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Full Length Research Paper

Incidence of potato blackleg caused by *Pectobacterium atrosepticum* in district Chiniot and its management through bio-products

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Potato is an important commercial crop of the world. It was reported to be affected by many biotic and abiotic factors. Among biotic factors is a blackleg, a bacterial disease of potato caused by *Pectobacterium atrosepticum* and is responsible for both quantitative and qualitative losses in the field and in the storage. This disease has been found to be more frequent in the districts of Chiniot. Therefore, a survey was conducted in field's conditions of district Chiniot of Pakistan, as well as cold storages to assess the disease severity in the potato field. For isolation, samples were collected and identification, purification and mass culturing of the isolated of pathogen was also performed. The efficacy of different chemicals, plant extracts and bio-products were studied against isolated pathogen. During the screening trial of fifteen varieties, Cardinal was found immune while Faisalabad white was found to be highly susceptible. Other varieties like Accent, Harmony, Faisalabad Red and Lady Rosette were found resistant, while Desiree, SH70, Everest, Hermes, Orla and Paramount were found moderately resistant. Varietals response against this bacterium was evaluated under natural conditions in the field on weekly basis. Evaluation of different bio-products and plant extracts was carried out under laboratory conditions. Under the laboratory conditions, bio-products produced maximum inhibition zone. Plant extracts namely neem (*Azadirachta indica*), garlic (*Allium sativum*), datura (*Datura alba*) and onion (*Allium sepa*) also produced a little inhibition zone against the growth of *Erwinia*.

Key words: Potato, *Pectobacterium atrosepticum*, control, bio-products.

INTRODUCTION

Potato (*Solanum tuberosum* L.) has a momentous place among the vegetable crops. In Pakistan, the total production of potato is 2939.5 thousand tons and area under potato cultivation is 249.6 thousand hectare during the year 2008-09 (Anonymous, 2008-09). Potato is a tasty, highly digestible vegetable and nutritive food in the world. Actually, numerous people in poor countries who could not manage to pay for high calorie diet such as meat, milk products and pulses used potato as their major source of calories in the seventeenth century.

Potato consists of about 20% organic matter and 80% water. It is an excellent source of carbohydrates and also gives vitamins, including thiamin, niacin, vitamin C and riboflavin (Cotton et al., 2004). Potato is a more efficient source of carbohydrate than its total protein contents. It is also a vital source of antioxidants (Chen et al., 2007), contains 22% dietary materials, 325 kcal food energy, 7.6 g protein, 0.04 g fat, 72.8 g carbohydrates, 42 mg calcium, 213 mg phosphorus, 2.7 mg iron, 70 IU vitamin A, 0.15 mg riboflavin, 4.4 mg niacin and 64 mg ascorbic acid. Mass spectrometry and liquid chromatography of wild and cultivated potatoes identify phenolic concentrations of 100 to 675 mg/100 g of dry weight (Vanaei et al., 2008).

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Potato is cool-season crops and has a wide range of seasonal adaptability. It is quite frost-tolerant. In a shorter period of time, the potato plant produces more nutritious food in colder environmental conditions than any other food crop. The most important factor influencing potato yield is a temperature. Blackleg disease of potato is relatively significant among the bacterial diseases. In India, losses have been estimated up to 45% (Singh, 1998). In Pakistan, the accurate losses due to this disease had not been investigated until now. The economic significance of blackleg is two-fold: Primarily it can decrease yield when the incidence of disease go over 5-10%, and secondly in the certification it causes downgrading of seed potato (Farran et al., 2006). In Pakistan, the average price of potato increased from about Rs. 350 / 40 kg in 2004-05 to Rs. 650 / 40 kg in 2009-10. During this period, exports of potato were 16.59 million kg, which are earning a foreign exchange worth Rs.183.2 million. Afghanistan, Malaysia and Sri Lanka are the main markets of Pakistani potato (Anonymous, 2006-07).

Erwinia carotovora, now called *Pectobacterium carotovora*, causes potato blackleg in field. The pathogens are generally present in or on the seed tubers when the disease development is started. The favorable environmental conditions increase the growth of pathogens in the stem or in the seed tuber. So *Pectobacterium* spp. are responsible for qualitative and quantitative losses in storage as well as in the field. In Pakistan, this disease was first reported in 1984 from Swat valley (Khan et al., 1985), and from the plains of Punjab and hilly areas, it was reported in 1986 and 1987 by Turkensteen (1988 and 1989).

In Pakistan potato-growing areas (such as districts of Sialkot, Okara, Faisalabad and Gujranwala), blackleg disease manifested high presence generally with an infection ranging between 0.3 and 3.5% in Ultimus, Cardinal, Desiree, Patrons and Multan varieties (Hafiz, 2003). The bacterium affects stems and causes soft rot in tubers. So the plant becomes contaminated from the soil and it leads to drooping and death of the above-ground parts. Shoots become stunted and frequently blackened at the bottom, recognized as blackleg.

Tuber rot may develop under storage or field conditions. Infectivity occurs through injuries or through the stolen end of the tuber and lenticels. Lesions related with lenticels emerge as a little water soaked, circular, tan to brown and sunken areas. When the incidence of this disease is boosted up, the yield decline is 0.9% after planting (Bain et al., 2004).

Objectives of the study

Keeping in view speculation of losses, no effective control measure for the disease and the importance of disease during storage under poor ventilation conditions or in field, this research was deliberated with the following objectives:

(i) To assess the current status of incidence of potato growing areas like Chiniot. Distt.
(ii) To assess the screening of potato germplasm against blackleg disease.

Concerning the managements of this disease, this study also aimed to:

(i) Assess the use of different chemicals particularly bio-products.
(ii) Assess antagonistic microbes are the potential source of bio-products.
(iii) Promote the use of bio-products as chemicals have health hazards effect on animals and plants.

MATERIALS AND METHODS

Isolation and identification of the pathogen

Potato fields and storage houses were visited in different parts of the District Chiniot. The plant and tubers showing typical symptoms of the disease were collected in polythene bags, brought to laboratory for isolation and placed in a refrigerator at 4°C for 24 h. For isolation of bacterium, nutrient glucose agar (NGA) medium was prepared from the following ingredients (Khan and Riaz, 2000): beef extract, 3.0 g; peptone, 5.0 g; glucose, 2.5 g; agar, 15.0 g. Beef extract and peptone were mixed slowly in 500 ml cold water and then volume was made up to 1 L. The contents were boiled and agar-agar was added slowly into the medium; the contents were thoroughly stirred so that these may not stick to the bottom of the pan. Glucose was added to the solution after some time, removing heating unit. The media was sterilized by autoclave at 121°C and 15 Lbs psi (1.8 kg/cm^2) for 20 min. The tubes and flask were taken out from the autoclave after the pressure came down to room temperature. About 15 ml of the sterilized medium was poured into the sterile Petri plates (90 mm) from the flasks before it solidifies and Petri plates stored in refrigerator.

