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Research Article

Effect of some plant extracts in controlling soft rot disease in some economic plants associated with molecular studies

Maryam M. ALOMRAN[1], Yaser HAFEZ[2], Rawia H. MEHANA[2], El-Sayed M. EL-KADY[2], Nadi Awad AL-HARBI[3], Khaled ABDELAAL[2*]

[1]*Princess Nourah bint Abdulrahman University, College of Science, Department of Biology, P.O. Box 84428, Riyadh 11671, Saudi Arabia; mmalomran@pnu.edu.sa*
[2]*Kafrelsheikh University, Faculty of Agriculture, Agricultural Botany Department, EPCRS Excellence Center, Plant Pathology and Biotechnology Laboratory, Kafrelsheikh, 33516, Egypt; hafezyaser@gmail.com; rawiamehannahanna@gmail.com; elsayedelkady2070@gmail.com; khaled.elhaies@gmail.com (*corresponding author)*
[3]*University of Tabuk, University College of Tayma, Biology Department, Tabuk 47512, Saudi Arabia; nalharbi@ut.edu.sa*

Abstract

Pectobacterium carotovorum strains were isolated from onion, pepper and carrot to investigate the molecular and physiological characters as well as efficiency of plant extracts, nano-particles and bioagents to control the bacterial pathogen. The results of molecular characterization revealed that *P. carotovorum* strain PB-1 (carrot), strain PB-2 (onion) and strain PB-3 (pepper). The results of phylogenetic tree displayed that the three strains were similar and characterized as *P. carotovorum,* and the genomic sequencing assured these results. Additionally, the pathogenicity test displayed that the strain PB-1 was very aggressive compared with strains PB-2 and PB-3. Antimicrobial activity using plant extracts, nanoparticles and bioagents were conducted. Application of fourteen plant extracts resulted that the plant extract of *Tamarindus indica* (fruit) showed highest diameter zone of inhibition (17.5 mm) against the *P. carotovorum* pathogen followed by *Hibiscus sabdariffa* (16 mm), *Rhus coriaria* (15.8 mm), *Punica granatum* (13.6 mm), *Citrus paradise* (11.6 mm), *Psidium guajava* (11.6 mm), *Citrus sinensis* (7.1 mm) and *Citrus limon* (6.6 mm) on carrot. Plant extract of *Punica granatum* (fruit peel) showed highest diameter zone of inhibition on *P. carotovorum* strains which isolated from onion and pepper followed with *Hibiscus sabdariffa* (14.25,13.75 mm) and *Rhus coriaria* (11.8,11.05 mm) compared with control (streptomycin 150 ppm). Nano-copper (Cu) and nano-silver (Ag) gave significant inhibition against the three strains. Nano-Cu showed 15.12, 7.6 and 14 mm inhibition however, nano-Ag showed 10.5, 10.7 and 9.75 mm inhibition on carrot, onion and pepper respectively. *Bacillus subtilis* as a biocontrol agent gave the best and significant results as antibacterial effect against *P. carotovorum* which showed 13.6 and 13 mm inhibition on carrot and onion respectively compared with *Streptomyces* spp., *Trichoderma* spp. and *Saccharomyces* spp.

Keywords: carrot; molecular characterization; onion; *Pectobacterium carotovorum*; soft rot

Introduction

Onion (*Allium cepa*) is one of the most important crops, the *Allium* genus comprises over 700 species, which can be found in 170 countries of the world (FAO, 2018). Onion contains phytochemicals such as

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polyphenols, flavonoids, organic acids, sugars, and sulfur compounds that are beneficial for human health (Vojnović *et al.,* 2023). There are three types of onion based on color; red, yellow, and white. In Egypt the onion is considered one of the greatest important economic crops, not only for local consumption but also for export. Several studies were conducted to improve the yield production of onion (Morsy *et al*., 2019; Ragab *et al*., 2019; Attaya *et al*., 2024). Onion bulbs are constantly confronted by the plant pathogens belonging to the soft rot species such as *P. carotovorum* leading to economic damage in yield (Mansour *et al*., 2011). Carrot is one of the most important vegetable crops, about 61.8% of world carrot production is realized in Asia, followed by Europe (22.6%), and the America (9.1%), while 5.4% is grown in Africa. It belongs to the family Apiaceae, the edible portion is the storage taproot, which contains high levels of carbohydrates (sugars) and a rich source of alpha and beta carotene, which also contain vitamins A, K and B6 and minerals (Char, 2017). Soft rot is a serious disease of carrots in the field that causes total loss, this disease is caused by *P. carotovorum* (Chandrashekar *et al*., 2022). Pepper (*Capsicum annuum*) is one of the common vegetables that belongs to the Solanaceae family (Dagnoko *et al*., 2013). It is very rich in vitamins C and a good source of vitamins B, potassium, magnesium and iron. Pepper is an important vegetable crop in the world, the worldwide production of pepper in 2016 was estimated at 38,415,621 tons. The top six pepper-producing countries in Africa in 2016 were Ethiopia, Tanzania, South Sudan, Kenya, Malawi and Rwanda (FAOSTAT, 2016). Bacterial disease is reported as the most serious threats in pepper production and cause significant economic losses by reducing both fruit quality and quantity (Waweru *et al*., 2019).

