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# In vitro antifungal activity of endophytic bacteria isolated from date palm (*Phoenix doctylifera* L.) against fungal pathogens causing leaf spot of date palm

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## Abstract

**Background:** Date palm (*Phoenix doctylifera* L.) suffers from several fungal diseases. The endophytic microorganisms present in higher plants generally offer protection to their host plants against invading phytopathogenic fungi and bacteria. In the present study, endophytic bacteria associated with date palm leaves were isolated and their in vitro antagonistic potential against fungal pathogens causing leaf spots in date palm was demonstrated.

**Results:** Endophytic bacteria were isolated from date palm leaves of 3 different cultivars viz., Nighal, Khalas and Khinaizi and evaluated for their inhibitory activity against leaf spot pathogens of date palm viz., *Fusarium solani*, *Alternaria* sp., *Nigrospora* sp., *Thielaviopsis* sp., *Curvularia subpapendrofii* and *Tilletiopsis minor* using an in vitro dual culture assay. Of the 24 endophytic bacterial strains tested, the endophytes designated B1, B7, B8 and B9 obtained from cv. Nighal showed inhibitory activities (more than 55% mycelial growth inhibition) against *F. solani* and *Alternaria* sp. None of the bacterial endophytes inhibited the growth of other fungal pathogens tested. These antagonistic bacterial strains were identified as *Pantoea septica* on the basis of 16S rRNA gene sequence analysis. The hyphae of *F. solani* and *Alternaria* sp. exhibited morphological abnormalities such as shrinkage and disintegration when grown in the presence of antagonistic bacterial endophytes. The cell-free culture filtrates of the bacterial endophytes caused inhibition of mycelial growth and induced leakage of electrolytes from the mycelia of *F. solani* and *Alternaria* sp. This is the first study that describes inhibition of the date palm pathogens *F. solani* and *Alternaria* sp. by *P. septica*.

**Conclusion:** Endophytic *Pantoea septica* strains isolated from date palm leaves inhibited the mycelial growth of *F. solani* and *Alternaria* sp. and induced morphological changes in their mycelia. The culture filtrates of these bacterial strains also inhibited the mycelial growth and caused leakage of electrolytes from the mycelia of *F. solani* and *Alternaria* sp. These promising bacterial strains can be exploited as biocontrol agents to control *F. solani* and *Alternaria* sp.-induced leaf spot diseases of date palm.

**Keywords:** *Alternaria* sp., Antifungal, Endophytic bacteria, Foliar pathogens, *Fusarium solani*, *Phoenix doctylifera*

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## Background

Date palm (*Phoenix doctylifera* L.) is the principal economic crop in Oman with a total yield of 360,917 tons annually, which makes Oman amongst the top 10 producers of dates globally (FAO 2019). The major constraints for date's production in Oman are drought, salinity, pests and diseases. Date palms are affected by many foliar, root and fruit diseases. The most serious disease of date palm in the world is Bayoud disease induced by the fungus *Fusarium oxysporum* f. sp. *albedinis* (Chao and Krueger 2007). Although it is limited in distribution to some areas, mostly in Morocco and Algeria, it resulted in the destruction of two-thirds of palm trees (El Hassni et al. 2007). Wilt, leaf spots, black scorch, root rots and fruit rots are the other most commonly found diseases in date palms. Leaf spot diseases cause lower economic losses compared to other diseases (Al-Nadabi et al. 2018). Many fungi are reported and proven to cause leaf spot diseases in date palms such as: *Alternaria*, *Mycosphaerella*, *Chaetosphaeropsis*, *Phoma* and *Phomopsis* (El-Deeb et al. 2007). In Oman, *Alternaria alternata*, *A. burnsii*, *A. arborescens*, *A. jacinthicola*, *A. tomato* and *A. slovacae* are the major fungal pathogens of leaf spot disease of date palm (Al-Nadabi et al. 2018). Despite these challenges, date palms withstand and show adaptations features.

