Evaluation of *Streptomyces* spp. against *Fusarium oxysporum* f. sp. *ciceris* for the management of chickpea wilt

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**Abstract:** In this study, about 112 isolates of *Streptomyces* were isolated from chickpea rhizospheric soils. Among the isolated strains, five showed strong inhibitory effects against chickpea Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* in vitro using plate assay and selected for further studies. The selected strains were identified as *Streptomyces* spp. based on morphological and biochemical characterization as well as 16S rDNA sequences analysis. Our results assigned them to genus of *Streptomyces*. In vitro, antagonistic effects of *Streptomyces* strains against the disease were evaluated through the dual-culture method, volatile and non-volatile metabolites, siderophore, protease and chitinase production. All bacterial strains inhibited mycelial growth of the pathogen ranging from 26 to 44.2% in dual culture assay. The non-volatile extract of five of the *Streptomyces* strains inhibited more than 50% growth of the pathogen, whereas volatile compounds were less effective on mycelial growth inhibition (20.2 to 33.4%). The ability of the biocontrol agents to produce siderophore and protease were varied, whereas, production of chitinase was detected for all strains. Results of the greenhouse assay indicated that all biocontrol agents reduced disease severity (ranging from 38.7 to 54.8%). Accordingly, strain KS62 showed higher control efficacy (54.8%). In addition, the biomass of chickpea plants (plant height and dry weight) significantly increased in plants treated with *Streptomyces* strains compared to non-bacterized control. The results of this study showed that it may be possible to manage chickpea Fusarium wilt disease effectively by using *Streptomyces* species, as biocontrol agents. Therefore, evaluating their efficiency under field conditions is needed.

**Key words:** biocontrol, chickpea, *Fusarium*, *Streptomyces*, 16S rDNA

**Introduction**

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato, is the most important disease in chickpea throughout the world, and it is the main yield-limiting factor in chickpea production in Iran (Navas-Cortés *et al.* 1998). Fungi survive saprophytically on crop debris in the soil. The pathogen infect the roots of a susceptible host, can colonize the vascular system of plants, produces toxins and kills plants by blocking xylem vessels that restricts water transport (Anjaiah *et al.* 2003). The uses of resistant cultivars are not always available or effective, because new races of the pathogen have appeared that overcome resistance in currently grown cultivars (Navas-Cortés *et al.* 1998). Furthermore, application of fungicides does not always prove economic against soil borne pathogens and it has led to environmental pollution, pathogen resistance, and increased risk to human and animal health (Li *et al.* 2012; On *et al.* 2015). In addition, excessive use of fungicides creates an imbalance in the microbial community in soil (On *et al.* 2015). The use of microbial biocontrol agents is an alternative to fungicides for the management of plant disease because biological control is one of the most environmentally viable and health-friendly approaches for replacing fungicides in management programmes to control fungal pathogens (Li *et al.* 2012; Jiménez-Díaz *et al.* 2015). Numerous biocontrol agents have been reported to control Fusarium wilt of chickpea, such as *Pseudomonas fluorescens* (Anjaiah *et al.* 2003), *Trichoderma* species (Dubey *et al.* 2007; Moradi *et al.* 2012) and *Bacillus subtilis* (Moradi *et al.* 2012). Different mechanisms have been implicated in the suppression of fungal root disease by biocontrol agents, such as the competition for nutrients and the production of biocontrol metabolites (extracellular antibiotics, volatile substances, siderophores, lytic enzymes, hydrogen cyanide, etc.) (Das *et al.* 2008; Naureen *et al.* 2009; Erdogan and Benlioglu 2010).

Species of *Streptomyces* have been evaluated against plant pathogens and have exhibited great potential in suppressing plant diseases caused by fungal pathogens (Li *et al.* 2012; Sadeghi *et al.* 2012; Elango *et al.* 2015). Some isolates of *Streptomyces* can produce compounds with antifungal activity for plant protection (Tanaka and Omura 1993). Hence, they have been selected as efficient antagonists against soil-borne fungal pathogens (Sabaratnam and Traquair 2002).

