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Efficacy of *Bacillus subtilis* V26 as a biological control agent against *Rhizoctonia solani* on potato

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ABSTRACT

The aim of this study is to evaluate the efficacy of the strain *Bacillus subtilis* V26, a local isolate from the Tunisian soil, to control potato black scurf caused by *Rhizoctonia solani*. The *in vitro* antifungal activity of V26 significantly inhibited *R. solani* growth compared to the untreated control. Microscopic observations revealed that V26 caused considerable morphological deformations of the fungal hyphae such as vacuolation, protoplast leakage and mycelia crack. The most effective control was achieved when strain V26 was applied 24 h prior to inoculation (protective activity) in potato slices. The antagonistic bacterium V26 induced significant suppression of root canker and black scurf tuber colonization compared to untreated controls with a decrease in incidence disease of 63% and 81%, respectively, and promoted plant growth under greenhouse conditions on potato plants. Therefore, *B. subtilis* V26 has a great potential to be commercialized as a biocontrol agent against *R. solani* on potato crops.

1. Introduction

Potato (*Solanum tuberosum L.*) is the third most important food crop in the world after wheat and rice (http://www.fao.org/potato-2008/en/potato/pdf.html) and occupies an important place in the diet of many countries in the world. In Tunisia, the potato production area is 25,000 ha, which represents more than 17% of the country's vegetables cultivated land (http://www.ctpt.com.tn), with a production of 360,000 tonnes/year. Potato production is threatened by many diseases, including black scurf caused by *Rhizoctonia solani*, which is one of the major diseases of potato around the world. The best known symptom of the disease is the appearance of sclerotia on the surface of potato tubers. Infections are caused by sclerotia boost in the number of malformed cracked tubers, an alteration in their size and distribution and the production of aerial tubers [1–3]. Brown, dry and usually sunken lesions may also develop on stems, stolons and roots [4]. This infection delays shoot emergence, decreases the number of stems and increases their variation height, and leads to stolon and sprout pruning [5,6]. Black scurf is responsible for economical losses and significant reduction in potato quality [7], especially for export-oriented potato. Effective strategies for pathogen control are necessary, especially as no resistant cultivars are yet available [8,9]. Moreover, control of *R. solani* is difficult because of its ecological behavior, its broad host

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range and the high survival rate of sclerotia under various environmental conditions [10,11]. In Tunisia, all registered fungicides applied to potato crops are often inefficient to control *Rhizoctonia* damping off under field conditions [12]. The applications of pencycuron and azoxystrobin in the Southwest of Tunisia have showed a decrease in the disease incidence in potato tubers [13]. Although some chemical fungicides have proved their effectiveness in controlling *Rhizoctonia* disease, they are not useful in organic farming. Interestingly, the use of biological agents seems to be a good alternative to protect crops against *R. solani*. In recent years, both bacterial and fungal agents have been described for the biocontrol of *R. solani* [10,14–17]. Nevertheless, most of them were not able to protect crops when applied under field conditions [15]. In this context, *Bacillus subtilis* has been widely studied as a potential biological agent against various plant diseases due to its ability to produce several antibiotics, lipoproteins, and hydrolytic enzymes [18–20]. This bacterium could be a promising alternative to control pathogens with a high ecological versatility such as *Rhizoctonia*. The aim of this work is to test the ability of *B. subtilis* V26, isolated from the Tunisian soil, to inhibit the growth of *R. solani in vitro*, to affect its hyphal morphology, and to protect potato plants against *Rhizoctonia* in pot experiments.

2. Materials and methods

2.1. Microorganisms

The strain V26, used throughout this study, was isolated from the rhizosphere of untreated almond tree (Bir El Malouli, Sfax, Tunisia) and identified by the laboratory of Biopesticides as *B. subtilis* [21]. The isolate of *R. solani* used in this study was obtained from potato, cv. Spunta, a tuber showing typical black scurf symptoms, from Essaïda, a Tunisian potato-growing area. The fungal pathogenicity was previously confirmed on potato plants by Daami-Remadi et al. [9]. The *R. solani* isolate was grown on Potato Dextrose Agar (PDA) at 25 °C for seven days and stored at 4 °C until use.

2.2. Inoculum preparation and fermentation conditions

A single colony of strain V26, picked from the agar plate, was transferred to a test tube containing 3 mL of LB medium and incubated overnight at 30 °C. The overnight culture was then seeded into a 1000-mL Erlenmeyer flask containing 100 mL of LB medium. The shake-flask cultures were maintained in a shaker set at 200 rpm for 24 h at 30 °C. The overnight cultures were then inoculated into 2-L shake flasks containing 500 mL of LB to achieve the initial cell density of 0.15 at $OD_{600\ nm}$. The inoculum culture was transferred into a 7-L Labfors (Infors, Switzerland) fully automatically controlled fermenter containing 5 L of culture medium. The production medium consisted of 25 g/L sucrose, 20 g/L peptone, 4.5 g/L yeast extract, 2 g/L KH_2PO_4, 0.6 g/L $MgSO_4$ and 6 g/L $MnSO_4$ [22]. The temperature was automatically controlled at 30 °C. The airflow rate and the impeller speed were set at 1 vvm and 200 rpm, respectively [23]. The pH was maintained at 7 with continuous regulation using 2 M HCl and 2 M NaOH. Foam production was controlled by automatic addition of a sterile antifoam solution.

