

Incidence of Pomegranate Wilt in Southern Karnataka, India

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ABSTRACT: Pomegranate (*Punica granatum*) is one of the commercially important fruit crops of India. Wilt caused by *Ceratocystis fimbriata* Ell. and Halts is one of the major production constraints affecting its cultivation in all major growing areas of the country. Therefore, roving survey was conducted during 2018-19 and 2019-20 in major pomegranate growing southern districts of Karnataka viz., Bengaluru Rural, Bengaluru Urban, Ramanagara, Chamarajanagara, Tumakuru and Chitradurga to pin down the status of the disease and results revealed that, wilt incidence was noticed in all the locations surveyed and it was ranged from 2.00 - 31.67 per cent. Maximum mean wilt incidence of 13.20 per cent was observed in Tumakuru district followed by Chitradurga (10.24 %) whereas; the least mean wilt incidence was recorded in Ramanagara district (3.20 %). Among nine taluks surveyed, the maximum wilt incidence was noticed in Sira taluk (22.50 %) of Tumakuru district followed by Hosadurga taluk (15.67 %) of Chitradurga district. Among the villages surveyed, the wilt incidence was found highest in Thogaragunte village (31.67 %) of Sira taluk of Tumakuru district followed by Kurubarahalli village (20.00 %) of Hosadurga taluk of Chitradurga district while, it was found lowest in Vijayapura village (2.00 %) of Devanahalli taluk of Bengaluru Rural district.

Keywords: Disease incidence, Pomegranate wilt, Survey, *Ceratocystis fimbriata*.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the important ancient fruit crops belonging to the botanical family punicaceae. It is a commercial fruit crop of both tropical and subtropical countries. Although native of Iran and adjoining areas, pomegranate has been widely cultivated throughout India and Mediterranean regions of Asia, Africa and Europe. It is extensively cultivated in Morocco, Spain and other countries around the Mediterranean, Iran, Afghanistan, Egypt, Arabia and Baluchistan (Sonyal, 2010).

India is one of the major producers of pomegranate in the world having annual production of 3034 thousand metric tonnes with 262 thousand hectare cultivable area and productivity of 11.58 metric tonnes per hectare. It is commercially cultivated in the states of Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Madhya Pradesh, Rajasthan and Tamil Nadu and Karnataka being the second leading producer of the country having an area

of 25.97 thousand hectare with a production of 268.23 thousand metric tonnes and productivity of 10.32 metric tonnes where the crop has extended across different districts such as, Chitradurga, Ballari, Tumakuru, Vijayapura, Bagalkote, Koppala, Belagavi, Davanagere, Bengaluru and Kalaburagi (Anon., 2018).

In spite of this, successful cultivation of pomegranate has met with different traumas mainly pest and diseases. Among the diseases, wilt caused by *Ceratocystis fimbriata* is one of the major production constraints in all major growing areas of the country and severity of wilting is increasing day by day at faster rate.

The disease was initially reported in India from Nasik district of Maharashtra during 1978 and subsequently from Kaladagi and Kanamadi areas of Karnataka on soft seeded Ganesh variety during 1988 (Somasekhara, 2006).

Later, wilt of pomegranate was recorded in two areas of Bijapur district during 1990. In 1993, rapid spread of the disease was observed in the entire Bijapur district but the cause was not known until 1995. The fungus, *Ceratocystis* sp., was isolated from the discolored stem, root and branch tissues on wilted plants which were formerly collected from various locations during 1996 and the fungus isolated from Bagalkot was confirmed by the International Mycological Institute (UK) as *C. fimbriata* Ellis & Halst. during 1997 (Somasekhara, 1999).

On the basis of microscopic examination of diseased plant parts, cultural studies and pathogenecity tests, the causal organism of pomegranate vascular wilt was identified as *Ceratocystis fimbriata* Ellis and Halsted as isolations from 77.00 per cent of wilt samples from various locations revealed growth of *Ceratocystis fimbriata*. Wilt was prevalent on all important cultivars of all ages from 2-20 years (Sharma *et al.*, 2010).

