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Use of Rhizobacteria for the Management of Soft Rot Disease of Potato

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Abstract

Potato is an important vegetable crop all over the world. It is vulnerable to many pathological problems, among these, potato soft rot caused by *Erwinia carotovora* has been reported to be most prevalent and destructive. Controlling the plant diseases by pollution free bio-control antagonists (Rhizobacteria) is desirable now a days. Samples of potato wilting plants were collected from different locations. *Erwinia carotovora pv Carotovora* was isolated, purified and identified. Rhizobacteria were also isolated using serial dilution method. Purified isolates were evaluated for their ability to antagonize the rot pathogen *in vitro.* These bacteria were selected for further biochemical and tests. After completion of the tests, these bacteria were evaluated for their possible ability for percentage disease reduction under storage conditions by applying Rhizobacteria as bio-control agents. Moreover, percentage loss in weight was calculated and change in the disease level and growth of the plant in the presence of pathogen and Rhizobacteria were also computedThe application of Rhizobacteria in combinations was proved to be the best one, which was represented by the reduction in the disease of potato.

Key words: Rhizobacteria, Potato soft rot, *Erwinia carotovora*

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Introduction

The potato (*Solanum tuberosum* L.) is an herbaceous, dicotyledonous, starchy, tuberous and annual vegetable crop. It is a member of the family *Solanaceae*or also commonly referred to the nightshade family of flowering plants (Korpan *et al.*, 2004), one of the largest and most diverse plant families. Potato occupies an important place among the vegetable crops grown all over the world as well as in Pakistan. The total area under potato production during the year 2008-09 in Pakistan was 249.3 thousand hectare with a total production of 2542 thousand tons (Anonymous, 2009). The major potato growing districts in Pakistan are Kalat, Pishin and Killa in Balochistan; Sialkot, Okara, Sahiwal, Jhang, Kasur and Gujranwala in Punjab; and Dir, Nowshehra and Mansehra in NWFP.

Potato shares the genus Solanum, the largest genus in the Solanaceae family, with over 1000 species (OECD, 2008). *S. tuberosum* is divided into two subspecies; subsp. tuberosum and subsp. andigena (Huaman*et al.*, 2002). *S. tuberosum* subsp. *andigena* is specifically suited to cultivation at high altitudes and short daylight hours whereas subsp. *tuberosum* prefers cultivation at lower altitudes and a longer day length (OECD, 2008). Potatoes are an important food source and also staple food of the people in many parts of the world. Potato remains an important food source today and is grown on every continent, in over 130 countries and in almost all climatic conditions (Wale *et al.*, 2008). In comparison with other roots and tubers the protein content of potato is very high but almost similar to that of cereals. Starch makes up about 85 percent of this solid mass and the rest is protein. After wheat, maize and rice the potato is ranked world's fourth most important food because of its nutritive richness. It provides a balanced source of starch, vitamins and minerals to many communities in the global village (Stevenson *et al.*, 2001). Pathogenic problems includes the fungal pathogens and their diseases; *Alternariasolani* (early blight) and *Phytophthorainfestans* (late blight); *Ralstonia (Pseudomones) solanacearum* (brown rot or bacterial wilt), *Erwiniacarotovora*sub sp*atroseptica* (black leg and Erwinia rot), *Clavibactermichiganense*sub sp. *sepedonicum*(ring rot) and *Streptomyces scabies* (common scab) (Kabei l*et al.*, 2008). Controlling the plant diseases by pollution free biocontrol agents are desirable now a days, which are alternative to the chemical pesticides for the control of plant diseases especially the soil-borne (Roberts *et al.*, 2005). Following were the specified objectives of the present study:

- Isolation, purification and pathogenicity of *Erwiniaspp.* From infected potato plant parts.
- Screening of the rhizobacterial isolates against *Erwinia* spp. in the laboratory through inhibition zone technique.
- Evaluation of selected rhizobacterial isolates towards diseases reduction against soft rot disease (*Erwinia* spp.) under storage conditions.