2.3. Antifungal and hydrolysis activities in vitro assays of B. subtilis V26

The antifungal activity was assessed *in vitro* by the well diffusion method [24]. *B. subtilis* V26 culture medium was centrifuged at 4000 × *g* for 20 min and the supernatant was recovered. The supernatant was sterilized through a Millipore filter (0.45 μm), then was serially diluted (1:4, 1:8, 1:16, 1:32) with sterile distilled water and used for activity assay. A suspension of *R. solani* was prepared by suspending loopfuls of hyphae from a Potato Dextrose Agar (PDA) (Lab M) slant. Hundred microliters of *R. solani* suspension were displayed on a plate filled with the PDA medium amended with chloramphenicol (30 μg/mL). Wells were drilled in the PDA, covered with the *R. solani* suspension, and then 80 μL of diluted supernatant were added to the wells. The inhibition zones were observed. Each diluted supernatant was assayed in triplicate and the diameter of the inhibition zone was measured after incubation at 25 °C for three days.

The ability of *B. subtilis* V26 to produce chitosanase was examined on Luria Broth (LB) agar plates: yeast extract 5 g/L, peptone 10 g/L, NaCl 5 g/L, and agar 15 g/L, containing 1.5% (v/v) of colloidal chitosan (Sigma, Deisenhofen, Germany). Protease activity was determined on agar containing 5% (v/v) skimmed milk. Wells were drilled in the PDA plates containing colloidal chitosan or skimmed milk, and then 80 μL of diluted supernatant were added to the wells. An equal volume of culture medium served as a control. The formation of clearing zones was detected after 1 day of incubation in the dark at 30 °C. Each experiment was replicated three times and the assay was repeated twice.

2.4. Evaluation of the antifungal activity in vitro on solid medium

The antagonistic effect of *B. subtilis* V26 against *R. solani* was evaluated *in vitro* by a growth inhibition assay [25]. Increasing volumes of *B. subtilis* V26 supernatant (1%, 3%, 5%, 7%, 9%; v/v) were mixed with molten PDA (cooled at 45 °C). After cooling, the plates containing the amended PDA were centrally inoculated with a 6-mm agar disc from a fresh culture of *R. solani*, and were incubated at 25 °C. An equal volume of PDB (Potato Dextrose Broth) was used as a control. Each treatment was done in triplicate and the antagonism assay was repeated twice. The colony's diameter was recorded after 72 h of incubation, in the dark, and the inhibition ratio was calculated using the following formula [26]:

$$\text{inhibition ratio}(\%) = [(\text{diameter of fungal colony in control} - \text{diameter of fungal colony in treatment})/\text{diameter of fungal colony in control}] \times 100.$$

2.5. Effect of B. subtilis V26 metabolites on R. solani mycelial growth

Two 6-mm agar disks of *R. solani* were transferred into a 250-mL flask containing 50 mL of the PDB medium, and each flask was treated with one of a series of sterile culture supernatants from 1 to 9% (v/v). The same volume of PDB was similarly treated as a negative control. The flasks were incubated at 25 °C under shaking at 100 rpm for 7 days. Each treatment was done in triplicate and the experiment was repeated twice. From each treatment, an aliquot of mycelia was picked to assess the presence of morphological/structural changes due to the presence of the *B. subtilis* V26 supernatant on the media by using a light microscope (Olympus Optical Co. LTD) operating with Olympus DP70 camera (magnification 40 ×).

2.6. Evaluation of antifungal activity on slices of potato tubers

Potato tubers were immersed for 5 min in a 0.5% sodium hypochlorite, and then rinsed several times with sterile distilled water, air-dried at room temperature and finally cut into 2-cm slices. Following a 72-h culture harvesting of *B. subtilis* V26, the spores and culture supernatant were separated by centrifugation at 8000 rpm for 20 min. Next, the pellet (spores) was washed twice with distilled water and bioassayed by dipping the potato slices into different cell suspensions, namely: 10^6 spores/mL washed cell suspension (SPO1), 10^9 spores/mL washed cell suspension (SPO2), 10^9 spores/mL unwashed cell culture mixture (SPO2-CM), commercial biofungicides Prevam (0.3%, v/v) (ORO Agri, International Ltd. USA), biofertilizer Nitrophoska (NPK: 2 g/L) (COMPO®) and sterile distilled water as a negative control (dipped for 30 min), prior and 24 h after the inoculation with a 6-mm agar disk of *R. solani*. The potato slices were sealed in polyethylene-lined plastic boxes to maintain high humidity and incubated at 25 °C, seven days in the dark. The incidence of decay was assessed by measuring the lesion area on each potato slice as compared to the total potato slice surface area using a visual scale from 0 to 4, where 0 = absence of lesion area, 1 = 1–25% potato slice surface with lesion area, 2 = 26–50% potato slice surface with lesion area, 3 = 51–75% potato slice surface with lesion area, 4 ≥ 75% potato slice surface with lesion area. Each treatment was replicated five times and the antagonism assays were repeated twice. The reduction of decay incidence was calculated according to the following formula:

$$\text{decay incidence (\%)} = [(\text{decay incidence of untreated potato slice} - \text{decay incidence of treated potato slice})/\text{decay incidence of untreated potato slice}] \times 100.$$

2.7. Evaluation of the protective activity of B. subtilis V26 against R. solani on potato

The ability of *B. subtilis* V26 to protect potato against *R. solani* infection was assessed on tubers of potato cv. Spunta (highly susceptible to black scurf) [9,13]. Initially, tubers were immersed in 0.5% sodium hypochlorite for 5 min, rinsed in running tap water and air-dried at room temperature. Then, the tubers were subjected to the following treatments during 30 min: 10^6 spores/mL (SPO1), 10^9 spores/mL (SPO2), 10^9 spores/mL culture mixture (SPO2-CM), commercial biofungicides Prevam (0.3% v/v) (ORO Agri, International Ltd. USA), biofertilizer Nitrophoska (NPK: 2 g/L) (COMPO®) and sterile distilled water as a negative control. Each treatment was replicated five times and the assay was repeated twice. Treated tubers were planted in plastic pots containing sterilized peat (Potgrond H), mixed or not with *R. solani* inoculum. The pathogen inoculum was obtained from 7-day-old cultures grown on PDA Petri dishes kept at 25 °C, and then it was scraped and blended with sterile distilled water at the rate of five plates per kilogram of peat. After planting, pots were placed in greenhouse for 60 days and irrigated daily to maintain high peat moisture.

Disease incidence and plant growth parameters were determined 60 days after planting. Plants were harvested, washed, and rated for root canker as described by Brewer and Larkin [27] as follows: 0 = no disease symptoms; 1 = brown discoloration of roots; 2 = cankers covering < 25% of the root circumference; 3 = 25–75% roots covered by cankers; 4 = 75% coverage by roots cankers; and 5 = root completely nipped off or death of the plant. The black scurf was assessed on a scale of 0–5, as described by Brewer and Larkin [27]: 0 = no visible sclerotia; 1 = sclerotia covering 1% of the tuber area; 2 = 2–5% of the tuber area affected; 3 = 5–10% of the tuber area affected; 4 = 10–15% of the tuber area affected and 5 or more then 15% of the tuber area affected. The reduction of disease incidence was calculated according to the following formula:

$$\text{disease incidence(\%)} = [(\text{disease incidence of untreated potato plant} - \text{disease incidence of treated potato plant})/\text{disease incidence of untreated potato plant}] \times 100.$$

The number, the height and the fresh weight of stems, and the fresh weight of roots were also recorded for each plant at harvesting time.

2.8. Statistical data analysis

Data were subjected to analysis of variance using SPSS software. Mean values were compared using Duncan's multiple range test at the 5% ($P < 0.05$) level of significance.

3. Results

3.1. Evaluation of antifungal and hydrolysis activities assays of B. subtilis V26

In dual culture assays of strain V26 against *R. solani*, a significant inhibition zone of the mycelia growth was shown, suggesting the presence of antifungal metabolites secreted by this strain. The *B. subtilis* V26 antagonistic effect was evaluated by serial dilutions and its culture supernatant was able to inhibit the growth of *R. solani* even after dilution to 1:32 (Fig. 1a). The strain V26 was also able to produce chitosanase and proteases (Fig. 1b and c), which

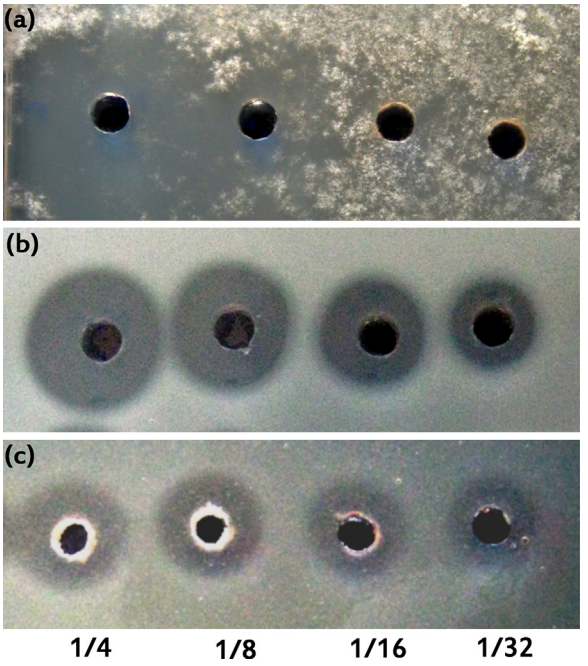

Fig. 1. (Colour online.) Evaluation of the antifungal activity against *R. solani* (a), chitosanase (b) and protease (c) activities of *B. subtilis* V26 for different culture supernatant dilutions.

may play a role in dissolving and penetrating the cell walls of *R. solani*, as reported by McQuilken and Gemmell [28].

