Effect of different bacteriophage isolates on managing potato soft rot caused by *Pectobacterium carotovorum subsp. carotovorum*

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ABSTRACT----Isolation trials from potato tubers showing bacterial soft rot collected from Giza, Kalubia, Sharkia and Gharbia governorates yielded 35 bacterial isolates. The isolated bacteria were purified and identified as *Pectobacterium* sp., *Bacillus* spp. and *Pseudomonas* spp. The frequency of the isolated bacteria was not greatly differed in the four governorates. 15 strains of the isolates proved to be *Pectobacterium carotovorum* subsp. *carotovorum.* The identification of the 15 isolates was confirmed by the Biolog system based on their sugar contents. Electron micrographs of the nine isolates of *P.carotovorum* bacteriophages were taken. Of the fifteen isolates of *P. carotovorum* (Pc) , only 4 isolates ,*i.e.* Pc_2, Pc_4, Pc_7 and Pc_{10}, were highly pathogenic to the five potato cultivars , *i.e.*. Belini , Cara, Hermes, Mondial and Spunta . On the other hand, the tested isolates of Pc1, Pc_3, Pc_5, and Pc_{11} showed moderate effect on potato tubers. Meanwhile, Pc_9, Pc_{13} and Pc_{14} isolates had low effect on potato tubers and the other isolates, *i.e.* Pc_6, Pc_8, Pc_{12} and Pc_{15} failed to cause any infection to four potato cvs. ,*i.e.*. Bilini, Cara , Hermes and Mondial, but infected cv. Spunta cv. Four isolates of the bacteriophages (1, 2, 8 and 9) inhibited all the isolates of the causal bacterium . In addition, isolate (3) inhibited two pathogenic isolates only (Pc_7 and PC_{10}). Meanwhile, the mixture of the nine isolates of the bacteriophage inhibited all isolates of the causal bacterium. All the isolated phages were specific for *P.carotovorum* isolates and formed circular, clear plaques with or without halo and ranged from 1.0 to 5.0 mm in diameter**.**

The tested 9 bacteriophages were effective in controlling bacterial soft rot on potato tubers comparing with the control treatment. In addition, the mixture of these isolates was more efficient in decreasing the incidence and severity of the disease as soft rot 2 and 4 days after the infestation with *P. carotvorum* isolates (Pc_2, Pc_4, $Pc_7$and Pc_{10}) than the efficient of each of them alone.

Key words: Potato, bacteriophage, Biolog system**,** *Pectobacterium carotovorum*, cultivars, management.

1 INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important edible plants, universally planted in moderate climate, more rarely in the warm one .On a global scale, potato is considered the fourth most cultivated food crop after wheat, rice, and maize. In Egypt, potato has an important position among all vegetable crops, and is used for human consumption, animal feed as a source of starch, carbohydrates, alcohol and protein, exporting and processing (Douches *et al.*, 1996 ; FAO, 2008 and El-Helaly *et al*., 2012).

Potato is liable to infection by bacterial , fungal . viral and viral like diseases in addition to physiological disorder and nemates (Stevenson *et al.*,2001). In this respect different bacterial diseases have been reported to attack potatoes: soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*), brown rot (*Ralstonia solanacearum*), ring rot (*Clavibacter michiganensis*) and potato scab (*Streptomyces scabies*). *Pectobacterium carotovorum* , the causal agent of potato soft rot is a post-harvest pathogen resulting in economic losses wherever it is stored (Elphistone, 1987)

Soft rot Enterobacteriaceae, *Pectobacterium carotovorum* subsp. *carotovorum* (Gardan *et al.*, 2003) [*Erwinia carotovora* subsp. *carotovora* (Jones) Bergey *et al.*1923] (Pcc), is responsible for causing potato tuber soft rots in storage and in transit as well as in the field worldwide. *P.c.* subsp. *carotovorum* is a Gram-negative phytopathogen responsible for soft rot disease, wilt, or blackleg in various crops, by producing plant cell wall degrading enzymes that are actively secreted by the bacterium. Several important crops, such as potato; Chinese cabbage and carrot, have been attacked by this pathogen, and large economic losses resulting from significant yield reductions in the field, in transit, and during storage have occurred (Gardan *et al*., 2003 and Marquez *et al*., 2011). However, effective control methods have not yet been developed. Various strategies have been developed to control plant diseases, such as chemical antibiotics and copper, which have been used for many years (McManus *et al*., 2002). In addition, disease management involving cultural practices, plant activators, and plant resistance genes are extensively used to control various bacterial diseases. However, copper resistance has been reported in many bacterial pathogens, few effective bactericides have been developed, and plasmid-encoded antibiotic resistance genes are common (Cooksey. 1990; Bender *et al*., 1990). Thus, novel strategies for the control of bacterial diseases are required. Particular attention has

been paid to biologically based strategies, such as acteriocins or bacteriophages (Byrne *et al*., 2005; Ji *et al*., 2006). Recently, several bacteriocins have been developed, including a novel bacteriocin, carocin D, which was isolated from *P. c.* subsp. *carotovorum* Pcc21 and used to control soft rot disease (Roh *et al*., 2010). However, another environmentally friendly biological control agent, bacteriophage, has not been applied to the control of soft rot disease. Bacteriophages are viruses that specifically infect bacteria. Typically, upon infection with a virulent phage, the bacterium is lysed and numerous progeny phages are released and infect neighboring bacteria. Therefore, the phage is amplified followed by bacterial lysis (Frampton *et al*., 2012), which is an advantage of use of bacteriophages over other treatments. In addition, the lytic properties of bacteriophages are bactericidal rather than bacteriostatic, and they are effective against antibioticor heavy-metal-resistant bacteria, and have high target specificity, and in some cases biofilm disruption activity (Frampton *et al*., 2012 and Gupta and Prasad 2011). Bacteriophages are natural components of the biosphere and nontoxic to the eukaryotic cell, and their preparation is easy and inexpensive (Jones *et al.,* 2007). These characteristics suggest bacteriophages to be promising bioagents. Bacteriophages specific for *P. c.* subsp. *carotovorum* have been isolated from diseased plant materials and their associated soils, fertilizer solutions, cull piles, and sewage. Bacteriophages are isolated from these locations at lower frequencies and have narrower host ranges than phages that infect other Enterobacteriaceae (Gross *et al*., 1991 and Ji *et al*., 2006). Based on previous bacteriophage collection data, we characterized the lytic bacteriophage PP1 in terms of its efficacy as a bioagent for *P. c.* subsp. *carotovorum*. The bacteriophage was characterized both morphologically and phenotypically, and its antibacterial activity was confirmed in vitro and in vivo. These days, bacteriophages (commonly called phages), viruses that infect bacteria, are increasingly used as part of the bacteria-fighting arsenal in the food industry. Their antimicrobial potential has been noticed not only by physicians treating bacterial infections in humans (including those that result from consuming microbiologically contaminated food or water, such as dysentery) (Brussow , 2005) but also by researchers trying to control food borne pathogens before they become a cause of major human diseases. Phages are present in almost every environment (Brussow and Kutter 2005 and Weinbauer *et al.*, 2007). They can even be present in municipal water supplies of large European cities, indicating resistance to physico-chemical methods of purification of drinking water (Weber-Dabrowska *et al.* ,2014). This example clearly shows the continuous direct contact of humans with phages. Such widespread and frequent occurrence of phages supports the view that phages are safe for humans and their environment and can be utilized efficiently in agriculture (Meaden and Koskella, 2013). Furthermore, phage particles are mostly stable after being suspended in a wide range of neutral solutions such as phosphate-buffered saline (PBS). This feature allows phages not to alter the flavour, texture or nutritional value of foods. Phages have also been proposed as agents to eliminate plant pathogens. Katznelson (1937) still before World War II described in his review article phages isolated from soil or parts of plants against phytopathogenic bacteria (e.g. *Agrobacterium tumefaciens* causing crown gall disease and *Erwinia carotovora* responsible for soft rot in many vegetables and fruits) and their therapeutic and prophylactic possibilities. Phages have been considered to be natural and safe plant disease control agents since the 1970s when they were found occurring extensively and continuously in unaffected plant environments (Dunleavy and Urs, 1973).

