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Antagonistic and Inhibitory Effect of Some Plant Rhizo-Bacteria Against Different Fusarium Isolates *on Salvia officinalis*

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Abstract: Rhizo-bacteria of high biocontrol potential against different Fusarium isolates were bio-assayed under both laboratory and greenhouse conditions. Eighty five rhizo-bacteria isolated from the rhizosphere of plants growing in various desert areas were screened for their antagonistic activity against eight Fusarium isolates (four isolates of *Fusarium solani* and four isolates of *Fusarium oxysporum*) they were isolated from root and stem of *Salvia officinalis* using dual culture technique. Fourteen bacterial isolates that recorded high antagonistic activity against the pathogens of Fusarium wilt and root rot diseases on *Salvia officinali*s were selected for further investigation. Most of these bacterial isolates belong to different genus of *Bacillus, Pseudomonas, Lactobacillus, Brevibacillus* and *Stenotrophomonas* spp. Assessment of inhibitory secondary metabolites as HCN, Chitinase and Siderophore production for the most active fourteen bacterial strains revealed the ability of all bacterial isolates to produce at least one of the bioactive metabolite. Inhibition of hyphal fungal growth using bacterial live cells and metabolites were conducted to select the most effective bacterial strains. Four strains were identified as *Brevibacillus brevis, Brevibacillus formosus, Brevibacillus agri* and *Stenotrophomonas maltophilia* were selected as highly effective biocontrol agents against all tested pathogenic fungi. The four selected strains were evaluated for their ability to control wilt and root rot diseases of *Salvia officinalis* under greenhouse conditions. Results showed that these strains could be a possible candidate for biological control of Fusarium wilt and root rot in some economical crops. Also, this report is thought to be the first on using of these four bacterial strains as biological control agents against Fusarium wilt and root rot diseases on *Salvia officinalis* in Egypt.

Key words: Biological control · Soil-borne fungal pathogens · Anti-fungal activity · *Fusarium* spp. · Rhizo-bacteria

INTRODUCTION

Common sage *Salvia officinalis* L. (Lamiaceae, common sage) is one of the oldest medicinal and aromatic plants that grow in different countries worldwide and well known for its medical properties and has a great industrial significance. Plant diseases control is necessary to maintain the health of plant and thus the quality of food, feed and fiber, which is produced through agricultural activity. Root rot and wilt of *Salvia officinalis* is a soil-borne diseases that are incited by several fungal pathogens including *Fusarium* spp. and *Rhizoctonia solani.* Fusarium wilt diseases caused by different species of the soil inhabiting fungi Fusarium is a major concern in many growing areas of these plants, also can cause severe losses in a wide variety of plants. There are species of Fusarium (*F. solani* and *F. oxysporum*). Both of these pathogens occur throughout most *Salvia officinalis*, growing areas and either can devastate a crop [1, 2]. Biological control of fungal diseases of plant is ecofriendly and is a potential component of integrated disease management. A variety of soil microorganisms have demonstrated activity in the control of various soil borne plant pathogens, including Fusarium wilt pathogens [3]. Although the agricultural practices and use of chemical fertilizers and pesticides have led to

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increase productivity and reduce plant diseases, the use of chemical pesticides has led to many of the environmental and health problems. So, some researches focused on the development of alternative inputs for chemical pesticides to control plant diseases through different approaches used to prevent or control plant diseases [4, 5]. Biological control of plant pathogens using non-pathogenic microorganisms is considered one of the strategies to reduce losses from these diseases and improve the health of plants. Also those microorganisms can be considered as alternatives to use of chemical pesticides which cause a lot of damage to the environment and the consumer [6, 7, 8, 9]. Many bacterial species associated with or surrounding the plant roots can be played effective roles in control of plant diseases [10]. Rhizobacterial strains of *Pseudomonas, Burkholderia* and *Bacillus* spp. also have been used to reduce disease caused by a variety of soil borne pathogens [11, 12, 13]. The effectiveness of bioagents as biocontrol may be due to secretion of one or more of inhibitory metabolites. The high antagonistic activities of *Bacillus* spp. are executed by secretion of a number of metabolites including antibiotics, volatile compound HCN, siderophores, enzymes chitinase and β-1, 3-glucanase [14-17]. Fluorescent pseudomonads with multiple mechanisms for biocontrol of phytopathogens and plant growth promotion are being used widely as they produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase and can solubilize phosphorous [18, 19]. The production of sidrophores and its role as a suppressive agent to soil borne root pathogens has been reported and considered as indicator to effectiveness of bacterial isolates as biological control agents [20, 21, 22].