3.2. Effect of antifungal activity of B. subtilis V26 on the growth and the hyphal morphology of R. solani

The antifungal activity of the strain V26 against *R. solani* was quantified by the incorporation of its culture supernatant in PDA plates. As shown in Fig. 2A, the antagonist V26 exhibited a significant inhibition of the pathogen growth compared to the untreated control. Mycelial growth reduction reached 80% at low percentages of supernatant (3% v/v) in comparison to the untreated control. An increase in the percentage of the V26 supernatant induced a significant increase in fungal growth inhibition. This reduction of mycelial growth caused by the V26 supernatant was also accompanied by morphological alterations of *R. solani* hyphae (Fig. 2B). Compared to *R. solani* treated by PDB, the supernatant culture of V26 (1% v/v) caused uncommon hyphae deformation and developed swelling at hyphal tips (Fig. 2B). Moreover, when *R. solani* hyphae were treated with increased supernatant percentage (9% v/v), hyphal deformation and enlargement of cytoplasmic vacuoles were increased, since its cell wall disintegrated, protoplasm leaked out, and the mycelia cracked (Fig. 2B).

3.3. Decay incidence in potato tuber slices assay

The efficacy of the *B. subtilis* strain V26 to inhibit the growth of *R. solani* was estimated on potato slices 24 h before and after being inoculated with *R. solani* (Fig. 3). The observations from treated potato tuber slices and untreated ones showed differences ($P < 0.001$) in decay incidence. A slight decrease in the incidence of the disease was shown on the potato slices when the application of the antagonist V26 (SPO1 and SPO2-CM) and of the biofertilizer nitrophoska was made 24 h after inoculating with the fungus (Fig. 3a). Like commercial biofungicide Prevam, V26 (SPO2) treatment significantly decreased the decay incidence in potato slices by only 30% in comparison to the inoculated but untreated control. However, the decay incidence observed in potato slices treated with *B. subtilis* V26 (SPO1, SPO2 and SPO2-CM), nitrophoska and Prevam 24 h prior to inoculation with the pathogen was significantly different from that developed in the control set treated with *R. solani* alone. The disease incidence was nearly zero for all V26 treatments compared to untreated potato tuber slices (Fig. 3b). Prevam treatment at (0.3% v/v) gave also a complete control of *R. solani* infection. However, the

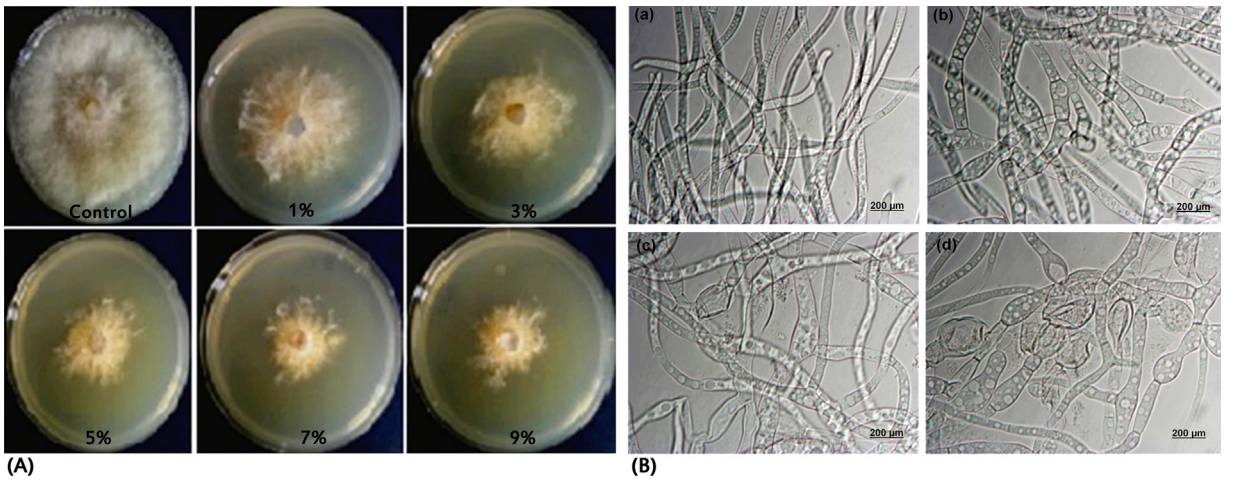

Fig. 2. (Colour online.) A. Inhibition of *R. solani* growth on solid medium amended with different percentages of *B. subtilis* V26 culture supernatant. B. Microscopic observations of *R. solani* hyphae growing on the PDB medium (a) without *B. subtilis* V26 culture supernatant, (b) with 1% *B. subtilis* V26 culture supernatant, (c) with 3% *B. subtilis* V26 culture supernatant, and (d) with 9% *B. subtilis* V26 culture supernatant. Magnification: 40 ×.

