EGYPTIAN JOURNAL OF BOTANY (EJBO)

Print ISSN: 0375-9237
Online ISSN: 2357-0350

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PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Fungal endophytes as promising antibacterial agents against ralstonia solanacearum, the cause of wilt disease in potato plants

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All over the world, vegetable crops are suffering, including potatoes, which have been severely affected by bacterial wilt disease caused by *Ralstonia solanacearum*. *Alternaria burnsii* (*A. burnsii*), *Aspergillus niger* (*A. niger*), and *Aspergillus niveus* (*A. niveus*) are endophytic fungi that were isolated from *Trifolium alexandrinum* and were applied to enhance potato growth and stimulate defense mechanisms against *Ralstonia solanacearum* wilt (RSW) disease. The findings showed that the endophytic fungal filtrate possesses antibacterial activity against *R. solanacearum in vitro*, with inhibition zones for *A. burnsii*, *A. niveus*, and *A. niger* at concentrations of 1 mg/ml measuring 38.0 mm, 30.0 mm, and 28.0 mm in size. In pot trials, the mixture of *A. burnsii*, *A. niveus*, and *A. niger* was the most effective treatment. It reduced the disease index to 17.5% and provided 78.7% protection, followed using *A. niger* and *A. niveus* which reduced the disease index to 30% and 35%, respectively, and provided protection of 63.6% and 57.5%. Applying the fungal mixture to RSW-infected plants resulted in a 132.5% increase in soluble carbohydrates and a 94.3% increase in protein levels. The application of the endophytic fungi, singly or in combination, resulted in an increase in the phenolic content and the activities of antioxidant enzymes. Based on the study results, we recommend using a mixture of these tested endophytic fungi as therapeutic agents against RSW and as plant growth stimulants for commercial purposes.

Keywords: *Ralstonia solanacearum*, endophytes, potato, wilt disease

ARTICLE HISTORY
Submitted: March 25, 2024
Accepted: August 31, 2024

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DOI: 10.21608/ejbo.2024.279261.2777

EDITED BY: N. Khalil

INTRODUCTION

There will be 8 billion people on the planet by 2025 and 9 billion by 2050. Agriculture production must increase to feed the world's constantly growing population. Food security is unfortunately at risk due to crop loss caused by disease attacks, particularly those caused by bacteria (Mwangi et al., 2023). Plant diseases cause around one-third of the global yearly crop reductions (Savary et al., 2012). Crop production is decreased annually by 20–40% by phytopathogens (Srivastava et al., 2016). Plant protection is hampered by vegetable diseases that cause either whole or partial crop loss (Shafique et al., 2016). Diseases have harmed all vegetables, but potatoes have been particularly affected. Each year, these diseases result in output losses of 70–95 percent (Charkowski et al., 2020). Bacterial wilt is one of the most damaging potato illnesses, caused by the bacterium RS, one of the most dangerous plant infections (Bereika et al., 2020; Soliman et al., 2024). Over 450 plant species in more than 50 families are affected by this pathogen's strains worldwide, including a variety of crop plants, ornamentals, and weeds (Geng et al., 2022).

They destroy host plants quickly and cause them to be fatally wilted, resulting in direct yield losses that vary greatly depending on host, cultivar, climate, type of soil, and cropping strategy (Karim and Hossain, 2018). The wilt disease was first discovered in potatoes in Egypt, when the bacterium was isolated from wilted tubers. The pathogen was then isolated from naturally infected potato tubers of three different cultivars (Mahdy et al., 2012), and the bacteria displayed characteristic morphological traits of *Ralstonia solanacearum* on Selective Medium South Africa (SMSA) (Balabel, 2006).

Developing more effective management techniques that guarantee environmental preservation is essential (Olalekan et al., 2019; Hashem et al., 2022). Biological control of plant diseases makes use of antagonist nonpathogenic microorganisms that can lessen the disease's potential hazards on a range of crops. Physiological immunity is a vital defense mechanism against plant bacterial diseases (Zehra et al., 2021). Resistance developed as a result of elevated phenolic compounds and pathogenesis-related proteins (Jain and Khurana, 2018).

Microorganisms may survive in plants, and when isolated and analyzed, they can reveal wild microorganisms, perhaps even rare or new strains of microorganisms (Weiner, 2010; Elbahnasawy et al., 2021; El-Debaiky et al., 2023; Aburagaegah et al., 2024). Where applicable, rare and new strains can have a wide range of applications, including pest control. It is well known that endophytic microorganisms can invade higher plant parts' intracellular spaces without obviously harming the

host plants (Waghunde et al., 2017; Khalil et al., 2021). The abundance of bioactive compounds has been frequently observed. Cooperation between endophytes and host plants can enhance both species' development (Faeth and Fagan, 2002; Abdelaziz et al., 2024).

By generating secondary chemicals that impede pests and harmful microbes, endophytes can provide direct chemical protection for plants. Because endophytes live and reproduce inside healthy plant tissues and may have undergone gene recombination with the host, it has been claimed that various endophytes have more biosynthetic abilities (Muthu Narayanan et al., 2022). Therefore, the main objective of the present work is to isolate and identify the endophytic fungal species associated with *Trifolium alexandrinum* and evaluate the antibacterial activity of these fungal species against *Ralstonia solanacearum* in potato plants *in vitro* and *in vivo* through the evaluation of morphological and metabolic resistance indicators in potato plants under pot conditions.

