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ORIGINAL PAPER

Influence of Certain Streptomyces Isolates on the Management of Gray Mold in Strawberries

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ABSTRACT

Strawberry is a very important crop in Egypt for local consumption and export. Gray mold, caused by *Botrytis cinerea*, represents one of the most severe diseases in pre- and post-harvest stages. In this study, twelve isolates of Streptomyces were obtained from healthy-looking strawberries collected from local markets. All isolates inhibited the linear growth of the pathogen on medium *in vitro*, and the most promising isolates identified based on their morphological and biochemical characteristics were *Streptomyces lavendulae, S. glaucescens, S. albus, S. violaceoruber*, and *S. flavotricini*. All five isolates effectively protected strawberry fruits from gray mold when applied as foliar treatments on Festival and Fortuna strawberry varieties. *S. glaucescens* provided 96.3 and 87.3% protection compared to 0.0% in the control, and reducing the infected area in infected fruits to 32.5 and 49.8% compared to 83.9 and 81.2% in the control for Festival and Fortuna, respectively. Soluble solids content, vitamin C, and color density showed no significant differences among treated strawberries with such *Strptomyces* isolates, or they decreased. However, the levels of firmness and titratable acidity varied depending on the varieties.

Key words: Biological control, *Botrytis cinerea*, post-harvest diseases, strawberry, gray mold

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INTRODUCTION

Strawberry (*Fragaria* X *ananassa* Duch.), a temperate crop, is one of the most economically significant fruit crops, offering substantial nutritional value and health benefits worldwide. Covering a total area of 29,944 feddan (12,579 hectares), Egypt produces 470,913.10 tons of strawberries annually (Anon., 2021), ranking it fourth globally, following the United States, Turkey, and Spain (Moussa *et al*., 2019). During pre- and post-harvest stages, gray mold caused by *Botrytis cinerea* is one of the most dangerous diseases on strawberry causing economic losses in production (Rhouma *et al*., 2022).

Botrytis cinerea Pers. Fr. is an airborne plant pathogen belonging to Sclerotiniaceae with a necrotrophic lifestyle attacking more than 200 species, particularly dicotyledonous fruits, vegetables, and ornamental plants, causing gray mold worldwide (Jarvis, 1977 and Fillinger and Elad, 2016). *B. cinerea* infects plants by producing a range of cell wall-degrading enzymes, toxins, and other low-molecular-weight compounds such as oxalic acid, where the fungus triggers the host to produce programmed cell death (Dean e*t al*., 2012).

B. cinerea, is essentially managed through the use of fungicides. However, increasing global consumer demand for decreased fungicide usage, along with the emergence of chemical-resistant pathogens, has underscored the need for alternative control methods to control gray mold disease (Kim *et al*., 2007). Biological control has emerged as an appropriate alternative to fungicides for *B. cinerea* control, particularly they do not show cross resistant and are safe for the environment (Iqbal *et al*., 2022). Biological control includes use of microorganisms and/or their secreted molecules to prevent diseases, making it an active alternative, work in numerous ways, such as parasitism, induced resistance, competition for nutrition,

and the formation of bioactive metabolites (Spadaro and Droby, 2016). Actinomycetes are recognized as promising sources of antibiotics and lytic enzymes, which have applications in both medical and industrial fields (Roberts *et al*., 2005). Although only a limited number of taxa, primarily from the genus Streptomyces, have been explored as potential biocontrol agents against fungal plant pathogens, *Streptomyces* spp. can promote plant growth and control pests, diseases, weeds and phytopathogenic microorganisms by producing phytohormones (mainly IAA), siderophores, enzymes, volatile organic compounds, antibiotics, and other secondary metabolites (Nazari *et al*., 2023). In addition, *Streptomyces* spp. can enhance nutrients bioavailability and alleviate abiotic stresses, such as salinity, drought, and organic and inorganic contaminants in soil. Several antibiotics have been isolated from different *Streptomyces* species and are commercially utilized to control plant diseases. For instance, natamycin, isolated from *Streptomyces natalensis*, is effective against postharvest gray mold (He *et al*., 2019). Lucensomycin and rapamycin, derived from various *Streptomyces* spp., are employed to manage gray mold on grapes (Shi *et al*., 2018). Wuyiencin, a soluble secondary metabolite produced by *Streptomyces albulus* CK-15, effectively prevents and controls a range of plant pathogens, including *B. cinerea* and *Sclerotinia sclerotiorum* on tomatoes and strawberries (Yang *et al*., 2021).

