Evaluation of the antibacterial activity of some plant essential oils against *Ralstonia solanacearum* and the molecular diagnosis of the bacterium

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Background
Evaluation of the antibacterial activity of some essential oils for controlling potato bacterial wilt.
Objective
In vitro and planta trials, five essential oils, lemongrass, eucalyptus, thyme, ginger, and peppermint, were evaluated for their aptitude to impede the progress of *Ralstonia solanacearum.*
Materials and methods
Five essential oils were assessed for their activity to inhibit *R. solanacearum* growth using the disc diffusion method. Certain volumes of each tested plant essential oil were added on filter paper discs using tetrazolium chloride agar medium, which contained the tested bacterium to obtain the proposed concentrations used to evaluate the most efficient oil for controlling potato bacterial wilt. Serological and molecular identification was also conducted.
Results and conclusion
R. solanacearum was characterized using cultural characteristics on the selective medium South Africa, "which is the most effective in suppressing the growth of contaminating microorganisms, and the highest recovery rate of the tested bacterium," immunofluorescence antibody staining tomato bioassay test and molecular identification via real-time PCR (Taq-man) assay. *In-vitro* studies showed that all tested plant essential oils (lemongrass, eucalyptus, thyme, ginger, and peppermint) significantly reduced *R. solanacearum* progress. Eucalyptus (*Eucalyptus globulus*) and peppermint (*Mentha piperita*) essential oils were the most efficient in suppressing *R. solanacearum* growth, where it showed the highest mean inhibition zone, followed by thyme oil (*Thymus vulgaris*), lemongrass oil (*Comybogen citratus*), and ginger oil (*Zingiber officinale*), respectively. Phenotypic variances in bacterial cell construction have been noticed via a transmission electron microscope. Eucalyptus oil led to cell wall lysis, cell laceration, bubble-like structures, and degraded cellular components in bacterial cells.

Keywords:
antibacterial activity, immunofluorescence antibody staining, plant essential oils, *Ralstonia solanacearum*, real-time PCR assay, transmission electron microscope

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Introduction

The plant pathogenic bacterium *Ralstonia solanacearum* causes vascular wilt disease in a vast domain of economic crops such as pepper, eggplant, ginger, and potato [1]. This bacterium is soil-borne and infects its plant host via injuries and natural openings, inhabits and prohibits water transport in the xylem, and lastly, leads to plant wilt and dying of the host plant [2]. Once this pathogen infects potato tubers through vascular tissue, it leads to the appearance of brown rot on tubers and also above-ground symptoms like yellowing, stunting, and wilting [3]. The disease was first recorded in Egypt at EL-Gemeiza farm by Briton-Jones [4]. Conventionally, *R. solanacearum* has been divided into strains depending on host range [5] and biovars based on carbohydrate utilization [6]. The causative in Egypt, the pathogen is identified as *R. solanacearum* race3 biovar 2 or phyllotype II, sequevar I, according to Fegan and Prior [7]. The pathogen has a broad host range of economic crops, including more than 450 plant species representing more than 50 different botanical families [3]. Some of them are of

significant economic value crops such as peanuts, bananas, tomatoes, and potatoes [8]. Potato bacterial wilt represents a stumbling bulk that impede the export of Egyptian potatoes to numerous foreign markets. Many attempts have been made to control potato bacterial wilt was trial for many years without any progress due to the extreme aggressiveness of the bacterium and wide host range [6]. Antibiotic usage and chemical control are inappropriate and ineffective forms of control [9]. Essential oils and their components have been shown to not only own broad-range antibacterial properties [10] but also their insecticidal, antioxidant, antifungal, and antiparasitic properties. The objective of the current study was to characterize the pathogen by means of serological and molecular methods and assessing the potential efficacy of some plant essential oils against *R. solanacearum*.

Materials and methods

Sample collection and pathogen isolation

Symptomatic samples were collected from different localities located in six Egyptian governorates (Beni Suef, Beheira, Gharbia, Giza, Ismailia, and Menufiya) during 2019–2020 growing season.

Potato tubers exhibiting internal brown rot symptoms were washed using tap water and then superficially sterilized by alcohol (70%) and flaming. Section 5–10 mm in diameter and 5 mm thick from the stolon end with the major cortical and vascular tissue were soaked in 10 ml sterile phosphate buffer (PB 0.05 M, pH 7.0). After 5–10 min, 100 µl of the resulting suspension was streaked on selective medium South Africa (SMSA) [11]. Plates were incubated at 28°C for 3 days and observed daily for the presence of bacterial growth compatible with the colony features of *R. solanacearum* on this medium.

