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Molecular Identification of Some Endophytic Bacteria Isolated from Leaves and Roots of Some Cultivars of Date Palm (*Phoenix Dactylifera*) and Its Anti-Fungal Activity Against *Macrophomina Phaseolina*

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ABSTRACT

Date palm tree (the blessed tree) considers a green treasure cause of its rich of nutrients carbohydrates, vitamins, and minerals, etc. Unfortunately, many bacterial and fungal diseases infected palm. Charcoal rot disease is one of the economic importance disease which caused by *Macrophomina phaseolina.* Fifty endophytic bacteria in both of leaves and roots in several cultivars of date palm were isolated and tested *in vitro* against *M.phaseolina*. Ten of the isolated bacteria got effect on growth of the fungus and these bacteria were identified as *Brevundimonas diminuta*, *Streptomyces murinus*, *Enterobacter cloacae* subsp. *Dissolven*, *Brucella intermedia, Sphingobacterium nematocida, Sphingobacterium spiritivorum, Sphingobacterium spiritivorum, Sphingobacterium tabacisoli*, *Sphingobacterium soli,* and *Brucella lupine*. Antagonistic *In vivo* using 10 bacteria versus *M.phaseolina* was carried out in green-house and the best results after 45 and 90 days were using *Strept.murinus*. many biochemical were estimated in *In vivo* experiment and the results showed total indol were high in infected plants, total phenol were high in *Sph.spiritivorum* strain AR2, and in pigments the high chlolrphyll a and b and carotenoids were in *Strept.murinus*, *Brucella intermedia*,and *Sphingobacterium tabacisoli* respectively

Keywords: Charcoal rot, Endophytic bacteria, date palm, Antagonistic

1. Introduction

One of the earliest cultivated plants in human history, the date palm (*Phoenix dactylifera* L.) is a tropical and subtropical fruit tree found in Egypt and the Arabian Peninsula (Abd Allah, 2018). Date palm is a monocotyledon, dioecious plant that considers a member of *Arecaceae* family that belongs to genus Phonix (Al-Shahib and Marshall, 2003). Date tree is very important in terms of diet, medicine, ornamentation, culture, and religion (Haq and Khan, 2020). According to FAO (2022), the whole world yield production was 9475420 tons; In Egypt, the yield production was 1685035 tons.

Date fruits are regarded as a source of some vitamins, proteins, carbohydrates, and a number of minerals. (Dayani *et al.,* 2012).

Numerous diseases that resemble fungi, has been observed to infect date palm trees in Egypt (Lewaa and Zakaria, 2023). Fungal causal disease like *Fusarium oxysporum*, *F. solani*, *Ceratocystis paradoxa*, *Diplodia phoenicum, Phytophthora* sp., *Macrophomina phaseolina, Graphiola phoenicis*, and *Rhizoctonia solani* (Mohamed *et al.,* 2016 and Ahmed 2018). Bacterial causal like *Erwinia chrysanthemi* and *Serratia marcescens* (Lewaa *et al.,* 2023).

Endophytic bacterial populations play a major role in the growth of plants. It is well known that endophytic bacteria can colonize internal plant tissues without causing diseases (Yaish *et al.* 2016). Numerous endophytic bacteria with demonstrated antibacterial properties have been employed to

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combat plant diseases (Mohamad *et al.* 2018). It prevents the plants that live inside it from getting infection cause of phytopathogenic bacteria and fungi (Al-Nadabi *et al.,* 2021).

One of the fungi that infect date palm that is thought to be economically significant is *Macrophomina phaseolina*. That causes charcoal rot disease. Plants affected by it experience a wilting of the foliage, older leaves dying, and a browning and eventually blackening of the crown EL-morsi *et al.,* (2012) and Mohamed *et al.,* (2016)

The aim of this research is isolation of endophytic bacteria in several date palm cultivars and study effect of their antibacterial to control *Macrophomina phaseolina* fungus

2.Materials and Methods

All the experiments of this research were executed in Plant Pathology Research Institute (PPRI) and the Central Laboratory for Date Palm Research and Development (CLDPRD), Agricultural Research Center (ARC), Giza, Egypt.

2.1. Endophytic bacteria isolation

Eight cultivars of date palm were collected and used as source of endophytic bacteria. The cultivars (as shown in table 1) including 4 wet cultivars (Amhat, Barhee, Samany, and Bent-aisha), 1 semi-dry cultivar (Siwi), 2 dry cultivars (Malkaby and Bartmoda), and 1 male cultivar. Leaves and root of each previous cultivar were used to isolate endophytic bacteria. Small pieces of leaves and roots which cut were sunk for 3 min. in sodium hypochlorite (5%) for sterilization and followed 3 times washing by distilled water and then dried using filter paper. The sterilized pieces were crushed well in sterile mortar and pestle. The crushed sap was streaked on nutrient agar (NA) with petri dish plates. Streaked plates were incubated at 28ºc for 24-72 hrs. The grown colonies of bacteria were selected based on the morphological properties (Sahpe, color, and size) and then streaked in separated plates to obtain pure isolate of bacteria. This technique was carried out according to Al-Nadabi *et al.* (2021).

Table 1: List of date palm cultivars and their locations.

Type	cultivar	Location	Geographic location
Wet	Amhat	Giza	30°01'20.4"N 31°12'40.1"E
Wet	Barhee	Giza	30°01'20.4"N 31°12'40.1"E
Wet	Samany	Giza	30°01'20.4"N 31°12'40.1"E
Wet	Bent-aisha	Giza	30°01'20.4"N 31°12'40.1"E
Semi-dry	Siwi	Giza	28°20'24.5"N 28°56'38.0"E
Dry	Malkaby	Aswan	24°03'04.4"N 32°51'33.0"E
Dry	Bartmoda	Aswan	24°03'04.4"N 32°51'33.0"E
-	Male	Giza	30°01'20.4"N 31°12'40.1"E

2.2. Pathogenic fungi source

Identified isolate of *Macrophomina phaseolina* was obtained kindly from Dr. Doaa A. Imara, Plant Pathology Research Institute, Dept. of Mycology Research and Disease Survey, Agric. Res. Center, Giza, Egypt.

2.3. Pathogenicity test of *M. phaseolina*

2.3.1. Preparation of date palm plantlets

Seeds of sewi cultivars were prepared as described by Abd Allah, (2018) as following: Seeds were collected and washed well with dH_2O and fully submerged in solution of Rhizolex-fungicide (3gm/L) and immersed in dH_2O for 10 days and the water was changed every 2 days. After that, seeds were incubated for 50 days/37ºc/50% humidity. After planting of seeds, the plantlets were fully submerged in 3% hypo-chlorite (5% conc.) for 10min. and followed washed using sterile dH_2O. Finally, the plantlets were planted in sterilized pots which contained sterilized clay soil: peat moss (1:1 w/w) and left for 6 months to ready for pathogenicity test.

2.3.2. Preparation of *M. phaseolina*

Mass production of *M. phaseolina* was obtained according to Abd El-Khair and El-Mougy (2003) method by culturing the *M. phaseolina* on in bottles (500 cc) contained sterilized sorghum grain medium and the bottles were incubated for7days at 27ºC.

2.3.3. Pathogenicity test

The sterilized soil (clay soil: peat moss) was infested with *M. phaseolina* with rate of 3% (w/w) and watered twice a day for a week prior to planting. Pots (10cm in diameter) were sterilized by submerging in formalin 5% for 15min. were filled with soil which inoculated with *M. phaseolina*. Date palm plantlets were planted in the pots. Nine replicates (pots) of the treatment were used, with one plantlet per pot.

2.3.4. Assessment of diseases

The percentage of affected plants was used to calculate the incidence of the disease. According to estimates of disease incidence at 45 and 90 days after planting, respectively, the virulence of the various pathogen isolates was evaluated as following:

Diseases incidence after 45 and 90 days from planting

$\%=\Sigma(c- t)/c \times 100$

Σ= sum

c= control (healthy number of plantlets)

t= treatment (number of infected seedlings in infested pots).

2.4. Antagonistic activity test

Fifty isolated endophytic bacteria from collected samples were tested *in vitro* for their antagonistic activity against *M. phaseolina* using dual culture technique according to Al-Nadabi *et al.* (2021) as following:

Colony of each bacteria was streaked on nutrient agar medium (NA) at the end side of 9-cm of petri dish, and the other side of same petri dish was cultured withdisc of *M. phaseolina* (6mm in diameter) which aged 7days that cultured on potato dextrose agar medium (PDA). Dishes were incubated at 27ºc till the growth of *M. phaseolina* in control dish was cover entire plate and then the linear growth of *M. phaseolina* growth in treated dishes were counted as Antagonistic factor as counted by Lewaa and Zakaria, (2023).

2.5. Identification of isolated endophytic bacteria

2.5.1. Bacterial DNA isolation

Twenty microliter of each 10 of efficiency endophytic bacteria was transferred to 2 ml microfuge tube with 1ml of nutrient broth and the tubes were incubated at 27ºc/ 24hrs. The cultures tubes were centrifuged at 11,500xg/ 5min. pellet was collected and about 400µl of extraction buffer (1% CTAB, 2% PVP, 0.2% β-mercaptoethanol, and 2% SDS) was added to pellet. Tubes were vortexed and incubated for 30min at 65ºc with inversion the tubes every 10min. then, 200µl of KOac (3M) was added and centrifugation at 10,000xg/5min. Supernatant was transferred to new tube and 500µl of chloroform: isoamyl alcohol (24:1) was added and tubes were vortexed well and centrifuged at 10,000xg/10min. Supernatant was transferred to new tube and 375 µL of isopropanol were added and tubes were shaken gently. Supernatant was discarded and the pellet was washed with 200µl of ethanol 70% and centrifugation at 7000xg/5min. Supernatant was discarded and the pellet was dried at air room temperature for 30min. Finally, the dried pellet was resuspended with 50µlof TE buffer and store at -20ºC till use. The previous method was carried out according to Dos Santos *et al.* (2019).