The parts of potato tubers affected with blackleg were disinfected with 0.5% mercuric chloride ($HgCl_2$) and then three washings were given with sterilized water to reduce the injurious effects of $HgCl_2$. Afterward, these pieces were placed on Petri plates and incubated at 30°C (Khan and Riaz, 2000). The grayish-white, circular and smooth colonies appeared after 96 h of incubation. Isolated colonies of the bacterium were picked and single colony transferred to each slant and incubated at 30°C for further use. Slide was prepared of the bacterium and characteristics were observed under the microscope (Khan and Riaz, 2000).

Purification of pathogen

Pathogen was purified by using streaking method, then the Petri plates were incubated at 30°C for 24 h until grayish white, circular and smooth bacterial colonies appeared. Isolated colonies of bacterium were picked and single colony was transferred to slants and incubated at 30°C for further use.

Gram staining for the bacteria

A drop of sterilized distilled water was placed in the middle of a clear slide. Then a loop of bacterial suspension (Young culture) was

transferred to the sterilized drop of water and a very thin film was prepared on the slide by spreading uniformly. The film was fixed by passing it over the gentle flame for two or three time. The slide was flooded with crystal violet solution and allowed to stand for 30 s and then washed thoroughly with gentle stream of tape water. The slide was then immersed in the iodine solution for 1 min and washed thoroughly with 95% alcohol for 10 s. The alcohol was drained off and washed thoroughly with gentle stream of tape water, and then the slide was the covered with safranin for 1 min. After washing with the tape water and blotting, slides were examined under the microscope.

Pathogenicity of *Erwinia*

Pathogenicity of *Erwinia* was tested by; (i) injecting the 4.2×10^8 cfu/ml solution of pathogen into shoot of the potato plants by syringe; (ii) soaking tuber/slices of potato in suspension of *Erwinia* and (iii) mixing the pathogen in soil and then planting potato seed in soil. The soil was sterilized by drenching the 5% formalin, then the bacteria were mixed into this soil and potato tuber was planted in the pots. All the three methods were completed by pathogen and morphological characteristics were compared with the culture of *Erwinia*. The bacteria showing similar colony as that of original culture were considered to be pathogenic.

In order to assess the disease incidence and severity of blackleg disease of potato, a survey was conducted in the potato fields of following localities of different tehsils of district Chiniot (Lasla Waris, Chak Bhandi, Thatha Thakir, Moza Talib and Chak Corala).

Effect of plant extracts and bio-products on pathogen (*Erwinia carotovora)* growth

The following extracts were used on the pathogen: *Allium sativum* L. (garlic); *Allium sepa* (onion); *Datura alba*; *Azadirachta indica* (neem); Vampire (bio-product); Biosal (Bio-product). The aforementioned treatments were evaluated against *E. carotovora* subsp. *carotovora.* For the preparation of aqueous extracts of aforementioned plant, bulbs and other parts (tubers, stems, and leaves) were washed gently with distilled water. Fresh parts were macerated in 250 ml of distilled water separately with the help of pestle and mortar to get extract of every treatment. The ground extracts were first passed through four layer muslin cloth and then filter through Whatman's filter paper No. 4 (it was considered standard (S)) and different dilution concentration were made from this standard these plant extracts were stored at -4°C to avoid from contamination and further evaluation plant extracts was followed by the using inhibition zone techniques in which measured quantities of plant extracts (SD) and bio-products were added to in the centre of every Petri plates in the well designed by sterilized cork borer. The commercially available bio-products were Vampire and Biosal (*Bacterium*) purchased from the market. These Petri plates were allowed to solidify; Petri plates containing NGA with distilled water instead of plant extracts served as check. All Petri plates were incubated at 27, 32 and 37°C and bacterial colony growth was recorded after 96 h of incubation.

Screening of potato varieties

Preparation of field

The field plot was prepared at the research area of the Department of Plant Pathology, University of Agriculture, Faisalabad, by applying all the agronomic practices including fertilization like nitrogen 100 kg, phosphorus 100 kg and potash 50 kg, out of which half quantity of nitrogen was applied at the time of sowing and other half one month after sowing and Stomp 330E (Pendimethalin) at the rate of one and a half liter per acre after the sowing was applied to remove the weeds from the plot.

Varieties used

Fifteen varieties namely, Cardinal, Faisalabad white, Faisalabad red, Accent, Harmony, Lady Rosette, Desiree, SH70, Everest, Hermes, Paramount, Fontane, Atlantic, Melody and Orla were grown during the year 2006 and 2007 in the experimental area of Department of Plant Pathology University of Agriculture Faisalabad for the screening purposes. These varieties were grown under field conditions temperature 28 to 32°C with 20 cm plant to plant and 75 cm from row to row distance. These varieties were inoculated by injecting 4.2×10^8 concentration of bacterial suspension after the twenty days of planting. On the appearance of first symptom of the disease, incidence was recorded as given by James (1969):

$$\text{Disease incidence} = \frac{\text{No. of affected plants/unit area}}{\text{Total no. of plants/unit area}} \times 100$$

Disease severity was assessed by visual rating scale (0 - 5) based on parent plant, tuber surface showing symptoms (Ahmad et al., 1995): 1, No symptoms; 2, 1 to 10% plant/leaf area affected; 3, 11 to 20% plant/leaf area affected; 4, 21 to 30% plant/leaf area affected; 5, 31 to 40% plant/leaf area affected; 6, 41 to 50% plant/leaf area affected; 7, 51% or more area affected. The susceptible and resistant varieties were screened against blackleg disease of potato by the above mentioned scale.

Management of blackleg/ *Erwinia*

The disease management studies were conducted by exploiting various disease management strategies like host resistance, plant extracts and bio-products means in laboratory, as well as under field conditions.

In vitro* evaluation of plant extracts against *Erwinia carotovora* sub sp. *carotovora

Aqueous neem (Azadirachta indica) extract (ANE) preparation

Fresh neem (*A. indica*) leaves 25 g in 75 ml of distilled water were ground in pestle and mortar, centrifuged, and filtered through Whatman No. 1 filter paper. This was taken as standard. Then further dilutions were prepared by adding distilled water.

Aqueous garlic (Allium sativum L.) extract (AGE) preparation

Fresh garlic (*A. sativum L.*) bulbs were peeled, weighed (100 g), and cleaned garlic were taken and surface sterilized using ethanol. The ethanol was allowed to evaporate in a sterile laminar flow chamber, and the garlic was homogenized aseptically using a sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. This extract was considered as standard (S) concentration of the extract. The concentrations, S/50, S/25 were made by diluting the concentrated extract with appropriate volumes of sterile distilled water.

Aqueous onion (Allium sepa) extracts (AOE) preparation

Fresh onions were purchased from a local market. They were peeled, weighed (100 g), cut into small pieces, cleaned and

surface-sterilized using ethanol. The small pieces were crushed in a blender pre-cooled at 4°C. The homogenate was filtered through cheesecloth followed by Whatman-40 filter paper. The filtrate was centrifuged at 2500 rpm for 30 min at 4°C. The supernatant of onion extract (AOE) was passed through a 0.45 µM filter, sterilized through a 0.22 µM Millipore filter and kept frozen at −20°C.