Isolation and identification of the causative bacterium

Bacteria were isolated from a diverse habitat from infected potato tubers, infested soil, irrigation water, and weeds associated with potato plants. Potato tubers exhibiting internal brown rot disease symptoms were washed using tap water and then superficially sterilized by alcohol (70%) and flaming. Section 5–10 mm in diameter and 5 mm thick, from the stolon end with the major cortical and vascular tissue, was soaked in 10 ml sterile phosphate buffer (PB 0.05 M, pH 7.0). After 5–10 min, 100 µl of the resulting suspension were streaked on SMSA casamino acid, 1.0 g, bactopeptone 10.0 g, glycerol 5.0 ml, agar 20.0 g, distilled water 1000.0 ml, pH 6.9 [11]. Plates were incubated at 28°C for 3 days to detect the possible variation in morphology and color of pathogenic and nonpathogenic.

Pathogen identification

Conventional identification

Bergey's Manual of Systemic Bacteriology [12] was used to determine the cultural, physiological, and biochemical features of the selected isolates. Disaccharides and hexose alcohols were used to verify the biovar of *R. solanacearum* [1].

Immunofluorescence antibody staining test

Immunofluorescence antibody staining (IFAS) serological test was used to identify the isolated bacteria [13]. IFAS can also be performed to identify pure cultures and have a high level of sensitivity for the detection of *R. solanacearum* in potato tissue. IFAS is a quick serological test used to identify bacteria with a high probability of identification. This technique depends on the polyclonal antibodies. The anti-rabbit antiserum is conjugated with fluorescein isothiocyanate [13].

Taq-Man real-time PCR assay

Molecular diagnosis of pathogen isolates through quantitative PCR was done on 10 selected isolates, as mentioned by Janse [13]. A specific primer and probe were used via Applied Biosystem (Singapore) Time apparatus in PBRP. All nominated isolates from various habitats were authenticated using this procedure.

Tomato bioassay

Pathogenic potential of the isolates of *R. solanacearum* was made by injecting tomato plantlets with three or four leaves with 10 µl of bacterial suspension in the leaf axis by a hypodermic syringe. The cell density of the suspension was adjusted by a spectrophotometer to give a dense bacterial suspension to 10^8 cell/ml. The control was made by injection of a little drops of sterilized water as an alternative to bacterial suspension [13]. Injected tomato seedlings were observed daily under the greenhouse for induced wilt symptoms followed by rapid death.

Influence of some plant essential oils on the growth of Ralstonia solanacearum, in vitro

Highly purified essential oils, that is lemongrass (*Comybogen citratus*), eucalyptus (*Eucalyptus globulus*), thyme (*Thymus vulgaris*), ginger (*Zingiber officinale*), and peppermint (*Mentha piperita*) were graciously obtained from Oils Extract Unite, National Research Centre (NRC), Giza, Egypt.

A standard disc diffusion method [14] was used to determine their antimicrobial activity. The identified bacterial isolate was grown for 24 h at 28°C in nutrient broth, and the cell density was verified at 10^8 CFU/ml. The bacterial solution was added to tetrazolium chloride agar (TZC) medium peptone 10.0 g, casein hydrolysate (casamino acid) 1.0 g, glucose 5.0 g, distilled water 1000 ml, agar 20.0 g, pH 7.2, and poured in petri dishes, as soon as the plates were inoculated with the bacterium, sterilized filter paper disks of 5 mm in diameter were located in the center of the dish in which a number of concentrations of the essential oils were used. Essential oils were applied at different concentrations from the original as the following: 1 : 1, 1 : 2, 1 : 3, 1 : 4, 1 : 5, 1 : 6, and 1 : 7 dilutions (v/v). Fifty microliters of each concentration of the essential oils were sited on the sterilized filter paper disks. Negative control by adding sterilized distilled water was used, mixed with tween 20 (1 : 1) to make an emulsion. After adding the different concentrations of essential oils on the filter paper disks, the plates were then incubated at 28°C for 48 h, and bacterial growth inhibition zone haloes were estimated in millimeters using a ruler.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined by preparing a dilution series of the essential oils added to agar, then inoculated with *R. solanacearum* and incubated at 28C for 48 h. The disc diffusion method was used to determine MIC.

