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RESEARCH

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Multi-species endophytic *Bacillus* for improved control of potato soilborne and tuber-borne diseases in Tunisia: from laboratory to field conditions

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Abstract

Background Due to the soilborne and tuber-borne nature of their causative agents, Fusarium wilt, Rhizoctonia root canker and black dot diseases are still leading to serious problems in potato production in Tunisia and worldwide. Among endophytic bacteria, the genus *Bacillus* is one of the most exploited microbial groups known as potent biocontrol agents against several potato diseases. In the present study, six strains belonging to five *Bacillus* species were screened for their abilities to inhibit root and wilt potato pathogens in vitro, in vivo and under natural conditions over three cropping seasons and to promote plant growth.

Results Based on the dual-culture assays, the whole-cell suspensions of SV39 and SV104 (*Bacillus tequilensis*), SV41 (*B. subtilis*), SV44 (*B. methylotrophicus*) and SV65 (*B. amyloliquefaciens* subsp. *plantarum*) strains exhibited potent antifungal activity against important potato soilborne phytopathogens with ~ 65 to 70% inhibition rates. Significant inhibition rates were also induced by the cell-free culture filtrates, the butanolic and the chloroformic extracts depending on the target pathogens, the concentration used and the *Bacillus* strain tested. In pot experiment, a decrease in Rhizoctonia root canker severity, ranging from 43 to 65% compared to the inoculated and untreated control, was induced by all *Bacillus* spp. strain-based treatments, while SV39-, S42- and SV65-based treatments were the most effective in suppressing by 50–53 and 65–52%, black dot severity and the relative vascular discoloration extent induced by *F. oxysporum* f. sp. *tuberosi*, respectively, relative to positive control. This biocontrol potential was associated with an enhancement of potato growth parameters. Field studies indicated that soil treatment with the most of the *Bacillus* spp. strains had significantly controlled all the target fungal soilborne diseases and improved at the least two growth and/or production parameters depending on the strain used and the cropping seasons. SV39-, SV41-, SV44- and SV104-based treatments resulted in a significant increase in tuber yield in one cropping season.

Conclusion These *Bacillus* spp. strains could be used in combinations and/or introduced with other existing practices in order to provide supplemental control of target diseases and yield promotion under organic or conventional potato production systems.

Keywords *Bacillus* spp., Biocontrol, Cropping season, Growth promotion, Soilborne fungi, *Solanum tuberosum*, Yield

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Background

Potato (*Solanum tuberosum* L.) is one of the most important and valuable worldwide food crops which ranks fourth, in terms of production and area harvested, after maize, wheat and rice (FAO 2019). It is regarded as a high-potential food security crop because of its adaptability, its high yielding ability and its nutritive value and as an important component of diversified cropping systems (Devaux et al. 2021).

In Tunisia, potato holds an important position among all vegetable crops where about 16% (21,880 ha) of total vegetable cultivation area has been devoted for potato production (ONARGI 2019). Soilborne and tuberborne fungal diseases induced by wilt and root rot causing agents are major constraints that could affect potato production, productivity and quality. Of special emphasis throughout main potato-growing areas in Tunisia, due to their high prevalence and severity, are Fusarium wilt caused mainly by *Fusarium oxysporum* f. sp. *tuberosi* (Mejdoub et al. 2020), Verticillium wilt due to *Verticillium dahliae* (Daami-Remadi et al. 2010b), Rhizoctonia canker and black scurf incited by *Rhizoctonia solani* (Djébali and Belhassen 2010) and black dot caused by *Colletotrichum coccodes* (Daami-Remadi et al. 2010a).

The biological control has gained increased interest in the last decades and is currently considered as a promising eco-friendly alternative method to manage potato soilborne diseases (Zhao et al. 2021). *Bacillus* genus is known for harboring the most widely studied active antagonists, which biocontrol potential relies on several mechanisms such as competition for nutrients and space, production of a wide range of antibiotics, hydrolytic enzymes, siderophores and/or inducing systemic resistance (Fira et al. 2018). Several potato tuber, root and wilt diseases have been successfully controlled by *Bacillus* spp., such as late blight, common scab, Rhizoctonia stem canker and black scurf, Fusarium wilt and dry rot. In fact, previous studies revealed the significant antifungal activity of *B. subtilis* (Alfiky et al. 2022), *B. amyloliquefaciens* (Lin et al. 2018), *B. cereus* (Donmez et al. 2015), *B. velezensis* (Asaturova et al. 2021), *B. altitudinis* (Li et al. 2019), *Bacillus* sp. (Ntemafack et al. 2022), *B. megaterium* (Wang et al. 2020) and *B. mojavensis* (Ghazala et al. 2022) against many potato pathogens such as *Phytophthora infestans, Streptomyces scabies, Rhizoctonia solani* and *Fusarium* spp. and showed that they are able to suppress mycelial growth and spore germination, to decrease disease incidence and severity and to promote potato growth. These *Bacilli* are considered as the predominant soil and rhizosphere microorganisms and among the most widespread endophytic bacteria (De Silva et al. 2019). Thus, a number of bacteria have to be screened in order to maximize the chance of finding an effective biocontrol agent with broad-spectrum antifungal activity to increase yield of potato (Devi et al. 2016).

Indeed, in our previous studies, several endophytic *Bacillus* strains from five wild Solanaceous species, *Nicotiana glauca*, *Datura stramonium*, *D. metel*, *Solanum nigrum* and *S. elaeagnifolium*, were isolated and identified (Aydi Ben Abdallah et al. 2021) that were shown effective in controlling tomato Fusarium wilt induced by *Fusarium oxysporum* f. sp. *lycopersici* and in promoting plant growth. However, the broad-spectrum activity of these wild plant-associated bacterial endophytes against other phytopathogens such as those infecting potato has never been reported neither in Tunisia nor worldwide.

Therefore, in the present study, six strains belonging to five *Bacillus* species, namely *B. subtilis*, *B. cereus*, *B. tequilensis*, *B. methylotrophicus* and *B. amyloliquefaciens* subsp. *plantarum*, were tested for the biocontrol of potato-associated pathogenic fungi, in vitro, in vivo and under natural conditions over three cropping seasons.

Methods

Plant materials

Potato cv. Spunta seed tubers used in this study were kindly provided by the Technical Center of Potato and Artichoke, Essaïda, Tunisia. Before use, tubers were superficially disinfected with a 10% sodium hypochlorite (NaOCl) solution (commercial bleach containing 12% of active chlorine) during 5 min, rinsed with tap water and air-dried at ambient temperature. They were kept for two weeks under 15–20 °C, 60–80% relative humidity and natural room light for pre-germination.

Fungal strains

The phytopathogens used in this study were *Fusarium oxysporum* f. sp. *tuberosi* (accession number KU253815), *Colletotrichum coccodes* and *Rhizoctonia solani*. These agents were originally isolated from roots, stems and tubers of diseased potato plants. For *R. solani* and *C. coccodes* isolates, identification was carried out based on cultural and morphological traits according to Parmeter (1970) and Bailey and Jejer (1992), respectively. These isolates were widely used in the pathogenicity tests and cited in several previous studies (Daami-Remadi et al. 2008, 2010a; Mejdoub-Trabelsi et al. 2020). They were deposited in the fungal culture collection of the laboratory of Phytopathology in the Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia. Cultures of each pathogen were maintained on potato dextrose agar medium (PDA; Difco, Detroit, MI, USA) and stored in PDA slants at 5 °C until further use.

For *F. oxysporum* f. sp. *tuberosi* and *C. coccodes*, liquid cultures used for substrate inoculation were

prepared on potato dextrose broth (PDB) and incubated at 25 °C under continuous shaking at 150 rpm for 4 to 5 days. Concentration of the conidial suspensions used was adjusted to 10^7 conidia/ml using a Malassez haemocytometer.

For *R. solani* inoculum production, ten PDA Petri plates (9 cm in diameter) covered with full mycelium growth of pathogen cultures previously grown on PDA for 5–6 days at 28 °C were macerated using a blender in one liter of sterile distilled water (SDW). *R. solani* inoculum was adjusted to 10^8 mycelial fragments per ml using a Malassez haemocytometer.

Bacterial strains

Bacterial strains belonging to five *Bacillus* species (*B. tequilensis* str. SV39, *B. subtilis* str. SV41, *B. cereus* str. S42, *B. methylotrophicus* str. SV44, *B. amyloliquefaciens* subsp. *plantarum* str. SV65 and *B. tequilensis* str. SV104), originally recovered from internal stem tissues of several wild Solanaceous species (Table 1) and exhibiting high suppressive potential against tomato Fusarium wilt (Aydi Ben Abdallah et al. 2021), were selected for use in this study. Before being used in the different bioassays, stock cultures maintained at 20 °C in nutrient broth (NB) supplemented with 40% (v/v) glycerol (Fisher Scientific, Pittsburgh, PA, USA) were grown on nutrient agar medium (NA, Difco Laboratories, Detroit, MI, USA) and incubated at 25 °C for 48 h. For each bacterial strain, cell suspension was prepared from a 2-day-old culture on NA broth and incubated at 25 °C under continuous shaking at 150 rpm and adjusted to the desired concentration (~ 10^8 cfu/ml) using a spectrophotometer.

In vitro assessment of the antifungal potential of *Bacillus* spp. against target pathogens

Antifungal activity of the selected endophytic stains was screened using their whole-cell suspensions, cell-free culture filtrates and butanolic and chloroformic extracts.