MATERIALS AND METHODS

Isolation of Plant Growth Promoting Fungi (PGPF)

Trifolium alexandrinum was collected from the southwest of Alexandria. Plant sections (leaves and stems) were washed with sterilized distilled water twice and then surface-sterilized with C_2H_5OH 70% for 60 s, then with 4% NaOCl for the same time. We then rinsed the plant sections three times in sterile distilled water. Sterilized plant sections were plated on potato dextrose agar (PDA) medium. The plates were incubated under dark conditions for 7 days at 25 ± 2°C. Then, the growing mycelium was subcultured and purified by the single-spore technique (Abdelaziz et al., 2023a).

Identification of the Most Potent Fungi

Morphological structures were observed, including color, texture, diameter of colonies, characteristics, and reproductive and vegetative components of the tested fungi. These were also documented and characterized using molecular identification techniques using the large subunit 28S. The fungal sequences were sent to the NCBI database's BLAST program to look for phylogenetic sequences that were remarkably similar to them, and accession numbers were obtained. The Mega 5.0 program's neighbor-joining approach was used to create the tree of phylogeny (Daigham et al., 2023; Khalil et al., 2021).

Source of Pathogen

R. solanacearum was obtained from the Potato Brown Rot Project (PBRP), Dokki, Egypt. The pathogenic bacterium inoculum was generated.

Extraction of Active Metabolites from Plant Growth Promoting Fungi

The endophytic fungi were cultured in a shaking incubator at 28°C for three weeks. After the growth period, the broth was extracted using acetic acid. The extracted fungal filtrate was mixed with an equal volume of ethanoic acid and shaken until two distinct layers formed. The organic layer was then separated from the aqueous layer using a separating funnel and evaporated (Hashem et al., 2023).

In Vitro Antagonistic Activity

On nutrient agar (NA, India), the antibacterial activity of ethyl acetate crude extracts of fungal isolates (EACE) was tested. On the surface of NA, the young culture of RS was spread (cultured for 24 hours). Wells (6 mm) were cut using a sterile corkborer, 100 µl of EACE was put into each well separately, and they were left in the refrigerator at 4°C for 120 min. The dishes were incubated at 37°C for one day. After incubation, inhibitory zones were detected and recorded (Sharaf et al. 2022).

Trial Design

The Potato Brown Rot Project, Ministry of Agriculture, Dokki, Giza, Egypt, provided potato tubers (Spunta) for this study. In a separate green house, uniform Spunta tubers were planted in pots (plastic bags) 40 cm in diameter, encompassing a combination of sand and clay (1:3 W/W), a total of 6.5 kg, in a plastic greenhouse. Pots stayed in the greenhouse at day/night temperature of 22/18°C and relative humidity of 70–85%. After planting, the seedlings were normally irrigated and left for 7 days without treatment. Treatments were applied one week after inoculation with RS by adding 25 ml of fungal extract to each seedling. The pots were set up with 6 replicates in a subsequent random order: (T1) healthy control, (T2) control infected, (T3) healthy seedlings treated with *A. burnsii*, (T4) healthy seedlings treated with *A. niger*, (T5) healthy seedlings treated with *A. niveus*, (T6) healthy seedlings treated with a combination of *A. burnsii, A. niger*, and *A. niveus*, (T7) infected seedlings managed with *A. burnsii*, (T8) infected seedlings handled with *A. niger*, (T9) infected seedlings treated with *A. niveus*, and (T10) infected seedlings managed with a

mixture of *A. burnsii*, *A. niger*, and *A. niveus.* The severity of the disease was assessed 15 days after the infection. The biochemical indications of resistance were discovered 70 days after inoculation.

Disease Symptoms and Disease Index

The disease indicators were recorded, and the severity of the disease and protection percentage were determined according to Attia et al. (2021). Protection % = A-B/A × 100%, where A = percent disease index (PDI) in diseased control plants and B = PDI in diseased-treated plants, as reported by Rabea et al. (2023).

Biochemical Defense Indicators

The soluble carbohydrate content of the dried shoot was calculated according to the method described by Irigoyen et al. (1992). The procedure of Lowry et al. (1951) was used to determine the soluble protein content of the dry shoot. The total phenol content of dry shoots was determined using the procedure of Dai et al. (1993).

Antioxidant Enzymes Assay

Peroxidase (POD) activity was assayed according to the method described by Verduyn et al. (1984). The activity of polyphenol oxidase (PPO) was calculated by the procedure used by Matta and Dimond (1963). Both cultivars were assayed on fresh potato leaves.

Statistical Analyses

The obtained results were subjected to one-way variance analysis (ANOVA). The significant variances between treatments were demonstrated by CoStat (CoHort, Monterey, CA, USA) utilizing the least significant difference (LSD) test at $p < 0.05$. The results were provided as means ± standard errors (n=3).

