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Efficacy of Orange, Cinnamon, and Ginger Oils in Emulsion and Nano-emulsion Forms on Potato Bacterial Wilt Caused by *Ralstonia solanacearum*

Eman O. Hassan [1*], Tahsin Shoala [2], Omnia A. M. Badr [3], Shereen A. Mohamed [3], and Ibrahim A. I. EL-Fiki [1]

[1]Department of Plant Pathology, Faculty of Agriculture, Benha University, Benha 13511, Egypt.
[2]Environmental Biotechnology Department, College of Biotechnology, Misr University for Science and Technology, Giza 12563, Egypt.
[3]Department of Genetics and Genetic Engineering, Faculty of Agriculture, Benha University, Benha 13511, Egypt.

*** Corresponding author**
Eman O. Hassan

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Abstract

Antibacterial activity of essential plant oils ex. orange, cinnamon, and ginger in normal nano-emulsion forms against potato bacterial wilt disease caused by *Ralstonia solanacearum* was screened in vitro and in vivo. All treatments revealed inhibitory effects in vitro on growth of R. solanacearum compared to the control treatment. In this respect, 0.05% ginger nano-emulsion oil (GIN-NE) increased the bacterial inhibition zone to 18.5 mm, followed by cinnamon essential oil ex. CIN-NE and CIN-EO (15.5 and 11.5 mm, respectively) while, the inhibition zone of bacterial growth recorded the greatest inhibition of 22.0 mm followed by 21.0 mm in reaction to 0.10% cinnamon nano-emulsion oil (CIN-NE) and ginger nano-emulsion (GIN- NE) oil comparing with control treatments. The bacterial inhibition zone of *R. solanacearum* was inhibited to 27.5, 25.8, and 21.5 mm in response to 0.20% GIN-NE, CIN-NE, and CIN-EO, respectively. Applying 0.20% concentration of each tested essential plant oil as foliar spray on Cara and Spunta cvs. reduced disease severity index % in comparison to control. All tested treatments decreased bacterial wilt disease assessment than control treatment. The treatment effect on the Spunta cultivar was more effective in decreasing disease severity index % of bacterial wilt disease than the Cara cultivar. All tested treatments increased potato yield. Relative expression profiles of Serine protease, glutathione S-transferase, catalase, and peroxidase genes in Cara and Spunta cultivars after inoculation with *R. solanacearum* showed positive transcriptome alterations in response to essential oils at 0.20% concentrations in normal and nano-emulsion form in comparison to the positive control.

Keywords: Ralstonia solanacearum, essential oils, gene expression, nano-emulsion.

Introduction

The potato (*Solanum tuberosum* L.) is one of the most substantial vegetable crops in the world, ranking fourth after wheat, maize, and rice. **Birch and colleagues (2012)** revealed that potato is a long-term nutrition safety crop with a potential future for reducing challenges and combating malnutrition in developing countries **(Devaux *et al.,* 2020)**. The entire value of Egyptian potato exports is currently changing due to the EU's quarantine ban on potato bacterial wilt caused by *Ralstonia solanacearum* **(Makled and Elkodosy, 2018)**. Bacterial wilt is the most constraining disease on Egyptian potato fields, particularly for exports. The disease is easily detected in potato tubers, with both inner and exterior signs **(Thabit, 1961)**.

The cause of potato brown rot and wilt, *R. solanacearum*, is one of the most significant challenges to potato production in Asia, Africa, Central and South America **(Charkowski *et al.,* 2020)**. Because this bacterium is a soil pathogen, it often penetrates the host plant via roots and colonizes xylem vessels **(García *et al.,* 2019)**. As the bacterium accumulates in the vascular bundles of the infected plant, it obstructs the transfer of water and essential minerals, resulting in symptoms such as leaf discoloration, redness of the vascular bundles, tissue damage, and eventually overall wilt, which is then accompanied by physiological changes in the diseased plants, such as increased respiratory rate and decreased water loss and photosynthetic activity **(Karim and Hossain, 2018)**.

Essential oils are fragrant compounds found in oil sacs or oil glands found at greater depths in the fruit peel, primarily in the flavedo section and the cuticle. These are insoluble in water but soluble in alcohol, ether, and natural oils **(Nannapaneni *et al.,* 2009)**. The active compounds in essential oils are very volatile and readily destroyed by oxygen, heat, and light **(Muriel-Galet *et al.,* 2015)**. Orange essential oil is composed of 85–99 percent volatile components and 1–15 percent nonvolatile compounds **(Fisher and Phillips, 2008)**. Monoterpene (limonene) and sesquiterpene hydrocarbons, as well as oxygenated derivatives such as aldehydes, ketones, acids, alcohols, and esters, are among the volatile components **(Flamini *et al.,* 2007)**. The major antibacterial element in this oil is d-limonene. This is a monocyclic monoterpene produced from orange peel that is easily absorbed into the polymer matrix and has significant antibacterial properties effects **(Arrieta *et al.,* 2013)**. Cinnamon essential oil is predominantly employed in the fragrance and essence sectors due to its aromatic qualities, which may be found in a range of foods, perfumes, and medicinal items **(Barbosa, 1992)**. Cinnamaldehyde and trans-cinnamaldehyde are the most important components of cinnamon, and they may be found in essential oils, where they contribute to the scent and the numerous biological activities associated with cinnamon **(Pires *et al.,* 2011)**. Ginger essential oil is a herbaceous plant from the Zingiberaceae family. It originated in the tropical regions of Southeast Asia and has since spread across China **(Alsherbiny *et al.,* 2019)**. It is high in bioactive elements such as bioactive phenols (gingerols, shogaols, and zingerones) **(Kieliszek *et al.,* 2020)**. Essential oil of ginger is a flammable oil derived from ginger root. Because of its characteristic odor and biological activity, it has a wide range of uses in the pharmaceutical, food, and cosmetics industries **(Mesomo *et al.,* 2013)**. Ginger essential oil has been intensively studied, with an emphasis on their antioxidant, antifungal, and antibacterial characteristics, as well as their growing usage in food preservation **(Ju *et al.,* 2019)**.

