



Efficacy of plant extracts and bio-fertilizers for the control of bacterial wilt and soft rot agents in potato

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Abstract

This study aims to assess the suppressive effect of plant crude extracts, bio-, and mineral fertilizers, plant-animal origin compost, and vermicompost for controlling potato bacterial wilt and soft diseases in in-vitro and in-vivo pots experiments. Nine plant crude extracts were screened in-vitro for their antibacterial effectiveness against *Ralstonia solanacearum*, *Pectobacterium carotovorum*, and *Pectobacterium atrosepticum*. Ethanolic and water crude extracts from peels and leaves of *Punica granatum* L. and *Eucalyptus camaldulensis* Dehnh respectively, showed the most growth inhibition against the three tested bacteria. In-vivo assessment, the peels ethanolic and water crude extract for *P. granatum* L. were the most effective treatments that exhibited a 91% and 89% decrease in the disease severity, area under disease progress curve of *R. solanacearum* wilt disease, population count, and enhanced the potato growth parameters, triterpenoids, and higher potato yield compared to other assessed mineral and organic fertilizers. In another in-planta experiment, treating potato tubers with these plant extracts significantly suppressed the maceration symptoms on the tubers, pre-inoculated by either *P. carotovorum* or *P. atrosepticum* with a treatment efficiency (TE) of 100% for each, except for the water extract of *E. camaldulensis* Dehnh that showed 22% of efficiency as compared to untreated potato tubers. Phytochemical characterization and Gas Chromatography-Mass Spectrometry (GC-MS) revealed the presence of phenols, tannins, and flavonoids that have antimicrobial activity in both extracts. These treatments are recommended to be applied individually or as an effective component of integrated pest management program to enhance potato tolerance against bacterial pathogens during the growing season and storage.

Keywords Bacterial wilt · Soft rot · *Punica granatum* · *Eucalyptus camaldulensis* · Plant crude extracts · Disease suppression · Bio-fertilizers · Potato

Abbreviations

AUDPC	Area under disease progress curve
Rs	<i>Ralstonia solanacearum</i> bacteria
CFU	Colony forming unit
DS	Disease Severity
EOs	Essential oils

FWt	Fresh weighed
GC-MS	Gas Chromatography-Mass Spectrometry
GA	Gallic acid
PCR	Polymerase chain reaction
SDW	Sterilized distilled water
TE	Treatment efficiency

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Introduction

In Egypt, potato is a substantial vegetable crop plowed to cover national food consumption, exordium, and manufacturing, and its export production to the European United (EU) has recently ranged from 70 to 90%. A steady increase in the annual rate of potato production (5.23 M Tons), as well as potato exportation (831,996 tons), was recorded in Egypt in the last three years 2020, 2021, and 2022. In 2022,

the value of potato exports from Egypt amounted to approximately 4.7 billion Egyptian pounds (FAO-STAT 2022).

The potato (*Solanum tuberosum* L.) crop might be exposed to various pathogens that negatively affect tubers' quality (economic value) and production scale. Bacterial diseases such as potato brown rot (bacterial wilt) etiologies by *R. solanacearum*, soft rot, and blackleg caused by pectinolytic bacterial agents, *Pectobacterium* sp. and recently several species of *Dickeya* accounted for the most critical losses in the Egyptian potato yield (Elhalag et al. 2015, 2020). The potato bacterial wilt pathogen negatively influenced the productivity of several crops in different countries (Hayward 2000). It is classified, depending on the zero-tolerance restrictions imposed in Egypt, as a severe quarantine disease, and its only recorded race in Egypt includes Phylo-type IIa, Sequevar 1, race 3, biovar 2 (Farag et al. 1999). No more efficacious chemical treatments are beneficial to enucleate this bacterial wilt pathogen from infected soil and water nor to recover the infected potato plants in the Nile Delta of Egypt. Therefore, planting certified and resistant cultivars could be one of the most effective approaches, though there are no currently available resistant ones. Another serious potato disease is bacterial soft rot, which is caused by either single or complex agents and results in greater economic loss in overall yield than other bacterial diseases. In Egypt, both *P. carotovorum* and *P. atrosepticum* influence the growing potato plants and tubers yield storage, aside from the negative economic impact during transportation (Rashid et al. 2012; Elhalag et al. 2020). Their potato yield damages have been recorded to range from 15 to 30% (Abo-Elyousr et al. 2010).

To promote the sustainability of Egyptian potato production and minimize yield losses, the control of their diseases should be handled in an environmentally friendly and safe way. The increasing desire for healthy food that is chemical-free and harvested from organic farming has led to the augmented application of bio-pesticides due to their advantages: low cost, less toxicity to humans, animals, plants, soil, and the environment, more quickly biodegradable, and highly specific targeting against pests (Popp et al. 2013).

Recently, phytopathologists have renewed their research interest in using organic and bio- or mineral fertilizers as antibacterial agents (Pinto et al. 2023). Recent research studies have identified several antimicrobial agents of plant origin (McMurray et al. 2020; Mulyaningsih et al. 2011). A wide range of biotic molecules, namely those that act as secondary metabolites, are generated by plants, thereby directing them as an opulent source for a variety of antibacterial agents (Abd-Elrahim et al. 2022; Mostafa et al. 2018; Khaleel et al. 2016). Concerning plant extracts that possess noticeable efficiencies versus *R. solanacearum* pathogen, the essential oils (EOs) of palmerosa and lemongrass plants

were reported to suppress its propagation cycles (Paret et al. 2010). Furthermore, in the literature, different research studies concluded that the EOs of thyme and palmerosa plants are able to achieve great suppression activities on tomatoes as bio-fumigants against bacterial wilt (Abd-Elrahim et al. 2022; Ji et al. 2005). Several distinct phytochemical components have been detected in the *E. camaldulensis* Dehnh leaves, particularly sterols, cineol, geraniol, cuminal, cardiac glycosides, saponins, phellandrene, aromadendral, valeraldehyde, cymene, isoprenoids, catechol, phenolics, flavonoids, terpenes and tannins (Babayi et al. 2004). These main compounds of eucalyptus are known to have antimicrobial effectiveness, as reported by Mulyaningsih et al. (2011). Moreover, in a previous in-vitro study, ethyl acetate peel extracts of *P. granatum* L. showed significant antibacterial activity versus different pathogenic plant bacteria including, *R. solanacearum*, *P. carotovorum* subsp. *carotovorum* and *Xanthomonas gardneri* (Khaleel et al. 2016). Other investigators have proposed that antimicrobial constituents of these plant extracts, including phenolic, alkaloid, and terpenoid compounds, might react with proteins and enzymes of the bacterial cell membrane, inciting its disorder and cell death, or might inhibit the enzymes requisite for its amino acids biosynthesis (Fagbemi et al. 2022; Jing et al. 2015; Askarne et al. 2012; Savchenko et al. 2010; du Plooy et al. 2009; Park et al. 2009; Sharma and Tripathi 2008; Burt 2004; Daferera et al. 2000;). The organic formulations containing microorganisms known as biofertilizers, or bio-inoculants, are beneficial to agricultural productivity in terms of nutrient delivery, especially with regard to N and P. They proliferate quickly and form a dense population in the rhizosphere whether given as a soil treatment, seed treatment, or seedling root dip. Through the process of biological nitrogen fixation, biofertilizers can fix atmospheric nitrogen (N₂), solubilize plant nutrients like phosphates, and promote plant development by synthesizing chemicals that encourage plant growth. Biofertilizers are becoming more and more important in agriculture because they are safe, non-toxic, and environmentally friendly products (Sharma et al. 2007; Wani et al. 2016). Antibiotics, cell wall hydrolases, and siderophores are known to be excreted by Bacilli as antagonistic metabolites. It has previously been reported that *Pseudomonas*, *Bacillus*, and *Paenibacillus* are plant-growth-promoting rhizobacteria (PGPR) in Morocco (Hashem et al. 2019; Bahadou et al. 2018).

In recent years compost and vermicompost have been widely used as a rich organic fertilizer for controlling several soil-borne pathogens, also known as plant growth promoters, and contain nutrients and beneficial microbes for plant health (Abada and Hassan 2017; Masoud et al. 2023).

The current research aims to assess the effectiveness of various plant crude extracts compared to other bio- and

mineral fertilizers, plant-animal waste-origin compost, and vermicompost, for enhancing potato tolerance against wilting and soft rot diseases in in-vitro and in-vivo pot experiments.

Materials and methods

Plant materials

Different plants' parts were used for preparing the crude plant extracts including the seeds and leaves of *Azadirachta indica* and *Azadirachta* Juss, collected from the Faculty of Agriculture, Cairo University, Cairo, Egypt; leaves of *Calotropis procera* (Aiton); *Plectranthus amboinicus* Lour.; *Ocimum basilicum* L. and *E. camaldulensis* Dehnh, obtained from Fac. of Agric., Al-Azhar Univ., Cairo, Egypt) and, peels of *P. granatum* L., *Citrus limon* L. and *Citrus sinensis* (L.), bought from the local market, Cairo, Egypt) (Table 1).

Potato tubers cultivar Spunta tested-free from bacterial pathogens were obtained from the Potato Brown Rot Project, Dokki, Giza, Egypt.

Bacterial strains and growth conditions

Virulent phytopathogenic bacteria, sourced from the culture collection of the Bacterial Diseases Research Dept., ARC, Giza, Egypt; referring to the authors' previous studies as; 2 strains of *R. solanacearum* K3 and K10; Phylotype IIa, Sequevar 1 for wilting disease (race 3, biovar 2) (Elhalag et al. 2015) and, 2 virulent strains of soft rot, *P. carotovorum* (100 H) and *P. atrosepticum* (MH3c) (Elhalag et al. 2020).

The bio-fertilizers consist of *Azospirillum brasilense* and *Azotobacter chroococcum* (nitrogen fixers), *Bacillus megaterium*, and Vesicular Arbuscular Mycorrhiza-VAM (P-dissolving agents). and *Bacillus cereus* (K-dissolving bacteria) were purchased from the Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Mineral fertilizers and compost

Mixed mineral fertilizers, including N₂, rock phosphate (16.2% P₂O₅), and Potash feldspar (8.1% K₂O) at a ratio of 2:1:3, according to El-Sayed et al. (2015) were bought from Al-Ahram Mining Company, Cairo, Egypt.

The plant-animal waste origin compost was obtained from Alfayrouz Company, El-Beheira Governorate, while vermicompost was originated from the organic experimental farm (Environment and Bio-Agriculture Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt).

Confirming the virulence of the phytopathogenic bacteria

The virulence of all pathogens was confirmed before being used in this study to ensure that they maintain their pathogenic activity on their relevant hosts after the period of storage and subculturing on artificial media. *R. solanacearum* was bio-assayed on tomato seedlings (Janse 1988), while *P. carotovorum* and *P. atrosepticum* were done using potato tuber maceration assay (Czajkowski et al. 2014).

