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Efficacy of antifungal substances of three *Streptomyces* spp. against different plant pathogenic fungi

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Abstract

Background: Soil-borne plant pathogenic fungi with a wide host range of crops cause a significant limitation on the global production of agronomic crops. Applications of synthetic pesticides are an important tool for managing plant diseases, but have deleterious influences on the environment as well as its incompatibility with organic agriculture. Recently, *Streptomyces* spp. became one of the best bio-control agents as a promising environmentally eco-friendly method for effective management of plant diseases.

Results: In a previous research, three species of *Streptomyces* spp., i.e., *S. griseus* (MT210913 "DG5"), *S. rochei* (MN700192 "DG4") and *S. sampsonii* (MN700191 "DG1" strains) were identified, as exhibiting potent antifungal activities against plant pathogenic fungus, *Sclerotinia sclerotiorum* in vitro and greenhouse. GC–Mass analysis revealed the presence of 44, 47 and 54 substances of *S. sampsonii* DG1, *S. griseus* DG5 and *S. rochei* DG4, respectively. GC–MS revealed substances, with bio-control activity, were categorized as volatile organic compounds (VOCs), fatty acids and plant growth regulators, etc. GC–MS analysis exhibited the presence of 7, 13 and 20 volatile compounds produced by *S. sampsonii, S. rochei* and *S. griseus,* respectively. These substances exhibited potent antifungal activity against various plant pathogenic fungi, i.e., *Botrytis cinerea, Macrophomina phaseolina, Rhizoctonia solani* and *S. sclerotiorum* in vitro, by dual-culture assay. The three strains inhibited all the pathogenic fungi in dual-culture assay in the range of 30–73.67%. Also, the produced substances were applied in vivo (in the field) and supported their potential biocontrol agent against *S. sclerotiorum* as well as possessed significant biological properties for plant health and growth. Applying *Streptomyces* spp. culture broth in the field enhanced physiological responses of phenols, sugar, chlorophyll, protein contents and parameters as well as the yield of bean plants.

Conclusion: In field experiments, foliar application of *Streptomyces* spp. and their metabolites proved to be a great potential, as promising biocontrol agents, for controlling *S. sclerotiorum* and enhanced plant growth and yield. *S. rochei* and *S. griseus* proved to be strong antifungal, plant growth promoters and environmentally eco-friendly fungicides.

Keywords: Antifungal activity, *Streptomyces* spp., *S. griseus*, *S. rochei*, *S. sampsonii*, GC–Mass analysis, Secondary metabolites

Background

Plant diseases caused by fungi and bacteria accounted for 42 and 27%, respectively, of the global crop production loss (14%) which represents $200 billion a year, and this destruction can worsen under climate changes (Elad and

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Pertol 2014). *Botrytis cinerea, Macrophomina phaseolina, Rhizoctonia solani* and *Sclerotinia sclerotiorum* are the most prevalent and destructive fungal diseases affecting crops in all growing regions. However, these soilborne pathogens are very difficult to control, because of their broad-host range and high survival rate of sclerotia under various environmental conditions. Excessive applications of fungicides lead to harmful effects, i.e., higher input costs, human health problems, environmental pollution, pathogens resistance and phytotoxicity (Le et al. 2022). Nowadays, researchers are focused on identifying and applying environmentally friendly alternatives for controlling plant diseases and improving crop productivity, which are recommended within an integrated crop management ecosystem (Boukhatem et al. 2022).

Last few decades, controlling plant diseases using biocontrol agent antagonists developed much attention. Recently, the most important bioagents used in biological control are the *Streptomyces* genus. *Streptomyces* spp. belonging to this genus are of great importance in the field of biotechnology, as producers of beneficial bioactive secondary metabolites with extensive industrial, medical and agricultural applications (Harir et al. 2018). The secondary metabolites produced by *Streptomyces* spp. are diversified in their biological activities and functions as antifungal, antibacterial, insecticidal, enzymes, antibiotics and activities (Katarzyna et al. 2018). *Streptomyces* spp. have the potential inhibitory effect on the growth of various plant pathogens, i.e., fungi (Le et al. 2022), bacteria (Guo et al. 2020) and viruses (Li et al. 2019). Moreover, *Streptomyces* spp. are known to improve the availability of nutrients and minerals, enhance the production of metabolites and promote plant growth regulators (Boukhatem et al. 2022). The objective of the present research is to: (a) evaluate three *Streptomyces* spp. in vitro on four plant pathogenic fungi (*B. cinerea; M. phaseolina*; *R. solani* and *S. sclerotiorum*), (b) identify their secondary metabolites by GC–MS analysis and (c) evaluate its ability in controlling *S. sclerotiorum* causative agent of bean white rot disease in vivo.

Methods

The experiments were carried out in two phases, firstly in vitro antagonism assay of microorganisms against various plant pathogenic agents and secondly in vivo biocontrol trials on susceptible bean plants cultivated in the producer's fields located in Dahshur Province, Giza Governorate, Egypt, during two successive seasons (2018–2019 and 2019–2020).

Isolation of microorganisms and in vitro antagonism assay

This experiment was conducted in the laboratory at the Central Lab. of Organic Agri., Agri. Research Center (ARC) and Plant Pathology Dept., Fac. of Agric., Cairo Univ., Giza, Egypt. Three *Streptomyces* species were previously characterized molecularly, using PCR technique, then sequenced and submitted to NCBI GenBank and gave accession numbers, i.e., *S. griseus* (MT210913"DG5"), *S. rochei* (MN700192"DG4") and *S. sampsonii* (MN700191"DG1") strains. *Streptomyces* spp. were cultured and maintained on starch casein (StC) medium as described in our previous report (Gebily et al. 2021).

Concerning the tested fungi, various plant pathogenic fungi, i.e., *B. cinerea, M. phaseolina, R. solani* and *S. sclerotiorum* were isolated from Strawberry, Sesame, Sweet pepper and Bean plants, respectively. These fungi were maintained and stored at the laboratory, using a potato dextrose agar (PDA) medium. Five-mm-diameter inoculum disks of the above phytopathogenic fungi were cut from the margin of the fresh colonies on PDA plates. Each disk was inoculated on the center of the PDA pour plate and incubated at 20 °C (*S. sclerotiorum* and *B. cinerea*) and 25 °C (*R. solani* and *M. phaseolina*) for 7 days. To evaluate bioagent activity, each of the three *Streptomyces* species was dually cultured with all above-mentioned phytopathogenic fungi. The growth inhibitory effects on the pathogenic fungi were represented as a percentage of pathogen growth inhibition (PIG) and calculated according to Zambrano et al. (2021) following equation:

$$\mathrm{PGI} = \frac{R1 - R2}{R1} \times 100$$

where $R1$ = Growth of pathogenic fungus in the control plate. $R2$ = Growth of pathogenic fungus interacting with the antagonist.