2.5.2. 16s rDNA amplification and sequencing

Each DNA of 10 of efficiency endophytic bacteria was amplified using PCR for 16s rDNA amplification using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTACGACTT-3′) as described by Al-Nadabi *et al.* (2021). Tubes of PCR had 25µl of PCR reaction mixture which contained of 1 µl of bacterial DNA (~4-250ng), 1 µl of each primer (10pmol), 12.5µl of supermix (OmniPCR, BIO-HELIX, LTD) and volume was filled to 25µl using dH2O. Program of PCR was 95ºc/2min. for initial denaturation and 35 cycle contained of 95ºc/30sec,

57ºc/30sec, and 72ºc/60sec. and final extension at 72ºc/10min. The program was carried out using MJ Research (PTC-200) thermo-cycler. 5µl of each PCR product was loading in 1.2% agarose gel for electrophoresis. Finally, PCR product was sent to Macrogen, Inc. (South Korean public biotechnology company) for purification and sequencing. The previous method was carried out according to Dos Santos *et al.* (2019).

2.6. Effect *in vivo* of endophytic bacteria on *M. phaseolina*

2.6.1. Prevention

Ten of efficiency isolated endophytic bacteria were used to resist *M. phaseolina in vivo*. Inocula of 10 isolated endophytic bacteria were prepared by culturing each 10 of efficiency endophytic bacteria with flask contained of nutrient broth to obtain concentration $1x10^{10}$cfu/ml. Sterilized soil (clay soil: peat moss) was mixed with the inocula of each endophytic bacteria with rate 3% (w/v) and then the soil were filled in sterilized pots (10 cm in diameter) and then the plantlets were planted in the pots. Three plantlets per pot and each treatment have 3 replicates. Finally, after 15 days the pots were watered with the incoula again (with 3% ratio). After 30 days the pots were infested with *M. phaseolina* (with 3% ratio). Disease was assessment after 45 and 90 days from infestation.

2.6.2. Preservation

The sterilized soil (clay soil: peat moss) was infested with *M. phaseolina* with rate of 3% (w/w) and watered twice a day for a week prior to planting. Before planting planlets, the isolated actinomyces bacteria (with concentration $1x10^{10}$cfu/ml) was added (with 3% in ratio) to the infested soil and the planlets were planted with 3 plantlets per pot and 3 replicates were used for the treatment. After 15 days the inocula of actinomyces. Disease was assessment after 45 and 90 days from infestation.

2.7. Biochemical changes assay

Biochemical change was assayed using 3 duplicates of each treatment. Five ml of 80% ethanol were added to 1 gram of each treatment sample. Then, each sample was homogenized with pestle and mortar and kept at 4ºc for 24hrs in the dark. After that, the sample was centrifuged at 13000xg/5min and supernatant was transferred to assay total phenol, total Indols, and total amino acids.

2.7.1. Determination of total phenol

Total phenol contents were estimated as described by Ainsworth and Gillespie (2007) and modified by Patel *et al.* (2010) as follows:

Each previously extracted sample, 1ml was put into a tube along with 5ml of dH_2O and 0.5 ml of the Folin-Ciocalteu reagent(F-C). After vortexing for 5min, the material was thoroughly combined. The volume increased to 10 ml after 1.5 ml of 20% sodium carbonate (Na2CO3) was added. After that, the mixture was let to settle for 2hrs at room temperature in the dark. Finally, samples were red on spectrophotometer (Orion AquaMate 8000) at 765nm.

2.7.2. Determination of total indol conents.

Estimation of total indol contents was carried out according to Selim *et al.* (1978) as following:

One ml of aliquot sample was transferred to tube and 4ml of Para di-amino benzoate (PDAB) was added. Then, the tubes were incubated for 1hour 37ºc. On Orion AquaMate 8000 spectrophotometer, samples were red at 530nm.

2.7.3. Determination of total amino acid conents.

One ml of extracted sample was added to test tube and the total volume was filled up to 4ml with dH_2O and 1 ml of ninhydrin as reagent was added. The tubes were covered and put in water path at 100ºc/15min. Then, the tubes were submerged in chilled water and 1ml of 50% ethanol was added. Finally, at wave length 570nm on Orion AquaMate 8000 spectrophotometer, samples were red.

2.7.4. Estimation of pigments

Chlorophyll a, chlorophyll b and carotenoids were assayed as described by Wettstein (1957) as following:

Fresh leaf sample (1gm) was grounded with 85% acetone using pestle and mortar in presence of traces of $CaCO_3$. The grounded mixture was filtrated and the filtrate volume was completed with acetone to 15ml. Using Orion AquaMate 8000 spectrophotometer, filtrate samples were red at 440, 640, and 660nm. According to Weinstein formula, Chlorophyll a, chlorophyll b and carotenoids were estimated as following:

Chlorophyll a = 9.784x E(660) – 0.99 x E (640) mg/g
Chlorophyll b = 21.426x E(640) – 4.65 x E (660) mg/g
Carotenoids = 4.695x E (440) – 0.268 x C (chl. a + chl. b) mg/g

2.8. Analytical Statistics

In accordance of Snedecor and Cochran, (1980), all the obtained results/data were analyzed statistically using one way of analysis of variance (ANOVA) using version 23 of SPSS program.

3. Results

3.1. Endophytic Bacteria isolation

Fifty endophytic bacteria strains were isolated from 8 different cultivars of date palm from their leaves and roots based on the bacterial morphology. Results that tabulated in table (2) showed that fifty isolated bacteria were distributed as follow: 6 isolated from Amhat, 7 from Barhee, 8 from Samany, 7 from Bent-aisha, 9 from Siwi, 3 from Malkaby, 6 from Bartmoda, and 4 from Male cultivar.

Table 2: List of source of endophytic bacteria

Cultivars	Isolated endophytic bacteria	
	From Leaves	From roots
Amhat	3	3
Barhee	5	2
Samany	3	5
Bent-aisha	2	5
Siwi	3	6
Malkaby	1	2
Bartmoda	4	2
Male	2	2
Total	**23**	**27**
	50	

3.2. Pathogenicity test of *M. phaseolina*

Pathogenicity test of *M. phaseolina* on date palm plantlets was carried out to assay virulence of fungi isolate. Results showed that the pathogenicity percentage was 55.5% after 45 days and reached to 66.6% after 90 days. Fig (1) showed the symptoms of fungi on plantlets appear as wilting of foliage, older leaves were died, the crown turned brown and then black.

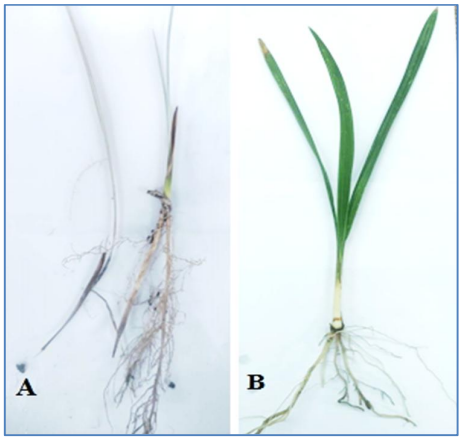


Fig 1: Symptoms of infected date palm plantlet with *M. phaseolina* . (A) infected plantlet and (B) control plantlet.

3.3. Antagonistic activity test

Antagonistic of 50 isolated endophytic bacteria against *M. phaseolina in vitro* was studied**.** The obtained data showed that antagonism for 10 of isolated endophytic bacteria against *M. phaseolina* were the most effective. As shown in table (3) the effective bacterial strains were 2 isolates from siwi root (with ID SR3 and Siwi-R), 2 isolates from bartmoda leaves (with ID BL3 and BL4), 1 isolate form bent-aisha leaves (with ID BNL2), 1 isolate from barhee leaves (with ID BHL5), 1 isolate from amhat roots (with AR2), 1 isolate from samany leaves (with ID SML2), 1 isolate from male leaves cultivar (with ID ML1), and 1 isolate from male roots cultivar (with ID MR1).

Table 3: List of effective bacteria against *M. phaseolina*

Bacterial source	ID
Siwi root	SR3
Siwi root	Siwi-R
Bartmoda leaves	BL3
Bartmoda leaves	BL4
Bent-aisha leaves	BNL2
Barhee leaves	BHL5
Amhat roots	AR2
Samany leaves	SML2
Male leaves	ML1
Male roots	MR1

Results of linear growth and percentage of growth reduction of *M. phaseolina* through the antagonistic test were tabulated and figured. Table (4) and fig (2) referred to the isolated bacteria (with ID: BL4, BNL2, AR2, SML2, ML1, and MR1) are the high of linear growth of *M. phaseolina* (in the range 4.7 to 4.9 cm) and in the growth reduction (in range 44.7 to 47.2%) and there are non-significant between them. There were non-significant also between isolates (ID SR3, BHL5, and AR2) in linear growth (in range 4.4 to 4.6 cm) and in growth reduction (in range 47.7 to 50.2 %). Finally, isolates no. 2 and 3 were the highly inhibition of linear growth of *M. phaseolina* (3.7 and 4.1 cm) and give the high growth reduction (58.9 and 53.6%).

Table 4: Effect of endophytic bacteria on growth of *M. phaseolina*.