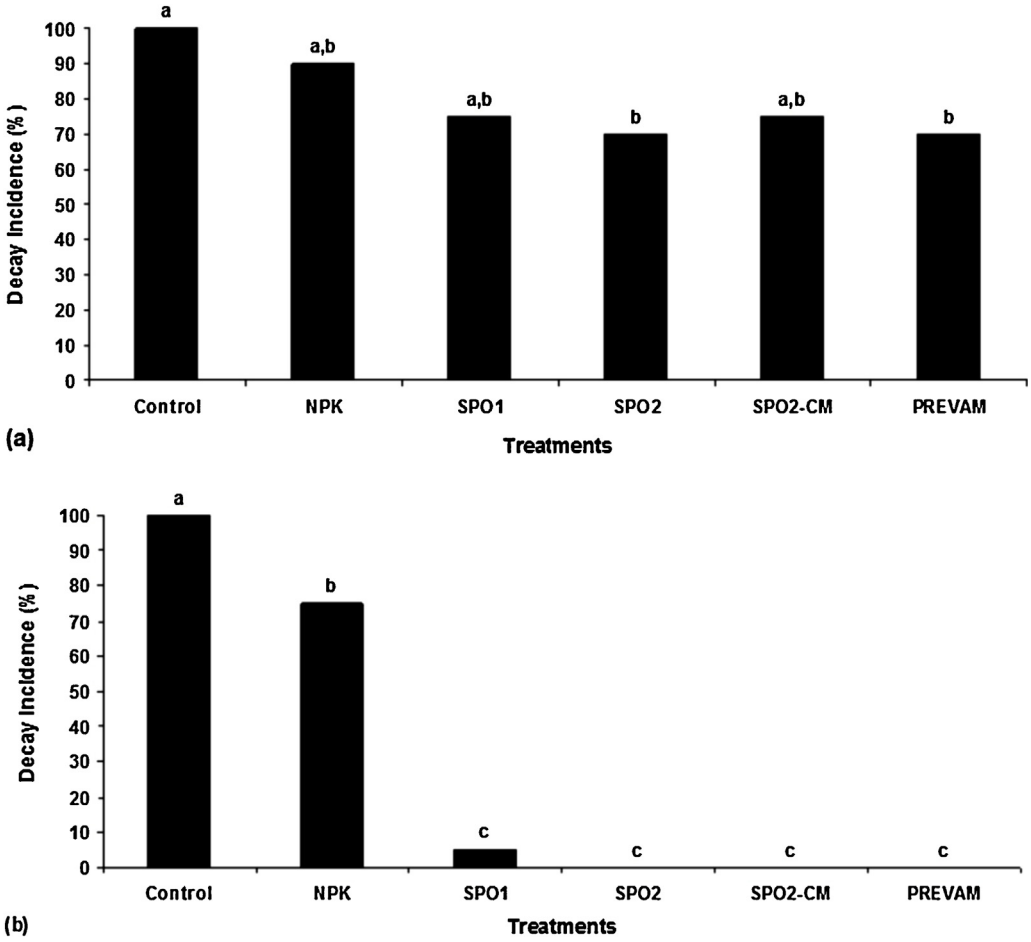

Fig. 3. Efficacy of protection against *R. solani* on potato tuber slices by various treatments with *B. subtilis* V26: 10^6 spores/mL (SPO1), 10^9 spores/mL (SPO2), 10^9 spores/mL culture mixture (SPO2-CM), commercial biofungicides (Prevam) and biofertilizer Nitrophoka (NPK); (a) 24 h after *R. solani* inoculation; (b) 24 h prior to *R. solani* inoculation.

Nitrophoska treatment at 2 g/L reduced decay incidence at only 25%, in comparison to the control (Fig. 3b).

3.4. *Biocontrol assay of* B. subtilis *V26 against* R. solani *on potato*

In pot experiments, data were collected 60 days after inoculation. The results of V26 biocontrol assays are presented in Figs. 4 and 5A. Disease incidence on roots of all treated potato plants was significantly lower than that of the untreated ones that were used as controls ($P < 0.001$). V26 (SPO2-CM) treatment resulted in low disease incidence on roots, with a reduction of 63% compared to the untreated one (Fig. 4). Moreover, the disease incidence on potato tuber caused by *R. solani* was also significantly ($P < 0.01$) reduced by the different treatments applied when compared to the controls. The application of V26 significantly reduced black scurf in potato tuber compared to the control (Fig. 5Ba), with a decrease in the incidence rate to 81% when using V26 (SPO2) treatment (Fig. 5Bb). No statistical differences were observed between (SPO2) treatment and the application of the commercial biofungicide, Prevam. No significant protection was noted on tubers treated by the biofertiliser "Nitrophoska", which was shown to be less effective in reducing the incidence of the disease, in comparison to the pathogen control. Compared to the untreated potato plants, the treated ones recorded a significant increase in the growth parameters (Table 1).