Endophytic microbial communities contribute significantly to the growth of the plants under regular and stress status. Endophytic bacteria are known for their colonization ability in inner plant tissues without leading to any disease (Yaish et al. 2016). Many endophytic bacteria have been proved to have antimicrobial activities and many of them have been utilized against plant diseases (Mohamad et al. 2018). Over the last twenty years, biological control has become an important alternative to control pests and diseases that affect plants, especially with the emergence of pest resistance to chemical pesticides and for the protection of the environment and to ensure sustainable agriculture (Huffaker 2012). Biocontrol agents control diseases by direct and/or an indirect interaction with the causal agents (Pal and Gardener 2006). The direct impact is mostly due to the antagonism of the biocontrol agent toward the pathogen resulting from nutrient competition or antibiosis and/or parasitism, while the indirect effect is due to stimulation of plant defence mechanisms against various pests and diseases (Heydari and Pessarakli 2010). Previous studies have attempted with *Streptomyces globosus* and *Trichoderma harzianum* for controlling black scorch disease in date palm (Saeed et al. 2017). Moreover, there are several studies on the uses of microorganisms such as *Bacillus amyloliquefaciens* and *Burkholderia cepacia* as biocontrol agents for controlling bayoud disease in date palm (El Hassni et al. 2007). To our knowledge, there

are no previous studies on the use of endophytic microorganisms for controlling foliar diseases of date palms.

In this research, endophytic bacteria were isolated from date palm leaves and their antagonistic potential against fungal pathogens causing leaf spots of date palm was demonstrated.

## Methods

### Isolation of endophytic bacteria

Twelve leaf samples were gathered from 3 date palm cultivars viz., Khalas, Nighal and Khinaizi (four samples from each location) at 3 different locations (GPS coordinates 23.232825,58.078146; 23.233959,58.082256; 23.233851,58.082413) in the village of Al-Fankh, Samael, Oman. Samples were collected in sterile paper bags and processed on the same day of collection. Leaves were ruptured into pieces and sterilized with 5% sodium hypochlorite for 3 min, followed by washing in distilled water for 3 times and dried by filter paper. The chopped leaves were then crushed in a sterile mortar and the sap was streaked on nutrient agar (NA) plate. The sterile water obtained from the last washing step was streaked on NA plate as a control. Followed by the incubation of the plates at 27 °C for 3 days. The selection of bacterial colonies was based on distinct morphological features (colour, size, shape) and pure cultures were obtained by streak-plate technique (Al-Hussini et al. 2019).

### Fungal pathogens

The isolates of different pathogenic fungi viz., *Fusarium solani* 2114, *Curvularia subpendrofi* 2315, *Tilletiopsis minor* 2110, *T. minor* 2111, *Alternaria* sp. 2372, *Nigrospora* sp. 2394 and *Thielaviopsis* sp. 2107, used in this work, came from the previous collections of Al-Nadabi et al. (2020). These fungal pathogens were isolated from date palm leaves infected with leaf spot disease collected from different governorates of Oman. All of them were cultured on PDA medium at 25±2 °C.

### In vitro testing for antagonistic activity

Twenty-four bacterial strains (13 strains from Nighal, 6 from Khinaizi and 5 from Khalas) were investigated in vitro for their antagonistic vitality against 7 foliar fungal pathogens of date palm by dual culture technique. A bacterial colony was streaked at one end of a 9-cm Petri dish containing NA medium. A 7-mm mycelial disc of a 7-day-old culture of the test fungal pathogens was placed on the other end and incubated at 27 °C until mycelial growth of the test fungus covers the entire Petri plate in the control. In the control plate, only a fungal disc was placed. At the end, the inhibition zone was measured by counting the distance between the 2 sides of the tested Petri dish (Al-Hussini et al. 2019).

## Molecular characterizations

### DNA extraction

The DNA of the bacterial strains was extracted using a foodproof StarPrep Two kit (BIOTECON Diagnostics, Germany). The quality and concentrations of DNA were measured by the NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA) in accordance with the manufacturer's direction.

### PCR amplification and sequencing

The identification of endophytic bacterial isolates was achieved by PCR amplification of 16S rRNA genes with universal primers viz., 27F (5'-AGAGTTT-GATCMTGGCTCAG-3'), 1429R (5'-TACGGYTACC TTACGACTT-3') and 534R (5'-ATTACCGCGGCTGC TGG-3') (Al-Hussini et al. 2019). PCR was carried out with 25 µl of PCR reaction mixture containing PuRe Ready-to-go PCR beads (GE Healthcare), 22 µl of sterile distilled water, 1 µl of each primer and 1 µl of DNA. PCR conditions included of initial DNA denaturation at 95 °C for 2 min, followed by 35 cycles of 30 s at 95 °C, 30s at 54 °C, 1 min at 72 °C and a final extension at 72 °C for 10 min. Finally, PCR products were sent to Korea for purification and sequencing at Macrogen Inc.