Bacterial ID	*M. phaseolina*	
	Linear growth (cm)	**Growth reduction (%)**
SR3	4.67bc	48.0ab
Siwi-R	3.7a	58.9c
BL3	4.17ab	53.6bc
BL4	4.78c	45.3a
BNL2	4.88c	45.8a
BHL5	4.48bc	50.2ab
AR2	4.7bc	47.7ab
SML2	4.98c	44.7a
ML1	4.78c	46.9a
MR1	4.75c	47.2a
P. value	0.0001	0.0001
LSD at 0.05	0.53	5.4

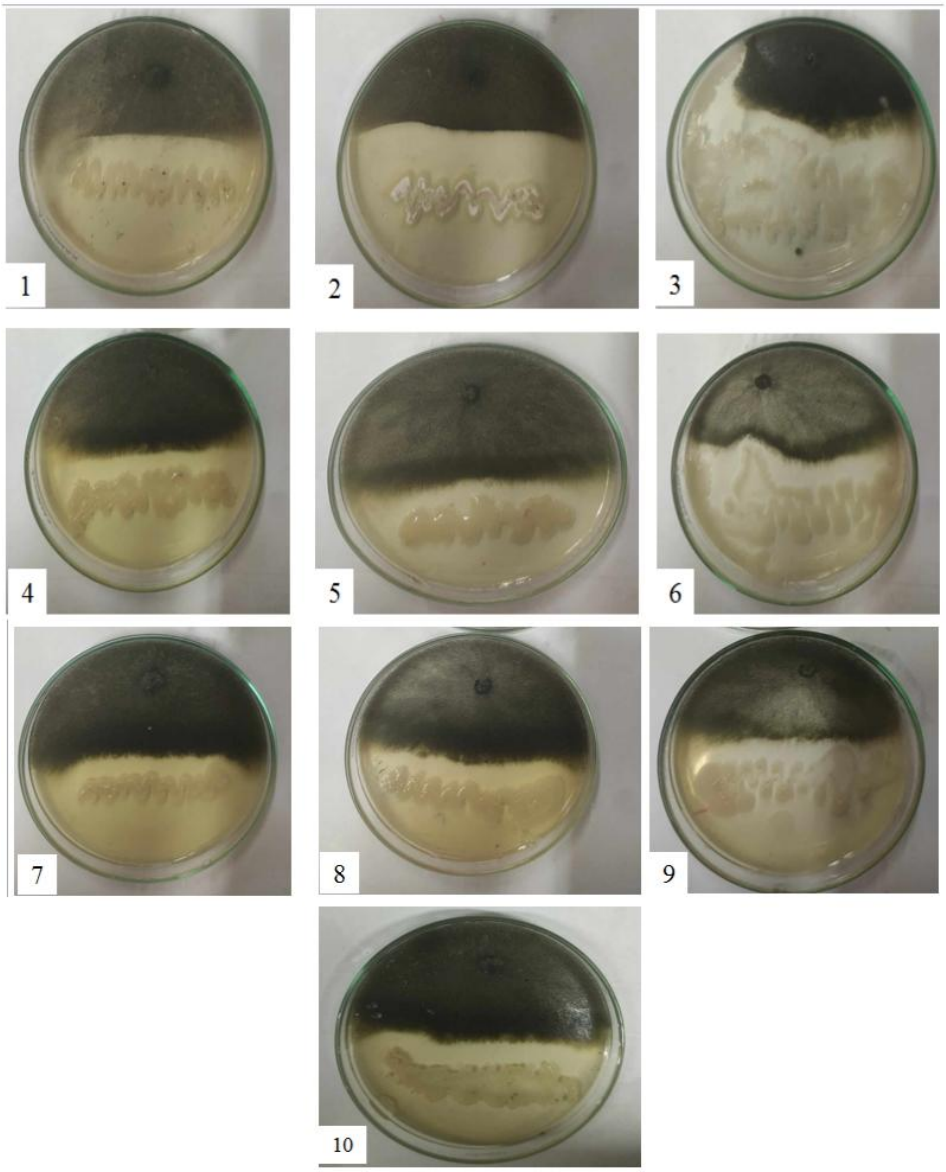


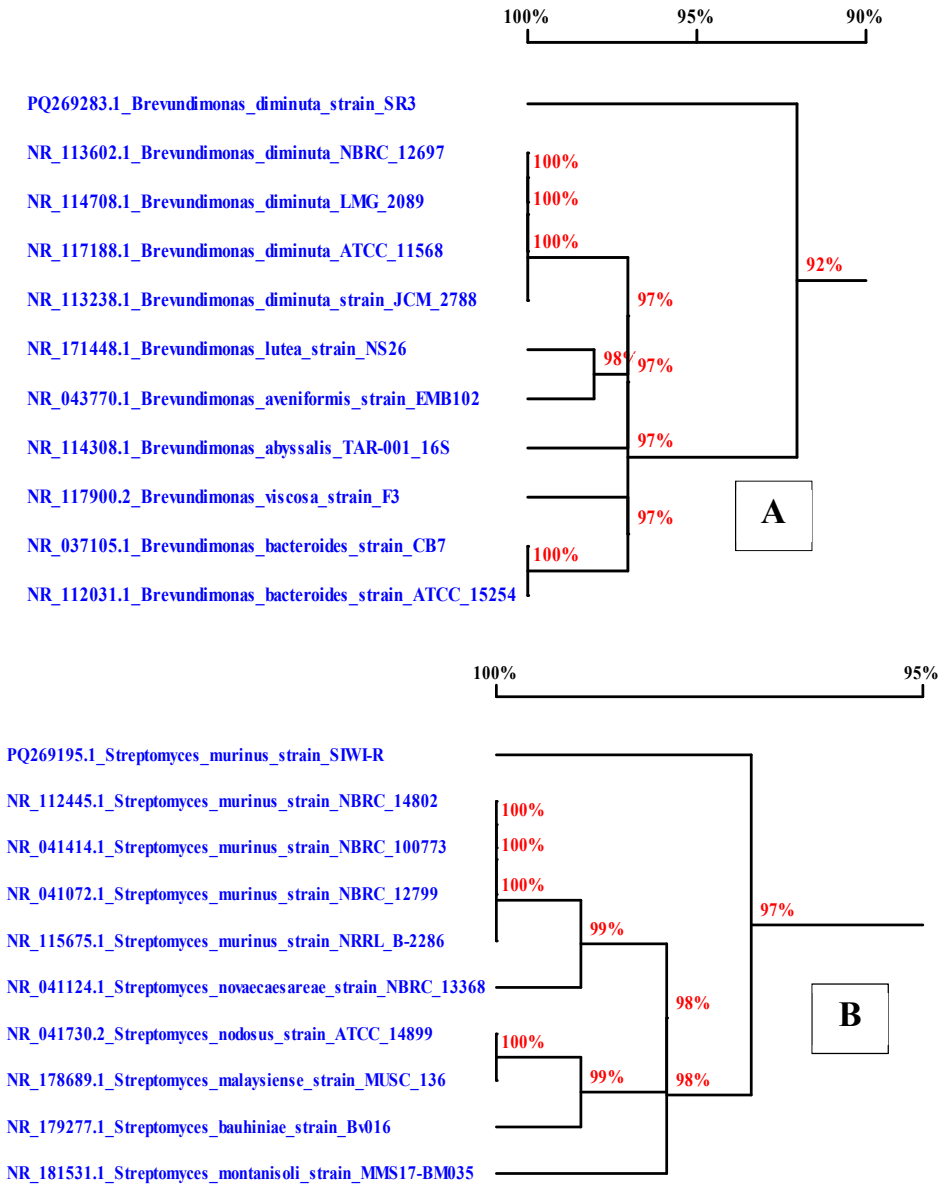
Fig 2: *In vitro* antagonistic effect of endophytic bacteria on *M. phaseolina* 1) SR3, 2) Siwi-R, 3) BL3, 4)BL4, 5) BNL2, 6) BHL5, 7) AR2, 8) SML2, 9) ML1, 10) MR1

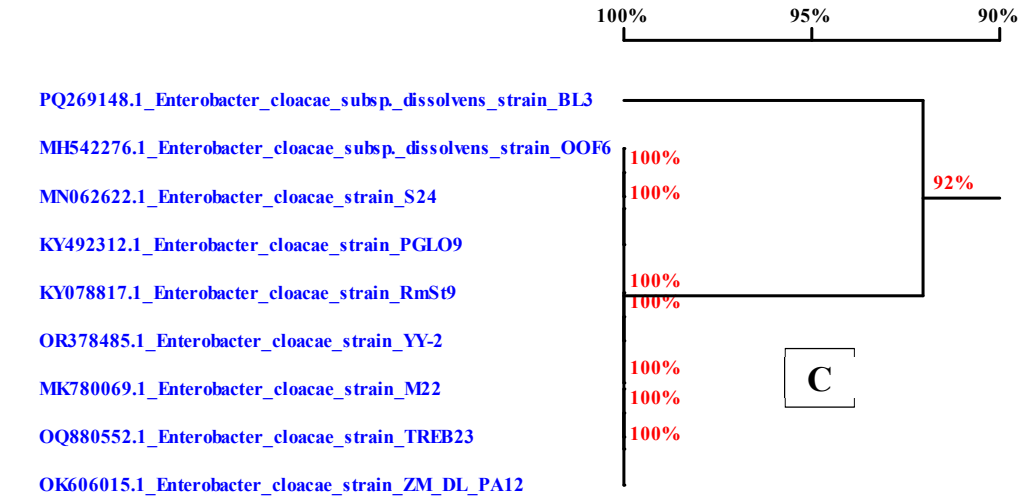
3.4. Molecular identification of effective isolated endophytic bacteria

Identification of effective 10 isolated endophytic bacteria using 16srDNA sequencing was carried out. The obtained sequence of 10 isolated endophytic bacteria was compared with referenced sequenced bacteria on GenBank. As shown in table (5) and phylogenetic tree in fig (3), results that obtained from GenBank showed bacteria with ID: SR3 is *Brevundimonas diminuta* with accession number **PQ269283** with identity 94.4% with recorded Japanese, German, Spanish, and Japanese isolates under accession numbers NR_113602.1, NR 114708.1, NR 117188.1, and NR 117188.1 respectively. Bacteria with ID: Siwi-R was identified as *Streptomyces murinus* with identity 97.66% with 3 Japanese and American isolates *Strept. murinus* which recorded with accession number NR 112445.1, NR 041414.1, NR 041072.1, and NR 115675.1 respectively. This isolate was recorded on GenBank under accession number **PQ269195**. GenBank referred to the bacteria with ID: BL3 that closes to *Enterobacter cloacae* subsp. *dissolvens* with identity 98.43% with recorded Indian isolate with the accession number MH 542276.1. This isolate noted with accession number **PQ269148**. Identity on GenBank for bacteria with

