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Bacterial root bark necrosis and wilt of pomegranate, hereto a new disease

Abstract

A disease with new symptomatology was observed on several pomegranate plants in pomegranate research orchard of Mahatma Phule Agriculture University, Rahuri, India. The disease symptoms include initial yellowing and drooping of plant leaves on one or two branches, followed by leaf fall and wilting of the affected branches. Subsequently, the same types of symptoms appear on other branches leading to its death. The entire plant wilts within a period of 2-3 months. There is no recovery of the wilted plant or wilted branches.

The roots of infected plants show root bark necrosis symptoms. Root bark necrosis is observed at several places on the root or at the site of infection on the root. Several necrosis lesions coalesce to form large necrotic patches on the root. The necrotic bark is easily peeled off from the root.

Microscopic diagnosis of infected root bark revealed the presence of bacteria, which were isolated and proved to be pathogenic on pomegranate plant producing wilting symptoms. No vascular infection is observed. The bacterial infection is limited to cortex tissues and eventually, the root bark is detached. The bacterium produces either browning reaction or induces chlorophyll loss in the bacterial infiltrated areas in leaves of *solanaceous* plants like chilli, tomato, eggplant and cause wilts in these plants when present in the soil. The bacterium is identified as *Klebsiella pneumoniae* by using 16 S rRNA sequence.

The root bark necrosis and wilt in pomegranate due to bacterial infection is a new disease report. It is here to an unknown disease in the pomegranate growing regions of the world. Therefore the disease symptoms and the causal bacterium were studied.

Keywords: *Klebsiella pneumonia*, pomegranate, root bark necrosis, wilt

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Introduction

Pomegranate is an important fruit crop grown in agro-climatic condition ranging from tropical to temperate[1] and in various countries like Israel, Afghanistan, Pakistan, Egypt, India and different counties of USA. The current total annual world production of pomegranate is estimated to be 1.5 million tonnes.[2] According to an estimate, more than 0.119 million tonnes pomegranate fruit was exported from Iran, India, Tunisia, U.S.A, Spain, etc to different countries.[2] The cultivation of pomegranate has expanded to five continents with India, Iran, China, Turkey, U.S.A, Spain, South Africa, Peru, Chile and Argentina as major players in production. Since 2013, India has been the world's largest pomegranate producer and one of the largest exporters of fresh and processed pomegranate with the production of 745,000 tonnes since 2013. Iran is second followed by China. India ranks first in the world with respect to pomegranate area (0.125 million hectares) and production (1.14 million tonnes). In India it is grown under the arid dry climate of Rajasthan, Maharashtra, Karnataka, Andhra Pradesh states which occupy a major area in this fruit crop for India's National Horticultural Mission, uplifting the economic status of the fruit crop growing farmers. Maharashtra contributes more than 75% of the total area alone followed by Karnataka and Andhra Pradesh.

The production in most of the cases is reduced by pest and diseases which are not controlled properly or for which no pesticidal advisory or label claim is available on the new emerging disease.

A disease with new symptomatology was observed on several pomegranate plants in pomegranate research orchard of Mahatma Phule Agriculture University, Rahuri, India. It is here to an unknown disease in the pomegranate growing regions of the world. The disease symptomatology and causative pathogen were studied in detail.

Materials and methods

Description of disease symptomatology

Standard terms were used to describe the disease symptomatology on leaf, twigs, branches, plant and root systems.

Isolation of disease-causing pathogen and Koch's postulates

The root bark necrosis portions on root systems were used for microscopic observations of the causative agent which revealed the presence of bacteria and therefore the same was used for isolation of these bacteria on nutrient agar media. The standard procedure of bacterial isolation was followed.[3] The bacterial colonies were purified, grown in nutrient broth and used for pathogenicity test under pot culture experiment in the glasshouse on pomegranate rooted cuttings. For this, the bacterial growth in nutrient broth was mixed with sterile soil in plastic pots and used for planting of pomegranate rooted cuttings. The pots were maintained at the 30±2^{0}c temperature, 87% relative humidity with misting for 20 seconds after every 2hrs. The development of wilting symptoms was recorded, re-isolation of

the bacterial pathogen from wilted plant roots were carried out and compared with original bacterial cultures.

Production of hypersensitive reaction and wilt on the *solanaceous* host

To test the hypersensitive reaction on the *solanaceous* host particularly tobacco, eggplant, chilli and tomato, 0.1ml of fresh bacterial growth (48hrs) suspension (0.1 OD at 620nm) was syringe infiltrated in the leaves of these plants and observed for the types of reaction developed in the inoculated areas of the leaves.

Similarly the 20 days old seedlings of eggplant, chilli and tomato were planted in the bacterial sick soil in plastic pots under glasshouse condition. The test seedlings pots were maintained at 30±2°C temperature, 87% relative humidity with misting for 20 seconds after every 2hrs. The development of wilting symptoms was recorded, re-isolation of the bacterial pathogen from wilted plant roots were carried out and compared with original bacterial culture.

