Asian Journal of Agricultural and Horticultural Research

7(4): 14-29, 2020; Article no.AJAHR.64302
ISSN: 2581-4478

Pomegranate (*Punica granata* L.) Inner Decay Caused by *Gluconobacter oxydans* Bacterium

Hanaa A. H. Armanious[1*]

[1]*Department of Plant Pathology, Faculty of Agriculture, Minia University, Egypt.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/AJAHR/2020/v7i430101
Editor(s):
(1) Dr. Magdaléna Valšíková, Slovak University of Agriculture (SUA), Slovakia.
Reviewers:
(1) Soumyadev Sarkar, Kansas State University, USA.
(2) Tariq Ahmad Lone, Government Degree College Beerwah, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64302

Original Research Article

Received 28 November 2020
Accepted 03 January 2021
Published 27 January 2021

ABSTRACT

During the autumn of 2018, inner fruit decay symptoms were observed in pomegranate fruits collected from markets in different localities and farms from Giza, Minia and Assuit Governorates, Egypt. Similar symptoms were observed in each location. The symptoms appeared as creamy bright growth of bacteria in the mesocarp layer, decayed both arils and seeds. Bacteria were isolated from these decayed fruits. The pathogenicity test for isolated bacteria was done. Also, the expressed symptom was compared with the original observed symptoms as followed in Koch postulates. Based on morphological characteristics, analysis of 16S rDNA Genes sequences, and pathogenicity test on pomegranate fruits, the causal agent was identified as *Gluconobacter oxydans*. Possible control attempts were implemented included applying of essential oils. The results revealed that essential oils of Marjoram, followed by Chamomile expressed the most effective against infection with the bacterium when compared with the control.

Keywords: Gluconobacter oxydans; host range; pomegranate; Punica granatum; essential volatile oils.

1. INTRODUCTION

Pomegranate (*Punica granatum* L., belong to family *Punicacae* [1]) is one of the important fruit crops which are cultivated in both arid and semiarid regions around the world; however is gaining lot of attention of total world over due to its high nutrients and economic values [2,3].

**Corresponding author: Email: hanaarmanious@gmail.com;*

Recently, there is an increase in the cultivation area of pomegranate in Egypt. Pomegranate is used in several medical purposes as diarrhea, ulcers [4], diabetes [5], male infertility [6] and antiparasitic agent [7]. Pomegranate fruit production has become limited due to many fungal pathogens that attack's in many several areas in the world. Boulos et al. [8] found that *Cercospora punicae* caused pomegranate leaf and fruit spots in Egypt. In Florida; USA, very aggressive six fungal pathogens on pomegranate fruits and leaves, causing foliar spotting and blighting, and fruit rot, were isolated. *Neofusicoccum parvum* and *Lasiodiplodia* sp., two species of *Colletotrichum*, *Pilidiella granati*; the fruit rot-causing fungus, and a fungus belonging to Order: *Diaporthales* [9].

Fruits of pomegranate are subject to infect with several biotic and abiotic diseases. *Alternaria alternata, Coniella granati*, *Aspergillus niger, Rhizopus stolonifer* and *Botrytis* spp. were reported as the major biotic agents whereas fruit cracks, sunburn and hail damage were the most commonly detected abiotic diseases in Turkey and Palestine [10,11]. *Penicillium implicatum* was found to be the causal of fruit rot of stored pomegranate [12]. *Penicillium expansum*, *P. sclerotiorum*, *P. glabrum* and *P. minioluteum* and *Pilidiella granati* were reported as mold pathogens of pomegranate (cv. Mollarde Elche) in Spain [13] and in Mexico [14]. Allam et al. [15] in Egypt isolated *Botrytis cinerea* from gray molded pomegranate fruits. *Alternaria alternata* was isolated from fruits (Wonderful cv.) infected with heart rot (black rot), whereas species of *Alternaria*, *Aspergillus*, *Botrytis*, *Penicillium* and *Rhizopus* were isolated from soft rotted pomegranate fruits in southern Italy [16]. Sherkhane *et al.* [17] reported that *Xanthomonas axonopodis* pv. *punicae,* the causal organism of bacterial blight of pomegranate in India, infect leaves, stems and reduce crop yield to 60 - 80%, while bacterial knot disease caused by *Pseudomonas savastanoi* pv. *savastanoi* on pomegranate trees isolated in Turkey by Bozkurt et al. [18].

Gluconobacter oxydans was the prominent suspected bacterial species. *Gluconobacter* strains bright in flowers and fruits, e.g. ripe grapes [19,20,21]; apples and dates, [20]. *Gluconobacter* strains also found in soil of the gardens, baker's soil, honeybees, fruits, cider, beer and wine, and juice of sugarcane [22-24], also tomato products, juices and nectars [25]. This bacterium species is featuring by regard to its nutritional requirements and optimal growth conditions [26], and is classified in distinct family: *Acetobacteriaceae* (as a member of the alpha-proteobacteria). No bacterial members of the family *Acetobacteriaceae* are known to be plant pathogenic, thus *G. oxydans* has been previously reported as specific plant pathogenic agent. Rohrbach and Pfeiffer [27], Kontaxis and Hayward [28] and Sherkhane [26] reported that *G. oxydans* is the causal agent of pink disease in pineapple. *Gluconobacter oxydans* strains are capable to induce apple and pear rots accompanied by various shades of browning [23]. *Acetobacter* and *Gluconobacter* were prevalent bacteria with gray mold and soft rot of postharvest diseases of tomato [29]. Essential oils (Eos) are a set of the most important natural products from medicinal and aromatic plants, due to their various biological, their medicinal and nutritional usages. In recent years, researchers of postharvest diseases used some essential oils as alternatives anti-pathogen agents to chemical applications. Most natural essential oils and their single constituents have been reported to inhibit the postharvest pathogens either *in vitro* or *in vivo* [29].

The aim of this study is to characterize and identify the decay bacterial pathogen of pomegranate fruits in Egypt, and to found a technique for its control. Studies included effect of different essential oils on growth and disease severity as a healthy and cheep alternative chemicals as well as include the host range of the organism.

2. MATERIALS AND METHODS

2.1 Samples

Mature apparently healthy sound fruits of pomegranate (*Punica granata,* cv. wonderful) were collected from commercial local markets in Minia, Giza and from pomegranate private orchards in Assuit and Minia Governorates, Egypt, in autumn 2018-2020. The surface sterilized by soaking in 3% sodium hypochlorite (NaOCl) for 3 minutes and followed by washing in several changes of distilled sterile water, fruits were cut using a sterile scalpel into two halves to show if there are healthy or decayed. Naturally inner decayed fruits (Fig. 1) were used to isolate the associate pathogen(s).