Molecular characterization of the bacterial isolates

The molecular PCR technique was handled to confirm all isolates of *R. solanacearum*, *P. carotovorum*, and *P. atrosepticum* after the DNA was extracted by a simple technique illustrated by Sambrook et al. (1989) from fresh pure cultures of individual bacterial broth growth (24 h). Amplification scheme cycles were adjusted using a thermal cycler (Applied Biosystems, UK). Specific primers (BioBasic, Canada), listed in Supplementary Table ST1, were used in 25 µL mixture of PCR reaction (5 µL, of 1Xready PCR master mix (My Taq- red DNA polymerase, Bioline, Germany) + 3 µL, template DNA + 1 µL, 10 µM forward primer (0.2 µM of final concentration) + 1 µL, 10 µM reverse primer (0.2 µM of final concentration) + 15 µL, sterile and ultra-pure H₂O) according to Pastrik et al. (2002), Kang et al. (2003) and de Boer and Ward (1995) respectively. PCR products were electrophoretically separated in agarose gel (1.5%). DNA ladder, 1 kb plus (Thermo Fisher Scientific, USA) as a reference was loaded in such gel and then all gels were directly run on scale 8 v/1 cm gel size by 80 V voltages at 400 mA. Finally, the PCR-developed bands were UV trans-illuminated at 355 nm. The 16S rRNA for all isolates used in this study was sequenced using universal primers according to Frank et al. (2008). A big-dye Terminator Cycle Sequencing Kit (Applied Biosystems, UK) was used for the sequencing. The Bacterial Diseases Research Department, Agricultural Research Center, Egypt, employed an 8-capillary Applied Biosystem genetic analyzer to perform 16S rDNA sequencing. The generated FASTA DNA sequences were compared to those in the Gene Bank database for sequence similarity (BLAST query coverage and identity) using the BLASTN tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic trees were generated for all strains using MEGA_11.0.13 software.

Preparation for plant extracts used in this study

The crude extracts for each selected part of nine plant species belonging to six different families (Table 1) were individually extracted according to Al-Anany et al. (2017). In

brief, the collected parts of each plant (leaves, peels, or seeds), were washed using Sterile Distilled Water (SDW), air-dried, and then grounded directly with an electric mill. The ethanolic extracts were simply prepared via moistening 50 g for every raw plant in 150 ml of 70% ethanol at a rate of 1:3 (w/v) with continuous shaking for 24 h. The ethanolic extracts were evaporated using a rotary evaporator until dryness and the crude extracts were obtained. Water extracts were obtained via drenching 50 g of every plant sample in 500 ml of SDW at a rate of 1:10 (w/v) for 1 h at 70 °C and then shaken for 24 h. All ethanolic and water extracts were individually centrifuged at 10,000 rpm/10 min, and then their supernatants were filtered through a syringe filter of 0.22 µm and preserved at 4 °C for additional studies. Both ethanolic and water extracts from different plant parts used in this study were assessed in-vitro for their suppressive activity against three pathogens of potato.

Antibacterial inhibitory bioassay of the plant extracts

The inhibitory effect of different plant crude extracts, listed in Table 1 was assayed in-vitro against *R. solanacearum*, *P. carotovorum* (100 H), and *P. atrosepticum* (MH3c), using 70% ethanolic and water extract types.

Discs of sterilized filter papers were saturated with 10, 20, and 30 µL of each plant extract and placed onto the surface of the king's agar plates (King et al. 1954), suspended with 6×10^8 CFU/mL of each tested bacterium as 3 plates for each tested bacterium/each extract and directly incubated for 48 h at optimum temperature for each targeted bacteria. Discs of sterilized filter papers saturated with 30 µL of SDW were used as a negative (−v) control (3 plates), while others saturated with 10 µL from Streptomycin sulfate (250 mg/mL) were used as the positive (+ve) control. The antibacterial inhibitory activities were identified through the development of a halo zone of clearing around the discs and their minimum inhibitory concentration (MIC) was defined as the lowest concentration that absolutely inhibited bacterial growth.

Assessment of the impact of different plant extracts, bio-fertilizers, and composting on controlling bacterial wilt caused by *R. solanacearum*

Experimental design

A pot experiment was executed to confirm the suppression effect of the most effective crude plant extracts of *P. granatum* L. and *E. camaldulensis* Dehnh resulting from the

in-vitro assessment conducted in this study in parallel with a comparative assay of other biotic and abiotic elicitors including bio- and mineral fertilizers, and organic compost (plant-animal waste compost, and vermicompost) for induction the potato tolerance against *R. solanacearum* infection under open air condition. The complete randomized design was utilized for pots experiment throughout the 2021 and 2022 potato growing winter seasons at an organic experimental farm for Environ. and Bio-Agric. Dept., Fac. of Agric., Al-Azhar Univ., Cairo, Egypt; in 2 ways (1st under *R. solanacearum* infection and 2nd for non-infected ones) using 3 replicates for nine treatments as follow: ethanolic peels extract from *P. granatum* L., SDW peels extract of *P. granatum* L., ethanolic leaves extract from *E. camaldulensis* Dehnh, SDW leaves extract from *E. camaldulensis* Dehnh, bio-fertilizers, mineral fertilizers, plant-animal waste origin compost, vermicompost treatment and untreated control (pathogen only). A sandy soil was collected from the Giza governorate disease-free area from potato bacterial wilt pathogen, and then leached using continuous irrigation with tap water for 5 days, and finally air dried. After that, the sandy soil was filled in sterilized pots (40 Cm in diameter) at a rate of 20 kg/each pot and then planted with individually well-sprouted potato tubers cv. Spunta. Representative samples of sandy soils, plant-animal waste origin compost, and vermicompost were physically and chemically analyzed at Soils, Water and Environment Research Institute, ARC, Dokki, Giza, Egypt, as represented in Supplementary Tables ST2 and ST3.

The application of each treatment was carried out at 3 separate times as 1st one as soil application type for each pot (10 mL of each plant extract, or 10 mL of mixed bio-fertilizers at 10^7 CFU/mL or 2.8 g of mixed mineral fertilizers and 150 g of each tested compost types (plant-animal waste origin compost mixture, and vermicompost), mixed with 20 kg of sandy soil before planting; 2nd time and 3rd application were done after 3 and 6 weeks from potato planting as a spray near the root system (rhizospheric soil) using knapsack pesticide sprayer at the same rate and using an adjusted concentration (1:10, v/v) except both compost types that applied using their filtrates after soaking 100 g of each one in 400 ml SDW for 24 h with an air pump (Eid and El-Sayed 2012).

For pathogen artificial inoculation, the potato roots of each pot were gently cut to ease pathogen permeation and enhance the artificial infestation success, and then they were inoculated with a mingled suspension (1:1, v/v) of 2 virulent strains K3 and K10 concerning *R. solanacearum* at a concentration of 5×10^8 CFU/g soil, 1:10 (v/w) after 3 weeks from potato planting (zero time pathogen inoculation).

Phytopathological and average yield assessments for the potatoes used in the greenhouse pots experiment

For phytopathological evaluation, the wilting symptoms were visually recorded once a day and scaled from 1 to 5 (1 = 1–10% of the leaves wilted, 2 = 11–30% of the leaves wilted, 3 = 31–60% of the leaves wilted, 4 = more than 60% but less than 100% of the leaves wilted and, 5 = all leaves wilted), to determine the disease severity according to Kempe and Sequeira (1983), and AUDPC was enumerated. The population level for *R. solanacearum* was determined in the rhizosphere at zero time, 40 days, and in the rhizosphere and via potato tissues of the crown area at 80 days after soil pathogen inoculation, via the serial dilution plating method on modified Semi Selective Medium of South Africa (SMSA), according to Elphinstone et al. (1996). The average fresh weight (FWt) of potato yield was determined for all tubers per pot at the experimental end time (80 days after the pathogen inoculation).

Biochemical analyses of potato leaves (plant health and induced resistance indicators) used in the greenhouse pots experiment

The photosynthetic pigments, including chlorophyll a, b, total chlorophyll, and carotenoids, were calculated and expressed as mg g FWt⁻¹ in 0.2 g FWt potato leaf dices as described by Şükran et al. (1998) at wavelengths of 666, 653 and 470 nm for chlorophyll a, b, and carotenoids respectively, using a UV VIS spectrophotometer (Model SM1200; Randolph, NJ, USA).

The triterpenoids were determined using the method described by Fan and He (2006) in 10 mg dry weight (DWt) of each leaf sample that was separately grounded in 1 ml methanol. The absorbance for each tested sample was read via a UV VIS spectrophotometer (Model SM1200; Randolph, NJ, USA) at 548 nm. The results were specified as mg/g DWt (ursolic acid/extract) compared to the reading of 0.025–0.5 mg/ ml, the standard ursolic acid/ methanol.

In planta efficacy of the ethanolic crude extract from *P. granatum* L. and *E. camaldulensis* Dehnh on controlling potato tuber maceration

According to the obtained results of the previous in-vitro screening of different plant extracts against tuber soft rot pathogens, *P. carotovorum* (100 H) and *P. atrosepticum* (MH3c) revealed that both ethanolic crude extracts of peels *P. granatum* L. and leaves of *E. camaldulensis* Dehnh were the most effective treatments, another confirmatory *in planta* test was used to individually verify their protective effect on maceration of potato tuber tissue

caused separately by either *P. carotovorum* or *P. atrosepticum* (Czajkowski et al. 2014). Both tested phyto bacteria were propagated in a broth of King's medium at 28 °C for *P. carotovorum* and 25 °C for *P. atrosepticum* for 16 h. After centrifugation, the cells were resuspended in sterilized water, and the cells' concentrations were adjusted to 10⁸ CFU/ ml. *In- planta* experiment was implemented in a complete randomized design in the laboratory using 45 potato tubers (cv. Spunta) for 9 treatments and 5 replicates per each treatment as: (*P. granatum* L. extract + *P. carotovorum*), (*E. camaldulensis* Dehnh extract + *P. carotovorum*), (*P. granatum* L. extract + *P. atrosepticum*), (*E. camaldulensis* Dehnh extract + *P. atrosepticum*), (non-treated) and (infected⁺v control (SDW + *P. carotovorum*), (non-treated and infected⁺v control (SDW + *P. atrosepticum*), (treated and uninfected⁻v control (*P. granatum* L. extract + SDW), (treated and uninfected⁻v control (*E. camaldulensis* Dehnh extract + SDW) and, (non-treated and uninfected blank control (SDW only). The surface of all tubers was sterilized for 10 min through Na hypochlorite then swilled in SDW and immediately dried with tissue paper, then every tuber was handled by one well through a sterilized cork-borer 8 mm in diameter to make a cylindrical shape hole in 15 mm depth from the tuber exterior surface. Then, each well was filled with 30 µL of each crude extract + 50 µL of each bacterial suspension. Five wells of 5 tubers were only filled with 30 µL SDW + 50 µL of bacterial suspension as non-treated and infected⁺v control. Another different 5 wells were filled with 30 µL of each crude extract + 50 µL SDW as treated and uninfected⁻v control. Another 5 wells were filled with 80 µL SDW only as uninfected and non-treated blank control. Finally, for each treatment, every cylinder of tuber tissues taken away was returned, consequently, the wounding was plugged via melting sterile wax of paraffin. Then, all tubers were put in plastic special boxes containing moistened filter papers and directly incubated in the dark for 14 days at room temperature with continuous monitoring for soft rot and maceration symptoms. Visual assessment and disease severity (DS%) was calculated referring to the ranking of every treated tuber depending on the scale from 0 to 5 (0 = no soft rot, 1 = 1–10% soft rot, 2 = 11–30% soft rot, 3 = 31–50% soft rot, 4 = 51–70% soft rot and 5 = complete rot) according to the formula defined by Colyer and Mount (1984):

$$DS (\%) = \sum \frac{(\text{Infection grade frequencies} \times \text{tubers number of each grade})}{(\text{total of observed tubers} \times \text{highest value of the evaluation grade})} \times 100.$$

The recovering impact of each crude extract on the maceration of potato tuber tissue was estimated as the rate of intermediated diameter for rotten tissues surrounded by the wells that were treated with crude extracts and infected⁺v control to the intermediated diameter for rotten wells tissues of the non-treated and infected⁺v control. The treatment efficiency

(TE%) was calculated using the formula reported by Kheirandish and Harighi (2015), where TE (%) = (DI in control pots - DI in treatment pots) / (DI in control pots) × 100.