Pectobacterium carotovorum belongs to the genus *Pectobacterium,* which causes many diseases to the important vegetables and fruits such as carrot, potato, tomato, pepper and onion. It is a Gram-negative and common soil-borne bacterium that causes soft rot disease (Lestari *et al*., 2022). Under infection with soft rot disease, vegetables and fruits become watery, blackish and unsuitable for consumption and further sale. The synthetic bactericides are a common way to control soft rot disease but also there are no efficient bactericides used to protect vegetables against *Pectobacterium* spp. (Baz *et al*., 2012). There are few bactericides can use for controlling soft rot disease, which are mostly considered as antibiotics such as streptomycin or copper compounds, which reduce the spread of the pathogens. Application of chemical bactericides for controlling is not favoured because of their side toxic effects, high cost as well as development of resistance in bacterial populations (Roshdy *et al*., 2018). Non-traditional treatments and biological control were studied to control many plant pathogens (Abdelaal *et al*., 2020; Hafez *et al*., 2020a). Biological control may be one of the good protection methods for controlling bacterial soft rot disease by application of *Bacillus* spp. (Alabouvette *et al*., 2006). *Bacillus subtilis* is one of the antagonistic microorganisms used as a biocontrol agent against soil-borne and foliar diseases (Prihatiningsih *et al*., 2015). *Bacillus* spp. has been shown to possess antifungal activity against plant pathogens (Lee *et al*., 2013). *Bacillus* spp. as a plant growth promoting rhizobacteria plays an important role under normal conditions and in controlling plant diseases (Porcel *et al*., 2014; AlKahtani *et al*., 2021; Alkilayh *et al*., 2024). Application of nanoparticles is very important method to improve the plant growth and productivity under natural and stressful conditions (El-Shawa *et al*., 2022; Al-Shammari *et al*., 2023; Abd-El-Aty *et al*., 2024). Nanoparticles as Nano-silver and Nano-capper have physicochemical and biological properties and it has activity against both Gram-positive and Gram-negative bacteria (Gargouri *et al*., 2017). The Nps have gained significant importance due to their ability to withstand adverse processing conditions. Green synthesis of NPs using plant extracts is emerging as an attractive alternative to chemical methods from an environmental standpoint. Plant extracts consist of several biomolecules belonging to various structural classes such as alkaloids, polysaccharides, vitamins, amino acids, proteins, enzymes, tannins, phenolic acids, saponins, terpenoids, etc. (Pradeep *et al*., 2022). This research aimed to molecularly characterized *P. carotovorum* bacteria and find out new control methods against the pathogen in which safe for human health and decreasing environmental pollution, consequently decreasing the cost.

Materials and Methods

Isolation of soft rot pathogen

The bacterial soft rot strains were isolated from some vegetables which showed bacterial soft rot symptoms (were composed from some marketing locations in Egypt).

Identification and characterization

Identification was carried out using morphological properties and biochemical tests like Gram reaction, catalase test, potato soft rot test, growth in 5% NaCl test, growth at 37 °C, Glucose production test and fructose production test (Akbar *et al.*, 2015).

Pathogenicity tests

Healthy immature fruits of host plants (pepper, carrot and onion) were washed with sterile water, sterilized with ethanol 70% and dried with tissue paper, then inoculated with 50 µL of *Pectobacterium carotovorum* isolates suspensions (1×10^8 CFU ml) placed in polyethylene bags with wet papers and these bags were followed for a week at 27-30 °C. Control treatment was treated with sterile distilled water. After 5-days existence of bacterial oozes was a positive response of *P. carotovorum* in contrast to control treatment (Dadaşoğlu *et al.*, 2017).

Koch's postulates

The pathogen was isolated from diseased samples (onion, pepper and carrot) showing typical soft rot symptoms were collected from the local markets from Kafrelsheikh. Infected plant materials were rinsed with tap water to remove the soil particles from the samples and soaked 70% ethyl alcohol for 5 minutes for surface sterilization. then; samples were washed with sterile distilled water and dried with tissue paper. Subsequently, Small pieces of plant material from both infected and healthy parts were cut off. these pieces were streaked onto nutrient agar and incubated at 27 °C. The bacterial colonies that formed were purified in king's B medium. Obtained isolates were kept on 2% glycerol nutrient agar slants, 50% glycerol and lyophilized for using later as well.

Molecular characterization, DNA extraction and PCR analyses

The DNA extraction was performed from *Pectobacterium* genomes (BYF DNA extraction mini kit). The PCR analysis was performed to amplify conserved 16S rRNA genes for each *Pectobacterium* using universal primers based on 16S rRNA gene.

PCR was conducted in an Eppendorf thermal cycler. Amplification was performed in a thermocycler (Applied Bio-Rad, USA). The initial denaturation at 94 °C for 5 minutes, then the cyclic condition was 35 cycles of denaturation at 94 °C for 30sec, primer annealing at 50 °C for 1 min and extension at 72 °C for 1 min. a final extension at 72 °C was given for 10 minutes. The expected DNA fragments were 1100 base pairs in length (Adioumani *et al.* 2022).

Genomic sequencing and bioinformatic analysis

The expected PCR-band was purified using the Extraction-Gel kit and sent (20 µg) to Macrogen Inc. of Seoul, South Korea, for sequencing. Sequence results were used as search queries against 16S rRNA gene sequences of *Pectobacterium* obtained from the NCBI database using BLAST (Table 1). The sequences were aligned using ClustalW and Phylogenetic tree analysis was carried out using the Neighbor-Joining Method and PHYLIP version 3.69 (*http://evolution.genetics.washington.edu/phylip.html*) with bootstrap values from 1,000 neighbor-joining bootstrap replicates.

Table 1. Sequences of universal primer based on 16S rRNA genes

Strains	Primer name Forward-revers	Nucleotide sequences (5'-3')	Length of amplified fragment(bp)	Reference
Strain PB-1 Strain PB-2 Strain PB-3	8F 1492R	Forward- AGAGTTTGATCCTGGCTCAG Reverse- GGTTACCTTGTTACGACTT	Approximately 1500bp	(Eden *et al.*, 2011; Sean *et al.*, 1999)

Screening of plant extracts, biocontrol agents and nano-particles for pathogen inhibiting

Fourteen plants extracts were obtained from herbal markets. Two bacterial agents namely *Bacillus subtilis*, *Streptomyces* spp., and two fungal agents namely *Trichoderma* spp. and *Saccharomyces* spp. were used to evaluate its antibacterial effect against *P. carotovorum* the causal agent of soft rot bacterial disease. The isolates were obtained from EPCRS Excellence Center, Faculty of Agriculture, Kafrelsheikh University, Egypt. Nano Cu and Nano Ag as well as ZnNo3 were obtained from Soil, Water and Environment Research Institute (SWERI). Standard check streptomycin (150 ppm) was obtained from Egypt Masters Co. (EMC), Dakahlia, Egypt.

Preparation of plant extracts

Fourteen plants extracts were as follow: lemon, orange and olive oils were obtained from Karnak company, Giza, Egypt, however pomegranate fruits, sumac seeds, fig leaves, hibiscus sepals, tamarind fruits, fenugreek seeds, guava leaves, black cumin seeds, garlic bulbs, thyme leaves and grapefruit fruit peel were obtained from herbal markets (Table 2).