Experiments under field conditions

Host resistance

Fifteen varieties namely, Cardinal, Faisalabad white, Faisalabad red, Accent, Harmony, Lady Rosette, Desiree, SH70, Everest, Hermes, Paramount, Fontane, Atlantic, Melody and Orla were grown during the year 2010 and 2011 in the experimental area of Department of Plant Pathology University of Agriculture, Faisalabad, and were screened out for the sources of resistance against *Erwinia*/blackleg.

Physiological data

Temperature

For the growth of bacteria at different temperatures, Petri plates containing the nutrient agar medium were inoculated with the bacterium and incubated at 27, 32 and 37°C. The diameter of colony was recorded daily for four days and for each temperature, two readings at right angle were recorded for each Petri plate. The data was analyzed statistically to visualize the difference among the treatments.

pH data

The growth of *Erwinia* at pH levels 6, 7 and 8 was studied on nutrient agar medium. Briefly, 250 ml of media was taken in a flask and adjusted to pH level 6, 7 and 8 by adding the appropriate amount of 1.0 normal HCl or NaOH solutions. The solidified medium was inoculated with bacterium inoculums in the center and the Petri plates were incubated at 32°C. The diameter of the colony at each pH level was recorded every day for four days of incubation by taking two readings at right angle for each Petri plate, and then mean of the two readings was taken. The data was analyzed statistically to visualize the difference among the treatments.

Pectinolytic activity test

Pectin substances are the primary constituent of the middle lamella and are the structural elements of the primary cell wall. The bacterial isolates were grown on Hankin's medium incubated at 32°C for four days and then changes in the plates were observed.

Biotest on potato tuber slices

Potato tubers of cultivar Faisalabad White were washed and sterilized with 96% ethanol and flaming, 8-10 mm slices were cut with a sterile knife and placed into Petri dishes. Then, 10-ml of 0.01 M magnesium sulphate was added to each Petri dish, and each slice was inoculated with a drop of 10 µL of 10^8 cfu/ml of *Erwinia* isolates already grown on King's medium B for 24 h and incubated at 27°C for 48 h. Each bacterial isolate was tested three fold. A positive reaction was recorded when the potato slices showed soft rotting within two days.

Statistical analysis

All the data on percent plant infection and disease severity in the field conditions were subjected to statistical analysis to determine the effect of plant extract and bio-pesticides, and treatment means when compared by Least Significant Difference (LSD) or Duncan's multiple range (DMR) test (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

Characteristics of the bacterium

The morphological characters of bacterium isolated from infected potato tubers with blackleg disease were Gram negative and rod shaped. The cultural characteristics were grayish-white, circular and smooth colonies, which appeared on nutrient glucose agar medium after 24 h of incubation at 30°C. The symptoms on plant were: Stem shows brown black or jet black color, upward curling of and the infected plant becomes pale green. On tuber water soaked, lesion appeared which later on become mushy and softened. Agar colonies were circular, smooth and grayish-white. Agar salt was Grayish-white; optimum temperature was 28 to 32°C. Rods were 0.6 to 0.8 by 1.25 to 2.5 µ, actively motile by means of peritrichous flagella. The rods are white and Gram-negative. The cause of blackleg disease in potato, also affects cucumber and other vegetables. The bacterium was identified as *E. carotovora subsp. carotovora*

Conventional physiological tests

The following physiological tests of *Erwinia* were conducted under the laboratory conditions.

Effect of different temperatures on the in vitro growth of Erwinia

The bacteria was inoculated on nutrient agar medium and incubated at 27, 32 and 37°C. The bacterial growth increased with the incubation period at each temperature. The maximum growth of 7.05 cm was observed at 32°C after 96 h of incubation. The data recorded at different temperatures for different intervals of time were analyzed statistically as shown in Table 1 and Figure 1. These values show the difference among growth at different temperatures and values showing similar letters do not differ from each other at alpha 0.05 and LSD for temperature and incubation period = 0.3241.

Growth at different pH levels

The growth of *E. carotovora* subsp*. carotovora* was studied at different pH levels on nutrient agar medium after adjusting the pH of medium at 6, 7 and 8 for

Table 1. Effect of different temperature ranges on *in vitro* growth (cm) of *Erwinia carotovora* at various incubation periods.

Incubation period (days)	Temperature ranges (°C)			Mean
	27	32	37	
1	3.317	3.6	2.55	3.156
2	4.5	4.817	3.05	4.122
3	5.617	5.917	4.4	5.311
4	6.817	7.05	5.25	6.372
Mean	5.063	5.346	3.813	

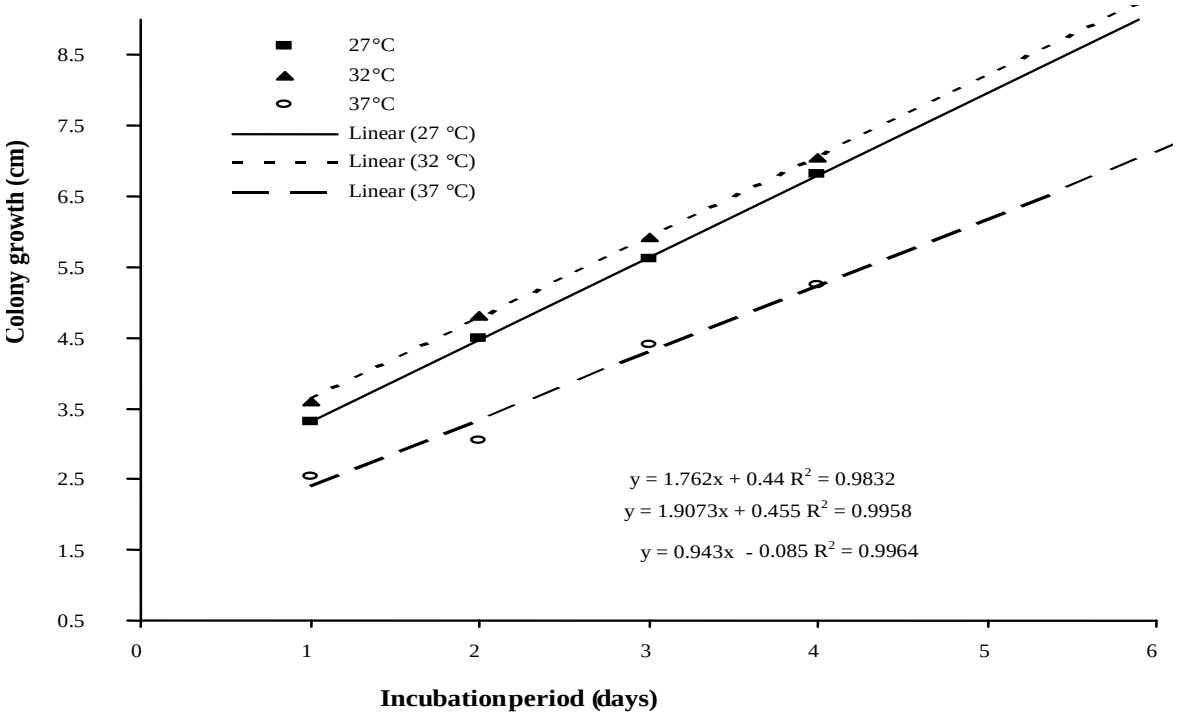

Figure 1. Effect of different temperature ranges on *in vitro* growth (cm) of *Erwinia carotovora* at various incubation periods.

different period of incubation of four days. The bacterial growth varied at each pH level but increased with the increase in the incubation period. The effect of pH level on bacterial growth was recorded maximum on forth at pH 7 and 8 as 8.133 and 7.25 cm, respectively. The comparison of means of treatment at all incubation periods showed a statistical difference between bacterial growths at all pH levels as shown in Table 2 and Figure 2. The values show the difference among growth at different temperatures and incubation period at alpha 0.05 and LSD for temperature and incubation period = 0.1294.