Reactive oxygen species (ROS) are unavoidable by-products of plant metabolic activities and considered one of the first events after elicitation in plants confronted with harmful microbes such as fungus, bacteria, and viruses **(Wojtaszek, 1997)**. Negative external factors, such as pathogen infection, disrupt this delicate equilibrium of

ROS creation and scavenging, resulting in a fast increase in intracellular ROS synthesis, often known as "oxidative burst" **(Apel and Hirt, 2004)**. The hypersensitive reaction during non-host contacts has been linked to disease resistance during of ROS buildup, which is frequently longer and more severe **(Lamb and Dixon, 1997)**. The oxidative burst in plant cells performs numerous roles under pathogen infection. ROS promote oxidative cross-linking of plant cell walls, which slows pathogen entrance and dissemination, and function as essential signal molecules in the promotion of plant responses and systemic resistance. Furthermore, due to their strong reactivity with cellular macromolecules such as DNA and proteins, ROS are efficient bactericidal agents capable of either killing or delaying pathogen development. To counteract ROS, it was discovered that oxidative stress response genes are present in plant-associated bacteria during interactions with their hosts **(Okinaka *et al.,* 2002; Saenkham *et al.,* 2007; Tamir-Ariel *et al.,* 2007)**.

During tomato plant pathogenesis, an *in vivo* expression technology (IVET) screen in *R. solanacearum* discovered that at least 15 of the 153 planta-expressed genes encoded proteins are present in the oxidative stress response, reinforcing the idea that plant infection is related to an oxidative challenge **(Brown and Allen, 2004; Flores-Cruz and Allen, 2009)**. The main ROS of the oxidative burst, hydrogen peroxide (H_2O_2), is an uncharged and stable species that may pass through cell membranes and disperse to perform distant physiological tasks **(Wojtaszec, 1997)**. Catalases (E.C. 1.11.1.6; H_2O_2:H_2O_2 oxidoreductase) are the principal scavengers of H_2O_2 by catalyzing its dismutation to water and oxygen among the bacterial enzymes designed to eliminate ROS and prevent toxicity. Phylogenetic analyses distinguish three distinct catalase families: conventional (mono-functional) heme catalases (KatEs), bi-functional heme catalase-peroxidases (KatGs), and manganese catalases (MnCats) **(Zámocky *et al.,* 2012)**. The great majority of sequenced bacterial genomes encode several catalase isozymes that work in a variety of physiological and environmental circumstances. When bacteria sense ambient ROS and reach the stationary phase, certain catalases are activated **(Mishra and Imlay, 2012)**. Furthermore, recent research has indicated that particular catalases play a role in pathogenesis by enhancing the capacity of bacteria to withstand host-induced oxidative bursts **(Tondo *et al.,* 2010; Mishra and Imlay, 2012)**. Numerous ROS-scavenging enzymes have been predicted in the genome of *R. solanacearum* GMI1000, including three putative catalases. RSc0775 (KatGb) and RSc0776 (KatGa) encode anticipated bi-functional catalase-peroxidases on the bacterial chromosome, but RSp1581 (KatE) codes for a projected typical mono-functional catalase and is located in the megaplasmid, which includes the bulk of *R. solanacearum* pathogenicity functions **(Salanoubat *et al.,* 2002; Genin and Denny, 2012)**.

Glutathione S-transferase (GST) was discovered in 1970 as the first plant glutathione S-transferase enzyme (GST, EC 2.5.1.18) that catalyzed the detoxification of the herbicide atrazine in sorghum and maize plants by conjugation to the endogenous tri-peptide glutathione (GSH, -L-glutamyl-L-cysteinyl-glycine). These early findings fueled a rush of GST research into the detoxification of various herbicides and other hazardous xenobiotic chemicals in plants **(Schröder *et al.,* 2007)**. GSTs have been shown to catalyze the conjugation of several xenobiotics with electrophilic centres and nucleophilic GSH, thereby marking the xenobiotic for vacuolar sequestration **(Martinoia *et al.,* 1993)**. GSH and homoglutathione conjugates (-L-glutamyl-L-cysteinyl—alanine) were less toxic and more water-soluble than the original xenobiotics **(Dixon *et al.,* 1998)**. Several GST enzymes have been found to exhibit glutathione peroxidase activity, hinting that these GSTs

can contribute in anti-oxidative defense **(Dixon *et al.,* 2009)**. It has been demonstrated that auxin-inducible GSTs bind auxins as non-substrate ligands (ligandin function) and engage in auxin transport **(Droog *et al.,* 1995)**. GSTs have also been discovered to be involved in the regular metabolism of plant secondary metabolites such anthocyanins **(Marrs, 1996)**. Despite significant knowledge of the detoxifying function of GSTs, understanding of their role in endogenous plant processes and their metabolic substrates is far from complete (**Dixon and Edwards, 2009; Dixon *et al.,* 2010)**.