The objective of this research was to isolate, characterize and evaluate the biocontrol potential of *Streptomy-
ces strains in chickpea Fusarium wilt causing *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* and greenhouse conditions.

**Materials and Methods**

**Pathogen and inoculum preparation**

Wilt-infected plants of chickpea were collected from the major chickpea-growing region of Kurdistan Province, Iran. The pathogen, *Fusarium oxysporum* f. sp. *ciceris* (FOC) was isolated from infected tissues and cultured on Potato Dextrose Agar (PDA) medium. Fungi identification was based on morphological characteristics and microscopic observation on PDA and Carnation Leaf Agar (CLA) (Leslie and Summerell 2006). A pathogenicity test for the pathogen was carried out under artificially inoculated conditions. Then, the pathogen was re-isolated from the diseased tissues of chickpea. The fungus was grown on PDA medium for 7 days in Petri dishes at 25–27°C for mycelial production. The microconidial inoculum of FOC was produced in flasks (250 ml) containing 150 ml of sterile Potato Dextrose Broth (PDB) medium. Each flask was inoculated with 7-days-old culture on PDA. The flasks were shaken at 150 rpm at 25–27°C for 7 days. Next, the culture was filtered through sterile glass wool to collect spores to be used as inoculum. The concentration of microconidia was adjusted to 1 × 10^6 spores · ml⁻¹.

**Bacterial isolates**

*Streptomyces* isolates were isolated from the rhizosphere of healthy chickpea plants using the dilution plate technique on Starch Casein Agar (SCA) medium (g · l⁻¹: starch – 10, casein – 0.3, CaCO₃ – 0.02, FeSO₄ – 0.01, K₂HPO₄ – 2, MgSO₄ – 0.05, NaCl – 2, Nystatin – 0.02 and agar – 15, pH 7.0±0.2) (Don et al. 1993; Dhanasekaran et al. 2005). For bacterial isolation, 5 g of rhizosphere soil of chickpea plants were suspended in 45 ml of sterile distilled water. For bacterial isolation, 5 g of rhizosphere soil of chickpea plants were suspended in 45 ml of sterile distilled water. After 48 h a 5 mm diameter of plug from the leader of the Petri dishes (9 cm diameter) containing PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The antifungal activity of the selected bacterial strains was assessed against FOC by dual culture method in Petri dishes on PDA media. The bacterial strains (3-days-old culture) were streaked on one side of the Petri dishes (9 cm diameter) containing PDA media. After 48 h a 5 mm diameter of plug from the leading edge of a 5-days-old fungal culture (FOC) was placed in the center of each Petri dish. Plates with FOC only were used as control. The Petri dishes were incubated at 28°C for 7 days (16 h light : 8 h dark). The percentage of growth

**Screening for antagonistic bacterial isolates *in vitro***

The assessment of the inhibitory effects of all the isolated bacteria on the chickpea Fusarium wilt was performed using plate assay (four spots per plates) as described by Weller and Cook (1985). Four different isolates of bacteria were spotted on the edge of PDA plates. Each plate was inoculated with four droplets of 20 µl bacterial suspension (10⁸ cfu · ml⁻¹). The droplets were placed on four sites at equal distances from the center of plates. After 24 h incubation at 28°C, a single 5 mm-diameter plug of the pathogen from dilute PDA was placed in the center. After five days, the radius of each fungus colony was measured and zones of inhibition were calculated. Antagonism isolates showing the greatest inhibition by this test were selected for further studies.

