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RESEARCH

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Integration of *Pseudomonas fluorescens* and *Rosemarinus officinalis* for controlling of potato bacterial wilt

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Abstract

Background Bacterial wilt caused by *Ralstonia solanacearum* is considered one of the most important diseases that cause economic losses to many economic crops and spread in many regions around the world, especially in the subtropical tropics and some warm temperate regions of the world. In this study the aqueous plant extract of *Rosemarinus officinalis* and *Pseudomonas fluorescens* for their antagonistic activity against bacterial wilt in vitro, greenhouse and population of *R, solanacearum* in plants was study.

Results Following intensive screening studies, aqueous plant extract of *R. officinalis* and *P. fluorescens* was selected to study their effectiveness against bacterial wilt of potato plants and their antimicrobial activity or induction of systemic resistance in plants. In greenhouse, all soil drenching treatments on potato plants significantly reduced disease incidence of potato bacterial wilt than infected control. *R. officinalis*, *P. fluorescens* mixture between plant extract and endophytic bacteria and streptomycin reduced profoundly the disease severity by 75.51, 65.0 77.9 and 81.00%, respectively, than the infected control (83.71%). The plant extract and microorganism significantly increased fresh and dry weight of potato plants (g) per plant compared to non-treated control plants. *R. officinalis*, *P. fluorescens* mixture between plant extract and endophytic bacterium and streptomycin increased fresh weight from 100.1 to 169.9 compared to infected control also increased dry weight from 68.4 in streptomycin to 170.4% in mixture of plant extract and endophytic bacterium. The populations of bacterial pathogen *R. solanacearum* were lowest in stem of potato plants treated with plant extracts than in inoculated control plants (50%). In general, the total phenols increased in both inoculated and non-inoculated potato plants.

Conclusions *Ralstonia officinalis* and *P. fluorescens* showed a strong in vitro activity in relation growth limitation of *R. solanacearum* as well as limiting the development of bacterial wilt disease on potato plants under greenhouse conditions.

Keywords *Ralstonia officinalis*, *Pseudomonas*, Plant extract, Biocontrol agents, Potato bacterial wilt

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Background

Potato (*Solanum tuberosum*) is the most important crop on family Solanaceae. Potato plants are attacked by many diseases among them the soil borne diseases which considered to cause loss of potato tuber yield (10–90%) annually. *Ralstonia solanacearum* is an important pathogen that spreads worldwide and infects hundreds of plant species such as pepper, potato, eggplant, tomato, banana and olive (Hossain, et al. 2021). In Egypt, it is considered one of the limiting factors of potato's production (Bereika et al. 2022). In the last few years, the disease has taken more attention as a serious problem for potato exportation to European and therefore plant quarantines in importing countries are quite alert for the Egyptian potatoes (Messiha and Elhalag 2019). Control of this bacterium is much difficult because it is a soil-borne pathogen; long survival period in the soil has a wide host range and a wide biological variation. In many studies, there are several reports on different methods of disease control including chemical control such as strarner 20% pesticide (Aguk et al. 2018) and biological control using *Trichoderma asperellum* T34 (Bereika et al. 2020) and plant extract *Punica granatum* (Hassan et al. 2009) essential oils thyme oil (Sallam et al. 2021). The plant interior is now recognized a prolific environment for the discovery of endophytes with new biological activities. Therefore, biological control using microorganisms can be an option for disease management. Many beneficial microbes such as *Pseudomonas fluorescens, Acinetobacter* spp. *P. putida, Bacillus* spp., *Paenibacillus macerans, Streptomyces, Pantoea* spp. and *Trichoderma* spp. have been documented as effective biocontrol agents against *Ralstonia solanacearum* (Ling et al. 2010).

