

Original Research Article

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Prevalence of *Phytophthora* spp. In Citrus Nurseries of Vidarbha Region

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ABSTRACT

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Phytophthora disease has been identified as one of the major causes of decline in citrus. It causes the most serious and economically important soil borne diseases to citrus crops. In year 2016 and 2017 soil samples were collected from Thirty eight (38) surveyed nurseries during August to November. Three species of *Phytophthora* viz. *P. parasitica*, *P. citrophthora* and *P. palmivora* found associated to cause damping off, collar rot and root rot in citrus. In the present studies these three of *Phytophthora* were isolated by using CMA-PARPH medium from collected soil samples. The occurrence of *Phytophthora* spp. was recorded in 35 out of 38 surveyed locations, with a range from 3.10 to 24.33 cfu / g of soil in the year 2016 and 2.33 to 24.99 cfu / g of soil in year 2017. Pooled data of infected nurseries indicate propagules in the range of 2.94 to 24.66 cfu/g of soil. The morphological characters like mycelium and its appearance, sporangial papillation and their shapes and presence of chlamydospores of different isolated species were recorded. Presence of chlamydospores was observed only in *P. nicotianae* var. *parasitica* which were present as intercalary with oval shape and thick wall.

Introduction

Citrus occupies the position of most remunerative fruit crops of India, having its own niche in the international trade and world finance. Nagpur mandarin, Sweet orange and Acid lime are the three important species of citrus having international acceptance. This is the second largest industry in India and third largest in production. Citrus is primarily grown in Andhra Pradesh, Maharashtra, Punjab, Karnataka, Uttaranchal, Bihar, Orissa, Assam and Gujarat (Lakshmi *et al*, 2014).

Phytophthora disease has been identified as one of the major causes of decline in citrus. It causes the most serious and economically important soil borne diseases to citrus crops. Three species of *Phytophthora* viz. *P. parasitica*, *P. citrophthora* and *P. palmivora* found associated to cause damping off, collar rot and root rot in citrus (Naqvi, 1988; Gade, 2012).

The pathogen attacks citrus plants right from nursery causing damping off of seedlings, decay of fibrous roots, crown rot, collar rot, foot rot and gummosis in mature orchards.

Nagpur mandarins face short life span and low productivity because of these three species of *Phytophthora* (Das *et al.*, 2011). Damping off in citrus seedlings occurred up to 24 per cent due to *Phytophthora*, thereafter, 5-15 per cent mortality occurred in secondary beds (Naqvi, 2006).

Survey have been undertaken to see the association of *Phytophthora* spp. in Vidarbha region where nearly 80L grafts are being raised for sale every year (Gade, 2012). It is very well known that infected nursery plants are one of the major ways of movement of *Phytophthora* spp. in orchards.

The soil borne pathogen '*Phytophthora*' causes tremendous losses in nurseries and orchards of Nagpur mandarin in Vidarbha region of Maharashtra and all over India is well. Unfortunately, since last 10 years or so, growing Nagpur Mandarin has no longer remained a profitable proposition, as it is being increasingly realized by citrus growers, beside with one or other problems. Nevertheless, the area under Nagpur mandarin has considerably increased mainly due to implementation of the different missions of the central government.

The purpose of current study is to estimate prevalence of *Phytophthora* spp. in selective citrus nurseries and orchards. An intensive rapid roving survey of nurseries in contributes to assess the present status, distribution of plant pathogens prevalent in particular areas. Such surveys are helpful to characterize local isolates of pathogen.

The survey and current status about availability of disease free planting material in study area is also one of the initial views of this research work. It is generally agreed that the environment is the driving force in the development of epidemics (Hardwick, 1998).

Materials and Methods

Survey of citrus nursery and collection of soil samples

An extensive survey of citrus nurseries of Amravati district has been made in year 2016 and 2017 and soil samples were collected from Thirty eight (38) surveyed nurseries during August to November. Samples from particular nursery were collected from rhizosphere and combined to make a composite sample (Naqvi, 1994; Timmer *et al.*, 1989).