Gas chromatography–mass (GC–Mass) analysis of *Streptomyces* spp. secondary metabolites

This experiment aimed to identify secondary metabolites produced by *Streptomyces* spp. which play an important role in their antagonistic activities against the phytopathogenic fungi in two steps as follows: Extraction of the secondary metabolites using ethyl acetate according to Parthasarathi et al. (2012) and gas chromatography–mass spectrometry (GC–Mass) analysis as described by El-Kareem et al. (2016). The obtained pellets of *Streptomyces* spp. (*S. griseus*, *S. rochei* and *S. sampsonii*) were categorized using GC–Mass and performed using Trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25-µm film thickness). The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST14 mass spectral databases.

Application of *Streptomyces* spp. on the control bean white rot disease in vivo

Nowadays, due to the harmful effect on the environment caused by excessive usage of pesticides, the urge to use an alternative method became precisely important and motivated toward managing the diseases in sustainable agriculture. For this reason, a field experiment was conducted to (a) apply an eco-friendly manner such as microbial bioagents and/or their mycoparasitic activity toward pathogen sclerotia for decreasing their number in the soil and infection percent; (b) improve the quality and quantity of bean pods by controlling white rot disease in the field under natural infection; (c) check the ability of the selected three *Streptomyces* spp. in controlling white rot disease caused by *S. sclerotiorum*, bean plants were sprayed by *Streptomyces* spp. as individual or mixture treatments and (d) determine the most effective *Streptomyces* spp. and their concentrations to control white rot disease and enhancing plant parameters components (phenols, sugars, chlorophyll and protein contents).

Effect of applying *Streptomyces* spp. under field conditions on disease reduction and enhancing plant growth

In vivo experiment was carried out to evaluate the effect of different concentrations of *Streptomyces* spp. to control *S. sclerotiorum* infecting bean. Field experiments were conducted in light sandy soil, irrigated by a flood system and naturally infested due to our observation from the previous plantations. All experiments were designed in complete randomized blocks design. Three replicates were used for each treatment; each plot (2.8×5 m) was used as a replicate. Each replicate contained 100 bean seeds cv. Paulista. All plants received the same fertilizers and irrigation regime. *Streptomyces* spp. were applied individually or in combinations under field conditions. All the used *Streptomyces* spp. were grown into liquid starch casein medium for 7 days. All media components of the *Streptomyces* spp. were prepared as culture broth (containing cell-free extract, mycelia + spores) + 5% acacia gum (Arabic gum) and 0.5% potassium soap. Each suspension was adjusted using sterilized distilled water to be contained (15×10^6 c.f.u./ml). The application was carried out 4 times (15 days intervals) on the plants after 30-day post-sowing. Corporal Max was used as a positive control (commercial fungicide produced by the Central Lab. for Organic Agric.). Control treatment plants were sprayed with water.

Impact of applying different concentrations of *Streptomyces* culture broth

To determine the most efficient and economic concentration of each *Streptomyces* spp., three different concentrations (diluted by water) were applied for each treatment 1/50, 1/100, and 1/150 (v/v). Plants were sprayed by three isolates of *Streptomyces* spp. either individually or their combinations to evaluate disease reduction, plant parameters and components as the following:

Evaluation of disease reduction and plant parameters

The percentages of disease incidence and reduction in white rot disease were calculated after the development of natural disease symptoms on the control plants (60-day post-sowing). Healthy plants of three-month-old were collected from each treatment, and the plant's dry matter and pods were dried using an electric oven at 70 °C. The total yield and number of pods/plant in the different treatments were calculated per feddan.

Impact of Streptomyces spp. treatments on plant components

Analyses of plant contents were carried out at the Central Lab. of Organic Agric. Samples of the treated plants were collected from different tested treatments to assess their contents (phenols, sugars and protein in pods as well as leaves chlorophyll). Collected samples were washed with tap water and then, prepared to determine reducing and non-reducing sugars using the method developed by AOAC (2005). Total phenols, free and conjugated were also determined using a method described by Simons and Ross (1971). Protein and leaf chlorophyll contents were also determined using the methods described by Bradford (1976) and Askar and Treptow (1993), respectively.

Statistical analysis

Data were statistically analyzed according to the general linear models procedure of Statistical Package for Social Sciences (SPSS 2008) Version 17.0.0 Software. Least significant difference test (LSD) at $\leq 5\%$ level of probability ($P \leq 0.05$) was used. The presented data were calculated for two successive seasons.

Results

Streptomyces spp. exhibited broad-spectrum antifungal activity against four phytopathogenic fungi, i.e., *B. cinerea, M. phaseolina, R. solani* and *S. sclerotiorum*. In vitro dual-culture assay revealed that the volatile organic compounds (VOCs) produced by the three strains of *Streptomyces* spp. showed inhibitory activity against the mycelial growth of all the tested phytopathogenic fungi in the range of 30–73.67%. *S. sampsonii* revealed the highest antifungal activity (73.67%) against *B. cinerea,* followed by *S. rochei* (70.83%) and *S. griseus* (70.33%), while *S. sampsonii* showed the lowest antifungal activity (35.33%) against *R. solani,* followed by *S. griseus* (33.33%) and *S. rochei* (30%) (Table 1 and Fig. 1).

