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Effectiveness of *Bacillus subtilis* in enhancing regulatory hormones and inhibiting the main fungus causing root rot in date palm offshoots (*Phoenix dactylifera* L.)

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Abstract

Root rot disease in date palm nurseries and new plantations has raised serious concern among farmers in Basrah Governorate, Iraq. Date palm offshoots (*Phoenix dactylifera* L.) are particularly vulnerable to this disease. Pathogenicity tests identified *Fusarium. oxysporum, Fusarium. proliferatum* S1, *Fusarium. proliferatum* S2, and *Fusarium fujikuroi* were the causative agents of the root rot disease in date palm offshoots. *Among them, F. oxysporum was the most virulent,*, causing a severity index of 84.2% of root rot and a recorded severity level of 4 (root discoloration of up to 76% or more than one wilted leaf). In contrast, *R. solani* was the least pathogenic, with a disease severity percentage of 16.2%. In a dual-culture test on a PDA medium, *Bacillus. subtilis* exhibited antagonistic effects on the growth of *F. oxysporum*, leading to an 86% inhibition of mycelial growth development (1.2 cm diameter of colony growth fungus). The control(fungus alone) showed typical fungal growth (9 cm diameter for colony growth). Results from the greenhouse experiment indicated that plantlets treated with *B. subtilis* in conjunction with *F. oxysporum* showed increased production of indole acetic acid (IAA) and indole butyric acid (IBA) while the level of abscisic acid (ABA) was decreased compared to plantlets treated only with *F. oxysporum*.

Keywords: Antagonistism; DSI; *Fusarium oxysporum*; Pathogenicity;; Phytohormones

Abbreviations: disease severity index (DSI), plant growth-promoting rhizobacteria (PGPR), inducing systemic resistance (ISR), colony forming units (CFUs), potato dextrose agar (PDA), fungal growth inhibition (FGI).

Introduction

Date palms (*Phoenix dactylifera* L.) are a valuable commodity with significant economic importance, serving as a primary revenue source for farmers across North Africa, the Arabian Peninsula, Iraq, and Iran. However, these trees face a serious threat from soil-borne pathogenic fungi, leading to substantial damage and yield losses globally, affecting both mature trees and offshoots (El-Morsi et al. 2012; Maitlo et al. 2013). Root rots and wilt diseases in date palms have been associated with various pathogenic fungal species, including *Fusarium oxysporum*, *F. solani, F. moniliforme F. equiseti, F. semitectum*, and *Rhizoctonia solani*, in different regions (Baraka et al. 2011; Arafat et al. 2012; Alwahshi et al. 2019).These infections can spread through contaminated irrigation water, transportation of infected palm wastes, use of contaminated soil as manure, and transmission of diseased offshoots (Abdullah et al. 2010). Historically, chemical methods have been utilized to manage soil-borne diseases. However, these methods pose several concerns, including potential pathogen resistance, adverse effects on human health and beneficial soil organisms, and environmental contamination (Zubair et al. 2019). Moreover, there might be future restrictions on using certain fungicides and fumigants in soil. Therefore, the quest for viable alternatives like beneficial microbes is essential for soil pathogen management (Gerhardson, 2002). According to Gravel et al. (2004), microbial antagonists, including bacteria and fungi, provide a practical, cost-effective, and environmentally friendly approach to combat soil-borne diseases. One such prominent plant growth-promoting rhizobacteria (PGPR) licensed for commercial use as a biological control agent against various plant diseases is the *Bacillus subtilis* strain (Punja et al. 2019). *B. subtilis* suppresses diseases through antibiotic production, competition for resources and space with pathogens, hyphal lysis of pathogens, and induction of systemic resistance (ISR) in host plants (Li et al. 2013; Chen et al. 2020). *B. subtilis* also plays a role in producing essential phytohormones like auxins and cytokinins crucial for plant growth and development. Additionally, it releases volatile compounds that impact plant development and trigger defense mechanisms, particularly related to systemic resistance (ISR) (Wang et al. 2018 ; Hashem et al. 2019). As noted by Collins & Jacobsen (2003), *Bacillus* species, including *B. subtilis*, form endospores, enhancing their survival in harsh environments. This characteristic, unlike non-endospore-producing plant growth-promoting bacteria (PGPB), boosts their persistence in soil over extended periods, thereby improving their efficacy in promoting plant growth (Yadav et al. 2021). Date palm farming may benefit from using *B. subtilis* as a biocontrol agent to manage soil-borne diseases. Farmers may lessen their dependency on chemical