Isolation purification of pathogen culture

Ten gram soil from each sample was diluted in 90 ml water having 0.25% agar. One ml aliquot was spread on each of 10 plates of Corn meal agar (CMA-PARPH) selective medium (Kannwischer and Mitchell, 1978). The plates were incubated at 28⁰C for 2-3 days and number of colonies of *Phytophthora* was counted. Soil in the second core was flooded with water, baited with pieces of citrus leaves and placed in the incubator for 48hr (Grimm and Alexander, 1973). The leaves were transferred to petri dishes and examined for the presence of papillate sporangia under compound microscope. Subculture of *Phytophthora* from infected leaf pieces was easily accomplished by submerging infected leaf pieces in PARPH medium and transferring hyphal tips as they grew in the medium. As soon as the growth of fungus was observed, small portion was transferred to the slants.

Composition of CMA-PARPH selective media (Mitchell *et al.*, 1986)

Corn meal agar- 17 g/ lit, Pimaricin- 10 mg/ lit, Ampicilin- 250 mg/ lit, Refampicin- 10 mg/ lit, PCNB- 100 mg/ lit, Hymexazole- 50 mg/ lit, Distilled water-1000 ml.

Production of sporangia

Sporangia are the cells or vessels in which zoospore are formed. Production of sporangia was carried out by the water culture method. A thin layer of sterile distilled water is added to the surface level of discs in Petri plates containing discs of fungus growth removed from culture. Plates are incubated under cool white fluorescent light for 1-3 days and observed daily under the dissecting scope for sporangia.

Morphological Identification and characterization of the pathogen

Phytophthora spp. was observed under 40X magnification on compound microscope and identified on the basis of their morphological characteristics like mycelium structure, shape of sporangia, presence of chlamyospore etc. and confirmed with the help of CMI publications.

Results and Discussion

Survey of citrus nurseries

Citrus nurseries were surveyed in Amravati district of Vidarbha region of Maharashtra. Citrus nurseries in this district were selected during 2016 and 2017 to record prevalence of *Phytophthora* spp. Soil samples from the nurseries were collected during prevalence of the disease in citrus nursery. Collected samples were subjected for isolation of *Phytophthora* spp. on selective medium (CMA-PARPH).

The occurrence of *Phytophthora* spp. was recorded in 35 out of 38 surveyed locations, with a range from 3.10 to 24.33 cfu / g of soil in the year 2016 and 2.33 to 24.99 cfu / g of soil in year 2017. Pooled data of infected nurseries indicate propagules in the range of 2.94 to 24.66 cfu/g of soil (Table 1). Three

Phytophthora spp. viz., *Phytophthora nicotianae* var. *parasitica*, *P. citrophthora* and *P. palmivora* were isolated from collected samples. *P. nicotianae* var. *parasitica* was found prominently involved in causing damping off, collar rot and root rot in surveyed citrus nurseries.

The isolation of *Phytophthora* was done by using leaf bait technique (Grimm and Alexander, 1973) and soil spreading (Kannwisher and Mitchell, 1978) method. *Phytophthora* was isolated on CMA-PARPH medium (Michell *et al.*, 1986). The use of selective medium was found effective in the detection of *Phytophthora* when low inoculum was present in the soil (Zitko *et al.*, 1987; Gade, 2009).

For propagule count soil spreading method was used accordingly 35 out of 38 soil samples showed presence of *Phytophthora* in citrus nurseries (Table 1). Presence of the pathogen may be because of raising of nurseries on same piece of land and to the roadside to attract the customer and free access to enter in to the nursery. Pathogen population can be build up even phytosanitary measures are taken. Ridings *et al.*, (1977) showed that even with strict sanitary practices, recontamination of disinfected areas occurred when it was present near to the nursery.

Gade (2009) and Gade (2012) reported that *Phytophthora parasitica* is associated with disease in citrus nurseries of Vidarbha in the range of 28 to 46 cfu/g soil. Present results of association of *Phytophthora* with citrus rhizosphere confirms the findings of Das *et al.*, (2016) who reported similar results and isolated *Phytophthora palmivora*, *P. parasitica* and *P. citrophthora* from the soil samples collected from citrus growing areas of Nagpur, Wardha and Amravati districts.

