

Leaf Spot and Dry Fruit Rot of Pomegranate: Biology, Epidemiology and Management

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Abstract

Pomegranate (*Punica granatum* L.) diseases often caused by a range of fungi and bacteria, pose direct significant financial, nutritional and postharvest losses. Common fruit rot pathogens of pomegranate include *Botrytis cinerea*, *Alternaria alternata*, *Penicillium implicatum*, *Coniella granati* and *Aspergillus niger*. Pomegranates are highly susceptible to fruit rot caused by *Coniella granati* (Sacc.) Petrak and Sydow and the disease is assuming importance in Himachal Pradesh due to its severity. This review discusses the leaf spot and dry fruit rot of pomegranate caused by *Coniella granati*, its symptomatology, epidemiology and management.

Keywords: *Coniella granati*, epidemiology, management, pomegranate, symptomatology.

1. Introduction

Pomegranate (*Punica granatum* L.) which belongs to the family Punicaceae, is a species of fruit bearing deciduous shrub or small tree growing up to 5–8 m long. Pomegranate is a favourite table fruit of Mediterranean, tropical and subtropical regions of the world, grown commercially for its sweet acidic fruits and also for its medicinal properties. According to De Candole (1967) pomegranate originated in South West Asia, probably Iran and some adjoining countries. It is extensively cultivated in Spain, Morocco, California, Florida, Mexico, South America and North-Western parts of Pakistan. In India, it attained the commercial status only after 1985–1986 and now its cultivation is done on scientific lines particularly in states of Arunachal Pradesh, Gujarat, Himachal Pradesh, Karnataka, Maharashtra, Nagaland and Rajasthan. The best fruits are produced in the areas with cool winters and hot dry summers (Saxena et al., 1984).

Pomegranate suffers from a variety of fungal and bacterial diseases due to high moisture content and richness in nutrients. Cultivation of this crop is adversely affected by various foliar (leaf spots), fruit (spots and rots) and soil borne (wilt) diseases resulting in huge losses to the growers. The extensive cultivation in different agro ecological situations under mid hill zone with introduction of new varieties directly by the growers from other states is further adding to the pathogenic fauna (Khosla and Bhardwaj, 2011).

Several fungi are reported to be associated with pomegranate and the important fruit rot pathogens prevailing are

Glomerella cingulata, *Penicillium expansum* and *Aspergillus nidulans*, but dry fruit rot caused by *Coniella granati* (Sacc.) Petrak and Sydow, had resulted in substantial losses after harvest and is threatening the upcoming pomegranate cultivation in Himachal Pradesh (Sharma, 1998). *Coniella granati* is a widespread pathogen of pomegranate recorded in Brazil, Cyprus, Italy, Korea, North Carolina, Netherland and Pakistan (Farr et al., 2007).

2. Occurrence and Distribution

Coniella granati originally named *Phoma granati* by Saccardo (1884), who later listed the fungus as *Macrophoma granati* (Sacc.) Berl and Vogl (Saccardo, 1892). Later Petrak and Sydow (1927) transferred the species to *Coniella* and described it as *Coniella granati*. In China, *Zythia versolana* Sacc. has been reported by Tai and Cheo (1934) to cause a dry rot of pomegranate fruits with symptoms very similar to those of the disease reported in Europe. Subsequently, the pathogen has been reported from other parts of the world i.e., North Carolina (Herbert and Clayton, 1963), Turkey (Yildiz and Karaca, 1973; Pala et al., 2009; Celiker et al., 2012), Kenya (Siboe et al., 1982), India (Sharma and Jain, 1978; Sharma, 1998), Korea (Kwon and Park, 2005), Northern Greece (Tziros and Klonari, 2007), Israel (Levy et al., 2011) and Iran (Mirabolfathy et al., 2012).

An extensive and comprehensive survey of various pomegranate growing areas of Himachal Pradesh was conducted and it was reported that the incidence of dry fruit rot varied from 0.55 to 22.7% with a mean incidence of



8.38%. The incidence was higher in Solan district where the infected fruits were often seen in the form of dark brown to black fruit mummies scattered on the orchard floor (Sharma and Tegta, 2011).