treatments, encourage healthy plant development, and protect the environment by utilizing this advantageous bacteria (Zubair et al. 2019). It is worth noting that *B. subtilis* has been classified as "generally recognized as safe" (GRAS) by the US Food and Drug Administration (USFDA), affirming its safety for use in the food processing industry. These properties make *B. subtilis* a promising candidate for enhancing plant health and combating pathogens.

The study aimed to investigate the pathogenicity of isolated fungi, assess the effectiveness of the bioagent *Bacillus subtilis* strain against *Fusarium oxysporum*, and examine the potential impact of *B. subtilis* on phytohormone levels in date palms. The ultimate objective of this research was to develop strategies for managing root rot diseases in date palm offshoots.

Materials and Methods

Pathogenicity test of isolating fungi

The research was conducted at the Date Palm Research Center, University of Basrah, Basrah. *Fusarium oxysporum*, *Fusarium proliferatum* S1, *Fusarium proliferatum* S2, and *Fusarium fujikuroi* strains, *Rhizoctonia solani* obtained from a previous study at the College of Science, University of Basrah by Kazaal (2019), we used these isolates in the study. The sequences of these isolates were deposited in GenBank under accession numbers MK751702.1, LT970774.1, KX582247.1, and KY29366.1, respectively. To assess the pathogenicity of the recovered fungal isolates, infection trials were conducted in a greenhouse at the date palm research center in Basra, Iraq. Ten-month-old date palm plantlets of the Halawi cultivar, grown from seeds, were planted in plastic pots (4 kg capacity) filled with sterilized soil (1:1 ratio of peat moss and sand by weight). The pathogenic fungi were inoculated onto the plantlets using homogenized culture techniques described by Muthomi et al. (2007). The experimental design included five replicate pots for each fungal isolate, along with a negative control group consisting of uninfected soil. Within each pot, three plantlets were planted, resulting in 15 plantlets per fungal isolate (including the control group). The irrigation of the potted plantlets was carried out as needed throughout the experiment. After 60 days of inoculation, the extent of root rot and wilt symptoms was assessed based on visual observations of root discoloration and leaf yellowing. A rating scale ranging from 0 to 5 was used to quantify the severity of symptoms: 0 = no discoloration of the root or yellowing of the leaves; 1 = 1–25% discoloration of the root or one yellowed leaf; 2 = root discoloration of 26–50% or more than one yellowed leaf; 3 = root discoloration of 51–75% plus one wilted leaf; 4 = root discoloration of up to 76% or more than one wilted leaf; and 5 = dead plants (Abdou et al. 2003). To calculate the disease severity index (DSI) for each replicate,

the following formula was used: DSI = (Σd / (dmax × n)) × 100, where d represents the ranking of each diseased plantlet, dmax is the overall maximum disease rating, and n is the total number of plantlets assessed in each replicate (Liu et al. 1995). The DSI provides a quantitative measure of disease severity within each replicate, considering the proportion of diseased plantlets and the maximum disease rating observed.

