Readers Insight
Publisher of open access books and journals
PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

| Research Article | Pak-Euro Journal of Medical and Life Sciences |
|---|---|
| DOI: 10.31580/pjmls.v6i3.2865 | Copyright © All rights are reserved by Corresponding Author |
| Vol. 6 No.3, 2022: pp. 277--284 | |
| www.readersinsight.net/pjmls | **Revised:** August 31, 2023 **Accepted:** September 03, 2023 |
| **Submission:** May 13, 2023 | **Published Online:** September 30, 2023 |


# ISOLATION, IDENTIFICATION AND *IN-VITRO* MANAGEMENT OF SOFT ROT OF ONION IN BALOCHISTAN

Mohammad Arif[1], Abdul Qadir[1]*, Basheer Ahmed[1], Muhammad Azhar Shafique[2], Tahira Nisa[1], Muhammad Waris[1], Zobia Jabeen[1], Syed Zulfiqar Ali[1], Muhammad Basharat[1], Atta Muhammad[3]

[1]**Department of Plant Pathology,** Balochistan Agriculture College, Quetta, Pakistan
[2]**Department of Entomology,** Balochistan Agriculture College, Quetta, Pakistan
[3]**Department of Agriculture Economics,** Balochistan Agriculture College Quetta, Pakistan

***Corresponding Author:** Dr. Abdul Qadir. E. mail: abdulqadirjan@gmail.com

**Abstract**

*The onion, a crucial vegetable crop widely utilized in households, is susceptible to various biotic and abiotic factors throughout cultivation and storage. The escalating prevalence of onion soft rot presents a substantial economic threat on a global scale, leading to considerable losses during post-harvest and storage phases, particularly in Asian regions. Therefore, early identification and effective management of this deteriorating disease are imperative. Through comprehensive biochemical and physiological assessments, including Gram staining, catalase testing, starch hydrolysis, NaCl tolerance (5.0%), pH tolerance (8.0), yeast dextrose chloramphenicol agar (YDC), growth at 37 ˚C, and potassium hydroxide (KOH, 3%), the causative agent of onion soft rot was identified as Dickeya dadantii spp. In the pursuit of di sease management, seven antibiotics were administered at concentrations of 100 ppm, 200 ppm, and 300 ppm. Tetracycline exhibited the highest efficacy, manifesting a notable 34.08% inhibition zone. Conversely, Penicillin-streptomycin displayed diminished effectiveness, with a minimal inhibition zone of 1.16%. Despite the in-vitro nature of the study, the compelling results advocate for the application of Tetracycline as a preferred option for the agricultural community in mitigating onion soft rot, emphasizing its minimal risk to human health.*

**Keywords:** *Management, Onion, Pathogenicity, Phenotypic diversity, Soft rot*

## INTRODUCTION

The onion (*Allium cepa L.*), renowned as the "Queen of Kitchen," stands as one of the paramount and extensively cultivated commercial plants worldwide, serving diverse culinary applications (1). As a biennial vegetable grown annually on a global scale, its monocotyledonous nature is characterized by a diploid chromosome number of 2n = 16 (2). The versatility of onions extends to their incorporation into various dishes, condiments, pickles, powders, pastes, and flakes (3).

Despite its culinary prominence, onions face susceptibility to numerous ailments and pests, with bulb soft rot emerging as a particularly detrimental issue during growth, transport, and storage (4). Storage-related onion bulb rots have been associated with 11 distinct bacterial plant diseases, primarily identified through saturated scales and a light yellowish tint in infected bulbs (5, 6). Storage-associated problems are predominantly attributed to microorganisms, with Pseudomonas and Pectobacterium identified as major contributors, leading to significant onion losses (7, 8). Bacterial soft rot, prevalent during storage or transit, commonly initiates through wounds caused by insect feeding or harvesting damage (9).

The soft-rotting genera responsible for postharvest diseases include Erwinia, Pseudomonas, Xanthomonas, Cytophaga, and Bacillus species (11). Among these, Dickeya dadantii, formerly known as *Erwinia chrysanthemi*, is a gram-negative bacillus within the Pectobacteriaceae family, exhibiting global prevalence, particularly in tropical, subtropical, and temperate climates (12-14). *D. dadantii* induces soft rot diseases in onions, characterized by sunken and cracked exterior lesions, with a predilection for the plant's xylem vessels (15, 16).