**Phenotypic and molecular characterization of selected strains**

Morphological and biochemical properties of bacterial strains were studied according to Shirling and Gottlieb (1966) and Schaad et al. (2001). Molecular characterization based on DNA sequencing of 16S rRNA gene was used for phylogenetic studies. Genomic DNA was extracted from pure cultures using the genomic DNA extraction kit from gram positive bacteria (QIAGEN) based on the manufacturer’s instructions. Nearly full-length 16S RNA gene sequences in target-specific screening strains were amplified with the universal primers RP1 (5’-AGAGTTTGATCAGGCTGCTACGG-3’) and FD2 (5’-AGGTTATCCTGGTACGGAC-3’) (Weisburg et al. 1991). Polymerase Chain Reaction (PCR) was carried out in a total volume of 50 µl. Reaction mixture containing 25 µl of Taq master mix (Genet Bio, Seoul, South Korea), 2 µl of each of forward and reverse primers (10 µM stock), 2 µl of template DNA (50 ng · µl⁻¹) and 19 µl of PCR grade pure water. The reaction mixture contained for 4 min at 95°C and then amplification was performed in 35 cycles. For each cycle, DNA was denatured at 94°C for 1 min, annealed at 51°C for 1 min, and then extended at 72°C for 2 min. The final extension was at 72°C for 10 min. PCR products were purified using the QIAGEN PCR purification kit. PCR-purified 16S rRNA genes of the five isolates were partially sequenced in both directions using the same two primers. The results obtained via sequencing were compared with other related taxa in the GenBank databases using the NCBI BLAST (www.ncbi.nlm.nih.gov). The nucleotide sequences were aligned with multiple sequence alignment program CLUSTALW (Thompson et al. 1994). The phylogenetic tree was constructed by using MEGA version 6.0 software based on the neighbour joining method and Kimura two-parameter distance with 100 bootstrap replicates (Tamura et al. 2013).

**In vitro antagonism assessment**

**Dual culture method**

The antifungal activity of the selected *Streptomyces* strains was assessed against FOC by dual culture method in Petri dishes on PDA media (Erdogan and Benlioglu 2010). The bacterial strains (3-days-old culture) were streaked on one side of the Petri dishes (9 cm diameter) containing PDA media. After 48 h a 5 mm diameter of plug from the leading edge of a 5-days-old fungal culture (FOC) was placed in the center of each Petri dish. Plates with FOC only were used as control. The Petri dishes were incubated at 28°C for 7 days (16 h light : 8 h dark). The percentage of growth
of FeCl$_3$ was prepared. Then, 100 µl of bacterial strains were suspended in medium B Agar (KB) containing 0, 100 and 1,000 mM FeCl$_3$ as described by Weller and Cook (1983). Firstly, King's medium B Agar was placed at the center of a Petri dish (90 mm diameter) containing PDA media, and a 5 mm disk of a 5-days-old FOC was placed at the center of another Petri dish containing PDA. Both half Petri dishes were placed face to face preventing any physical contact between the pathogen and the bacterial suspension. The pairs of each Petri dish were sealed together with parafilm. Bacterial suspension was replaced with sterile water in the control Petri dish. All Petri dishes were incubated at 28°C for 7 days (Naureen et al. 2009). Then, the percentage of growth inhibition was measured as mentioned above.

**Effect of volatile inhibitors**

To study the effects of volatile antibiotic on radial growth of FOC, 100 µl of antagonistic bacterial suspension (1 × 10$^6$ cfu · ml$^{-1}$, 3-days-old culture) was placed at the center of a Petri dish (90 mm diameter) containing PDA media, and a 5 mm disk of a 5-days-old FOC was placed at the center of another Petri dish containing PDA. Both half Petri dishes were placed face to face preventing any physical contact between the pathogen and the bacterial suspension. The pairs of each Petri dish were sealed together with parafilm. Bacterial suspension was replaced with sterile water in the control Petri dish. All Petri dishes were incubated at 28°C for 7 days (Naureen et al. 2009). Then, the percentage of growth inhibition was measured as mentioned above.