Various kinds of bacteriophage with characteristic features have been isolated recently (Yamada *et al*., 2007), and have paved the way for new methods of biocontrol of bacterial soft rot. These phages may be useful as tools for effective detection (diagnosis) of the pathogen in cropping ecosystems and in growing crops. They also have potential uses in eradication of the pathogen from contaminated soil or prevention of bacterial soft rot in economically important crops. Like other methods of biological control, one advantage of phage biocontrol is the reduction in the use of chemical agents against pathogens. This prevents the problems of multiple environmental pollution, ecosystem disruption, and residual chemicals on the crops. Phage biocontrol in agricultural settings was extensively explored 40–50 years ago as a means of controlling plant pathogens (Okabe & Goto, 1992).

The aim of the study is isolation of bacterial pathogens and tested with a group of varieties of potatoes and isolate bacteriophage and use it to resist bacterial pathogens in the store.

2 MATERIALS AND METHODS

2.1. **Isolation and** purification of **the associated bacteria**:

Samples of naturally infected potato tubers (S*olanum tuberosum* L.) by soft-rot were collected from four different farms located in Kalubia , Sharkia, Giza and Gharbia governorates. Infected tubers were thoroughly washed using tap water to remove adherent soil and then air-dried. Small pieces (1cm thick) were surface sterilized by dipping in 2% sodium hypochlorite solution for 2 min. and rinsed several times in sterilized distilled water then dried between folds of sterilized filter papers. The sterilized pieces were then transferred onto ready nutrient agar (NA) medium and incubated at 30±1°C for 48 hrs. Observations were daily recorded and any emerged colony was picked up and cultured onto fresh NA plates. All picked colonies were purified using the single colony technique.

2.2. Evaluation of four potato cultivar:

Tubers of five different potato cultivars ,*i.e.* Belini , Cara, Hermes, Mondial and Spunta were inoculated with any of the isolated fifteen bacterial isolates to assess their pathogenicity . Bacterial dilution (0.5 ml at the rate of 1.3×10^8 cfu/ml) of each isolate was pipette individually at the center of each slice. Three slices

were used for each tested isolates as well as control. All treatments were incubated at 30±°C for 4 days then examined. Disease readings were expressed using the description of Lelliott and Dickey (1984) as follows:
(-): Negative infection
(+): Low positive infection
(++): Moderate infection
(+++): High infection.

2.3. Identification of the pathogenic bacteria:

Identification of the bacterial isolates was conducted on the bases of their morphological, nutritional and physiological characteristics according to schemes suggested by Krieg and Holt (1984) and Leliott and Stead (1987). Identification test was carried out only for the pathogenic bacteria, which verified their abilities to infection as mentioned before as follows: Morphological characteristics Different morphological characteristics of the subjected bacterial isolates i.e. cell shape, Gram stain and spore formation was carried out. Cultural characteristics various cultural properties of the examined isolates, i.e. the growth colony shape on different media, oxygen requirements and growth at different temperatures were also studied. Physiological and biochemical characteristics:

The following physiological characters and biochemical activities were used as bases for bacterial classifications: Acid production from sucrose. & reducing compounds from sucrose. Degradation of macromolecules: - Gelatin hydrolysis test -Starch hydrolysis test. Other tests: - Catalase test. - Salt tolerance test. - Phosphates test. - Pigment production. - Soft rot symptoms on potato slices - Relation to free O_2. - Hydrogen sulfide production (H_2S). - Levan formation. - Reducing compounds from sucrose. - Acid production from carbohydrates. - Potato dextrose agar (PDA). - Yeast extract dextrose-$CaCO_2$ (YDC) - King's medium B agar (KB). - Gas from d-glucose. - Peptone yeast extract agar (PYEA). - Pectate degradation.

2.3.1. Biolog System:

Four pathogenic isolates of those isolated from infected potato tuber soft rot samples and identified as *Pectobacterium carotovorum* pv *carotovorum* by cultural, morphological, biochemical and physiological characters, as mentioned above, and it confirm by Biolog system. The isolates were identified in VACSERA.

2.4. Bacteriophages:

2.4.1. Bacterial strains and bacteriophages:

Bacterial strains were grown on nutrient agar (NA) medium (0.8% (w/v) nutrient broth (NB) and 1.5% (w/v) Bacto Agar (Difco) at 28°C. For bacteriophage detection and propagation either semisolid nutrient agar yeast extract medium (NYA), (0.8% Nutrient Broth, 0.6% Bacto Agar and 0.2% Yeast Extract (Difco, Becton Dickinson and Co., Sparks, MD) or liquid nutrient broth medium was used. Sterilized tap water or SM buffer (0.05 M Tris-HCl (pH 7.5), 0.1 M NaCl, 10 mM MgSO4 and 1% (w/v) gelatin) was used for preparing phage suspensions. Bacterial strains used in this study were stored at -80ºC in NB supplemented with 30% glycerol. Bacteriophages were stored at 4ºC and protected from light.

2.5. Phage isolation from diseased tuber tissue:

Bacteriophages were isolated from tuber tissue of potato. Phage was isolated from diseased tissue at the Laboratory of Department Plant Pathology, Bacteriology branch, Fac. Agric of Moshtohor Benha Univ. Diseased tissues were collected directly from infected tuber located then directly placed in flasks 125 containing 50 ml sterilized tap water were shaken for 20 min. Two milliliters were collected and centrifuged at 10,000 rpm for 10 min to remove debris. The supernatants then were either treated with chloroform or filter-sterilized and then were checked for the presence of bacteriophages by spotting 20 µL onto freshly prepared lawns of the indicator bacteria. Three isolates were used for detection of diverse origins plus isolates were used. If plaques were observed after 24 h post incubation at 28±1°C, the phage was purified by three successive single plaque isolations and then propagated and stored, as described before.