Living bacterial cells were counted at the end of the experiment for every treatment via plating on Logan's medium (Fahy and Hayward 1983). This experiment was duplicated twice in the same arrangement and the recorded results were illustrated in average/assay.

Biochemical analyses of crude extracts of *P. granatum* L. and *E. camaldulensis* Dehnh (most efficient ones)

Based on the obtained results of in-vitro and in-vivo assessments for the efficacy of different plant extracts and other biotic and abiotic elicitors against *R. solanacearum* wilting agent and tuber soft rot pathogens, *P. carotovorum* (100 H) and *P. atrosepticum* (MH3c), respectively, the ethanolic crude extracts for *P. granatum* L. and *E. camaldulensis* Dehnh were the most effective treatments. So these ethanolic crude plant extracts were biochemically analyzed for detection and identification of their bioactive components (total phenolics and triterpenoids) that may be correlated to their antimicrobial activities that were carried out at Plant Protection Research Institute, ARC, Dokki, Giza, Egypt.

Quantification of phenolics for the ethanolic crude extracts for *P. granatum* L. and *E. camaldulensis* Dehnh

The total phenolics concentration was estimated by Folin-Ciocalteu procedure that was modified by Singleton and Rossi (1965).

The results of total phenolics were specified as mg/ ml [gallic acid (GA)/treatment sample] compared to the reading of the standard 5% (w/v) GA.

Quantification of triterpenoids for the ethanolic crude extracts for *P. granatum* L. and *E. camaldulensis* Dehnh

The method was performed based on Fan and He (2006) to determine the triterpenoids in 10 ml of each individually crude plant extract that was dissolved in 1 ml of methanol.

GC-MS fractionation analysis for the ethanolic crude extracts for *P. granatum* L. and *E. camaldulensis* Dehnh

The GC-MS fractionation analysis was done throughout mass spectra operation timing (45 min) that was recorded at

70 eV according to Nasr-Eldin et al. (2019) for identification of the chemical composition of ethanolic crude extracts for peels and leaves from *P. granatum* L. and *E. camaldulensis* Dehnh, respectively. Each extract was prepared for injection after extraction procedures for 20 min with vigorous shaking in ethyl acetate, 1:1 (v/v), and then, the solvent phase containing the target formula (metabolites) was detached via a separating funnel and evaporable concentrated at 50 °C. The GC-MS injector was run at 230 °C, while the initial temperature setup of the oven was 60 °C/2 min with a gradient increasing rate (10 °C/min) up to 300 °C throughout 8 min by the carrier flow rate (1.5 ml/min) of helium gas in Trace 1310 with capillary column TG-5 (30 m Å~ 250 µm Å~ 0.25 µm).

Statistical analysis

All experiments were performed twice. The data from the replicated experiment is presented as means ± standard error (SE) of five biological replicates for each treatment. Standard Error bars were included in Figs. 2A, B, C, D, and Fig. 3. The mean value was compared using Duncan's multiple range test at a level of probability = 0.05 according to Gomez and Gomez (1984).

Results

Bacterial strains confirmation

The phylogenetic tree based on 16S analysis for the genomic DNA of all isolates used in this study confirmed that K10 isolate is related to *R. solanacearum* where its 16S rRNA sequences were added to the gene bank under the accession number OR533690-OR533691 (Fig. 1A) including 1207 bp fragment length, 100 H isolate confirmed to be *P. carotovorum* and deposited on the gene bank under accession number OP603322.1 (Fig. 1B), with 882 bp fragment length, while MH3c was confirmed as *P. atrosepticum* with accession number OR538566 (Fig. 1C), including 907 bp fragment length.

All tested potato phyto-bacterial strains under the current study were also assured via PCR amplification technique targeted by their specific primers. Specific primers, used for confirming *R. solanacearum* K3 and K10, amplified a specific visualized band at 718 bp (Supplementary Fig. S1A), while *P. carotovorum* 100 H isolates showed an identical band at 550 bp using specific primers (Supplementary Fig. S1B). Furthermore, *P. atrosepticum* MH3c isolates showed identical band at 690 bp using specific primers (Supplementary Fig. S1C).

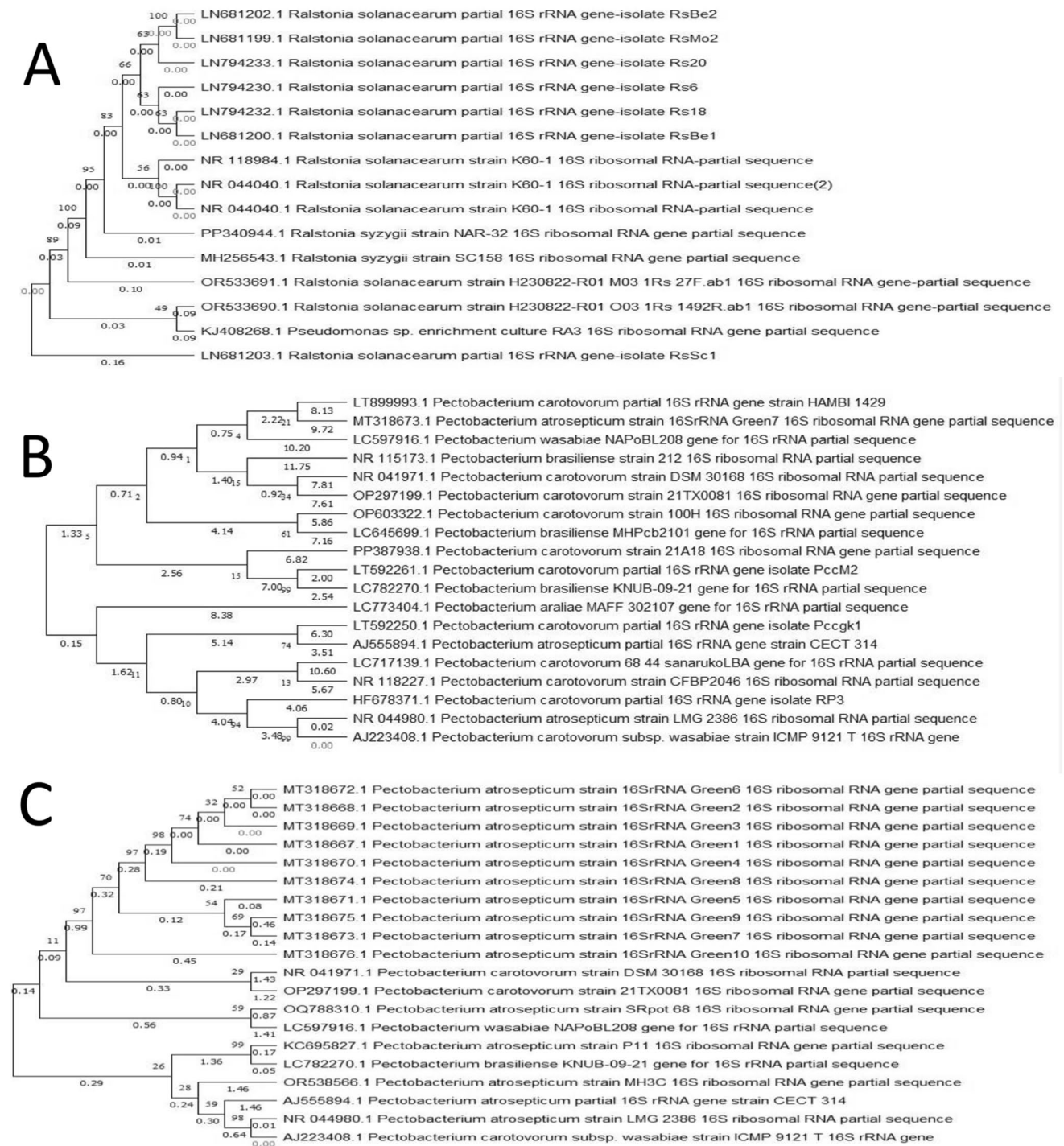


Fig. 1 Phylogenetic tree based on 16S analysis for the genomic DNA of *Ralstonia solanacearum* (A) GenBank accession No. OR533690-OR533691.; *Pectobacterium carotovorum* strain 100 H (B)

(OP603322.10) and *Pectobacterium atrosepticum* (C) (OR538566), used in this study using primers set 27 F and 1492R according to Frank et al. (2008)

In-vitro screening of the antibacterial inhibitory effectiveness of certain plant extracts

The in-vitro assessment of the biological activity of different plant extracts (Table 1) on various plant pathogenic

bacteria showed that the most efficient crude extracts on *R. solanacearum*, *P. carotovorum*, and *P. atrosepticum* were recorded for ethanolic and water extracts of the peels of *P. granatum* L. and leaves ethanolic extract of *E. camaldulensis* Dehnh as indicated by the highest diameter of the

Table 1 In vitro inhibitory activity of different plant extracts on various phytopathogenic bacteria used in this study