Table 2. Antibacterial plant extracts

Plant species	Common name	Plant part used
Punica granatum	Pomegranate	Fruit peel
Rhus coriaria	Sumac	Seeds
Ficus carica	Fig	Leaves
Hibiscus sabdariffa	Hibiscus	Sepals
Tamarindus indica	Tamarind	Fruit
Trigonella foenumgraecum	Fenugreek	Seeds
Psidium guajava	Guava	Leaves
Citrus limon	Lemon	Oil
Citrus sinensis	Orange	Oil
Nigella sativa	Black cumin	Seeds
Allium sativum	Garlic	Bulb
Olea europaea	Olive	Oil
Thymus vulgaris	Thyme	Leaves
Citrus paradise	Grapefruit	Fruit peel

The plant materials were dried and grinded into fine powder in an electrical blender, The extract was prepared by mixing powder of plants (10 g) with 100 ml of sterile distilled water. The flasks then, put in a water bath at 100 °C for 20 min. The suspensions were filtered through Whatmann filter paper and kept in sterilized flask under refrigerator conditions (Hafez *et al.*, 2022).

Preparation of the biocontrol agents

For preparation of each bacterial biocontrol agent was used sterilized nutrient glucose (2%) broth medium (peptone 5 g, beef extract 3 g, glucose 20 g, distilled water 1 L, pH 7.2) was prepared and sterilized in flasks. then Each flask was separately inoculated with (1 ml) culture of each *Bacillus subtilis*, *Streptomyces* spp.,

Trichoderma spp. and *Saccharomyces* spp. The inoculated flasks were incubated at 28 °C for 48 h. then was used to evaluate its antibacterial effect against *P. carotovorum* the causal agent of soft rot bacterial disease using agar well diffusion method (Abd-El-Khair *et al.*, 2021).

Preparation of the nano-articles

Nano-Cu were prepared in 70 ppm however, nano-Ag and nano-Zn were prepared in 60 ppm.

In vitro screening of antibacterial activity of plant extracts, bio agent and nano-particles

King′s B medium was inoculated with 24 hrs broth of *P. carotovorum* (10^8cfu ml^{-1}) poured into sterilized Petri-plates (10 cm) and was allowed to solidify then, a hole with a diameter of 10 mm is punched aseptically with a sterile Cork borer and filled with 100µl of plant extracts, 24 hrs broth of bioagents isolates and nano-particles. Four replications were made for each treatment. Control treatment was made by sterile water as negative control and antibiotic (Streptomycin 150 ppm) as positive control. Inoculated plates were incubated at 28 C˚ for 48 hrs and the inhibition zone diameter was measured in mm (Viswanath *et al.*, 2018).

Statistical analysis

Data represent the mean ± SD obtained from four replicates of each treatment. Student's t-test was used to determine whether significant difference ($P < 0.05$) existed between mean values according to O'Mahony (1986).

Results

Identification and Characterization

The results of the investigated morphological characters of *Pectobacterium. carotovorum* are given in (Table 3). The bacterium is a rod shaped facultatively anaerobic, gram negative and peritrichously flagellated. Obtained isolates PB-1, PB-2 and PB-3 of *P. carotovorum* subsp. *carotovorum* which obtained from different hosts namely, carrot, onion and pepper, respectively.

Table 3. Characterization of *P. carotovorum* subsp. *carotovorum* isolates obtained from different hosts

Isolate	Gram reaction	Catalase test	Potato soft rot test	Growth in 5% NaCl test	Growth at 37 °C	Glucose test	Fructose test
Isolate PB-1 (carrot)	-	++	+	+	+	+	+
Isolate PB-2 (onion)	-	+	-	+	+	+	++
Isolate PB-3 (pepper)	-	+	-	+	+	++	+

(+): positive reaction, (-): negative reaction, (++): strongly positive reaction

Figure 1. Pathogenicity test of *Pectobacterium carotovorum* (50 µL) on different hosts (carrot, pepper and onion) than were followed for a week at 27-30 °C. After 5-days existence of bacterial oozes was a positive response of *P. carotovorum* in contrast to control treatment

Molecular characterization of P. carotovorum

The bacterial pathogen which causes soft rot was isolated, purified, and Koch's postulated were applied, then DNA from the pathogen was isolated and PCR technique was conducted using universal primer of 16S rRNA gene (Table. 1). The results proved that the pathogen is: *Pectobacterium carotovorum* based on PCR results (Figure 2).

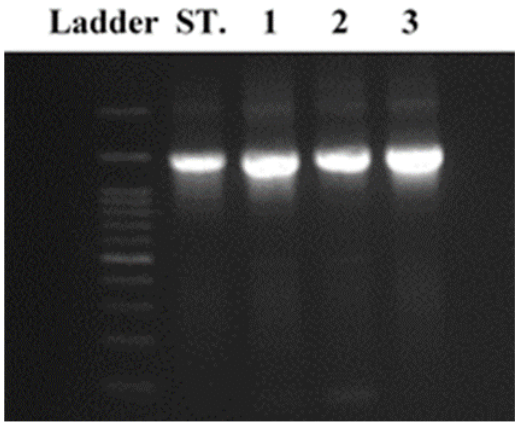

Figure 2. PCR Performing of *P. carotovorum* bacterial strains using 16S rRNA gene's universal primer Ladder: Marker. ST: standard isolate of *P. carotovorum* (1) *P. carotovorum* strain which isolated from carrot. (2) *P. carotovorum* strain which isolated from onion. (3) *P. carotovorum* strain which isolated from pepper sample

Sequences analysis

The Sequence results were used as queries for searching on GenBank database against 16S rRNA genes from different *Pectobacterium* spp. Results of comparative sequences analysis of 16S rRNA genes showed high similarity among the three strains (Figure 3).