As the disease prevails in every season with varying degrees of severity, a systematic survey for disease incidence in different growing areas is crucial to intend

appropriate management strategy. Hence, the present investigation was proposed to take up field survey in important growing areas of Southern Karnataka to generate the information on percentage disease incidence.

MATERIAL AND METHODS

Survey for the disease incidence and collection of isolates of *Ceratocystis fimbriata* causing pomegranate wilt in major pomegranate growing areas of Karnataka

Field survey and collection of samples. Roving field survey was undertaken in major pomegranate growing regions of Karnataka during the year 2018-19 and 2019-20. Majorly southern districts of Karnataka were covered such as Bengaluru rural, Bengaluru, Ramanagara, Chamarajanagara, Tumakuru and Chitradurga. Pomegranate growing regions surveyed was listed in Table 1. Per cent disease incidence in each orchard was assessed as per the formula given by Varnell and McCloud (1975)

$$\text{Per cent disease incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants observed}} \times 100$$

Plants were diagnosed on the basis of typical symptoms like yellowing and drooping of foliage of one or more branches of plant and drying. The suspected plant parts like roots and collar region from infected plants were

observed for vascular grayish brown discoloration and samples were collected, labeled and further studies were conducted under laboratory conditions.

Table 1: List of pomegranate growing regions surveyed during 2018-19 and 2019-20.

Sr. No.	Name of the place		
	District	Taluk	Village
1.	Bengaluru rural	Devanahalli	Koramangala
			Pura
			Devanahalli
			Harohalli
			Vijayapura
			Yaluvahalli
		Doddaballapura	Machagondahalli
			Hosahudya
2.	Bengaluru	Bengaluru North	Nagadasanahalli
			Kalenahalli
			GKVK, Hebbal
3.	Ramanagara	Kanakapura	Uyyalappanahalli
4.	Chamarajanagara	Kollegala	Jakkalli
			Thimmarajipura
5.	Tumakuru	Pavagada	K. T. Halli
			Tumakunte
			Arasikere
		Sira	Rangapura
			Thogaragunte
6.	Chitradurga	Hiriyur	Dharmapura
			Javagondanahalli
			Maskal
		Hosadurga	Ramajjanahalli
			Kurubarahalli

Isolation, purification and maintenance of *C. fimbriata*

Carrot baiting technique. Pathogen isolation was done by using carrot bait technique (Moller and Devay, 1968) where tissue bits were enclosed in a cavity hollowed out of the inner face of a pair of disks cut from carrots which had been washed in running tap-water previously. The disks were fastened together with a sterile rubber band and incubated at high Relative Humidity (RH) for 4 days at $26\pm1^{\circ}\text{C}$. The growth of *C. fimbriata* on carrots was started after 3-4 days of incubation. Initially, mycelium was whitish grey/light grey and later changed to dark grey color. Black colored perithecia were observed after 4-5 days of incubation. Growth of the fungus was then transferred onto potato dextrose agar medium. The pure culture of pathogen was maintained on potato dextrose agar slants at $26\pm1^{\circ}\text{C}$.

Tissue isolation method . The samples with typical wilt symptoms were cut into small bits of size 0.5 to 1.0

cm. The tissue bits were initially surface sterilized with one per cent sodium hypochlorite (NaOCl) for one minute and washed with sterile distilled water successively for 3 times (30 seconds each time). Later, the bits were placed on sterile tissue paper to remove excess moisture and finally placed on solidified potato dextrose agar medium in sterile Petri plate and incubated at $26\pm1^{\circ}\text{C}$ temperature until the growth initiates. Culture of *C. fimbriata* was purified by following standard hyphal tip method and then purified culture was maintained on potato dextrose agar slants and kept in refrigerator at 4°C for further use. Total of fifteen isolates of *C. fimbriata* were collected from different locations of Southern Karnataka. Isolation of pathogen using carrot bait technique and tissue isolation method is depicted in Plate 1. Designation of *C. fimbriata* isolates collected from different areas was given in Table 2.