Source of plant extracts					Diameter of bacterial inhibition zone (mm)		
Family	Scientific name	English name	Used raw plant parts	Extract type	<i>Ralstonia solanacearum</i>	<i>Pectobacterim carotovorum</i>	<i>Pectobacterium atro-septicum</i>
Asclepiadaceae	<i>Calotropis procera</i> Ait.	Sodom apple	Leaves	70% ethanol	0 d	0 f	0 f
Lamiaceae	<i>Plectranthus amboinicus</i> Lour.	Spanish thyme	Leaves	70% ethanol	0 d	0 f	0 f
			Fresh leaves	Water	0 d	0 f	0 f
			Dry leaves	Water	0 d	0 f	0 f
		<i>Ocimum basilicum</i> L.	Basil	Leaves	70% ethanol	0 d	8±0.02 d
Lythraceae	<i>Punicum granatum</i> L.	Pomegranate	Peels	70% ethanol	22±0.06 a	20±0.04 a	20±0.07 a
				Water	21±0.04 b	17±0.02 b	16±0.08 b
Meliaceae	<i>Azadirachta indica</i>	Neem	Seeds	70% ethanol	0 d	6±0.01 e	7±0.04 d
	<i>Azadirachta</i> Juss		Leaves	70% ethanol	0 d	0 f	0 f
Myrtaceae	<i>Eucalyptus camaldu-lensis</i> Dehnh	Eucllyptus	Leaves	70% ethanol	20±0.05 c	8±0.07 d	14±0.04 c
Rutaceae	<i>Citrus limon</i> L.	Lemon	Peels	70% ethanol	0 d	8±0.03 d	6±0.01 e
	<i>Citrus sinensis</i> L.	Navel oranges	Peels	70% ethanol	0 d	11±0.02 c	7±0.05 d
				Water	0 d	0 f	0 f
LSD (0.05)					0.066	0.093	0.081

Data are represented as the mean of three replicates ± Standard Error (SE). Mean values within columns followed by the same letter are statistically insignificant ($P > 0.05$). Shaded cells represent the most effective plant extracts based on the diameter of the inhibition zone produced around the filter paper discs, saturated with different kind of plant extracts, used in this study

clear zone with MIC value of 10 µL (Table 1; Fig. 2). Other tested crude plant extracts reacted with limited activity on all pathogenic phyto bacteria as 70% ethanol extract of *O. basilicum* L. possessed only inhibitory activity for *P. carotovorum* not for *R. solanacearum* and *P. atrosepticum*; 70% ethanol extract of individual *A. indica*, *C. limon* L. and *C. sinensis* L. exhibited a similarity of inhibitory activity for both pathogens of soft rot (*P. carotovorum* and *P. atrosepticum*) while reacted negatively for *R. solanacearum*. On the other hand, all ethanolic and water plant crude extracts for *C. procera* Ait. *P. amboinicus* Lour., *A. Juss*, and *C. limon* L., and water extract of *C. sinensis* L. did not show any suppression activity against all 3 tested phytopathogenic bacteria.

Impact of different plant extracts, bio-fertilizers, and composting on controlling potato bacterial wilt

The chemical composition of the soil used in this experiment showed that the soil texture is considered a sandy soil with NPK ratio of about 1:1:3 (20:16:56), respectively (Supplementary Table ST2). The pH of the soil is alkaline (8.1), while the soil soluble content of Ca^{++} , Mg^{++} , Na^{++} , K^{++} are 0.6, 0.2, 5.4, and 0.2 mol l⁻¹ respectively (Supplementary Table ST2). Furthermore, soluble anions content of HCO_3^- , Cl^- , and SO_4^- are 0.6, 2.5, and 3.3 mol l⁻¹ respectively. The available minerals detected in the soil are N, P, K, Fe, Cu, Zn, and Mn with a ratio of 20, 16, 56, 1.1, 0.08, 0.24, and 0.94 respectively. While the physical and

chemical properties of the two types of compost used in the greenhouse pots experiment revealed that the pH for both plant- animal waste origin compost and vermicompost are 7.8 and 7.1 respectively (Supplementary Table ST3). The percentages of organic carbon are 29.0 and 11.8 respectively, while for organic matter are 50.01 and 20.39 respectively. Furthermore, the humidity (%) is 35 and 13 respectively (Supplementary Table ST3). In general, the organic carbon, organic matter content, humidity, C/N ratio, total nitrogen, and total potassium of plant-animal waste origin compost are in general higher than the vermicompost (Supplementary Table ST3), where the total nitrogen (%) are 1.2 and 0.80 and total potassium is 2.68 and 0.11 respectively. Otherwise, the N (nitrate) (ppm) and total phosphorus (%) in plant-animal waste origin compost (51.0 and 1.24 respectively) are less than those in vermicompost (71.0 and 1.61 respectively). Both types of compost are free from nematodes or parasites.

The obtained results, represented in Table 2 showed that soil drenching of the ethanolic and water crude extract for peels of *P. granatum* L. and leaves of *E. camaldulensis* Dehnh induced a remarkable suppressive effect on the bacterial wilt disease by the causal *R. solanacearum* that was expressed by a remarkable reduction in the disease severity (Fig. 3A), AUDPC (Fig. 3B) as well as the count of bacteria in the rhizosphere and potato crown tissue compared to other tested mineral and organic fertilizers and the control untreated plants (Fig. 3C). The peels ethanolic and water

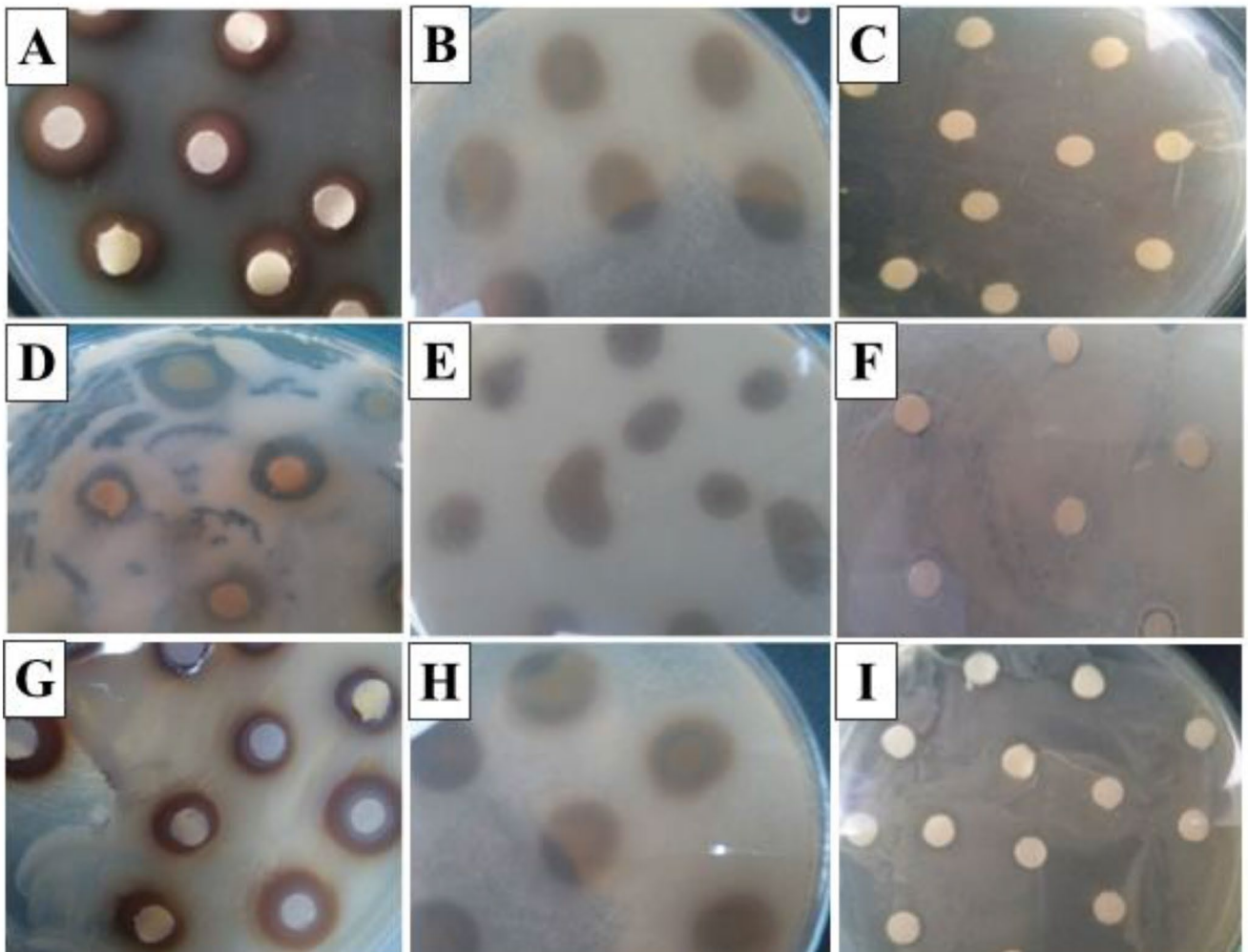


Fig. 2 Inhibition zones for the assessment of inhibition activity of *Punicum granatum* L. peel extract as representative treatment on *Ralstonia solanacearum* (A, B) compared to water control (C), *Pectobac-*

terium carotovorum (D, E) compared to water control (F) and *Pectobacterium atrosepticum* (G, H) compared to water control (I)

crude extract for *P. granatum* L. were the most effective treatments that exhibited a 91% and 89% decrease in the disease severity, AUDPC as well as the count of living *R. solanacearum* in potato crown tissues (Fig. 3A, B and C; Supplementary Fig. S2). While for the ethanolic extract from the leaves of *E. camaldulensis* Dehnh and the use of bio-fertilizers possessed a 60% reduction in the disease severity, AUDPC as well as a decrease in the count of the bacteria in the rhizosphere and potato crown tissues. Application of a water crude extract of *E. camaldulensis* Dehnh and vermicompost showed 38 and 28% reductions in disease severity and AUDPC, respectively. Individual treatment of the mineral fertilizers and plant-animal waste origin compost did not show any reduction effect on the bacterial wilt incidence as pointed for disease severity, AUDPC, and count of the pathogen compared to untreated control (Fig. 3A and B).

The average weight for potato yield per pot was in general higher in non-infested potatoes than in infested

ones with all applied treatments used in the current work (Fig. 3D). For the non-infested plant sets, application of all treatments caused a remarkable increase in potato yield per pot compared to untreated control. The highest potato yield was recorded with non-infested vermicompost treatment (210.2 g/pot) followed by both ethanolic and water peels crude extract for *P. granatum* L. treatments. For infested plants, the ethanolic and water crude extract for peels of *P. granatum* L. enhanced the highest potato yield at 190.66 and 175.00 g/pot, respectively, compared to other treatments and untreated control (Table 2). Application of bio-fertilizers, ethanolic extract from the leaves of *E. camaldulensis* Dehnh, vermicompost, and water extract of *E. camaldulensis* Dehnh increased the potato yield as compared with the untreated control, which showed no yield in infested potatoes (early death of plants). While application of compost and mineral fertilizers showed no yield of potatoes in the infested plants (Table 2; Fig. 3D).