RESULTS

Identification of the Most Potent Fungi

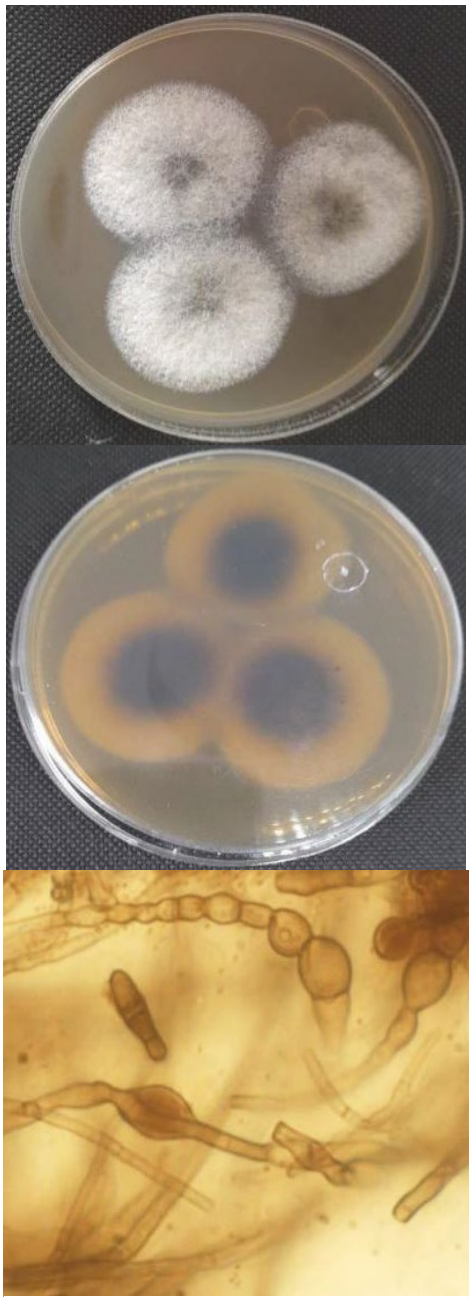
For 7 days at 25^{o}C, the fungus (*Alternaria burnsii*) was cultured on PDA. Colonies were 6.5 mm in diameter, white to buffer in appearance, and dark to buffer in reverse. Mycelium appeared with branches on the surface. Conidiophores were straight or curved, smooth-walled with septa, and pale brown. Conidia were found in short or somewhat long chains of 28 conidia, occasionally more ellipsoid, long ellipsoid, obclavate, or ovoid with 2 transeverse septa and four longitudinal septa, typically smooth in the conidial wall, or occasionally verruculose. Phylogenetic trees revealed that the fungal strain was identified genetically as *Alternaria burnsii*, which are related to the fungal strains *Alternaria* sp. isolate M24 (MK762667.1), *Alternaria alternata* strain OTA 69 (KM233353.1), *Alternaria* sp. strain QCC/M011/17 (KY744136.1), and *Alternaria alternata* isolate MT-5 (ON422111.1) that were deposited in the NCBI database (accession number OR578407) with similarity percentages 92.64%, 92.64%, 92.80%, and 92.80%, respectively (Figure 1).

The appearance of a fungal colony (*A. niger*) on PDA was black and yellow centered with a white border. The colony's backside was pale yellow (Figure 4). Conidial heads were dark brown to black, biseriate, and globose, with irregularly rough to finely rough conidia. The hyaline or pigmented conidiophores are 254-300 m long, erect, with a vesicle at each end. The vesicle, which develops at the apical end, has a variable shape ranging from spherical, hemispherical, globular, subglobose, or ellipsoidal, whereas conidiophore vesicles have a thick, spherical, globose-subglobose wall. Conidia are globular, 2.9–4 µm in diameter, ornate, brown, with rough walls that form at the end of the tubular phialides.

The fungal strain was genetically identified as *Aspergillus niger*, and it showed similarity with the fungal strains *Aspergillus niger* isolate T26 (MN198138.1), *Aspergillus niger* isolate CA05 (OP735644.1), *Aspergillus tubingensis* isolate CA42 (OP735610.1), and *Aspergillus tubingensis* isolate CA07 (OP735647.1) that were deposited in the NCBI database (accession number OR589346) with similarity percentages 99.31%, 98.97%, 98.97%, and 98.97%, respectively (Figure 2).

Microscopically, conidiophore stripes are smooth-walled, hyaline, and able to grow to be up to 1000 µm long. Conidial heads are small, radiate to a loose columnar shape, and white, turning dull ivory with age. Hemispherical vesicles have a diameter of 8–15 µm. Conidiogenous cells are severed. Metulae cover the top one-third to two-thirds of the vesicle. Conidia are spherical, hyaline, and smooth-walled, with a diameter of 2–2.5 µm (Figure 3).

Phylogenetic trees revealed that the fungal strain was identified genetically as *Aspergillus niveus* isolate AM11MSEA, which is related to the fungal strains *Aspergillus terreus var. terreus* (MH877766.1), *Aspergillus carneus* CBS (NG_069717.1), *Aspergillus niveus* CBS (NG_069613), and *Aspergillus terreus* strain *EUR1* (MF590163.1) that were deposited in