This study was carried out to isolate the new antagonistic bacterial strains from the rhizosphere of field-grown plants in various desert areas in Egypt to study their anti-fungal activities against eight Sage Fusarium isolates.

MATERIALS AND METHODS

Isolation of Biocontrol Agents from Rhizosphere: In 2012 eighty five rhizo-bacteria were isolated (described below) from the rhizosphere of seven different plants (Fennel, Wheat, Saliva, Rosemary, Solanum, Barley and Cucumber) from different locations. Bacterial isolates were maintained in 80% glycerol (v/v) at -80°C. In order to culture these bacteria, a loopful of inoculum was streaked on nutrient medium (NA) plates. After incubation for 24h at room temperature, single colonies were streaked on fresh nutrient plates. On the basis of their colony morphology, many colonies were randomly selected and further purified by streaking on nutrient medium. Each isolate was evaluated as potential antagonist agent against eight Fusarium isolates (stated below) causing wilt and root rot diseases of *Salvia officinalis* (Data not shown).

Morphological and Molecular Identification of Bacterial Strains: All bacterial isolates tested and screened in this study were identified to the genus level at Soil Microbiology Unit, Department of Soil Fertility and Microbiology, Desert Research Center (data not shown). The preliminary identification was carried out according to Bergey's Manual of Determinative Bacteriology [23]. Only preliminary identification of the most active fourteen antagonistic isolates was recorded. Four screened isolates used in greenhouse experiments were identified to molecular level using partial 16S rRNA gene sequence technique according to Berg *et al.* [24] in Sigma Scientific Services Co. Bacterial 16S rRNA gene sequences were amplified by PCR using the eubacterial primer pair 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-TAC GGY TAC CTT GTT ACG ACT T-3') [25]. The PCR was performed by using a total volume of 20µl containing 1 x Taq and Go (MP Biomedicals, Eschwege, Germany), 1.5 mM $MgCl_2$, 0.2 mM of each primer and 1µl of template DNA (95 °C, 5 min; 30 cycles of 95 °C, 30 s; 57°C, 30 s; 72°C, 90 s; and elongation at 72°C, 5 min). PCR product was sequenced with the Applied Biosystems 3130l Genetic Analyzer sequencer, Data Collection v3.0, Sequencing Analysis v5.2 (Foster City, USA) at the sequencing core facility ZMF. Obtained sequences were aligned with reference RNA sequences from National Center for Biotechnology Information (NCBI) data base.

Isolation and Identification of Soil and Root Pathogens: Soil and roots were collected from established *Salvia officinalis* field plots that had been grown in various desert areas and showing typical wilt symptoms from (El-Sheikh Zuweid (North Sinai); El-Maghara Experimental Station, Desert Research Center, (Middle Sinai); Matrouh (North Coastal Zone) and Nuweiba (South Sinai)) during seasons of 2012 and 2013. Segments of root systems and soil from rhizospher collected directly from the field were used.

