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INOCULUM SOURCES, DISEASE INCIDENCE AND SEVERITY OF BACTERIAL BLACKLEG AND SOFT ROT OF POTATO

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Abstract

Comprehensive surveys during spring, summer and fall, 2007-2009 were conducted in the major potato-growing areas of KPK to record the incidence, severity and distribution of blackleg and soft rot of potato caused by the different subspecies of Erwinia. Maximum disease severity (40%) and disease incidence (60-75%) was found in the spring and summer potato crop grown in Abbottabad, Swat and Mardan, Pakistan. The frequencies of *Erwinia carotovora* subsp. *atroseptica* (Eca), E. c. subsp. *carotovora* (Ecc) and *Erwinia chrysanthemi* (Ech), based upon the survey of 42 locations, were found to be 48%, 45%, and 7%, respectively. Regarding soil, diseased plant debris and seed potatoes as sources of inoculum for Eca, healthy plant material, potted un-sterilized soil and healthy seed potatoes were inoculated with Eca, kept in screen-house and monitored for changes in bacterial population over a period of three months. It was found that seed potatoes were the most important source of primary inoculum as the bacterial population considerably increased in numbers throughout the monitoring period. The bacterial population in the inoculated plant debris did decrease but the rate of decline was quite slow and therefore, diseased plant debris could be considered as a good source of primary inoculum for the appearance of black leg disease in the next season.

Introduction

Potato (*Solanum tuberosum* L.) is the most important vegetable crop of the world, including Pakistan, ranking number one among all vegetables both in production and consumption (Abbas *et al*., 2011). The climate of Pakistan, especially of KPK, is very suitable for the production of potatoes. In Pakistan, potato cultivation occupies an area of 112000 ha with a total production of 2024900 tons, while in KPK it occupies an area of 9600 ha with a total production of 125100tons (Anonymous, 2005). The average per hectare production of potato in KPK is well below its potential. One of the reasons for this low yield is the occurrence of different bacterial diseases including black leg and soft rot of potatoes. These bacterial diseases cause heavy losses to potato crop not only in the field but also in the storage where the bacteria are transmitted from the diseased tubers to the healthy ones and cause rotting of the stored potatoes.

Erwinia carotovora (Ec) is the causal agent of black leg and soft rot of potato which is a Gram-negative, facultatively anaerobic, rod-shaped bacterium with peritrichous flagella, that produces deep pits on crystal-violet-pectate (CVP) medium. Many economically important food crops including potatoes and tomatoes can be affected by this disease. Two subspecies of *Erwinia carotovora*, i.e., E. c. subsp. *atroseptica* (Eca) (Van Hall)/Dye, and E. c. subsp. *carotovora* (Ecc) (Jones)/Bergey, attack potatoes. Pathogenicity of Eca strains (mainly causing blackleg of potatoes) is usually restricted to potatoes in cool and temperate climates, while Ecc strains (causing potato soft rot and, in some cases blackleg too) have a wide distribution in both temperate and tropical zones, showing wider host ranges than those of the other subspecies (Wells & Moline, 1991). Eca (Van Hall)/Dye is usually considered as the major causal agent of potato black leg in cool and temperate regions (Oliveira *et al*., 2003). However, Ecc (Jones)/Bergey and *Erwinia chrysanthemi* (Ech) (Berkholder) have also been reported to cause similar symptoms (Gudmestad & Secor, 1993; Duarte, 1999). Stanghellini & Menely (1975) found that Ecc strains were associated with potato black leg in Arizona and Colorado, USA. Degefu *et al*., (2006) reported that Eca, Ecc and Ech are the different sub-species of *Erwinia* that cause the diseases commonly known as blackleg, aerial stem rot and soft rot on potato. Blackleg and aerial stem rot affect vines during the growing season, whereas soft rot affects tubers in the field and during transit and storage. The three species can cause soft rot under cool and moist conditions. Eca is the major cause of blackleg, a blackening of the stem base of potato plants, which originates from the mother tuber (Pérombelon & Kelman, 1980). Ecc mainly causes aerial stem rot (aerial blackleg), but under high temperatures it has been reported to cause blackleg like symptoms. Ech also induces blackleg-like symptoms (Degefu *et al*., 2006).