Screening of the antifungal potential of Bacillus spp. whole cells

The antifungal activity of whole-cell suspensions of the six bacterial strains, previously grown in NB medium (liquid culture), was evaluated using the dual-culture method on PDA medium amended with streptomycin sulfate (300 mg/l) (w/v), according to Pandey et al. (2023) with some modifications. An equal volume of 20 µl of each bacterial suspension (~ 10^8 cells/ml) was suspended into a well performed using a sterile Pasteur pipette (6 mm in diameter, 3 mm in depth) at one side of the Petri plate (90 mm in diameter). An agar plug (6 mm in diameter) removed from the growing edge of a 7-day-old culture of each tested fungal pathogen was placed at the opposite side of the plate.

Control plates were treated with the same volume of SDW. Each individual treatment was replicated three times. The colony diameter was measured after 3 (*R. solani*), 7 (*F. oxysporum* f. sp. *tuberosi*) and 8 (*C. coccodes*) days of incubation at 25 °C. The inhibition rate (IR) of the target pathogen was calculated using the formula of Tiru et al. (2013) as follows: IR = [(C2 − C1)/C2]*100, where C2 is the diameter of pathogen colony in control plates and C1 is the diameter of pathogen colony in the presence of bacterial strains.

Screening of the antifungal potential of Bacillus spp. cell-free culture filtrates

The antifungal activity of the cell-free culture filtrates of the selected bacterial strains was assessed according to Karkachi et al. (2010) protocol. Liquid cultures obtained, as described above, were centrifuged at 10.000 rpm for 10 min. The centrifugation was repeated three times. The cell-free supernatant fluids were sterilized by filtration through a 0.22-µm pore size filter. NB filtrate was used as control treatment. The filtrates were aseptically added to Petri plates containing cool molten PDA medium amended with streptomycin sulfate (300 mg/l) (w/v) at the concentrations of 10 and 20% (v/v). After the overlay complete absorption, one agar plug of each pathogen tested (6 mm in diameter) was placed in the center of

Table 1 Putative endophytic *Bacillus* spp. strains tested and their isolation sources and geographical origins

Strain	Species	Accession number	Plant	Locality	GPS locality
SV39	*Bacillus tequilensis*	KR818070	*Datura metel*	Chott-Mariem, Sousse, Tunisia	N35°56'20.451"; E10°33'32.028"
SV41	*B. subtilis*	KR818071	*D. metel*	Chott-Mariem, Sousse, Tunisia	N35°56'20.451"; E10°33'32.028"
SV44	*B. methylotrophicus*	KR818072	*D. metel*	Chott-Mariem, Sousse, Tunisia	N35°56'20.451"; E10°33'32.028"
SV65	*B. amyloliquefaciens* subsp. *plantarum*	KR818073	*Solanum nigrum*	Chott-Mariem, Sousse, Tunisia	N35°56'20.451"; E10°33'32.028"
SV104	*B. tequilensis*	KU976970	*S. elaeagnifolium*	M'saken, Sousse, Tunisia	N35°43'32.073"; E10°34'48.90"
S42	*B. cereus*	KP993206	*Nicotiana glauca*	Bekalta, Mahdia, Tunisia	N35°37'14,327"; E10°59'41,393"

the Petri plate. Each individual treatment was replicated three times. The colony diameter was measured after 3 (*R. solani*), 7 (*F. oxysporum* f. sp. *tuberosi*) and 8 (*C. coccodes*) days of incubation at 25 °C. The inhibition rate (IR) of the target pathogen was calculated using the formula of Tiru et al. (2013) as follows: IR = [(C2 − C1)/C2]*100, where C2 is the diameter of pathogen colony in control plates and C1 is the diameter of pathogen colony in the presence of bacterial filtrates.

Screening of the antifungal potential of Bacillus spp. n-butanol and chloroform extracts

Organic extraction was performed for the six bacterial strains using *n*-butanol (Romero et al. 2007) and chloroform (Bhoonobtong et al. 2012) as previously described in Aydi Ben Abdallah et al. (2017b). Sixty milliliters of cell-free culture filtrate of each strain was poured in a separating funnel, and 60 ml of solvent (chloroform or *n*-butanol) was added carefully. The funnel was reversed several times by degassing from time to time. The mixture was allowed to settle for few minutes with the cap open. The organic phase (the lowest phase for extraction with chloroform and the upper one with *n*-butanol) was recovered. The aqueous phase was replaced in the funnel, and the extraction was repeated two other times. The solvent was evaporated in a rotary evaporator at 35 °C for chloroform and 75 °C for *n*-butanol with a slight rotation at 150 rpm. The dry weights of crude extracts obtained using chloroform and *n*-butanol solvents were noted, and the yield extraction was determined (in mg/ml).

To assess their antifungal activity against target pathogens, *n*-butanol and chloroform extracts were suspended into ethanol (1:1) (mg/ml) and added separately at two concentrations (2.5 and 5% v/v) to molten PDA medium pre-cooled to about 45 °C and amended with streptomycin sulfate (300 mg/l) before being poured in Petri plates. Negative control cultures were treated with similar concentrations of ethanol. After the overlay complete absorption, one agar plug of each pathogen tested (6 mm in diameter) was placed in the center of the Petri plate. Each individual treatment was replicated three times. The colony diameter was measured after 3 (*R. solani*), 7 (*F. oxysporum* f. sp. *tuberosi*) and 8 (*C. coccodes*) days of incubation at 25 °C. The inhibition rate (IR) of the target pathogen was calculated using the formula of Tiru et al. (2013) as follows: IR = [(C2 − C1)/C2]*100, where C2 is the diameter of pathogen colony in control plates and C1 is the diameter of pathogen colony in the presence of bacterial extracts.

Disease suppression potential of *Bacillus* spp. against potato diseases (Pot experiment)

The whole-cell suspensions (~ 10^8 cells/ml) of the tested *Bacillus* strains were screened for their ability to suppress the main soilborne potato fungal diseases induced by *F. oxysporum* f. sp. *tuberosi*, *R. solani* and *C. coccodes*, in pot experiment. In order to imitate natural conditions, these fungi were added to the culture substrate as consortium preparation containing a mix of equal proportions of each pathogen inoculum. One week before potato planting, pots (25 cm diameter) were filled with sterilized peat and watered each with 100 ml of pathogenic consortium. Potato seed tubers, showing optimal germination, were planted into individual pots containing the previously inoculated peat and treated by drenching 100 ml of each bacterial strain liquid culture (~ 10^8 cells/ml) per pot. The three subsequent bacterial treatments were applied at 7-day intervals from the first one. For inoculated (IC) and non-inoculated (NIC) and untreated control plants, tubers were planted in pathogen challenged-substrate or not, respectively, and treated similarly with SDW only.

All potato plants were grown under greenhouse conditions at 21–26 °C, with 70–85% air relative humidity and 12-h photoperiod, and watered regularly as needed. Six replicate pots were used for each individual treatment, and the whole experiment was repeated twice.

At the end of the experiment, 60 days post-planting (DPP), plants were uprooted and the belowground organs were carefully removed from the pots and gently washed with running water to remove the remaining culture substrate. The effects of bacterial treatments were assessed based on disease severity and plant growth and production parameters.

Disease severity assessment

Foliar wilt severity was assessed based on the intensity of leaf wilting, yellowing and necrosis on the whole plant. This parameter representing the intensity of foliar symptoms was noted for each plant using a 0–5 foliar rating scale, where 0 = no symptoms, 1 = 1–25% plant wilting and yellowing, 2 = 26–50% wilting and yellowing, 3 = 51–75% wilting and yellowing, 4 = 76–99% and 5 = dead plant (modified scale of Amini 2009).

The vascular wilt severity was estimated based on the relative vascular discoloration extent which is the percentage of stem height exhibiting vascular discoloration. For each plant, each stem was longitudinally cut and visually examined for the presence of vascular discoloration. The extent of this vascular discoloration was measured per stem, and the average for all stems was calculated per plant.

For the assessment of black dot severity, the stem base, roots and stolons were estimated visually according to the scale adopted by Daami-Remadi et al. (2010a) based on necrotic lesions progress and percentage of area covered by black dot microsclerotia, where 0 = no microsclerotia, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 = 76–100% of plant tissue colonized by microsclerotia. The assessments of the disease severity were done for each stem individually, and the average for all stems was recorded for each plant.

Rhizoctonia root canker severity was assessed visually based on the presence of necrotic lesions and cankers on the stem bases, roots and stolons and estimated according to 0–5 scale (Daami-Remadi et al. 2008), where 0 = no lesion, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99% and 5 = 100% of plant tissue covered by necrotic lesions. The assessments of the disease severity were done for each stem individually, and the mean was recorded for each plant.

Potato growth and production parameters

The effects of inoculations and bacterial treatments tested were also evaluated based on plant growth and production parameters. In fact, the length of all stems from the ground level was measured and the average per plant was used to calculate the mean height. However, for the aerial and the belowground parts (belowground stems, roots and stolons) and tubers, the total fresh weight for each plant was recorded. For each individual treatment, plant stems and roots were mixed, surface-sterilized in 0.5% NaOCl, rinsed with sterile water, cut into 3–5 mm fragments, plated on potato dextrose agar (PDA) medium and incubated at 20 °C for 7 days for the isolation of fungal pathogens involved in the observed wilting symptoms.

Disease suppression potential of *Bacillus* spp. against soilborne potato diseases under field conditions

This study pursued the effect of the bacterial treatments on controlling vascular wilt and canker and root rot diseases in potato grown in naturally infested soil as well as on plant growth and yield parameters.