In planta treatments

Bacterial culture and inoculum preparation

The bacterium was grown on king's media for 48 h overnight, then transferred to nutrient broth and shaken at 100 rpm at 28°C. The bacterium was suspended in distilled sterilized water, the concentration of the suspension was measured at an absorbance of 600 nm to give 10^8 CFU/ml on a Jenway spectrophotometer [14,15]. A suspension of *R. solanacearum* was amended to each pot. The soil was carefully homogenized for uniform distribution of bacterial suspension. Roots of tomato plants were dipped or immersed in each essential oil emulsion of the previous five essential oils, then transplanted in infested soil. Five pots were used for each treatment. Negative control pots were kept in identical circumstances at 28–30°C, 75–85% RH without infestation with the pathogen. Positive control pots were made by adding the inoculum of *R. solanacearum* only.

Disease assessment

Disease severity was calculated after the first mark of disease incidence according to the Kempe [16] scale defining the wilt signs in the vegetative foliage as shown.

0=no leaf wilted; 1=up to 25%; 2=26–50%; 3=51–75%; 4=76–100%; 5=plant dead.

$$DI = \frac{\sum R.T}{5 * N} \times 100$$

T=total number of wilted plants with each group; R=disease rating scale R (R=0, 1, 2, 3, 4, and 5), N=whole number of tested plants.

$$\text{Essential oils efficacy \%} = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C}} \times 100.$$

C=control, T=treatment.

In a greenhouse experiment, vegetative growth parameters were determined by random sampling of five tomato plants treated with essential oils in each category.

Also, growth tomato plant parameters were recorded as plant height, plant shoot fresh weight, and dry weight.

Transmission electron microscope

A transmission electron microscope (TEM) was used to describe the phenotype of bacterial cells both before and after processing with the most dynamic essential oil "Eucalyptus." The bacterial broth culture was incubated overnight at 28°C. A negative stain TEM (Cairo University Research Park) operating at 80 kV was used to acquire TEM images and capture imaging and diffraction modes.

Statistical analysis

Analyzed using GenStat 12.1 and were submitted to Duncan's multiple range test at P=0.05 level [17].

Results

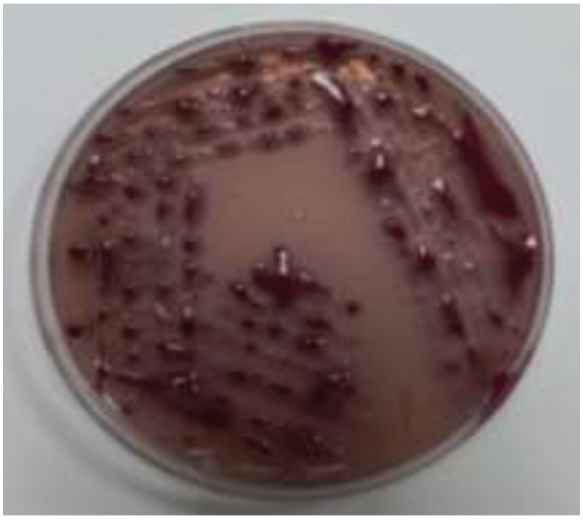
Isolation and identification of causal bacterium pathogen

Pure cultures of *R. solanacearum* were isolated using SMSA and TZC media. Colonies morphology of *R. solanacearum* on SMSA were slimy, mucoid, highly fluidal, irregular, and white colonies with pink–red centers after 3 days of incubation at 28°C (Fig. 1). Similar colony morphology appeared on TZC medium.

Biovar determination

Ten bacterial isolates were able to utilize disaccharides (maltose, lactose, and cellobiose) producing acids and were unable to utilize sugar alcohols (mannitol, sorbitol, and dulcitol) producing acids. The isolates