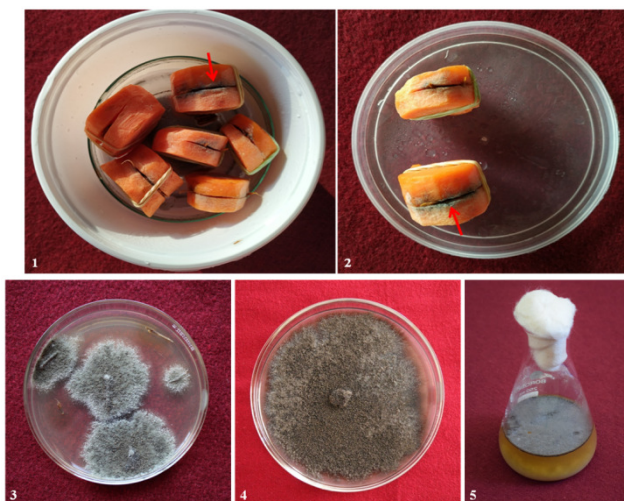


Plate 1. 1 & 2- Carrot bait technique; 3- Tissue isolation method; 4- Pure culture on Potato Dextrose Agar medium; 5- Broth culture of *Ceratocystis fimbriata*

Note: Arrow (1 & 2) indicates the fungal mycelia contains numerous spore mass

Table 2: Designation of *Ceratocystis fimbriata* isolates collected from different southern districts of Karnataka.

Sr. No.	Designation of the isolate	Name of the place		
		Village	Taluk	District
1.	Cf-1	Pura	Devanahalli	Bengaluru Rural
2.	Cf-2	Harohalli		
3.	Cf-3	Vijayapura		
4.	Cf-4	Yaluvahalli		
5.	Cf-5	Kalenahalli	Bengaluru North	Bengaluru
6.	Cf-6	GKVK, Hebbal		
7.	Cf-7	Uyyalappanahalli	Kanakapura	Ramanagara
8.	Cf-8	Jakkalli	Kollegala	Chamarajanagara
9.	Cf-9	Rangapura	Sira	Tumakuru
10.	Cf-10	Thogaragunte		
11.	Cf-11	Dharmapura	Hiriyur	Chitradurga
12.	Cf-12	Javagondanahalli		
13.	Cf-13	Maskal		
14.	Cf-14	Ramajjanahalli	Hosadurga	
15.	Cf-15	Kurubarahalli		

Fungal purification by hyphal tip method. Hyphal tip isolation was done on water plates. Dilute spore suspension of the pathogen was prepared in sterilized distilled water containing eight to ten spores per ml from 15 days old culture. One ml of such suspension was spread uniformly on two per cent solidified water agar plates and observed for spores under the microscope. Single spore was marked with a marker on backside of the Petri plate and it was allowed to germinate. Such plates were periodically observed for spore germination under microscope. The hyphae growing from each cell of the single spore was traced and marked with marker. The tip of the hyphae was cut carefully and transferred to PDA plates and incubated at $26\pm 1^{\circ}\text{C}$ for 15 days. Later, mycelial bits of the fungus were transferred into centre of Petri plates containing PDA and incubated at $26\pm 1^{\circ}\text{C}$ for 15 days.

Maintenance of the culture. The hyphal tip cultures of the fungus were sub-cultured on potato dextrose agar slants and kept in laboratory at $26 \pm 1^{\circ}\text{C}$ for 15 days. Such mother culture slants were preserved at 4°C in refrigerator. Further, these cultures were sub-cultured once in three months and used for future studies.

RESULTS AND DISCUSSION

Survey for the disease incidence and collection of isolates of *Ceratocystis fimbriata* causing pomegranate wilt in major pomegranate growing areas of Karnataka

Symptoms. In polyhouse condition, the disease appeared as yellowing of foliage of one or few branches of a plant. Later progression of disease resulted in complete yellowing of entire plant and finally leads to wilting. Commonly defoliation occurs after yellowing and drooping. In the present investigation, plants took few days to 2-3 months to reveal complete wilting. However, some plants exhibited sudden drooping of all the leaves resulted in complete wilting within one or two days after symptom initiation.