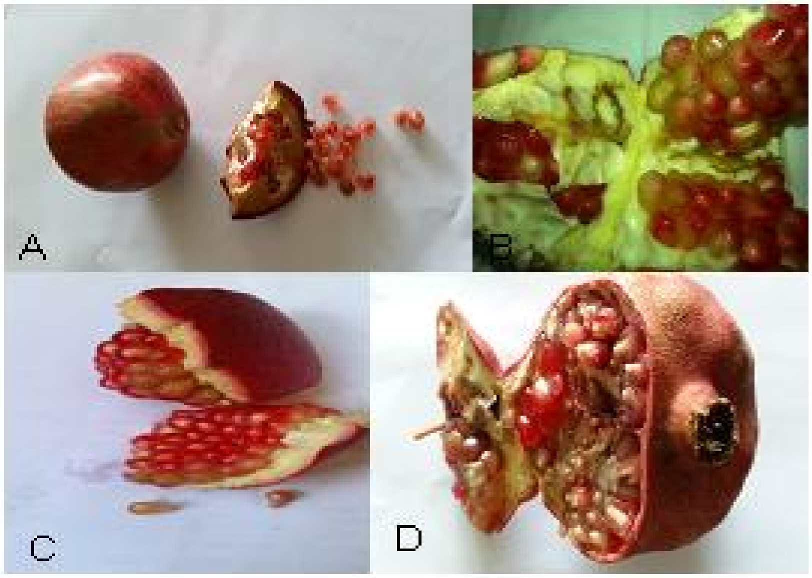

Fig. 1. (A, B, and C) natural infection at autumn 2018, 2019 and 2020, respectively, (D) Artificial infection

2.2 Isolation of the Pathogen(s)

Two methods were applied to isolate the pathogen, i) a loop of the bacterial growth grown on the inner tissues of the fruit was striated on nutrient glucose agar (NGA) medium, ii) Twenty-five gram of each collected sample was weighed in sterile conditions and homogenized in sterile saline water using pestle and mortar for five minutes. The samples were collected in sterile tubes and stored at -20°C for further use [30]. One ml of each sample was serially tenfold diluted in sterile water up to 10^{-5} dilution. The amount of 0.1 ml at 10^{-5} dilution was spread over Nutrient agar media (NA) using sterile spreaders. The plates were incubated at 30°C for 12-24 hours for the appearance of bacterial colonies. The pure bacterial colonies obtained were primary identified using morphological analysis. Pure cultures of isolated bacteria were maintained on GYC slants (glucose 5%, yeast extract 1%, $CaCO_3$ 3%, agar 1.5%, pH 6.3) at 4°C for further analysis [31].

Three isolates of rod-shaped creamy-white bacteria were secured from three different pomegranate fruits showing typical symptoms. They were designated PB1, PB2 and PB3 originated from decayed fruit mesophyll.

2.3 Inoculation with the Pathogen

The inoculum was prepared for inoculation from 48 hours old cultures on nutrient glucose agar medium (NGA) suspended in distilled sterilized water, the titer was through up $2x10^6$ cell ml^{-1}. Healthy apparent fruits were inoculated with sterile distilled water and served as control. Three methods of inoculating pomegranate fruits were compared, one by dipping them in an aqueous suspension of the tested bacteria, the other by inserting the end of a sterilized wooden toothpick charged with undiluted bacteria into the healthy fruits [32] and the third method was carried by placing a droplet of the bacterial suspension on the blossom end and then piercing the fruit repeatedly through the inoculum with the sterile needle.

2.3.1 Disease assessment

The disease severity percent **(DS %)** was determined using the following formula

Disease severity (DS %) = Weight (g) of the diseased area/ Weight of integrated fruit x 100

Re-isolation was made from inoculated fruits of pomegranate. A second inoculation was performed with the isolated bacteria to confirm pathogenicity.

2.4 Identification of Bacteria

The cultural, morphological and physiological characters (Listed in Table 1) of the three isolates under investigation were determined according to the methods described by [33], The culture characters were studied using Nutrient Glucose-sodium Carbonate agar; NGCA [23], nutrient glucose (1%) agar; NGA, nutrient sucrose (5%). Pigmentation was studied separately using NGA and potato slices. Biochemical tests (dextrose, maltose, lactose, sucrose, manitol, methyl red, Voges–Proskauer, H_2S and indole productions) were performed to identify the bacteria. The Bacterial presumptive identification was confirmed with 16S rDNA , sequence analysis [34] by methods of BioTech Research Lab, Sigma Scientific Service Technical Support Co. Cooperation with the Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485 (www. HardyDiagnostics.com).

2.5 Reaction of Pomegranate Varieties and Susceptibility of Different Fruits to Infection with *Gluconobacter oxydans*

The most pathogenic isolate (GP1) was tested. The reaction of 13 plant species, apple, pear, plum, peach, orange, lemon, mango, guava, grape, tomato, kaka, strawberry and cherry, belonging to 8 different families, were tested. Fruits of tested plants were inoculated using the methods described by [32]. Sets of 2 inoculated fruits were kept in plastic containers (25x7x7 cm), each replicated three times, at 30°C and observed daily to record the symptoms development up to 7 days. The weight of decayed areas was recorded. Sets of different fruits were inoculated with distilled sterilized water, were used as control.

2.6 Effect of Essential Oils of Some Ornamental Plants on Bacterial Growth and Pomegranate Decay Control

2.6.1 On bacterial growth

The inhibitory effect of the essential oils on the growth of *G. oxydans* was evaluated by *in vitro* assay. The essential oils of Chamomile (*Matricaria chamomilla)*, marjoram *(Origanum majorana)*, Rosemary (*Salvia rosmarinus*)*,* and thyme (*Thymus vulgaris)* were assessed at concentrations of 0.1, 0.25, 0.5, 1.0, 5 and 10% in 1.0% powdered milk. Powdered milk was added as an emulsifier agent for the oil-based substances [35]. The essential oils were added separately to a previously autoclaved aqueous solution (1.0%) of powdered milk. Control treatments with tetracycline sulfate 500 ppm, Copper sulfate 2.0 mg mL^{-1} (a copper fungicide), 1.0% powdered milk, and sterilized water was also evaluated. The experimental design was a completely randomized block, with three replicates (Petri dishes). Previously autoclaved filter paper disks (5.0 mm in diameter) were soaked in 20 µL of each treatment, dried at room temperature, and spread in Petri dishes with NGA medium containing 100 µL of the *G. oxydans* suspension ($2x10^6$ CFU mL^{-1}). The presence and the diameter of inhibition zones around the disks were measured, 48 hours of incubation at 30°C [36].

2.6.2 On disease severity

The essential oils of chamomile (*Matricaria chamomilla)*, marjoram *(Origanum majorana)*, rosemary (*Salvia rosmarinus*)*,* and thyme (*Thymus vulgaris)* were assessed at concentrations of 0.1 , 0.5 and 0.25 % in 1.0% powdered milk which was added as an emulsifier agent for the oil-based substances [35]. Mature and healthy pomegranate fruits cultivar wonderful were selected and washed by tap water then air drying at room temperature (20-25°C), fruits were surface-sterilized in 0.3% sodium hypochlorite for three minutes then they washed several times in sterilized distilled water. Holes (5 mm diameter and 4 mm deep) made into the fruits, using a cork borer, 1 ml of essential oils were sprayed separately into the holes, then kept to air drying. One ml, 48 hours old cultures of *Gluconobacter oxydans* were suspended in distilled sterilized water through up 2x106 cell ml^{-1} and sprayed into the holes which were plugged with the removed pieces [15]. Each treatment consisted of three replicates with four fruits per replicate control fruits were inoculated with sterilized water, 1.0% powdered milk, and tetracycline 500 ppm was used as a positive control. All treated fruits kept into plastic containers (25x7x7 cm), and incubated at 30°C up to one and three weeks when the weight of decayed areas were recorded.