This investigation aimed to study which species of the twelve *Streptomyces* isolates could protect strawberries from *B. cinerea* and how to maintain fruit quality within the framework of sustainable agriculture seeking to enhance the biodiversity of the agricultural system and ensuring a supply of high-quality food by minimizing the chemical toxicity in food contaminated through the use of fungicides.

MATERIALS AND METHODS

Botrytis cinerea

Isolation

Rotted strawberries showing typical symptoms of gray mold were collected from local markets. Fruits were surface disinfected with sodium hypochlorite (1% active chlorine) for 2 minutes, washed several times with sterilized distilled water and dried by sterilized filter papers, then left to dry in Laminar air-flow. Small portions were placed in 9 cm Petri dishes containing water agar medium and incubated at 23±1°C for 3-5 days. The emerging fungi were picked up, microscopically examined and purified using the hyphal tip or single spore technique. Fungi were incubated on potato dextrose agar plates and slants for 7 days on light and dark every 12 hours. Slants were stored at 4±1°C for further studies, while plates were used for identification and preparation of spore suspension.

Identification

Based on the morphological characteristics of the colony, mycelium, shape and measurements of the spores, the fungus identification was adopted with a light microscope according to Hennebert (1973).

Spores suspension

B. cinerea spore suspension was prepared using sterilized distilled water mixed with 0.05% Tween 20. Spores were harvested using a fine camel hair brush from the surface of the PDA Petri dishes. The spore concentration was adjusted to 5×10^4 spores/ml using a hemocytometer (Sinclair and Dhingra, 1995).

Streptomyces spp.

Isolation

Healthy-looking strawberry fruits were collected from markets, and each fruit was put into a one-liter beaker containing 200 ml of sterilized distilled water with 0.05% Tween 20. The beakers were placed on a rotary shaker at 120 rpm for 15 min. Serial dilutions were then performed up to 10^3, and 100 µl of each dilution was spread onto 9 cm Petri dishes containing inorganic salt starch agar medium (ISP4) (Shirling and

Gottlieb, 1966). Three plates were prepared for each dilution and incubated at 27±1°C for 4 to 7 days. Colonies showed up were selected and purified by streaking, as outlined by Williams and Davis (1965), and stored on ISP4 slants at 4±1°C for further studies.

Cultures filtrate and bacterial suspension

To prepare the bacterial suspension and culture filtrate, isolates were cultured in 250 ml conical flasks, each containing 125 ml of inorganic salt starch broth medium (ISS). The flasks were incubated on a rotary shaker at 120 rpm, 27±1°C for 7 days. After incubation, the cultures were blended for 5 minutes and then adjusted to 1×10^6 cfu/ml using a hemocytometer (Callan *et al*., 1990). For culture filtrate, the blended broth was filtered through double layers of muslin, centrifuged for 10 minutes at 3000 rpm, and then filtrated through a glass filter (0.2µm) to obtain the culture filtrate free from bacterial cells.

Disk-diffusion

The culture filtrate collected from each *Streptomyces* isolate was used to prepare antibiotic disks. Ten µl of the filtrate were added to 6 mm filter paper disc, then left to dry under aseptic conditions. One milliliter of *B. cineria* spore suspension was carefully spread over the surface of 9 cm Petri dishes containing NYDA medium. After allowing 15 minutes for the spores to settle, five discs of the filtrate representing each isolate were placed inside each plate, with each disc occupying a circle with a 3 cm diameter, incubated at 27±1 °C, with five plates as replications for each treatment. The diameters of the inhibition zones were measured after 48 hours (Jain and Jain, 2005).

Identification

As a result of the disk diffusion test, the taxonomic properties of the promising Streptomyces strains (St2, St3, St4, St7, and St9) were evaluated based on morphological and physiological criteria. This evaluation followed the methods outlined in the International Streptomyces Project (ISP) to reach a possible classification for the genus and species confirmation, as referenced in the works of Shirling and Gottlieb (1966), Holt *et al*. (1994), Cappuccino and Sherman (2005) and William *et al*. (2012). The color of the aerial and substrate mycelium, the shape of the sporophore, the growth rate (rated as negative (-), very weak (+/-), and from 1+ to 4+), and the Gram stain were examined using a light microscope for 14 days old cultures grown on different media including, yeast extract malt extract agar (ISP2), oatmeal agar (ISP3), inorganic salts starch agar (ISP4), glycerol asparagine agar (ISP5), tyrosine agar (ISP7), Gause' synthetic agar (GST), starch casein nitrate agar (SCNA) and glucose yeast-malt agar (GYM) . Furthermore, various carbon sources, including glucose, lactose, dextrin, inositol, cellulose, L-sorbose, D-xylose, sodium citrate, sodium acetate, and arabinose, were evaluated on ISP9 medium (carbon utilization medium). Additionally, ammonium, nitrate, and urea were tested as nitrogen sources. Also, the utilization of casein and gelatin, along with the production of melanin and lipase, was observed on ISP6 medium (peptone yeast extract iron agar).

