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Impact of *Streptomyces* on sesame plants under *Macrophomina phaseolina* infestation

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Abstract

Background Sesame is an ancient oil crop that has been cultivated for centuries. It is an important source worldwide for food, industry and edible oil. Sesame plants are attacked by many pathogens during different stages of growth. *Macrophomina phaseolina* is considerable one of the most dangerous fungi that attacks sesame plants during their different growth stages. The impact of *Streptomyces violaceoruber* and *Streptomyces hirsutus* in comparison with Topsin-M fungicide on damping-off and charcoal rot caused by *M. phaseolina* and also on the growth of sesame plants was studied.

Results In general, *S. hirsutus* was effective more than *S. violaceoruber*. *S. hirsutus* reduced the linear growth of *M. phaseolina* *in vitro* by 70.83%, while reduction with *S. violaceoruber* reached 53.89%. *S. hirsutus* isolate reduced pre-, post-emergence damping-off, and charcoal rot incidence by 58.3, 56.6, and 50%, under greenhouse conditions, in 2021 growing season, while the percentage reduction of disease was 43, 56.4, and 71.2% for 2021 and 2022 growing seasons, respectively, under field conditions at Fayoum Governorate, Egypt. *Streptomyces* treatments increased concentration of nitrogen, phosphorus, and potassium (NPK) in plant leaves, seed yield, and seed oil concentration more than Topsin-M treatment and untreated plants.

Conclusions *S. violaceoruber* and *S. hirsutus* have proven that they can be used to combat soil-borne diseases, as well as improve growth parameters and increase yields.

Keywords Biological control, Charcoal rot, Damping-off, *Macrophomina phaseolina*, Sesame, *Streptomyces*, *Streptomyces violaceoruber*, *S. hirsutus*

Background

In Egypt, the cultivated area reached 76,997 feddan (Feddan=40% Hectare) in 2019 with an average production of 0.519 tons/ feddan (Anonymous 2019). However, sesame plants suffer from many diseases; among those are damping-off and charcoal rot caused by *M. phaseolina* considered one of the most destructive diseases on sesame plants, especially in Upper Egypt where hot, dry weather and unfavorable environmental conditions stress the plant (Abdou et al. 2001).

Macrophomina phaseolina (Tassi) Goid is a worldwide soil-borne fungus that affects about 500 cultivated and wild plant species (Khan 2007). Collar rot, damping-off, charcoal rot, stem rot, root rot, and seedling blight are some of the most important diseases caused by *M. phaseolina* (Babu et al. 2007). Several methods were suggested to control soil-borne diseases of sesame include chemical control, resistant cultivars, and biological control (Amin and Shoukry 2017). Applying biotic and abiotic agents protects sesame plants from several soil-borne diseases for a long time and significantly increases the yield. Several bio-control agents such as *Streptomyces* spp., *Trichoderma* spp., *Bacillus subtilis*, and VA mycorrhizae were able to control root diseases of sesame in the field as reported by Amin and Shoukry (2017).

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Actinomycetes are filamentous bacteria widely distributed in soils and make up 10 to 50 percent of the total microbial community in both virgin and cultivated lands, producing several important secondary metabolites, antibiotics, and lytic enzymes (Khan et al. 2023). Many researchers recorded the antagonistic effect of *Streptomyces* or their active ingredient against several plant pathogenic fungi and play a role as plant-promoting bacteria (Amin and Shoukry 2017).

This investigation aimed to study the capability of *S. violaceoruber* and *S. hirsutus* as seed treatment to combat damping-off and charcoal rot incidence on sesame plants. Furthermore, the effect of treatments on the growth, the content of NPK in plant leaves, seed yield, and oil concentration was also studied.

Methods

The pathogen

Isolate of *M. phaseolina* was provided by Onion, Garlic and Oil Crops Disease Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt. Barley seed medium consisting of autoclaved 100 g seeds and 120 ml tape water was inoculated using seven-day-old *M. phaseolina* culture and incubated for 2 weeks at 30 ± 1 °C to use as inoculum (Van der Meer et al. 1983).

The host

Giza 32 sesame seeds were kindly provided from Oil Crops Research Department, Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt. Atrophied seeds showing any signs of disease were excluded, and apparently healthy were surface sterilized for 2 min. in sodium hypochlorite 3%; then, it was washed thoroughly with sterilized distilled water and dried under aseptic conditions.