### Phylogenetic analysis

The software MEGA v.7.0.26 was used for generating the multiple sequence alignments. Maximum likelihood (ML) was analysed through RAXMLGUI v. 1.3 (Silvestro and Michalak 2012). The search of optimal ML tree was performed with 1000 separate runs from a random starting tree for each run using the default algorithm of the program. Based on suboptimal trees from each run, the final trees were selected by comparing likelihood scores under the GTR+GAMMA substitution model evolution by MrModeltest 2.2. The software MEGA v.7.0.26 was used for printing the generated tree, while Adobe Illustrator CS v.6 was employed for making the layout.

### Morphological changes in the fungal pathogens due to antagonistic effects of endophytic bacterial strains

Scanning electron microscopy (SEM) was used to study the morphological changes of susceptible fungal species (*F. solani* and *Alternaria* sp.) due to antagonistic activity of bacterial endophytes. A small piece of fungal mycelia taken from the inhibition zone in the dual culture plate was transferred into a glass vial containing fixative buffer (2.0 g paraformaldehyde, 8.0 ml of 25% glutaraldehyde, 1 N NaOH, 1 N HCl, 10 ml of 0.1 ml sodium cacodylate, 2.0 g of sucrose, 1.0 ml of 1% CaCl<sub>2</sub>, 1.01% MgCl<sub>2</sub> and 100 ml of distilled water) and incubated at 36 °C. On the next day, buffer was removed and sufficient quantity of the osmium tetroxide was added and mixed in a rotor for one hour. The previous step was repeated by using

distilled water twice and kept in the rotor for 10 min, followed by washing with absolute ethanol four times for 10 min, 10 min, 20 min and 10 min. Then the samples were kept overnight and dried. Finally, samples were coated with platinum and observed using a JEOL scanning electron microscope model JSM-7800F (JEOL, MA, USA).

### Evaluation of cell-free culture filtrates of endophytic bacteria for antifungal activity

The evaluation of antifungal activity of cell-free culture supernatants from bacterial strains B1, B7, B8 and B9 against *F. solani* and *Alternaria* sp. was achieved by poisoned food technique as described by Kumar et al. (2008). Antagonistic bacterial strains were cultured in nutrient broth for 48 h at 28 °C on an incubator shaker (150 rpm). Followed by centrifugation of bacterial culture for 10 min at 12,000 rpm at temperature of 4 °C and the supernatant was collected. After that, the bacterial culture supernatant was filtered by a 0.2 µm sterilized syringe filter. PDA plates amended with different concentrations (2.5, 5.0, 7.5 and 10%) of cell-free culture supernatants were prepared. Then, 6-mm diameter of a mycelial disc cut out from 7-day-old culture and placed on the centre of agar medium. Next, incubated at 25 °C and the mycelial growth was measured after 7 days. The percentage of inhibition of mycelial growth was calculated, using the following formula (Pandey et al. 1982)

$$\text{Percentage of mycelial inhibition} = \frac{Dc-dt}{Dc} \times 100$$

where *Dc* = average diameter of fungal colony in control and *dt* = average diameter of fungal colony in treatment.

### Testing antifungal metabolites production by bacterial endophytes

The antifungal metabolites production by endophytic bacterial strains was evaluated using electrolyte leakage bioassay (Manhas and Kaur 2016). The supernatants of cell-free culture of the endophytic bacterial strains were prepared as described above. Then, 0.5 g of fungal mycelium (*F. solani* and *Alternaria* sp.) was added to 10 ml of cell-free bacterial culture supernatant in glass vials. Finally, the samples were placed at room temperature (25±2 °C) and then the conductivity of the mixture was assayed at 0 and 2 h by using the conductivity meter (Prapagdee et al. 2008).

### Statistical analysis

Data were analysed using PSPPiRE (PSPP+GUI). Treatment means ± SD were compared using one-way ANOVA and Tukey test.