Although infested crop residues and rotting tubers are among the important sources of inoculum, latent infections in seed tuber provide the major source of infection in potato production (Hannukkala & Segertedt, 2004). De Mendon & Stanghellini, (1979) investigated the endemic and soil-borne nature of *Erwinia carotovora* var. *atroseptica* (biotype KSB), a pathogen of mature sugar beets, in the Sulphur Springs Valley, Arizona, USA. During a 4-month period, biotype KSB was recovered from the rhizosphere of artificially infested sugar beets. The researchers found that the pathogen (biotype KSB) survived at soil depths of 12 cm or more. Moreover, they observed that the bacterium was able to survive in soil for longer time (135 days) at 0-10^{o}C but persisted only briefly when soil was dry. Powelson, (1980) concluded that blackleg of potatoes in a center pivot irrigated circle of Kennebec potatoes in Oregon’s (USA) Columbia Basin was caused by either Eca or Ecc. Eca was recovered only early in the growing season whereas Ecc was associated with blackleg symptoms later in the season. A soft rot, atypical of blackleg, was widespread throughout the circle and was most prevalent during the latter part of the growing season. Initially, the vines near the soil surface appeared translucent, watery and soft. The pathogen associated with these symptoms was exclusively Ecc.

Oliveira *et al*., (2003) determined the occurrence of Eca, Ecc and Ech in blackleg-affected potatoes under the conditions found in the State of Rio Grande do Sul (RS), Brazil. Four hundred strains were identified as either Eca, Ecc or Ech. Although the three erwinias were found in RS potato fields, only three strains of Ech were found in one field. Frequencies of Eca and Ecc were 55% and 42%, respectively. Black leg reportedly (Turkensteen, 1986) causes losses of 10-30% whereas soft rot causes 2-10% losses in KPK. As far as research on any aspects of bacterial diseases in KPK is concerned, except for a few yield-loss-estimate reports, absolutely no documented work has been done so far. Therefore, work needs to be done to record the incidence and distribution, to assess severity and to investigate the inoculum sources of these diseases. In this paper we report the incidence and severity of black leg and soft rot of potato in the major potato-growing areas of KPK as well as the possibility of soil, plant debris and seed potatoes as sources of inoculum for the causal organism of the disease.

Materials and Methods

Survey and sampling: In order to record the incidence, severity and distribution of black leg and soft rot of potatoes, and to collect diseased plant samples showing typical disease symptoms, several comprehensive surveys (spring, summer and autumn crop, 2007-2009) were conducted in the major potato-growing areas of KPK (Fig. 1). For this purpose, the main potato-growing areas were divided into five different agro-ecological zones (primarily based upon soil type and climate) i.e. Northern (Mingora, Madain, Behrain, Shangla, Swat, and Kalam), Southern (D.I. Khan and Bannu), Eastern (Abbottabad, Naran, Kaghan, Mansehra, Haripur, and Battagram), Western (Parachinar, South Waziristan, and Razmak) and Central (Mardan, Nowshera, Peshawar, Swabi, and Charsadda). A total of 42 locations in 11 districts (Table 1), with a minimum of two-three fields per location, were selected. Only fields with plants showing disease symptoms were surveyed. Within each field, two-three spots were randomly chosen. Each spot consisted of two rows (10-12 feet long) of potato plants. Total number of plants per spot and number of plants showing any disease symptoms were counted. Farmers, if present, were interviewed for their perceptions of the diseases, management strategies used and reasons for the prevalence of these diseases. Also, diseased plant specimens were collected in order to isolate the causal organisms. Samples (tubers, soil and debris) were put in paper bags, kept cool and processed as soon as possible (to reduce the chances of secondary invaders) in the KPK Agricultural University's Clinical Plant Pathology Laboratory.