2.6. Standard bacteriophage techniques:

2.6.1. Purification and storage:

Phages were purified by three subsequent single plaque isolations. Single plaque isolations were carried out by transferring phages from isolated plaques to a fresh lawn of the host bacterium using sterile toothpicks and then quadrant streaking them with sterile plastic transfer loops. Following purification the phages were propagated by mass streaking on fresh lawns of the host. After 24-h incubation at 28ºC, the phages were eluted by pouring 5 ml sterilized tap water into the Petri dishes and gently shaking the plates (20.000 rpm for 30 min). The eluate was centrifuged (10,000, rpm for 10 min), treated with chloroform or filter-sterilized, depending on the phage type, then quantified as described below, and stored in 2-ml plastic vials at 4°C in complete darkness. The concentrations of these suspensions were approximately 10^7 plaque-forming units (PFU) per ml determination of titer. Phage concentrations were determined by dilution-plating-plaque- count assay on NYA plates without bottom agar as previously described by (Rizvi and Mora, 1963). One hundred microliter aliquots of dilutions of phage suspensions were mixed with 100 µl of concentrated bacterial suspension in empty Petri dishes and then 12 ml warm (48°C) NYA medium was poured in each dish. The dishes were gently swirled to evenly distribute the bacteria and the phages within the medium. After the medium solidified, the plates were transferred to 28°C incubators and the plaques were counted on the appropriate dilutions after 24 or 48 hrs. The phage concentration was calculated from the plaque number and specific dilution and was expressed as PFU/ml.

2.6.2. Phage propagation:

Phages were recovered from storage, purified by

single plaque isolations and then mass streaked on the freshly prepared lawn of the propagating host. The next day phages were eluted from the plate, sterilized and enumerated, as described above. The elute used for infecting 500 ml actively growing culture of the propagating grown in NB liquid medium in 1 liter flasks, at 0.1 multiplicity of infection (MOI), (i.e., the phage concentration at the beginning of the incubation was 10^7 PFU/ml). After addition of the phage and 5-min incubation on the bench top, the culture was shaken at 150 rpm/min at 28ºC for 18 h. The resulting culture was sterilized; phages were enumerated and stored at 4 ºC in the dark until use. This method yielded phage titers of approximately 10^7 PFU/ml.

2.6.3. **.Phage concentration by high speed centrifugation:**

High titer phages *lysate* (10^7 PFU/ml) were concentrated and purified according to the method described by Ackermann (2005). The supernatant was discarded and replaced with 0.1 M ammonium acetate solution (pH 7.0). Following an additional centrifugation (60 min, 10,000 rpm) the supernatant was discarded and the pellet was resuspended in 1.5 ml SM buffer. The final phage concentration was approximately 10^{10} PFU/ml.

2.6.4. Evaluation of bacterial sensitivity to the bacteriophage:

Sensitivity of a bacterial strains to phages was determined based on the ability of the phage to produce plaques on the bacterial lawn, and the level of sensitivity was evaluated based on efficiency of plating (EOP) on the test strain in comparison with the propagating host strain of the phage as follows. A phage suspension of known concentration was plated simultaneously on the test and the host strains and EOP was calculated as the number of plaques on the test strain divided by the number of plaques on the host strain.

2.7. **Transmission Electron Microscope:**

Transmission Electron Microscopy (TEM) was carried out at Laboratory of the Microbiology Department, Faculty of Science, El- Azhar University. The phages were visualized using negative staining protocol with 1% aqueous uranyl acetate, as follows. A drop of the phage suspension was applied to a 300 mesh formvar- coated copper grid. After 2 min, the liquid was blotted away and the grid was rinsed with DI water. A 1% uranyl acetate solution was applied to the grid and blotted away after 1 min. The phages were observed and photographed on a Zeiss EM-10CA Transmission Electron Microscope operating at 100 K.volt.

2.8. **Bacteriophage *in vivo:***

Phage isolates which purified and identified as mentioned before, 3% corn flour + 5% sucrose was added to the liquid cultures of the tested phage isolates individually the other treatment was mixture of the nine phage isolates. Potato tuber (Spunta) were submerged with phage isolates as mixture treat individual and treatment before the inoculation with the four isolates of *Pectobacterium* carotovorum (Balogh *et al.*, 2003). Treated tuber were placed in humid chamber (70%) for treatments. Tuber box contained fifteen tuber were used as replicates per treatment.
Disease severity was recorded 2 and 4 days after inoculation as mentioned before.

2.9. **Experimental setup:**

After the treatment with the bacteriophage , the tubers were placed in surface sterilized plastic containers (about 30 x 40 x 10 cm) with lids at the rate of fifteen tubers per container, replicated two times for each treatment (method of bacteriophage application – tubers submerged, and exposure time). Moist tissue paper was placed at the bottom of each container to maintain high humidity. The control treatments were setup in the same manner. The containers were arranged in a completely randomized design on shelves in an incubator and incubated for 2 and 4 days at 27±1°C.

The experiments were evaluated based on the incidence and severity of the tuber soft rot. The tuber soft rot severity was assessed on a scale of 0–5 as described by Bdliya and Langerfeld (2005b) as follows:

0 = no symptom of rot
1 = 1–15% of tuber rotten
2 = 16–30% of tuber rotten
3=31–45% of tuber rotten
4 = 46–60% of tuber rotten
5 ≥ 61% of tuber rotten.

The severity was then computed using the following formula :

$$\% \text{ Disease severity} = \frac{\sum nxv}{5\,N} \times 100$$

where:
n = Number of infected tubers in each category.
v = Numerical values of each category.
N = Total number of the infected tuber
5 = Highest score on the severity scale.

3 RESULTS

3.1.Isolation and frequency of the isolated bacteria from potato tubers showing soft rot symptoms:

Isolation trials from potato tuber showing bacterial soft rot yielded 35 bacterial isolates (Table,1). The isolated bacteria were purified and identified *as Pectobacterium* sp., *Bacillus* spp. and *Pseudomonas* spp, being 15, 10 and 10 isolates, respectively. The frequency of the isolated bacteria was not greatly differed in the four governorates, being 6, 7 9 and 13 isolates at Giza, Kalubia , Sharkia and Gharbia, respectively.

Table 1. Frequency of the isolated bacteria from potato tubers showing soft-rot collected from four Governorates, during 2015.

The isolated bacteria	Frequency of the isolated bacteria from	Total	%Frequency

	Giza	Kalubia	Sharkia	Gharbia		
Pectobacterium sp.	2	3	4	6	15	42.9
Bacillus spp.	2	2	2	4	10	28.6
Pseudomonas spp.	2	2	3	3	10	28.6
Total	6	7	9	13	35	--

Fig .1: Four isolates of *P. carotovorum* high virulent to potato tubers (Spunta cv).