Field trials were conducted over two cropping years 2020–2021 and 2021–2022, at two experimental farms of the Regional Research Centre on Horticulture and Organic Agriculture in Téboulba (37° north, 10° south, altitude 28 m) and in Chott-Mariem (37° north, 10° south, altitude 28 m) regions. These sites, located in the center-east coast of Tunisia, were under conventional farming system and had a history of potato and other vegetables production practices. These two sites are characterized by a semi-arid climate with mild rainy winters, hot dry summers and a great annual and seasonal variability in precipitation. In Téboulba, the soil has a sandy clay texture, whereas in Chott-Mariem the soil is clay-loam. These two sites have a long history of potato soilborne fungal diseases, such as Fusarium wilts, black dot and Rhizoctonia root and stem canker, with consistent disease pressure throughout the field. Thus, no pathogen inoculum was added to the field, and development of soilborne diseases is dependent on natural inoculum and infection.

Three field experiments were conducted during 2020/2021 and 2021/2022 for three growing seasons. The first and second trials were set up in Téboulba for the mainseason and late season crops from February 19 to June 11, 2021, and from September 27, 2021, to January 20, 2022, respectively. The third experiment was carried out in Chott-Mariem region during the extra-early crop season from December 20, 2021, to March 28, 2022. The potato cultivar used in this experiment was Spunta being the most cultivated in Tunisia (representing 86% of the grown cultivars) and which tubers were not treated with fungicides before planting.

These trials were conducted in a randomized complete block design with three replicates and seven treatments (six bacterial treatments and untreated control) per replicate (plot). Each plot, consisting of seven rows (one line for each treatment), was 10 m long and 6 m width with 0.75 m row spacing. Thirty fully potato seed tubers were planted in each row with 0.33 m within row spacing. The first treatment was applied at planting (one bacterial treatment per line) through soil drenching of 100 ml of the whole-cell suspension (~ 10^8 cfu/ml)/tuber/replicate. The three subsequent applications were carried out every two weeks.

All the agronomical practices such as irrigation, fertilization and weeding were adopted as required. Fertilizers were applied according to the fertilization program established by the Technical Center of Potato and Artichoke, Tunisia (Nouna et al. 2016).

At 90 DPP (i.e., tuber maturation stage), ten whole plants per treatment and replication were randomly selected, uprooted and brought to laboratory. The belowground organs were carefully separated from the aerial parts and gently washed with water to remove the adhering soil. The root and aerial part fresh weights as well as the average tuber weight per plant were measured. At 110 DPP (at harvest), total tuber yield was measured (Marouani et al. 2015). The effects of bacterial treatments were assessed through disease severity parameters. The severity of foliar and vascular wilt, black dot and Rhizoctonia root canker was estimated as previously described above for pot experiments.

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA), using SPSS 16.0 (Statistical Package for the Social Sciences), using the general linear models (GLM). The in vitro assays were performed according to a completely randomized factorial model, and each individual treatment was repeated six times. For the evaluation of the antifungal activity of whole-cell suspensions, the independent variables were bacterial treatments tested and fungal pathogens. For the screening of the effects of the cell-free filtrate cultures and the organic extracts, the trial was carried out including three independent variables (bacterial treatments tested, target fungal pathogens and concentrations tested). For all in vitro tests, data were arcsin-transformed prior to statistical analysis.

The in vivo experiments were performed according to a completely randomized design where bacterial treatments (bacterial strains and control) represented the only independent variable and each individual treatment was replicated six times.

Field trials were analyzed according to randomized block factorial design with two independent variables (cropping seasons and bacterial treatments) with three replications and 30 repetitions per individual treatment.

For all the above-mentioned experiments, means were separated using LSD or Duncan multiple range tests to identify significant pair-wise differences at $P \leq 0.05$.

Results

Antifungal potential of *Bacillus* spp. whole cells

Analysis of variance revealed that the inhibition rate depended significantly (at $P \leq 0.05$) upon the target phytopathogens and the endophytic *Bacillus* spp. strains tested but non-significant interaction was recorded between both fixed factors. Indeed, using the dual-culture method, five *Bacillus* spp. strains, i.e. SV39 SV41 SV44, SV65 and SV104, had significantly reduced the mycelial growth of all pathogens combined by 65–70%, over the untreated control, than the 34% recorded using S42 strain (Fig. 1A). For all bacterial treatments combined, the highest radial growth inhibition (74%) was recorded in *C. coccodes* cultures, followed by 60 and 52% noted in those of *R. solani*, and *F. oxysporum* f. sp. *tuberosi*, relative to the untreated control (Fig. 1B).

Antifungal potential of *Bacillus* spp. cell-free culture filtrates

Tested using the poison food method, the cell-free culture filtrates of tested *Bacillus* strains had significantly ($P \leq 0.05$) decreased the fungal radial growth, in comparison with the untreated control. This inhibitory effect varied significantly depending on *Bacillus* spp. filtrates tested, concentrations used, target fungal pathogens and their interactions (Table 2).

Using SV39 cell-free culture filtrate at 10% v/v, fungal growth reduction varied from 9 to 35%, compared to 34–43% noted at 20% v/v. When applied at 10% v/v, inhibition rates induced by S42, SV44, SV65 and SV104 filtrates ranged from 8 to 22% compared to 19–32% recorded at 20% v/v. SV41filtrate showed growth reduction ranging from 10 to 24% when applied at 20% v/v, while mycelial reduction did not exceed 20% when this filtrate was used at 10% v/v (Table 2).

Regardless of the concentrations tested, the highest decrease in *F. oxysporum* f. sp. *tuberosi*, *R. solani* and *C. coccodes* mycelial growth by 25, 24 and 39%, respectively, was achieved using SV39 diffusible metabolites (Table 2).

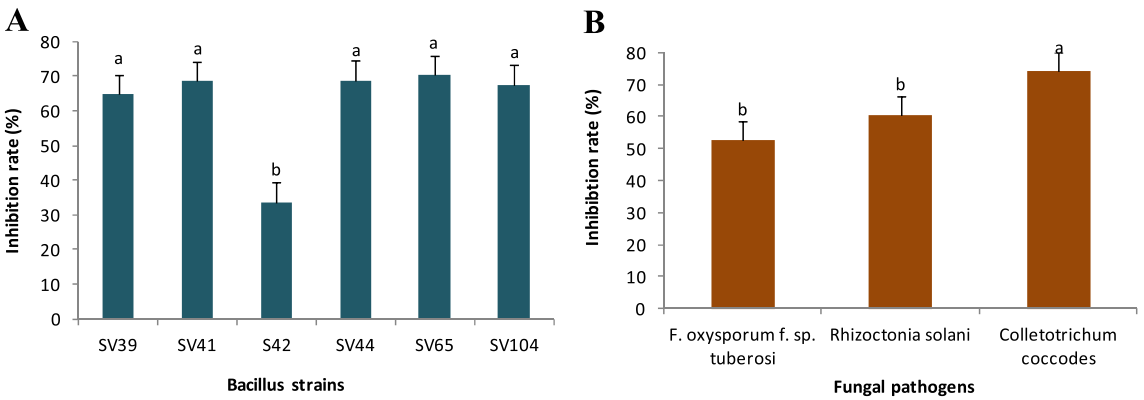

Fig. 1 Antifungal activity induced by *Bacillus* spp. whole cells (**A**) tested against three potato wilt-causing fungi (**B**) noted after incubation at 25 °C on PDA medium. Growth inhibition was noted after incubation for 3 days (*Rhizoctonia solani*), 7 days (*Fusarium oxysporum* f. sp. *tuberosi*,) and 8 days (*Colletotrichum coccodes*). SV39: *Bacillus tequilensis*, SV41: *B. subtilis*, S42: *B. cereus*, SV44: *B. methylotrophicus*, SV65: *B. amyloliquefaciens* subsp. *plantarum* and SV104: *B. tequilensis*. The results are presented as mean ± SE ($P \leq 0.05$). Bars sharing the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

Table 2 Growth inhibition rate (%) induced by *Bacillus* spp. cell-free culture filtrates tested at two concentrations against three potato wilt-causing fungi

Bacterial treatment	Concentration (%)	Fungi tested			
		Fusarium oxysporum* f. sp. *tuberosi	***Rhizoctonia solani***	***Colletotrichum coccodes***	**Average**
Bacillus tequilensis SV39	10	8.72	14.93	34.92	19.52 cd[d*]
	20	40.87	33.80	42.76	39.14 a
	Average[c*]	24.80 a[+]	24.37 a	38.84 a	29.33 A[a*]
B. subtilis SV41	10	7.85	17.75	19.59	15.06 e
	20	9.74	23.94	23.68	19.12 d
	Average	8.80 c	20.85 b	21.64 c	17.09 C
B. cereus S42	10	7.85	14.65	22.32	14.94 e
	20	19.85	22.82	31.52	24.73 b
	Average	13.86 b	18.73 bc	26.92 b	19.83 B
*B. methylotrophicus*SV44	10	9.74	12.68	18.23	13.55 e
	20	19.42	21.13	27.09	22.54 bc
	Average	14.58 b	16.90 c	22.66 bc	18.04 BC
B. amyloliquefaciens subsp. *plantarum* SV65	10	12.37	10.42	20.95	14.58 e
	20	19.42	22.25	31.86	24.51 b
	Average	15.90 b	16.34 c	26.41 b	19.54 B
B. tequilensis SV104	10	11.49	14.93	14.48	13.63 e
	20	21.19	21.69	28.45	23.77 b
	Average	16.35 b	18.31 bc	21.47 c	18.7 BC
Average[b*]		15.71 c	19.25 b	26.32 a	

[a] Mean mycelial growth inhibition per bacterial treatment for all concentrations and all fungal pathogens combined

[b] Mean mycelial growth inhibition per fungal pathogen for all bacterial treatments and all concentrations combined

[c] Mean mycelial growth inhibition per bacterial treatment and per fungal pathogen for all concentration combined

[d] Mean mycelial growth inhibition per bacterial treatment per concentration for all fungal pathogens combined

+ Means within columns followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

*For fungal pathogens, bacterial treatments tested and concentrations applied, values (means) followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

LSD (Fungal pathogens × Bacterial treatments × Cell-free culture filtrate concentrations) = 5.13% at $P \leq 0.05$

Growth inhibition was noted after incubation at 25 °C for 3 days (*R. solani*), 7 days (*F. oxysporum* f. sp. *tuberosi*) and 8 days (*C. coccodes*) on PDA medium

For all bacterial treatments and concentrations combined, the highest decrease in fungal mycelial growth, by 26% compared to control, was noted on *C. coccodes* cultures, followed by those of *R. solani* (19%), whereas only 16% were recorded on *F. oxysporum* f. sp. *tuberosi* cultures. Regardless of the concentration used and pathogens tested, SV39 cell-free filtrate was the most effective in reducing fungal radial growth by 29%, compared to 17–20% induced by the remaining filtrates.