Results

Disease symptomatology of root barks necrosis and wilt on pomegranate

The disease symptoms appear as yellowing of leaves of one or two twigs followed by the drooping and subsequent death of the infected twigs. Subsequently, other twigs also show infection and the entire plant die within 2-3months (Figure 1).

The root system of the infected plant shows root bark necrosis lesions spread on the infected root system. The necrosis lesions show dark brown blackish areas (Figure 2). The root bark of affected region separates out easily.

There is no infection of xylem vessels. The bacterial infection is limited to the cortex portion of the root and eventually, the root bark (cortex and epidermis of root) is detached from the root (Figure 3).

When such root bark necrosis showing roots were immersed in water in a beaker, the infected loci showed water bubbling on them (Figure 4) after 48 hours due to the presence of bacterial infection while healthy root does not show such water bubbling.

Disease-causing bacterium

The bacterium from diseased tissues was isolated on nutrient agar media, which produces creamy white, circular, raised, fluidal colonies of 3-4mm diameter on the 4th day, at the 28±2°C temperature (Figure 5).

As the culture aged further, there is frothing during the bacterial growth, characteristic of anaerobic nature of bacterial cultures (Figure 6).

The bacterial culture was used to prove its pathogenicity on pomegranate plant and was found pathogenic which caused wilting of test pomegranate plant. The wilting symptoms were observed by 25-30 days after planting (Figure 7) which was similar to initial field symptoms of wilt.

The bacterium was identified as *Klebsiella pneumoniae* by using 16S rRNA gene sequence which was deposited in NCBI (Gene accession number KY941097.1). The NCBI designate this bacterium as *Klebsiella pneumoniae* strain Borkar.

Figure 1 Symptoms of wilting on pomegranate plant.

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Figure 2 Symptoms of root bark necrosis on roots of wilted plant.

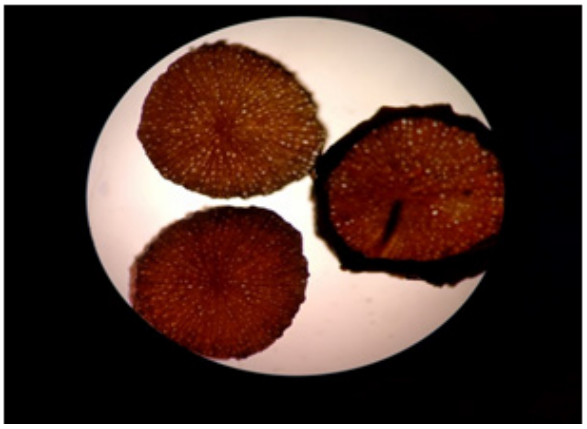

Figure 3 Disintegration of root bark from cortex tissues due to bacterial infection.

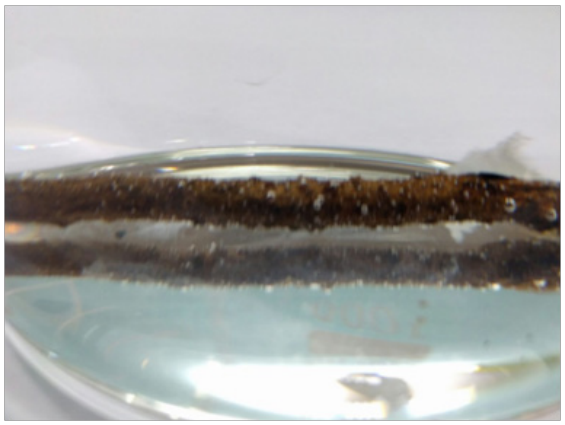

Figure 4 Water bubbling (In water immersed infected root) at the site of bacterial infections.

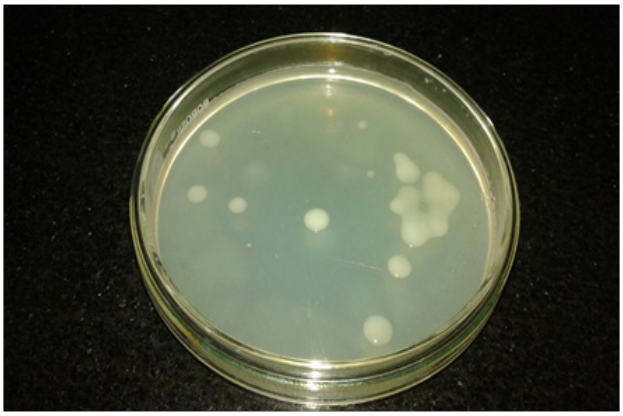

Figure 5 The isolated colonies of *Klebsiella pneumoniae* on nutrient sucrose agar medium.