Pectinolytic activity test of Erwinia

The bacterial isolates grown on Hankins's medium and incubated at 32°C for three days and clear zone around the colonies was observed, and results indicated the degradation of pectin due to secretion of pectate lyase by bacteria.

Biotest on potato tuber slices

The slices of potato cultivar Faisalabad white showed soft rotting within two days. The bacteria were identified as *Erwinia carotovora*, according to pathological, morphological and cultural characteristics.

Management of blackleg/*Erwinia*

The disease management studies were conducted by

Table 2. Effect of different pH levels on *in vitro* growth (cm) of *E. carotovora* at various incubation periods.

Incubation period	pH levels			Mean
	6	7	8	
1	2.533	4.783	4.533	3.95
2	4.533	6.283	5.633	5.483
3	5.8	7.216	6.516	6.511
4	6.616	8.133	7.25	7.333
Mean	4.87	6.604	5.983	

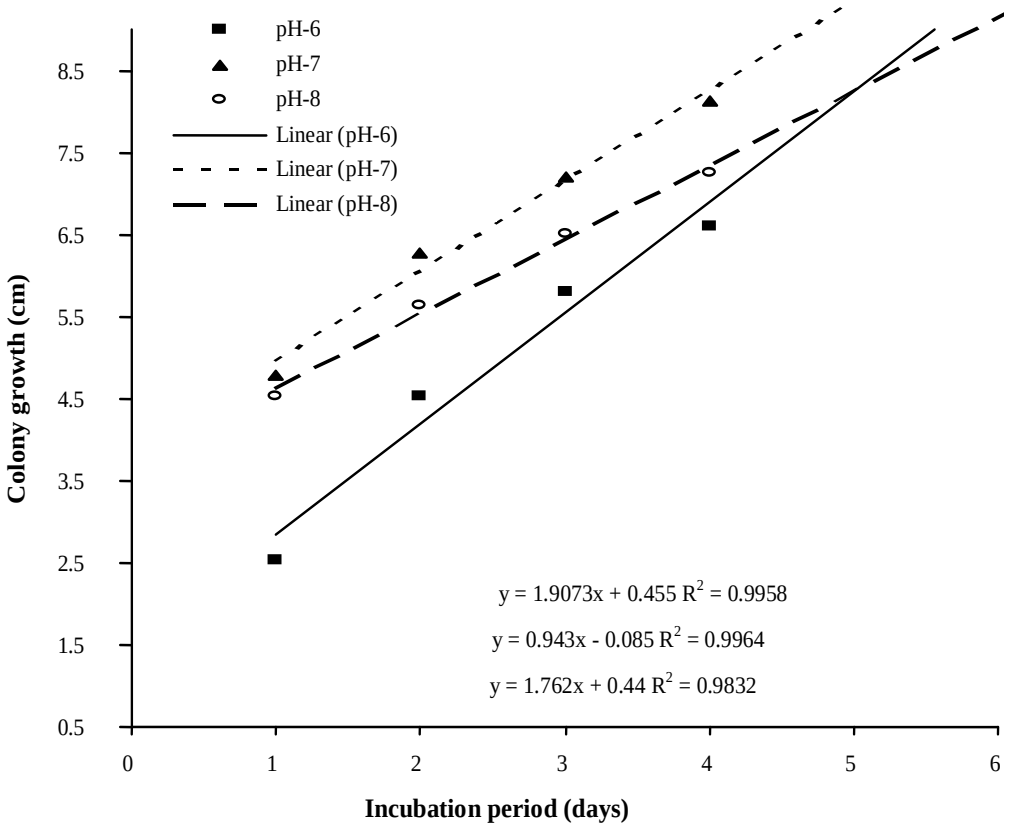

Figure 2. Effect of different pH levels on *in vitro* growth (cm) of *E. carotovora* at various incubation periods.

exploiting various disease management strategies like, host resistance, plant extracts and bio-products in laboratory as well as field conditions.

In vitro evaluation of plant extracts against of *E. carotovora* subsp. *carotovora*

Neem (A. indica) extract

The extract from neem (*A. indica*) was used against *Erwinia* at three different concentrations. These extracts inhibited the growth up to 1.58 cm on fifth day of incubation period as shown in Table 3 and Figure 3.

Means sharing similar letters in rows are statistically non-significant and represent comparison among interaction means at alpha 0.05 and LSD = 0.1157.

Garlic (A. sativum) extract

The extract from garlic (*A. sativum*) was used against *Erwinia* at three different concentrations. These extracts inhibited the growth up to 1.500 cm on the fifth day of incubation period as shown in Table 4 and Figure 4. Means sharing similar letters in rows are statistically non-significant and represent comparison among interaction

Table 3. *In vitro* antibacterial effect of Neem extract at three different concentrations and various incubation periods on growth (cm) of *E. carotovora* subsp. *carotovora.*

Incubation period (days)	Concentration				Mean
	S/50	S/25	S	Control	
1	0.85	1.1	1.35	0	0.825
2	1.017	1.233	1.433	0	0.921
3	1.133	1.37	1.5	0	0.987
4	1.2	1.383	1.567	0	1.037
5	1.217	1.383	1.583	0	1.046
Mean	1.083	1.283	1.487	0	

