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PLANT PATHOLOGY

An overview of bacterial blight disease: A serious threat to pomegranate production

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Abstract

Bacterial blight of pomegranate is among the most devastating natural calamity that inflicted huge losses to pomegranate crop productivity especially in India during the last 24 years. The dilemma of bacterial blight is still under discussion among the researchers since its appearance in 1952. Symptoms of the disease manifested as numerous, small, segregated, depressed, discoloured and typically water-soaked spots. The epidemiology of the disease remains prevalent in mild to moderate form throughout the year at higher temperature ranged between 20.0-43.0° C during April-July and become severe under highly humid conditions (>80 %) and moderate temperature (25-35° C) during rainy season. None of the genotypes exhibited resistant against bacterial blight. Some genotypes found moderately susceptible against bacterial blight of pomegranate. Management of the disease is only by various chemicals.

Highlights

- Many experiments and studies by various authors revealed that bacterial blight of pomegranate is caused by *Xanthomonas axonopodis* pv. *punicae*.
- Disease can occur in epidemic under highly humid conditions (>80 %) and moderate temperature (25-35° C) during rainy season.
- Disease can be managed by use of copper (copper oxychloride and copper hydroxide) salt with combination of any antibiotic.

Keywords: Bacterial blight, pomegranate, *Xanthomonas axonopodis* pv. *punicae*, epidemiology, screening, management

Pomegranate (*Punica granatum* L.) is one of the ancient fruits associated with several human cultures of the world. There is mention of pomegranate in the Bible, the Koran, and in Buddhist and Chinese arts. Based on evidence from archeo-botanical samples, literature, religious iconography etc., it is estimated that pomegranate might have been introduced into culture about 5000 years ago. According to various reports, wild pomegranate grows in Transcaucasia and Central Asia from Iran and Turkmenistan to northern India. Thus, it is considered to be native of these regions.

Pomegranate grows very well on the moderately alkaline soils as well as slightly acidic soils. It is common to the tropics, sub-tropics and sub-temperate regions and is well adapted to areas with hot and dry summers. Pomegranate has renewed interest as a commercial orchard crop because of the health benefits associated with its high level of antioxidants in the pulp or juice. Its nutritional, therapeutic and ornamental values were known to humans since antiquity. Although pomegranate was reported to have a narrow genetic base, its huge collections available in different parts of the world

indicate that it has high genetic diversity among the germplasm. Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family punicaceae. This family has a single genus Punica with two species viz., P. granatum and P. protopunica. (Chandra *et al.* 2010a).

Pomegranate is a good source of carbohydrates and minerals such as calcium, iron and sulphur. Malhotra *et al.* (1983) studied that pomegranate is rich in vitamin-C and citric acid is the most predominant organic acid. Glucose (5.46%) and fructose (6.14%) are the main sugars with no sucrose in fruits. Chandra *et al.* (2010b) reported that pomegranate is rich in several potentially active phytochemicals like sterols and terpenoids, fattyacids and triglycerides, simple gallyol derivatives, organic acids, flavonols, anthocyanins and anthocyanidins, catechin and procyanidins. At present, 187 (both exotic and indigenous) germplasms are available in its national field genebank. In the last 50 years, 10 pomegranate cultivars have been identified and released for commercial cultivation. Of the released cultivars, 'Bhagawa' and 'Ganesh' are popular among farmers.

It is extensively cultivated in Spain, Morocco and other countries around the Mediterranean, Egypt, Iran, Afghanistan, Arabia and Baluchistan. India ranks second with an annual export of 33,415 tons after Iran (67,000 tons) (Jadhav and Sharma 2009). In India, pomegranate was grown in area of 112.74 thousand hectare with production of 741.08 thousand MT in 2012-13 (Anonymous 2013).The fruits of pomegranate are known to possess pharmaceutical and therapeutic properties, but pomegranate production is associated with many problems. Inherent constraints are long dry spells, non-availability of resistant varieties, environmental conditions, nutritional deficiencies and physiological disorders. Biotic constraints are pest and disease problems which threaten pomegranate cultivation. Prominent diseases are leaf spots incited by *Colletotrichum gloeosporioides, Cercospora punicae, C. granati, Sphaceloma, Phyllosticta* sp. and *Xanthomonas punicae*, canker caused by *Ceuthospora phyllosticta* and soft rot incited by *Rhizopus arrhizusand R. stolonifer*. Among the various diseases, bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* is a wide spread disease affecting its successful production and every year results into 50-100 per cent economic losses depending upon disease severity. A disease of minor importance at one time has now emerged as a constraint of significance because of increased incidence over years in all the pomegranate growing regions (Anonymous 2002). It assumed epidemic form occurs in all pomegranate growing states resulting in abandoning of crop by the farmers. In recent years, due to severe/epidemic outbreak of the disease, many farmers have started uprooting the plants and destroying orchards and have incurred heavy losses.

