The Pharma Innovation Journal 2020; 9(4): 31-35

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(4): 31-35
© 2020 TPI
www.thepharmajournal.com
Received: 18-02-2020
Accepted: 20-03-2020

HS Viswanath
Division of Plant Pathology, Faculty of Agriculture, Wadura campus, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, India

Roaf Ahmad Rather
Division of Plant Pathology, Faculty of Agriculture, Wadura campus, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, India

KA Bhat
Division of Plant Pathology, Faculty of Agriculture, Wadura campus, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, India

SH Peerzada
Division of Plant Pathology, Faculty of Agriculture, Wadura campus, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, India

Corresponding Author:
HS Viswanath
Division of Plant Pathology, Faculty of Agriculture, Wadura campus, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, India

Management of post-harvest bacterial soft rot of potato caused by *Pectobacterium carotovorum* using bacterial bio-agents.

HS Viswanath, Roaf Ahmad Rather, KA Bhat and SH Peerzada

Abstract
Bacterial soft rot is one of the most destructive diseases worldwide causing heavy losses in field, storage and transit. Chemical control of this disease was not successful even in developed countries besides use of antibiotics to control this disease on stored produce causes severe health complications by inducing resistance to other human pathogenic bacteria. In present study, four different bacterial species viz., *Bacillus subtilis*, *Pseudomona fluorescens*, *Bacillus tequilensis* and *Bacillus* spp. were evaluated against *Pectobacterium carotovorum*, soft rot causing pathogen *in-vitro*. Best results were obtained by *Bacillus subtilis* with a mean diameter zone of inhibition of 14.33mm followed by *Pseudomonas fluorescens* with a zone 0f 14mm. Least zone of inhibition of 2.16mm was observed in case of Bacillus sp., whereas no zone of inhibition was observed in case of both control (sterile water) and *Bacillus tequilensis*. The two effective antagonists *in vitro viz*., *Bacillus subtilis* and *Pseudomonas fluorescens* were also evaluated against post-harvest soft rot disease on potato tubers at different times viz., 12 hours prior to the inoculation, simultaneously with the inoculation of the pathogen and 12 hours after the inoculation of the pathogen. Among the bio-agents evaluated, *Bacillus subtilis* exhibited highest control with the disease incidence of 84% and severity of 16.8% followed by *Pseudomonas fluorescens* with the incidence of 88% and severity of 17.6% when applied 12 hours prior to the inoculation of the pathogen even after 6 days of storage.

Keywords: *Bacillus subtilis*, *Pectobacterium carotovorum*, post-harvest bacterial soft rot, *Pseudomonas fluorescens.*

Introduction
Potato (*Solanum tuberosum* L.) is the one of the most important food crops worldwide and represents a valuable source of nutrients in a balanced diet. It is the third most important staple food source after rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Czajkowski *et al*., 2011) [9]. Potato is also one of the important food crops in India. India stands second in world's potato production where it is cultivated over an area of 2.13 million hectares with a production of 43.77 million metric tonnes (Anonymous, 2015) [4].

Post-harvest soft rot is one of the destructive diseases of vegetables including potato. It occurs worldwide wherever vegetables and ornamentals having fleshy storage tissues are found. The disease can be found on crops in the field, in transit and in storage or during marketing. Soft rot causes greater total loss of produce than any other bacterial disease. Post-harvest bacterial soft rot losses have been estimated to vary between 15-30% of the harvested crop (Agrios, 2007) [1]. Approximately 22% of potatoes are lost per year due to viral, bacterial, fungal diseases and pest attack to potato tubers and plants, incurring an annual loss of over 65 million tonnes and bacterial soft rot alone accounts for 30–50% of this huge loss (Czajkowski *et al*., 2011) [9]. Various causal organisms responsible for causing soft rot in potato but economically *Pectobacterium carotovorum* subsp. *carotovorum* is a very important pathogen in terms of postharvest losses and is a common cause of decay in stored fruits and vegetables (Perombelon, 2002) [12].