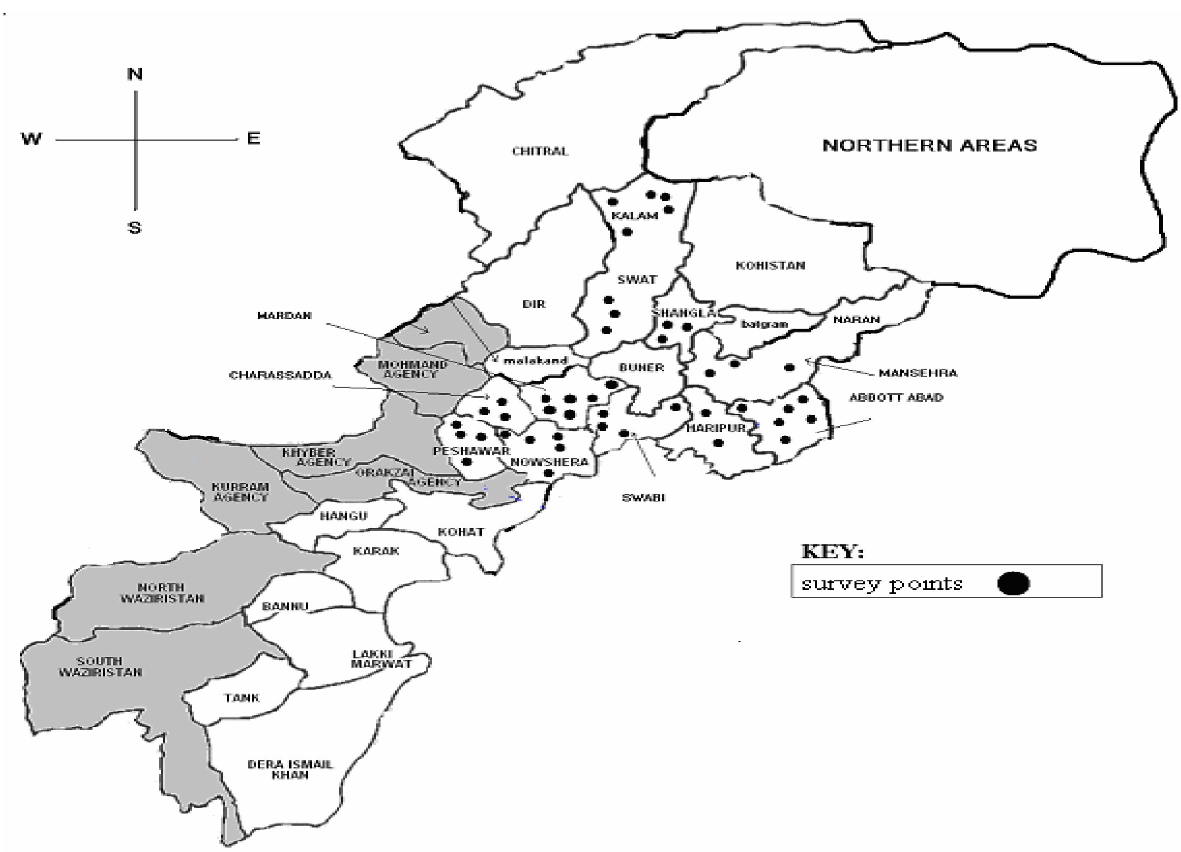

Fig. 1. Map of KPK showing locations surveyed (circles) for recording the incidence, severity and distribution of potato blackleg and soft rot.

Table 1. Blackleg and soft rot incidence, severity and distribution in KPK during spring and fall potato-growing seasons, 2007-09.