The bio-agent

Streptomyces violaceoruber and *S. hirsutus* were kindly obtained from Amin and Shokry (2017) were cultured in 250-ml conical flasks, each containing 125 ml of inorganic salt starch (ISS) broth medium. The flasks were carried out and incubated on a rotary shaker (120 rpm) at 30 ± 1 °C for 7 days. According to Callan et al. (1990), the bacterial count was adjusted to 1×10^9 cfu/ ml. For five min, sesame seeds were mixed with 1% bacterial suspension and 1% Arabic gum and left to dry just before sowing.

Fungicide

Topsin-M70% WP consists of thiophanate-methyl (dimethyl 4, 4'-o-phenylenebis [3-thioallophanate]), Nippon Soda Company, Japan, was used in this investigation as a check

control. The prepared sesame seeds were mixed with the fungicide at the rate of 0.3% supplemented with 1% Arabic gum for 5 min. and then left to dry just before sowing.

In vitro antagonistic effect

Seven-day-old cultures of *S. violaceoruber*, *S. hirsutus*, and *M. phaseolina* were used. A streak of bacteria was made at a 1 cm distance from the edge of 9-cm sterilized Petri dishes containing 10 ml of PDA medium, and on the other side, 6-mm disk of fungal growth was placed. Five plates were used for each isolate and control (only fungus). Plates were incubated at 30 ± 1 °C. Percentage of the fungal growth reduction was calculated after full growth of control plates (Amin and Ahmed 2023).

Greenhouse experiment

The pot experiment was performed under greenhouse conditions during the 2021 growing season. The autoclaved clay soil was potted into 30-cm plastic pots. Seven days before seed sowing, 20 g of *M. phaseolina* inoculum was added per kilogram of soil. Four pots were used as replicates for each treatment and control (untreated seeds). In each pot, five holes were made with a pencil, and then, 3 seeds were seeded in each hole on the 1st of June, and after 30 days from sowing, one plant was left for each hole. Plants were irrigated when needed.

Field experiment

The field experiment was carried out in clay soil naturally infested with *M. phaseolina* at Fayoum Governorate, Egypt, during the 2021 and 2022 growing seasons in complete randomized plots. Three plots were made for each treatment and control (untreated seeds). Each plot consisted of 12 m², 4 rows, 35 hills/ row seeded with 5 seeds/ hill on the 15th of June in each season, and after 30 days from seed sowing one plant was left for each hill. The recommended cultural practices were adopted until harvest.

Disease assessment

Under greenhouse and field conditions, the percentage of pre-, post-emergence damping-off, and charcoal rot incidence was estimated after 15 and 30 days from seed sowing and at the end of the season, respectively, according to the following formulas:

$$\begin{aligned} & \% \text{ pre-emergence damping-off} \\ &= \frac{\text{Number of non germinated seeds}}{\text{Number of sown seed}} \times 100 \end{aligned}$$

$$\begin{aligned} & \% \text{ post-emergence damping-off} \\ &= \frac{\text{Number of dead seedlings}}{\text{Number of survival seedlings}} \times 100 \end{aligned}$$

$$\% \text{ charcoal rot} = \frac{\text{Number of infected plants}}{\text{Number of examined plants}} \times 100$$

NPK content

In each season under field conditions at 60 days from seed sowing, one hundred leaves from the top of random plants were collected from each replicate. Three leaves were taken, and the content of NPK was measured as described by Jackson (1967).

Seed yield and oil concentration

Under field conditions at the end of both seasons, seed yield (Ton/ feddan) was determined and seed oil concentration was estimated according to A.O.A.C. (1980).

Statistical analysis

The obtained data were statistically analyzed, and the least significant difference (LSD) was assessed using SAS ANOVA program V.9 at 5% probability level (Anonymous 2014). The statistical model was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} represents observation, μ the overall mean, T_i effect of treatment (experimental group), and e_{ij} experimental error.

Table 1 Influence of *Streptomyces violaceoruber* and *S. hirsutus* on linear growth* of *M. phaseolina* on PDA medium at 30 ± 1 °C

Isolates	Linear growth (mm)	Reduction (%)
<i>Streptomyces violaceoruber</i>	41.50 ^b	53.89
<i>S. hirsutus</i>	26.25 ^c	70.83
Control	90.00 ^a	–

Means with the same letter are not significantly different at $p = 0.05$

*Linear growth was recorded when the control plates were filled

Table 2 Impact of Giza 32 sesame seed treatment by *Streptomyces violaceoruber*, *S. hirsutus*, and Topsin-M on pre-, post-emergence damping-off, and charcoal rot incidence* caused by *M. phaseolina* under greenhouse conditions in 2021 growing season