Various strategies, including hot water treatment, conventional drying, and balanced fertilization, have shown efficacy in mitigating soft rot incidence (17). Chemical methods and manipulation of environmental conditions, such as pH alterations, are employed to combat bacterial infections, with Streptomycin considered as a potential treatment for soft rot and blackleg infections (18, 19). Research on alternative bactericides, often explored in laboratory settings, aims to address the rapid proliferation and dissemination of Dickeya spp., emphasizing the need for effective control measures in both laboratory and field conditions (20). The primary objectives of the study encompassed (i) the isolation and identification of microorganisms through morphological and biochemical analyses, and (ii) the in vitro assessment of microbial susceptibility to antibiotics for potential management strategies.

# MATERIALS AND METHODS

## SAMPLE COLLECTION

Selection of diseased onion bulbs exhibiting distinct signs of soft rot and characteristic putrid odor was conducted following the criteria outlined by Shing 1985 (21). The survey spanned the years 2021–2022 and encompassed three districts in Balochistan, namely Ziarat, Killa Saifullah, and Quetta. Laboratory procedures involved the isolation, identification, morphological characterization, and management of the causative organisms using ecologically sound biochemical concentrations.

## ISOLATION AND PURIFICATION OF SAMPLES

A standard bacteriological medium comprising nutrient agar (NA) was formulated by dissolving yeast extract, sodium chloride, peptone, and sucrose in 500 ml of distilled water. The pH was adjusted to 6.5 to 7.2, followed by autoclaving at 12 °C, 15 lbs pressure for 15 minutes for sterilization. External sterilization of the decayed tissues was carried out using 1% sodium hypochlorite (NaOCl) for a brief duration. Purified tissue fragments were then smeared and transferred to NA plates containing 20g of peptone. Incubation at 27°C for 24 hours facilitated bacterial growth monitoring, with subsequent colony selection and streaking for purification. Only axenic colonies were utilized for subsequent identification trials (22).

## ANALYSIS OF HARMFUL BACTERIAL STRAINS

Physiological and biochemical characterization of the more pathogenic isolates was conducted through a series of assays. This included Gram staining-based physiological and biochemical investigations (23), catalase production test (24), amylase (starch hydrolysis) test (25), salt and pH tolerance test (26), potassium hydroxide (KOH) solubility test (27), growth at 37˚C (28), yeast dextrose calcium carbonate (YDC) test (29), and a pathogenicity test involving the inoculation of infected solutions onto healthy bulbs under dark room conditions (30).

## ANTIBIOTICS SUSCEPTIBILITY TEST

The *in-vitro* experiment aimed to assess the antibacterial efficacy of seven substances against *Dickeya dadantii*, the causative pathogen. Prior to injection, *Dickeya dadantii* was cultured on NA at 28˚C for 24 hours. The traditional paper disc technique (31) was employed for this investigation. Agar with nutrients was prepared, and bacterial culture was introduced. Filter paper discs (7 mm in diameter) were aseptically immersed in various chemical solutions, including Tetracycline, Oxytetracycline, Streptomycin, Gentamycin, Penicillin streptomycin, Kasugamycin, and Sodium hypochlorite. Each substance was tested at three different concentrations (100ppm, 200ppm and 300ppm), with a positive control. Petri plates were incubated at 27°C for 96 hours, and post-incubation, the zone of inhibition around the filter paper disc was measured to evaluate the efficacy of the compounds.

# RESULTS

## MORPHOLOGICAL CHARACTERIZATION OF BACTERIA

Visual and microscopic examinations were conducted after a 24-hour incubation period at 27°C to characterize the colony morphology.

