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Efficacy of *Bacillus subtilis* (Ehrenberg1835) Cohn1872, in suppressing *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, the causal agent of root rot of date palm offshoots (*Phoenix dactylifera* L.) in Iraq

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Efficacy of *Bacillus subtilis* (Ehrenberg1835) Cohn1872, in suppressing *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, the causal agent of root rot of date palm offshoots (*Phoenix dactylifera* L.) in Iraq1

Abstract: Date palm root rot disease is one of the most important diseases of date palms and offshoots. It is caused by many soil-borne pathogenic fungi. Pathogenicity assays of the isolated fungi showed that the major causative agents of root rot disease in date palm plantlets were *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg S1, *F. proliferatum* S2, *Gibberella fujikuroi* (Sawada) Wollenw., and *Rhizoctonia solani* J.G. Kühn. The most virulent fungus was *F. oxysporum* with a severity index of 82.16 % of root rot, while *R. solani* was the least harmful with a disease severity rate of 12.42 %. In laboratory tests, *Bacillus subtilis* reduced the radial mycelial growth of *F. oxysporum* on PDA medium by 86.6 %. The application of *B. subtilis* in combination with *F. oxysporum* substantially inhibited the severity of root rot disease relative to plantlets treated with only *F. oxysporum*. In addition, *B. subtilis* application in the presence or absence of *F. oxysporum* improved the plant physiology of plantlets, including total chlorophyll, total carotenoid, antioxidant enzyme levels (catalase and peroxidase), and total proline content.

Key words: *B. subtilis*; date palm; *F. oxysporum*; plant physiology

Učinkovitost bakterije *Bacillus subtilis* Ehrenberg 1835) Cohn 1872 pri zatiranju glive *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, kot povzročiteljice koreninske gnilobe pri dateljevi palmi (*Phoenix dactylifera* L.) v Iraku

Izvleček: Koreninska gniloba je najpomembnejša bolezen dateljeve palme. Povzročajo jo številne talne patogene glive. Preiskus patogenosti z izolati gliv na sadikah dateljeve palme je pokazal, da so bili glavni povzročitelji njene koreninske gnilobe naslednje glive: *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg S1, *F. proliferatum* S2, *Gibberella fujikuroi* (Sawada) Wollenw., and *Rhizoctonia solani* J.G. Kühn. Najbolj virulentna je bila gliva *F. oxysporum*, z indeksom virulentnosti 82,16 % med tem, ko je bila gliva *R. solani* najmanj škodljiva z indeksom povzročitve koreninske gnilobe 12,42 %. V laboratorijskem poskusu je bakterija *B. subtilis* na PDA gojišču zmanjšala radialno rast micelija glive *F. oxysporum* za 86,6 %. Uporaba bakterije *B. subtilis* je v kombinaciji z glivo *F. oxysporum* znatno zavrla razvoj koreninske gnilobe na sadikah dateljeve palme v primerjavi s sadikami, ki so bile tretirane samo z glivo. Dodatno je uporaba bakterije *B. subtilis* v prisotnosti ali odsotnosti glive *F. oxysporum* izboljšala fiziološke parametre sadik kot so vsebnost celokupnega klorofila in karotenoidov, aktivnost antioksidacijskih encimov katalaze in peroksidaze ter vsebnost celokupnega prolina

Ključne besede: *B. subtilis*; dateljeva palma; *F. oxysporum*; fiziološki parametri rastline

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1 INTRODUCTION

Date palm (*Phoenix dactylifera* L.), Palmaceae (Arecacea), is a tropical and subtropical plant native to southern Asia and Africa. Selective breeding over thousands of years has resulted in the 3,000 variants presently farmed across the world in areas where the date palm thrives in hot, dry climates (Zaid, 2002). Dates are high in nutrients and provide a wonderful source of energy. Date fruits are composed of 70 % carbohydrates, primarily sugars, and 15 %-30 % water. Dates are also a good source of minerals, including iron, potassium, and calcium, and are low in salt and fats (Thabet et al., 2010; Dayani et al., 2012).

Date palms are infected by many soil-borne pathogenic fungi that threaten mature trees and offshoots, resulting in substantial tree damage and yield losses across the world (El-Morsi et al., 2009; Maitlo et al., 2013). In several locations, pathogenic fungi of root rot and wilt disease caused by *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliform*, and *Rhizoctonia solani* have been isolated from young offshoots and adults of the date palm (*Phoenix dactylefera* L.) (Alwahshi et al., 2019; Arafat et al., 2012; Baraka et al., 2011).