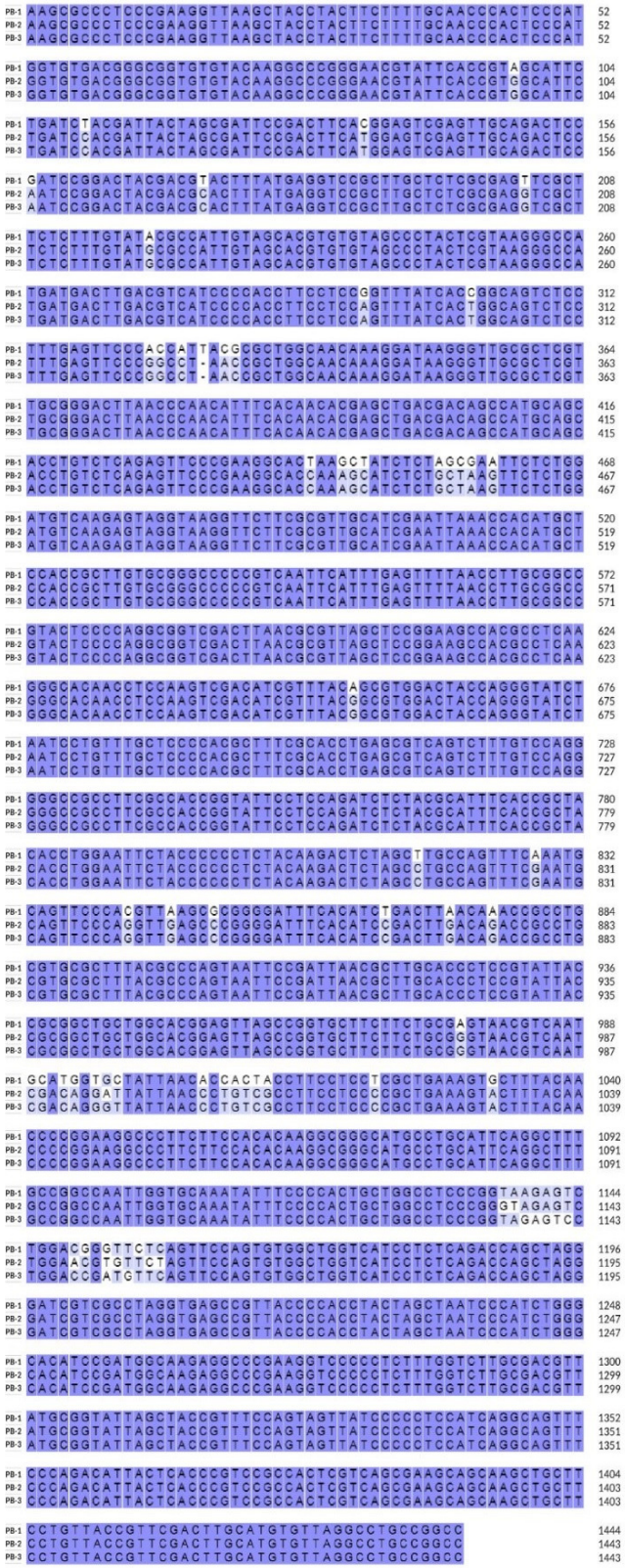

Figure 3. Comparative analysis among 16S rRNA genes' sequences from the new isolates

For phylogenetic analyses, was carried out using the Neighbor-Joining PHYLIP version 3.69 Method with 1000 bootstrap replicates. The results showed the cluster analysis of 16S rRNA genes of the new isolated against 16S rRNA genes of *Pectobacterium* spp. from NBCI GenBank database (S1-supplmantry file) and revealed that the new strains belonged to *Pectobacterium carotovoru m* (Figure 4). In addition, the percentage identity analysis among the PB samples and between the samples and database sequences showed high similarity (Figure 5).

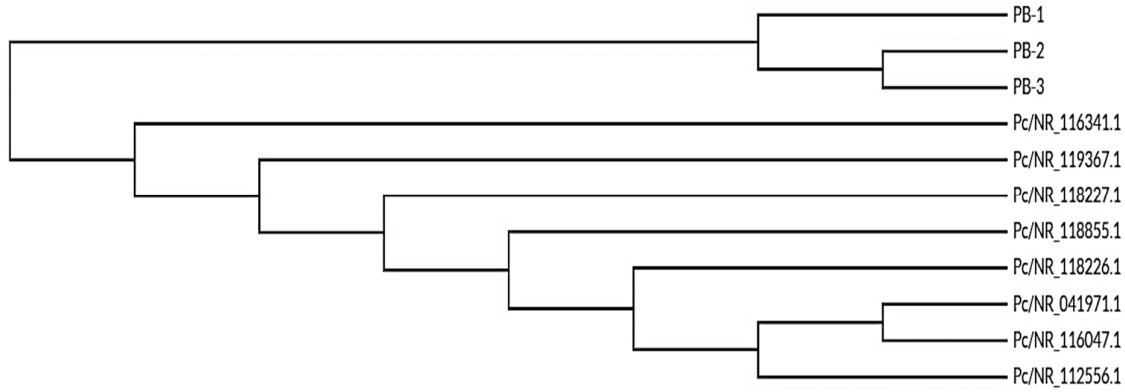

Figure 4. Phylogenetic tree results of PB strains sequences vs 16S rRNA relatives in databases Tree *P. carotovorum* subsp. *carotovorum*

PB-1	100.00%	96.05%	96.12%	43.47%	44.01%	44.05%	44.02%	44.13%	44.06%	44.34%	44.75%
PB-2	96.05%	100.00%	99.03%	41.99%	42.88%	42.92%	42.87%	43.00%	42.92%	43.09%	43.21%
PB-3	96.12%	99.03%	100.00%	42.46%	43.27%	43.31%	43.26%	43.39%	43.31%	43.52%	43.74%
Pc/NR_116341.1	43.47%	41.99%	42.46%	100.00%	94.44%	94.69%	94.60%	94.60%	94.60%	94.60%	94.31%
Pc/NR_119367.1	44.01%	42.88%	43.27%	94.44%	100.00%	99.73%	99.59%	99.73%	99.66%	99.70%	99.73%
Pc/NR_118227.1	44.05%	42.92%	43.31%	94.69%	99.73%	100.00%	99.79%	99.93%	99.87%	99.93%	99.91%
Pc/NR_118855.1	44.02%	42.87%	43.26%	94.60%	99.59%	99.79%	100.00%	99.86%	99.86%	100.00%	100.00%
Pc/NR_118226.1	44.13%	43.00%	43.39%	94.60%	99.73%	99.93%	99.86%	100.00%	99.93%	100.00%	100.00%
Pc/NR_041971.1	44.06%	42.92%	43.31%	94.60%	99.66%	99.87%	99.86%	99.93%	100.00%	100.00%	100.00%
Pc/NR_116047.1	44.34%	43.09%	43.52%	94.60%	99.70%	99.93%	100.00%	100.00%	100.00%	100.00%	100.00%
Pc/NR_112556.1	44.75%	43.21%	43.74%	94.31%	99.73%	99.91%	100.00%	100.00%	100.00%	100.00%	100.00%

Figure 5. Percent identity matrix

Effectiveness of antimicrobial agents and compounds against P. carotovorum inhibition (in vitro)

Most of plant extracts were effective to inhibit the *P. carotovorum*. Eight plant extracts from 14 extracts were effective enough (Table 4).