3.2. Confirmation the identification of *Pectobacterium carotovorum*:

The 15 strains of the presumptive pathogen were creamy Gram negative of short rods cant produced any pigment in Kings B medium (Table , 2) shows the following the tested isolates were positive for growth on Miller &Smith medium: Acid production from: lactose, growth at 37°C, growth in 5% NaCl , growth in 7% NaCl , starch hydrolysis , potato soft rot slice and catalase activity, but negative for KOH 3%, pigment in K.B, oxidase activity ,acid production from maltose, acid production from sorbitol, indole production, reducing substances from sucrose. The strains, also induced typical bacterial soft rot on Spunta potato cv.(Fig 1).

Table2. Identification and classification of isolated bacterium (*P. carotovorum*) by morphological, physiological and biochemical characteristics.

Identification Tests	Isolates location and number														
	Gi*.1	Gi 2	K 3	K 4	K 5	S 6	S 7	S 8	S 9	G 10	G 11	G 12	G 13	G 14	G 15
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
King's medium B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
size	All short														
Miller&Smith Media	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hyderolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid production from: lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid production from: maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid production from: sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Production (H_2S).	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Yeast extract dextrose-CaCO2 (YDC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pigment production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pectate degradation.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in 5% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in 7% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potato soft rot slice	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Relation to free O_2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Indol production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reducing substances from sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Genus and its code number	Pc_1	Pc_2	Pc_3	Pc_4	Pc_5	Pc_6	Pc_7	Pc_8	Pc_9	Pc_{10}	Pc_{11}	Pc_{12}	Pc_{13}	Pc_{14}	Pc_{15}

* Gi= Giza, K= Kalubia , S= Sharkia and G= Gharbia.

3.3. Identification of bacterial soft rot by Biolog system:

Fig.(2) shows the four bacterial isolates, *i.e.* Pc_2, Pc_4, Pc_7 and Pc_{10}, which selected according to their virulence as well as to represent the four different locations of isolation in Egypt were confirmed for their identification as *P. carotovorum* using the Biolog system based on their sugar contents.

3.4. Susceptibility of five potato cultivars to the infection by four isolates of *P. carotovorum* :

In this experiment, of the fifteen isolates of *P. carotovorum* , isolated from the different localities of Giza,Kalubia,Sharkia and Gharbia governorates were examined for their pathogenic capability to five potato cultivar, *i.e.*. Belini, Cara, Hermes, Mondial and Spunta. As shown in (Table,3) only 4 isolates ,*i.e.* Pc_2, Pc_4, Pc_7 and Pc_{10}, were the highly pathogenic to all the potato cultivars used. Therefore, they were used in the following experments. On the other hand,the tested isolates Pc_1,Pc_3,Pc_5 and Pc_{11} were moderately pathogenic to all tested potato cultivars. Meanwhile,cv. Spunta was infected by all the 15 isolates of *P.carotovorum.* No infection was observed on the un-inoculated potato tubers of all cultivars.

3.5.Evaluation of bacterial sensitivity to the bacteriophage:

Fig. (3) illustrates the electron micrographs of nine isolates of the bacteriophage isolated from potato tubers infected by soft rot collected from four governorates. **Table (4)** shows the effect of nine bacteriophages on the four different isolates of *P. carotovorum*, the highly pathogenic isolates on the four different isolates. Four isolates of bacteriophages (1, 2, 8 and 9) inhibited all the isolates of the causal bacterium. In addition, isolate (3) of the bacteriophage inhibited two isolates only ($Pc_7$and PC_{10}). Meanwhile, the mixture of the nine isolates of the bacteriophage inhibited all isolates.

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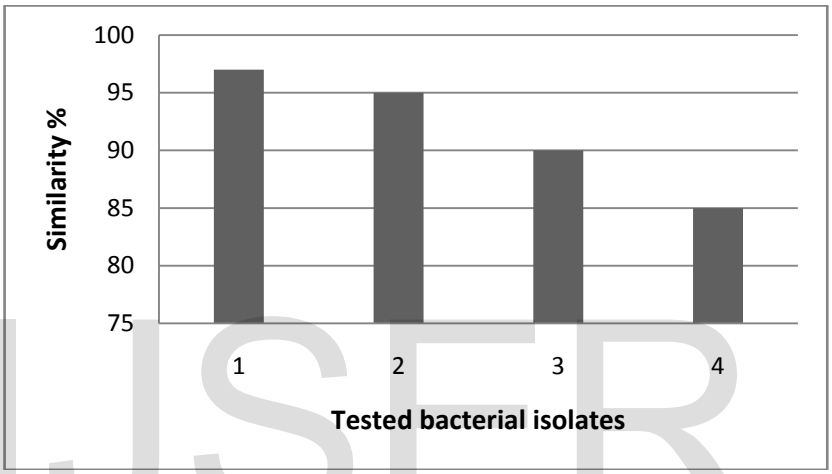

Fig. (2). The great similarities among the four tested *P. carotovorum* isolates.

Table 3. Evaluation of five potato cultivar to the infection by four *P. carotovorum* isolates

	Potato cvs.				
The tested isolates *	Belini	Cara	Hermes	Mondial	Spunta
Gi.1	++	++	++	++	++
Gi.2	+++	+++	+++	+++	+++
K.3	++	++	++	++	++
K.4	+++	+++	+++	+++	+++
K.5	++	++	++	++	++
S.6	-	-	-	-	++
S.7	+++	+++	+++	+++	+++
S.8	-	-	-	-	++
S.9	+	+	+	+	++
G.10	+++	+++	+++	+++	+++
G.11	++	++	++	++	++
G.12	-	-	-	-	++
G.13	+	+	+	+	++
G.14	+	+	+	+	++
G.15	-	-	-	-	++
Control	-	-	-	-	-

