evaluation of the bioagent *T. koningiopsis* against *F. solani*

The results in Table 5 and Figure 3 showed the effectiveness of the fungus *T. koningiopsis* in controlling the pathogenic fungus *Fusarium solani.* The bioagent fungus was significantly superior in controlling the pathogenic fungus with an inhibition percentage of 96.29% compared to the control treatment, which had an inhibition percentage of 0.00%. Thus, it achieved a score of 1 according to the Bell *et al*. (1982) scale. The results showed the effectiveness of bioagent *T. koningiopsis* in controlling the two pathogenic fungi *R. solani* and *F. solani*

Table (5): The antagonistic efficiency of the bioagent *T. koningiopsis* against *F. solani*

Treatments	Inhibition %
Fusarium solani	0.00
T. koningiopsis* + *F. solani	96.29
L.S.D 0.01	5.2324

Fig. (3) The antagonistic efficiency of the bioagent *T. koningiopsis* against the pathogenic fungus F. *solani*

The efficiency of Beltanol in controlling *F. solani*

The results of Table (6) illustrated the fungicide utilized against the pathogenic fungus. This led to the inhibition of fungal growth by 100%. The fungal growth was habited completely using the concentration (75ml/L). These results are consistent with Lahuf (2020a), who found that a complete inhibition of the *Fusarium* spp. occurred when using Beltanol at a concentration of 1 ml/1000ml. The reason for the efficiency of the fungicide is attributed to the active ingredient for its effectiveness against a wide range of fungi that are pathogenic to the plant.

Table (6) Effect of the fungicide Beltanol on the growth of *Fusarium solani*

Treatments	Inhibition %
1	100.00
0.75	100.00
Control	0.00

Table (7) The identified fungal isolate preserved in the GenBank database with serial codes

No.	Fungi	GenBank Accession Number
	Fusarium solani	PP319596

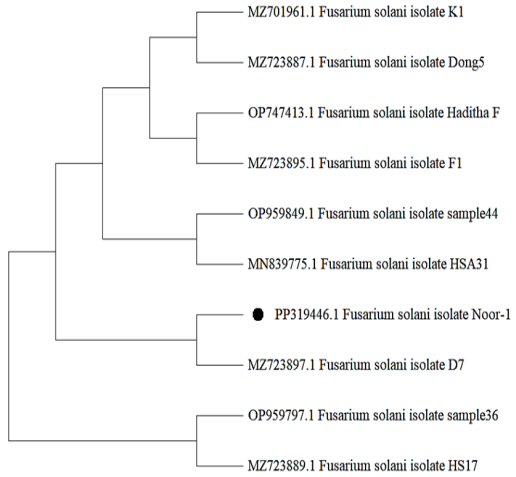

Fig. (4): The phylogenetic tree of the pathogenic *F. solani* isolate Noor-1 (indicated by a black dot) obtained from GenBank data repositories.

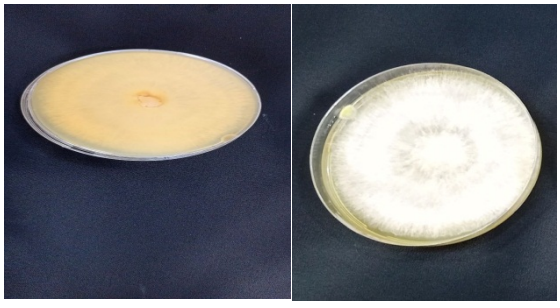

Fig. (5) Phenotype of *Fusarium solani*

Molecular identification

The results showed that the DNA of the isolated fungus in this study had its rDNA - ITS region successfully amplified using primers (ITS1-ITS4), producing a single double-stranded amplification of 500-1000 nitrogen bases in length. The results of bioinformatic analysis of the ITS region sequence using the BLAST program accompanied with morphological diagnosis confirmed the identity of *F. solani*. The nucleotide sequences of the identified isolate were deposited in NCBI under accession number PP319596 Table (6) Fig.(4).

Conclusion

F. solani was identified as the causal agent of root rot and damping-off of *Acacia mangium*. This identification was based on the morphological and molecular features of the pathogen. The bioagent *T. koningiopsis* and the fungicide Beltanol efficiently controlled this fungal pathogen. Thus, they can be a part of any control strategy against this pathogen in nurseries in Karbala Governorate and other Iraqi Governorate.

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Contributions of authors

N. A. Al-Ghazali: Work in laboratory experiments and suggest the research idea

B. A.N.: He proposed the research idea and the name of the research and conducted experiments

A. B. Abu-Duka: Worked in the field experiment and wrote the research

L.A. K.: Preparing plants and writing sources

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Conflicts of interest

The authors declare that they have no conflict of interests.

Ethical approval

All ethical principles and approvals related to plants have been implemented

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تسجيل أول للفطر *Fusarium oxysporum* كمسبب لمرض تعفن جذور وموت بادرات نبات الاكاسيا *Acacia mangium* ومكافحته مختبريا في محافظة كربلاء – العراق

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المستخلص: أجريت هذه التجربة لهدف عزل وتشخيص الفطر المسبب لمرض تعفن الجذور وموت البادرات الاكاسيا *Acacia mangium* في حدائق كلية الزراعة / جامعة كربلاء واختبار مقدرته الامراضية مختبريا وفي ظروف البيت البلاستيكي , أظهرت النتائج الحصول على أربعة عزلات تم تشخيصها مظهريا وهي (F1) *Fusarium sp* و *Fusarium sp* (F2) و *Fusarium sp* (F3) و *Rhizoctonia sp* (R1) وقد تفوقت العزلة F3 معنويا على العزلات الاخرى في خفضها لنسبة انبات بذور نبات الفجل الاحمر على الوسط الزرعي Water agar اذ بلغت نسبة الانبات فيها 0 % وبنسبة تثبيط 100 % قياسا لمعاملة المقارنة التي بلغت النسبة المئوية لانبات البذور فيها 100 % .كما اظهرت نتائج تجربة الأصص البلاستيكية في البيت البلاستيكي تطابق النتائج مع التجربة المختبرية اذ تفوقت العزلة F3 معنويا في خفضها نسبة الانبات اذ بلغت 0 % وبنسبة تثبيط 100 % عند معاملة بذور نبات الاكاسيا بالفطريات المعزولة قياسا بمعاملة المقارنة والتي بلغت النسبة المئوية للانبات فيها 100 % . واظهرت نتائج التشخيص الجزيئي أن العزلة الاكثر أمراضية هي F3 تعود للفطر *Fusarium solani* وتم ايداعها في بنك الجينات الامريكي تحت رقم الادخال PP 319596. كما اظهرت نتائج التجربة المختبرية فعالية الفطر *Trichoderma koningiopsis* في مكافحة الفطر*Fusarium solan* حيث تفوقت معنويا في مكافحة الفطر وبنسبة تثبيط 96.29 % قياسا بمعاملة المقارنة التي بلغت 0.00 %. كما وبينت النتائج تفوق مبيد Beltanolفي خفض نمو الفطر الممرض حيث كانت النسبة المئوية للتثبيط 100% قياسا بمعاملة المقارنة التي بلغت 0%.

الكلمات المفتاحية: *Fusarium solani* ، نبات الاكاسيا ،تعفن وموت الجذور