**Effect of non-volatile inhibitors**

PDA media Petri dishes, covered with a 0.2 µm cellophane membrane, were inoculated in the center with 200 µl of Streptomyces strains suspension (1 × 10$^6$ cfu · ml$^{-1}$, 3-days-old culture). Petri dishes were incubated at 28°C for 7 days. After 72 h, the membrane with the growth bacterial isolates was removed and a 5 mm disk of a pure culture of FOC (5-days-old) was placed in the center of the Petri dishes. Bacterial suspension was replaced with sterile water in control Petri dishes. Inhibition percentages were determined using the method previously described (Naureen et al. 2009).

**Siderophore production**

This experiment was undertaken based on the method described by Weller and Cook (1983). Firstly, King's medium B Agar (KB) containing 0, 100 and 1,000 mM of FeCl$_3$ was prepared. Then, 100 µl of bacterial strains were cultured on the surface of media and plates were incubated at 28°C for 5 days. Next, 0.5 ml suspension of Geotrichum candidum (1 × 10$^6$ cfu · ml$^{-1}$) was sprayed on the surface of the media and incubated at 28°C for 72 h. Clear zones surrounding the bacterial strains colonies suggested siderophore production by biocontrol agents that caused inhibition of mycelial growth of G. candidum.

**Protease production**

Bacterial strains were tested for production of protease by growing them on Skim Milk Agar (SKM) (Chantawannakul et al. 2002). Plates were incubated at 28°C for 24 h. An ability to clear the SKM suspension in the agar was taken as evidence of the secretion of protease. Non-bacteria inoculated plates were used as the control.

**Chitinase production**

To assay the production of chitinase, Streptomyces strains were grown on medium containing chitin (0.4%), KH$_2$PO$_4$ (0.3 g), K$_2$HPO$_4$ (0.7 g), MgSO$_4$ · 7H$_2$O (0.5 g), FeSO$_4$ · 7H$_2$O (0.01 g), ZnSO$_4$ · 7H$_2$O (0.001 g), MnCl$_2$ · 4H$_2$O (0.001 g) and Agar (20 g · l$^{-1}$), (pH was adjusted to 8.0–8.5). Petri dishes were incubated at 28°C for 7 days. Clear zones surrounding the bacterial colonies suggested chitinase activity on colloidal chitin agar plates (Hus and Lockwood 1975). All experiments were repeated twice with four replicates per treatment.

**Greenhouse studies**

Biocontrol efficacy tests were conducted in the greenhouse in pots (20 cm in diameter and 15 cm in height) with a pasteurized sand-peat-perlite mix (2 : 2 : 1, v/v/v). Chickpea seeds (cv. Kaka) were surface-sterilized with 2% sodium hypochlorite for 3 min, washed twice with sterile water and soaked in 30 ml suspension (1 × 10$^6$ cfu · ml$^{-1}$) of bacterial-methylcellulose for 30 min. Then the treated seeds were dried under a laminar flow hood and sown in sterile soil in pots infested with the FOC (30 ml of 10$^6$ spores · ml$^{-1}$ added to each pots before planting). For the control tests, seeds were soaked in sterile water-methylcellulose instead of the bacterial spore suspension. All pots were transferred to a greenhouse and incubated at 27±2°C with a 16-h light : 8-h dark cycle and 60–70% relative humidity. Three seeds were sown in each pot and the treatments were replicated five times in a completely randomized design. Plants were watered weekly with sterile tap water and fertilizer solution (NPK 1 : 1 : 1) was added to pots twice a week at a rate of 3 g · l$^{-1}$.

Disease severity was recorded for each plant 30 days after inoculation by a visual 0–4 scale according to the percentage of chlorosis, necrosis and wilt, as follows: 0 = healthy, 1 = 1–33%, 2 = 33.1–66%, 3 = 66.1–100%, 4 = dead plant (Campbell and Madden 1990). Disease incidence assessments were calculated using the method reported by Cao et al. (2011) with the following formula:

\[
\text{Disease incidence} \% = \left(\frac{\text{number of infected plants}}{\text{total number of plants}}\right) \times 100.
\]

Biocontrol efficacy was measured using the following formulas:

\[
\text{Control efficacy} \% = \left(\frac{\text{disease index of control}}{\text{disease index of treated}}\right) \times 100.
\]

At the end of the experiments, effects of bacterial strains on shoot length (cm), root length (cm), dry weight of shoot and root (g) were determined for each plant. The experiment was repeated twice.