Occurrence of the Disease

It has been reported to occur in all the major pomegranate growing countries of the world including South Africa, Pakistan, Egypt and Australia. The disease was recorded first time in Egypt by Boulos *et al.* (1968). Akhtar and Bhatti (1992) recorded first time this disease from Pakistan. Peterson *et al.* (2007) observed first time bacterial blight of pomegranate disease from South Africa.

First time in India, Hingorani and Mehta (1952) reported the occurrence of leaf spot of pomegranate. Microscopic examination of lesions revealed the presence of myriads of bacteria. The presence of *X. punicae* in pomegranate orchards was also confirmed by Vasudeva in 1956. Later Hingorani and Singh (1959) took thorough investigation of the disease and pathogen and reported the presence of disease in different parts of the country.

Rangaswami (1962) observed bacterial leaf spot of pomegranate at Annamalainagar of Madras state. The disease was reported by Sohi *et al*. (1964) in Solan region of Himachal Pradesh. Rani (1998) reported that disease caused by *X. axonopodis* pv. *punicae* manifested as numerous, small, segregated, depressed, discoloured and typically water-soaked spots on the surface of young fruits.

Economic Losses

Bacterial blight has developed into a very serious disease in India, where it causes very heavy losses in many pomegranate growing areas (Kumar et al. 2009). Mondal and Sharma (2009) recorded 60-80 per cent losses in India. Chand and Kishun (1991) have noticed the severe incidence of the disease causing 60-80 per cent losses at IIHR experimental plots in Bangalore. Pomegranate, the boon commercial fruit crop to the farmer turned as a big bane after

the outbreak of bacterial blight. Survey of 82 pomegranate orchards in Maharashtra revealed that bacterial blight was observed up to 100 per cent severity in some orchards (Anonymous 2007).

Subramanyam (2001) recorded 71.0 and 62.5 per cent disease severity on leaves and fruits, respectively. According to Rani (1998), the disease severity on fruits and leaves recorded on different cultivars varied from 18.43 to 45.67 per cent and from 15.32 to 37.81 per cent, respectively in Punjab. During 2002, outbreak of the disease was noticed in major pomegranate areas of Bellary and Bijapur districts of Karnataka (Anonymous 2002). Ravikumar *et al.* (2006) revealed 20-90 per cent disease severity in Bijapur and Bagalkot districts of Karnataka. Bacterial blight causes loss upto 70-80 per cent on pomegranate in Karnataka (Benagi *et al.* 2011).

Symptomatology

During 1952, Hingorani and Mehta described the symptoms as irregular spots varying from 2 mm to 5 mm in diameter, primarily appeared on the leaves. The spots were initially light brown in colour, surrounded by water-soaked margin, later turned to dark brown as the disease progressed. Rangaswami (1962) described the symptoms of bacterial blight on leaves as necrotic spots surrounded by chlorotic halos with translucent water-soaked appearance. Sohi *et al.* (1964) stated that the leaf spots of pomegranate due to *X. punicae* were circular to irregular in shape varying from less than 1 mm to more than 5 mm in diameter and light brown in colour. Kanwar (1976) observed symptoms of bacterial blight as small, brown, water-soaked spots on leaves, flowers and fruits of pomegranate in different orchards of Haryana. In the beginning, spots on leaves were small, circular with yellowish border and brown centre. Later on, number of adjacent spots coalesced with each other and formed elongated and irregular lesions.

Rani (1998) reported that disease caused by *X. axonopodis* pv. *punicae* manifested as numerous, small, segregated, depressed, discoloured and typically water-soaked spots on the surface of young fruits. The spots coalesced to form large, irregular, necrotic patches. Manjula and Khan (2002) described the symptoms as minute water-soaked lesions appeared both on leaves and fruits, which later turned brown to black coloured spots surrounded by diffused water-soaked margin. The necrotic lesion on the fruit increased as the fruit size increased with age leading to L, Y or star shaped cracking within the spots. Peterson *et al.* (2010) observed symptoms included leaf and fruit spots and cankers on stems, branches and trunks. Mulla *et al.* (2009) and Mondal *et al.* (2012) studied that initially symptoms on leaves were irregular, water-soaked spots (2 to 5 mm diameter) on foliage which later became necrotic with brown centre and finally turned dark brown. In severe infection diseased leaves distorted and shed off.