Field application

Streptomyces isolates, St2, St3, St4, St7, and St9 were used to evaluate their impact on *B. cinerea* under field conditions. Fresh strawberry transplants of the "Festival" and "Fortuna" varieties were planted under plastic tunnels on September 1st for the 2023 and 2024 seasons at Sadat City, Menofia Governorate, Egypt. The experiments were designed as complete randomized blocks, with three plots (20*0.5 m for each) used as replicates for each treatment and control group. All treatments received the same recommended agricultural practices. Three foliar sprays of the treatments were applied every other week, starting from October 15th.

Disease assessment and Physicochemical properties

Healthy, non-damaged, semi-ripe fruits (around 75% red coloration) were carefully harvested, pre-cooled at 0-2°C for 6 hours, and then 50 fruits were placed into a plastic box for each replicate, with 5 replicates for each treatment. All boxes were incubated at 0-2°C and 95% relative humidity for 30 days. After this period, the protection level and percentage of infected area in infected fruits were measured, along with firmness (F), soluble solid content (SSC), vitamin C (VC), titratable acidity (TA), and color density (CD) of the fruits as follows.

Disease assessment

After storage, both percentage of protection and infected area in infected fruits only were determined according to Yong *et al*. (2022).

Fruit firmness

Firmness was measured using a McCormick penetrometer (FTo-11) with a range of 0-11 lbs. A 5/16 plunger tip was used, and the measurement was expressed as gram per square inch (g/inch^2).

Soluble solid content

SSC was measured in fruit juice using a hand-held refractometer (model PZORR, m/m, 30-130-0Ochsle), and the measurement was expressed as a percentage (%).

Vitamin C

One hundred grams of fruits were blended with 100 ml of 6% oxalic acid for 3 minutes and filtered using double layers of cheesecloth. A 200 µl of the filtrate were diluted with 100 ml of 3% oxalic acid, vortexed for 2 minutes, and then centrifuged for 5 minutes at 4000 rpm. From the obtained supernatant, 10 ml were used for titration with 2,6-dichlorophenolindophenol stain until a pink color developed. The amount of vitamin C was calculated using the formula provided by Jemy and Kovacs (1968), and the measurement was expressed as mg ascorbic acid per 100 g fresh sample.

Titratable acidity

Ten grams of a 20-30 homogenate fruit mixture were combined with deionized water to achieve a total of 100 grams and titrated to a pH of 8.1 using 0.1 N. NaOH. The titratable acidity (TA) was calculated according to the formulas described by Morris *et al*. (1985), and the measurement was expressed as % citric acid.

Color density

The color density was visually determined using the following scale: (5) full red, (4) 75% red, (3) 50% red, (2) 25% red, and (1) no red color.

Statistical analysis

The obtained data were statistically analyzed and the significant differences among means were assessed by least significant difference (LSD) at 5% probability level using SAS ANOVA program V.9 (Anon., 2014). The statistical model used was: $Yij= \mu + Ti + eij$

Where: Yij = observed value, μ = overall mean, Ti = treatment effect (experimental group), eij = experimental error.

RESULTS

Identification of the isolated fungi

Various types of fungal growth were observed on PDA. The colonies appeared cottony to powdery, exhibiting either compact or radial growth patterns. Initially, the colonies are hyaline in color, transitioning to white or dirty white, and eventually to grayish white. Over time, they develop into light gray, dark gray or dark brown. The mycelium is hyaline to brown, branched, and septate. Conidiophores arise directly from the mycelium, mostly straight, septate, and branched at the apex, often in a dichotomous or trichotomous arrangement. Both the conidiophores and conidia have a grape bunch-like shape. Each conidium is a single cell hyaline with an egg-like shape.