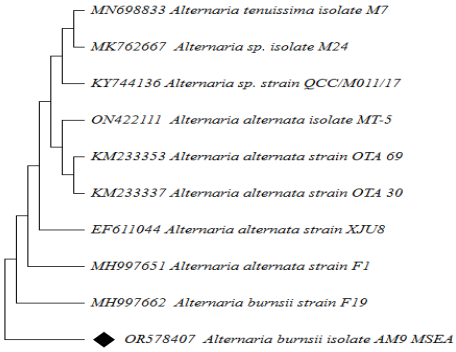
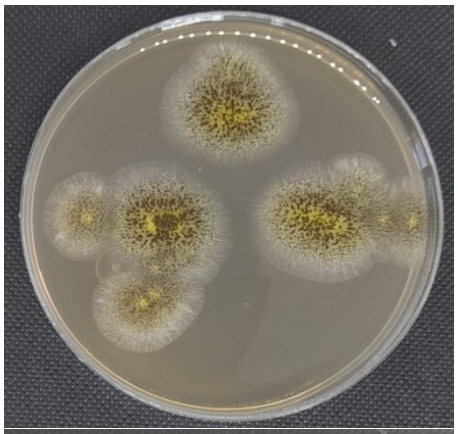
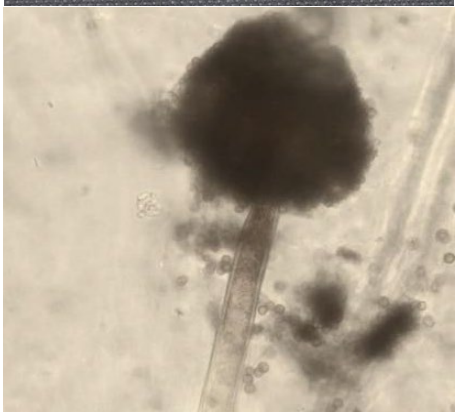


Figure 1. Morphological and phylogenetic tree of *A. burnsii*: (A) surface, (B) reverse, (C) conidia and conidiophore, and (D) phylogenetic tree.





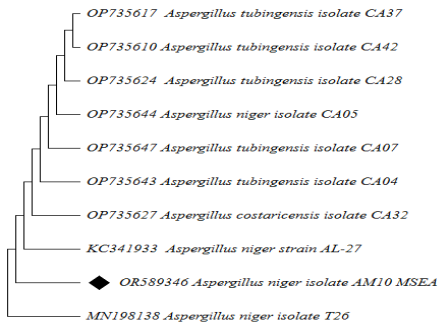


Figure 2. Morphological and phylogenetic tree of *A. niger*: (A) surface, (B) reverse, (C) conidia and conidiophore, and (D) phylogenetic tree. *A. niveus* colony characteristics revealed that colonies on PDA grew slowly, from white to light yellow.

Figure 3. Morphological and phylogenetic tree of *A. niveus*: (A) surface, (B) reverse, (C) conidia and conidiophore, and (D) phylogenetic tree.

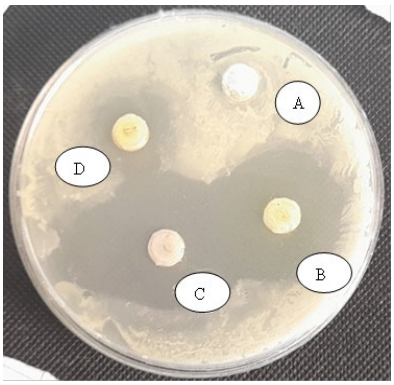


Figure 4. Antibacterial activity of endophytic fungi: (A) -ve control (ethyl acetate), (B) *A. niveus*, (C) *A. burnsii*, and (D) *A. niger*.

the NCBI database (accession number OR589345) with a similar percentage of 98.44% (Figure 3).

Antimicrobial Activity of Fungal Extract

The antibacterial activity of ethyl acetate fungal extracts of *A. burnsii*, *A. niger*, and *A. niveus* was determined using the agar well diffusion method (Figure 4). According to the findings, the four extracts have potential antibacterial activity against *R. solanacearum,* where the inhibition zones of the fungal extracts of *A. burnsii*, *A. niveus*, and *A. niger* at concentrations of 1 mg/mL were 38.0, 30.0, and 28.0 mm, respectively. The three extracts of *A. burnsii*, *A. niger*, and *A. niveus* showed *in vitro* antibacterial activity.

Disease Symptoms and Disease Index

As shown in the results of Table 1, the incidence of infection reached 82.5% due to *Ralstonia solanacearum*. On the other hand, a mix of plant growth promoting fungi (PGPF) was the best treatment in reducing PDI to 17.5% and increasing protection to 78.7%, followed by treatments with *A. niger* and *A. niveus*, where the PDI was 30% and 35% and protection was 63.6% and 57.5%, respectively, and the next treatment was with *A. burnsii*, where PDI was 60% and protection was 26.2%, respectively.

Total Soluble Carbohydrates

Results recorded in Figure 5A declared that infected plants established significant declines in the contents of total soluble carbohydrates by 57.4% in comparison with healthy controls. Treatment with a mixture of fungal biomasses significantly increased the contents of carbohydrates in *RS*-infected and healthy potato plants. Treatment of healthy plants with a fungal mixture gave a significant increase in potato total soluble carbohydrate by 15.03%,

followed by *A. niger* by 13.89%, *A. niveus* by 4.27%, and *A. burnsii* by 2.16%. Treatment of infected plants with a fungal mixture was the best treatment that showed the greatest significant increase in the contents of carbohydrates by 132.5%, in comparison with *A. niveus* by 127.06%, *A. niger* by 117.98%, and *A. burnsii* by 88.03%.

Total Soluble Protein

Results in Figure 5B illustrated a substantial decrease in the soluble protein content of RS-infected plants by 49.4% in comparison with healthy controls. On the other hand, treatment of healthy plants with a fungal mixture was presented, and the treatment combination gave a significant increase in potato plant height by 11.67%, followed by *A. niveus* by 5.98%, *A. niger* by 5.12%, and *A. burnsii* by 2.86%. Treatment of infected plants with a fungal mixture was found to be the best treatment that showed the greatest significant increase in the contents of protein by 94.3%, in comparison with *A. burnsii* by 84.02%, *A. niger* by 70.75%, and *A. niveus* by 70.33%.

