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Deciphering Components of Wilt Complex in Pomegranate: A Case Study Based on Survey in Northern Karnataka

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Abstract: Fixed plot survey of 47 orchards were undertaken in major pomegranate growing areas of northern Karnataka comprising Bagalkote, Belagavi, Gadag, Koppal, and Vijayapura districts during 2017 and 2018 for recording the prevalence of wilt complex. The wilt incidence was noticed in all the surveyed locations except one, thus depicting range from zero to 46.3%. Among the districts surveyed, the maximum mean wilt incidence was recorded in Bagalkote (19.2%), followed by Belagavi (17.8%), whereas the least wilt incidence was recorded in Koppal (7.0%) district. In addition to wilt, maximum root knot nematode population (*Meloidogyne incognita*) was observed in Gadag district with a mean population of 2.7 cc^{-1} soil sample. Out of 47 orchards, the association of *C. fimbriata* (C), *F. oxysporum* (F), *M. incognita* (M), and shot hole borer was observed in 13 places. Association of C + F + S at 11 places, C + F + M at 1 place, C + F at 10 places, C+M at 3 places, C + S and F + M at 1 place each, C alone in 7 places and F alone in 1 place was recorded. *M. incognita* alone did not cause wilting of plant. Infestation of shot hole borer alone or with *F. oxysporum* or *M. incognita* was not observed in this study. This study clearly indicated that where the per cent disease incidence was higher, there was an association of nematode and shot hole borer.

Key words: Pomegranate, survey, wilt complex, root knot nematode, Bagalkote.

The pomegranate (*Punica granatum* L.), commonly known as Dadam or Anar, belongs to the family Punicaceae and is regarded as the "Fruit of Paradise". The scientific name *Punica granatum* is derived from the Latin names Pomum (apple) and granatus (grain). It originated in Iran. Pomegranate is one of the first five domesticated edible fruit crops, along with olive, grape, fig, and date palm. It is widely cultivated in tropical, subtropical, and Mediterranean regions and is considered an excellent fruit crop growing in arid zones for its tolerance to drought conditions. India is the one and only country where pomegranates are available throughout the year. In India, it is regarded as a "vital cash crop", grown in an area of 2,76,060 ha with a production of 31,48,320 tons with an average productivity of 11.40 tons ha^{-1} (Anonymous, 2021).

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Among the states, Maharashtra is the largest producer of pomegranates, occupying about 2/3 of the total area in the country, followed by Gujarat, Karnataka, Andhra Pradesh, and Rajasthan. Pomegranate in Karnataka covers an area of 27,680 ha with a production of 2,89,600 tons and a productivity of 10.46 tons ha^{-1} under tropical conditions (Anonymous, 2021). In Karnataka, it is grown in different districts, viz., Bagalkote, Belagavi, Bangalore, Bellary, Chitradurga, Davangere, Gulbarga, Gadag, and Tumkur.

Owing to its wider adaptability, drought tolerance, salt tolerance, hardy nature, higher yield levels, excellent keeping quality of fruit, and remunerative prices in the domestic as well as export markets, the area under pomegranate is increasing worldwide. It grows very well even in low fertility soils (Rao and Khandelwal, 2001; Marathe *et al*., 2009). But pomegranate cropping is largely limited by biotic stresses. Among the various diseases of pomegranate, the wilt complex popularly known as 'sooragu roga', caused by *Ceratocystis fimbriata* Ell. & Halst., *Fusarium oxysporum* Schlecht., and *Meloidogyne incognita* Chitwood, is becoming a major threat to production. Somasekhara (1999) reported that the wilt of pomegranates was caused by *Ceratocystis fimbriata* in India. Chavan and Dake (2001) collected the roots of wilt-infected pomegranate plants from Ahemdnagar and Solapur districts of Maharashtra and identified the fungus as *Fusarium oxysporum*. Tziros and Tzavella-Klonari (2008) first reported that *Verticillium dahlia* caused pomegranate wilt in Greece. Khosla and Bhardwaj (2013) reported that in Himachal Pradesh the incidence level of wilt disease caused by *C. fimbriata* and *F. oxysporum* varied from 1.03 to 15.3 and 0.1 to 7.3%. The wilt incidence in Karnataka is increasing day by day. Therefore, the present investigation was undertaken to assess the extent of disease incidence and also to understand the association of wilt pathogens with (i) shot hole borer (ii) nematode infection, (iii) soil properties and (iv) age of orchards in the major pomegranate growing districts of Karnataka.