When applied at 20% v/v, diffusible metabolites from all *Bacillus* strains were significantly more active in inhibiting fungal radial growth (all target pathogens combined) by 19–39% compared to 14–20% noted at 10% v/v (Table 2).

Antifungal potential of *Bacillus* spp. n-butanol extracts

Growth inhibition rates varied significantly (at $P \leq 0.05$) depending on *Bacillus* spp. strains used for extraction, concentrations used, target fungal pathogens and their interactions. The results given in Table 3 showed that, using SV39 butanolic extract at 2.5% v/v, radial growth inhibition varied between 28 and 72% compared to 43 and 78%, achieved using the same extract at 5%.

Butanolic extracts of SV65 and SV104 strains applied at 2.5% v/v had inhibited by more than 47% the radial growth of two pathogens out of three tested, while at 5% v/v the inhibition rate ranged between 57 and 78%. S42 and SV44 butanolic extracts used at 5% v/v had suppressed by more than 42% the in vitro growth of two out of three fungal pathogens compared to 58 and 37% reached for only one pathogen using these extracts at 2.5% v/v, respectively.

Mycelial growth of the tested pathogens was inhibited by 47–65% using butanolic extract from SV41 at 5% v/v compared to 56% reached for only one pathogen (*R. solani*) using this extract at 2.5% v/v. Tested potato

Table 3 Growth inhibition rate (%) of *Bacillus* spp. butanolic extracts tested at two concentrations against the mycelial growth of various potato wilt-causing fungi

Bacterial treatment	Concentration (%)	Fungi tested			
		Fusarium oxysporum* f. sp. *tuberosi	***Rhizoctonia solani***	***Colletotrichum coccodes***	**Average**
*Bacillus tequilensis*SV39	2.5	28.43	71.55	40.72	46.90 cd[d*]
	5.0	42.97	78.31	67.97	63.08 a
	Average[c*]	35.70 c[+]	74.93 a	54.34 a	54.99 A[a*]
B. subtilis SV41	2.5	29.73	55.77	19.93	35.15 e
	5.0	47.12	64.51	59.11	56.91 b
	Average	38.43 bc	60.14 b	39.52 b	46.03 B
B. cereus S42	2.5	26.25	58.31	19.59	34.72 e
	5.0	37.39	66.48	46.17	48.33 c
	Average	29.30 d	62.39 b	32.88 c	41.52 C
*B. methylotrophicus*SV44	2.5	37.12	23.94	20.95	27.34 f
	5.0	48.43	42.82	38.67	43.31 d
	Average	42.78 b	33.38 c	29.81 c	35.32 D
B. amyloliquefaciens subsp. *plantarum* SV65	2.5	47.12	72.96	17.21	45.76 cd
	5.0	56.69	78.03	66.27	67.00 a
	Average	51.91 a	75.49 a	41.74 b	56.38 A
B. tequilensis SV104	2.5	47.56	58.03	29.47	45.02 cd
	5.0	57.12	65.35	78.19	66.89 a
	Average	52.34 a	61.69 b	53.83 a	55.95 A
Average[b*]		41.74 b	61.34 a	42.02 b	

[a] Mean mycelial growth inhibition per bacterial treatment for all concentrations and all fungal pathogens combined

[b] Mean mycelial growth inhibition per fungal pathogen for all bacterial treatments and all concentrations combined

[c] Mean mycelial growth inhibition per bacterial treatment and per fungal pathogen for all concentration combined

[d] Mean mycelial growth inhibition per bacterial treatment per concentration for all fungal pathogens combined

+ Means within columns followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

*For fungal pathogens, bacterial treatments tested and concentrations applied, values (means) followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

LSD (Fungal pathogens × Butanolic extracts × Concentrations) = 7.26% at $P \leq 0.05$

Growth inhibition was noted after incubation at 25 °C for 3 days (*R. solani*), 7 days (*F. oxysporum* f. sp. *tuberosi*) and 8 days (*C. coccodes*) on PDA medium

fungal pathogens responded differently to *Bacillus* spp. butanolic extracts. In fact, regardless of the concentrations tested, *F. oxysporum* f. sp. *tuberosi* and *C. coccodes* radial growth was inhibited by 43–57 and 46–78%, respectively, using five *Bacillus* spp. butanolic extracts out of six tested when applied at 5% v/v (Table 3). For *R. solani*, all butanolic extracts, excepting those of SV44, had significantly inhibited pathogen mycelial growth by more than 55%, regardless of the concentrations used.

The results (Table 3) revealed that for all butanolic extracts and concentrations combined, the highest mycelial growth inhibition rate (61%) was noted on *R. solani* cultures, followed by those of *C. coccodes* (42%) and *F. oxysporum* f. sp. *tuberosi* (42%).

For all tested pathogens and all concentrations combined, SV65, SV104 and SV39 butanolic extracts displayed the highest antifungal potential by inhibiting pathogens growth by 54–56%.

As for concentrations effects and for all fungal pathogens combined, butanolic extracts from *Bacillus* spp. strains applied at 5% v/v had suppressed fungal radial growth by 43–67%, compared to 27–47% noted when they were tested at 2.5% v/v (Table 3).

Antifungal potential of *Bacillus* spp. chloroform extracts

Amendment of PDA medium with *Bacillus* spp. chloroform extracts led to a significant decrease in the mycelial growth of the tested fungal pathogens in comparison with the untreated control (Table 4). The inhibition rate varied significantly ($P \leq 0.05$) depending on target pathogens, chloroform extracts tested, concentrations used and their three-way interaction. In fact, at 5% v/v, SV39, SV41 and SV65 chloroformic extracts had induced the highest inhibition rates estimated at 39–85, 31–71 and 43–66% compared to 20–53, 21–46 and 18–54% achieved using the same extracts at 2.5% v/v, respectively (Table 4).

Table 4 Growth inhibition rate (%) induced by *Bacillus* spp. chloroform extracts tested at two concentrations against three potato wilt-causing fungi

Bacterial treatment	Concentration (%)	Fungi tested			
		Fusarium oxysporum* f. sp. *tuberosi	***Rhizoctonia solani***	***Colletotrichum coccodes***	**Average**
Bacillus tequilensis SV39	2.5	20.17	53.52	38.67	37.45 ef[d*]
	5.0	38.86	85.07	50.94	58.29 a
	Average[c*]	29.51 a[+]	69.30 a	44.80 c	47.87 A[a*]
B. subtilis SV41	2.5	21.47	45.92	52.98	40.12 de
	5.0	31.38	70.70	69.68	57.14 a
	Average	26.42 ab	58.31 c	61.33 a	48.63 A
B. cereus S42	2.5	20.14	44.79	26.06	30.35 g
	5.0	26.41	67.89	41.74	45.30 c
	Average	23.27 b	56.34 c	33.90 d	37.83 D
B. methylotrophicus SV44	2.5	23.70	32.39	48.89	34.98 f
	5.0	34.14	52.96	55.03	47.21 c
	Average	28.92 a	42.68 d	51.96 b	41.09 C
B. amyloliquefaciens subsp. *plantarum* SV65	2.5	17.92	54.37	49.57	40.64 d
	5.0	42.58	66.20	60.82	56.16 a
	Average	30.25 a	60.28 c	55.20 b	48.40 A
B. tequilensis SV104	2.5	17.51	60.00	38.67	38.74 de
	5.0	35.99	69.58	50.60	51.85 b
	Average	26.75 ab	64.79 b	44.63 c	45.30 B
Average[b*]		27.31 c	58.62 a	48.64 b	

[a] Mean mycelial growth inhibition per bacterial treatment for all concentrations and all fungal pathogens combined

[b] Mean mycelial growth inhibition per fungal pathogen for all bacterial treatments and all concentrations combined

[c] Mean mycelial growth inhibition per bacterial treatment and per fungal pathogen for all concentration combined

[d] Mean mycelial growth inhibition per bacterial treatment per concentration for all fungal pathogens combined

+ Means within columns followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

*For fungal pathogens, bacterial treatments tested and concentrations applied, values (means) followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

LSD (Fungal pathogens × Chloroformic extracts × Concentrations) = 4.82% at $P \leq 0.05$

Growth inhibition was noted after incubation at 25 °C for 3 days (*R. solani*), 7 days (*F. oxysporum* f. sp. *tuberosi*) and 8 days (*C. coccodes*) on PDA medium

Even for the remaining chloroformic extracts tested, the inhibition rates were higher when they were applied at 5% than at 2.5% v/v. In fact, for those of SV104, SV44 and SV42 fungal radial growth was inhibited by 18–60, 24–49 and 20–45%, when they were used at 2.5% v/v but raised to 36–70, 34–55 and 26–68% when applied at 5% v/v, respectively.