2.7 Statistical Analysis

Data of all treatments were arranged and presented as mean from three replicates. The experimental designs of all experiments were completely randomized. Data were statistically analyzed for significance in the 8^{th} edition, Analytical Software, USA [37] using analysis of variance (ANOVA). Significance between means was compared by Duncan's multiple range test at p<0.05 probability according to the method of Gomez and Gomez [38].

3. RESULTS

Pomegranate (*Punica granata,* cv. wonderful) mature apparently healthy fruits were collected from commercial markets in Minia, Giza and from private orchards in Assuit and Minia Governorates, Egypt, in autumn 2018-2020 showed heart decay consisted in an internal decay of the arils, which usually confined to part of the fruit compartments, and some seeds (25%-50%) were discolored while the rind remained healthy and unaffected. Three isolates, PB1, PB2 and PB3, of non-capsulated, non-spored, rod-shaped, gram negative, creamy - like bacteria, were isolated from the inner decayed arils on NGA. The same symptoms had showed at autumn of 2019 and 2020.

Pathogenicity test revealed that all isolates of the pathogen under investigation were able to infect pomegranate fruits cv. wonderful. However, isolates differed as regards the severity of symptoms they initiated (Table 1). Data shows that isolate PB1 is the most pathogenic one, followed by PB2 and Pb3, which could be regarded as moderately pathogenic. Data in Table (1) shows also that the incidence and severity of infection differed due to the method of inoculation, whereas the infection using toothpick for wounding fruits caused the greatest infection, then inoculation through blossom end. No infection was appeared in sound fruits immersed in bacterial suspension after 7 days of incubation at 30°C.
The morphological and physiological properties (Table 2) of the bacterium on NGA, about 48 hours old at 30°C pointed to moderate growth develops, colonies are large, highly raised with regularly edges, slimy, milky white to yellowish, produce yellowish to pink change to dark brown pigment. Growth on nutrient sucrose (5%) agar is moderate and bacteria produced a low amount of mucoid substances. On potato slice, growth is moderate and the slice tissues appear brownish, dried and necrotic after 5 days. The three tested bacterial isolates grow well at a wide range of pH, from 4 to 6.5. Optimum temperature for growth was 25 -30°C, minimum was 5-10°C, but no growth at 40°C. Comparing the characters of the isolated bacteria with those reported by Gupta *et al.* [23], it is suggested that the isolated bacteria belonging to *Gluconobacter oxydans* and it is pathogenic to pomegranate. The identification of pathogenic isolated bacteria was confirmed applying the 16S rDNA Genes sequence analysis [34] by BioTech Research Lab, Sigma Scientific Service Technical Support cooperation with the Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485 in USA. their (PCR) technique, indicating the causal agent of inner decay of pomegranate disease was identified as *Gluconobacter oxydans* (Annon. 2018. *Gluconobacter*. HardyDiagnostics,USA. https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/Gluconobacter.htm)

Wikidata: Q17629105
Wikispecies: Gluconobacter
EoL: 83645
EPPO: 1GLUBG
GBIF: 3221591
iNaturalist: 356860
IRMNG: 1004163
ITIS: 957265
LPSN: gluconobacter.html
NCBI: 441
NZOR: 991eea32-a396-4745-a4e7-2b931903b570
uBio: 225328
WoRMS: 571169

Table 1. Disease incidence and disease severity on pomegranate cv. Wonderful fruits according to method of inoculation with 3 bacterial isolates, PB1, PB2 and PB3, 7 days after inoculation

Method of inoculation	DI%			DS%		
	PB1	PB2	PB3	PB1	PB2	PB3
Prickle (stick) with toothpick	100	100	100	92	61	77
Inoculation through the blossom end	88	52	50	55	36	38
Immersing the sound fruit in bacterial suspension	00	00	00	00	00	00

Table 2. The reported morphological, biochemical and physiological characters of *Gluconobacter oxydans* in comparison with those of the isolated organism

Character or test	*G. oxydans* strain reported by [23]	Isolated bacteria		
		PB1	**PB2**	**PB3**
Gram reaction	Gram-variable, more than likely negative	Mostly negative	negative	Negative
Shape of cell	Ellipsoidal to rod-shaped. Occurring singly, in pairs, and sometimes in short chains.	Short rod, singly, in pairs or in short chains	Short rod, singly, in pairs or in short chains	Short rod, singly,or in pairs
Size	0.5-1.0 µm X 2.6-4.2 µm	0.7-1.1 x2.4-4.1 µm	0.5-0.8 X 0.9-4.2 µm	0.5-0.8 X 0.9-4.2 µm
Capsules	None	None	None	None
Sporulation	None	None	None	None
Aerobiosis	+	+	+	+
Motility:	Motile and non-motile. When motility occurs, cells have 3-8 polar flagella	Motile	Motile	Motile
Color and Shape of colony	Large, slimy, pale colonies	Large, slimy, milky white to yellowish colonies	Large, slimy, milky white colonies	Large, slimy, pale white colonies
Edge of colonies	regularly	regularly	regularly	Regularly
Pigmentation	may produce pink or dark brown pigments	Produce yellowish to pink change to dark brown pigment	Produce yellowish to pink pigment	Produce pink change to dark brown pigment
Glucose oxidase	-	-	-	-
Voges-Proskaur (VP)	?	-	-	-
Methyl red	?	-	-	-
Indol formation	-	-	-	-
H_2S production	-	-	-	-
Levan test on NSA [(1)] medium	?	±	-	±
Catalase	Strongly-catalase-positive	positive	+	+
Oxidase	negative	negative	negative	Negative
Indole production	negative	negative	Negative	Negative
Nitrate reduction to nitrite	Does not reduce	Does not reduce	Does not reduce	Does not reduce
Aerobic	Obligate aerobic	Obligate aerobic	Obligate aerobic	Obligate aerobic

Character or test	*G. oxydans* strain reported by [23]	Isolated bacteria		
		PB1	**PB2**	**PB3**
Oxides ethanol into acetic acid	positive	positive	Positive	Positive
Utilization of carbon sources				
Starch hydrolysis	No growth or acid	-	-	-
Esculin hydrolysis	?	-	-	-
D-Mannitol	Grow but requires p-aminobenzoic acid as growth factor	delicate growth	delicate growth	delicate growth
sorbitol, glycerol	grow	+	+	+
D-Glucose, galactose, D-fructose,, mannose, sucrose,		Abundant growth and acid	Abundant growth and acid	Abundant growth and acid
pantothenic acid, niacin, thiamine	grow	**+**	**+**	**+**
Hypersensitive reaction in tobacco	?	positive	Positive	Positive
Temperature	Grow Opt. at range 25-30°C	Opt. 30°C	Opt. 25-30°C	Opt. 25-30°C
pH	pH 5.5 – 6.0.	4-6.5	4-6.0	4-6.0

(1)NSA = nutrient sucrose (30g/L) agar medium, (2) += poor growth, ±= moderate growth with low amount of mucoid substances

Furthermore, the pathogen was re-isolated from all inoculated fruits and was identified to be *G. oxydans* as described above, fulfilling Koch's postulates.