Figure 1



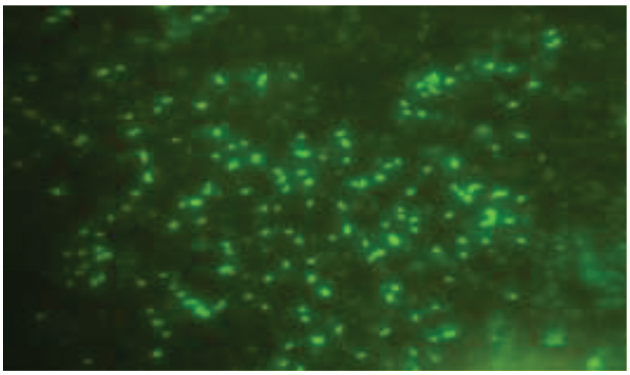
Typical colony of *Ralstonia solanacearum* on modified SMSA showed slime, mucoid, highly fluidal, irregular, and white colonies with pink–red centers. SMSA, selective medium South Africa.

showed characteristics confirming those described for the main strain in Egypt race 3 biovar 2 or phylotype II sequevar I.

Immunofluorescence antibody staining

The obtained isolates did not appear to have any serological variance in IFAS testing, both in cell morphology or the grade of fluorescence. Results showed that bacterial cells were short rod and appeared to emit bright green fluorescent after being stained by the specific fluorescein-labeled antiserum (Fig. 2).

Figure 2



Cell morphology of *Ralstonia solanacearum* in the immunofluorescence antibody staining test.

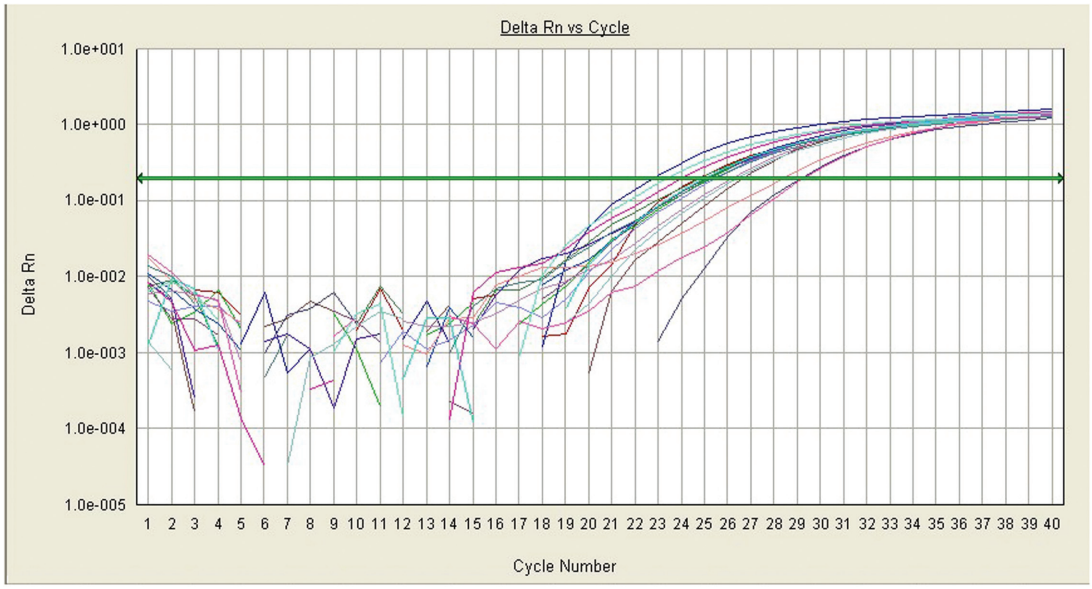
Real-time PCR

Real-time PCR was conducted to detect *R. solanacearum* isolates, which showed a typical curve over the threshold. Negative control appeared as noise under the threshold. The RS primers and probe detected all biovars and strains of *R. solanacearum*. Positive results were observed with cycle threshold (Ct) value detected where Ct less than 40 according to the manufacture protocol (Fig. 3).

Tomato pathogenicity test

Pathogenicity test revealed that the isolated bacteria induced wilt symptoms on inoculated tomato plants after 4–7 days of inoculation, followed by rapid plant death under greenhouse conditions at 28–30°C, 75–85% RH (Fig. 4).

Figure 3



Taq-Man real-time PCR detected isolates of *Ralstonia solanacearum*, which showed a typical curve over the threshold.

