Taha *et al.*
Egyptian Journal of Biological Pest Control (2023) 33:106
https://doi.org/10.1186/s41938-023-00752-6

Egyptian Journal of Biological Pest Control

RESEARCH

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Biological control of postharvest tomato fruit rots using *Bacillus* spp. and *Pseudomonas* spp.

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Abstract

Background Postharvest diseases cause a wide loss to tomato fruits during handling and storing from harvest to consumers. Fungicides are mainly used to control postharvest diseases. Biological control is the eco-friendly substitute strategy used for postharvest diseases management as which becoming promise worldwide. Six bacterial bioagent (i.e., *Bacillus subtilis*, *B. amyloliquefaciens, Pseudomonas resinovorans, P. alcaligenes, P. putida* and *P. stutzeri*) were tested to suppress both *Geotrichum candidum* and *Alternaria alternata* causal agents of tomato fruit rots during storage.

Results In vitro, most of bioagents significantly reduced mycelial growth rate of *G. candidum*. Both of *B. subtilis* and *P. stutzeri* were the most superior bacterial bioagents with values 67.03 and 72.2%, respectively. In addition, *B. subtilis* and *B. amyloliquefaciens* resulted in the most superior over all antagonists against *A. alternata*. The lowest percent of disease incidence and severity of *G. candidum* were obtained by *B. subtilis* and *P. stutzeri*. The maximum reduction percent of it on tomato fruits was recorded by applying *B. subtilis* and *P. stutzeri* with values (90 and 87%) and (91, 89%) in both seasons, respectively. Also, the highest reduction of *A. alternata* was obtained by using *B. subtilis,* which resulted in 85 and 84% in both seasons, respectively. The application of bioagents against both pathogens was significantly improved fruit quality aspects (weight loss, vitamin C, TSS and acidity %) during storage period compared to infected control fruits.

Conclusion The findings revealed that both of *B. subtilis* and *P. stutzeri* could be potential biological control agents against most postharvest pathogens of tomato fruits. This might be an alternative control strategy instead of fungicides which service the sustainable and organic farming.

Keywords Postharvest diseases, *Geotrichum candidum*, *Alternaria alternata*, *Bacillus* spp., *Pseudomonas* spp., Tomato fruit rots, Fruit quality

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Background

Tomato (*Solanum lycopersicum* L.) is a main vegetable crop that belongs to the *Solanaceae* family. It considered the first food vegetables worldwide, in a total harvested area (5,051,983 hectares), producing 189 million Mg; total cultivated area in Egypt was about 150,109 hectares with a total productivity of 6.73 million Mg and an average yield of 41.6 tons/ha in 2021 (FAOSTAT 2023). As a great worth crop, tomatoes are important revenue source for farmers; it is also a rich source of nutrients, beta-carotene, flavonoids, folate, ascorbic acid and many medicinal health benefits, which play a significant role in human health (FAO 2021).

A great quantity of tomato fruits is not reaching the customer mainly back to many postharvest losses. FAO report in 2018 indicated that postharvest losses in tomatoes were more than 50% in the development countries, with a huge loss of appreciated nutrients, income, and employment to millions of small growers along the chain (FAO 2018). Generally, tomato fruits in Egypt are vulnerable by various diseases caused by fungi, bacteria, and viruses during plant growing and post-harvesting period. Fungal and bacterial infections that highly decreased tomato quality and quantity include *Alternaria alternata, A. solani, Fusarium oxysporum, Geotrichum candidum, Sclerotium rolfsii, Septoria lycopersici, Verticilium dahlia, Erwinia carotovora, Ralstonia species complex (RSC), Staphylococcus aureus* (Pandey et al. 2022). The fruit injuries as fungal deteriorations have been assessed to be more than 90 % throughout harvesting and marketing (Bayoumi et al. 2023). Different strategies as fungicides, resistant hybrids, and cultural practice are generally used as commercial strategies to prevent postharvest diseases. However, the use of fungicides intensively may help pathogen to be resistant as well as fungicides released to the environment (Bakade et al. 2022); however, it may represent environmental and health hazards (Wu et al. 2023). Therefore, its application is becoming more limited in order to the consumer's anxieties and the human health importance (Wu et al. 2023).

Thereafter, based on the complications of fungicide applications led to use of the safe and natural substances (Pathak et al. 2022), one of these alternatives, biological control methods, depends on microorganisms with a strong fungal activity (Adeleke et al. 2022). Biocontrol strategy is unscathed for the ecology system and human health as well as district the fungicides resistance (Jaiswal et al. 2022). Biological control agents include both fungal and bacterial antagonists such as *Trichoderma harzianum, T. viride, Gliocladium roseum, P. stutzeri, P. putida, P. alcaligenes* and *B. amyloliquefaciens* that could be an alternative strategy to prevent postharvest decay on tomato fruits (Bonaterra et al. 2022). In this manner, Lastochkina et al. (2019) cleared that the applying many effective isolates of *Bacillus* spp. successfully controlled different postharvest pathogens of different vegetable fruits during storing period. Furthermore, *Bacillus* spp. are considered main promising bioagents, which have high antagonistic abilities for suppression various postharvest diseases of different horticultural crops (Wang et al. 2021).

The objective of this investigation was to study the role of various bacterial antagonists in controlling tomato fruits rots caused by postharvest pathogens (*G. candidum* and *A. alternata*), which cause various losses during storage period conditions and to, moreover, study the effects of these bioagents on the properties of tomato fruits quality during storing period in both trials.

Methods

Isolation and purification of the fungi related to rotten tomato fruits

Many vegetable markets from Kafr El-Sheikh city, Northern Egypt (31° 06′ 25.20″ N, 30° 56′ 26.99″ E), were surveyed for gathering the tomato fruits showing various kinds of rots during October 2021 season. Diseased fruits were sliced (1 cm) and sterilized with sodium hypochlorite (1%) two min and double-washed in disinfected distilled water. These sections were dried by filter paper and placed on plates contained water agar with incubation at 28 ± 2 °C until 48 h. Then, transferring the hyphal tips that occurred on Petri dishes contains Potato Dextrose Agar (PDA) medium with incubation till 5 days at 28 ± 2 °C (Carisse and Van Der Heyden 2015).