Every year samples from 25 plants of *Salvia officinalis* with disease symptoms were taken at fifteen days intervals from (January to March). Root and stem bases sections of approximately 0.2g were added to 100 ml

Table 1: The most active bacterial strains and its origin

Bacterial isolates	Symbols	Source
Pseudomonas sp.	PsF	Fennel
Bacillus sp.	BF	Fennel
Stenotrophomonas sp.	SW	Wheat
Bacillus sp.	BS	Salvia
Bacillus sp.	BF	Fennel
Brevibacillus sp.	BrF	Fennel
Bacillus sp.	BR	Rosemary
Not identifeid	S	Solanum
Pseudomonas sp.	PsS	Solanum
Bacillus sp.	BB1	Barley
Bacillus sp.	BB2	Barley
Brevibacillus sp.	BrB	Barley
Brevibacillus sp.	BrC	Cucumber
Pseudomonas sp.	PsC	Cucumber

sterile water in flasks and shaken on a rotary shaker at 150 rpm for 30 min. Root segments, as well as a 10-fold dilution series of the resulting water suspensions, were plated on various general and selective media to recover root organisms. *Fusarium* spp. was recovered using Potato dextrose agar (PDA). For purification of fungal isolates, isolation and purification procedures were carried out according to the method described by Dhingra and Sinclair [26] using the Rose Bengal medium (Dextrose, 10 g, Peptone, 5 g, KH_2PO_4, 1.0 g, $MgSO_4$.7HO, 0.5 g, Rose Bengal 0.05 g, Agar,15 g in 1 liter of distilled water and pH 7.2±0.2) [27]. The resulted fungi were purified using the hyphal tips technique on Rose Bengal medium and then subculture of each isolated fungus on slant medium for future studies. The fungi were identified according to cultural characters described by Nelson *et al.* [28]. In this study, nine isolates of Fusarium (*F. oxysporum, F. solani* and *F. culmorum*) were isolated from *Salvia officinalis* as showed in Table (1).

Antagonistic Effect of Biocontrol Agents: For selection of bacteria on the basis of their ability to inhibit *in vitro* growth of tested Fusarium isolates, among 85 bacterial isolates, fourteen biocontrol agents recorded the maximum inhibition of mycelial growth of eight Fusarium isolates were selected for further investigation. The antagonistic effect of tested bio-control agents against mycelial growth of Fusarium isolates were investigated *in vitro* using dual culture technique [29, 30]. For testing the antagonistic effect of bacterial isolates, each of bacterial bio-control agents was streaked in center of sterile Petri dish on potato dextrose agar (PDA). One disc (0.5 cm in diameter) of 7 days- old culture of each pathogenic fungus was separately placed on each side of the same Petri dish at 10 mm distance. Petri dishes containing of 15 ml PDA medium inoculated with fungal cultures and free of bacteria were used separately as control. All plates were incubated at 28±2°C until the growth of each pathogenic fungus in the control treatment reached to the edge of Petri dish. This screening experiment was repeated using both live bacterial cells and bacterial metabolites. For preparation of bacterial metabolites, bacterial strains were grown at 25°C for about 48 h on nutrient medium and supernatant was collected by centrifugation at 10000 rpm for 15 minutes and the supernatants were used as metabolites. The reduction of pathogenic fungi mycelial growth were calculated according to the sizes of the inhibition zones (distance between fungal mycelium and bacterial colony); whereas, (1), 0.1 to 0.4 mm inhibition zone; (2), 0.5 - to 1cm inhibition zone; (3), 1.1 to 1.5 cm, inhibition zone. (4), more than 1.5 cm and blanks indicate no inhibition.

Assessment of Secondary Metabolites Production of Bioagents

HCN Detection: Qualitative cyanide determination was carried out using the method of Alstrom [31]. Isolates sub cultured on NA medium were supplemented with glycine (4/4 g/l). The production of cyanide was detected 48h after inoculation, using picrate /Na_2CO_3 paper fixed to the under side of the Petri-dish lids which were sealed with parafilm before incubation at 28°C. A change from yellow to orange, red, brown, or reddish brown was recorded as an indication of weak, moderate, or strongly cyanogenic potential, respectively.