Natural plant products found in plants are among the most important sources of new chemicals extracted in order to combat plant diseases develop an integrated strategy for disease control programs (Abdallah et al 2019). Crude extract of *R. officinalis* (rosemary) was used to control of different plant diseases *e.g., Xanthomonas oryzae* pv. *oryzae* (Abdallah et al 2019), mycelial growth of *Colletotrichum graminicola, Alternaria alternata, Phytophthora* sp., *Rhizoctonia solani* and *Sclerotium rolfsii* (O¨zcab and Chalchat 2008). In this study, isolation of microorganisms from potato plants and plant extracts for their antagonistic activity against bacterial wilt in vitro, greenhouse and population of *R. solanacearum* in plants was studied.

Methods

Bacterial pathogen and growth conditions

Ralstonia solanacearum isolate PHYRS3 obtained from prior study was used (Bereika, et al. 2020). The pure culture of bacterial isolate was grown in 250-ml flasks, each containing 100 ml of NSA broth and incubated at 27 ± 2 °C for 48 h on a rotary shaker at 150 rpm; the bacterial growth was centrifuged at 10.000 × g for 8 min. The cells were pooled and re-suspended in tap water, and the cell density was then adjusted to 1×10^8 CFU/ ml, using a spectrophotometer at a wavelength of 620 nm (Kelman 1954).

Processing and cultivation of potato plants

Healthy potato tubers (*Solanum tuberosum* L.) cv. Berema were sterilized by soaking in 3% sodium hypochlorite for 5 min, washed thoroughly with SDW and planted directly into sterilized plastic pots (30 cm in diameter). Plastic pots and soil were sterilized by 5% formalin and left for 15 days before planting. The soil was carried out in plastic pots (30 cm) with a rate of 8-kg sterilized sandy-clay soil (3:1.w/w). The plastic pots were placed in an open greenhouse during the growing season. The cultivated plants were fertilized after germination every 10 days with 5 g per plastic pot of NPK (20-20-20) and irrigated with water when necessary. Subsequently, potato plants at 42-days age were used for all experiments according to Kelman and Winstead (1952).

Severity of the disease

Disease severity was recorded 42 days after inoculation using the scale described by Kempe and Siqueira, (1983).

Endophytic bacteria

The bioagent isolate was obtained from Department Plant Pathology, Assiut University, Egypt (*Pseudomonas fluorescens* ON202985). The pure isolates were kept on NA slants and stored at 4 °C for use in the following experiments.

Assessment of antagonistic activity of pseudomonas fluorescens against R. solanacearum

Pseudomonas fluorescens and *R. solanacearum* were individually grown in 100-ml conical flask containing 50 ml of nutrient sucrose ager and incubated at 28 °C for 48 h under agitated conditions by shaking at 100 rpm. After incubation, the bacterial growth in sterile micro-centrifuge tubes was centrifuged at 10,000 × g for 8 min. The supernatants were excluded and bacterial cells were individually collected. The bacterial cells were re-suspended and the density was adjusted to 2×10^8 cfu/ml, using a spectrophotometer (at 600 nm).

Antagonistic activity of bacterial isolate against *R. solanacearum* PHYRS3 was evaluated using the dual-culture method described by Algam et al. (2010) with some modifications. Using a sterile glass spreader, 0.1 ml of the cell suspension containing 1×10^8 CFU/ml of the 2-day-old culture of *R. solanacearum* was inoculation dispersed

onto the NSA medium in Petri dishes. Then the tested bacterial isolate was transferred onto 5-mm punches in the same agar inoculated with the pathogen. The inoculated Petri dishes were then incubated at 28 °C for 48 h after incubation. The antibacterial effect of the tested bacterial isolate was monitored by measurement of the inhibition zone (mm). Four replicates per treatment were used for all twice performed experiments.

Preparation of plant extracts

To prepare the aqueous extracts from the leaves of *R. officinalis*, plant material (200 g) was ground with pestle and mortar in 200 ml sterile water and the crushed was filtered through double-layered cheesecloth, followed by centrifugation at 5000 × g and room temperature for 10 min to obtain the stuck plant extract of 100% conc. Then six concentrations from plant extract were prepared by diluting the stock of each from plant extract with sterile water in sterilized flasks plugged with sterile cotton to obtain final concentrations of [1:10 (10), 1:15 (6.6%), 1:20 (5), 1:30 (3.3), 1:35 (2.9) and 1:50 (2%) w/v]. Finally, flasks containing plant extract were then kept in the dark at 5 °C until use (Kurucheve et al. 1997).