Pathogenicity test

Ten fungi and bacteria isolates were used to perform the pathogenicity during 2021 season. One mm diameter in 4-mm-deep wound was done on every separately selected fruit. Mycelial masses from old cultures of the fungal isolates (10 days) and bacterial suspension were injected in the wounds of fruits. Inoculated fruits were located in plastic packet containing disinfected paper wetted and stored till 12 days at 25–30 °C. The trials were separately arranged for all isolates in four replicates and repeated twice. The virulence rate of every isolate was assessed by calculating the lesion diameter of each treated fruit during the storage period (3, 6, 9 and 12 days).

The most aggressive isolates were identified by morphological and microscopic parameters and by molecular identification in Sigma Company, Cairo, Egypt, according to phylogenetic tree. The mycelia of the two destructive fungal isolates were cultured on disinfected potato dextrose broth. Consequently, the DNA was taken away according to Atallah et al. (2022) and sequencing of the ITS regions with reference to the GenBank.

In vitro antagonism test

Antifungal activity of bacterial isolates against the two virulence pathogens

Six bacterial isolates were obtained from Agricultural Botany Dept., Fac. Agric., Kafr Elsheikh Univ., Egypt. These bioagents were *B. subtilis*, *B. amyloliquefaciens, P. resinovorans, P. alcaligene, P. putida* and *P. stutzeri*.

The efficacy of the antagonistic bacterial isolates was tested using 2-cm-long side, on PDA medium within petri dishes. Five mm in diameter disks from pathogens were taken from culture (7 days old) and cultured at 1 cm distance of the edge of a Petri dish, while the opposite site of Petri dish was inoculated with the antagonistic bacteria. Dishes were stored at 28 ± 2 °C. The antifungal activity of bioagents was assessed as inhibition % of mycelial growth according to Ferreira et al. (1991).

Electron microscope

The investigation with a JEOL scanning electron microscope (SEM, T.330 A) has been performed at the Central Lab., Fac. Agric., Mansoura Univ., Egypt, to inspect and show the impact of the tested bioagents bacteria on the growth variations of both *G. candidum* and *A. alternata* after 7 days from inoculation on PDA media in comparison with pathogens without bioagent application as a control.

In vivo, applying of selected bacterial bioagents on tomato

Fresh and healthy tomato fruits (023 F1 hybrid) were washed, sanitized and treated with the selected four bacterial antagonists, and the trials were containing six treatments as follows:

1—*Bacillus subtilis*, 2—*Bacillus amyloliquefaciens*, 3—*Pseudomonas alcaligene*,

4—*Pseudomonas stutzeri*, 5—Infected control without bioagents and 6—Healthy fruits.

Spore suspensions of the two pathogens (*G. candidum* and *A. alternata*) were grown on potato dextrose broth medium for eight days at 28 ± 2 °C. The bacterial isolates were grown in nutrient broth for three days. The fruits were washed with sterilized distilled water and then air-dried. The fruits were dipped in the suspensions of the four bioagents for 20 min (1×10^8 CFU/ml). The treated fruits were air-dried and then dipped till 20 min in a conidial suspension of the pathogens (1×10^4 spores/ml). The untreated fruits were dipped only with sterilized distilled water. All fruits were then stored at room temperature (25–30 °C) until 12 days. The investigation was repeated in 2 years (2021 and 2022) with four replications (6 fruits in each replication) for every application. Disease severity (DS %) was recorded daily from 1 to 12 days after inoculation. The rots diameters were measured by the caliper, and DS % is recorded by the following formula as described by Lahlali et al. (2020):

$$\text{DS}\ (\%) = (D_{\text{T}})/(D_{\text{C}}) \times 100$$

where D_{T} = Mean diameter (mm) of inoculated fruits with bioagents, & D_{C} = Mean diameter (mm) of the control (infected only by *G. candidum* or *A. alternata*).

Fruit quality aspects

Some fruit quality aspects were also recorded during storage period in both seasons as follows: weight loss (%) at 12 days after treatment, firmness (kg mm^{-2}), vitamin C content, total soluble solids (T.S.S %) and total titratable acidity (TTA %) at 6 days after treatment according to AOAC (1990).

Statistical analysis

All results were analyzed using analysis of variance (ANOVA) method of the statistical software, and Duncan's multiple range tests was used to compare among the means, according to Snedecor and Cochran (1989). All analyses were achieved by Web Based Agricultural Statistics Software Package (WASP program), which was designed by ICAR.

Results

Isolation, identification and pathogenicity tests of the pathogens caused tomato fruits rots

The isolation trials appeared various kinds of decays, obtained from dissimilar markets in Kafr El-Sheikh city. Each isolate was aggressively examined using tomato hybrid 023 by artificial inoculation of robust fruits after 3, 6, 9 and 12 days of treatment under room temperature (25–30 °C).

According to pathogenicity test, both isolate numbers 2 and 7 were the most aggressive pathogens compared to other different isolates, and the differences were significant (Table 1). Both isolates caused completely rotted fruits after nine days of inoculation (7 cm), followed by isolate numbers 4 and 8; however, the isolate numbers 5 and 9 showed the lowest rotted fruit diameter. So, in the present study both isolate numbers 2 and 7 were used as an aggressive pathogen of tomato fruits, which caused fruit rots as well as identified those isolates to complete this work. Based on molecular characterizations of highly pathogenic isolates, the identification of first pathogen was confirmed as: *Geotrichum candidum* strain Elsharkawy by sequencing of the ITS regions with reference to the GenBank accession number OR351019, using phylogenetic trees of both pathogens (Fig. 1A, B), while the second pathogen was confirmed as *Alternaria alternata* strain Elsharkawy by sequencing of the ITS regions with reference to the GenBank accession number OR351018.