## Results

### Isolation and in vitro screening of endophytic bacteria

Twenty-four endophytic bacterial strains (B1–B24), with distinguished colony morphologies, were isolated from the leaves of date palm collected from Nighal (B1–B13), Khinaizi (B14–B19) and Khalas (B20–B24) cultivars. The antagonistic activities of the bacterial strains were tested against *F. solani*, *Alternaria* sp., *Nigrospora* sp., *Thielaviopsis* sp., *C. subpseudopendrofii*, *T. minor* and *Tilletiopsis* sp. in vitro by using a dual culture technique. Out of tested strains, the endophytic bacteria isolated from date palm cultivar Nighal alone showed inhibitory activity against the fungal pathogens tested. Of the 13 bacterial strains (B1–B13) from the date palm cv. Nighal, except B10, all the other strains inhibited the mycelial growth of *F. solani*. Four bacterial strains (B1, B7, B8 and B9) were found to be effective in restricting the mycelial growth of *Alternaria* sp. (Table 1). None of the bacterial strains tested was found effective against *Nigrospora* sp., *Thielaviopsis* sp., *C. subpseudopendrofii*, *T. minor* and *Tilletiopsis* sp.

### Molecular characterization of bacterial endophytes

The 4 bacterial strains (B1, B7, B8 and B9), which exhibited antagonistic potential against both *F. solani* and *Alternaria* sp., were identified by 16S rRNA gene sequencing. Based on the phylogenetic analysis, all the 4 strains were identified as *Pantoea septica* (Fig. 1). The nucleotide sequences of 4 bacterial strains (B1, B7, B8 and B9) were deposited in GenBank with accession numbers MT355663, MT355662, MT355664 and MT355665, respectively.

### SEM observation

Examination of the hyphae of *F. solani* and *Alternaria* sp. from the dual culture assay plate by SEM revealed morphological alterations. Shrinkage and distortion of *F. solani* hyphae were observed when the fungus was co-cultivated with the *P. septica* strain B1. The hyphae of *F. solani* exhibited loss of turgidity, shrinkage and fragmentation when exposed to *P. septica* strains B7, B8 and B9.

**Table 1** In vitro antagonistic activity of endophytic bacterial isolates against *Fusarium solani* and *Alternaria* sp.

Endophytic bacterial strains	% inhibition of mycelial growth	
	<i>Fusarium solani</i>	<i>Alternaria</i> sp.
B1	65.88±0.1c	55.54±0.48c
B7	61.18±0.39d	58.63±0.34b
B8	71.76±0.42b	58.14±0.26b
B9	75.31±0.67a	65.14±0.27a

Data are mean of three replications (±SD)

Values of the same letter have no significant differences from each other at  $P < 0.05$

The control hyphae were healthy, normal in growth with smooth surface (Fig. 2). The hyphae of *Alternaria* sp. also exhibited shrinkage and disintegration when treated with the bacterial endophytes (Fig. 2).

### Antifungal activity of cell-free culture supernatants of endophytic bacteria

The test of a poisoned food technique showed that the cell-free culture supernatants of *P. septica* strains B1, B7, B8 and B9 exhibited inhibitory activities against *F. solani* and *Alternaria* sp. The culture supernatants of all the 4 bacterial endophytes caused 100% inhibition of the mycelial growth of *F. solani* and *Alternaria* sp. at all the 4 concentrations tested (Table 2). The culture supernatants of *P. septica* strain B1 completely inhibited the mycelial growth of *Alternaria* sp. at all the 4 concentrations tested, whereas the other 3 strains of *P. septica* showed varying levels of inhibitory activities (Fig. 3).

### Production of antifungal metabolites by antagonists

The electrolyte leakage assay was used for testing the production of antifungal metabolites by *P. septica* strains. The culture supernatants of all the 4 *P. septica* strains caused damage and leakage from mycelium cellular electrolytes of *F. solani* and *Alternaria* sp. as proved by raised levels of electrical conductivity (Table 3). Amongst them, *P. septica* strain B9 induced the highest of electrolytes release from the mycelia of *F. solani* and *Alternaria* sp. (Table 3).

## Discussion

Endophytic microorganisms are distributed in a wide range of host plants including vegetable crops, ornamental plants, grasses, medicinal plants and tree crops (Rosenblueth and Martínez-Romero 2006). The involvement of endophytes in plant disease control, induction of resistance in plants against biotic and abiotic stresses, plant growth promotion, production of novel bioactive compounds and detoxification of toxic compounds has been well documented (Al-Badri et al. 2020). Endophytic fungi (Ben Mefteh et al. 2018) and bacteria (Yaish et al. 2015) have been isolated and characterized from date palm roots. Ben Mefteh et al. (2018) reported that endophytic fungi viz. *Penicillium citrinum* and *Geotrichum candidum* obtained from date palm tree roots showed antifungal and antibacterial activities. El-Deeb and Arab (2013) reported *Acremonium* spp. as date palm root endophytes. However, limited information is available in the literature regarding endophytes of date palm leaves (Siala et al. 2016) who isolated endophytic bacterial strains belonging to Enterobacteriaceae, Microbacteriaceae and Pseudomonadaceae from date palm leaves and demonstrated that *Arthrobacter agilis* and *Bacillus subtilis* showed antagonistic activities against several