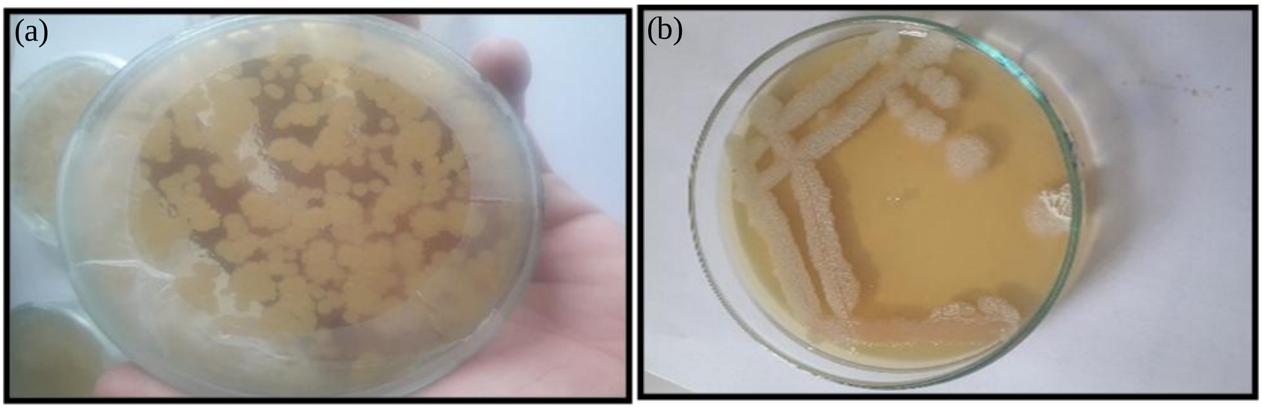


**Fig. 1.** (a) Isolation of bacteria on nutrient agar plate. (b) Bacterial streaks for pure culture

Results revealed that all isolates displayed a light brown colony color with medium to small size, exhibiting a rod shape and irregular form. The colonies had undulate margins and raised elevation.

## BIOCHEMICAL CHARACTERIZATION

For biochemical characterization, various tests were conducted on the pathogen isolates, and the results are presented in Fig. 2 and Table I.