Chemical pesticides used to manage soil-borne diseases can result in pathogen resistance, negative effects on people and beneficial soil organisms, and pollution. Several soil fumigants and fungicide compounds are expected to be phased out soon. However, in order to achieve optimal plant development and production, soil pathogens will still need to be managed (Gerhardson, 2002). As a result, efficient beneficial microbes that can be used as an alternate method for controlling soil pathogens need to be discovered. For managing many soil-borne diseases, microbial antagonists such as bacteria and fungi have the potential to be a low-cost, healthy, and ecologically friendly solution (Caron et al., 2002; Gravel et al., 2004).

Bacillus species proliferate quickly, are resistant to harsh environmental conditions, and have been identified as beneficial microorganisms. Antibiotics; competition with pathogens for space or resources; destruction of pathogen hyphae; synthesis of siderophores and phytohormones that stimulate plant development; and induced systemic resistance (ISR) in the host plant are all modes of action by which *Bacilus subtilis* suppresses plant pathogens (Cao et al., 2012; Chen et al., 2020; Li et al., 2013). By induction of systemic resistance in plants, *B. subtilis* generates volatile chemicals that influence plant development and activate the plant defense mechanism (Hashem et al., 2019; Wang et al., 2018). *Bacillus* spp. also produce endospores, which allow the bacteria to live in harsh environments, allow for germination in response to varied environmental circumstances, allow for long-period storage of biopesticide, and make the formulation process easier (Collins & Jacobsen, 2003). The US Food and Drug Administration (USFDA) classifies *B. subtilis* as "generally recognized as healthy" (GRAS) for use in the food processing industry.

This work aimed to measure the efficacy of *B. subtilis* strains against *F. oxysporum* under greenhouse conditions. Second, levels and activity of some physiological and biochemical components were measured in date palm during infection by *F. oxysporum* in the presence and absence of *B. subtilis*, and compared to the control treatments.

2 MATERRIALS AND METHODS

2.1 PATHOGENICITY ASSAY OF ISOLATED FUNGI

Representative fungal isolate strains used throughout this study were obtained from a previous study carried out in the Biology Department, College of Science, University of Basrah, Basrah by Kazaal (2019). These isolates are *Fusarium oxysporum*, *Fusarium proliferatum* S1, *Fusarium proliferatum* S2, and *Fusarium fujikuroi*. *Rhizoctonia solani* isolate obtained from the lab of date palm diseases/Date Palm Research Center/University of Basrah. Infection trials with the recovered isolates run in a greenhouse trial at the Date Palm Research Center. Six-month-old plantlets (grown from seeds of the Halawii cultivar) were planted into plastic pots (2 kg pots) filled with sterilized soil (1 : 1 peat moss + sand). To prepare the fungi inoculant, each isolate was cultivated on PDA for 5–15 days at 27 °C. The spore suspension of each isolate was made by flooding plates of 15-day-old cultures with sterile distilled water, scraping with a sterilized glass rod, filtering, and adjusting to a 10^6 spore ml^{-1} concentration using a Neubauer haemocytometer before adding to the potted soil. Potted soil was injected with each fungus inoculum at a concentration of 10^6 spores ml^{-1} with irrigation water (Al-Ani et al., 2012). Each fungus (treatment) has five pots (replicates) with three plantlets, and a control treatment (uninfected soil). The pots were in the greenhouse under favorable conditions. For three months, pots were kept at 90 % soil humidity. The pots were carefully watered every time at the level of the field capacity. The percentage of disease severity is calculated after 60 days from inoculation using the following scale (with little modulation) of 0–5: where 0 = healthy; 1 = 1-25 % of the plant has a few spots on the roots; 2 = 26-50 % of the roots have spots and one leaf is wilting; 3 = 51-75 % of the roots have big black spots and all leaves are

wilting; 4 = up to 76 % of roots are rotted and all leaves are wilting, and5 = dead plants (Abdou et al., 2003).

The disease severity index (*DSI*) of each replicate was calculated according to the method described by Liu et al. (1995) as follows: $DSI = \Sigma d/(d_{max} \times n) \times 100$, where d = the disease rating of each plantlet, d_{max} = the maximum rate of disease, and n = the total number of plantlets in each replication assessed.