Antibacterial activity of different concentrations of Rosemarinus officinalis against Pseudomonas fluorescens

The prepared concentrations of *R. officinalis* were tested in vitro for their antimicrobial activities against *P. fluorescens,* using sterile water as negative control, respectively, according to the method of impregnated filter paper disc described by Sholberg et al. (2001). One-ml cell suspension containing 1×10^8 cell/ml prepared from the 48-h-old culture from endophytic bacteria was transferred to sterile Petri dishes (9 cm diameter) and mixed with precooled NA medium. After the medium solidification, sterilized filter paper disks (9 mm diameter, 1 mm thick) were saturated with different concentrations of plant extract and then placed in the middle of inoculated plants. The Petri dishes were then incubated at 27 °C for 48 h. After incubation, the inhibition zone around each disk was measured in mm. Four replicates per treatment were used for all twice performed experiments.

Antibacterial activity of Rosemarinus officinalis against Pseudomonas fluorescens

From above experiment the concentration 1:10 w/v was used against bioagent. The prepared concentration 1:10 w/v of *R. officinalis* was tested in vitro for their antimicrobial activities against *P. fluorescens*; sterile water was used as a negative control, according to the method described above. After incubation, the inhibition zone around each disk was measured in mm. Four replicates per treatment were used for all twice performed experiments.

Effect of Rosemarinus officinalis and Pseudomonas fluorescens on severity of bacterial wilt disease of potato plants in greenhouse

Six weeks from planting, stem of the growing plants was injected with 100 µl bacterial suspension from bacterial pathogen contacting 1×10^8 CFU/ml, using a syringe 1 cm above the soil. After the inoculation, the plants were kept in a moist chamber at 25 °C for 2 days before being transferred to the greenhouse. Then 100 ml bacterial suspension containing 2×10^8 CFU/ml of tested bacteria strain and 100 ml of plant extract (at 1/10 w/v conc.) and 100 ml from mix between 50 ml endophytic bacteria and 50 ml plant extract were added to the plastic pots 48 h before inoculation with the bacteria pathogen. The disease severity was estimated, as described by Kempe and Siqueira, (1983). Four replicates per treatment were used for all twice performed experiments.

Fresh and dry weight of vegetable mass of potato plants

The fresh and dry weights of potato plants were estimated by harvesting the vegetable total of potato plants for the treatments used from above the surface of the soil, then tab water was used to remove impurities and dust from the vegetable mass, and then, the drying process was done using dry air and paper towels. The vegetable total was placed in paper bags to calculate the fresh weights of all the transactions used. To calculate the dry weights for the previous treatments, the paper bags containing the vegetable total of potato plants were placed in an oven for 4 days at a temperature of 70 °C; then, the weighting process was carried out to calculate the dry weight.

Effect of soil treatment with Rosemarinus officinalis and Pseudomonas fluorescens on population of bacteria pathogen in plant

To calculate the number of bacteria causing bacterial wilt disease in potato plant treated with the above-mentioned treatments, a portion of the stalks of potato plant weighting one gram of the stem tissues of each treatment after 42 days from were injected with bacteria pathogen and washed with tap water used to remove the impurities and suspended soil; then, it was dried using dry air and paper towels. The plant parts used were sterilized by ethyl alcohol at a concentration of 70% for 30 s for 3 consecutive times and then removed using sterile distilled water. The plant parts were placed in a sterile mortar, then added 10 of 0.1 M potassium phosphate buffer (ph 7.0), then grinded well until the solution was homogeneous and 1 ml was taken to make several concentrations by phosphate buffer with concentration of 1 to 10. The 200 µl

of each dilution was transferred on a selective medium (TTC) and spread by using a glass rod (Hassan et al. 2009). Petri dishes were placed in the incubator at 27 °C for 48 h, after which the number of bacterial colonies of the pathogen was counted (Roberto et al. 2002).