Several methods of the disease control such as hot water treatment (Shirsat *et al.*, 1991) [15], air drying of tubers (Bartz and Kelman, 1985) [6] have been tried with the varying scale of success. Chemical control of the disease has not been successful even in the developed countries, besides the use of antibiotics is not considered safe *in view* of human health considerations. Indiscriminate use of chemical pesticides to control various pests and pathogenic microorganisms of crop plants is causing health hazard both in terrestrial as well as aquatic lives through their residual toxicity (Ambridge and Haines, 1987; Anonymous, 1998) [2, 3].

Biological control using antagonistic microorganisms is an alternative strategy for the management of this disease. So, in this study four different antagonistic bacterial species were evaluated and tested for their ability to manage this disease.

Material and methods

Isolation of the causal pathogen: Diseased vegetables and potato tubers showing typical soft rot symptoms were collected from the local markets from Kashmir valley**.** Collected samples were surface sterilized with 0.1% sodium hypochlorite solution and the infected tissue was macerated in sterile water to make a bacterial suspension. A drop of resultant suspension was spread on Crystal violet pectate, a semi selective medium (CVP). The type of colonies which upon flooding with 1% hexadecyl trimethyl ammonium bromide (precipitant solution) formed halo zones around them on Crystal violet pectate medium (CVP) were selected for subculturing on nutrient agar and were tested for pathogenicity.

Pathogenicity test by Potato slice assay: Potato tubers were first surface sterilized with sodium hypochlorite solution (0.5%) and cut into slices (1.0 cm in thickness) with sterile blade. These Slices were inoculated by smearing a loop full of bacteria at the center, on the surface of healthy tuber slice. The inoculated tuber slices were incubated for 24-48 h at 28 ± 2ºC in Petri plates having sterile filter paper at the bottom of petri plate soaked in 5ml of sterile water, kept in such a way that the tuber slices should not come in direct contact with the water by placing a glass slide at the bottom of the slice. Tuber slice inoculated with sterile water in one petri plate was kept as control. Softening of the inoculated tuber slices was taken as a positive reaction. From the softened/macerated slice tissue, bacteria was re-isolated and compared with the original isolate of inoculated pathogen (Shashirekha *et al.*, 1987) [14]. The pathogen showing typical soft rot symptoms was reisolated from the infected slice and was further characterized by biochemical and other morphological tests to ascertain identity.

Identification of four different bacterial species and screening them against soft rot causing *Pectobacterium carotovorum*

Bacterial species screened against *Pectobacterium carotovorum*.

Two out of four antagonistic bacterial species *viz. Pseudomonas fluorescens*, and *Bacillus subtilis* were obtained from the division of plant pathology, Faculty of Agriculture, Sher-e-Kashmir university of agricultural sciences and technology, kashmir and another two *viz. Bacillus tequilensis* and *Bacillus* sp. were obtained from Advanced Mycology and Plant pathology Laboratory, Jai Narain Vyas University, Jodhpur, Rajasthan.

***In-vitro* screening of antibacterial activity by bio-agents**

Four Known and identified bacterial species viz. *Bacillus subtilis*, *Pseudomonas fluorescens*, *Bacillus tequilensis*, *Bacillus* sp. were screened against the growth of *Pectobacterium carotovorum* subsp. *carotovorum* by dual culture technique (Agar well diffusion method) measuring the diameter zone of inhibition. In this method, Suspensions containing approximately 10^8 cells/ml of bacterial bio-agents were dropped into the 6mm wells, punched by the cork borer on the agar plate which were equidistant to each other of the previously swabbed/seeded plate with the pathogenic bacteria *(Pectobacterium carotovorum*). In case of control, only sterile water was used instead of suspension. One treatment with Streptomycin 150 ppm was kept as positive control (15 µ g in 100µl). The plates were then incubated at 30±1ºC. Diameter Zone of inhibition around the wells were measured and recorded after 24 hours (Rashid *et al*., 2013) [13].