3.1 Effect of Some Essential Oils on Bacterial Growth *in vitro*

None of the four tested essential oils inhibited the growth of *G. oxydans in vitro*, at the concentration of 0.1% (Table 3). At the concentrations of 0.25 and 0.5%, the tested essential oils partially inhibited the growth of the bacterium. The pathogenic bacterium growth was highly inhibited at 5.0 and 10.0% of chamomile, marjoram, rosemary and thyme. However, at the concentrations of 5 and 10% of the essential oils, the inhibition growth of bacterium was almost like the effect of the tested antibiotic. Bacterial growth was observed on sterilized water, powdered milk and copper ~~sulfate,~~ whereas total bacterial inhibition occurred on tetracycline sulfate.

3.2 Effect of Temperature Degrees on Artificial Infection by *G. oxydans* of Pomegranate Fruit

This experiment was conducted to standardize the range and optimum temperatures for the pomegranate fruit decay which revealed that the disease could occur at all the temperatures from 5 to 35°C (Table 4). Data pointed to the highest significantly amount of decay was caused in fruits incubated at 30°C, followed by that incubation at 25°C, either 7 or 14 days of incubation period. Significant decrease in decay amount was observed when temperature of storage was decreased to 15°C. The lowest amount of decay occurred at 5°C. At 40°C no decay was observed. The maximum amount of pomegranate fruits decay (92.33%) was occurred after two weeks of incubation at 30°C.

3.3 Susceptibility of Different Fruit Hosts to Infection by *Gluconobacter oxydans* (PB1 isolate) under Laboratory Conditions

Data in Table (5) represented that kaka fruits were the most susceptible to infection by the pathogen (79.2 and 100% decay after 3 and 7 days of infection, respectively), followed by peach fruits (51.8 and 88.5%). Hosts can be classified into 4 groups depending on their susceptibility to infection

Group one: Fruits include highly susceptible hosts; kaka, peach, pear and apple, more than 50% infection,

Group two: Susceptible hosts, include tomato, grape and cherry, infection ranged between 25 and 50%,

Group three: include lowly susceptible hosts; strawberry, guava, plum and mango, infection was ranged between than 1-25%, Group one: Fruits include Group four include the most resistant hosts, i.e., lemon and orange, which no infected with the bacterium.

Table 3. Inhibition growth of *G. oxydans* (mm) *in vitro* due to essential oils treatment

Source of essential oil	Concentration of essential oils (%)						
	0.0	**0.1**	**0.25**	**0.50**	**1.0**	**5.0**	**10.0%**
Chamomile, (*Matricaria chamomilla)*	0.0k	0.0k	12.00hi	13.33ghi	23.33d	27.33b	31.33a
Marjoram**,** (*Origanum majorana*)	0.0k	0.0k	13.67gh	14.33g	16.33f	23.67cd	27.67b
Rosemary (*Rosmarinus officinalis)*	0.0k	0.0k	11.67i	12.33hi	13.67gh	21.33e	25.33c
Thyme (*Thymus vulgaris***)**	0.0k	0.0k	13.00g-i	16.33f	27.67b	30.33a	31.33a
powdered milk 1.0%(1)	0.0k	0.0k	0.0k	0.0k	0.0k	0.0k	0.0k
Water(1)	0.0k	0.0k	0.0k	0.0k	0.0k	0.0k	0.0k
Copper sulfate 2 mg $l^{-1(1)}$	5.0j	5.0j	5.0j	5.0j	5.0j	5.0j	5.0j
tetracycline sulfate 25 mg $mL^{-1(1)}$	32a	32a	32a	32a	32a	32a	32a

(1)The same concentration was applied in all trials without adding the essential oils; (2)Values in each column followed by the same letter are not statistically different P = 0.05

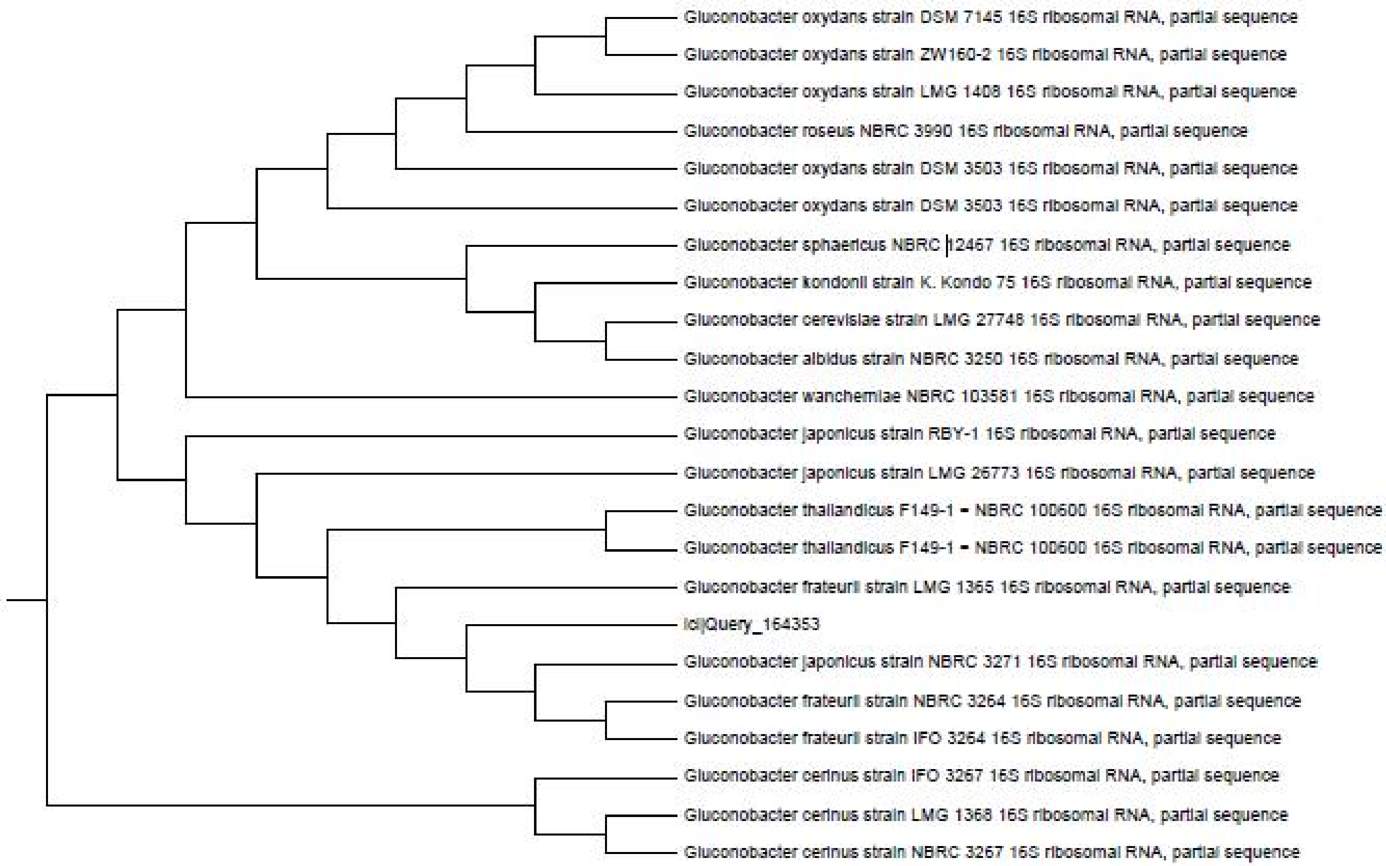

Fig. 2. Represent the hierarchical clustering of *Gluconobacter oxydans* strains and other species based on 16S rDNA Genes sequences identifies relevant pathogen subsets.