Table 1 Inhibitory activities of *Streptomyces* spp. against different plant pathogenic fungi

Fungus	Strain	% Reduction*
B. cinerea	*S. sampsonii*	73.67[a]
	S. rochei	70.83[b]
	S. griseus	70.33[b]
M. phaseolina	*S. sampsonii*	44.50[e]
	S. rochei	50.33[c]
	S. griseus	47.50[d]
R. solani	*S. sampsonii*	35.33[f]
	S. rochei	30.00[i]
	S. griseus	33.33[g]
S. sclerotiorum	*S. sampsonii*	47.33[d]
	S. rochei	32.40[h]
	S. griseus	44.87[e]
Control	0.00	0.00[j]
SE		0.22

*Values with the same letter, in the column, are not significantly different ($P \leq 0.05$)

Identification of potent bioactive compounds by GC–MS

The GC–MS analysis profiles of the active fraction and its constituents from the selected strains were presented in (Figs. 2, 3). A total of 44, 47 and 54 compounds for *S. sampsonii* DG1; *S. griseus* DG5 and *S. rochei* DG4, respectively, were detected and compared with those entries in the NIST14 database, and the nearest compound resembling those peaks was identified, as well as its molecular weight (MW), retention time (RT), and other characteristics. It was noticed that the fraction contained various types of major VOCs compounds including groups of alkenes, sulfides, ketones, benzenoids, aromatic hydrocarbons, odors, hormones and alcohols. The most abundant volatile compounds produced by the three *Streptomyces* spp. were phenol-2-methoxy4,2 propenyl (Eugenol); 1,2 benzenedicarboxylic acid; phenols and heptanol, 6-methyl; 1-hexadecanol, acenaphthenol, behenic alcohol, 2,4-di-tert-butylphenol; benzene acetic acid-butyl ester; 2-acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin; tetrahydroactinidiolide; 4-hydroxybenzaldehyde; benzene, 1,1-(1-butene-1,4-diyl)bis-(Z); N-(4-hydroxyphenethyl) acetamide, 7,9-Di-tertbutyl-1-oxaspiro decadienedione and 2-(3,4-dimethoxyphenyl)-2,3-dihydro-3-hydroxy-5,7-dimethoxy-3-phenyl-4H-1-benzopyranone. The results indicated the inhibitory activities of one or more volatile compounds produced by each *Streptomyces* strain on mycelial growth, suppressing spore germination or sclerotia formation of all pathogenic fungi. Also, the result of in vitro bioassay showed that the VOCs from the three *Streptomyces* spp. had a strong antifungal activity against *B. cinerea,* followed by *M. phaseolina* and *S. sclerotiorum* (Fig. 1).

Concerning the GC–MS chromatogram peak area percent, molecular weight and molecular formulae of three *Streptomyces* spp., the consequent compound were identified and shown in Fig. 2. Peak areas (%) were directly proportional to the degree of the bioactive compounds existing in the culture broth. GC–MS analysis revealed the presence of 7, 13 and 20 VOCs produced by *S. sampsonii, S. rochei* and *S. griseus,* respectively. Also, GC–MS analysis showed the existence of peak area percentages (0.87, 0.55 and 1.64%) of phthalate derivatives such 1,2- benzenedicarboxylic acid were produced by *S. sampsonii*, *S. rochei* and *S. griseus*, respectively. Further, GC–MS analysis indicated the presence of the highest peak area percentages of fatty acids for *S. sampsonii* strain. Peak area percentages were 6.63, 5.84, 3.74, 3.05 and 2.60% for oleic acid, palmitic acid, palmitoleic acid and hexadecanoic acid, respectively. Regarding *S. rochei* strain, data indicated that it was recognized by the highest peak area percentages of aromatic hydrocarbon derivatives, i.e., acenaphthene (10.25%), 1-acenaphthenol (2.59%), benzene acetic acid-butyl ester, (1.97%), 7,9-di-tert-butyl-1-oxaspirodeca-6,9-diene-2,8-dione, (1.81%). On the other hand, GC–MS chromatogram of *S. griseus* strain exhibited the highest peak area percentages of aromatic compound derivatives such as 1,4-benzenediol, 2-(1,1-dimethylethyl) 5, 2-propenyl (5.48%), 1-Acenaphthenol (3.83%), benzene,1,1-(2-butene-1,4-diyl) bis (2.25%), 1-benzoxirenol,2,2,5a-trimethyl-1a[2-(2-methyl)-1,3-dioxolanyl)1-ethenyl] hydro (1.82%), N(4-Hydroxyphenethyl) acetamide (0.83%), thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester (1.90%) and methanone, bis (4-phenoxyphenyl) (1.25%). The aforementioned results indicated that these derivatives and components played an important role individually or combined with others as biologically active and effective against the tested phytopathogenic fungi. Additionally, GC–MS showed that *S. rochei* (DG4) and *S. griseus* (DG5) were able to produce secondary metabolites as indolic compounds, i.e., phenylacetic acid (PAA), 1H-Indole and gibberellic acid (GA) at a very low area percent (1.87, 0.59 and 0.28%, respectively). Thus, the result suggested that the application of *Streptomyces* spp. represented a promising sustainable solution to improve plant growth, productivity and disease reduction as well as alternative pesticides.

Impact of culture broth concentrations

Streptomyces spp. culture broth was used at various concentrations to prove the most effective one in reducing bean white rot disease, enhancing plant vigor and components in the treated plants as follows:

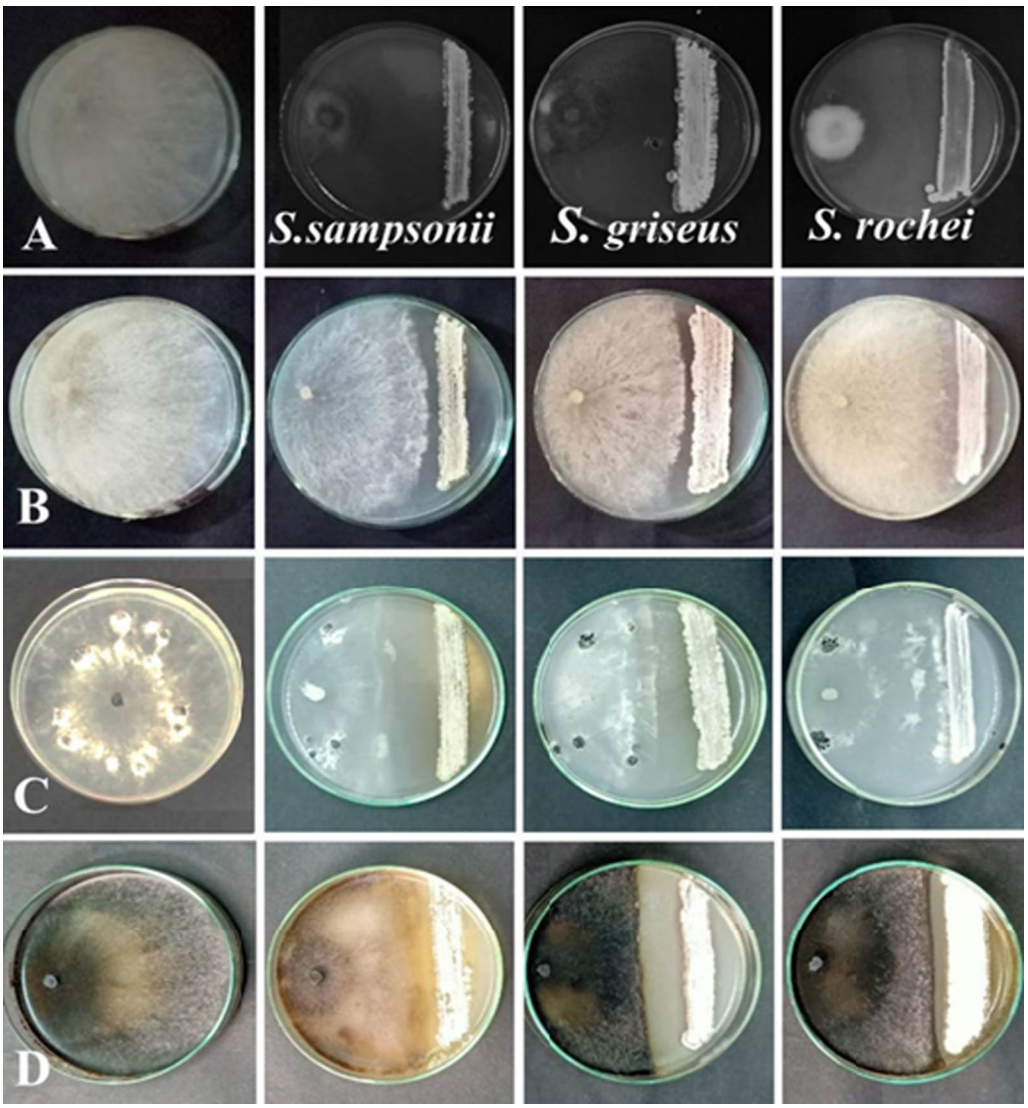

Fig. 1 In vitro growth inhibition (PGI) of various plant pathogenic fungi, *B. cinerea*, (**A**); *R. solani*, (**B**); *S. sclerotiorum* (**C**) and *M. phaseolina* (**D**) using three *Streptomyces* spp.

Streptomyces spp. efficacy on bean white rot disease reducing

To determine the most effective concentration of different *Streptomyces* spp. on disease control, different concentrations were tested as presented in Table 2. The various concentrations of each *Streptomyces* sp. led to a significant reduction in white rot disease compared with the control. In all cases, a clear positive correlation was noticed between the concentration of the antagonist and its efficacy. The highest reduction (61.33%) was obtained when used *S. rochei* (DG4) at the concentration (1:50). The highest reductions with the same concentration in the mixtures were noticed when *S. griseus* (DG5) mixed with *S. sampsonii* (DG1) or *S. rochei* (DG4), recording (86.67 and 84%) in disease reduction, respectively. Results also indicated that using a mixture of the three *Streptomyces* spp. together was the most effective treatment for disease control and resulted in a (92%) reduction when used at a concentration of 1:50 (v/v) compared with the control (0.00%) and Copral Max (74.67%).

Streptomyces spp. efficacy on enhancing green bean plant vigor

The used concentrations of each *Streptomyces* sp. culture broth exhibited significant differences in the percentages of plant and pods dry matter of the treated plants than

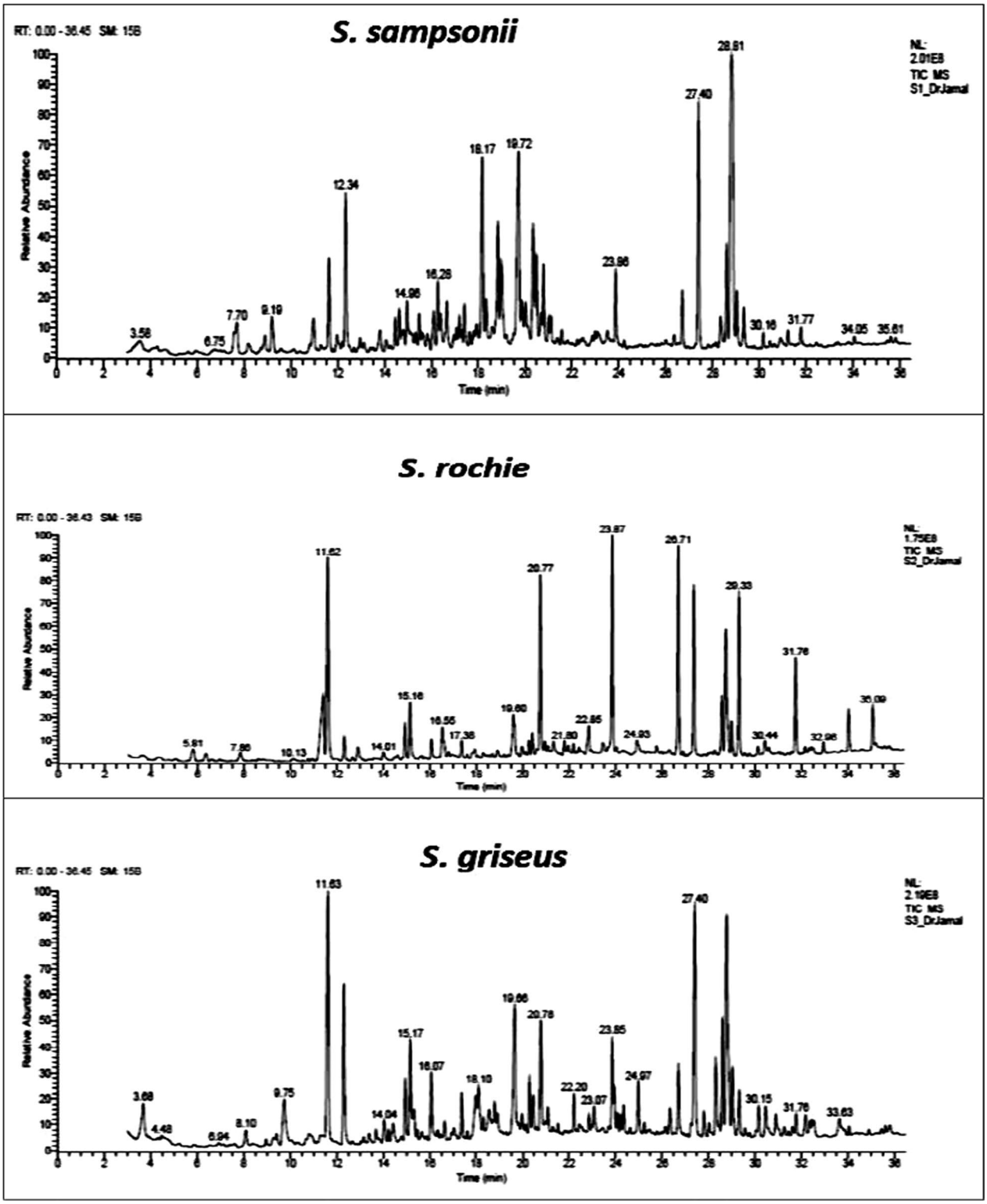

Fig. 2 GC–MS analysis of ethyl acetate extract of three *Streptomyces* spp.