Chitinase Detection: Colloidal chitin was prepared from the commercial chitin by the method of Mathivanan [32]. The colloidal chitin agar (CCA) medium of the following ingredients (in g/l): (NH_4SO_4, 7, K_2HPO_4, 1, NaCl, 1, $MgSO_4$ $5H_2O$ 0.1, yeast extract, 0.5) and amended with 0.5% colloidal chitin was prepared [33]. Chitinase detection was performed by spot inoculating bacterial isolates on CCA. After 5 days of incubation at room temperature, the zone of clearance due to chitin hydrolysis was recorded as positive chitinase- producing bacteria.

Siderophore Detection: Siderophore production was assayed by the plate method using Chrome Azurol S (CAS) described by Schwyn and Neilands [34]. For production of siderophores, 24 hours old bacterial culture was inoculated in iron free Succinate medium [35]. After 24 hrs incubation at 28°C with constant shaking at 120 rpm, media were centrifuged at 5000 rpm for 10 min;

the cell free supernatants were poured in the well of CAS plate. After incubation for 24 hrs at 28°C, formation of yellow orange color zone around the colonies in plate assay indicated the siderophore production.

Bacterial Inoculums Preparation for Plant Bacterization: Fresh cells were obtained from stock cultures stored at -80°C and grown in Nutrient broth medium at room temperature in a shaker. From this 100 ml of Nutrient broth in a 250 ml flask was inoculated and incubated for 48h at room temperature in a rotary shaker (100 round/min). The bacterial culture was centrifuged (10000 rpm for 10 min) and the supernatant was discarded. The cell pellet was resuspended in sterile 0.85% NaCl and centrifuged again under the same conditions. The supernatant was discarded and washed bacterial cells were resuspended in sterile distilled water (SDW). The concentration of cells in the suspension was spectrophotometricaly adjusted to 10^8CFU/ml and used for greenhouse pot experiments.

Greenhouse Experiments: Biocontrol Agents and Disease Assessment: This experiment was conducted to assess the pathogenic effect of Fusarium isolates, against *Salvia officinalis* seedlings under green-house conditions. One hundred ninety two plastic pots (30 cm - diameter) containing 5 kg of sterilized sandy soil were arranged in the green-house of Plant Protection Department, Desert Research Center, according to a completely randomized design. Four pots were used as replicates for each fungal isolate as well as the untreated control. This experiment was conducted ones using plant treatment with cell suspensions. For seedling soaking, 6 weeks old Sage seedlings were soaked with the suspension of bacterial cells (10^8cfu/ml) while control replicates were treated with water. After 24 hours, all transplants were transported into plastic pots (30 cm - diameter) under greenhouse conditions [36, 37]. For soil treatment, each fungal isolate was singly grown on sterilized Sorghum-Sand medium in conical flasks (500 ml) for two weeks at 28 ± 2°C. The soil was infested with 5g/kg soil of each fungus culture, separately. The infested soil was watered daily for 7 days to obtain the optimum fungal growth and distributing of the pathogenic fungal growth before planting. Roots of *Salvia officinalis* plantlets were sterilized by dipping in sodium hypochlorite solution (0.1%) for 2 min and then the roots were washed through serial sterilized distilled water before planting. Four plantlets were sown in each pot. The control pots were inoculated with the equal amount of uninoculated Sorghum medium [38]. Number of symptomatic leaves and dead plants were recorded for foliar wilt development ratings on plant using the scale of Gao *et al*. [39] as follows: 0 = no symptoms, 1 = <25%, 2= 25 to 50%, 3 = 51 to 75 %, 4 = 76 to 100% of leaves with symptoms and 5 = plant dead, The disease rating was calculated by the following formula:

$$\text{Desease index} = \frac{\Sigma(\text{rating no.x no. of plants in the rating})}{\text{Total no. of plants x highest rating}} \times 100$$

Length of vascular discoloration (cm) was determined as an internal symptoms based on scale of Szczech [40].

Statistical Analysis: Data were subjected to statistical analysis using the method described by Gomez and Gomez [41] with procedure "ANOVA". Treatment means were compared by Duncan's multiple Range Test at 0.05 level of probability.