Biochemical investigations

Following greenhouse trails, plant samples were taken two days before inoculation, at zero and two, four and six days after inoculation for determining total contents of phenolic and salicylic acids.

Determination of total phenolic and salicylic acid contents

The method described by Rapp and Zeigler (1973) was used for the preparation of plant samples. Plant leaves (1.0 g) of potato were ground in liquid nitrogen and homogenized in 10 ml of 80% methanol. The homogenate was centrifuged at 1.000 × g and 4 °C for 30 min. The pellet was discarded after the addition of ascorbic acid (0.1 g/5 ml). Then the homogenate was evaporated in a rotary evaporator at 65 °C, and the process was repeated three times each for 5 min. The residues were then dissolved in 5 ml of 80% methanol.

Total phenolic content

The phenolic content was determined according to the method described by Sahin et al. 2004. The reaction mixture was composed of 0.02 ml methanol extract, 0.5 ml Folin reagent, 0.75 ml of 20% Na_2CO_3 solution and 8 ml water. The mixture was incubated at 37 °C in water bath for 60 min. Methanol was used a negative control. Total phenol content was assayed spectrophotometrically at 767 nm as mg/g plant fresh weight using gallic acid as standard.

Total phenolic acid = mg gallic acid/g plant material.

Salicylic acid

Salicylic acid content was estimated by the method described by Dat et al. 2000 with some modifications. A 500 µl of homogenate sample was mixed with 250 µl of 10 N HCl and 1 ml methanol. The sample was incubated in a water bath at 80 °C for 2 h. The sample was then neutralized with 4–5 drops of 1 M $NaHco_3$, and 1 ml methanol was added to the mixture. The optical density (OD) was measured at 254 nm, and the salicylic acid content was calculated as µg salicylic acid per g plant fresh weight.

Statistical analysis

The complete randomized experimental design with four replicates per treatment was used for all twice performed experiments. All data obtained were analyzed using the statistical analysis system (SAS Institute Inc., 1996). Means were compared with L.S.D test at $P \leq 0.05$ levels. Means of standard deviation for four plants per treatment were also shown.

Table 1 Effect of *Rosemarinus officinalis* on growth of *Pseudomonas fluorescens* in vitro

Treatments	Concentration	Inhibition zone diameter (cm)
Rosemarinus officinalis	1:10 w/v	1.1c
R. officinalis	1:15 w/v	1.2 c
R.officinalis	1:20 w/v	1.0c
R. officinalis	1:30w/v	1.34 b
R. officinalis	1:35 w/v	1.34 b
R. officinalis	1:5 0 w/v	2.0 a
Water	0.0	0.0 d

Values in the column followed by different letters indicate significant differences among treatments according to a least significant differences test ($P = 0.05$)

Table 2 Effect of *Rosemarinus officinalis* and *Pseudomonas fluorescens* on growth of *Ralstonia solanacearum* in vitro

Treatments	Concentration	Inhibition zone diameter (cm)
Rosemarinus officinalis	(1:10 w/v)	2.5 b
Pseudomonas fluorescens	1×10^8 cfu/ml	2.2 c
Streptomycin	1.0 mg/ml	3.33 a
Water	0.0	0.0 d

Values in the column followed by different letters indicate significant differences among treatments according to a least significant differences test ($P = 0.05$)

Results

Effect of Rosemarinus officinalis on growth of Pseudomonas fluorescens in vitro

All concentrations from plant extract tested significantly inhibited the growth of *P. fluorescens* in vitro compared with negative control (water) but non-significantly among the concentrations (1:10, 1:15 and 1:20) and non-significantly between concentrations 1:30 and 1:35 v/ v). The highest inhibition zone was recorded by (1:50 W/V). Concentration of plant extract was 2.0 cm. Results showed no effect of plant extract on growth *P. fluorescens* in vitro (Table 1).