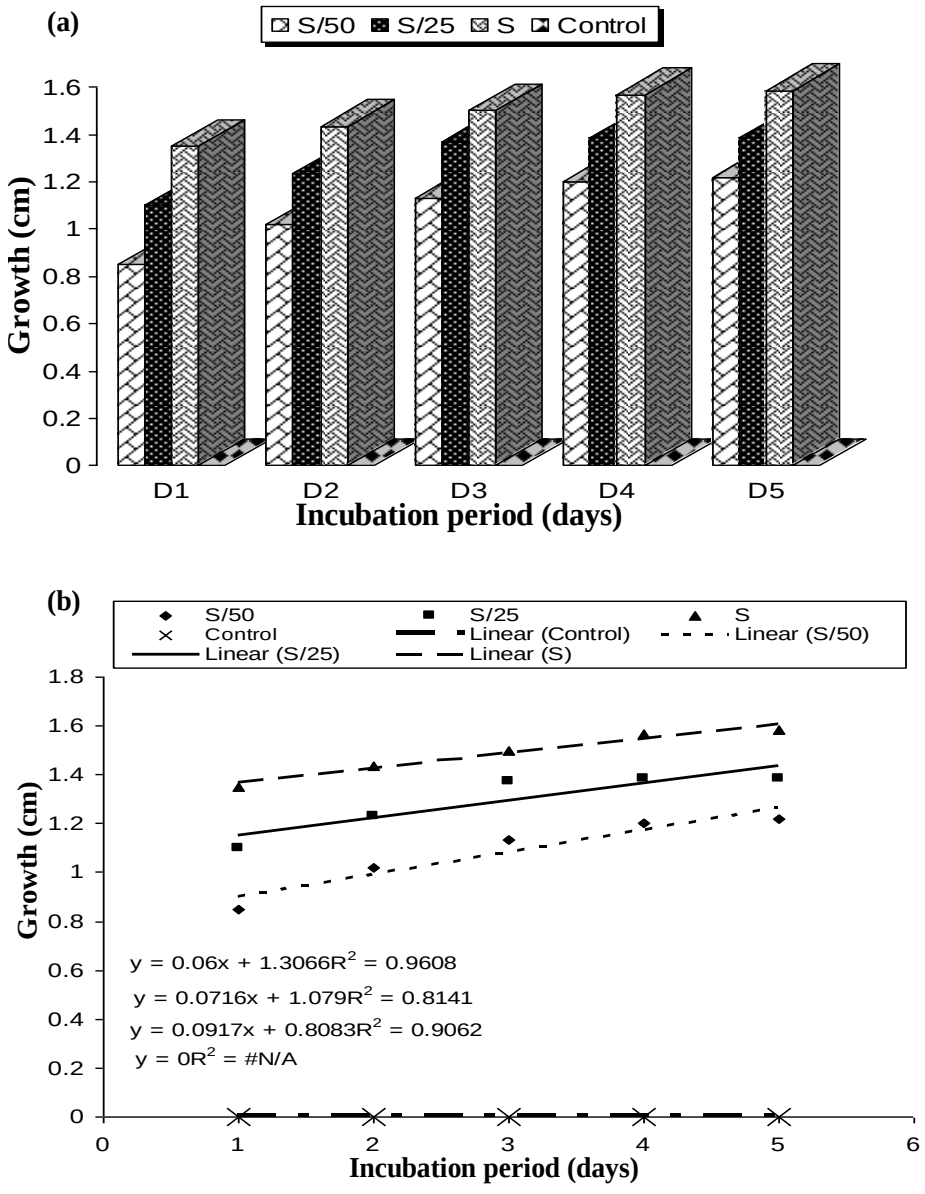

Figure 3. (a and b) Effect of neem at three different concentrations and various incubation periods on *in vitro* growth (cm) of *E. carotovora* subsp. *carotovora* the cause of blackleg disease of potato.

Table 4. *In vitro* inhibitory effect of Garlic extract at three different concentrations and various incubation periods on *invitro* growth (cm) of *E. carotovora* subsp. *carotovora*.

Incubation period (days)	Concentration				Mean
	S/50	S/25	S	Control	
1	0.65	0.983	1.300b	0	0.733
2	0.75	1.117	1.4	0	0.816
3	0.817	1.2	1.45	0	0.866
4	0.867	1.233	1.5	0	0.9
5	0.883	1.233	1.5	0	0.904
Mean	0.793	1.153	1.43	0	

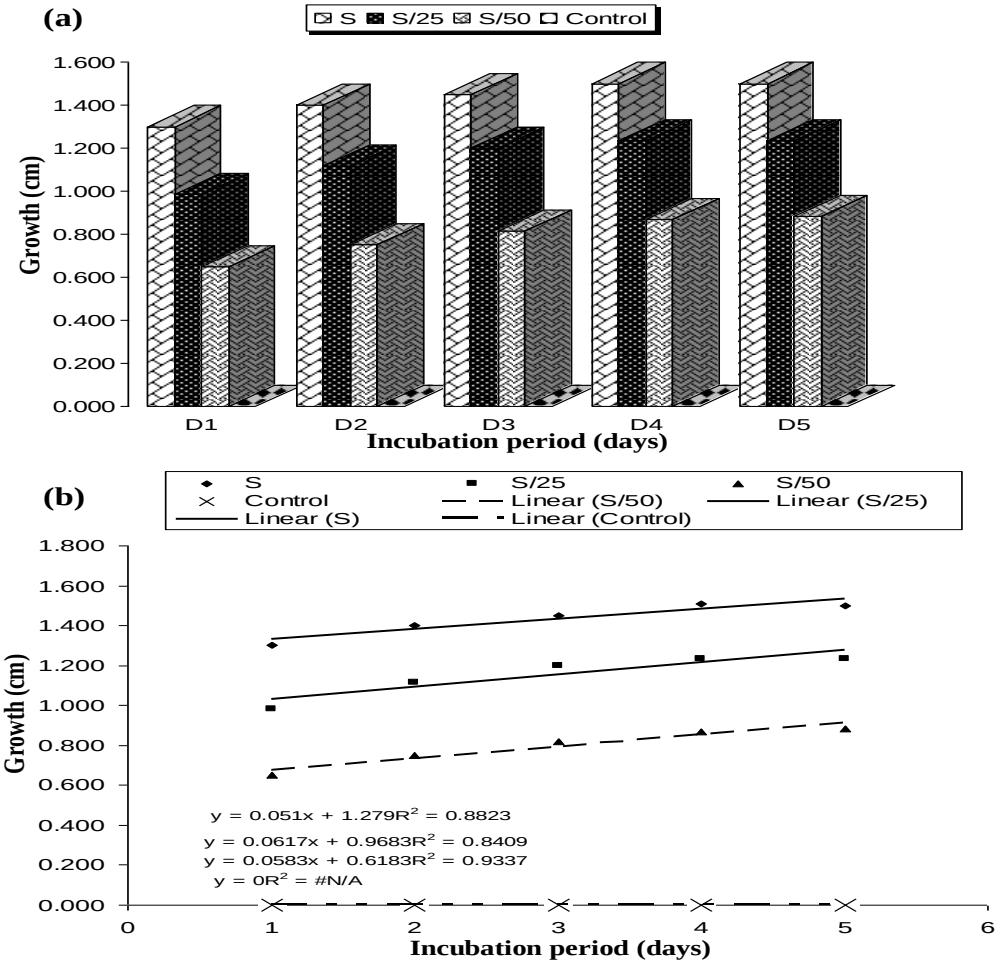

Figure 4. (a and b) Effect of garlic extract at three different concentrations and various incubation periods on *in vitro* growth (cm) of *E. carotovora* subsp. *carotovora* the cause of blackleg disease of potato.

means at alpha 0.05 and LSD=0.1005.

Onion (A. sepa) extract

The extract from onion (*A. sepa*) was used against *Erwinia* at three different concentrations. These extracts inhibited the growth up to 1.504 cm on the fifth day of incubation period as shown in Table 5 and Figure 5. Means sharing similar letters in rows are statistically non-significant and represent comparison among interaction means at alpha 0.05 and LSD=0.1005.

Table 5. Effect of Onion extract at three different concentrations and various incubation periods on *in vitro* growth (cm) of *E. carotovora* subsp. *carotovora*.