**Statistical analysis**

Experiments in vitro and greenhouse conditions were designed as a completely randomized design (CRD). All analyses were performed using SPSS (Statistical Product and Service Solutions) versions 12.0. The means were compared by Duncan's Multiple Range Test (DMRT) at p ≤ 0.05.
Results

Pathogenicity test

Among ten isolates of *F. oxysporum* f. sp. *ciceris*, isolate number one was found to be significantly more pathogenic in chickpea (cv. Kaka) as all five plants from each replicate were diseased and symptoms of wilting, yellowing and discoloration of the vascular tissue were observed on diseased plants. The diseased index for infected plants in the F1 isolate was expressed as 3 which represent 66% wilt. Therefore, the F1 isolate was selected and used in this research. Isolates number 5 was a weak pathogen and showed significantly lower pathogenicity than all the other isolates (Table 1). The pathogen was re-isolated from the infected plants.

In vitro antifungal activity

A total of 112 bacterial strains were isolated from the rhizosphere of healthy chickpea plants according to the isolation procedures described in material and methods. Of the 112 bacterial isolates, five (KS31, KS55, KS58, KS62 and KS112) were selected for further studies according to their high efficiency in *in vitro* antagonism (with inhibition zones > 25%) using the plate assay on PDA media (data not shown).

Identification of selected strains

Identification of five of the selected strains, designated KS62, KS55, KS112, KS58 and KS31 was based on the morphological, cultural and biochemical characteristics, as well as molecular phylogenetic analysis. Results showed that all strains produced chalky/white colonies on yeast extract malt extract agar (ISP2) plates. Spore masses in four of the five strains were gray with linear spore chain. The strain KS62 had a white spore mass color with spiral chain. The strains were strictly aerobic, Gram-positive, catalase positive and they were all able to hydrolyze starch and casein. They were negative for H2S test, liquefaction of gelatine and nitrate reduction test. The isolates were characterized by 16S rDNA sequencing and phylogenetic analysis. After sequencing DNA, the resulting sequences were submitted as BLAST queries to GenBank. BLAST search results indicated that related strains have relatively high homology (97–99%) with members of the *Streptomyces* genus.

To determine phylogenetic relationships between isolated strains and other related *Streptomyces* spp. that shared the highest sequence similarities, a phylogenetic tree was constructed (Fig. 1). The 16S rDNA sequences from strains KS62, KS55, KS112, KS58 and KS31 were deposited to Gene Bank databases under the accession numbers of KT358975, KT358977, KT358974, KT358978 and KT358976, respectively.

Dual culture assay

All of the five selected bacterial strains showed antifungal activities in dual culture assay on PDA medium. Strain KS62 had maximum inhibitory effect (44.2%), followed by KS31 (44%) and KS112 (36.7%) on PDA medium. Strain KS58 had minimum inhibitory effect (26%) (Table 2).

Effect of volatile compounds

Out of the five antagonistic bacterial strains, three strains (KS55, KS58 and KS112) were found to inhibit growth of FOC and gave maximum inhibition 33.4%, 32.2% and 27.7%, respectively. The other bacterial strains (KS62 and KS31) were weak for inhibition growth of the pathogen and reduced the radial growth of the pathogen which ranged from 20.2% to 23.4% (Table 2).

Effect of non-volatile compounds

The evaluation of antagonistic activity of five strains of *Streptomyces* against FOC showed that four strains inhibited more than 50% growth of FOC. The maximum percent inhibition of growth of FOC was observed by strains KS62 (61.1%) followed by strain numbers KS112, KS55 and KS31 (ranging from 58.1% to 50.4) (Table 2).