Materials and Methods

A fixed plot survey was undertaken in major pomegranate growing districts, viz., Bagalkote, Vijayapura, Koppal, Belagavi, and Gadag districts of northern Karnataka, during 2017 and 2018. In each district, important pomegranate-growing *taluks* (Administrative block within a District) were identified and forty-seven orchards were selected for the study. The selected orchards were inspected for incidences of pomegranate wilt for two consecutive years 2017 and 2018. Plants were diagnosed on the basis of typical symptoms like yellowing of single branches from top to bottom at early stages and later progression of disease resulting in complete wilting of plants. The association of shot hole borer was noted based on its typical shot hole symptoms from the affected plant**.** The suspected plant parts, like roots, the collar region, and other branches, were collected separately from the orchards, labelled, and further pathogen isolation using potato dextrose agar media and microscopic confirmation studies were conducted under laboratory conditions. The per cent disease incidence in each orchard was calculated by the following formula: Per cent disease incidence = (Number of plants infected/Total number of plants examined) x 100

Composite soil samples were collected randomly from a depth of 15-20 cm in the rhizosphere region of 10-15 plants with the help of a shovel. Each sample consisted of 200 cc of soil and 5 g of root. All the samples were stored in polythene bags, sealed tightly with a rubber band, and labelled. The soil and root samples were processed on the same day or kept in the refrigerator at 4^{o}C for two days. Subsequently the soil samples were processed for estimation of nematode population. For this the known quantity (200 cc) of soil from each sample was processed by combined Cobb Sieving (using 20, 60 and 325 mesh sieves) and Baermann's funnel technique (Ayoub, 1977). The nematode population in soil was counted under stereo microscope.

Results

Wilt incidence in northern Karnataka

Wilt infected plant and infection of *Ceratocystis fimbriata*, *Fusarium oxysporum*, root knot nematode and shot borer is shown in Fig. 1. Data presented in Table 1 revealed that disease incidence in all the surveyed areas ranged from 0.0-35.4% and 0.0-46.3% during 2017 and 2018 respectively. Maximum mean per cent wilt incidence was recorded in field-1 of Alliyabad village in Vijayapura

Table 1. Survey on wilt disease of pomegranate in different regions of northern Karnataka during 2017-18