Ashish (2014) observed that initially small, discoloured and water-soaked spots were noticed on the leaves. On the upper surface of leaves, diffused water-soaked zone was seen around the spot. Later on these spots increased in size (2.0–5.0 mm in diameter), coalesced and extended upto midrib within a week covering the major portion of the leaf lamina. The infected leaves lost their lush green colour and became yellow resulting into premature leaf fall.

Icoz *et al.* (2014) observed this disease from Turkey and reported that disease were characterized by dark brown, angular to irregular shaped spots on leaves and fruits, canker on stems, branches and trunks.

Identification of the Pathogen

Hingorani and Mehta (1952) and Hingorani and Singh (1959) designated the pathogen of leaf spot of pomegranate as *X. punicae* and described it as short rods with rounded ends, single or in pairs, sometimes in chains, measuring 1-2.5 × 0.5 µm in size, motile with a single polar flagellum, Gram-negative, non-endospore forming and capsules present. Growth on nutrient dextrose agar plates was slow, filiform with edges entire, glistening and colourless to pale yellow and butyrous. Rangaswami (1962) and Thirumalachar and Patel (1966) also isolated *X. punicae* from infected samples of pomegranate.

According to Kanwar (1976), the pathogen of bacterial blight of pomegranate occured in single pairs and also in chains, rod shaped with rounded ends, measuring 0.75-3.0 µm × 0.45 µm, Gram-negative with single polar flagellum. Kishun (1991) isolated the *X. campestris* pv. *punicae* from leaf, fruit and node. The colonies were mucoid, circular, convex, yellow, rounded, glistening and raised on nutrient agar medium. Rani (1998)

reported that colonies on nutrient agar were pale yellow, circular, convex, opaque, mucoid, butyrous and glistening with entire margins. Bacterial cells were straight rods, Gram-negative, motile, singly or in pairs. Manjula (2002) reported that seven isolates of the pomegranate bacterium were small rods, appeared singly, rarely in pairs, Gram-negative, capsulated and non-spore forming with monotrichous flagellation.

Mogle *et al.* (2009) observed that bacterium (*X. axonopodis* pv. *punicae*) was short rod with rounded ends, arranged singly or in pairs, 1.25-2.0 × 0.5-0.7 μm in size, motile with single polar flagellum, Gram-negative and capsulated. Mondal *et al.* (2012) also observed that bacterial blight of pomegranate is caused by a yellow pigmented, Gram-negative, rod shaped bacterium named as *X. axonopodis* pv. *punicae*. Ashish (2014) observed that bacterium produced colonies on nutrient agar medium were pale yellow, circular, convex, slightly raised, opaque and mucoid, named as *X. axonopodis* pv. *punicae*.

Host Range Studies

Hingorani and Singh (1959) inoculated 59 hosts with and without injury for determining the host range of *Xanthomonas punicae*. Out of these many hosts, the pathogen attacked only *Punica granatum* L. The host plants inoculated with bacterial culture were *Abelmoschus esculentus* Moench, *Amaranthus* viridis L., *Arachis hypogaea* L., *Begonia* sp., *Brassica campestris* var. *rapa* L., *Brassica oleracea* var. *botrytis* L., *Brassica oleracea* var. *capitata* L., *Cajanus cajan* L., *Capsicum frutescens* etc.

Manjula (2002) inoculated with bacterial culture, isolated from pomegranate to various crops such as maize, ragi, paddy, jowar, cowpea, beans, tomato, carrot and cabbage and found that none of these plants were infected.

Ravikumar *et al.* (2005) succeeded in establishing the host range of the pomegranate bacterium and reported the natural infection by pathogen on neem (*Azadirachta indica* L.) and bael (*Aegle marmelos* L.) grown nearby the infected pomegranate gardens. Samples were collected, analyzed for symptom similarity and associated pathogen. The results on pathogenicity and cross inoculation studies confirmed the pathogen.

Disease Cycle

Bacteria propagate in lesions in leaves, stems and fruit. When there is free moisture on the lesions, the bacteria ooze out and can be dispersed to infect new growth. Wind-driven rain is the main dispersal agent. Wind aids in the penetration of bacteria through the stomatal pores or wounds made by thorn or by birds and insects on fruit. Pruning cause severe wounding and can lead to infection. The bacteria remain alive in the margins of the lesions in leaves and fruit until they abcise and fall to ground. Bacteria have also been reported to survive in lesions on woody branches upto a few years of age.