Table 5: Accession number of effective endophytic bacteria in GenBank

Bacterial ID	GenBank identification	Accession number
SR3	*Brevundimonas diminuta*	**PQ269283**
Siwi-R	*Streptomyces murinus*	**PQ269195**
BL3	*Enterobacter cloacae* subsp. *dissolvens*	**PQ269148**
BL4	*Brucella intermedia*	**PQ269161**
BNL2	*Sphingobacterium nematocida*	**PQ269169**
BHL5	*Sphingobacterium spiritivorum*	**PQ269172**
AR2	*Sphingobacterium spiritivorum*	**PQ269287**
SML2	*Sphingobacterium tabacisoli*	**PQ269162**
ML1	*Sphingobacterium soli*	**PQ269168**
MR1	*Brucella lupini*	**PQ269288**

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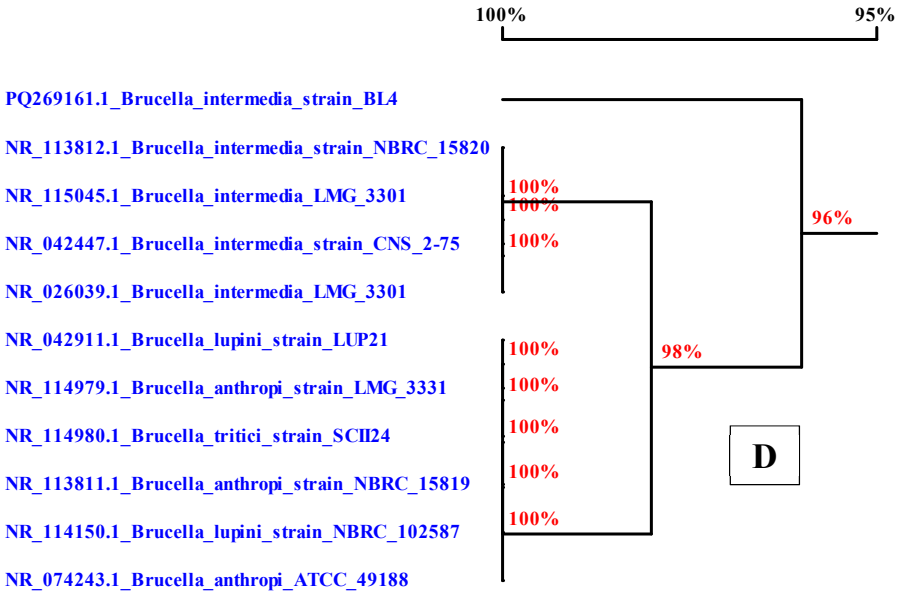
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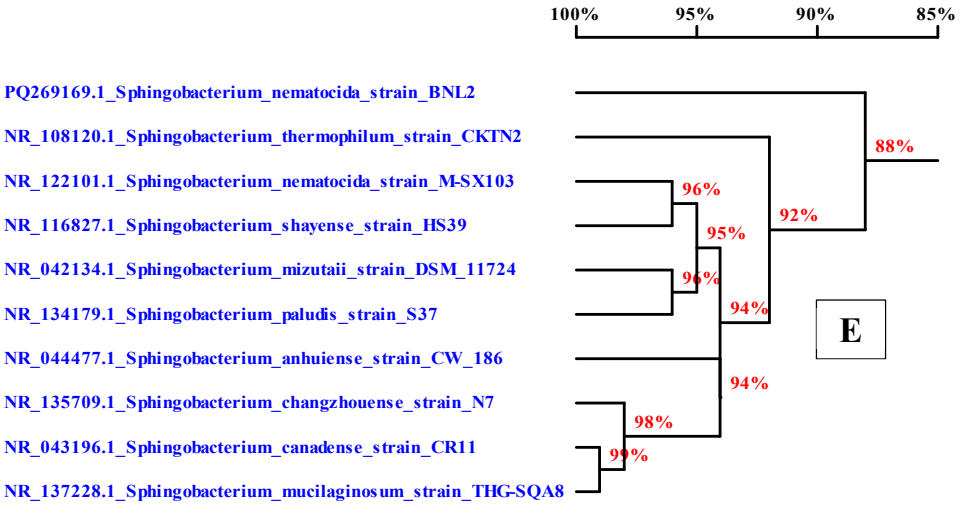
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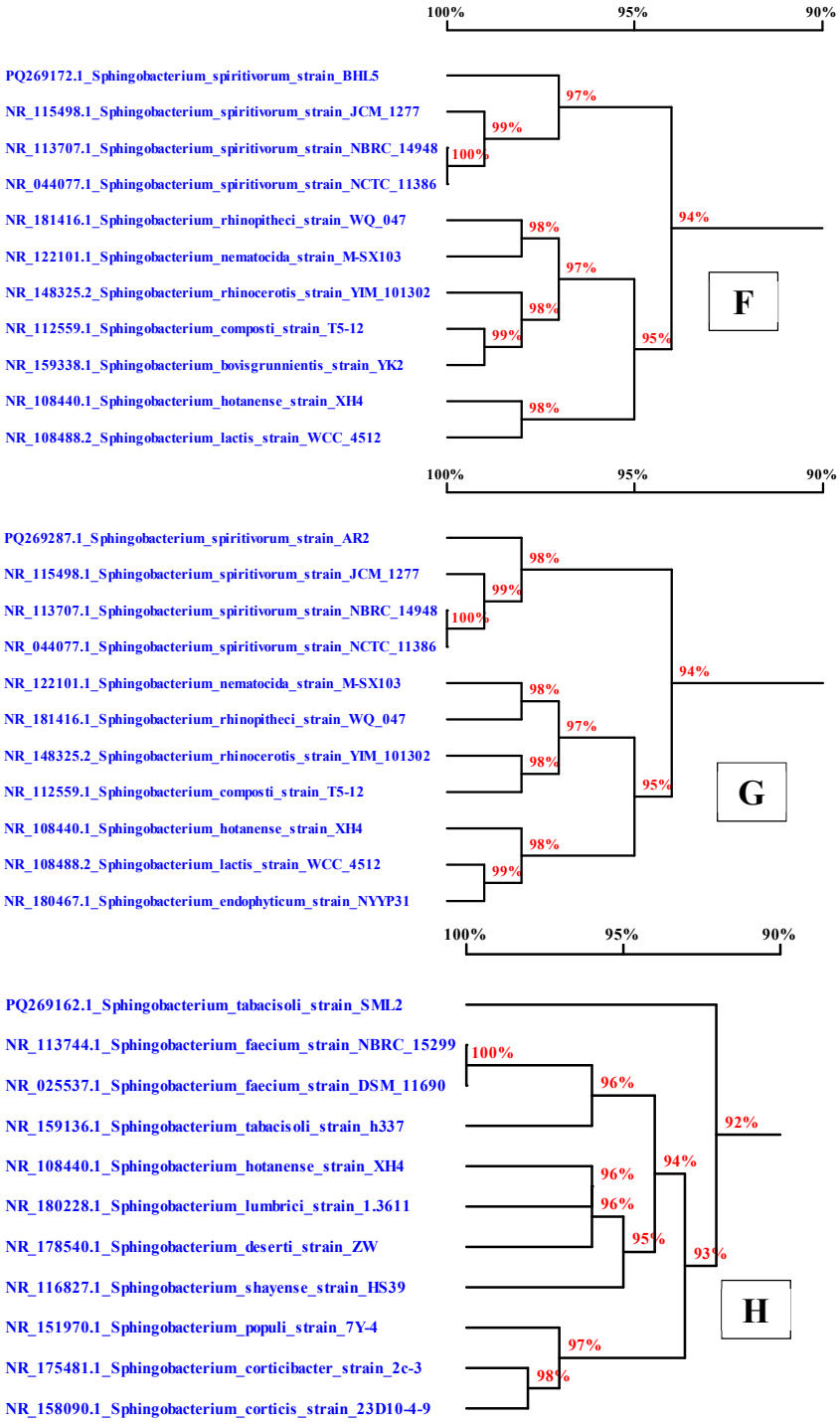
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NR_112559.1_Sphingobacterium_composti_strain_T5-12
NR_159338.1_Sphingobacterium_bovisgrunnientis_strain_YK2
NR_108440.1_Sphingobacterium_hotanense_strain_XH4
NR_108488.2_Sphingobacterium_lactis_strain_WCC_4512
97%
99%
100%
94%
98%
97%
98%
99%
95%
98%
F
100%
95%
90%
PQ269287.1_Sphingobacterium_spiritivorum_strain_AR2
NR_115498.1_Sphingobacterium_spiritivorum_strain_JCM_1277
NR_113707.1_Sphingobacterium_spiritivorum_strain_NBRC_14948
NR_044077.1_Sphingobacterium_spiritivorum_strain_NCTC_11386
NR_122101.1_Sphingobacterium_nematocida_strain_M-SX103
NR_181416.1_Sphingobacterium_rhinopitheci_strain_WQ_047
NR_148325.2_Sphingobacterium_rhinocerotis_strain_YIM_101302
NR_112559.1_Sphingobacterium_composti_strain_T5-12
NR_108440.1_Sphingobacterium_hotanense_strain_XH4
NR_108488.2_Sphingobacterium_lactis_strain_WCC_4512
NR_180467.1_Sphingobacterium_endophyticum_strain_NYYP31
98%
99%
100%
94%
98%
97%
98%
95%
98%
99%
G
100%
95%
90%
PQ269162.1_Sphingobacterium_tabacisoli_strain_SML2
NR_113744.1_Sphingobacterium_faecium_strain_NBRC_15299
NR_025537.1_Sphingobacterium_faecium_strain_DSM_11690
NR_159136.1_Sphingobacterium_tabacisoli_strain_h337
NR_108440.1_Sphingobacterium_hotanense_strain_XH4
NR_180228.1_Sphingobacterium_lumbrici_strain_1.3611
NR_178540.1_Sphingobacterium_deserti_strain_ZW
NR_116827.1_Sphingobacterium_shayense_strain_HS39
NR_151970.1_Sphingobacterium_populi_strain_7Y-4
NR_175481.1_Sphingobacterium_corticibacter_strain_2c-3
NR_158090.1_Sphingobacterium_corticis_strain_23D10-4-9
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96%
92%
94%
96%
96%
95%
93%
97%
98%
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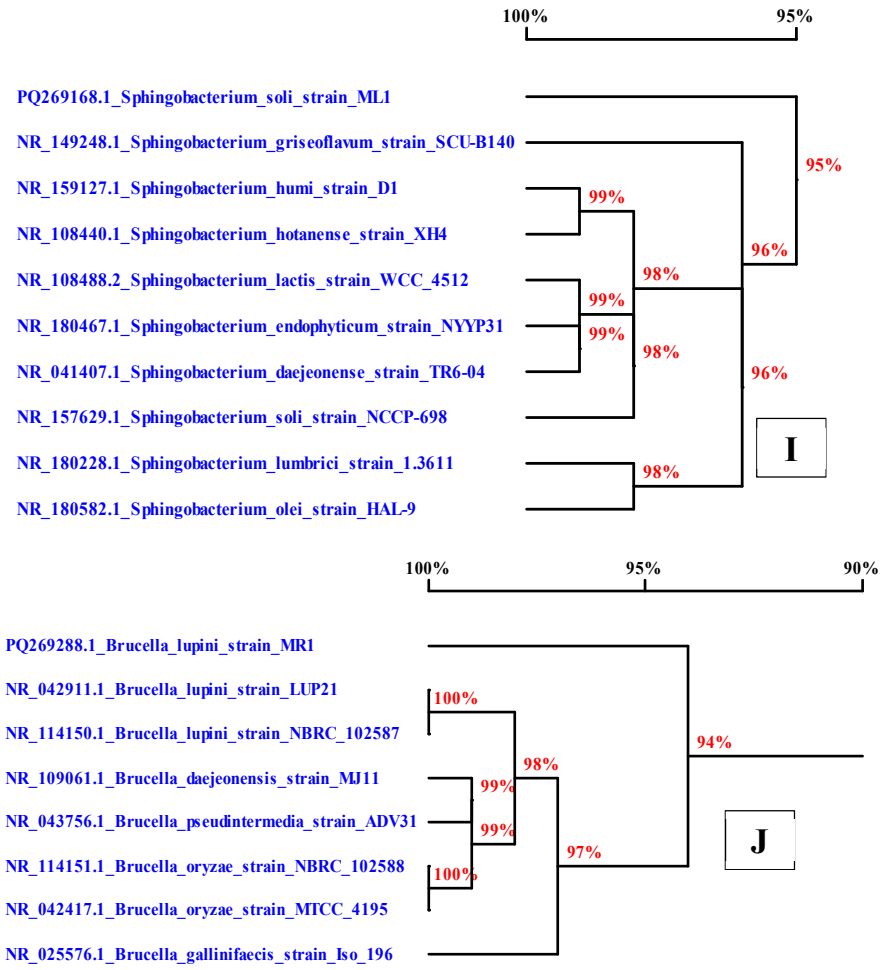