Table 2 Impact of different plant extracts, bio-fertilizers, and composting on controlling potato bacterial wilt and average yield of potatoes under greenhouse conditions

Treatments	Disease severity	AUDPC ^a	Population-level of <i>Ralstonia solanacearum</i> (Log10 of CFU/g)				Average weight for potato yield (g/pot)	
			Rhizosphere at zero time from inoculation	Rhizo-sphere at 40 days from inoculation	Rhizosphere at 80 days from inoculation	Crown potato tissues at 80 days from inoculation	Pathogen-Infected plants	Non-infected plants (pathogen free)
Rs ^b + Ethanollic peel extract of <i>Panicum granatum</i> L.	9.0 ± 0.09e	36.0 ± 2.1f	7.78 ± 0.0057d	4.2 ± 0.005i	1.1 ± 0.0057h	0.0 ± 0.0g	190.66 ± 3.46a	202.3 ± 2.23a
Rs + Water peel extract of <i>Panicum granatum</i> L.	11.0 ± 1.06e	55.0 ± 2.03f	7.89 ± 0.00579a	4.4 ± 0.005g	1.1 ± 0.0056h	0.0 ± 0.0g	175.0 ± 1.15b	209.0 ± 1.73a
Rs + Ethanollic leaves extract of <i>Eucalyptus camaldulensis</i> Dehnh	40 ± 2.9d	292.4 ± 3.5e	7.8 ± 0.0057cd	5.3 ± 0.0057e	3.44 ± 0.005e	1.45 ± 0.0115f	123.33 ± 2.88d	168.0 ± 2.308b
Rs + Water leaves extract of <i>Eucalyptus camaldulensis</i> Dehnh	62 ± 1.41c	822 ± 2.9d	7.81 ± 0.0057c	5.5 ± 0.005d	4.85 ± 0.0057c	2.35 ± 0.057e	86.66 ± 1.73e	146.0 ± 1.7c
Rs + Bio-fertilizers ^c	40 ± 1.7d	297.6 ± 7.5e	7.82 ± 0.00575c	4.3 ± 0.0057h	3.14 ± 0.0056f	2.21 ± 0.086e	145.0 ± 2.31c	174.0 ± 2.9b
Rs + Mineral fertilizers	100 ± 0a	1375.9 ± 5.2a	7.84 ± 0.0057b	6.62 ± 0.005b	5.55 ± 0.005b	6.82 ± 0.069b	0.0 ± 0.0f	86.0 ± 1.7d
Rs + Plant and animal origin compost	100 ± 0a	1339.1 ± 15.6b	7.63 ± 0.0057e	5.7 ± 0.006c	4.53 ± 0.0057d	6.23 ± 0.026c	0.0 ± 0.0f	91.33 ± 2.31d
Rs + Vermicompost	72 ± 1.15b	960 ± 8.7c	7.8 ± 0.0058cd	4.5 ± 0.0057f	2.85 ± 0.005g	3.15 ± 0.17d	118.9 ± 1.8d	210.2 ± 4.84a
Rs only (untreated control)	100 ± 0a	1366.7 ± 8.7a	7.9 ± 0.005a	6.7 ± 0.005a	6.9 ± 0.0056a	7.3 ± 0.173a	0.0 ± 0.0f	78.0 ± 2.31e
LSD (0.05)	3.71	22.07	0.017	0.017	0.017	0.27	5.698	7.769

Note Potato cv Spunta was used in this study. The applications of each treatment were carried out 3 separate times: 1st one as soil application type for each pot (10 mL of each plant extract, or 10 mL of mixed bio-fertilizers at 10⁷ cfu/mL or 2.8 g of mixed mineral fertilizers and 150 g of each tested compost types (a mixture of plant and animal origin compost, and vermicompost) mixed with 20 kg of sandy soil before planting; 2nd time 3rd application times were done after 3 and 6 weeks from potato planting as a spray near the root system (rhizosphere soil) using knapsack pesticide sprayer at the same rate and using adjusted concentration (1:10, v/v) except both compost types that foliar applied using their filtrates after soaking 100 g of each one in 400 mL SDW for 24 h with air pump (Eid and El-Sayed 2012). For pathogen artificial inoculation, potato roots of each pot were gently cut to easy pathogen permeation and enhance the artificial infestation success, and then were inoculated with a suspension (1:1, v/v) of 2 virulent strains K3 and K10 concerning *R. solanacearum* (Phylo type IIa, Sequevar I) at a concentration of 5 × 10⁸ cfu/g soil, 1:10 (v/v) after 3 weeks from potato planting (zero time pathogen inoculation)

^aAUDPC=Area under disease progress curve, ^b*Ralstonia solanacearum*, ^cbio-fertilizers consist of *Azospirillum brasilense* and *Azotobacter chroococcum* (nitrogen fixers), *Bacillus megaterium* and *Vesicular Arbuscular Mycorrhiza*-VAM (P-dissolving agents), and *Bacillus cereus* (K-dissolving bacteria), data are represented as the mean of three replicates ± Standard error (SE), Mean values within columns followed by the same letter are statistically insignificant ($P > 0.05$)

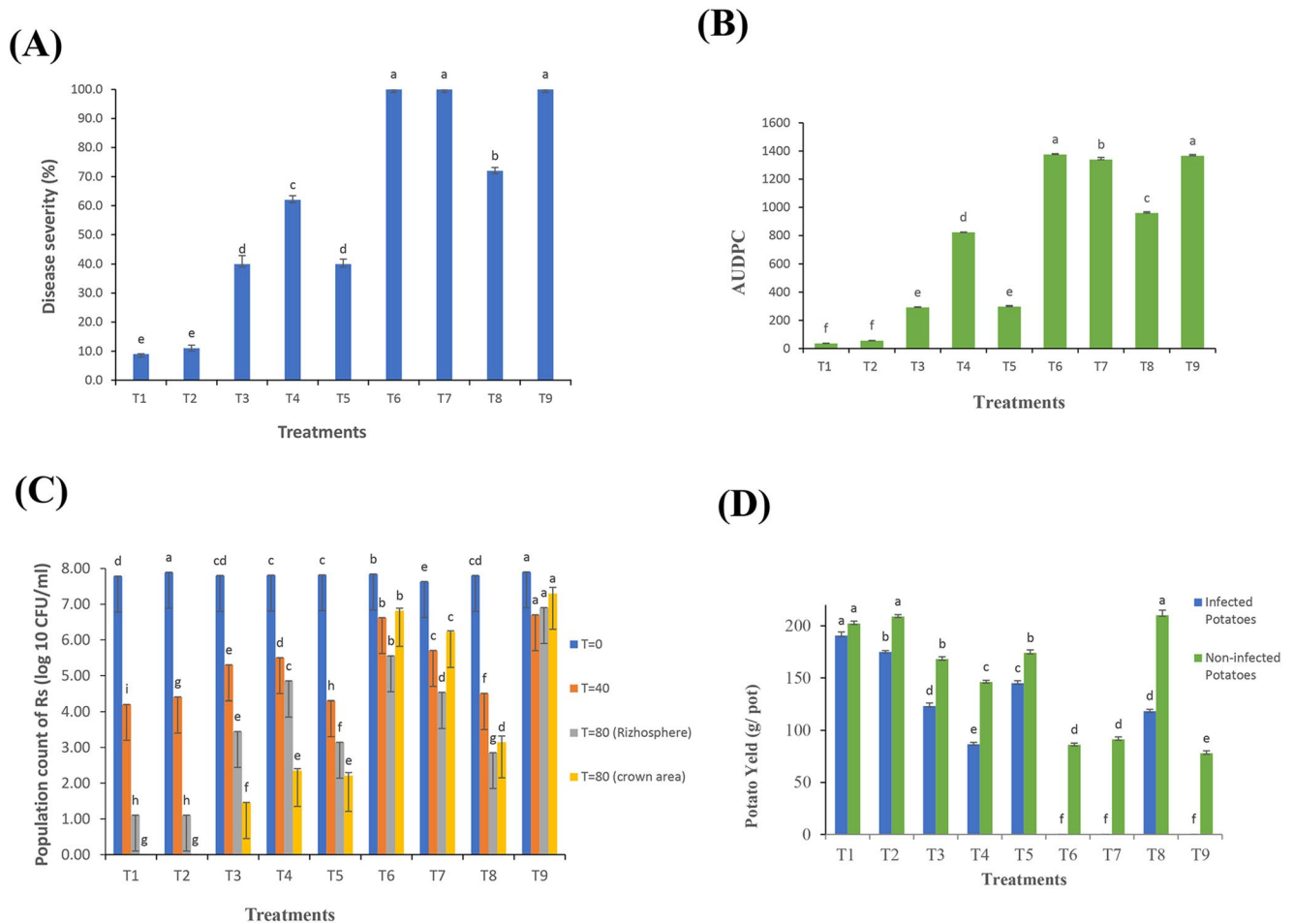


Fig. 3 Impact of different plant extracts, bio-fertilizers, and composting on controlling bacterial wilt caused by *Ralstonia solanacearum* (Rs). Note: disease severity (A); Area under Disease progress Curve, AUDPC (B); population level *R. solanacearum* (log₁₀ CFU/ml) (C); average weight for potato yield (g/pot) (D); Rs+ ethanolic peel extract of *Punicum granatum* L. (T1), Rs+ water peel extract of *Punicum*

granatum L. (T2), Rs+ ethanolic leaves extract of *Eucalyptus camaldulensis* Dehnh (T3), Rs+ water leaves extract of *Eucalyptus camaldulensis* Dehnh (T4), Rs+ bio-fertilizers (T5), Rs+ mineral fertilizers (T6), Rs+ plant and animal origin compost (T7), Rs+ vermicompost (T8), and Rs only (untreated control) (T9)

The recorded data in Table 3 revealed that the total content of both chlorophylls (a, b) and carotenoids was in general higher in non-infected potatoes than in infected plants. The treatment with ethanolic and water crude peel extracts of *P. granatum* L. as well as the application of vermicompost increased the total content of chlorophyll (a, b) and carotenoids when applied in both non-infested potato pots, and infested ones as compared to other treatments and untreated control (Table 3).

Total triterpenoids identified after each treatment were in general higher in infested potatoes pots than non-infested ones (Table 3). The highest triterpenoids concentration was obtained with application of peels ethanolic and water crude extract of *P. granatum* L., and ethanolic and water extract of *E. camaldulensis* Dehnh leaves (Table 3).