In vitro antagonism tests

The in vitro impacts of various antagonistic bacteria on the growth rate mycelium for both pathogens of *G. candidum* and *A. alternata* were noticeable (Figs. 2, 3 and Table 2). All bacterial isolates under test significantly

Table 1 Rot diameters of tomato fruits as affected by artificial inoculation with the different pathogens (ten isolates) at room temperature (25–30 °C)

Isolates number	Mean diameter of rots (cm) after inoculation (days)			
	3	6	9	12
1	0.0f	1.2cde	2.0d	2.5c
2	2.0b	4.5a	7.0a	7.0a
3	0.0f	0.4e	1.1e	2.0c
4	0.8c	2.2bc	3.4bc	5.0b
5	0.0f	0.0f	0.0g	0.1e
6	0.5e	0.9de	1.8d	2.5c
7	2.6a	5.5a	7.0a	7.0a
8	0.7d	1.8bcd	2.6c	3.9b
9	0.0f	0.0f	0.2f	0.5e
10	0.0f	0.5e	1.2e	1.5d
F. test	**	**	**	**

**Indicate significant differences at p values < 0.01, according to F test. Means followed by the same letter in same column are not significantly different at the 0.05 level, according to Duncan's multiple range test. The angular transformation was used for resistance to fruit rots percentage as outlined by Snedecor and Cochran (1989)

reduced mycelial growth rate of the fungus *G. candidum* relative to its control (Fig. 2). The maximum inhibition rate (%) was obtained by applying the bacteria *P. stutzeri,* followed *B. subtilis*, *B. amyloliquefaciens* and *P. alcaligens* by 72.2, 67.03, 65.2 and 62.95%, respectively. The remainder isolates *P. resinovorans* and *P. putida* occupied an intermediate position between above isolates and the control. So, the bacteria *B. subtilis* and *P. stutzeri* appeared to be the most superior over all the antagonists tested against *G. candidum*. The results also emphasize that the suppression averages of *A. alternata* radial growth were differed due to different bacterial isolates used. The highest inhibition rate was recorded by the bacteria *B. subtilis* and *B. amyloliquefaciens* (58.9 and 55.3%), while the isolates *P. resinovorans* and *P. putida* did not effect on the mycelial growth of *A. alternata* and insignificantly differed with the control (Fig. 3). Consequently, the bacteria *B. subtilis* and *B. amyloliquefaciens* appeared to be the most superior over all the antagonists tested against *A. alternata*.

Electron microscopic investigation

The changes in mycelial growth of both pathogens were examined 7 days after inoculation using a scanning electron microscope (SEM) (Figs. 4 and 5). The hyper-parasitic effects of the two bacterial bioagents (*B. subtilis* and *B. amyloliquefaciens*) in vitro conditions are given in Fig. 4 for *G. candidum* images, while the effect of the four bioagents (*B. subtilis* and *B. amyloliquefaciens, P. alcaligenes* and *P. stutzeri*) on *A. alternata* is resulted in Fig. 5 as SEM (scanning electron microscope). Using the bioagents caused some morphological modifications in the hyphae and mycelial growth of both pathogens. The mycelium of both *G. candidum* and *A. alternata* was

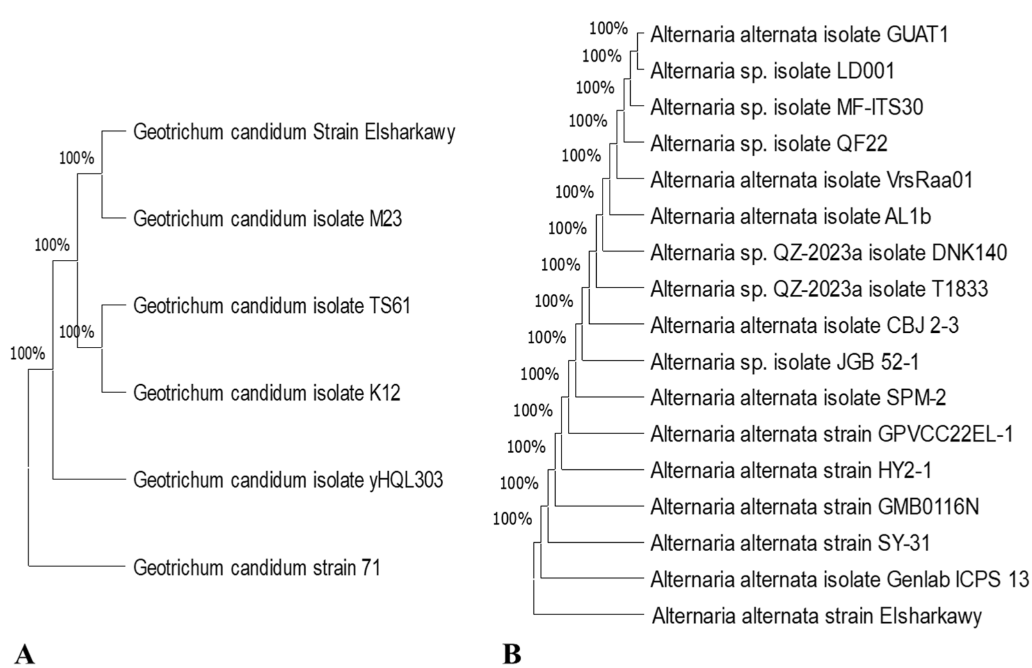

Fig. 1 Phylogenetic trees of both *Geotrichum candidum* (**A**) and *Alternaria alternata* (**B**)