R. solani and *C. coccodes* growth inhibition rates were estimated at 32–60 and 26–53% at the concentration 2.5% v/v compared to 53–85 and 42–70% noted at 5% v/v, and this for all cholоformic extracts combined. *F. oxysporum* f. sp. *tuberosi* was more inhibited by SV65 chloroformic extract applied at 5% v/v (43%), as compared to 26–39% achieved using the remaining extracts at the same concentration.

The results (Table 4) revealed that for all chloroformic extracts and concentrations combined, the highest decrease in mycelial growth, by 59% was noted on *R. solani* cultures, followed by 49 and 27% recorded on those of *C. coccodes* and *F. oxysporum* f. sp. *tuberosi*, respectively.

Independently of the concentration used and the pathogen tested, SV39, SV41 and SV65 chloroformic extracts induced the highest inhibition rates estimated at 48–49% compared to 38–45% noted using the remaining extracts.

Overall, for all fungal pathogens combined, chloroformic extracts from all *Bacillus* spp. strains tested at 5% v/v were significantly more effective in reducing fungal radial growth by 45–58% compared to 30–41% recorded at 2.5% v/v (Table 4).

Biocontrol potential of *Bacillus* spp. strains against potato fungal diseases (Pot experiment)

Diseases severity

The severity of the monitored diseases, recorded 60 DPP on potato cv. Spunta plants, varied significantly (at

$P \leq 0.05$) depending on bacterial treatments tested. SV39-based treatment was found to be effective in suppressing potato foliar wilt symptoms by 50% compared to pathogen-inoculated and untreated control. Moreover, wilt severity noted on SV39-treated plants was significantly comparable to the uninoculated and untreated controls (Fig. 2A). Potato plants treated with the remaining *Bacillus* spp. strains showed significantly similar wilt severity as all controls (Fig. 2A).

A significant (at $P \leq 0.05$) decrease in Rhizoctonia root canker severity, ranging from 43 to 65% compared to the inoculated and untreated control, was induced by all *Bacillus* spp. strains based treatments. Moreover, all biologically treated plants showed significantly similar disease severity as the negative control ones (Fig. 2B).

SV39-, S42- and SV65-treated plants showed 53, 50 and 51% lower black dot severity relative to the positive control. Moreover, potato plants treated with these strains had the same phytosanitary status as the negative controls. The remaining *Bacillus* spp. strains had similar effects on disease severity as both controls (Fig. 2C).

SV39-, S42- and SV65-based treatments were also the most active in reducing by 65, 63 and 52% the relative extent of the vascular discoloration (from collar) compared to that of the untreated and inoculated control. Vascular discoloration severity was significantly similar to that noted on disease-free control plants. The three remaining *Bacillus* spp. strains exhibited the same disease-suppressive effect as both controls (Fig. 2D).

Potato growth and production parameters

All potato growth and production parameters (plant height and root and tuber weights), except aerial part fresh weight, noted 60 DPP, varied significantly (at $P \leq 0.05$) depending on bacterial treatments tested. SV65-based treatment was the most effective in enhancing plant height by 55% as compared to both untreated controls. Potato plants treated with the remaining *Bacillus* spp. strains showed 35–49% improvement in their height in comparison with both controls (Fig. 3A).

SV39-, SV41- and to lesser SV44-based treatments were the most active in enhancing root growth by 45, 41

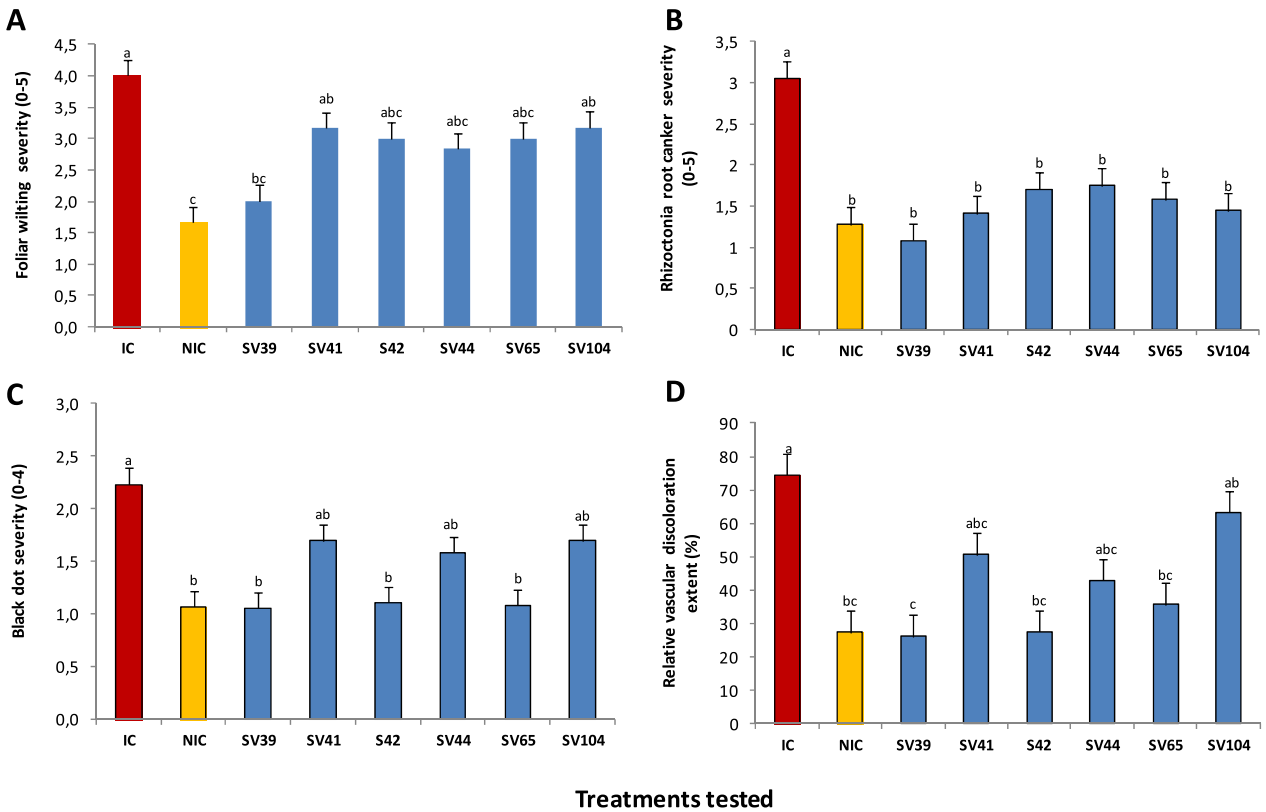

Fig. 2 Effectiveness of various bacterial treatments against foliar wilting (**A**), Rhizoctonia root canker (**B**), black dot (**C**) and vascular discoloration (**D**) on potato cv. Spunta plants inoculated with soilborne fungal pathogens as compared to controls. IC: inoculated and untreated control; NIC: uninoculated and untreated control; Parameters were noted 60 days post-planting. SV39: *Bacillus tequilensis*, SV41: *B. subtilis*, S42: *B. cereus*, SV44: *B. methylotrophicus*, SV65: *B. amyloliquefaciens* subsp. *plantarum* and SV104: *B. tequilensis*. The results are presented as mean ± SE ($P \leq 0.05$). Bars sharing the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