2.2 EFFECT OF *B. SUBTILIS* AGAINST THE GROWTH OF *F. OXYSPORUM IN VITRO*

The purpose of the experiment was to determine the antagonistic connection between the most virulent fungus, *F. oxysporum* and *B. subtilis* (*Bacillus subtilis* was isolated by serial dilution technique on nutrient agar medium (NAM). A 0.5 g of BioHealth biopesticide was separated and vortexed for 15 minutes in 10 ml of distilled water. From 10^{-1} to 10^{-6}, the suspension was serially diluted. 1 ml of suspension was pipette out and distributed with a glass rod in an L shape onto nutrient agar plates and incubated at 37 °C for 24 hours. For subsequent research, the most conspicuous colonies were separated and kept at 4 °C. The *in vitro* effect of *B. subtilis* on colony growth of *F. oxysporum* was assessed by the dual culture method. A 0.7 cm dia. disc from the *F. oxysporum* culture was chosen from the colony's edge (5 days) and was placed in the center of the PDA plate. After that, four-discs (0.7 cm dia. each) were taken from a three-day old *B. subtilis* colony on nutrient ager (NA) medium and placed at the periphery of the petri dish with equal dimension to each other and 1.5 cm from the edge of the petri dish. For the control treatment, a 0.7 cm dia. disc from the same pathogen colony was added to the sterilized PDA plate (without adding *B. subtilis*). For both the antagonism treatment and control, there were five plates (= replicates). All the plates were incubated at 28 °C. After incubation, in the antagonism treatment, the radial growth mycelium of the fungus was measured when the radial growth mycelium in the control reached the edge of the growth plates. The percentage of fungal growth inhibition (*FGI*) was calculated as the ratio of growth between fungal growth in the treatment opposite to the control: *FGI* % = [1 – (*FG* in antagonism/*FG* in control)] 100.

2.3 THE NATURE OF EXPERIMENT

This experiment was conducted in the greenhouse of the Date Palm Research Center, University of Basrah, during the 2019-2020 growing season. The experiment was repeated twice. Plantlets were 12 months old (they were grown from seeds of the Halawii cultivar) and were planted in plastic pots (4 kg each) filled with sterilized soil (1 : 1 peat moss + sand). The pots (=replicates) experiments were arranged and conducted in a completely randomized design. The pots in the experiment were divided into four treatments: (1) controls (no added any bacterial or fungal inoculation); (2) plantlets inoculated with *B. subtilis* only; (3) plantlets inoculated with *F. oxysporum* only; and (4) plantlets inoculated first with *B. subtilis* at a concentration of 10^8 spores ml^{-1} (*B. subtilis* inoculum concentration used according to the manufacture recommendation, 0.5 g of BioHealth biopesticide 10 mL of water, the concentration of *B. subtilis* was 10^8 spores ml^{-1}) and, after 48 hours, also inoculated with *F. oxysporum* at a concentration of 10^6 spores ml^{-1} (for both organisms, the inoculum was added at a rate of 150 ml/pot with irrigation water) (Al-Ani et al., 2012). All pots were placed in the greenhouse under favorable conditions (28–30 ˚C, watering and fertilization). Treatments were applied to the plantlets, which were then held for 28 days. Each treatment was replicated five times (each replicate having one pot with three plantlets). At the end of the experiment (day 28), we measured four response parameters: (1) photosynthetic pigment content, (2) antioxidant enzyme levels, and (3) total soluble proline, as described in detail below:

Photosynthetic pigment content was measured according to the protocol of Metzner et al. (1965).

The total chlorophyll and carotenoids were determined spectrophotometrically (CECL, 2021 spectrophotometer, UK).The absorbance was calculated against a blank of pure 85 % aqueous acetone at 452 and 663 nm, represented as mg. g fresh mass (FM). Using the following equations: total chlorophyll and total carotenoids: photosynthetic pigments represented as mg. g FM^{-1}.

For antioxidants, the activity of catalase (CAT) was determined according to Luck (1974), and the activity was expressed as a unit/mg of protein. Peroxidase (POD) activity was estimated according to Kara & Mishra (1976). The amount was evaluated by the absorbance at 420 nm, and the enzyme activity was expressed as a unit/mg of protein.

The protocol of Bates et al. (1973) was used to determine the total soluble proline leaf content. The toluene reagent was aspirated from the aqueous phase, and the solution absorbance was measured at 520 nm. Proline content was determined by measuring it from a standard curve and was calculated as mg. g dry $mass^{-1}$ (DM).