Table 4. Inhibitory activity of antimicrobial compounds against the bacterial growth of *P. carotovorum*.

Treatments	Zones of growth inhibition (mm)		
	Isolate 1 (carrot)	Isolate 2 (onion)	Isolate 3 (pepper)
Control	00	00	00
Antibiotic(streptomycin)	25.3	32.6	22.5
Plant extracts			
Tamarindus indica	17.5	00	00
Hibiscus sabdariffa	16	14.25	13.75
Rhus coriaria	15.8	11.8	11.05
Citrus paradise	11,6	00	00
Psidium guajava	11.62	00	11.75
Punica granatum	13.6	18.6	17
Citrus sinensis	7.1	00	00
Citrus Limon	6.6	00	00
Ficus carica	00	00	00
Trigonella foenumgraecum	00	00	00
Nigella sativa	00	00	00
Allium sativum	00	00	00
Olea europaea	00	00	00
Thymus vulgaris	00	00	00
Bio-agents			
B. subtilis	13.6	13	00
Streptomyces spp.	00	00	00
Trichoderma spp.	00	00	00
Saccharomyces spp.	00	00	00
Nano and chemical compounds			
Nano cu	15.12	7.6	14
$AgNo_3$	10.5	10.7	9.75
$ZnNo_3$	00	00	00

Tamarindus indica and *Citrus paradise* extracts showed 17.5 and 11.6 mm inhibition against the bacterial isolated from carrot (Figs. 6 and 7). *Psidium guajava* inhibit the bacterial growth of 2 tested strains except strain which isolated from onion (Figs. 6, 7, 10 and 11). However, *Hibiscus sabdariffa*, *Rhus coriaria* and *Punica granatum* showed strong inhibition against all three isolates (Figs. 6-11). *Citrus sinensis* and *Citrus limon* showed inhinition 7.1 and 6.6 mm inhibition only on bacterial isolated from carrot (Figs. 6 and 7).

As regards *B. subtilis* inhibited the bacterial growth of all tested *P. carotovorum* isolates except strain which isolated from pepper (Figs. 6, 7, 8 and 9). On the other hand, *Streptomyces* spp., *Trichoderma* spp. and *Saccharomyces* spp. had no noticeable effect on *P. carotovorum* strains in plates of well diffusion method. Nano copper and nano Ag were highly effective to suppress the bacterial growth of *P. carotovorum*. of all tested isolates (Figs. 6-11). But nano Zn did not show in effect of all isolates (Table 4). Moreover, the antibiotic streptomycin has high effects on bacterial growth compared to control (Figs. 6-11).

Among the plant extracts tested on *P. carotovorum* strain which isolated from carrot, the extract of *Tamarindus indica* (fruit) showed the highest diameter zone of inhibition followed by *Hibiscus sabdariffa* (sepals) and other extracts as follow: *Rhus coriaria* (seeds), *Punica granatum* (fruit peel), *Citrus paradise* (fruit peel), *Psidium guajava* (leaves), *Citrus sinensis* (oil) and *Citrus limon* (oil) (Figures 6 and 7).

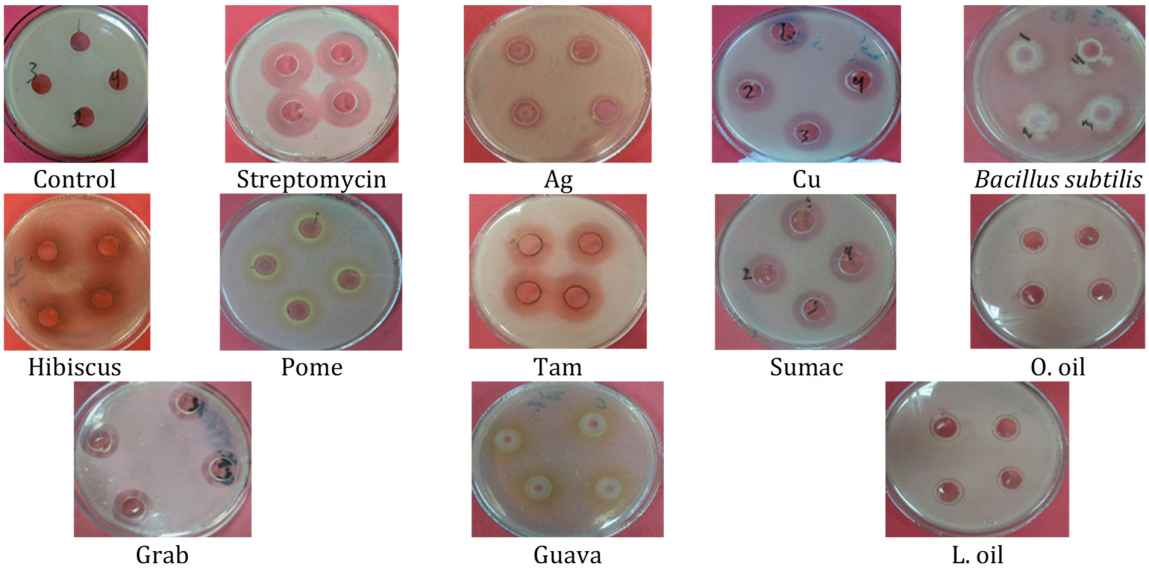

Figure 6. Effect of plant extracts, bioagents and nano particles on the growth of *P. carotovorum* isolated from carrot *in vitro* by well diffusion method