* Gi= Giza, K= Kalubia , S= Sharkia and G= Gharbia

** (-): Negative infection , (+): Low infection ,(++): Moderate infection and (+++): Highly infection.

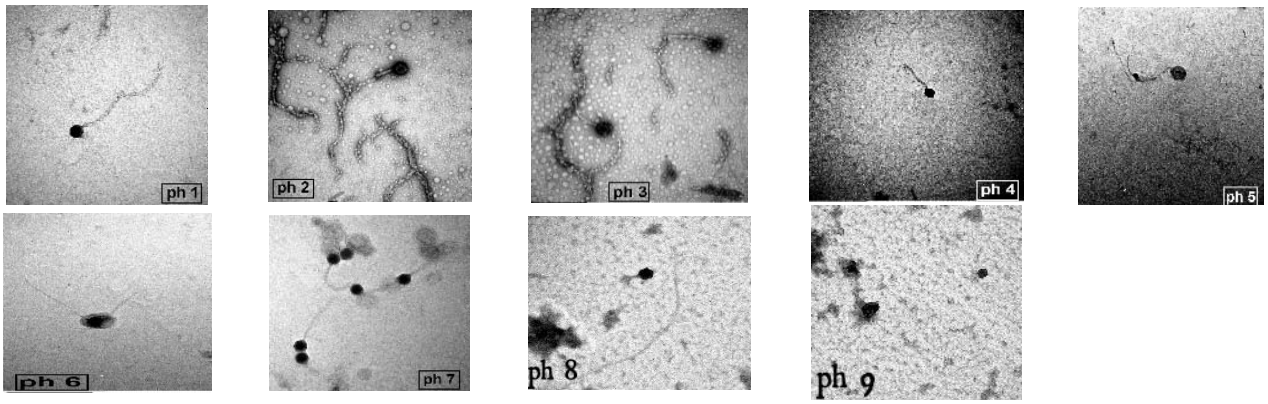

Fig.3. Electron micrographs of nine isolates of *P.carotovorum* bacteriophages isolated from potato tubers infected by soft rot collected from 4 governorates.

Table 4.The ability of nine different bacteriophage isolates to inhibit four *P.carotovorum* isolates.

	Number of the causal bacterium isolate			
No. of phages	2	4	7	10
1	+*	+	+	+
2	+	+	+	+
3	-	-	+	+
4	+	+	-	-
5	+	-	+	+
6	+	-	-	-
7	+	-	+	+
8	+	+	+	+
9	+	+	+	+
The mixture	+++	+++	+++	+++

* (-): No interaction with the phage ,(+) : Low interaction with the phage and (+++): High interaction with the phage.

3.5. Management of the disease by the bacteriophage :

Tables (5 and 6) indicate that all the tested 9 bacteriophages, each alone or in combination after 2 and 4 days after treatment, were effective in manging bacterial soft rot caused by the four pathogenic *P. carotvorum* isolates (Pc_2, Pc_4, Pc_7 and Pc_{10}) on potato tubers comparing with the control treatment. In this respect, these phages decreased the incidence and severity of the disease comparing with the control treatment, 4 days after the infestation.

Concerning of *P. carotovorum* isolate 2 , data presented in Tables (5 and 6) reveal that, phages of 1,2,4,5,6,7,8 and 9 isolates as well as the mixture of the nine phages were the most effective ones in reducing disease incidence, and disease severity ,2 and 4 days after the infestation with Pc_2 due to submerged the tubers in these phages. Meanwhile, the least recorded effect was recorded with using phage 3 to control bacterial soft rot on potato tuber using ($Pc_{2)}$, where it gave 6.7 % and 13.3% diseases incidence 1.5 % and 4.7 disease severity respectively. On the other hand, the control treatment (without phages) recorded high value of disease incidence (73.3%), disease severity (48.7%) after 2 days while 4 days after infestation of potato tuber with Pc_2.the disease incidence was93.3 and the disease severity was76.7%.

The effect of phage1, 2, 4, 8 and 9 isolates and the mixture of the nine phages on potato tubers after infestation with Pc_4 was studied. Tables (5 and 6) and Fig. (4) reveal that the mixture of the nine phages and phages of 1,2, 4,8 and 9 isolates were more effective than the other single phage isolates (3, 5, 6 and 7) on all disease parameters. In this regard, all the used phages either single or the mixture form were more effective on decreasing the disease parameters comparing to control treatment , 2 and 4 days after infestation with Pc_4.

Also, the effect of phage1, 2, 3 , 7, 8 and 9 isolates as well as the mixture of the nine phages on potato tubers after infestation with Pc_7 was studied. Data in Tables (5 and 6) indicate that the mixture of the nine phages was more effective than the other single phage isolates (4,5 and 6) on all disease parameters. In this respect, all the used phages either single or the mixture form were more effective on decreasing the disease parameters comparing to control treatment, 2 and 4 days after the infestation with Pc_7.

The effect of phage of 1, 2, 3 , 4, 6 , 7, 8 and 9 isolates as well as the mixture nine phages on potato

tubers after infestation with Pc_{10} was studied. Data in Tables (5 and 6) reveal that the mixture of the nine phages and the single of phages isolates 1,2,3,5,7,8 and 9 were more effective than the other single phage isolate (4 and 6) on all disease parameters. In this respect, all used phages either single or the mixture form were more effective on decreasing the disease parameters comparing to control treatment , 2 and 4 days after the infestation with Pc_{10}.

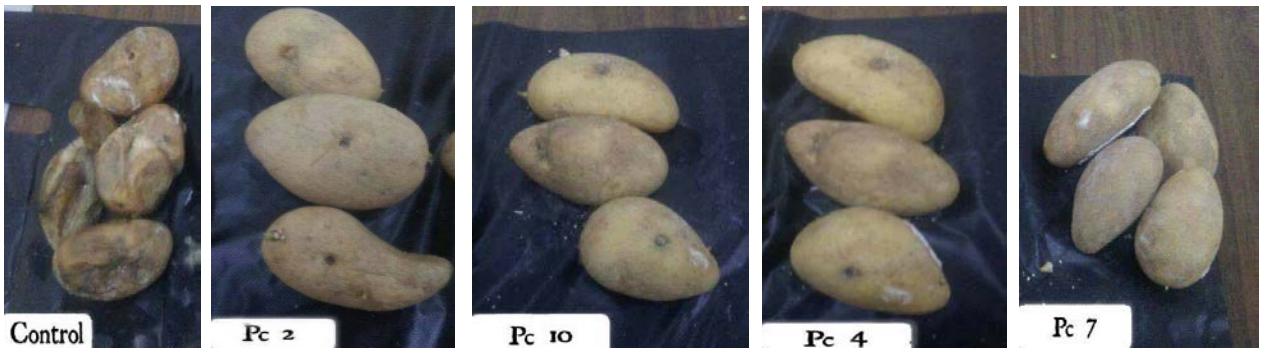

Fig.4. Potato tubers treated with the mixture of nine bacteriophage isolates to managing soft-rot caused by 4 *P. carotovorum* isolates (isolates 2,4,7 and 10) .

Table 5. Effect of nine bacteriophage isolates and their mixture on managing bacterial soft rot of potatoes in the store , 2 days after the treatment.

No. of phages	Bacterial isolates							
	2		**4**		**7**		**10**	
	% Ds *	**% DI****	**% Ds**	**% DI**	**% Ds**	**% DI**	**% Ds**	**% DI**
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	1.5	6.7	1.5	6.7	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	4.7	13.3	4.7	13.3
5	0.0	0.0	4.7	13.3	1.5	6.7	0.0	0.0
6	0.0	0.0	4.7	13.3	4.7	13.3	4.7	13.3
7	0.0	0.0	1.5	6.7	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
The mixture	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control	48.5	73.3	37.8	60.0	33.8	66.7	27.9	53.3

* % DS= Disease severity , ** % DI= disease incidence and *** % Rotting= The percentage of rot in the infected tubers .