Fig 3: Phylogenetic trees of effective isolated endophytic bacteria A) *Brevundimonas diminuta* **B)** *Streptomyces murinus* **C)** *Enterobacter cloacae* subsp. *Dissolvens* **D)** *Brucella intermedia* **E)** *Sphingobacterium nematocida* **F)** *Sphingobacterium spiritivorum* **G)** *Sphingobacterium spiritivorum* **H)** *Sphingobacterium tabacisoli* **I)** *Sphingobacterium soli* **J)** *Brucella lupini*

ID: BL4 was got 97.95% with Japanese, German, German, and Spanish isolate *Brucella intermedia* that were recorded under accession number NR 113812.1, NR 115045.1, NR 042447.1, and NR 026039.1 respectively. This isolate was recorded under accession number **PQ269161**. BNL2 Bacterial ID: was identified on GenBank as *Sphingobacterium nematocida* with similarity 96.82% with the Chinese isolate that take accession number NR 122101.1. This isolate was documented under accession number **PQ269169**. Identification of the bacteria with ID: BHL5 and AR2 were carried out on GenBank and the results referred to the bacteria are *Sphingobacterium spiritivorum* in both isolates with identity 98.47% and 98.33% respectively with recorded Japanese, Japanese, and American isolates with accession numbers NR 115498.1, NR 113707.1, and NR 044077.1 respectively for both isolates. That isolates take accession number **PQ269172** and **PQ269287** respectively. In bacteria with ID: SML2, the obtained data on GenBank showed that bacteria were closed to *Sphingobacterium tabacisoli* with identity 96.76% with the Pakistani isolate that recorded under accession number NR 159136.1 and recorded under accession number **PQ269162**. From the obtained data, the sequence of bacterial ID: ML1 and MR1 were identified on GenBank. Data referred that ML1 bacteria was *Sphingobacterium soli* with identity 97.16% with recorded Pakistani isolate that recorded under accession number NR 157629.1. This isolate was recorded under accession number **PQ269168**. Finally, MR1 bacteria got

95.65% in identity with *Brucella lupine* Spanish and Japanese isolates which recorded under accession number NR 042911.1 and NR 114150.1 respectively. The bacteria were recorded on GenBank under accession number and **PQ269288**.

3.5. *In vivo* effect of endophytic bacteria on *M. phaseolina*

Effect of 10 isolated effective bacteria on virulence of *M. phaseolina in vivo* under greenhouse condition was studied. Results as shown in table (6) that referred to after 45 days, the plantlet samples which treated with *Streptomyces murinus* and *Sphingobacterium tabacisoli* were the best treatments and there were non-significant between them with control and samples treated with Rhizolex and the samples which only treated with *M. phaseolina* was significant with all treatments when after 90 days, the treated samples with *Streptomyces murinus*, *Sphingobacterium soli* and *Brucella lupini* were the best samples and there were non-significant between them and there are significant between samples that treated with *M. phaseolina* and all treatments.

Table 6: *In vivo* effect of endophytic bacteria on *M. phaseolina* under greenhouse condition

Treatment	Disease incidence, % after 45 days	Disease incidence, % after 90 days
Brevundimonas diminuta	11.77^{b}	22.87^{bc}
Streptomyces murinus	0.00^{a}	11.77^{ab}
Enterobacter cloacae* subsp. *dissolvens	22.53^{c}	22.87^{bc}
Brucella intermedia	11.77^{b}	50.33^{d}
Sphingobacterium nematocida	11.77^{b}	22.87^{bc}
***Sphingobacterium spiritivorum* (BHL5)**	11.77^{b}	28.10^{c}
***Sphingobacterium spiritivorum* (AR2)**	22.53^{c}	33.67^{c}
Sphingobacterium tabacisoli	0.00^{a}	22.53^{bc}
Sphingobacterium soli	11.77^{b}	11.77^{ab}
Brucella lupini	22.87^{c}	11.77^{ab}
M. phaseolina	33.30^{d}	66.67^{e}
Rhizolex	0.00^{a}	11.77^{ab}
control	0.00^{a}	0.00^{a}
p-value	**0.0001**	**0.0001**
LSD at 0.05	**10.6**	**10.2**

3.6. Biochemical changes assay

Biochemical changes on treated plantlets (with 10 isolated endophytic bacteria only, with *M. phaseolina* and 10 isolated endophytic bacteria, with *M. phaseolina* only, with *M. phaseolina* and Rhizolex and control) were assayed. The obtained results as tabulated in table (7) and illustrated in fig (4) mentioned that the total indo in treated plantlets were changed based on the treatment with inoculum.

Results showed that the high concentration of total phenol that was in plants infected only with *M. phaseolina* and there is significant between this treatment and the others then there are significant in total indol contents in plantlets that treated with endophytic bacteria only and endophytic bacteria with *M. phaseolina* comparing with control plantlet.

Total phenol was assayed in all treatments, table (7) and fig (4) presented that total phenol in infected plantlets were decreased significantly comparing with control plantlets and all other treated plantlets.

In case of amino acids, total amino acids in infected plantlets has not changed. There is non-significant between infected plants with *M. phaseolina* and control plantlets, while it increased significantly comparing with other treated plantlets as shown in table (7) and fig (4).

Estimation of pigments in treated plantlets *In vivo* were carried out, the estimated pigments include Chlorophyll a, Chlorophyll b, and carotenoids. Data in table (7) and fig (5) showed that Chlorophyll a in infected plantlets were decreased significantly comparing to control samples and the other treated samples. On other hand, both of Chlorophyll b, and carotenoids were decreased gradually in infected plantlets. There are significant between infected plantlets and control when it were increased comparing with some treated samples.