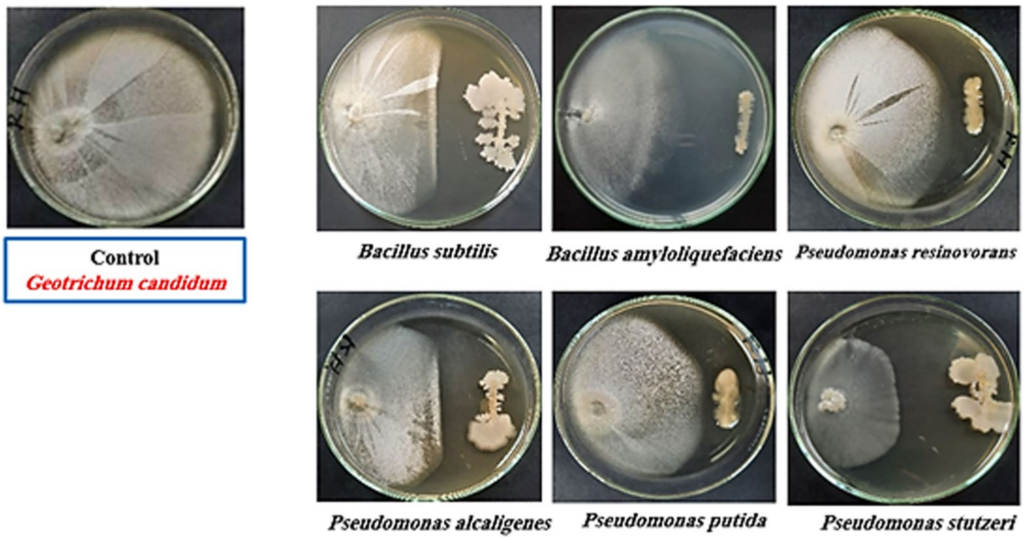

Fig. 2 Inhibition rate of radial growth for *Geotrichum candidum* by different bacterial bioagent isolates

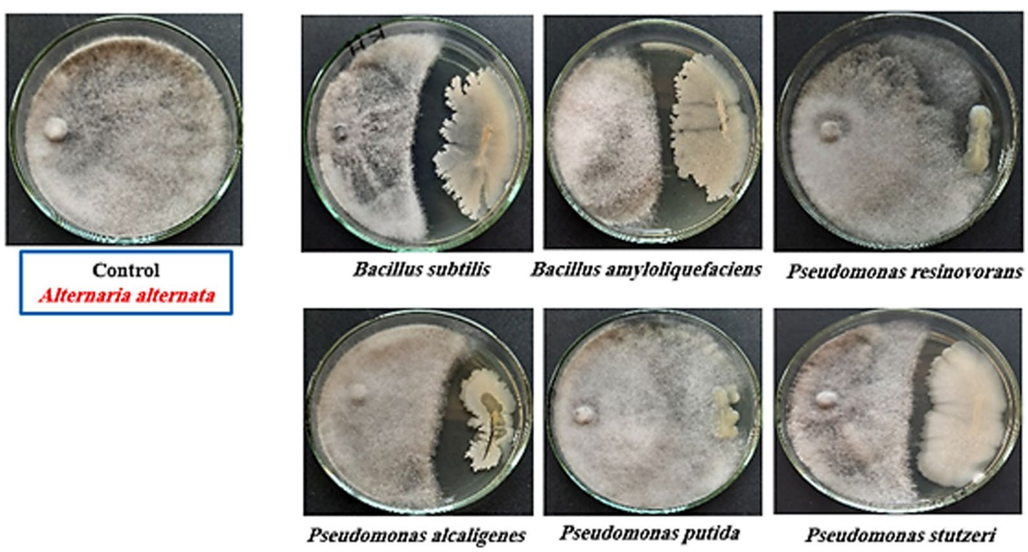

Fig. 3 Inhibition rate of radial growth for *Alternaria alternata* by different bacterial bioagent isolates

severely malformed in the presence of all bioagents specially *B. subtilis* and *B. amyloliquefaciens* as compared to the negative control that was uninoculated and showed typical mycelial structures for the both pathogens. Also, the abnormal features were resulted from applying *P. alcaligens* and *P. stutzeri* on *A. alternata* fungus.

Effect of selected bacterial bioagents on tomato fruits rot development

The results of biological control with bacterial antagonists on the fruit rot progress caused by *G. candidum* and *A. alternata* pathogens in in vitro tests under room temperature in both seasons are showed in (Tables 3, 4 and Fig. 6). The disease incidence (DI) and severity (DS) % were clearly significant differed during storage periods (4, 8 and 12 days after inoculation) in both seasons. The use of both *B. subtilis* and *P. stutzeri* achieved the lowest values of DI and DS % for *G. candidum* pathogen, followed by *B. amyloliquefaciens* and *P. alcaligenes* in both seasons. Infected control fruits (untreated with bacterial isolates) resulted the maximum DI and DS % at all days after inoculation; it gave 100% after 8 days from inoculation. Therefore, the maximum significant reduction % of *G. candidum* was recorded, using the bacterial isolates *P.*

Table 2 In vitro antagonistic effect of bacterial bioagents on the mycelial growth of both *Geotrichum candidum* and *Alternaria alternata*

Bacterial isolates	*G. candidum*	*A. alternata*
	Reduction %	
1. *Bacillus subtilis*	67.03a	58.90a
2. *Bacillus amyloliquefaciens*	65.20a	55.30a
3. *Pseudomonas resinovorans*	59.50a	1.95b
4. *Pseudomonas alcaligenes*	62.95a	45.50a
5. *Pseudomonas putida*	58.95a	2.05b
6. *Pseudomonas stutzeri*	72.20a	45.7a
7. Control	0.00b	0.00c
F. test	**	**

**Indicate significant differences at *p* values < 0.01 according to *F*. test. Means followed by the same letter in same column are not significantly different at the 0.05 level, according to Duncan's multiple range test. The angular transformation was used for resistance to fruit rots percentage as outlined by Snedecor and Cochran (1989)

alcaligenes and *B. subtilis* by 91, 89% and 90, 87%, respectively, in both years. The antagonistic role of bacterial isolates on *A. alternata* pathogen was differed (Table 4); the most bioagents resulted in the minimum disease incidence and severity % during all dates after inoculation (4, 8 and 12 DAI) compared to infected control fruits in both trials. Also, the highest reduction of *A. alternata* was obtained from using the bioagent *B. subtilis,* which resulted in 85 and 84% in both experiments, respectively.