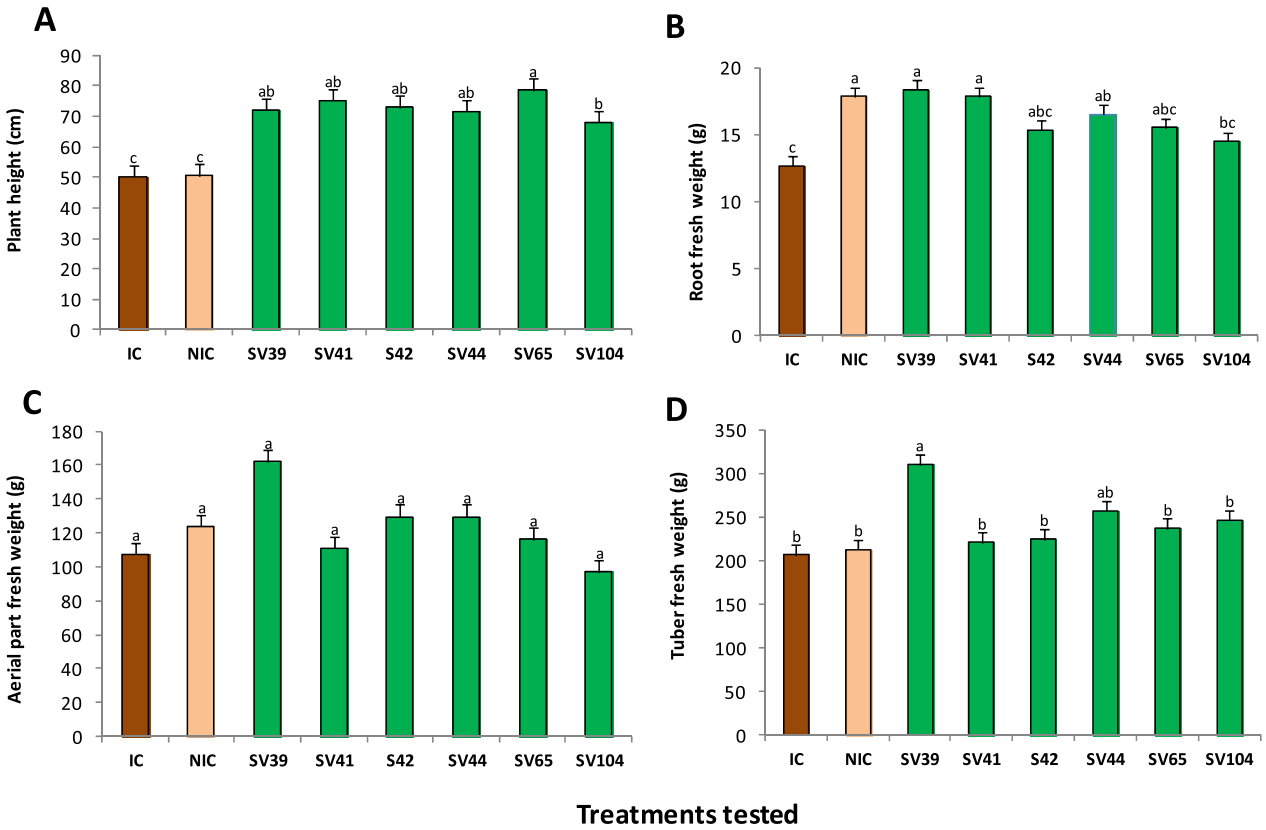

Fig. 3 Effects of various bacterial treatments tested on growth and production parameters of potato cv. Spunta plants inoculated with soilborne fungal pathogens as compared to controls. IC: inoculated and untreated control; NIC: uninoculated and untreated control; **A** plant height, **B**, **C** and **D** root, aerial part and tuber fresh weights, respectively, noted 60 days post-planting. SV39: *Bacillus tequilensis*, SV41: *B. subtilis*, S42: *B. cereus*, SV44: *B. methylotrophicus*, SV65: *B. amyloliquefaciens* subsp. *plantarum* and SV104: *B. tequilensis*. The results are presented as mean ± SE ($P \leq 0.05$). Bars sharing the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

and 30%, respectively, over the inoculated and untreated control (Fig. 3B). Potato plants treated with all *Bacillus* spp. strains showed aerial part fresh weights significantly comparable to those of disease-free and inoculated control plants (Fig. 3C). SV39-based treatment was found to be the most effective in enhancing tuber weight by 50 and 46% over the negative and positive controls, respectively (Fig. 3D).

Biocontrol potential of *Bacillus* spp. strains against potato-associated soilborne diseases under field conditions

During the three cropping seasons, moderate to severe levels of foliar wilting, Rhizoctonia root canker, black dot and vascular discoloration were noted on potato cv. Spunta plants at 90 DPP. For the management of these diseases commonly found in most potato-growing areas, the whole-cell suspensions (~10^8 cells/ml) from six *Bacillus* spp. strains were tested as soil drenching applied four times per cropping season.

Diseases severity

The severity of all monitored potato diseases was significantly ($P \leq 0.05$) reduced, with varying degrees, depending on bacterial treatments tested, cropping seasons and their interactions as detailed below.

Bacillus spp.-based treatments had significantly reduced yellowing and wilt symptoms intensity by 29–57 and 6–29%, relative to the untreated control, in the extra-early and late season crops, respectively, while only S42 was able to decrease it by 5% in the main season crop (Table 5).

In the extra-early season trial, treatments with SV39, SV65 and SV104 strains led to the highest wilt severity suppression, by 53, 57 and 50% versus control, while following treatments using SV41, S42 and SV44 strains, wilt severity was reduced by 29, 34 and 32%, respectively. As for their effects in the late planting season, SV39-, S42-, SV44- and to lesser extent SV41-based treatments induced decreases in wilt severity by 24, 27, 29 and 11%, respectively.

Table 5 Variation of the effectiveness of bacterial treatments against foliar wilting, root canker, black dot and vascular discoloration severities on potato cv. Spunta plants depending on cropping seasons

Cropping season	Bacterial treatment							Average[a*]
	Control	SV39	SV41	S42	SV44	SV65	SV104	
Foliar wilting severity (0–5)								
Late season	3.82 a[c+]	2.90 c	3.40 b	2.80 c	2.70 c	3.45 ab	3.60 ab	3.24 b
Extra-early season	3.07 a	1.43 c	2.17 b	2.03 b	2.10 b	1.33 c	1.53 c	1.95 c
Season	5.00 a	4.87 ab	4.93 a	4.73 b	5.00 a	5.00 a	5.00 a	4.93 a
Average[b*]	3.96 a	3.07 d	3.50 b	3.19 cd	3.27 bcd	3.26 bcd	3.38 bc	
Rhizoctonia root canker severity (0–5)								
Late season	1.73 a	1.28 c	1.33 bc	1.58 ab	1.31 bc	1.64 a	1.86 a	1.53 b
Extra-early season	1.52 a	1.18 b	1.25 ab	1.49 a	1.08 b	1.16 b	1.19 b	1.27 c
Season	1.93 a	1.85 a	1.76 a	1.65 a	2.16 a	1.74 a	1.84 a	1.85 a
Average	1.73 a	1.43 c	1.45 bc	1.57 abc	1.52 bc	1.51 bc	1.63 ab	
Black dot severity (0–4)								
Late season	2.51 a	2.13 ab	2.36 a	1.59 c	1.78 bc	2.49 a	2.26 a	2.16 b
Extra-early season	1.77 a	0.90 b	0.88 b	0.80 b	1.07 b	0.90 b	0.84 b	1.02 c
Season	2.77 a	2.79 a	2.74 a	1.68 b	2.31 a	2.43 a	2.75 a	2.50 a
Average	2.35 a	1.94 bc	1.99 b	1.36 d	1.72 c	1.94 bc	1.95 bc	
Relative vascular discoloration extent (%)								
Late season	58.01 a	17.83 b	14.69 b	12.29 b	19.77 b	24.79 b	25.71 b	24.73 c
Extra-early season	68.84 a	20.54 c	51.62 ab	39.41 bc	35.25 bc	29.56 c	29.02 c	39.18 b
Season	83.93 a	50.37 b	87.08 a	55.54 b	63.10 b	66.55 ab	61.56 b	66.88 a
Average	70.26 a	29.58 c	51.13 b	35.74 c	39.37 c	40.30 c	38.76 c	

[a] Means per cropping season for all bacterial treatments combined

[b] Means per bacterial treatment for all cropping seasons combined

[c] Means per bacterial treatment and per cropping season

+Values within lines followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

*For cropping seasons and bacterial treatments tested values and means followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

For wilt severity, LSD (Cropping seasons × Bacterial treatments) = 0.70 at $P \leq 0.05$

For Rhizoctonia root canker severity, LSD (Cropping seasons × Bacterial treatments) = 0.51 at $P \leq 0.05$

For Black dot severity, LSD (Cropping seasons × Bacterial treatments) = 0.71 at $P \leq 0.05$

For Relative vascular discoloration extent, LSD (Cropping seasons × Bacterial treatments) = 4.7% at $P \leq 0.05$

SV39: *Bacillus tequilensis*, SV41: *B. subtilis*, S42: *B. cereus*, SV44: *B. methylotrophicus*, SV65: *B. amyloliquefaciens* subsp. *plantarum* and SV104: *B. tequilensis*

For all cropping seasons, diseases severity was recorded at 90 DPP

For all cropping seasons combined, whole-cell suspensions of SV39 and to lesser extent those from S42 were the most efficient in reducing severity of wilt symptoms by 22 and 19%, in comparison with the untreated control, compared to 12–18% noted following treatments with the remaining bacterial strains.

Overall and for all bacterial treatments combined, foliar wilt symptoms noted in the main season crop were 1.6 and 2.6 times more severe than those recorded in the extra-early and late season crops (Table 5).

The results (Table 5) indicated also that, in the late season crop, *Bacillus* spp.-based treatments were shown able to suppress Rhizoctonia root canker severity on potato cv. Spunta plants by 5–26% compared to 2–29 and 4–14% recorded in the extra-early and main season crops, respectively.

When applied in the late season crop, SV39-, SV41- and SV44-based treatments had reduced significantly Rhizoctonia canker severity by 26, 23 and 24%, respectively, while in the extra-early season, SV39, SV44, SV65 and SV104 strains were the most effective in decreasing this disease severity by 22, 29, 23 and 21% than the untreated control, respectively.

For all cropping seasons combined, the highest decrease in Rhizoctonia root canker severity, as compared to untreated control, was achieved following treatments with SV39 (17%), SV41 (16%), SV44 (12%) and SV65 strains (13%).

For all bacterial treatments pooled, Rhizoctonia canker was 1.45 and 1.2 times less severe in the extra-early and late season crops, respectively, than at the main cropping season.

In the extra-early season crop, soil drench with *Bacillus* spp. whole cells at four times during the potato-growing cycle led to 40–55% decrease in black dot severity, relative to the untreated control, compared to 0.8–37 and 0.7–39% recorded in the late and main season crops, respectively (Table 5).

In the extra-early potato crop, all treatments with *Bacillus* spp. strains had significantly decreased black dot severity by 40 to 55% versus control, while the highest disease suppression ability, by 37 and 39% over control, was recorded, respectively, in the late and main season crops on potato plants treated with S42 strain.

Regardless of the cropping season, S42- and to lesser extent SV44-based treatments were the most effective in suppressing black dot severity, by 42 and 27%, respectively.

Black dot severity noted in the extra-early and late season crops was 2.45 and 0.2 times significantly lower compared to that recorded in the main cropping season.

Following bacterial treatments, the relative vascular discoloration extent was reduced in the late season crop by 56–79%, than control, while those noted on the extra-early and main season crops were estimated at 25–70 and 21–40%, respectively (Table 5).

SV39-based treatment had lowered by about 70% the vascular fungal development in the late and extra-early crops compared to about 40% decrease noted in the main season crop.

On potato plants treated with S42 and SV44 strains, the vascular discoloration extent was reduced by 79 and 66% in the late season crop compared to 43–34 and 49–25%, respectively, recorded in the extra-early and main season crops.