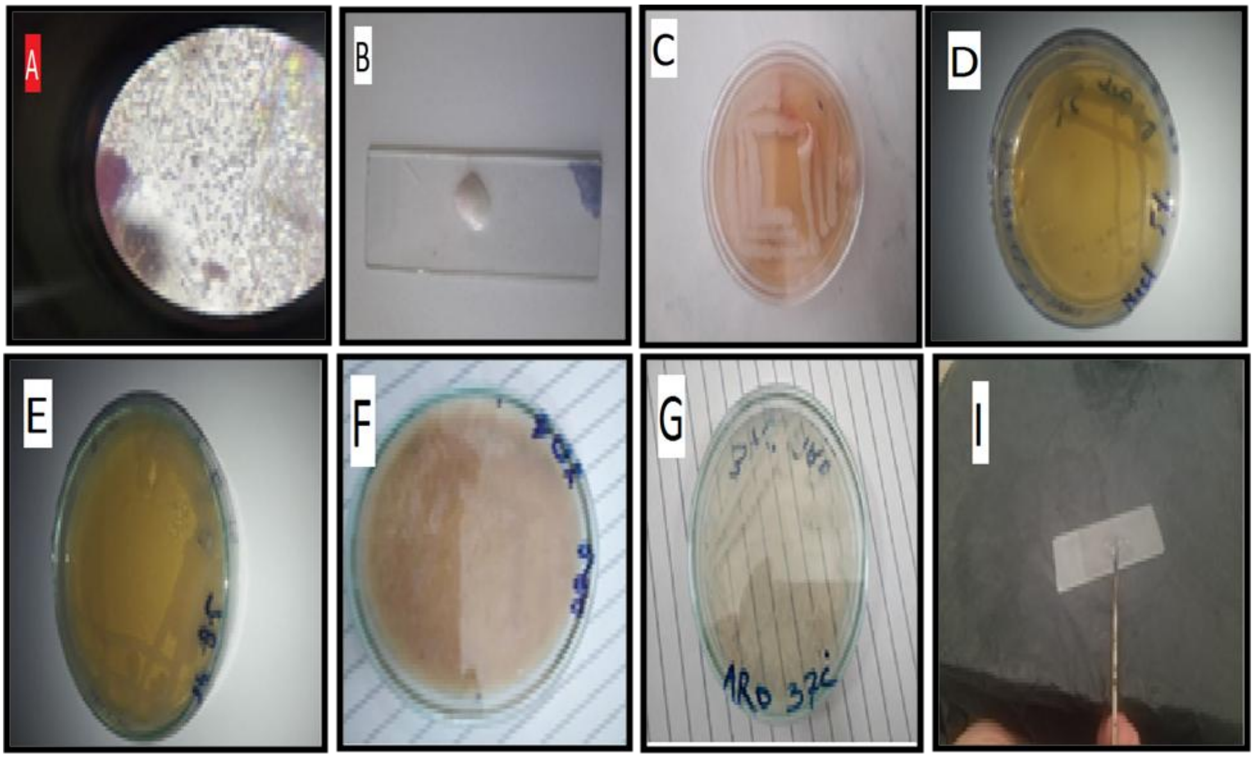


**Fig. 2.** Biochemical tests of bacterial isolates; A. pink color of bacterial cells after Gram's staining, B. Catalase test, C. Starch hydrolysis test, D. NaCl 5.0%, E. pH 8.0, F. YDC, G. Growth at 37°C, H. KOH (3%)

The bacterial isolates were identified as Gram-negative and rod-shaped (bacillus) with a scattered arrangement. Gram staining showed pink-colored bacterial cells. The Catalase test for soft rot bacteria demonstrated the presence of gas bubbles, indicating a positive result. In the Amylase test (Starch hydrolysis test), the soft rot bacteria did not utilize starch, resulting in a negative outcome.

The Salt (NaCl) test, conducted at concentrations of 1.0%, 3.0%, and 5.0%, showed positive growth. The pH test, performed at various pH levels (6.0, 6.5, 7.0, 7.5, 8.0), also demonstrated positive growth. The Yeast Dextrose Calcium Carbonate (YDC) test on agar revealed no yellow colonies, indicating a negative result. Under control conditions at 37°C, positive growth was observed. The Potassium Hydroxide (KOH 3%) test produced viscous suspension strands, resulting in a negative outcome.

**Table I.** Biochemical tests perfomed against soft rot bacteria isolates from onion

| Tests | Results |
|---|---|
| Gram staining | Gram negative (-) |
| Catalase test | Catalase positive (+) |
| Amylase test (satrch hydrolysis test) | Amylase Negative (-) |
| Salt and pH Tolerance | NaCl and pH Positive (+) |
| Yeast Dextrose Calcium Carbonate Test (YDC) | YDC Negative (-) |
| Potassium Hydroxide Solubility test (KOH) (3%) | KOH Positive (-) |
| Growth at 37°C | Growth at 37 °C Positive (+) |

## MANAGEMENT THROUGH ANTIBIOTICS

Every antibiotic assessed for the treatment of postharvest soft rot infection in onions demonstrated superior performance compared to the untreated control, exhibiting lower infection rates and a reduced percentage of yields lost to the illness.