Bacteria that ooze onto plant surfaces do not survive and begin to die upon exposure to rapid drying. Death of bacteria is also accelerated by exposure to direct sunlight. Survival of exposed bacteria is limited to a few days in soil and to a few months in plant refuse that is incorporated into soil. On the other hand, the bacteria can survive for years in infected plant tissues that have been kept dry and free of soil.

Survival of the Pathogen

The vital role of fallen leaves in the survival of the phytopathogenic bacterium causing leaf spot disease of various crops is well established (Burkholder 1948). Hingorani and Singh (1959) conduct studies to know about the survival of Xanthomonas bacteria in leaves under different conditions indicated that the pathogen survived on fallen leaves from December to mid March and reproduced from mid March to end of June.

According to Rangaswami (1962) the pathogenic bacterium causing leaf spot/oil spot of pomegranate (*X. axonopodis* pv. *punicae*) infects through wounds and stomatal openings and causes water-soaked lesion, which later develops into irregular spots. The organism spread by air borne cells, could survive in soil for four months and cause fresh infections on new flush. Rani and Verma (2002) reported from Punjab that the pathogen *X. axonopodis* pv. *punicae* survived in the infected fallen leaves kept protected under field condition upto 210 days and in canker lesions upto 80 days under Punjab conditions.

Epidemiology

According to Hingorani and Singh (1959), Kishun (1993) and Atulchandra *et al*. (1994) the disease caused by bacteria on pomegranate spreads very fast due to high temperature and low humidity from March to July months. Rani (1998) observed that bacterial blight of pomegranate assumed serious proportions from the time of its appearance in the first week of May till August in Punjab. The disease severity on fruits and leaves recorded on different cultivars varied from 18.43 to 45.67 per cent and from 15.32 to 37.81 per cent, respectively. The rate of increase in size of the spot was observed to be very slow during May to June but the spots witnessed a more than three-fold increase in size during July showing an increase from 3.25 mm to 12.5 mm. There was a rapid enlargement of the lesions produced on leaves as well as on branches.

Studies conducted by Yenjerappa *et al.* (2006) revealed that the pomegranate crop pruned during first and second fortnight of September was almost free from bacterial blight index from pruning to harvest except that very negligible disease intensity on foliage was noticed in the beginning of the crop period. The reason being the uncongenial weather such as low minimum temperature (10.8–19.4°C) and no rainfall received (November–March) during growth and development stage of the crop. On the contrary, the crop pruned in the month of November was absolutely free from bacterial blight infection at early stages of its growth from December to March owing to uncongenial weather prevailed but disease started progressing from April onwards with the receipt of unusual rains and prevalence of higher temperature during April and May (maximum temperature ranged between 36.5–42.9°C and minimum temperature between 20.8–24.2°C). The disease severity coincided with the fruit development and fruit maturity stage, where 90 per cent of developing fruits got infected resulting into the huge loss in yield and quality.

Jadhav and Sharma (2009) reported that the disease remains prevalent in mild to moderate form throughout the year at temperature of 9.0-43.0°C and lower humidities and become severe under highly humid conditions (>80 %) and moderate temperature (25-35°C) during rainy season.

Ashish *et al.* (2015) concluded that unit change in maximum/minimum temperature and mean minimum temperature exerted influence on disease index up to an extent of 12.96 units in positive direction and 12.16 units in negative directions, respectively, while mean morning and evening relative humidity had influence on disease index (4.03 units in positive and 1.92 units in negative directions, respectively) and unit change in the total rainfall influenced disease index by 0.12 unit in negative directions in the variety Ganesh and Kandhari.

Screening of Pomegranate Germplasm

Rani (1998) reported that Chawla-2, Ganesh and PS-75-K3 expressed highly susceptible reaction, where as Kandhari showed moderately susceptible reaction on fruits. All the other cultivars including Achikdana, Ekanar, Anar Shri Mohamad Ali, Assam Local, Basse-in-Seedless, Chawla-1, Co-1, G-137, Jodhpur White, Kandhari Ganganagar, Malas, Moga local, Nabha, Panipat Selection, P-26, PS-77, Russian Seedless and Shirin Anar were found to show susceptible disease reaction on fruits. None of the cultivars expressed highly susceptible disease reaction on leaves. All other cultivars showed susceptible reaction except the two cultivars, namely, Kandhari and Moga Local which expressed moderately susceptible disease reaction.