Screening of effective Bio-agents against the disease on stored potato tubers

Bio-agents which proved best *in vitro* were used for the treatment on stored potato tubers and applied at different times *viz.* 12 hours prior to the inoculation of the pathogen, simultaneously with the inoculation of the pathogen and 12 hours after the inoculation of the pathogen. Fresh potato tubers were surface sterilized by dipping in 0.1% solution of sodium hypochlorite followed by serial washings with sterile water and then dried under the hood of laminar air flow. One set of potato tubers was given 30 pinpricks and dipped in uniform suspensions of different bio-agents for 10 minutes and 12 hours afterwards inoculated with the pathogen by swabbing the bacterial suspension on them. In the second set of tubers, after giving the pinpricks, application of bio-agents was done simultaneously. In third case, the pinpricked tubers were first inoculated by the pathogen by swabbing bacterial suspension on them and 12 hours afterwards they were treated with bio-agents for 10 minutes.

One set of potato tubers which were inoculated with only pathogen (no treatment) served as inoculated control. Other set of tubers inoculated and treated with antibiotic (streptomycin@150ppm) were kept as positive control. Five potato tubers constituted 1 replication and total of 5 replications were maintained in each treatment. The tubers were kept in sterile air tight plastic bags and were stored at 30±1ºC. Observations on soft rot incidence and severity were recorded on 2nd, 4th and 6th day of incubation.

Incidence of soft rot disease = $\frac{\text{Number of tubers infected}}{\text{Total number of tubers assessed}} \times 100$

Tuber rot severity

Severity of the disease was calculated using 0-5 scale (Bdliya and Langerfeld, 2005b)

0	No symptoms of rot
1	1-15% tuber rot
2	16-30% tuber rot
3	31-45% tuber rot
4	46-60% tuber rot
5	≥61% tuber rot

The severity was calculated using formula

$$\text{Tuber rot severity} = \frac{\sum nv \times 100}{N \times G}$$

Where,

$\sum$ = Summation

v = Disease score

n = Number of tubers showing a particular score.

N = Number of tubers examined.

G = Highest score.

Data analysis

The collected data was subjected to the analysis of variance

using CRD (Complete Randomised Design) and transformed values of the data compared using critical difference (CD) at 5% level of significance using Statistical Package for Agricultural Research workers (OPSTAT).

Results and discussion

The causal Pathogen: The bacterium isolated from potato tubers was a short rod measuring 0.7-1.0µm in width, 1-2.5µm in length, Gram negative, facultative anaerobic, produced acid from D lactose, trehalose and maltose, did not hydrolyze the starch, reduced nitrates, liquefied gelatin, degraded pectin, produced H_2S from cysteine, was catalase positive, oxidase negative, urease negative, not sensitive to erythromycin and showed positive growth at 37ºC. Based on Morpho-cultural, biochemical and pathogenic characters, the pathogen was identified as *Pectobacterium carotovorum.* It was also identified as *Pectobacterium carotovorum* by ITCC, IARI, New delhi.