In fact, V26 (SPO2-CM) treatment significantly enhanced plant height, with an increase of 37.89%, comparatively to the control. In addition, the number of stems significantly increased more than 70% when potato tubers were treated by V26 (SPO2-CM), in comparison to the untreated control. Shoot fresh weights were significantly increased for all treatments, compared to the untreated control. The improvement in shoot fresh weight of plant growing in presence of V26 (SPO2-CM) is more than 80%, in comparison to the untreated control. V26 treatments also promoted root growth, with a maximal increase of 37.34% reached by SPO2-CM treatment (Table 1). It seems also that the use of the whole culture was the best treatment to promote potato plant growth, followed by spore suspension (Fig. 6).

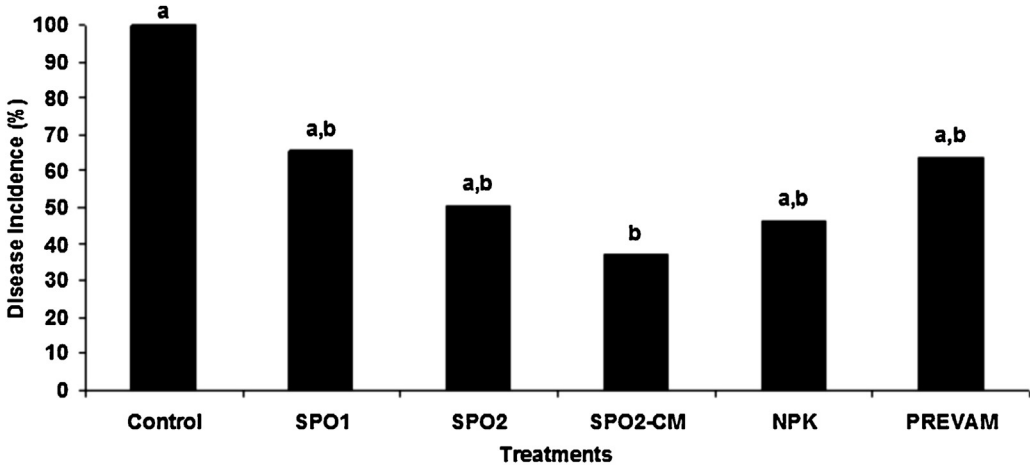

Fig. 4. Disease incidence of *R. solani* in roots after 60 days pathogen inoculation in pot experiments, treated by V26 strain: 10^6 spores/mL washed cell suspension (SPO1), 10^9 spores/mL washed cell suspension (SPO2), 10^9 spores/mL unwashed cell culture mixture (SPO2-CM), commercial biofungicide (Prevam), biofertilizer Nitrophoska (NPK), and sterile distilled water. All values are the means of five replicated pots. Bars with a same letter are not statistically different among the five treatments according to the Duncan test at $P = 0.05$.

4. Discussion

Several *B. subtilis* strains had proved their efficacy in the control of plant diseases, due to their capacities to produce a wide variety of antifungal compounds, including volatiles, enzymes, lipopeptides, and several small peptides [29,30]. In this study, *B. subtilis* V26 showed significant inhibitory effects on the growth of *R. solani in vitro* and *in vivo*. This result is in accordance with those of Yang et al. [31] and Solanki et al. [32], who reported the efficacy of *B. subtilis* in controlling *R. solani*.

The ability of V26 to control *R. solani* was confirmed by the determination of the inhibition ratio against this fungus and also verified by optical microscopic observation. The culture supernatant of *B. subtilis* V26 contained chitosanase [21] and proteases besides antifungal compounds. The