2.4 DATA ANALYSIS

The experimental design was completely randomized. The statistical analysis 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) ($p < 0.05$).

3 RESULTS

3.1 PATHOGENICITY TESTS OF ISOLATED FUNGI

Fusarium oxysporum, *F. proliferatum* S1, *F. proliferatum* S2, *F. fujikuroi*, and *R. solani*, were responsible for root rot infections in date palm plantlets (Table1). *F. oxysporum* was the most pathogenic fungus, causing 82.16 % of root rot severity, with highly significant differences compared with other fungi, followed by *F. proliferatum* S1, *F. proliferatum* S2, and *F. fujikuroi*, which caused 30.12 %, 24.26 %, and 18.56 % severity, respectively. *R. solani* was the least harmful species as it showed a disease severity 12.42 %.

Table 1: Pathogenicity of fungal isolates recovered from date palm plantlets after greenhouse inoculations, 60 days post-inoculation

	Disease severity of root rot disease		
Fungi tested	D	DSI	% Plant survival
F. oxysporum	4	82.16 a	12.82 a
F. proliferatum S1	3	30.12 b	68.64 b
F. proliferatum S2	3	24.26 c	76.10 c
F. fujikuroi	2	18.56 c	82.20 d
R. solani	2	12.42 d	90.00 d
Control(untreated)	0	_	100 e
LSD at p = 0.05	NA	2.86	4.93

Average scores for 15 plantlets for each treatment, where; D: disease rating scale and, DSI: disease severity index

3.2 EFFECT OF *B. SUBTILIS* AGAINST THE GROWTH OF *F. OXYSPORUM IN VITRO*

Bacillus subtilis reduced colony spread of *F. oxysporum* on PDA by 86.6 % (Fig.1) (1.2 cm mean radial colony growth with *B. subtilis* versus 9.0 cm mean colony growth without *B. subtilis*) after 6 days of incubation at 27 °C.

3.3 EFFECTS ON TOTAL CHLOROPHYLL AND TOTAL CAROTENOIDS

The results showed that date palm plantlets treated with *B. subtilis* in the presence and absence of *F. oxysporum* resulted in a highly significant increase in total chlorophyll and total carotenoid, in comparison to the pathogen alone (*F. oxysporum*). Plantlets treated with *B. subtilis* had the highest scores, while the pathogen treatment resulted in low values (Fig.2). *B. subtilis* significantly raised