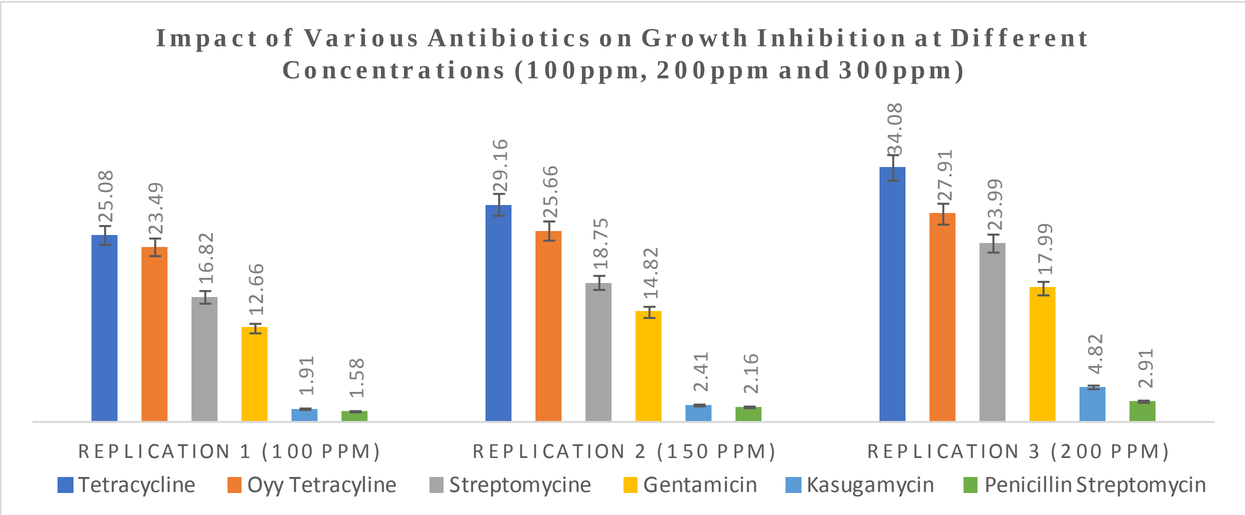


**Fig. 3.** Overall comparison of antibiotics; Tetracycline (Blue), Oxytetracycline (Orange), Streptomycin (Grey), Gentamycin (Yellow), Kasugamycin (skyblue), and Penicillin-streptomycin (Green)

In this study, while examining the impact of antibiotic treatment at various concentrations (100ppm, 200ppm, and 300ppm), as illustrated in Fig. 3, tetracycline exhibited the highest efficacy in controlling soft rot (34.08%), followed by oxytetracycline (27.91%), streptomycin (23.99%), gentamycin (17.99%), kasugamycin (4.82%), and penicillin-streptomycin (2.91%), the latter showing notably poorer control. The use of antibiotics during pathogen inoculation on onions revealed that all treatments significantly outperformed the control. Once again, the 300 ppm concentration of tetracycline demonstrated the highest efficiency. Notably, the control maintained without any drug application showed no inhibitory zone. This study underscores the practical potential of antibiotics for preventing soft rot disease. Tetracycline, recognized as an effective antibacterial drug against Gram-negative members of the Enterobacteriaceae family (32), has proven effectiveness against Dickeya dadantii ssp., as supported by the current investigation. Analysis of illness severity as a percentage after 4 days revealed the lowest control with penicillin-streptomycin (2.91%).

# DISCUSSION

The comprehensive evaluation of antibiotics was conducted to compare their reactions and impacts on the in vitro growth of bacterial colonies on agar plates. Seven treatments, including a control without antibiotic application, were employed to assess their efficacy. All antibiotics exhibited notable antibacterial activities against *Dickeya dadantii* across three concentrations. Notably, Tetracycline demonstrated superior results compared to the other six chemicals, exhibiting efficacy at concentrations of 100ppm (25.08%), 200ppm (29.16%), and 300ppm (34.08%). The highest efficacy was observed at the 300ppm concentration, indicating a concentration-dependent response. Tetracycline consistently displayed excellent performance in creating bacterial inhibition zones across all tested concentrations (31).

While antibiotic treatments have shown promise in mitigating soft rot diseases, it is essential to explore complementary strategies for post-harvest management. Packaging materials play a crucial role in controlling humidity and temperature during storage and transportation. Previous research supports our findings, emphasizing that higher levels of humidity (RH) accelerate soft rot development during storage (33, 34). Managing moisture levels, particularly in net bags, has been demonstrated to reduce postharvest soft rot by dehydrating rot tissue and impeding the progress of the disease in a dry environment. Additionally, substances like alum have shown effectiveness against soft rot (36).

Despite the potential benefits of chemical-based disease management, it is imperative to consider potential health concerns and the risk of antibiotic-resistant bacterial species. Further research is needed to thoroughly investigate the safety and long-term implications of employing antibiotics in onions or other food products. It is crucial to view chemical-based disease management as one option among many, and continuous research efforts should focus on identifying safer alternatives (37).

In the pursuit of sustainable agriculture, the emphasis on integrated disease management strategies becomes paramount. Biological control agents, such as beneficial microbes and antagonistic bacteria, present themselves as promising alternatives to chemical interventions. Harnessing the natural defense mechanisms of plants through the application of plant growth-promoting rhizobacteria (PGPR) and other biocontrol agents can contribute to enhanced disease resistance without the environmental and health concerns associated with certain chemicals.

Crop rotation and diversification strategies should also be considered as integral components of disease management. Breaking the disease cycle by alternating onion cultivation with non-host crops can disrupt the continuity of pathogens like Dickeya dadantii. Additionally, adopting resistant onion varieties through breeding programs can provide a sustainable long-term solution to combat soft rot diseases.

Research endeavors should extend to exploring the intricate interactions within the onion microbiome. Understanding the dynamics between the plant, pathogen, and the surrounding microbial community can uncover novel avenues for disease management. Moreover, advancements in molecular techniques, such as metagenomics and transcriptomics, offer powerful tools to unravel the complexities of plant-microbe interactions and devise targeted interventions.