Because of a lack of the most efficient chemical control treatments for *R. solanacearum*, which causes bacterial wilt disease in potatoes, management of *R. solanacearum* appears to have become extraordinarily difficult. Hence, the purpose of this research was to determine if orange, cinnamon, ginger essential plant oils in normal and nano-emulsion forms were efficient *in vitro* against the pathogenic bacterium *R. solanacearum*, as well as whether these plant oils might be employed *in vivo* to reduce potato bacterial wilt and increasing potato crop yield.

Material and Methods

Bacterial culture

Ralstonia solanacearum isolate has been isolated from potatoes have been kindly contributed by Dr. Rabab Mohamed, Bacterial Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Pathogenicity tests and Koch's postulates were successfully performed on potato seedlings (cv. Spunta) in the laboratory to confirm its pathogenicity, and re-isolated pure cultures of *R. solanacearum* were maintained on nutritional agar slants at 4°C.

Source of the essential oils

International Favors and Plant oils Inc. located in Giza, Egypt provided orange, cinnamon, and ginger essential oils. These essential oils were stored in dark bottles at 4°C until used.

Synthesis of the tested nano-emulsion oils and characterization

Orange, cinnamon, and ginger oils were synthesized with slight changes using the technique reported by **Hamouda *et al.,* (1999)**. In summary, nano-emulsions were created by mixing orange, cinnamon, or ginger oils (14% v/v), ethanol (3% v/v), and bio-surfactant non-ionic Tween 80 (3% v/v), which made up 20% (v/v) of the overall emulsion **(Rassem *et al.,* 2016)**. Finally, the emulsion was combined and churned for 1 hour at 86°C. As a consequence, the reaction product was mixed with water (80%), allowed to cool to ambient temperature (25°C), and centrifuged at 10,000 rpm. The droplet size distribution (analysis by volume) of the prepared nano-emulsions was determined using the dynamic laser light-scattering technique (DLS). Photon correlation spectroscopy was utilized to study the zeta potential and polydispersity index (PDI) using a ZetaPlus instrument (Malvern Zetasize Nano-zs90, Malvern Instruments Ltd., Enigma Business Park, Grovewood Road, Malvern, Worcestershire WR14 1XZ, UK) **(Hashem *et al.,* 2018)**.

Effect of tested treatments on the growth of *R. solanacearum in vitro*

Orange, cinnamon, and ginger oils were employed in normal and nano-emulsion forms at three distinct concentrations: 0.05, 0.10, and 0.20%. A nutritional agar medium was spread with 0.1 ml of bacterial suspension ($1X10^{-8}$ dilution, 24 hrs old cultures). Saturated filter paper discs (5 mm) of any of the specified treatments were put on the surface of inoculated plates at varying concentrations. As a control, discs with no treatment (just sterile water) were utilized. For each treatment, four disks/plate, and three replicates were conducted, and the plates were subsequently incubated at 25-28°C for 48 hrs. The impact of the studied compounds was assessed as an inhibitory zone around the discs **(Loo *et al.,* 1945)**.

Control of potato bacterial wilt disease under open field conditions

Field tests were carried out at the Experimental Station, Moshtohor, Faculty of Agriculture, Benha University, Egypt. This experiment was carried out on potato (*Solanum tuberosum* L.) plants (Cara and Spunta cultivars) under open field condition to evaluate the efficacy of three essential plant oils i.e., orange, cinnamon, and ginger oils in both normal and nano-emulsion forms at concentration 0.20% for each to ascertain its efficacy against bacterial wilt disease development caused by *R. solanacearum*. As usual, the planting area (sandy-clay soil) was readied for potato planting. Plot area was 42.0 m^2. On the 22nd of October, apparently healthy (free of any biotic infection) petite hole tubers of Cara and Spunta potato cultivars were planted in rows 25-30 cm apart. All recommended agronomic practices were carried out for cultivation of potato plants in accordance with the recommendations of Egypt's Ministry of Agriculture and Land Reclamation. Vegetative growth of the planted potato tubers began 10 days after culture. The Janse stem pierce technique was used to infect two-week-old healthy three-leafed seedlings of both tested potato cultivars with 100 ml of 10^7-10^8 CFU bacterial solution. Similarly, control treatment was performed using sterile water. Three sprayer doses of orange, cinnamon, and ginger of prepared normal and nano-emulsion of the tested oils were given 48 hours after infection.

Disease and total yield assessments

Disease severity index percentage was evaluated and calculated using a disease index scale ranging from 0 (no wilting symptoms) to 5 (all leaves wilted-dead plant) at harvesting time using the method described by **Winstead, (1952) and Cooke, (2006)**. Harvesting was done when 75% of the plants had reached senescence. The total potato yield (kg/plant) for each treatment was recorded.

Primer design

To provide maximal specificity and efficiency during qPCR amplification under a defined set of reaction conditions, Real-time PCR primers were designed using Allele ID 7.7 software. The internal housekeeping gene ITS was employed as a reference gene in this investigation **(Table 1)**.

Table1: Serine protease, glutathione S-transferase, catalase and peroxidase designed primers for QRT-PCR.

Gene name	Accession	Forward	Reverse	TM
Serine protease	XM_006345021.2	CGCAAGCTATTGTGGCAGAG	CCTCAGGCCTACAGATGAAGT	60
Glutathione S-Transferase	XM_006355737.2	CTAGCTGATTTGCACCACGC	ACCCATCCGCCACAAATCTT	60
Catalase	NM_001287934.1	TCCAAACAATGGACCCCGAG	TCGACCAACTGGGATCAACG	60
Peroxidase	XM_006347106.2	GGAGACTTGCCTCCATCCG	ACCAAAACCACATTCTGGAGC	60

Differential expression analysis using quantitative Real-Time PCR

Total RNA was isolated from treated and untreated (control) potato leaf tissues according to the manufacturer's procedure using the RNeasy® Plant Mini Kit (QIAGEN, Germany). The RNA was processed with DNase for 1 hour at 37°C to eliminate any DNA residues. To assess the concentration and purity of RNA, the Nano-Drop 2000C spectrophotometer was utilized (Thermo Scientific, USA). For the reverse transcription procedure, the High-Capacity cDNA reverse transcriptase kit was employed (Thermo Fisher Scientific, Waltham, MA, USA). The cDNA samples were then kept at 20°C until needed.