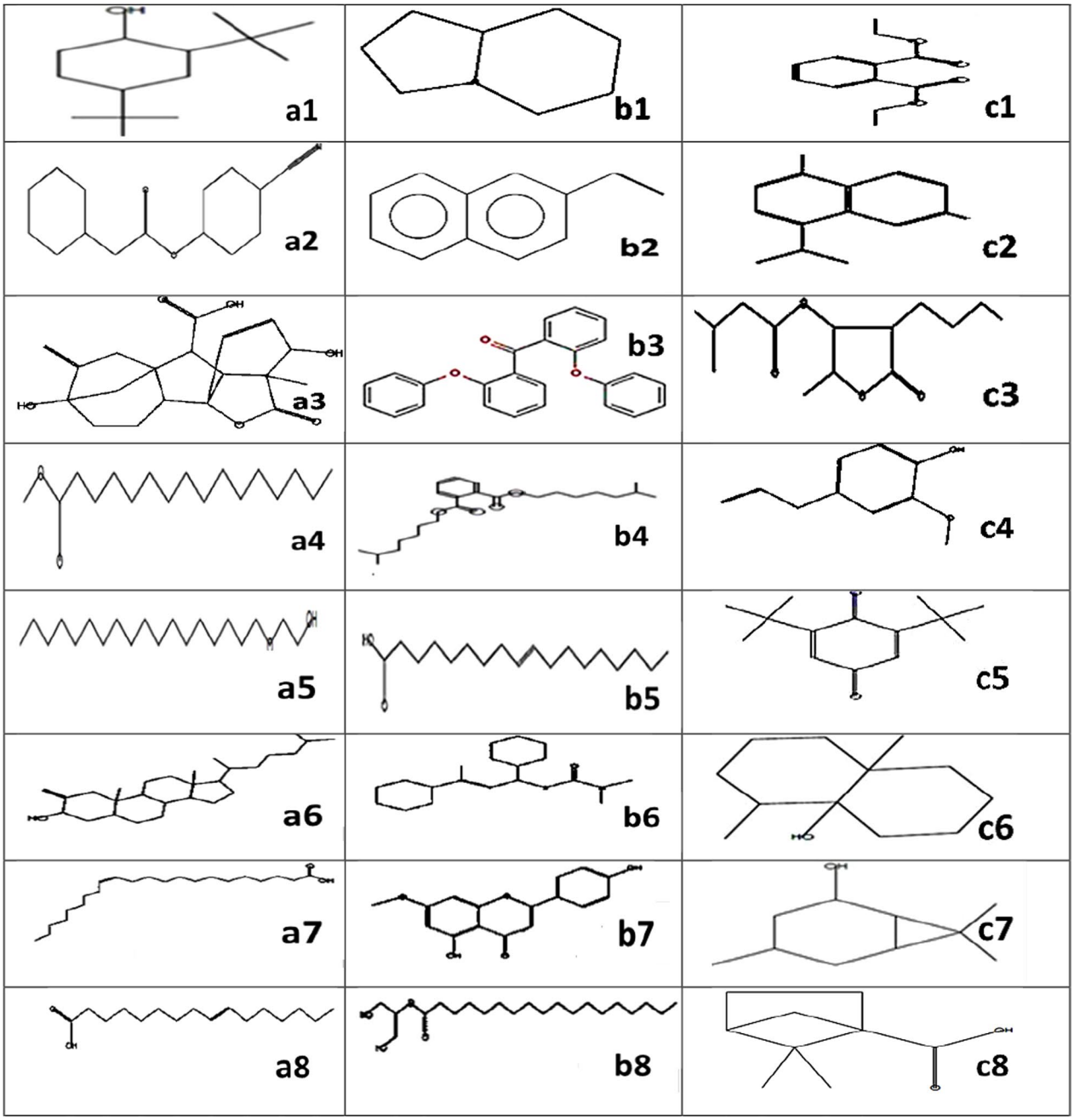

Fig. 3 Molecular structures of the representative compounds. Mass spectrum of the compounds obtained by GC–MS analysis of three *Streptomyces* spp. were compared with the WILEY 09 and NIST14 mass spectral databases. ***S. rochei*** (**a1–8**): (**a1**) 2,4-Di-Tert-butylphenol, (**a2**) Phenyl acetic acid, (**a3**) Gibberellic acid (GA), (**a4**) Palmitic acid, (**a5**) Ethanol, 2-(Octadecyloxy), (**a6**) Cholestanol, (**a7**) 2-methylene, & (**a8**) Hexadecenoic acid (Erucic acid). ***S. griseus*** (**b1–8**): (**b1**) Indole, (**b2**) Naphthalene, 2-ethenyl, (**b3**) Methanone, Bis-4-phenoxyphenyl, (**b4**) 1,2-benzenedicarboxylic acid (Phthalate), **b5**) Oleic acid, (**b6**) Thiocarbamic acid, (**b7**) O-Methylated Flavone, (Flavonoid or Genkwanin & (**b8**) 9-Octadecenoic acid (Oleic Acid). and ***S. sampsonii*** (**c1–8**): (**c1**) 1,2-Benzenedicarboxylic acid (Phthalic acid), (**c2**) Naphthalene, 1,6-Dimethyl-4-(1-Methylethyl), (**b3**) 3-Methyl-butyric acid (**b4**) Eugenol phenol,2-Methoxy-4-(2-Propenyl). (**c5**) 2,6-Ditert-butylbenzo-1,4-Quinone, (**c6**) 2 h-Naphthol, Octahydro-dimethyl, (**c7**) Heptanol and (**c8**) Hexane-1-carboxylic acid

Table 2 Impact of applying *Streptomyces* spp. at different concentrations on bean white rot disease