Phenol Content

As shown from the results in Figure 6, RS caused a markedly significant increase in total phenols of the infected plants by 65.06% as compared to the healthy control. It was noticed that healthy potato plants treated with *A. burnsii*, *A. niger*, and *A. niveus* revealed significant rises in the contents of phenol. Furthermore, treatment with a fungal mixture significantly increased the contents of phenol in RS-infected and healthy plants. Concerning the effect of *A. burnsii*, *A. niger,* and *A. niveus* on the plants infected with RS, it was found that the fungal mixture was the best treatment that showed the greatest significant increase in the contents of phenol by 25.5%.

Antioxidant Enzymes Activity

The activities of PPO were highly increased in diseased plants linked to healthy control plants, as shown in Figure 7. Also, the treatment with a mixture of *A. burnsii, A. niger,* and *A. niveus* significantly increased the contents of the PPO activity of potato plants. In healthy plants, this mixture gave a significant increase in potato PPO by 63.36%, followed by *A. niveus* by 51.43%, *A. niger* by 36.15%, and *A. burnsii* by 24.38%. It was found that a mix of *A. burnsii*, *A. niger,* and *A. niveus* was the best treatment that showed the greatest significant increase in the PPO activity of RS-infected potato by 37.31%, in comparison with *A. niger* by 27.11%, *A. burnsii* by 26.2%, and *A. niveus* by 23.65%.

The activities of POD were highly increased in diseased plants linked to healthy control plants, as shown in Figure 8. It was noticed that healthy potato plants treated with *A. burnsii*, *A. niger*, and *A. niveus* either individually or in combination revealed significant rises in POD activity. Treatment with a mixture of *A. burnsii*, *A. niger*, and *A. niveus* significantly increased the POD activity of RS-infected potato plants. In healthy plants, it was presented that treatment with *A. niveus* gave a significant increase in PPO by 63.37%, followed by the fungal mixture by 39.22%, *A. burnsii* by 26.79%, and *A. niger* by 15.85%. The fungal mixture was the best treatment that showed the greatest significant increase in the POD of RS-infected activity by 94.37%, in comparison with *A. niger* by 58.48%, *A. burnsii* by 45.64%, and *A. niveus* by 42.02%.

DISCUSSION

The goal of biological control is to maintain a balance in agrosystems such that the host suffers less damage in the presence of pathogens due to the regulatory action of nonpathogenic microorganisms that inhibit or antagonize plant diseases. Microorganisms are currently utilized to manage infections and pests to protect vital plants for humans (Khattab et al., 2022). The first and most important phase in biological control is the identification and selection of effective antagonistic species (Khalil et al., 2021). In the current study, the endophytic fungi *A. burnsii*, *A. niger*, and *A. niveus* were tested *in vitro* for antifungal effectiveness against RS. Our findings reported that the extracts of the fungal biomasses have promising antibacterial activity against RS, where the inhibition zones of the fungal extracts of *A. burnsii, A. niveus,* and *A. niger* at concentrations of 1 mg/mL were 38.0, 30.0, and 28.0 mm, respectively. Our findings agreed with the findings of a previous study by Savi et al. (2019). They stated that endophytic microorganisms have gained interest for use in biological control, where it is necessary to develop viable alternatives for reducing the use of chemicals, such as antimicrobials and pesticides, as well as the recurring problems of their indiscriminate use and could be used for plant growth promotion and bacterial infection prevention (Hashem et al., 2023).

The mechanism of action of endophytic fungal extracts against phytopathogenic bacteria involves three main mechanisms. Firstly, endophytic fungal