Table 7: Biochemical changes assaying

Treatment	Total indol mg/gm	Total phenol mg/gm	Total amino acid mg/gm	Pigments mg/gm		
				Chl. A	Chl. B	Carot.
Brev. diminuta*+ *M. phaseolina	0.47^{j}	33.11^{j}	1.62^{i}	26.67^{l}	11.95^{b}	5.25^{k}
Strept. Murinus* + *M. phaseolina	0.35^{e}	39.81^{t}	1.32^{efg}	29.48^{n}	41.33^{p}	1.79^{d}
Ent. cloacae* subsp. *dissolvens*+ *M. phaseolina	0.39^{f}	35.97^{n}	1.29^{ef}	31.74^{p}	35.33^{n}	0.73^{c}
Bruc. intermedia*+ *M. phaseolina	0.45^{i}	23.36^{e}	1.21^{de}	34.30^{q}	32.06^{m}	0.10^{a}
Sph. nematocida*+ *M. phaseolina	0.48^{j}	35.09^{k}	1.45^{gh}	21.29^{c}	17.96^{g}	5.54^{l}
Sph. spiritivorum* BHL5+ *M. phaseolina	0.29^{c}	36.50^{p}	1.29^{ef}	26.31^{j}	16.06^{d}	4.71^{i}
Sph. spiritivorum* (AR2)+ *M. phaseolina	0.52^{l}	50.74^{v}	1.45^{gh}	29.04^{m}	17.57^{f}	3.61^{g}
Sph. tabacisoli*+ *M. phaseolina	0.42^{gh}	18.36^{b}	0.64^{a}	21.59^{d}	25.07^{k}	7.67^{o}
Sph. soli* + *M. phaseolina	0.50^{k}	39.44^{s}	1.56^{hi}	29.42^{n}	23.49^{j}	2.79^{e}
Bruc.lupini*+ *M. phaseolina	0.25^{b}	29.21^{f}	1.04^{bc}	26.08^{i}	22.24^{h}	3.44^{f}
M. phaseolina	0.91^{a}	18.53^{c}	1.67^{i}	15.67^{a}	16.36^{e}	5.05^{j}
Rhizolex	0.39^{f}	36.32^{o}	1.50^{hi}	25.85^{h}	9.89^{a}	5.96^{n}
Brev. Diminuta	0.26^{b}	29.79^{h}	1.12^{cd}	23.34^{e}	15.40^{c}	5.27^{k}
Strept. Murinus	0.36^{e}	18.71^{d}	1.31^{efg}	26.46^{k}	15.40^{c}	5.07^{j}
Ent. cloacae* subsp. *dissolvens	0.29^{c}	29.51^{g}	0.99^{bc}	20.89^{b}	17.69^{f}	5.26^{k}
Bruc. intermedia	0.51^{kl}	15.97^{a}	0.93^{b}	23.39^{e}	23.27^{i}	4.19^{h}
Sph. nematocida	0.33^{d}	36.75^{q}	1.69^{i}	20.89^{b}	17.69^{f}	5.26^{k}
Sph. spiritivorum	0.28^{c}	39.04^{r}	1.08^{bcd}	25.08^{g}	12.10^{b}	5.74^{m}
Sph. spiritivorum	0.41^{g}	35.47^{l}	1.22^{de}	25.08^{g}	12.10^{b}	5.74^{m}
Sph. tabacisoli	0.43^{h}	39.99^{u}	1.54^{hi}	31.01^{o}	37.50^{o}	0.31^{b}
Sph. soli	0.38^{f}	35.64^{m}	1.08^{bcd}	23.39^{e}	23.27^{i}	4.19^{h}
Bruc.lupini	0.41^{g}	31.30^{i}	1.43^{fgh}	31.01^{o}	37.50^{o}	0.31^{b}
control	0.36^{e}	39.69^{t}	1.58^{hi}	24.32^{f}	29.10^{l}	3.31^{f}
p-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD at 0.05	0.02	0.15	0.09	0.28	0.3	0.2

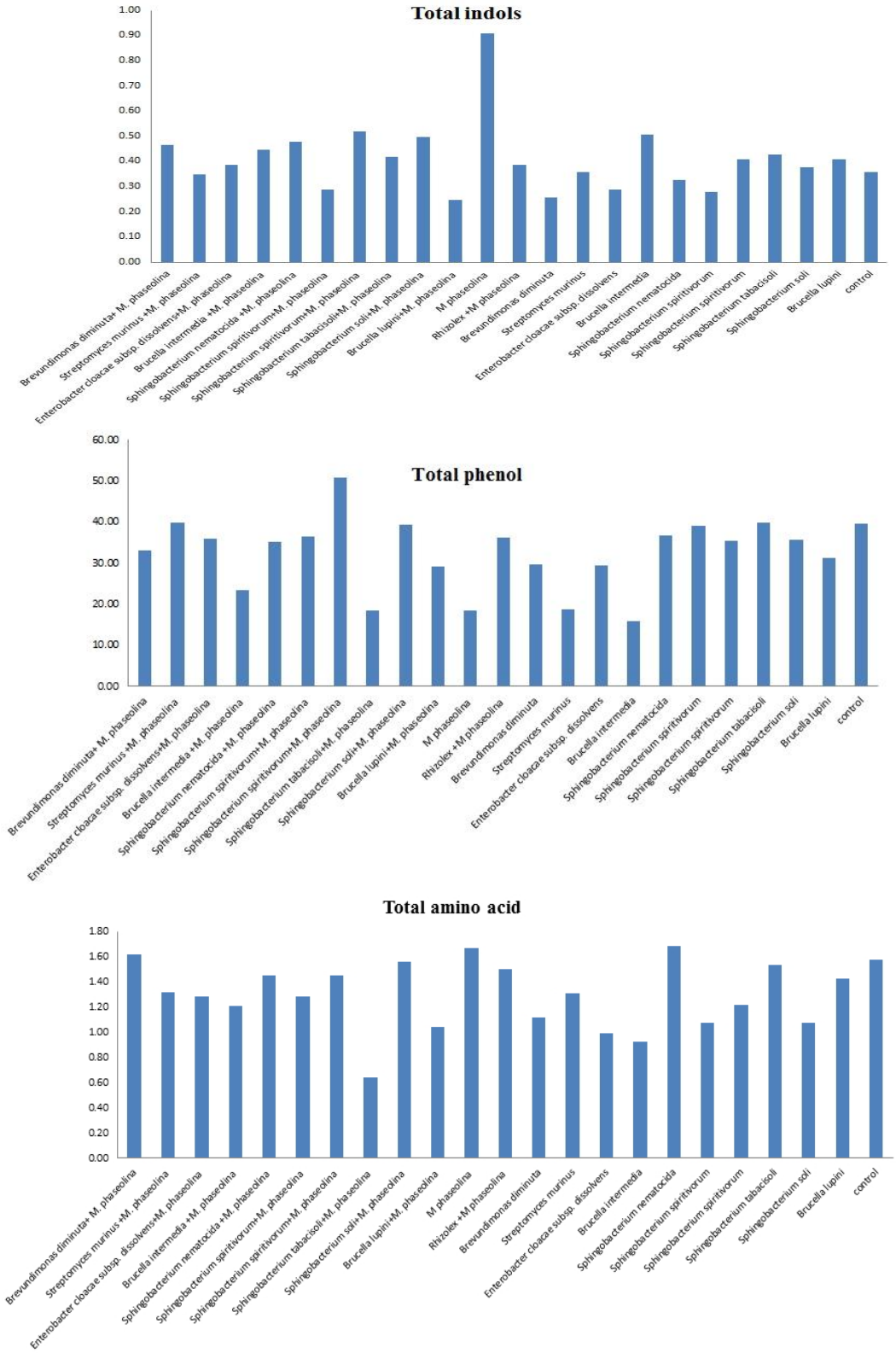

Fig. 4: Biochemical assaying: Total Indol, Total phenol, and total amino acids

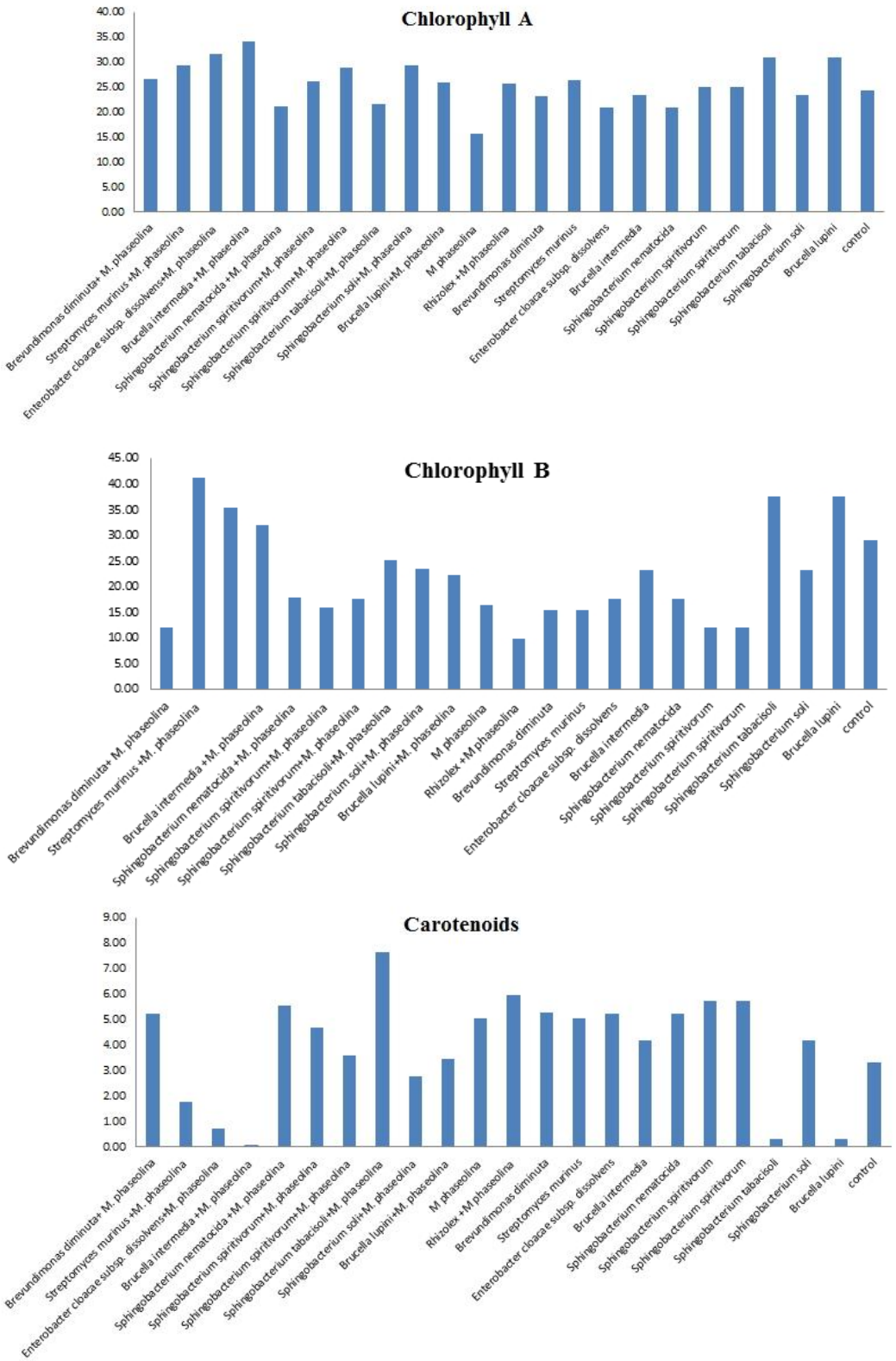

Fig. 5: pigments assaying: Chlorophyll a, Chlorophyll b, and carotenoids

3.7. Preservation experiment

Preservation experiment using *Streptomyces murinus* was carried out after infest the samples with *M. phaseolina*. Results showed that the pathogenicity percentage was 33.3% after 45 days and reached to 50% after 90 days. The obtained data showed that total indol, total phenol, total amino acids, chlorophyll a, and chlorophyll b were increased in the treated plantlets comparing with control. Results showed that it was 0.39 mg/g, 1.79mg/g, 1.79mg/g, 29.98mg/g, and 33.85mg/g in total indol, total phenol, total amino acids, chlorophyll a, and chlorophyll b in treated plantlets respectively when it was 0.36 mg/g, 1.58mg/g, 1.58mg/g, 24.32mg/g, and 29.10mg/g in control plantlets respectively.