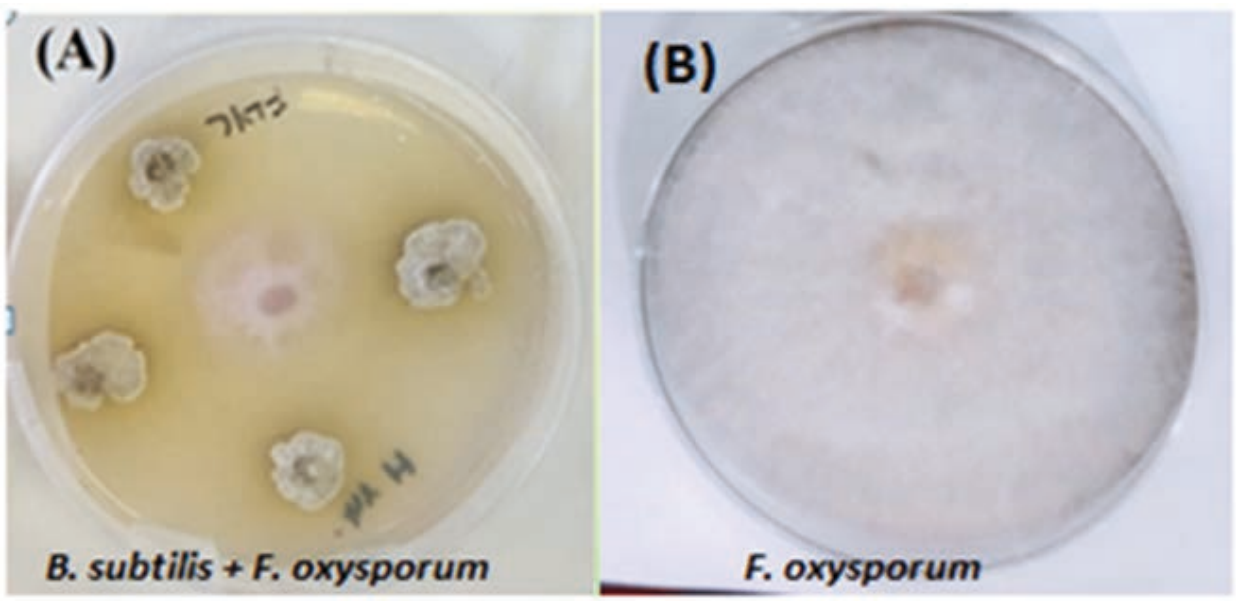

Fig. 1: *In vitro* inhibition of mycelial growth of *F. oxysporum* by *B. subtilis* on PDA (A: antagonism, B: *F. oxysporum*)

the total chlorophyll and total carotenoids in comparison with the *F. oxysporum* treatment. When plantlets infected with *F. oxysporum* were treated with the bacterium *B. subtilis*, chlorophyll levels were restored to roughly 90 % of control levels, and carotenoid levels were restored to control levels.

3.4 LEVELS OF CATALASE (CAT) AND PEROXIDASE (POD) ENZYMES

The obtained data showed that *B. subtilis* augmented the levels of the antioxidant enzymes CAT and POD significantly in *F. oxysporum*-treated plantlets, about 31.2 % and 5.2 %, respectively, compared to inoculated plantlets with *F. oxysporum* alone (Fig.3). Plantlets inoculated with *F. oxysporum* had lower levels of catalase (CAT) and peroxidase (POD) enzyme activity than those in the control. The data analyses showed highly significant enhancement of the antioxidant enzyme activities (CAT and POD) as a result of treating the plantlets with the *B. subtilis* strain in the presence and absence of the pathogenic fungus *F. oxysporum*.

3.5 EFFECT ON TOTAL PROLINE CONTENT

Data from our study revealed that total proline was reduced in response to inoculation with *F. oxysporum* compared with un-inoculated plantlets (control). Infection by *F. oxysporum* reduced proline levels, but co-application of *B. subtilis* restored proline to normal levels seen in the control (Fig.4). Data from the ANOVA table showed that the value of total proline content was increased in plantlets inoculated with *B. subtilis* in the presence of *F. oxysporum* by about 47.6 % compared with plantlets inoculated with *F. oxysporum* alone. *F. oxysporum* reduced total proline content by approximately 48.2 % (compared to the control (healthy).

4 DISCUSSION

4.1 PATHOGENICITY TESTS

The results of the pathogenicity test showed that *Fusarium* isolates were highly pathogenic to date palm plantlets. Species of *Fusarium* are known to produce toxins such as fumosisin, fusaric acid, fusaproliferin, fusarin, zearalenone, and others, which aid in the attack and parasitism of plant hosts (Hernandez et al., 2010). According to El Modafar & El Bostani (2000), *F. oxysporum* releases cell wall hydrolytic enzymes that hydrolyze host ingredients, allowing the pathogen to move into root tissues, and these enzymes are linked with disease progression. Our results agree with those of other researchers, showing that date palm trees and offshoots are attacked by many soil-borne pathogenic fungi capable of causing severe losses and degradation (Arafat et al., 2012; Baraka et al., 2011), including *F. oxysporum, F. solani, F. moniliforme, F. smitectum, R. solani,* and *Thielaviopsis paradoxa* (De Seynes) Höhn.(Ahmed, 2018; El-Morsi et al., 2012; Maitlo et al., 2013).