***Streptomyces* isolate**	**Concentration**	**% White rot***	
		% Disease incidence	**% Reduction**
S. sampsonii (DG1)	1:50	12.33^{fg}	50.67^{ef}
	1:100	15.00^{d}	40.00^{h}
	1:150	20.00^{b}	20.00^{j}
S. rochei (DG4)	1:50	9.67^{h}	61.33^{d}
	1:100	12.00^{fg}	52.00^{ef}
	1:150	17.00^{c}	32.00^{i}
S. griseus (DG5)	1:50	12.00^{fg}	52.00^{ef}
	1:100	13.00^{ef}	48.00^{fg}
	1:150	19.33^{b}	22.67^{j}
S. sampsonii (DG1) + *S. rochei* (DG4)	1:50	6.67^{i}	73.33^{c}
	1:100	9.00^{h}	64.00^{d}
	1:150	14.00^{de}	44.00^{gh}
S.sampsonii (DG1) + *S. griseus* (DG5)	1:50	3.33^{j}	86.67^{b}
	1:100	5.67^{i}	77.33^{c}
	1:150	12.00^{fg}	52.00^{ef}
S. rochei (DG4) + *S. griseus* (DG5)	1:50	4.00^{j}	84.00^{b}
	1:100	6.00^{i}	76.00^{c}
	1:150	11.33^{g}	54.67^{d}
S. sampsonii (DG1) + *S. rochei* (DG4) + *S. griseus* (DG5)	1:50	2.00^{k}	92.00a
	1:100	3.33^{j}	86.67^{b}
	1:150	11.67^{fg}	53.33^{d}
Copral Max		6.33^{i}	74.67^{c}
Control		25.00^{a}	0.00^{k}
SE		0.44	1.60

*Values with the same letter, in each separate column, are not significantly different at ($P \leq 0.05$)

the control. Results in Table 3 indicated that the most effective concentration in the individual treatment was *S. rochei*, since recorded the highest number of pods (22 pods/plant) and yield (3.9 ton/feddan). Also, it increased the dry matter of plants and pods to (67.33 and 18.67%), respectively. Also, the results revealed that the mixtures in all cases significantly enhanced plant vigor when compared with an individual treatment at the same concentration. Further, application of the triple mixture of *S. rochei* (DG4), *S. sampsonii* (DG1) and *S. griseus* (DG5) resulted in the highest control and increased all parameters related to plant vigor when using concentrations of (1:50 and 1:100), recording the highest number of pods (29 pods/plant) and yield (5.37 ton/feddan).

Streptomyces spp. efficacy on the treated plant components

Field experiments were conducted to determine the physiological responses of bean plants by applying an individual or mixture of *Streptomyces* spp. at different concentrations. Results of plant analyses included phenol, sugar, chlorophyll and protein contents are illustrated in Table 4. The presented data suggested that increased concentration of the tested *Streptomyces* spp. either individual or mixtures treatment increased its effectiveness which was reflected in the plants growth and changes in their components. Protein amount reached the highest content (9.18) when applying a triple mixture of *Streptomyces* spp., followed by the dual mixture of *S. rochei* (DG4) and *S. griseus* (DG5) (6.55), then *S. rochei* and *S. sampsonii* (DG1) (6.30) and *S. sampsonii* and *S. griseus* (5.80). Regarding phenols, all the treatments either individual or combination of *Streptomyces* spp. at a concentration of 1:50 increased the amount of total and free phenols than the control. On the contrary, the increase in conjugated phenols was correlated with the low concentration (1:150) in the different treatments. The mixture of three *Streptomyces* spp. resulted in the highest percentage of disease control and the highest amount of phenol content. A mixture of *S. sampsonii* (DG1), *S. rochei* (DG4) and *S. griseus* (DG5) reached the highest levels of free and total phenols (14.2 and 16.35, respectively) and 5.8 reduced sugars. Sugar contents were determined to clear positive correlation between the increase in the

Table 3 Impact of applying *Streptomyces* spp. at various concentrations on bean plant vigor

Streptomyces isolate	Concentration	Dry matter of plants (g.)	Dry matter of pods g./plant	No. of pods/plant	Weight of total yield Ton/fed
S. sampsonii (DG1)	1:50	64.00^{de}	16.00^{de}	16.00^{hi}	3.50^{gh}
	1:100	60.00^{hi}	13.00^{fg}	13.00^{j}	3.00^{j}
	1:150	57.33^{k}	10.43^{h}	9.00^{kl}	2.50^{k}
S. rochei (DG4)	1:50	67.33^{b}	18.67^{c}	22.00^{de}	3.90^{ef}
	1:100	63.00^{ef}	16.00^{de}	20.00^{f}	3.50^{gh}
	1:150	59.00ij	13.50^{f}	12.00^{j}	2.90^{j}
S. griseus (DG5)	1:50	61.33^{gh}	15.33^{e}	18.00^{g}	3.00^{j}
	1:100	59.33^{ij}	13.00^{fg}	17.00^{gh}	2.50^{k}
	1:150	55.00^{l}	8.00^{jk}	10.00^{k}	2.00^{l}
S. sampsonii (DG1) + *S. rochei* (DG4)	1:50	65.00^{cd}	16.00^{de}	23.00^{cd}	4.00^{e}
	1:100	62.33^{fg}	14.00^{f}	21.00^{ef}	3.90^{ef}
	1:150	57.00^{k}	10.43^{h}	15.00^{i}	3.20^{i}
S. sampsonii (DG1) + *S. griseus* (DG5)	1:50	66.33^{bc}	17.00^{d}	27.00^{b}	4.53^{c}
	1:100	63.00^{ef}	15.33^{e}	24.00^{c}	4.00^{e}
	1:150	59.00^{ij}	9.00^{ij}	20.00^{f}	3.60^{g}
S. rochei (DG4) + *S. griseus* (DG5)	1:50	67.00^{b}	19.33^{bc}	23.00^{cd}	4.30^{d}
	1:100	66.00^{bc}	17.00^{d}	22.00^{de}	4.00^{e}
	1:150	63.00^{ef}	10.00^{hi}	17.00^{gh}	3.40^{h}
S. sampsonii (DG1) + *S. rochei* (DG4) + *S. griseus* (DG5)	1:50	69.67^{a}	22.00^{a}	29.00^{a}	5.37^{a}
	1:100	69.33^{a}	20.00^{b}	27.00^{b}	5.00^{b}
	1:150	60.00^{hi}	12.00^{g}	22.00^{de}	3.83^{f}
Copral Max		58.33^{jk}	13.00fg	20.00^{f}	2.40^{k}
Control		40.17^{m}	7.00^{k}	8.00^{l}	1.53^{m}
SE		0.45	0.43	0.47	0.05

Values with the same letter, in each separate column, are not significantly different ($P \leq 0.05$)

concentration of *Streptomyces* spp. and the number of reduced sugars in all cases.