Evaluation of antagonistic activity of Rosemarinus officinalis and Pseudomonas fluorescens against Ralstonia solanacearum

The antagonistic activity *R. officinalis* and *P. fluorescens* against *R. solanacearum* PHYR3 was evaluated using dual-culture method (Table 2). The results indicated

that the highest inhibitory effect against the PHYR3 was attributed to the plant extract caused production of inhibition zone as 2.5 cm compared with 3.33 cm because of specific antibiotic streptomycin and 2.2 cm caused *P. fluorescens*.

Effect of Rosemarinus officinalis and Pseudomonas fluorescens on severity of bacterial wilt disease of tomato plants in greenhouse

The results illustrated in Table 3 showed that the all treatments used in experiments in vivo significantly reduced disease severity of bacterial wilt of potato than non-infected control, but non-significantly between treatments streptomycin and mixture of plant extract and *P. fluorescens*. The soil drenching with streptomycin at concentration 1.0 mg /ml significantly reduced the incidence of potato bacterial wilt disease compared with the infected control. Soil drenching applications with 100-ml pot of treatments plant extract *R. officinalis*, *P. fluorescens* mixture between plant extract and endophytic bacteria and streptomycin reduced profoundly the DSI by 75.51, 65.0 77.9 and 81.00%, respectively, than the infected control (83.71%).

Fresh weight (FW) and dry weight (DW) 0f potato plants after applications with Rosemarinus officinalis and Pseudomonas fluorescens under vivo trails

Effect of soil drenching of treatments on the FW and DW of potato plant was determined at 7 weeks after inoculation with *R. solanacearum* PHYR3. Data indicated that they were significantly lower than that of non-inoculated control plants. The treatment with mixture between plant extract and *P. fluorescens* caused the highest increase in both FW and DW of potato plants (170%), followed by *R. officinalis* (100.75–83.7%) as shown in Table 4.

Table 4 Effect of *Rosemarinus officinalis* and *Pseudomonas fluorescans* on fresh and dry weight of potato plants under open vivo condition

Treatments	FW of potato plant (g/plant)	DW of potato plant (g/plant)
Rosemarinus officinalis (Ro)	66.75 c	18.00 c
Pseudomonas fluorescens (Pf)	66.50 c	16.90 c
Ro + Pf	89.75 b	26.50 b
Streptomycin	65.2 c	16.5 c
Infected control	33.25 d	9.80 d
Non-infected control	100.50 a	29.30 a

Values in the column followed by different letters indicate significant differences among treatments according to a least significant differences test ($P = 0.05$)

Effect soil drenching with Rosemarinus officinalis and Pseudomonas fluorescens on number of bacterial pathogen in stem potato tissues under vivo conditions

The results in Table 5 showed effect on number of bacterial pathogens within artificially inoculated potato plant. Data were calculated after 49 days for injection with bacterial pathogen. Data were significantly reduced the numbers of *R. solanacearum* cells than the control. Results also indicated that the bacterial counts of the pathogen drastically decreased in stem sections than that of inoculated control plants. The population of *R. solanacearum* were lowest in potato plants treated with *R. officinalis* (4.13×10^8 cfu/ml), *P. fluorescens* (3.6×10^8 cfu/ml), the mixture (2.8×10^8 cfu/ml) and streptomycin (1.0×10^8 cfu/ml) than infected control plant (2.8×10^{10} cfu/ml).

Table 3 Effect of *Rosemarinus officinalis* 1:10 w/v and *Pseudomonas fluorescans* 1×10^8 cfu/ml on severity of bacterial wilt disease of tomato plants in greenhouse

Treatments	Disease index	Reduction of disease severity
Rosemarinus officinalis (Ro)	20.5 c	75.51
Pseudomonas fluorescens (Pf)	29.3 b	65.00
Ro + Pf	18.5 d	77.90
Streptomycin	15.9 d	81.00
Infected control	83.71 a	–
Non-infected control	0.00 e	0

Values in the column followed by different letters indicate significant differences among treatments according to a least significant differences test ($P = 0.05$)