Fruit quality

Fruits were highly significant influenced by bacterial bioagents application against both pathogens during storage period under room-temperature conditions (Fig. 7 and Table 5). Therefore, tomato fruits inoculated with the bacterial bioagents *B. subtilis, B. amyloliquefaciens* and *P. stutzeri* resulted to the lowest weight loss % without significant differences with healthy fruits at 12 days after inoculation during storage period (Fig. 7), while the maximum values were obtained from untreated fruits (infected only with each pathogen) in most cases.

Concerning chemical quality aspects (vitamin C, TSS and TTA contents) in fruits, the highest significantly values were recorded in the most cases in fruits treated with all bacterial bioagents without significant differences with healthy fruits in comparison with infected control treatment (both *G. candidum* and *A. alternata*), which gave the minimum values in most cases during the storage period. So, tomato fruits treated by the bacteria such as *B. subtilis, B. amyloliquefaciens* and *P. stutzeri* showed the highest contents of vitamin C, TSS and TTA at 6 days after inoculation during storage period under biotic challenge with both *G. candidum* and *A. alternata,* which caused fruit rots diseases.

Discussion

Tomato fruit rots caused by *G. candidum* and *A. alternata* are the most common postharvest pathogens that attack tomato fruits during handling and storage period until reaching the consumers (Lastochkina et al. 2019). One of the safest and eco-friendly control methods is using the antagonistic microorganisms, which are becoming common as biological control in the world (Fenta et al. 2023). Several potential antagonists of bacteria-active anti-fungi have been reconnoitered and used against numerous postharvest pathogens (*Geotrichum, Botrytis, Alternaria, Rhizopus, Colletotrichum, Penicillium, Monilinia*) and extend the shelf-life period of horticultural crops (Ali et al. 2022). Both *Pseudomonas* spp. and *Bacillus* spp. are familiar as eco-friendly and safety for users as well as highly suppressive effects on postharvest diseases (Peeran et al. 2014) as well as white rot disease in onions (Amin and Ahmed 2023).

The present investigation evaluated the efficacy of some bacterial bioagents (*Bacillus* spp. and *Pseudomonas* spp.) against both *G. candidum* and *A. alternata*, which caused tomato fruit rots. All bacterial bioagents of *Bacillus* spp.

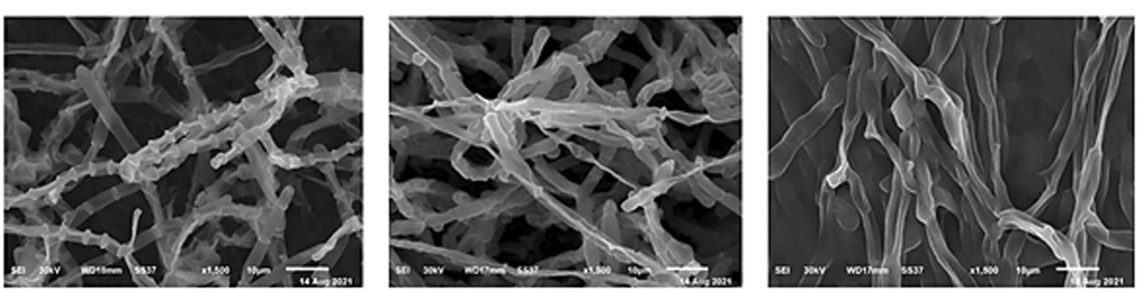

Fig. 4 Scanning electron microscope (SEM) images showing the effect of different bacterial bioagents on the development of mycelial growth of *Geotrichum candidum* on PDA for 7 days after inoculation

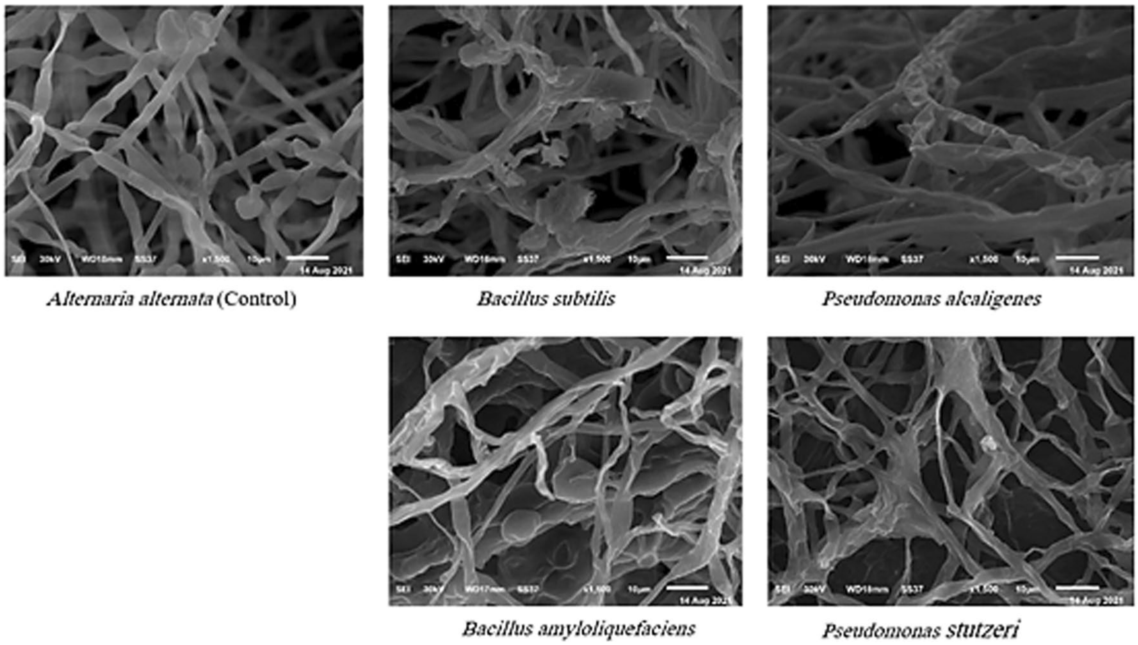

Fig. 5 Scanning electron microscope (SEM) images showing abnormalities in the hyphae of *Alternaria alternata* due to antagonistic effect of different bacterial bioagents grown on PDA for 7 days after inoculation