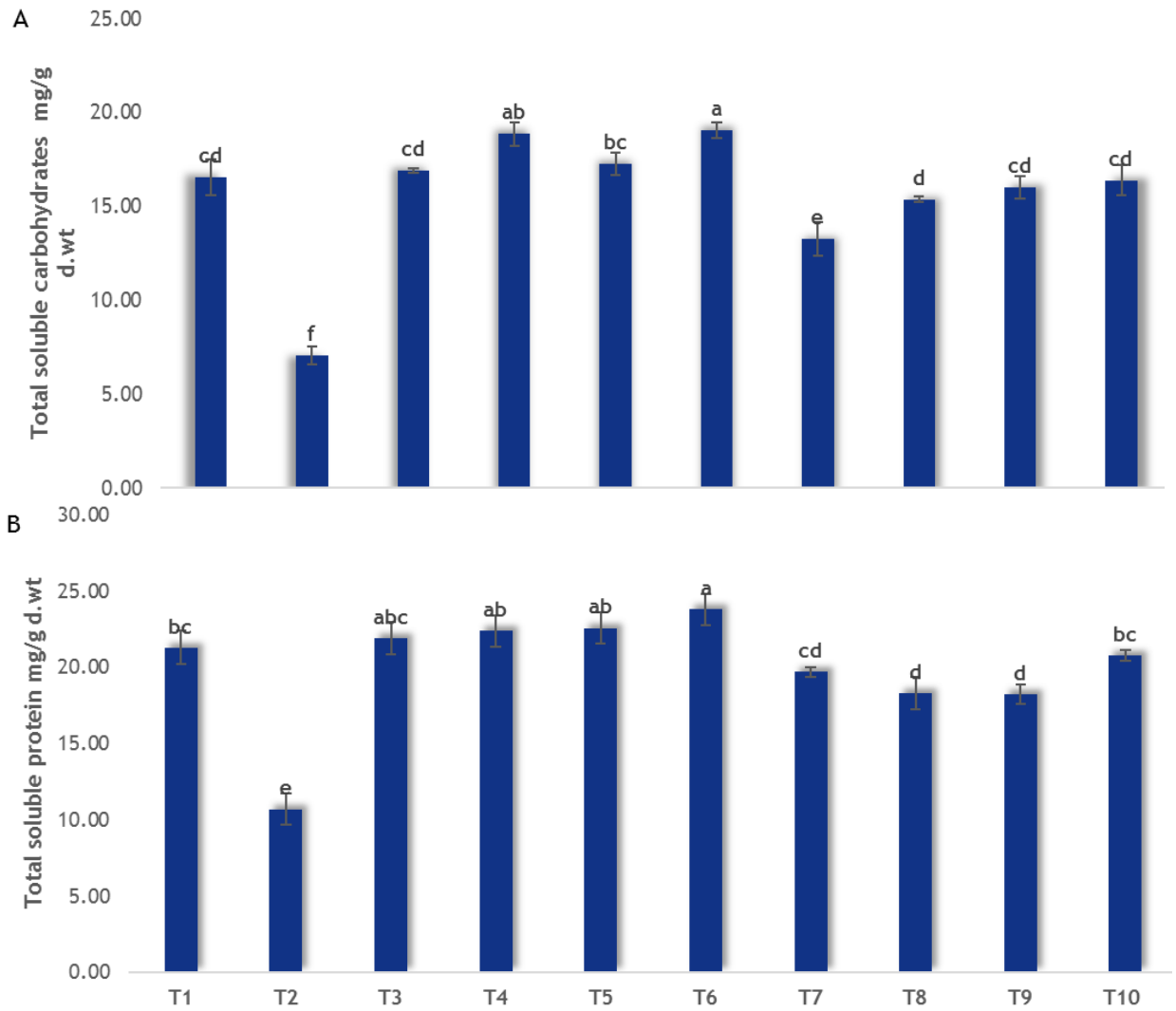


Figure 5. Effect of *A. burnsii*, *A. niger*, and *A. niveus* on (A) total soluble carbohydrates and (B) total soluble protein of potato plants. (T1) healthy control, (T2) control infected, (T3) healthy seedlings treated with *A. burnsii*, (T4) healthy seedlings treated with *A. niger*, (T5) healthy seedlings treated with *A. niveus*, (T6) healthy seedlings treated with a mixture, (T7) infected seedlings managed with *A. burnsii*, (T8) infected seedlings handled with *A. niger*, (T9) infected seedlings treated with *A. niveus*, and (T10) infected seedlings managed with a mixture.

Table 1. Effect of induced resistance elicitors on disease index and protection (%) in potato plants

Treatments	Disease symptoms Classes					PDI (disease index %)	Protection (%)
	0	1	2	3	4		
Control infected	0	0	1	5	4	82.5	0
A. burnsii	1	1	3	3	2	60.0	27.2
A. niger	3	3	3	1	0	30	63.6
A. niveus	2	4	2	2	0	35	57.5
Mix of *A. burnsii*, *A. niger*, and *A. niveus*	5	3	2	0	0	17.5	78.7

* 0 (no symptoms), 1 (slight yellowing of leaves), 2 (moderate yellow plant), 3 (wilted plant), 4 (plants severely stunted and destroyed).

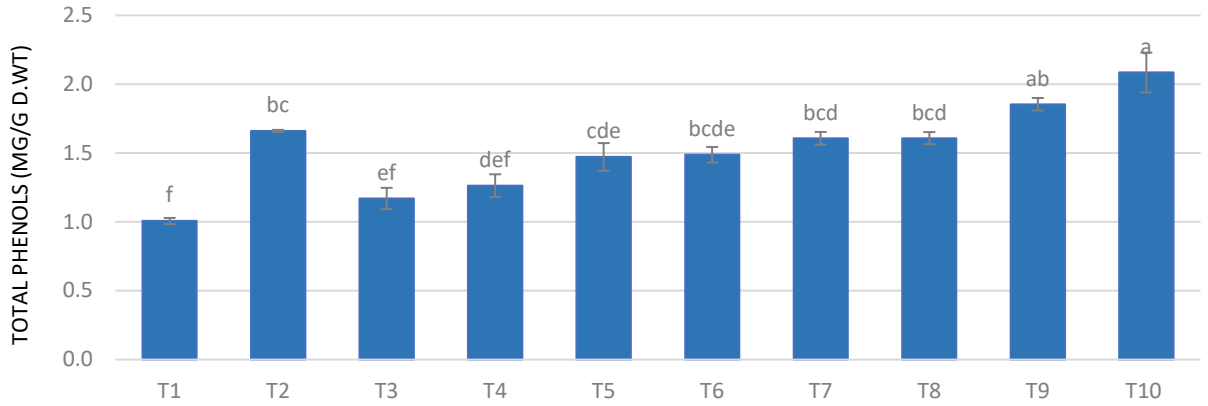


Figure 6. Effect of *A. burnsii, A. niger*, and *A. niveus* on phenol content of potato plants. (T1) healthy control, (T2) control infected, (T3) healthy seedlings treated with *A. burnsii*, (T4) healthy seedlings treated with *A. niger*, (T5) healthy seedlings treated with *A. niveus*, (T6) healthy seedlings treated with a mixture, (T7) infected seedlings managed with *A. burnsii*, (T8) infected seedlings handled with *A. niger*, (T9) infected seedlings treated with *A. niveus*, and (T10) infected seedlings managed with a mixture.