Incubation period (days)	Concentration				Mean
	S/50	S/25	S	Control	
1	0.651	0.987	1.304	0	0.731
2	0.752	1.115	1.405	0	0.814
3	0.815	1.199	1.456	0	0.868
4	0.866	1.231	1.504	0	0.904
5	0.881	1.231	1.504	0	0.908
Mean	0.793	1.153	1.43	0	

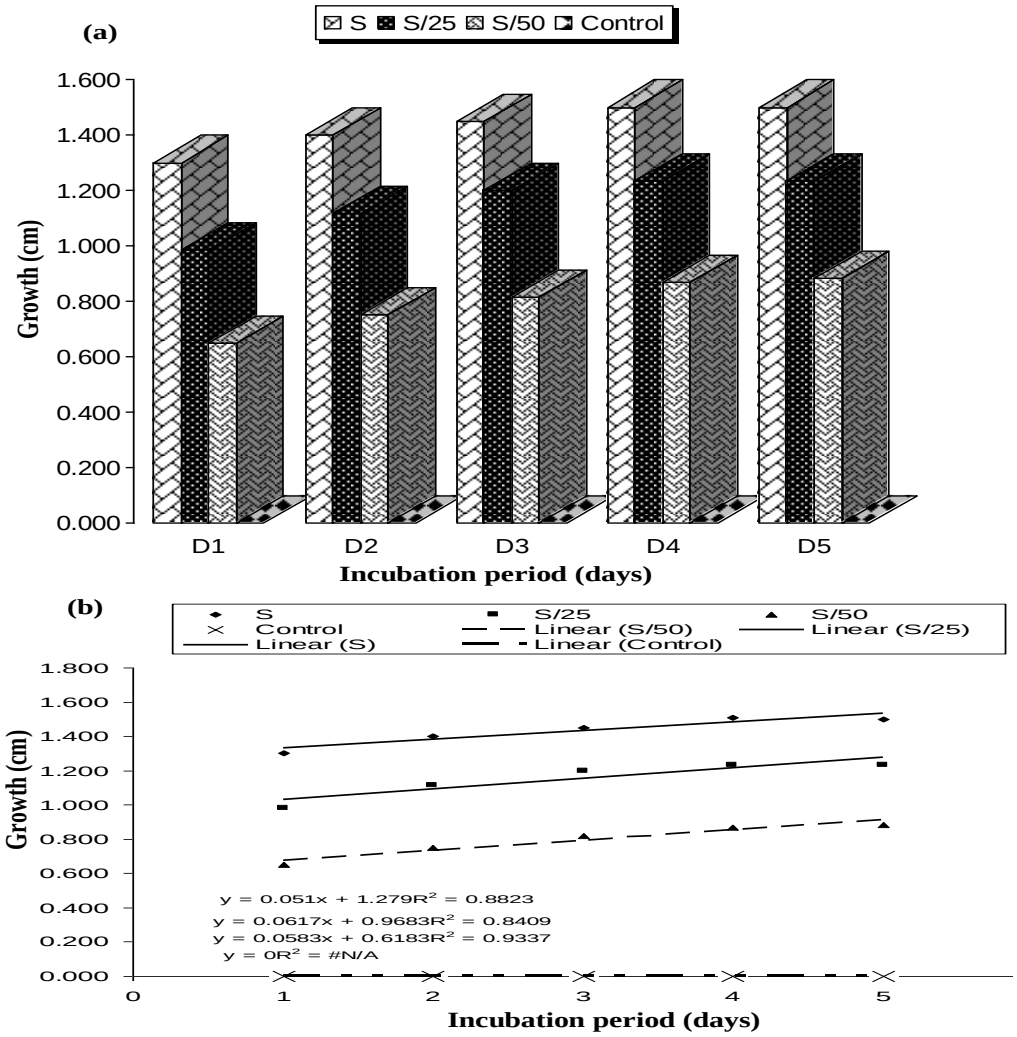

Figure 5. (a and b) Effect of onion extract at three different concentrations and various incubation periods on *in vitro* growth (cm) of *E. carotovora* subsp. *carotovora*.

Vampire (bio-product)

Vampire was also found very effective against the *E. carotovora* causing blackleg of potato and inhibited the growth of pathogen at all the three concentrations viz. 60, 80, 100 ppm and created the inhibition zone. The comparison of means showed a little differentiation among the effect of doses. However the higher dose (100 ppm) was found more effective than other two (60 and 80 ppm) as observed in Table 6 and Figure 6. Means

Table 6. Effect of Vampire (bio-product) at three different concentrations and various incubation periods on *in vitro* growth (cm) of *E. carotovora* subsp. *carotovora*.

Incubation period (days)	Concentration (ppm)				Mean
	60	80	100	Control	
1	2.267	2.87	3.25	0	2.096
2	2.46	2.97	3.4	0	2.208
3	2.56	3.083	3.5	0	2.287
4	2.7	3.183	3.6	0	2.371
5	2.75	3.25	3.667	0	2.417
Mean	2.55	3.07	3.483	0	

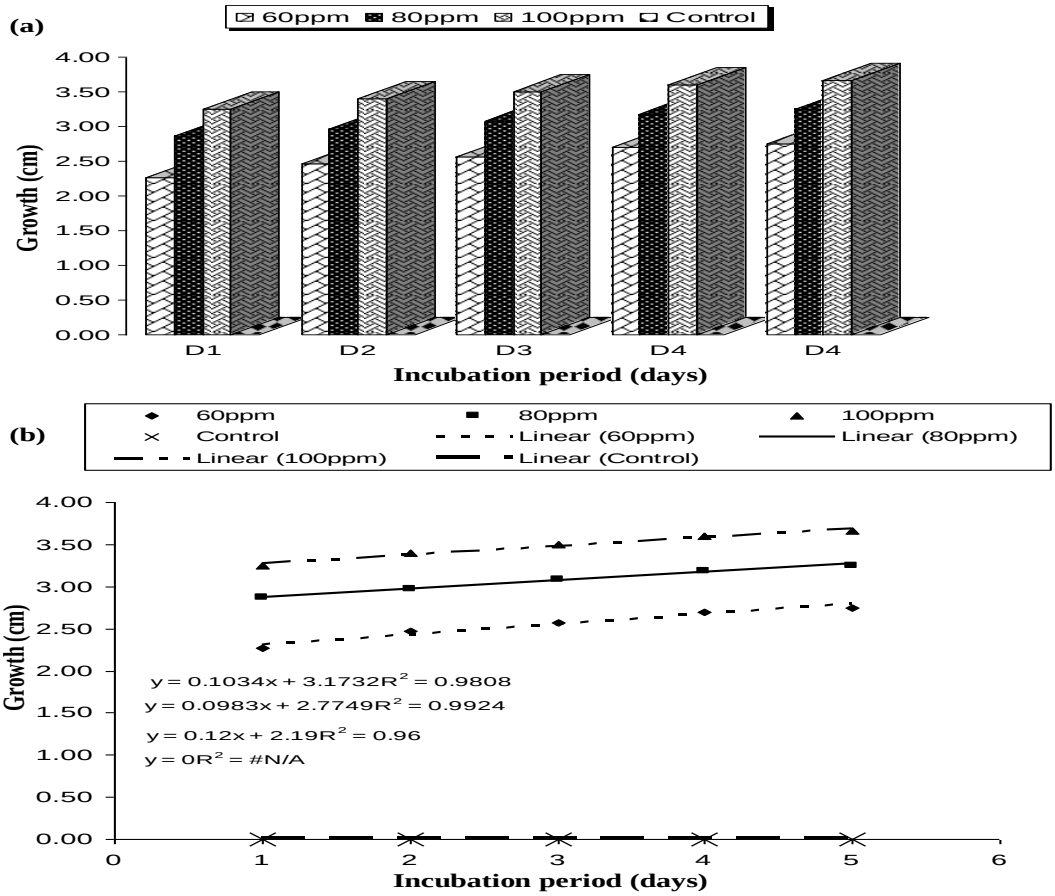

Figure 6. (a and b) Comparison of means of effect of Vampire (bio-product) at three different concentrations and various incubation periods on *in vitro* growth (cm) of *E. carotovora* subsp. *carotovora*.