Each PCR reaction comprised 2.5 µL of cDNA (except for the NTC and cDNA controls), 12.5 µL of SYBR Green Mix (Cat. No. 204143; Qiagen, Hilden, Germany), 0.3 M of each forward and reverse primer **(Table 2)**, 1 µL RNase inhibitor, and a final volume of 25.0 µL was adjusted by adding RNase-Free water. The AriaMx Real-Time PCR System (Agilent Technologies, Santa Clara, CA, USA) was utilized using a two-step cycling protocol under the following conditions: 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 60 seconds. The data were normalized by

utilizing ITS as an internal housekeeping gene. The following formula was used to compute the relative gene expression ratios (RQ) between the treatment and control groups: $\mathbf{RQ} = 2^{-\Delta\Delta CT}$

Statistical analysis:

The presented data were laid out in triplicates and were statistically analyzed for the least significant difference (L.S.D.) according to **Gomez and Gomez (1984)**.

Results

Characterization of tested synthesized nano-emulsion oils

The size distribution and stability of synthesized orange, cinnamon, and ginger nano-emulsions of the tested plant oils were examined using dynamic light scattering, and the findings were about 140, 150, and 170 nm, respectively, as shown in **Fig. (1)**. While the Zeta potential in orange, cinnamon, and ginger nano-emulsions was electrically stabilized **(Fig. 1)**.

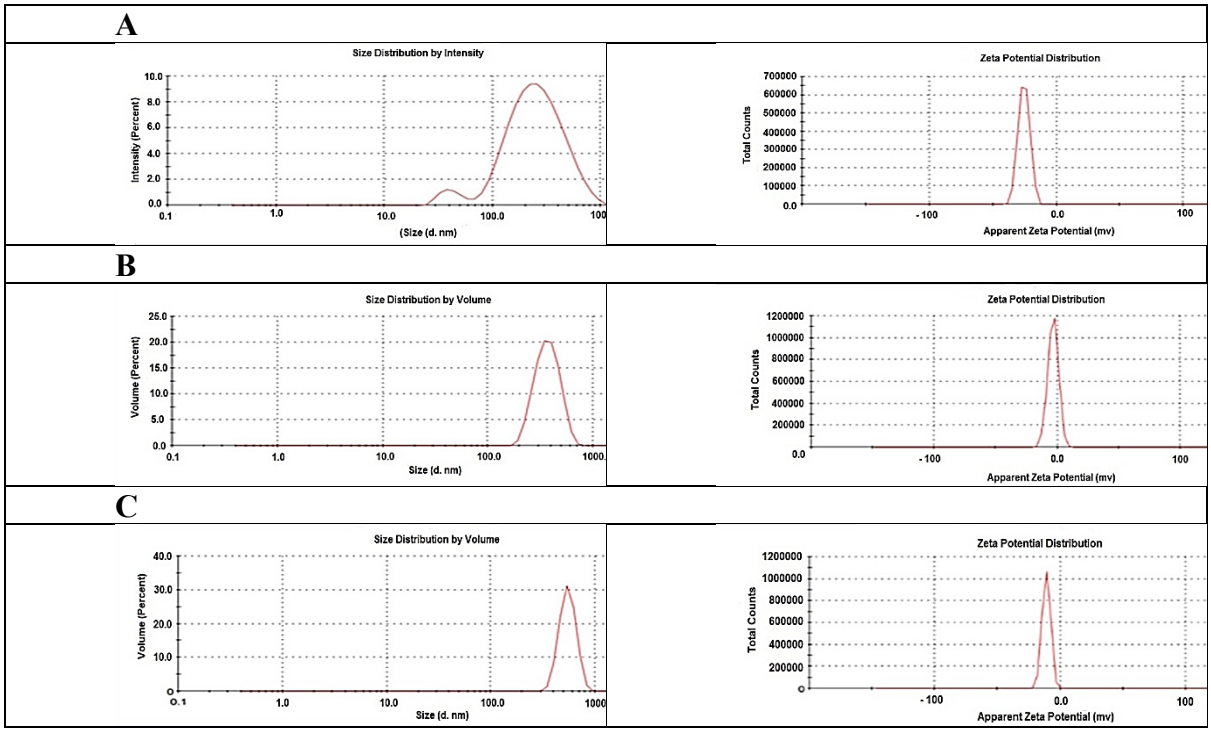

Figure 1: Z average size and Zeta potential of cinnamon (A) ginger (B), and orange (C) nano-emulsion of the tested plant oils.