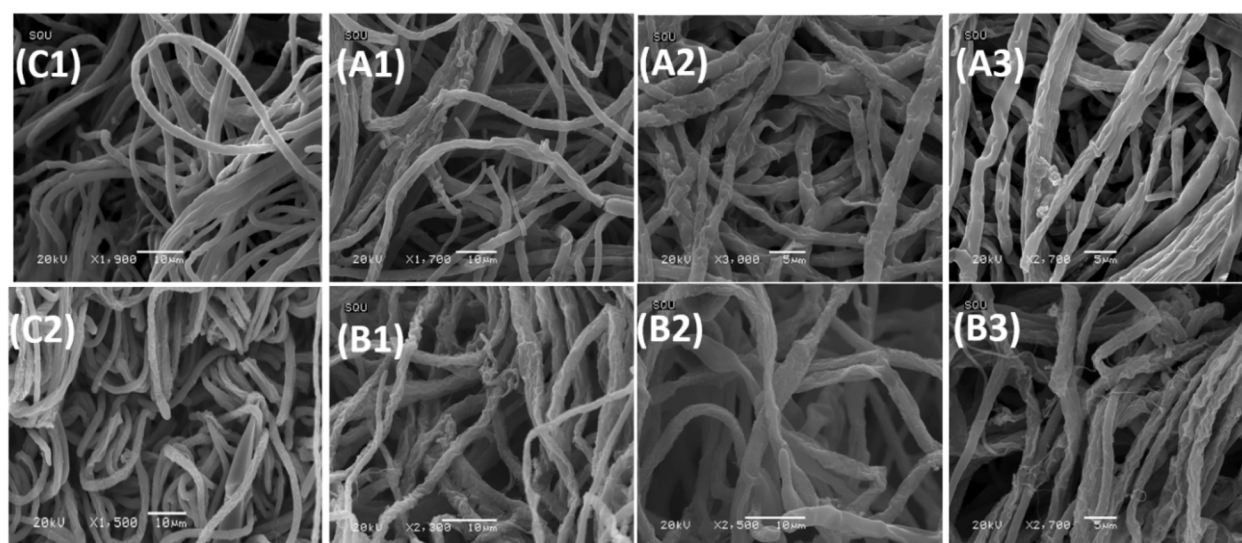




*Fusarium* species including *Fusarium oxysporum* f. sp. *albedinis*, the causal agent of Bayoud disease of date palm. In the present study, endophytic bacteria were isolated from the leaves of 3 different date palm cultivars. These endophytic bacteria might have entered into the roots of date palm through wounds caused by mechanical damage or herbivores and migrated to leaves through transpiration system (Compant et al. 2010) or entered into leaves via stomata (Frank et al. 2017).

In vitro evaluation of the bacterial endophytes for anti-fungal activity in this research revealed that the bacterial strains obtained from the date palm cultivar Nighal alone exhibited antifungal activity. Four endophytic bacterial strains (B1, B7, B8 and B9) showed inhibitory activity against both *F. solani* and *Alternaria* sp. On the basis of sequence analysis of 16S rRNA gene, all the 4 strains were identified as *Pantoea septica*. *Pantoea*

species, belonging to the family Enterobacteriaceae, have been isolated from soil and plants (Han-Jen et al. 2013). *Pantoea* spp. such as *P. agglomerans*, *P. vagans* and *P. dispersa* are widely used as biocontrol agents for controlling fungal and bacterial diseases of plants (Jiang et al. 2019). The antagonistic potential of *P. agglomerans* against *Fusarium culmorum*, *Puccinia recondita* f. sp. *tritici* (Kempf and Wolf 1989), *Botrytis cinerea*, *Penicillium expansum* (Bryk et al. 1998), *Penicillium digitatum* (Plaza et al. 2004) and *Macrophomina phaseolina* (Vasebi et al. 2015) has been reported. The commercial formulation of *P. agglomerans* strain C9-1 (Nufarm Americas, Burr Ridge, IL) is available for the control of fire blight of apple caused by *Erwinia amylovora* (Ishimaru et al. 1988). Jiang et al. (2019) demonstrated the potential of *P. dispersa* for controlling black rot of sweet potato caused by *Ceratocytis fimbriata*. A few species of