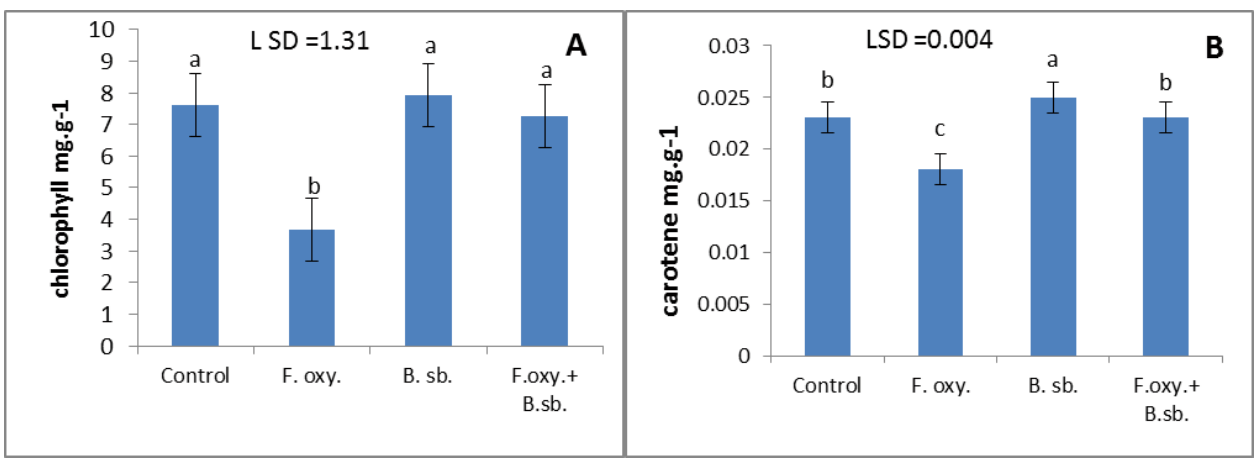

Fig. 2: Effectiveness of *B. subtilis* in presence and absence of *F. oxysporum* on: A: total chlorophyll content and, B: Total carotenoid content.(Each value is the mean of five replicates, means in the columns followed by the different letters are significantly different at $p < 0.05$ test)

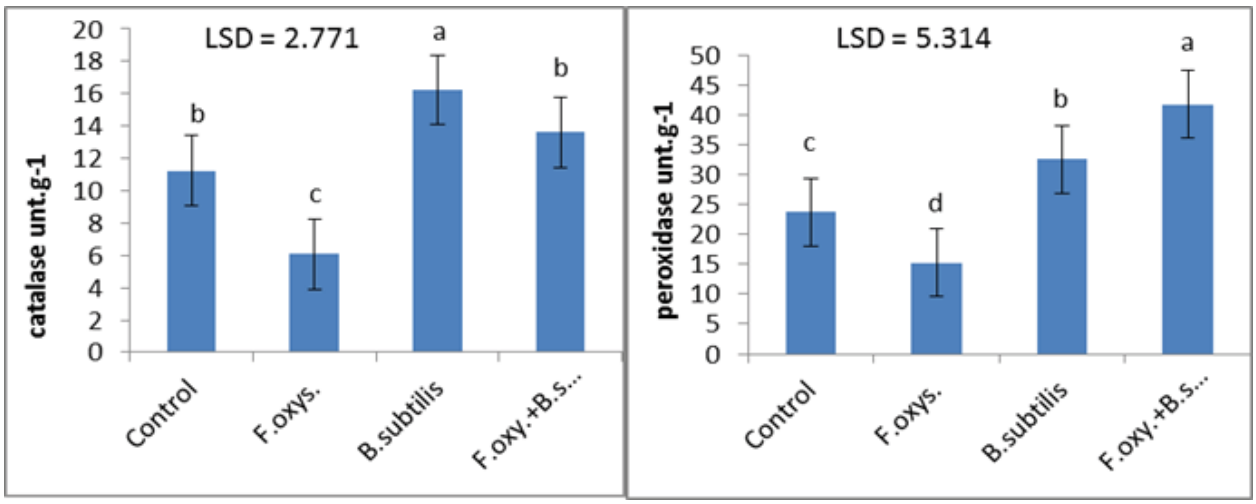

Fig. 3: Effectiveness of *Bacillus subtilis* in presence and absence of *Fusarium oxysporum* on catalase enzyme, and peroxidase enzyme (Each value is mean of five replicates, means in the columns followed by the different letters are significantly different at $p < 0.05$ test)