Table 1 ***In vitro*** **antagonistic activity of some plant essential oils against** ***Ralstonia solanacearum***

	Inhibition zone(mm)/concentrations								
Essential oil	Pure oil	1 : 1	1 : 2	1 : 3	1 : 4	1 : 5	1 : 6	1 : 7	Mean
Lemongrass	23.0^{bc}	16.0^{c}	12.7^{c}	11.0^{b}	10.0^{b}	9.0^{b}	8.0^{b}	0.0^{a}	11.21
Eucalyptus	36.0^{a}	29.0^{a}	26.7^{a}	19.0^{a}	17.0^{a}	13.7^{a}	11.0^{a}	10.0^{a}	20.30
Thyme	22.3^{bc}	22.3^{b}	17.7^{b}	12.3^{b}	9.3^{b}	8.7^{bc}	10.0^{ab}	8.0^{a}	13.83
Ginger	16.70^{c}	8.70^{d}	8.30^{d}	7.70^{c}	7.3^{c}	6.7^{c}	0.0^{c}	0.0^{b}	6.93
Peppermint	24.0^{b}	21.0^{b}	18.0^{b}	17.0^{a}	15.3^{a}	13.7^{a}	10.0^{ab}	9.0^{a}	16.00

Inhibition zone measured in millimeters. Within the same column, the mean with different superscripts is significantly different (Duncan's multiple range test, $P \leq 0.05$).

All the five plant essential oils tested reduced the growth of *R. solanacearum* at different concentrations and presented inhibition haloes as antimicrobial activity against the bacterium. The mean of antibacterial activity achieved from all concentrations was reported in Table 1.

It is obvious from the data in Table 1 that eucalyptus oil and peppermint oil are the two most efficient plant essential oils in suppressing *R. solanacearum* development, followed by thyme oil, lemongrass oil, and ginger oil in descending order.

Estimation of minimum inhibitory concentrations

MIC were calculated for all the five plant essential oils to determine the least concentration of the used essential oils showing clear inhibition the dilution 1 : 7 was taken as the MIC as shown in Table 2.

The value of MIC in this case was necessary to prevent bacterial growth after 48 h of incubation at 28°C.

In planta application

Treatment of tomato plants with the five essential oils resulted in a reduction in bacterial wilt occurrence (Table 3) and an increase in plant growth (Table 4) (plant height, plant shoot fresh weight, and dry weight) compared with the positive control. The five used essential oils were found to be effective in decreasing bacterial wilt of tomato plants grown in infested soil. It is evident from the data in Table 3 that eucalyptus and ginger are the most efficient essential oils in reducing bacterial wilt incidence, followed by lemongrass, peppermint, and thyme in descending order.

Table 2 Minimum inhibitory concentration of different essential oils against ***Ralstonia solanacearum***

No.	Essential oil	MIC
1	Lemongrass (*Cymbopogon citratus*)	1 : 6
2	Eucalyptus (*Eucalyptus globulus*)	1 : 7
3	Thyme (*Thymus vulgaris*)	1 : 7
4	Ginger (*Zingiber officinale*)	1 : 5
5	Peppermint (*Mentha piperita*)	1 : 7

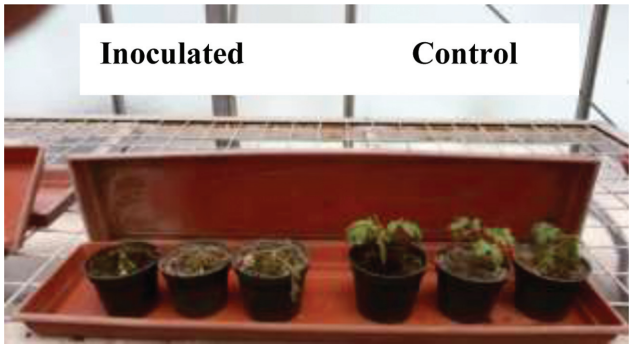
MIC, minimum inhibitory concentration.

Table 3 Effect of some essential oils on the severity of bacterial wilt disease of tomato, under artificial inoculation conditions

Essential oil	Disease index mean (%)	Essential oils efficacy (%)
Lemongrass	54.8^{c}	38
Eucalyptus	46.9^{d}	46
Thyme	77.6^{b}	15.2
Ginger	52.0^{cd}	40.8
Peppermint	58.6^{c}	32.4
Positive control	92.8^{a}	
Negative control	0.0^{e}	

Within the same column, the mean with different superscripts is significantly different (Duncan's multiple range test, $P \leq 0.05$).

Figure 4



Pathogenicity test on tomato plants.