RESULTS AND DISCUSSION

Bacterial Strains: Of the Eighty five bacterial strains tested and screened, the most active fourteen bacterial were preliminary identified by several morphological, physiological and biochemical tests to the genus level. As illustrated in Table 1 these bacterial isolates were identified as (*Bacillus* spp., *Pseudomonas* spp., *Brevibacillus* spp. and *Stenotrophomonas* spp.). Bacteria of diverse genera have been identified as PGPR or biocontrol agents, of which *Bacillus* and *Pseudomonas* spp. are predominant [42].

Fusarium Isolates and its Sources: Totally, 536 fungi isolates were obtained from four sage growing locations [El-Sheikh Zuweid (North Sainai); El-Maghar Experimental Station, Desert Research Center, (Middle Sinai); Matrouh (North coastal zone) and Nuweiba (South Sinai)] in two growing seasons (2012 and 2013). The highest numbers of cultures were obtained from Root and soil, while the less were from Stem bases (Table 2). Isolates obtained from second seasons were more than that obtained from the first one. Cultures from *Fusarium oxysporum* were most frequently compared with the others. The *Fusarium oxysporum* was isolated from root, soil and stem bases but *F. solani* was not isolated from stem bases in all samples obtained from the four locations. The isolates species constituted 73.3% of all fungi obtained from root and soil while 26.7 % isolated from Stem bases. *F. solani* and *F. oxysporum* were isolated from all tested locations.

Table 2: *Fusarium* spp. Isolated from the rhizosphere and salvia plants

			No of isolates/origin			
			2012/2013		2013/2014	
Fusarium spp.	Symbols	Location	Root and soil	Stem bases	Root and soil	Stem bases
Fusarium solani (Martius) Saccardo	Fs1	El-Sheikh Zweid	27	-	28	-
	Fs2	El-Maghara	23	-	25	-
	Fs3	Nuweiba	18	-	20	-
	Fs4	Matrouh	20	-	23	-
Fusarium oxysporum Schlechtendal	Fo1	El-Sheikh Zweid	30	22	32	25
	Fo2	El-Maghara	25	17	28	18
	Fo3	Nuweiba	20	12	24	15
	Fo4	Matrouh	23	16	27	18

The most frequently isolates were obtained from El-Sheikh Zuweid location (30.6%) and the lowest percentage was from Nuweiba (20.3%), while the other locations recorded (25.4% and 23.7% from El-Maghara and Matrouh, respectively (Table 2). These results are in line with those obtained by Zimowska [2] and Frużyńska-Jóźwiak and Andrzejak [43].

Selection of Bacteria for Ability to Inhibit *in Vitro* Growth of Fusarium Isolates: Most of the Eighty five bacterial strains inhibited *in vitro* growth of some *Fusarium* isolates belong to the genera *Bacillus* and *Pseudomonas*. These results are in agreement with Edwards *et al.* [44], who mentioned that, in most cases, the effective bacteria against fungal plant diseases belong to the genera *Bacillus, Pseudomonas* and *Streptomyces* spp. From the preliminary round of screening for antagonism in Petri plate assay, only fourteen bacterial isolates were selected to evaluate their inhibitory effects as live bacterial cells or as metabolites. Most of these strains were more effective as a bacterial metabolite than live cells except for the isolates (BF, BS and PsS) which were more effective as live cell. There were no difference with isolates (BR and PsC) in both of live bacterial cells or as metabolites (Table 3), Results from bioassays suggest that production of antifungal substances by these bacteria may be responsible for the inhibition of fungal isolates hyphal growth where there was no direct contact between bacterial colonies and mycelium of pathogenic fungi, so that the fungal growth inhibition was caused by diffusion of substances into the agar medium. On the other hand, most of bacteria that used as a biocontrol agents like *Bacillus* spp. produce antibiotics responsible for their antifungal activities. In addition, the PDA medium is rich in nutrients and thus competition between them might be excluded [3, 44-49].