Control: inoculated with *P. carotovorum* only. Streptomycin: inoculated and treated with streptomycin. Nano Ag: inoculated and treated with Nano $AgNo_3$. Nano cu: inoculated and treated with Nano cu. *Bacillus subtilis*: inoculated and treated with *B. subtilis*. Hibiscus: inoculated and treated with Hibiscus extract. Pome: inoculated and treated with pomegranate peel extract. Tamarind: inoculated and treated with Tamarind extract. Sumac: inoculated and treated with sumac extract. Orange oil: inoculated and treated with orange oil. Grapefruit: inoculated and treated with Grapefruit extract. Guava: inoculated and treated with Guava extract. Lemon oil: inoculated and treated with Lemon oil

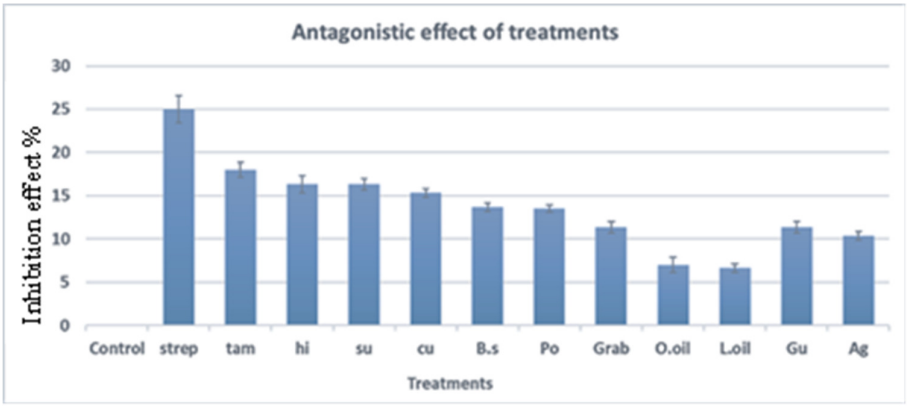

Figure 7. Inhibition effect of treatments against *P. carotovorum* isolated from carrot *in vitro* (the bars represent SD)

Control: inoculated media with *P. carotovorum* only. Strep: inoculated and treated with streptomycin. Tam: inoculated and treated with Tamarind extract. hi: inoculated and treated with *Hibiscus* extract. Su: inoculated and treated with sumac extract. Cu: inoculated and treated with Nano cu. B.S: inoculated and treated with *Bacillus subtilis*. Po: inoculated and treated with pomegranate peel extract. Grab: inoculated and treated with Grapefruit extract. O. oil: inoculated and treated with orange oil. Loil: inoculated and treated with Lemon oil. Gu: inoculated and treated with Guava extract. Ag: inoculated and treated with Nano $AgNo_3$

Also, application of plant extracts in controlling *P. carotovorum* strain which isolated from onion showed that the extract of *Punica granatum* (fruit peel) gave the highest diameter zone of inhibition followed by *Hibiscus sabdariffa* (Sepals), and *Rhus coriaria* (seeds) (Figures 8 and 9). The plant extracts tested on *P. carotovorum* strain which isolated from pepper showed that the extract of *Punica granatum* (fruit peel), *Hibiscus sabdariffa* (sepals), and *Rhus coriaria*.

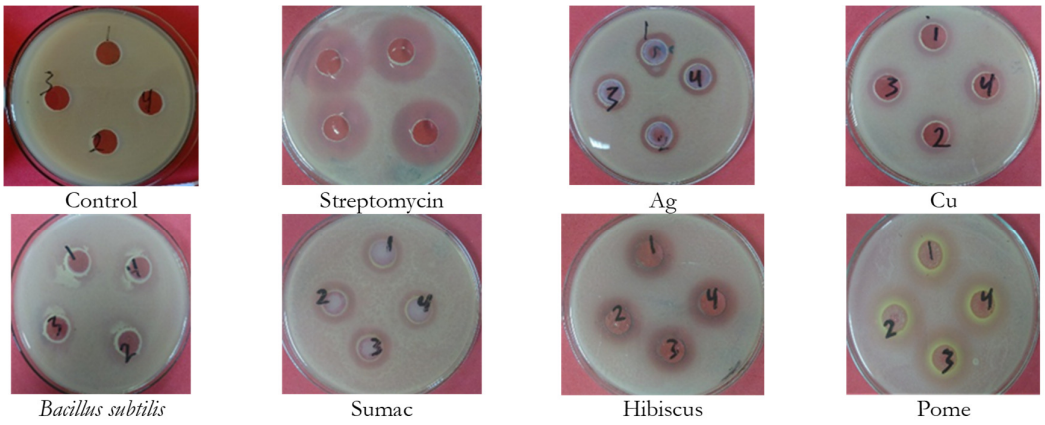

Figure 8. Effect of plant extracts, bioagents and nano particles on the growth of *P. carotovorum* isolated from onion *in vitro* by well diffusion method

Control: inoculated media with *P. carotovorum* only. Streptomycin: inoculated and treated with streptomycin. Ag: inoculated and treated with Nano $AgNo_3$. Cu: inoculated and treated with Nano cu. *Bacillus* subtilis: inoculated and treated with *B. subtilis*. Sumac: inoculated and treated with sumac extract. *Hibiscus*: inoculated and treated with *Hibiscus* extract. Pome: inoculated and treated with pomegranate peel extract

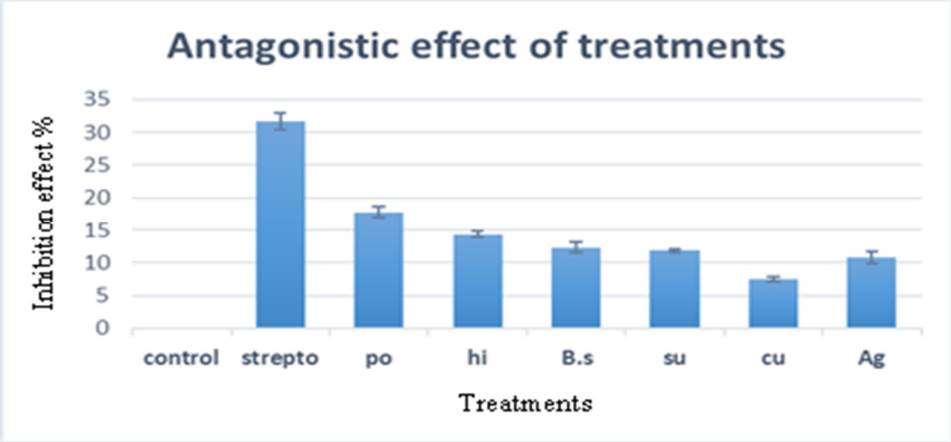

Figure 9. Inhibition effect of treatments against *P. carotovorum* which isolated from onion *in vitro* by well diffusion method. (The bars represent SD)