A roving survey was conducted during Mriga and Hasta Bahar (August to January) of 2008–2009 in major pomegranate growing areas of north Karnataka viz. Bagalkot, Bijapur, Belgaum, Bellary, Gadag and Koppal districts. Plantations of at least three years and above were selected for survey. Five major and two minor diseases were observed, they were bacterial blight, wilt, anthracnose, *Alternaria*, root knot, scab and fruit rot. Bacterial blight appeared to be in severe form in all the pomegranate growing areas, which ranged from 0.67 to 94.80% of severity on a tree. The disease was very severe in Bagalkot district with an average severity value of 74.08%. The least average severity (6.73%) was observed in Bellary district. The higher percent wilt incidence of 7.59 was observed in Bellary district. Anthracnose was recorded in the range of 15–25%, *Alternaria* 2–15%, root knot 2–15%, scab 1–5%, fruit rot 5–15% and *Cercospora* were also recorded in the range of 1–2% (Benagi et al., 2011).

Khosla and Bhardwaj (2011) conducted survey of pomegranate growing areas of Himachal Pradesh during June to September (2011) and revealed that the biggest threat to pomegranate cultivation is posed by wilt disease caused by *Ceratocystis fimbriata* and *Fusarium oxysporum* with their incidence varying from 1.03 to 15.3 and 0.1 to 7.3%, respectively. Similarly, fruit rot was caused by *Coniella granati* (1.0 to 14.8%), *Phomopsis aucubicola* (1.0 to 14.7%) and *Phytophthora* sp. (1.4 to 13.6%). The incidence of anthracnose (7.3%) caused by *Colletotrichum gloeosporioides* was recorded only at one location (village Samtana) in Hamirpur district. A bacterial pathogen *Xanthomonas axonopodis* was found causing leaf and fruit spots and subsequent fruit rotting ranging from 1.2 to 7.5%.

3. Symptomatology

Mummified fruits of pomegranate due to *Coniella granati* with outer surface of rind covered with partly immersed fruit bodies up to 1 mm in diameter were observed by Petrak and Esfandiari (1941). Herbert and Clayton (1963) observed a fruit rot caused by *C. granati* on half grown or mature fruits and abundant pycnidia were produced on such rotted fruits. In a few cases the infections extended through the rotted fruit pedicel into the branch. The leaves and stem from 8 to 10 inches below the diseased fruit to the tip of the branch were killed within a period of a week or two.

Sharma and Tegta (2011) noticed that the dry fruit rot of pomegranate started as brown, irregular, patchy lesions on the rind. Later the colour changed to dirty pinkish. The lesion increased very fast, producing fissures of differing length and depth. It was characterized by softening of the rind and underneath pulp and seed. The affected fruit rind turned light to dark brown. The infected fruit did not shrivel or loose its

shape. The pathogen invaded the whole fruit within 8-10 days at ambient temperature 25 ± 1 °C after which the fruit dropped to the ground. At advanced stage, fruit epicarp exhibited abundant, minute, erumpent fruit bodies (pycnidia) of the pathogen. Ultimately the fruit turned brown to black and was as firm as sound fruit. Crown rot symptoms were also reported to be caused by *C. granati* (Thomidis and Exadaktylou, 2011; Celiker et al., 2012).

Disease symptoms on leaves of pomegranate by *Coniella granati* was reported by Ram and Sharma (2013) as small necrotic angular lesions which started from the leaf tip region towards the proximal end. Gradually, lesions increased in size leading to desiccation and premature shedding of leaves. Leaves showed abundant gritty black, minute pycnidial bodies, which spread throughout on the leaf surface (Figure 1).



On Fruits

On Leaves

Figure 1: Symptoms of leaf spot and dry fruit rot of pomegranate caused by *Coniella granati*

4. Isolation and Identification

The fungus (*Coniella granati*) was readily isolated and sporulated by Herbert and Clayton (1963) on potato-dextrose agar medium in a 90 mm Petri plate within 7 to 10 days, which produced scanty light aerial mycelium (Figure 2). On



Figure 2: Pure culture of *C. granati* on PDA medium

pomegranate fruits, pycnidia were brownish in colour, had thin membranous wall and varied in diameter from about 50 to 170 μ . They occurred singly or in closely packed groups of two to six, which sometimes gave the impression of locules in stroma. An immature pycnidium was closed at first but at maturity a single pore 15–25 μ in diameter was present. When viewed singly, conidia appeared to be essentially hyaline, but masses of conidia were light brown in colour. They were single celled, elongated, straight or slightly curved, 12–19 μ long by 2.8–5 μ wide (mean 15 \times 3.5). The diameter of pycnidia as reported by Sharma and Jain (1978) was up to 135 μ and that of conidia was 9–14 \times 2.5 \times 3.2 μ , respectively (Figure 3). Siboe