In case of carotenoids, the level of carotenoids in control plantlets were increased comparing of treated plantlets.

4. Discussion

Macrophomina phaseolina consider one of the economically importance fungi that infecting date palm. It cause charcoal disease on plants that appear as wilting of foliage, older leaves were died, the crown turned brown and then black. This symptoms also were described by and EL-morsi *et al.,* (2012) and Mohamed *et al.* (2016).

Endophytic bacteria consider one of the means in plants which play a natural role in plant resistance to some diseases. In this syudy, we isolate number of endophytic bacteria from leaves and roots of different 8 cultivars (4 wet cultivars incuding Amhat, Barhee, Samany, and Bent-aisha, 1 semi-dry cultivar include Siwi, 2 dry cultivars incuding Malkaby and Bartmoda, and 1 male cultivar) were carried out. Fifty isolates of bacteria were isolated from the previous cultivars and the antangonsim *in vitro* against *M. phaseolina* were tested. Ten of the 50 isolates of endophytic bacteria were effective against *M. phaseolina*. These 10 isolates were identified using 16srDNA and the obtained sequences were identified and recorded on GenBank.

The identified isolates were *Brevundimonas diminuta* strain SR3 with accession number PQ269283, *Streptomyces murinus* strain Siwi-R under accession number PQ269195, *Enterobacter cloacae* strain BL3 with the accession number PQ269148, *Brucella intermedia* stain BL4 under accession number PQ269161, *Sphingobacterium nematocida* stain BNL2 under accession number PQ269169, *Sphingobacterium spiritivorum* strain BHL5 with accession number PQ269172, *Sphingobacterium spiritivorum* strain AR2 with accession number PQ269287, *Sphingobacterium tabacisoli* strain SML2 under accession number PQ269162, *Sphingobacterium soli* strain ML1 under accession number PQ269168, and *Brucella lupine* strain MR1 under accession number PQ269288.

The antagonism was carried out using endophytic bacteria by Al-Nadabi *et al.,* (2021) which using endophytic bacteria against *Alternaria* sp. and *Fusarium solani*. Hammia and Bouatrous (2023) on *Mauginiella scaettae*. While Mahmoud *et al.,* (2017) mentioned that 552 endophytic fungi isolates were isolated from date palm and tested against *Hypocreales* sp., *Fusarium* sp., and *Clonostachys* sp. and 30 of isolated fungi were more effective for their 3 fungi.

Dhillon and Chakrabarti (2023) reported that Streptomyces murinus were isolated from foxtail palm's ripe berries of (*Wodyetia bifurcata*) and using this isolate as antifungal against potential fungal pathogens of palms.

Ferjani *et al.* (2015) mentioned that *Sphingobacterium* was isolated from date palm rhizosphere. This bacteria was used as plant growth promoting bacteria. Yaish *et al.* (2015) isolated and identified endophytic plant growth promoting bacteria (PGPR) from date palm *Enterobacter cloacae* was identified as endophytic bacteria that increase the IAA level in date palm. Ahmed (2020) identified *Enterobacter cloacae* as endophytic bacteria and used as plant growth promoting bacteria (PGPR)

Li *et al*. (2021) represent that endophytic bacteria were isolated from some halophytic plants. *Brucella* sp. was isolated and identified from *Reaumuria soongorica* and *Artemisia carvifolia*. She *et al.* (2023) mentioned that *Brucella* lupini was isolated and identified from root nodules of *Lupinus honoratus*. While Shazmin *et al.* (2023) reported that *Brucella intermedia* was isolated and identified from cotton plants and found that this isolate help the plants by degradation the pesticide and consider multi-stress tolerance and PGPR.

Biochemical changes in *in vivo* experiment to study the effect of pathogenic and endophytic bacteria on plantlets. Total indol was assayed and the results showed that IAA was increased in infected plants with *M. phaseolina* comparing with other treatments. Rodríguez *et al.* (2023) reported that *M. phaseolina* excreted IAA and the level of IAA in infected plants with this fungus were increased.

Level of total phenol, chlorophyll a, and chlorophyll b were decreased in infected plantlets with *M. phaseolina* comparing with other treatments. Abd Allah (2018) also noted that the level phenol in infected date palm was decreased because of the metabolism of infected plants especially the shikimic acid pathway was degraded so that the phenol level was decreased. Also he noted that the pigments were decreased in infected plants because of the metabolism was degraded until the plant death comparing to the treated and control plants.

References

Abd Allah, L.L., 2018. Bio-control of some pathogens infecting date palm using viruses (Ph. D. Thesis, Ain Shams University, Cairo, Egypt).183.

Abd El-Khair, H. and N.S. El-Mougy, 2003. Field biological approach under organic cultivation conditions for controlling garlic black mould disease infection during storage. Egypt. J. Appl. Sci., 18 (6): 50-69.

Ahmed, E.A.S., 2020. Evaluation the interaction between some medicinal plants and their associated endophytic microorganisms (Doctoral dissertation, Ain Shams University). 136

Ahmed, M.F.A., 2018. Management of date palm root rot diseases by using some biological control agents under organic farming system. Novel Research in Microbiology Journal. 2(2): 37-47

Ainsworth, E.A., and K.M. Gillespie, 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols, 2(4): 875-877.

Al-Nadabi, H.H., N.S. Al-Buraiki, A.A. Al-Nabhani, S.N. Maharachchikumbura, R. Velazhahan, and A.M. Al-Sadi, 2021. *In vitro* antifungal activity of endophytic bacteria isolated from date palm (*Phoenix doctylifera* L.) against fungal pathogens causing leaf spot of date palm. Egyptian Journal of Biological Pest Control, 31: 1-8.

Al-Shahib, W. and R.J. Marshall, 2003. The fruit of the date palm: it's possible use as the best food for the future? Int. J. Food Sci. Nutr. 24: 247–259.

Dayani, O., A. Khezri and A.G. Moradi, 2012. Determination of nutritive value of date palm by products using *in vitro* and *In situ* measurements. Small Rum. Res. 105:122-125.

Dhillon, B., and S. Chakrabarti, 2023. Draft genome sequence of *Streptomyces murinus* strain SPC1 with antimicrobial potential against fungal pathogens of palms. Microbiology Resource Announcements, 12(12): e00826-23.

Dos Santos, H.R.M., C.S. Argolo, R. C. Argôlo-Filho, and L.L. Loguercio, 2019. A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. BMC microbiology, 19: 1-14.

EL-morsi, M.E., A. Rehab, E. Mohsen, and S.A. EL-Morsy, 2012. Survey and control trials of root rot/wilt of date palm offshoots in New Valley Governorate. Egyptian Journal of Agricultural Research, 90(4): 1403-1414.

FAO 2022. FAOSTAT. Food and Agriculture Statistics. www.fao.org.

Ferjani, R., R. Marasco, E. Rolli, H. Cherif, A. Cherif, M. Gtari, and H.I. Ouzari, 2015. The date palm tree rhizosphere is a niche for plant growth promoting bacteria in the oasis ecosystem. BioMed research international, 2015(1): 153851.

Hammia, H., and Y. Bouatrous, 2023. Biocontrol potential of inflorescence rot of date palm caused by Mauginiella scaettae in the Biskra region (Algeria). Journal of Biological Research-Bollettino della Società Italiana di Biologia Sperimentale, 96(2).

Haq, I.U., and N.A. Khan, 2020. Fungal Diseases of Date Palm (*Phoenix dactylifera*): Etiology and Management. Etiology and Integrated Management of Economically Important Fungal Diseases of Ornamental Palms, 169-196.

Lewaa, L.M., and H. Zakaria, 2023. *Chaetomium globosum*: a potential biocontrol agent for root rot of date palm seedlings. Egyptian Journal of Phytopathology, 51(2): 114-128. http://doi.org/10.21608/EJP.2024.353068.

Lewaa, L.M., B.A. Othman, A.M. Metwaly, and K.A. El-Dougdoug, 2023. Molecular and physical characterization of the phage specific for bacterial-pathogen causing pink-rot inflorescence disease in date-palm. Egyptian International Journal of Palms, 3(1): 53-68.

Li, L., L. Gao, Y. Liu, B. Fang, Y. Huang, O.A. Mohamad, and J. Ma, 2021. Diversity of cultivable endophytic bacteria associated with halophytes in Xinjiang of China and their plant beneficial traits. Journal of Arid Land, 13: 790-800.

Mahmoud, F.M., Z. Krimi, J.G. Maciá-Vicente, M.B. Errahmani, and L.V. Lopez-Llorca, 2017. Endophytic fungi associated with roots of date palm (*Phoenix dactylifera*) in coastal dunes. *Revista iberoamericana* de Micologia, 34(2), 116-120.