Treatment	Damping-off (%)				Charcoal rot	
	Pre-emergence	Efficacy	Post-emergence	Efficacy	(%)	Efficacy
<i>Streptomyces violaceoruber</i>	13.3 ^b	33.5	13.5 ^b	19.3	20.0 ^{ab}	33.3
<i>S. hirsutus</i>	8.4 ^c	58.3	7.3 ^c	56.6	15.0 ^b	50.0
Topsin-M	5.0 ^c	74.9	7.0 ^c	58.1	10.0 ^b	66.7
Control	20.0 ^a	–	16.7 ^a	–	30.0 ^a	–

Means with the same letter are not significantly different at $p = 0.05$

*At 15 and 30 days from seed sowing for pre- and post-emergence damping-off. And at the harvest for charcoal rot

Results

In vitro antagonistic effect

Data presented in Table 1 show that the two tested isolates significantly reduced the linear growth of *M. phaseolina* on PDA medium. Linear growth of *M. phaseolina* reduced by 53.89% in the presence of *S. violaceoruber* and 70.83% with *S. hirsutus* compared to control.

Disease incidence

Data presented in Table 2 demonstrate the role of *S. violaceoruber* and *S. hirsutus* as biological control in comparison with Topsin-M fungicide on controlling pre-, post-emergence damping-off, and charcoal rot disease on sesame plants during the 2021 season under greenhouse conditions. All tested treatments led to a significant decrease in pre-, post-emergence damping-off, and charcoal rot incidence than the control. Infection decreased from 20, 16.7, and 30% in control to 8.4, 7.3, and 15% with *S. hirsutus*, followed by 13.3, 13.5, and 20% with *S. violaceoruber*, while Topsin-M treatment gave 5, 7, and 10% infection for pre-, post-emergence damping-off, and charcoal rot, respectively. In general, *S. hirsutus* treatment was superior *S. violaceoruber* treatment in control tested diseases with non-significant difference with the recommended fungicide.

Regarding the effect of *S. violaceoruber*, *S. hirsutus* as bio-agents in comparison with Topsin-M fungicide on controlling pre-, post-emergence damping-off and charcoal rot in sesame plants under field conditions, the data presented in Table 3 showed that all treatments significantly reduced damping-off as well as charcoal rot disease incidence. *S. hirsutus* was more powerful in controlling damping-off and charcoal rot in sesame plants under field conditions than *S. violaceoruber*. According to the mean values, infection decreased from 10.9, 19, and 57.8% with control to 6.2, 7.4, and 16.7% with *S. hirsutus*, followed by 8.3, 9, and 19.6% with *S. violaceoruber*, while Topsin-M gave 4.9, 6.3, and 13.3% infection for pre-, post-emergence damping-off and charcoal rot, respectively.

NPK content

As a result of treatment with *S. violaceoruber*, *S. hirsutus*, and Topsin-M, the concentration of NPK in plant leaves increased significantly than untreated plants as shown in Table 4. According to the mean values, *S. hirsutus* recorded a 39.02, 168.18, and 31.1% increase, followed by 16.46, 81.82, and 17.7% with *S. violaceoruber*, while the least increase was recorded with Topsin-M than the control treatment; which means that bio-treatments outperformed Topsin-M, and *S. hirsutus* also outperformed *S. violaceoruber* in the same context.

Seed yield and oil concentration

Table 5 shows that all treatments increased seed yield and the oil concentration more than control. *S. violaceoruber* and *S. hirsutus* treatments were more effective than Topsin-M. According to the mean values, seed yield (Ton/ feddan) and its oil concentration increased from 0.32 and 49% with untreated plants to 0.44 and 53.1% with *S. hirsutus*, followed by 0.39 and 53.1% oil with *S. violaceoruber*, while Topsin-M treatment gave 0.37 and 50.6%, respectively.

Table 3 Impact of Giza 32 sesame seed treatment by *Streptomyces violaceoruber*, *S. hirsutus*, and Topsin-M on pre-, post-emergence damping-off, and charcoal rot incidence* in soil naturally infested by *M. phaseolina*, in 2021 and 2022 growing seasons at Fayoum Governorate, Egypt

Treatment	Damping-off (%)								Charcoal rot(%)			
	Pre-emergence				Post-emergence				2021	2022	Mean	Efficacy
	2021	2022	Mean	Efficacy	2021	2022	Mean	Efficacy				
<i>Streptomyces violaceoruber</i>	9.5 ^b	7.1 ^b	8.3	23.2	8.6 ^b	9.4 ^b	9.0	47.2	20.6 ^b	18.5 ^b	19.6	66.2
<i>S. hirsutus</i>	6.6 ^c	5.7 ^c	6.2	43.0	7.7 ^b	7.2 ^c	7.4	56.4	18.5 ^b	14.8 ^b	16.7	71.2
Topsin-M	4.5 ^d	5.2 ^c	4.9	55.0	6.4 ^c	6.3 ^d	6.3	62.8	15.6 ^b	11.1 ^b	13.3	76.9
Control	11.3 ^a	10.4 ^a	10.9	–	17.3 ^a	16.8 ^a	19.0	–	60.0 ^a	55.6 ^a	57.8	–