District	Taluk	Village	Variety	Age (Yrs)	Bahar	Soil type	Wilt PDI (2017)	Pathogens involved (2017)	Shot hole borer (2017)	Wilt PDI (2018)	Pathogens involved (2018)	Shot hole borer (2018)
Bagalkote	Bagalkote	Devanal	Bhagwa	4	Mirg	Sandy loam	10.2	C, F	-	12.4	C, F	-
		Govindgoppa	Bhagwa	7	Mirg	Sandy loam	16.0	C, F, M	+	18.4	C, F, M	+
		Kaladagi-1	Bhagwa	6	Mirg	Sandy loam	18.9	C, F	+	21.9	C, F	+
		Kaladagi-2	Bhagwa	5	Mirg	Red	4.8	C, F	+	8.4	C, F, M	+
		Sokanadgi	Bhagwa	4	Mirg	Sandy loam	3.4	C, F	-	4.3	C, F	-
		Tulasigere-1	Bhagwa	3	Mirg	Red	1.3	C	+	5.8	C,F	+
		Tulasigere-2	Bhagwa	4	Mirg	Red	4.0	C, F	-	5.7	C, F	-
		Tulasigere-3	Bhagwa	3	Mirg	Red	2.3	C, F	-	5.4	C, F	-
						Mean	7.6		Mean	10.3		
	Mudhol	Mudhol	Bhagwa	8	Mirg	Sandy loam	27.2	C, F, M	+	31.6	C, F, M	+
		Mahalingapur	Bhagwa	6	Mirg	Sandy loam	28.3	C, F	-	33.8	C, F	-
		Lokapur	Bhagwa	7	Mirg	Sandy loam	25.5	C, F	+	29.9	C, F	+
						Mean	27.0		Mean	31.8		
Belagavi	Athani	Bedarahatti	Ganesh	9	Mirg	Sandy loam	7.9	C,F	+	9.3	C,F	+
		Kottalgi-1	Bhagwa	8	Mirg	Red	9.7	C,F	+	11.4	C,F	+
		Kottalgi-2	Bhagwa	6	Mirg	Red	6.7	C	-	8.4	C	-
						Mean	8.4		Mean	9.7		
	Chikodi	Examba-1	Bhagwa	11	Mirg	Sandy loam	22.5	C,F,M	+	24.8	C,F,M	+
		Examba-2	Bhagwa	12	Mirg	Sandy loam	24.3	C,F,M	+	27.3	C,F,M	+
						Mean	23.4		Mean	26.0		
	Savadatti	Chachadi	Bhagwa	6	Mirg	Red	3.2	C,F	-	6.2	C,F	-
		Hirebudanur	Bhagwa	11	Mirg	Red	32.8	C,F,M	+	36.8	C,F,M	+
						Mean	18.0		Mean	21.5		
Gadag	Mundargi	Mundargi-1	Bhagwa	8	Hasta	Sandy loam	32.3	F, M	-	38.6	F, M	-
		Mundargi-2	Bhagwa	7	Hasta	Sandy loam	15.3	C, M	-	17.0	C, M	-
		Naharahalli-1	Bhagwa	2	Hasta	Red	0.6	C	-	0.8	C	-
		Naharahalli-2	Bhagwa	2	Hasta	Red	0.0	-	-	0.0	-	-
		Ramenahalli-1	Bhagwa	3	Hasta	Red	1.1	F	-	1.6	F	-
		Ramenahalli-2	Bhagwa	4	Hasta	Red	4.2	C, F	-	6.6	C, F	-
						Mean	8.9		Mean	10.8		
	Ron	Kotabal-1	Bhagwa	5	Hasta	Sandy loam	11.9	C, F, M	+	12.8	C, F, M	+
		Kotabal-2	Bhagwa	3	Hasta	Red	5.4	C,M	-	7.4	C, M	-
						Mean	8.7		Mean	10.1		

PDI-Per cent disease incidence, C-*Ceratocystis fimbriata,* F- *Fusarium oxysporum,* M- *Meloidogyne incognita,*+ Present, - Absent
Bahar: Season of flowering and fruiting (Mirg: June-July; Hasta: Sept. - Oct.)

Table 1. Continued ...

District	Taluk	Village	Variety	Age (yr)	Bahar	Soil type	Wilt PDI (2017)	Pathogens involved (2017)	Shot hole borer (2017)	Wilt PDI (2018)	Pathogens involved (2018)	Shot hole borer (2018)
Koppal	Kushtagi	Hanamsagar-1	Bhagwa	6	Hasta	Red	5.7	C, F	+	7.3	C, F	+
		Hanamsagar-2	Bhagwa	7	Hasta	Red	6.6	C, F	+	9.2	C, F,	+
		Katapura	Bhagwa	3	Hasta	Red	3.4	C,M	-	5.7	C,M	-
						Mean	5.2		Mean	7.4		
	Yelburga	Bevoor-1	Bhagwa	4	Hasta	Red	2.2	C	-	4.1	C	-
		Bevoor-2	Bhagwa	5	Hasta	Red	4.8	C	+	6.2	C	+
		Kalkbandi-1	Bhagwa	8	Hasta	Red	5.3	C, F, M	+	6.6	C, F, M	+
		Kalkbandi-2	Bhagwa	7	Hasta	Red	4.6	C, F, M	+	5.6	C, F, M	+
		Mandalmari-1	Bhagwa	5	Hasta	Red	13.4	C, F	+	15.4	C, F	+
		Mandalmari-2	Bhagwa	6	Hasta	Red	10.4	C, F, M	-	13.6	C, F, M	-
						Mean	6.8		Mean	8.6		
Vijayapura	Indi	Atharga-1	Bhagwa	7	Mirg	Sandy loam	4.1	C,F.M	+	5.5	C, F, M	+
		Atharga-2	Bhagwa	3	Mirg	Red	0.5	C	-	1.3	C	-
		Indi	Bhagwa	6	Mirg	Sandy loam	5.7	C,F	+	7.6	C, F	+
						Mean	3.4		Mean	4.8		
	Vijayapura	Aliyabad-1	Bhagwa	5	Mirg	Sandy loam	35.4	C, F, M	+	46.3	C, F, M	+
		Aliyabad-2	Bhagwa	3	Mirg	Red	5.8	C, F	-	8.2	C, F	-
		Babaleshwar-1	Bhagwa	6	Mirg	Sandy loam	28.4	C, F	-	32.1	C, F	-
		Babaleshwar-2	Bhagwa	7	Mirg	Sandy loam	25.8	C, F, M	+	29.4	C, F, M	+
		Jambagi	Bhagwa	5	Mirg	Sandy loam	4.1	C, F, M	+	5.6	C, F, M	+
		Kumatagi-1	Bhagwa	7	Mirg	Sandy loam	17.3	C, F	+	21.40	C, F	+
		Kumatagi-2	Bhagwa	6	Mirg	Sandy loam	27.1	C, F	+	30.07	C, F	+
		Nagathan-1	Bhagwa	5	Mirg	Sandy loam	15.3	C, F	-	18.73	C,F	-
		Nagathan-2	Bhagwa	4	Mirg	Sandy loam	15.5	C	-	19.60	C	-
						Mean	19.4		Mean	**23.4**		