Protective efficacy of the ethanolic crude extract of *P. granatum* L. and *E. camaldulensis* Dehnh on controlling tuber maceration pathogens under storage conditions

Application of ethanolic peels crude extracts of *P. granatum* L. and leaves extract of *E. camaldulensis* Dehnh showed a remarkable suppressive effect on maceration of potato tubers tissue caused separately by either *P. carotovorum* or *P. atrosepticum* compared with the pathogen-inoculated control that reacted identically to maceration symptoms after 48 h from incubation as 100% of DS was recorded (Fig. 4; Supplementary Fig. S3). All crude extracts showed a 100% decrease in tuber maceration for both phyto-bacterial pathogens based on DS (%), the diameter of rotted tissue around the inoculated part of the tuber. No symptoms of soft rot were recorded for 14 days after being inoculated by

Table 3 Impact of different plant extracts, bio-fertilizers, and composting on potato photosynthetic pigments and triterpenoids content in relation to bacterial wilt caused by *Ralstonia solanacearum*

Treatments	Infected potato plants						Non-Infected potato plants					
	Chlorophyll a (mg/g FWt)	Chlorophyll b (mg/g FWt)	Total Chloro- phyll (mg/g FWt)	Carotenoids (mg/g FWt)	Total triterpenoids (mg urosolic acid/g DWt)		Chlorophyll a (mg/g FWt)	Chlorophyll b (mg/g FWt)	Total Chloro- phyll (mg/g FWt)	Carotenoids (mg/g FWt)	Total triterpenoids (mg urosolic acid/g DWt)	
Rs ^a + Ethanolic peel extract of <i>Punicum</i> <i>granatum</i> L.	0.86 ± 0.0057a	0.17 ± 0.0057cde	1.21 ± 0.023a	0.22 ± 0.11bc	35.73 ± 0.4c		0.96 ± 0.011c	0.35 ± 0.0057b	1.28 ± 0.028c	0.38 ± 0.011b	31.57 ± 0.4a	
Rs + Water peel extract of <i>Punicum</i> <i>granatum</i> L.	0.74 ± 0.011b	0.14 ± 0.0057de	0.94 ± 0.0057b	0.21 ± 0.11bc	36.80 ± 0.23b		1.11 ± 0.017a	0.42 ± 0.011a	1.53 ± 0.017a	0.47 ± 0.023e	27.07 ± 0.23c	
Rs + Ethanolic leaves extract of <i>Eucalyptus camaldul-</i> <i>ensis</i> Dehnh	0.58 ± 0.0057c	0.20 ± 0.0011abc	0.76 ± 0.0115c	0.25 ± 0.017ab	38.37 ± 0.46a		0.80 ± 0.023e	0.32 ± 0.0057b	1.03 ± 0.017e	0.33 ± 0.011b	21.07 ± 0.04e	
Rs + Water leaves extract of <i>Eucalypt-</i> <i>us camaldulensis</i> Dehnh	0.54 ± 0.017cd	0.16 ± 0.006de	0.70 ± 0.017d	0.21 ± 0.0057bc	31.03 ± 0.23d		0.76 ± 0.017e	0.22 ± 0.011c	0.98 ± 0.011e	0.33 ± 0.0057b	14.23 ± 0.034h	
Rs + Bio-fertilizers	0.54 ± 0.009cd	0.15 ± 0.011de	0.71 ± 0.015d	0.18 ± 0.0057c	27.10 ± 0.23e		0.81 ± 0.0057e	0.23 ± 0.017c	1.04 ± 0.023e	0.32 ± 0.0057b	18.70 ± 0.115f	
Rs + Mineral fertilizers	0.50 ± 0.011d	0.21 ± 0.011ab	0.69 ± 0.017d	0.28 ± 0.017a	25.40 ± 0.28f		0.60 ± 0.011f	0.19 ± 0.0057c	0.79 ± 0.028f	0.24 ± 0.023c	18.87 ± 0.57f	
Rs + Vegetarian and animal compost	0.56 ± 0.017c	0.18 ± 0.0057bcd	0.64 ± 0.011e	0.28 ± 0.011a	20.77 ± 0.346g		0.88 ± 0.017d	0.25 ± 0.017c	1.13 ± 0.023d	0.35 ± 0.023b	16.47 ± 0.05g	
Rs + Vermicompost	0.71 ± 0.017b	0.23 ± 0.017a	0.91 ± 0.0057b	0.20 ± 0.0057bc	27.10 ± 0.115e		1.01 ± 0.017b	0.37 ± 0.023b	1.38 ± 0.029b	0.38 ± 0.023b	29.43 ± 0.17b	
Rs only (untreated control)	0.43 ± 0.011e	0.13 ± 0.0057e	0.56 ± 0.017f	0.21 ± 0.0057bc	27.90 ± 0.63e		0.82 ± 0.0057e	0.25 ± 0.0173c	1.06 ± 0.011e	0.31 ± 0.0057b	25.00 ± 0.034d	
LSD (0.05)	0.0376	0.029	0.044	0.033	1.065		0.045	0.042	0.066	0.0495	0.767	

^a*Ralstonia solanacearum* (suspension (1:1, v/v) of 2 virulent strains K3 and K10), the assessment was conducted under greenhouse with the same setup clarified in the footnote of Table 2, data are represented as the mean of three replicates ± standard error (SE), mean values within columns followed by the same letter are statistically insignificant ($P > 0.05$), the whole experiment was repeated twice

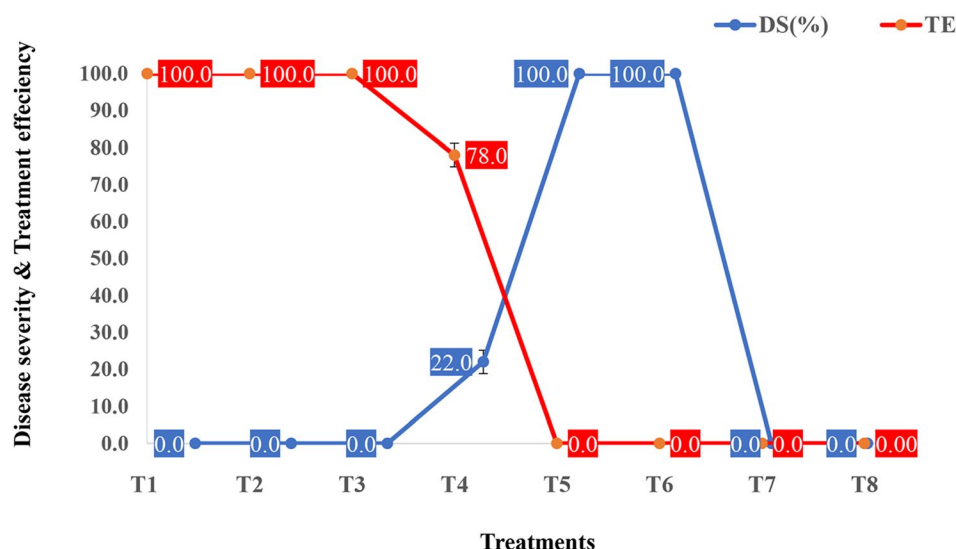


Fig. 4 Protective effect of peels ethanolic crude extract from *Punica granatum* L. and ethanolic extract from the leaves for *Eucalyptus camaldulensis* Dehnh on disease severity % (DS) and treatment efficiency % (TE) of potato tuber maceration caused by *Pectobacterium carotovorum* and *Pectobacterium atrosepticum*. Note: (T1) [*P. granatum* L. extract + *P. carotovorum*], (T2) [*E. camaldulensis* Dehnh

extract + *P. carotovorum*], (T3) [*P. granatum* L. extract + *P. atrosepticum*], (T4) [*E. camaldulensis* Dehnh extract + *P. atrosepticum*], (T5) [SDW + *P. carotovorum* (+ve control)], (T6) [SDW + *P. atrosepticum* (+ve control)], (T7) [SDW + *P. granatum* L. extract (-ve control)], (T8) [SDW + *E. camaldulensis* Dehnh extract (-ve control)] and (T9) [SDW only (blank control)]

Table 4 Biochemical analyses of the ethanolic crude extract of *Punica granatum* L. and *Eucalyptus camaldulensis* Dehnh

Biochemical analyses	<i>P. granatum</i> L.	<i>E. camaldulensis</i> Dehnh
Total phenols (mg/mL)	138 ± 0.028a	76 ± 0.87b
Tannins (mg tannic acid/mL)	33.1 ± 0.57d	22.4 ± 0.33c
Flavonoids (mg catechin/mL)	49.03 ± 1.154c	20.6 ± 0.692c
Alkaloids (mg atropine/mL)	125 ± 1.73b	156 ± 2.309a
LSD (0.05)	3.55	4.21

Data are represented as the mean of three replicates ± standard error (SE). Mean values within columns followed by the same letter are statistically insignificant ($P > 0.05$)

either *P. carotovorum* or *P. atrosepticum* with a treatment efficiency of 100% for each except for the *E. camaldulensis* Dehnh treatment, which showed 22% DS with TE 78% against *P. atrosepticum* only (Fig. 4). Living bacterial cells could not be detected via isolation on logan's medium in crude extract treated tubers. The handled potato tubers by SDW did not possess any rotting visible symptoms.

Quantification of phenolics and triterpenoids in ethanolic crude extract for *P. granatum* L. and *E. camaldulensis* Dehnh

The biochemical analysis of peels ethanolic crude extract for *P. granatum* L. and ethanolic extract from the leaves of

E. camaldulensis Dehnh showed that the concentrations of total phenols, tannins, and flavonoids were in general higher in the peels crude extract of *P. granatum* L. than ethanolic crude extract of the leaves from *E. camaldulensis* Dehnh, while the concentrations of alkaloids were lower in *P. granatum* L. ethanolic crude extract than *E. camaldulensis* Dehnh (Table 4).

GC-MS mass analysis for the ethanolic crude extract of *P. granatum* L. and *E. camaldulensis* Dehnh

The outputs results of the operated GC-MS mass analysis cited in Table 5 showed that the most abundant bioactive compounds identified in the peels crude extract of *P. granatum* L. were 3 chemical compounds with different M.Wt including; 5-hydroxymethylfurfural (83.39%), 2,3-dihydro-3,5-dihydroxy-6methyl-4 H-pyran-4-one (13.5%) and hexadecanoic acid (palmitic acid) (1.28%) (Fig. 5). While 13 total majority numbers of chemical compounds with different M.Wt were identified in the leaves crude extract from *E. camaldulensis* Dehnh and the most abundant bioactive compounds were caryophyllene oxide (33.55%), eucalyptol (1,8-cineole) (22.23%), 2-cyclohexen-1-one, 4-(1-methylethyl) (7.13%), aromadendrene (5.75%), L-à-terpineol (5.11%), 6-methyl-cyclodec-5-enol (4.32%) and caryophyllene (2.85%) (Table 5; Fig. 5).