Materials and Methods

Site of Experiment

The experiments were carried out at the laboratories of Department of Plant Pathology, Department of Soil Science and Soil & Water Conservation, Faculty of Crop and Food Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi.

Collection of Lentil Rhizospheric Soil

Rhizospheric soil samples were collected from four potato planted fields i.e. NARC field, NARC potato greenhouse soil, Sihala potato field, and from Taxila field. The samples were collected by uprooting healthy plants along with adhering soil. Diseased samples were collected on the basis of symptomatology. Samples were labeled and were stored at 4 ^{o}C.

Isolation and culturing of Erwinia carotovora

Nutrient Agar (NA) medium was used for isolation, culturing and purification of fungal pathogen. After the preparation of petriplates and medium the infected potato collar region stem or tubers samples were washed with tap water and cut into small pieces. Following this, these pieces were dipped in a petriplate containing Clorox for surface disinfection. Subsequently these pieces were washed twice with distilled water to remove excessive Clorox. After washing these pieces were placed in test tubes containing 5 ml of sterile distilled water and shaked vigorously for standard isolation. Bacteria were allowed to flow from the infected pieces for 5 to 10 minutes. One loop full of the bacterial suspension will be streaked on Nutrient Agar (NA) plates and incubated at 28^{o}C for 48 h. After 48 h bacterial growth appeared and was purified (Khan et al., 1999).

Purification of Erwinia carotovora

Each bacterial colony was purified on separate nutrient agar plate using streaking method. The activity was carried out in sterilized laminar flow near the spirit lamp in sterile conditions. Purified cultures were stored at 4 ^{0}C for further use.

Pathogenicity test by tuber slice assay

The suspension of the antagonistic PGPR isolates (10^{8}cfu/ml) and the Erwinia suspensions of the tested plant species (10^{6}cfu/ml) were prepared under sterile conditions. All of the selected bacterial isolates were tested for their ability to cause soft rot on potato tubers following procedure of Lelliot et al. (1966). Potato tubers were sterilized with 70% ethyl alcohol, rinsed with sterile distilled water, and aseptically cut into slices (1 cm). The potato slices were put in petridishes containing sterilized filter paper impregnated with 2 ml of sterile distilled water. The potato slices were inoculated with needle pricking method. The inoculated slices were maintained in moistened petridishes according to Togashi, (1988) and Nabhan *et al.,* (2006) and incubated at 30°C for 2 to 3 days. After 72 h, re-isolation of the bacteria from the tooted potato slice was performed using the isolation procedure.

Isolation and purification of Rhizobacteria

For isolation and culturing of rhizobacteria, nutrient agar (NA) medium was used and plates were prepared.

Serial dilution

The non-rhizospheric soil was removed from plant roots by gentle shaking. Serial dilution method was used for the isolation of rhizobacteria. For this method following procedure was adopted: **1.** The rhizosphere soil adhered to roots was collected by dipping and gentle shaking in sterilized water under aseptic conditions. Soil sample was taken in 9 ml distilled water in test tube. 2. Solution was shook well on vortex mixer for 30 sec to make it homogeneous. **3.** Then second dilution was prepared by taking 1 ml from the first dilution (i.e. 10^{-1}) and adding it to a fresh 9 ml of diluent in a sterile universal tube, thus making dilution of 10^{-2}. Tube was capped tightly and was mixed thoroughly on vortex mixer. **4.** By adopting the same procedure, solution was diluted upto 10^{-7} dilution. **5.** After proper shaking, dilution number 2, 5 and 7 (i.e. 10^{-2}, 10^{-5} and 10^{-7} respectively) were spread inoculated on petri plate containing solidified nutrient agar media. **6.** Plates were taped with paraffin tape so that to avoid any contamination. **7**. Plates were incubated at 35±2 ^{o}C. After purification pure cultures were stored at 4 ^{o}C for further use. From the composite culture of bacteria that resulted from the spreading inoculation of dilutions 10^{-2}, 10^{-5} and 10^{-7}, each bacterial colony was purified on separate nutrient agar plate using the streaking method.