Means with the same letter are not significantly different at $p = 0.05$

*At 15 and 30 days from seed sowing for pre- and post-emergence damping-off. And at the harvest for charcoal rot

Table 4 Impact of Giza 32 sesame seed treatment by *Streptomyces violaceoruber*, *S. hirsutus*, and Topsin-M on NPK* content in leaves of sesame plants growing in soil naturally infested by *M. phaseolina* in 2021 and 2022 growing seasons at Fayoum Governorate, Egypt

Treatment	N (%)				P (%)				K (%)			
	2021	2022	Mean	Increase	2021	2022	Mean	Increase	2021	2022	Mean	Increase
<i>Streptomyces violaceoruber</i>	0.95 ^b	0.96 ^b	0.96	16.46	0.18 ^b	0.22 ^b	0.20	81.82	1.18 ^b	1.28 ^b	1.23	17.70
<i>S. hirsutus</i>	1.11 ^a	1.29 ^a	1.14	39.02	0.29 ^a	0.30 ^a	0.30	168.18	1.34 ^a	1.40 ^a	1.37	31.10
Topsin-M	0.81 ^{bc}	0.86 ^b	0.84	1.83	0.14 ^c	0.16 ^c	0.15	36.36	1.17 ^b	1.22 ^c	1.20	14.35
Control	0.77 ^c	0.87 ^b	0.82	–	0.11 ^d	0.11 ^d	0.11	–	0.95 ^c	1.14 ^d	1.05	–

Means with the same letter are not significantly different at $p = 0.05$

*At 60 days from seed sowing

Table 5 Impact of Giza 32 sesame seed treatment by *Streptomyces violaceoruber*, *S. hirsutus*, and Topsin-M on seed yield and its oil concentration of sesame plants growing in soil naturally infested by *M. phaseolina*, in 2021 and 2022 growing seasons, at Fayoum Governorate, Egypt

Treatment	Seed yield (Ton/ feddan)				Oil concentration (%)			
	2021	2022	Mean	Efficacy	2021	2022	Mean	Efficacy
<i>Streptomyces violaceoruber</i>	0.40 ^b	0.38 ^{ab}	0.39	20.4	52.6 ^a	53.5 ^a	53.1	8.2
<i>S. hirsutus</i>	0.46 ^a	0.43 ^a	0.44	37.0	53.0 ^a	54.1 ^a	53.1	9.2
Topsin-M	0.37 ^a	0.36 ^{bc}	0.37	12.7	50.3 ^b	50.8 ^b	50.6	3.1
Control	0.32 ^c	0.32 ^c	0.32	–	48.9 ^c	49.2 ^c	49.0	–

Means with the same letter are not significantly different at $p = 0.05$

Discussion

Macrophomina phaseolina is one of the most important pathogens that attack sesame plants at all stages of growth all over the world and cause many diseases such as pre-, post-emergence damping-off, and charcoal rot.

This study clarified the inhibitory effect of *S. hirsutus* and *S. violaceoruber* against *M. phaseolina* *in vitro*, and considerable protection of sesame plants against *M. phaseolina* from pre-, post-emergence damping-off, and charcoal rot during the season as seed dressing. The biological activities of *Streptomyces* against numerous plant pathogenic fungi such as *Rhizoctonia solani*, *Fusarium* sp., *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, and *Fusarium oxysporum* f. sp. *lycopersici* was recorded and described (Olanrewaju and Babalola 2019). Mujoko et al. (2014) found eight isolates of *Streptomyces* inhibited *F. oxysporum* f.sp. *lycopersici*. Also, results are comparable with Yuan and Crawford (1995) against a wider range of pathogenic fungi. Chung and Hong (1991) recorded a reduction in infection by *F. oxysporum* f.sp. *vasinfectum* and *Phytophthora nicotiana* var. *parasitica* on tomato plants as soil treatment with *Streptomyces* St-11. As well, *Streptomyces lydicus* and *Streptomyces* sp. were used to control damping-off on tomatoes and peas caused by *R. solani* and *Pythium ultimum*, respectively, as described by Yuan and Crawford (1995). El-Abyad et al. (1993) stated that using *S. pulcher*, *S. canescens*, and *S. citreofluorescens* controlled the diseases caused by *Fusarium oxysporum*, *Verticillium albo-atrum*, *Alternaria solani*, *Pseudomonas solanacearum*, and *Clavibacter michiganensis* sub sp. *michiganensis* in tomatoes and significantly improved the growth.