District	Location	Variety	Disease incidence (%)	Disease severity (%)	Lab. results	Observations
Abbottabad	Kakool	Raja	10	2	Ecc	Clay loam soil, Poor crop management
Abbottabad	Hazara	Raja	30	13	Eca	Clay loam soil
Abbottabad	Kakool	Desiree	75	40	Eca	Clay loam soil, Poor crop management,
Abbottabad	Kakool	NK	35	10	Eca	Clay loam soil, Good crop management
Abbottabad	Logan colony	Desiree	22	10	Ecc	late blight losses 20%
Battagram	Pagora	Rodio	10	3	Ecc	Sandy soil, poor crop
Charsadda	Umarzai	Desiree	10	7	Ecc	crop age 2½ months
Charsadda	dawoodzai	Raja	10	3	Ecc	crop age 2 monthsLate blight 50%
Charsadda	mandani	Raja	30	10	Ecc	crop age 2 months
Haripur	Dobandi	NK	5	3	Ecc	Well managed cropping practices
Harripur	Saraye sala	NK	20	12	Ecc	Clay Loam soil, Good crop management
Swat	Behrain	Raja	20	14	Eca	late blight losses 30%
Swat	Behrain	Raja	40	10	Eca	Crop age 2 months, late blight losses 60%
Swat	Madain	Raja	30	5	Ech	Crop age 2 months, late blight losses 40%
Swat	Utrore (Kalam)	Raja	40	15	Eca	late blight losses 80%
Swat	Kanai (Kalam)	Raja	20	13	Eca	late blight losses 90%
Swat	Boyan (Kalam)	Raja	10	5	Eca	Late blight losses 70%, poor managment
Swat	Kalam	Raja	60	40	Eca	late blight losses 50%
Swat	Kas kili (Kalam)	Raja	10	3	Ecc	late blight 30%, Crop age 2 months
Mansehra	kalgan	NK	10	3	Eca	Clay loam soil, Good management
Mansehra	Chatti ghetti	Desiree	10	5	Ecc	Insect attack, poor crop management
Mansehra	Bafa	Kuroda	15	5	Ech	Good crop management
Mardan	Sher ghar	Desiree	25	18	Eca	Sandy loam soil, 20% late blight
Mardan	Torbhati	Desiree	45	20	Ech	Colocacia intercropping
Mardan	Torbhati	Desiree	50	30	Eca	Sandy loam soil,
Mardan	Torbhati	Raja	20	10	Eca	Clay loam soil, poor crop management
Mardan	Taus(Ijara korona)	Desiree	70	40	Eca	Clay loam soil, 25% late blight
Mardan	Katlang	Desiree	10	5	Eca	Clay loam soil, poor crop management
Nowshehra	Dag baisood	Desiree	25	15	Ecc	Clay loam soil, Viral problem,
Nowshehra	Dag baisood	Desiree	20	10	Eca	Clay loam soil, late blight losses 70%
Nowshehra	Aza kheil bala	Raja	20	10	Eca	Clay loam soil, poor crop management
Nowshehra	Wazir gari pubbi	karora	40	15	Ecc	Clay loam soil
Peshawar	Machni	Desiree	15	5	Eca	Clay loam soil
Peshawar	Kohat road	Desiree	20	5	Ecc	Clay loam soil, poor crop management
Peshawar	Ghari sarfaraz	karora	30	10	Eca	Sandy loam soil
Peshawar	Palosay	Desiree	40	15	Ecc	Clay loam soil , late blight losses 10%
Shangla	Koat kili (bower)	Raja	10	3	Ecc	crop age 2½ months
Shangla	Shangla top	Raja	30	18	Ecc	crop age 2 months
Shangla	Shangla	Raja	50	20	Ecc	crop age 2 months
Swabi	Manirri	karora	20	10	Ecc	Clay loam soil
Swabi	Adena,	Desiree	15	5	Ecc	Sandy loam soil, late blight losses 80%
Swabi	Jhangira	Desiree	15	10	Eca	Sandy loam soil

Late blight crop losses were visually estimated
Eca = *Erwinia carotovora* subsp. *atroseptica*
Ecc = *Erwinia carotovora* subsp. *carotovora*
Ech = *Erwinia chrysanthemi*

Disease incidence and severity: To calculate disease incidence (%), the number of potato plants showing symptoms of the disease were divided by the total number of plants observed (per spot) multiplied by one hundred. The values of all random spots (per field) were averaged together. Disease severity was assessed on a scale of 0-3 as reported by Wright *et al*., (2005) where 0 = no disease symptoms on plant, 1 = less than 50 % of the plant has disease symptoms, 2 = more than 50% of the plant has disease symptoms, and 3 = plant totally dead. Disease severity (%) for each random spot was then computed according to Bdliya & Dahiru (2006) $S = 100\sum n/Nx3$ where S = black leg severity (%), $\sum n$ = summation of individual ratings, N = total number of plants assessed, and 3 = highest score on the severity scale. Values of all spots per field were averaged.

Isolation of bacteria: Tubers and plant samples showing disease symptoms were cleaned, surface-sterilized with 0.5% sodium hypochlorite solution (for 30 seconds), washed with sterile distilled water, and ground in sterile 0.85% saline solution using sterile mortar under aseptic conditions. The resulting bacterial suspension was left undisturbed for a few minutes. A loop ful of this suspension was then streaked on to plates containing nutrient agar (NA) (Bacto Agar 10gm, NaCl 5.0 gm, K_2HPO_4 5 gm, KH_2PO_4 2gm, Bacto-peptone 1.0gm), and incubated at 28^{o}C for 24 h. Individual colonies (transparent, circular, raised, shiny and creamy white) growing on NA were selected, re-suspended in 0.85 % saline, streaked on NA plates, and then incubated at 28^{o}C for another 24 h. This was repeated several times in order to obtain pure cultures. In order to avoid contamination, green pepper fruits were used as an enrichment host for the soft rot *Erwinias* which were subsequently isolated on NA. Initially, green pepper fruits were surface-disinfested with 70% alcohol for 30 seconds, followed by treatment with 1% Sodium hypochlorite (NaOCl) for 30 seconds and then washed with sterile distilled water. Next, sterile toothpicks were stabbed into soft-rotten tubers or the margin of blackleg lesions on potato stems. The same toothpicks were then inserted into green pepper (*Capsicum annuum* L.) fruits (Takatsu *et al*., 1981).