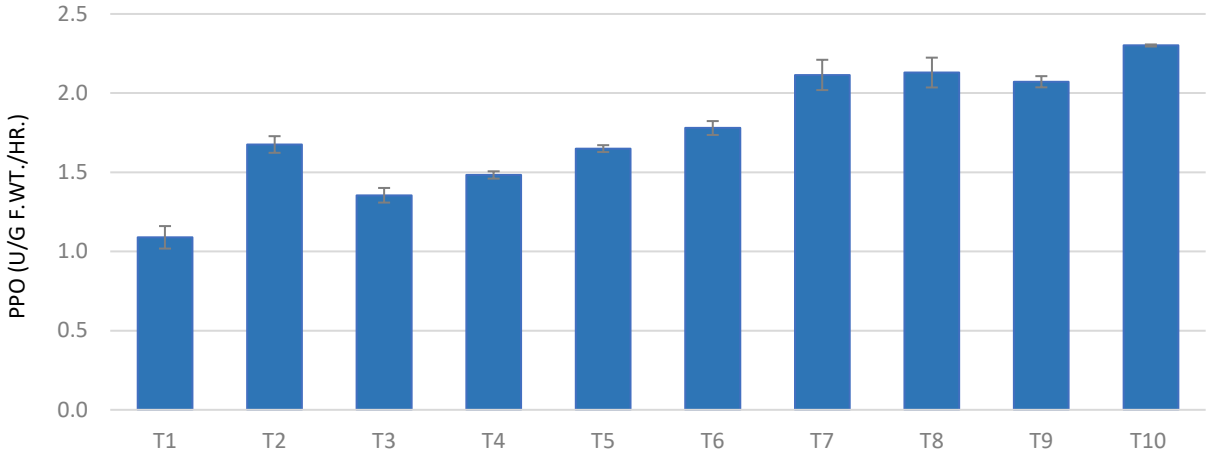


Figure 7. Effect of *A. burnsii*, *A. niger*, and *A. niveus* on the PPO activity of the potato plant. (T1) healthy control, (T2) control infected, (T3) healthy seedlings treated with *A. burnsii*, (T4) healthy seedlings treated with *A. niger*, (T5) healthy seedlings treated with *A. niveus*, (T6) healthy seedlings treated with a mixture, (T7) infected seedlings managed with *A. burnsii*, (T8) infected seedlings handled with *A. niger*, (T9) infected seedlings treated with *A. niveus*, and (T10) infected seedlings managed with a mixture.

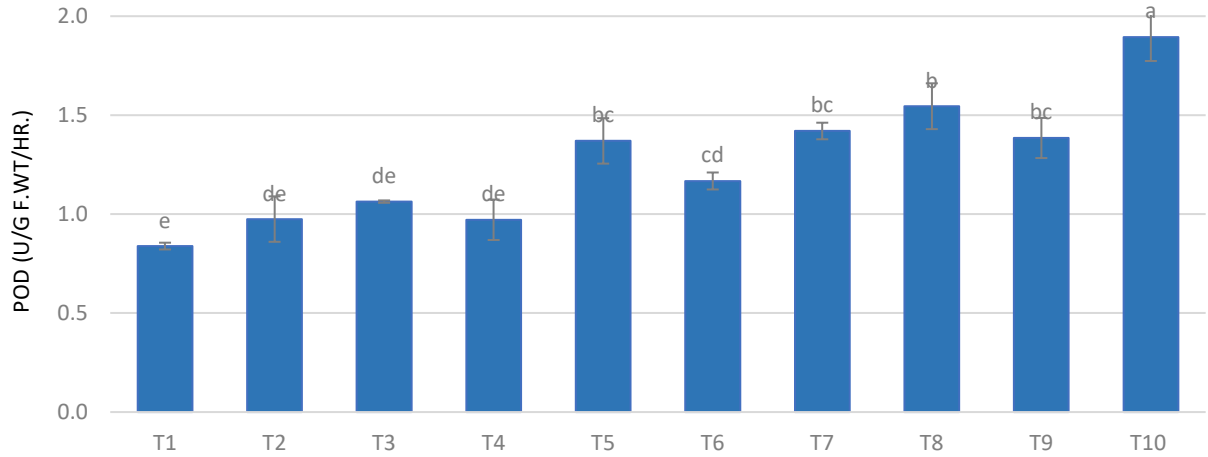


Figure 8. Effect of *A. burnsii, A. niger,* and *A. niveus* treatments on the POD activity of potato plants. (T1) healthy control, (T2) control infected, (T3) healthy seedlings treated with *A. burnsii*, (T4) healthy seedlings treated with *A. niger*, (T5) healthy seedlings treated with *A. niveus*, (T6) healthy seedlings treated with a mixture, (T7) infected seedlings managed with *A. burnsii*, (T8) infected seedlings handled with *A. niger*, (T9) infected seedlings treated with *A. niveus*, and (T10) infected seedlings managed with a mixture.