Table 3 Effect of bacterial antagonists on tomato fruit rot development (disease incidence and disease severity %) caused by *Geotrichum candidum* under room-temperature conditions

Bacterial bioagents	Days after inoculation						Reduction %
	4		8		12		
	DI%	DS%	DI%	DS%	DI%	DS%	
2021 season							
1. *Bacillus subtilis*	0.0c	0.0b	1.0d	4.0c	2.0b	11.0d	89.0b
2. *Bacillus amyloliquefaciens*	0.0c	0.0b	2.0c	10.0c	2.0b	17.0c	83.0c
3. *Pseudomonas alcaligenes*	1.0b	2.0b	3.0b	9.0c	3.0b	22.0b	78.0d
4. *Pseudomonas stutzeri*	0.0c	0.0b	1.0d	4.0c	2.0b	9.0e	91.0b
5. Infected control	6.0a	35.0a	8.0a	100.0a	12.0a	100.0a	00.0e
6. Healthy fruits	0.0c	0.0b	1.0d	4.0c	2.0b	5.0f	95.0a
F. test	*	**	*	**	*	**	**
2022 season							
1. *Bacillus subtilis*	0.0c	0.0c	2.0b	4.0d	4.0b	13.0d	87.0b
2. *Bacillus amyloliquefaciens*	0.0c	0.0c	1.0b	8.0c	3.0b	18.0c	82.0c
3. *Pseudomonas alcaligenes*	2.0b	4.0b	3.0b	10.0b	4.0b	24.0b	76.0d
4. *Pseudomonas stutzeri*	0.0c	0.0c	1.0b	5.0d	2.0b	10.0e	90.0ab
5. Infected control	10.0a	42.0a	14.0a	100.0a	20.0a	100.0a	00.0c
6. Healthy fruits	0.0c	0.0c	2.0b	5.0d	3.0b	7.0f	93.0a
F. test	**	**	*	**	*	*	**

* and **Indicate significant differences at p values < 0.05 and < 0.01, respectively according to *F*. test. Means followed by the same letter in same column are not significantly different at the 0.05 level, according to Duncan's multiple range test. The angular transformation was used for resistance to fruit rots percentage as outlined by Snedecor and Cochran (1989)

Table 4 Effect of bacterial antagonists on tomato fruit rot development (disease incidence and disease severity %) caused by *Alternaria alternata* under room-temperature conditions

Bacterial isolates	Days after inoculation						Reduction %
	4		8		12		
	DI%	DS%	DI%	DS%	DI%	DS%	
2021 season							
1. *Bacillus subtilis*	0.0c	0.0c	2.0b	4.0d	2.0c	15.0c	85.0b
2. *Bacillus amyloliquefaciens*	0.0c	0.0c	2.0b	6.0d	3.0bc	18.0bc	82.0b
3. *Pseudomonas alcaligenes*	1.0b	5.0b	3.0b	10.0c	4.0b	25.0b	75.0c
4. *Pseudomonas stutzeri*	1.0b	6.0b	3.0b	18.0b	4.0b	31.0b	69.0d
5. Infected control	4.0a	12.0a	6.0a	48.0a	12.0a	100.0a	00.0e
6. Healthy fruits	0.0c	0.0c	2.0b	4.0d	2.0c	10.0d	90.0a
F. test	*	*	*	**	**	**	**
2022 season							
1. *Bacillus subtilis*	0.0c	0.0c	2.0b	5.0c	3.0b	16.0d	84.0b
2. *Bacillus amyloliquefaciens*	0.0c	0.0c	3.0b	8.0c	5.0b	20.0c	80.0b
3. *Pseudomonas alcaligenes*	2.0b	6.0b	4.0b	10.0bc	5.0b	27.0b	73.0c
4. *Pseudomonas stutzeri*	0.0c	0.0c	2.0b	15.0b	3.0b	25.0b	75.0c
5. Infected control	5.0a	14.0a	8.0a	46.0a	13.0a	100.0a	00.0d
6. Healthy fruits	0.0c	0.0c	2.0b	5.0c	2.0b	12.0e	88.0a
F. test	*	**	*	**	*	**	**

* and **Indicate significant differences at p values < 0.05 and < 0.01, respectively, according to *F*. test. Means followed by the same letter in same column are not significantly different at the 0.05 level, according to Duncan's multiple range test. The angular transformation was used for resistance to fruit rots percentage as outlined by Snedecor and Cochran (1989)

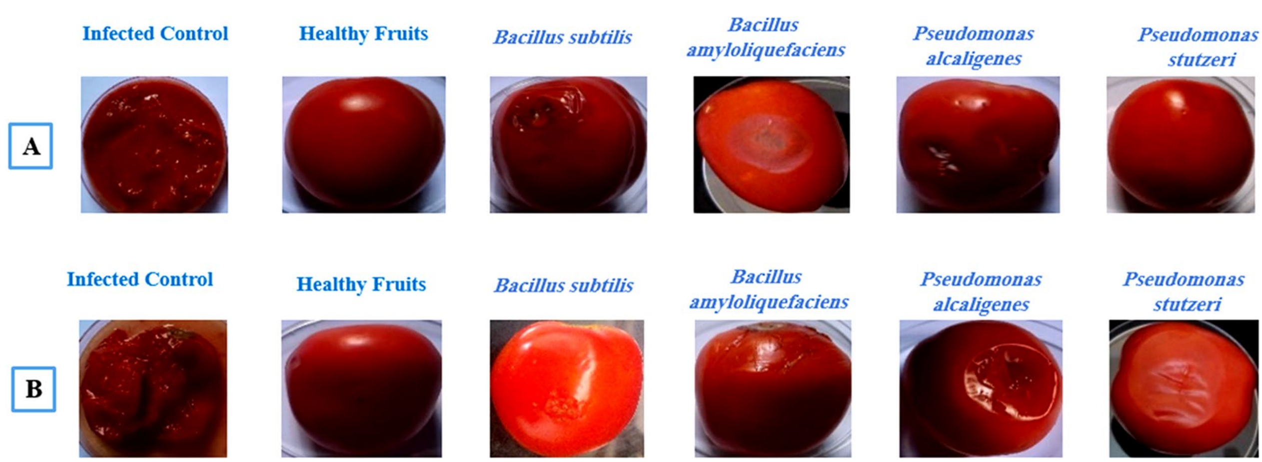

Fig. 6 Biocontrol effects of bacterial isolates on tomato fruit rots development at 12 DAI with (**A**) *Geotrichum candidum* (**B**) *Alternaria alternata*