The inoculated fruits were maintained in a humid chamber at 28^{o}C for 24-48 h. Decayed tissue was peeled off with a sterile scalpel and crushed in 0.85% saline as described previously. A loop ful of bacterial suspension was used to streak the surface of NA plates. Single colonies were harvested and purified as described before. Pure colonies were preserved in 70% glycerol solution and stored at -20^{o}C or -80^{0}C. Cultures were also preserved in 0.85% sterile saline solution and stored at 4^{o}C. When required, each bacterial strain was cultured on LB (Trypton 10gm, yeast extract 5gm, NaCl 10 gm, agar 15 gm, distilled water 1 liter) and incubated at 28°C for 2 days.

Identification of bacteria: Identification of the causal organism as Eca, Ecc or Ech was done using different tests corresponding to the genus *Erwinia* according to Dye, 1969; Schaad, 1980; Lelliott & Dickey (1984), and Lelliott & Stead (1987) (data not shown).

Population dynamics of Eca: To know the population dynamics of Eca, the major causal organism of potato blackleg, we artificially inoculated seed-potato tubers, stems of potato plants and un-sterilized potted soil with the suspension (A_{540} = 0.52 or 5x10^8 cells/ml) of a spontaneous ampicillin-resistant strain of Eca. Inoculations were done (with a syringe) at multiple points in the potato stems and tubers whereas the potted soil was drenched with 200 ml of the bacterial suspension. Inoculated potato stems were cut into pieces and mixed with un-sterilized soil (kept in a trays) to mimic diseased plant debris left in field after harvest. The inoculated material was kept in screen-house under natural conditions. To monitor the changes in the population of the pathogen, a known amount (5 g) of each inoculated material i.e., soil, plant debris and whole tubers was used (in case of potato stems and tubers, the tissue was first macerated in a few ml of 0.85% saline) for dilution plating (using nutrient agar medium plus ampicillin) after every ten days. The NA plates were incubated at 27^{o}C for 24h and the resulting Eca colonies were counted. Data were taken for about three months, converted to $\log_{10}$ for ease and summarized as bar graph.

Results and Discussion

Incidence, severity and distribution of blackleg and soft rot: The disease severity and incidence of 42 locations visited at different times ranged from 2-40% and 5-75%, respectively (Table 1). Potato crop at some locations was so badly damaged by potato late blight that it was very difficult to get any estimates or to collect potato plants/tubers showing disease symptoms. For the same reason some of our collected samples did not yield any bacterial or Gram-negative bacterial cultures and were therefore not included in the study. Maximum blackleg severity (40%) was found at Kakool, Abbottabad (potato variety ‘Desiree’); Kalam, Swat (potato variety ‘Raja’) and at Taus (Ijara Koruna), Mardan (potato variety ‘Desiree’). Likewise, quite high disease incidence was recorded at Kakool (75%), Kalam (60%) and Taus (70%). Abbotabad and Swat are both high elevation hilly areas where temperature remains considerably low during the whole growing season of the crop. The main causal organism of potato blackleg and soft rot is *Erwinia carotovora* subsp. *atroseptica* (Eca) and this pathogen favors low temperature. So, high incidence and severity of the disease at low temperature areas is not un-usual. Similar results were reported by Perombelon *et al*., 1979. However, Mardan is a low elevation plain area where the normal temperature range during the growing season of the crop is not low enough to support the growth of Eca, hence the incidence and severity of blackleg and soft rot of potato was expected to be lower than what we found. Similar un-expected results were found by Oliveira *et al*., (2003).

There could be a few reasons to explain this result. First, there is possibility that the potato blackleg and soft rot in this case and some other similar cases is not due to Eca but due to Ecc which has been reported to be equally important in causing the disease and which favors relatively higher temperature (Oliveira ***et al.,*** 2003). This possibility is supported by our findings that some of the bacterial strains isolated from samples collected from high temperature areas do show characteristics belonging to Ecc group. Second possibility is that usually Eca and Ecc both are present latently in potato crop and if temperature remains low for a considerable length of time during the growing season, Eca will outgrow and if the temperature remains high, Ecc will outgrow.