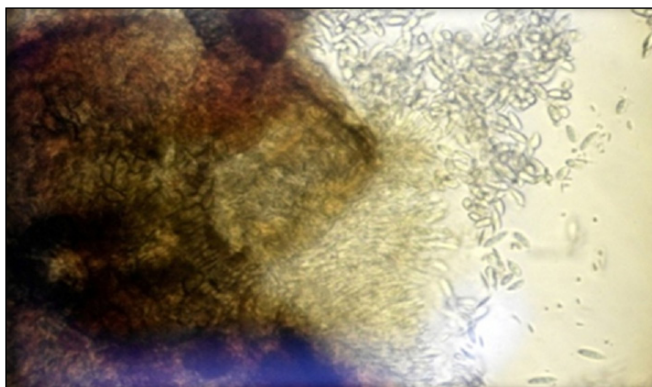


Figure 3: Ruptured pycnidium of *C. granati* liberating conidia

et al. (1982) found pycnidia 13.0 μ –17.7 μ in diameter and 11.1 μ –20.6 μ in height and were more or less submerged in the host. Pycniospores were olivaceous to hyaline, navicular to oblong and measure 1.95 μ in length and 0.32 μ in breadth.

Sharma (1998) isolated the fungus from diseased tissue on potato-dextrose agar medium and identified it as *Coniella granati*. Sub-surface, cream white mycelium which was scanty and slow growing developed throughout the medium. Abundant dark brown coloured pycnidia developed in concentric rings throughout the petriplate within 15 days at 24 °C. In cultures, pycnidia occurred singly or in closely packed groups of 2–5. Microscopic examination of diseased tissue revealed that pycnidia were gregarious, sub-epidermal, globose or somewhat compressed, thin walled, membranous, dark brown to black, measure 64.8–225.0 \times 63.0–198.0 μ m (Av. 138.9 \times 122.6 μ m). Average diameter of pycnidium was slightly more on host (198–63 μ m) than in cultures (184–58 μ m). Conidiophores were simple, slender, hyaline, arised only at the base of pycnidium in tufts, measure 22.5–7.6 μ m. Conidia were single-celled, hyaline to light brown in colour, elongate, straight or slightly curved, 16.2–9.0 μ m long and 3.6–1.8 μ m wide (Av. 13.3 \times 2.6 μ m). Subsequently, the pathogen was isolated and identified by Kwon and Park (2005) and Tziros and Klonari (2007).

The potential causal agents differing in their symptomatology in causing postharvest fruit rot of pomegranate in Spain were transferred to PDA, purified and identified as *Penicillium* spp. and *Coniella granati* by morphological observation of colonies.

This identification was confirmed by the amplification and subsequent sequencing of the ribosomal DNA intragenic spacer regions ITS1 and ITS2, along with the 5.8s rRNA gene (Palou et al., 2010). Based on mycelium and spore morphology and ribosomal ITS sequence data (GeneBank Accession No. FN908875), the pathogen producing symptoms of crown rot on pomegranate was identified as *Pilidiella granati* (*Coniella granati*) (Thomidis and Exadaktylou, 2011). Mirabolfathy et al. (2012) also identified the causal pathogen of dieback and fruit rot of pomegranate as *Coniella granati* on the basis of cultural, morphological, genetic (ITS) and pathogenicity analysis.

The causal agent of crown rot of pomegranate in Turkey was isolated from the lower margins of the necrotic area by plating tissues of approximately 3mm diameter onto potato dextrose agar. Plates were incubated at 25 °C for 7 days. The pathogen was identified as *Coniella granati* (Saccardo) Petrak & Sydow (synonym *Pilidiella granati* Saccardo) based on morphological characteristics. Yellowish cream-coloured fungal colonies with abundant dark brown to black spherical pycnidia (average 171.5–245 μ m) were consistently isolated. Hyphae were septate. Ellipsoid to fusiform single-celled hyaline conidia (average 10–17.5 \times 2.5–5 μ m) were observed (Celiker et al., 2012).

5. Pathogenicity

Herbert and Clayton (1963) proved pathogenicity of *Coniella granati* on flowers, fruits of pomegranate and mature Golden Delicious apples by inoculating them with mycelium in needle punctures. Flowers were completely rotted by the fungus within 3 to 4 days and typical symptoms developed on fruits. Moderately firm tan-coloured lesions which averaged about 1 inch in diameter developed in 1 week on Golden Delicious apples. The fruits were completely rotted within 15 to 20 days. Sharma (1998) proved the pathogenicity of *Coniella granati* by inoculating healthy pomegranate fruits with mycelial plugs of actively growing cultures, which after 7–8 days produced symptoms similar to those observed under field conditions. Subsequently, pathogenicity of the fungus was proved by Kwon and Park (2005), Tziros and Klonari (2007) and Palou et al., (2010).