Table 1. Pathogenicity of *Fusarium oxysporum* f. sp. *ciceris* isolates under greenhouse conditions

<table>
<thead>
<tr>
<th>Isolates of pathogen</th>
<th>Disease severity</th>
<th>Disease incidence [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>3.00 a</td>
<td>100.0 a</td>
</tr>
<tr>
<td>F2</td>
<td>2.58 a</td>
<td>100.0 a</td>
</tr>
<tr>
<td>F3</td>
<td>1.33 bc</td>
<td>91.5 ab</td>
</tr>
<tr>
<td>F4</td>
<td>1.66 b</td>
<td>100.0 a</td>
</tr>
<tr>
<td>F5</td>
<td>1.00 c</td>
<td>91.5 b</td>
</tr>
<tr>
<td>F6</td>
<td>1.41 bc</td>
<td>91.5 ab</td>
</tr>
<tr>
<td>F7</td>
<td>1.58 b</td>
<td>91.5 ab</td>
</tr>
<tr>
<td>F8</td>
<td>1.66 b</td>
<td>100.0 a</td>
</tr>
<tr>
<td>F9</td>
<td>1.25 bc</td>
<td>91.5 ab</td>
</tr>
<tr>
<td>F10</td>
<td>1.25 bc</td>
<td>100.0 a</td>
</tr>
<tr>
<td>Control water</td>
<td>0 d</td>
<td>0 c</td>
</tr>
</tbody>
</table>

Different letters within columns indicate significant differences at p ≤ 0.05, according to Duncan multiple-ranges test (DMRT). Data are means of five replicates.
Biocontrol of chickpea Fusarium wilt by *Streptomyces* strains

Siderophore, protease and chitinase production by *Streptomyces* strains

Strains KS55, KS58, KS112 and KS31 produced siderophores in media containing 100 µM FeCl₃. Two of the five *Streptomyces* strains, KS55 and KS58 produced protease. All of the five selected strains produced chitinase in media containing chitin 4% and produced a clear halo zone on media (Table 2).

**Table 2.** In vitro activity of biocontrol agents against *Fusarium oxysporum* f. sp. *ciceris* and production of antimicrobial metabolites

<table>
<thead>
<tr>
<th>The bacterial strains</th>
<th>DC% ± SD</th>
<th>Va% ± SD</th>
<th>NVa% ± SD</th>
<th>Siderophore</th>
<th>Protease</th>
<th>Chitinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS31</td>
<td>44±0.16a</td>
<td>23.4±0.08c</td>
<td>50.4±0.12d</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>KS55</td>
<td>28.4±0.14c</td>
<td>33.4±0.11a</td>
<td>57.4±0.21c</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>KS58</td>
<td>26±0.15d</td>
<td>32.2±0.08a</td>
<td>36.3±0.14e</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>KS62</td>
<td>44.2±0.11a</td>
<td>20.2±0.12d</td>
<td>61.1±0.14a</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>KS112</td>
<td>36.7±0.08b</td>
<td>27.7±0.09 b</td>
<td>58.1±0.17b</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

Different letters within columns indicate significant differences at p ≤ 0.05, according to Duncan multiple-ranges test (DMRT). Data are means of four replicates (“±” = standard deviation): DC = percent growth inhibition in dual culture method, Va = percent growth inhibition in volatile antibiotics, NVa = percent growth inhibition in non-volatile antibiotic, “+” = positive, “−” = negative