Population dynamics of Eca: Although the initial amount of inoculum was not the same in the three cases (i.e., in tubers, in potato stems and in soil), we still could study the population dynamics of Eca as we were just interested in finding out the general trend of the population changes i.e., we wanted to know whether or not the population would increase, decrease, or stay the same beyond the initial numbers. As obvious from Fig. 2, the population of Eca rapidly declines when the bacteria are released freely in soil. Our results are in line with those of De Mendon *et al*., (1979). However, the population of the pathogen in diseased plant debris gradually goes up, stays un-changed for a few weeks and then gradually declines. The pathogen population in inoculated seed-potato tubers gradually went up and kept increasing for the whole monitoring period. Eca is a soil invader and cannot live freely in soil for a longer period of time as it is unable to successfully compete with other microbes naturally present in soil. Moreover, high temperatures and low moisture/dry conditions are especially damaging for the survival of these bacteria in soil. Therefore, it is expected that its population would quickly go down when the pathogen is released freely in soil (Agrios, 2005). Eca population can go up in inoculated plant debris for a short period of time as the debris is fresh and plenty of photosynthate is available. When the debris start dis-integrating and the competition from the soil microorganisms get stronger, the Eca population starts declining. However, the rate of decline is quite slow and therefore, the bacteria present in plant debris might act as a good source of primary inoculum for the appearance of blackleg disease in the next season. Seed-potato is the best source of primary inoculum for the disease to occur in the next season. Eca in seed-potatoes can increase in numbers and may reach a very high population. This population may decline slightly but it still provides a huge primary inoculum which, under favorable environmental conditions, can cause a severe disease problem if the diseased seed-potato is used for sowing.

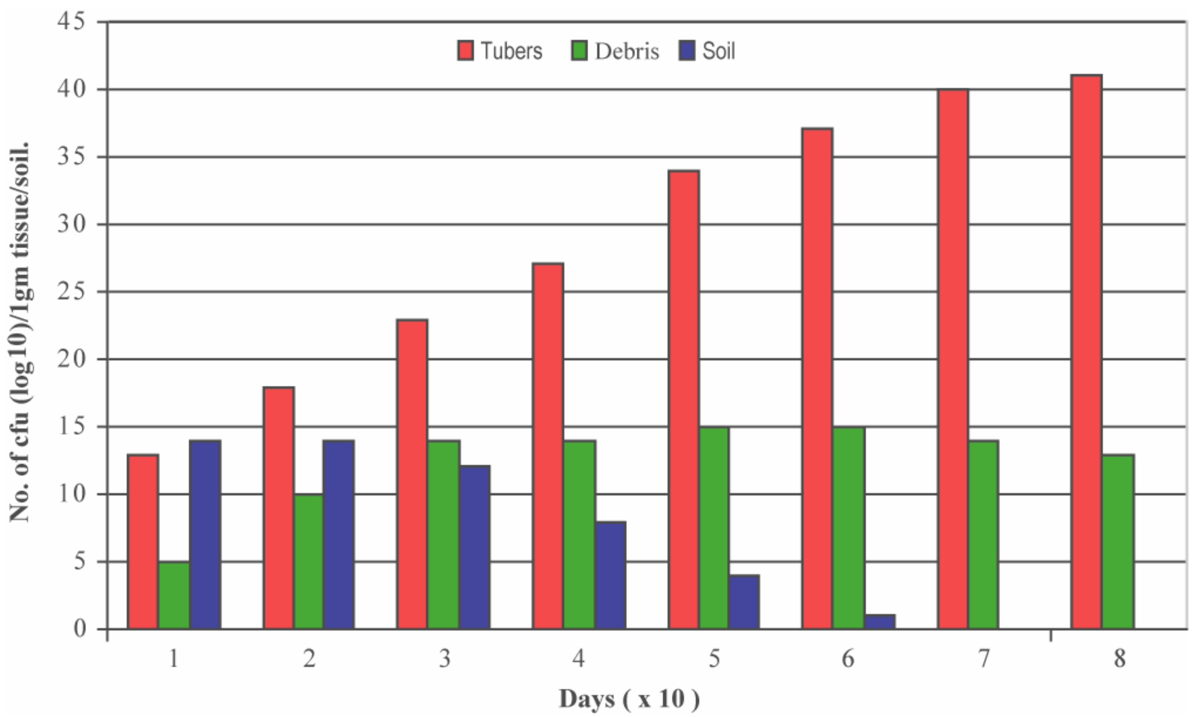

Fig. 2. Survival of black leg and soft rot bacteria (Eca) in soil, diseased plant debris and diseased tubers.

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