Sharma *et al.* (1990) revealed that 'Daru' (wild form) was resistant to bacterial blight. Mondal *et al.* (2012) recorded that cultivar Bhagwa was highly susceptible to bacterial blight. Jalikop et al (2006) observed that hybridization of Daru with susceptible cultivar Ganesh recorded a mean disease score of 3.8 per cent indicating that the resistance in Daru was controlled by recessive alleles while another variety Nana, a miniature pomegranate having ornamental value (Nath and Randhawa 1959) also showed resistance but its hybridization with Ganesh exhibited little susceptibility (0.42 per cent). These findings revealed the role of incomplete dominant alleles in determining the bacterial blight resistance. Daru and Nana may be used as valuable donors for bacterial blight resistance.

Ashish *et al.* (2014) studied that three genotypes namely Chawla-1, Ichakdana and Moga local showed moderately susceptible reaction under natural conditions. Whereas, the genotypes including Anar-Shirin, Chawla-2, G-137, Jodhpur White and Kali-Shirin expressed susceptible reaction on leaves and

fruits under natural as well as artificial inoculation conditions except Chawla-2 and Kali-Shirin, which were found to be highly susceptible on fruits under artificial inoculation conditions. The Ganesh and Kandhari graded as highly susceptible as those had >40 per cent disease index on leaves and fruits under both natural as well as artificial inoculation conditions.

Integrated approach for Disease Management

Suriachandraselvan *et al.* (1993) reported that 3 sprays of paushamycin (0.05%) + copper oxychloride (0.2%) at fortnightly intervals was most effective in controlling the bacterial blight on pomegranate caused by *X. campestris* pv. *punicae*. Atulchandra *et al.* (1994) stated that same bacterium could be controlled by spraying of Bordeaux mixture (5:5:50) and other copper fungicides at an interval of 15 days. Ravikumar *et al.* (2002) reported that pruning of infected parts along with one spray of copper oxychloride followed by four sprays of streptocycline (100 ppm) + copper oxychloride (0.3%) was found very much promising in reducing the incidence of bacterial canker of acid lime.

Ravikumar and Yenjerappa (2005) found that five sprays of bactrinashak (500 ppm) + copper oxychloride (2000 ppm) were significantly effective in reducing the bacterial blight of pomegranate. Highest yield and maximum benefit cost ratio was recorded with the same treatment. Method demonstration in farmers field comprising practices like clean sanitation, removing water shoots, spraying streptocycline, pasting of stem and branches with copper oxychloride + carbaryl + sticker etc. were carried out in a campaign organized against oily spot disease of pomegranate (*X. axonopodis* pv. *punicae*), which benefited growers at large (Anonymous 2005b). Jamadar *et al.* (2009) studied that copper oxychloride (2000 ppm) + streptocycline (500 ppm) or bactrinashak had been found most effective against bacterial blight of pomegranate.

Raju (2010) studied that among the different chemicals evaluated to prevent the spread of disease from stem infection revealed that copper oxychloride and copper hydroxide (0.25%) each in combination with streptocycline (0.05%) recorded the least disease incidence and severity. The highest yield was recorded in streptocycline + copper oxychloride (6.12 tons/ha) followed by streptocycline + copper hydroxide (5.91 tons/ha).

Ashish *et al.* (2014) revealed that bacterial blight can be effectively managed by pruning of the diseased twigs followed by four sprays of Blitox + streptocycline or Kocide + streptocycline starting from mid June to end July at 15 days interval. It concluded that Blitox + streptocycline and Kocide + streptocycline have shown superiority over other agro-chemicals in reducing the per cent disease index on leaves and fruits of pomegranate. The more effectiveness of these agro-chemicals might be due to their more potentiality to reduce inoculum and fresh infection of the pathogen.

Conclusion

It is evident from various studies that bacterial blight is devastating disease on pomegranate and resulted economic losses upto 100 per cent when spread in epidemic form. Symptoms of bacterial blight on pomegranate is appeared as small, circular to irregular, water-soaked spots on leaves, flowers and fruits. On fruits, the spots developed into typical cankers with cracks on the necrotic areas. High humidity and rainfall is favourable for development of bacterial blight. The bacteria are mainly spread/dispersal through Wind-driven rain. Not even single variety of pomegranate showed resistance against bacterial blight. Management of the disease can be possible through the spray of various agro-chemicals and destruction of debris (diseased fallen leaves).

Future Studies to be Required

1. Management of bacterial blight of pomegranate through transgenic needs to be studied.
2. Need to develop resistant varieties against bacterial blight.
3. Detail research need to be concentrated on role of botanicals and bio-agents in managing the disease.
4. There is also an important need to develop integrated disease management practices in order to save the crop.

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