PDI-Per cent disease incidence, C-*Ceratocystis fimbriata,* F- *Fusarium oxysporum,* M- *Meloidogyne incognita,* + Present, - Absent
Bahar: Season of flowering and fruiting (Mirg: June-July; Hasta: Sept. - Oct.)

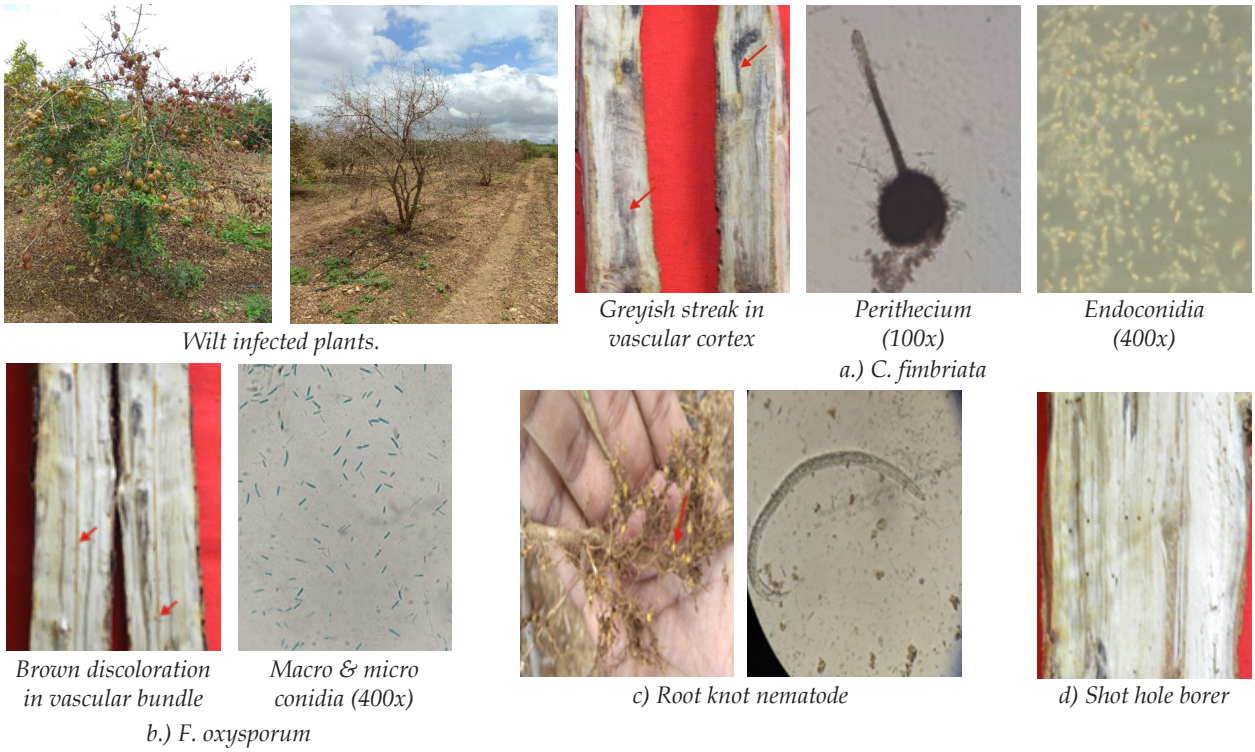

Fig. 1. Etiology of pomegranate wilt complex-recorded from survey.