Table 4. Effect of temperature degrees on artificial infection by *G. oxydans* of pomegranate fruit

Temperature (°C) of incubation	% of rot weight, period of storage	
	a week	**two weeks**
5	17.67[(1)]e[(2)]	22.33f
10	24.50d	33.67e
15	24.67d	31.67e
20	27.67d	42.17d
25	71.67b	82.00b
30	81.00a	92.33a
35	41.00c	52.50c
40	0.00f	0.00g

[(1)]Data are presented as mean of three replicates each contains two pomegranate fruits. [(2)]Values in each column followed by the same letter are not statistically different P = 0.05

Table 5. Susceptibility of different fruit hosts to infection by *Gluconobacter oxydans* (PB1 isolate), at 30°C

Host	Scientific name	Family	% of rot weight after,	
			3 days of incubation at 30°C	**7 days of incubation at 30°C**
Apple	*Malus domestia*	*Rosaceae*	30.33[(1)]c[(2)]	57.17c
Pear	*Pyrus communis*	*Rosaceae*	31.50c	62.17c
Plum	*Phoenix dactylifera*	*Plamaceae*	15.17e	22.17f
Peach	*Prunus sp.*	*Rosaceae*	51.83b	88.50b
Orange	(*Citrus. × sinensis*)	*Rutaceae*	0.00f	0.00g
Lemon	(*Citrus × limon*)	*Rutaceae*	0.00f	0.00g
Mango	*Mangifera* sp.	*Anacardiaceae*	12.50e	18.00f
Guava	*Psidium guajava*	*Myrtaceae*	12.67e	23.50f
Grape	*Vitis vinifera*	*Vitaceae*	27.83c	34.17e
Tomato	*Solanum lycopersicum*	*Solanaceae*	20.83d	44.00d
Kaka	*Diosyros kaki*	*Ebenaceae*	79.17a	100.00a
Strawberry	*Fragaria ananassa*	*Rosaceae*	16.33de	23.00f
Cherry	*Prunus avium*	*Rosaceae*	20.33d	31.33e

[(1)]Data are presented a mean of three replicates each contains two pomegranate fruits. [(2)]Values in each column followed by the same letter are not statistically different P = 0.05

3.4 Effect of Essential Oils of Some Ornamental Plants on Decay Occurrence

Data in Table (6) indicate that marjoram (oregano) essential oil, followed by chamomile essential oil is the most affected against infection with the bacterium when compared with the control, 7 and 21 days after inoculation. The decay was significantly decreased with increasing the oil concentration. The least percent of decay was detected when marjoram (oregano) essential oil was applied by 0.5 and 1 ml L^{-1} (12.5% and 8.53%, respectively). Rosemary essential oil showed the lowest effective one against decay (22.8 and 26.7%) was occurred 7 and 21 days after inoculation, respectively. Tetracycline at 500 ppm prevent pomegranate fruits against the bacterium infection, significantly decreasing the decay percent to 14.3 and 18.0%, comparing with control (80 and 87%), after 7and 21 days from inoculation.

Table 6. Effect of essential oils on infection of rot % pomegranate caused by *Gloconbacter oxydans* on 30°C after one and two weeks

Essential oil source	Concentration (ml L-^{1})	One week after inoculation	After 3 weeks of inoculation
Thyme	1ml L^{-1}	13.$^{2(1)}$e$^{(2)}$	16.67e
	0.5ml \ L	21.33d	22.33d
	0.25 ml\L	25.00bc	25.00c
	mean	19.94	21.33
Oregano L Marjoram	1ml\ L	8.53f	11.50f
	0.5ml \ L	12.50e	16.83e
	0.25 ml\L	14.33e	17.67de
	mean	11.79	15.33
Chamomile	1ml\ L	12.00ef	13.50ef
	0.5ml \ L	15.5e	16.67e
	0.25 ml\L	20.5d	24.67c
	mean	16	18.28
Rosemary	1ml\ L	20.17d	23.33c
	0.5ml \ L	22.50cd	25.50c
	0.25 ml\L	26.17b	31.33b
	mean	22.78	26.72
Tetracycline	500 ppm	14.27e	18.00de
Control (water)	0.0	80.17a	87.33a

(1)Data are presented a mean of three replicates each contains two pomegranate fruits. (2)Values in each column followed by the same letter are not statistically different P = 0.05

4. DISCUSSION

The losses from postharvest diseases are significant important for overall agribusiness activities and it can result into rise the consumer prices and low incomes to farmers, processors and traders [39]. FAO organization reported that about half of the yield losses of crop production over the world [40], about 10-30% of crop yields, especially in developing countries, destroy due to postharvest diseases [41,42]. Different postharvest diseases reduce the quantity, quality and postharvest life of pomegranate. In autumn of 2018-2020 seasons, inner decay symptoms were observed in pomegranate fruits collected from different markets of Giza, Minia and Asuit Governorates, Egypt. A creamy bright bacterial growth was observed in the mesocarp layer, decayed both arils and seeds while the rind remained healthy and unaffected. These symptoms could make confusion with physiological disease caused due to the high temperature during storage which disappear (discoloration) for all seeds in the pomegranate fruits.

Three isolates, PB_1, PB_2 and PB_3, of non-capsulated, non-spored, short rod-shaped, gram negative, creamy - like bacteria were isolated from the inner decayed arils on NGA. The morphological and physiological tests of the isolated bacterium on NGA, about 48 hours old at 30°C, and verifying the results by molecular (PCR) and biochemical methods (VITEK 2) pointed to a *Gluconobacter oxydans* is the pathogen.

Gluconobacter, earlier known as *Acetobacter oxydans* [43], has been featured as having the pronounced capability by glucose oxidation to gluconate and weak ability for oxidation ethanol to acetate [44]. Also, *Gluconobacter* strains grows well in sugary media, e.g. ripe grapes, apples, dates, garden soil, baker's soil, honeybees, fruit, cider, beer and wine [23]. They found also that the bacterium strains are capable to cause rot of apples and pears fruits accompanied by various shades of browning.

There are no external symptoms were observed but when the fruit cuts into two halves, the inner arils appear decayed. This suggests that the infection occurs through the flowers. Sometimes, the diseased fruits were heavy in weight. These results are in agreement with Hine [45] who reported that pink disease of pineapple fruit, caused by strains of acetic acid bacteria, has no external symptoms but, during the canning process, infected fruit develop a brownish-pink discoloration after heating. He mentioned also that when flowering occurs during dry, high temperature stress conditions, followed by wetblooming cycles in November and December, led to increase the percentage of disease incidence in March.