Table.1 Prevalence of *Phytophthora* spp. in citrus nurseries of Amravati district

Sr. No.	Location	GPS (Latitude/Longitude)	Average cfu / g soil		
			2016	2017	Pooled
1	Badnera	20.844719/77.721848	10.44	14.21	12.33
2	Shivaji Agri. College	20.950424/77.775459	8.88	13.10	11.00
3	Govt. Nursery	20.948980/77.781731	6.77	9.0	7.89
4	Jarud (Hole Nursery)	21.457016/78.228668	12.22	21.77	17.00
5	Tembhurcheda	21.504112/78.215836	11.77	14.77	13.27
6	Warud	21.496664/78.266365	10.11	6.88	8.50
7	Wai mauje (S. ghat)	21.503023/78.268936	4.11	2.33	3.22
8	Wai mauje (S. ghat) B	21.503023/78.268936	5.88	5.99	5.94
9	Tivsa ghat	21.511379/78.271576	00	00	0.00
10	Pimpalsheda A	21.527672/78.262978	10.33	7.99	9.17
11	Pimpalsheda B	21.526918/78.258087	4.22	3.66	3.94
12	Zatamziri A	21.541759/78.237930	8.55	2.99	5.77
13	Zatamziri B	21.534712/78.246094	7.10	7.33	7.22
14	Rawala A	21.528708/78.275536	6.33	12.55	9.44
15	Rawala B	21.559467/78.276146	7.55	10.32	8.94
16	Satnur	21.566528/78.283066	5.33	5.88	5.61
17	Shendurjana ghat (Bhavesh Nursery)	21.538845/78.296150	6.10	7.10	6.61
18	Shendurjana ghat A	21.521938/78.290787	3.10	2.77	2.94
19	Shendurjana ghat B	21.525549/78.295952	00	00	0.00
20	Mauja jamtal	21.530968/78.303642	3.66	2.88	3.28
21	Wai	21.567837/78.335106	4.88	7.88	6.39
22	Jamtal	21.537741/78.308250	6.33	2.55	4.44
23	Shendurjana ghat C	21.514324/78.297485	4.44	5.44	4.94
24	Malakapur (S. ghat)	21.508900/78.299469	4.77	5.99	5.39
25	Loni A	21.381285/78.174507	10.99	13.21	12.11
26	Loni B	21.374554/78.174927	24.33	24.99	24.66
27	Bargaon	21.438925/78.132545	5.77	10.99	8.39
28	Jarud B	21.455263/78.206528	12.66	13.21	12.94
29	Zolamba	21.381041/78.118736	10.77	15.88	13.33
30	Hiwarkheda A	21.395351/78.069649	9.88	11.77	10.83
31	Hiwarkheda B	21.407637/78.077736	9.10	10.66	9.89
32	Govt. farm Umarkhed	21.425966/78.105476	00	00	0.00
33	Hiwarkheda C	21.412468/78.087799	10.66	11.77	11.22
34	Bhaypur	21.377542/78.054153	6.77	8.22	7.50
35	Govt farm Yerla	21.335150/78.003914	5.10	9.66	7.39
36	Bhumiputra nursery Khed	21.278652/77.862617	16.99	10.22	13.61
37	Govt. nursery Tivsa	21.093372/78.090927	6.44	11.00	8.72
38	Govt. nursery Chandur	20.816896/77.981674	4.88	4.66	4.78

Table.2 Morphological characteristics of *Phytophthora* spp.

Sr. No.	Characters	Species		
		<i>Phytophthora palmivora</i>	<i>Phytophthora nicotianae</i> var. <i>parasitica</i>	<i>Phytophthora citrophthora</i>
Mycelium and chlamydospore				
1	Mycelium	Coenocytic, cottony mycelium	Coenocytic, dense cottony Mycelium	Coenocytic hyaline to slightly brownish
2	Colony colour	Puffy dull white with stellate pattern	creamy white	White rosette and slightly cottony, Pettaloid pattern
3	Chlamydospore	Not recorded	Intercalary	Not recorded
Sporangia				
4	Shape	Mainly ovoid	Speroid (spherical), prominent papilla	Varies from spherical, ovoid
5	Papillation	distinctly pappillate	prominent papilla	Papillate

Fig.1 Microphotographs of sporangia of *Phytophthora* spp.