taluk of Vijayapura district, which was followed by Hirebudanur in Savadatti *taluk* of Belagavi district. Least per cent wilt incidence (0.0%) was recorded in Naharahalli-2 field in Mundargi *taluk* of Gadag district. Among the *taluks* surveyed, the maximum incidence was noticed in Mudhol *taluk* of Bagalkote district followed by Chikodi *taluk* of Belagavi district and the least incidence was noticed in Indi *taluk* of Vijayapura district. Among the districts surveyed, the maximum incidence was noticed in Bagalkote district followed by Belagavi district and the least incidence was recorded in Koppal district.

The pooled data on disease incidence presented in Fig. 2 revealed that maximum wilt incidence was recorded in Bagalkote (19.2%) followed by Belagavi (17.8%) district, whereas least wilt incidence was recorded in Koppal (7.0%) district. Among the *taluks* surveyed the maximum disease incidence (29.3%) was

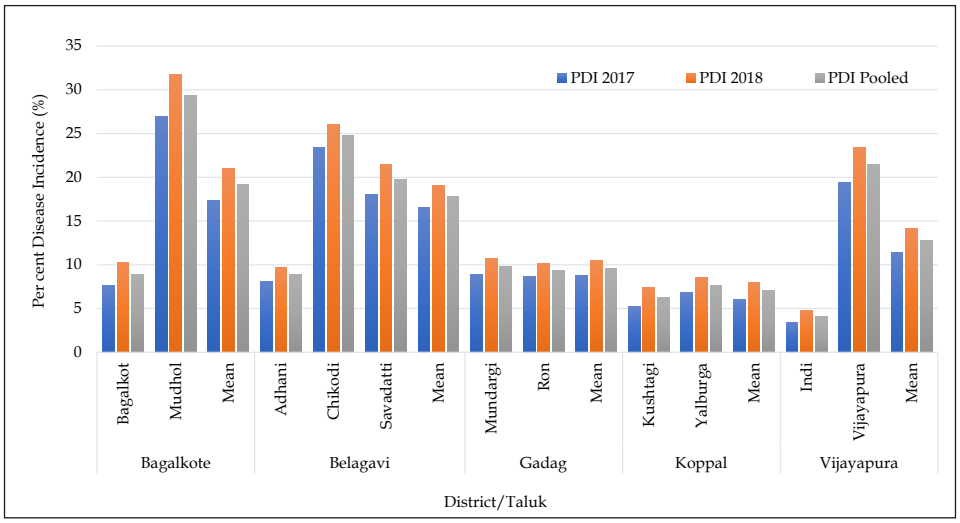

Fig. 2. Prevalence of incidence of wilt complex in different districts and Taluks of northern Karnataka during 2017 and 2018.

Table 2. Enumeration of Root knot nematode in northern Karnataka (2017)

District	Taluk	Village	No. of *M. incognita* (cc^{-1} of soil sample)	District mean
Bagalkote	Bagalkote	Devanal	0.0	1.1
		Govindgoppa	3.3	
		Kaladagi	1.5	
		Tulasigere	0.0	
		Sokanadgi	0.0	
	Mean		0.95	
	Mudhol	Lokapur	0.0	
		Mahalingapur	0.0	
		Mudhol	4.1	
	Mean		2.0	
Belagavi	Athani	Bedarhatti	0.0	1.1
		Kottalgi	0.0	
	Mean		0.0	
	Chikodi	Examba	2.1	
	Mean		2.1	
	Savadatti	Chachadi	0.0	
		Hirebudanur	3.6	
	Mean		1.8	
Gadag	Mundargi	Mundargi	2.6	2.7
		Naharahalli	0.0	
		Ramanahalli	0.0	
	Mean		2.1	
	Ron	Kotabal	8.1	
	Mean		8.1	
Koppal	Kushtagi	Hanamsagar	0.0	1.1
		Katapura	5.1	
	Mean		2.6	
	Yelburga	Bevoor	0.0	
		Kalkbandi	0.5	
		Mandalmari	0.1	
	Mean		0.2	
Vijayapura	Vijayapura	Aliyabad	4.3	0.3
		Babaleshwar	1.3	
		Jambagi	1.4	
		Kumatagi	0.0	
	Mean		1.7	
	Indi	Atharga	0.1	
		Indi	0.0	
	Mean		0.1	

noticed in Mudhol *taluk* of Bagalkote district followed by Chikodi (24.7%) *taluk* of Belagavi district and the least incidence (4.1%) was recorded from Indi *taluk* of Vijayapura district.