Antagonistic Activity of *Rhizobacteria* against *Erwinia Sp* by Zone Inhibition Method

Zone Inhibition Method

Suspension was made by adding small amount of distilled sterilized water to the media containing the pure culture of the pathogen and was collected in test tube. Sterilized cotton swab was touched to the suspension and spread evenly over the face of a sterile nutrient agar plate and the test rhizobacteria was applied at the center of the agar plate in such a way that it does not spread out. Plates were incubated and examined after 2 days. Zone of inhibition was measured in millimeter using a ruler with bright light in order to avoid any error. Rhizobacterial isolates that exhibited considerable antagonistic activity against the pathogen, were selected for further tests.

Biochemical tests of Bacteria

Gram Staining Test

A loop full of bacterial suspension was spread on a glass slide and fixed by heating on low flame. Half percent aqueous crystal violet solution was spread over the smear. After 30 seconds, the slide was washed thoroughly with tap water for one minute. The slide was then flooded with iodine for one minute and rinsed with tap water. Ninety five percent ethanol was used for decolorization for approximately 30 seconds. The specimen was then counter

stained with safranin after washing for about 10 seconds. Subsequently after washing, it was dried and observed under microscope at 10, 40 and 100 X using mineral oil (Schaad, 1980).

Potassium hydroxide (Loop) Test

Bacteria were removed from petri plates with inoculating wire loop and were placed on glass slide containing a drop of 3 percent KOH solution. It was then stirred for 10 seconds using quick circular motion of the hand and observed the formation of slime threads (Suslow *et al.*, 1982).

Catalase Oxidase Test

A loop full of bacterium was put on a glass slide in a drop of hydrogen peroxide, mixed it and observed for the production of gas bubbles with aid of naked eye. A dissecting microscope at a magnification of 25 X was also used (Schaad, 1980).

Indole Acetic Acid (IAA) Test

A modified colorimetric method was used for determination of IAA (Asghar *et al.*, 2000). For this purpose, King's B broth medium was prepared according to King *et al.* (1954).

Preparation of King B broth medium

Following were the ingredients of the medium:

Peptone	20 gm
Glycerol	15 ml
K_2HPO_4	1.5 gm
$MgSO_4.7H_2O$	1.5 gm

All of the ingredients were added in 500 ml of water and mixed thoroughly. After the complete dissolution, volume was made 1 liter. Twenty five ml of media was poured in each 50 ml flasks and flasks were plugged. Flasks were autoclaved at 15 lbs psi and 121 ^oC for 20 minutes and allowed to cool.

Culturing of Rhizobacteria

Pure colonies of isolates obtained were grown in 50 ml conical flask containing 25 mL King's B (King *et al.*, 1954) broth with and without L-TRP (0.5 percent) solution and incubated at 28±2 °C for 24 h on a shaker. The cultures were then centrifuged at 4000 rpm for 20 min. One-milliliter culture supernatant was placed in a test tube and mixed with 2 mL Salkowski reagent A (2 percent of 0.5M ferric chloride in 35 percent per chloric acid). After 20-25 min, the color of supernatant was observed.

Phosphorus Solubilizing Test

The ability of isolates to solubilize phosphate was assessed qualitatively using Potato-Dextrose Yeast Extract Agar (PDYA) containing freshly precipitated calcium phosphate, that is 50 mL sterile 10 percent (wt. vol^{-1}) disodium hydrogen phosphate and 100 mL sterile 10 percent (wt. vol^{-1}) calcium chloride were added per liter sterile PDYA to produce a precipitate of calcium hydrogen phosphate (Katznelson and Bose, 1959). Each bacterial culture was spot inoculated in the center of a PDYA-CaPplate and incubated at 28±2 °C for 10 days. Phosphate solubilization was assessed by observing the clear/halo zone. The greater the diameter of the halo zone, greater was the ability of the bacterium to solubilize phosphate (Freitas *et al.,* 1997).