Assessment of Secondary Metabolites Production of Bioagents: Detection of secondary metabolites of the bioagents tested in this study revealed that all of these bacterial isolates have the ability to produce at least one of the bioactive metabolite assayed. The ability of bacteria to produce these active metabolites may explain why they have suppression or inhibition activities against *Fusarium* spp. This is compatible with that of Gupta *et al.* [50], who found that bacteria applicability as biocontrol agents have great attention because of production of secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes and phytohormones. Regarding to the data presented in Table 4, there are distinct variations in the ability of bacterial isolates for metabolites secretion. Results showed that low percent of isolated bacteria were capable of producing HCN (about 35.7%) and that the HCN producing isolates were belonging only to the genera *Pseudomonas* and *Stenotrophomonas*. Defago and Haas [51] presented the evidence that HCN is beneficial to biological control of diseases; hence indirectly it plays a role in plant growth. Also, the volatile compound can easily degrade fungi cell wall [52]. For chitinase production, most of the bacterial agents (about 60%) represented with all genera used (*Bacillus, Brevibacillus Pseudomonas and Stenotrophomonas*) showed different degrees of enzyme production (Table 4). The chitinolytic enzymes are considered important in the biological control of soil-borne pathogens as it play a crucial role in hydrolyzing fungal cell walls [53, 54].

Concerning to siderophore production, all of bacterial bioagents except BB2 have the ability to produce siderophores which clarify its important role in antagonistic activity of the biocontrol agents. Tian *et al.* [55] showed that distribution of siderophore producing isolates according to amplified ribosomal DNA restriction

Table 3: Growth inhibition of different *Fusarium* spp. In response to some bacterial strains *in vitro.*

	Inhibition of hyphal growth of plant pathogens by bacterial isolates													
	PsF		BF		SW		BS		BF		BrF		BR	
Fungal isolates	c	m	c	m	c	m	c	m	c	m	c	m	c	m
Fs1	4*	4	3	3	4	4	0	1	3	4	2	4	2	4
Fs2	1	2	3	2	3	3	2	2	3	1	2	3	2	3
Fs3	1	3	4	2	2	4	3	4	2	2	4	3	4	3
Fs4	3	2	0	2	3	4	3	3	2	4	2	3	3	0
Fo1	0	3	3	3	0	3	3	2	2	3	0	3	3	4
Fo2	4	4	2	1	3	2	3	2	1	2	2	3	0	3
Fo3	0	1	1	2	1	2	2	0	1	2	2	3	2	1
Fo4	1	3	2	2	0	3	1	2	2	3	0	3	1	1
Mean	2.25	2.4	2.4	1.6	1.1	2.1	2.2	1.8	2.4	2.1	3.1	1.8	2.6	2

Table 3: Continued

	Inhibition of hyphal growth of plant pathogens by bacterial isolates													
	S		PsS		BB		BB2		BrB		BrC		PsC	
Fungal isolates	c	m	c	m	c	m	c	m	c	m	c	m	c	m
Fs1	2	1	2	3	3	2	1	3	3	4	1	3	4	3
Fs2	3	3	4	2	3	3	4	2	3	3	4	4	3	3
Fs3	2	3	2	0	2	2	2	3	2	3	0	4	2	4
Fs4	3	3	3	1	1	3	2	3	1	3	2	3	2	4
Fo1	1	2	2	0	1	3	4	1	2	3	0	4	2	1
Fo2	1	1	3	2	2	3	3	3	2	3	1	3	3	2
Fo3	1	2	1	0	1	1	1	1	1	2	0	3	2	1
Fo4	1	3	0	1	0	2	2	2	1	3	1	3	1	3
Mean	2	2	3	2	2	2.3	2.8	1.8	2.6	2.4	3.4	1.1	3.0	19

*figures refer to the area of the inhibition zones (distance between fungal mycelium and bacterial colony); (1), 0.1 to 0.4 mm inhibition zone; (2), 0.5 - to 1cm inhibition zone; (3), 1.1 to 1.5 cm, inhibition zone. (4), more than 1.5 cm, (0), indicate no inhibition.