The dimensions of the conidia ranged from 12 to 16µm in length and 9 to 12µm in width. According to the aforementioned cultural and morphological characteristics ;the isolated fungus was identified as *Botrytis cinerea* Pers. Fr.

Disk-diffusion

As a result of the disk diffusion test, all *Streptomyces* isolates displayed varying levels of inhibition against *B. cinerea* mycelial growth, as shown in Table (1). The diameters of the inhibition zones ranged from 21.5 mm to 1.0 mm. The most effective isolate was St3, which produced a 21.5 mm inhibition zone, followed by St4 with 16.0 mm, St7 with 15.0 mm, St2 with 12.4 mm, and St9 with 11.5 mm. The inhibition zones for the other isolates ranged from 1.0 to 5.0 mm.

Identification of *Streptomyces* spp.

In this part of the study, *Streptomyces* isolates St2, St3, St4, St7, and St9, as the most promising candidates against *B. cinerea*, were identified in the laboratory according to their morphological and biochemical characteristics as shown in Tables (2, 3 and 4). Data in Table (2) indicate that all isolates were Gram-positive (G+) and exhibited strong growth (4+) on all tested media. The types of sporophores varied from rectus shape with St2, spiral with St3 and St7, flexible with St4, and formed hooks with St9. The color of both substrate and aerial mycelium also varied among the isolates in different media, ranging from white to black.

Table 1. Effect of *Streptomyces* isolates (St) culture filtrate on growth of *B. cinerea* on NYDA medium after 48 hours incubation at 27±1° C

Average inhibition zone diameter (mm)												SE
St1	St2	St3	St4	St5	St6	St7	St8	St9	St10	St11	St12	
2.0^{i}	12.4^{d}	21.5^{a}	16.0^{b}	3.0^{h}	3.9^{f}	15.0^{c}	5.0^{f}	11.5^{e}	1.0^{j}	4.5^{f}	3.2^{gh}	0.26

Table 2. Color of aerial (A) and substrate mycelium (B), sporophore shape, growth rate on different media and gram stain of the St2, St3, St4, St7 and St9 *Streptomyces* isolates

Media	St2		St3		St4		St7		St9	
	A	B	A	B	A	B	A	B	A	B
ISP2	Yellowish white	Yellowish brown	Gray	Yellowish gray	White	Yellowish gray	White	Yellowish brown	White	Yellowish brown
ISP3	Whitish gray	Yellowish brown	Dark gray	Dark green	Whitish gray	Yellowish gray	Dark green	Yellowish brown	Gray	Yellowish brown
ISP4	White	Yellowish brown	Dark gray	Dark brown	Purple	Yellowish gray	Whitish gray	Brownish red	White	Yellowish brown
ISP5	White	Yellowish brown	Bluish gray	Brownish gray	Whitish gray	Yellowish brown	Gray	Yellowish red	Gray	Yellowish brown
ISP7	White	Yellowish brown	Gray	Yellowish brown	Purplish gray	Yellowish green	Dark green	Yellowish brown	Gray	Yellowish brown
GST	White	Yellowish brown	Whitish gray	Yellowish brown	White	Yellowish brown	White	Yellowish olive	Gray	Yellowish brown
SCNA	Whitish gray	Yellowish brown	Dark green	Dark green	White	Yellowish green	Whitish gray	Greenish brown	White	Yellowish brown
GYM	White	Yellowish brown	Gray	Yellowish brown	Brownish gray	Yellowish brown	Yellowish green	Yellowish brown	White	Yellowish brown
Sporephore shape	Rectus		Spiral		Flexibilis		Spiral		Hooks	
Growth rate	4+									
Gram stain	G+									

The utilization of various carbon and nitrogen sources differed among the isolates, as described in Table (3). The growth rate ranged from negative (-) to strong positive (4+) depending on the carbon and nitrogen sources used in the ISP9 medium. Only St3 and St4 were able to utilize L-sorbose, while the other isolates could not. Furthermore, St4 was unable to use sodium citrate as a carbon source or urea as a nitrogen source, and utilized cellulose as a carbon source with a very weak rate (+/-). Also, utilization of urea as a nitrogen source by isolate St2 was very weak (+/-).

Data presented in Table (4) indicate that all isolates utilized casein and gelatin, produced melanin pigment in various colors, and also exhibited lipase production. The growth rates ranged from 1+ to 4+. The zone diameter for casein utilization varied from 1.4 mm for St9 to 7.8 mm for St3, while the zone diameter for lipase production ranged from 17 mm for St4 to 31 mm for St3. The liquefaction rate of gelatin for all isolates rated as 3+ except for St7, which was rated as 2+. The yellow melanin pigment was produced by St2 and St9, brown by St3 and St7, and red-brown by St4.