Control: inoculated media with *P. carotovorum* only. Strepto: inoculated and treated with streptomycin. Po: inoculated and treated with pomegranate peel extract. hi: inoculated and treated with Hibiscus extract. B.S: inoculated and treated with *Bacillus subtilis*. su: inoculated and treated with sumac extract. cu: inoculated and treated with Nano cu. Ag: inoculated and treated with Nano $AgNo_3$

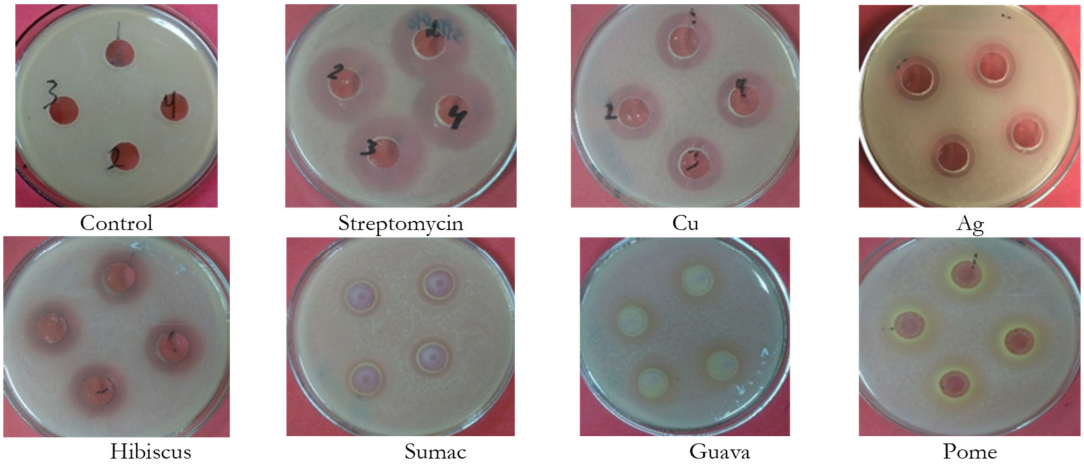

Figure 10. Effect of plant extracts and nano particles on the growth of *P. carotovorum* isolated from pepper *in vitro* by well diffusion method

Control: inoculated media with *P. carotovorum* only. Streptomycin: inoculated and treated with streptomycin. Cu: inoculated and treated with Nano cu. Ag: inoculated and treated with Nano $AgNo_3$. Hibiscus: inoculated and treated with Hibiscus extract. Sumac: inoculated and treated with sumac extract. Guava: inoculated and treated with Guava extract. Pome: inoculated and treated with pomegranate peel extract

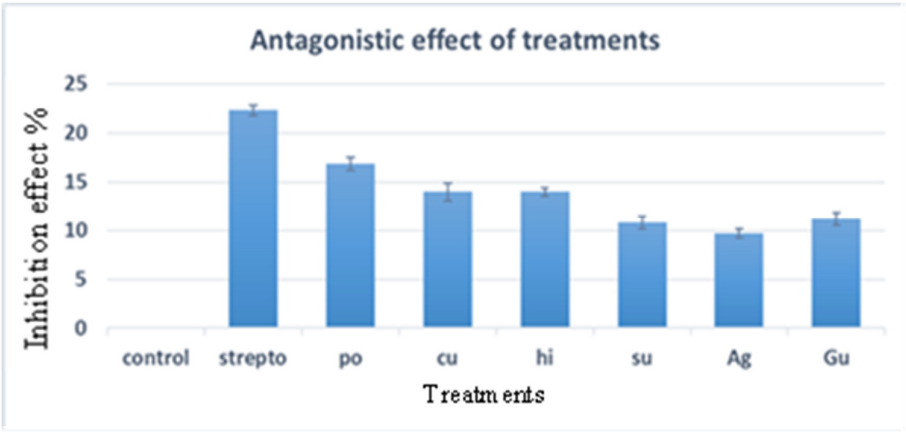

Figure 11. Inhibition effect of treatments against *P. carotovorum* isolated from pepper *in vitro* by well diffusion method

Control: inoculated media with *P. carotovorum* only. strepto: inoculated and treated with streptomycin. po: inoculated and treated with pomegranate peel extract. cu: inoculated and treated with Nano cu. hi: inoculated and treated with *Hibiscus* extract. su: inoculated and treated with sumac extract. Ag: inoculated and treated with Nano $AgNo_3$. Gu: inoculated and treated with Guava extract. (The bars represent SD)

Discussion

The bacterial soft rot disease caused by *P. carotovorum*, causes severe losses in a lot of vegetables such as pepper, carrot and onion in fields and storages. Using the chemical bactericides for controlling bacterial soft rot is a common practice as well as they cause the potential health damage to human, animals and environment. Application of biological control agents and antimicrobial compounds may replace the chemicals for more safety (Abd-El-Khair *et al.*, 2012). The results in this research prove that some plant extracts were effective enough to inhibit the *P. carotovorum.* Actually, the Plant-derived compounds from plant parts such as root,