Association of pathogens and shot hole borer in wilt complex disease of pomegranate from surveyed areas

The data presented in Fig. 3 revealed that infection of *Ceratocystis fimbriata* alone led to wilt in five orchards and of Fusarium *oxysporum* alone in one orchard. Wilt was caused by combination of *C. fimbriata* + *M. incognita* in three orchards; Combination of *, F. oxysporum* + *M. incognita* and of *C. fimbriata* + shot hole borer was responsible for wilt in single orchard. *C. fimbriata* + *F. oxysporum* combination was found in ten orchards, *C. fimbriata* + *F. oxysporum* + shot hole borer in eleven orchards, *C. fimbriata* + *F. oxysporum* + *M. incognita* in one orchard and *C. fimbriata* + *F. oxysporum* + *M. incognita*

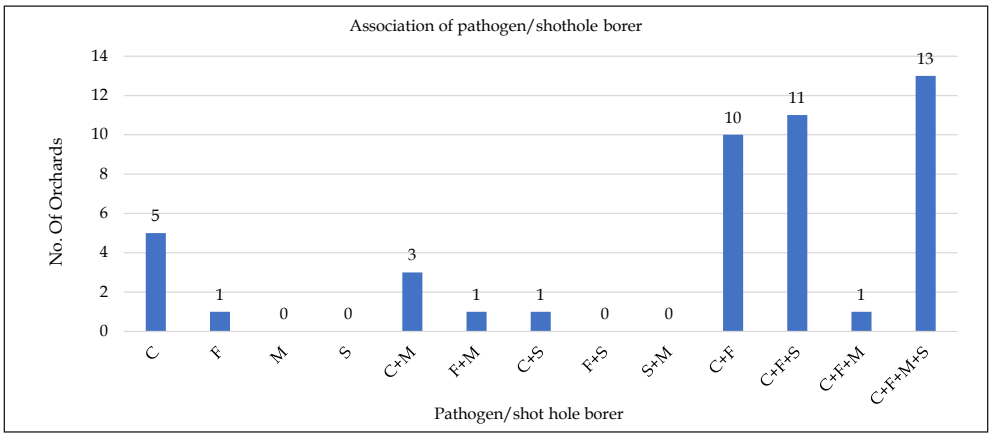

Fig. 3. Association of the pathogens and shot hole borer in wilt complex of pomegranate from surveyed areas (2018).

+ shot hole borer respectively in thirteen orchards. *M. incognita* alone did not cause wilting of plant. Either shot hole borer alone or with *F. oxysporum* or *M. incognita* was not observed in this survey.

Prevalence of wilt complex of pomegranate as influenced by type of soil

In northern Karnataka pomegranate is mostly growing in red and sandy loam soil. The data presented in Fig. 4 depicts that, among two type of soil the maximum disease incidence was noticed in sandy loam soil with mean incidence of 18.3% in 2017 and 22.1% in 2018 survey. In red soil, the recorded mean per cent wilt incidence was 4.0 and 7.4%, respectively, in 2017 and 2018.

Influence of age of pomegranate on per cent wilt incidence

The data presented in Fig. 5 depicts that the age of pomegranate influences the severity of wilt complex. Wilting was observed in plants with all age groups. However, as age of the crop increased the disease incidence also increased. While comparing the plant with below 5 years age group, the plants with more than 5 years had shown more wilt incidence.

Enumeration of root-knot nematode

The data presented in the Table 2 revealed that among districts surveyed maximum root knot nematode population was observed in Gadag district with mean population of 2.7 cc^{-1} soil. In case of *taluks*, maximum population was observed in Ron *taluk* with 8.1 cc^{-1} of soil sample, followed by Kushtagi (2.6 cc^{-1} soil). Among all the villages s maximum number of *Meloidogyne incognita* (8.1 cc^{-1}soil) was recorded in Kotabal of Gadag district followed by Katapura of (5.1 cc^{-1} soil) of Koppal district. Root knot nematode was not found in 14 out of 29 villages surveyed.