**Greenhouse studies**

All treatments significantly decreased disease severity and increased control efficacy compared to untreated control (pathogen alone) (Table 3). Fifty days after treatment, strains KS62, KS112 and KS31 significantly (p ≤ 0.05) reduced disease severity by 54.8%, 51.6% and 48.4%, respectively. Strains KS55 and KS58 had the least effect on disease severity 45.1% and 38.7%, respectively (Table 3). All
The present study was conducted to evaluate the efficacy of *Streptomyces* strains against chickpea Fusarium wilt. In this study, all bacterial isolates were selected from the rhizosphere of healthy chickpea plants at different locations of chickpea-growing regions. The use of microorganisms isolated from the rhizosphere of a specific crop might be better adapted to that crop. Hence, it may provide better control of diseases than organisms that are isolated from other plant species (Lucy et al. 2004). Of the 112 isolates, five isolates were selected according to their high efficiency in the plate assay. These results have been demonstrated in previous research (Tjamos et al. 2004; Zheng et al. 2011).

The sequence analysis of 16S rDNA region of selected isolates showed that all of the five strains belonged to the *Streptomyces* genus. *In vitro*, selected bacterial strains showed different degrees of antagonism. In dual-culture assay, the inhibition zone values of selected strains ranged from 26 to 44.2%. The *Streptomyces* strains inhibited the growth of pathogen significantly (more than 50%) by the production of non-volatile compounds (p ≤ 0.05). This result showed that non-volatile substances produced by *Streptomyces* strains were more inhibitory to *Fusarium oxysporum* f. sp. *ciceris* than production of volatile substances (Table 2). The inhibitory effects of volatile and non-volatile compounds on different soil borne fungal pathogens were observed (Sabaratnam and Traquair 2002; Elango et al. 2015). The results of the present study demonstrate that non-volatile substances produced by *Streptomyces* strains have a significant effect on the mycelial growth of pathogen. In other studies, the effects of these compounds on different soil borne fungal pathogen of chickpea have also been observed (Abo-Elyousr and Mohamed 2009). Non-volatile antifungal substances produced by *Streptomyces globisporus* JK-1 can inhibit the growth of *Magnaporthe oryzae* causing rice blast of rice (Li et al. 2011). Production of chitinase was observed in all selected *Streptomyces* strains. Production of non-volatile enzymes, such as chitinase by *Trichoderma harzianum* and *Talaromyces flavus* against a wide range of fungal species of *Aspergillus*, *Rhitizopus*, *Moucor* and *Sclerotinia* have been reported (Menendez and Godeas 1998; Nampoothiri et al. 2002). Moreover, chitinase production by *Trichoderma harzianum* and *T. flavus* was effective for controlling soybean stem white rot and bean stem rot diseases caused by *Sclerotinia sclerotiorum* and *S. rolfsii*, respectively (Madi et al. 1997; Menendez and Godeas 1998). Chitinase inhibited the fungal growth through damaging cell walls (Xiao et al. 2002).

### Table 3. Effect of *Streptomyces* strains on *Fusarium oxysporum* f. sp. *ciceris* under greenhouse conditions after 50 days

<table>
<thead>
<tr>
<th>The bacterial strains</th>
<th>Disease severity</th>
<th>Control efficacy [%]</th>
<th>Disease incidence [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS31</td>
<td>1.6±0.07 cd</td>
<td>48.4</td>
<td>100</td>
</tr>
<tr>
<td>KS55</td>
<td>1.7±0.16 d</td>
<td>45.1</td>
<td>100</td>
</tr>
<tr>
<td>KS58</td>
<td>1.9±0.15 e</td>
<td>38.7</td>
<td>100</td>
</tr>
<tr>
<td>KS62</td>
<td>1.4±0.10 b</td>
<td>54.8</td>
<td>100</td>
</tr>
<tr>
<td>KS112</td>
<td>1.5±0.07 bc</td>
<td>51.6</td>
<td>100</td>
</tr>
<tr>
<td>Non infested control</td>
<td>±0.00 a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control pathogen</td>
<td>3.1±0.10 f</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Different letters within columns indicate significant differences at p ≤ 0.05, according to Duncan multiple-ranges test (DMRT). Data are means of five replicates (“±” = standard deviation).