Table 4 Effect of some essential oils on growth parameters of tomato, under artificial inoculation conditions

Essential oil	Plant height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Lemongrass	12.4^{b}	1.78^{abc}	0.076^{b}
Eucalyptus	23.0^{a}	2.86^{ab}	0.308^{a}
Thyme	11.2^{b}	1.34^{bc}	0.082^{b}
Ginger	17.2^{ab}	2.02^{abc}	0.180^{ab}
Peppermint	12.8^{b}	1.96^{abc}	0.114^{b}
Positive control	8.8^{b}	1.04^{c}	0.100^{b}
Negative control	24.6^{a}	3.16^{a}	0.272^{a}

Within the same column, the mean with different superscripts is significantly different (Duncan's multiple range test, $P \leq 0.05$).

It is obvious from the data in Table 4 that there is an increase in most vegetative growth parameters of treated tomato plants compared to positive control. All applied treatments increased plant height (cm), shoot fresh weight (g), and dry weight (g) compared to positive control.

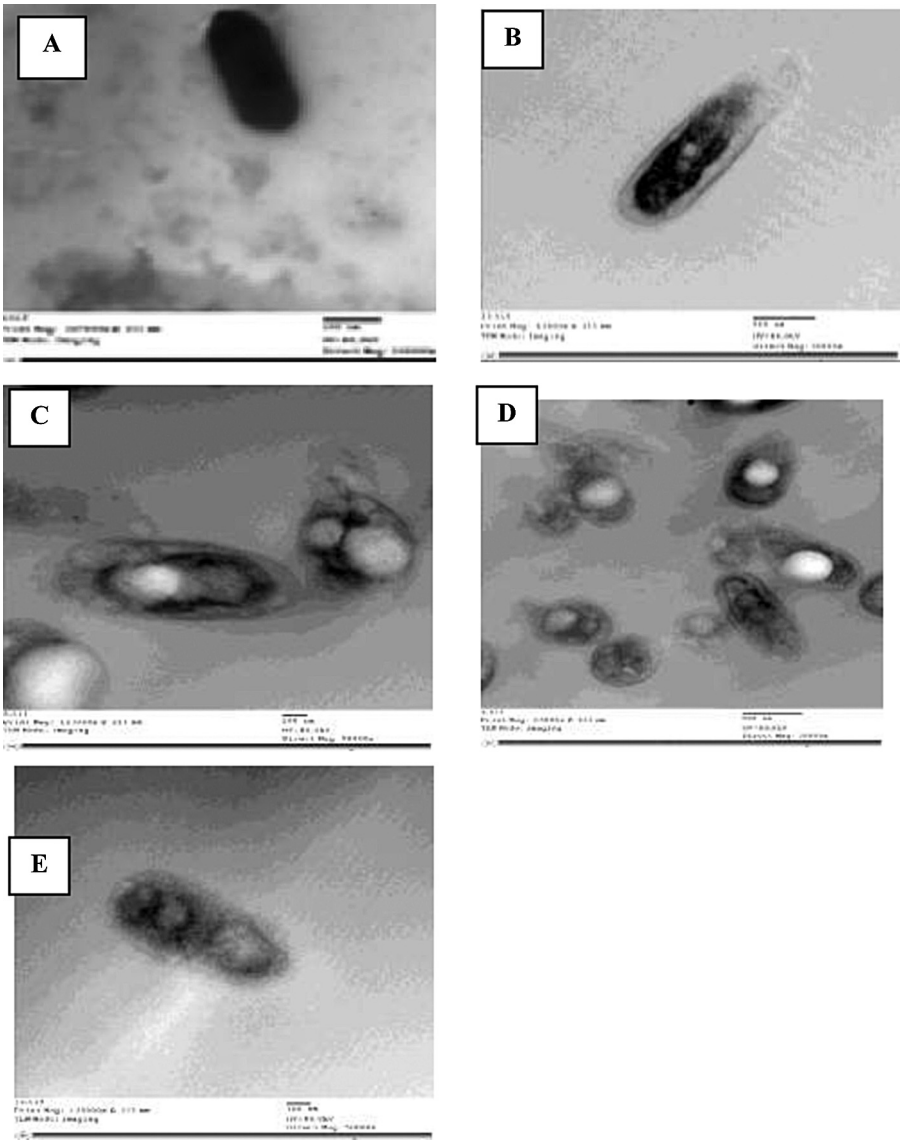
Transmission electron microscopy

TEM was used to inspect the relationship between eucalyptus essential oil and the bacterial cells to associate the cellular variation of the treated and untreated cells of *R. solanacearum*. As obvious in TEM micrographs, untreated cells of *R. solanacearum* with the examined essential oil had a soft texture, while the treated cells with eucalyptus essential oil were enveloped up in form, and damage was observed in the bacterial cell membrane compared to the control: cell wall lysis, loss of flagella, and bubble-like structures. Also, the degraded cellular component and the used oil caused cytoplasmic condensation and vacuolization in bacteria. Lacerated cells and crooked texture were also noticed in treated cells (Fig. 5).