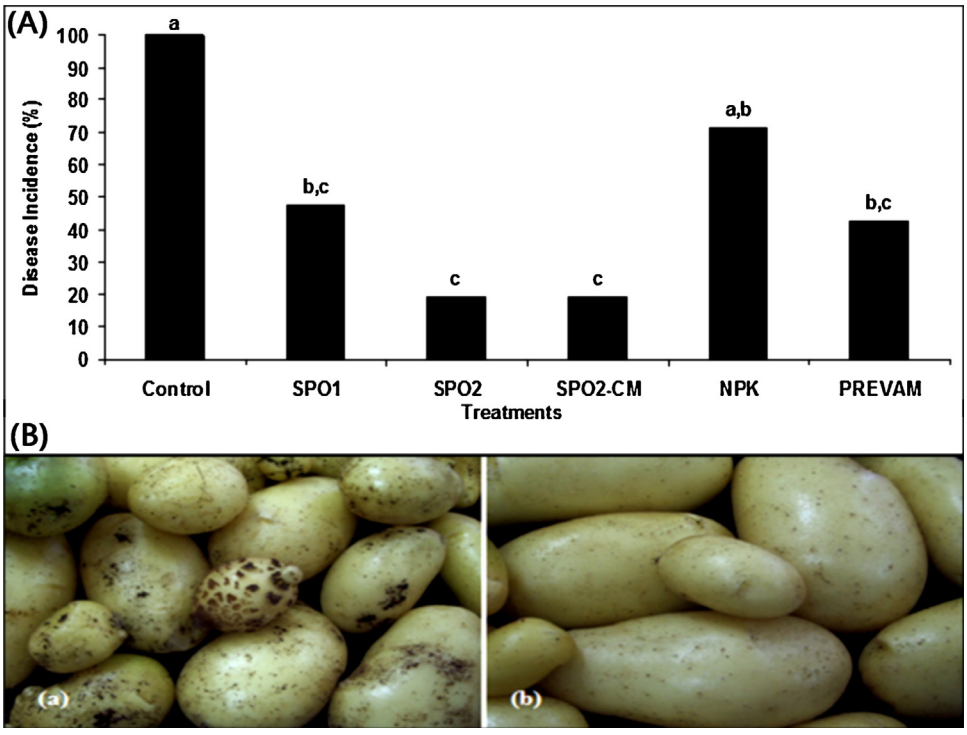

Fig. 5. (Colour online.) **A.** Disease incidence of *R. solani* in potato tubers after 60 days of pathogen inoculation in pots experiments, treated by V26 strain (10^6 spores/mL washed cell suspension (SPO1)), 10^9 spores/mL washed cell suspension (SPO2) (**Bb**), 10^9 spores/mL unwashed cell culture mixture (SPO2-CM), commercial biofungicides (Prevam), biofertilizer Nitrophoska (NPK), and sterile distilled water (**Ba**). All values are the means of five replicated pots. Bars with a same letter are not statistically different among the five treatments according to the Duncan test at $P = 0.05$.

Table 1
Effect of V26 biocontrol treatments on potato plant growth parameters.

Treatments	Maximal stem height (cm)	Stems number	Fresh shoot weight (g/plant)	Fresh roots weight (g/plant)
Control	35.1 ± 11.56^{c}	2.8 ± 0.63^{b}	95.6 ± 41.72^{c}	8.3 ± 2.31^{b}
Biofertilizer Nitrophoska (NPK)	$39.8 \pm 6.17^{b,c}$	3 ± 1.33^{b}	130.7 ± 28.23^{b}	11.7 ± 2.31^{a}
10^{6} spores/mL washed cell suspension (SPO1)	$39.8 \pm 10.64^{b,c}$	$3.4 \pm 1.07^{a,b}$	126.8 ± 32.68^{b}	11.4 ± 2.63^{a}
10^{9} spores/mL washed cell suspension (SPO2)	$44.7 \pm 12.94^{a,b}$	$4 \pm 2^{a,b}$	129.7 ± 20.36^{b}	$9.6 \pm 3.74^{a,b}$
10^{9} spores/mL unwashed cell culture mixture (SPO2-CM)	48.4 ± 8.84^{a}	4.85 ± 1.29^{a}	173.35 ± 23.38^{a}	11.92 ± 2.98^{a}
Commercial biofungicides (Prevam)	$45.6 \pm 10.84^{a,b}$	$4.1 \pm 1.66^{a,b}$	138.6 ± 17.85^{b}	$10.5 \pm 2.36^{a,b}$
$F_{treatments}$	5.391	2.872	9.701	3.338
P-value	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.05$

The mean values with standard errors in the same column followed by the same letter are not significantly different according to the Duncan test at $P = 0.05$.

ability of this strain to produce chitosanase and proteases suggested that it can act on *R. solani* growth by antibiosis. In fact, chitosanase activity hydrolyses chitosan, which is among the main constituents of fungi cell wall. Proteases also play an important role in antifungal activity by degrading the protein linkage in the fungal external layers and/or the utilization of the fungal proteins for nutrition [33,34]. *B. subtilis* V26 supernatant caused vacuolization and deformation in *R. solani* hyphae similar to those observed after treatment of *R. solani* with *Bacillus* spp. [35,36]. Our results are also in accordance with the detrimental effect exhibited by *B. subtilis* strain in controlling *R. solani* [37].

The potato slices were treated by strain V26 before or after fungal inoculation in order to reveal whether V26 has curative or protective activities. Although both treatments were effective in reducing fungal infection, the most effective control was achieved when V26 was applied 24 h prior inoculation (protective activity). This could be due to the fact that *B. subtilis* endospores need time to germinate before they become ready to inhibit the germination of *R. solani*. This behaviour is commonly observed with other antagonists such as *Serratia plymuthica*, which inhibited green and blue mould infections of orange fruit [38] and *Enterobacter cloacae*, which inhibited *Rhizopus stoloninfer* spores on peach [39], when they are applied before the pathogen.