### Table 4. The effects of *Streptomyces* strains on growth parameters of chickpea plants in seed treatments under greenhouse conditions

<table>
<thead>
<tr>
<th>The bacterial strains</th>
<th>Shoot length [cm]</th>
<th>Root length [cm]</th>
<th>Shoot dry weight [g]</th>
<th>Root dry weight [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS31</td>
<td>32.41±0.14 b</td>
<td>15.31±0.10 c</td>
<td>0.26±0.01 a</td>
<td>0.18±0.01 b</td>
</tr>
<tr>
<td>KS55</td>
<td>33.23±0.15 c</td>
<td>15.31±0.09 c</td>
<td>0.31±0.04 b</td>
<td>0.18±0.02 b</td>
</tr>
<tr>
<td>KS58</td>
<td>30.1±1.42 a</td>
<td>13.5±0.20 a</td>
<td>0.24±0.02 a</td>
<td>0.12±0.01 a</td>
</tr>
<tr>
<td>KS62</td>
<td>32.45±0.19 b</td>
<td>14.38±0.18 b</td>
<td>0.31±0.04 b</td>
<td>0.21±0.01 c</td>
</tr>
<tr>
<td>KS112</td>
<td>34.98±0.19 d</td>
<td>17.65±0.25 d</td>
<td>0.37±0.01 c</td>
<td>0.21±0.01 c</td>
</tr>
<tr>
<td>Control</td>
<td>30.18±0.21 a</td>
<td>13.55±0.15 a</td>
<td>0.25±0.01 a</td>
<td>0.13±0.01 a</td>
</tr>
</tbody>
</table>

Different letters within columns indicate significant differences at p ≤ 0.05, according to Duncan multiple-ranges test (DMRT). Data are means of five replicates (“±” = standard deviation).
Four strains produced siderophore and two strains produced protease.

Streptomyces strains can protect plants from pathogens by different mechanisms, including antibiosis (Smith et al., 1990), competition for space and nutrients and (Tanaka and Omura, 1993), like dimethyl disulfide, dimethyl trisulfide, acetophenone and 2-methylisoborneol (Li et al., 2010), competition for iron through production of siderophores (Sadeghi et al., 2012), induction of systemic resistance (Tarkka et al., 2008), production of extracellular enzymes such as chitinase (Srividya et al., 2012) and protease that can lyse cell walls of pathogens (Xiao et al., 2002; Srividya et al., 2012), and parasitism (Loliam et al., 2013).

Our study indicated that Streptomyces strains applied as seed treatment significantly reduced disease severity of chickpea Fusarium wilt caused by F. oxysporum f. sp. ciceris under greenhouse conditions compared to control (38.7 to 54.8%). Strains S62 and S512 showed the highest control efficacy 54.8 and 51.6%, respectively. Previous studies have illustrated that Streptomyces spp. effectively suppressed red root-rot disease of tea plants and red rust disease caused by Poria hypolateritia and Cephaleuros parasitica, respectively (Elango et al., 2015). Of the five Streptomyces strains tested in the greenhouse, the highest degree of increment of plant biomass was observed by strains Streptomyces KS112 and KS55 (Table 4). Moreover, the Streptomyces strains significantly increased plant height and dry matter compared to the untreated control (Gopalakrishnan et al., 2013). Thus, these biocontrol agents have the capability to enhance the growth of plants by producing plant growth regulator hormones including cytokinins, gibberellins and auxins (Ajay et al., 2004).

Conclusions

Our findings demonstrated that Streptomyces spp. have great potential to be used as biocontrol agents for management of F. oxysporum f. sp. ciceris causing chickpea Fusarium wilt as well as many other fungi pathogens that have been previously reported (Wan et al., 2008; Li et al., 2010; Elango et al., 2015). These biocontrol agents not only reduced the disease severity but also increased growth parameters of chickpea plant. Therefore, further study is needed to verify their effectiveness under different field conditions.

Acknowledgements

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References