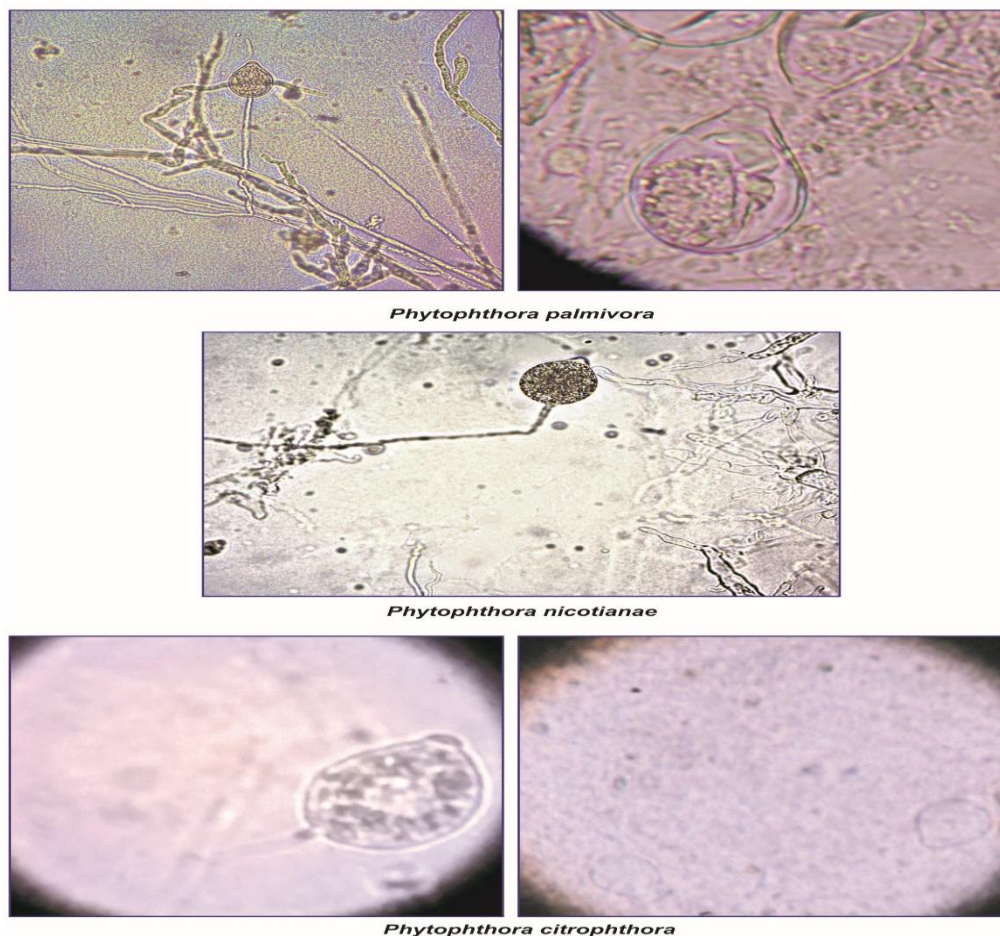
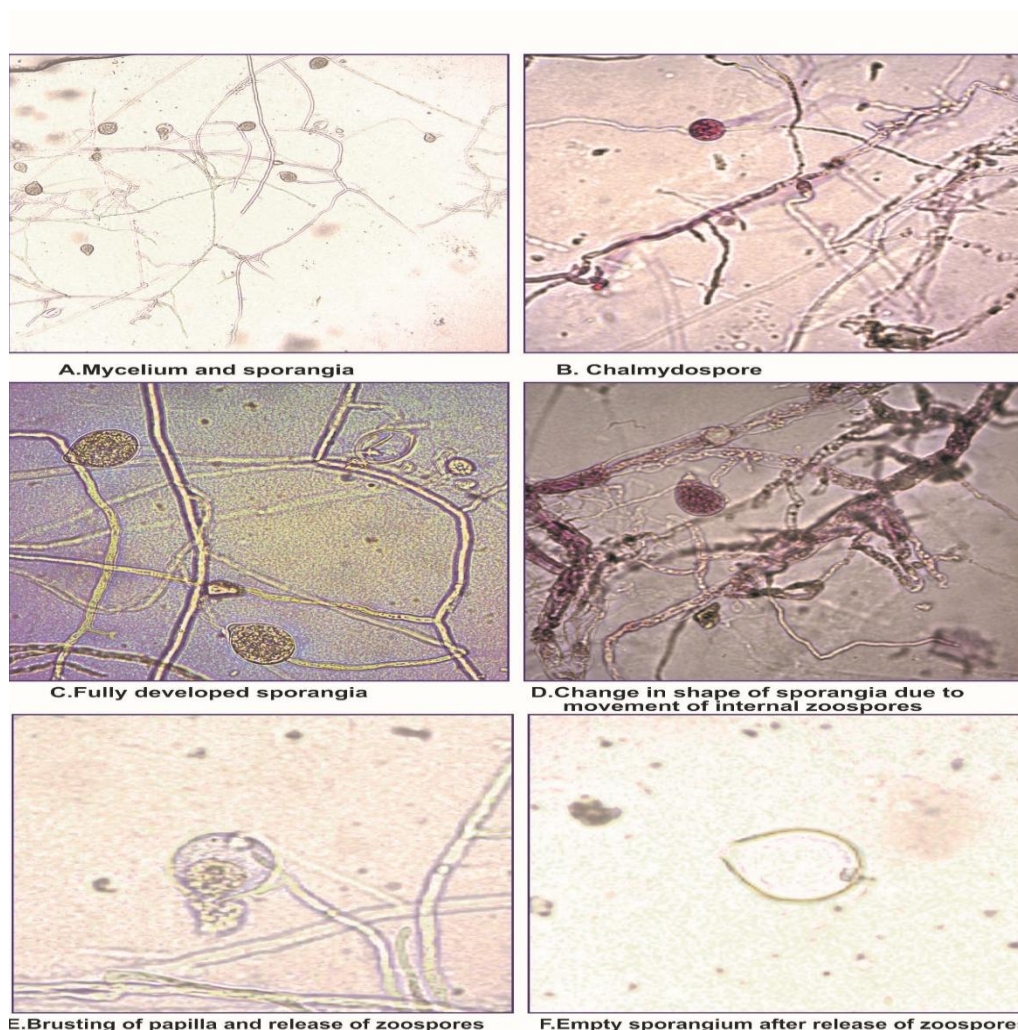