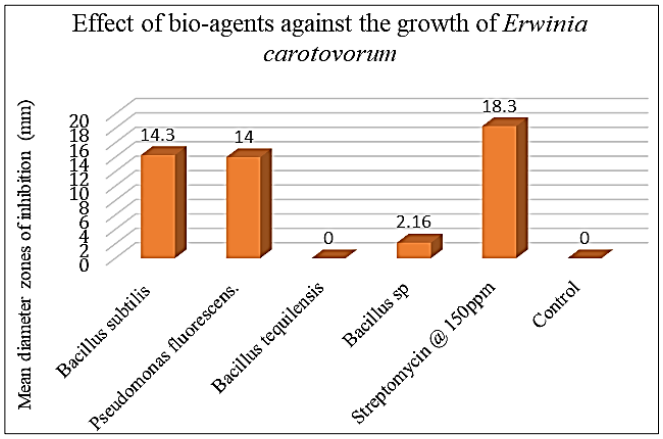
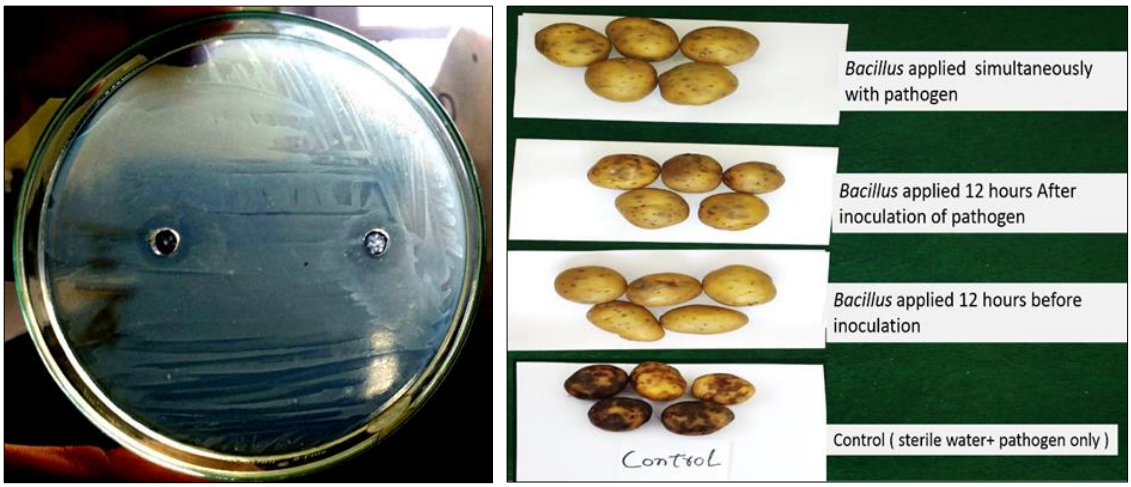


Fig 1: Evaluation of bacterial species against the growth of *Pectobacterium carotovorum in vitro* by agar well diffusion assay



i) **ii)**

Fig 2: Effect of *Bacillus subtilis* against soft rot causing bacterium *Pectobacterium carotovorum*

i) *Bacillus subtilis* forming zone of inhibition against *Pectobacterium carotovorum in-vitro.*
ii) Effect of *Bacillus subtilis* on potato tubers against soft rot disease after 2 days of storage

Laboratory evaluation of different bacterial species against the growth of *Pectobacterium carotovorum*

Among the four bacterial bio-agents tested, best control was exhibited by *Bacillus subtilis* with highest diameter zone of inhibition of 14.3mm, immediately followed by *Pseudomonas fluorescens* with 14mm. Another strain of *Bacillus* sp. showed less diameter zone of inhibition, whereas both *Bacillus tequilensis* and control (sterile water) did not gave any zone of inhibition. Standard check streptomycin showed 18.3mm zone of inhibition (Fig:1). These results were similar to the findings of Backman *et al.* (1997) [5] who reported that *Bacillus subtilis* shows biological activity against phyto-pathogenic bacteria including *P. carotovorum*. Soil fluorescent and non-fluorescent *Pseudomonas* spp. have shown biological control of soft rot disease of potato by producing a variety of secondary antibacterial metabolites including siderophores, antibiotics and surfactants was previously reported by (Compant *et al.*, 2005) [8]. Rashid *et al.* (2013) [13] also reported that among the evaluated bio-agents against *Pectobacterium carotovorumin-vitro*, *Bacillus subtilis* showed best results against the causal bacterium. All these previous findings, more or less, confirmed the present studies.

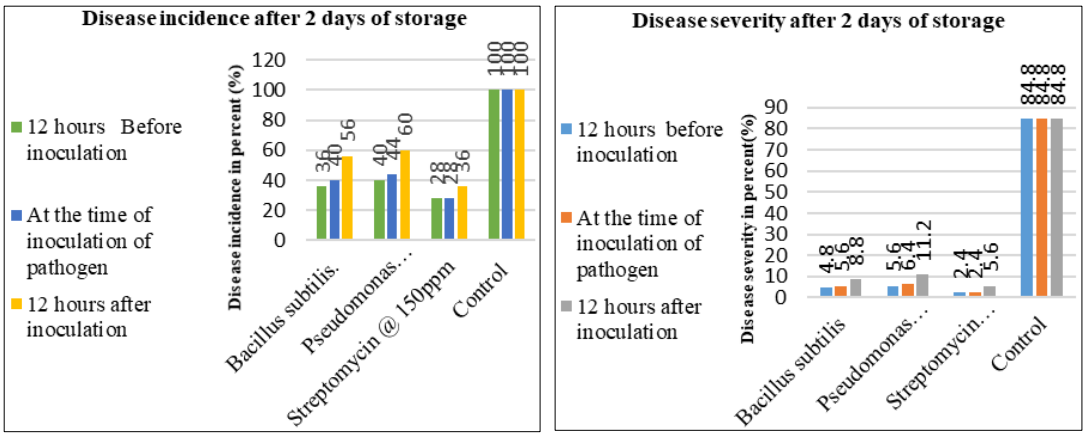
Effect of bioagents on incidence and severity of soft rot disease caused by *Pectobacterium carotovorum* on stored potato tubers