Discussion

Among the *taluks* surveyed, the mean maximum incidence was noticed in Bagalkote district followed by Belagavi district. Hence the place Bagalkote can be considered as hotspots for pomegranate wilt. The wilt incidence increased as the age of the crop increased,

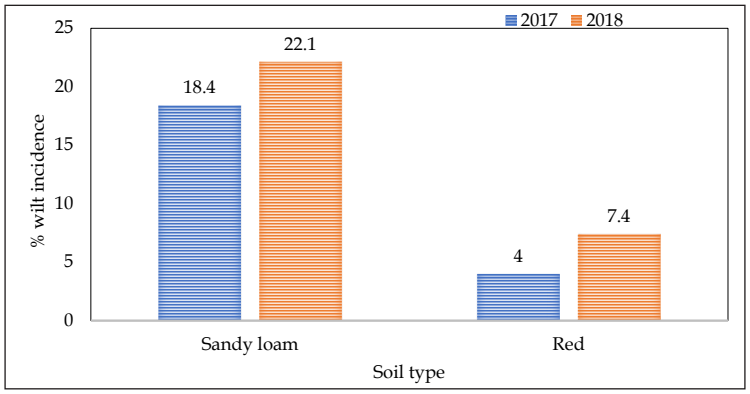

Fig. 4. Prevalence of pomegranate wilt complex as influenced by soil type.

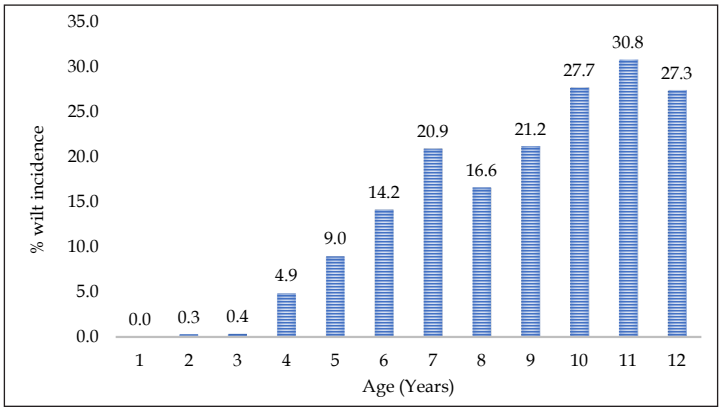

Fig. 5. Per cent wilt incidence of pomegranate in relation to age of the crop.

it may be due to incidence of nematode and shot hole borer (Somu *et al.,* 2018). The disease incidence varied from locality to locality which may be due to different soil, environmental conditions and build up of inoculum. Our results are in agreement with studies conducted by Jadhav and Sharma (2009), Balaganur (2016) and Somu *et al.* (2018).

In the surveyed locations it was also observed that, where the disease incidence was higher, there was association of nematode and shot hole borer. The presence of plant parasitic nematode has increased the severity of wilt disease by pre-disposing the host roots for easy colonization by *Ceratocystis fimbriata* and *Fusarium oxysporum*. It is in accordance with the study conducted by Sonyal *et al.* (2016). The incidence of shot hole borer increases wilt disease by secondary spread from already infected plant. It was noticed only in five and above year age old plants. Even the incidence of root-knot nematode was also observed in five and above year old plant. However, wilt disease was reported in the absence of shot hole borer and nematode association. These findings are in good agreement with the results of Balaganur (2016) and Sonyal *et al.* (2016). Since the new varieties like Bhagwa and Kesar have replaced the earlier varieties and also continuous mono-cropping of pomegranate might have resulted in the increase of wilt incidence. In case of type of soil, cultivation in sandy loam soil showed higher disease incidence as compared to red soil.

Conclusion

The survey brought out that the wilt disease of pomegranate is caused by (i) either *Ceratocystis fimbriata* or *Fusarium oxysporum* (ii) a complex involving both fungi and (iii) combination of both fungi with the nematode *Meloidogyne incognita*. It was also observed that infestation of insect pest (shot hole borer) helps in the dissemination of fungus. The severity of diseases increases when the number of pathogen involved increased and orchards of more than 5 years age are more vulnerable.

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