4 DISCUSSION

Potatoes is one of the most important vegetable crops belonging to family Solanaceae . Potato tubers are liable to infection by bacterial soft rot in the field, storage and during propagation by or divided tubers (Lelliott and Stead 1987 and Stevenson *et al.*,2001). In addition, *Pectobacterium carotovorum* causes soft rot disease to many plants including potato (Czajkowski *et al.*, 2009 and Tsror *et al*., 2009). Isolation trials from potato tubers showing soft-rot collected from Giza, Kalubia, Sharkia and Gharbia governorates yielded 35 bacterial isolates belonging to 3 genera and 3 species. The isolated bacteria were purified. and identified as: *Pectobacterium* sp., *Bacillus* spp.and *Pseudomonas* spp. The highest isolation number and frequency % of

P. carotovorum was recorded in Giza followed by Kalubia then Sharkia and Gharbia governorates, respectively. *P. carotovorum* is the most pathogenic bacteria inducing rotting of potato tubers, where the infection area increased gradually after the second day of infestation

Table 6. Effect of nine bacteriophage isolates and their mixture on managing bacterial soft rot of potato tubers in the store, 4 days after the treatment.

No. of phages	Bacterial isolates							
	2		4		7		10	
	% Ds%	% DI**	% Ds	% DI	% Ds	% DI	% Ds	% DI
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	4.7	13.3	4.7	13.3	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	11.9	20.0	11.9	20.0
5	0.0	0.0	11.9	20.0	4.7	13.3	0.0	0.0
6	0.0	0.0	11.9	20.0	4.7	13.3	14.5	20.0
7	0.0	0.0	4.7	13.3	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
The mixture	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control	76.7	93.3	65.5	86.7	61.3	80.0	44.3	66.7

* % DS= Disease severity , ** % DI= disease incidence and *** % Rotting= The percentage of rot in the infected tubers .

Phytopathogens that infect important agricultural plants can reduce plant growth and the crop yield subsequently. Currently, these phytopathogens are controlled through management strategies, which can include the conventional methods and the chemical application of antibiotics and copper. However, the emergence of resistant bacteria and the desire to reduce usage of toxic products that accumulate in the environment mean there is a great need to develop alternative control agents. An attractive interst is the use of specific bacteriophages, small viruses that specifically kill bacteria, providing a more targeting approach. Typically, phages that target the bacterial pathogens were isolated from different sources and characterized to determine that they have a features required for using a biocontrol agents. In addition, suitable formulation and delivery to affected plants are necessary for ensuring the phages survival in the environment and do not have a deleterious effect on the plant. (Frampton *et al.*,2012).

Bacteriophages are natural components of the biosphere, which can be readily isolated from everywhere where bacteria are found, including soil, water, plants, sewage, animals and the human body (Adams, 1959 and Vinod et al., 2006).Therefore, in the present study nine phage isolates specific for Pc have been isolated from different sources as rotted potato tubers sample and have been used successfully as a therapeutic agent for elimination of bacteria.

The bacterial isolates were identified based on morphological, biochemical and molecular characters. Morphological and biochemical characters of colonies were similar to the standard *P. carotovorum* according to Bergy,s Manual of Systematic Bacteriology (Brenner *et al.*, 2007 and De Vos *et al.*, 2009). It was found that *P. carotovorum* belongs to Enterobacteriaceae family that possessed circular, creamy, convex, smooth and entire margine, Gram negative ,rod shaped, non-forming spores , motile with peritrichous flagella and cannot produce pigments ,they grown ,creamy colored colonies with no fluorescent pigment on King B medium, grow on Miller&Smith Medium (Kettani-Halabi *et al.*,2013 and Mohamed and selman,2013) . Based on biochemical tests *P. carotovorum* isolates gave positive reaction for KOH, catalase, and gelatin hydrolysis, and acid production from lactose, and sorbitol, tests. On the other hand, all *P. carotovorum* isolates can grow at 37^{o}C, and 5-7% NaCl, but all isolates gave a negative reactions for oxidase, indole, reducing sugars from sucrose, starch hydrolysis, acid production from maltose tests .These results compatible with those obtained by (Terta et al., 2010; Kettani-Halabi *et al.*, 2013; and Nazerian *et al.*, 2013).

Identification of the selected four bacterial isolates ,*i.e.* Pc_2, Pc_4, Pc_7 and Pc_{10} ,which selected according to their high pathogenic abilities as well as to represent the four different locations of isolation in Egypt was confirmed using the Biolog system to reveal that there are of great similarity.

The isolates of 6, 8 , 12 and 15 of *P.carotovorum* failed to infect cvs. Bilini. Cara, Hermes and Mondial . Meanwhile, cv. Spunta was infected by all the 15 isolates of *P.carotovorum.* Moreover, *P.carotovorum* isolates of 3,4, 7 and 10 were the most virulent ones. The evaluation test of the 5 potato cultivars is in agreement with *in vitro* assays and approved their reliability (Allefs *et al.*, 1996). The screening for soft-rot resistance could be a

good method for evaluation of resistance against *P. carotovorum.*

As for the isolated phages of *P. carotovorum* from infected potato tuber samples collected from Giza, Kalubia, Sharkia and Gharbia governorates . The isolated nine phages produced different types of plaques.

Adriaenssens *et al*. (2012) isolated bacteriophages from soil samples from a potato field plants infected with Dickeya spp. or Pectobacterium spp. they found that all phage isolates produced small clear plaques of 1 mm in diameter on Dickeya solani strains.

All the isolated phages were specific for *P.carotovorum* isolates and formed circular, clear plaques , clear plaques with or without halo and ranged from 1.0 to 5.0 mm in diameter. These results are in agreement with those reported by Jee et al. (2015) which found that soil samples showed bacteriophage with different sizes of plaques on solid media(largest clear plaque, medium sizes plaques and smallest plaques with almost all *P. carotovorum* subsp. *carotovorum* strains and this similarly with those found by (Delafn *et al*.(2012); Czajkowski *et al* . (2015) and Mohamed *et al*.(2016).

As for the effect of the nine tested bacteriophages and theie mixture , all the tested phages were effective in managing bacterial soft rot of potato tubers comparing with the check treatment. Also, these phages were effective in decreasing disease incidence, disease severity, rotting comparing with the control treatment ,2 and 4 days after the infestation with *P. carotovorum* isolates (2,4,7 and 10). The obtained results by Jones et al. (2007and Tarasi et al. (2010) supported the obtained results. Potato yield increase with phage treatment up to 93.3% when all tubers were inoculated with the tested four isolates. These results are in agreement with the obtained results by Balogh (2003).Flaherty *et al.* (2000) used a mixture of specific bacteriophages to control the bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*) as biological control of bacterial spot on 'Sunbeam' tomato (*Lycopersicon esculentum* Mill.) transplants and field-grown plants for two seasons.