sharing similar are statistically non-significant (P>0.05) and represent comparison among interaction means at LSD=0.1031 and alpha = 0.050.

Biosal (bio-product)

Biosal (bio-product) inhibited the growth of *E. carotovora subsp. carotovora* and created a zone of inhibition at all the three concentrations. The maximum inhibition was recorded at concentration 100 ppm (4.03cm) on the fifth day of incubation (Table 7). The inhibition was more and frequent in diameter but in later days the rate of inhibition became slower as shown in Figure 7b. Means sharing similar letters in a row are statistically non-significant (P>0.05) and represent comparison among interaction

Table 7. Effect of Biosal (Bio-product) at three different concentrations and various incubation periods on *in vitro* on growth (cm) of *E. carotovora* subsp. *carotovora* the cause of blackleg of potato.

Incubation period (days)	Concentration (ppm)				Mean
	60	80	100	Control	
1	3.367^{f}	3.383^{f}	3.483^{ef}	0.00^{j}	2.558
2	3.450^{ef}	3.500^{ef}	3.667^{cde}	0.00^{j}	2.654
3	3.583^{def}	3.650^{de}	3.750^{bcd}	0.00^{j}	2.746
4	3.733^{bcd}	3.817^{bcd}	3.883^{f}	0.00^{j}	2.858
5	3.783^{bcd}	3.917^{ab}	4.033^{a}	0.00^{j}	2.933
Mean	3.583	3.653	3.763	0	

Means sharing similar letters in a row are statistically non significant (P>0.05) and represent comparison among interaction among overall means. LSD= 0.2173 alpha = 0.050.

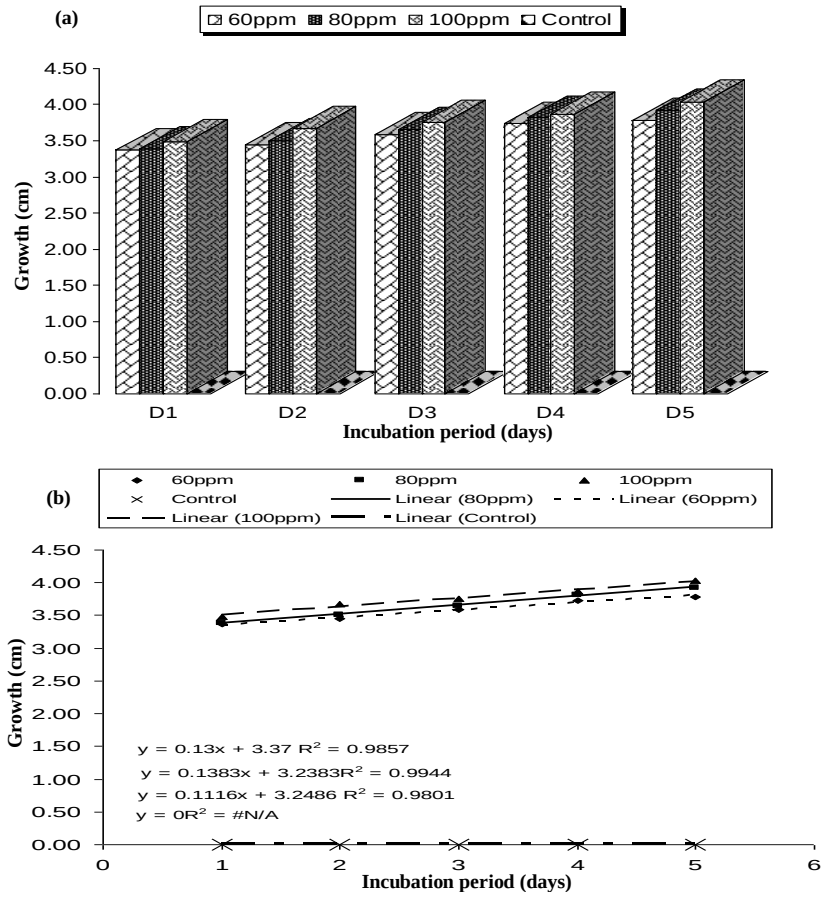

Figure 7. (a and b) Comparison of means of effect of Biosal (Bio-product) and various incubation periods on *in vitro* growth (cm) of *Erwinia carotovora* subsp. *carotovora* at three different concentrations.

among overall means. LSD=0.2173 alpha=0.050.

Field experiment

Host resistance (screening of potato germplasm against the blackleg disease)

Fifteen varieties namely, Cardinal, Faisalabad white, Faisalabad red, Accent, Harmony, Lady Rosette,Desiree, SH70, Vales Everest, Hermes, Paramount, Fontane, Atlantic, Melody and Orla were grown during the year 2010 and 2011 in the experimental area of the Department of Plant Pathology University of Agriculture Faisalabad for the screening purposes. All these varieties showed different response to the disease, Cardinal was found immune while Faisalabad white was highly

Table 8. Disease incidence, level of resistance/susceptibility of 15 potato varieties against blackleg disease in 2010.

S/N	Variety	Disease incidence mean	Rating	Response
1	Cardinal	0.093[g]	1	Immune
2	Harmony	41.657[c]	6	S
3	Lady Rosetta	46.632[b]	6	S
4	Paramount	28.087[c]	4	MR
5	Orla	18.687[e]	3	R
6	Melody	28.120[e]	4	MR
7	Atlantic	29.433[e]	4	MR
8	Vales Everest	38.780[d]	5	MS
9	Hermes	19.377[f]	3	R
10	Fontane	28.617[e]	4	MR
11	Accent	47.417[b]	6	S
12	Faisalabad White	63.887[a]	7	HS
13	Faisalabad Red	19.657[f]	3	R
14	SH70	37.35[7]	5	MS
15	Desiree	38.663[d]	5	MS

Means sharing similar letters in a row are statistically non significant (P>0.05) and represent comparison among interaction among overall means. LSD= 0.2173 alpha = 0.050."LSD=2.3233 and alpha= 0.05.

Table 9. Disease incidence, level of resistance/susceptibility of 15 potato varieties against blackleg disease in 2011.