In field condition, symptoms initiated as yellowing of leaves of one or more branches and plants appeared devitalized, leaves turned pale green starting from lower branches and progressed upwards. The partial wilting of the plant with drying and death of some branches were the common symptoms. In severe cases, the defoliation and complete wilting of plants were observed within 2-3 months. The entire plant wilted from top to bottom and wilt-infected plants often seen with dried foliage and fruits being attached to the branches for several months. Sometimes, fruit drop was also observed. Vertical sections of affected plant parts near the root region revealed dark grayish brown discoloration in the vascular tissue and this is the typical symptom of pomegranate wilt disease. Similar symptomatology was also reported by earlier workers (Jadhav and Sharma, 2009; Chaudhari *et al.*, 2016; Raja, 2017 and Somu, 2017). Symptoms of pomegranate wilt observed under field condition are depicted in Plate 2 and 3.



A-Yellowing; B- Partial wilting; C-Complete wilting

Plate 2. Different stages of pomegranate wilt under field condition



1-Infected root; 2 & 3- Vertical section of diseased root showing dark grayish brown discoloration in vascular bundle and adjoining tissues

Plate 3. Vascular discoloration in pomegranate roots due to the *Ceratocystis fimbriata*

Field survey. A roving survey was carried out in a major pomegranate growing southern districts of Karnataka during 2018-19 and 2019-20 to assess the incidence of wilt in different locations.

Survey was conducted in twenty four different locations of Southern Karnataka from six districts viz., Bengaluru Rural, Bengaluru Urban, Ramanagara, Chamarajanagara, Tumakuru and Chitradurga. Field view of pomegranate orchards visited during survey was depicted in Plate 4. The wilt incidence was noticed in all the locations surveyed and it was ranged from 2.00 - 31.67 per cent and the data is presented in Table 3b.

Results of Table 3b revealed that, the maximum mean wilt incidence was observed in Tumakuru district (13.20 %) followed by Chitradurga (10.24 %) whereas, the least mean wilt incidence was recorded in Ramanagara district (3.20 %). Among nine taluks surveyed, the maximum wilt incidence was noticed in Sira taluk (22.50 %) of Tumakuru district followed by Hosadurga taluk (15.67 %) of Chitradurga district. Among the villages surveyed, the wilt incidence found highest in Thogaragunte village (31.67 %) of Sira taluk of Tumakuru district followed by Kurubarahalli village (20.00 %) of Hosadurga taluk of Chitradurga district while, it was found lowest in Vijayapura village (2.00 %) of Devanahalli taluk of Bengaluru Rural district.

The present results were found similar with Sonyal *et al.* (2016) where they reported that, maximum wilt complex was recorded in Tumakuru district with 38.43 per cent incidence followed by Chitradurga district (31.68 %) and least wilt incidence was recorded in Hassan district (25.74 %). Among fifteen talukas surveyed, maximum wilt complex was noticed in Sira (39.86 %), Pavagada (37.68 %) and Madugiri (37.77 %) talukas of Tumakuru district. Among 49 villages, wilt incidence noticed was highest in Pattanayakana halli (59.37 %) of Sira taluk of Tumakuru district followed by Shravanagere (57.84 %) of Hiriyur taluk of Chitradurga district and Kodihalli (53.84 %) of Madugiri taluk of Tumakuru district.

In the present study, the disease incidence was varied from locality to locality and higher incidence was observed in Tumakuru followed by Chitradurga district. This might be due to the type of cropping pattern that farmers have been practicing, environmental conditions prevailing and hence buildup of pathogen inoculum and monocropping of susceptible variety Bhagwa that also aggravated the disease occurrence. Apart from this, following improper management practices in the initial period of the crop by the farmers and lack of knowledge regarding the prophylactic management practices also made it difficult to manage the wilt disease and even due to usage of local planting material which are neither certified nor completely ensured that are from disease free orchards. Thus, initial inoculum through planting material, accompanied with susceptible variety and prevailing congenial soil and climatic conditions help the disease to aggravate in the field.