Siderophore Production Test

Siderophores are low molecular weight organic molecules that have high affinity for iron (Fe^{3+}). By producing siderophore, bacteria could chelate the iron (Fe) thus causing iron deficiency for the pathogenic organisms. Greater the ability of bacteria to produce siderophore, lesser will be attack of pathogens.

The bacterial cultures were streaked onto the Nutrient agar media with and without (50 μgL^{-1}) ferric chloride and incubated at 28±2 °C for 48 h. The fluorescence pigment of the bacterial colonies and that diffuse in the surrounding agar was evaluated using an ultraviolet lamp. Fluorescence pigment formed was considered as an indication of siderophore production (Teintze *et al.,* 1981).

Control of soft rot disease under storage conditions
Tuber inoculation with Erwinia spp.

21 fresh tubers of potatos were dipped in suspensions of antagonistic bacterium (107–108 cfu/mL), for 30 min and air-dried separately, then from these some treated potato tubers were inoculated with soft rot bacteria E. *carotovora* subsp. *carotovora* by spraying with inoculum suspensions (107–108 cfu/mL) with an atomizer.

Inoculated potato tubers bulbs were air-dried and stored separately in net bags at room temperature. Data on soft rot incidence was recorded after 4 and 8 weeks of inoculation. Number and weight of soft rot infected tubers were recorded and expressed in percentage using the following formula described by Abd- El-Khair and Karima (2007).

$$\text{Loss of weight \%} = \text{Initial weight} - \text{weight} \quad \text{---------- Initial weight-}$$

Percentage of disease reduction (PDR) was calculated according to the following formula described by Hajhamed*et al*., (2007).

$$\text{Infection \%} = \frac{\text{Number of infected tubers}}{\text{Total number of tubers}} \times 100$$

$$\text{PDR} = \frac{\text{Ack} - \text{Atr}}{\text{Ack}} \times 100$$

where Ack and Atr represent the severity of the disease in control and treated specimens, respectively.

Experimental Design

The tests were carried out in Randomized Complete block Design (RCBD) with three replications of each treatment.

T 0 = No treatment
T 1 = P-I
T 2 = P-II
T 3 = P-I + P-II
T 4 = *Bacillus* sp.
T 5 = P-I + *Bacillus* sp.
T 6 = P-II + *Bacillus* sp.
T 7 = P-I + P-II + *Bacillus*sp

Data Collection

Disease Incidence

Symptoms were produced by *Erwinia spp.* on potatoes The tuber soft rot severity was assessed on a scale of 0–5 as described by Bdliya and Langerfeld, (2005b).i.e. 30 days from placing into storage. The final readings were analyzed statistically and compared to the control.

Statistical Analysis

The data were analyzed statistically using One Way ANOVA and LSD tests (Steel and Torrie, 1997) using the software "Statistix 8 (version 8.1)".

Results and Discussion

Collection of Potato rhizospheric soil

All collected samples, showing the prevalence of the soft rot symptoms. 12 samples were collected with varying degree of rot symptoms. Rot index varied from 1 to 5 i.e. 1 to 15 % rotting to more than 61% rotting.

Morphology of ErwiniaColonies

According to morphology of *Erwinia* colonies on NA medium they were: transparent, circular, shining, raised and creamy white. These colony characteristics helped in the identification of the isolates to be belonged to Erwinia spp. and in particular *Erwinia carotovora* pv *carotovora*.

Pathogenicity Test

Results deliberately explained that isolate was pathogenic, symptoms of disease appeared as rotting and maceration of punctured area on the potato slice which progressively increased in diameter from initial point of inoculation. Most of the tuber slices showed severe maceration and decay while in some slices the macerated area is there but not as much as compared to others.

Isolation and Culturing of Rhizobacteria

From the samples, 15 isolates of rhizobacteria were isolated that varied in colony color, size of the colony and shape and elevation of the colony.

Morphology of the Colony

Morphology of the colony is the distinctive characteristic of each bacterium. There could be slight difference among the strains of same bacteria but the colony characteristics of different bacterium vary considerably. Thus it is an important source of confirmation of bacterium. Among the 15 isolates, colony color, colony shape and colony size of each isolate was observed and recorded (Table 1). According to the color, shape, elevation and size of the colony, it was presumed that most of the bacteria were Pseudomonads i.e. 2, 3, 5, 7, 8, 14, 15 while some colonies exhibited the characteristic of *Bacillus* i.e. 1, 4 and 9.