extracts can cause bacterial cell lysis, which is the rupture of the cell wall and cytoplasmic membrane, leading to bacterial death. This is achieved by the entry of active metabolites into the bacterial cell through porins and subsequent disruption of bacterial enzymes and cell structure (de Andrade et al., 2024). Secondly, endophytic fungi produce a wide range of antibiotics, which inhibit the growth of bacteria. These antibiotics can bind to specific sites on bacterial cell membranes, disrupt their permeability, or interfere with metabolic processes (Soliman et al., 2024). Thirdly, some endophytic fungal extracts have been found to exhibit antioxidant properties, which can help protect against oxidative stress and damage caused by free radicals (Abdelaziz et al., 2023b). The severity of the disease was the first guide to govern systemic resistance in plants treated with endophytic fungus. According to the data presented in this study, RS has a highly destructive effect on potato plants, causing typical wilt symptoms with a PDI of 82.5%, which is consistent with other investigations on the same pathogenic bacteria. On the other hand, a mixture of *A. burnsii*, *A. niveus*, and *A. niger* was the best treatment in reducing PDI to 17.5% and increasing the protection to 78.7%, then followed by treatments with *A. niger* and *A. niveus*, where PDI was 30% and 35% and protection was 63.6% and 57.5%, and the next treatment with *A. burnsii*, where PDI was 60% and protection was 26.2%, respectively. These results are consistent with the fact that endophytic fungi are capable of producing a vast array of chemically distinct secondary metabolites that have many functions, including their use as antimicrobials, antifungals, and antivirals (Abdelaziz et al., 2023a).

Our findings suggest that RS infectivity causes significant decreases in the contents of soluble carbohydrates and soluble proteins. These findings were explained by the fact that RS caused a reduced photosynthetic rate and an excessive respiration rate in infected plants, resulting in lower concentrations of both soluble carbohydrates and protein (Youssef and Tartoura, 2013). The results of the current investigation showed that the application of *A. burnsii*, *A. niger*, and *A. niveus* had a substantial impact on the level of total soluble sugar and soluble protein, which may operate as a marker of resistance, in both healthy and diseased plants. The noticed increase in potato plant soluble sugar and protein could be attributed to the ability of these endophytic fungi to fix nitrogen, produce plant growth regulators, and solubilize phosphate, which enhances nutrient uptake from the root rhizosphere (Priyadharsini and Muthukumar, 2017). Besides, phenolic chemicals were able to strengthen cellular membranes by restricting membrane flexibility, which lowers the ability of free radicals to cross membranes and causes membrane peroxidation (Stanley and Parkin, 1991). Data generated in the present study exhibited that RS caused a markedly significant increase in total phenols of the infected plants by 65.06% when compared to healthy control. These results are supported by many previous findings (Attia et al., 2021). The importance of phenolic compounds in controlling the spread of diseases is critical because they either produce multiple metabolic products, including those engaged in host defense mechanisms or reduce pathogen toxicity while enhancing host defensive pathways (Shalaby and Horwitz, 2015). Our results indicated that treatment with a mixture of *A. burnsii, A. niveus*, and *A. niger* significantly increased the contents of phenols in potato plants. This was the case for RS-infected and healthy plants.

These results were supported by many previous findings (Reverchon and Méndez-Bravo, 2021). The plant demonstrated a variety of defense strategies against infection, including increasing the activity of antioxidant enzymes to keep ROS levels in plant cells low. POD and other antioxidant enzymes contribute to the conversion of H_2O_2 to H_2O (Sharma et al., 2012). Increasing the activity of antioxidant enzymes plays an important role in plant physiological immunity and protecting cells from oxidation caused by infection. The antioxidant enzyme levels in RS-infected plants handled with fungal biomass extracts (*A. burnsii*, *A. niger,* and *A. niveus*) were greater than those in the control. The infected potato plants treated with *A. burnsii*, *A. niger*, and *A. niveus* displayed the highest level of PPO and POD activities compared to other treatments. The outcomes demonstrate how treatments protect potato plants from RS infection in an ameliorative manner, which is agreed with by Ezzat and Moussa (2016).

CONCLUSION

The present investigation used a promising approach that focused on applying extracts of endophytic fungal biomass to promote systemic resistance in potato plants against RSW disease. Three fungi *A. burnsii*, *A. niger*, and *A. niveus* were isolated from the *Trifolium alexandrinum* plant to boost the systemic resistance in potato plants and reduce the

severity of the RSW disease. These fungal extracts demonstrated potent antibacterial activity against RS *in vitro* and *in vivo*. Potato plants pretreated with fungal biomass extracts had significantly higher levels of total soluble proteins, total carbohydrates, and phenols. Interestingly, the harmful impact of RSW disease on potato plants was significantly decreased. In considering this, endophytic fungal biomass is a promising isolate for application in agriculture, as effective biological management against RSW disease and for the induction of immune responses in potato plants.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, for promoting this research.

AUTHORS' CONTRIBUTIONS

Mohamed S. Attia, Essam A. Soliman, Mennat-Allah El Dorry, and Amer M. Abdelaziz contributed to conceptualization, methodology, data analysis, preparation of figures and tables, writing the original draft, writing, review, and editing of resources. All authors have read and approved the published version of the manuscript.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study can be made available by the corresponding author on reasonable request**.**

COMPETING INTERESTS

The authors declare that they have no conflict of interests.

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