4.2 EFFECT OF *B. SUBTILIS* AGAINST THE GROWTH OF *F. OXYSPORUM IN VITRO*

The results of a double culture in Petri dishes containing PDA medium revealed that *Bacillus subtilis* has the ability to suppress radial mycelial growth to a large extent. *Bacillus subtilis* suppresses pathogen growth directly by the synthesis of many secondary metabolites, such as hormones, cell wall degrading enzymes, and antioxidants. Our results were in agreement with studies by Cao et al. (2012), who observed that *B. subtilis* produces many antibiotic compounds, including fengycin, iturin, and bacillomycin, and these compounds inhibit mycelial growth and spore germination of the fungal pathogen *F. oxysporum*. Jassim (2015) showed that *B. subtilis* completely inhibited the mycelial growth of the pathogen *F. moniliforme* in the PDA medium. Siala et al. (2016) found that an endophytic strain of *B. subtilis* slowed the growth of *Fusarium* species on PDA and that this strain produced proteases, contributing to the degradation of the cell walls of fungal pathogens. Isolate *B. subtilus* 174 has been shown to have strong biocontrol activity and to cause significant suppression of disease severity in Fusarium wilt disease in tomato plants caused by *F. oxysporum*, likely due to induced resistance (Akarm and Anjum, 2011). Several important plant pathogens, including *Fusarium* sp. (Zhao et al., 2013), *Rhizoctonia solani* (Kumar et al., 2012), and *Verticillium dahliae* Kleb (Li et al., 2013), can be suppressed by *B. subtilis*. Bhusal & Mmbaga (2020) examined three *Bacillus* spp. isolates as biological control agents against the pathogen *Phytophthora capsici* Leonian. These isolates suppressed the mycelial growth of *P. capsici in vitro* and reduced the incidence of disease in plants grown in soil infested with *Phytophthora* inoculum under greenhouse conditions.

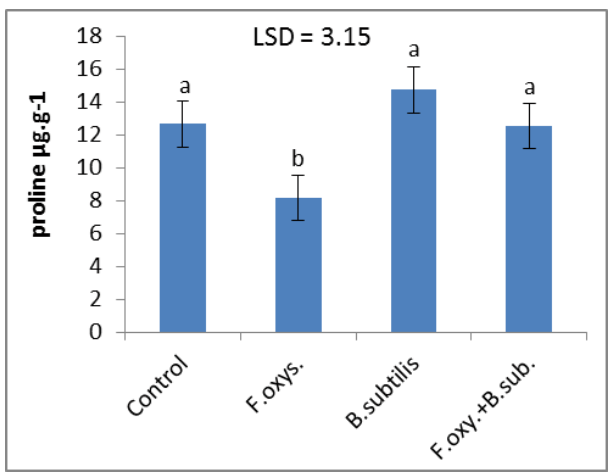

Fig. 4: Effectiveness of *Bacillus subtilis* in the presence or and absence of *Fusarium oxysporum* on proline content (Each value is mean of five replicates: means in the columns followed by the different letters are significantly different at $p < 0.05$ test)

4.3 EFFECTS ON TOTAL CHLOROPHYLL AND TOTAL CAROTENOIDS

Our results show that plantlets inoculated with *F. oxysporum* reduced photosynthetic activity (mostly photosynthesis), perhaps due to reduced levels of key proteins in the thylakoid membranes and/or the reduction of RuBPC-specific leaf soluble proteins (Weintraub & Jones,

2010). Huang et al. (2012) found that stress and infection by fungi led to a decline in leaf chlorophyll that was due to increased chlorophyllase activity, increased active oxygen products, and destabilization of ionic equilibrium. Reduction in chlorophyll may be due to the toxic action of compounds released by pathogenic fungus; such compounds lead to necrosis and chlorosis due to their toxic effects on chloroplasts in the host cells (Bashan et al., 1995). The reduction in the absorption of minerals (e.g., magnesium) required for chlorophyll synthesis can also indirectly reduce the chlorophyll content in plants infected by pathogens (Murkute et al., 2006; Sheng et al., 2008).

Nevertheless, in response to *F. oxysporum* infection, *B. subtilis* greatly increased the production of antioxidant enzymes, several secondary metabolites, growth regulator hormones, and enzymes to degrade cell walls (Hashem et al., 2019). According to Cazorla et al. (2007), because *B. subtilis* can emit antibiotics and hydrolytic enzymes, it may change its environment for the better and develop resistant endospores to survive in harsh environments. In mung beans, *B. subtilis* alleviated symptoms of infection by the fungal pathogen *Macrophomina phaseolina* (Tassi) Goid., reducing charcoal rot disease and enhancing total chlorophyll (Hashem et al., 2017). 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. In date palms, *B. subtilis* increased the total chlorophyll and total carotenoid in plants under abiotic stress and prevented the harmful effects of stress (Jassim et al., 2020). *F. oxysporum* infection in date palm plantlets generates reactive oxygen species (ROS), such as radicals of superoxide(O^{-2}), hydroxyl (OH^{-}), and molecules of hydrogen peroxide (H_2O_2). The accumulation of ROS in infected plant cells causes major and important injuries in all plant functions (Manhas & Kaur, 2016).