Effect of tested essential oils on the growth of *R. solanacearum in vitro*

As seen in **Fig. (2)**, three different essential plant oils were tested in normal and nano-emulsion forms against *R. solanacearum* bacterium for their antibacterial properties at three distinct concentrations: 0.05, 0.10, and 0.20% for each. Data in **Fig. 3** and **Fig. 4** reveal that all tested treatments had clear inhibitory effects on *R. solanacearum* in comparing with control treatments. In this respect, 0.05% ginger nano-emulsion oil (GIN-NE) increased the bacterial inhibition zone to 18.5 mm, followed by cinnamon essential oil *ex.* CIN-NE and CIN-EO (15.5 and 11.5 mm, respectively). In addition, the inhibition zones for ginger (GIN-EO), orange nano-emulsion (OR-NE), and orange essential oil (OR-EO) were 10.3, 9.0, and 5.5 mm, respectively. According to **Fig. (3)**, the inhibition zone of bacterial growth recorded the greatest inhibition of 22.0 mm in reaction to 0.10% cinnamon nano-emulsion oil (CIN-NE)

followed by 21.0 mm in response to 0.10% ginger nano-emulsion (GIN-NE) oil comparing with control treatments. Other treatments with 0.10% essential oils; cinnamon (CIN-EO), ginger (GIN-EO), and orange (OR-EO) lowered bacterial inhibition zones of 18.8, 13.5, and 8.8 mm, respectively, while 0.10% orange nano-emulsion oil inhibited bacterial growth by 11.5 mm. The bacterial inhibition zone of *R. solanacearum* was inhibited to 27.5, 25.8, and 21.5 mm in response to 0.20% GIN-NE, CIN-NE, and CIN-EO, respectively, as shown in **Fig. (4)**. The other treatments, which included 0.20% GIN-EO, OR-NE, and OR-EO, reduced the bacterial inhibition zone to 17.0, 16.0, and 12.5 mm, respectively.

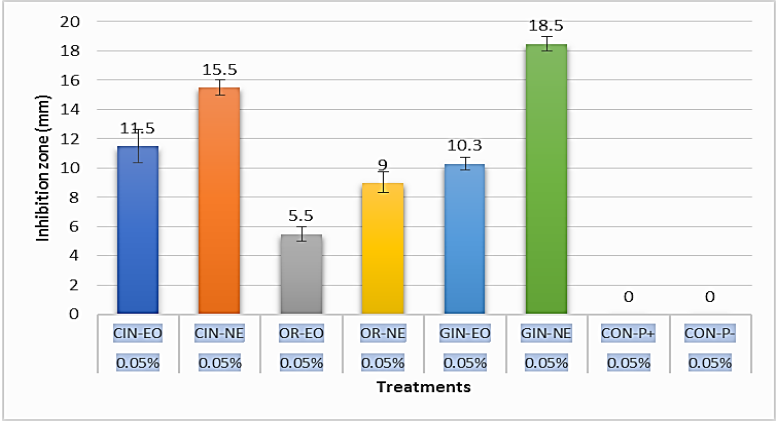

Figure 2: The inhibitory effect of tested essential oils of cinnamon (CIN), orange (OR), and ginger (GIN) in the normal (EO) and nano-emulsion (NE) forms at concentration 0.05% against bacterium *R. solanacearum in vitro*.

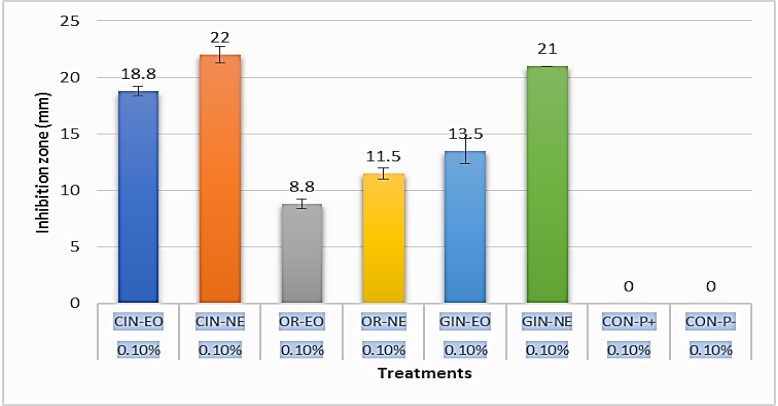

Figure 3: The inhibitory effect of tested essential oils of cinnamon (CIN), orange (OR), and ginger (GIN) in the normal (EO) and nano-emulsion (NE) forms at concentration 0.10% against bacterium *R. solanacearum in vitro*.

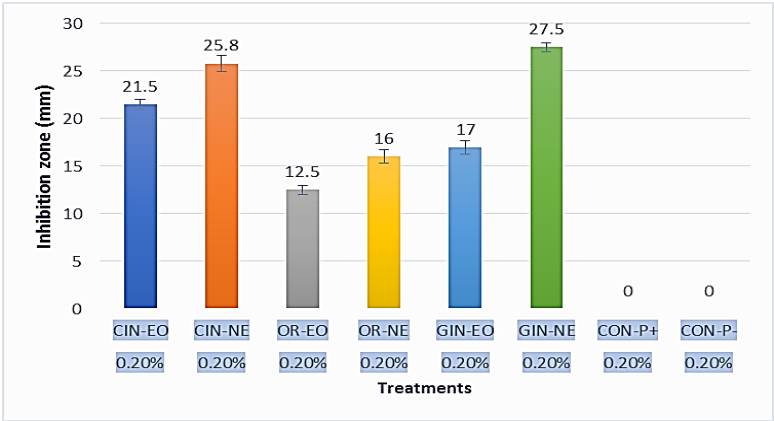

Figure 4: The inhibitory effect of tested essential oils of cinnamon (CIN), orange (OR), and ginger (GIN) in the normal (EO) and nano-emulsion (NE) forms at concentration 0.20% against bacterium *R. solanacearum in vitro*.