In the late and extra-early season crops, potato plants treated with SV65 and SV104 showed 55–57% lesser vascular discoloration extent, compared to 21 and 27% recorded in the main season, respectively. The vascular discoloration severity was reduced by 75% using SV41-based treatment in the late crop compared to 25% recorded in the extra-early season; however, non-significant reduction was noted in the main crop (Table 5).

Regardless of the cropping season, rhizosphere soil drench with SV39, S42, SV44, SV65 and SV104 strains and to lesser extent with SV41 had induced significant reductions of relative vascular discoloration extent by 58, 49, 44, 43, 45 and 27%, respectively, in comparison with the untreated control.

For all bacterial treatments combined, relative vascular discoloration extent noted on potato plants in the late and extra-early season crops was 2.7 and 1.7 times lower than that recorded in the main cropping season.

Potato growth and production parameters

All potato growth and production parameters (root and aerial part fresh weights, average tuber weight per plant and total tuber yield), noted 90 DPP, varied significantly depending on bacterial treatments tested and cropping seasons. A significant interaction was also noted between these two fixed factors, except for potato yield parameter. *Bacillus* spp.-based treatments induced increment in root fresh weight, relative to the untreated control, by 16–90% in the late season, compared to 1–95 and 14–48%, noted in the extra-early and the main season crops, respectively (Table 6).

When applied in the late and extra-early season crops, SV39-based treatment had enhanced root weight by 90 and 79%, respectively, while only 24% increase was recorded in the main season. SV41-based treatment improved this parameter by 95% when applied in the extra-early crop, compared to 23 and 42% recorded in the late and main season crops, respectively.

In the late crop, potato plants treated with S42 and SV65 strains showed 53 and 59% higher root weight, relative to the untreated control, compared to 48.17 and 32%, respectively, recorded in the main season crop (Table 6).

For all cropping seasons combined, SV39 and SV41 treatments had induced the highest root fresh weight increase, by 70 and 58% over control. Also, S42- and SV65-based treatments had increased this parameter by about 26%, while effects of SV44 and SV104 on this parameter were significantly similar to the untreated control and the recorded increments ranged between 3 and 15%.

Bacterial treatments tested had improved the potato aerial part fresh weight by 13 to 67%, over control, in the late season crop and by 20–76 and 14–82%, in the extra-early and main season crops, respectively (Table 6).

SV39-based treatment had improved this growth parameter by 76% when applied in the extra-early crop, compared to 13 and 42%, recorded, respectively, in the late and main season crops.

In the main season crop, potato plants treated with S42 showed 82% increase in their aerial part fresh weight, relative to control, compared to 61 and 20%, recorded in the late and extra-early crops, respectively (Table 6).

The highest increase in aerial part fresh weight was induced by S42-based treatment (59%), followed by SV44 (54%), SV41 (44%), SV65 (39%) and SV104 (38%), while the least increment 36% was induced by SV39 treatment and this regardless of the cropping season.

When applied in the late season crop, *Bacillus* spp.-based treatments led to 8–65% increment in the average

Table 6 Effect of bacterial treatments tested on growth and production parameters of potato cv. Spunta plants depending on cropping seasons

Cropping season	Bacterial treatment							Average[a*]
	Control	SV39	SV41	S42	SV44	SV65	SV104	
Root fresh weight (g)								
Late season	17.32 e[c+]	32.95 a	21.35 cde	26.55 bc	20.15 de	27.60 b	23.00 bcd	24.13 a
Extra-early season	21.67 b	38.83 a	42.30 a	19.93 b	18.43 b	21.17 b	22.00 b	26.33 a
Season	12.33 a	15.33 a	17.47 a	18.27 a	14.07 a	16.27 a	14.13 a	15.41 b
Average[b*]	17.11 c	29.04 a	27.04 a	21.58 b	17.55 c	21.68 b	19.71 bc	
Aerial part fresh weight (g)								
Late season	170.82 c	192.55 bc	232.85 ab	275.05 a	285.25 a	255.10 a	257.40 a	238.43 a
Extra-early season	79.83 c	140.73 a	125.07 ab	95.82 bc	129.13 ab	106.73 abc	114.80 abc	113.16 c
Season	114.00 c	161.67 b	165.73 ab	207.27 a	145.80 bc	145.60 bc	130.40 bc	152.92 b
Average[b]	121.55 c	164.98 b	174.55 ab	192.71 a	186.73 ab	169.14 ab	167.53 ab	
Tuber fresh weight (g)								
Late season	535.93 c	609.80 bc	665.15 b	594.35 bc	886.65 a	576.85 bc	697.80 b	658.08 b
Extra-early season	482.20 a	554.30 a	538.63 a	428.07 a	555.80 a	524.37 a	444.67 a	504.00 c
Season	895.80 a	1087.80 a	924.27 a	933.27 a	1007.00 a	956.47 a	946.13 a	964.39 a
Average[b]	637.98 c	763.97 ab	709.35 bc	651.89 c	816.48 a	685.89 bc	696.20 bc	
Yield (T/ha)								
Late season	33.16 d	40.21 bc	41.16 bc	36.78 bcd	54.86 a	35.69 cd	43.18 b	40.72 b
Extra-early season	29.84 a	34.30 a	33.33 a	26.49 a	34.39 a	32.45 a	27.51 a	31.19 c
Season	55.43 a	67.31 a	57.19 a	57.75 a	62.31 a	59.18 a	58.54 a	59.67 a
Average[b]	39.47 c	47.27 ab	43.89 bc	40.34 c	50.52 a	42.44 bc	43.08 bc	

[a] Means per cropping season for all bacterial treatments combined

[b] Means per bacterial treatment for all cropping seasons combined

[c] Means per bacterial treatment and per cropping season

+Values within lines followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

* For cropping seasons and bacterial treatments tested values and means followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

For root fresh weight, LSD (Cropping seasons × Bacterial treatments) = 9.24 g at $P \leq 0.05$

For aerial part fresh weight, LSD (Cropping seasons × Bacterial treatments) = 70.73 g at $P \leq 0.05$

For tuber fresh weight, LSD (Cropping seasons × Bacterial treatments) = 217.67 g at $P \leq 0.05$

SV39: *Bacillus tequilensis*, SV41: *B. subtilis*, S42: *B. cereus*, SV44: *B. methylotrophicus*, SV65: *B. amyloliquefaciens* subsp. *plantarum* and SV104: *B. tequilensis*

For all cropping seasons, growth parameters were recorded at 90 DPP while yield was noted at 110 DPP

tuber fresh weight over the untreated control, compared to 12–15% and 3–21% noted in the extra-early and main season crops, respectively (Table 6). SV44- and SV104-based treatments and at lesser extent SV41 improved this production parameter by 65, 30 and 24% in the late season crop compared to slight and insignificant enhancements recorded in the other cropping seasons.

For all cropping seasons combined, potato plants treated with SV44 and SV39 strains showed significant increases in tuber weights, by 28 and 20% over control, respectively. The remaining *Bacillus* spp. strains did not improve this parameter compared to the untreated control.

For all cropping seasons combined, SV44- and SV39-based treatments induced the highest increases in tuber yields, with by 28 and 20% over control, respectively. Treatments using the remaining *Bacillus* spp. strains did affect this yield parameter as compared to the untreated control (Table 6).

Discussion

The present study aimed to assess the antifungal activity of six endophytic *Bacillus* strains isolated from five wild Solanaceous species, *N. glauca*, *D. stramonium*, *D. metel*, *S. nigrum* and *S. elaeagnifolium*, against the causal agents of potato wilt, root rot and canker diseases and their subsequent effects on plant growth and production.

Based on the in vitro dual-culture assays, significant inhibition rates were induced by the whole-cell suspensions, the cell-free culture filtrates, the butanolic

and the chloroformic extracts, depending on the target pathogens, the concentration used and the *Bacillus* strains tested. Overall, SV39 and SV104 (*B. tequilensis*), SV41 (*B. subtilis*), SV44 (*B. methylotrophicus*), SV65 (*B. amyloliquefaciens* subsp. *plantarum*) and to a lesser extent S42 (*B. cereus*) exhibited potent antifungal activity against *F. oxysporum* f. sp. *tuberosi*, *R. solani* and *C. coccodes*. This significant broad spectrum of antimicrobial activity has been widely reported in previous studies (Baard et al. 2023), where *B. subtilis* and *B. amyloliquefaciens* have received the most attention, but less is known about the potential of the other *Bacillus* species included in the current investigation.

In fact, *B. subtilis* and *B. amyloliquefaciens*, the most studied species among *Bacillus* genus, have been shown active against many soilborne pathogens such as *R. solani*, *Fusarium* spp., *Colletotrichum* spp., *Sclerotinia* and *Phytophthora*, inducing inhibition rates varying from 30 to 90% (Alfiky et al. 2022). However, *B. tequilensis* has recently been reported to possess the ability to inhibit the growth of several soilborne phytopathogens. For instance, the endophytic *B. tequilensis* PBE1 strain was found to be the most efficient in suppressing *F. oxysporum* f. sp. *lycopersici* through direct antagonism (Bhattacharya et al 2019). Recently, Baard et al. (2023) studies asserted that *B. tequilensis* isolates significantly inhibited the mycelial growth of four *Fusarium* species (namely *F. oxysporum*, *F. culmorum*, *F. proliferatum* and *F. verticillioides*), with *F. oxysporum* being the least inhibited pathogen by 35–44%. *B. tequilensis* strain GYUN-300 was also found able to suppress *C. acutatum* mycelial growth, to decrease its conidial germination potential and to induce gall-like formations in its mycelium (Kwon et al. 2022). Although recent significant advances on the use of *B. tequilensis* as a biocontrol agent are increasing, the present work is the earliest report of its antagonism against phytopathogenic fungi inducing wilt and root rot diseases of potato plants. In fact, in the present study, *B. tequilensis* cell-free culture filtrate and organic extracts displayed the most important antifungal activities against all target phytopathogens. However, very little has been reported about the antagonism of *B. methylotrophicus* toward fungal plant pathogens, and to the best of our knowledge, this the first report of its biocontrol ability against the target soilborne potato pathogens.