Table 5 Major phytochemical bioactive compounds identified by GC-MS mass in the ethanolic peels crude extract of *P. granatum* L. and leaves of *E. camaldulensis* Dehnh

Crude extract	Name of the detected compounds	Retention time (min)	Peak area %	Chemical formula	Molecular weight (g/mol)
<i>Punica granatum</i> L. (Fig. 4A)	2,3-Dihydro-3,5-dihydroxy-6methyl-4 H-pyran-4-one	8.14	13.5	C ₆ H ₈ O ₄	144
	5-Hydroxymethylfurfural	10.82	83.39	C ₆ H ₆ O ₃	126
	Hexadecanoic acid (palmitic acid)	23.44	1.28	C ₁₆ H ₃₂ O ₂	256
<i>Eucalyptus camaldulensis</i> Dehnh (Fig. 4B)	Eucalyptol (1,8-cineole)	6.94	22.23	C ₁₀ H ₁₈ O	154
	1-Heptatriacotanol	8.83	0.21	C ₃₇ H ₇₆ O	536
	Terpinen-4-ol	10.02	1.39	C ₁₀ H ₁₈ O	154
	2-Cyclohexen-1-one, 4-(1-methylethyl)	10.17	7.13	C ₉ H ₁₄ O	138
	L-à-Terpineol	10.40	5.11	C ₁₀ H ₁₈ O	154
	2 H-Pyran, 2-(7 heptadecynyloxy) tetrahydro	11.39	1.09	C ₂₂ H ₄₀ O ₂	336
	6-Methyl-cyclodec-5-enol	13.3	4.32	C ₁₁ H ₂₀ O	168
	Caryophyllene	14.29	2.85	C ₁₅ H ₂₄	204
	Aromadendrene	14.64	5.75	C ₁₅ H ₂₄	204
	Caryophyllene oxide	17.54	33.55	C ₁₅ H ₂₄ O	220
	Epiglobulol (Globulol)	17.7	0.09	C ₁₅ H ₂₆ O	222
	Isoaromadendrene epoxide	19.23	1.23	C ₁₅ H ₂₄ O	220
	Methyl arachidonate	19.64	1.27	C ₂₁ H ₃₄ O ₂	318

Shaded cells represent the compounds that have the highest abundance (high peaks) among the others within each plant extract

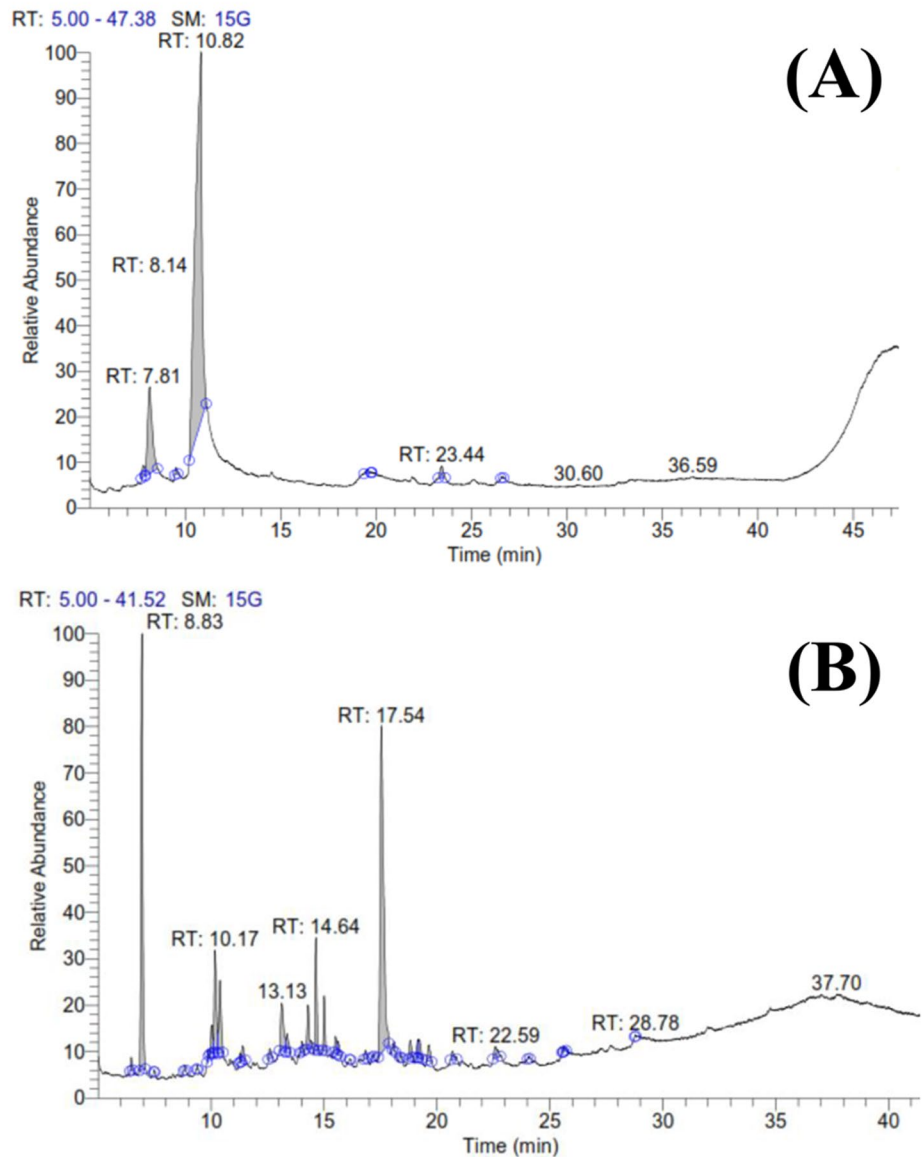
Discussion

In this study, the in-vitro assessment for biological efficiency of different plant extracts on different plant pathogenic bacteria used in the current investigation showed that ethanolic and water peels crude extracts of *P. granatum* L. and, *E. camaldulensis* Dehnh water leaves ethanolic crude extract are the most efficient crude extracts on *R. solanacearum*, *P. carotovorum*, and *P. atrosepticum* growth inhibition, as indicated by their highest diameter of the clear zone, while other tested crude extracts reacted different limited activity. This is in accordance with previous in-vitro assessment data recorded by Mohamad and Khalil (2014) which concluded that the peels methanolic extract from *P. granatum* L. possessed an inhibitory effect in tomato against various phytopathogenic fungi, including *Colletotrichum dematium*, *Botrytis cinerea*, *Phoma* sp., *Fusarium solani*, *Fusarium oxysporum*, and *Rhizoctonia solani*, and also in agreement with the obtained results by Khaleel et al. (2016) that ethyl acetate extracts of peels from *P. granatum* L. previously revealed an in-vitro antibacterial activity against *R. solanacearum*, *X. gardneri* and *P. carotovorum*. So, the application of peel extracts from *P. granatum* L. could be the felicitous procedure for controlling a variety of dangerous agricultural diseases (Mostafa et al. 2018).

In our study, the *in planta* assessment for all treatments revealed that the peels ethanolic crude extract from *P. granatum* L. and ethanolic extract from the leaves of *E. camaldulensis* Dehnh exhibited a remarkable suppressive effect

on *R. solanacearum* wilting disease that expressed a noticeable reduction in the disease severity, AUDPC as well as the count of bacteria in the rhizosphere and potato crown tissues after artificial infection compared to other tested minerals, organic fertilizers and the control untreated plants. In the current study, the results obtained from the pots assessment, for *R. solanacearum* infection concluded that the ethanolic and water peels crude extract from *P. granatum* L. induced the highest potato yield compared with other tested treatments and the untreated control. This is in accordance with Mohamad and Khalil (2014) who documented that, under greenhouse conditions, handling tomato seedlings before sowing or soil treated with methanolic peel extract from *P. granatum* L. possessed perfect protection versus damping-off caused by *Fusarium oxysporum*. Moreover, a previous study reported that the aqueous extract of a dried leaf of *E. globulus* exhibited an in-vitro antibacterial activity against *Pseudomonas syringae* pv. *tomato*, *X. euvesicatoria*, and *Clavibacter michiganensis michiganensis* (Pinto et al. 2023). Furthermore, a previous study revealed that the ethanolic extract from the leaves of *E. camaldulensis* var. *brevirostris* Dehnh has possessed an antioxidant efficacy (El-Ghorab et al. 2003). Many investigators have documented the antimicrobial activity of ethanolic extract for *P. granatum* L. versus food poisoning bacteria against both Gram⁺ve (*B. cereus* and *S. aureus*) and Gram⁻v (*Escherichia coli*) bacteria (Nuamsetti et al. 2012), so they suggested that these plant extracts could be applied as preservatives for

Fig. 5 GC-MS mass analysis for the ethanolic peels crude extract of *Punica granatum* L. (A) and crude leaves extract of *Eucalyptus camaldulensis* Dehnh (B)



preventing foodborne pathogens through food industrial processing (Mahboubi et al. 2015).

Furthermore, *E. camaldulensis* Dehnh bark extract exhibited an inhibition for Gram⁻ bacterial growth such as *E. coli*, *klebsiella pneumoniae*, and *Salmonella* spp., and also possessed a suppressive influence on Gram⁺ve such as *Staphylococcus epidermidis*, *Staph. aureus* and *Streptococcus mutans* (Sidkey and Omran 2017). Leaves extract from *E. camaldulensis* Dehnh is renowned for having several biological impacts as an antimicrobial (Ghalem and Mohamed 2014), and antioxidant (Singab et al. 2011). In our study, the ethanolic and water crude extracts of the peels of *P. granatum* L. and ethanolic and water extracts of *E. camaldulensis*

Dehnh leaves triggered the accumulation of triterpenoids in potato leaves as a response to *R. solanacearum* infection.

A previous study by Huang et al. (2012) revealed the existence of capsidiol as a kind of terpenoid and greater phytoalexin generated in *Solanaceae* crops as an induced reaction to bacterial and fungal infection. They reported that the terpenoids capsidiol might participate in the fungal resistance of *Nicotiana plumbaginifolia* to *B. cinerea*. Terpenoids and phytoalexins of potatoes are known as low molecular-weight (MWt) compounds, acting as defense substances versus a wide series of pathogens and pests that are synthesized and accumulate in plants after exposure to microorganisms (Huang et al. 2012). Additionally, sesquiterpenoid phytoalexin, capsidiol 3-acetate were reported to

act as an antiviral in response to potato virus X (PVX) RNA in *N. benthamiana* (Ran et al. 2015). The total content of chlorophyll (a, b) and carotenoids was determined in potato leaves after each treatment used in this study as the greenness degree of potato leaves. The total content was found to be higher in non-infected potato plants than non-infected ones, and the ethanolic and water crude extract for the peels of *P. granatum* L., besides the treatment by vermicompost increased the total content of chlorophyll (a, b) and carotenoids in both non-infested and non-infected potato as compared to untreated control.

Our obtained results illustrated that the application of mixed bio-fertilizers (*A. brasilense*; *A. chroococcum*; *B. megaterium* and VAM) reduced the disease severity of *R. solanacearum* by 60% and improved the potato yield as compared to untreated potato in pots experimental study. These nitrogen-fixing bacteria are suggested to increase plant growth and crop yield, decomposing organic matter, enhancing nutrient availability, and reducing the susceptibility to plant pathogens infection, like viruses, bacteria, fungi, and nematodes (Megahed et al. 2013; Zhang et al. 2021). According to Messiha et al. (2023), the biofertilizer, which included nitrogen-fixing bacteria *Azotobacter*, phosphate-solubilizing bacteria *Bacillus megaterium*, and potassium-solubilizing bacteria *Bacillus circulans*, was effective in controlling *R. solanacearum* in Egyptian field conditions while also increasing potato yield and improving soil properties. Individual amendments of the mineral fertilizers and compost in our study did not show any reduction effect on the disease incidence caused by *R. solanacearum*, and did not enhance the potato yield per pot as compared to untreated control potato plants. The opposite results were obtained by Youssef and Tartoura (2013), who reported that under greenhouse pot experiment conditions, the application of compost showed a substantial improvement in plant growth and a remarkable reduction in the disease incidence and bacterial population of *R. solanacearum*. In a different study published by Messiha et al. (2023), the use of compost treatment of plant-animal origin in conjunction with a crop rotation program including, the use of corn, wheat, corn, and potatoes has significantly decreased the population of *Ralstonia* spp. in the soil under experimental field conditions.