Kado [46] reported that no organism belonging to family *Acetobacteriaceae* are known to be plant pathogens, thus neither *Gluconobacter oxy dans* nor *A. aceti* have been previously reported as plant pathogens. *Gluconobacter oxydans* brings about the incomplete oxidation processors of sugars, alcohols, aldehydes and acids. Incomplete oxidations lead to nearly quantitative results of the oxidation products making this organism important for industrial use. Strains of *Gluconobacter* can be used industrially to produce L-sorbose from D-sorbitol; D-gluconic acid, 5-keto- and 2-ketogluconic acids from D-glucose; and dihydroxy acetone from glycerol. It is primarily known as a ketogenic bacterium due forming 2,5- di-ketogluconic acid from D-glucose [23]. *Gluconobacter oxydans* was reported by Rohrbach and Pfeiffer [27], Kontaxis and Hayward [28] and Kado [46] as the causal agent of pineapple pink disease, also, it was reported as the causal agent of apple and pear rots accompanied by various shades of browning [23]. Also, the obtained results are in agree with that obtained by Buddenhagen and Dull [47] who mentioned that the strains of *G. oxydans* differences occur, but all strains produce the disease when injected into fruit.

The temperature is one of the most important factors for destructive nature of soft rots during growing, storage and transportation of fruits and vegetables This study revealed that the lowest amount of decay was recorded when artificially pomegranate fruits was incubated at 5°C for 7 or 14 days (17.67 and 22.3%, respectively), whereas the highest significantly amount of decay was recorded in fruits incubated at 30°C (81and 92.3%), followed by that incubated at 25°C (71.6 and 82%), either for7 or 14 days of incubation period. At 40°C no decay was observed, thus may be due to this temperature not favored bacterial growth. In 1973, Kanwar *et al*. [48] found that soft rot of pomegranate fruits occurred by *Rhizopus arrhizus* occurred between 10 and 40°C with maximum infection (100%) at 20, 25 and 30°C. Bhat *et al*. [49] during their study on effect of temperature on cabbage soft rot caused by *Erwinia carotovora* sub sp. *carotovora,* found that 30-35°C mostly favor the soft rot in cabbage and thus emphasis is to be given to prevent the disease during the prevailing temperatures in the region, in order to prevent losses due to the disease different hosts of the same pathogen. A high humidity coupled with a temperature of 80°F the pathogen is capable to cause the greatest injury. The optimum temperature for its growth was 85°F the maximum slightly over 100°F [50]. The highest severity of radish rot caused by *E. carotovora* subsp. *carotovora* was recorded when radish discs were incubated at 35°C and 100% relative humidity [51]. Farrar *et al*. [52], also, revealed that a range of 30-37°C was optimum for soft rot development in different vegetable plants. For this reason much of the loss due to decay of pomegranate occurs during middle of the summer. Under temperate conditions of Egypt, maximum damage was there only during the summer months viz., June- August as the temperature remains quite high. This also aggravated damage due to decay considerably during this time.

The present study revealed that kaka, peach, pear, apple, tomato, grape, cherry, strawberry, guava, plum and mango are susceptible to infection by *G. oxydans.* These hosts can be classified into 4 categories depending on their susceptibility to infection: Group one: Fruits include highly susceptible hosts; kaka, peach, pear and apple, more than 50% infection, Group two: Susceptible hosts, include tomato, grape and cherry, infection ranged between 25 and 50%, Group three: include lowly susceptible hosts; strawberry, guava, plum and mango, infection was ranged between than 1-25%, Group four include the most resistant hosts, i.e., lemon and orange, which no infected with the bacterium. These results are agreement with that obtained by Blackwood *et al*. [19], Passmore and Carr, [20], and Ameyama [21], on grapes, Passmore and Carr [20] on apples and dates and De Ley [22] and Gupta *et al*., [23] on honeybees, fruits, cider, beer and wine as well as capable to cause rot of apple and pear and cause pink disease in pineapple. Lambert *et al*. [53] reported that *Gluconobacters* are capable for causing rot of apples and pears which were accompanied by various shades of browning. The bacteria penetrate the apples through wounds in the cuticle and then to the tissue. Strains of *G. oxydans* are also the causative agent of "pink disease" of pineapple fruit; the disease fruit turns pink or pink-brown to deep brown after heating [46].

Majority of postharvest diseases could be controlled successfully by using fungicide compounds, their use is becoming increasingly restricted due to regulations regarding chemical residue levels. Essential oils coating, for the keeping fresh produce quality, is an environmentally friendly treatment that may be an alternative to chemical fungicide applications. However, there is little information about the chemical control of *P. granati* fruit diseases [54].

Our study showed that pathogenic bacterium growth was highly inhibited at 5.0 and 10% of marjoram, chamomile, rosemary and thyme. However, the inhibition growth of bacterium was almost like the effect of the tested antibiotic (tetracycline), but no inhibition was observed at 0.1%.

Essential oils of Marjoram (oregano) , followed by Chamomile proved the most affected against infection with the bacterium when compared with the control, 7 and 21 days after inoculation. In general, the decay severity was significantly decreased with increasing the oil concentration. Rosemary essential oil showed the lowest effective one against decay, either 7 or 21 days after inoculation. Tetracycline at 500 ppm prevents pomegranate fruits against the bacterium infection when compared with control.

In 1977, Kanwar and Thakur [55] tested 16 preservatives before and after pomegranate fruits inoculation with *Rhizopus arrhizus,* they found that Potassium metabisulphite (3%) was the best for inhibition the fungal spore germination, and causing the minimum soft rot incidence at room temperature for ten days.

Martins *et al.* [56] reported the inhibitory effects of 1.0, 2.0, 4.0,8.0, and 100% citronella and lemongrass oilson the development of the bacterium *Ralstonia solanacearum,* antibacterial activity of clove oil against seven different species of plant pathogenic bacteria i.e. *Agrobacterium tumefaciens, Erwinia carotovora* pv. *carotovora, Pseudomonas syringae* pv. *syringae, R. solanacearum, Xanthomonas campestris* pv. *pelargonii, Rhodococcus fascians,* and *Streptomyces* spp. [57]. They reported also that both Gram (+) and Gram (-) bacteria were sensitive to to clove essential oil (0.1 and 0.5%), with *R. solanacearum* being the most sensitive one. *Marjorana hortensis* showed antifungal activity against *C. acutatum* and *B. cinerea*, and antibacterial activity against two strains of Gram positive (*Bacillus megaterium* and *C. michiganensis*) and five strains of Gram negative (*Escherichia coli*, *X. campestris*, *B. mojavensis*, *P. savastanoi* and *P. syringae* pv. *phaseolicola*) [58]. The antifungal and antibacterial activity of oregano essential oil against a number of plant pathogens, including fungi; *Aspergillus niger*, *A. flavus*, *A. ochraceus*, *Fusarium oxysporum*, *F. solani* var. *coeruleum* , *Penicillium* sp., *Phytophtora infestans* and *Sclerotinia sclerotiorum*, and bacteria; *Pseudomonas aeruginosa* , *Staphylococcus aureus*, , *Clavibacter michiganensis*, *Xanthomonas vesicatoria*, has been reported by Adebayo *et al*. [59]. Lucas *et al.* [35] found all tested essential oils (EOs) from citronella, clove, cinnamon, lemongrass, eucalyptus, thyme, and tea tree showed direct toxic effect on the *X. vesicatoria* at a 10% concentration in laboratory test. They mentioned also that tested of clove and tea tree, and streptomycin sulfate promoted loss of electron-dense material and alterations in the cytoplasm, whereas essential oil of tea tree effect on cell vacuoles, and essential oils of tea tree, clove, citronella, and lemongrass caused damage to the bacterial cell wall. Several studies have showed that there seems to be a synergetic effect between the individual Eos chemical constituents. This synergism in the aromatic plants components functions to make them more effective and reduces the developing resistance of any pathogenic pathogen. In particular, some single constituents such as carvacrol, γ-terpinène and p-cymene become more effective when they are combined together and act synergistically [59]. Also, p-cymene component is efficient facilitator of the transport of carvacrol across cell wall components and the cytoplasmic membrane of the pathogen [60]. Another hypothesis suggested by Soylu *et al*. [61,62], is that the observed diameter reduction and lysis of the hypha wall, may be attributed to the enzymatic reactions within the essential oil which make to regulate synthesis of the wall components. Furthermore, the lipophilic characters of the above mentioned components might have the ability to damage the plasma membrane, and thus to increase the permeability of the cytoplasm.