Antagonistism of *B. subtilis* on *F. oxysporum*

In the described experiment, *Fusarium oxysporum* and the biocontrol agent bacteria *B. subtilis* were evaluated for *B. subtilis* antagonistic impact using the dual culture technique. Here are the steps and calculations involved: A 0.7 cm diameter disc from the *F. oxysporum* culture was selected from the colony's margin (5 days of age) and placed in the center of a test Petri dish containing sterilized potato dextrose agar (PDA) medium. A suspension of *B. subtilis* was streaked on the PDA medium using a sterilized loop, positioning the line 3 cm away from the *F. oxysporum* inoculum. For the control dishes, a 0.7 cm diameter disc from the same *F. oxysporum* culture was added to sterilized PDA dishes without adding *B. subtilis*. There were five Petri dishes for the treatment and control, serving as replicates. All the dishes were incubated at a temperature of 25±2°C. After incubation, the mean diameter of the fungal growth colony on the culture plates in the presence of the bacterium (*B. subtilis*) was measured when the mycelial growth colony in the control (fungus alone) arrived at the edge of the dishes. The fungal growth inhibition (FGI) was calculated using the following equation: FGI % = [1 – (FG in bacteria treatment / FG in control)] × 100. The FG in bacteria treatment represents the mean diameter of the fungal growth colony in the presence of *B. subtilis*. The FG in control represents the mean diameter of the fungal growth colony without *B. subtilis*. The FGI percentage indicates the extent of inhibition of fungal growth due to the presence of *B. subtilis* compared to the control (without *B. subtilis*).

Greenhouse experiment

The study described was conducted at the Date Palm Research Center, University of Basrah in Basrah, Iraq, during the 2022-2023 growing season. The experiment was carried out in a greenhouse and laboratories. The experiment was repeated twice to ensure reliability. The plant material used in the study consisted of twelve-month-old plantlets, which were grown from seeds of the Halawi cultivar. The plantlets were planted in plastic pots, each weighing 4 kg, and filled with autoclaved soil. The soil was a mixture of peat moss and sand in a 1:1 ratio (w/w). Three plantlets were placed in each pot, and the pots served as replicates for the experiment (Each

treatment had five replicates, with one pot containing three plantlets considered one replicate). The arrangement of pots and treatments followed a completely randomized design. The experiment included four treatments: Control (without any bacterial or fungal inoculation) *B. subtilis* inoculation(plantlets inoculated with *B. subtilis* only, using a concentration of 10^8 CFU (colony forming units). *F. oxysporum* inoculation(plantlets inoculated with *F. oxysporum* only, using a concentration of 10^6 spores/mL) Combined inoculation(plantlets sequentially inoculated with *B. subtilis* first and then, after a 48-hour interval, with *F. oxysporum*) (Al-Ani et al. 2012). The pots were placed under controlled growing conditions in the greenhouse, with a temperature range of 28-30±2°C. Further details regarding watering and fertilization conditions are not mentioned in the provided information. The experiment lasted 60 days, during which the treatments were applied to the plantlets. At the end of the 60-day experiment, the levels of plant growth regulators were measured using specific methods: Abscisic acid (ABA) quantification: The way described by Kamboj et al. (1999) was used, with ABA as the standard reference compound. Indol acetic acid (IAA) and indol butyric acid (IBA) quantification: The method described by Kelen et al. (2004) was employed for both analyses. Ten grams of fresh plant leaf tissue were collected and crushed in 70% (v/v) methanol alcohol. The mixture was then held overnight at 4°C and filtered using Whitman No.1 filter paper. The filtrate pH was adjusted to 8.5 using a phosphate regulator and an absorbent pump. The aqueous phase was extracted using ethyl acetate, and its pH was adjusted to pH 2.5 using HCl (1N). The plant hormones present in this layer were washed three times with diethyl ether, and the diethyl ether was subsequently removed using a rotary evaporator. The remaining solution was dissolved in 1 mL of methanol alcohol and stored at 4°C for further analysis.

These methods allowed for plant growth regulators, specifically ABA, IAA, and IBA, based on the respective references mentioned. The levels of plant hormones in the final solution were detected by high-performance liquid chromatography (HPLC) (Shimadzu Company LC10AVP). Analyses were done at 208, 265, and 280 nm to determine levels of plant hormones. The IAA, IBA, and ABA concentrations were calculated as $\mu g.g^{-1}$ FW from the standard curve. SBA/ST.BA S.conc. N = SBA/ST.BA S.conc. N (Damodaran & Strader, 2019).