Discussion

Until now, white rot disease of bean caused by *S. sclerotiorum* remains a significant challenge to recognize and control its infection mechanisms. To develop effective biocontrol agents to control the pathogen, three strains of *Streptomyces* spp., i.e., *S. sampsonii* (MN700191 "DG1"), *S. rochei* (MN700192 "DG4") and *S. griseus* (MT210913 "DG5"), were isolated, tested in vitro and molecularly characterized in the previous research (Gebily et al. 2021). *Streptomyces* sp. is well-known for producing strong VOCs, odors, fatty acids and plant growth regulators. In the dual-culture assay, *Streptomyces* spp. induced inhibition of all the tested phytopathogenic fungi, which indicated the production of antifungal compounds that may be involved in the inhibition of mycelial growth, as there was no direct contact between the phytopathogenic fungi and *Streptomyces* spp. The result revealed that volatile substances of *Streptomyces* spp. had a potential for usage as a bio-fumigant to control plant fungal diseases. Such results are consistent with those described by other investigators working with *Streptomyces* species that often produce a wide diversity of secondary metabolites inhibiting the growth of phytopathogenic fungi and bacteria (Le et al. 2022).

Thus, the progress and exploitation of biological agents, such as antagonistic *Streptomyces* spp. and their secondary metabolites, became a promising alternative way to control plant diseases. Nowadays, GC–MS developed a novel technique to identify the secondary metabolites produced by *Streptomyces* species, and its analysis is very reliable to identify the compound in the complex biochemical products. The resultant GC–MS peak area, molecular weight, formula and the derived compounds were identified in the presented results. The peak area was directly relational to the level of the bioactive compounds prevailing in the composites. GC–MS analysis showed the presence of (44, 47 and 54) compounds for *S. sampsonii* DG1; *S. griseus* DG5 and *S. rochei* DG4, respectively. Obtained results of the antifungal effect of *Streptomyces* spp. culture broth on mycelial growth of different pathogens revealed that all bioagent strains

Table 4 Effect of applying *Streptomyces* spp. at different concentrations on the treated plant components

***Streptomyces* isolate**	**Concentration**	***Plants composition (mg/g)**						
		Sugars		**Protein**	**Total phenols**	**Free phenols**	**Conjugated phenols**	**Chlorophyll**
		Reduced	**Non-red**					
S. sampsonii (DG1)	1:50	4.35	1.11	4.43	12.02	9.12	2.9	25.03
	1:100	3.04	0.37	3.57	10.21	7.50	2.7	23.54
	1:150	2.76	0.24	3.30	9.30	6.00	3.3	22.54
S. rochei (DG4)	1:50	4.80	1.60	4.79	14.54	12.24	2.3	24.89
	1:100	3.94	0.98	4.18	13.34	10.84	2.5	24. 68
	1:150	2.46	0.47	3.92	11.20	7.5	3.7	23.97
S. griseus (DG5)	1:50	4.80	1.50	4.04	13.25	11.35	1.9	24.47
	1:100	4.50	0.14	3.06	11.30	9.20	2.1	23.93
	1:150	2.58	0.28	2.43	10.20	6.92	3.28	22.85
S. sampsonii (DG1) + *S. rochei* (DG4)	1:50	4.90	1.40	6.30	15.32	14.14	1.18	26.03
	1:100	3.47	0.86	6.06	14.21	11.14	3.07	25.20
	1:150	2.2	0.23	4.55	12.01	7.65	4.36	24.20
S. sampsonii (DG1) + *S. griseus* (DG5)	1:50	5.20	1.20	5.80	14.30	11.80	2.50	25.03
	1:100	4.83	0.89	5.43	13.40	10.62	2.78	24.22
	1:150	3.45	0.25	3.79	11.35	7.71	3.64	23.08
S. rochei (DG4) + *S. griseus* (DG5)	1:50	4.80	1.10	6.55	14.45	13.46	0.99	27.52
	1:100	3.96	1.79	6.30	14.55	13.16	1.39	26.49
	1:150	2.2	0.27	2.49	10.23	7.73	2.50	24.89
S. sampsonii (DG1) + *S. rochei* (DG4) + *S. griseus* (DG5)	1:50	5.80	1.60	9.18	16.35	14.20	2.15	30.98
	1:100	5.03	1.17	8.60	15.75	13.49	2.26	30.00
	1:150	3.20	0.90	3.67	11.23	8.52	2.71	25.27
Copral Max		4.11	1.22	4.81	12.02	4.30	6.72	22.29
Control		1.25	0.12	2.20	6.70	1.63	5.07	18.79

*Data represent one replicate

tested produced several substances, i.e., alcohols, alkenes, aromatic hydrocarbons, benzenoids, fatty acids and alcohols, ketones, sulfides and steroids with varying efficiencies. GC–MS analysis showed various types of VOCs compounds produced by *Streptomyces* spp. and play an important role as one of the biocontrol mechanisms against plant diseases. Further, GC–MS analysis of three *Streptomyces* spp. revealed similarities in their producing many volatiles and other substances as mentioned before.

Results indicated significant differences among the number and components of the VOCs (7, 13 and 20) produced by *S. sampsonii* "MN700191 DG1," *S. rochei* "MN700192 DG4" and *S. griseus* "MT210913 DG5," respectively. The presented results of the three *Streptomyces* spp. were recorded as major aromatic compounds, i.e., phenol, acenaphthene, 1,4-benzenediol and phthalate derivatives (PAHs such benzenedicarboxylic acid or phthalic acid) with the highest number and area percentage, which supported their role in bioactivities against the tested pathogens. This result is resembling those obtained by Danaei et al. (2014) who mentioned that the antifungal activity of *S. griseus* cultures can be attributed to carvacrol, benzaldehyde, naphthalene and phenol as well as other compounds. Also, the results were in harmony with Ahsan et al. (2017) who reported that *Streptomyces* strain KX852460 produced major aromatic compounds with the highest peak number and had a good antifungal activity as well as potential biocontrol agent against *R. solani*. Furthermore, Memic et al. (2017) tested various polycyclic aromatic hydrocarbons (PAHs) against two ligninolytic fungi *Hypoxylon fragiforme* (wood white rot) and *Coniophora puteana* (wood brown rot). They found that the antifungal activity of PAHs depended on the number of aromatic or benzene rings and area percentage of the compound as well as molecular structure. Nevertheless, the VOCs components were identified as antimicrobial, antibacterial and antifungal to control bacterial and fungal diseases in vitro by destroying, deformation, and degrading cell membrane integrity and mycelial ultrastructure of the pathogens (Le et al. 2022); and in vivo (fields) and postharvest as alternative pesticides as well as environmentally friendly options (Kaari et al. 2022).