5 REFERENCES

[1] Ackermann, H. (2005). Bacteriophage classification. in: Bacteriophages: biology and applications. in: Kutter E, and Sulakvelidze A, eds. CRC Press, Boca Raton, Florida. pp. 67-89.

[2] Adams, M.H. (1959). Bacteriophages. Interscience Publishers, New York, USA.

[3] Adriaenssens, E. M.; Vaerenbergh, J.V.; Vandenheuvel, D.; Dunon, V.; Ceyssens, P.; De Proft, M.; Kropinski, A. M.; Noben, J.; Maes, M. and Lavigne, R. (2012): T4-related bacteriophage LIM Estone isolates for the control of soft rot on potato caused by "*Dickeya solani*". PLoS ONE,7(3) Article ID e33227.

[4] Allefs, J.J.H.M.; Van Dooijeweert, W.; Prummel, W.; Keizer, L.C.P. and Hoogendoorn, J. (1996). Components of partial resistance to potato blackleg caused by pectolytic *Erwinia carotovora* subsp. *atroseptica* and *E. chrysanthemi*. Plant Pathol., 45: 486- 496.

[5] Balogh, B.; Jones, J.B.; Momol, M.T.; Olson, S.M.; Obradovic, A.; King, P. and Jackson, L.E. (2003). Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. Plant Dis., 87: 949–954

[6] Bdliya, B.S. and Langerfeld, E. (2005b). Soft rot and Blackleg [*Erwinia carotovora* subsp. *atroseptica* (Van Hall) Dye] of potato as affected by inoculum density and variety. Nigerian J. Plant Protec., 22: 65–75.

[7] Bender, C.L.; Malvick, D.K.; Conway,K.E.; George, S. and Pratt, P. (1990). Characterization of pXV10A, a copper resistance plasmid in *Xanthomonas campestris* pv. *vesicatoria*. App. Environ. Microbiol.,56 : 170- 175.

[8] Bergey D.H.; Harrison F.C.; Breed R.S.; Hammer B.W. and Huntoon F.M.(1923). Bergey's Manual of Determinative Bacteriology. 1st Ed.. Williams and Wilkins Co., Baltimore, MD, USA.

[9] Brenner, D. J.; Krieg, N. R. and Staley, J. T. (2007). Bergey's Manual of Systematic Bacteriology. Second Edition. Volume Two. The Proteobacteria.

[10] Brüssow, H. (2005). Phage therapy: The Escherichia coli experiment. Microbiology 151(Pt 7):2133-40.

[11] Brüssow ,H. and Kutter, E. (2005). Phage Ecology. In Bacteriophages – Biology and Applications. ed. Kutter E., Sulakvelidze A. pp. 129-163. Boca Raton: CRC Press.

[12] Byrne, J.; Dianese, A.; Ji, P.; Campbell, H.; Cuppels ,D. and Louws, F.(2005). Biological control of bacterial spot of tomato under field conditions at several locations in North America. Biol. Control 32: 408-418.

[13] Cooksey, D.A. (1990) Genetics of bactericide resistance in plant pathogenic bacteria. Ann. Rev. Phytopathol., 28: 201-219.

[14] Czajkowski, R; van Veen ,J.A. and van der Wolf, J.M.(2009). New biovar 3 *Dickeya* spp. strain (syn. *Erwinia chrysanthemi*) as a causative agent of blackleg in seed potato in Europe. Phytopathology, 99: S27.

[15] Czajkowski, R.; Ozymko1, Z.; de Jager, V.; Siwinska, V.; Smolarska, A.; Ossowicki, A.; Narajczyk, M. and Lojkowska, E. (2015). Characterization of Two Novel Broad Host Lytic Bacteriophages ΦPD10.3 and ΦPD23.1 Infecting Pectinolytic *Pectobacterium* spp. and *Dickeya* spp. PLOS ONEDOI:10.1371/ journal. pone. 0119812.

[16] De Vos, P. and Trüper, H.G.(2000). Judicial Commission of the International Committee on Systematic Bacteriology, IXth International (IUMS) Congress of Bacteriology and Applied Microbiology. Inter. J. of Syst. and Evol. Microbiol. 50: 2239-2244.

[17] Delfan, A. S.; Etemadifar, Z.; Bouzari, M. and Emtiazi, G. (2012). Screening of novel bacteriophage infection in *Pseudomonas putida* isolated from potato disease. Jundishapur J. of Microbiol., 5(4), 550-554.

[18] Dunleavy, J.M. and Urs, N.V.R. (1973) . Isolation and characterization of bacteriophage SBX-1 and its bacterial host, both endemic in soybeans. J .Virol., 12(1):188-93.

[19] El-Helaly, M. A. (2012). Propagation of potato (*Solanum tuberosum* L.) by seedlings. American-Eurasian J. of Agricultural & Environmental Sciences; 12(9):1117-1121 (C.F. CAB Abst. 2013, AN: 20133130999).

[20] Elphinstone, J. G. (1987). Soft rot and blackleg of potato: *Erwinia* spp. Technical Information Bulletin, 21. International Potato Center, Lima,

[21] Flaherty, J. E., Jones; J. B., Harbaugh; B. K., Somodi;G. C., and Jackson, L. E. (2000). Control of bacterial spot on tomato in the greenhouse and field with h-mutant bacteriophages. Hort. Sci., 35: 882-884.

[22] Frampton RA; Pitman AR; Fineran PC. (2012). Advances in bacteriophage-mediated control of plant pathogens. Int. J. Microbiol. DOI: 10.1155/2012/326452.

[23] Food and Agriculture Organization of the United Nations (FAO) (2008). International Year of the Potato. The Nutrition and Consumer Protection Division of FAO. Photo: © FAO / Giulio Napolitano.

[24] Gardan L.; Gouy C.; Christen R.; Samson R.; (2003). Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. Inter. J. of Syst. and Evolu. Microbiol., 53: 381-391.

[25] Gross D; Powelson M; Regner K; Radamaker G. (1991). A bacteriophage-typing system for surveying the diversity and distribution of strains of *Erwinia carotovora* in potato fields. Phytopathology 81: 220-226.

[26] Gupta R and Prasad Y. (2011) P-27/HP endolysin as antibacterial agent for antibiotic resistant Staphylococcus aureus of human infections. Curr. Microbiol. 63: 39-45

[27] Jee, S.; Malhotra, S.; Roh, E.; Jung, K.; Lee, D.; Choi, J.; Yoon, J. and Heu, S. (2015): Isolation of Bacteriophages Which Can Infect *Pectobacteirum carotovorum* subsp. *Carotovorum*. Plant Pathol. J., 31(1), 83-89.