Celiker et al. (2012) confirmed the pathogenicity of *Coniella granati* by inoculating 6 branches (8mm in diameter) of three 2-year-old plants of pomegranate cultivar Izmir 1513. Using a 4-mm-diameter cork borer, a wound was created in the middle of each branch by removing the bark. A 4-mm-diameter agar plug bearing mycelia from a 7-day-old culture of *C. granati* was inserted into each wound. The wound was covered with transparent tape to prevent desiccation. Four branches (7–8 mm in diameter) of 2 trees were inoculated with sterile potato dextrose agar plugs to serve as controls. All plants were incubated at 22 °C for 17 days, at which time necrosis was observed on inoculated plants. Koch' postulates were satisfied after reisolating the fungus from inoculated plants. Control plants produced no symptoms of the disease.

6. Epidemiology

Growth of *Coniella granati* was best at 25–30 °C, being nil at 35 °C and very slight at 5 °C (Yildiz and Karaca, 1973). Sharma and Jain (1978) reported *C. granati* causing fruit rot of pomegranate during late of August. Lukose and Singh (1997) studied the climatic factors affecting the severity of pomegranate fruit rot caused by *C. granati*. Results revealed that the fungus required a good rainfall, high humidity around 80% and a temperature range of 22–32 °C for its initial spread and development. Under optimum conditions, *C. granati* caused complete rotting of fruit within a week.

Sharma (1998) reported *Coniella granati* on ten to twelve years old trees cultivated in a private orchard in Mandi district of Himachal Pradesh showed unusual fruit lesions and fruit fall after moderate rains during the first week of July when temperature was around 21–35 °C. The disease appeared in an epidemic form and caused about 80% fruit loss.

Sharma and Tegta (2011) reported that the fruit rot of pomegranate appeared maximum during the first week of August after an extended period of warm and foggy weather. Optimum temperature for vegetative growth of *C. granati* was 25 °C whereas, a range of 4.0–5.0 pH favoured better growth of the fungus.

7. Management

7.1. *In vitro* efficacy of fungicides against *Coniella granati*

Bisiach and Battino (1973) while studying the *in vitro* efficacy of some chemical compounds observed that spore germination of *Coniella diplodiella* causing white rot of grapevine was inhibited most effectively by a mixture of manam, thiram, benzothiazil mercaptan and dithiobisbenzothiazol, followed by captan and dichlofluanid. Thakur (2009) found carbendazim, thiophanate methyl, hexaconazole, mancozeb and carbendazim+mancozeb most effective under *in vitro* conditions against *C. granati*. Ma et al. (2012) isolated and purified Chitinase from *Gliocladium catenulatum* through sodium dodecyl sulfate polyacrylamide gel electrophoresis and it was found to have inhibitory effect on the hyphal growth, conidial germination and sclerotial germination of various plant pathogenic fungi including *Coniella diplodiella* causing white rot of grapes.

Because of natural occurrence, biological activity, and industrial applications of phenols extensive attention has been paid to prepare new derivatives by chemical modification and to explore the new application of phenols. A novel synthetic approach towards 5-fluoro-2-hydroxy butyrophenone was reported by Xin et al. (2013) and it was found to have potent antifungal activities against *Valsa mali*, *Coniella diplodiella* and other agricultural plant fungi. The inhibitory effect of 5-fluoro-2-hydroxy butyrophenone was tested *in vitro* against six popular plant pathogenic fungi. It was dissolved in acetone and added to PDA medium (potato 200 g, dextrose 20 g and agar 17 g L⁻¹) immediately before it was poured into the petri

dishes at 40–45 °C. 5-fluoro-2-hydroxy butyrophenone was tested at 100, 50, 25, 12.5 and 6.25 mg L⁻¹ against *Valsa mali* but at the double doses against *Coniothyrium diplodiella* (*Coniella diplodiella*).

7.2. *In vitro* efficacy of plant extracts and animal products against *Coniella granati*

Cong et al. (2005) observed the fungistatic effect of different extracts from stems and leaves of tomato with six different kinds of solvents against *Coniella diplodiella* and 100% inhibition of fungus was reported. Pre-inoculation treatment with plant extracts of darek (*Melia azadirach*), dedonea (*Dedonea viscosa*), lantana (*Lantana camara*) and pepper mint (*Mentha piperita*) provided significant control of fruit rot of pomegranate caused by *Coniella granati* (Sharma and Tegta, 2011).