Fig. 6. (Colour online.) Promotion plant growth effect on potato by the application of *B. subtilis* V26. Treatments: 10^{9} spores/mL (SPO2), 10^{9} spores/mL culture mixture (SPO2-CM) and sterile distilled water (control).

The *in vivo* experiments proved the biocontrol ability of the antagonist V26 against *R. solani*. Indeed, potato tuber (seeds) treatment with V26 significantly suppressed *R. solani* growth and enhanced plant growth, when compared to the untreated plants. Likewise, Szczech and Shoda [40] and Idris et al. [41] reported that *Bacillus* strains significantly enhanced plant growth and inhibited soil-borne pathogen when using an antagonist-treated soil. Statistical analysis of data on the root canker and black scurf disease caused by *R. solani* indicated that there are significant differences between treatments. The strain V26 was able to control black scurf disease (81% biocontrol efficacy) as well as root canker (63% biocontrol efficacy) more efficiently than the commercial biofungicides Prevam. Interestingly, strain V26 seems to be more effective in suppressing black scurf than many other fungi and bacteria antagonists. Indeed, Tariq et al. [42] reported that *Pseudomonas* spp. StT2 and StS3 reduced potato black scurf disease caused by *R. solani* with 65.1% and 73.8% efficacy, respectively. *Pseudomonas* sp. strain S8.Fb11 reduced the proportion of infected tubers by *R. solani* to 40% for cv. Spunta and to 74% for cv. Nicola [17]. Unlike the V26 effect, *Rhizoctonia zeae* and *Laetisaria arvalis* reduced black scurf severity caused by *R. solani* at only 54–60% [27]. The pre-treatment of potato tubers with α-1,3-glucan induced 40% of protection against *Rhizoctonia* canker [43]. In addition to its ability to protect potato plants against *R. solani* more efficaciously than *Pseudomonas* spp, *B. subtilis* V26 has the advantage to form endospores that facilitate its production and recovery at a large scale, its formulation and its use in pots and in field treatments, in comparison to the others antagonists previously reported. Moreover, V26 significantly promoted the growth of potato plants. Indeed, *B. subtilis* V26 inoculation into potato tuber in the presence of the pathogen increased the number, the height and the weight of stems, and the weight of roots significantly, in comparison to the untreated control. The promotion of plant growth can involve direct and indirect mechanisms. Direct growth promotion is due to bacterial secretion of phytohormones, and to volatile metabolites that can impact root architecture by overproduction of root hairs and lateral roots and subsequently increase nutrient and water uptake, thus contributing to growth [44]. The indirect promotion of

plant growth can be due to antibiosis, competition for space and nutrients, parasitism or lysis of pathogen hyphae, inhibition of pathogen-produced enzymes or toxins, and through induced systemic resistance [45]. In the *in vivo* experiment, the improvement in fresh root weight by V26 treatment is statistically similar to that obtained by the biofertiliser "Nitrophoska". Moreover, the fresh root weight of potato plants treated with V26 in the absence of *R. solani* was statistically higher than those of potato plants treated with V26 and inoculated by the pathogenic fungus (data not shown). These results suggested that the promotion effect of V26 may be a direct effect. According to the ANOVA analysis on the length, the number and the fresh weight of stems means, there were no significant differences between plants treated with V26 and inoculated with *R. solani* and plants treated with V26, in the absence of the fungus (data not shown). Taking into account the detrimental effect of V26 on fungus hyphae, this suggests that the promotion of plant growth by V26 may be mainly associated with an indirect effect. Additional studies on the mechanisms of action of the new antagonist V26 are necessary to understand its potential role to control potato crop.

This study suggested that bacterization of potato tuber seeds with *B. subtilis* V26 significantly improves plant growth and successfully decreases the incidence of the disease caused by *R. solani*, which could be a good strategy to avoid black scurf disease. This finding agrees with those reported by Milus and Rothrock [46] that bacterization of wheat seeds with *B. subtilis* and *B. pumilus* significantly reduced plant stand compared to the control. On the other hand, coating of tomato seeds with *B. subtilis* RB14-C did not protect tomato plants against *R. solani* [40]. Consequently, the efficacy of seed bacterization by *B. subtilis* was not established for all model plants.

Therefore, our results show that the local strain V26 of *B. subtilis* is an efficient tool to control black scurf in potato. Further field experiments are needed to validate the efficacy of this strain to control *Rhizoctonia* disease in natural conditions and confirm whether *B. subtilis* V26 could be a promising candidate for the development of a new biocontrol agent, either alone or in combination with other biological agents or in rotation treatments, for use in organic farming in Tunisia.

Disclosure of interest

The authors declare that they have no competing interest.

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