Table 3. Effect of different carbon and nitrogen sources on growth of *Streptomyces* isolates after 7 days incubation at 27±1° C on ISP9 medium

Source		Isolates				
		St2	St3	St4	St7	St9
Carbon	Glucose	4+	4+	3+	4+	3+
	Lactose	3+	4+	3+	3+	3+
	Dextrin	3+	4+	3+	3+	3+
	Insole	2+	3+	3+	3+	3+
	Cellulose	2+	3+	+/-	3+	2+
	L- Sorbose	-	3+	2+	-	-
	D-Xylose	3+	4+	3+	3+	3+
	Sodium citrate	2+	4+	-	2+	3+
	Sodium acetate	2+	3+	3+	2+	3+
	Arabinose	3+	3+	2+	3+	3+
Nitrogen	NH4	3+	4+	3+	2+	2+
	NO3	3+	3+	3+	2+	2+
	Urea	+/-	3+	-	1+	1+

Table 4. Utilization of casein and gelatin, production of melanin and lipase after 7 days incubation at 27±1° C on ISP6 medium by certain Streptomyces isolates

Isolates	Casein		Gelatin		Melanin		Lipase	
	Growth rate	Zone diameter (mm)	Growth rate	Liquefaction rate	Growth rate	Pigment color	Growth Rate	Zone diameter (mm)
St2	3+	4.3	3+	3+	3+	Yellow	3+	22
St3	4+	7.8	4+	4+	3+	Brown	4+	31
St4	3+	6.8	3+	2+	3+	Red brown	3+	17
St7	3+	4.7	2+	2+	2+	Brown	3+	19
St9	2+	1.4	1+	1+	3+	Yellow	3+	22

The data mentioned before in Tables (2, 3 and 4) indicated that the isolates St2, St3, St4, St7, and St9 were identified closely related to *Streptomyces lavendulae*,

Streptomyces glaucescens, *Streptomyces albus*, *Streptomyces violaceoruber*, and *Streptomyces flavotricini*, respectively.

Disease incidence

Data presented in Table (5) show that all treatments were able to protect strawberries of both varieties from infection by gray mold. 96.3 and 87.3% of Festival and Fortuna fruits, respectively, were protected when treated with *S. glaucescens* compared to 100% infection in untreated fruits. *S. albus* ranked second in Festival with 92.8% protection, while in Fortuna, *S. violaceoruber* was in second place with 83.6% protection. The lowest treatment, *S. flavotricini*, recorded 77.0% and 65.1% protection in Festival and Fortuna, respectively. In terms of infected area, all treatments significantly reduced the percentage of infected area in infected fruits compared to untreated fruits, with no significant difference among most of treatments. *S. glaucescens* and *S. albus* were recorded as the best treatments with Festival, while *S. violaceoruber* and *S. glaucescens* were noted as the best treatments for Fortuna.

Table 5. Impact of pre-harvest application* of certain *Streptomyces* isolates on the percentage of protection and infected area in infected fruits, varieties Festival and Fortuna, grown under plastic tunnels during 2023 and 2024 season in Sadat City, Menofia Governorate, Egypt

Treatment (Strains)	Festival		Fortuna	
	Protection (%)	Infected area (%)	Protection (%)	Infected area (%)
S. lavendulae	82.2^{abc}	66.1^{ab}	75.9^{ab}	54.7^{ab}
S. glaucescens	96.3^{a}	32.5^{b}	87.3^{a}	49.8^{b}
S. albus	92.8^{ab}	32.5^{b}	82.8^{a}	62.9^{ab}
S. violaceoruber	78.6^{bc}	55.0^{ab}	83.6^{a}	48.3^{b}
S. flavotricini	77.0^{c}	61.4^{ab}	65.1^{b}	65.5^{ab}
Control	00.0^{d}	83.9^{a}	00.0^{c}	81.2^{a}
SE	4.84	14.58	4.84	9.13

* Three foliar sprays starting from October 15th every other week.