**Fig. 2** Scanning electron micrographs showing morphological abnormalities in the hyphae of *F. solani* and *Alternaria* sp. due to the antagonistic effect of endophytic bacterial strains from date palm (cv. Nighal) (C1 and C2, control for *F. solani* and *Alternaria*, respectively; A1–A3, *F. solani* exposed to B1, B7 and B8; B1–B3, *Alternaria* sp. exposed to B1, B7 and B8)

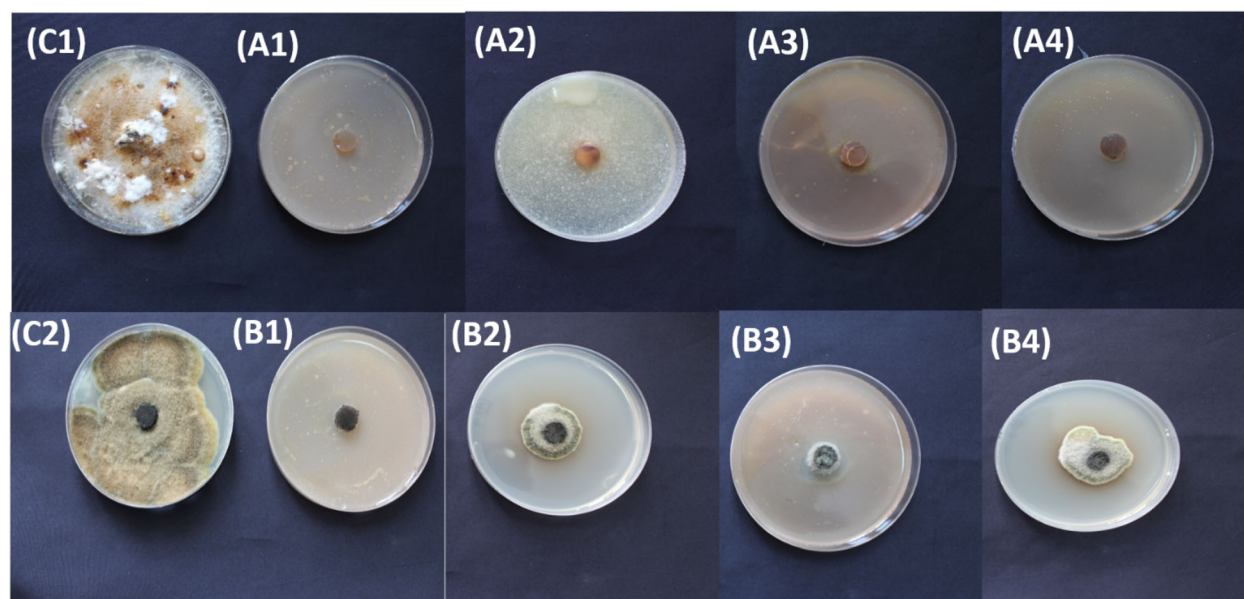
*Pantoea* have been reported to possess plant growth-promoting ability. For instance, Chen et al. (2017) demonstrated that an isolate of *P. alhagi* enhanced the growth of wheat and improved its resistance to drought stress. To our knowledge, this is the first report of antifungal activity of *P. septica* against fungal pathogens of date palm. The endophytic *P. septica* present in the leaves of date palm cv. Nighal might have involved in the resistance of host plants against foliar pathogens. However, the reaction of date palm cv. Nighal to foliar fungal pathogens has not been reported so far.

In the present study, the culture supernatants of the endophytic bacterial strains caused inhibition of mycelial growth of *F. solani* and *Alternaria* sp. Furthermore, the culture supernatants of the antagonistic endophytic bacterial strains caused leakage of cellular electrolytes from

the mycelium of *F. solani* and *Alternaria* sp. suggesting alterations in the permeability of fungal cell membrane (Halo et al. 2018). The leakage of electrolytes from fungal membranes has previously been reported as an indicator of cell membrane damage (Manhas and Kaur 2016; Halo et al. 2018). Many *Pantoea* spp. are known to produce antimicrobial metabolites (Vasebi et al. 2015). The inhibitory activity of *P. septica* might be due to the production of such diffusible antifungal compounds (Kotze et al. 2011). This was further confirmed by SEM analysis of the fungal hyphae from the inhibition zone, which revealed morphological alterations such as shrinkage, loss of turgidity and distortion of *F. solani* hyphae. The loss of cytoplasmic contents usually results in the shrinkage of hyphae (Garg et al. 2010). The results of the present study suggest the production of antifungal compounds