Discussion

Potato brown rot (potato bacterial wilt) disease is caused by *R. solanacearum* [18]; it was first recorded in Egypt many years previously by Briton-Jones [4], although the first introduction of the disease is not well authenticated. This bacterium is considered a major problem and is among the extremely devastating

Figure 5



TEM images: (a) untreated cell of *Ralstonia solanacearum*, (b, c, d, e) the overnight broth of bacterial culture. (b) Cell wall lysis and loss of flagella. (c) Degraded cellular components and damaged cytoplasm. (d) Bubble-like structures and lacerated cell wall. (e) The cells have a crooked texture. TEM, transmission electron microscope.

pathogens of potatoes [19]. Potato has a special position among all vegetable crops cultivated in Egypt. This crop is economically important, and any trouble in its production greatly affects its local and export impact [20]. The causative agent in Egypt and European countries is *R. solanacearum* race 3 biovar 2 newly, depending on the origin of the genomic sequencing phylotype II Segevar I [7].

Potato bacterial wilt is described as a vascular bacterium that is carried by soil and water. It can live in potato tuber roots, in the rhizosphere, in infected plant debris, and nonhosts [21]. The bacterium is frequently described as a tuber-borne pathogen as well [22]. Microscopic observation via IFAS and detection by means of Taq-Man PCR clearly indicated that the organism was *R. solanacearum*. The morphological and cultural of specific SMSA medium [11] and TZC medium [3] using tomato plants as a biological and rapid test for diagnosis of the bacteria was the most accurate to detect as few as 10^4 cells/ml [11,13]. Tomato plants exhibit wilt signs within 7 days from injection, relying on the initial suspension and environmental conditions. If the temperature is less than 21°C, the infection occurs without symptoms produced, and the wilt may fail to progress [23]. Based on biovar determination results, it was proved that all inspected isolates belong to biovar 2, depending on the biochemical reaction. Furthermore, the outcomes approving that the race 3 biovar 2 (phyllotype II sequvar I) is the main race in Egypt. A similar trend was reported by other researchers [24]. One of the most used serological assays for detecting latent infections in potato tubers is IFAS. This technique with the polyclonal antisera was defined as a fast and inexpensive manner, but the deficiency in sensitivity besides giving a false positive result due to cross-reactions with further bacteria with additional microorganisms [25]. Typically, this technique is usually carried out in conjugation with the tomato bioassay testing [26]. Taq-Man PCR is a molecular detection technique that integrates PCR with fluorescent detection of the amplicon [27]. A lot of researchers have elucidated that these methods vary in their grade of sensitivity [28].

Nowadays, the use of chemical control and antibiotics are inadmissible control techniques due to the dangerous effects of chemical control residues [9]. *In vitro* studies have shown that lemongrass, eucalyptus, thyme, ginger, and peppermint plant essential oils have potential antibacterial activity to minimize the growth of *R. solanacearum* and their influence depending on the concentrations used. The breakdown of the cell wall, disruption of the membrane proteins, cytoplasmic membrane coagulation damage, and enhanced infiltration that results in cell contents penetration are among the action mechanisms of essential plant oils [29]. The lemongrass essential oil transmits a quantity of several biodynamic compounds such as citral, isogeranial, geranyl acetate, citronellal, germacrene-D, and elemol, in addition to other biodynamic composites [30]. Moreover, Hindumathy [31] showed that the inhibiting effect of *C. citratus* is perhaps attributed to phenols and alkaloids such as c-terpinene, linalool, a-terpinene a-pinene, which have antimicrobial properties. It was reported that using neem solvent extracts can shift the antibiotics to control bacterial infection. This kind of biological attitude would be efficient and environmentally friendly. Likewise, these plant products are available adequately, and farmers can use them for control of wilt in many crops [32].