Physicochemical properties

Variety may influence treatment regarding the impact of pre-harvest application on fruit characteristics after 30 days of post-harvest storage, as indicated in Table (6) and Fig. (1). In both varieties, SSC, VC, and CD show no significant differences compared to untreated fruits or decreased. About firmness, only the *S.glaucescens* treatment maintained higher fruit firmness in Festival, while in Fortuna, all treatments significantly enhanced firmness in treated fruits compared to the control during storage. Regarding TA, all kept more TA in Festival, whereas there was no significant difference or values lower than the control in Fortuna.

Table 6. Impact of pre-harvest application* of certain *Streptomyces* isolates on the firmness (F), soluble solid content (SSC), vitamin C (VC), titratable acidity (TA) and color density (CD) of strawberry fruits, varieties Festival and Fortuna, grown under plastic tunnels during 2023 and 2024 season in Sadat City, Menofia Governorate, Egypt

Treatment (Strains)	Festival					Fortuna				
	F	SSC	VC	TA	CD	F	SSC	VC	TA	CD
	g/inch²	(%)	mg ascorbic acid/ 100 g fresh sample	% citric acid	1-5 Scale	g/inch²	(%)	mg ascorbic acid/ 100 g fresh sample	% citric acid	1-5 Scale
S. lavendulae	5.2^{b}	7.2^{b}	51.7^{a}	1.8^{a}	3.8^{a}	4.6^{ab}	8.2^{ab}	53.7^{b}	1.5^{b}	3.8^{a}
S. glaucescens	5.7^{a}	7.3^{ab}	52.3^{a}	2.0^{a}	3.8^{a}	4.8^{a}	8.1^{bc}	61.7^{a}	1.8^{ab}	3.7^{a}
S. albus	5.1^{b}	7.0^{b}	51.3^{a}	1.8^{a}	3.8^{a}	4.4^{ab}	7.9^{c}	58.7^{ab}	2.0^{a}	3.8^{a}
S. violaceoruber	5.1^{b}	6.8^{b}	51.3^{a}	1.9^{a}	3.8^{a}	4.4^{ab}	8.0^{bc}	62.0^{a}	1.9^{a}	3.8^{a}
S. flavotricini	5.2^{b}	7.3^{ab}	52.0^{a}	2.0^{a}	3.7^{a}	4.5^{ab}	8.1^{bc}	58.7^{ab}	2.0^{a}	3.8^{a}
Control	5.0^{b}	8.3^{a}	54.0^{a}	1.4^{b}	3.8^{a}	4.1^{b}	8.4^{a}	63.0^{a}	1.9^{a}	3.8^{a}
SE	0.12	0.35	0.92	0.08	0.17	0.18	0.10	1.71	0.14	0.16

* Three foliar sprays starting from October 15th every other week.