[28] Ji P;Campbell H; Kloepper J;Jones J;Suslow T; Wilson M. (2006). Integrated biological control of bacterial speck and spot of tomato under field conditions using foliar biological control agents and plant growth-promoting rhizobacteria. Biol. Cont., 36: 358-367.

[29] Jones J; Jackson L; Balogh B; Obradovic A; Iriarte F; Momol M. (2007). Bacteriophages for plant disease control. Ann. Rev. Phytopathol. 45: 245-262.

[30] Katznelson H. (1937) Bacteriophage in relation to plant diseases. The Botan. Rev., 10:499-521.

[31] Kettani-Halabi, M.; Terta, M.; Amdan, M.; Elfahime, E.; Bouteau, F., and Ennaji, M. M. (2013). An easy, simple inexpensive test for the specific detection of Pectobacterium carotovorum subsp. carotovorum based on sequence analysis of the pmrA gene. BMC Microbiology, 13, 176.

[32] Krieg, N.R. and Holt, J.R. (1984). Bergey's manual of systematic bacteriology. Baltimore, Williams and Wilkins, 38-46 .

[33] Lelliott, R.A. and Dickey, R.S. (1984): Genus VII, *Erwinia* spp., pages 469-476, In: Krieg, N.R. and Holt, J.G. (eds.), Bergey's Manual of Systemic Bacteriology, Vol. 1, Williams and Wilkins, Baltimore, London.

[34] Lelliott, R.A. and Stead, D.E. (1987): Methods for diagnosis of bacterial disease of plants. Published on British Society for Plant Pathology, pp. 24, 106 and 107.

[35] Marquez-Villavicencio, M. D. P.; Groves R.L. and Charkowski A. O., (2011). Soft rot disease severity is affected by potato physiology and *Pectobacterium* taxa. Plant Dis., 95: 232-241

[36] McManus PS; Stockwell VO; Sundin GW; Jones AL (2002). Antibiotic use in plant agriculture. Ann. Rev. Phytopathol., 40: 443-465

[37] Meaden, S. and Koskella, B. (2013). Exploring the risks of phage application in the environment. Front Microbiol., 4:358.

[38] Mohameda, S. H.; Gado, E. A.; Gomaa, H. and Sadik, A. S. (2016). characterization of bacterial soft rot strains and their specific phages isolated from soil at Taif Pak. J. Biotechnol.,13 (1), 111-116.

[39] Mohammed, M. J. and Selman, E. D. (2013). Detection of local Erwinia isolates causing diseases in potato by using DNA Amplification by Polymerase Chain Reaction technique (PCR). Al-Nahrain Univ.,16 (3): 224-229.

[40] Monk, A.B.; Rees, C.D.; Barrow, P.; Hagens, S. and Harper, D.R. (2010). Bacteriophage applications: where are we now. Letters in App. Microbiol., 51(4): 363–369.

. [41] Nazerian, E., Sijam, K., Ahmad, Z. A. M., and Vadamalai, G. (2013). Characterization of *Pectobacterium carotovorum* subsp. *carotovorum* as a new disease on Lettuce in Malaysia. Australas. Plant Dis. Notes 8: 105–107. doi: 10.1007/s13314-013-0107-9.

[42] Narulita, E.; Kawasaki, T.; Fujie, M. and Yamada, T. (2015): Isolation of *Ralstonia solanacearum*-infecting bacteriophages from tomato fields in Chiang Mai, Thailand, and their experimental use as biocontrol agents. J. of App.Microbiol., 118(4): 1023-1033.

[43] Okabe, N., and Goto, M. (1963). Bacteriophages of plant pathogens. Ann, Rev. Phytopathol., 1: 397-418.

[44] Rizvi, S. and Mora, P.T. (1963): Bacteriophage plaque-count assay and confluent lysis on plates without bottom agar layer. Nature, 200:1324-

1325.

[45] Roh, E; Park, TH; Kim, MI; Lee, S; Ryu, S; Oh, C.S.(2010). Characterization of a new bacteriocin, Carocin D, from *Pectobacterium carotovorum* subsp. *carotovorum* Pcc21. App Environ. Microbiol., 76: 7541-7549.

[46] Stevenson , W.R. : Loria, Rosemary ; Franc, Gary D. and Weingartner , D. P. (2001). Compendium of Potato Diseases. Second Edition .The American Phytopathological Society.144 pp.

[47] Tarasi, G.; Manana, L.; Elena, K.; Konstantin, S.; Natela C.; Kakha D. and Giorgi, M. (2010). The Use of Bacteriophages for Controlling Bacterial Blight in Rice. Virus Microbes, pp.139.

[48] Terta, M.; Karkouri, A.; Ait M'hand, R.; Achbani, E.; Barakate, M.; Amdan, M.; Annajar, B.; El Hassouni, M.; Val, F.; Bouteau, F. and Ennaji, M. M. (2010). Occurrence of *Pectobacterium carotovorum* strains isolated from potato soft rot in Morocco. Cell Mol. Biol. (Noisy-le-Grand), 13(Suppl) 1324–1333.

[49] Tsror Lahkim, L.; Erlich, O.; Lebiush, S.; Hazanovsky, M.; Zig, U., Slawiak, M.; Grabe, G.; van der, Wolf, J.M.and van de Haar, J.J.(2009). Assessment of recent outbreaks of Dickeya sp. (syn. *Erwinia chrysanthemi*) slow wilt in potato crops in Israel. Eur. J. of Plant Pathol. 123: 311-320.

[50] Vinod, M. G.; Shivu, M. M.; Umesha, K. R.; Rajeeva, B. C.; Krohne, G.; Karunasagar, I. and Karunasagar, I. (2006). Isolation of *Vibrio harveyi* bacteriophage with a potential for biocontrol of luminous vibriosis in hatchery environments. Aquaculture, 255,117-124.

[51] Weber-Dąbrowska, B.; Żaczek, M.; Dziedzic, B.; Łusiak-Szelachowska, M.; Kiejzik, M.; Górski, A.; Gworek, B.; Wierzbicki, K. and Eymontt, A. (2014) .Bacteriophages in green biotechnology - the utilization of drinking water. In Industrial, medical and environmental applications of microorganisms: current status and trends. ed. MéndezVilas A. pp. 500-504. Wageningen Academic Publishers.

[52] Weinbauer, M.G.; Agis, M.; Bonilla-Findji, O.; Malits, A. and Winter, C. (2007). Bacteriophage in the environment. In Bacteriophage. Genetics and Molecular Biology. ed. McGrath S., van Sinderen D. pp. 61-91. Norfolk: Caister Academic Press.

[53] Yamada, T.; Kawasaki, T.; Nagata, S.; Fujiwara, A.; Usami, S., and Fujie, M. (2007). New bacteriophages that infect the phytopathogen *Ralstonia solanacearum*. Microbiology, 153: 2630-2639.