Control of potato bacterial wilt disease under field conditions

Data illustrated in **Fig. (5)** demonstrate that applying of 0.20% concentration of each tested essential plant oil (orange, cinnamon, and ginger oils) as foliar spray on both tested potato cultivars (Cara and Spunta cvs.) considerably reduced bacterial wilt disease development (disease severity index %) in comparison to control. All tested treatments were far better in decreasing bacterial wilt disease assessment than control treatment. Also, it clear that treatment results on the Spunta cultivar were more effective in decreasing disease severity index % of bacterial wilt disease than the Cara cultivar. The percentage of disease severity for the Cara cultivar was decreased to 4.0% in response to both treatments with 0.20% CIN-NE and GIN-NE, compared to 63.2% in the control treatment, as shown in **Fig. (5)**. However, the treated potato Spunta cultivar with the same CIN-NE and GIN-NE concentrations had disease severity index % of 3.6 and 5.6%, respectively, compared to 74.3% in the control.

Effect of tested essential oils on potato crop yield

As for the effect tested oils on total yield weight (kg tuber/plant) of potato, data in **Fig. (6)** reveal that all tested treatments had a great effect in increasing yield of potato comparing with the positive control treatment (only Ralstonia-inoculated). Data in the same figure demonstrate that applying of 0.20% concentration of each tested essential plant oil as foliar spray on both tested potato cultivars (Cara and Spunta cvs.) considerably increased potato crop yield (kg tuber/plant) in comparison to control. All tested treatments were far better in increasing crop yield assessment than control treatment. Also, it clear that treatment results on the Cara cultivar were more effective in potato crop yield/plant than the Spunta cultivar. According to **Fig. (6)**, crop yield of the Cara cultivar reached 5.06, 4.02, 3.96 and 3.70 Kg/plant in response to 0.20% CIN-NE, GIN-NE, OR-NE and CIN-EO compared to 3.1 and 2.3 Kg tubers/plant in untreated (negative control) plants and infected plants (positive control) with *R. solanacearum*, respectively. Meanwhile, Cara cultivar treated with 0.20% OR-EO and GIN-EO yielded 3.02 and 2.98 tubers/plant, respectively. In potato Spunta cultivar, crop yield production reached 5.06, 4.30, 3.56, 3.34 and 3.18 Kg/plant in response to 0.20% GIN-NE, GIN-EO, CIN-NE and CIN-EO compared to 3.22 and 1.8 Kg tubers/plant in un-treated (negative control) plants and infected plants (positive control) with *R. solanacearum*, respectively.

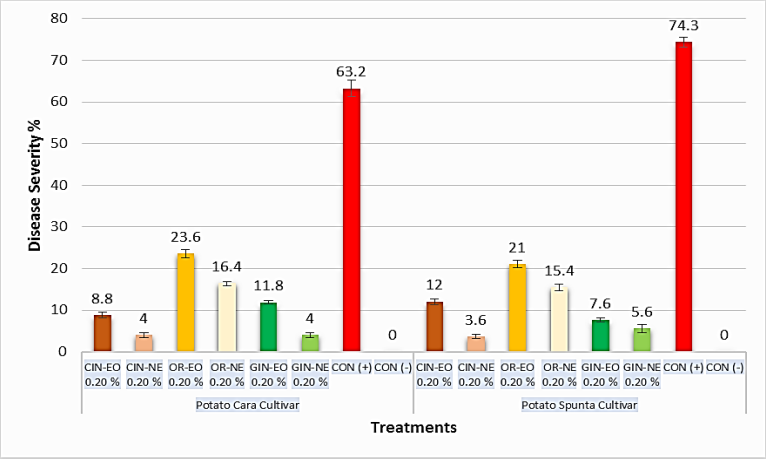

Figure 5. Effect of tested essential oils of cinnamon (CIN), orange (OR), and ginger (GIN) in the normal (EO) and nano-emulsion (NE) forms at concentration 0.20% on potato (Cara and Spunta cvs.) bacterial wilt disease severity under field conditions.

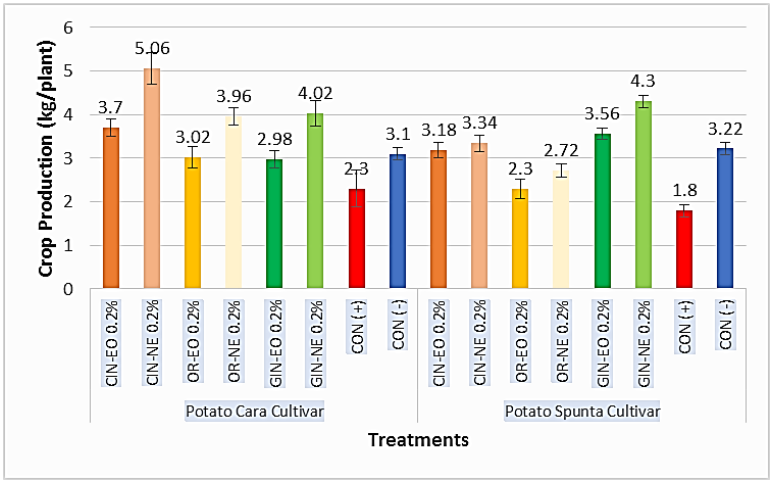

Figure 6. Effect of tested essential oils of cinnamon (CIN), orange (OR), and ginger (GIN) in the normal (EO) and nano-emulsion (NE) forms at concentration 0.20% on potato (Cara and Spunta cvs.) crop production under field conditions.