As per the present recommendation, farmers have to drench infected plant roots and surrounding healthy plants with suitable pesticides after observing first symptoms of wilt and also should repeat the drenching 3-4 times at 15-20 days interval. But this is not actually happening in the fields because of unawareness of the control measures. Moreover, infected plants are not removing timely from the fields and are not burning properly by the farmers. The dead trees have to be removed and fresh planting has to be done after the treatment of infected soil with formalin. But this is also not strictly practicing by farmers. This results in more pathogen survivability and occurrence of disease in higher proportions.

With respect to age of the crop, orchards of all ages (2-6 years) showed wilt incidence. Findings are in agreement with Sharma *et al.* (2010). They reported that, wilt was prevalent on all important cultivars of all ages from 2-20 years.

With respect to soil type, the maximum incidence of wilt disease was observed in red loamy soils (31.67 %) followed by red sandy loam soils (13.33 %) and black soils (12.30 %) whereas, the minimum disease incidence was recorded in red (2.00 %) and sandy soils (2.50 %). Results are presented in Table 3a. The results are in accordance with Somasekhara (2006) who analyzed the soil type and wilt disease occurrence and reported that, wilt incidence was 8.42, 5.06, 4.35 and 2.60 per cent in loamy, black, sandy loam and red soils respectively. Similarly, Sonyal (2015) studied soil characteristics and wilt disease incidence in pomegranate plants and reported that, maximum incidence was recorded in red sandy soils (31.30 %) followed by red loamy soils (30.30 %), red sandy loam soils (28.19 %) and red soils (27.96 %). Marathe *et al.* (2016) studied the identification of suitable soils for cultivation of pomegranate cv. Ganesh and inferred that, incidence and severity of wilt disease was found higher in the plants grown on clay textured soils compared to light textured soils and this might be attributed to very high water holding capacity of the soils that led to high humidity in the microclimate of the plants. The disease is more prevalent in heavy soil with high moisture content (Sharma *et al.*, 2010). Similarly, Pegg *et al.* (2019) studied on epidemiology of Fusarium wilt of banana and reported that, after infection, disease development will depend on sufficient water being available for pathogen growth and dispersion in the xylem fluid. Rishbeth (1957) and Stover (1962) reported that, a higher incidence of wilt disease was seen in poorly drained soil when there was temporary flooding of the root zone. Stover (1962) also suggested that, water-saturated, oxygen-deficient conditions predispose the host to infection.

The pomegranate orchards were also associated with other diseases such as bacterial blight, anthracnose, leaf spot, fruit rot *etc.* and insect pests like borers, thrips, aphids, mites *etc.* (Table 3a).

Table 3a: Survey for the incidence of Pomegranate wilt in pomegranate growing southern districts of Karnataka during 2018-19 and 2019-20.