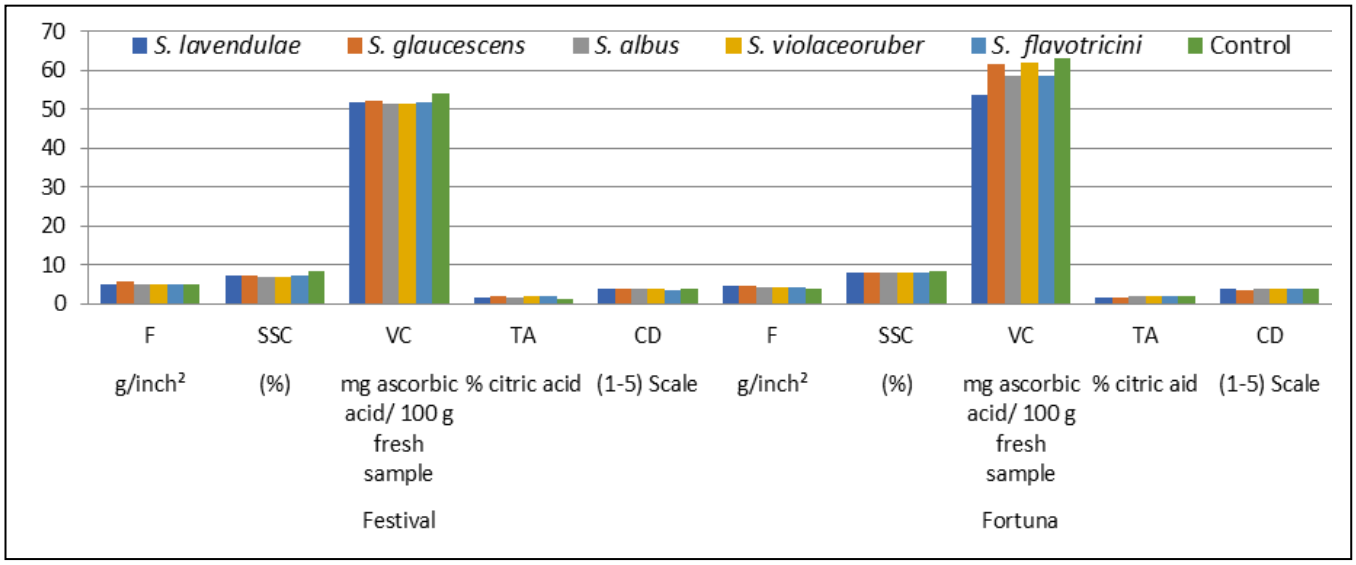

Fig. 1 Impact of pre-harvest application of certain *Streptomyces* isolates on the firmness (F), soluble solid content (SSC), vitamin C (VC), titratable acidity (TA) and color density (CD) of strawberry fruits, varieties Festival and Fortuna, grown under plastic tunnels during 2023 and 2024 season in Sadat City, Menofia Governorate, Egypt

DISCUSSION

Botrytis cinerea Pers. Fr. is a phytopathogenic fungus that causes gray mould on over 230 hosts (Vallejo *et al*., 2002). However, *B. cinerea* was found to be the most destructive pathogen deteriorating strawberries in Egypt particularly during cold storage. Given the significance of this pathogen and the considerable damage it inflicts on agricultural products, effective environmentally safe management strategies are essential.

Based on the morphological characteristics data of isolates on PDA medium, all isolates in the study were identified as *Botrytis cinerea* Pers. Fr. Traditionally, the identification of *B. cinerea* has relied on morphological and cultural traits, along with host specificity (Jarvis, 1977). Although Menzinger (1966) noted that cultural conditions could significantly alter taxonomic features such as the size and shape of conidia, morphological traits continue to be used for *B. cinerea* identification. Only in recent years molecular markers have been employed to confirm traditional classifications, as noted by Staats *et al*. (2005) and Khazaeli *et al*. (2010).

The selection strategy for microbial antagonists to control postharvest disease of fruit and vegetables has been followed by Wilson and Wisniewski (1989). *Streptomyces* isolates were selected to be investigated as biological control tools in the current study. The isolation, of Streptomyces from fruit surface, with different levels of antagonistic biocontrol efficacy against postharvest pathogens has been reported in several studies (Taqarort *et al*., 2008). The *in vitro* studies revealed that tested *Streptomyces* isolates have the antagonistic activity against *B. cinerea*. The culture filtrate of all tested isolates potently inhibited the mycelium growth of *B. cinerea*, and the isolates St2, St3, St4, St7, and St9 were the most promising candidates. This result suggested that these isolates inhibited *B. cinerea* mycelial growth through the production of extracellular antifungal antibiotics. Production of secondary antifungal substances such as antibiotics has been also reported in many species of *Streptomyces* (Kim *et al*., 2015). In addition, according to a literature survey, many *Streptomyces* spp. are able to inhibit a broad range of pathogens such as *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium oxysporum*, and *Sclerotinia homeocarpa* (Chamberlain and Crawford, 1999).

As a result of the disk diffusion assay, five promising actinomycetes isolates were selected for further identification. The collected data on colony morphology, growth rates in various media, the color of both aerial and substrate mycelium, the presence of soluble pigments, and Gram stains indicated that the isolates St2, St3, St4, St7, and St9 were classified as *Streptomyces* according to Bergey's Manual of Systematic Bacteriology (Locci, 1989). Furthermore, the arrangement of spore chains has been historically important for species descriptions, and research suggests that various genes influence spore formation (Keiser *et al*., 2000). Additionally, the ability to utilize a wide range of carbon and nitrogen sources, as well as the capacity to break down casein and gelatin and produce melanin and lipase, are crucial for differentiating species among bacteria, including Actinobacteria. Goodfellow *et al*. (1987) explored the use of rapid biochemical tests for the taxonomy of *Streptomyces*. Based on these criteria, the isolates St2, St3, St4, St7, and St9 were identified as *Streptomyces lavendulae, Streptomyces glaucescens, Streptomyces albus, Streptomyces violaceoruber,* and *Streptomyces flavotricini*, respectively. This classification follows the guidelines in Bergey's Manual of Systematic Bacteriology (William *et al*., 2012) and the International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966).