- Fungal Isolates, Fs1, Fs2, Fs3 and Fs4, isolates of *F. solani* and Fo1, Fo2, Fo3 and Fo4, isolates of *F. oxysporum*.

- c: bacterial live cells, m: Bacterial metabolites"

Table 4: Assessment of secondary metababolites production of bioagents

	Bacterial strains													
Bacterial Secondary metabolites	PsF	BF	SW	BS	BF	BrF	BR	S	PsS	BB	BB2	BrB	BrC	PsC
HCN	+		++				+		++					+
Chitinase		+	+		+	++		++			+	+	++	+
Siderophore	++	++	++	+++	+	+	++	+	++	++		++	+++	+

For HCN: + yellow to orange, ++ red,+++ reddish brown

For chitinase: + 0.5 to 1 cm, ++ 1 to 1.5 cm, +++1.5-2cm inhibition zone of clearance

For siderophore: + 0.5to 1 cm, ++ 1to1.5 cm, +++1.5-2cm yellow orange color zone

analysis groups reveals that most of the biocontrol isolates produce siderophores belong to genera *Pseudomonas, Enterobacter, Bacillus* and *Rhodococcus* spp. All *S. maltophilia* isolates produced catechol-type siderophores, but hydroxamate-typesiderophores were not detected [56].

Molecular Identification of Bacterial Strains: The most active four isolates recorded the maximum inhibition of mycelial growth were identified using partial 16S rDNA sequence based molecular identification as: *Brevibacillus brevis* "strain NBRC 15304", *Brevibacillus formosus* "strain DSM 9885", *Brevibacillus agri* "strain DSM 6348" and *Stenotrophomonas maltophilia* "strain IAM 12423". Shida *et al*. [57] reclassified nine species of the genus *Bacillus,* i.e., *Bacillusbrevis, Bacillusagri* and *Bacillusformosus* and placed them within a new genus, *Brevibacillus*. Numerous *Brevibacillus* species have potential as biocontrol agents in agricultural production and some of these strains have become research hotspots [58].

Table 5: Effect of antagonistic bacterial strains against *Salvia officinalis* wilt disease incidence, under greenhouse conditions.

	Fusarium Isolates														
	Fo1			Fo2			Fo3			Fo4			Mean		
Bact. Strain	IRB (cm)	FWR	DI (%)	*IRB* (cm)	*FWR*	*DI* (%)	*IRB* (cm)	*FWR*	*DI* (%)	*IRB* (cm)	*FWR*	*DI* (%)	*IRB* (cm)	*FWR*	*DI* (%)
Brevibacillus brevis	0.68b	0.50e	12.20d	0.35c	0.50e	9.20e	1.18bc	0.70d	15.50de	0.89bc	0.80d	13.80d	0.78c	0.63e	12.68e
Brevibacillus agri	0.61b	1.20d	21.70c	0.55c	1.40d	14.50d	0.60e	1.40c	20.50d	0.82bc	1.70c	23.43c	0.65d	1.43d	20.03d
Stenotrophomonas maltophilia	1.32a	2.00c	33.80b	0.95b	2.30c	26.50c	1.43ab	3.50ab	53.10b	1.15ab	2.50b	34.87b	1.21b	2.58c	37.07b
Brevibacillus formosus	1.35a	2.90b	35.50b	1.13b	2.70b	32.80b	0.94cd	3.13b	27.47c	0.97bc	3.10b	37.10b	1.09b	2.96b	33.22c
mix.	0.40b	0.30d	10.07d	0.31c	0.30e	7.90e	0.85de	0.50d	11.10e	0.62c	0.40d	9.80d	0.55d	0.38f	9.72f
Cont.	1.70a	3.50a	63.00a	1.80a	3.80a	55.00a	1.50a	3.80a	60.00a	1.50a	4.00a	57.00a	1.63a	3.78a	58.75a

IRB; Internal Root Browning (Length of vascular discoloration) and DI; Disease Incidence

FWR; foliar wilt rating: development ratings on plant using the scale of Gao *et al*. [39] as follows: 0 = no symptoms, 1 = < 25%, 2= 25 to 50%, 3 = 51 to 75 %, 4 = 76 to 100% of leaves with symptoms and 5 = plant dead.