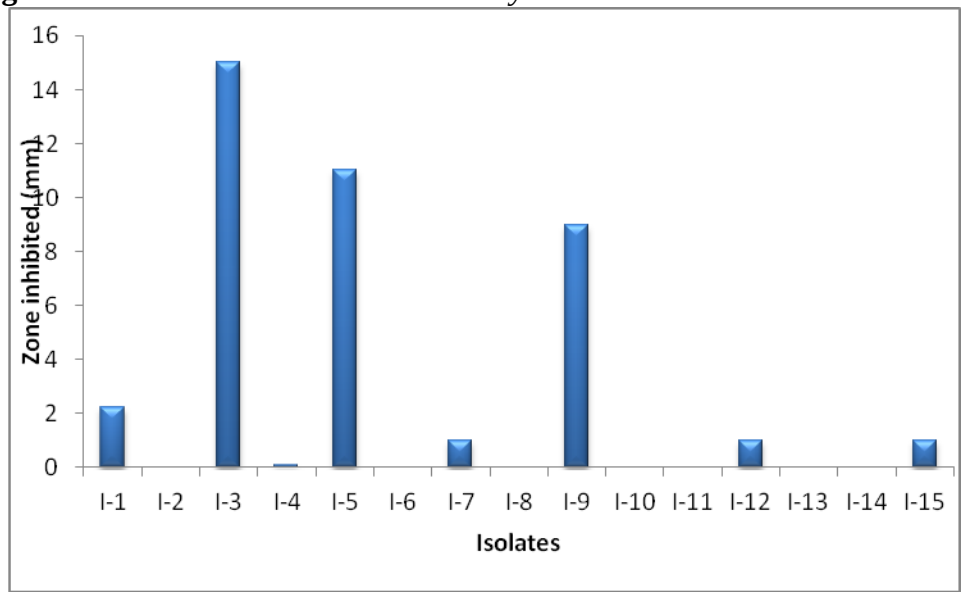
Table 1. *Colony characteristics of Rhizobacteria*

Serial No	Isolates	Colony color	Colony shape	Colony Size (mm)
1	I-1	creamy white	Round, medium, raised	3
2	I-2	off white	Large, round, flattened helical	3
3	I-3	pale yellow, shiny	circular, convex	3
4	I-4	creamy white	Large, flat, wavy margin	4
5	I-5	pale yellow, shiny	circular, convex	3
6	I-6	Reddish	Circular	2
7	I-7	pale yellow	Circular, convex	0.5
8	I-8	pale yellow	Circular, convex	1
9	I-9	whitish creamy	Flat, larger, circular and margin undulated	5
10	I-10	white colony	Round, Sharp, elevated	0.7
11	I-11	pale orange	Wavy, medium, flat	3
12	I-12	lightly brown	Round, small, raised,	0.5
13	I-13	Creamy	Round, small, smooth,	0.5
14	I-14	Yellow	Small, round, raised	1
15	I-15	light yellow	Round, small, smooth	1

In Vitro evaluation of antagonistic activity of Rhizobacteria against Erwinia sp Zone Inhibition Method

Among 15 isolates tested, I-3, I-5 and I-9 showed considerable antagonistic activity against the pathogen and were selected for further tests. I-3 produced better results than I-5 and I-9 as they respectively inhibited at 15 mm, 11 mm and 9 mm of the radius (Figure: 1). This inhibition of the growth of pathogen is attributed to the antibiotic production, siderophore production and antifungal compounds by the rhizobacteria (Figure:1).

Figure 1. *Zone inhibition in the isolates by Rhizobacteria*



Biochemical Tests of Bacteria

Gram Staining Test

Results revealed that two of the bacteria that exhibited the antagonistic activity against the pathogen were gram negative while one isolate responded positively to the test (Table 2).