The antibacterial efficacy of eucalyptus essential oil is related to its lipophilic profile, enabling it to penetrate the cell and cause cell leakage [33]. A range of monoterpenes, sesquiterpenes, ethers, oxides, aromatic phenols, alcohols, esters, aldehydes, and ketones, including 1,8 cincole (eucalyptol), citronellol, citronellyl acetate, a-pinene, limonene, and aromadendrene are also present in Eucalyptus oil [34].

Various bioactivities, such as antioxidant, antimicrobial, and antibacterial, have also been noticed for essential oils produced by eucalyptus [35]. The main constituents of thyme include thymol, carvacol, and flavonoids geraniol, gamma-terpineol, and linalool [36,37]. They have antimicrobial properties on widespread food-linked bacteria and fungi via the disk diffusion method. *T. vulgaris* essential oil has a vigorous antimicrobial effect and, probably in the future, clarifies a novel basis of eco-friendly antiseptics in the applications of the pharmaceutical and food industry. Essential oils with a phenolic structure, such as thymol, possess great bioactivity against bacteria, as elucidated in several studies [38]. The bioactivity of the essential oils of thyme was settled up to be counting ordinarily on their phenolic components. The quantitatively extremely significant composition is the carvacrol and phenols thymol. These two phenolic mixtures have great antibacterial characteristics [39].

The antimicrobial activity of ginger essential oil was attached to the main chemical constituents, that is ginger oil exhibited a significant inhibitory effect

against many tested pathogens [40]. Ginger oil consists of a combination of ingredients as follows monoterpenes, that is phellandrene, camphene, cineole, linalool, limonene, citral, geraniol, and zingiberol, along with some aliphatic aldehydes and alcohols [41,42]. The ginger essential oil exhibited significant antimicrobial activity against pathogenic microorganisms, while peppermint essential oil has a high concentration of natural pesticides and demonstrated antioxidant activity in a model emulsion system. The main constituents in this respect were menthol, menthone, methyl acetate, cineole, limonene, beta-pinene, and beta-caryophyllene [43]. The results regarding the decreasing impact of bacterial wilt *in vivo* are in harmony with those recorded by Pradhanang *et al.* [44]. They recorded that the oil of lemongrass and palmarosa decreased populations of *R. solanacearum* race1, the cause of tomato bacterial wilt.

Application of essential oils of eucalyptus, lemongrass, ginger, peppermint, and thyme under greenhouse conditions enhanced plant growth parameters and reduced bacterial wilt of tomato plants compared with the control. Our findings agree with Pradhanang *et al.* [44], who indicated that lemongrass and thymol oils can repress *R. solanacearum* count in soil and decrease bacterial wilt occurrence in greenhouse pot experiments. In addition Paret *et al.* [45] concluded that using essential oils from lemongrass has revealed that the oil prohibits *R. solanacearum* race 4 strain *in vitro* and pot tests, and they decrease wilt occurrence and severity in the greenhouse. Also, Young-Hee *et al.* [46] mentioned that thyme oil effectively reduced the occurrence of tomato bacterial wilt and enhanced vegetative development in comparison with the untreated control. Comparably, the outcomes of Orzali *et al.* [47] who resulted in testing of thyme and other essential oils caused a significant inhibition of bacterial growth caused by *R. solanacearum* and *Clavibacter michiganensis* subsp. *michiganensis*. Furthermore, an *in vivo* germination test revealed no main decrease in seed germination when the components were used as seed treatment. Similar results were obtained in the previous study for Tomazoni *et al.* [48], who concluded that essential oils of eucalyptus were able to control early blight disease for both *in vitro* and *in vivo* approaches.

Finally, we could infer that the tested essential oils possibly be the nominee for future eco-friendly antibacterial agents, controlling bacterial wilt disease of the Solanaceae and minimalizing risks and hazards for the environment.

Conclusion

To control *R. solanacearum*, we tested five plant essential oils, that is lemongrass, eucalyptus, thyme, ginger, and peppermint that have a potential antibacterial activity to repress the growth of *R. solanacearum*. Eucalyptus and peppermint oils were the most efficient in suppressing *R. solanacearum* growth and showed the greatest inhibition zone haloes of the bacterium, followed by thyme, lemongrass, and ginger in descending order.

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Conflicts of interest

There are no conflicts of interest.

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