Table 5 Effect of soil drenching with *Rosemarinus officinalis* and *Pseudomonas fluorescans* on number of bacterial pathogen in stem potato tissues under *vivo* conditions

Treatments	Bacterial pathogen population (cfu/g stem tissue)
Rosemarinus officinalis (Ro)	4.13×10^8 b
Pseudomonas fluorescens (Pf)	3.6×10^8 c
Ro + Pf	2.80×10^8 c
Streptomycin	1.0×10^8 d
Inoculated control	2.8×10^{10} a
Non-inoculated control	0.00 e

Values in the column followed by different letters indicate significant differences among treatments according to a least significant differences test ($P = 0.05$)

Effect of Rosemarinus officinalis and Pseudomonas fluorescens on production of biochemical precursors caused resistance induction in potato plant

Total content of phenols

The total content of phenols in potato plants injected with the diseased pathogen of bacterial wilt and treated with the treatments used in the research. A significant increase was shown in the total content of phenols compared to the infected and healthy potato plants. The increase in the total content of phenols started after 2 days of adding the treatments of potato plants and continued to increase until 6 days of treatment, which to the accumulation of phenol concentrations in potato plants (Fig. 1).

Salicylic acid

The results indicate that there was a significant difference in the total content of salicylic acid content in potato plants treated with the *R. officinalis* and *P. fluorescens* than either only potato plant infected with *R. solanacearum* or healthy plants. The total content of SA was estimated after 2 days from treatment with the up to 8 days. It was found that the use of the treated with streptomycin gave the highest increase in the total content of salicylic acid compared than those treated with *R. officinalis* and *P. fluorescens* (Fig. 2).

Discussion

Bacterial wilt and brown rot diseases are the most destructive ones of potato caused by *R. solanacearum*. The pathogen has an extensive host range of 200 plant species in over 50 families, and the family Solanaceae is one of the most economically essential host plants (Hassan et al. 2009). Antibacterial activity of certain plant extract of *R. officinalis, P. fluorescens* and streptomycin against *R. solanacearum* was investigated in vitro. Data showed the treated ones were able to inhibit the growth of the causal pathogen in vitro. Data obtained revealed that the treated concentrations (1:10, 1:15, 1:20, 1:30, 1:35 and 1:50 W/V) inhibited the *P. fluorescens* growth at all concentrations, except the concentration 1:50 W/V. Results reported here in indicated that non-significant inhibitory effect of *R. officinalis* on the bioagent growth in vitro, similarly to those obtained by Wagura et al. (2011). In greenhouse trails, the all treatments selected to control the bacterial wilt showed a significant reduction in disease severity; the results are in agreement with those obtained by Abo-Elyousr and Asran (2009), who mentioned that the use of different plant extract could be inhabited the disease severity of tomato bacterial wilt. Rosemary (*Rosemarinus officinalis* L.) contains secondary metabolites that can control different pathogens (Rožman & Jeršek 2009). The use of all treatments caused increasing the