4.4 LEVELS OF CATALASE (CAT) AND PEROXIDASE (POD) ENZYMES

Antioxidant enzymes mitigate the damage level from reactive oxygen species (ROS) in plants, which is a source of oxidative stress from pathogen infection (Asada, 1999). Catalase (CAT) is one of the most prevalent detoxifying enzymes in plants, and it plays an important role in regulating ROS generation and buildup. In contrast, the catalase enzyme converts H_2O_2 into H_2O and O_2, so any increase in CAT activity is more likely to result in a decrease in H_2O_2 generation, as shown at low concentration in the plantlet treatments inoculated with both *B. subtilis* in the presence or absence of *F. oxysporum*. At high concentrations, plantlets inoculated with *F. oxysporum* alone showed the opposite effect, with an increase in H_2O_2 production accompanying a decrease in CAT activity. The increase in antioxidant enzyme activity levels enhances disease resistance in the host plants (Shi et al., 2010). Selvaraj & Chellappan (2006) explain that POD enzymes play an important role in producing ethylene, resisting disease, promoting wound healing, and lignin formation, as well as in building cell walls by changing polymerizing hydroxyl and methoxy cinnamic alcohols into lignin.

Xie et al. (2021) found that *B.subtilis* strain LZ88 induced plant resistance with an enhanced expression in tobacco leaves of the antioxidant enzymes peroxidase (POD) and polyphenol oxidase (PPO). *B. subtilis* induced systemic resistance and alleviated the harmful effects of pathogens by increasing the activity of antioxidant enzymes on plants (Hashem et al., 2019).In a recent study on date palms, Jassim et al. (2020) showed that *B. subtilis* protected date palms from the harmful effects of salt stress and increased the activity of antioxidant enzymes (CAT and POD). In our study we found that *F. oxysporum*, in the absence of *B. subtilis,* increased the levels of CAT and POD activity, which confirms that oxidative damage is associated with ROS scavenging, while the *B. subtilis* bacteria inhibited the activity of the pathogen fungus *F. oxysporum* and significantly reduced the negative effects on the vital processes in the plant, which reflected its effect on the plant, and that led to mitigation in the antioxidant enzymes compared to the pathogen treatment alone.

4.5 EFFECT ON TOTAL PROLINE CONTENT

There were significant differences in the total proline content among plants treated with *B. subtilis* and *F. oxysporum* compared with *F. oxysporum.* This can be attributed to the ability of *B. subtilis* to limit the activity of the fungus pathogen through the ability to excrete antibiotics such as subtilin, bacteriocins, iturins, and bacilomycin, which act to inhibit the growth of fungus (Meena & Kanwar, 2015; Wang et al., 2015). Proline is essential in plants and accumulates during pathogen attacks in a variety of species (Rehman et al., 2014). According to the study by Wang et al. (2012), the inoculation of cucumber plants (*Cucumis sativa* L.) with a mixture of three plant growth promoting rhizobacteria (PGPR) strains (*B. cereus* AR156, *B. subtilis* SM21, and *Serratia* sp. XY21) elevated leaf proline content by 3–4 fold compared to uninoculated plants. Proline catabolism plays an essential role in controlling the cellular ROS balance and can also control various other regulatory pathways. It has also

been demonstrated that proline accumulation activates the pathways of alternative detoxification by the maintenance and duration of ROS-eliminating enzymes (Hayat et al., 2012). Plants inoculated with *B. subtilis* showed less damage from the harmful effects of *M. phaseoline* and an increase in the accumulation of sugars, proline, and free amino acids, which are considered to be the key osmolytes for sustaining the content of cellular water to protect the structures and functions of cell organelles (Hashem et al., 2017).

5 CONCLUSION

Pathogenicity studies showed that *Fusarium oxysporum* is the most causative agent of root rot disease in date palm plantlets, with a severity index of 82.16 % for root rot. The *Bacillus subtilis* strain reduced the mycelial growth of *F. oxysporum in vitro* as well as *in vivo*. Our results show that *B. subtilis* is a beneficial microorganism for controlling the root rot disease of date palm plantlets caused by *F. oxysporum*. *B. subtilis* inhibited oxidative damage caused by the pathogenic fungus and significantly improved all measured physiological characteristics that were adversely affected by the fungus pathogen. More work is needed to determine the potential of *B. subtilis* for biological control of this pathogenic fungus and regulation of plant growth.

6 CONFLICT OF INTEREST

Authors declare no conflict of interest.

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