In conclusion, the management of soft rot diseases in onions demands a multifaceted approach that integrates chemical, biological, and cultural practices. While antibiotics exhibit efficacy, their usage should be judiciously evaluated in conjunction with environmentally friendly alternatives. Sustainable disease management strategies not only preserve crop health but also safeguard the ecosystem and public health. Continuous interdisciplinary research efforts are imperative to address emerging challenges in agriculture and pave the way for resilient and sustainable food production systems.

# CONCLUSION

Based on the findings of the current investigation, it can be deduced that Tetracycline exhibits higher efficacy against the soft rot bacteria Dickeya dadantii. The application of Tetracycline on early infected bulbs is recommended. This suggestion aims to encourage the broader community to consider Tetracycline as a preferable option for combating soft rot due to its minimal adverse effects on both human health and the environment.

## Acknowledgments:

We would like to express our gratitude to the Plant Pathology Lab at Balochistan Agriculture College, Quetta, for generously providing the laboratory space and essential equipment that facilitated the successful completion of this research.

## Declaration of Competing Interest:

The authors declared no conflicts of interest.

## References:


1. Necola S. Allium cepa variety evaluation for organic farming in the 5th int. symposium on Edible Allianceae. Dronten the Nether lands. ISHS. 2007 45.
2. Khokhar KM. Environmental and genotypic effects on bulb development in onion–a review. The Journal of Horticultural Science and Biotechnology. 2017;92(5):448-54.
3. Griffiths G, Trueman L, Crowther T, Thomas B, Smith B. Onions: a global benefit to health. Phytother Res. 2002;16(7):603–615.
4. Ponvizhi Ramya V, Gunasekaran S, Senthil Kumar M. Genetic Diversity of Soft Rot Pathogens in Potato of South Indian Origin. Research Journal of Biotechnology Vol. 2014;9:5.
5. Schwartz HF, Mohan SK. Compendium of onion and garlic diseases and pests. Compendium of onion and garlic diseases and pests. 2007(Ed. 2).
6. Wright PJ, Clark RG, Hale CN. A storage soft rot of New Zealand onions caused by Pseudomonas gladioli pv. alliicola. New Zealand journal of crop and horticultural science. 1993;21(3):225-7.
7. Agrios GN. Plant pathology 5th edition: Elsevier academic press. Burlington, Ma. USA. 2005:79-103.
8. Kumar V, Neeraj SS, Sagar NA. Post harvest management of fungal diseases in onion—a review. International Journal of Current Microbiology and Applied Sciences. 2015;4(6):737-52.
9. Kim YK, Lee SD, Park CS, Lee SB, Lee SY. Soft rot of onion bulbs caused by Pseudomonas marginalis under low temperature storage. The Plant Pathology Journal. 2002;18(4):199-203.
10. Gupta SK, Thind TS. Disease problems in vegetable production. Scientific Publishers; 2018 Apr 1.
11. Liao CH, Wells JM. Diversity of pectolytic, fluorescent pseudomonads causing soft rots of fresh vegetables at produce markets. Phytopathology. 1987;77(5):673-7.
12. Samson R, Legendre JB, Christen R, Saux MF, Achouak W, Gardan L. Transfer of Pectobacterium chrysanthemi (Burkholder et al. 1953) Brenner et al. 1973 and Brenneria paradisiaca to the genus Dickeya gen. nov. as Dickeya chrysanthemi comb. nov. and Dickeya paradisiaca comb. nov. and delineation of four novel species, Dickeya dadantii sp. nov., Dickeya dianthicola sp. nov., Dickeya dieffenbachiae sp. nov. and Dickeya zeae sp. nov. International Journal of Systematic and Evolutionary Microbiology. 2005;55(4):1415-27.
13. Costechareyre D, Balmand S, Condemine G, Rahbé Y. Dickeya dadantii, a plant pathogenic bacterium producing Cyt-like entomotoxins, causes septicemia in the pea aphid Acyrthosiphon pisum. PLoS One. 2012;24;7(1):e30702.
14. Cother EJ, Gilbert RL. Presence of Erwinia chrysanthemi in two major river systems and their alpine sources in Australia. Journal of Applied Microbiology. 1990; 1;69(5):729-38.
15. Grenier AM, Duport G, Pages S, Condemine G, Rahbé Y. The phytopathogen Dickeya dadantii (Erwinia chrysanthemi 3937) is a pathogen of the pea aphid. Applied and Environmental Microbiology. 2006;72(3):1956-65.
16. Sławiak M, Łojkowska E, Van der Wolf JM. First report of bacterial soft rot on potato caused by Dickeya sp.(syn. Erwinia chrysanthemi) in Poland. Plant Pathology. 2009;58(4).
17. Abdalla ME, Hamza S, Fayzalla SA, Seeda SE. Post harvest control of bacterial soft rot pathogens of onion bulb in storage. Journal of Plant Protection and Pathology. 2013;4(11):945-57.
18. Bartz JA, Kelman A. Reducing the potential for bacterial soft rot in potato tubers by chemical treatments and drying. American potato journal. 1986;63:481-93.
19. Bonde R, de Souza P. Studies on the control of potato bacterial seed-piece decay and blackleg with antibiotics. American Potato Journal. 1954;31(10):311-6.
20. Harris, R. I. Chemical control of bacterial soft-rot of wounded potato tubers. Potato Research. 1979;22, 245-249.
21. Shing R S. Diseases of Vegetable crops. Oxford and IBA Publishing Company Pvt. Ltd. New Delhi. 1985;331pp.
22. Coventry MJ, Gordon JB, Wilcock A, Harmark K, Davidson BE, Hickey MW, Hillier AJ, Wan J. Detection of bacteriocins of lactic acid bacteria isolated from foods and comparison with pediocin and nisin. Journal of Applied Microbiology. 1997; 83(2):248-58.
23. Suslow TV, Schroth MN, Isaka M. Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology. 1982;72(7):917-8.
24. Reiner K. Catalase test protocol. American society for microbiology. 2010;11:1-6.
25. Cowan ST, Steel KJ. Manual for the identification of medical bacteria. (No Title). 1974.
26. Patel A, Prajapati JB, Holst O, Ljungh A. Determining probiotic potential of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented food products. Food Bioscience. 2014; 1(5):27-33.