7.3. *In vitro* efficacy of fungal and bacterial antagonists against *Coniella granati*

A possible method of attacking the fungus (*Coniella diplodiella*) in its resting phase is to promote the growth of antagonistic fungi. Certain species of *Chaetomella*, *Fusarium* and *Penicillium* were found to show antibiotic activity against *C. diplodiella*, but their activity consists more in preventing germination of spores than in their destruction. Other fungi normally present in the straw of stable manure; *Trichoderma*, *Chaetomium* and *Chaetomella* exercise a destructive action on *Coniella* spores (Turian, 1954). Sesan et al. (2003) reported *T. viridae* to be the most effective antagonist against *Coniella diplodiella* causing white rot of grapes followed by *Verticillium tenerum*, *Coniothyrium minitans* (isolates IVT, C15, C18 and CR), *Epicoccum nigrum* (isolates Ep.5, Ep.6, Ep.3 and Ep.4) and *Gliocladium roseum* (isolates GI.1 and GI.2).

Fungal antagonists viz., *Trichoderma virens*, *T.harzianum*, *T.hamatum* and *T.polysporium* gave maximum inhibition of mycelial growth of *Coniella granati*. Antagonist protection was higher when applied at a concentration of 10⁸ spores ml⁻¹ before inoculation (Sharma and Tegta, 2011). An isolate, AFB22, of *Bacillus subtilis* was identified from pomegranate and was found to have antifungal properties against fruit rot pathogens (Gajbhiye et al., 2013).

7.4. *Field* efficacy of fungicides against *Coniella granati*

Bolay (1977) reported good results against *Coniella diplodiella* with vinclozolin, rovril and sumisolex, all based on 3,5 dichloroniline. During the fungicide trial against white root rot (*Coniella diplodiella*) on grapevines, Chernyak (1978) observed that spray with sodium bicarbonate (0.5–1%) reduced infection by 5–7 times. Lukose and Singh (1997) found thiophanate methyl (0.1%) and mancozeb (0.2%) effective against three fruit rot/spot pathogens viz., *Colletotrichum gloeosporioides*, *Coniella granati* and *Pseudocercospora granati*. Field experiments were conducted during the fruiting seasons of 1994–1997 in Maharashtra to determine fungicide combinations for the control of leaf and fruit spot diseases of pomegranate. 0.1% carbendazim, 0.3% mancozeb,



0.1% benomyl, 0.4% copper oxychloride, 0.1% thiophanate-methyl, 0.2% chlorothalonil, 1% bordeaux mixture, 0.1% benomyl+0.2% mancozeb and 0.1% carbendazim+0.2% mancozeb were sprayed on pomegranate cv. Ganesh (first spray at fruit set and 6 subsequent sprays at 10 days interval). Fungicides combinations i.e., carbendazim+mancozeb or benomyl+mancozeb were found effective for controlling leaf and fruit spots caused by *Cercospora punicae*, *Coniella granati*, *C. lythracearum*, *Colletotrichum gloeosporioides*, *Alternaria alternata* and *Dreschlera rostrata*. For single fungicide application, 0.1% carbendazim proved to be as effective as combined fungicide applications (Gaikwad, 2000). Sesan and Stefan (2003) reported the efficacy of various fungicides named vinclozolin, carbendazim and tebuconazole against *Coniella diplodiella* causing white rot of grapes.

Raghuwanshi et al. (2005), evaluated the efficacy of eight fungicides (captan, 0.2%; mancozeb, 0.1%; ziram, 0.25%; carbendazim, 0.1%; copper oxychloride, 0.4%; thiophanate-methyl, 0.1%; Bordeaux mixture, 0.1% and chlorothalonil, 0.2%) against the leaf and fruit spot of pomegranate cv. Ganesh. Carbendazim, mancozeb and Bordeaux mixture were found most effective to control the pathogens and further spread of the disease in the field. According to Sharma and Tegta (2011) pre-harvest sprays with Bavistin and Stop among systemic fungicides gave significant control of dry rot of pomegranate caused by *C. granati* whereas, among non-systemic fungicides, spray of Indofil M-45 showed some promise.

8. Conclusion

Pomegranate is highly susceptible to fruit rot caused by *Coniella granati* and this disease is assuming importance because of its severity. Different fungicides have been found effective against *C. granati* which can be recommended to pomegranate growers to manage this disease. Besides, some work has also been done on biological control of pomegranate diseases but further development and effective adoption will require a greater understanding of the complex interactions among plants, human and environment

9. References

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