and *Pseudomonas* spp. significantly inhibited mycelial growth rate of *G. candidum* relative to its control, especially *B. subtilis* and *P. stutzeri*. These were the most superior bioagents over all the tested antagonists against *G. candidum*. However, *B. subtilis* and *B. amyloliquefaciens* were appeared the most superior over all the antagonists against *A. alternata*. This effect may be related to antagonistic bacteria secrete numerous compounds, namely antibiotic-like phytotoxins with probable antifungal potencies led to suppression role on pathogens (Li et al. 2020). High suppression of growth rate for postharvest fungi has been succeeded by applying various bacterial bioagents (Kim et al. 2016). This suppression area inter-pathogen and bioagents may back to antimicrobic constituents, which resulted by bacterial bioagents (Ghazanfar et al. 2016). For example, *Bacillus cereus*

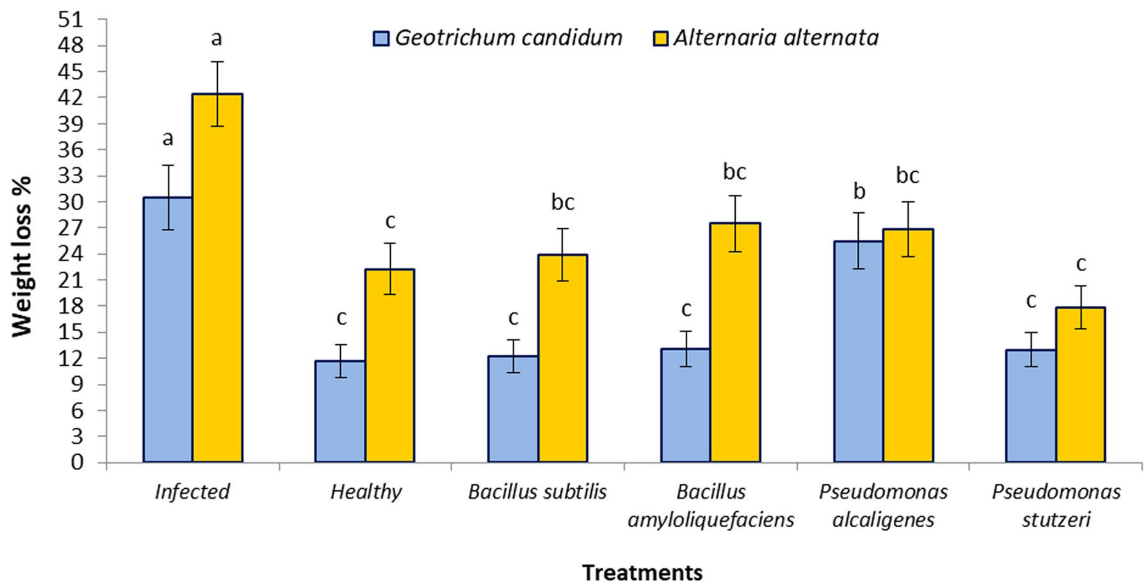

Fig. 7 Effect of bacterial bioagents on weight loss % of tomato fruits at 12th day after inoculation against *Geotrichum candidum* and *Alternaria alternata* pathogens during storage period at room-temperature conditions. Within each date, different letters indicate significant differences among treatments at $p < 0.05$ according to Duncan's multiple rang test

Table 5 Effect of bacterial bioagents on some quality aspects of tomato fruits at 6th day after inoculation against both *Geotrichum candidum* and *Alternaria alternata* pathogens during storage period at room-temperature conditions

Bacterial isolates	Firmness (kg mm^{-2})	Vit. C (mg 100 g^{-1})	TSS (%)	TTA (%)
Geotrichum candidum				
1. *Bacillus subtilis*	273.3a	17.16b	4.56 b	0.095 b
2. *Bacillus amyloliquefaciens*	269.0a	17.97b	4.70 ab	0.066 c
3. *Pseudomonas alcaligenes*	240.0b	17.50b	4.50 b	0.055 c
4. *Pseudomonas stutzeri*	270.0a	20.40a	4.81 ab	0.164 a
5. Infected control	216.70c	17.50b	3.78 c	0.068 c
6. Healthy fruits	279.0a	20.67a	5.03 a	0.185 a
F. test	**	*	**	*
Alternaria alternata				
1. *Bacillus subtilis*	245.0b	19.72b	4.83a	0.094b
2. *Bacillus amyloliquefaciens*	244.3b	20.16ab	4.75a	0.098b
3. *Pseudomonas alcaligenes*	233.3c	17.60c	4.87a	0.078c
4. *Pseudomonas stutzeri*	260.0a	19.67b	4.90a	0.102a
5. Infected control	223.3d	15.02d	3.33b	0.073c
6. Healthy fruits	262.7a	21.68a	4.97a	0.106a
F. test	**	*	**	**

* and **Indicate significant differences at *p* values < 0.05 and < 0.01, respectively according to *F*. test. Means followed by the same letter in same column are not significantly different at the 0.05 level, according to Duncan's multiple range test

applied as antifungal bacteria generated substances on PDA media as well as repressed the Fusarium growth (Pazarlar et al. 2022). Additional possibility of creation of suppression area is nutrient reduction by bioagents, encircled the pathogen that reticented the *G. candidum* and *A. alternata* growth. The inhibition area occurrence is back to creation of antibiotic bacteria (Reygaert 2018). In this manner, Zhou et al. (2011) revealed that appreciable inhibition of mycelium formation of *R. stolonifera* as a result of adding spores of *B. subtilis* to PDA medium. Numerous *Pseudomonas* spp. isolates have great influence on controlling vegetables and fruits diseases after harvesting and during storage period (Sharma et al. 2009). In this investigation, *Pseudomonas* spp. obviously showed the maximum suppression level for *G. candidum* and *A. alternata* mycelial growth. The results approved the conclusions of Ghazanfar et al. (2016) as they indicated the variable rate of efficacy of different *Pseudomonas* spp. anti-various pathogens, which attacks vegetables after harvests.