Sr. No.	District	Taluk	Village	Latitude	Longitude	Age of the crop (Years)	Soil type	Other disease/pest
1	Bengaluru rural	Devanahalli	Koramangala	13.290722	77.751381	2.5	Red sandy	Anthraxnose, Bacterial blight
			Pura	13.308675	77.796326	3.0	Red sandy	Bacterial blight, Leaf spot
			Devanahalli	13.336963	77.711318	2.5	Red	Bacterial blight, Fruit rot
			Harohalli	13.315439	77.749044	4.0	Red loamy	-
			Vijayapura	13.245214	77.718415	2.0	Red	Leaf Spot
			Yaluvahalli	13.293557	77.822294	3.0	Red	Bacterial Blight
		Doddaballapura	Machagondahalli	13.346673	77.623100	2.0	Red	Leaf spot, Anthracnose
			Hosahudya	13.232192	77.537920	4.0	Red loamy	-
2	Bengaluru Urban	Bengaluru North	Nagadasanahalli	13.159507	77.578614	2.0	Red	Borers, Aphids
			Kalenahalli	13.147152	77.512071	4.0	Red sandy loam	Bacterial blight, Anthracnose
			GKVK, Hebbal	13.088367	77.567373	4.5	Red loamy	-
3	Ramanagara	Kanakapura	Uyyalappanahalli	12.686633	77.465412	3.0	Red sandy	Bacterial blight, Anthracnose
4	Chamarajanagara	Kollegala	Jakkalli	12.097187	77.139798	4.0	Black	Bacterial blight
			Thimmarajipura	12.107448	77.144762	2.5	Red	Bacterial blight
5	Tumakuru	Pavagada	K. T. Halli	14.114274	77.124571	2.5	Red sandy	-
			Tumakunte	14.058642	77.056395	3.0	Red sandy loam	Leaf blight, Anthracnose
			Arasikere	14.085778	77.044244	4.0	Red loamy	Bacterial Blight, Borer, Thrips, Mites
		Sira	Rangapura	13.792429	76.913186	4.0	Red sandy loam	Bacterial blight, Anthracnose, Fruit rot
			Thogaragunte	13.813368	76.911796	6.0	Red loamy	Bacterial blight, Anthracnose, Fruit rot
6	Chitradurga	Hiriyur	Dharmapura	14.057167	76.861711	3.0	Red sandy	Bacterial blight, Leaf spot
			Javagondnahalli	13.836875	76.745992	2.5	Red	-
			Maskal	13.988954	76.691627	4.0	Red loamy	Anthraxnose,
		Hosadurga	Ramajjanahalli	13.802282	76.289879	4.0	Red sandy loam	-
			Kurubarahalli	13.803818	76.307231	5.0	Red loamy	Bacterial blight

Table 3b: Survey for the incidence of pomegranate wilt in pomegranate growing southern districts of Karnataka during 2018-19 and 2019-20.

Sr. No.	District	Taluk	Village	Total number of plants observed	No. of infected plants	Wilt incidence (%)		
						Village	Taluk	District
1.	Bengaluru rural	Devanahalli	Koramangala	1200	50	4.16	5.03	7.36
			Pura	650	22	6.25		
			Devanahalli	800	25	3.12		
			Harohalli	980	120	11.50		
			Vijayapura	1000	20	2.00		
		Doddaballapura	Yaluvahalli	750	20	2.67		
			Machagondahalli	700	50	7.14	9.69	
			Hosahudya	980	120	12.24		
2.	Bengaluru urban	Bengaluru North	Nagadasanahalli	2100	55	2.61	9.35	9.35
			Kalenahalli	320	20	11.71		
			GKVK, Hebbal	80	11	13.75		
3.	Ramanagara	Kanakapura	Uyyalappanahalli	750	24	3.20	3.20	3.20
4.	Chamarajanagara	Kollegala	Jakkalli	650	80	12.30	8.77	8.77
			Thimmarajipura	800	42	5.25		
5.	Tumakuru	Pavagada	K. T. Halli	1200	30	2.50	3.91	13.20
			Tumakunte	650	22	3.38		
			Arasikere	1100	70	6.36		
		Sira	Rangapura	600	80	13.33	22.50	
			Thogaragunte	600	190	31.67		
			Dharmapura	2000	82	4.10		
6.	Chitradurga	Hiriyur	Javagondanahalli	1500	50	3.33	4.81	10.24
			Maskal	1000	70	7.00		
			Ramajjanahalli	1500	170	11.33		
		Hosadurga	Kurubarahalli	900	180	20.00		



Plate 4. Field view of pomegranate orchards visited during survey.

CONCLUSION

— Wilt affected pomegranate plants produced typical symptoms of yellowing and drooping of foliage of one or few branches of plants. In severe cases, the defoliation and complete wilting of plants were observed within 2-3 months.

— Pomegranate wilt incidence was varied from 2.00-31.67 per cent in the areas surveyed. The mean wilt incidence (13.20 %) was more in Tumakuru district followed by Chitradurga (10.24 %) whereas; the least incidence was recorded in Ramanagara district (3.20 %).

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Conflict of Interest. None.

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