Where SBA denotes sample bundle area, S.conc. denotes standard concentration, N denotes the number of dilutions and ST.BA denotes standard bundle area.

Data analysis: All experiments were repeated twice, and the experimental design was completely randomized. The statistical analysis of the data was carried out by analysis of variance (ANOVA)

using SPSS-21 software. The differences in the means were determined by the least significant difference test (LSD) ($P < 0.05$).

Results

Pathogenicity tests

The pathogenicity tests indicate that root rot diseases in date palm plantlets are attributable to all tested fungi (Table 1). The most pathogenic fungus was *F. oxysporum*, which caused 84.2% of root rot severity and recorded the degree of quantifying the severity of the symptoms 4 (root discoloration of up to 76% or more than one wilted leaf), followed by *F .proliferatum* S1, *F .proliferatum* S2, and *F. fujikuroi*, which caused 28.2%, 20.2% and 19.5% of disease severity, respectively. *R. solani* was the least pathogenic of the fungi recovered with the lowest percentage of 16.2% of disease severity. Other fungi associated with root rot in date palms were *Alternaria alternata* and *Aspergillus* spp.

Table 1. Confirmation of pathogenicity of fungal isolates recovered from date palm plantlets using greenhouse inoculations, as measured 60 days post-inoculation

Fungi tested	Disease severity of root rot disease		
	D	DSI	% Plant Survival
F. oxysporum	4	84.18 a*	15.82 a
F. proliferatum S1	3	28.18 b	71.64 b
F. proliferatum S2	3	20.22 c	79.10 c
F. fujikuroi	2	19.50 c	81.29 d
R. solani	2	16.22 d	84.00 d
Control(untreated)	0	_	100 e
LSD at $P \geq 0.05$	NA	2.86	4.93

*Each value is a mean of five replicates; Means in the columns followed by the different letters are significantly different at $p < 0.05$ test.

Antagonism assay

In a dual-culture test on a PDA medium, *B. subtilis* had antagonistic effects on the growth of *F. oxysporum.* An inhibition zone was visible, which was caused by the antifungal activity of *B. subtilis*, leading to the inhibition of mycelial growth. It was clear that an inhibitory zone had

formed as a result of *B. subtilis* antifungal action inhibiting mycelial development by 86% (1.2 cm diameter of colony growth fungus) (Fig. 1A). On the other hand, the control(fungus alone) showed typical fungal growth(9 cm diameter for colony growth) (Fig. 1B).

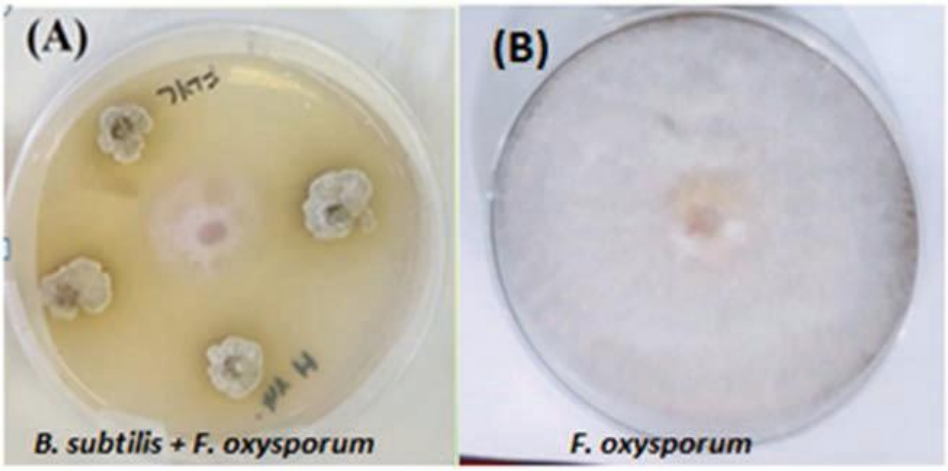

Fig. 1 *In vitro* anti-fungal antagonism on inhibition of mycelial growth of *F .oxysporum* by *B. subtilis* on PDA media in Petri dishes, A; Antagonism, B; Control(fungus alone).