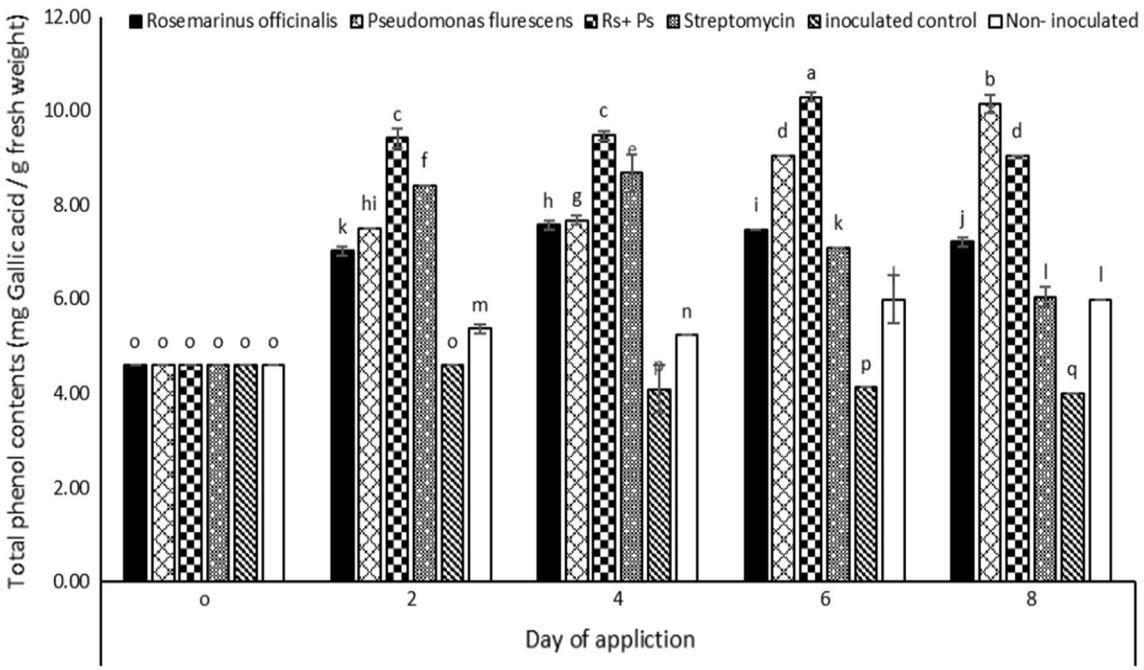

Fig. 1 Effect of *Rosemarinus officinalis* and *Pseudomonas fluorescans* on total phenol contents in inoculated potato plants. Values followed by different letters indicate that means are significantly different, while identical letters indicate that means are not significantly different according to Fisher's least significant difference at $p = 0.05$