Among 4 bacterial bio-agents tested in-vitro against the growth of *Pectobacterium carotovorum*, twobioagents *Bacillus subtilis* and *Pseudomonas fluorescens* showing highest efficacy with respect to growth inhibition are selected for this experiment. Best results were obtained when treatments were given 12 hours prior to inoculation or simultaneously with the inoculation of the pathogen than when treatments were given 12 hours after the inoculation of the pathogen

Results obtained after 2 days of storage exhibited that among bioagents, *Bacillus subtilis* gave least soft rot incidence of 36% and 40%and severity of 4.8% and 5.6% when applied 12 hours prior to and simultaneously with the inoculation of the pathogen respectively, followed by *Pseudomonas fluorescens* with disease incidence of 40% and 44% and severity of 5.6% and 6.4% respectively. Disease Incidence and severity recorded in case of these extracts was significantly less than that of the inoculated control (pathogen only and no treatment), which showed 100% soft rot incidence and 84.8% disease severity. Results are in Fig-3 (i, ii).

After 4 days of storage, there was a rapid progression of disease in case of inoculated control. Disease progression was less in case of treatments. Least disease incidence of 64% and 68% and severity of 9.6% and 10.4% respectively, were recorded in case of treatment with *Bacillus subtilis*, when applied 12 hours prior to and simultaneously with the inoculation of the pathogen, followed by *Pseudomonas fluorescens* with incidence of 68% and 72% and severity of 11.2% and 12.8% when applied 12 hours prior to pathogen inoculation or simultaneously with it, respectively, whereas in standard check streptomycin, incidence of 56% and severity of 7.2% were recorded in both casesi.e when applied 12 hours prior to and simultaneously with the inoculation of the pathogen. Whereas in the inoculated control disease severity of 100% was recorded Fig-4 (i, ii).