Effect of *B. subtilis* on plant hormones in infected plantlets

The study findings (Table 2) reveal that infection of date palm plantlets with *F. oxysporum* altered the levels of plant growth hormones, affecting Indol Acetic Acid (IAA), Indole Butyric Acid (IBA), and Abscisic Acid (ABA). Infected plantlets showed a decrease in auxin production (IAA and IBA) and an increase in ABA, a stress-inducing hormone. However, the plantlets were treated with *B. subtilis*, which enhanced auxin production (IAA and IBA) and inhibited ABA biosynthesis. This resulted in increased levels of IAA and IBA to 0.077 and 1.562 $\mu g.g^{-1}$ respectively, and decreased levels of ABA to 0.0443 $\mu g.g^{-1}$ compared to the *F. oxysporum* treatment alone, which recorded 0.032, 0.228, and 0.962 $\mu g.g^{-1}$ respectively. Co-inoculation with both *B. subtilis* and *F. oxysporum* further boosted IAA and IBA levels to 0.079 and 1.488 $\mu g.g^{-1}$ respectively, while reducing ABA levels to 0.585 $\mu g.g^{-1}$, approaching levels seen in the control group (0.054, 1.464, and 0.608 $\mu g.g^{-1}$ for IAA, IBA, and ABA, respectively). Overall, the results suggest that *B. subtilis* treatment positively impacted hormone levels, highlighting its potential as a beneficial bacterium for enhancing stress tolerance in date palm plantlets.

Table 2. Effectiveness of *B.subtilis* in the presence and absence of *F.oxysporum* on IAA, ABA, and IBA in date palm plantlets of date palm.

Treatments	Sample(g)	IAA (µg.g^{-1})	ABA(µg.g^{-1})	IBA (µg.g^{-1})
Control	4	0.054 b*	0.608 b	1.464 a
F. oxysporum	4	0.032 c	0.962 a	0.228 b
B. subtilis	4	0.077 a	0.443 c	1.562 a
F. oxy+ B. sub	4	0.079 a	0.585 b	1.488 a
LSD at $P \geq 0.05$	-	0.015	0.023	0.088

*Each value is a mean of five replicates; means in the columns followed by the different letters are significantly different at $p \geq 0.05$ test.

Discussion

Pathogenicity tests

Several species of *Fusarium* spp. are frequently found on distinct regions of sick plant material. According to Nelson et al. (1994), some of these species operate as pathogens, while others serve as saprophytes or endophytes that may play no part at all or a secondary pathogenic role in the disease process. All of the *Fusarium* isolates showed strong pathogenicity toward date palm plantlets, according to pathogenicity testing (Jassim & Jaafar, 2023). It has been discovered that some species of the *Fusarium* genus create a variety of hazardous substances, such as fumonisin, fumaric acid, fumaproliferin, fumarin, zearalenone, and others. According to Hernandez et al. (2010), these substances help in the assault and parasitism of plant hosts. El Modafar & El Bostani (2000) claim that the fungal pathogen *F. oxysporum* breaks down host components by releasing enzymes that hydrolyze cell walls. These enzymes facilitate the pathogen's invasion into root tissues and are linked to the development of the disease.

Our findings align with those of other researchers, demonstrating that date palm trees and their offshoots are vulnerable to a variety of other soil-borne pathogenic fungi, such as *F. oxysporum*, *F. solani, F. moniliforme, F. equiseti, F. smitectum, R. solani,* and *Thielaviopsis paradox* (El-Morsi et al. 2012; Maitlo et al. 2013; Ahmed, 2018).