Fig.2 Microphotographs of morphological changes in zoosporangia and release of zoospores from sporangia of *Phytophthora* spp.



Present results are helpful to show current status of citrus nurseries regarding *Phytophthora* population and accordingly to plan management strategies in study area on the basis of detected inoculum level present in the soil.

Morphological characteristics of *Phytophthora* spp.

In the present studies three *Phytophthora* spp. viz., *Phytophthora palmivora*, *P. nicotianae* var. *parasitica* and *P. citrophthora* were isolated by using CMA-PARPH medium from collected soil samples from experimental

fields. The morphological characters like mycelium and its appearance, sporangial papillation and their shapes and presence of chlamydospores of different isolated species were recorded.

The results presented in Table 2 showed that *P. palmivora* produced a puffy, stellate pattern whereas, *P. nicotianae* var. *parasitica* produce dense cottony mycelium having creamy white colony colour whereas, hyaline to slightly brownish mycelium and white rosette to slight cottony colonies with petaloid pattern were produced by *P. citrophthora*. Coenocytic mycelium was the

particular characteristic found in all three species. Ovoid sporangia were observed prominently in *P. palmivora* whereas, prominent papillation was observed in *P. nicotianae* var. *parasitica* and spherical to ovoid sporangia was observed in *P. citrophthora*. Presence of chlamydospores was observed only in *P. nicotianae* var. *parasitica* which were present as intercalary with oval shape and thick wall (Figure 1 & 2).

Morphology of *Phytophthora* has been well determined in past (Brasier and Griffin, 1979). More than 50 species have been identified based on morphological characteristics (Stamps *et al.*, 1990). The observations recorded in respect of mycelium, sporangial shape and papillation are in agreement with the findings of Drenth and Sendall (2001) who reported that, sporangium shape, papillation, presence of chlamydospores are the morphological characters upon which identification of *Phytophthora* was based and did morphological study of commonly found *Phytophthora* spp. of southeast Asia. Results were also correlate with the work of Das *et al.*, (2016) who reported that, morphology of *P. nicotianae* isolate produced spheroid sporangium that were non caduceus and papillate, whereas *P. palmivora* isolates produce ovoid papillate sporangia. Gupta *et al.*, (2012) also observed creamy white cultural characters in most of studied *P. nicotianae* isolates.

More diversity was observed in the colony morphology of isolated *Phytophthora* spp. A different colony patterns were observed in these three species of *Phytophthora* indicating a high level of morphological diversity within the species. Diverse sporangial shapes and structures observed in various *Phytophthora* spp. were ovoid to spherical/ globose. These findings are in agreements with Mounde *et al.*, (2012) who studied morphological and

cultural characters like colony diameter, size, shape, length breadth ratio of sporangia, hyphae diameter, production of chlamydospores and found differences in these characters of *P. citrophthora*, *P. nicotianae* and *P. syringae*.

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