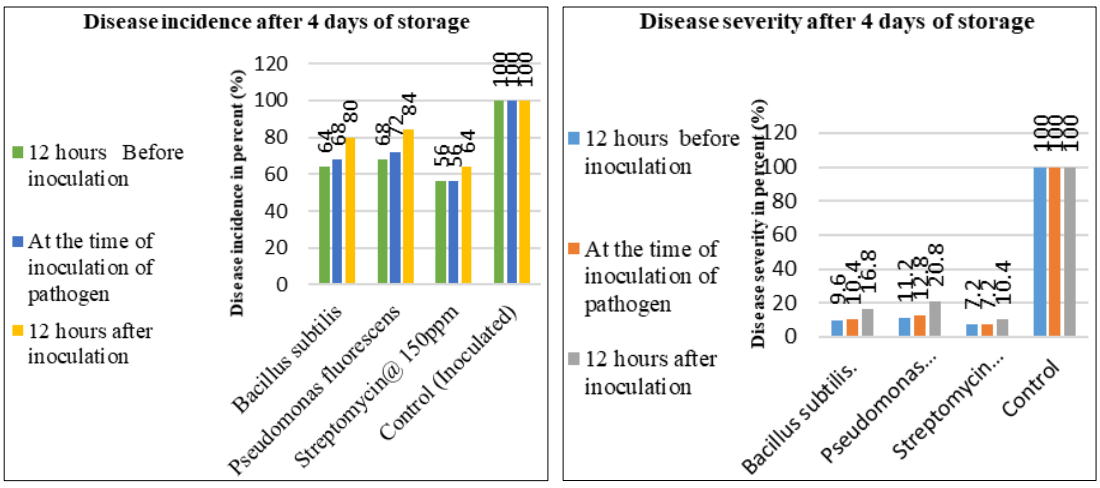
Results obtained after 6 days of storage almost revealed the same trend. Positive check streptomycin showed a similar incidence of 76% in both cases i. e when applied 12 hours prior to or simultaneously with the inoculation of the pathogen even after 6 days of storage. There was no hike in the severity of the disease in case of tubers treated with bio-agents. Treatments with bioagents protected the tubers from speedy spoilage thereby significantly reducing the severity of disease even after 6 days of storage. *Bacillus subtilis* stands best in preventing the severity of disease by exhibiting least severity of 16.8% when applied 12 hours before inoculation and 17.6% when applied simultaneously with the inoculation of the pathogen, followed by *Pseudomonas fluorescens*with severities of 17.6% and 20.8% respectively, when applied 12 hours prior to and simultaneously with the inoculation of the pathogen. Streptomycin recorded 12.8% disease severity in both cases i.e prior and simultaneous treatment. All these bioagents were highly significant and superior to the inoculated control (only pathogen and no treatment Fig-5 (i, ii). Although as far as *in vitro* evaluation studies of bacterial bioagents against E carotovorum is concerned there are many previous reports but we have not been able to find many reports of their use on potato tubers to manage storage rot except that of Hajhamed *et al*. (2007) [10] who reported similar findings where they evaluated *Pseudomonas fluorescens* and *Bacillus subtilis* as bio-agents against postharvest bacterial soft rot at different inoculation times *viz.*, 24 hours before inoculation, simultaneously and 24 hours after inoculation under artificial inoculation condition. They reported that all tested agents decreased the disease compared to the control, when *P. fluorescens* and *B. subtilis* were applied after or before or at the same time of inoculation with the pathogen, significantly reducing the disease.



i) ii)

Fig 3: Effect of bio-agents on post-harvest tuber soft rot after 2 days of storage

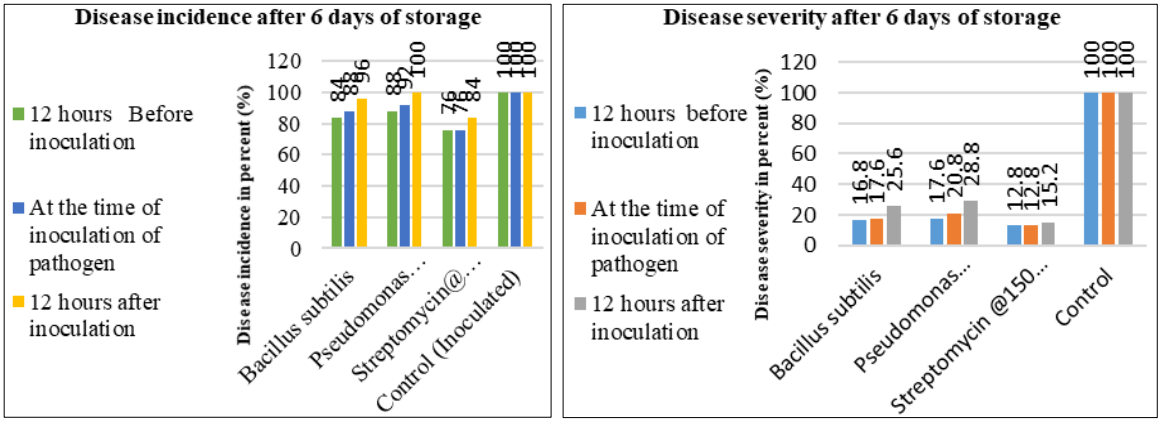
i) Disease incidence after 2 days of storage on stored potato tubers.
ii) Disease severity after 2 days of storage on stored potato tubers.



i) ii)

Fig 4: Effect of bio-agents on post-harvest tuber soft rot after 4 days of storage

i) Disease incidence after 4 days of storage on stored potato tubers.
ii) Disease severity after 4 days of storage on stored potato tubers.



i) ii)

Fig 5: Effect of bio-agents on post-harvest tuber soft rot after 6 days of storage

i) Disease incidence after 6 days of storage on stored potato tubers.
ii) Disease severity after 6 days of storage on stored potato tubers.