S/N	Variety	Disease incidence mean	Rating	Response
1	Cardinal	0.017[g]	1	Immune
2	Harmony	41.387[d]	6	S
3	Lady Rosetta	48.780[b]	6	S
4	Paramount	29.410[f]	4	MR
5	Orla	19.619[g]	3	R
6	Melody	29.463[f]	4	MR
7	Atlantic	28.690[f]	4	MR
8	Vales Everest	39.913[de]	5	MS
9	Hermes	19.473[g]	3	R
10	Fontane	29.900[f]	4	MR
11	Accent	46.090[c]	6	S
12	Faisalabad White	65.460[a]	7	HS
13	Faisalabad Red	19.673[g]	3	R
14	SH70	39.277[e]	5	MS
15	Desiree	39.540[e]	5	MS

Means sharing similar letters in a row are statistically non significant (P>0.05) and represent comparison among interaction among overall means. LSD= 0.2173 alpha = 0.050."LSD =1.5306 and alpha= 0.05.

susceptible. The varieties like Faisalabad Red, Hermes and Orla resistant, Lady Rosette, Ascent and Harmony, susceptible while Paramount, Melody, Atlantic and Fontane were found moderately resistant and three other varieties like Desiree, SH-70 and Vales Everest were found moderately susceptible (Tables 8 and 9).

DISCUSSION

Erwinia is a rod shaped bacterium that infects a variety of vegetables and plants, including carrots, potatoes, cucumbers, onions, tomatoes, lettuce and ornamental plants like iris. These widespread microbes can be found in soil, guts of insects, water and suspended aerosols in air. A major problem in agriculture, the microbes ceaselessly invade crops of potatoes and other vegetables in the fields or in storage that cause plant tissues to become soft and watery, and eventually turn slimy and foul-smelling. The pathogenicity of *E. carotovora* subsp. *atroseptica* is restricted to potatoes in temperate temperatures, while *E. carotovora* subsp.

carotovora infects a much broader host of plants, including potatoes, in warmer climates.

Physiological characterization

The Physiological properties and behavior of an organism can be applied for its taxonomic identification and differentiation which aids in understanding the life of an organism as a whole since the fundamental phenomena of life are essentially same throughout the whole series of living organisms. Temperature has a large effect on the specific growth rate of *E. carotovora* subsp. *carotovora.* The growth of *Erwinia* increased at all the three temperatures with the passage of incubation period on the media and the maximum growth 7.05 cm (Table 1) was recorded at 32°C on the fourth day (96 h) of incubation and these results are in accordance with the results reported by Serfontein et al. (1991) that both pathogen *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp*. carotovora* are virulent at the range of 28 to 32°C. During the present study, the growth of *Erwinia* was also was recorded at 37°C similar to Nielson (1978), Helias et al. (1998) and Smadja et al. (2004) also reported that *Erwinia* grew at 37°C, These results are also correlate with the results of field experiment of Molina and Harrison (1989) that the seed piece decay increased with the increase in soil temperature.

One of the important factors responsible for the bacterial growth is the hydrogen ion concentration (pH) of the medium upon which the bacteria grow. The pH exerts a decided effect on the rate of growth, the bacteria grown under different pH levels determined that *Erwinia* grow well under the slightly alkaline pH. The pH 7 and 8 were found most suitable as there was maximum growth 8.133 and 7.25 cm after four days of incubation, which resembles with the results of Shrestha et al. (2005). The physiological tests were applied to differentiate *E. carotovora* subsp. *carotovora.* It was concluded from earlier studies that the tubers obtained from apparently blackleg free tubers were also contaminated with *E. carotovora* subsp. *carotovora* (De Boer et al., 1975; Nielson, 1978), where the relative proportion of *E. carotovora* subsp. *carotovora* varied widely on the tubers. *E. carotovora* subsp. *carotovora* grew at 37°C, while *E. carotovora* subsp. *atroseptica* did not and it produced reducing substances from sucrose; this is a physiological characteristic for differentiation.

Management of disease

Heavy losses have been reported in different zones of Pakistan as Geddes (1997) reported that blackleg disease of potato was well distributed in different production zones of Pakistan. Similarly, Ahmad et al. (1995) found blackleg disease of potatoes in five out of eight zones of Pakistan. For this purpose, different plant extracts and bio-products were evaluated against the *Erwinia* isolated from infected potato for its management. Serrano et al. (2000) also reported that the bacterium *E. carotovora* subsp. *carotovora* produced blackleg and soft rot diseases in potato plants and tubers. Sixty-three and sixty-nine transgenic potato clones were evaluated in the greenhouse for resistance to blackleg and soft rot diseases, respectively.

In vitro evaluation of plant extracts against *E. carotovora* subsp. *carotovora*

The three plants' extracts namely neem (*Azadirachta indica*), onion (*Allium sepa*) and garlic (*Allium sativum*) extracts were used against *E. carotovora* subsp. *carotovora* under the controlled conditions. These inhibition zone recorded from these three extract were found to be 1.583 cm (Table 3) by neem extract, 1.500 cm (Table 4) by garlic extract and onion extract 1.504 cm (Table 5) after five days of incubation. These results are in accordance with the results of Jahan et al. (2007), they used the neem oil against some pathogenic bacteria and observed growth inhibition of the bacteria. Bdliya and Dahiru (2006) also reported that neem aqueous extracts reduced significantly the incidence and severity of tuber soft rot, and could therefore be used to reduce losses due to this disease in storage, while ironweed and *Siamese cassia* aqueous leaf extracts gave moderate control of the disease. El Astal (2004) used garlic extract against bacteria and reported that the aqueous extract of garlic has high antibacterial activity.

In vitro evaluation of Vampire and Biosal as bio-products against *E. carotovora* subsp. *carotovora*

The two bio-products, Vampire and Biosal, were used against *E. carotovora* subsp. *carotovora* under the controlled conditions. These inhibition zone recorded from these two bio-products were found to be 3.667 ppm (Table 6) by Vampire and 4.033 ppm (Table 7) by Biosal at the higher dose (100 ppm). This dose was found more effective than other two doses (60 and 80 ppm), after five days of incubation. These results are in accordance with the results of Jahan et al. (2007). Biosal and Vampire showed excellent work in inhibiting the pathogen growth.

Field experiment

The Genetic resistance is regarded as one of the best methods for the control of bacterial blackleg of potato tubers (Pasco et al., 2006) and according to Rousselle-Bourgeois and Sylvie (1995), the major cause of blackleg/soft rot of stored potatoes is *E. carotovora* subsp. *carotovora* and resistant varieties play the major role for the control of this disease. Out of fifteen varieties

namely, Cardinal, Faisalabad white, Faisalabad red, Accent, Harmony, Lady Rosette, Desiree, SH70, Everest, Hermes, Paramount, Fontane, Atlantic, Melody and Orla, the variety Cardinal was found immune while Desiree and Faisalabad white were susceptible. These results are similar to the results of Mairaj (2004). Bain and Perombelon (1988) ranked eight potato cultivars for resistance to tuber soft rot caused by *E. carotovora* subsp. *carotovora* and assessed these using single site, infectivity titration and vacuum infiltration tests. Similarly many potato varieties were found resistant during the experiments conducted by Pasco et al. (2006).

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