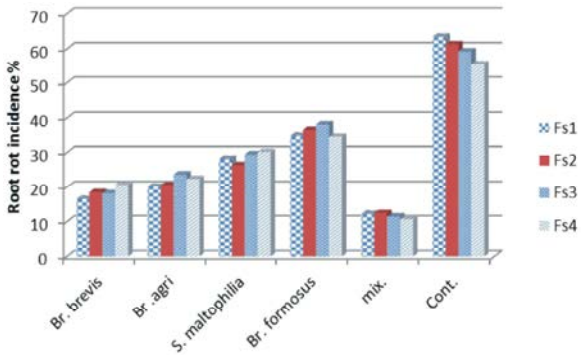

Fig. 1: Effect of antagonistic bacterial strains on root rot incidence of salvia plants under greenhouse conditions

Plant Protection Ability of Biological Control Agents under Greenhouse Conditions: According to the results obtained from *in vitro* screening and bioassay experiments, the most antagonistic bacterial strains "*Brevibacillus brevis, Brevibacillus formosus, Brevibacillus agri* and *Stenotrophomonas maltophilia"* were selected for greenhouse pot experiments and evaluated for their ability for controlling wilt and root rot diseases of *Salvia officinalis* as seedling soaking treatment, under green house conditions. Effect of antagonistic bacterial strains in suppression of *Salvia officinalis* wilt disease control were recorded (Table 5). *Brevibacillus brevis* and Mixture of four strains significantly reduced wilt disease incidence in soil infested with four isolates of *F. oxysporum*. The disease incidence were reduced to 12.7%, 20.2%, 37.1%, 33.3% and 9.7% due to bacterial strains (*Brevibacillus brevis, Brevibacillus agri, Stenotrophomonas maltophilia, Brevibacillus formosus* and Mix.), respectively compared with control (58.8%). Meanwhile, foliar wilt rating were reduced to (0.63, 1.43, 2.58, 2.95 and 0.38) compared with control (3.78) as well as the internal root browning rating of sage seedlings was reduced also into (0.77, 0.65, 1.21, 1.09, 0.55) compared to control (1.63) for the same manner as showed in Table 5. Sangita and Shah [59] showed that *Brevibacillus brevis* has significant potential as a gramicidin-producing biocontrol agent against *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani, Colletotrichum acutatum* and *Fusarium oxysporum*. From the conducted experiments under greenhouse conditions to detect the role of bacterial strains in root rot reduction, all tested isolates of *F. solani* were affected and the root rot incidence reduced. The mixture of bacterial metabolites recorded the highest significant lowest root rot value (59.90%) with all pathogens which followed by the most effective strain (*Brevibacillus brevis*) by rate (18.48%) compared with all other bacterial strains *Brevibacillus agri, Stenotrophomonas maltophilia* and *Brevibacillus formosus* which caused reduction by 21.78, 28.58, 36.0%, respectively (Fig. 1). Sunita *et al.* [60] showed that development of symptoms on glasshouse-raised tomato plants was markedly reduced in co-inoculations of *F. oxysporum* with *B. brevis*, compared to inoculations with the pathogen alone.

CONCLUSION

In the present study, Rhizo-bacterial strains namely *Brevibacillus brevis Brevibacillus agri, Stenotrophomonas maltophilia* and *Brevibacillus formosus* found to be highly effective in suppression of *Salvia officinalis* wilt and root rot diseases caused by different isolates of *F. oxysporum* and *F. solani* when applied as seedlings treatment. This may be attributed to the production of several inhibitory metabolites like HCN, chitinase and siderophore. However the further studies about their application in natural field condition are necessary to be applied in the future.

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