**Table 2** Antifungal activity of cell-free culture supernatants of endophytic bacteria against *Fusarium solani* and *Alternaria* sp. as assessed by poisoned food technique

Fungal pathogen	Percent mycelial growth inhibition					
	Concentration (%)	Distilled water control	B1	B7	B8	B9
<i>Fusarium solani</i>	2.5%	0±0b	100±0a	100±0a	100±0a	100±0a
	5%	0±0b	100±0a	100±0a	100±0a	100±0a
	7.5%	0±0b	100±0a	100±0a	100±0a	100±0a
	10%	0±0b	100±0a	100±0a	100±0a	100±0a
<i>Alternaria</i> sp.	2.5%	0±0e	100±0a	26.7±0.44d	56.7±0.3b	53±0.56c
	5%	0±0e	100±0a	30±0.96c	60±0.7b	56.7±0.6c
	7.5%	0±0e	100±0a	90±0.44c	66.7±0.56d	70±0.6b
	10%	0±0e	100±0a	36.7±1.4c	66.37±0.55c	73±0.4b



**Fig. 3** Inhibition of mycelial growth of *F. solani* and *Alternaria* sp. by cell-free culture supernatants of endophytic bacterial strains (C1 and C2, control for *F. solani* and *Alternaria*, respectively; A1–A4, *F. solani* exposed to B1, B7, B8 and B9; B1–B3, *Alternaria* sp. exposed to B1, B7, B8 and B9)

as one of the probable mechanisms of action of these endophytic bacterial strains on the tested fungal pathogens of date palm.

### Conclusions

Obtained results showed that the endophytic *P. septicum* strains obtained from the leaves of date palm cultivar Nighal suppressed the growth of *F. solani* and *Alternaria* sp. and caused morphological abnormalities in their mycelia. Further, cell-free culture filtrates of these bacterial strains suppressed the growth of these pathogens and induced electrolyte leakage from the mycelia. To our knowledge, this is the first detailed study on endophytic

bacteria of date palm leaves. Further experiments are needed to evaluate the efficacy of these bacterial strains to control *F. solani* and *Alternaria* sp.-induced leaf spot diseases of date palm.

### Abbreviations

NA: Nutrient agar; PDA: Potato dextrose agar; PCR: Polymerase chain reaction; SEM: Scanning electronic microscope; SD: Standard deviation

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"Not applicable" in this section.

### Authors' contributions

HH. Main author (PhD student) and contributed in all sections of the manuscript. NS. Contributed in sample collection and methodology section. AA. Supervised and contributed in the part of scanning electronic microscope. SN. Co-supervisor and prepare the phylogenetic tree and helped in all analysis of molecular work. RV Co-supervisor and contributed in all sections of the manuscript. We confirm that the manuscript has been read and approved by all named authors. We confirm that the order of authors listed in the manuscript has been approved by all named authors.

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### Availability of data and materials

Presented in the main manuscript.

### Declarations

### Ethics approval and consent to participate

We work on plant samples, so there is no need for ethical approval.

### Consent for publication

"Not applicable" in this section.

**Table 3** Leakage of electrolytes from the mycelia of *Fusarium solani* and *Alternaria* sp. due to antifungal activity of cell-free culture supernatants of endophytic bacterial isolates

Bacterial strains		Electrolyte leakage (mS)	
		0 h	2 h
<i>Fusarium solani</i>	B1	8.92±0.0a	9.65±0.01a
	B7	8.92±0.0a	9.67±0.07a
	B8	8.92±0.0a	9.47±0.06b
	B9	8.92±0.0a	9.72±0.05a
	Distilled water control	0.39±0.29b	0.55±0.01c
<i>Alternaria</i> sp.	B1	8.92±0.0a	9.65±0.06a
	B7	8.92±0.0a	9.41±0.13b
	B8	8.92±0.0a	9.63±0.04a
	B9	8.92±0.0a	9.71±0.04a
	Distilled water control	0.39±0.29b	0.55±0.01c

### Competing interests

No competing interest exists. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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