Antagonistism of *B. subtilis* on *F. oxysporum*

The results of dual culture experiments using Petri dishes containing PDA medium revealed that *B. subtilis* significantly suppresses the radial mycelial growth of pathogens. This suppression occurs through the direct synthesis of various secondary metabolites by *B. subtilis*, including hormones, cell wall degrading enzymes, and antioxidants. Cao et al. (2012) found that *B. subtilis* produces antibiotics, including fengycin, iturin, and bacillomycin, which prevent the mycelial growth and spore germination of the fungal pathogen *F. oxysporum*. Furthermore, *B. subtilis* completely inhibited the mycelial growth of the pathogen *F. moniliforme* in the PDA medium (Jassim, 2015). Siala et al. (2016) demonstrated that an endophytic strain of *B. subtilis* retarded the growth of *Fusarium* species in the PDA medium by producing proteases that contribute to the degradation of fungal pathogen cell walls.

Gong et al. (2014) reported that *B. subtilis*, based on its antibiotic production, causes severe damage to the cell walls and membranes of spores and mycelia of *Aspergillus flavus*. Moreover, the isolate *B. subtilis* 174 exhibited strong biocontrol activity, significantly reducing the disease severity of Fusarium wilt in tomato plants caused by *F. oxysporum* (Akarm & Anjum, 2011). *B. subtilis* has been found to suppress several important plant pathogens, including various *Fusarium* species (Cao et al. 2011), *Rhizoctonia solani* (Kumar et al. 2012), and *Verticillium dahliae* (Li et al. 2013). Bhusal & Mmbaga (2020) investigated three *Bacillus* sp. isolates as biological control agents against *Phytophthora capsici*. These isolates inhibited the mycelial growth of *P. capsici in vitro* and reduced disease incidence in plants grown in soil infested with *Phytophthora* inoculum under greenhouse conditions.

Effect of *B. subtilis* on plant hormones in infected plantlets

The inoculation of *B. subtilis* in date palm plantlets infected with the pathogenic fungus *F. oxysporum* resulted in a significant increase in the synthesis of auxins containing IAA and IBA. This increase in auxin production played a crucial role in protecting the plantlets against root rot disease. *Bacillus* species, including *Bacillus subtilis* was known to produce various phytohormones such as abscisic acid (ABA), gibberellins (GAs), cytokinins (CKs), and indole acetic acid (IAA) (Poveda & Gonzales-Andres, 2021). IAA, in particular, is a potent signaling molecule that plays a vital role in plant-microbe interactions. It promotes plant growth by increasing the auxin pool, leading to cell elongation, vascular tissue development, and apical dominance (Poveda & Gonzales-Andres, 2021). Many beneficial microorganisms in the rhizosphere synthesize auxins, including IAA, as reported by Patten & Glick (1996). IAA affects

various aspects of plant physiology, including cell division, pigment and photosynthesis production, elongation, apical dominance, root initiation, and growth (Ahemad & Kibret, 2014). Among the well-studied IAA producers, *Bacillus subtilis* has shown the potential to enhance plant growth by reducing the ABA content and mitigating the effects of growth inhibitors (Patten & Glick, 2002).

Based on our results, the inoculation of date palms with *B. subtilis* in the field led to increased IAA production and inhibition of ABA production, resulting in enhanced growth of date palm offshoots. Additionally, root rot disease caused a significant decrease in endogenous IAA production and an increase in ABA production. These findings align with a previous study showing that ABA accumulation was induced by the pathogenic fungus *R. solani*, and this accumulation was reduced in plants after inoculation with *B. amyloliphene* (Srivastava et al. 2016). *B. subtilis* alleviated the effects of pathogen-induced stress by reducing ABA synthesis in date palm plantlets infected with the pathogenic fungus *F. oxysporum*. Reetha et al. (2014) observed the presence of both *Pseudomonas fluorescens* and *B. subtilis* isolates in the onion rhizosphere, indicating their occurrence in the same ecological niche. The study of these bacteria in vitro found that these bacteria can produce indole acetic acid (IAA), which enhances onion growth. Shi et al. (2010) showed that *B. subtilis* elevated the photosynthetic capacity and total content of chlorophyll of sugar beet, resulting in a consequently enhanced synthesis of carbohydrates; furthermore, these changes were stimulated by IAA produced by the bacteria. These microbes also play an important role in the promotion of plant growth by increasing the biosynthesis of plant hormones such as gibberellic acid (GA3) and isoamyl acetate (IAA), which are closely linked to the availability of plant nutrients (Harman, 2011; Bhattacharya et al. 2019). Important medicinal plants inoculated with *B. subtilis* experienced increased plant growth by increasing phytohormone production, including gibberellins, auxins, and cytokinins (Egamberdieva et al.2016). *Bacillus spp* improved thermo tolerance in wheat and soybean by increasing salicylic acid (SA) and jasmonic acid (JA) expression and decreasing ABA production (Kang et al. 2019; Oleńska et al. 2020). Plant growth-promoting rhizobacteria are boosted by the production of phytohormones such as auxin, cytokinin, and gibberellins and by the reduction of ethylene concentration (Glick et al. 1999).