All isolates under this study gave a considerable protection to strawberries against *B. cinerea* during the storage for 30 days of incubation at 0-2° C and 95% relative humidity, following a pre-cooling at 0-2°C for 6 hours, these result are in agreement with those reported by Kim *et al*. (2015) who stated that postharvest strawberry gray mold and root rot of ginseng disease caused by *B. cinerea* were controlled up to 58 and 73.9%, respectively, upon treatment with culture broth of *Streptomyces* sp. BS062. Yanxuan *et al*. (2024) stated that *Streptomyces*

noursei C27 had excellent potential to be a commercial biocontrol agent of plant diseases caused by pathogenic fungi, especially *B. cinerea* on grape leaves and fruit reducing infection by 72.5 and 71.9%, respectively. *Streptomyces* species are recognized as an excellent source of biocontrol agents due to their ability to produce a variety of antimicrobial secondary and volatile metabolites (Saeed *et al*., 2017), For example, the volatile compounds produced by *Streptomyces philanthi* RM-1-138 effectively control rice sheath blight disease caused by *Rhizoctonia solani* PIRRC-9 (Boukaew *et al*., 2013). Gas chromatography-mass spectrometry analysis revealed that thirty alkenes, alcohols, esters, and alkanes are among the volatile organic compounds secreted by *Streptomyces* C27, which contribute to the inhibition of *Botrytis cinerea* growth (Yanxuan *et al*., 2024). Several fungicides derived from Streptomyces antibiotics are already in commercial use. For example, Jinggangmycin, an antibiotic produced by *S. hygroscopicus* var. *jinggangensis*, is used in China to manage rice sheath blight caused by *Rhizoctonia solani*. Likewise, Mycostop, which contains the mycelium and spores of *S. griseoviridis*, is utilized to control Fusarium wilt and Botrytis gray mold in vegetables and ornamental plants in Finland (Goodfellow and Williams, 1983 and Roberts *et al*., 2005).

The application had a varied effect on the characteristics of the fruits. There were no significant differences in soluble solid content (SSC), vitamin C (VC), and color density (CD) when compared to the control, or these values showed a decrease. In contrast, firmness (F) and titratable acidity (TA) varied depending on the tested strawberry varieties. These results suggest that the varieties respond differently to the treatment in terms of changes in certain physical and biochemical characteristics. However, other researchers, such as El-awady *et al*. (2017), found that applying *Saccharomyces cerevisiae* b4 and *Trichoderma viride*, in combination with heat and chitosan treatment, resulted in significant improvements in fruit firmness, total soluble solids, and total sugar content. Additionally, this combination delayed qualitative changes in color, titratable acidity, and ascorbic acid levels. Also, Freeman *et al*. (2004) suggested that biological control following heat treatment could be effective in reducing decay. Meanwhile, Wszelaki and Mitcham (2003) observed that strawberries subjected to heat treatment tended to have slightly drier calyces, and in some instances, displayed mild water-soaked areas compared to those that were not heated. Qaderi *et al*. (2023) found that there are variations in the nutritional content among different cultivars. Additionally, the phytochemical composition declines as storage time increases.

CONCLUSION

Based on their ability to inhibit the linear growth of *Botrytis cinerea*, five strains of *Streptomyces*, namely *S. lavendulae, S. glaucescens, S. albus, S. violaceoruber*, and *S. flavotricini*, were selected and identified from twelve isolates collected from healthy-looking strawberries in the markets. All the five isolates effectively protected the Festival and Fortuna strawberry fruits from gray mold when applied as foliar treatments, following a pre-cooling at 0-2°C for 6 hours, and then stored for 30 days at 0-2°C and 95% relative humidity. *S. glaucescens* provided protection rates of 96.3% for Festival and 87.3% for Fortuna, compared to 0.0% in the control. Also, infected area in infected fruits were decreased to 32.5% for Festival and 49.8% for Fortuna, compared to 83.9 and 81.2% in the control, respectively. The effectiveness of the treatment also influenced the Physicochemical properties of the strawberries, while soluble solids content, vitamin C levels, and color density either remained unaffected or decreased,

the firmness and titratable acidity varied based on the tested varieties.

AUTHOR CONTRIBUTIONS

Majority contribution for the whole article belongs to the author(s). The authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

STATEMENT AND ETHICS DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article. Ethical approval was not required for this study, as it involved only plant and microbial samples, which are exempt from human or animal ethical review regulations.

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