Streptomyces have important features as they belong to the *Actinomycetes*, which make up 10 to 50% of the total microbial community in both virgin and cultivated lands (Ajijur Rahman et al. 2011). ISP recognized 450 species of *Streptomyces* and *Streptoverticillium* (Kim et al. 2004). *Streptomyces* can utilize the available organic molecules via extracellular hydrolytic enzymes; some species are thermophilic; furthermore, in comparison with other bacteria that are less motile, the motility of vegetative filaments has a significant advantage in colonizing the soil; and its resistance to desiccation and nutrient stress via sporulation. Most *Streptomyces* are efficient rhizosphere and rhizoplane colonizers. They can also be endophytes colonizing the inner tissues of host plants (Sousa and Olivares 2016).

Suppression by bacteria is generally attributed to one or more mechanisms. These mechanisms include competition, parasitism, cross-protection and induced resistance via produce extracellular inhibitory substances, antibiotics; and other cell-wall lysis enzymes (Olanrewaju and Babalola 2019). According to many

reports, *Streptomyces* remains a significant source of antifungal substances such as Levorin, DPTB16, 24-Demethylbafilomycin C1, Phenyl-1-naphthyl-phenyl acetamide, and (6S, 8aS, 9S, 11S, 12aR)-6-hydroxy-9,10-dimethyldecahydrobenzo [d] azecine-2,4,12(3H)-trione (Wu et al. 2009). *Streptomyces* produce approximately 100,000 antibiotic compounds, representing 70–80% of all natural bioactive products used in pharmacological or agrochemical applications (Abdel-Razek et al. 2020). In addition, various *Streptomyces* release extracellular enzymes such as β -glucosidases, α -amylase, chitinases, cellulases, so it can utilize fungi cell-wall components in dead and living mycelia (Chater et al. 2010).

Sesame plants under treatment with *S. hirsutus* and *S. violaceoruber* in this investigation showed an increase in NPK content in leaves when tested 60 days after seed sowing and also showed an increase in seed yield and oil concentration compared to untreated plants or Topsin-M treatment. These microorganisms can enhance plant health and nutrient availability, as emphasized by Qiu et al. (2019). Gopalakrishnan et al. (2015) stated that *Streptomyces* sp. enhanced nodule number, nodule weight, root weight, shoot weight, pod number, pod weight, leaf area, leaf weight and stem weight of chickpea plants (*Cicerarietinum* L.) and significantly increased *Arabidopsis* and *Brassica* sp. vegetative growth and enhanced tolerance to abiotic stress (Manullang and Chuang 2020). Also, Doolotkeldieva et al. (2015) recorded increases in germination and seed vigor in wheat and soybean by *Streptomyces fumanus* (Gn-2) as a seed treatment. *S. lydicus* strain WYEC 108 increased shoot and root length, and root wet weights in pea seedling. Also, root nodulation, nodule size, number of *Rhizobium* spp. per nodule, nitrogenase activity and nodular assimilation of iron were observed (Tokala et al. 2002). The promoting effect of *Streptomyces* on plant growth may be back to their capability to produce promoting substances such as indole acetic acid, siderophores, dehydrogenase, lytic enzymes, vitamins, alkaloids, enhance soil biological and mineral nutrients, total nitrogen, available phosphorous and organic carbon and finally control different plant pathogenic fungi as mentioned (Tokala et al. 2002).

Conclusion

It was concluded that *Streptomyces hirsutus* and *S. violaceoruber* showed promising results in controlling pre- and post-emergence damping-off and charcoal rot in sesame plants caused by *Macrophomina phaseolina*. They also increased the leaf content of NPK, seed yield, and oil concentration when used as seed treatment along with Topsin-M fungicide for comparison. Both isolates

inhibited the linear growth of *M. phaseolina* on PDA medium. They effectively reduced pre- and post-emergence damping-off compared to the untreated plants. Additionally, *S. hirsutus* exhibited no significant difference compared to Topsin-M fungicide under greenhouse conditions. Furthermore, the incidence of charcoal rot was significantly controlled compared to the untreated plants, with no significant difference compared to Topsin-M. On the other hand, both isolates increased the leaf content of NPK, seed yield, and oil concentration more than Topsin-M. Overall, *S. hirsutus* demonstrated greater efficiency than *S. violaceoruber*.

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Author contributions

All authors contributed to the study conception and design, and commented on and approved the final manuscript.

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Availability of data and materials

The data and materials are available for other researchers.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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