Mohamad, O.A.A., L. Li, J.-B. Ma, S. Hatab, L. Xu, J.-W. Guo, B.A. Rasulov, Y.-H. Liu, B.P. Hedlund, and W.J. Li, 2018. Evaluation of the antimicrobial activity of endophytic bacterial populations from Chinese traditional medicinal plant Licorice and characterization of the bioactive secondary metabolites produced by *Bacillus atrophaeus* against *Verticillium dahliae*. Front Microbiol., 9:924. https://doi.org/10.3389/fmicb.2018.00924.

Mohamed, A.M., K.A. El Mangoury, and A.F. Abdelsalam, 2016. Plant regeneration in date palm (Phoenix dactylifera) cv. Khalas overcoming four different fungus contaminations by using biological control. *Arab J. Biotech., 19 (2): 135-148.*

Patel, A., A. Patel, A. Patel, and N.M. Patel, 2010. Estimation of flavonoid, polyphenolic content and *in vitro* antioxidant capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). Int J Pharma Sci. and Res., 1(1): 66-77.

Rodríguez, L.A., C.C. Rodríguez, E.G. Cano, L.R. Quintanilla, A.O. Hernández, C.L. Ortega and, J.L.H. Mendoza, 2023 Production of Acetic Indole Acid in Macrophomina phaseolina. International Journal of Plant and Soil Science, 35:(3). 129-136

Shazmin, Ahmad, S.A., T.A. Naqvi, M.F.H. Munis, M.T. Javed, and H.J. Chaudhary, 2023. Biodegradation of monocrotophos by Brucella intermedia Msd2 isolated from cotton plant. World Journal of Microbiology and Biotechnology, 39(6): 141.

She, R., C. Anglewicz, K. Jerke, R. Relich, M. Glazier, L. Filkins, and A. Schuetz, 2023. Brucella and Ochrobactrum taxonomic updates for laboratories. American Society for Microbiology (Guidelines).

Selim, H.H., M.A. Fayek, and A.M. Sewidan, 1978. Reproduction of Bircher apple cultivar by layering. Annals of Agric. Sci. Moshtohor, 9: 157-165.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7thed. The Iowa State Univ. Press, Ames., Iowa, USA. 75-78.

Wettstein, D., 1957. Formula of chlorophyll determination. Exp. Cell Res. 12(3): 427-489.552916.

Yaish, M.W., I. Antony, and B.R. Glick, 2015. Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (Phoenix dactylifera L.) and their potential role in salinity tolerance. Antonie Van Leeuwenhoek, 107: 1519-1532.

Yaish, M.W., I. Al-Harrasi, A.S. Alansari, R. Al-Yahyai and B.R. Glick, 2016. The use of high throughput DNA sequence analysis to assess the endophytic microbiome of date palm roots grown under different levels of salt stress. Int Microbiol., 19(3):143–155. https://doi.org/10.2436/20.1501.01.272.

Appendix
Supplementary Data
Standard isolates obtained from Gene Bank used in the phylogenetic analysis

***Brevundimonas diminuta* SR3**

Isolate	**NCBI accession**	**Country**
Brevundimonas diminuta SR3	PQ269283.1	Egypt
Brevundimonas diminuta NBRC 12697	NR_113602.1	Japan
Brevundimonas diminuta LMG 2089	NR 114708.1	Germany
Brevundimonas diminuta ATCC 1156	NR 117188.1	Spain
Brevundimonas diminuta JCM 2788	NR 113238.1	JAPAN
Brevundimonas viscosa F3	NR 117900.2	China
Brevundimonas bacteroides CB7	NR 037105.1	Germany
Brevundimonas bacteroides ATCC 15254	NR 112031.1	Japan
Brevundimonas lutea NS26	NR 171448.1	China
Brevundimonas aveniformis EMB102	NR 043770.1	Korea
Brevundimonas abyssalis TAR-001 16S	NR 114308.1	Japan

***Streptomyces murinus* SIWI-R**

Isolate	**NCBI accession**	**Country**
Streptomyces murinus SIWI-R	PQ269195.1	Egypt
Streptomyces murinus NBRC	NR 112445.1	Japan
Streptomyces murinus NBRC 100773	NR 041414.1	Japan
Streptomyces murinus NBRC 12799	NR 041072.1	Japan
Streptomyces murinus NRRL B-2286	NR 115675.1	USA
Streptomyces novaecaesareae NBRC 13368	NR 041124.1	Japan
Streptomyces nodosus ATCC 14899	NR 041730.2	Singapore
Streptomyces malaysiense MUSC 136	NR 178689.1	Malaysia
Streptomyces bauhiniae Bv016	NR 179277.1	Thailand
Streptomyces montanisoli MMS17-BM035	NR 181531.1	South Korea

***Enterobacter cloacae* subsp. *dissolvens* BL3**

Isolate	**NCBI accession**	**Country**
Enterobacter cloacae subsp. *dissolvens* BL3	PQ269148.1	Egypt
Enterobacter cloacae subsp. *dissolvens* OOF6	MH 542276.1	India
Enterobacter cloacae RmSt9	KY078817.1	Malaysia
Enterobacter cloacae YY-2	OR378485.1	China
Enterobacter cloacae PGLO9	KY492312.1	India
Enterobacter cloacae S24	MN062622.1	India
Enterobacter cloacae M22	MK780069.1	China
Enterobacter cloacae TREB23	OQ880552.1	India
Enterobacter cloacae ZM DL PA12	OK606015.1	India

***Brucella intermedia* BL4**

Isolate	**NCBI accession**	**Country**
Brucella intermedia BL4	PQ269161.1	Egypt
Brucella intermedia NBRC 15820	NR 113812.1	Japan
Brucella intermedia LMG 3301	NR 115045.1	Germany
Brucella intermedia CNS 2-75	NR 042447.1	Germany
Brucella intermedia LMG 3301	NR 026039.1	Spain
Brucella lupini LUP21	NR 042911.1	Spain
Brucella anthropi LMG 3331	NR 114979.1	Germany
Brucella tritici SCII24	NR 114980.1	Germany
Brucella anthropi NBRC 15819	NR 113811.1	Japan
Brucella lupini NBRC 102587	NR 114150.1	Japan
Brucella anthropi ATCC 49188	NR 074243.1	USA

***Sphingobacterium nematocida* BNL2**

Isolate	**NCBI accession**	**Country**
Sphingobacterium nematocida BNL2	PQ269169.1	Egypt
Sphingobacterium thermophilum CKTN2	NR 108120.1	Japan
Sphingobacterium nematocida M-SX103	NR 122101.1	China
Sphingobacterium shayense HS39	NR 116827.1	China
Sphingobacterium mizutaii DSM 11724	NR 042134.1	Germany

Sphingobacterium paludis S37	NR 134179.1	China
Sphingobacterium anhuiense CW 186	NR 044477.1	China
Sphingobacterium changzhouense N7	NR 135709.1	China
Sphingobacterium canadense CR11	NR 043196.1	Canada
Sphingobacterium mucilaginosum THG-SQA8	NR 137228.1	Korea
***Sphingobacterium spiritivorum* BHL5**		
Isolate	**NCBI accession**	**Country**
Sphingobacterium spiritivorum BHL5	PQ269172.1	Egypt
Sphingobacterium spiritivorum JCM 1277	NR 115498.1	Japan
Sphingobacterium spiritivorum NBRC 14948	NR 113707.1	Japan
Sphingobacterium spiritivorum NCTC 11386	NR 044077.1	USA
Sphingobacterium rhinopitheci WQ 047	NR 181416.1	China
Sphingobacterium nematocida M-SX103	NR 122101.1	China
Sphingobacterium rhinocerotis YIM 101302	NR 148325.2	China
Sphingobacterium composti T5-12	NR 112559.1	Korea
Sphingobacterium bovisgrunnientis YK2	NR 159338.1	India
Sphingobacterium hotanense XH4	NR 108440.1	China
Sphingobacterium lactis WCC 4512	NR 108488.2	Germany
***Sphingobacterium spiritivorum* AR2**		
Isolate	**NCBI accession**	**Country**
Sphingobacterium spiritivorum AR2	PQ269287.1	Egypt
Sphingobacterium spiritivorum JCM 1277	NR 115498.1	Japan
Sphingobacterium spiritivorum NBRC 14948	NR 113707.1	Japan
Sphingobacterium spiritivorum NCTC 11386	NR 044077.1	USA
Sphingobacterium nematocida M-SX103	NR 122101.1	China
Sphingobacterium rhinopitheci WQ 047	NR 181416.1	China
Sphingobacterium rhinocerotis YIM 101302	NR 148325.2	China
Sphingobacterium composti T5-12	NR 112559.1	Korea
Sphingobacterium hotanense XH4	NR 108440.1	China
Sphingobacterium lactis WCC 4512	NR 108488.2	Germany
Sphingobacterium endophyticum NYYP31	NR 180467.1	China
***Sphingobacterium soli* ML1**		
Isolate	**NCBI accession**	**Country**
Sphingobacterium soli ML1	PQ269168.1	Egypt
Sphingobacterium griseoflavum SCU-B140	NR 149248.1	China
Sphingobacterium humi D1	NR 159127.1	Korea
Sphingobacterium hotanense XH4	NR 108440.1	China
Sphingobacterium_lumbrici 1.3611	NR 180228.1	China
Sphingobacterium olei HAL-9	NR 180582.1	China
Sphingobacterium lactis WCC 4512	NR 108488.2	Germany
Sphingobacterium endophyticum NYYP31	NR 180467.1	China
Sphingobacterium daejeonense TR6-04	NR 041407.1	South Korea
Sphingobacterium soli NCCP-698	NR 157629.1	Pakistan
***Brucella lupini* MR1**		
Brucella lupini MR1	PQ269288.1	Egypt
Brucella_lupini LUP21	NR 042911.1	Spain
Brucella lupini NBRC 102587	NR 114150.1	Japan
Brucella daejeonensis MJ11	NR 109061.1	Korea
Brucella pseudintermedia ADV31	NR 043756.1	France
Brucella oryzae_NBRC 102588	NR 114151.1	Japan
Brucella oryzae MTCC 4195	NR 042417.1	Germany
Brucella gallinifaecis Iso 196	NR 025576.1	Germany