Also, the results of the present study indicated that the secondary metabolites such as fatty acids might contribute to VOC's inhibitory antagonistic effect on the mycelial growth and sclerotia formation of the tested phytopathogenic fungi. This result had already been described by Liu et al. (2008) on *S. griseus*, who evaluated in vitro inhibition of nine fatty acids against four phytopathogenic fungi. They detected that all fatty acids suppressed the mycelial growth of one or more tested fungi excluding oleic acid. Similarly, many researchers discussed that various *Streptomyces* spp. produced secondary metabolites as antibacterial, antifungal and antimicrobial bioactivities (Sholkamy et al. 2020).

Concerning the production of plant growth regulators, *S. rochei* "MN700192 DG4" followed by *S. griseus* "MT210913 DG5" proved to be the greatest effective producers with an area percentage of (1.87, 0.59 and 0.28%), respectively. Also, the result indicated that *S. rochei* was able to promote plant growth due to its secretion of some secondary metabolites as plant growth regulators such as auxin and gibberellin. Thus, they should be a great biological agent to control phytopathogens and promote plant growth. Such results were confirmed by several authors who applied *Streptomyces fradiae* NKZ-259 (Myo et al. 2019), *Streptomyces tricolor* HM10 (Rehan et al. 2021) and *Streptomyces lydicus* M01 (Wang et al. 2020). Similarly, Vurukonda et al. (2018) recorded that the *S. rochei* was able to promote plant growth due to its secretion of some secondary metabolites, i.e., auxin, gibberellin and cytokinin.

In the present research, the application of *Streptomyces* spp. to control bean white rot disease explained that the use of a mixture containing the three biological agents was the most efficient treatment due to their multiple weapons and mechanisms such as hyper-parasitism and secretion of secondary metabolites, which plays a role as potential biocontrol agents for controlling *S. sclerotiorum* pathogen in the field. The result agreed with Muiru et al. (2007) who tested secondary metabolites from three *Streptomyces* spp. isolates coded CS35, 28P, 14P against *Pythium* spp. The highest degree of activity was obtained from isolate 14P, it could be attributed to the presence of higher amounts and concentrations of antibiotics compared to metabolites from the other *Streptomyces* isolates.

Conferring *Streptomyces* spp. application to enhance plant vigor, the triple mixture of *S. Streptomyces* spp. proved to be the most efficient in increasing parameters (number of pods per plant, dry matter of plants (g.) and pods (g.)/plant as well as the weight of total yield [Ton/fed]) when concentrations of 1:50 and 1:100 were used. Similarly, Gopalakrishnan et al. (2015) tested six strains of *Streptomyces* spp. on the growth promotion and grain yield in chickpea (*Cicer arietinum* L.). Of six *Streptomyces* strains, CAI-85, CAI-93 and KAI-180 were found superior to CAI-155, CAI-140 and CAI-13, in terms of their effects on root and shoot development, nodule formation and crop productivity. These strains significantly enhanced the pod number and weight, leaf area and weight as well as stem weight and crop maturity, grain yield, total dry matter, pod weight seed number and seed weight compared with the untreated plants. Nevertheless, Mun et al. (2020) evaluated the ability of *Streptomyces* sp. LH4, a plant growth-promoting rhizobacteria (PGPR), when applied to colonize cucumber plant roots and enhance resistance against *S. sclerotiorum*. The results indicated that LH4 successfully colonized the root area promoting plant growth and proliferations. Applying *Streptomyces* spp. in the present paper resulted in changes including physiological responses of phenol, sugar, chlorophyll and protein contents of bean plants, indicating that their metabolites may have an excessive role as plant-growth-promoting, involvement in plant development and health. These results were consistent with those quoted by Nithya et al. (2020) who mentioned that *Streptomyces* applied plants had increased parameters such as carotenoid, sugars, nitrogen, amino acids, chlorophyll and protein contents. In the same respect, Cherif et al. (2007) mentioned that the percentage of chickpea blight disease (*Ascochyta rabiei*) reduction was correlated with the increase in free and total phenols. Further, Le et al. (2022) proved that *Streptomyces* sp. JCK-6131 revealed antagonistic activity in vitro against apple fire blight, tomato bacterial wilt cucumber, Fusarium wilt diseases caused by *Erwinia amylovora*, *Ralstonia solanacearum* and *Fusarium oxysporum f. sp. cucumerinum*, respectively. In vivo, JCK-6131 had suppressed the development of tomato bacterial wilt, cucumber Fusarium wilt and apple fire blight (on the detached leaf). Foliar application of tomato had induced pathogenesis-related (PR) genes and increased levels of salicylate (SA) as well as jasmonate (JA) in the treated plants compared with the untreated ones. They suggested that JCK-6131 played an efficient dual mechanism of antibiosis and induced resistance.

Conclusions

Obtained results confirmed that the foliar application of *S. griseus* (MT210913 "DG5"), *S. rochei* (MN700192 "DG4") and *S. sampsonii* (MN700191 "DG1") revealed a strong antifungal activity and inhibited the mycelial growth against various plant pathogenic fungi in vitro. It should be a valuable tool for controlling various plant pathogens, enhancing plant growth and yield of bean crop as well as reducing heavy usage of chemical

fertilizers and fungicides. *S. rochei* and *S. griseus* proved to have a strong antifungal activity and plant growth promotion.

Abbreviations
DG1: *S. sampsonii*; DG4: *S. rochei*; DG5: *S. griseus*; GA: Gibberellic acid; PAA: Phenylacetic acid; PAHs: Phthalate derivatives; VOCs: Volatile organic compounds.

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Author contributions
DG carried out laboratory works and analyses; GG supervisor, conceived, designed, led the study design, followed-up laboratory works and edited the manuscript. AA followed on some laboratory experiments. MR supervised some of the laboratory work. Two authors (NS and TM) are late/passed away. All authors read and approved the final manuscript.

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