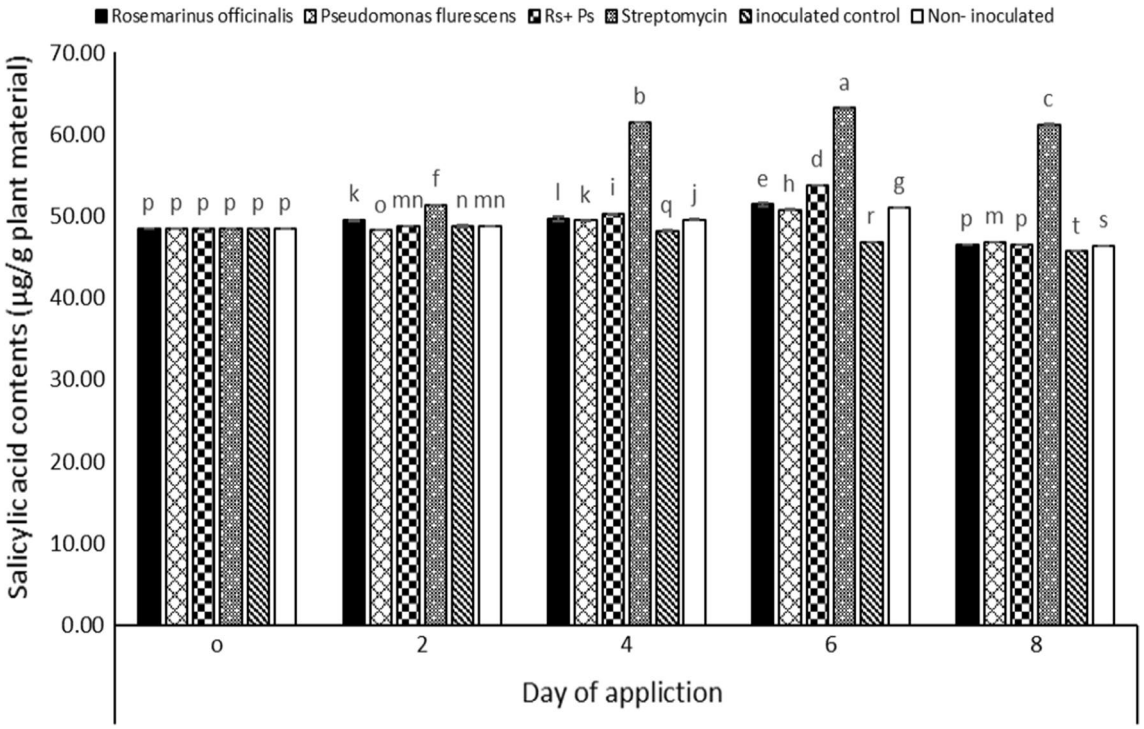

Fig. 2 Effect of *Rosemarinus officinalis* and *Pseudomonas fluorescans* on salicylic acid contents in inoculated potato plants. Values followed by different letters indicate that means are significantly different, while identical letters indicate that means are not significantly different according to Fisher's least significant difference at $p = 0.05$

productivity of the FW and DW by treatments than untreated control. This may be due to reduction of the disease incidence in addition to the increase of vegetative characters. Such results are in agreement with those reported by Sallam et al. (2021). In the present study, all treatments were tested for the presence of diseased xylem cells in the lower parts of potato plants treated with *R. officinalis* and *P. fluorescens* and streptomycin and it was determined 6 weeks after inoculation and the numbers of *R. solanacearum* were lower in infected and untreated potato plants. On the basis of obtained results, it is possible that the non-proliferation of diseased causative cell in plants treated is due to the presence of defensive components inside plant cells, especially in xylem due to the development of resistance through the use plant extracts. Salicylic acid may have a great effectiveness at the level of local or systemic resistance in plant diseases. It may include a direct effect on the effectiveness of some pathogens that infect plants as reported by (Sticher et al. 1997). In the present study, sometimes the presence of salicylic acid is necessary to determine the patterns and types of plant resistance to the diseases that attack plants. Also, salicylic acid is considered the defensive line used by the plant to resistance all pathogens present in the plant parts. The accumulation of increased concentration of salicylic acid inside the plant is an essential condition for the development of acquired resistance against *R. solanacearum* in potato plants (Abo-Elyousr et al. 2017). The results showed that the plant extracts and endophytic bacteria stimulate the resistance of infected plants to pathogens by activating the SA-dependent signaling pathway or by activating some other new factors that do not depend on SA jasmine acid or ethylene (Zimmerli et al. 2000). Total phenolic contents were higher in treated potato plants than the infected and healthy control plants. The presence of phenolic compounds at the site of infection is closely related to the reduction of the development of pathological symptoms because these compounds are toxic to pathogens. Also, one of the most important factors that increases resistance to disease is the increase in the PH inside the affected cells due to the increase in the total content of phenolic substances, which leads to the inability of the pathogen in patients to grow and develop symptoms due to the lack of optimal conditions for the growth of the pathogen. In the present study tuber treatment and soil application with treatment resulted in increasing the amount of phenolic substances as a result of infection with the pathogen (Abd-El-Kareem et al. 2001).

Conclusions

Rosemarinus officinalis and *P. fluorescens* showed a strong in vitro activity in relation growth limitation of *R. solanacearum* as well as limited the development of bacterial wilt disease on potato plants under greenhouse conditions. In addition to the association of some enhancing activities such as SA content and phenol content with the process of resistance to induction against *R. solanacearum.*

Abbreviations

NSA	Nutrient sucrose ager
TZC	2,3,5-Triphenyltetrazolium chloride
OD	Optical density
FW	Fresh weight
DW	Dry weight

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Not applicable.

Author contributions

All authors contributed equally in the manuscript; MHA suggested the idea of the work and contributed to data curation and their validation. MFFB performed the experiments and prepared the draft and contributed to the formal analysis of the data. KAMA, NMA, MHA and MFFB contributed to the reviewing and editing the manuscript. All authors reviewed and approved the final version of the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

This manuscript is in accordance with the guide for authors available on the journal's website. Also, this work has not been published previously and is approved by all authors and host authorities.

Consent for publication

Not applicable.

Competing interests

No potential competing interests were reported by the authors.

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