Acknowledgment: We are very much thankful and hereby sincerely acknowledge the hospitality extended to us besides providing lab facilities and cultures to carry out our research activities in Advanced Mycology and Plant pathology Laboratory, Jai Narain Vyas University, Jodhpur, Rajasthan.

References

1. Agrios GN. Bacterial Soft Rots. 5th Edn., Academic Press, San Diego, 2007, 656.
2. Ambridge EM, Haines TH. Some aspects of pesticide use and human safety in South East Asia, in Proceedings of 11th International Congress of Plant Protection, October, Manila Philippines, E. D. Magallona, Ed., 1987, 219-224.
3. Anonymous. Pesticides incidents up for 1996/1997 compared with previous year," International Journal of Pest Control. 1998; 40:1-8.
4. Anonymous. 3rd Advance Estimate of Area and Production of Horticulture Crops. Statistics and Market Information. National Horticulture Board, Ministry of Agriculture and Farmers Welfare Govt. Of India, Gurgaon, Haryana, 2015. (http//nhb.gov.in)
5. Backman PA, Wilson M, Murphy JF. Bacteria for biological control of plant diseases. In: Environmentally Safe Approaches to Crop Disease Control. Eds. N. A. Rechcigl and J. E. Rechcigl. Boca Rota: CRC Press, 1997, 95-109.
6. Bartz JA, Kelman A. Infiltration of sentinels of potato tubers by *Pectobacterium carotovorum* subsp.*caratovora* under hydrostatic pressure in relation to bacterial soft rot. Plant Disease. 1985; 69:69-74.
7. Bdliya BS, Langerfeld E. Soft rot and Blackleg [*Pectobacterium carotovorum* ssp. *atroseptica* (Van Hall) Dye] of potato as affected by inoculum density and variety. Nigerian Journal of Plant Protection. 2005b; 22:65-75.
8. Compant S, Duffy B, Nowak J, Clement C, Barka EA. Use of plant growth-promoting bacteria for bio-control of plant diseases: principles, mechanisms of action and future prospects. Applied and Environmental Microbiology. 2005; 71:4951-4959.
9. Czajkowski R, Perombelon MCM, Van veen JA, Van der wolf JM. Control of blackleg and tuber soft rot of potato caused by Pectobacterium and Dickeya species: a review. Plant Pathology. 2011; 10:1365-3059.
10. Hajhamed AA, Wafaa MA, Abou El-Yazied A, Abd El-Ghaffar NY. Suppression of bacterial soft rot disease of potato. Egyptian Journal of Phytopathology. 2007; 35(2):69-80.
11. Krebs H, Jaggir W. Effect of plant extracts against soft rot of potatoes: *Pectobacterium carotovorum* Flora and Fauna in Industrial Crops. Agrarforschung. 1999; 6(1):17-20.
12. Perombelon MCM. Potato diseases caused by soft rot *Pectobacterium*: an overview of pathogenesis. Plant Pathology. 2002; 51:1-12.
13. Rashid M, Chowdhury MSM, Sultana N. *In-vitro* Screening of some Chemicals and Biocontrol Agents against *Pectobacterium carotovorum* subsp. *carotovorum*, the Causal Agent of Soft Rotof Potato (*Solanum tuberosum*). The Agriculturists. 2013; 11(2):1-9.
14. Shashirekha MN, Karanth NGK, Narasimham P. "Surface microflora of seed potatoes (*Solanum tuberosum* L., Kufri Jyoti): Isolation and identification of organisms responsible for spoilage of potatoes grown at Devanahalli", Journal of Food Science and Technology. 1987; 24:261-263.
15. Shirshat SG, Paul T, Nair PM. Evaluation of treatment with hot water, chemicals and ventilated containers to reduce microbial spoilage in irradiated potatoes. Potato Research. 1991; 34:227-231.