Table 2. *Isolates response to Gram staining and loop test*

Serial No.	Isolate	Gram stain reaction	Cell morphology	KOH test reaction
1	I-3	-	Rod shaped	+
2	I-5	-	Rod shaped	+
3	I-9	+	Rod shaped	-

Potassium hydroxide (Loop) Test

Isolates that found gram negative in gram staining were found positive to potassium hydroxide (loop) test (Table 2). Formation of slimy threads was observed when bacterial cultures were mixed with 3 percent potassium hydroxide solution over the glass slide manifesting the dislocation of membrane.

Catalase Oxidase Test

All the isolates i.e. I-3, I-5 and I-9 when subjected to this test produced gas bubbles showing that these bacteria are aerobic. Based on the tests like; gram staining that revealed the morphology of bacterial cell, and loop test, it could be said that the bacteria may belong to *Pseudomonas* and *Bacillus*.

Indole Acetic Acid (IAA) Test

IAA was produced more in isolate I-3 than I-9 and I-5. Red color was more prominent and dark in I-5. Darkness of red color is directly related to the concentration of IAA. This was evident from the fact that red color got fade later in I-3 as the time passed while in I-5 and I-9, fadedness was more rapid.

Phosphorus Solubilization Test

I-9 results were much better than I-3 and I-5 in this respect because the halo zone was of greater diameter. I-9 halo zone was 14 mm in diameter thus explaining its ability to solubilize more Ca-P than I-3 and I-5 isolates which produced 5 mm and 8 mm of halo zone respectively.

Siderophore Production Test

The production of siderophore was recognized by the distinct yellow-green fluorescent pigment produced by the bacteria. Fluorescent pigment could be produced on the low Fe medium, as it has high affinity iron (Fe^{+3}) chelator (Teintze*et al*., 1981). All the strains i.e. I-3, I-5 and I-9 were found to be positive for siderophore production (Table 3). The color was diffused into the medium around the colony. I-3 and I-5 produced yellow to greenish color while I-9 produced orange to brown. On the basis of above characteristics and tests results, we could assume that I-3 and I-5 belonged to *Pseudomonas* while I-9 to *Bacillus*.

Table 3. *Phosphate Solu*
bilization, IAA, Catalase oxidation and Siderophore test response of the isolates

Serial No.	Isolate	P Solubilization test	IAA test	Catalase oxidase test	Siderophore test
1	I-3	+	+	+	+
2	I-5	+	+	+	+
3	I-9	+	+	+	+

Evaluation of selected Rhizobacterial isolates towards disease reduction against Soft Rot disease (Erwinia spp) under storage conditions.
Effect on Percentage disease reduction

Diseases reduction was 58.56 % after storage period of 4 weeks while in same treatment after interval of 8 weeks disease reduction was 76.16 % as compared to control while no other treatment showed such level of disease reduction. Along with the T8 other treatment i-e T2, T4, T5, T7 were also found considerably effective in Diseases reduction in both intervals of 4 and 8 weeks.T3, T6 were not be as efficient as compared to all other treatments i-e T2, T4, T5, T7 and T8, though they were significantly different from each other but the percentage disease reduction was not so effective in case of both treatments. The ability of these isolates to suppress the growth of various phytopathogenic bacteria makes them potential bio control agents (Table: 4).

Table 4. *Percentage of Disease Reduction after 4 and 8 week*

Treatment	Composition	% Disease Reduction after 4 weeks	% Disease Reduction after 8 weeks
T1	Control	0m	0m
T2	P-I	31.23k	56.96d
T3	P-II	23.03l	29.50e
T4	P-I + P-II	41.96i	67.66b
T5	*Bacillus* sp.	38j	50.56g
T6	P-I + *Bacillus* sp.	22.13l	25.13h
T7	P-II + *Bacillus* sp.	30.96k	52.66f
T8	P-I + P-II + *Bacillus* sp.	58.56b	76.16a

Effect on Percentage weight loss

At the 4th week of storage, the weight loss due to soft rot infection ranged 15.67 to 40.73% in different treatments, while, in control treatment the loss was 66.30%. The lowest loss was recorded in the treatment T8 which is 15.67 % and highest in treatment T7 which is 40.37%. The weight losses were always higher in control treatment. At the 8th week of storage , the percentage weight loss due to infection varies from 23.40 percent to 62.87 in different treatments while as compared to the control in which only pathogen is applied without any antagonistic rhizobacteria and weight loss is 100 percent. The lowest percentage of weight loss is in treatment T8 while highest percentage is in treatment T2 (Table: 5).