Conclusion

The most pathogenic fungus was *F. oxysporum*, which caused root rot severity, followed by *F .proliferatum* S1, *F .proliferatum* S2, and *F. fujikuroi*. *Bacillus subtilis* significantly inhibited the

mycelial growth *of F. oxysporum* and effectively suppressed the disease *in vitro*. *B. subtilis* had a significant positive impact on all plant hormones, which were adversely affected by the fungal pathogen. *B.subtilis* significantly ameliorated all plant hormones in infected plantlets, which were significantly affected by a fungus pathogen. More work is needed to determine the potential of *B. subtilis* for biological control of this pathogenic fungus and the regulating plant growth.

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فعالية *Bacillus subtilis* في تعزيز الهرمونات التنظيمية وتثبيط الفطر الرئيسي المسبب لتعفن الجذور في فسائل نخيل التمر (Phoenix dactylifera L.)

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الخلاصة

أثار مرض تعفن الجذور في مشاتل نخيل التمر والمزارع الحديثة قلقًا كبيرًا بين المزارعين في محافظة البصرة، العراق، إذ تُعد فسائل نخيل التمر (Phoenix dactylifera L.) عرضة بشكل خاص للإصابة بهذا المرض. أظهرت اختبارات التخصص المرضي أن الفطريات *Fusarium oxysporum* و *Fusarium proliferatum* (السلالتان S1 و S2)، بالإضافة إلى *Fusarium fujikuroi*، هي العوامل المسببة الرئيسة لتعفن الجذور في فسائل النخيل، وكان *F. oxysporum* الأكثر ضراوة، إذ سبب مؤشر شدة إصابة بلغ 84.2% ودرجة شدة إصابة مقدارها 4 (تلون جذري بنسبة تصل إلى 76% أو وجود أكثر من ورقة واحدة ذابلة). في المقابل، أظهر الفطر *Rhizoctonia solani* أقل درجة مرضية، حيث بلغت شدة الإصابة 16.2%. وأظهرت نتائج اختبار التثبيط المزدوج على وسط PDA أن البكتيريا *Bacillus subtilis* تمتلك تأثيرًا مضادًا واضحًا على نمو *F. oxysporum*، إذ بلغ معدل التثبيط في نمو الفطر الميسيلي 86% (بقطر مستعمرة بلغ 1.2 سم فقط)، مقارنة بمعاملة السيطرة (الفطر فقط) التي أظهرت نمواً فطريًا طبيعياً بقطر مستعمرة بلغ 9 سم. كما أظهرت نتائج تجربة البيت الزجاجي أن النباتات المعاملة بالبكتيريا *B. subtilis* مع الفطر *F. oxysporum* قد سجلت زيادة في إنتاج الأوكسينات مثل IAA (حمض الإندول أسيتيك) و IBA (حمض الإندول بيوتيريك)، في حين لوحظ انخفاض في مستوى حمض الأبسيسيك (ABA)، مقارنة بالنباتات المعاملة بالفطر فقط.

الكلمات المفتاحية: الاعراض المرضية؛ التحمل المرضي (DSI)؛ التعارض الحيوي؛ الفطر الممرض (*Fusarium oxysporum*)؛ الهرمونات النباتية.