Several mechanisms have been proposed to explain the inhibition effects induced by *Bacillus* spp. against the target fungal pathogens, such as the production of antimicrobials, the secretion of hydrolytic enzymes, the competition for nutrients or a combination of these mechanisms (Calvo et al. 2010). *Bacillus* strains are able to produce various antimicrobial metabolites including a range of polyketides and cyclic lipopeptides such as surfactins, iturins, fengycins and bacillomycins, known to exhibit antifungal activity against various fungi including *Rhizoctonia*, *Fusarium*, *Aspergillus* and *Penicillium* species (Ongena and Jacques 2008). In this regard, the six *Bacillus* strains tested in the present study are known to produce lytic enzymes, such as chitinase and protease (Aydi Ben Abdallah et al. 2017a), leading to cell wall degradation during antagonism toward *F. oxysporum* f. sp. *lycopersici*. Furthermore, among the tested *Bacillus* spp. strains, three were able to produce surfactins, iturins and fengycins. In fact, SV39 and SV104 (*B. tequilensis*) are surfactin-producing strains; SV65 (*B. amyloliquefaciens* subsp. *plantarum*) and SV104 (*B. tequilensis*) are able to produce fengycins, while only SV44 (*B. methylotrophicus*) had the ability to produce bacillomycins. In fact, these last cyclic peptides were capable of inducing damage in the membranes of pathogen mycelia (Zhang et al. 2013). This could explain the lowest antifungal activity displayed by S42 (*B. cereus*) against the tested phytopathogens, as it is not able to produce any of the cited peptides.

Given that more than one mechanism may be involved, a complex response with a range of antagonistic effects among *Bacillus* strains and distinct responses by different pathogens could be expected, as was observed in the current investigation.

In this study, endophytic bacterial strains were able to significantly reduce, with varying degree, *R. solani*, *C. coccodes* and *F. oxysporum* f. sp. *tuberosi*, tested in mixed infection, on potato plants. In fact, the inhibition of *R. solani* infecting potato by *Bacillus* species, mainly *B. subtilis* and to a lesser extent *B. amyloliquefaciens*, had already been reported in numerous studies; nonetheless, the efficacy of *B. tequilensis* (SV39 and SV104), *B. cereus* (S42) and *B. methylotrophicus* (SV44) is first reported in this study. In fact, present findings are positively coupled with several previous reports where soil or tuber treatments with *B. subtilis* had effectively controlled Rhizoctonia stem canker and black scurf diseases on potato under greenhouse experiments leading to up to 80% decrease of disease severities (Abeer et al. 2017). Similarly, in Del Ángel et al. (2017) study, a consortium of two *B. amyloliquefaciens* strains applied on potato plants had reduced the severity of *R. solani*-induced disease by 85%.

Our finding is the first report on the use of *Bacillus* strains against the causative agent *C. coccodes*, while there are limited reports on *Bacillus*-based control tools against Fusarium wilt of potato. For instance, *B. amyloliquefaciens* was able to decrease by 75% *F. oxysporum*-inducing potato wilt, under pot experiment conditions (Xu et al. 2020). In this context, Eljounaidi et al. (2016) mentioned that bacterial endophytes being able

to colonize an ecological niche similar to that of vascular wilt pathogens and to compete for growth, favors them as potential biocontrol agents against wilt diseases.

Moreover, the potential of our *Bacillus* strains to control potato diseases was associated with an enhancement of potato growth parameters. Similar results were also reported describing the promoted growth and development of the crop by increasing chlorophyll content, biomass fresh weight, root weight, stem diameter, plant height and crop yield under greenhouse conditions (Xu et al. 2020).

In fact, it has been widely reported that *Bacillus* strains effectively enhance the plant growth (Aydi Ben Abdallah et al. 2016) as they possess the ability to synthesize phytohormones such as indole-3-acetic acid (IAA), gibberellin and cytokines, to produce siderophores and to carry out phosphate solubilization which are directly involved in the primary and secondary growth of plants at cellular level. Thus, this increment in plant growth parameters was possibly attributed to the plant growth-promoting traits of our *Bacillus* strains as they were all able to produce IAA and siderophores. They were also demonstrated as phosphate-solubilizing strains, excepting SV39 and SV41.

Although there are many *Bacillus* strains with similar functions (i.e., biocontrol and promotion of plant growth), most do not show these traits under field conditions; thus, the consistent and predictable performance of our tested *Bacillus* strains was assessed under different field conditions over three consecutive cropping seasons.

The results from field studies indicated that soil treatment with the most of the *Bacillus* spp. strains had significantly controlled all the target fungal soilborne diseases. However, there was a variation in the magnitude of disease reduction between the strains and the different cropping seasons. For instance, all *Bacillus* strains were shown able to significantly reduce foliar wilting and black dot severities under the extra-early season, while under the main season conditions, only S42 was found the most active against these diseases. Furthermore, of all the strains tested, S42 (*B. cereus*) consistently reduced foliar wilting, black dot and vascular discoloration extent under all planting seasons conditions. However, this strain has not been found to have disease-suppressive activities against Rhizoctonia root canker for all cropping seasons.

It should be noted that the positive response of potato cv. Spunta plants to the bacilli based treatments was more pronounced under late and extra-early cropping seasons compared to the main season. This could be explained by the highest disease's severity observed under main season conditions which could be attributed to the field's ever-changing biotic (e.g., presence and competition with native microbiota) and abiotic conditions (temperature, humidity, ultraviolet radiation, etc.) affecting biocontrol agent fitness and performance (Berini et al. 2018; Parnell et al. 2016). These conditions fluctuate in soil and may significantly influence bioactive metabolites production *in planta*.

Our study revealed also the control efficiency of SV44 (*B. methyltrophicus*) to suppress all the target potato soilborne diseases under late and extra-early cropping seasons, while only vascular discoloration extent was limited in the main season crop. Thus, of special interest in the present investigation is the fact that tested *Bacillus* strains were able to significantly reduce, with varying degrees, all the target potato diseases for at the least one cropping season.

In fact, *Bacillus* strains have been shown to effectively control *R. solani* disease on potato in different geographic regions around the world (Asaturova et al 2021). Accordingly, under field conditions, about 40–45% of potato stem canker severity was reduced over multiple trials by using *Bacillus subtilis* (Larkin, 2020).

The present study highlighted also the ability of almost all the *Bacillus* strains to significantly decrease the vascular discoloration extent, induced by *F. oxysporum* f. sp. *tuberosi*, in the late and extra-early seasons, while only SV39, S42, SV44 and SV104 performed more efficiently under the main season conditions. In fact, specific agents must compete with other soil- and root-associated microbes to survive, propagate and express their antagonistic potential during those times when the targeted pathogens pose an active threat to plant health. As reported by Baysal et al. (2013), soilborne pathogens such as *Fusarium* species that infect through mycelial contact are, in general, more susceptible to competition from other soil- and plant-associated microbes than those pathogens that germinate directly on plant surfaces and infects through appressoria and infection pegs. This could explain in part the modest inhibition percentages engendered by the tested *Bacillus* strains against Rhizoctonia root canker under field conditions. Nonetheless, Larkin (2020) affirmed that for these types of soilborne diseases, any significant reductions were improvements over existing conditions and may improve production.

The results from the present study indicated also that the tested *Bacillus* strains were found able to improve at least two growth and/or production parameters depending on the strain used and cropping seasons. Only SV39, SV41, SV44 and SV104 were able to significantly increase tuber yield in one cropping season. These data are in accordance with other studies indicating that *B. subtilis*-based treatments are able to increase yield by 11–37% over all three cropping seasons (Larkin 2020). In addition, for the remaining tested strains, reductions in

soilborne diseases severities were not necessarily associated with increased tuber yield.

Conclusions

In the present investigation, *Bacillus* strains isolated from five wild solanaceous species could be considered as potentially effective biocontrol agents as they displayed a broad-spectrum antifungal activity against several economically important potato soilborne pathogens, with consistent and predictable performance under changing field conditions. These *Bacillus* spp. strains could be used in combinations and/or introduced with other existing practices in order to provide supplemental diseases control and yield promotion under organic or conventional potato production systems. Thus, under Tunisian conditions of high soil inoculum level, the use of *B. tequilensis* (SV39 and SV104) and *B. methylotrophicus* (SV44) could be an eco-friendly alternative as these excellent biocontrol agents could be used as biofungicide formulations for the valuable reduction of multiple potato soilborne diseases and promotion of tuber yield.

Abbreviations

PDA	Potato dextrose agar
PDB	Potato dextrose broth
SDW	Sterile distilled water
NB	Nutrient broth
NA	Nutrient agar
CFU	Colony-forming unit
IR	Inhibition rate
IC	Inoculated control
NIC	Non-inoculated control
DPP	Days post-planting
ANOVA	Analysis of variance
SPSS	Statistical Package for the Social Sciences

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Author contributions

H.J.-K. was involved in data acquisition, methodology, formal analysis, investigation, data curation, validation, writing—original draft, writing—review and editing and visualization. R.A.B.A. was involved in conceptualization, methodology, Validation and investigation. M.D.-R. was involved in conceptualization, methodology, investigation, validation, writing—original draft, writing—review and editing and supervision. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated and/or analyzed during the present study are available in the manuscript, and the corresponding author has no objection to the availability of data and materials upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no conflict of interest.

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