27. AL-Saleh MA. Pathogenic variability among five bacterial isolates of Xanthomonas campestris pv. vesicatoria, causing spot disease on tomato and their response to salicylic acid. Journal of the Saudi Society of Agricultural Sciences. 2011;10(1):47-51.
28. Pina-Pérez MC, González A, Moreno Y, Ferrús MA. Helicobacter pylori growth pattern in reference media and extracts from selected minimally processed vegetables. Food Control. 2018 Apr 1;86:389-96.
29. Schaad NW, Jones JB, Chun W. Laboratory guide for the identification of plant pathogenic bacteria. American Phytopathological society (APS press). 2001.
30. Naqqash T, Hameed S, Imran A, Hanif MK, Majeed A, van Elsas JD. Differential response of potato toward inoculation with taxonomically diverse plant growth promoting rhizobacteria. Frontiers in plant science. 2016;7:144.
31. Thornberry HH. A paper-disk plate method for the quantitative evaluation of fungicides and bactericides. Phytopathology. 1950;40(5).
32. Anonymous. Antibiotics. In: Drug Today. P.O. Box No. 9116 Delhi 110091, 2003; 218-232.
33. Bhattacharya I, Mukherjee N. Soft rot of storage tissue due to some uncommon bacteria: some epidemiological aspects. Indian Agriculturist. 1986;30(1); 75-82.
34. Raju MR, Pal V, Jalali I. Inoculation method of Pectobacterium carotovorum subsp. carotovorum and factors influencing development of bacterial soft rot in radish. J. Mycol. Plant Pathol. 2008;38:311-5.
35. Walker JC. Bacterial soft rots of carrot. Diseases of Vegetable Crops. Discovery Publishing Company, New Delhi. 1998;184-215.
36. Mills AA, Hurta RA. Sensitivity of Erwinia spp. to salt compounds in vitro and their effect on the development of soft rot in potato tubers in storage. Postharvest Biology and Technology. 2006; 41(2):208-14.
37. HIGASHIO H, Yamada M. Control of soft rot after harvest of cabbage in Indonesia. Japan Agricultural Research Quarterly: JARQ. 2004;38(3):175-8.