Table 5. *Percentage of weight loss after 4 and 8 Week*

Treatment	Composition	% Weight loss after 4 weeks	% Weight loss after 8 weeks
T1	Control	66.30b	100a
T2	P-I	32.53i	62.53cd
T3	P-II	31.50i	58.27e
T4	P-I + P-II	38.53h	62.87c
T5	*Bacillus* sp.	25.50j	42.17f
T6	P-I + *Bacillus* sp.	21.57l	39.63i
T7	P-II + *Bacillus* sp.	40.73g	61.40d
T8	P-I + P-II + *Bacillus* sp.	15.67m	23.40k

Discussion

Rhizobacterial isolates I-3, I-5 and I-9, that were identified as P-I, P-II (*Pseudomonas*) and *Bacillus* sp. respectively, were found to be antagonistic not only *in vitro* but also in pot experiments. They have considerably reduced the incidence of *Erwinia carotovora* pv *carotovora* (Table:4). These results were confirming the earlier findings for the reduction of disease by the PGPR (Whipps, 2001). Reduction of Bacterial growth by certain PGPR and formation of inhibition zones were presumably due to the materials (antibacterial or antibiotic substances) released by the bacteria into the culture medium. I-3, I-5 and I-9 inhibited the zone of fungal growth to 15 mm, 11 mm and 9 mm respectively as compared to the other isolates. While in storage experiments, mixture of these isolates was more efficient than individual isolate (Figure:1). It has previously been reported that application of mixture of isolates inhibits pathogen growth more efficiently than single isolate (Marjan *et al*., 2003). The reason why application of single isolate does not control disease in better way might be related to insufficient root colonization. Therefore, these mechanisms by applying a mixture of the isolates lead to more effective or at least more reliable bio control of soft rot pathogens of potato.

The results of the present experimental findings strongly correlated with those of the earlier findings (Abd-El-Khair and Karima, 2007; Cladera-Oliver*aet al.,* 2006 and Raju *et al.,* 2006).The pretreatment of potato tubers with antagonistic bacteria successfully prevented the initial infection and reduce soft rot disease of potato and multiplication of soft rot bacteria (Table: 4). Earlier studies reported that antagonistic rhizospheric bacteria have significant antagonistic activity against plant pathogenic bacteria including soft rot *Erwinia* genera (Long *et al.,* 2003 &Raju *et al.,*2006). Long *et al.,* (2003) reported that the genus. *Bacillus* and *fluorescent Pseudomonads* have antagonistic activity against various plant pathogenic bacteria including soft rot bacterium *E. carotovora* subsp. *carotovora* (Ecc) in vitro

The present study demonstrates that use of rhizobacteria as an effective agent in control of soft rot disease in potato in vitro and as well as in storage and the results showed that rhizobacteria are efficient in controlling the

diseases and percentage weight loss. Previously similar work have been reported by Gamliel and Katan, 1993 in which they reported involvement of direct antagonism of pathogen, antibiotic production, or competition with pathogens for essential nutrients. Many rhizobacteria have been shown to produce antibiotics that inhibit the growth of an antagonistic bacterium. *P. fluorescens* (Trevisan) Migula F113, has been shown to control the soft rot potato pathogen *Erwinia carotovora* by producing the antibiotic 2,4-diacetylphloroglucinol (DAPG). Some of our bacterial isolates represented Siderophore production (Table:3). These findings are in agreement with the studies of Whipps, 2001 who also reported Siderophore production by *P. fluorescens*.

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