leaves and seeds acquired attention in the treatment against plant pathogenic Gram-negative as *P. carotovorum*. The mechanism of action based on plant phytochemicals which can be divided into alkaloids, polyphenols, terpenoids and polysaccharides that have biological actions against many pathogens (Jubair *et al.*, 2021). Fourati *et al.* (2019) reported that the plant extract of pomegranate peel produces biological compounds, phytochemical contents (TPC, TFC, and TAC) and inhibit the growth of Gram-negative bacteria. Rayen and Mazza (2007) reported that extract of *R. coriaria* fruits had a good effect against Gram negative bacteria because of the presence of natural antioxidants. Hafez *et al.* (2021) reported that *H. sabdariffa* contains phenolic acids, flavonoids, and polysaccharides that have biological effects against many pathogens. Our results *in vitro* showed *B. subtilis* had variable antagonistic effects against *P. carotovorum*, *Bacillus* sp. showing AHL-lactonase activity, provided significant preventive and curative biocontrol against the soft rot caused by *P. carotovorum*. *Bacillus* sp. has many different mechanisms as biocontrol agents of plant diseases including antagonism, competition and increased the growth parameters as well as increased defense-related enzymes such as (lipopeptides, polyketides and bacteriocins) that play important roles in plant disease control. Shkryl *et al.*, (2017) reported that synthesized AgNPs had the stronger antagonistic activity against plant pathogenic Gram-negative as *P. carotovorum*. The mechanism of the AgNPs effect is not known precisely, but it is currently known that the first step in its effect attach to the surface of the bacteria causing the formation of irregularly shaped pits, disruption in membrane permeability and penetrate the bacteria and interact with phosphorus-containing elements like DNA, Sulphur-containing proteins and disulphide bonds of enzymes. The mode of action of CuNPs is not yet completely understood, but one of the most main theories is that CuNPs damage the cellular membrane by changing its permeability, therefore causing cellular death (Rai *et al.*, 2018; Varympopi *et al.*, 2020). Also, Yadav *et al.* (2017) reported different mechanisms of Nano copper to cope with the oxidative damage, lipid peroxidation and DNA degradation by the overproduction of reactive oxygen species (ROS). Antibiotic function by either killing or stopping bacterial growth such as Streptomycin is common antibiotic with activity against a lot of bacteria. The streptomycin antibiotic functions as an inhibitor of protein synthesis and binds within the ribosome to four nucleotides of the 16S RNA and the ribosomal protein (Hafez *et al.*, 2022).

Recent work has confirmed that a variety of botanical extracts can suppress *P. carotovorum*. For example, a green tea (Camellia sinensis)–based coating (CMC+GTE) completely killed the pathogen *in vitro* and greatly reduced potato tuber maceration (coated tubers remained firm for days and showed no loss of efficacy over 8 weeks). Likewise, Elhalag *et al.*, (2025) showed that ethanolic peel extracts of *Punica granatum* (pomegranate) completely prevented soft rot on potato tubers (100% treatment efficiency, with no disease observed for 14 days). Essential oils also exhibit strong activity: Jílkova *et al.*, (2025) found that oils from cinnamon, mint, oregano, thyme, etc., each produced large inhibition zones and significantly reduced tuber rot *in vivo*. Víchová *et al.* (2024) showed that individual EO components such as cinnamaldehyde, carvacrol and menthone had very low MICs (≤0.5-10 µL/mL) and nearly abolished soft rot on potato discs. These effects are attributed to phenolic compounds in the extracts (e.g. thymol, carvacrol, catechins) which disrupt bacterial membranes and proteins, consistent with the rapid kill rates observed. *Bacillus* biocontrol agents. Multiple recent studies report that *Bacillus* spp. strongly antagonize *P. carotovorum*. In one storage trial, *B. pumilus* applied before inoculation completely prevented soft rot on potato tubers, whereas untreated controls lost about 80-85% of tubers to disease. Similarly, native potato rhizosphere isolates of *Bacillus* yielded broad inhibition zones (up to 26 mm), and tuber inoculation tests showed disease severities as low as about 7% with a *B. velezensis* strain (versus about 70-80% in controls). Maung *et al.* (2022) found that *B. velezensis* CE100 produced a secreted antibiotic (identified as macrolactin A) and that culture filtrate (50% v/v) completely eradicated *P. carotovorum* cells within 4 h. *In vivo*, CE100 significantly controlled soft rot on cucumber. These data confirm that *Bacillus* biocontrol (notably *B. velezensis*, *B. pumilus*, and related species) can protect tubers by producing antibiotics and lytic enzymes that inhibit or kill *P. carotovorum*. Metal nanoparticles. Silver and copper nanoparticles (NPs) have emerged as effective anti-soft-rot agents. For example, oak-extract–synthesized AgNPs showed clear antibacterial zones (about8 mm) against *P. carotovorum* and an MIC/MBC

around 150–200 µg/mL. In storage simulations, these AgNPs cut soft-rot incidence dramatically: treated potato tubers had only about 22% rot (versus nearly 100% in controls), and inoculation after AgNP "curative" treatment still gave about 74% disease reduction. Likewise, green-synthesized AgNPs and CuNPs from citrus peel extract were "quite effective against soft rot pathogens" – they prevented rot on potato slices and other vegetables both *in vitro* and *in vivo*. Egusa *et al.* (2023) prepared copper-NP–chitin composites (CuNPs/CNF) that exhibited "strong antimicrobial activity" against *P. carotovorum* and substantially reduced soft-rot on cabbage (surpassing a chitin-only or Bordeaux treatment). The mode of action for metal NPs is thought to involve release of Ag^+ or Cu^2+ ions, which bind bacterial proteins and membranes (through thiol groups), causing oxidative damage, leakage of contents, and cell death.

Conclusion

The results concluded that the isolate *P. carotovorum* race PB-1 (carrot), was very aggressive compared with isolates race PB-2 (onion) and race PB-3 (pepper). Application of plant extracts, nano particles and bioagents were effective enough as antimicrobial agents and compounds. The extract of *Tamarindus indica* gave the highest inhibition against *P. carotovorum* on carrot. Also, the plant extract of *Punica granatum* gave the highest inhibition on pathogen strains which isolated from onion and pepper compared with control (streptomycin 150 ppm). Furthermore, *B. subtilis* as a bio-agent was effective as antibacterial against *P. carotovorum* compared with *Trichoderma* spp., *Streptomyces* spp., and *Saccharomyces* spp. Nano silver (Ag) and nano copper (Cu) gave a significant inhibition against the three strains. These alternative agents and compounds could be recommended for *P. carotovorum* control.

Authors' Contributions

Y.H., Kh.A. Methodology, visualization, analysis, conceptualization, resources, methodology, data curation, writing-original draft preparation, writing-review and editing. Y.H., Kh.A., R.M., E.E., N.A. methodology, investigation, software, resources, data curation. M.Al.: writing—review and editing, funding acquisition. writing-original draft preparation, writing-review and editing.

All authors read, revised, and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.The authors declare that there are no conflicts of interest related to this article.

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