5. CONCLUSION

This study submitted approve that internal decay of pomegranate fruits due to a bacterial pathogen (*Gluconobacter oxydans*), while it was thought that internal decay of pomegranate due to high temperature only. Also the use of resistant varieties and cultivars is by far the most economical and sustainable applications for managing the pomegranate fruit decay. Even as research progresses, eventually is leading to pomegranate varieties with improved levels of resistance by using essential oils of Marjoram (oregano) and Chamomile at 0.5 or 1 ml L^{-1}, lowering disease levels. Essential oils are cheap, healthy and environmentally friendly treatment that may be an alternative to chemical fungicide applications.

ACKNOWLEDGEMENT

The authoress would like to thank Dr. Marzouk R. Abdellatif, Prof of Plant Pathology, Faculty of Agriculture, Minia University, Minia, Egypt, for his helpful advice on pathogenicity test and identification of the isolated fungi, also, for his endless assistance to get this manuscript so organized and well formatted. The authoress also would like to thank the anonymous reviewers for their insightful suggestions and careful reading of the manuscript.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Bailey LH. Manual of cultivated plants. Macmillan Co., New York. 1954;1116.
2. Jurenka JS. Therapeutic applications of pomegranate (*Punica granatum* L.). A Review. Altern. Med. Rev. 2008*;*13:128-144.
3. Mondal KK, Sharma J. Bacterial blight: An emerging threat to pomegranate export. Indian Farming. 2009*;*59:22-23.
4. Caceres A, Giron LM, Alvarado SR, Torres MF. Screening of antimicrobial activity of plants popularly used in Guatemala for treatment of dermatomucosal diseases. J Ethnopharmacol. 1987*;*20:223-237.
5. Saxena A, Vikram NK. Role of selected Indian plants in management of type 2 diabetes: A review. J. Altern. Complement Med. 2004;10:369-378.
6. Turk G, Sonmez M, Aydin M, Yuce A, Gur S. Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity, and testosterone level in male rats. Clin. Nutr. 2008;27:289-296.
7. Naqvi SAH, Khan MSY, Vohora SB. Antibacterial, antifungal, and antihelminthic investigations Indian medicinal plants. Fitoterapia. 1991;62:221-228.
8. Boulos ZY, Amr KM, Assawah MW. Cercospora leaf spot of pomegranate in the U.A.R. (Egypt). Alex. J. Agric. Res. 1968;16(1):209-216.
9. Nepal (Achala) KC, Vallad GE. Pomegranates in Florida: Current status and future possibilities. Citrus Industry. 2016;24-27.
10. Pala H, Tatli A, Yilmaz C, Özgüven AI. Important diseases of pomegranate fruit and control possibilities in Turkey. Acta hortic. 2009;818:258- 290.
11. Ezra D, Kirshner B, Hershcovich M, Shtienberg D. Heart rot of pomegranate: Disease etiology and the events leading to development of symptoms. Plant Disease. 2015;99(4):496-501.
12. Labuda R, Hudec K, Piecková E , Mezey J, Bohovi R, Mateova S, Lukác SS. *Penicillium implicatum* causes a destructive rot of pomegranate fruits. Mycopathologia. 2004;157(2):217-23.
13. Palou L, Guardado A, Montesinos-Herrero C. First report of *Penicillium* spp. and *Pilidiella granati* causing postharvest fruit rot of pomegranate in Spain. New Disease Reports. 2010;22:21. DOI:10.5197/j.2044-0588.2010.022.021
14. Erika A, Cintora-Martínez,Santos G, Leyva-Mir,Victoria Ayala- Escoba,Graciela D, Ávila-Quezada ,Moisés Camacho-Tapia, Juan M, Tovar-Pedraza. Pomegranate fruit rot caused by *Pilidiella granati* in Mexico. Australasian Plant Dis. Notes. 2017;12: 4.
15. Allam (Samar), A, Elkot A, Elzaawely G, Abdelnaser A, El-zahaby HM. Potential control of postharvest Gray Mold of pomegranate fruits caused by *Botrytis cinerea*. Env. Biodiv. Soil Security. 2017;1: 145-156.
16. Faedda R, Granata G, Pane A., Evoli M, Lo Giudice V, Magnano di San Lio, G, Cacciola SO. Heart rot and soft rot of pomegranate fruit in southern Italy. Acta Hortic. 2016;1144:195-198
17. Sherkhane AS, Suryawanshi HH, Mundada PS, Shinde BP. Control of bacterial blight disease of pomegranate using silver nanoparticles. J. Nanomed. Nanotechnol. 2018;9(3):500.
18. Bozkurt A, Soylu S, Mirik M, Ulubasserce C, Baysal O. Characterization of bacterial knot disease caused by *pseudomonas savastanoi* pv*. Savastanoi* on pomegranate *(Punica granatum* L) trees : A new host of the pathogen. Applied Microbiology. 2014;59:520-527.