In the last recent years, vermicompost has been widely used as a rich organic fertilizer for controlling several soil-borne pathogens, also known as a plant growth promoter and contains nutrients and beneficial microbes for plant health (Abada and Hassan 2017; Masoud et al. 2023).

Vermicompost was tested in the current investigation to be used as an environmentally friendly approach and an efficient alternative against bacterial wilt disease and to be compared with the peels crude extract for *P. granatum* L.

and leaves of *E. camaldulensis* Dehnh. Otherwise, in our study, the application of vermicompost showed only a 28% reduction in disease severity and AUDPC for bacterial wilting caused by *R. solanacearum*. It also increased the potato yield per pot, but to a lesser extent than the crude extract of peels of *P. granatum* L., leaves of *E. camaldulensis* Dehnh, and biofertilizers did. In organic farming, the implementation of compost and vermicompost perform a key role in raising soil fertility, crop nourishment, and controlling plant diseases (Passarini et al. 2014) as well as promoting soil biodiversity by ameliorating microbial biomass (Hernandez et al. 2014). A different finding was reported by Schönfeld et al. (2003) that the addition of household compost was able to suppress *R. solanacearum* propagation slope rates and decreased the diseased plant numbers in a bioassay inhibitory test.

The *in-planta* assessment of these plant extracts on potato soft rot symptoms revealed that the maceration was not recorded on pre-treated potato tubers with all types of ethanolic crude extracts of peels and leaves for *P. granatum* L. and *E. camaldulensis* Dehnh, respectively, for 14 days after being inoculated by either *P. carotovorum* or *P. atrosepticum* with a treatment efficiency of 100% for each as compared to untreated potato tubers except extract of *E. camaldulensis* Dehnh for *P. atrosepticum* which reacted with 22% disease intensity. Limited research studies in the literature discussed the use of the crude extract of peels of *P. granatum* L. and *E. camaldulensis* Dehnh to control potato soft rot agents. Three new EOs separated from various native plants (*Zataria multiflora*, *Satureja khuzistanica*, and *Hyssopus officinalis*) showed an in-vitro and an in-vivo suppressive effects against *P. carotovorum* subsp. *carotovorum* (Habibeh et al. 2019). In a previous investigation reported by Rahman et al. (2012), the dried leaf extracts of *Corchorus capsularis* L. and *Swertia chiraita* Ham. had extreme growth inhibition of *P. carotovora* subsp. *carotovora* via in-vitro and the storage assay. Furthermore, Ashmawy et al. (2014) reported that *Tecoma stans* leaves and branches extracts were masterly effective against soft rot bacteria namely *Dickeya chrysanthemi* (DSM 4610), *P. carotovorum* subsp. *carotovorum* (ipp038), *Pectobacterium wasabiae* (ipp041), *P. atrosepticum* (1007) and *Dickeya dianthicola* (IPO 2114). Pretreating susceptible potato tubers with the tested EOs possessed complete and perfect suppression of their soft and dry rot diseases by *P. carotovorum* and *Fusarium solani* (Awadalla et al. 2008).

The biochemical analysis of the ethanolic crude extract for the peels of *P. granatum* L. and leaves of *E. camaldulensis* Dehnh used in the current study showed that the concentrations of total phenols, tannins, and flavonoids were mostly higher in the crude extract of *P. granatum* L. than *E. camaldulensis* Dehnh, while the concentrations of alkaloids

were lower in the peels crude extract from *P. granatum* L. than the leaves crude extract of *E. camaldulensis* Dehnh. In this context, tannins were documented to exhibit in vitro toxicity against various fungi, including *A. niger*, *B. cinerea*, *C. graminicola*, and *Penicillium* sp (Lattanzio et al. 2006). Flavonoids, are secondary metabolites produced by plants as a reaction to microbial infectious and often act as antimicrobial agents against a broad range of microorganisms like *Launaea resedifolia*, which is known to possess good antimicrobial activity against *Streptococcus* sp., *Candida albicans*, and *Enterobacter* sp. (Moussaoui et al. 2010). Some researchers attributed the inhibitory effectiveness of the extract from *P. granatum* L. to the hydrophobicity behavior of this extract that interacts with the proteins of the cell membrane and mitochondria for pathogenic bacteria, causing a disturbance in their structures, and altering their permeability function (Tiwari et al. 2009). Therefore, the antibacterial efficacy of *P. granatum* L. may be attributed to polyphenol content because the polyphenols may have a negative impact on the bacterial cell wall, block enzyme function by oxidizing substances, react with proteins, and disorder the coaggregation of microorganisms (Naziri et al. 2012).

Moreover, the GC-MS mass analysis conducted in our study revealed that, the total majority of identified numbers of chemical compounds with different M.Wt were 3 in the peels crude extract for *P. granatum* L. and the most abundant bioactive substances were 5-hydroxymethylfurfural, 2,3-dihydro-3,5-dihydroxy-6methyl-4 H-pyran-4-one and hexadecanoic acid (palmitic acid). The phytochemical examination of ethyl acetate peel extracts from *P. granatum* L., reported by Omoregie et al. (2010) specified the presence of alkaloids, flavonoids, balsams, tannins, saponins, resins, sterols, terpenes, free-reducing sugar, carbohydrates, volatile oils, and glycosides. Similar findings were reported by Mohamad and Khalil (2014) with different ratios. Hexadecanoic acid (palmitic acid, PA) is a long-chain saturated fatty acid that is vastly detected in the seeds of beans, sunflowers, and cotton (Zhao et al. 2019). Previous investigations have revealed that PA can suppress soil-borne pathogens' growth and enhance plant seedlings' growth (Ding et al. 2019). On the other hand, 4 H-pyran-4 one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (DDMP) as flavonoid fractions and bioactive compounds produced by *Schizophyllum commune* revealed antifungal efficiency to the rubber wood-lysis fungi, as reported by Teoh et al. (2011). 1, 8-cineole as EO from *Mentha longifolia*, exhibited strong antifungal and anti-aflatoxigenic activities towards *A. flavus* (Dehghanpour-Farashah and Taheri 2016). The GC-MS mass analysis conducted in our study confirmed the presence of phenols, tannins, and flavonoids in both crude extracts used in this study that were detected individually by spectrophotometry biochemical analysis. While, the most abundant bioactive

compounds, identified in the leaves crude extract from *E. camaldulensis* Dehnh, were 13 chemical compounds with different M.Wt including; caryophyllene oxide, eucalyptol (1,8-cineole), 2-cyclohexen-1-one, 4-(1-methylethyl), aromadendrene, L- α -terpineol, 6-methyl-cyclodec-5-enol, and caryophyllene. Analogous results were also mentioned by several researchers that within the flowers EO for *E. camaldulensis* Dehnh exhibited the presence of thymol, γ -terpinene, carvacrol, α -pinene, 1, 8 cineole, α -terpineol, terpinen-4-ol and *pcymene* as the most important components (Siramon and Ohtani 2007). Other findings documented that the most extreme components for the fruit EO were cubenol, α -pinene, drimenol, and aromadendrene (El-Ghorab et al. 2002).

Some chemical ingredients were detected in different *E. camaldulensis* Dehnh parts including flavonoids (Abd-Alla et al. 1980), acylated pentacyclic triterpenoids (Siddiqui et al. 1997), eucalyptanoic acid (Begum et al. 2002) and EO (Ghalem and Mohamed 2014). Aromadendrene is a potent antimicrobial compound that can disturb the forming of proteins, alkylate proteins, and is highly lipophilic, which may negatively alter the biomembrane's fluidity and permeability (Wink 2008). 2 H-Pyran, 2- (7-heptadecyloxy) tetrahydro- is considered a flavonoid fraction, and it was suggested to play a role as an antioxidant, anti-inflammatory, and antimicrobial (Amutha and Kottai 2014). Caryophyllene possesses anti-inflammatory, cytotoxic, and antimicrobial activity (Peng et al. 2013). Terpinen-4-ol has strong antibacterial activity against biofilms formed by some pathogens, such as *Streptomyces mutans* and *Lactobacillus acidophilus* (Bordini et al. 2018). Former studies have been characterized the antifungal effectiveness of l- α -terpineol (Park et al. 2009). Furthermore, 2-Cyclohexen-1-one, 4-(1-methylethyl) is a derivative of limonene (Olivera et al. 2001) and limonene has been implicated as an effective antimicrobial agent (Magwa et al. 2006). Also, it has been recorded that, (E)- β -caryophyllene as a king of terpenoids directly prevents the *P. syringae* pv. *tomato* growth. Methyl arachidonate is a derivative of arachidonic acid and has been shown to exhibit strong antimicrobial effectiveness versus different microorganisms and inhibit bacterial growth of *S. aureus* (Zheng et al. 2005). All chemical constituents identified in the tested crude extracts in our study may be accounted for their inhibitory effect against the phytopathogenic bacteria used in our investigation.

Conclusions

Finally, based on the proved in-vitro and in-vivo antibacterial activity of the ethanolic crude extracts from *P. granatum* L. peels and *E. camaldulensis* Dehnh leaves against

R. solanacearum, *P. carotovorum*, and *P. atrosepticum*, it can be concluded that they are potent to be used as an efficient and environmentally secure for effective reduction of these diseases incidence and induction of the resistance of potatoes against bacterial diseases for substitution of the γ impacts of bactericides.

It is recommended to apply such safe treats by repeating the applications more than once to raise potato tolerance to the challenge of bacterial pathogens during the growing season, transit, and storage. Moreover, it requires more future investigations to easily apply these treatments as individuals or in a mixed way on a commercial scale, as a future vision for our research.

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Data availability All created and/or analyzed data during the present study are included in the manuscript, and the corresponding author has no interception to the availability of data and materials upon request.

Declarations

Ethics approval and consent to participate The study was conducted on pots experiment of potato wilting and in-vivo for soft rot diseases and usage of the crude plant extracts, bio- and mineral fertilizers, and organic compost (plant and animal-waste origin compost, and vermicompost) that are ready and obtainable in the environment and the ethical consent is not needed. This study does not contain any studies with human participants or s performed by any of the authors.

Consent for publication All authors consent to this submission.

Conflict of interest The authors declare that they have no conflict of interests.

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