Expression analysis of plant defense response genes against *R. solanacearum*

Real-Time Reverse Transcription RT-qPCR analysis was used to determine the relative expression of Serine protease, glutathione S-transferase, catalase, and peroxidase genes in Cara and Spunta cultivars 48 hours after inoculation with *R. solanacearum*. In comparison to the positive control group, all of the genes tested showed positive transcriptome alterations in response to essential oils at 0.20% concentrations in normal and nano-emulsion form. The susceptible Spunta potato cultivar had the lowest gene expression levels, while the resistant Cara potato cultivar had intermediate values, as shown in **(Fig. 7)**. When compared to the other treatments, plants treated with cinnamon oil nano-emulsion had the lowest transcript levels of defense genes (glutathione S-transferase, catalase, and peroxidase). The serine protease gene displayed a distinct pattern in the Cara cultivar, with a greater transcriptome level than the positive control. In general, the expression levels of the four investigated genes were much lower in the susceptible cultivar than in the resistant cultivar.

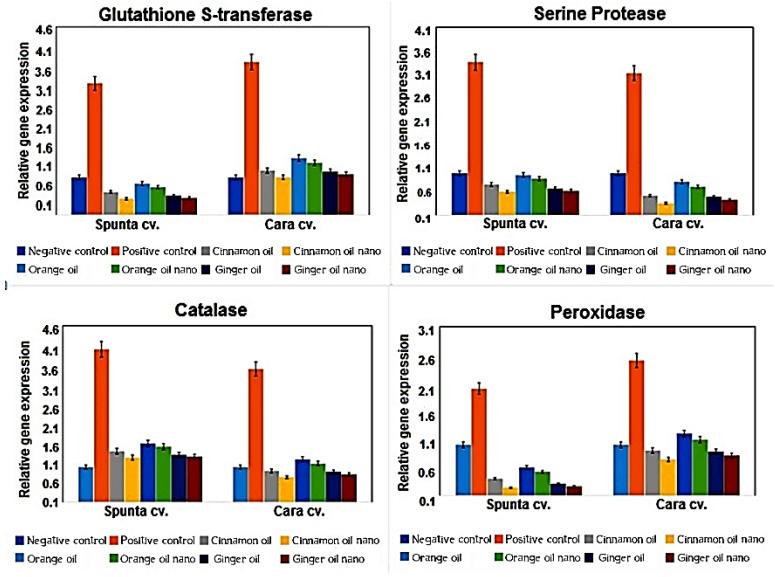

Figure 7. Relative gene expression of serine protease, glutathione S-transferase, catalase, and peroxidase genes in the leaves of two potato cultivars (the susceptible cv. Spunta and the resistance cv. Cara) after 48 hours post-inoculation with *R. solanacearum*.

Discussion

The most reputable option for battling *R. solanacearum* is the genetic platform to generate resistant variants **(Lebeau *et al.*, 2011)**; however, this strategy is unavailable in Egypt since all potato seeds are imported from several European nations. Therefore, the purpose of this research was to determine if cinnamon, orange, ginger essential plant oils in normal and nano-emulsion forms were efficient *in vitro* against the pathogenic bacterium *R. solanacearum*, as well as whether these plant oils might be employed *in vivo* to reduce potato bacterial wilt. These results showed that cinnamon, orange, and ginger emulsions and nano-emulsions can prevent *R. solanacearum* infection *in vitro*.

The results of the study on the *in vivo* impacts of bacterial wilt are comparable with previous findings **(Pradhanang *et al.*, 2003)** who discovered that palmarosa and lemongrass oils decreased *R. solanacearum* race-1 *in vitro* and tomato bacterial wilt populations *in vivo*. Furthermore, **Paret *et al.*, (2010)** and **Hong *et al.*, (2011)** revealed bacteriostatic efficacy of palmarosa, thymol, and lemongrass against *R. solanacearum* race 4, which causes bacterial wilt of tomato and ginger. *In vivo* studies, **Huang and Lakshman (2010)** found that clove oil may relieve *R. solanacearum* inhabitants and reduce the occurrence of geranium wilt and tomato bacterial wilt. **Hosseini Nezhad *et al.*, (2012)** demonstrated that the antibacterial activity of thyme oil against *R. solanacearum* and *Pectobacterium carotovorum* yielded comparable results.

By classifying the components of these essential oils and their effects on the bacterial growth and metabolic activities after implementation, the precise mechanism of action of emulsions and nano-emulsions in suppressing microbial activity and infection control was determined. Several more research have been undertaken in this field to clarify the therapeutic impact of different essential plant oils, which varies depending on the pathogen, host plant, oil, and their connection. **Rota *et al.*, (2008)** and **Hindumathy (2011)** discovered that essential oils' inhibitory impact on *R. solanacearum* development is due to efficient physiologically active components such as phenolic chemicals, terpenoids, alkaloids, citral, flavonoids, and tannins. Essential plant oils have been revealed to have mechanisms of action that include cell wall breakdown, destroying cytoplasm coagulation, notably cytoplasmic membrane **(Gustafson *et al.*, 1998)**, harmful and destructive membrane proteins, and permeability leading to cell substance seepage **(Lambert *et al.*, 2001)**. Essential oils, on the other hand, change membrane permeability by destroying the electron transport system **(Tassou *et al.*, 2000)**, and several essential oil components, such as carvon, thymol, and carvacrol, increase intracellular ATP levels **(Helander *et al.*, 1998; Tassou *et al.*, 2000)**. Electron transport, protein translocation, and the production of cellular components are all hindered. Finally, all physiological changes that might lead to cell lysis and death are considered **(Gill and Holley, 2006; Ben Arfa *et al.*, 2006)**. Because essential oil molecules are so small, they may easily permeate cell membranes and impact a number of metabolic pathways **(Huang *et al.*, 2005)**.