19. Blackwood A, Guimberteau C, Peynaud, E. Sur les bactéries acetiques isolies de raisins. R. Hebd Séances Acad. Sci. Ser. D. 1969;269:802-804
20. Passmore SM, Carr JG. The ecology of acetic acid bacteria with particular reference to Cider manufacture. J. Appl. Bacteriol. 1975;38:151- 158.
21. Ameyama M. *Gluconobacter oxydans* subsp. *sphaericus*, new subspecies isolated from grapes. Int. Syst. Bacteriol. 1975;25:365-370.
22. De Ley J. Comparative carbohydrate metabolism and a proposal for a phylogenetic relationship of the acetic acid bacteria. J. Gen. Microbiol. 1961;24:31-50.
23. Gupta A, Singh VK, Qazi GN, Kumar A. Gluconobacter oxydans: Its biotechnological applications. J Mol Microbiol Biotechnol. 2001; 3(3):445-456.
24. Tanasupawat S, Kommanee J, Malimas T, Yukphan P, Nakagawa Y, Yamada Y. Identification of *Acetobacter, Gluconobacter,* and Asaia strains isolated in Thailand based on 16S-235 rRNA Gene Internal transcribed spacer restriction and 16S r RNA sequence analyses. Microbes Environ. 2009;24(2):135-143.
25. Previdi MP, Vicini E, Scaramuzza N. Behavior of acetic-acid and related bacteria in culture media and in fruit and tomato products. Industria Conserve. 1994;69(2):118-122.
26. Cho JJ, Hayward AC, Rohrbach KG. Nutritional requirements and biochemical activities of pineapple pink disease bacterial strains from Hawaii. Anton. Van Leeuwenhoek. 1980;46:191-204.
27. Rohrbach KG, Pfeiffer JB. The interaction of four bacteria causing pink disease of pineapple with several pineapple cultivars. Phytopathology. 1976; 66:396-399.
28. Kontaxis DG, Hayward AC. The pathogen and symptomatology of pink disease of pineapple fruit in the Philippines. Plant Dis. Reptr. 1978;62:446-450.
29. Firas A, Slipes BS, and Alvarez (Anne) M. Postharvest diseases of tomato and natural products for disease management. African Journal of Agricultural Research. 2017;12(9):684-691.
30. Uzeh RE, Alade FA, Bankole M. The microbial quality of pre-packed mixed vegetables salad in some retail outlets in Lagos, Nigeria. Afr. J. Food Sci. 2009;3(9): 270-272.
31. Bibek R. Fundamental food microbiology. Third ed. Londan: CRC Press; 2004.
32. Hossain M, Logan CA. Comparison of inoculation methods for determining potato cultivar reaction to black leg. The Annals of Applied Biology. 1983;103(1):63-70.
33. De Ley J, Gillis M, Swings J. Family VI. Acetobacteraceae. Bergey's Manual of Systematic Bacteriology. ed. Krieg NR, Holt JG:. Baltimore: Williams and Wilkins Company Press. 1984;1: 267–278.
34. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J. Bacterial. 1991; 173:697-703.
35. Lucas GC, Alves E, Pereira RB, Perina FJ, de Souza RM. Antibacterial activity of essential oils on *Xanthomonas vesicatoria* and control of bacterial spot in tomato. Pesquisa Agropecuária Brasileira, *versão impressa.* 2012;47:3. ISSN 0100- 204X, Available:http://dx.doi.org/10.1590/S0100-204X2012000300006
36. Hudzicki (Jan). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology ©; 2016.
37. Steel RGD, Torrie JH, Dicky DA. Principles and procedures of statistics: A biometrical approach, 3rd ed., Mc Graw Hill, Inc. Book Co., New York, USA. 1997; 352-358.
38. Gomez KA, Gomez AA. Statistical procedures for agricultural research, Second Edition. John Wiley and Sons, New York. 1984;20-29:329-389.
39. SBP. The state of Pakistan's economy: Third quarterly report for FY 08. State Bank of Pakistan, Pakistan; 2008.
40. FAO. Global food losses and food waste. FAO, Rome; 2011.
41. Agrios NG, Plant Pathology. Academic Press Inc. 2000;803.
42. Ilyas MB, Ghazanfar MU, Khan MA, Khan CA, Bhatti MAR. Post-harvest losses in apple and banana during transport and storage. Pakistan Journal of Agricultural Sciences. 2007;4(3):534-539.
43. Asai T. Taxonomic studies on acetic acid bacteria and allied oxidative bacteria isolated from fruits. A new classification of the oxidative bacteria. J. Agr. Chem. Soc. Jpn. 1935;11:499-513,610-620,674-708.
44. Kluyver AJ, Boezaardt AGJ. The oxidation of glucose by *Acetobacter suboxydans*. Rec. Trav. Chim. 1938;57:609-615.

45. Hine R,B. Epidemiology of pink disease of pineapple fruit. Phytopathology. 1976;66: 323-327.
46. Kado CI. Pink Disease of Pineapple. APS*net* Features. Online; 2003. DOI:10.1094/APSnetFeature-2003-0303 Available:https://www.researchgate.net/publication/275073431
47. Buddenhagen IW, Dull GG. Pink disease of pineapple fruit caused by strains of acetic acid bacteria. Phytopathology. 1967;57: 806 (abstract).
48. kanwar ZS, Thakur DP, Kadian OP. A note on the effect of temperature and relative humidity on the development of soft rot of pomegranate fruits due to *Rhizopus arrhizus* Fischer. Indian Phytopathology. 1973;26(4):742-743.
49. Bhat KA, Masoodi SD, Bhat NA, Ahmad M, Zargar MY, Mir SA, Bhat AM. Studies on the effect of temperature on the development of soft rot of cabbage (*Brassica oleracea* var. *capitata*) caused by *Erwinia carotovora* sub sp. *carotovora*. Journal of Phytology. 2010;2(2):64–67.
50. Walker JC. Bacterial soft rots of carrot. In: Diseases of vegetable crops. Discovery Publishing House Ansari road, New Delhi. 1998; 78.
51. Raju MRB, Pal V, Jalali I. Inoculation method of *Pectobacterium carotovorum* sub-sp. *carotovorum* and factors influencing development of bacterial soft rot in radish. J. Mycol. Pl. Pathol. 2008; 38(2):311-315.
52. Farrar jj, Nunez JJ, Davis RM. Influence of soil saturation and temperature on. *Erwinia chrysanthemi* soft rot of carrot. Plant Diseases; 2000.
53. Lambert B, Kersters K, Gossele F, Swings J, De Ley J. *Gluconobacters* from honeybees. Antonie Van Leeuwenhoek. 1981;47:147-157.
54. Thomidis T. Pathogenicity and characterization of *Pilidiella grana* causing pomegranate diseases in Greece. Eur. J. Plant Pathol. 2015;141:45–50.
55. kanwar ZS, Thakur DP. Use of certain preservative chemicals for controlling soft rot of pomegranate (*Punica granatum* L.) fruits caused by *Rhizopus arrhizus* Fischer. Haryana Journal of Horticultural Sciences. 1977;6(3/4):140-144.
56. Martins ESC, da S Santos M, da S Barros HMM, Farias MA de A. Antibacterial activity of essential oils of citronella, alecrim and erva-cidreira does not control *in vitro* the bacteria *Ralstonia solanacearum* on tomato. Tecnologia e Ciência Agropecuária. 2009;3:29-34.
57. Huang Q, Laksman DK. Effect of clove oil on plant pathogenic bacteria and bacterial wilt of tomato and geranium. Journal of Plant Pathology. 2010;92:701-707.
58. Elshafie H, Camele S. Investigating the effects of plant essential oils on post-harvest fruit decay. Fungal pathogenicity, INTECH, Chapter . 2016;5:16.
59. Adebayo O, Dang T, Bélanger A, Khanizadeh S. Antifungal studies of selected essential oils and a commercial formulation against *Botrytis cinerea*. J. Food Res. 2013;2(1):217-226.
60. Elshafie HS, Mancini E, De Martino L, Pellegrino C, De Feo V, Camele I. Antifungal activity of some constituents of Origanum Vulgare L. essential oil against post-harvest disease of peach fruit. J. Med. Food. 2015;18(8):929-934.
61. Soylu S, Yigitbas H, Soylu EM, Kurt S. Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum.* J. Appl. Microbiol. 2007;103: 1021-1030.
62. Soylu EM, Kurt S, Soylu S. *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. International J. Food Microbiol. 2010; 143(3):183-189.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64302