In vivo investigation cleared that *Bacillus* spp. and *Pseudomonas* spp. as bioagents bacteria resulted in appropriate biocontrol against *G. candidum* and *A. alternata* on tomato fruits. The difference in the ability of the bioagents on tomato fruits might be related to keeping of high-density *Bacillus* spp. and *Pseudomonas* spp., in the infested area (Zong et al. 2010), wherever they compete for both area and nutrients as action mechanism of bioagents. In this concern, Zhou et al. (2011) found that applying *B. subtilis* on peach fruits minimized the growth of *R. stolonifera* pathogen. These results confirm that *Bacillus* spp. minimized the rotted area on tomatoes compared to only infected ones with the pathogens. In this manner, *Bacillus* spp. are considered perfect bioagents for pathogen-infected fruits after harvest, which may be in order to that *Bacillus* spp. live well under various temperatures (Singh and Daverall 1984).

The SEM remarks of the mycelium of both *A. alternata* and *G. candidum* displayed abnormal formation like deformation in the current examination. These findings were confirmed by Minaxi (2010) who found hydrolysis in mycelium of *Macrophomina phaseolina* back to antagonistic role of *P. aeruginosa*. Furthermore, the application of *Aspergillus terreus* against *Pythium aphanidermatum* led to alterations and lack of cytoplasm content in mycelial growth as noticed by Al-Shibli et al. (2019). Furthermore, Al-Badri et al. (2020) confirmed that *B. subtilis* and *B. cereus* caused some pits, retractility and lack of turgidity of the mycelium of both *R. solani* and *N. keratoplastica*. The use of *Enterobacter roggenkampii* and *P. aeruginosa* as bioagents against *A. alternata* led to malformations in the mycelium (Al-Maawali et al. 2021). The hyphae shrink of *A. alternata* in the current examination proposes a probable lack of cytoplasm size (Garg et al. 2010). The current study suggested that *B. subtilis*, *B. amyloliquefaciens*, *P. alcaligenes* and *P. stutzeri* use their antagonism against both *G. candidum* and *A. alternata* pathogens throughout excretion of antifungal components and hydrolytic enzymes that destroy its cell membrane and cytoplasm leakage (Chaurasia et al. 2005).

In the present trials, postharvest application of tomatoes by bacterial challengers considerably minimized both disease incidence and severity of tomato fruit rots. Among the tested antagonistic microorganisms, *P. alcaligenes* and *B. subtilis* were the greatest efficient for suppressing *Geotrichum* rots of tomatoes, while *Bacillus* spp. had the most effective role on *Alternaria* rots, followed by *Pseudomonas* spp. in most cases. Similar results were stated that *Pseudomonas* spp. revealed aggressive effect consequent to produce siderophores, antibiotics and volatile compounds (Fenta et al. 2023). Numerous *Pseudomonas* spp. have active function in preventing postharvest rots of vegetables and fruits (Sharma et al. 2009). Use of *Pseudomonas* spp., in the present examination, obviously suppressed the mycelium progress of both *G. candidum* and *A. alternata* with extreme reduction percentage. Furthermore, the inhibition size was found when *Pseudomonas* spp. was applied, which may be created restrained compounds. The results of our tests verify the conclusions of Ghazanfar et al. (2016).

In the present study, some of bacterial bioagents tended to make good inhibition for *G. candidum* and *A. alternata* at the infected area of fruits without rot lesion observations. This could be back to perfect colonization of injured part by bioagents, antagonism for minerals (Senthil et al. 2011).

Results indicated that a regular rise in weight loss percent observed with an increasing storage period. This increment might be related to respiration rate and moisture evaporation (Bayoumi et al. 2023). Fruits inoculated with bioagents proficiently maintained fruit firmness, when compared to infected control. All bacterial agents were having significant effect and showed that both *B. subtilis* and *P. stutzeri* were the greatest effective bioagents in keeping the firmness of tomato fruits during storage period under pathogen infection. Biological control with bacteria maintained vitamin C and TSS % contents in comparison with infected control fruits without using bacterial bioagents.

Conclusion

In biological control, the production of microorganisms has the potential to suppress postharvest tomato fruit rots. The in vivo results indicated that some bioagents of *Bacillus* spp. and *Pseudomonas* spp. were efficient in managing tomato fruit rots caused by *G. candidum* and *A. alternata*. The findings may provide an alternative control strategy for postharvest tomato fruits pathogens in sustainable and organic farming instead of fungicides. Two bioagents were also improved some physiological traits of tomato fruits during storage period (weight loss, Vit. C, TSS, acidity). Further studies are needed on these bioagents in terms of its toxicity and other environmental impacts before using them at large scale.

Abbreviations

TSS Total soluble solids
SEM Scanning electron microscope

PDA Potato dextrose agar medium

Acknowledgements
The authors greatly acknowledge the staff of Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, Kafr-Elsheikh, Egypt.

Author contributions
NT contributed to formal analysis, investigation, writing, reviewing and editing. ME contributed to conceptualization, investigation, resources, supervision, visualization, reviewing and editing. AS contributed to methodology, writing original draft. MKE contributed to conceptualization and supervision. AK contributed to visualization, investigation and supervision. All authors read and approved the final manuscript.

Funding
Funding is by the authors.

Availability of data and materials
All data and materials are available.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 7 September 2023 Accepted: 25 October 2023
Published online: 30 October 2023

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