Vanitha and Umesha (2008) showed that defense system enzymes including polyphenol-oxidase and lipoxygenase are completely implicated in bacterial wilt recurrence tolerance, which has the ability to reduce *R. solanacearum* spread. Resistance responses in plant-bacterial interactions have been shown to have higher peroxidase functionalities. Peroxidase activity rises and falls in proportion to the rate of bacterial reproduction, showing that peroxidase is crucial in plant resistance. During the host-pathogen interaction, peroxidase activity was shown to be elevated in several systems **(Kartashova *et al.*, 2000)**. The host creates protective enzymes in response to invading infections, which help in resistance mechanisms. Plant immune defense relies

heavily on defensive system enzymes. Polyphenol-oxidase, peroxidase, and-1, 3-glucanase are defensive enzymes found in plants. Both H_2O_2 scavenging and phenol oxidative stress are caused by peroxidase enzymes **(Polle *et al.*, 1994)**. Peroxidase is an enzyme involved in the oxidation of phenols and the lignification of host plant cells during pathogen defense. Polyphenol-oxidase is essential for the oxidation of polyphenols into quinones and the lignification of plant cells during microbial infection **(Mohammadi and Kazemi, 2002)**.

Our results are in agreement with those of **El-Argawy and Adss (2016)**, who discovered that catalase activity was usually greater in the susceptible cv. Spunta than in the resistant cv. Cara. Catalase is an important H_2O_2 detoxifying enzyme in plants that regulates the equilibrium of active oxygen species such as H_2O_2 during plant defense. H_2O_2 is connected to a hypersensitive reaction during systemic acquired resistance. In tomato-Ralstonia interactions, resistant tomato cultivars produce more H_2O_2 than susceptible tomato cultivars **(Mandal *et al.*, 2011)**. Another study discovered that the antibacterial properties of H_2O_2, which is highly concentrated around bacterial cells, may be restricting the proliferation of *R. solanacearum*. As a result, cultivars with high catalase susceptibility restrict H_2O_2 activity against *R. Solanacearum*. Catalase expression and activity may also change during plant-pathogen interactions, with resistant plants seeing a decrease in catalase activity in response to viral infection. This is hypothesized to allow H_2O_2 to accumulate, resulting in antimicrobial action via plant cell wall strengthening, defense gene activation, hypersensitive cell death, and a stop to pathogen invasion. Elicitors of the fungal pathogen *Verticillium alboatrum* were discovered to variably activate peroxidase gene expression in resistant and susceptible tomato lines **(El-Argawy and Adss, 2016)**.

By activating glutathione peroxidase, initiating glutathione conjugation processes, binding auxin and phenylpropanoid, and trafficking anthocyanin into the vacuole, glutathione S-transferase protects cells from oxidative stress. The serine protease was also induced by *R. solanacearum*, showing that these proteases are engaged in innate defense against this pathogen. Finally, a potato metallocarboxy peptidase inhibitor, which is activated by jasmonic and abscissic acids, was found among the up-regulated genes **(Zuluaga *et al.*, 2015; Han-Wei Jiang *et al.*, 2010)**.

Conclusion

Applying of 0.20% concentration of each tested essential plant oil as foliar spray on both tested potato (Cara and Spunta cvs.) considerably reduced bacterial wilt disease development. All of the genes tested showed positive transcriptome alterations in response to essential oils at 0.20% concentrations in normal and nano-emulsion form.

Abbreviation

ROS= Reactive oxygen species.
DNA= Deoxyribonucleic acid.
E.C.= Electric conductivity.
R. solanacearum= *Ralstonia solanacearum*
GST= Glutathione S-transferase
GSH= The endogenous tri-peptide glutathione (GSH, -L-glutamyl-L-cysteinyl-glycine)
cv.= Cultivar
°C= Degree Celsius
v/v= Volume/Volume
DLS= Dynamic laser light-scattering technique
PDI= Zeta potential and polydispersity index
hrs= Hours
mm= millimeter
i.e.,= For example
%= Percentage
m^2= Meter square
cm= Centimeter
CFU= Cell forming unit
ml= Milliliter
qPCR= Quantitative PCR
RNA= Ribonucleic acid
µL= Microliter
cDNA= Complementary DNA
rpm= Round/minuet
nm= Nanometer
GIN-NE= Ginger nano-emulsion oil
CIN-NE= Cinnamon nano-emulsion
CIN-EO= Cinnamon essential oil

GIN-EO= Ginger essential oil
OR-NE= Orange nano-emulsion
OR-EO= Orange essential oil
RT-qPCR= Real-time quantitative PCR

Authors' contributions

Conceptualization, Eman O. Hassan and Ibrahim A. El-Fiki; Data curation, Eman O. Hassan; and Ibrahim A. El-Fiki; Formal analysis, Eman O. Hassan, Ibrahim A. El-Fiki and Tahsin Shoala; Methodology, Eman O. Hassan, Omnia badr, Shereen A. Mohamed, Tahsin Shoala and Ibrahim A. El-Fiki; Resources, Eman O. Hassan, Ibrahim A. El-Fiki and Tahsin Shoala; Supervision, Eman O. Hassan and Ibrahim A. El-Fiki; Validation, Ibrahim A. El-Fiki, Omnia badr and Shereen A. Mohamed; Visualization, Ibrahim A. El-Fiki, Omnia badr, Shereen A. Mohamed and Tahsin Shoala; Writing – original draft, Eman O. Hassan and Ibrahim A. El-Fiki; Writing – review & editing, Eman O. Hassan, Omnia badr, Shereen A. Mohamed, Tahsin Shoala and Ibrahim A. El-Fiki.

Competing interests

The authors declare that they have no competing interests.

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