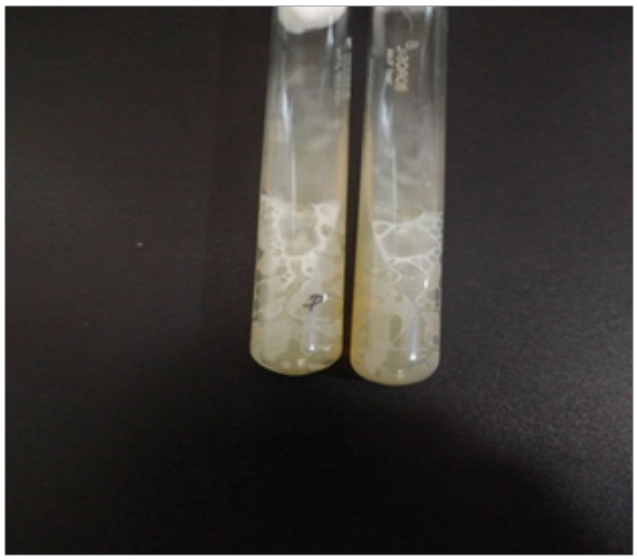

Figure 6 Frothing of Klebsiella pneumoniae in pure culture NAS slants.

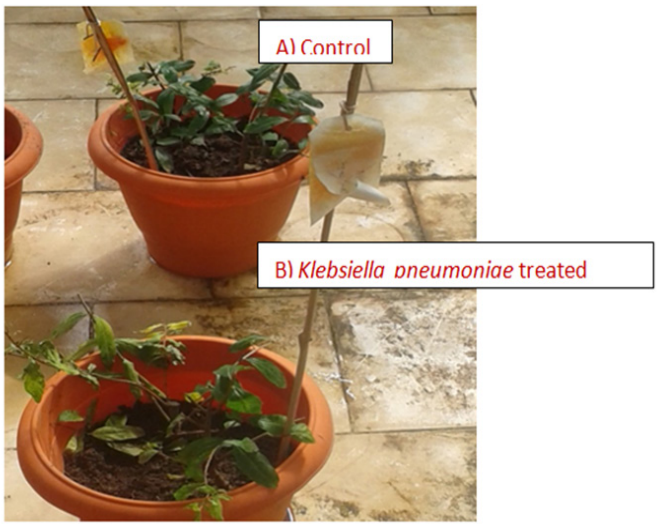

Figure 7 Wilting of pomegranate plants in pathogenicity test of *Klebsiella pneumoniae*.

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Discussion

Root bark necrosis and wilt in pomegranate is a new disease not reported earlier.[4] It is here to an unknown disease in the pomegranate growing regions of the world. Therefore the disease symptoms and the causal bacterium were studied. The association of bacterial pathogen *Klebsiella pneumoniae* makes it a special disease as *Klebsiella pneumoniae* is a human pathogen causing pneumonia.[5] It is not reported as a plant pathogen though its association is reported with roots of maize, wheat, and poa, probably as a nitrogen fixer.[6,7] The association of *Klebsiella pneumoniae* with pomegranate root-induced root bark necrosis symptoms on roots which results in yellowing of leaves on twigs with ultimate wilting of branches and the whole plant.

The bacterial infection was observed only on the root bark and not in the vascular tissues as reported in other bacterial wilt diseases.[8–11]

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Conflict of interest

The authors declare there is no conflict of interest.

References

1. Levin GM. *Pomegranate*. 1st ed. East Libra Drive Tempe, AZ, USA: Third Millennium Publishing; 2006:1–129.
2. Holland D, Bar-ya-akov I. The pomegranate; New interest in an ancient fruit. *Chronica Horticulturae*. 2008;48(3):12–15.
3. Borkar SG. *Laboratory techniques in plant bacteriology*. USA: CRC Press; 2017. 256 p.
4. Borkar SG, Yumlembam RA. *Bacterial diseases of crop plants*. USA: CRC Press; 2016. 594 p.
5. Podschun R, Ullman U. *Klebsiella spp*. As nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factor. *Clin Microbiol Rev*. 1998;11(4):589–603.
6. Chelius MK, Triplett EW. Immunolocalization of dinitrogenase reductase produced by *Klebsiella pneumoniae* in-association with Zea mays. *Appl Environ Microbiol*. 2000;66(2):783–787.
7. Haahtela K, Kari K. The role of root-associated *Klebsiella pneumoniae* in the nitrogen nutrition of *poa pratensis* and *Triticum aestivum* as estimated by the method of 15N isotope dilution. *Plant and soil*. 1986;90:245–254.
8. Janse JD, Arulappam F, Schans J, et al. Experiences with bacterial brown rot Ralstonia solanacearum biovar 2, race 3 in the Netherlands. In: Allen PP, Elphinstone J. editors. *Bacterial wilt disease: molecular and ecological aspects*. Berlin (DE), Germany: Springer; 1998:146–152.
9. Allen CP, Prior P, Hayward AC. *Bacterial wilt disease and the Ralstonia solanacearum species complex*. St Paul, Minnesota USA: APS Press; 2005.
10. Alvarez B, Vasse J, Le-Courtois V, et al. Comparative behaviour of *Ralstonia solanacearum* biovar 2 in diverse plant species. *Phytopathology*. 2008b;98(1):59–68.
11. Peeters N, Guidot A, Vailleau E, et al. *Ralstonia solanacearum*, a widespread bacterial plant pathogen in the post-genomic era. *Mol Plant Pathol*. 2013;14(7):651–662.

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